

UDK 615 (497.11)

ISSN 0004-1963 (Štampano izd.)

ISSN 2217-8767 (Online)

ARHIV ZA FARMACIJU

Godina 69

Broj 6

Beograd, 2019.

ČASOPIS SAVEZA
FARMACEUTSKIH
UDRUŽENJA SRBIJE

TEMATSKI BROJ

VAKCINE: DILEME, IZAZOVI I PERSPEKTIVE

6/2019

SADRŽAJ – CONTENTS

Reč gostujućeg urednika

Pregledni radovi – Review articles

- **Nevena Arsenović Ranin** 385
Perspektive u razvoju profilaktičkih vakcina
New vaccines on the horizon

- **Brankica Filipić, Zorica Stojić-Vukanić** 406
Adjuvansi u vakcinama registrovanim za primenu kod ljudi
Adjuvants in vaccines registered for human use

- **Danina Krajšnik, Tanja Ilić, Ines Nikolić, Snežana Savić** 420
Established and Advanced Adjuvants in Vaccines' Formulation: Mineral Adsorbents, Nanoparticulate Carriers and Microneedle Delivery Systems
Konvencionalni i napredni adjuvansi u formulacijama vakcina: mineralni adsorbenti, nanočestični nosači i sistemi tipa mikroigala

- **Srdja Janković** 452
**Childhood Vaccination in the Twenty-First Century:
Parental Concerns and Challenges for Physicians**
**Vakcinacija dece u dvadeset prvom veku:
nedoumice za roditelje i izazovi za lekare**

- **Biljana Bufan** 469
Primena profilaktičkih vakcina kod starih
Application of prophylactic vaccines in the elderly

- **Brankica Filipić, Zorica Stojić-Vukanić** 490
Aktivna imunoterapija malignih tumora: pregled terapijskih vakcina
Active immunotherapy of cancer: an overview of therapeutic vaccines

Prilozi – Contributions

- **120 GODINA OD ROĐENJA PROVOG SRPSKOG TOKSIKOLOGA, VELIKANA U FARMACIJI -
Sećanja na profesora Momčila St. Mokranjca (1899-1967)** 507

Reč gostujućeg urednika

Uprkos činjenicama koje nedvosmisleno kazuju da je vakcinacija omogućila eradicaciju variole, eliminaciju poliomijelitisa (sa izuzetkom par zemalja) i morbila (u značajnom delu sveta) i dovela do značajnog smanjenja morbiditeta i mortaliteta od zaraznih bolesti kod dece, posebno u razvijenim zemljama, a da je vakcinacija sezonskom vakcinom protiv gripe postala ustaljena praksa da se redukuju često teške neželjene posledice infekcije virusom gripe, opravdanost primene vakcina je predmet čestih diskusija, ne samo u okviru struke, već i u najširoj populaciji, koje periodično kulminiraju u prave anti-vakcinalne kampanje (poput one koja se proteklih godina vodila i našoj zemlji) nanoseći nemerljivu štetu, ne samo pojedincima, već i društvu kao celini. Imajući u vidu prethodno iznete činjenice, jasno je da razumevanje dometa vakcinacije, intervencije za koju se smatra da predstavlja najveće dostignuće medicine 20. veka, i konsekutivno angažovanje svih raspoloživih potencijala na širenju svesti o značaju ove medicinske intervencije, odnosno razvezavanju svih zabluda koje je prate, od izuzetnog značaja za pojedince i društvo u celini. S druge strane, svedoci smo da svakodnevni napori istraživača širom sveta rezultuju stalnim poboljšanjima postojećih (npr. kroz primenu novih adjuvansa) i razvojem novih tehnologija (DNK vakcine, žive rekombinantne vakcine) za koje se očekuje da će u najskorijoj budućnosti obezbediti još efikasnije, bezbednije, jeftinije, pa samim tim i dostupnije, profilaktičke vakcine. Osim toga, istraživački timovi širom sveta ulaze napore da osmisle: a) nove načine za davanje vakcine (kao što je lokalna primena posredstvom matriksa sa tankim iglicama), koji ne samo da isključuju „iglu“ kao izuzetno neprijatno sredstava za aplikaciju, već, možda važnije, koje ne zahtevaju obučeno medicinsko osoblje i b) vakcina koje će biti bez posebnih zahteva u pogledu čuvanja (otporne na visoke temperature), tako da mogu biti lako transportovane i primenjene i u najzabitijim delovima sveta. Konačno, važno je napomenuti da dugogodišnja istraživanja da se vakcinacijom spreče neke teške infekcije koje ovim putem nije bilo do sada moguće efikasno sprečiti (HIV, malarija, lešmanijaza), počinju da daju obećavajuće rezultate. Poseban izazov kada je pitanju vakcinacija jeste formulacija vakcina za primenu kod dece, s obzirom na karakteristike njihovog imunskog sistema, koje će biti još efikasnije. Drugi, ne manji izazov, je da se poboljša efikasnost vakcina koje se daju starim osobama (npr. vakcina protiv gripe, koja se pokazala neefikasnom u 60% primalaca koji pripadaju ovoj populaciji), s obzirom na dokazane neželjene efekte starenja na imunski sistem, zbog čega je Svetska zdravstvene organizacije u svoje prioritete za 2010-2020 godinu uvrstila i razvoj vakcina za primenu u gerijatrijskoj populaciji. S obzirom na sve što je prethodno izneto verujem da je sasvim jasno da sistematski osvrt na ovu važnu temu, posebno na ono što je novo i čemu se stremi u ovaj oblasti bi mogao da bude značajan materijal ne samo za istraživače koji se bave vakcinama, već i za sve one koji su direktno ili indirektno uključeni u sprovođenje vakcinacije kao medicinske intervencije kojoj u prilog idu svi

naučni argumenti. Imajući u vidu činjenicu da smo poslednjih decenija svedoci intenzivnih istraživanja na polju razvoja ne samo profilaktičkih, već i terapijskih vakcina, posebno onih namenjenih lečenju malignih tumora, od koji su neke ušle i u kliničku upotrebu, to su u okviru ovog tematskog broja i ove vakcine doobile mesto.

Na kraju, kao urednik ovog broja Arhiva za farmaciju, želim da kažem da su neke teme (npr. adjuvansi) našle mesto u više članaka, s namerom da se jasno iskristališe ono u čemu postoji konsenzus, ali da bi se ukazalo i ono u čemu eventualno postoji neslaganje.

Zahvaljujem se svim autorima na naporu koji su učinili da iznesu svoje viđenje ove kompleksne problematike i želim da ukažem da su uredničke intervencije bile samo u domenu jasnoće, ali bez uticaja na stavove i razmišljanja autora.

Gostujući urednik

Prof. dr Gordana Leposavić,

Univerzitet u Beogradu - Farmaceutski fakultet,

Katedra za patobiologiju

Perspektive u razvoju profilaktičkih vakcina

Nevena Arsenović Ranin

Univerzitet u Beogradu - Farmaceutski fakultet, Katedra za mikrobiologiju i imunologiju, Vojvode Stepe 450, 11221 Beograd, Srbija

Autor za korespondenciju, e-mail: nevena.arsenovic-ranin@pharmacy.bg.ac.rs

Kratak sadržaj

Vakcine se smatraju jednim od najvećih javnozdravstvenih dostignuća prošlog veka. Zahvaljujući vakcinaciji u svetu su potpuno iskorenjene velike boginje, a incidencija drugih infektivnih bolesti, kao što su dečija paraliza, male boginje, tetanus i difterija, drastično je smanjena. Današnje licencirane vakcine, koje pretežno sadrže žive atenuisane ili mrtve patogene ili njihove delove, su uspešne zahvaljujući tome što stimulišu produkciju neutrališućih antitela. Sa druge strane, ove vakcine mnogo teže indukuju čelijski-posredovanu imunost, koja je važna za eliminaciju intračelijskih patogena (koji često dovode do hroničnih infekcija). Trenutno se u pretkliničkim i kliničkim studijama ispituju brojne profilaktičke vakcine zasnovane na vektorima i nukleinskim kiselinama (DNK i iRNK), sposobne da indukuju snažan odgovor T-ćelija na vakinalni antigen, sa obećavajućim rezultatima. U ovom radu su date osnovne informacije o vakcinama sa vektorima i nukleinskim kiselinama, opisani su mehanizmi kojima one pokreću imunski odgovor, njihove dobre i loše strane, kao i problemi vezani za njihovu bezbednu primenu.

Ključne reči: tipovi vakcina, vektorske vakcine, DNK vakcine, iRNK vakcine

Uvod

Vakcinacija predstavlja najefikasniji metod zaštite od infektivnih bolesti i smatra se jednim od najvećih dostignuća imunologije, u medicini. Zahvaljujući vakcinaciji u svetu su potpuno iskorenjene velike boginje, a incidencija drugih infektivnih bolesti, kao što su dečija paraliza, male boginje, tetanus, rubela, hepatitis i druge, drastično je smanjena, čime su spašeni životi miliona ljudi (1, 2). Zbog toga se vakcinacija smatra najekonomičnijom i najuspešnijom merom za poboljšanje i unapređenje javnog zdravlja (1, 2).

Iako su danas dostupne vakcine za prevenciju više od trideset infektivnih bolesti (1), perzistentne (hronične ili latentne) infekcije, infekcije izazvane patogenima sa kompleksnim životnim ciklusom, kao i one koje izazivaju antigenski varijabilni i/ili novi patogeni, i dalje predstavljaju izazov (3). Među njima, infekcije izazvane virusom HIV, bakterijom *Mycobacterium tuberculosis* i protozoama iz roda *Plasmodium* koje godišnje dovode do smrti četiri miliona ljudi u svetu (4), imaju najveći prioritet. Nedavno je Evropska agencija za lekove (engl. European Medicines Agency, EMA) dala pozitivno mišljenje o prvoj vakcini protiv malarije (RTS, S), mada je njen efikasnost svega oko 30% (5). Postojeća vakcina Bacillus Calmette-Guérin (BCG) protiv *Mycobacterium tuberculosis* obezbeđuje zaštitu od diseminovanih oblika tuberkuloze kod male dece, ali pruža nepotpunu i varijabilnu zaštitu od plućne tuberkuloze, pogotovo kod odraslih (6). Takođe, neke od vakcina (npr. vakcina protiv gripe ili malih boginja) nisu dovoljno efikasne u svim populacijama. Odojčad i starije osobe, kao i imunokompromitovani, generalno slabije odgovaraju na vakcinaciju (3). Da bi odgovorili ovim izazovima, istraživači rade na razvoju novih i poboljšanju postojećih vakcina. Ovo zahteva bolje poznавање patogeneze i epidemiologije bolesti, kao i relativnog značaja pojedinih tipova imuniteta (urođeni, humoralni, ćelijski, mukozni, sistemski) u odbrani od patogena. Takođe, potrebni su novi pristupi u formulaciji vakcinalnih antigena, koji bi očuvali ili povećali njihovu imunogenost i stabilnost, i omogućili stvaranje protektivnog imunskog odgovora (7).

Značajna dostignuća u proteklom periodu u imunologiji, molekularnoj biologiji, genomici, proteomici, biohemiji i bioinformatici, pokrenula su napredak u oblasti dizajna i proizvodnje vakcina. Novi pristupi u razvoju vakcina se zasnivaju na unošenju gena od interesa i njegovoj ekspresiji u ćelijama domaćina. Ovi pristupi pružaju mnogo bolje mogućnosti za kontrolu indukcije i održavanja specifičnog imunskog odgovora, koji je najpogodniji za uspešnu odbranu protiv pojedinih patogena. Geni koji kodiraju antigene mogu se uneti u organizam domaćina u obliku DNK (8), informacione (i)RNK (9), ili modifikovanih bakterijskih ili virusnih vektora (10). Očekivanja od ovih vakcina kada je u pitanju prevencija i kontrola infektivnih bolesti je velika, pa se stoga one nalaze u fokusu ovog rada.

Vakcinacija

Vakcine su biološki preparati koji sadrže atenuisane ili mrtve mikroorganizme ili antigene koji potiču od njih, a koji se primenjuju u cilju prevencije infektivnih bolesti. Vakcine stimulišu imunski sistem dovodeći do imunskog odgovora i stvaranja imunske memorije kroz indukciju dugoživećih plazma ćelija i memorijskih T- i B- ćelija na sličan način kao prirodna infekcija, ali ne izlažu primaoca oboljenju i njegovim mogućim komplikacijama. Kada vakcinisana osoba kasnije bude izložena patogenu koji je prisutan u okruženju, cirkulišuća antitela koja se produkuju od strane dugoživećih plazma ćelija obezbeđuju neposrednu zaštitu od patogena, dok memorijske ćelije uspostavljaju sekundarni imunski odgovor koji je brži, snažniji i efikasniji u eliminaciji patogena, što može da spreči uspostavljanje infekcije i razvoj bolesti (11).

Imunitet protiv patogena (ili vakcine) rezultat je integrisane aktivnosti urođenog i adaptivnog imunskog sistema (12). Urođena imunost se aktivira nakon prepoznavanja molekulskih obrazaca patogena (strukture zajedničke za mikroorganizme istog tipa, otuda naziv obrasci, koje ispoljavaju mikroorganizmi, a ne ispoljavaju ćelije domaćina; engl. pathogen-associated molecular patterns, PAMPs) od strane receptora za prepoznavanje obrazaca (engl. pattern recognition receptors, PRRs) na površini ili u različitim subodeljcima ćelija urođene imunosti (fagociti, dendritske ćelije) u kojima mikroorganizam može da se nađe (13). Aktivacija profesionalnih antigen-prezentujućih ćelija (APĆ), kao što su dendritske ćelije, koje u sklopu molekula glavnog kompleksa tkivne podudarnosti (engl. major histocompatibility complex, MHC) prezentuju peptidne fragmente antiga T limfocitima, ključna je za pokretanje adaptivnog imunskog odgovora koji dovodi do generisanja efektorskih T ćelija i visoko afinitetnih antitela koji uspešno eliminišu patogen. Imunska memorija koja nastaje tokom ovog antigen-specifičnog imunskog odgovora perzistira i obezbeđuje bržu reakciju u odgovoru na ponovne infekcije istim patogenom (11, 14).

Prečišćeni proteinski antigeni, npr. oni koji se koriste u vakcinama, ne mogu da izazovu odgovor T-ćelija ako se ne daju zajedno sa adjuvansima, supstancama koje aktiviraju APĆ, posebno dendritske ćelije (11). Adjuvansi su uglavnom produkti mikroorganizma ili supstance koje ih imitiraju i one se vezuju za receptore za prepoznavanje obrazaca, npr. za receptore slične Tollu (engl. Toll-like receptors, TLR). Na taj način imunski sistem može da odgovori i na prečišćene proteinske antigene u vakcinama kao da su delovi infektivnih mikroorganizama (15).

Tipovi vakcina

U zavisnosti od tipa antiga, odnosno od tehnologije dobijanja antiga koje sadrže, vakcine se dele na žive (atenuisane), mrtve (inaktivisane), subjedinične-toksoidne, proteinske, polisaharidne, i konjugovane (1, 16).

Žive, atenuisane vakcine sadrže cele, žive mikroorganizme koji su specifičnim procesima kultivacije izgubili virulentnost tj. sposobnost izazivanja bolesti (17). Živi atenuisani mikroorganizmi mogu da se razmnožavaju u organizmu domaćina i pokreću imunski odgovor koji je praktično identičan onom do kojeg dovodi prirodna infekcija (18). Stoga su ove vakcine veoma efikasne, podstiču dugotrajan, često doživotan imunitet. Neke od ovih vakcina se mogu unosti u organizam tako da imitiraju prirodni put infekcije (npr. Sejbinova vakcina protiv poliomijelitisa se aplikuje oralno), što dovodi do stimulacije lokalnog imunskog odgovora. Ipak, primena živih vakcina nije pogodna za imunosuprimirane pacijente i trudnice kod kojih i nizak nivo virulencije može predstavljati problem. Osim toga, iako je to retko, postoji opasnost da atenuisani soj povrati virulenciju (19).

Inaktivisane vakcine sadrže hemijskim (npr. formaldehid, beta-propiolakton, fenol) ili fizičkim agensima (zagrevanje, UV zraci) inaktivisane cele mikroorganizme (15). Inaktivisani (mrtvi) mikroorganizmi nemaju sposobnost replikacije, pa je imunski odgovor znatno slabiji i traje kraće u odnosu na žive vakcine. Zbog toga je neophodno davanje više doza i dodavanje adjuvanasa (20, 21). Ove vakcine ne stimulišu lokalni imunski odgovor jer se primenjuju isključivo putem injekcija. Inaktivisane vakcine su bezbednije u odnosu na žive vakcine jer povratak virulencije nije moguć. Manje su osetljive na uslove skladištenja i čuvanja od živih vakcina (15, 16).

Subjedinične vakcine sadrže produkte ili delove mikroorganizama koji imaju antigenska svojstva (16). One se dalje mogu podeliti na vakcine koje sadrže proteinsku komponentu (modifikovane toksine-toksoide bakterija ili strukturni protein mikroorganizama) ili polisaharid. Dobijaju se na dva načina, klasičnim putem, prečišćavanjem mikroorganizama ili kada su u pitanju proteinske subjedinične vakcine, i savremenim metodama, korišćenjem genetičkog inženjeringu, tj. rekombinantne (r)DNK tehnologije (21). Rekombinantne vakcine su vakcina protiv hepatitisa B (22) i vakcina protiv humanih papiloma virusa (23). Kod rDNK tehnologije se gen koji kodira željeni protein (vakcinalni antigen) ugrađuje u vektor za kloniranje (plazmid), i tako nastala rekombinantna DNA se unosi u odgovarajuće ćelije (često su to bakterije *E. coli*, ili kvasnice), koje se kultivisu na hranjivim podlogama, čime se obezbeđuje sinteza velikih količina proteina od interesa (proteina za vakcinu). Protein od interesa se zatim izoluje i prečišćava, kombinuje sa adjuvansom i drugim ekcipijensima, i inkorporira u vakcinu. Polisaharidne subjedinične vakcine sadrže samo polisaharide (obično iz kapsule bakterija, npr. vakcine koje sprečavaju bolesti izazvane inkapsuliranim patogenima: *Streptococcus pneumoniae*, *Haemophilus influenza*, *Neisseria meningitidis*). Polisaharidi su slabo imunogeni, pa su stoga napravljene **konjugovane vakcine**, kod kojih je polisaharid jednog patogena vezan za proteinski nosač koji je imunogen, najčešće difterijski ili tetanusni toksoid ili neki površinski protein bakterija (21, 24). Pošto ne sadrže sve delove mikroorganizma, subjedinične vakcine imaju manje

neželjenih efekata od celoćelijskih. Međutim, mora im se dodati adjuvans i daju se u više doza da bi kod primaoca indukovale dobar zaštitni imunitet (15, 21). Subjedinične vakcine su veoma efikasne, ali su i skuplje od celoćelijskih, pogotovo ako je vakcina dobijena rDNK tehnologijom.

Skoro sve danas licencirane vakcine pripadaju prethodno opisanim tipovima vakcina. Ove vakcine su se pokazale veoma uspešnim u prevenciji mnogih infektivnih bolesti. Svoj uspeh duguju činjenici da su usmerene na patogene koji imaju nisku antigensku varijabilnost i za koje zaštita zavisi od imunosti posredstvom antitela. To se, između ostalih, odnosi na izazivače poliomijelitisa, tetanusa, difterije, morbila, rubele, hepatitisa B (25, 26). Posledično, vakcine koje stimulišu produkciju neutrališućih ili opsonizujućih antitela protiv ovih patogena ili njihovih produkata, pokazale su se kao izuzetno uspešne. Međutim, ako patogen pokazuje visok stepen genetskih (antigenskih) varijacija, kao što je npr. virus influence i HIV, cirkulišuća antitela generisana u odgovoru na vakcinu (kao i na infekciju) neće prepoznavati patogen pri narednim infekcijama.

S druge strane, aktuelne vakcine slabo stimulišu citotoksični odgovor CD8+T limfocita, koji je važan za eliminaciju intracelularnih mikroorganizama, koji u većini slučajeva dovode do perzistentnih, hroničnih (npr. HIV) i latentnih infekcija (npr. *Mycobacterium tuberculosis*, herpes virusi, citomegalovirus) (25, 26). Žive atenuisane vakcine, koje imaju sposobnost da stimulišu ovaj tip odgovora, nose potencijalni rizik od ispoljavanja virulencije patogena kod osetljivih primaoca, kao i mogćnost reverzije attenuacije. Iako mali, rizik od ovih događaja se ne može zanemariti.

Prema tome, jedan od glavnih izazova u razvoju novih strategija imunizacije je dizajniranje vakcina koje će stimulisati odgovarajući tip imunskog odgovora koji može da obezbedi imunost uglavnom na intraćelijske patogene, naročito na one koji dovode do razvoja hroničnih, često doživotnih infekcija. Poznavanje biologije visoko konzerviranih antigena koji su uključeni u patogenezu bolesti, kao i imunskih mehanizama koje treba stimulisati da bi se indukovala zaštita, je neophodno za racionalno osmišljavanje vakcinalnih strategija kojima bi se mogao obezbediti bolji protektivni imunitet u odnosu na onaj koji se prirodno indukuje (27).

Poslednjih godina ulažu se veliki napor da bi se identifikovali protektivni antigeni. Novije tehnologije, kao što je **reverzna vakcinologija** koja se bazira na bioinformatičkoj analizi genomske sekvene, može biti ključna za selekciju potencijalnih vakcinalnih antigena (28). Reverzna vakcinologija uključuje sekvenciranje celog genoma patogena i kompjutersko skeniranje gena koji mogu biti iskorišćeni u proizvodnji vakcine, kao što su oni koji kodiraju površinske proteine patogena, mada je pokazano da antigenski konzervirani proteini, kao i citoplazmatski proteini, mogu biti imunogeni. Zatim se kompjuterski odabrani proteini kloniraju i eksprimiraju u *E.coli*, prečišćavaju, i ako se pokažu imunogeni u eksperimentalnim modelima, dalje testiraju u

kliničkim studijama. Reverzna vakcinologija je omogućila identifikaciju kompletног antigenskog repertoara patogena, pa i onih koje je teško ili za sada nemoguće kultivisati. Pored toga, ovaj pristup može pomoći u otkrivanju antiga na koji se ne ispoljavaju velikoj količini, ne mogu da se eksprimiraju *in vitro*, ili su manje imunogeni u toku infekcije, pa se ne otkrivaju konvencionalnim pristupom (29). Vakcina protiv meningokoka serogrupe B je prva vakcina napravljena pomoću reverzne vakcinologije (30). I na kraju, mada ne i manje značajno, ovaj pristup značajno skraćuje vreme pronalaženja vakcinalnog antiga, a samim tim i vreme pojave novih vakcina. Za razvoj konvencionalnih vakcina, do ulaska u kliničku fazu ispitivanja potrebno je približno 20 godina, dok se primenom reverzne vakcinologije ovaj period skraćuje na približno 5 godina (29).

Nove generacije vakcina

Razvoj molekularne biologije i genetičkog inženjeringu omogućio je dobijanje novih, savremenih vakcina, vektorskih i DNK/iRNK vakcina (16). One su dizajnirane tako da se protektivni imunski odgovor indukuje unošenjem iRNK ili gena mikroorganizma koji kodira sintezu antiga značajnih za stimulaciju imunskog odgovora (16).

Vektorske vakcine

Vektorske vakcine sadrže atenuisani virus ili bakteriju koja služi kao vektor (nosač, isporučioc) za DNK sekvencu (gen) patogena. Gen patogena koji kodira protein odgovoran za pokretanje imunskog odgovora, ugrađuje se u genom atenuisanog virusa ili bakterije tehnikom rDNK, pa se ove vakcine nazivaju i **rekombinantne vektorske vakcine ili hibridne vakcine** (31). Kada vektor uđe u ćelije domaćina, insertovani (vakcinalni) gen se u ćelijama domaćina prepisuje i prevodi u antigen kao intrizična vektorska komponenta.

Mehanizam delovanja vektorskih vakcina

U toku poslednje tri decenije različite bakterije (*Mycobacterium bovis* BCG, *Listeria monocytogenes*, *Salmonella* spp. i *Shigella* spp.) i virusi (adenovirusi, parvovirusi, paramiksovirusi, poksvirusi) su ispitivani kao vakcinalni vektori. U eksperimentalnim modelima, invazivne intracelularne bakterije, kao što su *Salmonella typhimurium* i *Listeria monocytogenes*, su se pokazale kao najbolji nosači vakcinalnih DNK (32). U kliničkim studijama prevashodno se koriste virusni vektori, pa su oni u fokusu ovog rada. Virusni vektori mogu da se koriste za zaštitu od različitih infektivnih bolesti, npr. protiv onih koji su izazvani protozoama (malaria) (33), mikobakterijama (tuberkuloza) (34, 35) ili virusima (HIV, Denga virus) (36, 37). Od virusnih vektora najčešće se primenjuju adenovirusni vektori, za koje je pokazano da indukuju izuzetno snažan CD8+ T ćelijski odgovor kao i produkciju antitela (38). Efikasnost ovih vektora

u zaštiti od različitih patogena (npr. HCV, HIV, *Plasmodium* spp, Ebola virusa) pokazana je u brojnim pretkliničkim i kliničkim studijama (39).

Virusni vektori mogu da budu živi, a ne moraju da imaju sposobnost replikacije. Živi vektori su biološki aktivni i produkuju virusno potomstvo u vakcinisanom domaćinu, ali im je virulencija atenuisana zbog mutacija u vektoru, himerne prirode same vakcine, korišćenja vektora u heterologom domaćinu, ili usled kombinacije ovih faktora (40). Vektori, međutim, mogu da budu tako snažno atenuisani da ne podležu kompletном ciklusu replikacije u inficiranim ćelijama (40). Kada se himerna virusna vektorska vakcina unese u organizam, nije ni neophodno da se genom vektorskog virusa umnožava, već samo da se promoviše ekspresija insertovanog gena, čiji produkt (antigen) će pokrenuti imunski odgovor. Vektori koji su izgubili sposobnost deobe su mnogo više testirani u kliničkim studijama, delom i zbog njihove veće bezbednosti (41).

Karakteristike virusnih vektora, kao što je njihov ćelijski tropizam, sposobnost prenošenja heterolognog gena, količina ekspresije heterolognih gena, sposobnost izazivanja različitih tipova imunskih odgovora i perzistencija kod domaćina, neki su od faktora koji utiču na izbor određenog virusnog vektora za njegovu specifičnu primenu. Virusi su razvili visoko efikasne mehanizme za ulazak u ćeliju i korišćenje njene biosintetske mašinerije za ekspresiju virusnih proteina (42). Ovo svojstvo učinilo ih je veoma privlačnim za transportere gena od interesa u ćeliju. Virusni vektori izazivaju u ciljnim ćelijama stimuluse koji oponašaju prirodnu infekciju, i stimulišu produkciju antitela, ali što je još važnije aktiviraju odgovor CD8+ T-ćelija koji je važan za eliminaciju intracelularnih patogena (42, 43).

Putevi primene vektorskih vakcina

Putevi primene virusnih vektora mogu da budu različiti. U brojnim kliničkim studijama, ispitivana je intramuskularna, intradermalna (44, 45), intranasalna (46) i oralna vakcinacija (47) različitim virusnim vektorima. Izbor načina imunizacije, pored sposobnosti datog virusa da inficira određena tkiva, zavisi i od toga koji tip imunskog odgovora želimo da postignemo. Ako je za protективni odgovor potreban mukozni odgovor, oralna ili nazalna primena vakcinalnih vektora ima prednost u odnosu na parenteralnu primenu.

Dobre i loše strane vektorskih vakcina

S obzirom na veliki broj dostupnih različitih virusnih vektora i veliko stečeno znanje o mogućnostima manipulacije ovim vektorima, virusni vektori predstavljaju dragocenu i vrlo svestranu platformu za razvoj novih vakcina. U virusne genome može se ugraditi bilo koji gen koji kodira ekspresiju željenog antigena, što omogućava razvoj velikog broja vakcina. Upotrebom vektora, imunski odgovor se može usmeriti na određeni protein ili čak epitop, koji je antigenski konzerviran između različitih sojeva patogena, što ih čini korisnim za stvaranje univerzalnih heterosubtipskih vakcina (npr.

za grip) (48). U jedan virusni genom mogu se ugraditi i geni različitih patogena, što bi obezbedilo nastanak polivalentnih vakcina (49). Virusni vektori indukuju snažan imunski odgovor, pa nema potrebe za korišćenjem adjuvanasa (50).

Međutim, virusni vektori mogu imati nekoliko slabosti. Pre svega, atenuisani virusi mogu steći virulenciju *in vivo*, što čini vektorske vakcine manje bezbednim. Takođe, virusni vektori mogu da se rekombinuje sa endogenim virusima i steknu njihovu virulenciju (51). Već postojeći imunitet protiv virusa koji najčešće inficiraju ljude može biti glavni problem, jer prethodno nastala anti-vektorska antitela mogu da se vežu za vektor i onemoguće mu ulazak u ćeliju. Sličan problem postoji i ako se pokaže potreba za dodatnim (buster) imunizacijama (51). Neka od mogućih rešenja su korišćenje različitih serotipova virusnih vektora i bolji dizajn „prime-boost” pristupa (51).

U pogledu proizvodnje vakcina, svaki virusni sistem zahteva različite ćelijske sisteme za propagaciju, što zahteva različite proizvodne pogone za svaku platformu virusnog vektora. Kako tokom proizvodnje može doći do rekombinacije, mora se voditi računa da ne dođe do kontaminacije ćelijskih kultura nekim virusom koji bi mogao da dovede do pojave rekombinovanog i neokarakterisanog patogena (40). Budući da je proizvodnja virusnih vektorskih vakcina složen proces koji često zahteva mnoštvo komponenti humanog ili životinjskog porekla, poput ćelijskih linija, svinjskog tripsina ili goveđeg seruma, potreba za isključivanjem kontaminanata zahteva opsežno testiranje tokom različitih koraka proizvodnog procesa. Kontaminacija rotavirusne vakcine svinjskim cirkovirusima, ukazuje na realnost ovog rizika (52). Zbog svega navedenog, proizvodnja vakcina zasnovanih na korišćenju virusnih vektora je kompleksna i relativno skupa.

Do 2019. godine su samo dve virusne vektorske vakcine odobrene za primenu kod ljudi, Imojev®, protiv japanskog encefalitisa i Dengvaxia® protiv denga groznice (40). U obe vakcine se kao vektor koristi virus žute groznice, generički poznat kao ChimeriVax, u kojem su geni za strukturne proteine M i E viriona žute groznice zamenjeni homolognim genima za virus japanskog encefalitisa (Imojev®), odnosno Denga virusa (Dengvaxia®). Brojne druge vakcine, u kojima se koristi širok spektar vektora, i koje su usmerene na različite patogene, nalaze se u različitim fazama istraživanja i razvoja. Iako je trenutno broj virusnih vektorskih vakcina koji je odobren za primenu kod ljudi veoma mali, raznovrsne vektorske vakcine su licencirane za primenu u veterinarskoj praksi (53), što obećava i pruža nadu za šиру primenu ovih vakcina u budućnosti kod ljudi.

DNK vakcine

DNK vakcine su plazmidi bakterijskog porekla koji su tehnikama genetičkog inženjeringu tako modifikovani da kodiraju ekspresiju antiga u cilju indukcije adaptivnog imuniteta (54, 55). Proizvode se tako što se gen koji kodira antigen od

interesa (antigen patogena) insertuje u plazmid, i nastali rekombinantni plazmid se unosi u bakteriju, obično *E. coli*. Transformisane bakterije se kultivišu, radi dobijanja klonova tj. brojnih kopija rekombinantnog plazmida, koji se ekstrahuje, prečišćava i inkorporira u vakcinu. Budući da su se javila pitanja vezana za bezbednost nefunkcionalnih sekvenci u pazmidu, posebno markera rezistencije na antibiotike (plazmid se selektuje na osnovu rezistencije na antibiotike), za primenu kod ljudi, u novim generacijama DNK vakcina, marker je zamenjen ili uklonjen (56). Pored toga, razvijeni su minimalni DNK konstrukti, poput polusintetskih (57) ili potpuno sintetskih DNK konstrukta (58) koji isključivo kodiraju ciljni antigen. Kada plazmid preuzme ćelija domaćina, započinje sinteza antigaena patogena. Ideja koja stoji u osnovi DNK vakcinalnog sistema je da se antigen eksprimira u ćeliji domaćina na sličan način kao u toku virusne infekcije. Kao rezultat, antigeni mogu da se prerađuju kao proteini sintetisani u citoplazmi, i nastali peptidi prezentuju imunskom sistemu u sklopu molekula I klase MHC. Pored toga, ako se protein oslobodi ili sekretuje, može se preraditi i ispoljiti u sklopu II klase MHC molekula, što dovodi do produkcije specifičnih antitela (55).

Putevi primene i formulacija DNK vakcina

Razvoj DNK vakcina započeo je devedesetih godina prošlog veka, kada je uobičajeni put primene bio intramuskularna ili intradermalna imunizacija korišćenjem konvencionalnih igala. Iako su se ovako aplikovane DNK vakcine pokazale imunogenim kod miševa, kod velikih životinjskih modela i ljudi nisu indukovale protektivni imunitet. Slaba imunogenost može se objasniti činjenicom da se nakon aplikacije konvencionalnim iglama, DNK deponuje u međućelijskom prostoru, a ne ulazi u ćelije domaćina. Takođe, da bi se protein eksprimirao, DNK vakcina mora da prođe dve ćelijske membrane, citoplazmatsku membranu, kao i jedarnu membranu (51, 59). Zbog toga su razvijene metode koje mogu poboljšati preuzimanje, ekspresiju i imunogenost DNK vakcina. One uključuju različite uređaje koji mehanički povećavaju unos DNK u ćelije, kao što su genski pištolj, mlazni injektori i *in vivo* elektroporacija, čija je primena dovela do povećanja imunogenosti ovih vakcina i u pretkliničkim i u kliničkim studijama (60-64). Treba pomenuti da je u nekim eksperimentalnim modelima, aplikacija DNK vakcina (npr. DNK vakcine protiv gripe) putem transdermalnih flastera takođe pokazala odlične rezultate (65, 66). Pored toga, razvijene su različite formulacije DNK vakcina, npr. inkapsulacija u lipozome, sferične vezikule koje se sastoje od fosfolipida i holesterola, adsorpcija na polimere kao što je polietilenimin i adsorpcija ili inkapsulacija u biorazgradive nanočestice (67). Ove metode su prevashodno usmerene na poboljšanje preuzimanja DNK od strane ćelija i posledično povećanje ekspresije antigaena. Takođe, u cilju modifikacije i poboljšanja DNK-posredovanog imunskog odgovora koristi se nekoliko različitih pristupa. Oni uključuju uvođenje tzv. molekularnih adjuvanasa, kao što su ligandi za receptore

molekulske obrazace (CpG nukleotidi) i razliciti citokini, uglavnom IL-12, koji mogu biti inkorporirani u plazmid u koji je inkorporirana vakcinalna DNK ili drugi plazmid koji se daje istovremeno sa vakcinalnim. Strategije kojima se antigen usmerava u odredene celije (npr. APĆ) ili u odredene celijske subodeljke (npr. endoplazmatski retikulum ili lizozome) značajno mogu povećati preradu i prezentaciju antiga i stimulisati željeni imunski odgovor (68).

Mehanizam delovanja DNK vakcina

Iako je veliki broj studija pokazao da DNK vakcine stimulišu i humoralni i celijski imunski odgovor, posredstvom aktivacije CD4+ pomoćničkih i CD8+ citotoksičnih T celija, tačan mehanizam delovanja još uvek nije razjašnjen. Nakon ulaska u celiu, DNK vakcina se prepoznaće od strane receptora urođene imunosti, jer su plazmidski elementi DNK bakterijskog porekla koji deluju kao PAMPs. Prepostavlja se da TLR-9 nije kritičan za efikasnost DNK vakcina, dok je signalni put koji uključuje stimulator interferonskih gena (engl. stimulator of IFN genes, STING) i TANK-vezujući kinazu 1 (engl. TANK binding kinase 1, TBK1), kao i AIM2 (engl. absent in melanoma 2) receptor koji aktivira inflamazom, važan za mehanizam njihovog delovanja (69). Brojne studije ukazuju da imunski odgovor na DNK vakcincu značajno zavisi od toga koji će tip celijskih (somatskih, npr. miociti, keratinociti, fibroblasti ili APĆ, npr. dendritske celijske) preuzeti DNK, što zavisi od brojnih faktora, kao što su put primene, uređaj koji je korišćen za isporuku vakcine, formulacija vakcine i upotreba adjuvansa. Intramuskularna primena konvencionalnim iglama uglavnom rezultira u transfekciji miocita, dok intradermalna aplikacija pomoću komprimovanog gasa čestica obloženih molekulima DNK (genski pištolj) dovodi do transfekcije i keratinocita i profesionalnih APĆ, kao što su Langerhansove celijske (70, 71). Langerhansove celijske su glavne APĆ u koži, koje internalizuju antigen i migriraju u limfne čvorove gde prezentuju antigen T celijskim. Pošto su profesionalne APĆ ključne za aktivaciju CD8+ T celija, najverovatniji mehanizam pokretanja imunskog odgovora je unakrsna prezentacija odnosno fagocitoza transfektovanih somatskih celijskih od strane profesionalnih APĆ celijskih i sledstvena prezentacija peptida u sklopu I i II klase MHC molekula na profesionalnim APĆ (72, 73).

Dobre i loše strane DNK vakcina

DNK vakcine indukuju mukozni i sistemski imunski odgovor, humoralni i celijski, jeftine su, lako se konstruišu, stabilne su na sobnoj temperaturi, što pojednostavljuje transport i distribuciju tako da mogu biti dostupnije zemljama u razvoju (74). Mogu se davati u više doza, za razliku od rekombinantnih vektorskih vakcina kod kojih je primena u više doza otežana zbog stvaranja anti-vektorskog imuniteta (75).

Međutim, primena DNK vakcina potencijalno nosi neke rizike, uglavnom vezane za dugotrajnu perzistenciju transfektovane DNK u jedru domaćina, i potencijalnu

integraciju u genom domaćina, što može dovesti do mutogeneze i onkogeneze. U tom kontekstu treba pomenuti da je integracija DNK u genom domaćina detektovana nakon intramuskularne elektroporacije DNK vakcine kod miša (76, 77). Svetska zdravstvena organizacija (SZO) preporučuje studije integracije kao deo pretkliničkog programa prilikom procene bezbednosti DNK vakcina (78). Ubrizgavanje bakterijske DNK potencijalno može dovesti do stvaranja antitela protiv DNK i razvoja autoimunosti, mada ova antitela nisu nađena kod miševa, pacova, zečeva ili majmuna nakon imunizacije DNK vakcinama (76). Potencijalni razvoj rezistencije na antibiotike kod vakcinisanih takođe predstavlja jedno od mogućih bezbednosnih problema, ali do sada takvi događaji nisu dokumentovani. Konačno, ekspresija citokina ili kostimulatornih molekula koji se koriste za pojačavanje imunogenosti DNK može dovesti do neželjenih štetnih efekata uslovljenih ekspresijom i oslobođanjem citokina, kao što su generalizovana imunska supresija, hronična inflamacija ili autoimunost. U cilju bezbednosti DNK vakcina, SZO preporučuje praćenje postojanosti plazmida koji eksprimiraju citokine, kao i odgovarajuće pretkliničke modele, kao što su životinjski modeli koji reaguju na odgovarajuće humane citokine (79).

Trenutno su u toku brojne kliničke studije u kojima se ispituju DNK vakcine protiv različitih humanih patogena (hepatitis B i C virusa, HIV-a, virusa influence, Ebola virusa, respiratornog sincicijalnog virusa, herpes simpleks virusa, uzročnika malarije itd.) (55). Nijedna DNK vakcina još nije odobrena za humanu upotrebu, ali je četiri licencirano za veterinarsku primenu (npr. konjska vakcina protiv virusa Zapadnog Nila) (69), što pruža nadu da će ovaj tip vakcina uskoro postati efikasno sredstvo za prevenciju bolesti i kod ljudi.

iRNK vakcine

Ove vakcine sadrže informacionu (i)RNK koja služi kao matrica za sintezu endogenog proteina kod vakcinisanih osoba. Za profilaktičke vakcine protiv patogena, razvijene su dve vrste iRNK: nereplikujuće (konvencionalne) iRNK, i samoamplifikujuće (engl. self-amplifying) iRNK virusnog porekla (51, 80). Vakcine sa konvencionalnom iRNK kodiraju samo antigen od interesa. Vakcine sa samoamplifikujućom iRNK baziraju se u većini slučajeva na genomu alfa virusa u kojem su metodom genetskog inženjeringu geni koji kodiraju strukturne proteine zamenjeni genom od interesa, dok su geni koji kodiraju RNK replikacionu mašineriju i dalje prisutni (tako dobijen genom se naziva replikon). Ove vakcine diriguju svoju sopstvenu replikaciju (samoamplifikujuće), kroz sintezu od RNK-zavisnog RNK polimeraza kompleksa, što dovodi do stvaranja brojnih kopija iRNK koja kodira antigen od interesa. Na ovaj način je omogućena veća ekspresija proteina od interesa, uz primenu relativno male doze vakcine (51, 80). Sa druge strane, prednost korišćenja konvencionalnih iRNK vakcina u odnosu na samoamplifikujuće je jednostavnost

konstrukcije, mala veličina RNK, i odsustvo bilo kog dodatnog kodiranog proteina koji bi mogao dovesti do neželjenog imunskog odgovora (81).

Putevi primene i formulacija iRNK vakcina

Da bi delovala kao vakcina, egzogena iRNK mora ući u citoplazmu gde se odvija ekspresija proteina, što znači da mora proći kroz citoplazmatsku ili endozomalnu lipidnu membranu. Takođe, iako iRNK ima imunostimulatorne osobine (aktivira urođeni imunski sistem), one se mogu pojačati različitim formulacijama iRNK. Stoga su razvijeni različiti pristupi koji imaju za cilj da poboljšaju unos, odnosno preuzimanje iRNK od strane ćelija domaćina kao i adjuvantne osobine iRNK vakcina.

U eksperimentalnim modelima je pokazano da fizičke metode isporučivanja, kao što su genski pištolj i elektroporacija, povećavaju oslobađanje iRNK vakcina u citoplazmu (82, 83). Pored toga, jedna od najčešće korišćenih strategija za povećanje ekspresije i imunogenosti iRNK vakcina je povezivanje iRNK sa dodatnim komponentama. U tom kontekstu, jedan od prvih pristupa bio je povezivanje iRNK sa katjonskim peptidom, protaminom, koji štiti iRNK od degradacije (84, 85). Noviji pristupi uključuju stvaranje kompleksa sa lipidnim i polimernim nanočesticama. Danas se lipidne nanočestice najviše koriste za *in vivo* isporučivanje iRNK vakcina, i vrsta su agensa koji najviše obećava (86). Treba napomenuti da je jedan od načina isporuke nereplikujućih iRNK vakcina i putem dendritskih ćelija, ali se ovako primenjene iRNK koriste primarno za lečenje tumora (87).

Pored formulacije, put primene ima uticaja na kvalitet i jačinu imunskog odgovora. iRNK vakcine se mogu primenjivati sistemski ili lokalno, u zavisnosti od toga gde je potrebno indukovati ekspresiju antigena. U pretkliničkim studijama, iRNK vakcine protiv infektivnih bolesti su aplikovane intramuskularno, intradermalno i subkutano. Nakon intradermalne aplikacije, iRNK vakcine formulisane sa protaminom ili lipidnim nanočesticama uspešno indukuju imunski odgovor, uključujući produkciju antitela, kao i odgovor CD4+ i CD8+ T ćelija (88, 89).

Mehanizam delovanja iRNK vakcina

Egzogena iRNA je imunostimulatorna, zato što se prepoznaje od strane različitih membranskih, endozomalnih (TLR3, TLR7 i TLR8) i citoplazmatskih (RIG-I, MDA-5 i PKR) receptora urođene imunosti (90). Aktivacija ovih receptora rezultira u robusnom urođenom imunskom odgovoru koji na mestu inokulacije dovodi do oslobađanja hemokina i citokina kao što su IL-12 i TNF, koji su ključni za sazrevanje dendritskih ćelija, i otuda za indukciju efikasnog adaptivnog imunskog odgovora protiv kodiranog antigaena. Međutim, postoje podaci da prepoznavanje iRNK od strane receptora urođene imunosti, putem IFN α i β signalnog puta, može da dovede i do inhibicije ekspresije antigaena što negativno utiče na imunski odgovor (91).

Iako mehanizmi koji leže u osnovi indukcije imunskog odgovora koji pokreću iRNK vakcine nisu do kraja razjašnjeni, smatra se da ekspresija i prezentacija kodiranih antigena sledi slična pravila kao kod DNK vakcina. Ukratko, najvažniji tip ćelija za iRNK vakcine su profesionalne APĆ, najverovatnije dendritske ćelije, koje nakon transfekcije sintetišu antigen kodiran iRNK u nativnoj formi. Sintetisani protein se potom obrađuje do antigenskih peptida i prezentuje u sklopu I i II klase MHC molekula zajedno sa kostimulatornim signalima CD8+ i CD4+ T ćelijama. Antigen koji se eksprimira u nativnoj formi prepoznaće se od strane B ćelija koje potom produkuju antitela protiv antiga (51).

Dobre i loše strane iRNK vakcina

„Ogoljena” iRNK je u fiziološkim uslovima jako nestabilna (zbog prisustva ekstraćelijskih ribonukleaza koje katalitički hidrolizuju RNK). Takođe, usled hidrofilnosti i jakog negativnog naelektrisanja, nakon aplikacije *in vivo*, RNK se ne preuzima efikasno od strane ćelija domaćina. Ipak, ovi nedostaci su su prevaziđeni vezivanjem iRNK za visoko efikasne nosače, kao što su nove generacije lipidnih nanočestica, koje štite iRNK od degradacije ribonukleazama i omogućavaju prolongiranu ekspresiju antiga, što dovodi do indukcije snažnog humorалnog i ćelijskog imunskog odgovora nakon *in vivo* primene.

iRNK vakcine, slično DNK vakcinama, indukuju i humorali i ćelijski imunski odgovor i mogu se koristiti za ekspresiju bilo kog želenog antiga. U pogledu proizvodnje, obe platforme omogućavaju dobijanje različitih vakcina korišćenjem istih uspostavljenih proizvodnih procesa i postrojenja. Međutim, budući da se proizvodni proces iRNK zasniva na *in vitro* sistemima i ne zahteva amplifikaciju u bakterijama ili ćelijskim kulturama, proizvodnja iRNK vakcina, u poređenju sa DNK vakcinama, traje kraće i relativno se lakše kontroliše (51, 80). S obzirom na to da se translacija u protein odvija u citoplazmi, dodatna prednost u odnosu na DNK vakcine je izbegavanje potencijalnog rizika od integracije u genom domaćina. Ne indukuju stvaranje anti-vektorskih antitela, kao što je primećeno za izvesne virusne vektorske vakcine (92, 93), i zbog toga se mogu primenjivati više puta.

U pretkliničkim studijama, i konvencionalne i samoamplifikujuće iRNK vakcine protiv različitih patogena (npr. HIV, virus influence, Zika i Ebola virus), pokazale su dobre rezultate (94-97). Neke od iRNK vakcina trenutno se testiraju u kliničkim studijama i generalno su pokazale ohrabrujuće rezultate u pogledu bezbednosti i imunogenosti (51), pružajući podršku za dalja klinička istraživanja.

Zaključak

Prevencija mnogih infektivnih bolesti i dalje predstavlja izazov u oblasti vakcinologije. U toku proteklih godina postalo je jasno da vakcine za ove bolesti nije

moguće dobiti sledeći klasične pristupe uspešnih konvencionalnih vakcina. Napredak u oblasti molekularne biologije i genetičkog inženjeringu omogućio je razvoj novih vakcina, kao što su vakcine sa vektorima i nukleinskim kiselinama (DNK i iRNK vakcine), koje ispunjavaju preduslove za rešenje ovih izazova. Svaki od ovih tipova vakcina ima svoje prednosti i nedostatke vezane za mogućnost indukcije različitih tipova imunskog odgovora, proizvodne kapacitete i bezbednost primene kod ljudi. Virusne vektorske vakcine efikasno stimulišu imunski sistem, slično kao i kod prirodne infekcije, i indukuju snažan imunski odgovor protiv kodiranog ciljnog antigena. Međutim, proizvodnja ovih vakcina je relativno kompleksna, i otuda skupa. Anti-vektorska antitela, već postojeća ili stvorena nakon prve imunizacije mogu ometati imunski odgovor, odnosno onemogućiti revakcinaciju istom vektorskog vakcinom. Pored toga, vezano za primenu ovih vakcina, postoji zabrinutost zbog rizika od neželjenih efekata i rezidualne replikacije virusa *in vivo*. U pogledu proizvodnje, DNK (naročito sintetski DNK konstrukti) i iRNK vakcine imaju prednost jer omogućavaju relativno jednostavan, sintetski proizvodni proces. Iako se u ranim kliničkim studijama DNK vakcine nisu pokazale dovoljno imunogenim, novija klinička ispitivanja ukazuju da ove vakcine mogu indukovati protektivni imunitet. Međutim, mogućnost dugotrajne perzistencije i integracije u genom domaćina, zavisnost od uređaja za ubrzgavanje ili elektroporacije, neki su od važnih nedostataka DNK vakcina. iRNK vakcine su najnovija tehnologija i stoga još uvek nisu mnogo ispitivane kod ljudi. Poslednjih godina pojatile su se publikacije pretkliničkih i ranih kliničkih ispitivanja u kojima su objavljeni obećavajući rezultati. Nemogućnost genomske integracije i nedostatak perzistencije u ćelijama vakcinisanih čini primenu iRNK vakcina bezbednijom u odnosu na primenu DNK vakcina.

Vektorske vakcine i DNK vakcine protiv infektivnih bolesti već su licencirane za primenu u veterini. Iako malobrojne, vektorske vakcine su odobrene i za primenu kod ljudi. Obećavajući rezultati dobijeni u kliničkim studijama sa DNK vakcinama, a nedavno i u ranim kliničkim studijama sa iRNK vakcinama, ukazuju da bi one mogle postati moćna sredstva u prevenciji infektivnih bolesti.

Zahvalnica

Ovaj rad je finansijski podržan sredstvima Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije (projekat broj 175050).

Literatura

1. Delany I, Rappuoli R, De Gregorio E. Vaccines for the 21st century. EMBO Mol Med. 2014;6(6):708–20.
2. Greenwood B. The contribution of vaccination to global health: past, present and future. Philos Trans R Soc Lond B Biol Sci. 2014;369(1645):20130433.

3. Stanberry LR, Strugnell R. Vaccines of the future. In: Garçon N, Stern PL, Cunningham AL, editors. *Understanding modern vaccines: perspectives in vaccinology*. Vol. 1. Amsterdam: Elsevier; 2011. p. 151–99.
4. Murray C, Ortblad K, Guinovart C, Lim S, Wolock T, Roberts DA, *et al*. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: A systematic analysis for the global Burden of Disease Study 2013. *Lancet* 2014;13;384(9947):1005-70.
5. Kaslow DC, Biernaux S. RTS, S: Toward a first landmark on the Malaria Vaccine Technology Roadmap. *Vaccine* 2015;3(52):7425-32.
6. Hazel M, Dockrell HM, Smith SG. What Have We Learnt about BCG Vaccination in the Last 20 Years? *Front Immunol*. 2017;8:1134.
7. Cunningham AL, Garçon N, Leo O, Friedland LR, Strugnell R, Laupèze B, *et al*. Vaccine development: From concept to early clinical testing. *Vaccine* 2016;34(52):6655-64.
8. Flingai S, Czerwonko M, Goodman J, Kudchodkar SB, Muthumani K, Weiner DB. Synthetic DNA vaccines: Improved vaccine potency by electroporation and co-delivered genetic adjuvants. *Front Immunol*. 2013;4:354.
9. Kallen KJ, Heidenreich R, Schnee M, Petsch B, Schlake T, Thess A, *et al*. A novel, disruptive vaccination technology: Self-adjuvanted RNAActive® vaccines. *Hum Vaccin Immunother*. 2013;9(10):2263–76.
10. Skenderi F, Jonjic S. Viral vaccines and vectors-some lessons from cytomegaloviruses. *Period. biol.* 2012;114(2):201-10.
11. Pasquale AD, Preiss S, Silva FT, Garçon N. Vaccine adjuvants: from 1920 to 2015 and beyond. *Vaccines (Basel)*. 2015;3(2):320–43.
12. Moser M, Leo O. Key concepts in immunology. *Vaccine* 2010;28(Suppl 3): C2–C13.
13. Ishii KJ, Koyama S, Nakagawa A, Coban C, Akira S. Host innate immune receptors and beyond: making sense of microbial infections. *Cell Host Microbe* 2008;3(6):352–63.
14. Siegrist C-A. Vaccine immunology. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*. 6th ed. Philadelphia, United States: Elsevier/Saunders; 2013. p. 14–32.
15. Vetter V, Denizer G, Friedland LR, Krishnan J, Shapiro M. Understanding modern-day vaccines: what you need to know. *Ann Med*. 2018;50(2):110-20.
16. Kallerup RS, Foged C. Classification of vaccines. In Subunit vaccine delivery, Foged C, Rades T, Perrie Y, Hooks S, editors. Springer. 2015. p. 15-29.
17. Hajj Hussein I, Chams N, Chams S, El Sayegh S, Badran R, Raad M, *et al*. Vaccines through centuries: major cornerstones of global health. *Front Public Health*. 2015;3:269.
18. Pulendran B, Ahmed R. Immunological mechanisms of vaccination. *Nat Immunol*. 2011;12(6):509–17.
19. Esteves K. Safety of oral poliomyelitis vaccine: results of a WHO enquiry. *Bull World Health Org*. 1988;66(6):739–46.

20. Strugnell R, Zepp F, Cunningham A, *et al.* Vaccine antigens. In: Garcon N, Stern PL, Cunningham AL, editors. Understanding modern vaccines: perspectives in vaccinology. Vol. 1. Amsterdam: Elsevier; 2011. p. 61–88.
21. Vučković Opavski N. Bakterijske vakcine. U: Medicinska mikrobiologija, udžbenik za student medicine, urednici Savić B, Jovanović T, Mitrović S. Univerzitet u Beogradu - Medicinski fakultet, Beograd, 2019;p. 121-31.
22. Michel ML, Tiollais P. Hepatitis B vaccines: protective efficacy and therapeutic potential. *Pathol Biol (Paris)*. 2010;58(4):288–95.
23. Roldão A, Mellado MC, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. *Expert Rev Vaccines* 2010;9(10):1149–76.
24. Pichichero ME. Protein carriers of conjugate vaccines: characteristics, development, and clinical trials. *Hum Vaccin Immunother*. 2013;9(12):2505–23.
25. Plotkin S.A. Correlates of protection induced by vaccination. *Clin Vaccine Immunol*. 2010;17(7):1055–65.
26. Robinson HL, Amara RR. T cell vaccines for microbial infections. *Nat Med*. 2005;11(4 Suppl):S25–S32.
27. Lemaire D, Barbosa T, Rihet P. Coping with genetic diversity: the contribution of pathogen and human genomics to modern vaccinology. *Braz J Med Biol Res*. 2012;45(5):376-85.
28. Sette A, Rappuoli R. Reverse vaccinology: developing vaccines in the era of genomics. *Immunity* 2010;33(4):530-41.
29. Becker PD, Guzmán CA. Community-acquired pneumonia: paving the way towards new vaccination concepts. In: Community-Acquired Pneumonia, ed. by N. Suttorp, T. Welte and R. Marre, 2007 BirkhäuserVerlag Basel/Switzerland, p.201-45.
30. Vernikos G, Medini D. Bexsero® chronicle. *Pathog Glob Health*. 2014;108(7):305–16.
31. Bull JJ, Smithson MW, Nuismier SL. Transmissible Viral Vaccines. *Trends Microbiol*. 2018;26(1):6-15.
32. Yurina V. Live Bacterial Vectors—A Promising DNA Vaccine Delivery System. *Med Sci. (Basel)* 2018;6(2):27.
33. Douglas AD, Williams AR, Illingworth JJ, Kamuyu G, Biswas S, Goodman AL, *et al.* The blood-stage malaria antigen PfRH5 is susceptible to vaccine-inducible cross-strain neutralizing antibody. *Nat Commun*. 2011;2:601.
34. Satti I, Meyer J, Harris SA, Thomas Z-RM, Griffiths K, Antrobus RD, *et al.* Safety and immunogenicity of a candidate tuberculosis vaccine MVA85A delivered by aerosol in BCG-vaccinated healthy adults: a phase1, double-blind, randomised controlled trial. *Lancet Infect Dis*. 2014;14(10):939–46.
35. Jeyanathan M, Thanthrige-Don N, Afkhami S, Lai R, Damjanovic D, Zganiacz A, *et al.* Novel chimpanzee adenovirus-vectorized respiratory mucosal tuberculosis vaccine: overcoming local anti-human adenovirus immunity for potent TB protection. *Mucosal Immun*. 2015;8(6):1373–87.

36. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, *et al.* Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med*. 2009;361(23):2209–20.
37. Monath TP, McCarthy K, Bedford P, Johnson CT, Nichols R, Yoksan S, *et al.* Clinical proof of principle for ChimeriVax: Recombinant live, attenuated vaccines against flavivirus infections. *Vaccine* 2002;20(7-8):1004–18.
38. Tan WG, Jin HT, West EE, Penalosa-Macmaster P, Wieland A, Zilliox MJ, *et al.* Comparative analysis of simian immunodeficiency virus gag-specific effector and memory CD8+ T cells induced by different adenovirus vectors. *J Virol*. 2013;87(3):1359–72.
39. Lee CS, Bishop ES, Zhang R, Yu X, Farina EM, Yan S, *et al.* Adeno virus mediated gene delivery: potential applications for gene and cell-based therapies in the new era of personalized medicine. *Genes Dis*. 2017;4(2):43–63.
40. Condit RC, Williamson AL, Sheets R, Seligman SJ, Monath TP, Excler JL, *et al.* Collaboration Viral Vector Vaccines Safety Working Group (V3SWG). Unique Safety Issues Associated with Virus Vectored Vaccines: Potential for and Theoretical Consequences of Recombination with Wild Type Virus Strains. *Vaccine* 2016;34(51):6610–6.
41. Nascimento IP, Leite LCC. Recombinant vaccines and the development of new vaccine strategies. *Braz J Med Biol Res*. 2012;45(12):102–11.
42. Rollier CS, Reyes-Sandoval A, Cottingham MG, Ewer K, Hill AV. Viral vectors as vaccine platforms: deployment in sight. *Curr Opin Immunol*. 2011;23(3):377–82.
43. Liu MA. Gene-based vaccines: Recent developments. *Curr Opin Mol Ther*. 2010;12(1):86–93.
44. Frey SE, Wald A, Edupuganti S, Jackson LA, Stapleton JT, El Sahly H, *et al.* Comparison of lyophilized versus liquid modified vaccinia Ankara (MVA) formulations and subcutaneous versus intradermal routes of administration in healthy vaccinia-naïve subjects. *Vaccine* 2015;33(39):5225–34.
45. Meyer J, Harris SA, Satti I, Poulton ID, Poyntz HC, Tanner R, *et al.* Comparing the safety and immunogenicity of a candidate TB vaccine MVA85A administered by intramuscular and intradermal delivery. *Vaccine* (2013) 31:1026–33.
46. Green CA, Scarselli E, Voysey M, Capone S, Vitelli A, Nicosia A, *et al.* Safety and immunogenicity of novel respiratory syncytial virus (RSV) vaccines based on the RSV viral proteins F, N and M2-1 encoded by simian adenovirus (PanAd3-RSV) and MVA (MVA-RSV); protocol for an open-label, dose escalation, single-centre, phase 1 clinical trial in healthy adults. *BMJ Open* 2015;5:e008748.
47. Liebowitz D, Lindblom JD, Brandl JR, Garg SJ, Tucker SN. High titre neutralising antibodies to influenza after oral tablet immunisation: a phase 1, randomised, placebo-controlled trial. *Lancet Infect Dis*. 2015;15(9):1041–8.
48. Vemula SV, Sayedahmed EE, Sambhara S, Mittal SK. Vaccine approaches conferring cross-protection against influenza viruses. *Expert Rev Vaccines* 2017;16(11):1141–54.
49. Lauer KB, Borrow R, Blanchard TJ. Multivalent and multipathogen viral vector vaccines. *Clin Vaccine Immunol*. 2017;24(1):e00298-16.

50. Venkatraman N, Anagnostou N, Bliss C, Bowyer G, Wright D, Lovgren-Bengtsson K, *et al.* Safety and immunogenicity of heterologous prime boost immunization with viral-vectored malaria vaccines adjuvanted with Matrix-M. *Vaccine* 2017;35(45):6208–17.
51. Rauch S, Jasny E, Schmidt KE, Petsch B. New Vaccine Technologies to Combat Outbreak Situations. *Front Immunol.* 2018;9:1963.
52. Petricciani J, Sheets R, Griffiths E, Knezevic I. Adventitious agents in viral vaccines: lessons learned from 4 case studies. *Biologicals* 2014;42(5):223–36.
53. Meeusen EN, Walker J, Peters A, Pastoret PP, Jungersen G. Current status of veterinary vaccines. *Clin Microbiol Rev.* 2007;20(3):489–510.
54. Ferraro B, Morrow MP, Hutnick NA, Shin TH, Lucke CE, Weiner DB. Clinical applications of DNA vaccines: current progress. *Clin Infect Dis.* 2011;53(3):296–302.
55. Hobernik D, Bros M. DNA Vaccines-How Far From Clinical Use? *Int J Mol Sci.* 2018;19(11). pii: E3605.
56. Walters AA, Kinnear E, Shattock RJ, McDonald JU, Caproni LJ, Porter N, *et al.* Comparative analysis of enzymatically produced novel linear DNA constructs with plasmids for use as DNA vaccines. *Gene Ther.* 2014;21(7):645–52.
57. Williams JA. Vector design for improved DNA vaccine efficacy, safety and production. *Vaccines* 2013;1(3):225–49.
58. Chen ZY, He CY, Ehrhardt A, Kay MA. Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression *in vivo*. *Mol Ther.* 2003;8(3):495–500.
59. John J, Suschak, James A, Williams, Connie S, Schmaljohn. Advancements in DNA vaccine vectors, non-mechanical delivery methods, and molecular adjuvants to increase immunogenicity. *Hum Vaccin Immunother.* 2017;13(12): 2837–48.
60. Grant-Klein RJ, Van Deusen NM, Badger CV, Hannaman D, Dupuy LC, Schmaljohn CS. A multiagent filovirus DNA vaccine delivered by intramuscular electroporation completely protects mice from ebola and Marburg virus challenge. *Hum Vaccin Immunother.* 2012;8(11):1703–6.
61. Vasan S, Hurley A, Schlesinger SJ, Hannaman D, Gardiner DF, Dugin DP, *et al.* In vivo electroporation enhances the immunogenicity of an HIV-1 DNA vaccine candidate in healthy volunteers. *PLoS One* 2011; 6(5):e19252.
62. Roy MJ, Wu MS, Barr LJ, Fuller JT, Tussey LG, Speller S, *et al.* Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. *Vaccine* 2000;19(7-8):764–78.
63. Ledgerwood JE, Hu Z, Gordon IJ, Yamshchikov G, Enama ME, Plummer S, *et al.* Influenza virus h5 DNA vaccination is immunogenic by intramuscular and intradermal routes in humans. *Clin Vaccine Immunol.* 2012;19(11):1792–7.
64. Aguiar JC, Hedstrom RC, Rogers WO, Charoenvit Y, Sacci JB Jr, Lanar DE, *et al.* Enhancement of the immune response in rabbits to a malaria DNA vaccine by immunization with a needle-free jet device. *Vaccine* 2001;20(1-2):275–80.

65. Fernando GJ, Zhang J, Ng HI, Haigh OL, Yukiko SR, Kendall MA. Influenza nucleoprotein DNA vaccination by a skin targeted, dry coated, densely packed microprojection array (Nanopatch) induces potent antibody and CD8 (+) T cell responses. *J Control Release* 2016;237:35–41.
66. Song J-M, Kim Y-C, Eunju O, Compans RW, Prausnitz MR, Kang S-M. DNA vaccination in the skin using microneedles improves protection against influenza. *Mol Ther*. 2012;20(7):1472–80.
67. Donnelly JJ, Wahren B, Liu MA. DNA vaccines: progress and challenges. *J Immunol*. 2005;175(29):633–9.
68. Li L, Petrovsky N. Molecular mechanisms for enhanced DNA vaccine immunogenicity. *Expert Rev Vaccines* 2016;15(3):313–29.
69. Gómez LA, Oñate AA. Plasmid-Based DNA Vaccines. In: Plasmid, Edited by Munazza Gull, Published: June 19th 2019, eBook (PDF), Published by IntechOpen, 2019.
70. Manam S, Ledwith BJ, Barnum AB, Troilo PJ, Pauley CJ, Harper LB, *et al.* Plasmid DNA vaccines: tissue distribution and effects of DNA sequence, adjuvants and delivery method on integration into host DNA. *Intervirology* 2000;43(4-6):273–81.
71. Porgador A, Irvine KR, Iwasaki A, Barber BH, Restifo NP, Germain RN. Predominant role for directly transfected dendritic cells in antigen presentation to CD8+ T cells after gene gun immunization. *J Exp Med*. 1998;188(6):1075–82.
72. Kutzler MA, Weiner DB. DNA vaccines: ready for prime time? *Nat Rev Genet*. 2008;9(10):776–88.
73. Kanthesh M, Loide N, Raghu N, Gopenath TS, Chandrashekappa GK, Murugesan K, *et al.* DNA Vaccines. *Vaccines Vaccin*. 2018;3(2):000122
74. Jorritsma SHT, Gowans EJ, Grubor-Bauk B, Wijesundara D. K. Delivery methods to increase cellular uptake and immunogenicity of DNA vaccines. *Vaccine* 2016;34(46):5488–94.
75. Frahm N, DeCamp AC, Friedrich DP, Carter DK, Defaww OD, Kublin JG, *et al.* Human adenovirus-specific T cells modulate HIV-specific T cell responses to an Ad5-vectored HIV-1 vaccine. *J Clin Invest*. 2012;122(1):359–67.
76. Schalk JA, Mooi FR, Berbers GA, Van Aerts LA, Ovelgonne H, Kimman TG. Preclinical and clinical safety studies on DNA vaccines. *Hum Vaccin*. 2006;2(2):45–53.
77. Wang Z, Troilo PJ, Wang X, Griffiths TG, Pacchione SJ, Barnum AB, *et al.* Detection of integration of plasmid DNA into host genomic DNA following intramuscular injection and electroporation. *Gene Ther*. 2004;11(8):711–21.
78. http://www.who.int/biologicals/publications/trs/areas/vaccines/dna/Annex%201_DNA%20vaccines.pdf?ua=1
79. World Health Organization. WHO Expert Committee on Biological Standardization 54th report ed. Geneva: World Health Organization, 2005
80. Zhang C, Maruggi G, Shan H, LiJ. Advances in mRNA Vaccines for Infectious Diseases. *Front Immunol*. 2019;10:594.
81. Schalke T, Thess A, Fotin-Mleczek M, Kallen KJ. Developing mRNA-vaccine technologies. *RNA Biol*. 2012;9(11):1319–30.

82. Aberle JH, Aberle SW, Kofler RM, Mandl CW. Humoral and cellular immune response to RNA immunization with flavivirus replicons derived from tick-borne encephalitis virus. *J Virol* (2005) 79(4):15107–13.
83. Johansson DX, Ljungberg K, Kakoulidou M, Liljestrom P. Intradermal electroporation of naked replicon RNA elicits strong immune responses. *PLoS ONE* 2012;7(1):e29732.
84. Scheel B, Teufel R, Probst J, Carralot JP, Geginat J, Radsak M, *et al.* Toll-like receptor-dependent activation of several human blood cell types by protamine-condensed mRNA. *Eur J Immunol.* (2005) 35(5):1557–66.
85. Schnee M, Vogel AB, Voss D, Petsch B, Baumhof P, Kramps T, *et al.* An mRNA vaccine encoding rabies virus glycoprotein induces protection against lethal infection in mice and correlates of protection in adult and newborn pigs. *PLoS Negl Trop Dis.* 2016;10(6):e0004746.
86. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. *Nat Rev Drug Discov.* 2018;17(4):261–79.
87. Benteyn D, Heirman C, Bonehill A, Thielemans K, Breckpot K. mRNA-based dendritic cell vaccines. *Expert Rev Vaccines* 2015;14(2):161–76.
88. Kowalczyk A, Doener F, Zanzinger K, Noth J, Baumhof P, Fotin-Mleczek M, *et al.* Self-adjuvanted mRNA vaccines induce local innate immune responses that lead to a potent and boostable adaptive immunity. *Vaccine* 2016;34(33):3882–93.
89. Pardi N, Hogan MJ, Pelc RS, Muramatsu H, Andersen H, Demaso CR, *et al.* Zika virus protection by a single low-dose nucleoside modified mRNA vaccination. *Nature* 2017;543(7644):248–51.
90. Chen N, Xia P, Li S, Zhang T, Wang TT, Zhu J. RNA sensors of the innate immune system and their detection of pathogens. *IUBMB Life* 2017; 69(5):297–304.
91. Kariko K, Muramatsu H, Ludwig J, Weissman D. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. *Nucleic Acids Res.* 2011; 39(21):e142
92. Pinschewer DD. Virally vectored vaccine delivery: medical needs, mechanisms, advantages and challenges. *Swiss Med Wkly.* 2017;147:w14465.
93. De Bruyn G. Cofactors that may influence vaccine responses. *Curr Opin HIV AIDS* 2010;5(5):404–8.
94. Richner JM, Himansu S, Dowd KA, Butler SL, Salazar V, Fox JM, *et al.* Modified mRNA vaccines protect against Zika virus infection. *Cell* 2017;168(6):1114–25.
95. Pardi N, Secreto AJ, Shan X, Debonera F, Glover J, Yi Y, *et al.* Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge. *Nat Commun.* 2017;8:14630.
96. Brazzoli M, Magini D, Bonci A, Buccato S, Giovani C, Kratzer R, *et al.* Induction of broad-based immunity and protective efficacy by selfamplifying mRNA vaccines encoding influenza virus hemagglutinin. *J Virol.* 2016;90(1):332–44.
97. Meyer M, Huang E, Yuzhakov O, Ramanathan P, Ciaramella G, Bukreyev A. Modified mRNA-based vaccines elicit robust immune responses and protect guinea pigs from Ebola virus disease. *J Infect Dis.* 2018; 217(3):451–5.

New vaccines on the horizon

Nevena Arsenović Ranin

University of Belgrade - Faculty of Pharmacy, Department of Microbiology and Immunology, Vojvode Stepe 450, 11221 Belgrade

Corresponding author, e-mail: nevena.arsenovic-ranin@pharmacy.bg.ac.rs

Summary

Vaccines are considered to be one of the greatest public health achievements of the last century. As a result of widespread vaccine use, the smallpox virus has been completely eradicated and the incidence of other diseases such as polio, measles, tetanus and diphtheria has been drastically reduced. Current licensed vaccines, predominantly composed of either live attenuated or killed pathogens, pathogen subunits, owe their success to their ability to elicit neutralizing antibodies against pathogens. On the other side, cell-mediated immunity, which plays a central role in elimination of intracellular pathogens (which in most cases leads to chronic infections) is much more difficult to obtain using current vaccines. Currently, numerous vector and nucleic acid (DNA and mRNA)-based prophylactic vaccines, capable of inducing substantial vaccine-specific T cell responses, are investigated in preclinical and clinical studies, with promising results. This review focuses the background of vector and nucleic acid-based vaccines, their strengths and weaknesses and safety issues.

Key words: vaccine types, vector vaccines, DNA vaccines, mRNA vaccines

Adjuvansi u vakcinama registrovanim za primenu kod ljudi

Brankica Filipić*, Zorica Stojić-Vukanić

Univerzitet u Beogradu - Farmaceutski fakultet, Katedra za mikrobiologiju i imunologiju, Vojvode Stepe 450, 11221 Beograd, Srbija

*Autor za korespondenciju: Brankica Filipić, e-mail: brankica.filipic@pharmacy.bg.ac.rs

Kratak sadržaj

Vakcinacija je jedna od najznačajnijih strategija za prevenciju infektivnih oboljenja, a razvoj subjediničnih vakcina doveo je do potrebe za primenom adjuvanasa u vakcinama. Naziv adjuvans potiče od latinske reči *adjuvare* što znači „pomoći“. Adjuvansi su supstance koje se primenjuju u vakcinama više od 90 godina, a dodaju se da bi se povećala imunogenost antigena koji imaju nizak imunostimulatorni potencijal. U vakcinama za primenu kod ljudi, najduže se kao adjuvansi koriste soli aluminijuma, ali je poslednjih decenija nekoliko novih adjuvanasa uključeno u vakcine koje su odobrene za primenu. Adjuvantni sistem AS04, ulazi u sastav vakcina protiv humanog papiloma virusa (Cervarix®) i hepatitis B virusa (Fendrix®) i sadrži aluminijum hidroksid i TLR4 agonist, monofosforil lipid A. Zatim, adjuvansi na bazi emulzije (MF59 i AS03) su sastavni deo vakcina protiv gripa (Fluad®, Focetria® i Pandemrix®). Kombinacija dva imunostimulatorna molekula, označena kao AS01, ulazi u sastav vakcine protiv herpes zoster virusa i malarije dok se adjuvansi na bazi virozoma koriste u vakcinama protiv hepatitisa A (Epaxal®) i gripa (Inflexal® V, Invivac®). Daljim razvojem adjuvanasa, i ispitivanjem njihovog mehanizma delovanja oni će se umesto empirijski, sve više koristili racionalno i ciljano, čime će se postići bolji imunogeni profil vakcina, bez narušavanja njihovog bezbednosnog profila.

Ključne reči: adjuvansi, vakcine, imunogenost

1. Uvod

Vakcinacija predstavlja jedno od najznačajnijih dostignuća u medicini čija je primena u velikoj meri doprinela kontroli i prevenciji infektivnih bolesti. Osnovni princip vakcinacije je indukcija imunskog odgovora domaćina prema antigenu koji se nalazi u vakcini i obezbeđivanje dugotrajne specifične zaštite koja sprečava nastanak infekcije ili razvoj bolesti (1). Prema podacima Svetske zdravstvene organizacije imunizacijom se sačuva 5 života svakog minuta (2). Istorijски, početak razvoja vakcina vezuje se za 18. vek i engleskog lekara Edvarda Dženera koji je prvi sproveo vakcinaciju protiv velikih boginja (3). Prvobitne vakcine zasnivale su se na primeni živih atenuisanih ili inaktivisanih mikroorganizama (4). Međutim, iako se zbog dobre efikasnosti i visoke imunogenosti žive atenuisane i inaktivisane vakcine primenjuju i u 21. veku, postoji rizik od povratka virulencije patogena ili nekompletne inaktivacije mikroorganizama, što dovodi u pitanje bezbednosni aspekt primene ovih vakcina, zbog čega se ne primenjuju kod imunokompromitovanih osoba, osoba sa transplantiranim organima, trudnica ili populacije starijeg doba (5). Usled toga, nove strategije u oblasti vakcinacije usmerene su ka, takozvanim, subjediničnim vakcinama, koje sadrže samo deo patogena, čime je postignuta veća bezbednost, ali slabija imunogenost i posledično manja efikasnost ovakve imunizacije (2). Kako primena samo određenog dela patogenog mikroorganizma najčešće nije dovoljna da se postigne adekvatna zaštita, vakcinama su dodati adjuvansi koji imaju sposobnost da pojačaju imunski odgovor (6).

Inicijalni razvoj adjuvanasa u periodu između 1920. i 1940. godine bio je usmeren na primenu u vakcinama koje su sadržale bakterijske toksoide. Različite supstance, poput agara, lecitina, skroba, saponina, soli kalcijuma i magnezijuma, čak i mrvica hleba, testirane su sa ciljem da se ispita njihov imunostimulatorni efekat (7).

Sam termin adjuvans potiče od latinske reči *adjuvare* što u prevodu znači „pomoći” i prvi put ga je upotrebio francuski veterinar Gaston Ramon, koji je definisao adjuvans kao „supstancu koja primenjena u kombinaciji sa specifičnim antigenom indukuje jači imunski odgovor nego sam antigen” (8). On je uočio da je nivo antitela na tetanus i difteriju bio viši kod konja kod kojih je na mestu injektovanja inaktivisanog toksina indukovana apses. Razvoj apsesa Ramon je postigao injektovanjem skroba ili mrvica hleba, i time je potvrđena hipoteza da supstance koje indukuju razvoj lokalne inflamacije na mestu injektovanja antigena doprinose povećanoj produkciji antitela (5). Negde u isto vreme, 1926. godine, Glenny i saradnici su otkrili adjuvantni efekat soli aluminijuma (9). Soli aluminijuma smatraju se jednim od najznačajnijih adjuvanasa koji su razvijeni, i prvi put je aluminijum kao adjuvans upotrebljen u humanim vakcinama 1932. godine.

Sa istorijskog aspekta, mogu se razdvojiti četiri razdoblja u razvoju adjuvanasa: (1) inicijalni razvoj adjuvanasa za vakcine sa bakterijskim toksoidima u periodu između 1920. i 1940. godine; (2) šira upotreba ulja i aluminijuma kao adjuvanasa u periodu

između 1940. i 1970. godine; (3) razvoj sintetskih adjuvanasa i depo sistema između 1970. i 1990. godine i (4) racionalni dizajn adjuvanasa koji aktiviraju urođeni imunski sistem od 1990. godine do danas (7). Tek početkom 1990-ih godina registrovane su vakcine, prvenstveno namenjene za primenu kod životinja, sa novim adjuvansima, koji pripadaju različitim klasama jedinjenja kao što su mineralne soli, produkti mikroorganizama, emulzije, saponini, citokini, polimeri, mikročestice i lipozomi (10, 11). Dodavanje adjuvana vakcinama ima za cilj da brže i jače indukuje zaštitni imunski odgovor kao i da smanji količinu antiga i broj imunizacija potrebnih da se postigne efikasan imunski odgovor (12). Izbor adjuvana koji će biti adekvatan u formulaciji određene vakcine zavisi od brojnih parametara, kao što su fizičke i hemijske karakteristike antiga, tip imunskog odgovora koji se želi postići imunizacijom, starost ciljne populacije i put primene vakcine (13).

2. Klasifikacija adjuvanasa

Podela adjuvanasa može se izvršiti na osnovu nekoliko kriterijuma, a najčešće se uzimaju u obzir fizičkohemijske karakteristike, poreklo i mehanizam dejstva. Prema mehanizmu dejstva, adjuvansi su najčešće podeljeni u dve glavne klase, nosače (oslobađajuće sisteme) i imunostimulatore (14), ali postoje i adjuvansi koji su kombinacija ove dve klase.

Nosači (oslobađajući sistemi) se definišu kao komponente koje prikazuju antigene iz vakcine imunskom sistemu na optimalan način u cilju pojačanja imunskog odgovora prema antigenu. Pored toga, nosači dovode do lokalnog proinflamatornog odgovora i nakupljanja ćelija urođene imunosti na mestu injektovanja vakcine. Primeri adjuvanasa nosača su mineralne soli (soli aluminijuma), emulzije (MF59, AS03), lipozomi (AS01) i virozomi (11, 15, 16).

Imunostimulatori su supstance koje aktiviraju urođeni imunski sistem, najčešće vezivanjem za receptore koji prepoznaju molekulske obrasce patogenih mikroorganizama (engl. *pattern-recognition receptors*; PRR). Ovi receptori obuhvataju nekoliko različitih familija proteina, od kojih su najzastupljeniji receptori slični Tollu (engl. *Toll-like receptors*; TLRs). PRR su ispoljeni na površini, u endozomu ili citozolu ćelija urođenog imunskog sistema, kao što su dendritske ćelije (DĆ), makrofagi, urođenoubilačke ćelije i neutrofili, ali ih ima i na B ćelijama i mnogim drugim ćelijama u organizmu. Imunostimulatori su lipid A, monofosforil lipid A (MPL), saponini (QS-21), bakterijski egzotoksini i drugi (2, 11, 17).

Mnogi novi adjuvansi u vakcinama koje su u različitim fazama kliničkih ispitivanja su kombinacija i nosača i imunostimulatora (11). U Tabeli I dat je pregled adjuvanasa registrovanih za primenu u humanim vakcinama (18).

Tabela I Adjuvansi registrovani za primenu u humanim vakcinama.
 Table I Adjuvants in licensed vaccines for human use.

Adjuvans	Sastav	Vakcina	Mehanizam dejstva	Referenca
Aluminijum	Soli aluminijskog pomešane sa antigenom (adsorpcija)	Brojne vakcine registrovane za primenu kod ljudi (DTP, Hib, Hepatitis B, pneumokokna vakcina)	Stimulacija lokalnog inflamatornog odgovora; poboljšana prezentacija antiga od strane APC; povećana produkcija antitela	19, 20, 21
AS04 („Adjuvant System 04”)	Kombinacija adjuvanasa: Al-hidroksid i monofosforil lipid A (MPL) dobijen iz LPS-a <i>Salmonella minnesota</i>	Vakcina protiv humanog papiloma virusa (Cervarix®); vakcina protiv hepatitisa B virus (Fendrix®)	Aktivacija APC; lokalno povećanje sinteze i sekrecije citokina; povećana produkcija antitela	20, 22
MF59	Skvalen, polisorbat 80, sorbitan trioleat	Vakcina protiv gripa (Fluad®), H1N1 vakcina (Focetria®)	Povećana aktivacija APC; stimulacija preuzimanja antiga i migracije ćelija do limfnih čvorova	21, 23
AS03 („Adjuvant System 03”)	Skvalen, vitamin E (α -tokoferol), polisorbat 80	Vakcina protiv gripa (Pandemrix®)	Podstiče lokalnu produkciju citokina i nakupljanje ćelija urođene imunosti	5, 24
AS01 („Adjuvant System 01”)	Na bazi lipozoma; sadrži dva imunostimulatora- MPL i saponin QS-21	Vakcina protiv Herpes zoster virusa (Shingrix®); Vakcina protiv malarije (Mosquirix®)	Lokalno nakupljanje ćelija urođene imunosti	11, 25
Virozomi	Vežikule kod kojih je antigen ugrađen u fosfolipidni dvoslojni omotač ili može biti unutar vežikule	Vakcine protiv gripa (Inflexal® V i Invivac®); vakcina protiv hepatitisa A (Epaxal®)	Podstiče preuzimanje antiga od strane APC	20, 21, 26

LPS-lipopolisaharid; APC-antigen prezentujuće ćelije; DTP-vakcina protiv difterije, tetanusa i velikog kašla; Hib-vakcina protiv oboljenja koje izaziva *Haemophilus influenzae* tip b.

2.1 Jedinjenja aluminijuma kao adjuvansi

Jedinjenja aluminijuma kao adjuvansi u humanim vakcinama se koriste od 1932. godine (27). Prvi upotrebljen adjuvans na bazi aluminijuma bio je aluminijum kalijum sulfat, koji je zbog slabe reproducibilnosti u potpunosti zamenjen aluminijum hidroksidom i aluminijum fosfatom, koji se mogu pripremiti standardizovanim metodama i za koje se antigen vezuje direktnom adsorpcijom (28). Adjuvansi na bazi aluminijuma često se zajedničkim imenom označavaju kao *alum*, iako je zapravo termin alum hemijsko ime za so aluminijum kalijum sulfata (29). Ovi adjuvansi ulaze u sastav vakcina koje se primenjuju protiv hepatitisa A, hepatitisa B, difterije/tetanusa/pertusisa (DTP), humanih papiloma virusa (HPV), *Haemophilus influenzae* tip b (Hib) i pneumokoka (2).

Mehanizam dejstva aluminijuma, kao i većine adjuvanasa, nije u potpunosti razjašnjen. Istraživanja na miševima pokazala su da adjuvansi na bazi aluminijuma pre svega indukuju imunski odgovor koji je posredovan pomoćničkim T (engl. *T helper*; Th) limfocitima tipa 2 koji karakteriše sinteza i sekrecija antitela i većinski je usmeren ka uklanjanju ekstraćelijskih patogena. Sa druge strane, adjuvansi na bazi aluminijuma nisu efikasni u indukciji Th1 imunskog odgovora koji je zajedno sa citotoksičnih T limfocitima usmeren ka uklanjanju intraćelijskih patogena, zbog čega se adjuvansi na bazi aluminijuma ne mogu primeniti u svim vakcinama (30). Ipak, uprkos nedovoljnom razumevanju efekta aluminijumskih adjuvanasa, upotreba aluminijuma u vakcinama je opravdana time što se pokazao kao bezbedan i stabilan adjuvans, lako se priprema, a pored toga, ne postoji još uvek odgovarajuća zamena (31).

2.2 Aluminijumove soli kao nosači za nove adjuvanse: primer AS04

Tokom poslednje dve decenije Glaxo Smith Kline (GSK) je razvio nove adjuvantne sisteme sa ciljem da se postigne brža, bolja i duža zaštita indukcijom visokog i perzistentnog titra antitela i aktivacijom ćelijski-posredovane imunosti (32). Jedan takav sistem, označen kao adjuvantni sistem AS04, je već u kliničkoj praksi kao sastavni deo registrovanih profilaktičkih vakcina protiv HPV i hepatitis B virusa (18,32). AS04 se sastoji iz dva adjuvansa: aluminijumove soli i TLR4 agonista, MPL, prečišćenog derivata lipopolisaharida (LPS) ekstrahovanog iz R595 soja *Salmonella minnesota* (18). LPS je kompleksni molekul prisutan samo u spoljašnjoj membrani Gram-negativnih bakterija, a za biološke efekte LPS-a odgovorna je lipidna komponenta tj. lipid A. Snažna adjuvantna aktivnost LPS-a/lipida A je odavno poznata, međutim, visoka toksičnost je ograničavala njegovu primenu u vakcinama (33). Međutim, 80-ih godina dvadesetog veka detoksifikacijom LPS molekula intenzivnim tretmanom soja R595 *Salmonella minnesota* pomoću kiselina i baza dobijen je MPL koji je posedovao imunostimulatorni efekat kao i lipid A, ali je imao bolji bezbednosni profil nego polazni molekul (34). Danas, MPL se koristi kao adjuvans u brojnim

profilaktičkim i terapijskim vakcinama koje su u razvoju i čija efikasnost se ispituje u kliničkim studijama (32).

Studije na miševima su pokazale da je MPL agonist TLR4, i da u potpunosti zadržava svoju imunostimulatornu aktivnost posredstvom TLR4 i kada se adsorbuje na soli aluminijuma (22). Nakon primene vakcine koja sadrži AS04, aktivacija TLR4 dovodi do brze (u okviru 3-6 h) sinteze i sekrecije citokina i nakupljanja ćelija na mestu primene vakcine (mišićno tkivo). U okviru prvog dana od injektovanja, uočava se povećana aktivacija monocita i dendritskih ćelija, koja je praćena aktivacijom antigen-specifičnih T i B ćelija i indukcijom snažnog i perzistentnog, kako humorалног (sekrecija antitela) tako i ćelijskog imunskog odgovora (18).

Poređenjem aktivnosti samog MPL-a sa AS04 pokazano je da dodavanje aluminijumove soli ne dovodi do sinergističkog efekta, već da se samo produžava sinteza i sekrecija citokina koja je indukovana MPL-om na mestu injektovanja (18).

2.3 Adjuvansi na bazi emulzije: MF59 i AS03

Emulzije imaju dugu istoriju primene kao adjuvansi u vakcinama za humanu i veterinarsku primenu. Freund je prvi pokazao adjuvantni efekat emulzije tipa voda-u-ulju u kombinaciji sa mikobakterijom ubijenom topotom i ovaj adjuvans je nazvan „kompletan Freundov adjuvans” (35). Kompletan Freundov adjuvans je dugo bio zlatni standard kada su u pitanju adjuvansi na bazi emulzije. Međutim, jedan od glavnih nedostataka pri primeni ovog adjuvansa jeste indukcija jakog, dugotrajnog lokalnog inflamatornog odgovora na mestu injektovanja, usled čega je prestala njegova primena kod ljudi i životinja i čime je izgubio i status standarda za poređenje sa drugim adjuvansima (36).

Emulzija voda-u-ulju bez mikobakterije koja je poznata kao „inkompletan Freundov adjuvans” je nakon toga primenjivana u veterinarskim vakcinama. Iako je ovaj adjuvans korišćen pedesetih godina prošlog veka i u humanim vakcinama (vakcina protiv gripa), pokazano je da zbog snažnog, lokalnog inflamatornog odgovora nije pogodan za rutinsku upotrebu (37, 38).

Iako emulzije imaju dugotrajnju primenu kao adjuvansi u vakcinama, tek krajem dvadesetog veka su zvanično odobrene za primenu kod ljudi (18). Prva generacija vakcina sa emulzijama kao adjuvansima imala je u svom sastavu mineralna ulja koja se nisu mogla metabolisati, i iako su bila snažni aktivatori produkcije antitela, uzrokovala su nastanak apsesa (39). Razvojem emulzija tipa ulje-u-vodi, poput MF59 i adjuvantnog sistema AS03, u kojima se koristi mineralno ulje koje se u potpunosti metaboliše, prevaziđen je problem prethodno korišćenih emulzija i omogućena je primena adjuvanasa na bazi emulzije u inaktivisanoj vakcini protiv sezonskog gripa, zatim u vakcini protiv ptičjeg gripa (H5N1, a kasnije i drugih sojeva) i u vakcini protiv pandemijskog gripa 2009. godine (H1N1) (40).

Glavna komponenta emulzija tipa ulje-u-vodi (MF59 i AS03) je *skvalen* (Tabela I), organsko jedinjenje koje se potpuno metaboliše, a prekursor je u biosintezi holesterola kod ljudi (41). Pored stabilizatora emulzije, AS03, za razliku od MF59, sadrži i imunostimulator α -tokoferol (vitamin E) (Tabela 1), zbog čega je i dobio naziv adjuvantni sistem (21).

2.4 Kombinacija imunostimulatora: primer AS01

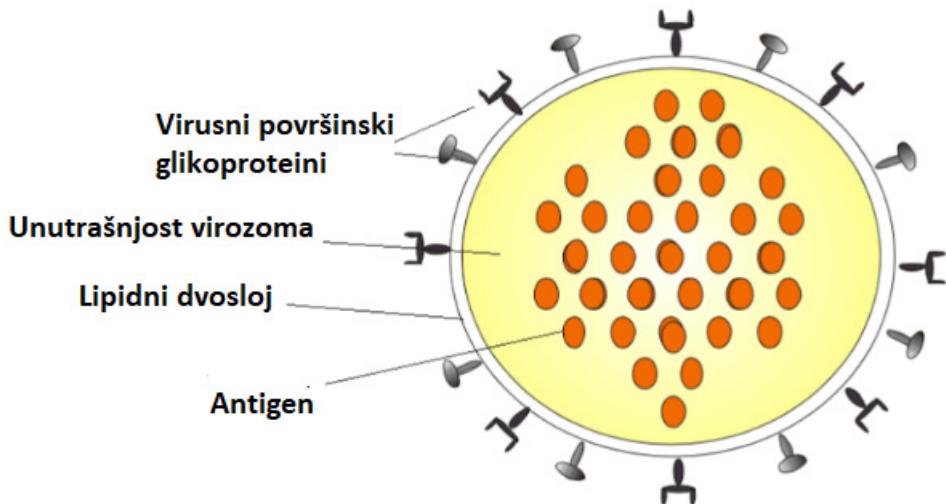
Adjuvantni sistem AS01 je jedinstven među adjuvansima jer sadrži dva različita imunostimulatorna molekula: MPL i saponin QS-21. QS-21 je triterpenski glikozid izolovan iz kore čileanskog drveta *Quillaja saponaria* Molina, za koji su studije na eksperimentalnim životinjama pokazale da indukuje sintezu antigen-specifičnih antitela i stimuliše nastanak citotoksičnih T-ćelija (42). QS-21 je rastvorljiv u vodi, amfifilne prirode, i poseduje hemolitičku aktivnost. Sa ciljem da se neutrališe njegova hemolitička aktivnost, napravljena je formulacija koju čine MPL, QS-21 i lipozom i tako je dobijen adjuvantni sistem AS01 (25).

AS01 je inicijalno razvijen da bi se uz indukciju sinteze antitela podstakao i dugotrajan ćelijski imunski odgovor, sa ciljem da se dobiju vakcine efikasne protiv patogena kod kojih ćelijska imunost ima ključnu ulogu u zaštiti. Kao adjuvans, AS01 je komponenta vakcine protiv *Plasmodium falciparum* koji je uzročnik malarije (Mosquirix[®]) i vakcine protiv herpes zoster virusa (Shingrix[®]) koje se klinički primenjuju, a ispituje se njegova primena u razvoju vakcina protiv virusa humane imunodeficijencije i tuberkuloze (18). Ono što AS01 čini različitim od ostalih adjuvanasa i što je ključna prednost kombinacije ova dva imunostimulatora, je sinergistički efekat koji se postiže kada se MPL i QS-21 koriste zajedno (43).

2.5 Virozomi

Tehnologija koja obuhvata primenu čestica koje se nazivaju virozomi razvijena je sa ciljem da se omogući proizvodnja efikasnih i bezbednih subjediničnih vakcina.

Almeida i saradnici su 1975. godine prvi generisali virozome ugrađivanjem proteina hemaglutinina i neuraminidaze poreklom od virusa influence u lipozome (44). Virozomi suštinski predstavljaju polu-sintetski kompleks veličine 150-200 nm u prečniku, koji je dobijen rekonstituisanjem virusne čestice. Sastoje se od glikoproteina virusnog omotača ugrađenih u dvoslojni fosfolipidni omotač, ali ne sadrže genetički materijal virusa od kojeg vode poreklo, usled čega nemaju sposobnost replikacije (Slika 1) (45).



Slika 1. Struktura virozoma

Figure 1. The structure of the virosome

Čitav spektar jedinjenja, uključujući antigene, nukleinske kiseline, lekove, tumorske antigene, antitela, može biti inkapsuliran unutar virozoma ili adsorbovan za njegovu površinu (46). Adjuvantni efekat virozoma zasniva se na njihovoj sposobnosti da pojačaju proces preuzimanja i prerade antiga od strane APĆ. Usled prisustva virusnih proteina omotača, virozom može „inficirati“ ćeliju domaćina i isporučiti antigen u citoplazmu ili može biti fagocitovan od strane APĆ (47). Prednost upotrebe virozoma je u tome što su biodegradibilni, biokompatibilni i netoksični, usled čega je njihova primena odobrena od strane Američke agencije za hranu i lekove (engl. *Food and Drug Administration*) za primenu kod ljudi jer imaju visoki bezbednosni profil. Pored toga, virozomi ne dovode do razvoja autoimunosti ili anafilakse. Virozomi se mogu primeniti intravenski, intramuskularno, subkutano, inhalacionim putem, oralno ili topikalno tj. površinski (48).

Prva generacija virozoma već je u primeni u komercijalnim vakcinama protiv hepatitisa A (Epaxal®) i gripe (Inflexal® V i Invivac®). Pored toga, vakcine protiv malarije, hepatitisa C, karcinoma dojke, *Candida* vrsta u kojima se koriste virozomi kao adjuvansi su u različitim fazama prekliničkih i kliničkih studija (2).

3. Bezbednost adjuvanasa

Adjuvansi su od ključnog značaja za efikasnost mnogih vakcina i poslednjih godina ulazu se napor i kako bi se razumeo mehanizam njihovog dejstva. Međutim, istorijski gledano, istraživanja u oblasti adjuvanasa uvek su bila više usmerena na

izučavanje njihove efikasnosti da indukuju protektivnu imunost, dok se manje napora ulagalo u razumevanje mehanizama njihove toksičnosti (49). Poznato je da su neki od imunostimulatornih efekata adjuvanasa, u isto vreme odgovorni i za njihova neželjena dejstva. U tom smislu, teško je postaviti jasnou granicu između imunostimulatornog efekta i imunotoksičnosti adjuvanasa, kako bi se postigla idealna ravnoteža po pitanju efikasnosti/bezbednosti. U prošlosti, stotine imunostimulatornih adjuvanasa je testirano u vakcinama, međutim, usled toksičnosti, mnogi potencijalni adjuvansi su odbačeni, a svega nekoliko je danas registrovano za primenu u humanim vakcinama (50). Trenutno, jedan od najvećih izazova u razvoju vakcina jeste da se primeni visoko efikasana kombinacija antigen-adjuvans koja će uzrokovati minimalna neželjena dejstva.

Najčešći neželjeni efekti primene adjuvanasa su blagi i lokalnog karaktera i manifestuju se pojmom bola na mestu injektovanja, crvenila i lokalne inflamacije, a mogu biti posledica hemijske iritabilnosti ili direktne citotoksičnosti adjuvansa (51). Sistemske posledice primene adjuvanasa najčešće se manifestuju kao groznica, glavobolja, mučnina, dijareja i bol u mišićima (49).

Jedna od najvećih dilema kada su u pitanju neželjena dejstva adjuvanasa odnosi se na moguću indukciju ili pojačavanje već postojećeg autoimunskog oboljenja. Međutim, čak i ako kombinacija vakcina/adjuvans indukuje autoimunsko oboljenje, to je teško dokazati (49). U toku 2010. godine, prijavljeni su slučajevi razvoja autoimunskog oboljenja narkolepsije koji su dovođeni u vezu sa primenom vakcine protiv gripa Pandemrix® koja kao adjuvans sadrži AS03. Ipak, istraživanja su pokazala da ne postoji veza između narkolepsije i Pandemrix® AS03 vakcine, ni kod dece ni kod odraslih osoba (52).

Kada se govori o bezbednosti adjuvanasa, zlatni standard predstavljaju soli aluminijuma, koje su izuzetno efikasne u pojačavanju imunskog odgovora, najduže se primenjuju i imaju najbolji bezbednosni profil kada su u pitanju humani adjuvansi (49). U tom smislu, preporučuje se da se svaki novi adjuvans testira i poredi sa ovim adjuvansima.

Iako ne postoji apsolutna garancija kada je u pitanju bezbednost vakcina i adjuvanasa, bilo kakav rizik od teške neželjene reakcije uzrokovane modernim vakcinama je ekstremno nizak i pacijenti treba da budu potpuno sigurni u bezbednost dostupnih vakcina (49).

4. Zaključak

Već gotovo vek adjuvansi se primenjuju sa ciljem da se poveća efikasnost vakcina. Do nedavno, izbor adjuvanasa je uglavnom bio empirijski, ali značajan napredak u razvoju novih adjuvanasa i stalno izučavanje i težnja da se utvrde molekularni mehanizmi njihovog delovanja postepeno doprinose razvoju njihove racionalne i ciljane upotrebe. Adjuvansi su danas postali neophodna komponenta

mnogih vakcina, jer se umesto živih atenuisanih patogena, mnogo češće koriste prečišćeni antigeni mikroorganizama sa niskim stepenom imunogenosti. Iako broj adjuvanasa u registrovanim vakcinama nije veliki, novi adjuvansi značajno su doprineli većoj efikasnosti pojedinih vakcina, bez narušavanja njihove bezbednosti. Dobar izbor kombinacije antigena i adjuvansa imaće u budućnosti ključnu ulogu u dizajnu vakcina koje će biti efikasne i bezbedne.

Zahvalnica

Autori se zahvaljuju COST akciji CA16231 „European Network of Vaccine Adjuvants- ENOVA”, koja im je svojim aktivnostima omogućila unapređenje znanja iz oblasti adjuvanasa.

Literatura

1. Zepp F. Principles of vaccine design-Lessons from nature. *Vaccine*. 2010;28:C14–C24.
2. Apostólico Jde S, Lunardelli VA, Coirada FC, Boscardin SB, Rosa DS. Adjuvants: Classification, Modus Operandi, and Licensing. *J Immunol Res*. 2016;1459394. doi: 10.1155/2016/1459394.
3. Gross CP, Sepkowitz KA. The myth of the medical breakthrough: Smallpox, vaccination, and Jenner reconsidered. *Int J Infect Dis*. 1998;3(1):54-60.
4. Bonanni P, Santos J. Vaccine evolution. *Perspective in Vaccinol*. 2011;(1):1–24.
5. Di Pasquale A, Preiss S, Tavares Da Silva F, Garçon N. Vaccine Adjuvants: from 1920 to 2015 and Beyond. *Vaccines (Basel)*. 2015;3(2):320–43.
6. Marciani DJ. Vaccine adjuvants: role and mechanisms of action in vaccine immunogenicity. *Drug Discov Today*. 2003;8(20):934-43.
7. Ott G, Van Nest G. Development of vaccine adjuvants: ahistorical perspective. In: Singh M (ed) *Vaccine adjuvants and delivery systems*. Wiley, London, 2007;pp 1–31
8. Ramon G. Sur la toxine et sur l'anatoxine diphtheriques. *Ann Inst Pasteur*. 1924;38:1–10.
9. Glenny AT, Pope CG, Waddington H, Wallace U. Immunological notes. XVII–XXIV. *Journal Pathol Bacteriol*. 1926;29(1):31–40.
10. Guy B. The perfect mix: recent progress in adjuvant research. *Nat Rev Microbiol*. 2007;5:505–17.
11. Christensen D. Vaccine adjuvants: Why and how. *Hum Vaccin Immunother*. 2016;12(10):2709–11.
12. Coffman RL, Sher A, Seder RA. Vaccine adjuvants: putting innate immunity to work. *Immunity*. 2010;33(4):492-503.
13. Reed SG, Orr MT, Fox CB. Key roles of adjuvants in modern vaccines. *Nat Med*. 2013;19(12):1597-608.

14. Pashine A, Valiante NM, Ulmer JB. Targeting the innate immune response with improved vaccine adjuvants. *Nat Med.* 2005;11(4 Suppl):S63-8.
15. García A, De Sanctis JB. An overview of adjuvant formulations and delivery systems. *APMIS.* 2014;122(4):257-67.
16. Kaurav M, Madan J, Sudheesh MS, Pandey RS. Combined adjuvant-delivery system for new generation vaccine antigens: alliance has its own advantage. *Artif Cells Nanomed Biotechnol.* 2018;46(sup3):S818-31.
17. Lee S, Nguyen MT. Recent advances of vaccine adjuvants for infectious diseases. *Immune Netw.* 2015;15(2):51-7.
18. Del Giudice G, Rappuoli R, Didierlaurent AM. Correlates of adjuvanticity: A review on adjuvants in licensed vaccines. *Semin Immunol.* 2018;39:14-21.
19. Kool M, Pétrilli V, De Smedt T, Rolaz A, Hammad H, van Nimwegen M, et al. Cutting edge: alum adjuvant stimulates inflammatory dendritic cells through activation of the NALP3 inflammasome. *J Immunol.* 2008;181: 3755-59.
20. Awate S, Babiuk LA, Mutwiri G. Mechanisms of action of adjuvants. *Front Immunol.* 2013;4:114.
21. Di Pasquale A, Preiss S, Tavares Da Silva F, Garçon N. Vaccine Adjuvants: from 1920 to 2015 and Beyond. *Vaccines (Basel).* 2015;3(2):320-43.
22. Didierlaurent AM, Morel S, Lockman L, Giannini SL, Bisteau M, Carlsen H, et al. AS04, an aluminum salt- and TLR4 agonist-based adjuvant system, induces a transient localized innate immune response leading to enhanced adaptive immunity. *J Immunol.* 2009;183(10):6186-97.
23. Calabro S, Tortoli M, Baudner BC, Pacitto A, Cortese M, O'Hagan DT, et al. Vaccine adjuvants alum and MF59 induce rapid recruitment of neutrophils and monocytes that participate in antigen transport to draining lymph nodes. *Vaccine.* 2011;17;29(9):1812-23.
24. Morel S, Didierlaurent A, Bourguignon P, Delhaye S, Baras B, Jacob V, et al. Adjuvant system AS03 containing α -tocopherol modulates innate immune response and leads to improved adaptive immunity. *Vaccine.* 2011;29(13):2461-73.
25. Didierlaurent AM, Laupèze B, Di Pasquale A, Hergli N, Collignon C, Garçon N. Adjuvant system AS01: helping to overcome the challenges of modern vaccines. *Expert Rev Vaccines.* 2017;1:55-63.
26. Khoshnejad M, Young PR, Toth I, Minchin RF. Modified influenza virosomes: recent advances and potential in gene delivery. *Curr Med Chem.* 2007;14: 3152-6.
27. Park WH, Schroder MC. Diphtheria Toxin-Antitoxin and Toxoid: A Comparison. *Am J Public Health Nations Health.* 1932;22(1):7-16.
28. Marrack P, McKee AS, Munks MW. Towards an understanding of the adjuvant action of aluminium. *Nat Rev Immunol.* 2009;9(4):287-93.
29. HogenEsch H, O'Hagan DT, Fox CB. Optimizing the utilization of aluminum adjuvants in vaccines: you might just get what you want. *NPJ Vaccines.* 2018;(10)3:51.

30. Lindblad EB. Aluminium compounds for use in vaccines. *Immunol Cell Biol*. 2004;82(5):497-505.
31. Djuricic S, Jakobsen JC, Petersen SB, Kenfert M, Gluud C. Aluminium adjuvants used in vaccines versus placebo or no intervention. *Cochrane Database Syst Rev*. 2017;(9):CD012805.
32. Garçon N, Tavares F. Development and Evaluation of AS04, a Novel and Improved Adjuvant System Containing 3-O-Desacyl-4'- Monophosphoryl Lipid A and Aluminum Salt. *Immunopotentiators in Modern Vaccines*. 2017;287-309.
33. Johnson AG, Gaines S, Landy M. Studies on the O antigen of *Salmonella typhosa*. V. Enhancement of the antibody response to protein antigens by the purified lipopolysaccharide. *J Exp Med*. 1956;103(2):225-46.
34. Myers KR, Truchot AT, Word J, Hudson Y, Ulrich JT. A critical determinant of lipid A endotoxic activity. In: Nowotny A, Spitzer JJ, Ziegler EJ, editors. *Cellular and molecular aspects of endotoxin reactions*. New York, NY: Elsevier Science Publishing Co; 1990. p. 145e56.
35. Freund J, Casals J, Hosmer E. Sensitization and antibody formation after injection of Tubercl Bacilli and paraffin oil. *Proc Soc Exp Biol Med*. 1937;37(3):509 – 13.
36. Billiau A, Matthys P. Modes of action of Freund's adjuvants in experimental models of autoimmune diseases. *J Leukoc Biol*. 2001;70(6):849-60.
37. Salk JE, Laurent AM. The use of adjuvants in studies on influenza immunization. I. Measurements in monkeys of the dimensions of antigenicity of virus-mineral oil emulsions. *J Exp Med*. 1952;95(5):429-47.
38. Miller LH, Saul A, Mahanty S. Revisiting Freund's incomplete adjuvant for vaccines in the developing world. *Trends Parasitol*. 2005;21(9):412-4.
39. Lattanzi M. Non-recent history of influenza pandemics, vaccines, and adjuvants. *Influenza Vaccines for the Future*. 2008;245-59.
40. O'Hagan DT, Tsai TF, Brito LA. Emulsion based vaccine adjuvants. *Hum Vaccin Immunother*. 2013;9(8):1698–1700.
41. Del Giudice G, Rappuoli R. Inactivated and adjuvanted influenza vaccines. *Curr Top Microbiol Immunol*. 2015;386:151-80.
42. Zhu D, Tuo W. QS-21: A Potent Vaccine Adjuvant. *Nat Prod Chem Res*. 2016;3(4):e113.
43. Coccia M, Collignon C, Hervé C, Chalon A, Welsby I, Detienne S, van Helden MJ, Dutta S, Genito CJ, Waters NC, Deun KV, Smilde AK, Berg RAVD, Franco D, Bourguignon P, Morel S, Garçon N, Lambrecht BN, Goriely S, Most RV, Didierlaurent AM. Cellular and molecular synergy in AS01-adjuvanted vaccines results in an early IFN γ response promoting vaccine immunogenicity. *NPJ Vaccines*. 2017;2:25.
44. Almeida JD, Edwards DC, Brand CM, Heath TD. Formation of virosomes from influenza subunits and liposomes. *Lancet*. 1975;2(7941):899-901.
45. Trovato M, De Berardinis P. Novel antigen delivery systems. *World J Virol*. 2015;4(3):156–68.

46. Kaneda Y. Virosome: a novel vector to enable multi-modal strategies for cancer therapy. *Adv Drug Deliv Rev.* 2012;64(8):730-8.
47. Moser C, Müller M, Kaeser MD, Weydemann U, Amacker M. Influenza virosomes as vaccine adjuvant and carrier system. *Expert Rev Vaccines.* 2013;12(7):779-91.
48. Rathore P, Swami G. Virosomes: a novel vaccination technology. *IJPSR.* 2012;3(10):3591–97.
49. Petrovsky N. Comparative safety of vaccine adjuvants: a summary of current evidence and future needs. *Drug Saf.* 2015;38 (11): 1059–74.
50. Batista-Duharte A, Martínez DT, Carlos IZ. Efficacy and safety of immunological adjuvants. Where is the cut-off? *Biomed Pharmacother.* 2018;105:616-24.
51. Gupta RK, Relyveld EH, Lindblad EB, Bizzini B, Ben-Efraim S, Gupta CK. Adjuvants--a balance between toxicity and adjuvanticity. *Vaccine.* 1993;11(3):293-306.
52. Weibel D, Sturkenboom M, Black S, et al. Narcolepsy and adjuvanted pandemic influenza A (H1N1) 2009 vaccines - Multi-country assessment. *Vaccine.* 2018;36(41):6202–11.

Adjuvants in vaccines registered for human use

Brankica Filipić*, Zorica Stojić-Vukanić

University of Belgrade - Faculty of Pharmacy, Department of microbiology and immunology, Vojvode Stepe 450, 11221 Belgrade, Serbia

*Corresponding author: e-mail: brankica.filipic@pharmacy.bg.ac.rs

Summary

Vaccination is one of the most efficient strategies for prevention of infection diseases, but with introduction of sub-unit vaccines with lower immunogenicity adjuvants were needed to enhance the immune response. The term adjuvant is from Latin verb *adjuvare* which means „to aid”. Adjuvants have been used in vaccines for more than 90 years. The longest adjuvant history belongs to aluminium salts, but novel adjuvants have been introduced in licensed vaccines in last 30 years. These novel adjuvants are AS04, which consists of aluminium hydroxide and Toll-like receptor 4 (TLR4) agonist monophosphoryl lipid A and is used in hepatitis B vaccine Fendrix® and HPV vaccine Cervarix®, emulsion based adjuvants which are part of several influenza vaccines-MF59 (Fluad® and Focetria®) and AS03 (Pandemrix®), AS01 liposomal adjuvant which is combination of two distinct immunostimulatory molecules and is component of herpes zoster and malaria vaccine and virosomes included in hepatitis A vaccine (Epaxal®) and influenza vaccines (Inflexal® V and Invivac®). Adjuvant development and better insight into their mechanism of action are of great importance in order to replace empirical with rational use of adjuvants, without affecting vaccine safety.

Key words: adjuvants, vaccines, immunogenicity

Established and Advanced Adjuvants in Vaccines' Formulation: Mineral Adsorbents, Nanoparticulate Carriers and Microneedle Delivery Systems

Danina Krajišnik, Tanja Ilić, Ines Nikolić, Snežana Savić*

University of Belgrade – Faculty of Pharmacy, Department of Pharmaceutical Technology and Cosmetology, Vojvode Stepe 450, 11221 Belgrade, Serbia

*Corresponding author: Dr Snežana Savić, Tel.: +381-11-3951288

E-mail: snezana.savic@pharmacy.bg.ac.rs

Summary

In the era of modern vaccinology, limited immunogenicity of the most commonly used antigens has enforced the use of various adjuvants in vaccine formulations to achieve desired immune response. Aluminum-containing adjuvants have been, historically, the most widely used mineral immunostimulants, generally regarded as safe to use in human vaccines. The great academic progress in inorganic (nano)materials synthesis, structure control and functionalization design has led to a growing interest in innovative adjuvants such as clays, mesoporous silica nanoparticles, zinc oxide, iron oxide and iron hydroxide nanoparticles, etc. On the other hand, there has been an intention to use specific nanoparticulated antigen delivery systems, such as nanoemulsions, in order to protect antigens from premature proteolytic degradation and/or to improve antigen immunogenicity by facilitating antigen uptake and processing by antigen presenting cells. Simultaneously, numerous research efforts have been focused on the development of innovative technologies for antigen delivery into the skin (such as microneedles), with the aim to improve vaccine efficacy alongside with enhanced patient adherence, particularly in children population (noninvasive or minimally invasive administration). Therefore, this review deals with each of these approaches in more detail, with the special emphasis on examples of their use in vaccine formulations as well as on the factors influencing their efficacy and safety.

Keywords: vaccine formulation, adjuvants, mineral adsorbents, nanoemulsions, Microneedles

1. Introduction

It has been well established that effective and safe vaccines present an irreplaceable tool continually contributing to public and animal health worldwide, while the ongoing development of vaccines points out to the certain technological achievements during the last decades of biomedical research advancement (1). Currently, European Pharmacopoeia (supplement Ph. Eur. 9.8) counts 144 monographs of different types of vaccines intended for human and veterinary use, from which 64 monographs are those for application in human population (2). In accordance with Ph. Eur. 9, *Vaccines for human use (Vaccina ad usum humanum)* are biological products that represent preparations containing antigens capable of inducing a specific and active immunity in man against an infecting agent, or the toxin or antigen elaborated by it. Immune responses include the induction of the innate and adaptive (cellular, humoral) parts of the immune system. Vaccines for human use have been shown acceptable immunogenic activity and safety within the human population taking into account the vaccination schedule (2).

Further, within the general monograph, the European Pharmacopoeia describes types of vaccines intended for human use and their different characteristics including basic principles of their production. Thus, vaccines for human use may contain: whole micro-organisms (MOs) (bacteria, viruses or parasites), inactivated by chemical and physical means that maintain adequate immunogenic properties; whole live MOs that are naturally avirulent or that have been treated to attenuate their virulence whilst retaining adequate immunogenic properties; antigens extracted from MOs or secreted from MOs or produced by genetic engineering or chemical synthesis. The antigens may be used in their native state or may be detoxified or modified by chemical or physical means, and may be aggregated, polymerised or conjugated to a carrier in order to enhance their immunogenicity. Vaccines may contain some specific adjuvant or adjuvants. Where the antigen is adsorbed on a mineral adjuvant, the vaccine is referred to as „adsorbed” (2). Ph. Eur. 9 differs following types of vaccines: 1) *Bacterial vaccines containing whole cells*; 2) *Bacterial vaccines containing bacterial components*; 3) *Bacterial toxoids*; 4) *Viral vaccines*; 5) *Synthetic antigen vaccines*; 6) *Combined vaccines* and 7) *Adsorbed vaccines* (2).

Whatever the vaccine type is, vaccines authorized for the human use are clear, colourless liquids or they may be coloured or vary in opacity according to the type of preparation; further vaccines' formulation may be suspension of various degree of opacity in colourless liquid, or they may be powders produced by freeze-drying/lyophilization procedure, which should be reconstituted by convenient vehicle just before the administration (2). In the most cases, vaccines are intended for parenteral administration route (intradermal vaccines), when they must be sterile, but they are also formulated for oral and nasal application (e.g. vaccine against influenza authorized for

intranasal administration by U.S. Food and Drug Administration (FDA) in 2003 and intended for human population aged 5 – 49 years) (3). Besides, a series of intensive researches, including clinical trials, are ongoing in order to investigate and consequently to enable the application of vaccines using a transdermal route and generally skin pathways mainly involving the concept of microneedles, or sublingual as well as buccal routes and inhalation pathways. In their formulations, vaccines usually contain different pharmaceutical excipients, at the first place adjuvants and adsorbents (as a specific type of adjuvants), some stabilizers, buffers or preservatives (2, 3).

Although the modern vaccines are formulated in such way to possess an acceptable safety profile as much as possible, thanked to the refined purification techniques and well defined nature of antigens, it could be happened that due to the high level of purity and specificity, resulted antigens have less immunogenicity (1, 4). Therefore, the investigation of more effective and specific adjuvants and their incorporation into vaccines' formulations has become a standard consideration during their development.

Adjuvants are substances that when mixed with vaccines antigens, may impact through certain aspects to the enhancement of the immune responses: enabling sustained release of the given antigen dose, or allowing the antigen dose sparing, which can dramatically reduce vaccine-manufacturing costs; some of the adjuvants can adjust the vaccine formulation and make them particularly relevant to the elderly, young children and patients with chronic diseases (1). Emulsion systems of oil-in-water type (e.g. MF59 composed of squalene and two surfactants, Tween 80 and Span 85) and mineral components – adsorbents (aluminum hydroxide, aluminum phosphate and amorphous aluminum hydroxyphosphate sulfate) are used as common choice for vaccine adjuvants. Simultaneously, there is an intention to apply specific advanced antigen- delivery systems (e.g. microneedles) which may improve an antigen immunogenicity, i.e. a vaccine efficacy alongside with adherence increase, particularly in children population (noninvasive or minimally invasive administration). The prospective aims of nanoparticulate systems as antigen carriers (nanoparticle-based vaccines) are based on assumptions that they could protect antigens from premature proteolytic degradation, facilitate antigen uptake and processing by antigen presenting cells, enable control release and should be safe for human use (5). Nanocarriers composed of lipids, proteins, metals or polymers are in focus and there is a need for some modification/adjustment of the critical attributes to functionalize them in order to reach an effective antigen delivery and satisfying immune response. In addition, it is of great importance to improve the performance of mineral adjuvants/adsorbents, through usage of micro- and mesoporous aluminosilicates. Apart different types of nanoparticulate carriers known as useful drug delivery systems, and therefore are considered for longer as prospective adjuvants in the vaccines' formulation development, further, a technology of controlled

antigen release and penetration/permeation through the skin applying solid or soluble microneedles is a subject of this review (4, 5).

2. Inorganic compounds as vaccine adjuvants

Aluminum-containing adjuvants, as representatives of inorganic compounds, have been historically served as immunostimulants in vaccines and continue to be the most widely used adjuvants (6) generally regarded as safe to use in human vaccines (7). During the last decade, great academic progress in inorganic nanomaterials for vaccine adjuvants in terms of nanometer-scale synthesis, structure control, and functionalization design was achieved (8). The following section summarizes the structure, physico-chemical and functional properties of currently used mineral adjuvants, relevant for their application in vaccine formulation. Additionally, a brief review of other mineral materials and novel inorganic nanoparticles which have been investigated as possible antigen carriers and adjuvants for vaccines is presented.

2.1 Mineral Adjuvants

Aluminum compounds and calcium phosphate are representatives of inorganic mineral compounds, which have been applied as immunological adjuvants in vaccine formulation. Of these two, the aluminum compounds have the longest history and by far the most comprehensive record of use (7).

Aluminum salts, as representatives of mineral adjuvants, have been used in human vaccines for over 70 years. Table I summarizes examples of available vaccines currently licensed for human use that employ aluminium adjuvants. A.T. Glenny and coworkers were the first to demonstrate the adjuvant effect of aluminum compounds (9). The first aluminum-adjuvanted vaccines were prepared by the addition of base to a solution of antigen (diphtheria toxoid) mixed with aluminum potassium sulfate, resulting in precipitation of the antigen and aluminum salt. Glenny observed that injecting the diphtheria toxoid as an alum precipitate led to a significant increase in the immune response against the toxoid. Although the term alum is used colloquially to refer to all aluminum adjuvants, it is technically name for aluminum potassium sulfate $[AlK(SO_4)_2 \cdot 12H_2O]$, which has not been widely used as an adjuvant in human vaccines (10). Vaccine preparation in accordance with this principle referred to as *alum-precipitated vaccines*, has been almost completely replaced by the adsorption of antigens onto preformed aluminum gels referred as *aluminium-adsorbed vaccines*, since this method provides a more reproducible production process, control of the adsorption and the absence of a base environment that can affect antigen stability (7, 9).

Table I Examples of licensed aluminium-adjuvanted vaccines for human use (6, 9, 16)**Tabela I** Primeri odobrenih vakcina sa adjuvansima na bazi aluminijuma za humanu upotrebu (6, 9, 16)

Adjuvant	Vaccine	Trade name	Manufacturer
Aluminum hydroxide	TdaP	Boostrix®	GSK
	DTaP	Infanrix®	GSK
	DTaP, Hepatitis B, polio	Pediatrix®	GSK
	DTaP, polio	Kinrix®	GSK
	Human papilloma virus	Cervarix®	GSK
	Hepatitis A	Havrix®	GSK
	Hepatitis B	Engerix®	GSK
	Meningococcus B	Bexsero®	GSK
Aluminum phosphate	Tetanus and Diphtheria Toxoids, Adsorbed	Tenivac®	Sanofi-Pasteur
	Pneumococcus	Prevnar 13®	Pfizer
	Meningococcus B	Trumenba®	Pfizer
	DTaP	Daptacel®	Sanofi-Pasteur
	DTaP, polio	Quadracel®	Sanofi-Pasteur
Amorphous aluminum hydroxyphosphate sulfate	Human papilloma virus	Gardasil®	Merck
	Hepatitis A	VAQTA®	Merck
	Hepatitis B	Recombivax HB®	Merck
	Haemophilus influenzae B,	PedVaxHIB®	Merck
Aluminum hydroxide and aluminum phosphate	Hepatitis A and Hepatitis B	Twinrix®	GSK

Legend: TdaP (tetanus toxoid & reduced diphtheria toxoid & acellular pertussis); DTaP (diphtheria toxoid & tetanus toxoid & acellular pertussis)

Aluminum compounds used in the licensed vaccines (Table I) are aluminum hydroxide, aluminum phosphate and amorphous aluminum hydroxyphosphate sulfate, although these commonly used names are scientific misnomers (6). They have distinctive physicochemical properties, which have important implications for their immunomodulatory effects (11, 12). These adjuvants are prepared in house by vaccine companies or purchased from manufacturers by their tradenames, such as Alhydrogel®, Rehydragel™, and Adju-Phos® (9).

Aluminum hydroxide adjuvant and aluminum phosphate adjuvant are composed of very small primary particles. However, the primary particles form aggregates that are the functioning units in vaccines (11). Commercially available aluminum hydroxide adjuvant is not Al(OH)_3 , but rather a crystalline aluminum oxyhydroxide AlO(OH) with mineralogical name of boehmite, as confirmed by X-ray diffraction and infrared spectroscopy. This difference is important because crystalline aluminum hydroxide has a low surface area (approximately 20 to 50 m^2/g) and as such a poor adsorbent properties, while crystalline aluminum oxyhydroxide has a surface area of approximately 500 m^2/g , which makes it an excellent adsorbent. This high surface is a result of its morphology, since the primary particles of adjuvant are fibers with average dimensions of $4.5 \times 2.2 \times 10 \text{ nm}$. Aluminum oxyhydroxide is a stoichiometric compound, and it is composed of Al-OH and Al-O-Al groups. The Al-OH surface groups can accept or donate a proton, resulting in a positive or in a negative surface charge, which may affect the surface characteristics of this adjuvant (6).

Aluminum phosphate adjuvant is a chemically amorphous aluminum hydroxyphosphate $[\text{Al(OH)}_x(\text{PO}_4)_y]$ in which some of the hydroxyl groups of aluminum hydroxide are replaced by phosphate groups. The disordered, amorphous state is responsible for the high surface area and high adsorptive capacity of this adjuvant whose plate-like particles have a diameter of approximately 50 nm (11, 12). The nanoparticles of this aluminium adjuvant form loosely connected porous aggregates that vary in size from 1 to about 20 μm depending on the adjuvant, the method used for measurement of particle size, and the experimental conditions (13, 14). Another commercially available adjuvant is amorphous aluminum hydroxyphosphate sulfate, which contains residual sulfate residues because alum was used instead of aluminum chloride for its synthesis. Transmission electron microscopy revealed mesh-like structure of aluminum hydroxyphosphate sulfate (15).

Depending on the types of aluminum salts, their surface charges have notable differences (16). The isoelectric points (IEPs) of these adjuvants are 4.6–5.6, 7.4 and 11.1 for aluminum phosphate, aluminum hydroxyphosphate sulfate and aluminum hydroxide, respectively (9, 15). Under physiological conditions ($\text{pH} = 7.4$), aluminum hydroxide is positively charged, whereas aluminum phosphate, and aluminum hydroxyphosphate sulfate are negatively charged (6, 9, 11), which can be of great significance for interaction with antigen.

The size of the aggregate adjuvant particles can be decreased (i.e. during exposure to shear forces and ultrasonication (13) or increased when these particles are suspended in saline solution (14). The morphology of aluminum-containing adjuvants contributes to the uniform distribution of antigen in vaccines (11). The adjuvant aggregates undergo a de-aggregation and re-aggregation process during mixing. These de-aggregation and re-aggregation processes provide a mechanism that distributes antigens in mono-valent

or combination vaccines throughout all of the adjuvant aggregates in the vaccine. Thus, even though quantities of antigen as low as 10 µg are combined with quantities of adjuvant up to 0.85 mg Al during the production of a vaccine, the nature of the adjuvant aggregates provides a mechanism to uniformly distribute the antigen as long as adequate mixing procedures are followed (11). During storage at room temperature, aluminum adjuvants become more ordered due to deprotonation and dehydration. Once the physical properties of the adjuvant are known, an antigen interaction with the adjuvant can be determined (17). As a general rule, the antigen should be adsorbed onto the adjuvant prior to immunization and the adsorption should be carefully monitored (7). Adsorption of antigens to aluminum adjuvants may contribute directly to the immune-enhancing effect of aluminum adjuvants. In addition, adsorption to the adjuvant may prevent adsorption of antigens to the wall of the vial or syringe, thus ensuring injection of the full dose of antigen (9). Owing to the complex structure of antigens, it is not surprising that a number of attractive mechanisms may contribute to their adsorption (11). These mechanisms include electrostatic attraction, hydrogen bonding, hydrophobic interactions, ligand exchange and van der Waals forces (11, 17), while each binding force in a given antigen-adjuvant combination depends on the nature of the antigen and the chemical environment: pH, ionic strength, presence of surfactants, etc. (7). The surface hydroxyls of aluminum hydroxide adjuvant provide the basis for the major mechanisms of adsorption of antigens: electrostatic adsorption when the antigen and adjuvant have opposite charges and ligand exchange when the antigen contains a phosphate group that is able to exchange with a hydroxyl group at the surface of the adjuvant. Electrostatic attraction is probably the most frequently used adsorption mechanism, while ligand exchange produces the strongest adsorption (6).

Determination of antigen adsorption capacity of the adjuvant is highly recommended and it can be completed by a variety of analytical methods. It is usually done by comparing the protein content in the aqueous phase of the antigen solution before and after adsorption onto the adjuvant. The amount of protein in the supernatant is simply subtracted from the amount that was initially added, to determine the amount adsorbed (7, 9). The adsorption of protein antigen has been typically analyzed according to the Langmuir adsorption model, which enables calculation of several adsorptive parameters, including the adsorptive coefficient, which corresponds to the strength of the antigen–adjuvant interaction. In order to correct the limitations of the Langmuir isotherm, other models (e.g. Toth isotherm, a hybrid model combining the Langmuir and the Freundlich isotherms) were later developed, that are better suited to fit data from heterogeneous systems (7).

2.2 Formulation of vaccines with aluminium adjuvants

When formulating a vaccine with aluminium-containing adjuvants, it is important to understand both nature of the surface of the adjuvant and how the antigen interacts

with the surface (17). Therefore, detailed characterization of aluminum-containing adjuvants with reference to properties that affect adsorption, such as surface area, surface charge, chemical composition, structure and morphology, stability, with other colloidal behavior is essential for consistent adjuvant effect (18, 19). Quality specifications for aluminium adjuvants usually list parameters based on compendial testing of identity, strength, purity, etc., according to their pharmacopoeial monographs (Table II).

Table II The tests listed in general monographs (Ph. Eur. 9.0) of aluminium hydroxide adjuvant

Tabela II Ispitivanja navedena u opštoj monografiji (Ph. Eur. 9.0) aluminijum hidroksida kao adjuvansa

Aluminum hydroxide, hydrated, for adsorption (<i>Aluminii hidroxidum hydricum ad adsorptionem</i>)	
Test	Ph. Eur. 9.0
Indentification	+
Characters	+
Solution	+
pH	5.5 – 8.5
Adsorption power	+
Sedimentation	+
Chlorides	≤ 0.33%
Nitrates	≤ 100 ppm
Sulfates	≤ 0.5%
Ammonium	≤ 50 ppm
Iron	≤ 15 ppm
Bacterial endotoxins	Less than 5 IU of endotoxin per milligram of aluminium, if intended for use in the manufacture of an adsorbed product without a further appropriate procedure for the removal of bacterial endotoxins.
Assay	90 – 110%
Storage: at a temperature not exceeding 30 °C. Do not allow to freeze. If the substance is a sterile, airtight, tamper-proof container.	
Labelling: The label states the declared content of aluminium.	

Care must be taken in selecting a buffer for an aluminum hydroxide adjuvant-containing vaccine (6). Phosphate anions are adsorbed by both aluminum hydroxide adjuvant and aluminum phosphate adjuvant by ligand exchange (19). Although the adsorption of antigens onto aluminum adjuvants is heavily dependent on electrostatic attraction, ligand exchange occurs with phosphorylated antigens and is the strongest adsorption force (10). The adsorption of phosphate anion present in a vaccine formulation as buffer can affect the adsorption of an antigen by affecting both the surface charge and the number of surface hydroxyls available for ligand exchange with phosphate groups of the antigen (19). Iyer et al. (20) determined the extent (adsorptive capacity) and strength (adsorptive coefficient) of adsorption for three phosphorylated proteins (alpha casein, dephosphorylated alpha casein, and ovalbumin), by the phosphate-treated aluminum hydroxide adjuvants, and found that it was inversely related to the degree of phosphate substitution of the aluminum hydroxide adjuvant. It is recommended that exposure of vaccines containing aluminum hydroxide adjuvant and phosphorylated antigens to phosphate ion in the formulation or during manufacture, should be minimized to produce maximum adsorption of the antigen. Therefore, phosphate buffer should be avoided in the formulation of vaccines with aluminium adjuvants, unless there is a specific rationale for its use (9).

Other anions of commonly used buffers that can affect adsorption include citrate, carbonate and succinate (9). Citrate buffer may lead to an increase in the soluble aluminum concentration. It was demonstrated that the citrate anion in an α -hydroxycarboxylic acid adsorbs to an aluminum-containing adjuvant and solubilizes the aluminum by formation of a soluble aluminum–citrate complex. Buffers that do not appear to alter the properties of aluminium-containing adjuvants include acetate, histidine, MOPS (3-(N-morpholino) propanesulfonic acid) and TRIS (tromethamine) buffers (6, 9, 19). Vaccines should be isotonic to reduce pain upon parenteral application; therefore, addition of excipients to adjust the tonicity is necessary. It has been demonstrated that ionic strength of sodium chloride solution can reduce the adsorption of electrostatically adsorbed adjuvants (21), so it is more appropriate to use a polyol rather than sodium chloride to adjust the tonicity of a vaccine containing electrostatically adsorbed antigen (11). Surfactants are frequently added to vaccine formulations to increase antigen stability. It was reported that nonionic surfactant produced less elutability of proteins from aluminium adjuvants in model vaccines compared to anionic and cationic surfactants. Although it is likely that nonionic surfactants such as polysorbate 20 and polysorbate 80 also do not affect the adsorption of antigens on aluminum hydroxide and phosphate, this needs to be further evaluated (9).

Aluminum hydroxide adjuvant and aluminum phosphate adjuvant both exhibited an increase in order during autoclaving, which resulted in a decrease in protein

adsorption capacity. Therefore, autoclaving conditions should be selected minimizing exposure time to elevated temperatures, and procedures requiring repeated autoclaving of the same samples should be avoided (19).

Vaccines adjuvanted with aluminum salts are formulated as liquid suspensions and must remain stored in cold chain at 2–8 °C from manufacturing to being administered to patients, due to aluminium adjuvant sensitivity to freezing and freezing induced irreversible coagulation or aggregation of the gel particles (10, 22). Vaccines containing aluminum hydroxide adjuvant or aluminum phosphate adjuvant should not be allowed to freeze and should not be used if suspected of having been exposed to freezing temperatures (6, 23). Aluminum salts are suspensions of hydrated colloid particles with slow sedimentation in water due the oriented water molecules that give buoyancy and the charges on the salts, enabling electrical repulsion among the particles in the suspension (10). Freezing brings changes in the structure and morphology of the adsorbed vaccines, whether monovalent or combined. It has been proposed that ice crystals formed during freezing force aluminum particles to overcome repulsion, thereby producing strong interparticle attraction resulting in aluminum particle coagulation/agglomeration. Thus, these particles agglomerates become bigger and sediment faster than particles in non-frozen vaccines. The size of the agglomerates seems to increase on repeated freezing and thawing cycles. Adsorbed vaccines kept at the optimal temperature (2–8 °C) show a fine-grain structure. In contrast, large conglomerates of massed precipitates with a crystalline structure are observed in vaccines affected by freezing (23). During the last two decades several technologies have been developed to overcome aluminium salt-adjuvanted vaccines sensitivity to freezing conditions (10, 22, 24).

Calcium phosphate was initially developed by the Pasteur Institute in hydroxyapatite form ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). X-ray diffraction, Fourier transform infrared spectroscopy, and thermal analysis of commercially available calcium phosphate (from Reheis Inc. NJ, USA) indicated that calcium phosphate adjuvant, with the suggested formula of $\text{Ca}_3(\text{PO}_4)_2$, could be described as nonstoichiometric hydroxyapatite, $\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$, where x varies from 0 to 2 (7). Calcium phosphate was used as an adjuvant in diphtheria, tetanus, pertussis and poliomyelitis vaccines in France, but it was completely substituted by alum salts in the late 1980s. However, it still remains as an approved adjuvant for the World Health Organization for human vaccination (1,4,25), EMA (4) and Ph. Eur. (1). Adsorption capability of calcium phosphate is similar to aluminium adjuvants, and it depends on preparation method (concentration of reactants and rate at which the reactants are mixed), Ca/P ratio, presence of hydroxyl groups, zeta potential and specific surface. The point of zero charge of calcium phosphate is 5.5, so it has a good adsorption capacity of positively charged and phosphorylated antigens. In addition, it is a biodegradable and biocompatible adjuvant

even at a nanoscale. According to Masson et al. (25), within the next 5 years, studies and clinical trials necessary for the calcium phosphate reuse in human vaccination could be completed. This wider range would enable patients to choose an adjuvant, thus ensuring a great vaccinal coverage.

In the general monograph „Vaccines for human use” (Ph. Eur. 07/2018:1053) aluminium (Al) content is maximum 1.25 mg per human dose, unless otherwise stated (1). In the United States, the limit is 0.85 mg aluminum per dose if determined by assay, 1.14 mg if determined by calculation on the basis of the amount of aluminum compound added, and 1.25 mg if safety and efficacy data justify it (9). The maximum allowed amount of calcium (Ca) is 1.3 mg per human dose, unless otherwise stated (1).

2.3 Novel inorganic nanoadjuvants as potential antigen carriers and vaccine adjuvants

Application of clay minerals in drug delivery has been intensively investigated in last two decades due to their advantagenous physico-chemical and functional properties, such as high specific surface area, adsorption, and ion exchange, thixotropy, swelling property, chemical inertness, low toxicity and possibility of surface modification (26–28). Additionally, clays have been recognized as alternative immunogen support in vaccine formulations due to their ability to increase the stabilization of immunogens that can enable easier administration and protection from proteolysis, and improved thermal and storage performance. However, it has also been observed in several studies that adsorption of antigens on pristine clays can alter their protein structure which can result in decayed immunogenicity of the antigen and eventually compromise the vaccination efficacy (29, 30). In order to overcome important issues related to the thermostability of vaccines and stability problems related to accidental freezing during transportation and storage, sepiolite-lipid bio-nano hybrids as novel adjuvants in thermostable influenza A vaccines were explored. In brief, these hybrids were obtained by modification of sepiolite fibres (Pangel S9 from Tolsa S.A., Spain) with a bilayer lipid membrane by contacting a 0.2 wt% sepiolite suspension with a 0.8 mM liposome dispersion. Liposomes were prepared from phosphatidylcholine (EmulmetikTM, Lucas Mayer GmbH, Germany) by the extrusion method (31). Improved thermal stability within functional studies at elevated temperatures up to 48 °C was shown with enhanced resistance against lyophilization-induced antigen denaturation as often seen for alum-stabilized antigens (32). This improvement in thermal stability was suggested to be related to creation of a chemical microenvironment by the sepiolite–lipid biohybrid forming a somewhat thermally protective scaffold for the adsorbed influenza virions. Fujimori et al. (33) fabricated ultrathin multilayer films of adsorbed biological molecules (enzyme lysozyme) by means of the modified Langmuir–Blodgett method using an organo-modified aluminosilicate (dimethyldioctadecylammonium modified montmorillonite platelets). The thermo-protecting effect of organoclays toward

sensitive biological species was reported since surprisingly high thermal stability of lysozyme showing enzymatic activity until 160 °C was determined. Clay nanoparticles, for example, layered double hydroxide (LDH) and hectorite nanoparticles, have shown their potent adjuvanticity in generating effective and durable immune responses in animal models. These findings suggest that both clay nanoadjuvants can serve as active vaccine platforms for sustained and potent immune responses (34).

The use of mesoporous silica nanoparticles (MSNs) has gained significant attention as potential delivery vehicles for various biomolecules (35). The main advantages of these materials are well-defined surface properties, high porosity, large surface area, low density, excellent biocompatibility, thermal and chemical stability (36, 37). It has been shown that introduction of MSNs as an immunoadjuvants can effectively enhance both cellular and humoral immunity in animal models (8, 38, 39). The factors like nanoparticle architecture, antigen type, antigen loading/encapsulation, dose administered, and immunization route can influence the adjuvant properties of MSNs (35). The capacity of MSNs to induce both humoral and cell mediated immune responses over traditional adjuvants is a great advantage, and in the future, more studies are expected to evaluate the biocompatibility, stability, efficacy and biological interactions of MSNs based protein delivery system (40). Other representatives of inorganic nanoparticles such as zinc oxide, iron oxide and iron hydroxide, cobalt oxide, titanium dioxide, nanodiamond, luminescent porous silicon, quantum dots have been also in focus of academic research towards next-generation vaccines (8). In the past few years, numerous studies have also demonstrated the great potential of nanoparticles of metal-organic frameworks (nanoMOFs) at the preclinical level for biomedical applications, among other as vaccine adjuvant delivery systems (41).

3. Nanotechnology in the development of vaccine adjuvants

In the era of modern vaccinology, efficacious adjuvants and appropriate delivery systems for antigens are needed. In this context, nanotechnology offers some solutions (42). The need for safer and potent adjuvants resulted in the administration of an antigen within the nanoparticulated delivery systems (43). Owing to their specific features (size, shape and surface functionalities), a variety of nanoparticulated structures have been introduced as adjuvants and/or antigen delivery/presenting systems, opening the door to nanovaccinology. Among them, inorganic nanoparticles, liposomes, nanoemulsions, polymeric nanoparticles, self-assembled proteins, immunostimulating complexes and virus-like particles have been underlined as promising, and some of them have already been approved for human use (44, 45). In this section, nanoemulsions, as lipid-based nanostructures will be specially addressed.

3.1 Nanoemulsions as vaccine adjuvants

As already described above in details, traditionally, aluminum salts are used as vaccine adjuvants with a view to boost the immune response in the presence of an antigen. Within the last two decades, nanoemulsion-based vaccine adjuvants have been shown to provide an alternative in terms of specific vaccine immunogenicity (46).

Nanoemulsions represent heterogeneous liquid systems, consisting of oil and water, stabilized by a surfactant – an amphiphilic molecule. Surfactants can be defined by their hydrophilic-lipophilic balance (HLB) value, which gives information on their relative affinity for the both phases. Oil and water form two distinct compartments, one being dispensed in the other, forming nanodroplets. They can be water-in-oil or oil-in-water (47, 48). These colloidal systems have been widely investigated as drug delivery systems for various administration routes: parenteral, dermal, oral, ocular, nasal (49–52). In addition, nanoemulsions as excipient systems have been introduced as efficient modality for improved oral bioavailability of hydrophobic nutrients from food and food supplements (53, 54). Small average droplet size (generally in the range 50 - 300 nm), high solubilization capacity, good kinetic stability, existence of several different methods for their preparation from biocompatible ingredients are just some advantages that distinguish them among other colloidal systems (48, 55–57).

As vaccine adjuvants in licenced vaccines, nanoemulsions were introduced in the 1990s, when the first emulsion-based adjuvant was registered in Europe, as a product of the company *Novartis* (Basel, Switzerland) (58). Nanoemulsion-based adjuvants used today in the approved vaccines are summarized in Table III.

Table III List of nanoemulsion-based adjuvants in licensed vaccines**Tabela III** Lista emulzionih adjuvanasa u vakcinama odobrenim za humanu upotrebu

Name	Type	Components	Size of dispersed phase (nm)	Producer	Year licensed	Clinical use
MF59	Oil-in-water	squalene; polysorbate 80; sorbitantrioleate; citrate buffer pH 6.5	160	Novartis	1997	seasonal influenza, pandemic influenza, avian influenza
ASO3	Oil-in-water	squalene; α -tocopherol; polysorbate 80; PBS pH 6.8	160	GSK	2009	pandemic influenza, avian influenza
AF03	Oil-in-water	squalene; polyoxyethylenecetostearyl ether; mannitol; sorbitan oleate; PBS pH 7.1	90	Sanofi	2010	pandemic influenza
Montanide TM ISA 51VG	Water-in-oil	mineral oil, mannide monooleate (Prior to use, it is mixed with the conjugated antigen in the PBS, forming water-in-oil emulsion)	< 1 μ m	Seppic	2008	lung cancer

More than eighty years ago, the adjuvant effect of mineral oil (in a water-in-oil emulsion) in combination with thermally killed mycobacteria cells was demonstrated by Freund, and this adjuvant was marked as *Freund's complete adjuvant* (59). Afterwards, water-in-oil emulsions without the bacterial cells (*Freund's incomplete adjuvant*) were used as adjuvants both in human and veterinary vaccines. Additionally, there was a little knowledge on their mechanism of action, even though it was suggested that they might act as depot systems. However, despite their indisputable efficacy, they were too reactogenic for routine use (45, 58).

Acquired experience through the years pointed out some important characteristics that nanoemulsion-based adjuvants should possess, representing a milestone for further development. In general, efficacy of an adjuvant is determined by droplet size, oil content (oil-to-water ratio), droplet surface properties and viscosity (44, 61). It is preferred that oil phase is biodegradable, and surfactants should be recognized as safe for human use, with many years of application. Tolerability can be highly improved by

lowering the oil content and shifting from water-in-oil to oil-in-water emulsions, which is followed by reduced viscosity, as well (43, 62).

Moreover, subsequent refinement of the base materials has resulted in the new versions of water-in-oil emulsions. Among the ones undergoing clinical trial assessment, the *Montanide*TM emulsions can be underlined (products of the company *Seppic* (Paris, France) – especially for therapeutic vaccines. A vaccine against non-small-cell lung cancer, the *CimaVax EGF*[®], using this adjuvant technology, has already been licensed (60).

3.1.1 Physicochemical properties of nanoemulsion-based adjuvants

Physicochemical properties of nanoemulsion direct its efficacy and safety profile as adjuvant. This includes the type of the nanoemulsion, viscosity, droplet size and surface properties (63).

The viscosity of a nanoemulsion is closely linked to the surfactant structure, its HLB value, and the ratio between the two phases. Too viscous formulations are not only difficult to be injected, but they also represent a safety issue. In general, oil-in-water nanoemulsions are characterized by lower viscosity. However, fluid water-in-oil nanoemulsions, with viscosity comparable to the viscosity of hydrophilic nanoemulsions, can be obtained if the stabilizers have optimized HLB value, corresponding to the type of selected oil (63).

Even though there are crossing opinions, it is generally accepted that droplet size may be one of the key features. Smaller size enables sterile filtration, safer parenteral administration, gives rise to more stable formulations, and finally, it reduces chances for local inflammation (51, 61, 64, 65). Much effort has been put in order to identify the ideal size of an adjuvant to elicit the highest responses. However, this topic cannot be generalized, and findings are highly dependent on the specific class of adjuvants (64). Considering nanoemulsions, in animal studies with a set of different mice strains and various antigen types, it was noted that with increase in the droplet size of the nanoemulsion, the adjuvant efficacy increases. Contrastingly, nanoemulsions with smaller droplet size are inferior adjuvants, but with better safety profile (64). In the study of Shah et al. (66) it was highlighted that there was no benefit in reducing nanoemulsion droplet size below 160 nm – both for humoral and cellular immune response. Taken all together – efficacy and safety profile of nanoemulsion-based adjuvants, their stability and possibility to undergo sterile filtration, it may be concluded that „smaller is not always the better”. Nevertheless, size of the nanoemulsion droplets is affected by the components, their ratio in the nanoemulsion and the preparation technique, so all these aspects should be carefully addressed during preparation of the final formulation.

3.1.2 Mechanism of action of nanoemulsion-based adjuvants

On the one hand, nanoemulsions represent delivery systems, which protect the antigen and release it at the target site, enhancing the uptake of the antigen by the antigen presenting cells. On the other hand, they create an inflammation at the injection site and act as immunopotentiators, activating specific pathways (firstly of innate, and then of adaptive immunity) important for improved antigen processing (45, 60, 62). There is also a rational hypothesis that effective immune activation by nanoemulsion adjuvants may be, at least partially, mediated by the presence of surfactant on the oil droplets. However, it was proved that none of the nanoemulsion components solely can induce appropriate response, but the complete formulation is responsible for the effect (67). In the study of Giusti et al. (68), in *in vitro* and *in vivo* experiments, it was described that nanoemulsion droplets of an adjuvant are internalized by the lymph node cells. It is still unknown whether internalization in the target cell is required for immune response. However, it is suggested that surfactant molecules contribute to the uptake of nanoemulsion droplets, because they induce cell membrane perturbation, but this is probably not the only mechanism (66, 67).

3.1.3 Antigen-adjuvant interactions

There are several ways how nanoemulsions can be loaded with an antigen. Antigen can be entrapped in the core of the droplet (encapsulated), chemically conjugated, or it can simply be adsorbed to the surface (61). The main advantage of the entrapment is antigen protection. Also, surface of the droplet can be further decorated (PEGylation, targeting ligands) in order to provide precise delivery. Adsorption considers weak interactions between the antigen and the droplet, which are mainly hydrophobic. It is critical in terms of antigen stability, but it is favorable as it needs less processing steps. Chemical conjugation may induce some changes on the antigen epitopes, but it can ensure that the antigen and the nanodroplet will reach the antigen presenting cell at the same time (45). Recent investigations have shown that soft matter particles, such as nanoemulsion droplets, are able to provide efficient immune response even if they are applied independently. Such finding highlighted the potential of nanoemulsions to act as immunopotentiators even in the situations with minimal association with the antigen, e.g. after simple mixing prior to the administration (45, 69).

3.1.4 Stability of nanoemulsion-based adjuvants

Nanoemulsion stability is one of the primary concerns for the formulators. Instability can be a consequence of many factors, such as droplet flocculation, coalescence, creaming, Ostwald ripening, as well as chemical degradation, eventually leading to the phase separation. Stability can be optimized by appropriate selection of

oil, surfactants and aqueous components, along with processing conditions (70). In general, it is checked at 4°C, room temperature, and at 37 °C (63).

Oil-in-water nanoemulsions usually show better stability compared to the water-in-oil ones. For instance, stability of water-in-oil *Montanide*TM emulsions at room temperature is only several weeks (71). This certainly represents a drawback, but it is not the unique case in the context of pharmaceutical preparations, and it can be overcome by mixing the water phase with the surfactant-oil blend prior to the administration to the patients. Such approach is provided by through and appropriate selection of the oil, surfactants, and their ratio, so that the low-energy mixing can render the emulsion with desired properties. Contrastingly, stability of the oil-in-water nanoemulsions is far better - at room temperature it is more than 2 years (63, 71)

3.1.5 Safety profile

It is often very difficult to define the exact boundaries between immunostimulation and immunotoxicity, in order to reach the perfect balance of efficacy/safety and to comply with strict regulatory standards. Safety of nanoemulsion-based adjuvants is related to the components, their origin and concentration in the formulation (70, 72).

Traditional oil adjuvants may induce local and general reactions, such as granuloma, abscesses or fever. Indeed, highly purified non-mineral oils are well tolerated as they are rapidly metabolized and eliminated from the injection site, inducing weaker inflammation (63). Mineral oil stays longer at the injection site and it is eliminated by macrophages, and partially metabolized in fatty acids, triglycerides, phospholipids or sterols. Bollinger et al. (73) demonstrated that 30% of the mineral oil disappears during the first month and most of the oil found outside the injection site is in the liver and fatty tissues in the form of phospholipids and fatty acids. Stewart-Tull et al. (74) investigated the direct influence of the oil hydrocarbon chain length on the safety of adjuvants, pointing out that small chains are efficient, but induce local reactions, whereas longer chains (more than 14 carbon atoms) are safer, but less efficient. Nevertheless, due to the overall properties (biocompatibility, biodegradability, accessibility and immunostimulation ability), squalene is still the most widely used oil component in nanoemulsion-based adjuvants (70).

The quality of surfactants, as inevitable components of nanoemulsion-based adjuvants, is also important. Up to now, licensed vaccine adjuvants are mostly based on the combination of sorbitan esters and polyoxyethylated sorbitan esters, because, in the long run, they have shown to be safe for human use in parenteral formulation and very efficient emulsifiers, rendering stable emulsions with small droplet size.

It should be underlined that development of any vaccine involves secondary processing steps, such as sterilization and usually lyophilization. It is important to

investigate the influence of these procedures since they can have a major impact on the final vaccine formulations in terms of droplet size, size distribution, stability, and consequently immune responses (61).

4. Microneedles for vaccine delivery into the skin

In recent years, numerous disadvantages of parenterally formulated vaccines (e.g., cold storage and transportation system requirements, pain and discomfort during administration, hazardous waste, risk of needle-stick injuries and needle re-use, poor patient compliance and frequently, lack of potent immune response), have enforced the development of innovative technologies to deliver vaccine into the skin, primarily owing to excellent skin immunocompetence and ease of access (75–77). The skin has a dense network of antigen presenting cells – Langerhans cells and dermal dendritic cells, located in epidermis and dermis, respectively – that capture antigens, and upon a proper activation mature, migrate to the draining lymph nodes to activate T lymphocytes, inducing an adaptive immune response. Also, epidermal keratinocytes, when are in danger, produce a wide variety of cytokines and chemokines (e.g., TNF- α and IL-1 β) as well as antimicrobial peptides, enhancing the maturation of antigen presenting cells and their migration to the lymph nodes (75, 78, 79). As a result, cutaneous vaccination has been shown to cause similar or better immune response compared to intramuscular injections, even, in some cases, lower antigen doses were used (due to a relatively sparse population of antigen presenting cells in the skeletal muscle compared to the skin) (76, 77). However, it should not be forgotten that the superficial skin layer, stratum corneum (SC), which protects the human body against entry of pathogen or toxic substances, represents tremendous physical barrier for efficient vaccine transport into the skin (76, 80). Among different strategies investigated to bypass this barrier resistance and to improve the transcutaneous immunization, considerable attention has been recently focused on microneedles.

Microneedles are needle-like structures with lengths in the micrometer range (typically less than 1500 or 2000 μm) that can pierce the SC and create transient micro-channels for the antigen delivery into the skin, but without disturbing the nerve endings and blood vessels in the inner viable skin layers (81, 82). Consequently, the application of microneedles provides the improved passage for the vaccine antigens towards the immune-competent skin layers in a minimally invasive and painless manner (75). In addition, due to the small size of needles, microneedles administration does not require the professional training and the healthcare personnel and simultaneously, reduces the risk of accidental needle-stick injuries (83). Most importantly, direct targeting of dense network of skin antigen presenting cells using microneedles generally produces higher humoral immune responses compared to conventional intramuscular injections (77). Moreover, it was shown that delivering a subunit protein antigen, ovalbumin, to the

skin's abundant immune cell population using the coated Nanopatch microneedle array ($>21,000/\text{cm}^2$ over $4 \times 4 \text{ mm}$ area; length $110 \mu\text{m}$) significantly enhanced the resultant CD8 $^+$ T cell response when compared to standard, intramuscular delivery of both antigen-only and its adjuvanted form, implying its potentially usefulness for improving immunogenicity of vaccine against tuberculosis, HIV and malaria, for which the current vaccine approaches that induce only antibody response are inadequate (84).

In addition, appropriately designed microneedles can generate the strong immune responses without any chemical-based adjuvant or with considerable lower adjuvant doses than required for the intramuscular injection, thus alleviating potential tolerability and safety issues (76, 84). Precisely, many of the commonly used vaccine adjuvants may not be compatible with the skin delivery approaches. For example, it was reported that alum, although the most frequently used vaccine adjuvant at the global market, when administered intradermally, might induce the serious side effects, such as persistent intradermal granulomas. On the other hand, advanced biphasic adjuvants (e.g., liposomes, oil-in-water emulsions), may not be sufficiently stable to withstand microneedle production procedure (coating and drying process) (76, 85). Hence, if necessary, a wide variety of substances including bacterial ADP-ribosylating exotoxins (Cholera toxin, Escherichia coli heat-labile toxin or their subunits), specific ligands of toll-like receptors [CpG oligodeoxynucleotides, imiquimod, polyinosinic:polycytidylic acid (poly I:C)], Quillaja saponins and others have been utilized in the combination with microneedles as immune enhancing additives to achieve the desirable immune responses (76). However, it should be kept in mind that skin permeability and the degree of vaccine delivery using microneedles can be also influenced by numerous parameters related to the physical properties of microneedle arrays (e.g., height, density, patch area and microneedle geometry) as well as to properties of applicator used for microneedle insertion into the skin (78, 86). For example, the length of the microneedles could be an important factor in the selective activation of specific antigen presenting cell population – shorter microneedles could predominately activate Langerhans cells in the epidermis, while longer could mainly affect dermal dendritic cells in the dermis (87). Also, the higher microneedle densities may be beneficial for improving the vaccine immunogenicity, since the skin treatment with microneedles induces the minor damage and cell death and consequently the release of damage-associated molecular patterns, which initiate activation of innate immune response acting as „natural immune enhancer” (78). On the other hand, the proper applicator is necessary to ensure the effective, consistent and depth-controlled microneedle penetration into the skin, without the microneedle fracture. Various applicators have been developed (e.g., applicators based on hand-operated rotary application for single microneedle, vibration-based microneedle insertion devices, impact-insertion devices, etc.) and their selection directly depends on the geometry, sharpness and density of the microneedles and the intended use (86, 88).

Up to now, microneedles have been manufactured out of different materials, in a variety of shapes and sizes, depending on the application purpose. In general, all investigated microneedles can be classified in four major groups: solid, coated, dissolving and hollow microneedles (Figure 1) (82, 86). The next section deals with each type of microneedles in more detail, along with examples of their use in the vaccine development.

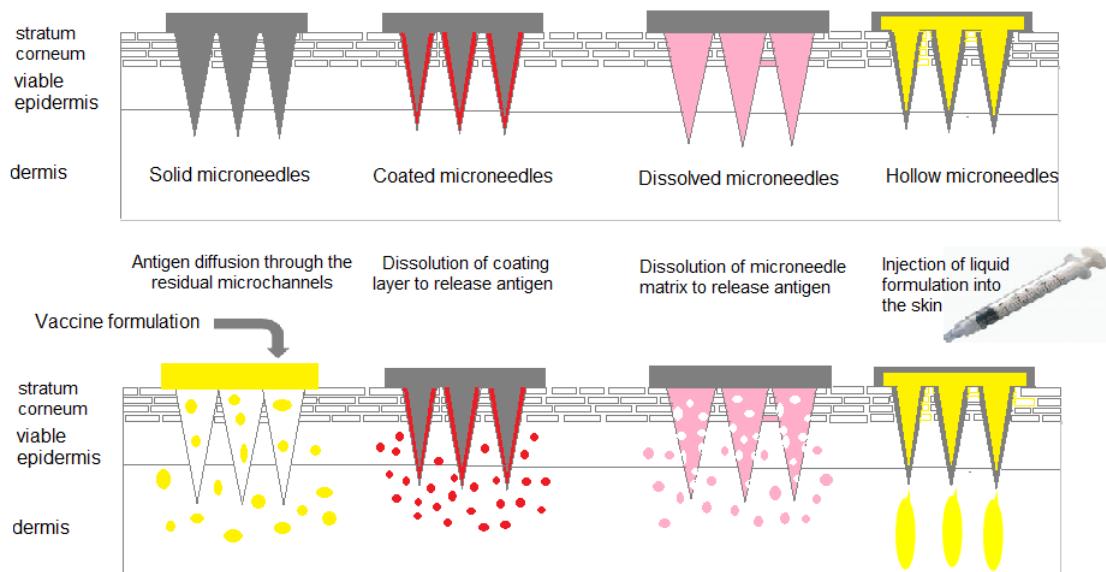


Figure 1. Schematic representation of different microneedle types and methods of antigen delivery into the skin (adopted according to 82, 88, 89, 94)

Slika 1. Šematski prikaz različitih tipova mikroigala i metoda za isporuku antiga u kožu (prilagođeno prema 82, 88, 89, 94)

4.1 Types of microneedles

Solid microneedles, as the simplest form of microneedles, were originally used for the skin pretreatment, in the „*poke and patch*” approach. After inserting and removing, solid microneedles form transient micropores in the skin surface, enabling the vaccine applied topically, on the treated area, to reach the immunogenic antigen presenting cells located in epidermis and dermis (82, 86, 89). The first solid microneedles were produced from silicon, but due to its fragile nature and risk of microneedle breaking into the skin, the various materials, such as metals (stainless steel, titanium, tantalum and nickel), ceramics, biodegradable and non-degradable polymers have been studied to ensure the sufficient mechanical strength of microneedles (82, 86). Detailed literature review revealed that solid microneedles have been successfully used to deliver the

various vaccines into the mouse skin *in vivo*, such as vaccine for diphtheria, influenza, hepatitis B and malaria (79). Among the first, Ding et al. (90) observed that skin perforation with stainless steel microneedles (4×4 array on a polymer plate with the surface area of around 0.5 cm²; microneedle length of 300 µm) led to almost 1,000-fold increase in diphtheria toxoid -specific serum IgG levels after its topical application in mice, compared to intact skin. Furthermore, immune response was further boosted by co-administration of Cholera toxin, indicating that addition of appropriate adjuvant may lower antigen dose required. Interestingly, the same authors observed that microneedle pretreatment had no effect on the immune response to topically applied plain influenza subunit vaccine (91), suggesting that the immune response depends primarily on vaccine type, but also, to certain extent, on the administration procedure. Here, it is worth noting that solid microneedles were also used in the alternative „*scrape and patch*” approach, involving skin scrapping with blunt-tipped microneedles to generate microabrasions of the SC before the vaccine application onto the skin surface (79, 86). This approach was tested with Onvax™ microneedles (Becton Dickinson, USA) (an array of plastic microprojections with a height of ~200 µm) to improve delivery of a rabies vaccine in humans, but no protective immune responses were detected, probably due to its inefficient delivery into the epidermis (79, 86, 92). However, it should be noted that, despite some promising results, the popularity of solid microneedles for improving vaccine delivery into the skin has declined in recent years, predominately due to cumbersome two-step administration process, inconsistent results and consequently, increased interest in other types of microneedles (79, 82, 93).

Advancement on classical solid microneedles was development of „*coat and poke*” approach, which involves pre-coating of microneedle arrays by suitable, water-soluble vaccine formulation that rapidly dissolves upon microneedles insertion into the skin (before the removal from the skin). In this way, solid microneedles are utilized not only to perforate the skin, but also, as vehicles to carry and deliver antigens directly into this skin, offering simplified (one-step) vaccination procedure (79, 82, 86, 94). However, it should be kept in mind that small antigen dose (typically less than 1 mg) can be administered via coated microneedles, due to the limited dimensions of microneedles shaft and tip – thick coatings are generally accompanied with a very low skin delivery efficacy because of a reduced sharpness of microneedle arrays (82, 86). Composition of coating solution depends on the nature and type of antigen investigated and commonly includes viscosity enhancer (e.g., carboxymethylcellulose (CMC), methylcellulose) to achieve sufficient thickness of coating layer, stabilizing saccharide (e.g., trehalose, sucrose) to retain activity of antigen during drying/storage and surfactant (e.g., poloxamers) to decrease the surface tension and thus to assure uniform coating efficiency (95, 96). Additionally, Choi and co-workers (97) observed that addition of viscosity enhancers was necessary to reduce the osmotic stress (caused by high concentrations of sugars) and to preserve activity of completely inactivated

influenza virus during the microneedle coating. Furthermore, the presence of viscosity enhancer improved systemic immune response and ensured better protection against the viral challenge (95, 97). On the other hand, owing to inherent stability and viscosity, DNA vaccines can be coated onto microneedles without stabilizers and viscosity enhancers (98). For example, Song et al. (98) observed that metal microneedles (length of 750 µm) coated with solution of plasmid DNA vaccine encoding the influenza hemagglutinin (excipient free coatings) induced higher humoral and cellular immune responses as well as better protective immunity compared to conventional i.m. immunization. Alternatively, in order to improve the coating efficiency, it was proposed to perform the pre-coating of microneedle surface with SiO₂, polylactic-co-glycolic acid (PLGA) or polyvinylpyrrolidone (PVP) or by the formation of polyelectrolyte coatings with microencapsulation properties (e.g., polydi(carboxylatophenoxy)phosphazene, chitosan and CMC). Interestingly, it was observed that some of these pre-coatings were capable to improve the vaccine stability and exhibit adjuvant effect (85, 86). Finally, it is worth mentioning that although coated microneedles were successfully used to deliver various antigens into the skin of different animal models (e.g., influenza, human papillomavirus, West Nile virus, rotavirus, herpes simplex virus, hepatitis B and C, bacillus Calmette-Guerin, measles and polio viruses), inducing better or equal immune response compared to parenteral needle-based immunization, literature review revealed no clinical trials pertaining to vaccine delivery via coated microneedles (79).

Opposite to the solid/coated microneedles, dissolving microneedles consist of water soluble or biodegradable materials (e.g., polymers, sugars), enabling the antigen encapsulation within the microneedle matrix. After insertion into the skin, dissolving microneedles completely dissolve/degrade into the skin, simultaneously releasing incorporated antigen (*“poke and release”* approach) (82, 86). Consequently, this approach offers numerous distinct advantages such as improved antigen stability, self-administration and absence of biohazardous sharp waste and safe disposal of remaining part (78, 79, 82, 86). However, there are also important challenges in the microneedle preparation which limit their widespread use, such as antigen wastage (due to low volume filling of the microcavities used for micromolding preparation procedure), insufficient antigen and adjuvant loading (due to low volumes of microneedle tips), antigen degradation and sterility (78, 86). The dissolving microneedles have been usually fabricated from sodium hyaluronate and CMC, as inactive materials approved by FDA for parenteral drug products, but also from other polymers (e.g., poly(vinylalcohol), PVP, methylvinylether-co-maleic anhydride) and low molecular weight sugars (e.g., maltose, trehalose). Biodegradable polymers such as PLGA, polylactic acid and polyglycolic acid are not advisable as the matrix material due to their slow dissolution rate in skin and a fabrication method involving high temperatures and organic solvents (78). Up to now, different vaccine types, ranging from proteins and peptides to DNA vectors encoding antigenic proteins and attenuated or inactivated

viruses, have been encapsulated in the microneedle matrix by direct dispersing into polymer, or by incorporating into nanoparticles or into a cross-linked structure to boost immune response. Numerous studies have shown that dissolving microneedles, depending on the antigen type, induced comparable or better humoral and/or cellular immune responses than s.c., i.m. or traditional i.d. injection of the same antigen dose. Furthermore, a considerable improvement of immune response was observed when adjuvants, compatible with skin administration route (e.g., CpG, poly(I:C), Quil-A, monophosphoryl lipid A and imiquimod) were added (78). Finally, it is important to emphasize that dissolving microneedles represent a relatively new vaccine delivery platform and there are no authorized products on the market. However, there are a few clinical studies examining (i) the effect of microneedle patch on local skin reactions, reliability of use and acceptability to patients (99, 100) as well as (ii) efficacy of novel transcutaneous influenza vaccine based on dissolving microneedles containing trivalent influenza hemagglutinins relative to subcutaneous injections of each influenza antigen (101). Obtained results suggested that investigated microneedle patches were generally well tolerated in the skin, with only mild erythema localized to treated site that resolved fully within seven days (78, 99). Furthermore, administration of influenza vaccines to human subjects via dissolving microneedles induced high level of immunity, proving that dissolving microneedles are promising for practical use as an easy and effective method to replace conventional injections systems (100).

Hollow microneedles have an empty space inside and hole at the tip, enabling the passive diffusion, or alternatively, active pressure-driven flow of a liquid formulation into the skin (*„poke and flow”* approach). The latter can be achieved by combining microneedles with external pressure device, such as syringe, pump, or pressurized gas (86). Hollow microneedles have been commonly made from silicon, glass, polymer and metal, in two designs, as a single microneedle (mimicking the conventional hypodermic needle) and an array of multiple microneedles. Increase in the number of microneedles per patch enables the simultaneous application of vaccine formulation over the wider skin area, thus potentially enhancing the likelihood of lymphatic uptake of presented antigens (86). The important advantage of this approach lies in a possibility of using the existing injectable formulations, but there are no opportunities to administer dry-state vaccine formulations which permit to improve the antigen stability and avoid cold storage and transportation system. Additional limitation of this approach is relatively low diffusion rate that can be potentially overcome by partial retraction or by adding hyaluronidase to the infusion solution (to degrade the hyaluronic acid within skin collagen fibers) (86, 102). Also, hollow microneedles are associated with less patient convenience compared to other microneedle types (82, 94). Hollow microneedles have been used to immunize human volunteers with polio and influenza vaccines, but also other antigens have been successfully delivered into the skin of various animal models (79). In this sense, it is interesting to note that, currently, there are few commercially

available hollow microneedle devices: (i) Soluvia™ Microinjection System (Becton Dickinson, USA), consisting of single silicon needle (length 1500 µm) coupled with a pre-fillable injection system, has been marked for delivery of influenza vaccination (other trade names are Intanza®; IDflu® and Fluzone Intradermal® by Sanofi Pasteur) (ii) MicronJet® (NanoPass, Israel), consisting of four hollow silicon microneedles (length 600 µm) arranged on a plastic adaptor for the use with a standard syringe, has also obtained FDA approval (iii) 3MTM Hollow Microstructured Transdermal System (hMTS) (3M, USA) comprising 12 polymer microneedles (length 1500 µm), is intended for clinical studies (81, 88, 103).

To conclude, in the era of modern vaccinology, it is *a must* to define optimal balance between efficacy and safety of an adjuvant, in order to provide potent and safe vaccines. Relying on already established approaches, some innovative adjuvants and vaccine delivery systems are presented in this work. It remains that intense multidisciplinary research provides new perspectives in this field.

Acknowledgement

This work was supported by the project TR34031, funded by Ministry of Education, Science and Technological Development, Republic of Serbia and through the activities within the framework of the COST Action 16231 European Network of Vaccine Adjuvants (ENOVA).

References

1. European Pharmacopoeia, 9th ed. Strasbourg: Council of Europe, 2016.
2. Memorandum of Understanding for the implementation of the COST Action "European Network of Vaccine Adjuvants" (ENOVA) CA16231. COST Association AISBL, 23.06.2017.
3. Allen LVJ, Ansel HC. Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. 10th ed. Philadelphia: Lippincott Williams & Wilkins; 2014.
4. Committee for medicinal products for human use (CHMP). Guideline on adjuvants in vaccines for human use, EMEA/CHMP/VEG/134716/2004, 2004.
5. Pati R, Shevtsov M, Sonawane A. Nanoparticle Vaccines Against Infectious Diseases. Front Immunol. 2018;9:2224.

6. Garçon N, Friede M. Evolution of adjuvants across the centuries. In: Plotkin S, Orenstein W, Offit P, Edwards KM, 7th, editor(s). Plotkin's Vaccines (Seventh Edition). Elsevier; 2018; p. 61-74.e4
7. Lindblad EB, Duroux L. Mineral Adjuvants. In: Schijns VEJC, O'Hagan DT, 2nd, editors. Immunopotentiators in modern vaccines. Elsevier; 2005; p. 347-375.
8. Li X, Wang X, Ito A. Tailoring inorganic nanoadjuvants towards next-generation vaccines. *Chem Soc Rev.* 2018;47(13):4954-4980.
9. HogenEsch H, O'Hagan DT, Fox CB. Optimizing the utilization of aluminum adjuvants in vaccines: you might just get what you want. *NJP Vaccines* 2018;3(1):51.
10. Thakkar SG, Cui Z. Methods to Prepare Aluminum Salt-Adjuvanted Vaccines. *Methods Mol Biol.* 2017;1494:181-199.
11. Hem SL, HogenEsch H. Relationship between physical and chemical properties of aluminum-containing adjuvants and immunopotentiation. *Expert Rev Vaccines.* 2007;6(5):685-98.
12. Sun B, Xia T. Nanomaterial-based vaccine adjuvants. *J Mater Chem B.* 2016;4(33):5496-5509.
13. Kolade OO, Jin W, Tengroth C, Green KD, Bracewell DG. Shear effects on aluminum phosphate adjuvant particle properties in vaccine drug products. *J Pharm Sci.* 2015;104(2):378-87.
14. Shardlow E, Mold M, Exley C. From stock bottle to vaccine: elucidating the particle size distributions of aluminum adjuvants using dynamic light scattering. *Front Chem.* 2016;4:48.
15. Caulfield MJ, Shi L, Wang S, Wang B, Tobery TW, Mach H, et al. Effect of alternative aluminum adjuvants on the absorption and immunogenicity of HPV16 L1 VLPs in mice. *Hum Vaccin.* 2007;3(4):139-45.
16. Shi S, Zhu H, Xia X, Liang Z, Ma X, Sun B. Vaccine adjuvants: understanding the structure and mechanism of adjuvanticity. *Vaccine.* 2019;37(24):3167-3178.
17. Morefield GL. A rational, systematic approach for the development of vaccine formulations. *AAPS J.* 2011;13(2):191-200.
18. Dey AK, Malyala P, Singh M. Physicochemical and functional characterization of vaccine antigens and adjuvants. *Expert Rev Vaccines.* 2014;13(5):671-85.
19. Hem SL, HogenEsch H. Aluminum-containing adjuvants: properties, formulation, and use. In: Singh M, editor. *Vaccine Adjuvants and Delivery Systems.* John Wiley & Sons, Inc.; 2007; p. 81-114.
20. Iyer S, HogenEsch H, Hem SL. Effect of the degree of phosphate substitution in aluminum hydroxide adjuvant on the adsorption of phosphorylated proteins. *Pharm Dev Technol.* 2003;8(1):81-6.
21. Ahl PL, Wang SC, Chintala R, Mensch C, Smith WJ, Wenger M2, Blue J3. Quantitative analysis of vaccine antigen adsorption to aluminum adjuvant using an automated high throughput method. *PDA J Pharm Sci Technol.* 2018;72(2):149-162.
22. Kristensen D, Chen D, Cummings R. Vaccine stabilization: research, commercialization, and potential impact. *Vaccine* 2011;29(41):7122-4.

23. Galazka A, Milstein J, Zaffran M. Thermostability of vaccines. Geneva, Switzerland: World Health Organization; 1998.
24. Li X, Thakkar SG, Ruwona TB, Williams RO 3rd, Cui Z. A method of lyophilizing vaccines containing aluminum salts into a dry powder without causing particle aggregation or decreasing the immunogenicity following reconstitution. *J Control Release*. 2015;204:38-50.
25. Masson JD, Thibaudon M, Bélec L, Crépeaux G. Calcium phosphate: a substitute for aluminum adjuvants? *Expert Rev Vaccines*. 2017;16(3):289-299.
26. Yang JH, Lee JH, Ryu HJ, Elzatahry AA, Alothman ZA, Choy JH: Drug-clay nanohybrids as sustained delivery systems. *Appl Clay Sci*. 2016;130:20–32.
27. García-Villen F, Carazo E, Borrego-Sánchez A, Sánchez-Espejo R, Cerezo P, Viseras C, Aguzzi C. Clay minerals in drug delivery systems. In: Mercurio M, Sarkar B, Langella A, editors. *Modified Clay and Zeolite Nanocomposite Materials, Environmental and Pharmaceutical Applications*. Elsevier; 2019; p.129-166.
28. Krajišnik D, Daković A, Janićijević J, Milić J. Natural and Modified Silica-Based Materials as Carriers for NSAIDs. In: Čalija B, editor. *Microsized and Nanosized Carriers for Nonsteroidal Anti-Inflammatory Drugs: Formulation Challenges and Potential Benefits*. Elsevier Academic Press; 2017; p. 219-258.
29. Pilar A, Darder M, Wicklein B, Rytwo G, Ruiz-Hitzky E. Clay–Organic Interfaces for Design of Functional Hybrid Materials. In: Delville MH, Taubert A, 1st, editors. *Hybrid Organic–Inorganic Interfaces: Towards Advanced Functional Materials*. Wiley-VCH Verlag GmbH & Co. KGaA; 2017; p. 1-84.
30. Ruiz-Hitzky E, Darder M, Fernandes FM, Wicklein B, Alcántara ACS, Aranda P. Fibrous clays based bionanocomposites, *Prog Polym Sci*. 2013;38 (10–11):1392-1414.
31. Wicklein B, Martín del Burgo MÁ, Yuste M, Darder M, Escrig Llavata C, Aranda P, Ortín J, del Real G, Ruiz-Hitzky E. Lipid-based bio-nanohybrids for functional stabilisation of influenza vaccines. *Eur J Inorg Chem*. 2012;5186–91.
32. Clapp T, Siebert P, Chen D, Jones Braun L. Vaccines with aluminum-containing adjuvants: optimizing vaccine efficacy and thermal stability. *J Pharm Sci*. 2011;100(2):388–401.
33. Fujimori A, Arai S, Soutome Y, Hashimoto M. Improvement of thermal stability of enzyme via immobilization on Langmuir–Blodgett films of organo-modified aluminosilicate with high coverage. *Colloids Surf A Physicochem Eng Asp* 2014; 448,45–52.
34. Chen W, Zuo H, Li B, Duan C, Rolfe B, Zhang B, Mahony TJ, Xu ZP. Clay Nanoparticles Elicit Long-Term Immune Responses by Forming Biodegradable Depots for Sustained Antigen Stimulation. *Small*. 2018;14(19):e1704465.
35. Mody KT, Popat A, Mahony D, Cavallaro AS, Yu C, Mitter N. Mesoporous silica nanoparticles as antigen carriers and adjuvants for vaccine delivery. *Nanoscale*. 2013;5(12):5167-79.

36. Xu W, Riikonen J, Lehto VP. Mesoporous systems for poorly soluble drugs. *Int J Pharm.* 2013;453(1):181-197.
37. Milic J, Čalija B, Đorđević S. Diversity and Functionality of Excipients for Micro/Nanosized Drug Carriers. In: Bojan Čalija, editor. *Microsized and Nanosized Carriers for Nonsteroidal Anti-Inflammatory Drugs: Formulation Challenges and Potential Benefits*. Elsevier Academic Press; 2017; 95-132.
38. Mahony D, Cavallaro AS, Stahr F, Mahony TJ, Qiao SZ, Mitter N. Mesoporous silica nanoparticles act as a self-adjuvant for ovalbumin model antigen in mice. *Small.* 2013;9(18):3138-46.
39. Kim J, Li WA, Choi Y, Lewin SA, Verbeke CS, Dranoff G, Mooney DJ. Injectable, spontaneously assembling, inorganic scaffolds modulate immune cells in vivo and increase vaccine efficacy. *Nat Biotechnol.* 2015;33(1):64-72.
40. Xu C, Lei C, Yu C. Mesoporous Silica Nanoparticles for Protein Protection and Delivery. *Front Chem.* 2019;7:290.
41. Simon-Yarza T, Mielcarek A, Couvreur P, Serre C. Nanoparticles of metal-organic frameworks: on the road to in vivo efficacy in biomedicine. *Adv Mater.* 2018;30(37):e1707365.
42. Mamo T, Poland GA. Nanovaccinology: the next generation of vaccines meets 21st century materials science and engineering. *Vaccine.* 2012;30: 6609-6611.
43. Saroja CH, Lakshmi PK, Bhaskaran S. Recent trends in vaccine delivery systems: a review. *Int J Pharm Ivest.* 2011;1(2):64-74.
44. Wallis J, Shenton DP, Carlisle RC. Novel approaches for the design, delivery and administration of vaccine technologies. *Clin Exp Immunol.* 2019;196(2):189-204.
45. Zhao L, Seth A, Wibowo N, Zhao CX, Mitter N, Yu C, Middelberg A P. Nanoparticle vaccines. *Vaccine.* 2014;32(3):327-337.
46. Iyer V, Cayatte C, Guzman B, Schneider-Ohrum K, Matuszak R, Snell A. et al. Impact of formulation and particle size on stability and immunogenicity of oil-in-water emulsion adjuvants. *Hum Vaccin Immunother.* 2015;11(7):1853-1864.
47. Singh Y, Meher JG, Raval K, Khan FA, Chaurasia M, Jain NK, Chourasia MK. Nanoemulsion: Concepts, development and applications in drug delivery. *J Control Release.* 2017;252: 28-49.
48. Tadros T, Izquierdo P, Esquena J, Solans C. Formation and stability of nano-emulsions. *Adv Colloid Int Sci.* 2004;108 -109: 303-318.
49. Bonferoni MC, Rossi S, Sandri G, Ferrari F, Gavini E, Rassu G, Giunchedi, P. Nanoemulsions for „Nose-to-Brain” Drug Delivery. *Pharmaceutics.* 2019;11(2): 84.
50. Ilić T, Savić S, Batinić B, Marković B, Schmidberger M, Lunter D. et al. Combined use of biocompatible nanoemulsions and solid microneedles to improve transport of a model NSAID across the skin: In vitro and in vivo studies. *Eur J Pharm Sci.* 2018;125:110-119.

51. Zhang J, Miao J, Han X, Lu Y, Deng B, Lv F. et al. Development of a novel oil-in-water emulsion and evaluation of its potential adjuvant function in a swine influenza vaccine in mice. *BMC Vet Res.* 2018;14(1), 415-426.
52. Đorđević SM, Santrač A, Cekić ND, Marković BD, Divović B, Ilić TM et al. Parenteral nanoemulsions of risperidone for enhanced brain delivery in acute psychosis: Physicochemical and in vivo performances. *Int J Pharm.* 2017;533(2):421-430.
53. Yao K, McClements DJ, Xiang J, Zhang Z, Cao Y, Xiao H, Liu, X. Improvement of carotenoid bioaccessibility from spinach by co-ingesting with excipient nanoemulsions: Impact of oil phase composition. *Food & Funct.* 2019;10: 5302-5311.
54. Aboalnaja KO, Yaghmoor S, Kumosani TA, McClements D. J. Utilization of nanoemulsions to enhance bioactivity of pharmaceuticals, supplements, and nutraceuticals: Nanoemulsion delivery systems and nanoemulsion excipient systems. *Expert Opin Drug Deliv.* 2016;13(9):1327-1336.
55. Helgeson ME. Colloidal behaviour of nanoemulsions: Interactions, structure, and rheology. *Curr Opin Colloid Interface Sci.* 2016; 25: 39-50.
56. Komaiko, JS, McClements DJ. Formation of food-grade nanoemulsions using low-energy preparation methods: A review of available methods. *Compr Rev Food Sci F.* 2016;15(2): 331-352.
57. Anton N, Vandamme TF. Nano-emulsions and Micro-emulsions: Clarifications of the Critical Differences. *Pharm Res.* 2009;28(5), 978–985.
58. O'Hagan DT, Ott GS, Nest GV, Rappuoli R, Giudice GD. The history of MF59® adjuvant: a phoenix that arose from the ashes. *Expert Rev Vaccines.* 2013; 12(1):13-30.
59. Freund J, Casals J, Hosmer EP. Sensitization and antibody formation after injection of tubercle bacilli and paraffin oil. *Proc Soc Exp Biol Med.* 1937;37:509-513.
60. Leroux-Roels G. Unmet needs in modern vaccinology: adjuvants to improve the immune response. *Vaccine.* 2010; 28: C25-C36.
61. Oyewumi MO, Kumar A, Cui Z. Nano-microparticles as immune adjuvants: correlating particle sizes and the resultant immune responses. *Expert Rev Vaccines.* 2010;9(9):1095-1107.
62. Del Giudice G, Rappuoli R, Didierlaurent AM. Correlates of adjuvanticity: a review on adjuvants in licensed vaccines. *Semin Immunol.* 2018; 39:14-21
63. Aucouturier J, Dupuis L, Ganne V. Adjuvants designed for veterinary and human vaccines. *Vaccine.* 2001;19(17-19):2666-2672.
64. Shah RR, O'Hagan DT, Amiji MM, Brito L. A. The impact of size on particulate vaccine adjuvants. *Nanomedicine.* 2014;9(17):2671-2681.
65. Niikura K, Matsunaga T, Suzuki T, Kobayashi S, Yamaguchi H, Orba Y. et al. Gold nanoparticles as a vaccine platform: influence of size and shape on immunological responses in vitro and in vivo. *ACS nano.* 2013; 7(5): 3926-3938.

66. Shah RR, Taccone M, Monaci E, Brito LA, Bonci A, O'Hagan DT. et al. The droplet size of emulsion adjuvants has significant impact on their potency, due to differences in immune cell-recruitment and-activation. *Sci Rep.* 2019; 9(1), 1-9.
67. Calabro S, Tritto E, Pezzotti A, Taccone M, Muzzi A, Bertholet S. et al. The adjuvant effect of MF59 is due to the oil-in-water emulsion formulation, none of the individual components induce a comparable adjuvant effect. *Vaccine.* 2013;31(33):3363-3369.
68. Giusti F, Seubert A, Cantisani R, Tortoli M, D'Oro U, Ferlenghi I. et al. Ultrastructural visualization of vaccine adjuvant uptake in vitro and in vivo. *Microsc Microanal.* 2015;21(4):791-795.
69. Morel S, Didierlaurent A, Bourguignon P, Delhaye S, Baras B, Jacob V. et al. Adjuvant System AS03 containing α -tocopherol modulates innate immune response and leads to improved adaptive immunity. *Vaccine.* 2011;29(13):2461-2473.
70. Fox C. Squalene emulsions for parenteral vaccine and drug delivery. *Molecules.* 2009;14(9):3286-331.
71. Aucouturier J, Dupuis L, Deville S, Ascarateil S, Ganne V. Montanide ISA 720 and 51: a new generation of water in oil emulsions as adjuvants for human vaccines. *Expert Rev Vaccines.* 2002;1(1): 111-118.
72. Batista-Duharte A, Lindblad EB, Oviedo-Orta E. Progress in understanding adjuvant immunotoxicity mechanisms. *Toxicol Lett.* 2011;203(2): 97-105.
73. Bollinger J. N. Metabolic fate of mineral oil adjuvants using ^{14}C -labeled tracers I: mineral oil. *J Pharm Sci.* 1970; 59(8): 1084-1088.
74. Stewart-Tull DES, Shimono T, Kotani S, Knights BA. Immunosuppressive Effect in Mycobacterial Adjuvant Emulsions of Mineral Oils Containing Low Molecular Weight Hydrocarbons. *Int Arch Allerg Immunol.* 1976;52(1-4): 118-128.
75. Ita K. Transdermal delivery of vaccines - Recent progress and critical issues. *Biomed Pharmacother.* 2016;83:1080-1088.
76. Engelke L, Winter G, Hook S, Engert J. Recent insights into cutaneous immunization: How to vaccinate via the skin. *Vaccine.* 2015;33(37):4663-74.
77. Suh H, Shin J, Kim YC. Microneedle patches for vaccine delivery. *Clin Exp Vaccine Res.* 2014;3(1):42-9.
78. Leone M, Mönkäre J, Bouwstra JA, Kersten G. Dissolving Microneedle patches for dermal vaccination. *Pharm Res.* 2017;34(11):2223-2240.
79. Marshall S, Sahm LJ, Moore AC. The success of microneedle-mediated vaccine delivery into skin. *Hum Vaccin Immunother.* 2016;12(11):2975-2983.
80. Gill HS, Kang SM, Quan FS, Compans RW. Cutaneous immunization: an evolving paradigm in influenza vaccines. *Expert Opin Drug Deliv.* 2014 Apr;11(4):615-27.

81. Duarah S, Sharma M, Wen J. Recent advances in microneedle-based drug delivery: special emphasis on its use in paediatric population. *Eur J Pharm Biopharm.* 2019; 136:48-69.
82. Kim YC, Park JH, Prausnitz MR. Microneedles for drug and vaccine delivery. *Adv Drug Deliv Rev.* 2012;64(14):1547-68.
83. Hiraishi Y, Nandakumar S, Choi SO, Lee JW, Kim YC, Posey JE, Sable SB, Prausnitz MR. Bacillus Calmette-Guérin vaccination using a microneedle patch. *Vaccine.* 2011;29(14):2626-36.
84. Ng HI, Fernando GJ, Kendall MA. Induction of potent CD8⁺ T cell responses through the delivery of subunit protein vaccines to skin antigen-presenting cells using densely packed microprojection arrays. *J Control Release.* 2012;162(3):477-84.
85. Andrianov AK, DeCollibus DP, Gillis HA, Kha HH, Marin A, Prausnitz MR, Babiuk LA, Townsend H, Mutwiri G. Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. *Proc Natl Acad Sci U S A.* 2009;106(45):18936-41.
86. van der Maaden K, Jiskoot W, Bouwstra J. Microneedle technologies for (trans)dermal drug and vaccine delivery. *J Control Release.* 2012;161(2):645-55.
87. Romgens AM, Bader DL, Bouwstra JA, Oomens CW. Predicting the optimal geometry of microneedles and their array for dermal vaccination using a computational model. *Comput Methods Biomech Biomed Engin.* 2016;19(15):1599–609.
88. Kolli CS. Microneedles: bench to bedside. *Ther Deliv.* 2015;6(9):1081-8.
89. Shin CI, Jeong SD, Rejinold NS, Kim YC. Microneedles for vaccine delivery: challenges and future perspectives. *Ther Deliv.* 2017;8(6):447-460.
90. Ding Z, Van Riet E, Romeijn S, Kersten GF, Jiskoot W, Bouwstra JA. Immune modulation by adjuvants combined with diphtheria toxoid administered topically in BALB/c mice after microneedle array pretreatment. *Pharm Res.* 2009a;26(7):1635-43.
91. Ding Z, Verbaan FJ, Bivas-Benita M, Bungener L, Huckriede A, van den Berg DJ, Kersten G, Bouwstra JA. Microneedle arrays for the transcutaneous immunization of diphtheria and influenza in BALB/c mice. *J Control Release.* 2009b;136(1):71-8.
92. Laurent PE, Bourhy H, Fantino M, Alchas P, Mikszta JA. Safety and efficacy of novel dermal and epidermal microneedle delivery systems for rabies vaccination in healthy adults. *Vaccine.* 2010;28(36):5850-6.
93. Hao Y, Li W, Zhou X, Yang F, Qian Z. Microneedles-based transdermal drug delivery systems: a review. *J Biomed Nanotechnol.* 2017;13(12):1581-1597.
94. Rejinold NS, Shin JH, Seok HY, Kim YC. Biomedical applications of microneedles in therapeutics: recent advancements and implications in drug delivery. *Expert Opin Drug Deliv.* 2016;13(1):109-31.
95. Tomar J, Born PA, Frijlink HW, Hinrichs WL. Dry influenza vaccines: towards a stable, effective and convenient alternative to conventional parenteral influenza vaccination. *Expert Rev Vaccines.* 2016;15(11):1431-1447.

96. Zaric M, Ibarzo Yus B, Kalcheva PP, Klavinskis LS. Microneedle-mediated delivery of viral vectored vaccines. *Expert Opin Drug Deliv.* 2017;14(10):1177-1187.
97. Choi HJ, Song JM, Bondy BJ, Compans RW, Kang S-M, Prausnitz MR. Effect of osmotic pressure on the stability of whole inactivated influenza vaccine for coating on microneedles. *PLoS One.* 2015; 10(7):e0134431.
98. Song JM, Kim YC, O E, Compans RW, Prausnitz MR, Kang SM. DNA vaccination in the skin using microneedles improves protection against influenza. *Mol Ther.* 2012;20(7):1472-80.
99. Arya J, Henry S, Kalluri H, McAllister DV, Pewin WP, Prausnitz MR. Tolerability, usability and acceptability of dissolving microneedle patch administration in human subjects. *Biomaterials.* 2017;128:1-7.
100. Hirobe S, Azukizawa H, Matsuo K, Zhai Y, Quan YS, Kamiyama F, et al. Development and clinical study of a self-dissolving microneedle patch for transcutaneous immunization device. *Pharm Res.* 2013;30(10):2664-74.
101. Hirobe S, Azukizawa H, Hanafusa T, Matsuo K, Quan YS, Kamiyama F, et al. Clinical study and stability assessment of a novel transcutaneous influenza vaccination using a dissolving microneedle patch. *Biomaterials.* 2015;57:50–8.
102. Martanto W, Moore JS, Kashlan O, Kamath R, Wang PM, O'Neal JM, Prausnitz MR. Microinfusion using hollow microneedles. *Pharm Res.* 2006;23(1):104-113.
103. Waghule T, Singhvi G, Dubey SK, Pandey MM, Gupta G, Singh M, Dua K. Microneedles: A smart approach and increasing potential for transdermal drug delivery system. *Biomed Pharmacother.* 2019;109:1249-1258.

Konvencionalni i napredni adjuvansi u formulacijama vakcina: mineralni adsorbenti, nanočestični nosači i sistemi tipa mikroigala

Danina Krajišnik, Tanja Ilić, Ines Nikolić, Snežana Savić*

¹Univerzitet u Beogradu - Farmaceutski fakultet, Katedra za farmaceutsku tehnologiju i kozmetologiju, Vojvode Stepe 450, 11221 Beograd, Srbija

*Autor za korespondenciju: Prof. dr Snežana Savić, Tel.: +381-11-3951288
e-mail: snezana.savic@pharmacy.bg.ac.rs

Kratak sadržaj

U doba savremene vakcinologije, ograničena imunogenost većine korišćenih antigena podstakla je primenu različitih adjuvanasa u formulacijama vakcina radi postizanja željenog imunskog odgovora. Mineralni adjuvansi na bazi aluminijuma su istorijski najčešće korišćeni imunostimulansi u vakcinama i smatraju se generalno bezbednim za humanu upotrebu. Značajni napredak na polju sinteze, kontrole strukture i funkcionalnog dizajna neorganskih (nano)materijala uslovio je povećano interesovanje za primenom inovativnih adjuvanasa kao što su gline, mezoporozne silika nanočestice, nanočestice cink-oksida, gvožđe oksida i gvožđe hidroksida, i dr. Sa druge strane, uočava se i sve veće interesovanje za primenom specifičnih nanonosača antigena, kao što su nanoemulzije, kako bi se antigeni zaštitili od preuranjene proteolitičke degradacije i/ili poboljšala njihova imunogenost, olakšavanjem preuzimanja i obrade od strane antigen-prezentujućih ćelija. Takođe, brojni istraživački naporci tokom poslednjih nekoliko godina usmereni su ka razvoju inovativnih tehnologija za isporuku antigena u kožu (kao što su mikroigle) radi poboljšanja efikasnosti vakcinacije uz istovremeno povećanje adherence pacijenata, posebno u pedijatrijskoj populaciji (neinvazivan ili minimalno invazivan način primene). Otuda, u ovom preglednom radu dat je detaljan pregled karakteristika svakog od navedenih pristupa za poboljšanje efikasnosti vakcina, sa posebnim osvrtom na primere njihove primene u formulacijama vakcina i faktore koji određuju efikasnost i bezbednost.

Ključne reči: formulacija vakcina, adjuvansi, mineralni adsorbenti, nanoemulzije, mikroigle

Childhood Vaccination in the Twenty-First Century: Parental Concerns and Challenges for Physicians

Srdja Janković

University Children's Hospital, Tiršova 10, 11000 Belgrade

Email: srdja.jankovic@udk.bg.ac.rs

Summary

Vaccination is one of the most important methods of prevention of infectious disease, saving millions of lives worldwide and protecting people from potentially debilitating complications. It is therefore hailed as one of the greatest advances of science-based medicine of all times. Although repeatedly proven safe and effective, vaccination has been questioned and resented throughout its long history. Vaccine hesitancy and refusal are once again on the rise in this century, due to a complex interplay of numerous factors and circumstances. A decline in childhood vaccination rates has already resulted in resurgence of hitherto eliminated vaccine-preventable diseases in many countries, and is now perceived as a major public health threat. This decline is closely related to increasing spread of misinformation regarding vaccine safety and effectiveness, coupled with a growing anti-vaccine activism. This, among other issues, underscores the need to improve communication between healthcare workers and parents, as well as devise a more comprehensive approach to boosting vaccine confidence, where scientists, physicians, media and the general public all have important roles to play. In this paper, we discuss the phenomenology and causal structure of vaccine hesitancy and refusal, and briefly review some widespread vaccine-related questions of everyday practical importance.

Keywords: vaccination, child, vaccine hesitancy, public health

1. Introduction

Vaccination is arguably the greatest triumph of science and science-based medicine. For more than two centuries, vaccines have been protecting millions of people from dangerous and deadly infectious diseases, one of which (smallpox) has been successfully eradicated (Simmons *et al.*, 2015), while another (poliomyelitis) is now close to eradication (Merten, 2019). However, due to a complex interplay of factors, both vaccination rates and vaccine coverage are currently in considerable decline in many countries: vaccine hesitancy and refusal are on the rise (Dubé *et al.*, 2013). In Serbia, some degree of vaccine hesitancy was recently self-reported by approximately 19% of parents who responded to a poll performed by Ipsos on behalf of UNICEF; however, people who profess firm anti-vaccine attitudes amounted to no more than 1% of respondents (UNICEF, 2017). The return of measles, a hitherto eliminated disease, claimed fifteen human lives in the season 2017/2018 (Institute of Public Health of Serbia, 2019). Decline of immunization rates and resurgence of vaccine-preventable diseases have been reported across Europe, though with considerable variation among countries (Rechel *et al.*, 2018). This phenomenon is partly caused by increasing activity of individuals and groups advocating anti-vaccine attitudes, *i. e.*, questioning safety and effectiveness of vaccines, scientific evidence, and often also motives and intentions of professionals involved in vaccination (Poland & Jacobson, 2012; Janković, 2014). Coupled with the advent of social networks and other means of rapid sharing of information, and unfortunately also misinformation (Stahl *et al.*, 2016), this trend is increasingly wreaking confusion among parents and caregivers. In this paper, we discuss the phenomenology and causes of vaccine hesitancy and refusal, and briefly review some of the issues most frequently raised by parents or caregivers as cause of their concern.

2. History of Vaccination: A Brief Reminder

The fact that some infectious diseases endow their survivors with lifelong protective immunity was well known since antiquity, as testified by the account of the „plague of Athens” (430 BC) written by Thucydides (Littman, 2009). The great Greek historian noted that people who recovered from this epidemic disease, caused by an unknown pathogen, were subsequently protected from contracting the same disease again, and thus were safe to tend the sick and dying fellow citizens. Similar knowledge was exploited for centuries in Chinese traditional medicine, where dried powder taken from the lesions of people who recovered from smallpox used to be inhaled or scratched into the skin of healthy persons, protecting them from the disease, albeit at the cost of usually moderate, but sometimes life-threatening, and even fatal adverse reactions. The practice later became widespread in Asia. It was brought to Europe by Lady Mary Wortley-Montagu (wife of the British ambassador to Istanbul), where it became known

as variolation (Stone & Stone, 2002). Variolation offered life-saving protection against smallpox, a disease with a mortality rate of about 30% in European populations at the time, that also often caused permanent blindness and disfiguration of survivors. At the turn of the nineteenth century, variolation was replaced by Edward Jenner's first vaccine. As widely known, the name was derived from cowpox, or vaccinia, a mild disease in humans, mostly affecting milkmaids, who frequently came into contact with infected cattle. Smallpox and vaccinia are caused by closely related viruses that share many antigens and thus elicit cross-reactive immunity (Rusnock, 2016). Although initially greeted with skepticism, some moral criticism, and even derision, vaccination soon proved to be the most effective means of protection against infectious disease. In the nineteenth century, Louis Pasteur successfully developed a vaccine against rabies, an invariably fatal disease, as well as a (veterinary) vaccine against anthrax (D'Amelio *et al.*, 2016). With the advance of immunology and ever-improving understanding of underlying principles of acquired immunity and immune memory, many new vaccines have been (and are still being) developed. Even by most conservative assessments, the number of lives saved by vaccines worldwide is on the order of millions. The accumulation of scientific knowledge has also enabled vast improvements in vaccine safety and effectiveness over time, as well as the design of many new types of vaccines. Today this can be achieved using modern tools of molecular biology, vastly expanding the realm of possibilities (Plotkin, 2014). This includes emerging technologies, such as gene libraries or fully synthetic molecular constructs.

3. Vaccine-Preventable Diseases

Vaccination has so far eliminated or drastically reduced the risk of many diseases that, historically, exerted a terrible toll on humanity (Weil, 2016). Apart from the above example of smallpox, children (and adults) in most parts of the world are now safe from diphtheria, tetanus, and poliomyelitis (Vitek, 2006; Roush *et al.*, 2007; Merten, 2019). The incidence of whooping cough, a bacterial infection that is often fatal in newborns and infants, has also been significantly curbed, although there is some resurgence due to a number of factors (insufficient lifelong immunity, possible development of resistance, decrease in vaccine uptake; please see Domenech de Cellés *et al.*, 2018). Immunization against *Mycobacterium tuberculosis*, although only modestly effective in preventing infection, has shown near-total effectiveness against most severe and life-threatening forms of the disease (miliary tuberculosis, tuberculous meningitis; Trunz *et al.*, 2006; Mangtani *et al.*, 2014). Recent introduction of conjugate vaccines against *Hemophilus influenzae* type B, *Pneumococcus*, and (in some countries) *Neisseria meningitidis* caused a considerable drop in the incidence of complications caused by these pathogens, endowed by a protective polysaccharide capsule as a factor of their virulence (Vella & Pace, 2015; Rappuoli, 2018). Measles, mumps and rubella have been eliminated in many countries (including Serbia), but have been „brought back” from the brink of

eradication by the infamous autism scare (Porter & Goldfarb, 2019; see also below). Another common childhood viral disorder accompanied by rash and fever, chickenpox, has been suppressed by vaccination in many countries, which is now recommended in Serbia as well. Rates of hepatitis B are steadily declining in countries that adopted recommended or compulsory vaccination (Nelson *et al.*, 2016), while the vaccination against human papillomavirus (HPV), causative agent of cervical carcinoma and a number of other tumors in both sexes, resulted in significant reduction in precancerous lesions caused by vaccine-preventable oncogenic strains of this DNA virus; the latent period from infection to malignancy is rather long, but countries that had been among the first to adopt HPV vaccine on a large scale, such as Australia, are already registering a drop in the incidence of HPV-related cancer, raising hopes of potential global elimination of these types of cancer towards the end of next decade (Canfell, 2019). Cervical cancer rates are also declining in many countries that introduced HPV vaccination more recently, notably Finland, which is partly due to highly efficient screening programs, highlighting the synergy between vaccination and other methods of disease prevention. Many more vaccines are used against infectious diseases in specific contexts or areas, such as vaccines against rabies, yellow fever, dengue, or, most recently, ebola. There are also many vaccines that are currently in the process of development.

4. Vaccine Hesitancy and Vaccine Refusal

It may seem as a bit of a paradox that vaccine hesitancy and refusal are increasing right in the face of incontrovertible successes of vaccination. However, apprehensive, reluctant, and potentially negative attitudes of patients and caregivers toward vaccination spring from a complex mixture of motives (Meyer & Reiter, 2004): fear of the unknown (or poorly understood); rarity of encounters with vaccine-preventable diseases (where vaccination is, in a way, „victim of its own success”); philosophical views on „natural” vs. „artificial”, associated with various misconceptions regarding health and disease; growing mistrust of the so-called „official” (or „Western”) medicine and science; view that healthcare providers could be corrupt or subject to their own biases and vested interests, not necessarily matching the interests of patients and/or public health; wide exposure to contradictory claims (and, indeed, viral misinformation) regarding vaccine safety and effectiveness, with insufficient level of rationality and critical thinking. A model developed by the SAGE Working Group clusters many factors contributing to vaccine hesitancy into three overlapping areas: complacency, confidence and convenience (MacDonald *et al.*, 2015). All three clusters of factors are strongly connected with emotional responses. Unsurprisingly, studies have demonstrated a consistent link between appeal to emotions and vaccine hesitancy (Tomljenović *et al.*, 2019). Vaccine hesitancy is also associated with viral spread of misinformation (Yiannakoulias *et al.*, 2019; Broadbent, 2019), particularly relating to

media coverage of high-profile court cases centered on harm allegedly caused by vaccines (Carrieri *et al.*, 2019). Interestingly, mistrust and avoidance of vaccines appears to be generally more prevalent in areas with higher level of socioeconomic advantage, and in persons with higher level of education, which has been dubbed „the privilege paradox” (Bryden *et al.*, 2019).

Analytical studies have demonstrated that complacency (not perceiving the risk of infectious disease as important), constraints (systemic and psychological obstacles), calculation (such as extensive information searching), and some aspects pertaining to collective responsibility (willingness to protect others) all play important roles in explaining vaccination behavior (Betsch *et al.*, 2018). A recent Cochrane meta-analysis of ten studies found that face-to-face interventions for educating parents about early childhood vaccination may improve children’s vaccination status to some degree. Less encouragingly, however, the effect has mainly been observed in settings of insufficient awareness or understanding of vaccine issues, while the impact of intervention is far less clear when vaccine hesitancy or refusal is thought to be the main barrier (Kaufman *et al.*, 2018). In any case, the approach to tackling vaccine hesitancy needs to be, above all, child/parent-centered and based on general education and communication skills (MacDonald & Dubé, 2018). Importantly, for most parents, including those that are vaccine-hesitant, physicians (and other healthcare workers) still constitute the principal – and most trusted – source of information regarding vaccines (Kennedy *et al.*, 2011; Mergler *et al.*, 2013; Kundi *et al.*, 2015). Most parents who voice concerns about safety of vaccination actually seek reassurance rather than confrontation. Thus pediatricians, and other health professionals, are typically presented with a golden opportunity to allay any unwarranted (or overmagnified) fears, regain the parents’ confidence and positively influence their decision. This once again highlights the importance of communication skills (Nayar *et al.*, 2019; Possenti *et al.*, 2019). In fact, parents usually respond favorably to clear, concise, and honest messages of physicians, and like to have their questions answered as plainly as possible (Healy & Pickering, 2011), which, in turn, points to the need for good knowledge base and personal integrity of helthcare professionals, as well as for systematically offering these professionals adequate training and support.

Since both the roots and the consequences of the problem of vaccine hesitancy concern society as a whole, apart from physicians and other healthcare workers, many other instances and institutions need to contribute to improving outcomes. Some good examples of social mobilization as a key factor in securing vaccine acceptance have been detailed by Jalloh and coworkers (Jalloh *et al.*, 2019). Some studies provided evidence that the internet, including social networks, can be used creatively to enhance the message about the importance of vaccines for public health, contributing to positive parents’ attitudes (Daley *et al.*, 2018), although this carries some risk of increased

polarization and enhanced „echochamber effect”, or even backfire. It is crucial to keep in mind at all times that true opponents of vaccination comprise only a minority of the population, while the vast majority of those parents/caregivers who hesitate, postpone, or deny some or all vaccines to their children state that they are not against vaccination in principle, but refer to safety concerns and other issues broadly related to (rather skewed) public perception of vaccines. In particular, risks of serious adverse events following vaccination, although extremely small in terms of frequency, tend to receive disproportionately more public attention compared to risks of vaccine-preventable diseases, since the reduction or elimination of the latter, and their consequent absence (or near-absence) from most or all communities, often gets taken for granted, in accordance with well-known findings of cognitive psychology that people typically fail to consider what they do not see. Naturally, this situation tends to change in times of epidemics. For instance, in our experience, during the epidemic of measles that recently struck Serbia (together with several other countries), some parents who had previously rejected the measles, mumps and rubella (MMR) vaccine, mostly out of ill-founded fear of autism-spectrum disorders (please see below), rapidly changed their minds and accepted their children to be vaccinated; indeed, quite a few of them even actively requested from their pediatricians to immunize the child as soon as possible.

There is one more ethical and practical dimension to vaccine hesitancy: apart from the protection afforded to the individual, an important aspect of vaccination is attached to collective immunity – prevention of spread of infection once a certain threshold of immunization coverage is reached in a given population (the exact threshold level depends on how contagious the disease in question is). Collective immunity is crucial for keeping infectious disease away, and thus protecting people who cannot be vaccinated due to medical contraindications, as well as babies and small children not yet vaccinated (depending on the specific vaccine timetable recommendations). Compromising collective immunity by faltering vaccine acceptance can therefore endanger many more lives. It is very important to keep in mind that collective immunity operates at the community level: even if coverage is, on the average, adequate nationwide, „pockets” of poor coverage may (and, indeed, do) give rise to local epidemics (Giubilini *et al.*, 2018).

5. Vaccine Safety: Global Evidence Base

Since vaccines have been, with constant improvement, used for a very long time, overwhelming evidence has accumulated as to their safety. Even though no medical agent or procedure can be absolutely devoid of risk, severe adverse effects of vaccination are extremely rare (WHO, 2012). There is, however, still some speculation about the possibility that vaccines may trigger a wide range of disorders, including allergic and autoimmune diseases. While this possibility must never be taken lightly,

comprehensive analyses confirmed that, for most disorders, there is no difference in incidence between vaccinated and unvaccinated persons. However, one may easily get a very different picture by reading raw data from passive surveillance registries such as VAERS (Vaccine Adverse Events Reporting System), or reports of individual patients or patient series. Crucially, such reports, although very important as data sources for further analysis, require additional scrutiny in order to determine the existence of a causal connection (or lack thereof); in other words, without proper comparison, and adequate control for relevant confounding factors, there is no way to differentiate a mere temporal connection (*i. e.*, a coincidence) from a causal relation. For instance, one study found that probable causality could be established in less than a quarter of investigated VAERS reports, and these were dominated by mild reactions, allergic reactions and symptoms specifically associated with vaccine administered (Loughlin, 2012).

As with any agents, allergic (and, more often, pseudoallergic) reactions to vaccines are certainly possible (Barbaud *et al.*, 2013). Indeed, they probably constitute the best documented category of potentially severe, and even life-threatening adverse effects of vaccination. However, such occurrences are still extremely rare and, although presently unpredictable, generally are amenable to treatment. Also, since vaccines are designed to trigger an immune response accompanied by a (minimal) inflammatory reaction needed to elicit the mechanisms ultimately leading to immune memory, there is a certain potential of triggering an autoimmune reaction in persons with unusually (idiosyncratically) high susceptibility. However, proven autoimmune adverse reactions to vaccination are quite rare and mostly limited to a few clinical entities, such as Guillain-Barré syndrome, autoimmune thrombocytopenia and narcolepsy. (For a review of documented, putative and speculative instances of autoimmunity associated with vaccination, please see Janković, 2017.)

Some of the most prevalent concerns of parents regarding vaccination are discussed below.

6. Do Vaccines Contain „Toxins”?

One of common concerns stated by parents (and widely reflected in the media and general public) is that vaccines contain many toxic substances, potentially harmful to children. As a rule, these concerns are tied to „bad reputation” of certain substances (for example, their known toxic effects in other contexts), but with more or less complete disregard of key principles of toxicology, including, first and foremost, the fact that for any toxic effect there is a threshold of dose and exposure. In this light, even though some components of vaccines indeed have the *potential* to be toxic, they are not *actually* toxic, since their concentration and total amount in vaccines are very strictly regulated, and never allowed to approach the toxicity threshold. Indeed, there is usually

a safety factor of at least an order of magnitude between the acceptable level of exposure and the lowest exposure that could entail toxicity. The key insight that no discussion of toxicity can be meaningful without explicitly considering dose is traditionally credited to Philippus Aureolus Theophrastus Bombastus von Hohenheim, a fifteenth/sixteenth century Swiss alchemist, astrologer, physician and proto-scientist, better known as Paracelsus.

As an example, aluminium is used in many vaccines, usually in the form of hydroxide, as an adjuvant – component intended to stimulate the immune response to a certain degree, in order to elicit long-term immune memory of target antigens. Aluminium is, as pointed out *ad nauseam* by vaccination opponents, a known neurotoxin. However, even though exact amounts of aluminium-containing adjuvants per vaccine dose had initially been defined empirically, the quantity of aluminium present in any vaccine does not exceed the established threshold of toxicity (Ameratunga *et al.*, 2017; Principi & Esposito, 2018). A single dose of vaccine against hepatitis B, for instance, contains 0.5 mg of aluminium (in the form of hydroxide). This quantity is equivalent to the amount that is usually ingested with food (including mother's milk) over a brief period of time, since aluminium is an abundant metal in the composition of Earth's crust and therefore present in our living environment (please see Corkins, 2019 and references therein). Correspondingly, aluminium levels in biological samples of vaccinated persons have not been found to differ from those in unvaccinated persons, while rare vaccine adverse effects that had been attributed to aluminium-based adjuvants, such as macrophagic myofasciitis, do not appear to be caused by aluminium toxicity *per se* (Goullé & Grangeot-Keros, 2019). In brief, while ongoing research directed at further optimization of vaccine adjuvants, including the design of new compounds and formulations guided by the accumulating knowledge of immunological mechanisms is unquestionably warranted, aluminium-based adjuvants remain the golden standard for such research (HogenEsch *et al.*, 2018).

For a second example, many vaccines contain formaldehyde, known to be toxic in a sufficient dose. However, all vaccines a child typically receives during childhood combined contain no more than 1 mg of formaldehyde (which is, furthermore, quickly degraded and does not accumulate); this is much lower than the amount normally present in the human body at any given time, or indeed the amount present in many common food items, such as bananas, apples, pears or apricots, as well as many vegetables, not to mention shiitake mushrooms, containing considerable, yet still harmless amounts. It is estimated that 1 kg of bananas, on the average, contains 16.3 mg of formaldehyde, while 1 kg of apricots contains about 9.5 mg (Centre for Food Safety, 2019). An analysis performed in USA by the Center for Biologics Evaluation and Research revealed that the amount of formaldehyde received from a single dose of a typical vaccine does not exceed 1% of the amount of formaldehyde normally present in

human body, and that this formaldehyde is metabolically degraded within 30 minutes (Mitkus *et al.*, 2013). There is, therefore, no risk of toxic effects.

On the other hand, concerns about „toxins” present in vaccines are sometimes merely a consequence of poor general understanding of chemistry. The most salient example is the claim that vaccines contain hydrochloric acid: this compound is actually added to vaccines just in order to adjust their final pH to neutral or physiological value. Hydrochloric acid itself is no longer present as such in the vaccine at time of its application, since it gets neutralized by other, slightly alkaline vaccine components; indeed, without the addition of acid to adjust the pH, vaccine would not be usable – for then it would indeed be toxic! To conclude, fears of „toxins” in vaccines are misplaced. No vaccine can be licensed without undergoing a comprehensive process of verification that none of its components exhibit toxicity at doses or levels of exposure involved (Gorski, 2008).

7. Are Vaccines Safe to Combine?

A common reason of stated concern is the notion that children are exposed to „too many” vaccine antigens. However, the immune system of every person daily encounters millions of antigens ever since (and even before) birth, with antigens associated with pathogenic organisms comprising only a tiny part of this number. Adaptive immune response has developed through evolution in order to provide a highly specific immune response to every individual antigen. More importantly, since different clones of lymphocytes respond to different antigens, a theoretically unlimited number of such responses may (and does) occur simultaneously, without placing any undue burden on the resources of the immune system. There is also ample evidence that combined vaccines, which have already been used for many decades, never produced any signs of „immune overload” (Offit *et al.*, 2002; Iqbal *et al.*, 2013). In addition, more recent vaccine designs are generally more purified. Therefore modern vaccines tend to contain an ever smaller number of antigens, thus reducing the overall antigenic burden associated with vaccines awaiting current and future generations, compared with the number of vaccine antigens children encountered in the past.

8. Can Vaccines Somehow „Weaken” the Immune System?

Many parents will ask their healthcare providers whether vaccinated children will have a less well developed immune systems, due to reduced stimulation by infections. This concern is wholly unfounded, although it is now known that exposure to a wide range of microorganisms, including some that may be pathogenic under some circumstances, is indeed beneficial, and even protects from development of allergies and autoimmune disorders (according to the well-known „hygiene hypothesis”, increasingly supported by evidence). A crucial point is, however, that pathogens

targeted (and hopefully eliminated) by vaccination are generally neither part of healthy microbiome nor beneficial stimulants of the immune system. Protection from such agents is therefore no loss but a great gain for a person's health, as testified by many studies that found no increase, and even a slight decrease, in the incidence of allergic conditions in children who received vaccines. Also of note, immune responses of vaccinated children to unrelated pathogens have not been found to be reduced in any way (Nicoli & Appay, 2017).

9. Vaccine Concerns: Some Common Examples

Autism Spectrum Disorders

Probably the most (in)famous vaccine-related concern in most parts of the world is the association between MMR vaccine and autism spectrum disorders (ASD). The existence of a link between the two, hypothesized in the 1998 Lancet article by Andrew Wakefield, has been extensively researched during the last two decades, and no such link has been found. The conclusion that the association is merely temporal (in other words, spurious) is attested by many large-scale, methodologically rigorous, independent studies. It is true that the first signs of ASD are very often noticed at the age when children are due to receive the MMR vaccine; however, children immunized with MMR (or any other vaccine) do not develop ASD more often than children that were not vaccinated. Meanwhile, the original study linking MMR vaccine with ASD has been retracted due to both methodological and ethical flaws (see, for example, Taylor *et al.*, 2014; Jain *et al.*, 2015; Hvid *et al.*, 2019; for a comprehensive review, please see DeStefano & Shimabukuro, 2019).

Another consequence of the vaccines-ASD story is the reluctance of many pediatricians to vaccinate children with perceived developmental delay of any kind (or a supposed risk thereof), with the tendency to postpone vaccination for months, or even years, „until the child has safely learned to walk and talk”. This is not only scientifically unfounded, but also brings children under risk of infection, which may cause serious complications. Ironically, this delay is particularly likely to occur in children with neurological conditions that also make them especially vulnerable to infection. And since diseases that had been eliminated by MMR vaccine have now returned to many countries due to falling vaccination rates, some of these vulnerable children are now at risk of contracting these diseases, notably measles, which carries a considerable complication rate even in healthy children, and is all the more dangerous in those with a weakened immune system, neurodevelopmental disorders, or both. Because of all this, it is imperative that all healthcare workers be armed with facts and well-prepared to offer concerned parents a thorough explanation and reassurance (with consultation of a pediatric neurologist and immunologist where necessary).

Egg Allergy

Some vaccines, such as MMR, are manufactured using viruses grown in chick embryos. For this reason, initial recommendations on vaccination included special precautions for persons with known or suspected egg protein allergies. However, it was found that there is no cross-reactivity with antigen epitopes that may potentially remain in the vaccine after purification, and that allergic reactions to MMR (or any other vaccine), including anaphylactic shock, are not more frequent in persons allergic to eggs than in the general population (Andersen & Jørgensen, 2013; Sánchez *et al.*, 2018; Kara Elitok *et al.*, 2019; Czajka *et al.*, 2019). For this reason, withholding vaccination from such persons is no longer recommended, and should be discouraged. Previous anaphylactic reaction to vaccine (not egg proteins) is still a contraindication, although it is possible to test immediate sensitivity to vaccine itself before applying the latter, as well as to vaccinate in hospital settings with appropriate precautions (Tuncel *et al.*, 2017).

Immune thrombocytopenia

Immune thrombocytopenia (ITP) is a known rare autoimmune adverse effect of some vaccines, notably MMR, whereupon it occurs with an incidence of 1:40,000 to 1:35,000 doses. However, since infections (including measles) are most often (in 80-90% of cases) the trigger of ITP, vaccine-triggered ITP comprises only about 10% of all childhood ITP. In addition, vaccine-triggered ITP is most often (>90%) self-limited and resolves without any harm to the child. The risk of ITP ensuing after vaccination is therefore not considered a reason not to vaccinate, since the child would be exposed to a much greater risk of the same condition if left unvaccinated. Even in children who previously suffered an episode of acute ITP, including vaccine-triggered ITP, MMR (or any other) vaccine is not strictly contraindicated. Children with chronic ITP can (and should) also be vaccinated, albeit with the precaution of choosing the time when disease activity is low and platelet number reasonably recovered (O’Leary *et al.*, 2012; Cecinati *et al.*, 2013).

11. Concluding Remarks

Scientists designing and testing vaccines, doctors and nurses administering them, media reporting on the whole process, and the general public, including traditional decision-makers, constitute the four corners of a „communication quadrangle”. Stable and effective communication conductive to good outcomes requires all four corners to be stable. In other words, overcoming the threat of vaccine hesitancy and protecting vulnerable lives will require all parts of society to play their roles – at community, national, regional and global levels. Only then may we hope to benefit from the full protective potential of the most powerful tool of medical science.

Acknowledgments

The author received support from the Ministry of Education, Science and Technological Development No. 41004. The author declares no conflict of interest.

References

1. Ameratunga R, Gillis D, Gold M, Linneberg A, Elwood JM. Evidence refuting the existence of autoimmune/autoinflammatory syndrome induced by adjuvants (ASIA). *J Allergy Clin Immunol Pract.* 2017; 5(6):1551-1555.
2. Andersen DV, Jørgensen IM. MMR vaccination of children with egg allergy is safe. *Dan Med J.* 2013; 60(2):A4573.
3. Barbaud A, Deschildre A, Waton J, Raison-Peyron N, Tréchot P. Hypersensitivity and vaccines: an update. *Eur J Dermatol.* 2013; 23(2):135-41.
4. Betsch C, Schmid P, Heinemeier D, Korn L, Holtmann C, Böhm R. Beyond confidence: development of a measure assessing the 5C psychological antecedents of vaccination. *PLoS One* 2018; 13(12): e0208601.
5. Broadbent JJ. Vaccine hesitancy: misinformation on social media. *BMJ* 2019; 366: I4457.
6. Bryden GM, Browne M, Rockloff M, Unsworth C. The privilege paradox: geographic areas with highest socio-economic advantage have the lowest rates of vaccination. *Vaccine* 2019; 37(32):4525-32.
7. Canfell K. Towards the global elimination of cervical cancer. *Papillomavirus Res.* 2019 (in press); DOI: 10.1016/j.pvr.2019.100170.
8. Carrieri V, Madio L, Principe F. Vaccine hesitancy and (fake) news: quasi-experimental evidence from Italy. *Health Econ.* 2019 (in press); doi: 10.1002/hec.3937.
9. Cecinati V, Principi N, Brescia L, Giordano P, Esposito S. Vaccine administration and the development of immune thrombocytopenic purpura in children. *Hum Vaccine Immunother.* 2013; 9(5):1158-62.
10. Centre for Food Safety. The Government of Hong Kong Special Administrative Region. Food Additives. Available at:
https://www.cfs.gov.hk/english/programme/programme_rafts/programme_rafts_fa_02_09.html; last accessed on October 14th, 2019.
11. Corkins MR; Committee on Nutrition. Aluminum effects in infants and children. *Pediatrics* 2019; 144(6): e20193148.

12. Czajka H, Czajka S, Dylag KA, Borek E, Kuchar E. Vaccination against measles, mumps, and rubella in the light of current epidemic threats: unjustified postponement. *Adv Exp Med Biol.* 2019; 1153:101-7.
13. D'Amelio E, Salemi S, D'Amelio R. Anti-infectious human vaccination in historical perspective. *Int Rev Immunol.* 2016; 35(3):260-90.
14. DeStefano F, Shimabukuro TT. The MMR vaccine and autism. *Annu Rev Virol.* 2019; 6(1):585-600.
15. Domenech de Cellès M, Magpantay FMG, King AA, Rohani P. The impact of past vaccination coverage and immunity on pertussis resurgence. *Sci Transl Med.* 2018; 10(434): pii: eaaj1748.
16. Dubé E, Laberge C, Guay M, Bramadat P, Roy R, Bettinger J. Vaccine hesitancy: an overview. *Hum Vaccin Immunother.* 2013; 9(8):1763-73.
17. Giubilini A, Douglas T, Savulescu J. The moral obligation to be vaccinated: utilitarianism, contractualism, and collective easy rescue. *Med Health Care Philos.* 2018; 21(4):547-560.
18. Gorski D. Toxic Myths About Vaccines. Science-Based Medicine 2008; available at: <https://sciencebasedmedicine.org/toxic-myths-about-vaccines/>; last accessed: October 15th, 2019.
19. Goullié JP, Grangeot-Keros L. Aluminum and vaccines: current state of knowledge. *Med Mal Infect.* 2019 [in press]; S0399-077X(18)30844-8.
20. Healy CM, Pickering LK. How to communicate with vaccine-hesitant parents. *Pediatrics* 2011; 127 Suppl 1:S127-33.
21. HogenEsch H, O'Hagan DT, Fox CB. Optimizing the utilization of aluminum adjuvants in vaccines: you might just get what you want. *NPJ Vaccines* 2018 [in press]; DOI: 10.1038/s41541-018-0089-x.
22. Hviid A, Hansen JV, Frisch M, Melbye M. Measles, Mumps, Rubella vaccination and autism: a national cohort study. *Ann Intern Med.* 2019; 170(8):513-520.
23. Institute of Public Health of Serbia „Dr. Milan Jovanović Batut”: Aktuelna epidemiološka situacija malih boginja (morbila) u Republici Srbiji. Available at:
<http://www.batut.org.rs/index.php?content=1629>; last accessed on October 14th, 2019.
24. Iqbal S, Barlie JP, Thompson WW, DeStefano F. Number of antigens in early childhood vaccines and neuropsychological outcomes at age 7-10 years. *Pharmacoepidemiol Drug Saf.* 2013; 22(12):1263-70.
25. Jain A, Marshall J, Buikema A, Bancroft T, Kelly JP, Newschaffer CJ. Autism occurrence by MMR vaccine status among US children with older siblings with and without autism. *JAMA* 2015; 313(15): 1534-40.
26. Jalloh MF, Wilhelm E, Abad N, Prybylski D. Mobilize to vaccinate: Lessons learned from social mobilization for immunization in low and middle-income countries. *Hum Vaccin Immunother.* 2019 (in press); DOI: 10.1080/21645515.2019.1661206.
27. Janković S. Anti-vakcinalni pokreti i naučna medicina. *Biomedicinska Istraživanja* 2014; 5(1):59-65.
28. Janković S. Vaccination and autoimmune phenomena. *Central Eur J Paed.* 2017; 13(1): 12-23.

29. Kara Elitok G, Celikboya E, Bulbul L, Kaya A, Toraman T, Bulbul A, et al. Does food allergy require any change in measles-mumps-rubella vaccination? *Indian J Pediatr.* 2019; 86(10):915-20.
30. Kaufman J, Ryan R, Walsh L, Horey D, Leask J, Robinson P, et al. Face-to-face interventions for informing or educating parents about early childhood vaccination. *Cochrane Database Syst Rev.* 2018; 5: CD010038.
31. Kennedy A, Basket M, Sheedy K. Vaccine attitudes, concerns, and information sources reported by parents of young children: results from the 2009 HealthStyles survey. *Pediatrics* 2011;127(Suppl 1); S92-99.
32. Kundi M, Obermeier P, Helfert S, Oubari H, Fitzinger S, Yun JA, et al. The impact of the parent-physician relationship on parental vaccine safety perceptions. *Curr Drug Saf.* 2015; 10(1):16-22.
33. Littman RJ. The plague of Athens: epidemiology and paleopathology. *M Sinai J Med.* 2009; 76(5):456-67.
34. Loughlin AM, Marchant CD, Adams W, Barnett E, Baxter R, Black S, et al. Causality assessment of adverse events reported to the Vaccine Adverse Events Reporting System (VAERS). *Vaccine* 2012; 30(50):7253-9.
35. MacDonald NE; SAGE Working Group on Vaccine Hesitancy. Vaccine hesitancy: Definition, scope and determinants. *Vaccine* 2015; 33(34):4161-4.
36. MacDonald NE, Dubé E. Addressing vaccine hesitancy in immunization programs, clinics and practices. *Paediatr Child Health* 2018; 23(8):559-60.
37. Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PE, Rodriguez LC, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. *Clin Infect Dis.* 2014; 58(4):470-80.
38. Mergler MJ, Omer SB, Pan WK, Navar-Boggan AM, Orenstein W, Marcuse EK, et al. Association of vaccine-related attitudes and beliefs between parents and health care providers. *Vaccine* 2013; 31(41):4591-5.
39. Merten M. Polio: getting ready for the day after eradication. *BMJ* 2019; 366:I5235.
40. Meyer C, Reiter S. Impfgegner und impfskeptiker. *Bundesgesundheitsblat Gesundheitsforschung Gesundheitsschutz* 2004; 47:1182-88.
41. Mitkus RJ, Hess MA, Schwartz SL. Pharmacokinetic modelling as an approach to assessing the safety of residual formaldehyde in infant vaccines. *Vaccine* 2013; 31(25):2738-43.
42. Nayar RK, Nair AT, Shaffi M, Swarnam K, Kumar A, Abraham M, et al. Methods to overcome vaccine hesitancy. *Lancet* 2019; 393(1077): 1203-4.
43. Nelson NP, Easterbrook PJ, McMahon BJ. Epidemiology of hepatitis B virus infection and impact of vaccination on disease. 2016; 20(4):607-628.
44. Nicoli F, Appay V. Immunological considerations regarding parental concerns on pediatric immunizations. *Vaccine* 2017; 25(23):3012-9.
45. Offit PA, Quarles J, Gerber MA, Hackett CJ, Marcuse EK, Kollman TR, et al. Addressing parents' concerns: do multiple vaccines overwhelm or weaken the infant's immune system? *Pediatrics* 2002; 109:124-129.

46. O'Leary ST, Glanz JM, McClure DL, Akhtar A, Daley MF, Nakasato C. The risk of immune thrombocytopenic purpura after vaccination in children and adolescents. *Pediatrics* 2012; 129(2):248-55.
47. Plotkin S. History of vaccination. *Proc Natl Acad Sci USA* 2014; 111(34):12283-7.
48. Poland GA, Jacobson RM. The clinician's guide to the antivaccinationists' galaxy. *Human Immunol.* 2012; 73:859-66.
49. Porter A, Goldfarb J. Measles: a dangerous vaccine-preventable disease returns. *Cleve Clin J Med.* 2019; 86(6): 393-8.
50. Possenti V, Luzi AM, Colucci A, De Mei B. Communication and basic health counselling skills to tackle vaccine hesitancy. *Ann Ist Super Sanita* 2019; 55(2):195-199.
51. Principi N, Esposito S. Aluminium in vaccines: Does it create a safety problem? *Vaccine* 2018; 36(39):5825-5831.
52. Rappuoli R. Glycoconjugate vaccines: principles and mechanisms. *Sci Transl Med.* 2018; 10(456): pii: eaat4615
53. Rechel B, Richardson E, McKee M (Eds). The organization and delivery of vaccination services in the European Union. Prepared for the European Commission. European Observatory on Health Systems and Policies 2018. Available at: https://ec.europa.eu/health/sites/health/files/vaccination/docs/2018_vaccine_services_en.pdf; last accessed on October 15th, 2019.
54. Roush SW, Murphy TV; Vaccine-Preventable Disease Table Working Group. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. *JAMA* 2007; 298(18):2155-63.
55. Rusnock AA. Historical context and the roots of Jenner's discovery. *Hum Vaccin Immunother* 2016; 12(8):2025-28.
56. Sánchez J, Ramírez R, Cardona R. The frequency of allergic reactions to the triple viral vaccine in 94 patients with egg allergy. *Biomedica* 2018; 38(4):514-520.
57. Simmons BJ, Falto-Aizpurua LA, Griffith RD, Nouri K. *JAMA Dermatol.* 2015; 151(5):521.
58. Stahl JP, Cohen R, Denis F, Gaudelus J, Martinot A, Lery T, et al. The impact of the web and social networks on vaccination. New challenges and opportunities offered to fight against vaccine hesitancy. *Med Mal Infect.* 2016; 46(3):117-22.
59. Stone AF, Stone WD. Lady Mary Wortley Montagu: medical and religious controversy following her introduction of smallpox inoculation. *J Med Biogr.* 2002; 10(4):232-6.
60. Taylor LE, Swerdfeger AL, Eslick GD. Vaccines are not associated with autism: an evidence-based meta-analysis of case-control and cohort studies. *Vaccine* 2014; 32(29):3623-9.
61. Tomljenović H, Bubić A, Erceg N. It just doesn't feel right – the relevance of emotions and intuition for parental vaccine conspiracy beliefs and vaccination uptake. *Psychol Health* 2019 (in press); DOI: 10.1080/08870446.2019.1673894.
62. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and milliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *Lancet* 2006; 367(9517):1173-80.

63. Tuncel T, Sancakli O, Ozdogru E. Successful administration of measles-rubella-mumps vaccine by graded challenge in a case with anaphylaxis after prior vaccination. *Arch Argent Pediatr.* 2017; 115(2):e89-e91.
64. UNICEF. Knowledge, attitudes and practices in relation to immunisation of children in Serbia. UNICEF 2017. Available at: https://www.unicef.org-serbia/sites/unicef.org-serbia/files/2018-12/Knowledge_Attitudes_Practices_Immunisation.pdf; last accessed: October 11th, 2019.
65. Vella M, Pace D. Glycoconjugate vaccines: an update. *Expert Opin Biol Ther.* 2015; 15(4):529-46.
66. Vitek CR. Diphteria. *Curr Top Microbiol Immunol.* 2006; 304:71-94.
67. Weil AR. Eliminating vaccine-preventable diseases around the world. *Health Aff. (Millwood)* 2016; 35(2):190-4.
68. World Health Organization. Global Vaccine Safety Blueprint. 2012; available at: https://apps.who.int/iris/bitstream/handle/10665/70919/WHO_IVB_12.07_eng.pdf;jsessionid=2F0AE6700D9600340B84C4D0172D434D?sequence=1; last accessed on October 15th, 2019.
69. Yiannakoulias N, Slavik CE, Chase M. Expressions of pro- and anti-vaccine sentiment on You Tube. *Vaccine* 2019; 37(15):2057-64.

Vakcinacija dece u dvadeset prvom veku: nedoumice za roditelje i izazovi za lekare

Srđa Janković

Univerzitetska Dečija klinika, Tiršova 10, 11000 Beograd, Srbija

Email: srdja.jankovic@udk.bg.ac.rs

Kratak sadržaj

Vakcinacija je jedna od najznačajnijih metoda prevencije zaraznih bolesti, čuvajući na milione života u celom svetu i pružajući zaštitu od potencijalno onesposobljavajućih komplikacija. Stoga se vakcinacija smatra jednim od najvećih dostignuća naučne medicine svih vremena. Uprkos brojnim dokazima delotvornosti i bezbednosti, vakcinacija je kroz čitavu svoju istoriju bila praćena otporima i sumnjama. Oklevanje i odbijanje vakcinacije su u ovom veku ponovo u porastu, što je uzrokovano složenim spletom činilaca i okolnosti. Opadanje obuhvata dece vakcinacijom je u mnogim zemljama već dovelo do povratka prethodno eliminisanih oboljenja predupredivilih vakcinacijom i nosi značajnu pretnju po narodno zdravlje. Pomenuto opadanje obuhvata tesno je povezano s uvećanim širenjem netačnih informacija o bezbednosti i delotvornosti vakcina, zajedno sa narastanjem antivakcinalnog aktivizma. To, između ostalog, podvlači potrebu za poboljšanjem komunikacije između zdravstvenih radnika i roditelja, kao i za obuhvatnjim pristupom podizanju nivoa pouzdanja u vakcine, pri čemu važne uloge pripadaju kako naučnicima i lekarima, tako i medijima i društvenoj zajednici. U ovom radu razmatramo fenomenologiju i kauzalnu strukturu oklevanja i odbijanja vakcinacije, uz kratak pregled nekih rasprostranjenih pitanja u vezi sa vakcinama, od značaja za svakodnevnu praksu.

Ključne reči: vakcinacija, deca, oklevanje u vezi s vakcinacijom, narodno zdravlje

Primena profilaktičkih vakcina kod starih

Biljana Bufan

Univerzitet u Beogradu - Farmaceutski fakultet, Katedra za mikrobiologiju i imunologiju, Vojvode Stepe 450, 11221 Beograd, Srbija

Autor za korespondenciju, e-mail: bbiljana@pharmacy.bg.ac.rs

Kratak sadržaj

Producenje ljudskog veka i povećanje broja starih (stariji od 65 godina) na svetskom nivou nosi sa sobom izazove za javno-zdravstveni sistem jer se radi o populaciji podložnoj infektivnim bolestima. Vakcinacija je jedan od načina prevencije ovih bolesti. U cilju poboljšanja kvaliteta života, smanjenja komplikacija bolesti, hospitalizacija i mortaliteta starih, mnoge evropske države i Sjedinjene Američke Države, kod starih osoba, preporučuju vakcinaciju protiv gripa, pneumokoka, varičela-zoster virusa (VZV), kao i „booster“ vakcinacije protiv tetanusa, pertusisa i difterije. Razlog veće podložnosti infektivnim bolestima i manjoj efikasnosti vakcina kod starih su starenjem uslovljene promene koje pogodaju imunski sistem, a koje utiču na njegovu funkciju. Sezonska vakcina protiv gripa se u većini evropskih zemalja preporučuje starijima od 65 godina. Budući da je njena efikasnost niža kod starih nego kod odraslih osoba, razvijaju se strategije koje bi prevazišle ovaj problem (uključivanje adjuvansa u formulaciju, povećanje doze, promena puta unošenja antiga, razvoj vektorskih vakcina). Protiv pneumokoka su dostupne polisaharidna i konjugovana vakcina, a podaci o njihovoj efikasnosti su nekonzistentni. Vakcina protiv VZV je atenuisana, živa vakcina i pokazala se efikasna u smanjenju incidencije herpes zostera i post-herpetične neuralgije.

Podizanje svesti o značaju vakcinacije starih osoba i razvoj vakcina prilagođenih ovoj populaciji su od velikog značaja za očuvanje njihovog zdravlja.

Ključne reči: stare osobe, sezonska vakcina protiv *Virus influenzae*,
vakcina protiv *Streptococcus pneumoniae*,
vakcina protiv *Varicella-zoster virus*

Uvod

Starenje svetske populacije je globalni trend. Prema podacima Ujedinjenih nacija (UN) broj osoba starijih od 60 godina u svetu u 2017. godini iznosio je 962 miliona, što je dvostruko više od onog zabeleženog pre 37 godina. Pretpostavka je da će se ovakav trend starenja populacije nastaviti i da će se do 2050. godine broj starih osoba udvostručiti i čak premašiti broj adolescenata i mlađih (1). Kada je u pitanju zastupljenost stare populacije, u 2017. godini, posmatrano na svetskom nivou, jedna od osam osoba bila je starija od 60 godina, dok je u Evropi udeo starih osoba iznosio oko 20 %. Prema projekcijama UN-a, 2050. godine oko 20 % svetske populacije će činiti stariji od 60 godina, dok će taj procenat u Evropi iznositi oko 35 % (1).

Producenje ljudskog životnog veka i starenje populacije, nastalo kao rezultat napretka medicine i poboljšanja socio-ekonomskih prilika, donelo je i nove izazove zdravstvenom sistemu svake zemlje, između ostalog i sa stanovišta kontrole i prevencije infektivnih bolesti. Naime, poznato je da stare osobe češće obolevaju od infektivnih bolesti, da su kod njih češći teži oblici bolesti, hospitalizacije i smrtni ishodi, u poređenju sa zdravim, mlađim odraslim osobama (1, 2, 3). Razlog za ovo jesu intrinzične promene kojima podleže imunski sistem tokom fiziološkog starenja organizma i koje stare osobe čine podložnijim infektivnim bolestima, ali i hronične bolesti i primenjena terapija koji takođe mogu da kompromituju imunski sistem (5, 6, 7).

Prevencija infektivnih bolesti u populaciji starih jeste važna mera koju treba preduzeti sa ciljem postizanja zdravog starenja i unapređenja kvaliteta života u starosti (3, 6, 8). Najefikasnija mera prevencije infektivnih bolesti je vakcinacija i dok se ova mera uspešno primenjuje kod dece, programi vakcinacije tokom života i vakcinacija stare populacije često su potcenjeni (8). Mnoge evropske države i Sjedinjene Američke Države (SAD) preporučuju vakcinaciju odraslog stanovništva i starih osoba protiv infekcija koje su značajan uzrok mortaliteta i morbiditeta u populaciji starijih od 65 i 85 godina (6). Preporuke se odnose na primenu sezonske vakcine protiv *Virus influenzae* i vakcine protiv *Streptococcus pneumoniae* i preporučuju se odraslima koji boluju od hroničnih bolesti i starijima od 50, 60 ili 65 godina, zavisno od države. Takođe, preporučuje se i primena „booster” doze vakcine protiv tetanusa, difterije i pertusisa svakih 10 godina, a u nekim državama i skraćenje intervala njihove primene kod starijih od 65 godina zbog bržeg opadanja nivoa zaštitnih antitela u ovoj populaciji (8). U nekim državama (Austrija, Francuska, Češka, Grčka, Italija, Ujedinjeno Kraljevstvo, SAD) stariim osobama se savetuje i vakcinacija protiv *Varicella-zoster virus* (VZV) radi prevencije njegove reaktivacije (5, 6, 8, 9). Nažalost, imunogenost i efikasnost većine danas dostupnih vakcina je niža kod starih osoba u poređenju sa mlađima. Razlog za to je isti kao i za povećanu osjetljivost na infekcije, tj. starenje imunskog sistema (6, 8, 10).

Starenje imunskog sistema

Starenje imunskog sistema je proces koji se odvija tokom starenja organizma i on ne podrazumeva progresivno smanjenje funkcije imunskog sistema, već se ogleda u složenom remodelovanju njegovih funkcija, pri čemu dolazi do progresivnog smanjenja efikasnosti nekih funkcija, dok druge ostaju nepromjenjene ili se čak pojačavaju (11). Istraživanja na ovom polju su intenzivirana poslednjih godina, i očekuje se da bi mogla biti važna za dizajniranje vakcina koje bi bile efikasne kod starih (10).

Promene povezane sa starenjem pogađaju gotovo sve komponente imunskog sistema: a) ćelije urođenog i adaptivnog imuniteta, b) mikrosredinu u limfnim i drugim organima u kojima se nalaze ćelije imunskog sistema i c) solubilne faktore koji interaguju sa ćelijama imunskog sistema i mikrosredinom, a koji su važni za započinjanje, održavanje i završetak imunskog odgovora, i za homeostazu imunskog sistema (7).

Kada je reč o ćelijama urođenog imuniteta, nađeno je da granulociti i makrofagi starih jedinki imaju smanjenu sposobnost fagocitoze i produkcije mikrobicidnih supstanci (reaktivnih kiseoničnih radikala i azot-monoksida), što se dovodi u vezu sa lošijom prognozom bakterijskih infekcija i sepsom u ovoj populaciji (12, 13). Urođenoubilačke (*engl.* natural killer, NK) ćelije, koje imaju ulogu u ubijanju inficiranih ćelija, kod starih osoba pokazuju smanjenu citotoksičnu aktivnost i sposobnost produkcije citokina (14). Dendritske ćelije (DĆ) su profesionalne antigen-prezentujuće ćelije i ključne su za pokretanje i usmeravanje adaptivnog imunskog odgovora. Promene kojima podležu ove ćelije tokom starenja kod ljudi i glodara, ogledaju se u smanjenom preuzimanju antiga i/ili mikroorganizama, smanjenoj sposobnosti migracije i smanjenom ispoljavanju kostimulatornih molekula i produkciji citokina, što su sve aktivnosti važne za stimulaciju T-ćelijskog odgovora (15, 16, 17, 18). Takođe, opisana je i smanjena sposobnost ovih ćelija da vrše unakrsnu prezentaciju tj. prezentaciju antiga zaraženih ćelija CD8+ T-limfocitima (19). Starenjem DĆ i makrofagi pokazuju smanjenu sposobnost da odgovore na molekulske obrasce patogena, tj. molekule mikroorganizama (16, 20). Tačnije, DĆ i makrofagi prepoznaju patogene zahvaljujući receptorima na svojoj površini koji detektuju molekulske obrasce koji su zajednički za patogene (*engl.* pathogen-associated molecular patterns), a koji su nazvani receptori za prepoznavanje obrazaca (*engl.* pattern recognition receptors, PRR). Ovi receptori obuhvataju: receptore slične Tollu (*engl.* Toll-like receptors, TLR), receptore slične NOD-u (*engl.* NOD-like receptors), receptore slične RIG-u (*engl.* RIG-like receptors) i druge (12, 13, 20). Smanjenje nivoa ispoljenosti ovih receptora i/ili poremećaji u prenosu signala koje ćelija dobija posredstvom ovih receptora mogli bi da budu značajni za efikasnost odgovora na vakcine i za slabiji ishod vakcine kod starih osoba (7, 20, 21).

Sveukupno, promene koje pogađaju ćelije urođenog imuniteta tokom starenja utiču na smanjenje preuzimanja antiga na mestu aplikacije vakcine, smanjenje aktivacije antigen-prezentujućih ćelija i stimulacije antigen-specifičnog adaptivnog imuniteta, što za rezultat ima smanjenu efikasnost vakcina (22, 23).

T- i B-limfociti, ćelije adaptivnog imuniteta, podležu značajnim promenama tokom starenja. Pre svega, promene se dešavaju na nivou primarnih limfnih organa: timusa, u kome sazrevaju T-limfociti, i kostne srži, u kojoj sazrevaju B-limfociti (24, 25), gde dolazi do smanjenog stvaranja naivnih limfocita. Ovo ima za posledicu povećanje broja memorijskih T-limfocita, te se odnos naivnih i memorijskih T-limfocita u okviru pula ukupnih T-limfocita kod starih menja u korist memorijskih limfocita (7, 26). Sa smanjenjem broja naivnih T-limfocita kod starih osoba, smanjuje se efikasnost imunskog odgovora na nove antigene (27).

Pored populacionih promena, starenjem dolazi do promena i na nivou pojedinačnih T- i B-limfocita. Neke od promena koje pogađaju T-limfocite su: smanjena sposobnost da odgovore na stimulaciju antigenom, defekt u prenosu signala posredstvom T-ćelijskog receptora, smanjeno ispoljavanje koreceptorskih molekula, aktivacionih markera i markera diferencijacije, smanjena sekrecija interleukina (IL)-2 i proliferacija nakon stimulacije od strane antigen-prezentujućih ćelija (7, 28).

Humoralni imunski odgovor, koji je posredovan B-limfocitima, takođe je kompromitovan kod starih osoba i miševa i on je i kvantitativno (smanjena produkcija antigen-specifičnih antitela, smanjeno trajanje protektivnog imuniteta) i kvalitativno (povećan/smanjen aviditet antigen-specifičnih antitela, promjenjen izotipski profil antitela) promenjen u odnosu na odgovor mlađih jedinki (29, 30, 31). Ove promene mogu biti rezultat intrinzičnih defekata B-limfocita starih osoba i/ili poremećene interakcije sa pomoćničkim T-limfocitima, što je naročito važno kada su u pitanju T-zavisni, proteinski antigeni (7). Do poremećaja interakcije sa pomoćničkim T-limfocitima može doći zbog intrizičnih promena koje pogađaju ove ćelije tokom starenja, uključujući i smanjenje sposobnosti ovih ćelija da migriraju u sekundarne limfne organe (32).

Uticaj na migratornu sposobnost ćelija imunskog sistema, a samim tim i na razvoj i oblikovanje imunskog odgovora, mogu da imaju i promene koje se dešavaju u sekundarnim limfnim organima (7, 28). U limfnim čvorovima starih miševa uočen je smanjen broj stromalnih ćelija (pre svega fibroblastnih retikularnih ćelija) koje imaju ulogu u transportu hemokina i usmeravanju kretanja ćelija i njihovim međusobnim interakcijama (DĆ/T-limfociti, B-limfociti/T-limfociti) (7, 28). Takođe, u limfnim čvorovima starih uočavaju se i fibrozne promene koje mogu negativno da utiču na migraciju i interakcije imunskih ćelija tokom imunskog odgovora (28).

Važna karakteristika starenja, koja ima značajan uticaj na podložnost zaraznim bolestima i na imunski odgovor na vakcine je i progresivna, sistemska, sterilna,

hronična inflamacija niskog intenziteta (*engl. inflammaging*) (33). Ovo stanje se odlikuje hroničnom aktivacijom ćelija urođenog imuniteta, pre svega makrofaga i karakterišu ga povišeni nivoi pro-inflamatornih citokina (IL-1, IL-6, faktor nekroze tumora- α), ali i drugih pro-inflamatornih medijatora (hemokini, proteini akutne faze) u krvi i tkivima (33). Pretpostavlja se da je ovakvo stanje posledica nakupljanja oštećenih i izmenjenih molekula i ćelijskog debrisa (zbog povećanog stvaranja ili smanjenog uklanjanja), koji za uzvrat stimulišu ćelije urođenog imuniteta (pre svega makrofage) (33, 34). Drugi faktori koji doprinose nastanku i održavanju ovakvog stanja su i hronična infekcija koju izaziva *Cytomegalovirus*, poremećaj funkcije mitohondrija, promene na nivou crevne mikrobiote koje su uslovljene starenjem, hronični stres, da pomenemo samo neke (34, 35). Povezanost hronične inflamacije niskog intenziteta i slabijeg odgovora na vakcine pokazali su McElhaney i saradnici (36), te se smatra da bi ovaj faktor trebalo uzeti u obzir prilikom razvoja strategija za povećanje efikasnosti vakcina za stare (35).

Većina vakcina koje se danas primenjuju stimuliše humoralni imunitet, tj. sintezu neutrališućih antitela. Razvoj humornog imunskog odgovora je proces koji uključuje: aktivaciju antigen-prezentujućih ćelija, njihovu migraciju u drenirajuće limfne čvorove gde aktiviraju pomoćničke T-limfocite, interakciju pomoćničkih T-limfocita sa B-limfocitima, formiranje germinativnih centara i sazrevanje B-limfocita u plazma ćelije i memorijске ćelije (10).

Vakcine koje se danas primenjuju su dizajnirane uglavnom za decu i mlade i nisu sasvim efikasne u zaštiti starih (6, 35). Naime, u poređenju sa mladim osobama, imunski odgovor na vakcinaciju je kod starih slabiji, uz čest izostanak dugotrajnog zaštitnog imuniteta, što ove osobe dovodi u povećan rizik od obolenja od infektivnih bolesti (28). Razlog za to su, prethodno navedene starenjem-uslovljene promene, pre svega adaptivnog imuniteta, koji bi trebao da obezbedi antigen-specifičan i dugotrajan imunitet (28). Prema tome, jasno je da postoji potreba da se strategija vakcinacije i vakcine prilagode stariim osobama, odnosno da se poveća njihova efikasnost kod starih.

U nastavku će se razmatrati vakcine čija se upotreba preporučuje stariim osobama, sa akcentom na sezonsku vakciju protiv gripe, zbog značaja ove infekcije za ovu vulnerabilnu populaciju.

Profilaktičke vakcine čija se upotreba preporučuje kod starih

Sezonska vakcina protiv gripe

Grip je akutna zarazna bolest respiratornog trakta i epidemije se javljaju sezonski. Izazivač je *Virus influenzae*. Najveći broj slučajeva obolenja (oko 80 %) beleži se u periodu godine koji obično traje 8 do 10 nedelja i karakterističan je za region. U umerenom klimatskom pojasu obe hemisfere sezona gripe se javlja u periodu od kasne

jeseni do ranog proleća kada niske temperature i vlažnost pogoduju širenju virusa (22, 37). Pandemije gripe se javljaju sporadično i sa nepredvidivom učestalošću. U poslednjih sto godina zabeležene su četiri pandemije (1918, 1957, 1968 i 2009. godine) i one su odnele milione života širom sveta (37).

Svetska zdravstvena organizacija (SZO) procenjuje da 5-15 % populacije bude inficirano virusom gripe u epidemijama. Smatra se da gotovo 1 milijarda osoba godišnje oboli od ove bolesti, od toga tešku formu bolesti razvije 3 do 5 miliona, dok 250 000 do 500 000 infekcija ovim virusom ima smrtni ishod (22).

Povećani rizik od obolevanja od težih formi bolesti imaju: deca mlađa od 5 godina, trudnice, osobe sa hroničnim bolestima i posebno osobe starije od 65 godina (38). Kod zdravih starih (65 godina starosti i starijih) osoba, za razliku od zdravih odraslih, sezonska infekcija virusom gripe može da predstavlja ozbiljan zdravstveni problem, koji se ogleda u povećanom riziku obolevanja od teških oblika gripe, ali i većim rizikom da se ova infektivna bolest završi letalno (22). Većina smrtnih ishoda (preko 90 %) koji su direktno ili indirektno povezani sa infekcijom ovim virusom u razvijenim zemljama beleži se u populaciji starih. Takođe, rizik od smrtnog ishoda koji je povezan sa infekcijom virusom influence značajno se povećava posle 65. godine života (39).

Imajući u vidu ove podatke, vakcinacija starih (stariji od 65 godina) protiv gripe se preporučuje u mnogim državama sa ciljem smanjenja komplikacija, hospitalizacija i mortaliteta od ove bolesti (5, 8, 22). Vakcina protiv gripe se preporučuje i kao mera prevencije kardiovaskularnih bolesti budući da je pokazana efikasnost od 15-40 % u prevenciji akutnog infarkta miokarda (40). Međutim, uprkos preporukama, pokrivenost stare populacije vakcinom protiv gripe značajno varira od države do države (22).

Virus influenzae i vakcina

Virus influenzae pripada porodici *Orthomyxoviridae*. Od četiri tipa ovog virusa (A, B, C i D), od značaja za humanu patologiju su tipovi A i B, dok se tip C javlja endemski i sporadično izaziva blaže infekcije gornjeg respiratornog trakta kod dece (41-43). Influenca virusi A i B su slične strukture. Virion se sastoji od nukleokapsida koji je izgrađen od nukleoproteina (NP) i oko koga se nalazi omotač poreklom od citoplazmatske membrane ćelije. Sa unutrašnje strane omotača nalazi se virusni specifični protein – matriksni (M) protein. Od M proteina polaze spoljni glikoproteinski izdanci – hemaglutinin (HA) i neuraminidaza (NA). Hemaglutinin je glavni antigen virusa i omogućava njegovo vezivanje za receptor na ćeliji domaćina i infekciju. Varijabilnost ovog antiga obezbeđuje kontinuiranu evoluciju virusa i nastanak epidemija i pandemija (41, 44). Antitela domaćina uperena protiv HA neutrališu njegovu aktivnost i sprečavaju infekciju (43). Neuraminidaza ima ulogu na

kraju replikacije virusa i olakšava oslobađanje virusa iz ćelije. Antitela uperena protiv ovog antigena ne sprečavaju infekciju već ograničavaju širenje virusa (44).

Odlika ovog virusa je da neprekidno menja svoje antigenske karakteristike što mu omogućava da izbegne imunski odgovor domaćina. Glavni mehanizmi izmene antigenskih svojstava su antigensko skretanje (antigenski „drift”) i antigenske izmene (antigenski „shift”) (45). Antigensko skretanje predstavljaju manje antigenske promene nastale usled tačkastih mutacija u genomu virusa tokom njegove replikacije i često se javljaju. One dovode do promene aminokiselina u strukturi proteina i odgovorne su za pojavu novih sojeva i epidemisko širenje virusa (45, 46). Značajnije promene u genomu virusa, koje nisu tako česte, označene su kao antigenske izmene. One mogu nastati usled rekombinacije genetskog materijala između dva ili više virusa u inficiranim ćelijama ptica ili svinja, kada virus stiče antigenski potpuno novi HA (44, 47). Najznačajnije promene nastaju rekombinacijom humanih i životinjskih (najčešće ptičjih) virusa gripa i mogu dovesti do pojave novog i izuzetno virulentnog soja virusa i nastanka pandemija (43, 48). Ovakve antigenske izmene su karakteristične za tip A virusa (43, 44).

Virus influenzae tipa A može se podeliti na subtipove na osnovu varijacija u HA i NA antigenima (44). Do danas je poznato 18 subtipova HA i 11 subtipova NA (49). Trenutno cirkulišu subtipovi virusa influence A/H1N1 i A/H3N2. Influenca B virusi se dele na linije i trenutno su u cirkulaciji linije Yamagata i Victoria (43).

Sezonska vakcina protiv gripa bi trebalo da štiti od cirkulišućih epidemijskih sojeva virusa. Ona obično sadrži tri različita soja virusa (trovalentna vakcina): A/H1N1, A/H3N2 i B. Pošto su poslednjih nekoliko godina paralelno cirkulisala dva različita soja influenca B virusa, od nedavno se preporučuje četvorovalentna vakcina. U njen sastav ulaze, pored dva soja influenca A virusa i dva soja B virusa (50). Budući da virus konstantno menja svoja antigenska svojstva i da sezonska vakcina obezbeđuje samo sojno-specifičnu zaštitu, optimalna zaštita se postiže godišnjom vakcinacijom (47, 48). Iz tog razloga, SZO svake godine u februaru (za severnu hemisferu) i u septembru (za južnu hemisferu) daje preporuku za sastav vakcine za nastupajuću sezonomu gripa, a na osnovu predikcije koji sojevi će cirkulisati (8, 22).

U najširoj upotrebi su inaktivisane vakcine, koje postoje u tri formulacije: celovirusna, split i subjedinična vakcina (Tabela I). One se međusobno razlikuju prema strukturnoj organizaciji i virusnim komponentama koje sadrže (51). Tradicionalno, vakcine se pripremaju u oplođenim kokošijim jajima koja su inokulisana pojedinačnim sojevima virusa, mada danas postaje aktuelna i upotreba ćelijskih kultura u ove svrhe (44). Celovirusne vakcine se dobijaju prikupljanjem virusa iz alantoisne tečnosti, nakon čega slede postupci inaktivacije formalinom ili β -propiolaktonom, koncentrisanje i prečišćavanje (44, 51). U proizvodnji split vakcina, osim navedenih, postoji i dodatni tretman deterdžentom sa ciljem da se razbije lipidni omotač i oslobode virusni proteini

koji ulaze u sastav vakcine (51). Kod subjediničnih vakcina, HA i NA se dodatno prečišćavaju i uklanaju se drugi virusni proteini (43, 44, 51). Primena celovirusnih vakcina je uglavnom napuštena 70-tih godina prošlog veka, budući da su se split i subjedinične vakcine, u poređenju sa celovirusnom, pokazale podjednako imunogene, a sa manje lokalnih i sistemskih neželjenih reakcija (bol i crvenilo na mestu aplikacije, povišena telesna temperatura, bolovi u mišićima, slabost) (44). Inaktivisane vakcine obezbeđuju zaštitni imunitet indukujući humoralni imunitet, tj. produkciju neutrališućih antitela specifičnih za, prevashodno, HA antigene (44).

Tabela I Vakcine protiv gripa namenjene staroj populaciji registrovane u Evropskoj uniji

Table I Influenza vaccines licensed for elderly in the European Union

Vakcina (naziv)	Tip	Preporučeni uzrast	Proizvođač
Vaxigrip	Inaktivisana, split, trovalentna,	6 meseci i stariji	Sanofi Pasteur
Vaxigrip Tetra	Inaktivisana, split, četvorovalentna	3 godine i stariji	Sanofi Pasteur
Intanza 15 µg	Inaktivisana, split, trovalentna	60 godina i stariji	Sanofi Pasteur
Fluarix	Inaktivisana, split, trovalentna	6 meseci i stariji	GlaxoSmithKline
Fluarix Tetra	Inaktivisana, split, četvorovalentna	3 godine i stariji	GlaxoSmithKline
Fluad	Inaktivisana, subjedinična, adjuvantna (MF59) trovalentna	65 godina i stariji	Novartis
Optaflu	Inaktivisana, subjedinična, trovalentna (dobijena u ćelijskim kulturama sisara)	18 godina i stariji	Novartis
Agrippal	Inaktivisana, subjedinična, trovalentna	6 meseci i stariji	Novartis
Fluvirin	Inaktivisana, subjedinična, trovalentna	4 godine i stariji	Novartis
Influvac	Inaktivisana, subjedinična, trovalentna	6 meseci i stariji	Abbot BGP Products B.V.
Foclivia	Inaktivisana, subjedinična, adjuvantna (MF95), monovalentna pandemiska (H5N1)	18 godina i stariji	Seqirus

Preuzeto iz Smetana et al. Influenza vaccination in the elderly. Hum Vaccin Immunother. 2018;14(3):540-9 i modifikovano.

Živa atenuisana vakcina protiv gripa, koja se aplikuje intranasalno, kreirana je sa ciljem da imitira prirodan put infekcije i da indukuje i celularni (posredovan CD4+ i CD8+ T-limfocitima) i humoralni imunski odgovor. Indukovanje celularnog imunskog odgovora vakcinacijom je poželjno jer se on razvija, između ostalih, i na virusne antigene koji su smešteni u unutrašnjosti viriona i koji su zajednički za sve sojeve virusa. Ovakav imunitet bi obezbedio zaštitu od različitih sojeva virusa, što je značajno u slučaju pandemija i nepoklapanja sojeva sadržanih u vakcini i cirkulišućih sojeva (44). Za razliku od celularnog, humoralni odgovor je uglavnom uperen protiv površinskih HA i NA antiga koji su izuzetno varijabilni, tj. sojno-specifični (44). Živa atenuisana vakcina se pokazala efikasna u dečijem uzrastu i u toj populaciji je preporučena njena primena. Zbog rizika od imunizacije živim virusom, ova vakcina se ne preporučuje imunokompromitovanim osobama, niti osobama koje dolaze u kontakt sa njima (44). Kod odraslih i starih osoba ova vakcina nije efikasna i prepostavlja se da je to posledica postojanja antitela sintetisanih u prethodnim kontaktima sa *Virus influenzae* (4).

Efikasnost vakcine protiv gripa u populaciji starih

Efikasnost i imunogenost vakcine protiv gripa je smanjena kod starih u poređenju sa mladim zdravim osobama (52). O tome govore rezultati meta-analize koju su sproveli Goodwin i saradnici (53). Oni su obradili rezultate 31 studije koje su se odnosile na antitelni odgovor mlađih odraslih i starih osoba na inaktivisanu vakcincu protiv gripa (split, celovirusnu ili subjediničnu) i koje su spovedene u periodu 1986 - 2002. godine na području Severne Amerike, Japana, Izraela i devet evropskih zemalja (53). Rezultati ove analize su pokazali da je, generalno, kod starih (stariji od 65. godine života) smanjena efikasnost vakcine u poređenju sa mlađim odraslim osobama (<65 godine) (53). Tačnije, serokonverzija (četvorostruki porast titra specifičnih antitela) je detektovana u većem procentu mlađih odraslih nego starih osoba, i to protiv H1N1 (60 % kod mlađih odraslih vs 42 % kod starih), H3N2 (62 % kod mlađih odraslih vs 51 % kod starih) i B (58 % kod mlađih odraslih vs 35% kod starih) virusa. Isti trend je pokazan i kada je u pitanju bila seroprotekcija (titar specifičnih IgG antitela u testu inhibicije hemaglutinacije veći od 1:40 nakon vakcinacije) i iznosio je: za antitela specifična za H1N1 virus – 83 % kod mlađih odraslih vs 69 % starih, za H3N2 virus – 84 % kod mlađih odraslih vs 74 % starih i za B virus – 78 % kod mlađih odraslih vs 67 % starih (53). Pokazano je da su mlađi odrasli, u odnosu na stare, imali 3-4 puta bolji odgovor na H1N1 i B soj i 2 puta bolji odgovor na H3N2 soj virusa gripa (53). Odgovor nije zavisio od tipa vakcine. Vakcina je kod 70 % - 90 % mlađih odraslih bila efikasna u sprečavanju gripa koji je serološki potvrđen, dok je taj procenat kod starih osoba bio niži i iznosio je od oko 17% - 53% (53). Takođe, budući da populaciju starih odlikuje heterogeno zdravstveno stanje, ispitivana je korelacija između zdravstvenog stanja i nivoa zaštitnih antitela. Pokazano je da su zdrave stare osobe imale veći titar specifičnih antitela u odnosu na stare osobe sa hroničnim bolestima (54).

Trajanje zaštite nakon vakcinacije kod starih nije sasvim poznato. Naime, postoji bojazan da se imunitet kod starih osoba brže gubi nego kod mlađih i da se ne održava tokom cele sezone gripa (22, 48). Meta-analiza koju su uradili Skowronski i saradnici obuhvatila je 8 studija koje su ispitivale nivo seroprotekcijske zaštite kod osoba starih 65 godina i više (55). Nađeno je da se adekvatan nivo seroprotekcijske zaštite održavao četiri meseca i duže, za H3N2 soj u svih 8 studija i za H1N1 i B sojeve u 5 od 7 studija (55).

Važno je i napomenuti da na efikasnost vakcine protiv gripa utiču, pored njene imunogenosti i poklapanje sojeva sadržanih u vakcini i cirkulišućih sojeva (43). Kao jedan od primera nepoklapanja može se navesti podatak da se u periodu od 11 godina (2000 - 2011. godine) sojevi influenca B virusa u vakcini i cirkulišući sojevi nisu poklapali u 6 sezona gripa (56).

Imajući u vidu prethodno rečeno, intenzivno se razvijaju strategije sa ciljem optimizacije efikasnosti vakcina za stare osobe. Predložene strategije podrazumevaju primenu adjuvansa, povećanje doze antigaena i promenu puta primene vakcine (8, 10).

Strategije za povećanje efikasnosti vakcina za stare

Upotreba adjuvansa

Upotreba adjuvansa u vakcinama jeste dobro poznata metoda za povećanje njihove imunogenosti. Soli aluminijuma, najpoznatiji i najčešće korišćeni adjuvansi u humanim vakcinama, u vakcinama protiv gripa su pokazale varijabilan efekat i potvrđile potrebu za adjuvansom koji bi omogućio da se kompenzuje smanjenje imunskog odgovora kod starih (10).

Adjuvansi tipa emulzija ulje u vodi testirani su u različitim vakcinama, između ostalog i u vakcini protiv gripa. Reprezentativni predstavnik ove grupe je MF59. On je prvi adjuvans primenjen u troivalentnoj inaktivisanoj vakcini protiv gripa (6) i sastavljen je od skvalena, polisorbata 80 i sorbitan-trioleata (43). Na mišijem modelu je pokazano da MF59 povećava ekspresiju gena koji su uključeni u pokretanje inflamatornog odgovora i odgovora ćelija urođenog imuniteta, ali i gena koji su odgovorni za njihovu kontrolu (supresiju) (60). Ovakvi rezultati upućuju na to da ovaj adjuvans, ne samo da je potentan u pokretanju inflamatornog odgovora, već i da je uspešan u kontroli njegovog razvoja i okončanja (6). Ovo je značajno radi postizanja optimalne efikasnosti ovog odgovora i izbegavanja rizika od razvoja patološkog inflamatornog odgovora (6). Kod starih osoba, vakcina protiv gripa sa MF59 je pokazala dobru imunogenost i dobar bezbednosni profil (58-60). Ona je imala i veći zaštitni efekat u ovoj populaciji u odnosu na vakcincu bez adjuvansa. Tačnije, kod starih osoba koje su primile ovakvu vakcincu rizik od hospitalizacije je bio smanjen za 25-50 % u odnosu na one koji su primili neadjuvantnu vakcincu (61, 62). Adjuvantna vakcina je indukovala stvaranje za oko četiri puta višeg nivoa zaštitnih antitela, kao i unakrsnu reaktivnost protiv heterologih sojeva virusa gripa u poređenju sa split ili virozomalnom vakcincu kod

starih (63, 64). Adjuvanta, subjedinična vakcina protiv gripe koja sadrži MF59 (Fluad®) odobrena je za upotrebu za stare u Evropi 1997. godine.

Drugi adjuvans iz grupe emulzija ulje u vodi, AS03, sastavljen je od skvalena, polisorbata 80 i α -tokoferola. Ovaj adjuvans je razvijen sa ciljem da se primeni u prepandemijskoj vakcini protiv H5N1 virusa influence i pokazalo se da ovakva vakcina indukuje snažan imunski odgovor protiv homologog i heterologog soja virusa kod odraslih nakon primene dve doze vakcine (8). AS03 je korišćen i u pandemijskoj vakcini protiv H1N1 (pdm2009), koja je registrovana i korišćena tokom pandemije 2009 i 2010. godine (9). Pokazano je da ovakva vakcina indukuje veći titar specifičnih antitela i nivo seroprotekcije kod odraslih (uključujući i starije od 65 godina) u poređenju sa inaktivisanom neadjuvantnom celovirusnom vakcinom. Ovakav efekat je postignut sa dvostruko manjom količinom HA u adjuvantnoj u odnosu na neadjuvantnu vakciju (65). Takođe, studija koja je obuhvatila više od 40 000 osoba starijih od 65 godina je pokazala da sezonska inaktivisana vakcina sa AS03 ima bolji zaštitni efekat u odnosu na neadjuvantnu vakciju (66).

U adjuvanse se ubrajaju i virozomi - sistemi za isporuku antígena koji imitiraju virusnu česticu. Oni se sastoje od rekonstituisanog omotača virusa sa HA i NA antigenima, ali bez jezgra virusa i genetičkog materijala (9, 43). Virozomi se već dve decenije upotrebljavaju u vakcinama protiv gripe i u kliničkim studijama je pokazano da imaju nešto veću imunogenost u odnosu na standardnu vakciju protiv gripe (9, 67). Inflexa V® trovalentna vakcina sadrži u svojoj formulaciji virozome.

Agonisti TLR se razmatraju kao potencijalni adjuvansi za različite vakcine, profilaktičke i terapeutske (68). Njihov adjuvantni potencijal baziran je na zapažanju da aktivacija TLR dovodi do produkcije pro-inflamatornih citokina od strane antigen-prezentujućih ćelija (69) i do pospešivanja reakcije germinativnog centra i produkcije antitela (70). Agonisti TLR koji indukuju produkciju pro-inflamatornih citokina i hemokina i interferona tipa I, te se smatraju kandidatima za adjuvanse u antivirusnim vakcinama su: lipopeptidi Pam2Cys and Pam3Cys (agonisti TLR2), analog dvolančane RNK poly:IC (agonist TLR3), monofosforil lipid A i glukopiranozil lipid A (agonisti TLR4), flagelin (agonist TLR5), imikvimod (agonist TLR7/8) i CpG oligodeoksinukleotidi (agonist TLR9) (6, 9). Dosadašnja ispitivanja upotrebe agonista TLR kao adjuvanasa u vakcinama za staru populaciju, sprovedena na mišjem modelu, dala su ohrabrujuće rezultate. Međutim, potrebno je pokazati njihovu efikasnost u humanoj populaciji (6).

Interesantno je da je imikvimod, korišćen, između ostalog, i u terapiji bazocelularnog karcinoma, topikalno primenjen pre vakcinacije povećao imunogenost efikasnost intradermalno aplikovane vakcine protiv gripe kod starih osoba (71).

Takođe, emulzije ulje u vodi su se pokazale kao dobri nosači za sintetski agonist TLR4 (E6020) (72), što ukazuje da bi kombinacija ova dva načina pojačanja imunskog

odgovora kod starih mogla da dâ još bolje rezultate nego pojedinačna primena svakog od njih (10).

Povećanje doze antiga

Budući da tokom starenja imunskog sistema dolazi i do smanjenja efikasnosti prezentacije antiga i poremećaja pri formiranju imunološke sinapse, jedna od strategija povećanja efikasnosti vakcine protiv gripe je i povećanje doze antiga (6, 10). Ovakav pristup je testiran i pokazano je da četiri puta veća doza antiga ($60 \mu\text{g}$ HA) može da poveća imunski odgovor kod starih, ali da on ipak nije na nivou koji indukuje dozu od $15 \mu\text{g}$ HA (standardna doza) kod mladih (73, 74).

Promena puta unosa antiga

Put unosa antiga ima značajnu ulogu u uspostavljanju imuniteta. Optimizacija načina primene vakcine kod starih jeste jedan od načina da se poveća imunogenost vakcine (6). Intradermalna aplikacija vakcine protiv gripe za staru populaciju, za razliku od standardne intramuskularne aplikacije, čini se atraktivnom jer favorizuje preuzimanje i prezentaciju antiga od strane antigen-prezentujućih ćelija koje su brojne u koži (DĆ, Langerhanske ćelije, makrofagi). Ono što bi moglo da predstavlja problem kada je u pitanju efikasnost ovako primenjene vakcine jesu promene koje nastaju u koži tokom starenja npr. promenjena fiziologija kože, vaskularizacija, smanjen broj i sa starošću promenjena funkcija antigen-prezentujućih ćelija (6). Rezultati ispitivanja intradermalnog puta davanja vakcine su oprečni. U studijama koje su obuhvatile osobe starije od 60 godina, intradermalni put davanja se pokazao superionijim od klasičnog, intramuskularnog (75, 76). Kod mladih odraslih osoba put primene vakcine nije uticao na njenu imunogenost (77).

Intranazalna primena žive atenuisane vakcine protiv gripe, kao što je ranije rečeno, nije se pokazala efikasnom kod starih.

Vektorske vakcine

Inaktivisane vakcine protiv gripe indukuju humoralni imunski odgovor posredovan CD4+ T-limfocitima, ali ne i odgovor posredovan citotoksičnim CD8+ T-limfocitima koji se ostvaruje samo prirodnom infekcijom (22). U tom kontekstu, s obzirom da se imunski odgovor protiv virusa posredovan CD8+ T-limfocitima ne menja tokom starenja (78), razmišlja se o razvoju vektorskih vakcina (*engl. vector-based vaccines*) koje bi stimulisale virus-specifične CD4+ i CD8+ T-limfocite (6, 22)*. Primer jedne takve vakcine je vakcina gde je korišćen modifikovani *Vaccinia virus Ankara* kao vektor za visoko konzervirani NP i M1 protein influenca virusa (79). Budući da NP sadrži imunodominantne epitope i za CD4+ i za CD8+ T-limfocite (80), jasno je da ovakva vakcina može da indukuje nastanak i specifičnih antitela i dobar T-limfocitni odgovor (6). U poređenju sa inaktivisanom vakcinom, prednost ove vakcine je upravo pokretanje imunskog odgovora koji je posredovan CD8+ T-limfocitima i koji je

usmeren prema antigenima zajedničkim za sve sojeve virusa gripe, što bi isključilo potrebu za sezonskom vakcinom (6). Ova vakcina se pokazala imunogenom, budući da indukuje nastanak specifičnih CD8+ T-limfocita i kod mlađih, ali i kod ljudi starosti 50 – 85 godina (81). Međutim, sposobnost da indukuje produkciju specifičnih antitela nije određivana u ovoj studiji, što je i njen veliki nedostatak.

*Napomena: Videti rad Arsenović-Ranin iz ovog broja časopisa.

Vakcina protiv *Streptococcus pneumoniae*

Streptococcus pneumoniae je čest izazivač pneumonije, maningitisa i bakterijemije kod starih osoba, te se savetuje prevencija ovih stanja vakcinacijom (5, 8). Dugi niz godina je u upotrebi 23-valentna polisaharidna vakcina. Podaci o njenoj efikasnosti kod starih osoba su kontradiktorni (5). Drugi tip vakcine koja je dizajnirana za primenu kod dece, ali se primenjuje i kod starih osoba je 13-valentna konjugovana vakcina, o čijoj efikasnosti kod starih osoba takođe nema dovoljno podataka, mada se veruje da je imunogenija (5).

Preporuke za upotrebu ovih vakcina se razlikuju od države do države. Neke države preporučuju vakcinaciju starog stanovništva samo polisaharidnom vakcinom (Nemačka, Mađarska, Kipar, Irska, Norveška, Švedska i dr.), neke samo konjugovanom (Grčka, Malta, Poljska, Slovačka), dok je preporuka u nekim zemljama (Austrija, Belgija, Danska, Finska, SAD i dr.) da se prvo primeni konjugovana, a zatim, nakon godinu dana i polisaharidna vakcina (8). U Srbiji je preporučena 13-valentna konjugovana polisahardina vakcina za vakcinaciju osoba starijih od 65 godina (82).

Vakcina protiv *Varicella-zoster virus*

Gotovo sve odrasle osobe su latentno inficirane virusom *Varicella-zoster* (VZV). Primarna infekcija se najčešće događa u detinjstvu i manifestuje se varičelom (ovčje boginje), nakon čega se uspostavlja latencija virusa. Pretpostavlja se da se delimična reaktivacija virusa događa često tokom života, ali da je obično dobro kontrolisana od strane T-limfocita specifičnih za virus. Međutim, usled gubitka kontrole od strane imunskih ćelija, reaktivacija virusa može voditi razvoju herpes zostera (83). Incidencija herpes zostera se povećava sa godinama starosti i procenjuje se da osobe starije od 85 godina čine do 50 % obolelih (84, 85). Epizode herpes zostera mogu kod nekih pacijenata da budu praćene post-herpetičnom neuralgijom (PHN) koju karakterišu dugotrajan, jak bol nakon povlačenja kožnih promena. Incidencija ove komplikacije je veća kod starih pacijenata i javlja se kod oko trećine obolelih (86). Vakcina protiv VZV je atenuisana živa vakcina. Ona je registrovana za primenu kod starih osoba 2006. godine i preporučena je u nekim državama (Austrija, Češka, Francuska, Grčka, Italija, Ujedinjeno Kraljevstvo i SAD) (8). Ova vakcina indukuje i T-ćelijski i antitelni odgovor (87). Rezultati dobijeni u studiji koja je obuhvatila osobe starije od 60 godina pokazuju da ona smanjuje incidenciju herpes zostera za 51,3 % i incidenciju PHN za 66,5 % u

odnosu na placebo (88). Zaštitni efekat vakcine je veoma nizak kod jako starih osoba i zaštita se gubi sa vremenom (89, 90), te se preporučuje ponavljanje vakcinacija (8).

Nedavno je u SAD-u i Kanadi registrovana vakcina koja sadrži rekombinantni glikoprotein VZV gE i adjuvans AS01b. Ova vakcina je pokazala dobru efikasnost u svim uzrasnim grupama, pa i kod osoba starijih od 70 i 80 godina (91). Prednost ove vakcine je što se može primenjivati i kod imunokompromitovanih osoba koje su pod velikim rizikom da razviju klinički manifestnu bolest (9).

Zaključak

Stare osobe su pod velikim rizikom obolenja od infektivnih bolesti, stoga vakcinacija predstavlja važnu preventivnu meru i meru koja obezbeđuje zdravo starenje. Međutim, svest o značaju vakcinacije starih osoba nije dovoljno razvijena. O tome govori i podatak da cilj koji je postavila SZO, da do 2014/2015. godine 75 % starog stanovništva (starijeg od 65 godina) bude vakcinisano protiv gripe, u mnogim zemljama nije ostvaren. Kada je reč o Srbiji, nedostaju podaci o pokrivenosti stare populacije vakcinom protiv gripe, mada je njihov udeo u ukupnom broju vakcinisanih u 2018. godini bio 66% (92). Takođe, nedostaju slični podaci koji se odnose na pokrivenost stare populacije vakcinom protiv *S. pneumoniae* i VZV. Takođe, vakcine protiv drugih izazivača infekcija kod starih (*Clostridium difficile*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, respiratori sincicijalni virus) bi mogле da unaprede zdravstveno stanje ove populacije i neke od njih se nalaze u fazama pre-kliničkih i kliničkih ispitivanja.

Značajan problem kada je u pitanju vakcinacija starih vakcinama koje se primenjuju kod dece i mlađih je njihova manja efikasnost zbog promena koje pogadaju imunski sistem tokom starenja. Sa tim u vezi, intenzivno se razvijaju strategije sa ciljem povećanja efikasnosti vakcina za stare (upotreba adjuvansa, povećanje doze antigena, promena puta unošenja antigena, razvoj vektorskih vakcina). Osim toga, neophodna su dodatna istraživanja koja bi rasvetlila još uvek nedovoljno proučene mehanizme koji su u osnovi procesa starenja imunskog sistema, a koji bi bili iskorišćeni sa ciljem da se koriguju ovakve promene (upotreba blokatora inhibitornih receptora na T-limfocitima, istovremena upotreba više različitih adjuvanasa radi sinergističkog delovanja na TLR, modulacija aktivacije intracelularnih signalnih puteva, pospešivanje timusne funkcije i drugi) (5, 28, 93).

Zahvalnica

Ovaj rad je finansijski podržan sredstvima Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije (projekat broj 175050).

Literatura

1. www.un.org/en/development/desa/population/publications/pdf/ageing/WPA2017_Highlights.pdf (26.9.2019.)
2. Gavazzi G, Krause KH. Ageing and infection. *Lancet Infect Dis.* 2002;2(11):659-66.
3. Esme M, Topeli A, Yavuz BB, Akova M. Infections in the elderly critically-ill patients. *Front Med (Lausanne).* 2019;6:118. doi: 10.3389/fmed.2019.00118.
4. Amanna IJ. Balancing the efficacy and safety of vaccines in the elderly. *Open Longev Sci.* 2012;6(2012):64-72.
5. Prelog M. Differential approaches for vaccination from childhood to old age. *Gerontology.* 2013;59(3):230-39.
6. Boraschi D, Italiani P. Immunosenescence and vaccine failure in the elderly: strategies for improving response. *Immunol Lett.* 2014;162(1 Pt B):346-53.
7. Nikolich-Žugich J. The twilight of immunity: emerging concepts in aging of the immune system. *Nat Immunol.* 2018;19(1):10-9.
8. Weinberger B. Vaccines for the elderly: current use and future challenges. *Immun Ageing.* 2018;15:3.
9. Weinberger B. Adjuvant strategies to improve vaccination of the elderly population. *Curr Opin Pharmacol.* 2018;41:34-41.
10. Lefebvre JS, Haynes L. Vaccine strategies to enhance immune responses in the aged. *Curr Opin Immunol.* 2013;25(4):523-28.
11. Burkle A, Caselli G, Franceschi C, Mariani E, Sansoni P, Santoni A, et al. Pathophysiology of ageing, longevity and age related diseases. *Immun Ageing.* 2007;4:4.
12. Hazeldine J, Lord JM. Innate immunosenescence: underlying mechanisms and clinical relevance. *Biogerontology.* 2015;16:187-201.
13. Montgomery RR, Shaw AC. Paradoxical changes in innate immunity in aging: recent progress and new directions. *J Leukoc Biol.* 2015;9:937-43.
14. Solana R, Tarazona R, Gayoso I, Lesur O, Dupuis G, Fulop T. Innate immunosenescence: effect of aging on cells and receptors of the innate immune system in humans. *Semin Immunol.* 2012;24(5):331-41.
15. Stojic-Vukanic Z, Bufan B, Arsenovic-Ranin N, Kosec D, Pilipovic I, Perisic Nanut M et al. Aging affects AO rat splenic conventional dendritic cell subset composition, cytokine synthesis and T-helper polarizing capacity. *Biogerontology.* 2013;14(4):443-59.
16. Bufan B, Stojic-Vukanic Z, Djikic J, Kosec D, Pilipovic I, Nacka-Aleksic M et al. Aging impairs endocytic capacity of splenic dendritic cells from Dark Agouti rats and alters their response to Tlr4 stimulation. *Acta Veterinaria-Beograd.* 2015;65(1):30-55.
17. Cumberbatch M, Dearman RJ, Kimber I. Influence of ageing on Langerhans cell migration in mice: identification of a putative deficiency of epidermal interleukin-1beta. *Immunology.* 2002;105:466-77.

18. Desai A, Grolleau-Julius A, Yung R. Leukocyte function in the aging immune system. *J Leukoc Biol.* 2010;87:1001-9.
19. Chougnet CA, Thacker RI, Shehata HM, Hennies CM, Lehn MA, Lages CS et al. Loss of phagocytic and antigen cross-presenting capacity in aging dendritic cells is associated with mitochondrial dysfunction. *J Immunol.* 2015;195(6):2624-32.
20. Metcalf TU, Cubas RA, Ghneim K, Cartwright MJ, Grevenynge JV, Richner JM et al. Global analyses revealed age-related alterations in innate immune responses after stimulation of pathogen recognition receptors. *Aging Cell.* 2015;14(3):421-32.
21. van Duin D, Mohanty S, Thomas V, Ginter S, Montgomery RR, Fikrig E et al. Age-associated defect in human TLR-1/2 function. *J Immunol.* 2007;178(2):970-5.
22. Smetana J, Chlibek R, Shaw J, Splino M, Prymula R. Influenza vaccination in the elderly. *Hum Vaccin Immunother.* 2018;14(3):540-9.
23. Liu WM, van der Zeijst BA, Boog CJ, Soethout EC. Aging and impaired immunity to influenza viruses: implications for vaccine development. *Hum Vaccin.* 2011;7 Suppl:94-8.
24. Chinn IK, Blackburn CC, Manley NR, Sempowski GD. Changes in primary lymphoid organs with aging. *Semin Immunol.* 2012;24(5):309-20. doi: 10.1016/j.smim.2012.04.005.
25. Kline GH, Hayden TA, Klinman, NR. B cell maintenance in aged mice reflects both increased B cell longevity and decreased B cell generation. *J Immunol.* 1999;162:3342-9.
26. Pawelec G. Age and immunity: What is "immunosenescence"? *Exp Gerontol.* 2018;105:4-9. doi: 10.1016/j.exger.2017.10.024
27. Schulz AR, Malzer JN, Domingo C, Jurchott K, Grutzkau A, Babel N et al. Low thymic activity and dendritic cell numbers are associated with the immune response to primary viral infection in elderly humans. *J Immunol.* 2015;195(10):4699-711.
28. Crooke SN, Ovsyannikova IG, Poland GA, Kennedy RB. Immunosenescence and human vaccine immune responses. *Immun Ageing.* 2019;16:25. doi: 10.1186/s12979-019-0164-9
29. Frasca D, Diaz A, Romero M, Blomberg BB. The generation of memory B cells is maintained, but the antibody response is not, in the elderly after repeated influenza immunizations. *Vaccine.* 2016;34:2834-40.
30. Frasca D, Diaz A, Romero M, Landin AM, Blomberg BB. Age effects on B cells and humoral immunity in humans. *Ageing Res Rev.* 2011;10(3):330-5.
31. Arsenović-Ranin N, Petrović R, Živković I, Bufan B, Stoiljković V, Leposavić G. Influence of aging on germinal centre reaction and antibody response to inactivated influenza virus antigens in mice: sex-based differences. *Biogerontology.* 2019;20(4):475-96.
32. Richner JM, Gmyrek GB, Govero J, Tu Y, van der Windt GJ, Metcalf TU et al. Age-dependent cell trafficking defects in draining lymph nodes impair adaptive immunity and control of West Nile virus infection. *PLoS Pathog.* 2015;11(7):e1005027. doi: 10.1371/journal.ppat.1005027
33. Franceschi C, Bonafe M, Valensin S, Olivieri F, Luca MD, Ottaviani E, Benedictis GD. Inflammaging: an evolutionary perspective on immunosenescence, *Ann NY Acad Sci.* 2000;908:244-54.
34. Oishi Y, Manabe I. Macrophages in age-related chronic inflammatory diseases. *NPJ Aging Mech Dis.* 2016;2:16018. doi: 10.1038/npjAMD.2016.18

35. Ciabattini A, Nardini C, Santoro F, Garagnani P, Franceschi C, Medaglini D. Vaccination in the elderly: the challenge of immune changes with aging. *Semin Immunol.* 2018;40:83-94. doi: 10.1016/j.smim.2018.10.010.
36. McElhaney JE, Kuchel GA, Zhou X, Swain SL, Haynes L. T-cell immunity to influenza in older adults: a pathophysiological framework for development of more effective vaccines. *Front Immunol.* 2016;7:41.
37. Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, Doherty PC et al. Influenza. *Nat Rev Dis Primers.* 2018;4:3.
38. Rasmussen SA, Jamieson DJ, Uyeki TM. Effects of influenza on pregnant women and infants. *Am J Obstet Gynecol.* 2012;207(3 Suppl):S3-8.
39. Rothberg MB, Haessler SD, Brown RB. Complications of viral influenza. *Am J Med.* 2008;121:258-64.
40. MacIntyre CR, Heywood AE, Kovoor P, Ridda I, Seale H, Tan T et al. Ischaemic heart disease, influenza and influenza vaccination: a prospective case control study. *Heart.* 2013;99:1843-8.
41. Taubenberger JK, Morens DM. The pathology of influenza virus infections. *Annu Rev Pathol.* 2008;3:499-522.
42. Moriuchi H, Katsushima N, Nishimura H, Nakamura K, Numazaki Y. Community-acquired influenza C virus infection in children. *J Pediatr.* 1991;118:235-8.
43. Tregoning JS, Russell RF, Kinnear E. Adjuvanted influenza vaccines. *Hum Vaccin Immunother.* 2018;14(3):550-64.
44. Wong SS, Webby RJ. Traditional and new influenza vaccines. *Clin Microbiol Rev.* 2013;26(3):476-92.
45. Kim H, Webster RG, Webby RJ. Influenza virus: dealing with a drifting and shifting pathogen. *Viral Immunol.* 2018;31(2):174-83.
46. Chen R, Holmes EC. Avian influenza virus exhibits rapid evolutionary dynamics. *Mol Biol Evol.* 2006;23:2336-41.
47. Dhakal S, Klein SL. Host factors impact vaccine efficacy: implications for seasonal and universal influenza vaccine programs. *J Virol.* 2019;93(21). pii: JVI.00797-19. doi: 10.1128/JVI.00797-19
48. Wilhelm M. Influenza in older patients: a call to action and recent updates for vaccinations. *Am J Manag Care.* 2018;24(2 Suppl):S15-24.
49. <https://www.cdc.gov/flu/avianflu/influenza-a-virus-subtypes.htm> (26.9.2019.)
50. Shaw MW, Xu X, Li Y, Normand S, Ueki RT, Kunimoto GY et al. Reappearance and global spread of variants of influenza B/Victoria/2/87 lineage viruses in the 2000-2001 and 2001-2002 seasons. *Virology.* 2002;303:1-8.
51. Soema PC, Kompier R, Amorij JP, Kersten GF. Current and next generation influenza vaccines: Formulation and production strategies. *Eur J Pharm Biopharm.* 2015;94:251-63.
52. Petrović R, Bufan B, Arsenović-Ranin N, Živković I, Minić R, Radojević K, Leposavić G. Mouse strain and sex as determinants of immune response to trivalent influenza vaccine. *Life Sci.* 2018;207:117-26. doi: 10.1016/j.lfs.2018.05.056

53. Živković I, Petrović R, Arsenović-Ranin N, Petrušić V, Minić R, Bufan B, Popović O, Leposavić G. Sex bias in mouse humoral immune response to influenza vaccine depends on the vaccine type. *Biologicals*. 2018;52:18-24. doi: 10.1016/j.biologicals.2018.01.007
54. Živković I, Bufan B, Petrušić V, Minić R, Arsenović-Ranin N, Petrović R, Leposavić G. Sexual diergism in antibody response to whole virus trivalent inactivated influenza vaccine in outbred mice. *Vaccine*. 2015;33(42):5546-52. doi: 10.1016/j.vaccine.2015.09.006
55. Lambert ND, Ovsyannikova IG, Pankratz VS, Jacobson RM, Poland GA. Understanding the immune response to seasonal influenza vaccination in older adults: A systems biology approach. *Expert Rev Vaccines*. 2012;11(8):985-94.
56. Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: a quantitative review. *Vaccine*. 2006;24(8):1159-69.
57. Mysliwska J, Trzonkowski P, Szmith E, Brydak LB, Machala M, Mysliwski A. Immunomodulating effect of influenza vaccination in the elderly differing in health status. *Exp Gerontol*. 2004;39(10):1447-58.
58. Skowronski DM, Tweed SA, De Serres G. Rapid decline of influenza vaccine-induced antibody in the elderly: is it real, or is it relevant? *J Infect Dis*. 2008;197(4):490-502.
59. Tricco AC, Chit A, Soobiah C, Hallett D, Meier G, Chen MH, et al. Comparing influenza vaccine efficacy against mismatched and matched strains: a systematic review and meta-analysis. *BMC Med*. 2013;11:153.
60. Mosca F, Tritto E, Muzzi A, Monaci E, Bagnoli F, Iavarone C, et al. Molecular and cellular signatures of human vaccine adjuvants. *Proc Natl Acad Sci USA*. 2008;105:10501-6.
61. Cheong HJ, Song JY, Heo JY, Noh JY, Choi WS, Park DW et al. Immunogenicity and safety of the influenza A/H1N1 2009 inactivated split-virus vaccine in young and older adults: MF59-adjuvanted vaccine versus nonadjuvanted vaccine. *Clin Vaccine Immunol*. 2011;18:1358-64.
62. Bihari I, Panczel G, Kovacs J, Beygo J, Fragapane E. Assessment of antigen-specific and crossreactive antibody responses to an MF59-adjuvanted A/H5N1 prepandemic influenza vaccine in adult and elderly subjects. *Clin Vaccine Immunol*. 2012;19:1943-8.
63. Ruf BR, Colberg K, Frick M, Preusche A. Open, randomized study to compare the immunogenicity and reactogenicity of an influenza split vaccine with an MF59-adjuvanted subunit vaccine and a virosome-based subunit vaccine in elderly. *Infection*. 2004;32:191-8.
64. Mannino S, Villa M, Apolone G, Weiss NS, Groth N, Aquino I et al. Effectiveness of adjuvanted influenza vaccination in elderly subjects in northern Italy. *Am J Epidemiol*. 2012;176:527-33.
65. Puig-Barbera J, ez-Domingo J, Perez HS, Belenguer VA, Gonzalez VD. Effectiveness of the MF59-adjuvanted influenza vaccine in preventing emergency admissions for pneumonia in the elderly over 64 years of age. *Vaccine*. 2004; 23:283-9.
66. Baldo V, Baldovin T, Pellegrini M, Angioletti G, Majori S, Floreani A et al. Immunogenicity of three different influenza vaccines against homologous and heterologous strains in nursing home elderly residents. *Clin Dev Immunol*. 2010;2010:517198.

67. Sindoni D, La FV, Squeri R, Cannavo G, Bacilieri S, Panatto D et al. Comparison between a conventional subunit vaccine and the MF59-adjuvanted subunit influenza vaccine in the elderly: an evaluation of the safety, tolerability and immunogenicity. *J Prev Med Hyg.* 2009;50:121-6.
68. Nicholson KG, Abrams KR, Batham S, Clark TW, Hoschler K, Lim WS et al. Immunogenicity and safety of a two-dose schedule of whole-virion and AS03A-adjuvanted 2009 influenza A (H1N1) vaccines: a randomised, multicentre, age-stratified, head-to-head trial. *Lancet Infect Dis.* 2011;11:91-101.
69. McElhaney JE, Beran J, Devaster JM, Esen M, Launay O, Leroux-Roels G et al. AS03-adjuvanted versus non-adjuvanted inactivated trivalent influenza vaccine against seasonal influenza in elderly people: a phase 3 randomised trial. *Lancet Infect Dis.* 2013;13(6):485-96.
70. Herzog C, Hartmann K, Kunzi V, Kursteiner O, Mischler R, Lazar H et al. Eleven years of inflexal V-a virosomal adjuvanted influenza vaccine. *Vaccine.* 2009;27:4381-7.
71. Duthie MS, Windish HP, Fox CB, Reed SG. Use of defined TLR ligands as adjuvants within human vaccines. *Immunol Rev.* 2011;239:178-96.
72. Huang H, Ostroff GR, Lee CK, Wang JP, Specht CA, Levitz SM. Distinct patterns of dendritic cell cytokine release stimulated by fungal beta-glucans and Toll-like receptor agonists. *Infect Immun.* 2009;77:1774-81.
73. DeFranco AL, Rookhuizen DC, Hou B. Contribution of Toll-like receptor signaling to germinal center antibody responses. *Immunol Rev.* 2012;247:64-72.
74. Hung IF, Zhang AJ, To KK, Chan JF, Li P, Wong TL et al. Topical imiquimod before intradermal trivalent influenza vaccine for protection against heterologous non-vaccine and antigenically drifted viruses: a single-centre, double-blind, randomised, controlled phase 2b/ 3 trial. *Lancet Infect Dis.* 2016;16:209-18.
75. Baudner BC, Ronconi V, Casini D, Tortoli M, Kazzaz J, Singh M et al. MF59 emulsion is an effective delivery system for a synthetic TLR4 agonist (E6020). *Pharm Res.* 2009;26(6):1477-85.
76. DiazGranados CA, Dunning AJ, Jordanov E, Landolfi V, Denis M, Talbot HK. High-dose trivalent influenza vaccine compared to standard dose vaccine in elderly adults: safety, immunogenicity and relative efficacy during the 2009-2010 season. *Vaccine.* 2013;31(6):861-6.
77. Couch RB, Winokur P, Brady R, Belshe R, Chen WH, Cate TR et al. Safety and immunogenicity of a high dosage trivalent influenza vaccine among elderly subjects. *Vaccine.* 2007;25:7656-63.
78. Ansaldi F, Orsi A, de Florentiis D, Parodi V, Rappazzo E, Coppelli M et al. Head-to-head comparison of an intradermal and a virosome influenza vaccine inpatients over the age of 60: evaluation of immunogenicity, cross-protection, safety and tolerability. *Hum Vaccin Immunother.* 2013;9:591-8.
79. Holland D, Booy R, De LF, Eizenberg P, McDonald J, Karrasch J et al. Intradermal influenza vaccine administered using a new microinjection system produce superior immunogenicity in elderly adults: a randomized controlled trial. *J Infect Dis.* 2008;198:650-8.
80. Patel SM, Atmar RL, El Sahly HM, Guo K, Hill H, Keitel WA. Direct comparison of an inactivated subvirion influenza A virus subtype H5N1 vaccine administered by the intradermal and intramuscular routes. *J Infect Dis.* 2012;206:1069-77.

81. Lelic A, Verschoor CP, Ventresca M, Parsons R, Evelegh C, Bowdish D, et al. The poly functionality of human memory CD8+ T cells elicited by acute and chronic virus infections is not influenced by age. *PLoS Pathog.* 2012;8:e1003076.
82. Berthoud TK, Hamill M, Lillie PJ, Hwenda L, Collins KA, Ewer KJ, et al. Potent CD8+ T-cell immunogenicity in humans of a novel heterosubtypic influenza A vaccine, MVA-NP+M1. *Clin Infect Dis.* 2011;52:1–7.
83. Doucet JD, Forget MA, Grange C, Rouxel RN, Arbour N, von MV, et al. Endogenously expressed matrix protein M1 and nucleoprotein of influenza A are efficiently presented by class I and class II major histocompatibility complexes. *J Gen Virol.* 2011;92:1162–71.
84. Antrobus RD, Lillie PJ, Berthoud TK, Spencer AJ, McLaren JE, Ladell K, et al. AT cell-inducing influenza vaccine for the elderly: safety and immunogenicity of MVA-NP+M1 in adults aged over 50 years. *PLoS One.* 2012;7:e48322.
85. <http://www.batut.org.rs/download/publikacije/SMU%20imunizacija.pdf> (18.12.2019.)
86. Oxman MN. Herpes zoster pathogenesis and cell-mediated immunity and immunosenescence. *J Am Osteopath Assoc.* 2009;109:S13–7.
87. Schmader K. Herpes zoster in older adults. *Clin Infect Dis.* 2001;32:1481–6.
88. Pinchinat S, Cebrian-Cuenca AM, Bricout H, Johnson RW. Similar herpes zoster incidence across Europe: results from a systematic literature review. *BMC Infect Dis.* 2013;13:170.
89. Mallick-Searle T, Snodgrass B, Brant JM. Postherpetic neuralgia: epidemiology, aetiology, and pain management pharmacology. *J Multidiscip Healthc.* 2016;9:447–54.
90. Levin MJ, Oxman MN, Zhang JH, Johnson GR, Stanley H, Hayward AR et al. Varicella-zoster virus-specific immune responses in elderly recipients of a herpes zoster vaccine. *J Infect Dis.* 2008;197:825–35.
91. Oxman MN, Levin MJ, Johnson GR, Schmader KE, Straus SE, Gelb LD et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. *N Engl J Med.* 2005;352:2271–84.
92. Schmader KE, Oxman MN, Levin MJ, Johnson G, Zhang JH, Betts R, et al. Persistence of the efficacy of zoster vaccine in the shingles prevention study and the short-term persistence substudy. *Clin Infect Dis.* 2012;55:1320–8.
93. Morrison VA, Johnson GR, Schmader KE, Levin MJ, Zhang JH, Looney DJ et al. Long-term persistence of zoster vaccine efficacy. *Clin Infect Dis.* 2015;60:900–9.
94. Lal H, Cunningham AL, Godeaux O, Chlibek R, Diez-Domingo J, Hwang SJ et al. Efficacy of an adjuvanted herpes zoster subunit vaccine in older adults. *N Engl J Med.* 2015;372:2087–96.
95. <http://www.batut.org.rs/download/izvestaji/Godisnji%20imunizacija%202018.pdf> (1.10.2019.)
96. Aiello A, Farzaneh F, Candore G, Caruso C, Davinelli S, Gambino CM et al. Immunosenescence and its hallmarks: how to oppose aging strategically? A review of potential options for therapeutic intervention. *Front Immunol.* 2019;10:2247. doi: 10.3389/fimmu.2019.02247

Application of prophylactic vaccines in the elderly

Biljana Bufan

University of Belgrade - Faculty of Pharmacy, Department of microbiology and immunology, Vojvode Stepe 450, 11221 Belgrade, Serbia

Corresponding author, e.mail: bbiljana@pharmacy.bg.ac.rs

Summary

Extended lifespan and increasing number of the elderly (>65 years) worldwide carries the challenges for the public health system, as this is the population particularly susceptible to infectious diseases. One way to prevent these diseases is vaccination. In order to improve the quality of life of the elderly, to reduce complications, hospitalizations and mortality, many European countries and the United States recommend the vaccination of the elderly with the influenza vaccine, vaccines against *Streptococcus pneumoniae* and *Varicella-zoster virus* (VZV), and booster vaccination against tetanus, pertussis and diphtheria. The reason for the greater susceptibility to infectious diseases, and lower efficacy of vaccines in the elderly, are age-associated changes of the immune system. In most European countries seasonal influenza vaccine is recommended for the individuals over 65 years of age. Its efficacy is lower in the elderly than in adults, so strategies are being developed to overcome these problems (including adjuvants in the formulation, increasing the antigen dose, changing the route of immunization, development of vector-based vaccines). Polysaccharide and conjugated vaccines are available against *S. pneumoniae*, but data regarding their efficacy are inconsistent. The VZV vaccine is an attenuated, live vaccine and has been shown to be effective in reducing the incidence of herpes zoster and post-herpetic neuralgia.

Raising awareness of the importance of vaccination in the elderly and the development of vaccines tailored for this population is of great importance for the preservation of their health.

Key words: the elderly, seasonal influenza vaccine,
vaccine against *Streptococcus pneumoniae*,
vaccine against *Varicella zoster virus*

Aktivna imunoterapija malignih tumora: pregled terapijskih vakcina

Brankica Filipić*, Zorica Stojić-Vukanić

Univerzitet u Beogradu - Farmaceutski fakultet, Katedra za mikrobiologiju i imunologiju, Vojvode Stepe 450, 11221 Beograd, Srbija

*Autor za korespondenciju: Brankica Filipić, e-mail: brankica.filipic@pharmacy.bg.ac.rs

Kratak sadržaj

Maligni tumor (rak, karcinom) je jedan od vodećih uzroka obolenja i smrtnosti pa se veliki naporovi ulažu u razvoj novih terapijskih pristupa. Savremena imunoterapija malignih tumora obuhvata primenu antitumorskih antitela i autologih T ćelija koje uništavaju ćelije tumora (pasivna imunoterapija) i pojačanje slabog antitumorskog imunskog odgovora domaćina (aktivna imunoterapija) vakcinacijom i primenom antitela koja blokiraju inhibitorne receptore (kontrolne tačke) T limfocita. Vakcinacija pacijenata obolenih od tumora njihovim sopstvenim tumorskim ćelijama, antigenima tih ćelija ili dendritskim ćelijama koje su inkubirane sa tumorskim antigenima stimuliše imunski sistem pacijenta da prepozna tumorske antigene i eliminiše maligne ćelije. Međutim, razvoj terapijskih tumorskih vakcina suočen je sa brojnim izazovima vezanim za njihov dizajn, u smislu optimalne kombinacije antiga, adjuvansa i nosača, kao i za način primene. Pored toga, savremena aktivna imunoterapija treba da prevaziđe nisku imunogenost tumora i imunosupresivne mehanizme mikrosredine tumora kod pacijenata sa klinički ispoljenom bolesti. U ovom radu prikazani su rezultati novijih kliničkih studija u kojima su ispitivane različite terapijske vakcine za karcinom i diskutovano je o njihovoj mogućoj primeni u kliničkoj praksi, kako samih, tako i u kombinaciji sa drugim imunoterapijama.

Ključne reči: maligni tumori, aktivna imunoterapija, tumorske vakcine

1. Uvod

Maligni tumor (rak, karcinom) je jedan od vodećih zdravstvenih problema u celom svetu i jedan od glavnih uzroka obolenja i smrtnosti kako dece tako i odraslih osoba (1). Prema podacima Svetske zdravstvene organizacije u 2018. godini 9,6 miliona ljudi je umrlo od posledica malignih tumora (www.who.int). Smatra se da je visoka stopa smrtnosti pacijenata sa malignim tumorima posledica poremećene regulacije proliferacije malignih ćelija, njihove otpornosti na programiranu ćelijsku smrt i sposobnosti da oštećuju zdravo tkivo, bilo na mestu nastanka, ili u udaljenim organima nakon metastaziranja (2). Takođe, na osnovu novih i upotpunjениh saznanja o mehanizmima imunskog odgovora na tumore sa jedne strane i terapijskog uspeha imunoterapije tumora sa druge strane, postalo je jasno da je jedna od najvažnijih osobina malignih ćelija njihova sposobnost da izbegnu uništenje posredovano imunskim mehanizmima domaćina (3, 4). Naime, sposobnost malignih ćelija da uspore ili zaustave reakcije imunskih ćelija omogućava im da se razvijaju, rastu, metastaziraju i na kraju, u nekim slučajevima, dovedu i do smrtnog ishoda (3).

Pre više od jednog veka, uočeno je prisustvo infiltrata mononuklearnih ćelija oko i unutar tumorskih lezija (5, 6). Kasnije, 50-tih godina dvadesetog veka Burnet je postavio hipotezu da neoantigeni tumora indukuju imunsku reakciju protiv tumora i uveo u imunologiju teoriju imunskog nadzora (7, 8, 9). U osnovi ove teorije je prepostavka da je fiziološka uloga stečenog imunskog sistema da spreči preterani rast maligno-transformisanih ćelija i da ih uništi pre nego postanu štetni tumori (8, 9, 10, 11). Iako je bilo dokaza i za i protiv ove teorije, ona je nesumnjivo imala veliki uticaj na dalji tok istraživanja vezanih za imunologiju tumora i posebno na ispitivanja celularnih i molekulskih mehanizama koji učestvuju u antitumorskom imunskom odgovoru (12).

1.1. Imunski odgovor protiv tumora

Glavni imunski mehanizam za uklanjanje tumora je ubijanje malignih ćelija CD8+ citotoksičnim T limfocitima (engl. cytotoxic T lymphocytes, CTL) (13). Pored toga, pokazano je da se i druge ćelije stečenog [CD4+ pomoćnički T (Th) limfociti i B limfociti] i urođenog (makrofagi i urođenoubilačke ćelije) imunskog odgovora aktivisu kod ljudi koji su oboleli od malignih tumora, međutim zaštitna uloga tih efektorskih mehanizama još uvek nije jasno utvrđena (14, 15, 16). Da bi se pokrenuo efikasan CTL odgovor potrebno je da dendritska ćelija (DĆ) preuzme tumorsku ćeliju ili tumorski antigen, preradi ih i prikaže na svojoj površini u kompleksu sa molekulama glavnog kompleksa tkivne podudarnosti (engl. major histocompatibility complex, MHC) I klase. DĆ sazreva i migrira u regionalne limfne čvorove gde prikazuje tumorske antigene naivnim CD8+ T-ćelijama. Pored toga, DĆ ispoljavaju kostimulatorne molekule koji zajedno sa aktiviranim CD4+ T limfocitima obezbeđuju signale neophodne za diferencijaciju naivnih CD8+ T ćelija u tumor specifične CTL (13). Efektorski CTL

napuštaju limfni čvor i odlaze u tkiva gde prepoznaju tumorski antigen i ubijaju maligne ćelije koje ga eksprimiraju (13, 17).

Paradoksalno, imunski sistem može i da podstiče rast tumora. Naime, odavno je primećeno da hronična inflamacija može da bude jedan od faktora za razvoj tumora u različitim tkivima, naročito onim koja su „pogođena” hroničnim inflamatornim bolestima (18, 19). Sa druge strane, sami tumori formiraju antiinflamatornu mikrosredinu koja inhibiše antitumorski imunski odgovor i stimuliše rast tumora. Naime, tumorska mikrosredina stimuliše razvoj DĆ koje indukuju nastanak regulatornih T ćelija ili antiinflamatornih Th2 ćelija (20, 21, 22). Ove T ćelije suprimišu antitumorski imunski odgovor i indukuju nastanak i nakupljanje antiinflamatornih makrofaga i supresorskih ćelija mijelodnog porekla koje mogu da blokiraju efektorske funkcije antitumorskih CTL i Th1 ćelija i/ili da sekretuju faktore neophodne za rast tumorskih ćelija i krvnih sudova tumora (20, 21, 22).

1.2. Kako tumori izbegavaju imunski odgovor

Kao što je prethodno navedeno, sposobnost tumora da izbegnu imunski odgovor domaćina je jedno od glavnih bioloških obeležja tumorskih ćelija. Glavni cilj naučnika koji se bave imunologijom tumora je da rasvetle te mehanizme sa ciljem da se razviju terapije koje će onemogućiti tumorima da izbegnu imunski odgovor i koje će povećati imunogenost tumora, kao i antitumorski imunski odgovor domaćina (21). Generalno, tumori koriste dve glavne strategije da izbegnu imunski odgovor: aktivno inhibišu antitumorski imunski odgovor ili izbegavaju prepoznavanje od strane CTL (21). Prva strategija podrazumeva da tumori angažovanjem inhibitornih receptora (tzv. kontrolne tačke imunskog odgovora) na T limfocitima, kao što su antigen 4 koji je udružen sa citotoksičnim T limfocitima (engl. cytotoxic T lymphocytes-associated protein 4, CTLA-4) i protein 1 programirane ćelijske smrti (engl. programmed cell death protein 1, PD-1), inhibišu njihovu aktivaciju (21). Tako, na primer, mnogi tumori ispoljavaju ligand za PD-1 (PD-L1) i uspešno mogu da inhibišu aktivaciju CTL specifičnih za tumor. Takođe, kontinuirano prisustvo tumora uzrokuje ponavljanu stimulaciju CTL specifičnih za tumor što za posledicu ima njihovo iscrpljivanje koje se karakteriše ispoljavanjem visokih nivoa CTLA-4 i PD-1 molekula i nemogućnošću da odgovore na aktivaciju (3, 21, 22). Takođe, neki tumori sekretuju imunosupresivne citokine, na primer faktor transformacije rasta β (engl. transforming growth factor β , TGF- β), koji inhibišu proliferaciju i efektorske funkcije T limfocita i makrofaga (21, 23). Konačno, već opisana imunosupresivna mikrosredina koju stvaraju tumori značajno doprinosi inhibiciji aktivacije T ćelija.

Kada je u pitanju druga strategija, tumori izbegavaju imunski odgovor tako što prestaju da ispoljavaju specifične tumorske antigene i/ili snižavaju/prestaju da

eksprimiraju MHC molekule I klase na svojoj površini tako da više ne prikazuju tumorske antigene i CTL ne mogu da ih prepoznaju (21, 24).

2. Imunoterapija malignih tumora

Razlog zbog kojeg sve više raste interesovanje za imunoterapiju malignih tumora je činjenica da lekovi koji deluju na ćelije koje se intenzivno dele ili blokiraju ćelijsku deobu, imaju brojne neželjene efekte na zdrave, proliferišuće ćelije. Usled toga, terapija malignih tumora uzrokuje značajan morbiditet i mortalitet. Sa druge strane, imunski odgovor protiv tumora je specifičan za tumorske antigene, što znači da neće oštetiti većinu normalnih ćelija, zbog čega imunoterapija ima veliki potencijal da postane najspecifičniji pristup u terapiji tumora (25, 26). Takođe, jedan od važnih razloga za dalja istraživanja i napredak na polju imunoterapije tumora je činjenica da citotoksični lekovi u većini slučajeva ne ostvaruju dugotrajne povoljne efekte u lečenju većine karcinoma koji su metastazirali (4). Suprotno, glavna osobina stečenog imunskog odgovora je memorija i sistemsko delovanje pa se smatra da bi antitumorski imunski odgovor, jednom kada se uspostavi, mogao da traje duže vreme i da bude efikasan u celom organizmu (4).

Savremena imunoterapija malignih tumora obuhvata primenu antitumorskih monoklonskih antitela i autologih T ćelija koje prepoznaju tumorske antigene i uništavaju tumorske ćelije, kao i pojačanje slabog antitumorskog imunskog odgovora domaćina vakcinacijom tumorskim antigenima i primenom antitela koja blokiraju kontrolne tačke (4).

2.1. Vakcinacija tumorskim antigenima

Stimulisanje aktivne imunosti protiv tumora moguće je vakcinacijom pacijenata obolelih od tumora njihovim sopstvenim tumorskim ćelijama, antigenima tih ćelija ili DĆ koje su inkubirane/stimulisane tumorskim antigenima. Vakcine mogu da sadrže nedefinisane tumorske antigene, ili pak tačno definisani tumorski antigen ili grupu antigena. Imajući ovo u vidu, od izuzetnog je značaja definisati tumorske antigene, što je za mnoge tumore i urađeno, jer će se time omogućiti stvaranje i upotreba tih antigena za vakcinaciju pacijenata protiv njihovih sopstvenih tumora (27).

Da bi se povećala imunogenost tumorskih antigena i indukovao zadovoljavajući imunski odgovor, tumorski antigeni se kombinuju sa adjuvansima. Dodatak adjuvansa nekim vakcinama može posledično dovesti do smanjenja količine antigena i/ili broja imunizacija potrebnih da se postigne željeni imunski odgovor (28). Kod vakcina koje se koriste u prevenciji infektivnih bolesti kod ljudi, najčešće korišćeni adjuvansi su soli aluminijuma koje su efikasne u indukciji nastanka antitela, tj. stimulišu humoralni imunitet koji zavisi od Th2 ćelija, dok retko indukuju nastanak Th1 ćelija, koje zajedno sa CTL učestvuju u antitumorskom imunskom odgovoru (29). Glavni cilj u razvoju

terapijskih vakcina protiv tumora jeste da indukuju aktivaciju, ekspanziju i održavanje CTL specifičnih za tumorski antigen. Takođe, formulacije adjuvansa koje u kombinaciji sa tumorskim antigenom indukuju snažan Th1 odgovor, su od centralnog značaja za razvoj efikasnih terapijskih vakcina protiv karcinoma (30, 31).

Dve glavne klase adjuvanasa su nosači i imunostimulatori. Nosači su komponente koje prikazuju antigene vakcine imunskom sistemu na optimalan način uz kontrolisano oslobođanje (različite mineralne soli, emulzije, lipozomi, virozomi, mineralna ulja). Imunostimulatori direktno stimulišu komponente imunskog sistema i pojačavaju odgovor na antigene. Adjuvanasi ovog tipa obuhvataju agoniste receptora sličnih Tollu (engl. Toll-like receptors, TLR) kao što je monofosforil lipid A, zatim citokine kao što su faktor koji stimuliše nastanak kolonija granulocita i makrofaga (engl. granylocite-macrophage colony-stimulating factor, GM-CSF) i interleukin-12, bakterijske egzotoksine i drugo (31, 32).

2.2. Tumorske vakcine

2.2.1. Karcinom prostate

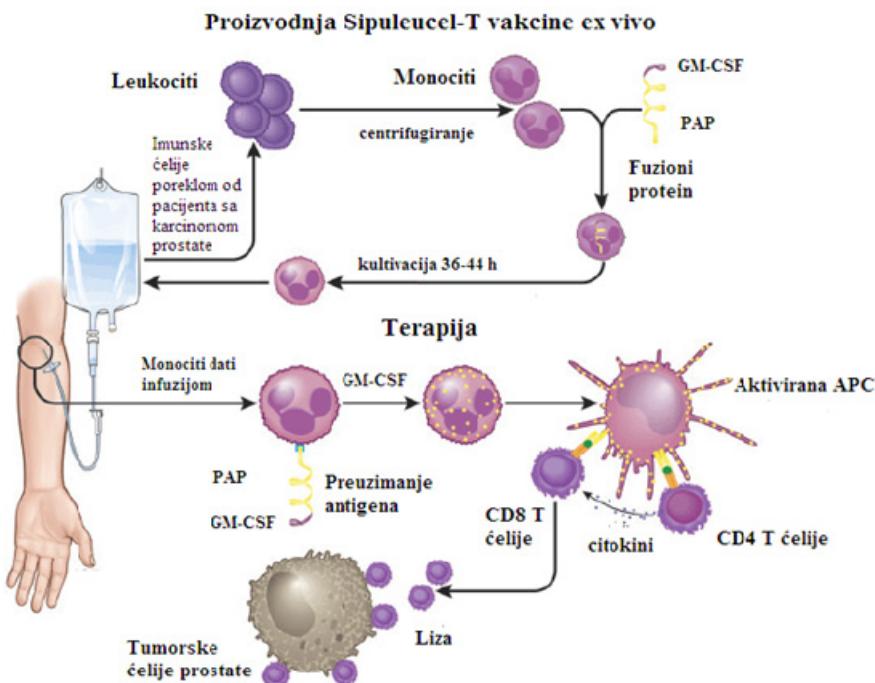
Karcinom prostate je, posle karcinoma pluća, jedan od glavnih uzročnika smrtnosti izazvanih karcinomom. Najčešći je karcinom kod muškaraca, sa preko 160.000 dijagnostikovanih obolelih i oko 26.000 umrlih u 2017. godini u Sjedinjenim Američkim Državama (33).

Prisustvo nekoliko specifičnih tumorskih antigena, poput specifičnog antigena prostate (engl. prostate-specific antigen, PSA), kisele fosfataze prostate (engl. prostatic acid phosphatase, PAP) i prostata-specifičnog membranskog antigena (engl. prostate-specific membrane antigen, PSMA), čini karcinom prostate pogodnim za ispitivanja primene imunoterapije (34).

U aprilu 2010. godine odobrena je prva terapijska vakcina za karcinom, *Sipuleucel-T* (Provenge), od strane Američke agencije za hranu i lekove (engl. Food and Drug Administration, FDA), koja je namenjena za terapiju karcinoma prostate i do danas je ostala jedina odobrena vakcina za terapiju ovog tipa karcinoma (35). Sipuleucel-T je vakcina koja sadrži DĆ i koja omogućava personalizovan tretman pacijenta koji boluje od karcinoma prostate (36, 37).

Dobijanje ove vakcine i njena primena obuhvata tri glavna koraka. Prvo se iz periferne krvi pacijenta sa karcinomom prostate izdvoje ćelije, pre svega DĆ i monociti. Zatim se one inkubiraju (36/48h) sa rekombinantnim fuzionim proteinom koji čine PAP i GM-CSF. PAP je glikoproteinski enzim koji se sintetiše u epitelnim ćelijama prostate i njegova sinteza se značajno povećava sa razvojem karcinoma. Istraživanja su pokazala da PAP primjenjen u formi fuzionog proteina indukuje PAP-specifičan humorálni i ćelijski imunitet, dok GM-CSF aktivira DĆ (36). Nakon inkubacije, aktivirane DĆ (5×10^7) se vraćaju nazad pacijentu (Slika 1). Postupak se ponavlja tri puta i navedeni

broj DĆ se pacijentu daje u vidu intravenske infuzije u razmacima od dve nedelje (38, 39).



Slika 1. Šematski prikaz dobijanja i primene Sipuleucel-T vakcine. (1) DĆ/monociti poreklom od pacijenta inkubiraju se *ex vivo* sa PAP i GM-CSF (fuzioni protein). (2) DĆ date pacijentu prikazuju PAP i aktiviraju anti-PAP CTL koji specifično prepoznaju tumorske ćelije prostate i ubijaju ih (40).

Figure 1 The diagram illustrates the two steps involved in Sipuleucel-T vaccine production. (1) Patient's DC/monocytes were pulsed *ex vivo* with recombinant fusion protein made of PAP and GM-CSF. (2) Infused DC activate anti-PAP CTL that recognize target prostate tumor cells and kill them (40).

Bezbednost i efikasnost Sipuleucel-T vakcine ispitana je u dve identične randomizirane, placebo-kontrolisane studije (D9901 i D9902A). Pacijenti (ukupno 225) nasumično izabrani za D9901 ili D9902A studiju dobijali su Sipuleucel-T ($n=147$) ili placebo ($n=78$) u vidu tri intravenske infuzije u razmaku od dve nedelje. Kod pacijenata koji su primali Sipuleucel-T, za 21% je smanjen rizik od napretka bolesti, dok je za 33% smanjen rizik od smrti u poređenju sa pacijentima koji su primali placebo. Prosečno preživljavanje pacijenata koji su primali Sipuleucel-T je bilo 4 meseca duže u poređenju

sa grupom koja je primala placebo. Nakon 36 meseci, 33% (Sipuleucel-T grupa) odnosno 15% (placebo grupa) pacijenata je preživelo (41).

Prostvac-VF, poznata još i pod nazivom PSA-TRICOM, je vakcina u trećoj fazi kliničkih studija, i jedna je od kandidata za primenu u terapiji karcinoma prostate. Prostvac-VF sadrži virusni vektor (rekombinantni pox virus) koji eksprimira PSA i tri kostimulatorna molekula označena kao TRICOM: CD80 (aktivator T-limfocita), intercelularni adhezionalni molekul 1 (ICAM-1), površinski adhezionalni molekul koji ima značajnu ulogu u regulaciji migracije i aktivacije i DĆ i T ćelija i LFA-3 (pojačava aktivaciju T limfocita). Virusni vektor se primenjuje u kombinaciji sa GM-CSF (42).

Prostvac-VF ne zahteva složenu individualizovanu terapiju i proizvodnja ove vakcine je relativno jednostavna. II faza kliničkih studija, u kojoj su poređeni efekti ove vakcine u odnosu na efekte primene praznog vektora i placebo, pokazala je pozitivne rezultate (43). Međutim, iako III faza kliničkih studija kod pacijenata sa metastatskim oblikom karcinoma prostate nije pokazala značajan napredak u preživljavanju, postoje podaci da Prostvac-VF može imati bolju efikasnost u ranim fazama bolesti. Usled toga, u toku je II faza randomizirane studije u kojoj se Prostvac-VF primenjuje kod pacijenata sa lokalizovanim oblikom karcinoma prostate (44).

Takođe, u toku je i II faza kliničke studije u kojoj se ispituje efikasnost Prostvac-VF vakcine u kombinaciji sa blokatorima kontrolnih tačaka, ipilimumabom (anti-CTLA-4 antitelo) i nivolumabom (anti-PD-1 antitelo) (45).

GVAX vakcina za karcinom prostate predstavlja imunoterapiju baziranu na primeni celih tumorskih ćelija. Kod ovog pristupa, dve ćelijske linije karcinoma prostate, LNCaP (izvedena iz metastaza limfnih čvorova) i PC3 (izvedena iz metastaza kostiju), su genetički modifikovane tako da eksprimiraju i sekretuju imunostimulatorni citokin GM-CSF. Iako je tokom ranih faza ispitivanja pokazana efikasnost i bezbednost ove vakcine kod pacijenata sa karcinomom prostate, dve studije u III fazi kliničkih ispitivanja obustavljene su usled odsustva terapijskog efekta (VITAL-1 studija) i povećane smrtnosti (VITAL-2 studija) (46, 47).

Na modelu kod miševa, pokazano je da je kombinacija GVAX vakcine sa CTLA-4 blokatorom efikasnija nego sam tretman GVAX, kao i da anti-CTLA-4 monoklonsko antitelo treba primeniti nakon vakcinacije, da bi se postigao aditivni imunski efekat (48).

2.2.2. Karcinom dojke

Uprkos brojnim dostignućima u dijagnozi, prognozi i terapiji, karcinom dojke se i dalje smatra drugim uzročnikom smrtnosti kod žena širom sveta. Iako do danas ne postoji vakcina za karcinom dojke odobrena od strane FDA, brojne vakcine i različite strategije za imunoterapiju karcinoma dojke su predmet kliničkih istraživanja (49, 50).

Jedna vakcina za terapiju karcinoma dojke (*NeuVax*) je prošla III fazu kliničkih studija (50, 51), a nekoliko terapijskih vakcina se nalazi u različitim fazama razvoja. *NeuVax* se sastoji iz sintetisanog peptida E75, koji je deo ekstracelularnog domena receptora za humani epidermalni faktor rasta 2 (engl. human epidermal growth factor receptor 2, HER2), u kombinaciji sa GM-CSF, koji ima ulogu adjuvansa. Nakon primene vakcine, peptid E75 se prerađuje, vezuje za MHC molekule I klase i stimuliše aktivaciju i nastanak CTL, koji zatim prepoznaju i uništavaju ćelije karcinoma koje eksprimiraju HER2 molekul (51). Pored terapijske efikasnosti same vakcine, ispituje se i njena efikasnost u kombinaciji sa pasivnom imunoterapijom anti-HER2 monoklonskim antitelom (trastuzumab, Herceptin®) (52).

2.2.3. Karcinom pluća

CIMAvax EGF vakcina je terapijska anti-tumorska vakcina u potpunosti razvijena i odobrena na Kubi za primenu kod odraslih pacijenata koji boluju od karcinoma pluća (stadijum IIIb/IV) (53). Na Kubi, karcinom pluća je maligna bolest sa najvećom incidencijom i vodeći uzročnik smrtnosti. Kubansko Ministarstvo javnog zdravlja je prepoznalo ovaj veliki problem i pristupilo razvoju novih terapija.

Gen koji kodira EGF receptor (EGFR) je dobro poznat onkogen. Pojačana aktivacija ovog gena može dovesti do maligne transformacije normalne ćelije, uzrokujući ćelijsku proliferaciju, inhibiciju apoptoze, angiogenezu i tumorom indukovane proinflamatorne i imunosupresivne procese (54). Generisanje signala posredstvom EGFR može biti smanjeno snižavanjem nivoa EGF ili direktnom blokadom receptora primenom specifičnih monoklonskih antitela. Snižavanje nivoa EGF aktivnom imunoterapijom je koncept koji su razvili kubanski istraživači. Vakcina stimuliše imunski sistem pacijenta da sintetiše i sekretuje sopstvena efektorska anti-EGF antitela koja vezuju i neutrališu EGF, što posledično dovodi do smanjivanja veličine tumora ili sprečava njegov dalji razvoj (54, 55, 56).

Prekid tolerancije na sopstvene proteine, tj. bolja imunogenost ove vakcine postignuta je hemijskim vezivanjem EGF za produkt bakterije, a u formulaciju vakcine je uključen i adjuvans (Montanide ISA51). Dodatno, niske doze ciklofosfamida (200 mg/m^2 telesne površine) koje se daju 72h pre primene vakcine takođe doprinose smanjenju tolerancije na sopstvene proteine (57).

Komercijalna CIMAvax EGF terapijska vakcina sastoji se od dva proizvoda, i svaki od njih se nalazi u zasebnom pakovanju. Jedan sastojak je rekombinantni humani EGF (rhEGF) koji je hemijski konjugovan za transportni protein p64K, koji se takođe dobija rekombinantnom tehnologijom, a poreklom je od bakterije *Neisseria meningitidis*. Drugi proizvod je visoko prečišćeno mineralno ulje Montanide ISA51 koje deluje kao adjuvans (57). Da bi vakcina indukovala efikasan imunski odgovor

potrebno je da se pre primene napravi emulzija između hemijskog konjugata i adjuvansa.

Kod svake imunizacije pacijent primi dozu od ukupno 2,4 mg EGF, raspoređenu na 4 mesta. Cilj ovakve primene je da se EGF iz vakcine transportuje u što veći broj regionalnih limfnih čvorova i da se na taj način poveća aktivni imunski odgovor pacijenta. Vrše se 4 imunizacije na svakih 14 dana, a potom se mesečno daje ista doza sve dok se ne promeni kliničko stanje pacijenta (58).

2.2.4. Karcinom pankreasa

Karcinom pankreasa je oboljenje sa malom petogodišnjom stopom preživljavanja (1,2-6%) (59). Poslednjih 30 godina incidencija karcinoma pankreasa je povećana u razvijenim zemljama, kao i stopa smrtnosti kod obolelih oba pola. Kod pacijenata sa razvijenim metastazama, preživljavanje je kraće od 12 meseci, čak i kada su na terapiji najaktivnijim hemioterapijskim agensima. Radioterapija je takođe pokazala slabe efekte, usled čega se nameće postreba za efikasnijim terapijskim strategijama, a imunoterapija je jedan od obećavajućih pristupa (60).

Aktivacija enzima telomeraze je ključni korak koji obezbeđuje besmrtnost tumorskoj ćeliji (61). Telomere su nekodirajuće, repetitivne DNK sekvene na kraju hromozoma. Kod većine somatskih ćelija, DNK telomera se gubi tokom deobe ćelije, dovodeći do ograničenog potencijala proliferacije ćelije (61). Nasuprot tome, kod tumorskih ćelija aktivira se enzim telomeraza, koji dovodi do obnavljanja telomera, i reaktivacija telomeraze je ključni korak u onkogenoj transformaciji ćelije, koji se javlja kod gotovo svih oblika karcinoma pankreasa (62, 63). Humana telomerazna reverzna transkriptaza (engl. human telomerase reverse transcriptase, hTERT) je katalitička subjedinica enzima telomeraze, koja je predložena kao ciljni molekul u terapiji karcinoma pankreasa i glavna je komponenta vakcine. **GV1001** peptidna vakcina sadrži sekvencu hTERT peptida dugu 16 aminokiselina i GM-CSF kao adjuvans (64). Međutim, iako je II faza kliničkih studija pokazala zadovoljavajući imunski odgovor kod 24 od 38 pacijenata (63%), druge dve studije u III fazi su imale razočaravajuće rezultate. Jedna studija je prekinuta usled odsustva pozitivnog efekta, a druga nije pokazala značajno poboljšanje preživljavanja, čak ni kada je GV1001 davan u kombinaciji sa hemioterapijom (65, 66).

Algenpantucel-L (HyperAcuteTM Pancreas) vakcina predstavlja imunoterapiju celim tumorskim ćelijama i sadrži dve ozračene alogene ćelijske linije karcinoma pankreasa (HAPa-1 i HAPa-2) koje su genetički modifikovane da eksprimiraju enzim α-1,3-galaktoziltransferazu (αGT) (67).

αGT enzim je odgovoran za sintezu α-galaktozila (αGa1), koji je ugljeni hidrat prisutan na površini ćelija. Iako je prisutan na većini ćelija sisara, αGa1 nije ispoljen na humanim ćelijama, usled inaktivacije αGT gena (68). Kao posledica kontinuirane

antigenske stimulacije normalnom crevnom florom, koja eksprimira α Gal, kod ljudi dolazi do sinteze velikog broja antitela koja su za njega specifična. Anti- α Gal antitela učestvuju sa 1% u ukupnim cirkulišućim imunoglobulinama, što ih čini jednim od najbrojnijih antitela u humanom serumu (68). Ova antitela su od kliničkog značaja jer mogu posredovati u hiperakutnom odbacivanju organa nakon transplantacije, budući da njihovo vezivanje za α Gal na ćelijama alografta dovodi do aktivacije komplementa i antitelima-posredovane destrukcije transplantiranog alografta (68).

Kada se pacijentu da Algenpantucel-L vakcina, prirodna anti- α Gal antitela prepoznaju α Gal epitope na tumorskim ćelijama iz vakcine i posreduju u hiperakutnom odbacivanju i fagocitozi. Prema tome, inicijalni imunski odgovor, nakon primene ove vakcine, je usmeren ka α Gal, ali tokom ovog odgovora nastaju imunske efektorske ćelije koje mogu da prepoznaju i druge antigenske molekule tumorskih ćelija, kroz proces koji se naziva „širenje“ epitopa. Na ovaj način, imunski odgovor se širi na tumorske antigene koje eksprimiraju dve ćelijske linije prisutne u vakcini Algenpantucel-L. HAPa-1 i HAPa-2 ćelijske linije eksprimiraju takođe i antigene koji su prisutni u većini ćelija karcinoma pankreasa, usled čega se očekuje da će kod pacijenata koji prime ovu imunoterapiju doći do razvoja odgovora prema sopstvenim ćelijama – ćelijama karcinoma pankreasa (69).

2.2.5. Metastatski melanom

Melanomi predstavljaju agresivan oblik tumora kože. Iako se rani stadijumi melanoma mogu lečiti hirurškim putem, prognoza kod pacijenata koji imaju metastaze je dosta loša. FDA je 2015. godine prvi put odobrila primenu onkolitičkog virusa u terapiji tumora i terapijsku vakcinu *Talimogene laherparepvec* (T-VEC, IMLYVICR[®]) koja se direktno ubrizgava u tumor pacijenta (70). T-VEC je ubrzo nakon toga odobrena za primenu i od strane Evropske agencije za lekove (engl. European Medicine Agency, EMA), Ministarstva zdravlja Izraela, kao i u Australiji i Švajcarskoj (71). T-VEC sadrži živ atenuisani herpes simplex virus tip 1 (HSV-1) koji je genetički modifikovan kako bi se omogućila njegova selektivna replikacija samo u tumorskim ćelijama i time poboljšao bezbednosni profil. Delecijom gena za faktor ICP34.5 smanjen je neurovirulentni potencijal virusa i afinitet za zdravo tkivo, čime je povećana specifičnost virusa za tumorske ćelije (71). Pored toga, delecija gena za ICP47 pojačava replikaciju modifikovanog HSV-1 u tumorskim ćelijama, pojačava lizu ćelija karcinoma i prezentaciju tumorskih antigena. I na kraju, insercija gena za GM-CSF omogućava njegovu lokalnu ekspresiju u tumorskom tkivu i posledično povećanu aktivaciju antigen-prezentujućih ćelija (71).

T-VEC se injektuje direktno u promene na koži i genetički modifikovan HSV-1 virus se replikuje unutar malignih ćelija dovodeći do njihove lize, što za posledicu ima oslobođanje tumorskih antigena (72). Lokalno sekretovanje GM-CSF dovodi do

nakupljanja DĆ i makrofaga i njihovog sazrevanja. Zrele DĆ i makrofagi prezentuju tumorske antigene T-ćelijama u regionalnim limfnim čvorovima i stimulišu tumor-specifične CTL koji prepoznaju tumorske ćelije na periferiji i ubijaju ih (72).

U III fazi kliničkih studija efikasnost T-VEC vakcine je poređena sa primenom samog GM-CSF. Pacijenti (n=436) su primali T-VEC vakcinu direktno u tumor, a GM-CSF subkutano. Ukupno preživljavanje u grupi koja je primala vakcincu je bilo 4 meseca duže u poređenju sa grupom koja je primala sam GM-CSF (71).

Pored toga, rane faze kliničkih studija pokazuju da kombinacija T-VEC sa blokatorima kontrolnih tačaka daje obećavajuće rezultate, usled aditivnog ili čak sinergističkog efekta (73).

3. Zaključak

Rezultati kliničkih ispitivanja velikog broja različitih tumorskih vakcina pokazali su veliku neusaglašenost i ukazali na nisku efikasnost ovog vida terapije karcinoma. Međutim, tokom dizajniranja i interpretacije kliničkih studija u kojima se ispituju mehanizmi i efikasnost aktivne imunoterapija tumora, treba uzeti u obzir glavne razlike između ovog vida savremene terapije tumora i klasične hemioterapije. Naime, dok hemioterapija u osnovi dovodi do rezultata brzo, odmah nakon primene, kod aktivne imunoterapije može proći i nekoliko meseci dok se imunski odgovor u potpunosti ne razvije. Pored toga, primena hemioterapije kao i pasivne imunoterapije obično uključuje direktno dejstvo na maligne ćelije, dok aktivna imunoterapija deluje indirektno, aktivacijom imunskog sistema.

Iz svega navedenog, može se zaključiti da će kombinacija različitih terapijskih pristupa biti jedan od naprednih koncepta lečenja malignih tumora u budućnosti. Smatra se da će tumorske vakcine biti veoma važan dodatak konvencionalnoj terapiji ili nekoj drugoj savremenoj imunoterapiji, ali je potrebno prevazići različite izazove vezane za njen dizajn, u smislu optimalne kombinacije antigena, adjuvansa i nosača i načina primene. Kao prvo, vakcine moraju da se prilagode svakom pacijentu ponaosob i treba da indukuju efikasan CTL odgovor što do sada nije uvek bilo moguće. Kada se koriste vakcine koje sadrže DĆ njihova priprema je vezana sa brojnim tehničkim izazovima/problemima i često taj proces nije moguće standardizovati. Dalje, pod selektivnim pritiskom imunskog odgovora indukovanim vakcinom tumori mogu da izgube MHC molekule I klase ili ciljne tumorske antigene i postanu nevidljivi za imunski sistem. I na kraju terapijska vakcina treba da prevaziđe imunosupresivne mehanizme koje tumor razvija kod pacijenata sa klinički ispoljenim tumorom (74, 75, 76). Uspeh terapije tumora blokadom kontrolnih tačaka uliva nadu da bi vakcinacija u kombinaciji sa blokadom inhibitora imunskog odgovora indukovala snažan i dugotrajan imunski odgovor na tumore i da bi to moglo značajno da unapredi lečenje tumora (45). Konačno, kako bi se što veći broj terapijskih tumorskih vakcina našao u kliničkoj praksi

neophodna su i velika finansijska ulaganja u klinička istraživanja ovog oblika terapije tumora.

Zahvalnica

Autori se zahvaljuju COST akciji CA16231 „European Network of Vaccine Adjuvants-ENOVA“, koja im je svojim aktivnostima omogućila unapređenje znanja iz oblasti terapijskih vakcina.

Literatura

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer.* 2019;144(8):1941-1953.
2. Leber MF, Efferth T. Molecular principles of cancer invasion and metastasis (review). *Int J Oncol.* 2009;34(4):881-95.
3. Messerschmidt JL, Prendergast GC, Messerschmidt GL. How Cancers Escape Immune Destruction and Mechanisms of Action for the New Significantly Active Immune Therapies: Helping Nonimmunologists Decipher Recent Advances. *Oncologist.* 2016;21(2):233-43.
4. Abbas AK, Lichtman AH, Pillai S. Basic immunology: functions and disorders of the immune system, 6th edition, Philadelphia, Pa.Saunders; 2018. 397 p.
5. Ehrlich P. Collected Studies on Immunity. New York: J. Wiley & Sons; 1910.
6. Virchow R. Cellular pathology as based upon physiological and pathological histology. Philadelphia: J. B. Lippincott; 1863
7. Baar HS. From Ehrlich-Pirquet to Medawar and Burnet; a revolution in immunology. *J Maine Med Assoc.* 1963;54:209-14.
8. Burnet FM, Cancer. A biological approach. 1. The process of control. *Br Med J.* 1957;1: 779-82.
9. Burnet FM. Immunological surveillance, Oxford, Pergamon Press 1970.
10. Simpson E. Reminiscences of Sir Peter Medawar: in hope of antigen-specific transplantation tolerance. *Am J Transplant.* 2004;4(12):1937-40.
11. Foley EJ. Antigenic properties of methylcholanthrene induced tumors in mice of the strain of origin. *Cancer Res.* 1953;13: 835-837.
12. Ribatti D. The concept of immune surveillance against tumors. The first theories. *Oncotarget.* 2017;24;8(4):7175-80.

13. Durgeau A, Virk Y, Corgnac S, Mami-Chouaib F. Recent Advances in Targeting CD8 T-Cell Immunity for More Effective Cancer Immunotherapy. *Front Immunol.* 2018;9:14.
14. Wu J, Lanier LL. Natural killer cells and cancer. *Adv Cancer Res.* 2003;90:127-56.
15. Whitworth PW, Pak CC, Esgro J, Kleinerman ES, Fidler IJ. Macrophages and cancer. *Cancer Metastasis Rev.* 1990;8(4):319-51.
16. Ostroumov D, Fekete-Drimusz N, Saborowski M, Kühnel F, Woller N. CD4 and CD8 T lymphocyte interplay in controlling tumor growth. *Cell Mol Life Sci.* 2018;75(4):689–713.
17. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature.* 2011;480(7378):480-9.
18. Multhoff G, Molls M, Radons J. Chronic inflammation in cancer development. *Front Immunol.* 2012;2:98.
19. Korniluk A, Koper O, Kemona H, Dymicka-Piekarska V. From inflammation to cancer. *Ir J Med Sci.* 2017;186(1):57–62.
20. Wang M, Zhao J, Zhang L, et al. Role of tumor microenvironment in tumorigenesis. *J Cancer.* 2017;8(5):761–773. Published 2017 Feb 25. doi:10.7150/jca.17648
21. Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. *Genes Dev.* 2018;32(19-20):1267–84.
22. Farhood B, Najafi M, Mortezaee K. CD8+ cytotoxic T lymphocytes in cancer immunotherapy: A review. *J Cell Physiol.* 2019;234(6):8509-8521.
23. Thomas DA, Massagué J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell.* 2005;8(5):369-80.
24. Garrido F, Aptsiauri N, Doorduijn EM, Garcia Lora AM, van Hall T. The urgent need to recover MHC class I in cancers for effective immunotherapy. *Curr Opin Immunol.* 2016;39:44-51.
25. Oiseth JS, Aziz SM. Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead. *J Cancer Metastasis Treat.* 2017;3:250-61.
26. Coventry BJ. Therapeutic vaccination immunomodulation: forming the basis of all cancer immunotherapy. *Ther Adv Vaccines Immunother.* 2019;7.
27. Kawakami Y, Fujita T, Matsuzaki Y, Sakurai T, Tsukamoto M, Toda M, Sumimoto H. Identification of human tumor antigens and its implications for diagnosis and treatment of cancer. *Cancer Sci.* 2004;95(10):784-91.
28. Kanzler H, Barrat FJ, Hessel EM, Coffman RL. Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. *Nat Med.* 2007;13:552–9.
29. Hogenesch H. Mechanism of immunopotentiation and safety of aluminum adjuvants. *Front Immunol.* 2013;3:406.
30. Dubensky TW Jr, Reed SG. Adjuvants for cancer vaccines. *Semin Immunol.* 2010;22(3):155-61.
31. Temizoz B, Kuroda E, Ishii KJ. Vaccine adjuvants as potential cancer immunotherapeutics. *Int Immunol.* 2016;28(7):329-38.
32. Melero I, Gaudernack G, Gerritsen W, Huber C, Parmiani G, Scholl S, Thatcher N, Wagstaff J, Zielinski C, Faulkner I, Mellstedt H. Therapeutic vaccines for cancer: an overview of clinical trials. *Nat Rev Clin Oncol.* 2014;11(9):509-24.

33. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65(1):5-29.
34. Madu CO, Lu Y. Novel diagnostic biomarkers for prostate cancer. J Cancer. 2010;1:150–77.
35. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW, Schellhammer PF; IMPACT Study Investigators. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411-22.
36. Cheever MA, Higano CS. PROVENGE (Sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. Clin Cancer Res. 2011;17(11):3520-6.
37. Graff JN, Chamberlain ED. Sipuleucel-T in the treatment of prostate cancer: an evidence-based review of its place in therapy. Core Evid. 2014;10:1-10.
38. Anassi E, Ndefo UA. Sipuleucel-T (provenge) injection: the first immunotherapy agent (vaccine) for hormone-refractory prostate cancer. P T. 2011;36(4):197-202.
39. Silvestri I, Cattarino S, Giantulli S, Nazzari C, Collalti G, Sciarra A. A Perspective of Immunotherapy for Prostate Cancer. Cancers (Basel). 2016;8(7).
40. Garcia JA, Dreicer R. Immunotherapy in castration-resistant prostate cancer: integrating sipuleucel-T into our current treatment paradigm. Oncology. (Williston Park). 2011;25(3):242-9.
41. Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, Provost N, Frohlich MW. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer. 2009;115(16):3670-9.
42. Madan RA, Arlen PM, Mohebtash M, Hodge JW, Gulley JL. Prostvac-VF: a vector-based vaccine targeting PSA in prostate cancer. Expert Opin Investig Drugs. 2009;18(7):1001–11.
43. Gulley JL, Madan RA, Tsang KY, et al. Immune impact induced by PROSTVAC (PSA-TRICOM), a therapeutic vaccine for prostate cancer. Cancer Immunol Res. 2014;2(2):133–141.
44. Parsons JK, Pinto PA, Pavlovich CP, Uchio E, Kim HL, Nguyen MN, Gulley JL et al. A Randomized, Double-blind, Phase II Trial of PSA-TRICOM (PROSTVAC) in Patients with Localized Prostate Cancer: The Immunotherapy to Prevent Progression on Active Surveillance Study. Eur Urol Focus. 2018;4(5):636-638.
45. Mougel A, Terme M, Tanchot C. Therapeutic Cancer Vaccine and Combinations With Antiangiogenic Therapies and Immune Checkpoint Blockade. Front Immunol. 2019;10:467.
46. Nemunaitis J. Vaccines in cancer: GVAX, a GM-CSF gene vaccine. Expert Rev Vaccines. 2005;4(3):259-74.
47. Madan RA, Mohebtash M, Schlom J, Gulley JL. Therapeutic vaccines in metastatic castration-resistant prostate cancer: principles in clinical trial design. Expert Opin Biol Ther. 2010;10(1):19–28.
48. Wada S, Jackson CM, Yoshimura K, et al. Sequencing CTLA-4 blockade with cell-based immunotherapy for prostate cancer. J Transl Med. 2013;11:89.
49. Mittendorf EA, Peoples GE. Injecting Hope—A Review of Breast Cancer Vaccines. Oncology (Williston Park). 2016;30(5):475-81.

50. Behravan J, Razazan A, Behravan G. Towards Breast Cancer Vaccines, Progress and Challenges. *Curr Drug Discov Technol.* 2019;16(3):251-8.
51. Schnable EJ, Berry JS, Trappey FA, Clifton GT, Ponniah S, Mittendorf E, Peoples GE. The HER2 peptide nelipepimut-S (E75) vaccine (NeuVaxTM) in breast cancer patients at risk for recurrence: correlation of immunologic data with clinical response. *Immunotherapy.* 2014;6(5):519-31.
52. Clifton GT, Peace KM, Holmes JP, Vreeland TJ, Hale DF, Herbert GS, Litton JK, Murthy RK, Lukas J, Peoples GE, Mittendorf Elizabeth A. Initial safety analysis of a randomized phase II trial of nelipepimut-S + GM-CSF and trastuzumab compared to trastuzumab alone to prevent recurrence in breast cancer patients with HER2 low-expressing tumors. *Clin Immunol.* 2019;201:48-54.
53. Rodríguez PC, Rodríguez G, González G, Lage A. Clinical development and perspectives of CIMAvax EGF, Cuban vaccine for non-small-cell lung cancer therapy. *MEDICC Rev.* 2010;12(1):17-23.
54. Saavedra D, Crombet T. CIMAvax-EGF: A New Therapeutic Vaccine for Advanced Non-Small Cell Lung Cancer Patients. *Front Immunol.* 2017;8:269.
55. Gonzalez G, Montero E, Leon K, et al. Autoimmunization to epidermal growth factor, a component of the immunological homunculus. *Autoimmun Rev.* 2002;1(1-2):89-95.
56. González G, Lage A. Cancer vaccines for hormone/growth factor immune deprivation: a feasible approach for cancer treatment. *Curr Cancer Drug Targets.* 2007;7(3):229-41.
57. Crombet Ramos T, Rodríguez PC, Neninger Vinageras E, Garcia Verdecia B, Lage Davila A. CIMAvax EGF (EGF-P64K) vaccine for the treatment of non-small-cell lung cancer. *Expert Rev Vaccines.* 2015;14(10):1303-11.
58. Macias, Amparo E. et al. P2.34: Vaxira and CIMAvax-EGF Therapeutic Vaccines Combination in the Advanced NSCLC Treatment. *J Thorac Oncology.* 2016;11(10):S236 - S237.
59. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin.* 2017;67:7-30.
60. Young K, Hughes DJ, Cunningham D, Starling N. Immunotherapy and pancreatic cancer: unique challenges and potential opportunities. *Ther Adv Med Oncol.* 2018;10.
61. Zvereva MI, Shcherbakova DM, Dontsova OA. Telomerase: structure, functions, and activity regulation. *Biochemistry (Mosc).* 2010;75(13):1563-83.
62. Artandi SE, DePinho RA. Telomeres and telomerase in cancer. *Carcinogenesis.* 2009;31(1):9-18.
63. Zisuh AV, Han TQ, Zhan SD. Expression of telomerase & its significance in the diagnosis of pancreatic cancer. *Indian J Med Res.* 2012;135(1):26-30.
64. Kyte JA. Cancer vaccination with telomerase peptide GV1001. *Expert Opin Investig Drugs.* 2009;18(5):687-94.
65. Middleton G et al. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): an open-label, randomised, phase 3 trial. *Lancet Oncol.* 2014;15(8):829-40.
66. Staff C, Mozaffari F, Frodin JE, Mellstedt H, Liljefors M. Telomerase (GV1001) vaccination together with gemcitabine in advanced pancreatic cancer patients. *Int J Oncol.* 2014;45:1293-1303.

67. Hardacre JM, Mulcahy MF, Small W, et al. Effect of the addition of algenpantucel-L immunotherapy to standard adjuvant therapy on survival in patients with resected pancreas cancer. *J of Clinic Oncology*. 2011;29:4_suppl, 236-236.
68. Coveler AL, Rossi GR, Vahanian NN, Link C, Chiorean EG. Algenpantucel-L immunotherapy in pancreatic adenocarcinoma. *Immunotherapy*. 2016;8(2):117-25.
69. McCormick KA, Coveler AL, Rossi GR, Vahanian NN, Link C, Chiorean EG. Pancreatic cancer: Update on immunotherapies and algenpantucel-L. *Hum Vaccin Immunother*. 2016;12(3):563-75.
70. Johnson DB, Puzanov I, Kelley MC. Talimogene laherparepvec (T-VEC) for the treatment of advanced melanoma. *Immunotherapy*. 2015;7(6):611-9.
71. Raman SS, Hecht JR, Chan E. Talimogene laherparepvec: review of its mechanism of action and clinical efficacy and safety. *Immunotherapy*. 2019;11(8):705-723.
72. Rehman H, Silk AW, Kane MP, Kaufman HL. Into the clinic: Talimogene laherparepvec (T-VEC), a first-in-class intratumoral oncolytic viral therapy. *J Immunother Cancer*. 2016;20:4:53.
73. Seremet T, Planken S, Schwarze JK, Jansen Y, Vandeweerd L, van den Begin R, Tsechelidis I, Lienard D, Del Marmol V, Neyns B. Successful treatment with intralesional talimogene laherparepvec in two patients with immune checkpoint inhibitor-refractory, advanced-stage melanoma. *Melanoma Res*. 2019;29(1):85-8.
74. Guo C, Manjili MH, Subjeck JR, Sarkar D, Fisher PB, Wang XY. Therapeutic cancer vaccines: past, present, and future. *Adv Cancer Res*. 2013;119:421–475.
75. Hollingsworth RE, Jansen K. Turning the corner on therapeutic cancer vaccines. *NPJ Vaccines*. 2019;4:7.
76. Coventry BJ. Therapeutic vaccination immunomodulation: forming the basis of all cancer immunotherapy. *Ther Adv Vaccines Immunother*. 2019;7:2515135519862234.

Active immunotherapy of cancer: an overview of therapeutic vaccines

Brankica Filipić*, Zorica Stojić-Vukanić

University of Belgrade - Faculty of Pharmacy, Department of microbiology and immunology, Vojvode Stepe 450, 11221 Belgrade, Srbija

*Corresponding author: e-mail: brankica.filipic@pharmacy.bg.ac.rs

Summary

Cancer is one of the leading causes of morbidity and mortality worldwide and great efforts are underway to develop new therapeutic protocols. One of the approaches is immunotherapy which uses the immune system and its components to fight against cancer. The two main axes of cancer immunotherapy refer to passive and active treatments. Passive immunotherapy includes administration of tumor-specific antibodies and autologous T cells which destroy tumor cells, while active immunotherapy is directed at inducing the patient's own antitumor immune responses and refers to cancer vaccines and immune checkpoint inhibitors. Vaccination of tumor-bearing individuals with tumor cells/antigens or autologous dendritic cells pulsed with tumor antigens may result in enhanced antitumor immune response. However, vaccine design is a complex, multi-component task, and the optimal combinations of antigens, adjuvants, delivery methods and routes of administration need to be precisely defined. Active immunotherapy also addresses the immunosuppressive and tolerogenic mechanisms developed by tumors. This review provides an overview of new results from clinical studies of therapeutic cancer vaccines and discusses their implications for the clinical use, alone or in combination with other immunotherapeutic strategies.

Key words: cancer, active immunotherapy, cancer vaccines

Prilozi – Contributions

120 GODINA OD ROĐENJA PRVOG SRPSKOG TOKSIKOLOGA, VELIKANA U FARMACIJI

Sećanja na profesora Momčila St. Mokranjca (1899-1967)

Danijela Đukić-Ćosić, Vesna Matović

Katedra za toksikologiju „Akademik Danilo Soldatović“

Univerzitet u Beogradu – Farmaceutski fakultet

U godini kada Farmaceutski fakultet Univerziteta u Beogradu obeležava 80 godina studija farmacije, možemo se pohvaliti brojnim velikanima, značajnim ličnostima koje su bile prvi profesori svojih oblasti u okviru farmaceutskih nauka, ali se profesor Mokranjac dodatno izdvaja iz dva razloga. Prvi je da se u godini jubileja obeležava 120 godina od njegovog rođenja, a drugi, da je potomak slavne srpske porodice. Otac mu je bio čuveni kompozitor i horovođa Stevan St. Mokranjac, a majka, Marija Mokranjac (rođ. Predić), bratanica poznatog slikara Uroša Predića. Iako je potomak slavnih srpskih umetničkih porodica, životni put ga je odveo na drugu stranu, daleko od muzike i umetnosti. Znanje na prestižnim francuskim školama pretočio je u osnove obrazovanja u toksikologiji u Srbiji i tadašnjoj jugoslovenskoj državi. Izuzetan doprinos je ostvario kao dekan Farmaceutskog fakulteta u Beogradu i ukazao na značaj farmaceutske profesije i jačanje struke kroz saradnju fakulteta sa farmaceutskim društvima i drugim institucijama u zemlji. Kao član brojnih udruženja i organizacija pružio je doprinos široj akademskoj zajednici, a iznad svega, vrednim i predanim naučnoistraživačkim radom, ostvario rezultate koji su nagrađeni od strane prestižnih francuskih akademskih institucija. Ovaj prilog ima za cilj da, iznoseći podatke iz života i dela profesora Mokranjca osvetli njegov lik i ukaže na značaj koji je imao u obrazovanju farmaceuta i razvoju farmaceutske struke u našoj zemlji.

Roden je 1899. godine u Beogradu, gde je završio osnovnu školu i pet razreda gimnazije. Po izbijanju Velikog rata povlači se sa roditeljima u Skoplje, odakle savezničkim brodovima sa ostalom srpskom omladinom odlazi u Francusku i nastavlja školovanje. Završava maturu u Nici (1917) a potom licej hemije na Pariskom naučnom

fakultetu u sastavu Sorbone (1920). U prestižnom Pasterovom institutu radi na doktorskoj disertaciji, koju će odbraniti 1922. godine kod čuvenog francuskog profesora, Gabriela Bertrana, „oca mikroelemenata”. Po povratku u Beograd, radio je najpre kao hemičar u Državnoj hemijskoj laboratoriji i šef Hemijske laboratorije beogradske opštine, da bi period radnog veka od 1935. do 1946. godine proveo u Narodnoj banci Kraljevine Jugoslavije kao šef Odeljenja za ispitivanje plemenitih metala. Prof. Mokranjac je postavio temelje kovanim novčanicama, posebno srebrnim kovanicama od 20 i 50 dinara, baveći se hemijskim analizama srebrnih pločica za njihovu izradu.

Na zahtev ministra narodnog zdravlja 1946. godine iz Narodne banke prelazi na Farmaceutski fakultet gde biva izabran za vanrednog profesora za predmet Toksikološka hemija. Ovaj predmet bio je predviđen prvim nastavnim planom studija farmacije u četvrtoj godini studija i izučavao se jedino na Farmaceutskom fakultetu u Beogradu u celoj bivšoj Jugoslaviji. Ubrzo po zaposlenju na Fakultetu, za nepune tri godine objavio je prvi udžbenik iz toksikološke hemije na srpskom jeziku, „Toksičkolaška hemija” (1949). Udžbenik je pripremio po uzoru na udžbeničku literaturu francuske škole. Godine 1951. prof. Mokranjac je izabran za redovnog profesora na Farmaceutskom fakultetu u Beogradu gde je uporedo sa utemeljenjem toksikologije i razvojem naučnoistraživačkog rada Instituta za toksikološku hemiju, obavljao i funkciju prodekana (1947-1949), kao i dekana Farmaceutskog fakulteta (1949-1952). Bio je član više stručnih organizacija i udruženja, pružajući svuda maksimalni doprinos za razvoj kako struke, tako i nauke. Posebno je proučavao cink, nikl i kobalt, izučavao toksikologiju olova i bavio se istraživanjem biljnih otrova u organima. Objavio je oko 60 naučnih i stručnih radova. Izabran je za dopisnog člana Pariske akademije farmacije 1953. godine i 1954. godine nagrađen Lavoazijeovom medaljom za istraživački doprinos na polju hemije. Nositelj je i ordena javnog zdravlja (1955) koji dodeljuje predsednik Francuske.

Obavljajući funkciju trećeg po redu dekana, tek osnovanog Farmaceutskog fakulteta intenzivno radi na razvoju nastave na fakultetu i obezbeđivanju prostorija za rad u okviru instituta fakulteta. Pred kraj 1950. godine u časopisu Univerzitetski vesnik objavljuje članak pod naslovom „Pet godina Farmaceutskog fakulteta u Beogradu”, osvrnuvši se na sve segmente rada fakulteta, od nastavnog kadra, prostorija za rad, broja studenata (733 u tom momentu i 295 apsolvenata), preko dostupne udžbeničke literature, do naučnog rada, za koji kaže da su temelji postavljeni i da se nastoje proširiti, kao i „da se asistenti pravilno rasterete od posla, da bi mogli više naučno raditi i da svoj rad koordiniraju s radom drugih naučnih radnika u istom području”. Iznoseći rezultate petogodišnjeg rada fakulteta, završava izlaganje rečima: „Iz ovih izlaganja vidi se, da rad na Farmaceutskom fakultetu u Beogradu napreduje i da će uroditи dobrim plodom.”

Prof. Mokranjac se ističe zalaganjem za studije farmacije pri čemu ukazuje na značaj obrazovanja farmaceuta. Iako hemičar po struci, kako je i sam zapisao, ukazivao je na to da se ne sme izgubiti iz vida da su studije farmacije u Zapadnoj Evropi, posebno u Francuskoj, oduvek bile na zavidnom nivou i da među najvećim imenima nauke ima priličan broj farmaceuta. Ukazuje na to da imamo studije na visokom nivou, prema uzoru na francuske univerzitete, ali i da je neophodna tešnja saradnja između farmaceutskih fakulteta i farmaceutskih društava kako se na farmaceute ne bi gledalo „kao na univerzitetski obrazovane kadrove, ali ipak nešto nižeg ranga”. Profesor Mokranjac zapisao je i sledeće „...kako će ljudi van struke imati visoko mišljenje o farmaceutima i farmaciji, ako osete da u tom pogledu ni nosioci struke – svršeni farmaceuti i oni koji na fakultetima formiraju te farmaceute nisu jedinstveni.”

Ukazuje na značaj saradnje farmaceutskih fakulteta sa farmaceutskim društvima i 1952. Godine u Skoplju na Trećem plenumu Saveza farmaceutskih udruženja FNRJ drži referat na ovu temu koji je u celosti objavljen u Farmaceutskom glasniku 1952. „Zašto je potrebna saradnja između farmaceutskih društava i fakulteta? Sa fakulteta izlaze budući članovi farmaceutskih društava, budući farmaceutski radnici, budući rukovodioci raznih grana farmaceutske struke. Oni će davati pravac i karakter razvoja struke na svojim radnim mestima. I oni za to treba da budu sposobljeni.” Dalje u tekstu, prof. Mokranjac navodi da je saradnja korisna pri diskusiji o nastavnim planovima i programima. „Stručna društva će u tom pogledu doneti jasne potrebe terena, potrebe bazirane na poznavanju farmaceutske prakse i struke kao celine. Fakulteti te sugestije treba da iskoriste, vodeći naravno računa o osnovnim zadacima opšteg, univerzitetskog obrazovanja. Škola treba da da lik farmaceuta koji odgovara stvarnim potrebama današnjice, vodeći pri tome računa da onaj ko završi fakultet mora imati jasno izražen i izgrađen lik farmaceuta – to jest mora imati onaj specifičan karakter koji daje značaj i smisao jedne struke kao celine. Tu bi sad možda ljudi iz prakse, svršeni farmaceuti, mogli zameriti nastavnicima na fakultetima, naročito nastavnicima nefarmaceutima, da oni ne ulaze dovoljno, ili čak i da ne mogu da uđu dovoljno, u suštinu farmacije kao takve, da zbog toga možda često preteruju u svojim, da tako kažem, užim stručno partikularističkim tendencijama. I tu svakako da ima nešto tačnog. Ali eto to je baš opet jedan razlog više za ostvarenje što prisnije i dublje saradnje između farmaceutskih društava i fakulteta i između pojedinih predstavnika fakulteta i članova društva.” Saradnju farmaceutskih fakulteta i farmaceutskih društava prof. Mokranjac je opisao kroz tri zadatka, odnosno tri oblasti. Prvi zadatak predstavlja usklađivanje nastavnih planova i programa na fakultetima, vodeći pritom računa o specifičnim potrebama i karakteru univerzitetskog obrazovanja kao takvog i o interesima farmacije kao celine. Druga oblast saradnje ogleda se, ili treba da se ogleda, kako je kazivao prof. Mokranjac, u publikacijama društva, budući da nastavnici fakulteta svoje rezultate objavljaju u tim časopisima. „...dalje usavršavanje i razvoj svih naših časopisa treba da bude opšta briga

i društva i fakulteta. Tu dakle mora da dođe do najjačeg izražaja saradnja između fakulteta i farmaceutskih društava.”

Treći segment saradnje prof. Mokranjac je istakao kroz pojedinačna predavanja koja nastavnici fakulteta drže u okviru udruženja i sekcija udruženja, kao i „održavanje seminara za farmaceute iz prakse, a u cilju da se i njima pruži mogućnost da se upoznaju sa najnovijim tekovinama nauke i struke”. Profesor Mokranjac u svom izlaganju ideja o potrebi, formama i načinima saradnje fakulteta i farmaceutskih društava nije upotrebio termin „kontinuirana edukacija” i „kursevi kontinuirane edukacije”, ali ih je doslovce opisao, a sve sa ciljem da saradnja „mora biti za opšte unapređenje farmacije, na opšte dobro sviju nas i naše društvene jedinice.”

Jubilej 120 godina od rođenja ovog velikana u farmaciji bio je povod da istražimo arhive, osvetlimo lik i delo prof. Mokranjca, „čoveka od nauke”, oca srpske i jugoslovenske toksikologije i više, ukažemo široj i naučnoj javnosti na značaj potomka slavnog srpskog kompozitora i da iza imena „Mokranjac” stoje velikani u različitim oblastima ne samo muzici, već i toksikologiji i farmaciji.

Napomene: Pojedini delovi teksta su preuzeti iz kataloga Momčilo St. Mokranjac – hemičar, profesor, toksikolog, štampanog za izložbu o prof. Mokranjcu koja je predstavljena u Beogradu u Galeriji nauke i tehnike SANU od 16. aprila do 4. maja 2019. i u Negotinu, u rodnoj kući Stevana St. Mokranjca, profesorovog oca, od 16. septembra do 2. oktobra 2019. godine u okviru manifestacije 54. „Mokranđevi dani”.

Korišćena literatura

Arhiva Farmaceutskog fakulteta Univerziteta u Beogradu

Arhiv Narodne banke Srbije (ANB), Fond narodne banke, I/II, AK 60.

Arhiva Saveza farmaceutskih udruženja Srbije (Farmaceutski glasnik: 2/1951; 6/1952; 10/1956)

Arhiv Srpske akademije nauka i umetnosti – SANU, 14455-II/7.

Đukić-Ćosić D, Matović V. Meet prof. Momčilo Mokranjac – the Father of Serbian Toxicology. CTDC10 and 12th SCT. Belgrade, April 18-21, 2018. Book of abstracts: 124.

Medenica M, Ivanović D. prof dr Momčilo St. Mokranjac u: 60 godina Farmaceutskog fakulteta u Beogradu (1945-2005). Univerzitet u Beogradu – Farmaceutski fakultet, Beograd 2006; pp.750-753.

Momčilo St. Mokranjac, hemičar, profesor, toksikolog. Galerija nauke i tehnike SANU, broj 38. Beograd, 2019.