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The vaccine and its dosage form

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List of important abbreviations used in the lecture

CTB – cholera subunit toxin
BDT – diphtheria toxoid
(DTaP) – diphtheria and tetanus toxoid together with subunit pertussis vaccine
EP – penetration accelerant
H – hemagglutinin
HepB – hepatitis B vaccine
HiB – hepatitis B virus
HIV – human immunodeficiency virus
HPV – human papillomavirus
ISCOM – immunostimulating complex
IPV - inactivated poliovirus vaccine
KA – killed antigen
LAV – live attenuated vaccine
MPs – polymer microparticles
N – neuramidase
NPs – polymer nanoparticles
OMP – membrane-encased microparticles
OPV – oral poliomyelitis vaccine
OV – oral vaccines
PCV – pneumococcal conjugate
PMV - oral herbal vaccine
rCTB - recombinant cholera subunit toxin
BrHBsAg – recombinant hepatitis B antigen
RSV – respiratory syncytial virus
RV – recombinant vaccineSUV - subunit vaccine
SV – split vaccine
T – toxoid (anatoxin)
TB – tuberculosis
TCI – transdermal immunization
Td - combined vaccine against diphtheria and tetanus
TT – tetanus toxoid
VLP - virus-like particle
VV – vector vaccine
wP - inactivated pertussis vaccine
YF – yellow fever

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Vaccine - definition

- A vaccine is a biological preparation designed to induce or increase specific and active immunity against an infectious agent
- It has antigenic specificity and can induce an immune response in the host or antibody production or cell-mediated immunity
- It is usually produced from weakened or killed forms of pathogens - bacteria and viruses or their parts, or derived agents such as toxoids, proteins, polysaccharides, DNA, RNA
- It must not be toxic to the organism
- It stimulates the body's immune system to recognize and destroy the pathogen as a foreign element and at the same time "remember" its antigen, which the immune system could more easily eliminate in the future without developing an infectious disease
- Reactivity
- Immunogenity

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A brief history of vaccination

- **Ancient times** - Indians ingested snake venoms to immunize themselves against snake bites
- **1695 -** in China they used tampons soaked in smallpox lesions
- **1767 -** Attempts at widespread variolization in India
- **1796 –** Edward Jenner applied crushed scab from cowpox (Latin vacca)
- **1885 -** Louis Pasteur prepared a vaccine from a weakened rabies virus
- **1830 -** the largest vaccination against smallpox in our territory took place
- **1961 -** polio (poliomyelitis) disappeared in our country thanks to the Sabin vaccine
- **1979 -** the last outbreak of smallpox in Somalia
- **1953 - 2010 -** the number of TBC cases in the Czech Republic dropped up to forty times compared to the rest of the world
- **2002 -** eradication of rabies in foxes in the Czech Republic

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Historical milestones

Weakened toxines
first toxoids

Collective immunity of monastic orders caring for the sick
inhalation vaccination

Killing of Native Americans during the settlement of the US with the first biological weapons (blankets of the infected)
absence of collective immunity

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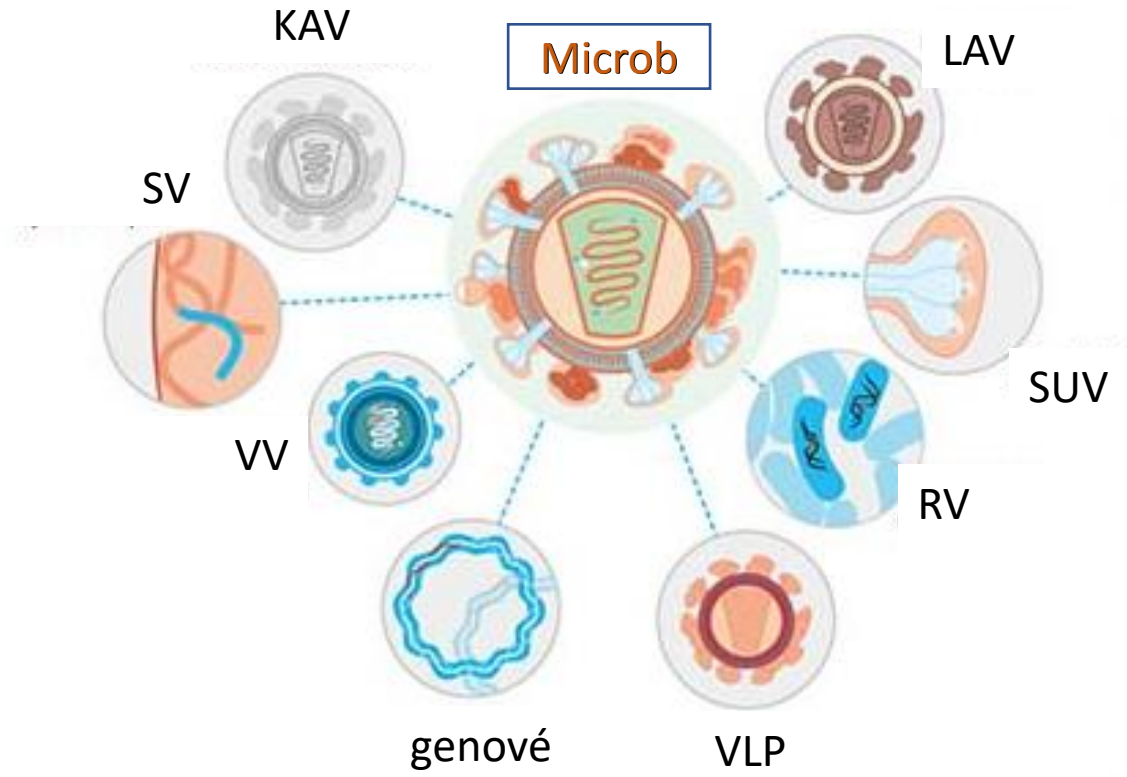
Types of vaccines

- **LAV** - Live attenuated vaccines
- **KAV** - Killed antigen vaccines
- **SUV** - Subunit vaccines
- **SV** - Splitting vaccines
- **T** - Toxoids
- **RV** - Recombinant vaccines
- **PMV** - Plant made vaccines
- **VLP** - Virus like particles
- **VV** - Vector vaccines
- **Gene vaccines** - DNA resp. RNA vaccines



Source: ARIAL S. Microbe Notes 2018 (modified)

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Attenuated vaccines (LAV)

- Made from whole weakened bacteria or viruses
- High immunogenicity and reactivity
- They can cause infectious diseases
- The oldest technology
- Deactivation by formaldehyde, phenol, sodium hydroxide, saponins, temperature or UV radiation
- Multiple passage through tissue cultures
- Retained antigenic specificity
- One dose is enough
- Cultivation on animal or human tissue or cells
- For viruses, fertilized eggs, animal embryos or animal or human cultures, fibroblasts
- There are five LAV vaccines recommended by the WHO. *BCG Bacillus Calmette-Guérin, reduced virulence Mycobacterium bovis, oral polio vaccine (OPV), rubella, rotavirus and yellow fever (YF)*

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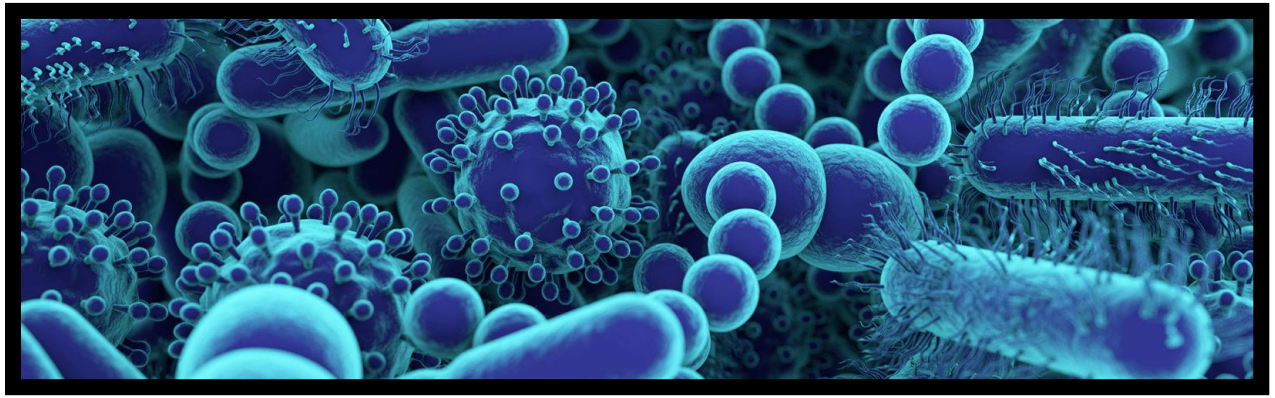
Inactivated (KAV)



- Made from whole killed bacteria or viruses
- Suitable for extracellular pathogens
- Lower immunogenicity and reactivity
- They cannot cause infectious diseases
- Killed by ascorbic acid solution, beta-aminophenyl ketone, ethylenimine, formaldehyde, phenol, propiolactone or their mixtures, elevated pressure, temperature, UV radiation
- Retained antigenic specificity
- More doses are usually needed
- Cultivation as LAV
- WHO currently recommends *inactivated pertussis (wPa) poliomyelitis vaccine (IPV)*

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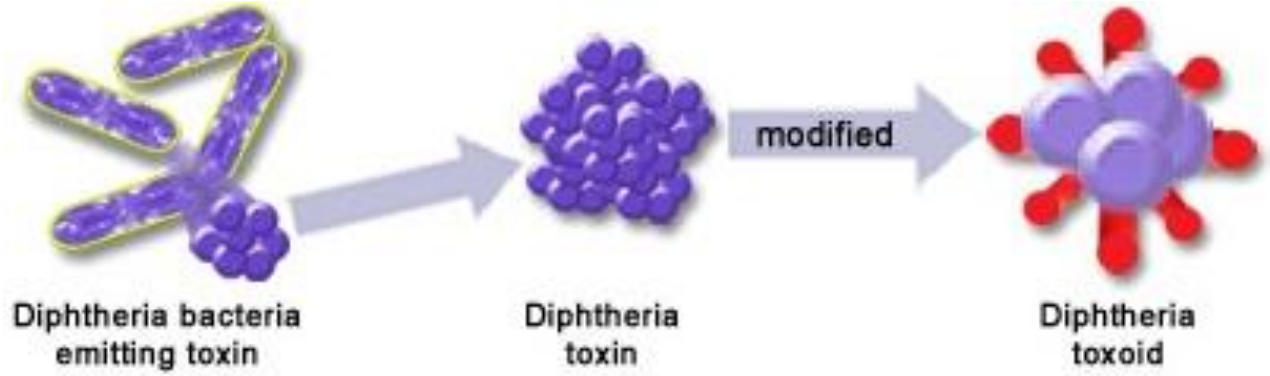
Toxoids (T)



- It is usually produced by inactivating toxins

by formaldehyde

- They are non-toxic
- Cultivation of bacteria's and production of toxins
- They can be liquid or adsorbed on a sorbent
- Lower reactivity (immune adjuvant additive needed)
- They are less immunogenic
- Usually not enough for a lifetime (after some time of repetition)
- WHO recommends vaccination against *diphtheria and tetanus* in the form of *TT* and *DT* vaccines

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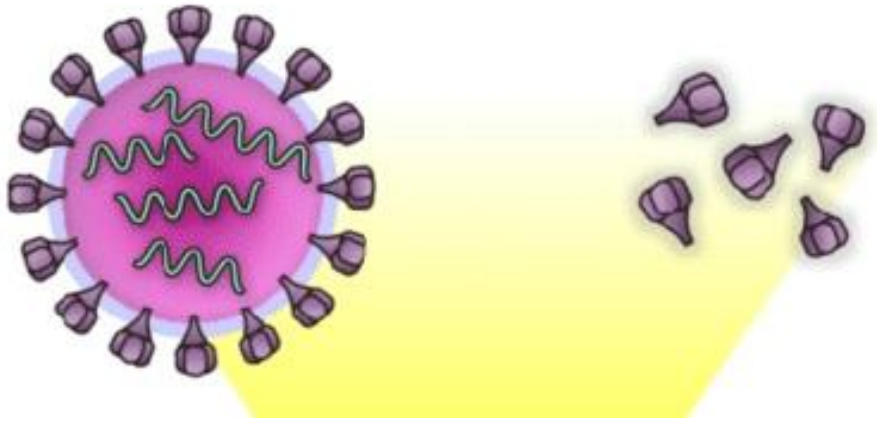
Subunit vaccines (SUV)



- They contain only microbial components
- They cannot cause infectious diseases
- They contain only surface antigens
- Lower reactivity
- Lower immunogenicity
- The killed pathogen is decomposed here, its components are separated and further used to prepare the vaccine.
- Usually not enough for a lifetime (after some time of repetition)
- **Types:**
- **Proteins:** from hepatitis B virus surface protein antigen (*HBsAg*) and acellular pertussis vaccine (*aP*)
- **Polysaccharides:** *Pneumo23*, which protects against 23 types of pneumococci (stimulates B cells)
- **Conjugated:** conjugation of e. g. polysaccharide with protein increases immunogenicity, e.g. with *DT* and *TT Haemophilus influenzae (HIb)* or seven-valent *(PCV-7)*, ten-valent *(PCV-10)* or thirteen-valent (*PCV-13*) pneumococcal conjugate against pneumococcus

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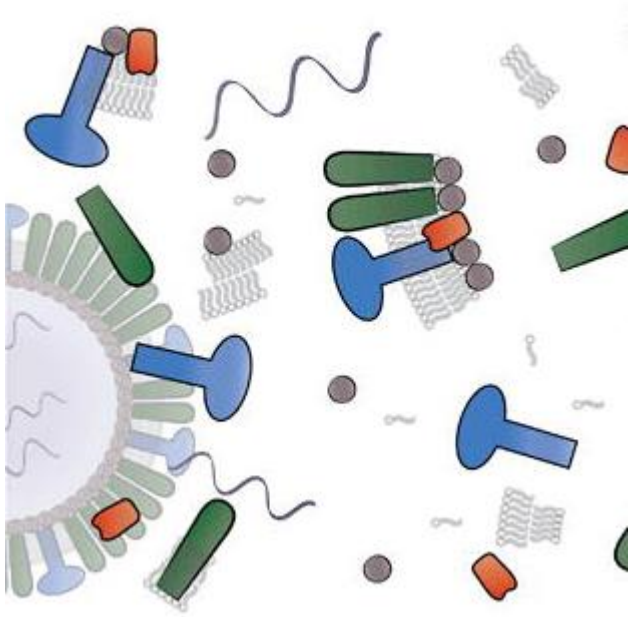
Splitting vaccines (SV)

- They only contain components of influenza viruses - *hemagglutinin* (H), *neuraminidase* (N), *RNA* and *nucleoproteins*
- Cleavage takes place, for example, with organic solvents
- They cannot cause infectious diseases
- They are more efficient than SUVs
- Reactivity low
- Immunogenicity acceptable
- Due to the mutations of the virus, it must be repeated
- They are used in the production of vaccines to prevent influenza



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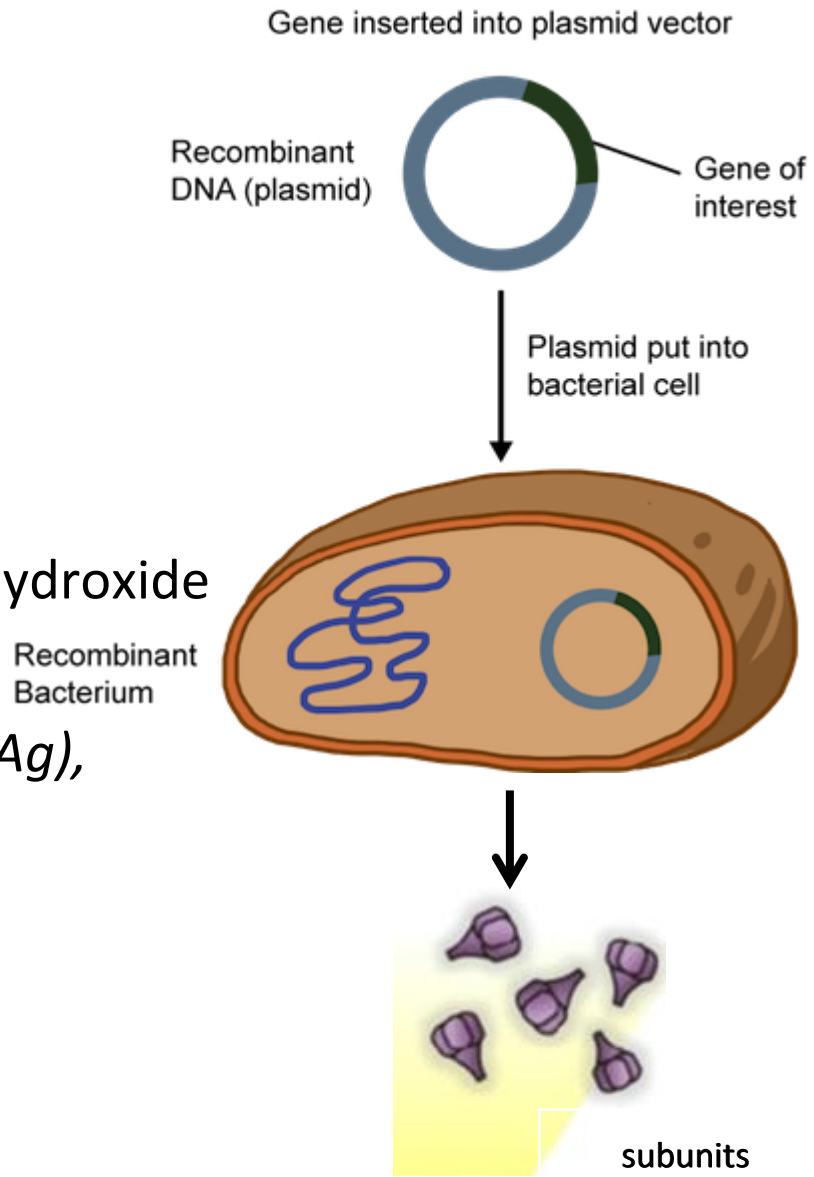
Recombinant vaccines (RV)

- They usually contain viral particles, or fragments (similar to SUVs)
- They are produced by genetic engineering methods
- Production of yeast, *Escherichia coli* or mammalian cells
- There is no need to cultivate the pathogen
- After cultivation digestion and chromatographic purification
- Usually, formaldehyde inactivation and absorption on aluminum hydroxide
- In general, the same applies as for SUVs
- From commercial vaccines: recombinant hepatitis *B vaccine (rHBsAg), human papillomavirus (HPV) vaccine*



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Virus like particles (VLP)

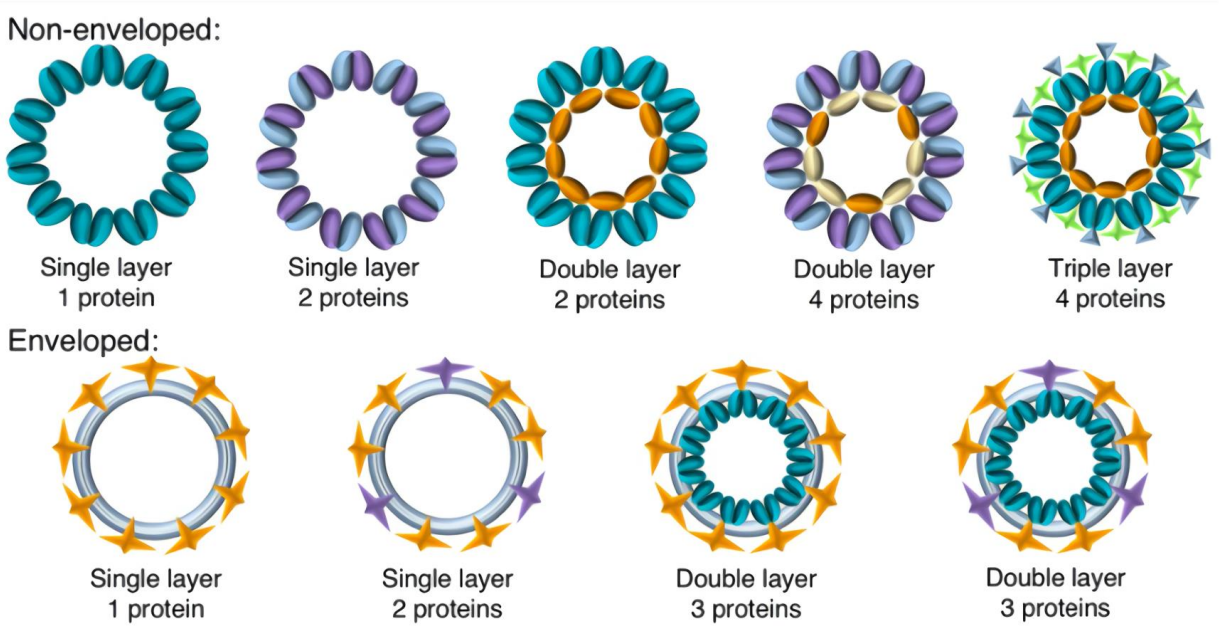
- Structures with dimensions of 30-90 nm
- Produced by r-bacteria and r-yeast
- The production phase of the SUV
- They are formed by self-stacking
- They do not contain nucleic acids,

often not even lipids

- They contain, for example, N and H

and nuclear proteins M1,2

- They cannot cause infectious diseases
- Induction of humoral and cellular immunity
- They cannot replicate (difference of LAV and VV)
- Reactivity lower
- Immunogenicity acceptable
- FDA approves *hepatitis B (HepB)* and *human papillomavirus (HPV)* vaccines



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Plant made vaccines (PMV)

- This is like the RV
- The host is a transgenic plant
- The gene carrier is *Agrobacterium tumefaciens*
- A plant is applied, e. g. a lyophilizate in an enteric capsule
- It should be completely safe
- Activation of intestinal M cells
- Increased activity of IgA and IgG
- Risk of low immunogenicity and development of tolerance
- To date, over 700 PMVs have been developed
- FDA approved: *B-subunit CTB*, which is a subunit of *B-cholera t*oxin, produced by rice seeds

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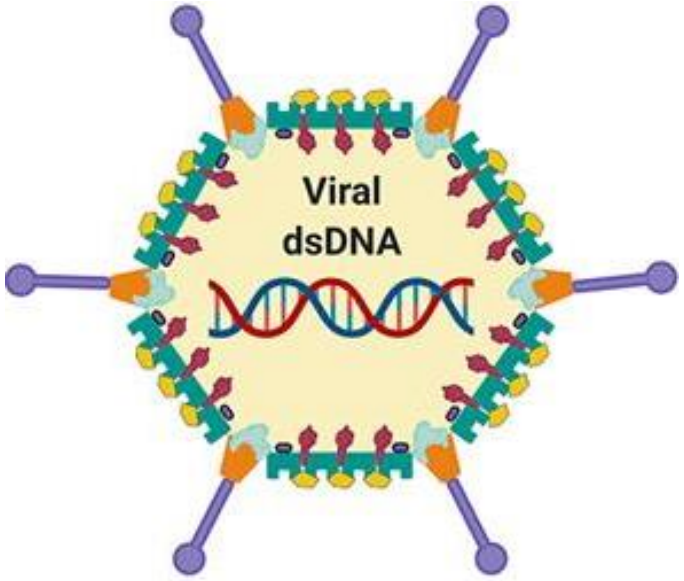
Vectoral DNA vaccines (VV)

- Antigen introduced into a non-pathogenic microbe
- Viral vectors: *adenoviruses, poxviruses, herpesviruses, picornaviruses, cytomegaloviruses* and *RNA retroviruses* and *flaviviruses*
- Most often *Vaccinia virus* (accommodates up to 30 genes)
- From several bacteria: *Typhimurium, Clostridium* or *Salmonella*
- They are replicating and non-replicating
- An epitope is formed on the surface, stimulating immunity
- They induce humoral and cellular immunity in essence,



the same applies to LAV, however, they do not cause disease infection

- Purification technologies are falling away
- One dose is usually sufficient
- Risk of altering the host genome
- Risk of destructive autoimmunity in cross-immune reaction to human DNA
- For example, human (*ad5*) and simian (*ad26*) Sputnik V (*Covid 19*) adenovirus vectors

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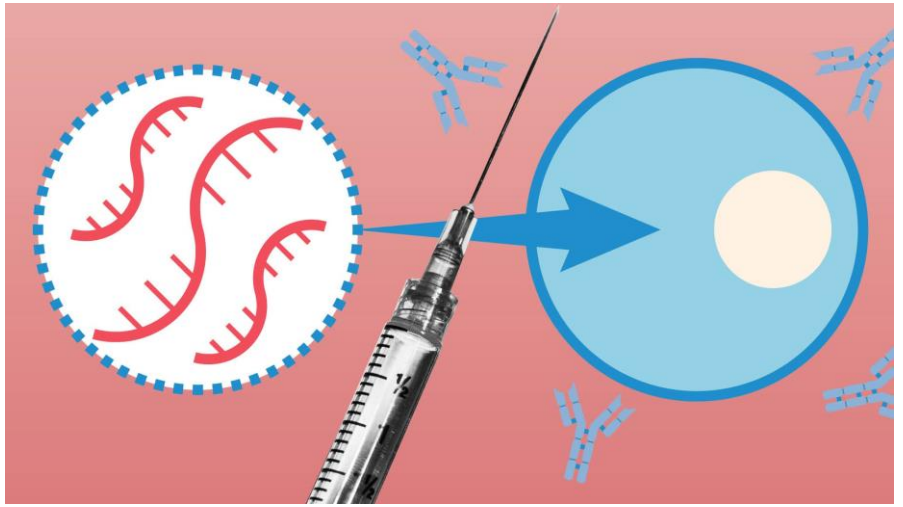
Gene (mRNA/DNA) vaccines



- Nucleic acid is introduced
- Plasmid DNA or mRNA
- Protein synthesis in ribosomes
- An epitope is formed on the surface(activation of T-cells and B cells), an antibody is also produced
- RNA bypasses the nucleus
- Possibility to hit non-mutating parts of viral RNA (conservative epitopes)
- The promise of a single dose option
- Reactivity (data varies) sometimes requires immunoadjuvant
- So far, there is no long-term clinical evaluation on humans
- Risk of host genome alteration in DNA
- Risk of destructive autoimmunity directly cross-immune reaction to DNA
- A series of vaccines for *Covid-19*, in the clinic *Zika, HIV*

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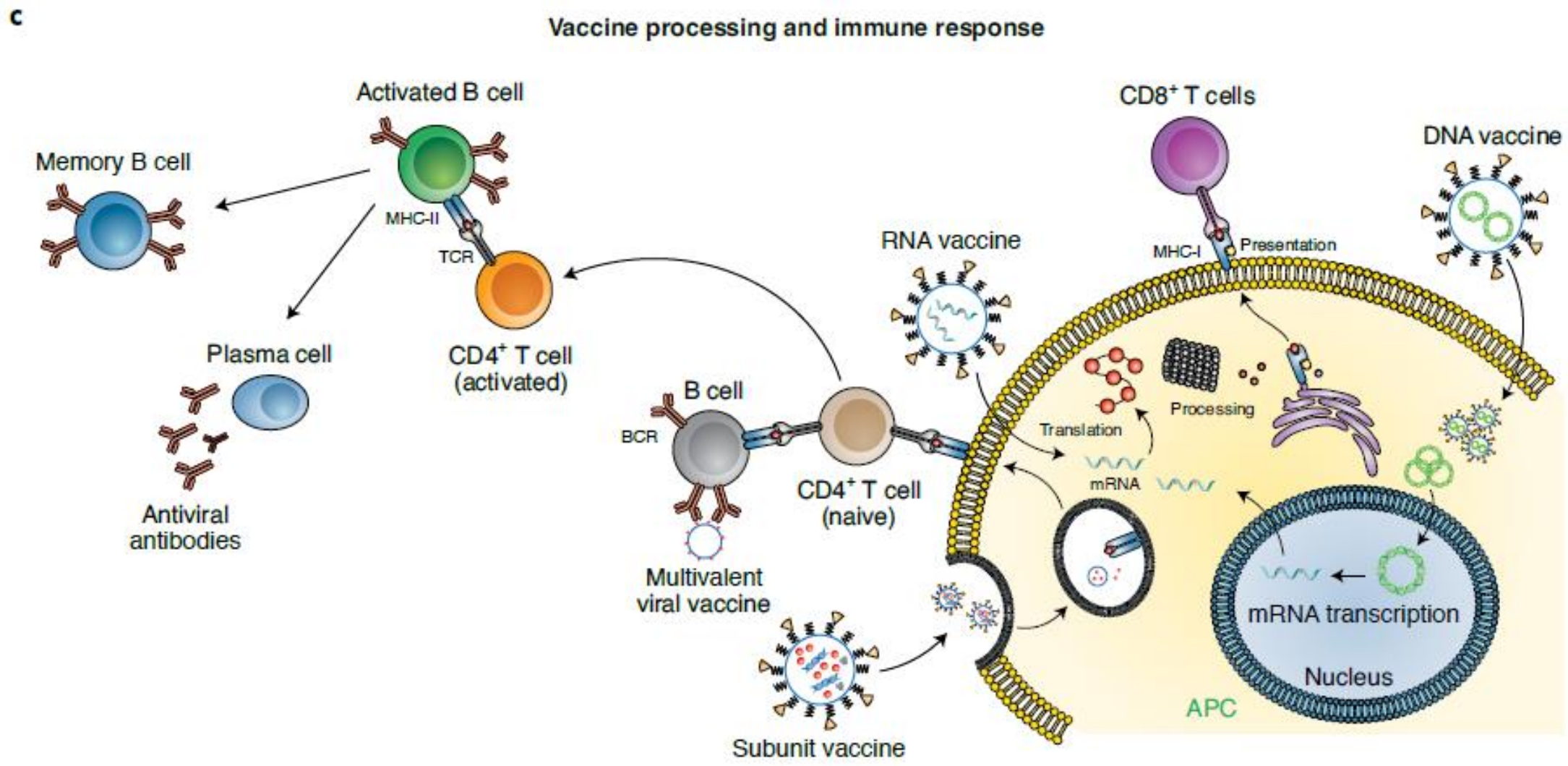
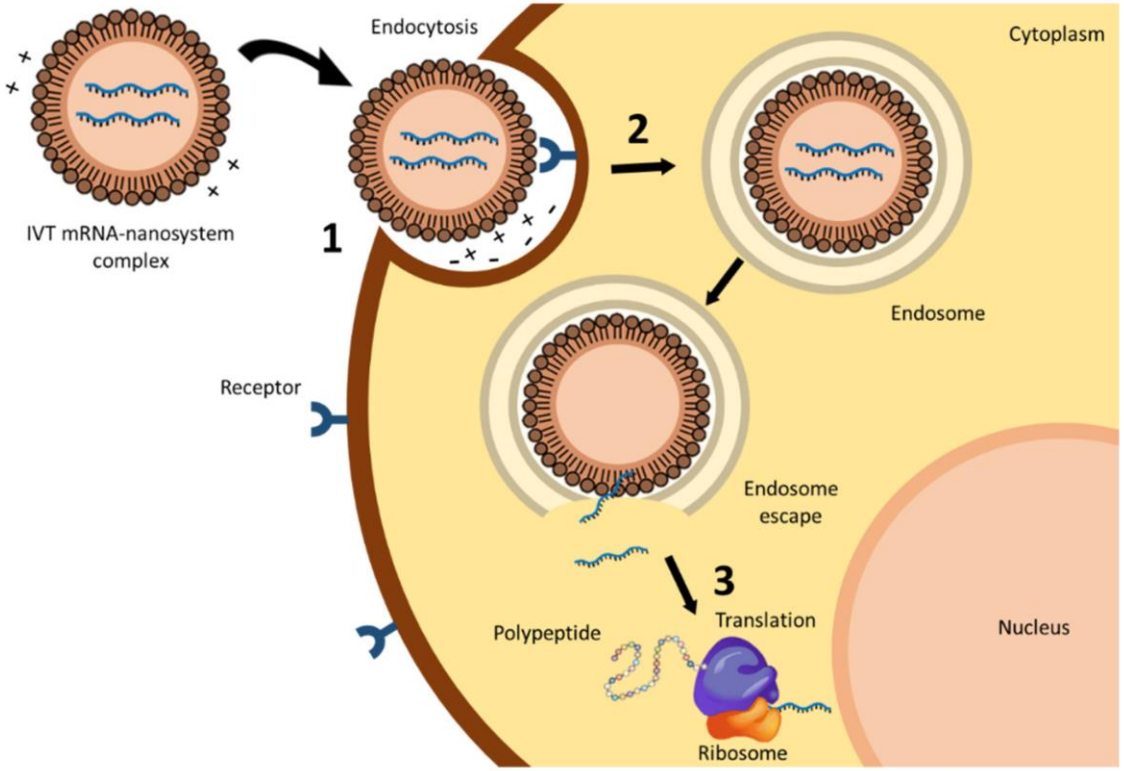
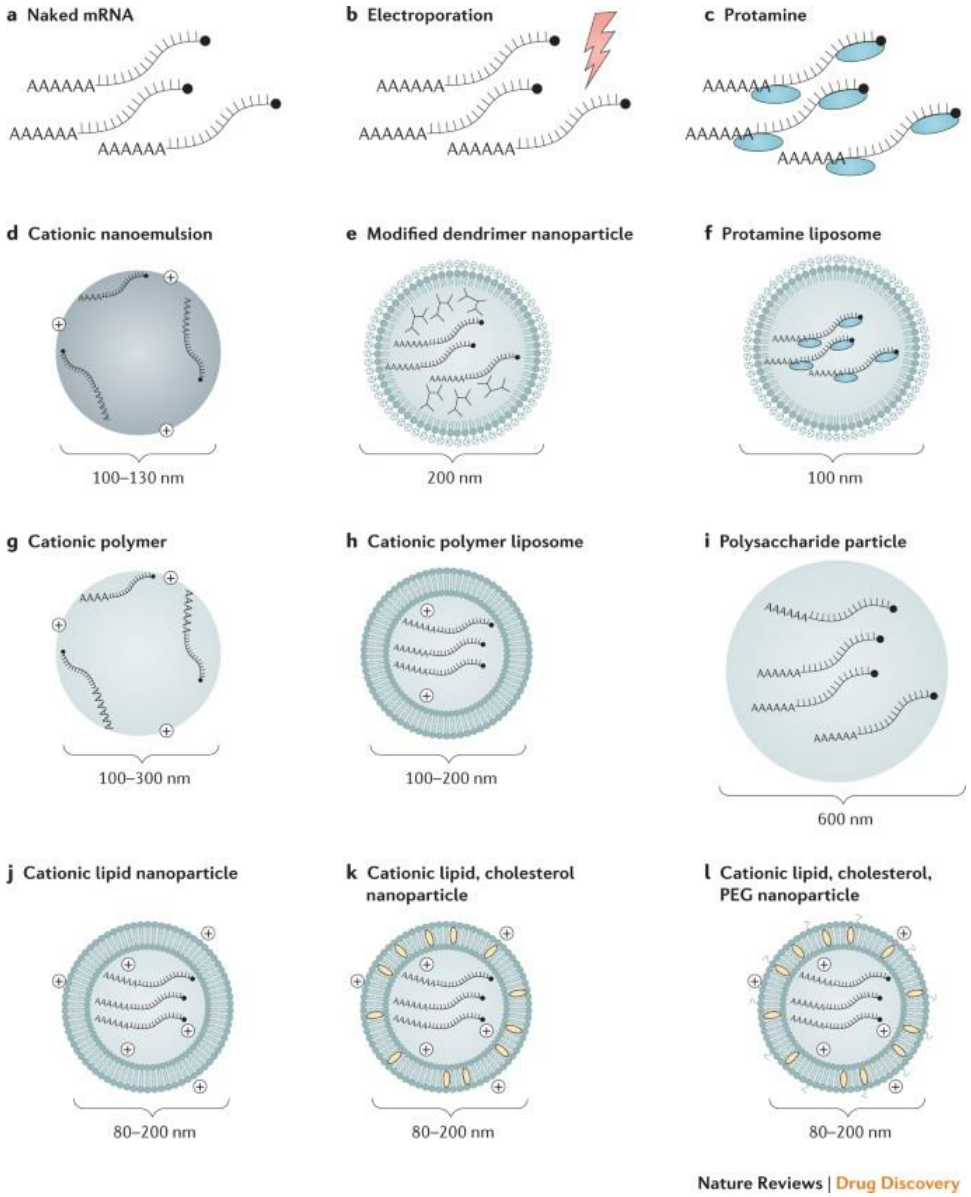
Gene (mRNA/DNA) vaccines



Immunity creation mechanism (Matthew D. Shin, et al.)

Gene vaccine transport systems





Translation (Gómez-Aguado et al.)

Translation systems mRNA (Pardi et al.)

Summary

Vaccine type	Infection
KAV (killed antigen vaccine)	wP, IPV, ccIIV3, eIPV, JE-MB, JE-VC, ViCPS
LAV (live attenuated vaccine)	LAIV, LAIV4, BCG, OPV, YF
SUV (subunit vaccines)	aP, HBsAg
CV (conjugate vaccine)	HIb, PCV-7, PCV-10, PCV-13
SV (splitting vaccines)	TIV, QIV, IIV
T (toxoid)	TT, DT, DTP, Td, DTaP
RV (recombinant vaccines)	RIV3, RZV, rHBsAg, HPV
PMV (plant made vaccines)	CTB
VLP (virus like particles)	HepB, HPV
VV (vector vaccines)	YFV 17D* SARS-COV-2**
DNA/RNA	ZIKV, HIV SARS-COV-2

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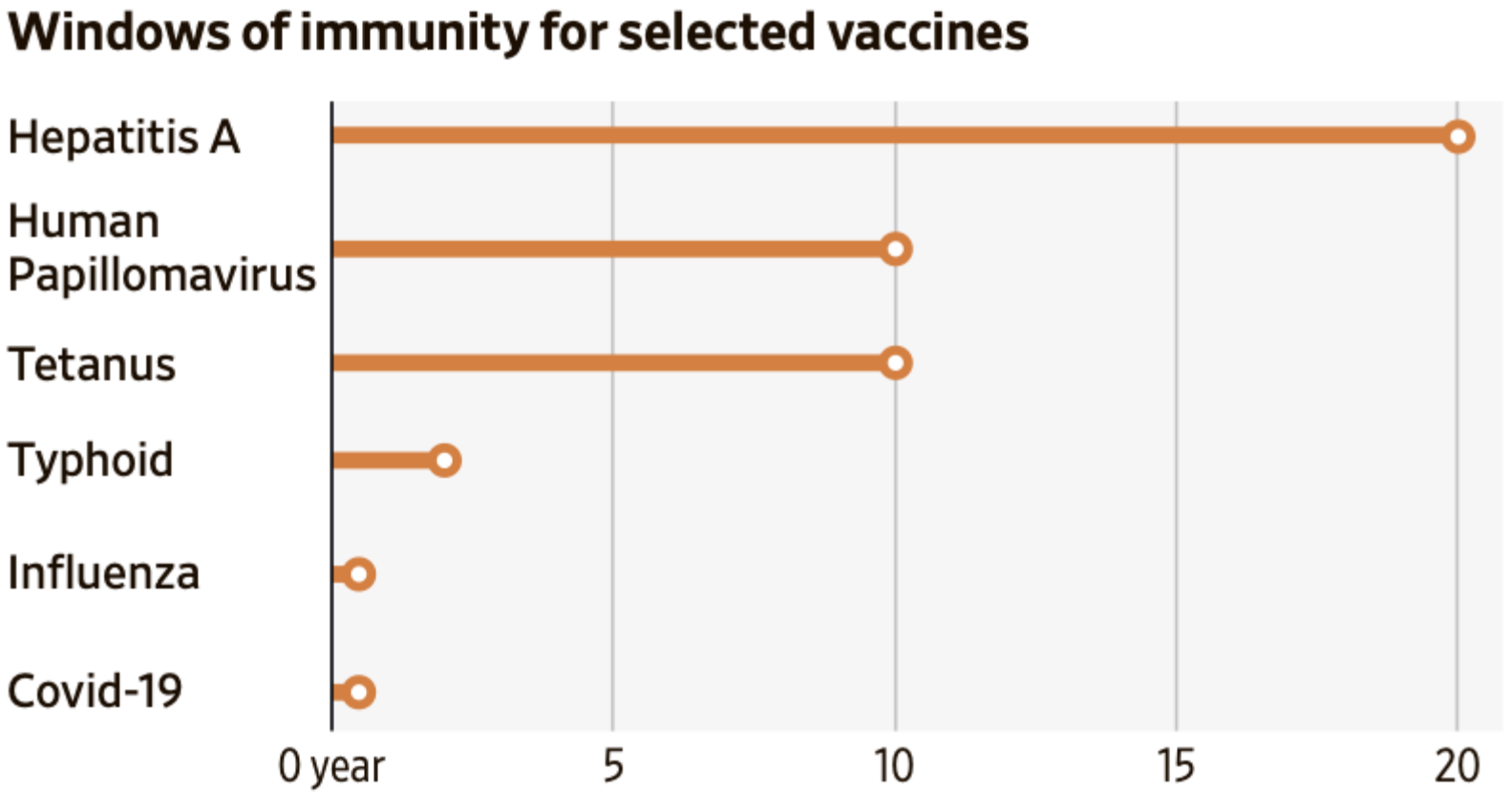
Duration of immunity



Sources: San Francisco Department of Public Health (hepatitis A); National Institutes of Health (human papillomavirus); Centers for Disease Control and Prevention (tetanus, typhoid, influenza, Covid-19)

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Contraindication

- **Anaphylactic reaction** after a previous dose or any component of the vaccine (*BCG, DTwP, DTaP, OPV, IPV, HepB, rotavirus, morbillivirus, HiB, PCV-7* and *YF*, residual proteins, antibiotics, possibly preservatives (egg protein, contained in chicken eggs).
- **Autoimmune disease** or during more **severe diarrhea and viral respiratory disease.**
- Vaccines containing thiomersal are **not recommended during pregnancy**.
- Pertussis-containing vaccines (Infarix® and Hexainfarix®) in encephalopathy one week after the previous. dose, progressive **encephalopathy, progressive neurological disorders including infantile spasms, or uncontrolled epilepsy**.
- Vaccine with a live microbe (against measles, mumps and rubella (e.g. Priorix® and Priorix tetra®) contraindicated **in case of pregnancy and severe immunodeficiencies**.
- TBC vaccination is contraindicated in case of **history of tuberculosis, positive tuberculin skin test, immunodeficiency, generalized skin disease and pregnancy.**

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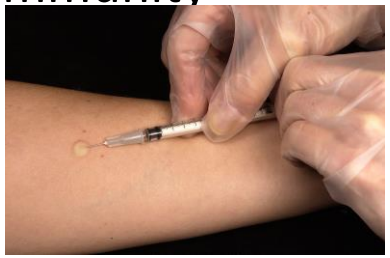
Advantages and complications

Enhancement of non-specific immunity

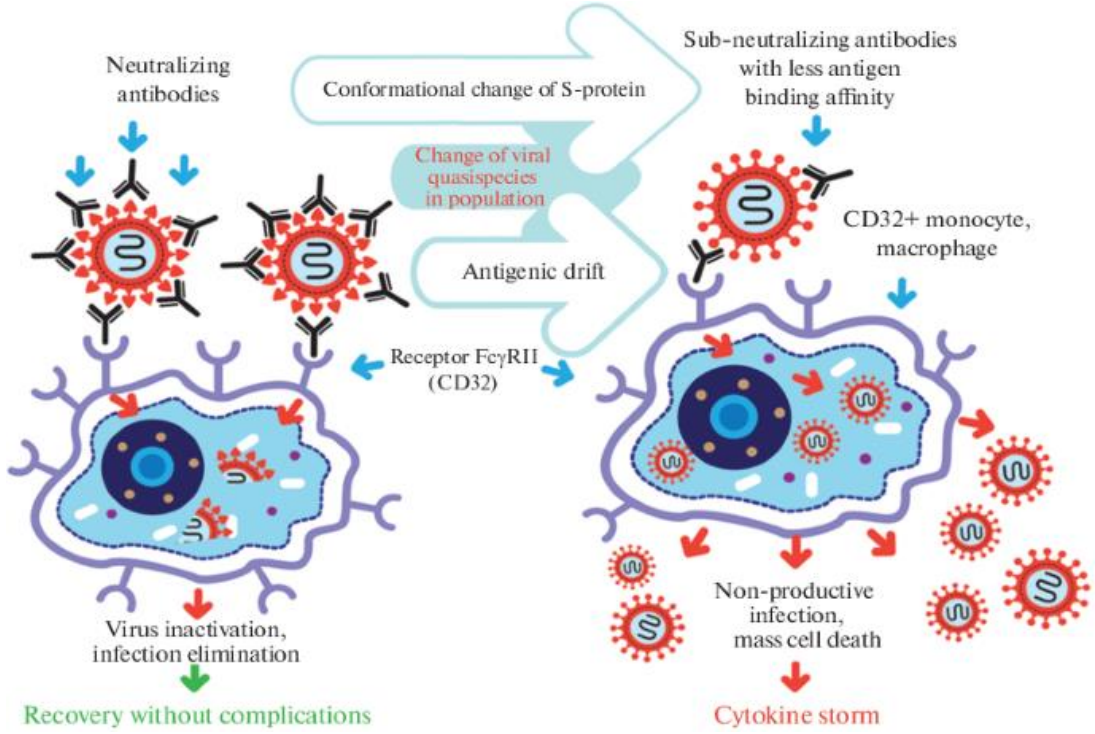
- increase of non-specific immunity
- BCG calmetization
- part survives after injection
- chemotaxis of immune cells
- formation of a tubercular nodule
- emergence of cellular immunity
- after 3-4 weeks redness (even necrosis)
- **non-specific immunity also occur**
- tuberculin test to check immunity

post-vaccination



tuberculine test

Antibody dependent enhancement (ADE)
Vaccine associated enhanced disease (VAED)



Zaichuk, T.A., Nechipurenko, Y.D., Adzhubey, A.A. *et al.* The Challenges of Vaccine Development against Betacoronaviruses: Antibody Dependent Enhancement and Sendai Virus as a Possible Vaccine Vector. *Mol Biol* **54,** 812–826 (2020). https://doi.org/10.1134/S0026893320060151

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Immuno adjuvants

Type	Antigen	Adjuvant
LAV	variola, polio, morbili, parotitis, ruboella, poxvirus, rotavirus, Shigela, influenza, YF, TBC, typhus	No
KA	polio, Japan encephalitis, hepatitis A, influenza, rabies, pertussis	Yes/No
T	tetanus, anthrax, diphteria	Yes
SUV polysacharide	pneumoccocus for adults with DT	No
SUV conjugated	pneumoccocus for children, haemophilus B, bacterial meningitis	Yes/No
SUV proteins	pertussis	Yes/No
VLP	hepatitis B, muman papilomavirus	Yes
mRNA/DNA	Covid-19	Yes/No
VV with DNA	Covid-19	No

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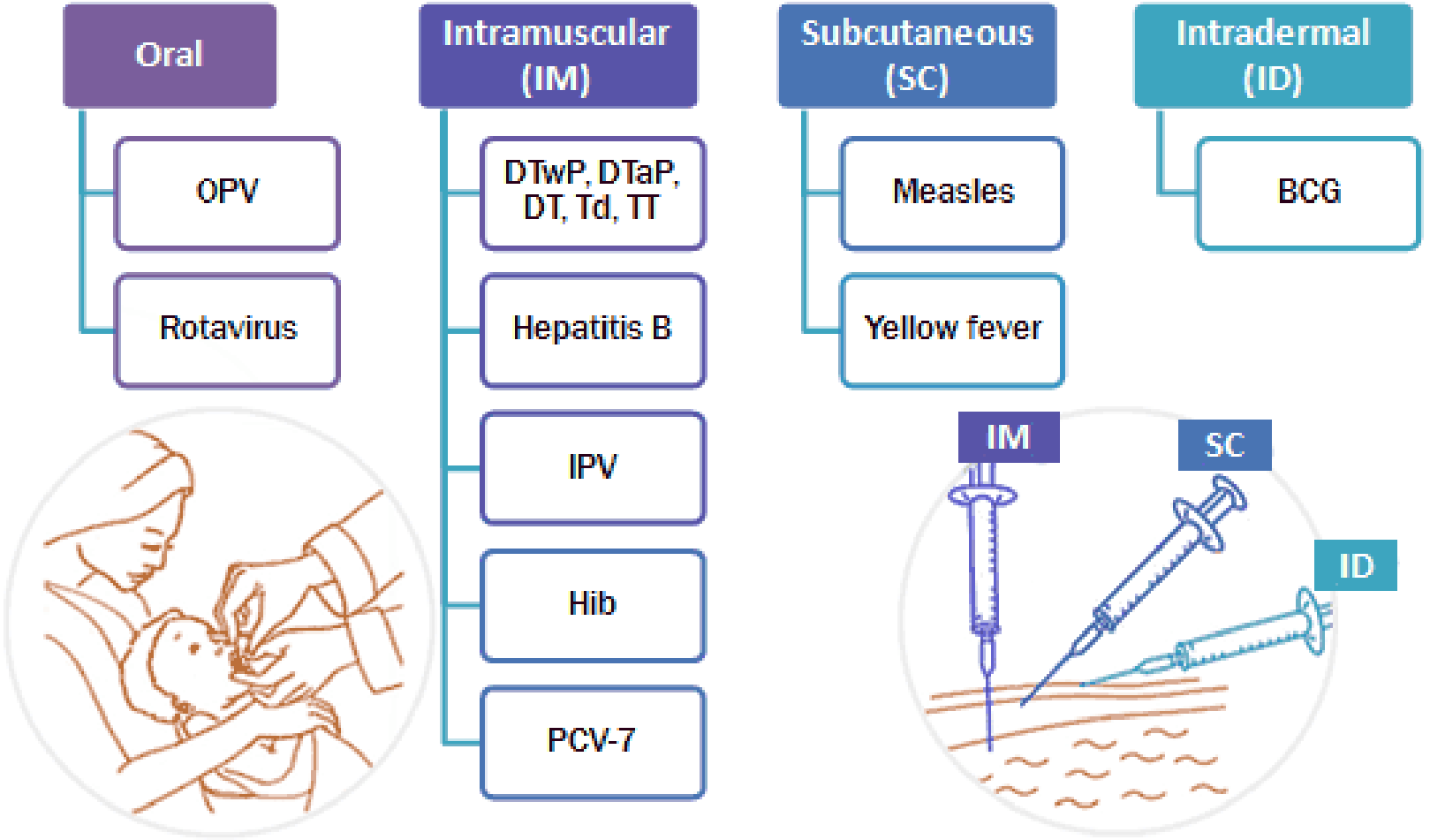
Dosage forms

Transdermalia (BCG)
ultrasound, iontophoresis, microneedles, liposomes, solid nanoemulsions, transferosomes, ethosomes, cubosomes, niosomes, dispersion in oil

Nasalia (LAIV A, B)
membrane-enveloped microparticles (OMP), liposomes, immunostimulatory complexes (ISCOM) or, for example, PLGA-based microparticles

Peroralia (OPV, Ty21A)
niosomes, liposomes, bilosomes, ISCOM, polymer microparticles (MPs) and nanoparticles (NPs) from PLA and PLGA, phospholipids, chitosan, etc. The particles can then be coated with enteric or colonic polymers (Eudragit FS)



Zdroj: WHO – Vaccine Safety Basic

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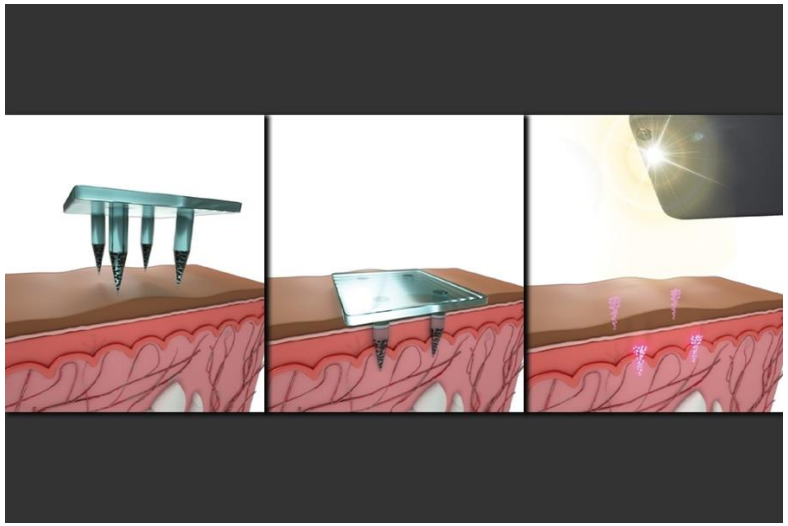
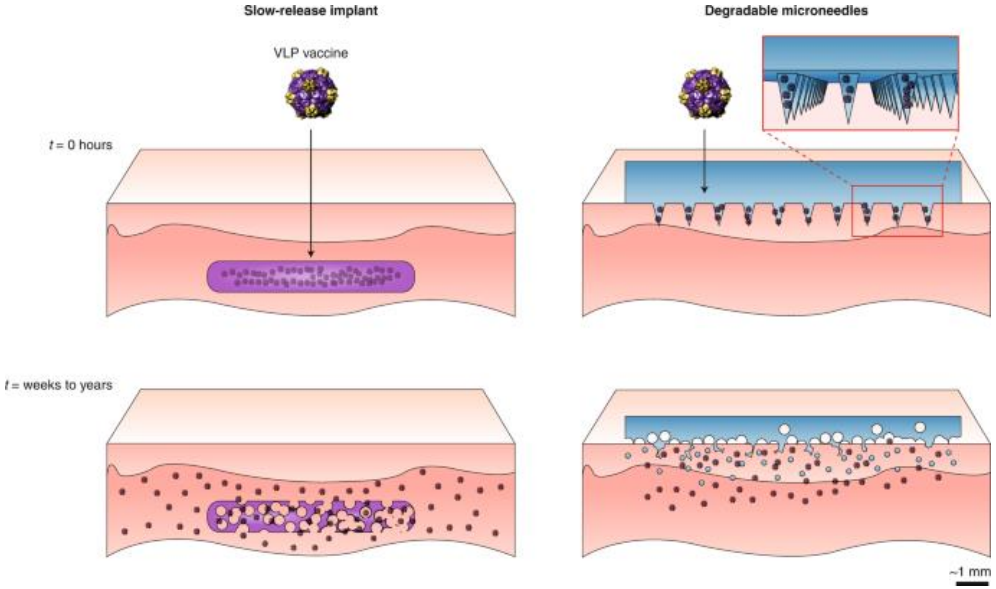
Progess in vaccine development



Bill and Melinda Gates Foundation and the Koch Institute Support (core) Grant from the National Cancer Institute.

2018



Possibility of controll release of vaccines (Shin et al.)

2020

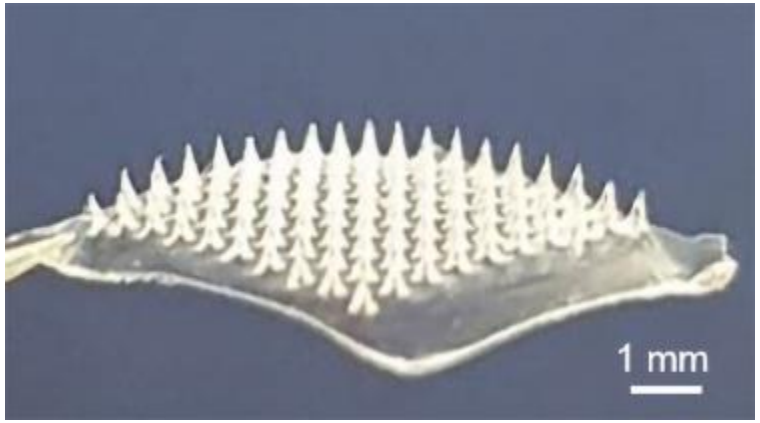


Image of the COVID-19 DNA vaccine delivering microneedle patch [Credit: Adapted from ACS Nano 2021, DOI: 10.1021/acsnano.1c03252].

2021

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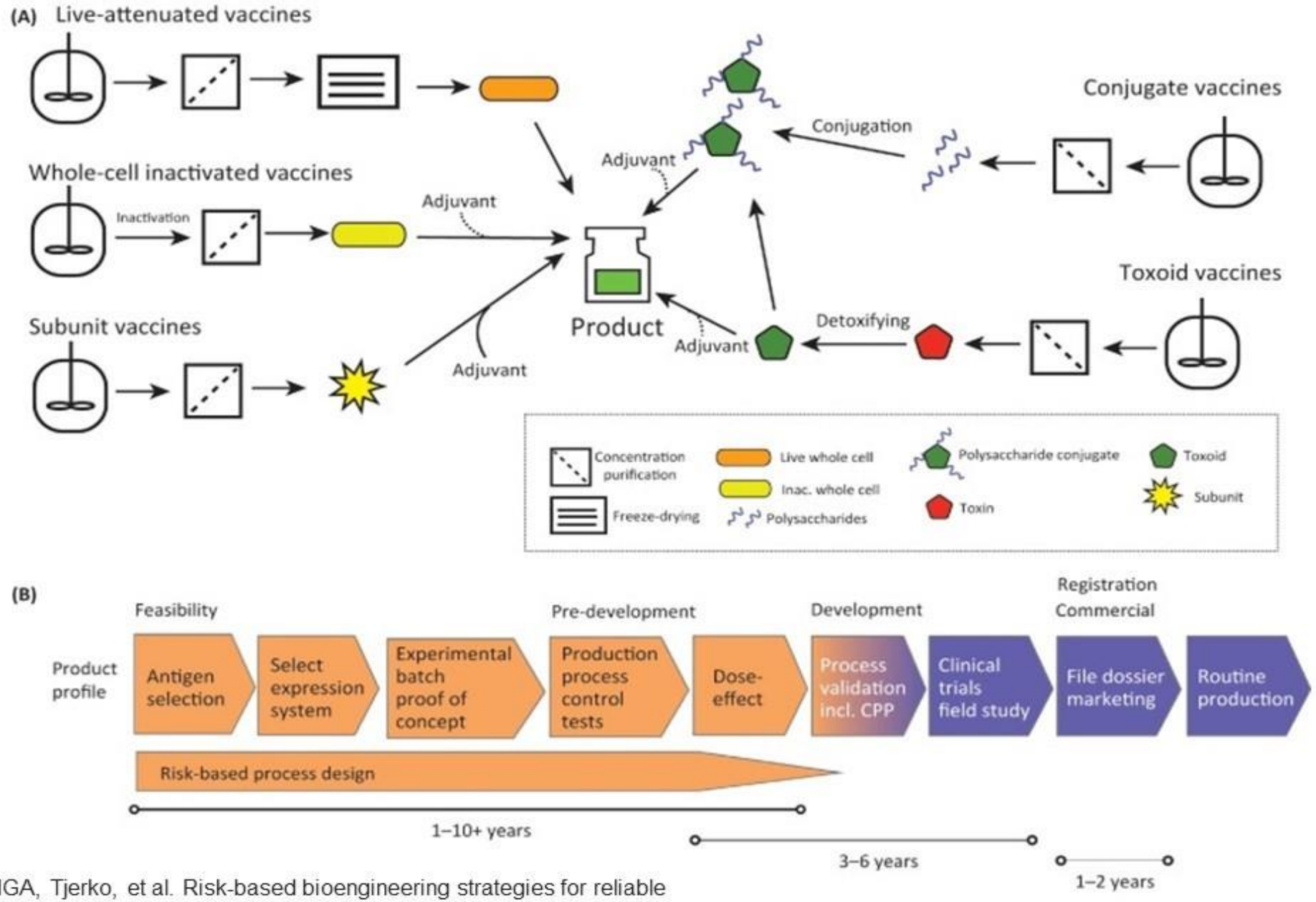
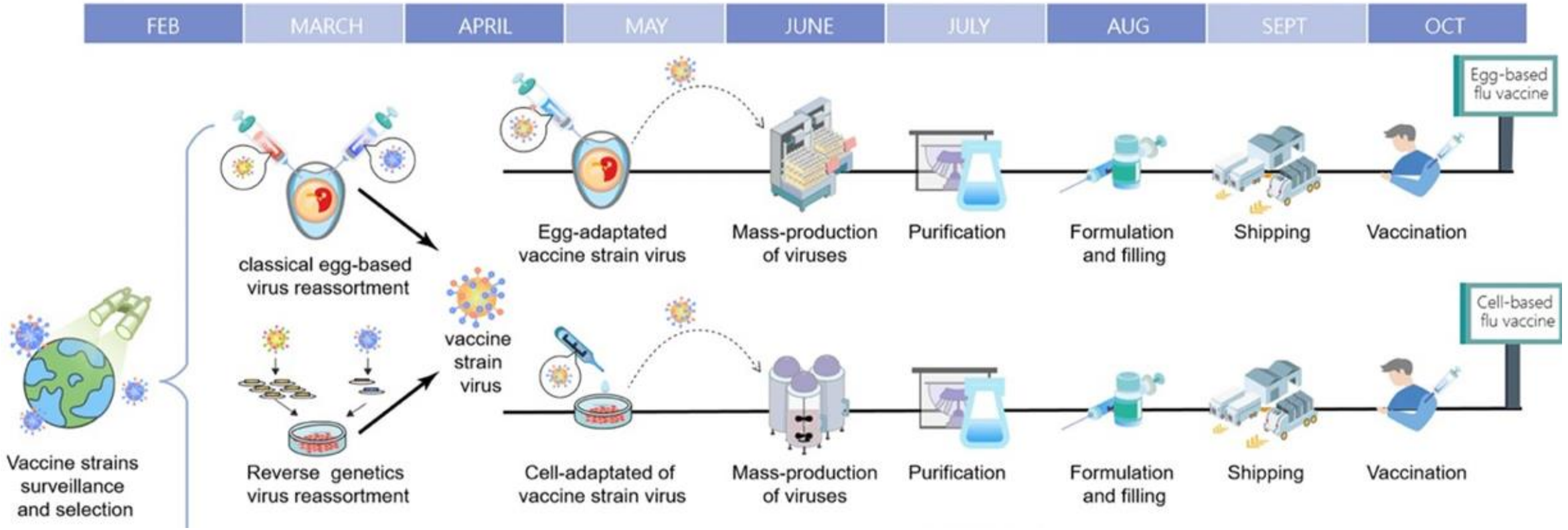
Production scheme



Zdroj: KAMMINGA, Tjerko, et al. Risk-based bioengineering strategies for reliable bacterial vaccine production. *Trends in biotechnology*, 2019, 37.8: 805-816.

Principle of production influenza vaccines



Zdroj: CHEN, Juine-Ruey, et al. Better influenza vaccines: an industry perspective. Journal of Biomedical Science, 2020, 27.1: 1-11.

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Vaccine development

EMMA Int. Cons. Group

- **Identification and isolation of antigen**

(preclinical and dosage form development)

- **Clinical evaluation:**

I. Phase (cca 10 – 100 participants) safety and immunogenity
II. Phase (cca 100 – 1000 participants) adverse effects and dosage
III. Phase (cca 1 000 – 10 000 participants, multicentric studies) clinical evaluation
IV. Phase – postmarked – adverse effects and consequent clinical studies

- **Stability:** short and long term
- **Pharmacopoeial evaluation:** pH; Al, Ca, formaldehyde, fenol, endotoxins content
- **Technology transfer:** (PAT technology, QbD a MVDA)
- **Registration:** national authority approval
- **Time of development:** 10 – 16 years

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Vaccination challenge

Present:

- **Cancer:** 9 600 000 (2018) – some vaccines now exist
- **TBC:** 1 700 000 (2006); infected up to 25 % population; 10 000 000 new cases, 5 – 10 % manifested
- **Influenza:** cca 300 000 – 600 000 deaths per year; efficiency 10 – 60 %
- **RSV:** cca 60 000 death children to 5 years; 3 000 000 hospitalized
- **Malaria:** cca 770 000 deaths; 38 000 000 infected
- **Spanish flue:** 40 – 50 000 000 deaths (1918 – 1919); after calculating for the current population: 160 – 200 000 000, cca to 2.5 % deaths (recalculation: 1.25 % deaths per year)

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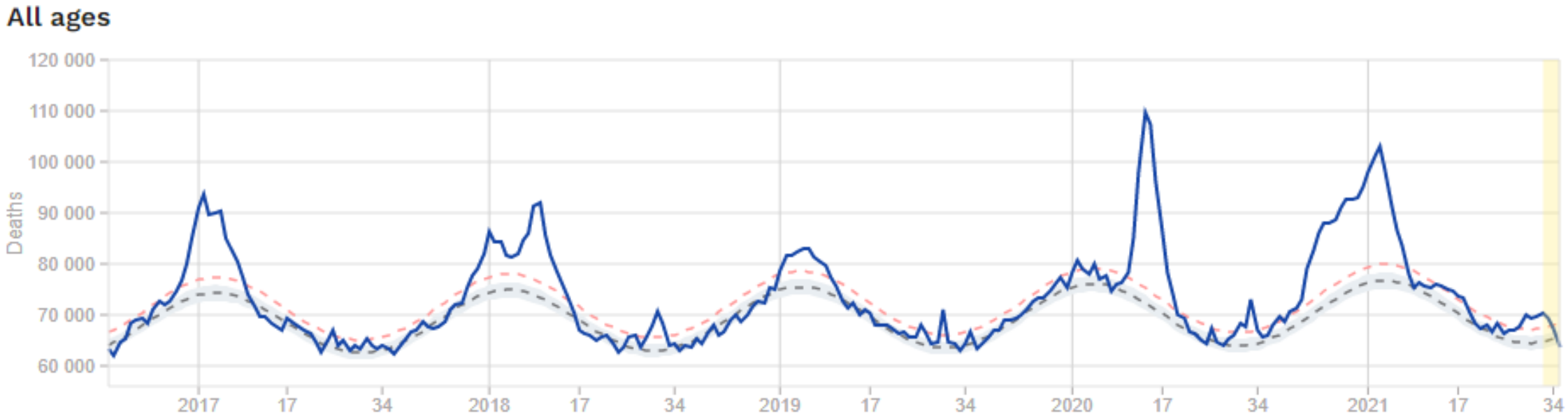
Vaccination challenge



Deaths during years https://www.euromomo.eu/graphs-and-maps

The number of epidemics is 1600 - 1800 cases per 100.000 inhabitants

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Vaccination challenge

Zdroj: Kyncl, J., et al. "A study of excess mortality during influenza epidemics in the Czech Republic, 1982–2000." *European Journal of Epidemiology* 20.4 (2005): 365-371.

Table 1. Estimated excess number of annual deaths attributable to influenza in the Czech Republic, 1982 – 2000

Season	Peak weekly ARI morbidity (per 100,000 population)	Duration of epidemic (weeks)	Predominant influenza virus strain	Difference between observed and expected annual deaths from all causes		Difference between observed and expected annual deaths from diseases of the circulatory system		No. of deaths from all causes above tolerance level (epidemic periods)
				absolute number	rate per 100,000 population	absolute number	rate per 100,000 population	
1982–1983	3874	9	A/Bangkok 1/79 (H3N2)	5507	53.68	3148	30.69	4259
1983–1984	2108	13	A/Brazil 11/78 (H1N1) B/Singapore/222/79	4841	47.18	3202	31.20	649
1984–1985	1671	9	A/Philippines 2/82 (H3N2)	2584	25.17	2041	19.88	781
1985–1986	3323	12	A/Philippines 2/82 (H3N2)	4570	44.50	2174	21.17	2612
1986–1987	2634	5	A/Singapore 6/86 (H1N1)	1124	10.94	1132	11.02	0
1987–1988	1820	4	A/Singapore 6/86 (H1N1)	−918	−8.93	−1020	−9.93	0
1988–1989	3885	8	A/Sichuan 2/87 (H3N2) A/Singapore 6/86 (H1N1)	1605	15.61	976	9.50	692
1989–1990	1622	13	A/Sichuan 2/87 (H3N2)	3481	33.86	2632	25.60	667
1990–1991	2955	6	B/Victoria 2/87 B/Yamagata 16/88	2631	25.59	1668	16.22	796
1991–1992	3005	5	A/Beijing 353/89 (H3N2)	2604	25.47	1599	15.64	1168
1992–1993	2234	8	B/Panama 45/90	1823	17.81	1372	13.41	708
1993–1994	1496	7	A/Shandong 9/93 (H3N2)	2209	21.57	1745	17.04	218
1994–1995	1055	11	A/Johannesburg 33/94 (H3N2) B/Beijing 184/93	−597	−5.83	−730	−7.13	0
1995–1996	3647	15	A/Wuhan 359/95 (H3N2) A/Johannesburg 33/94 (H3N2)	6172	60.41	4501	44.05	3247
1996–1997	2935	12	B/Harbin 7/94 A/Bayern 7/95 (H1N1)	3035	29.75	2032	19.92	590
1997–1998	1852	14	A/Wuhan 359/95 (H3N2) A/Bayern 7/95 (H1N1)	352	3.45	176	1.73	0
1998–1999	3258	6	A/Sydney 5/97 (H3N2) B/Harbin 7/94	3478	34.20	2459	24.17	1651
1999–2000	2803	5	A/Sydney 5/97 (H3N2) A/Moscow 10/99 (H3N2)	3399	33.47	2422	23.85	2216
Mean				2661	25.99	1752	17.11	1125

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Pandemics in history

Name	Time period	Type / Pre-human host	Death toll
Antonine Plague	165-180	Believed to be either smallpox or measles	5M
Japanese smallpox epidemic	735-737	Variola major virus	1M
Plague of Justinian	541-542	Yersinia pestis bacteria / Rats, fleas	30-50M
Black Death	1347-1351	Yersinia pestis bacteria / Rats, fleas	200M
New World Smallpox Outbreak	1520 – onwards	Variola major virus	56M
Great Plague of London	1665	Yersinia pestis bacteria / Rats, fleas	100,000
Italian plague	1629-1631	Yersinia pestis bacteria / Rats, fleas	1M
Cholera Pandemics 1-6	1817-1923	V. cholerae bacteria	1M+
Third Plague	1885	Yersinia pestis bacteria / Rats, fleas	12M (China and India)
Yellow Fever	Late 1800s	Virus / Mosquitoes	100,000-150,000 (U.S.)
Russian Flu	1889-1890	Believed to be H2N2 (avian origin)	1M
Spanish Flu	1918-1919	H1N1 virus / Pigs	40-50M
Asian Flu	1957-1958	H2N2 virus	1.1M
Hong Kong Flu	1968-1970	H3N2 virus	1M
HIV/AIDS	1981-present	Virus / Chimpanzees	25-35M
Swine Flu	2009-2010	H1N1 virus / Pigs	200,000
SARS	2002-2003	Coronavirus / Bats, Civets	770
Ebola	2014-2016	Ebolavirus / Wild animals	11,000
MERS	2015-Present	Coronavirus / Bats, camels	850
COVID-19	2019-Present	Coronavirus – Unknown (possibly pangolins)	4,700 (as of Mar 12, 2020)

Number of inhabitans

1. Century were cca 200 millions

1 000 – 1 500 cca 400 – 460 millions
1 500 – 1 800 cca 460 – millions
1 800 – 1 900 cca 1 – 1.65 billions
1 900 – 1 950 cca 1.65 – 2.52 billions
1 950 – 2 000 cca 2.52 – 6.10 billions
2 000 – 2 020 cca 6.10 – 7.70 billions

History of pandemics (WoF, 2020)

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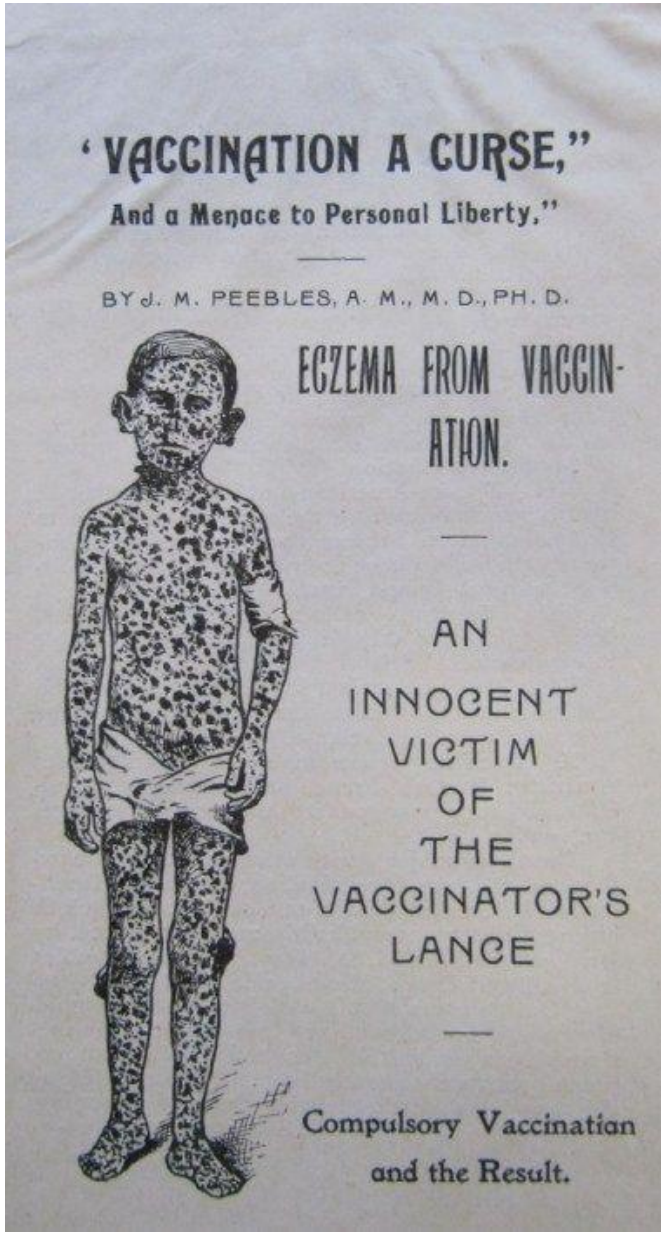
Arguments against vaccination

- The presence of heavy metals
- The presence of residues from cultivation
- Culture cells from human embryos
- Alteration of host DNA in gene vaccines
- Penetration of nanoparticles into body tissues, including the brain
- Autoimmunity
- Insufficient clinical evaluation
- Involuntary vaccination
- Pressure from pharmaceutical companies
- Conflict of interest of government officials



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