Peptides in vaccine research

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(updated: March 2017)

Table 1: Summary of WHO Position Papers - Recommendations for Routine Immunization

Antigen		Children (see Table 2 for details)		Adolescents	Adults	Considerations (see footnotes for details)
Recommendations for all immunization programmes						
BCG[1]		1 dose				Exceptions HIV
Hepatitis B[2]		3-4-doses (see footnote for schedule options)		3 doses (for high-risk groups if not previously immunized) (see footnote)		Birth dose Premature and low birth weight Co-administration and combination vaccine Definition high-risk
Polio[3]		3-4 doses (at least one dose of IPV) with DTP				bOPV birth dose Type of vaccine Transmission and importation risk criteria
DTP-containing vaccine[4]		3 doses	2 Boosters 12-23 months (DTP-containing vaccine) and 4-7 years (Td)	1 Booster 9-15 yrs (Td)		Delayed/interrupted schedule Combination vaccine Maternal immunization
***Haemophilus influenzae* type b**[5]	**Option 1**	3 doses, with DTP				Single dose if > 12 months of age Not recommended for children > 5 yrs old Delayed/interrupted schedule Co-administration and combination vaccine
	Option 2	2 or 3 doses, with booster at least 6 months after last dose				
Pneumococcal (Conjugate)[6]	**Option 1**	3 doses, with DTP				Vaccine options Initiate before 6 months of age Co-administration HIV+ and preterm neonates booster
	Option 2	2 doses before 6 months of age, plus booster dose at 9-15 months of age				
Rotavirus[7]		Rotarix: 2 doses with DTP RotaTeq: 3 doses with DTP				Vaccine options Not recommended if > 24 months old
Measles[8]		2 doses				Combination vaccine; HIV early vaccination; Pregnancy
Rubella[9]		1 dose (see footnote)		1 dose (adolescent girls and/or child bearing aged women if not previously vaccinated; see footnote)		Achieve and sustain 80% coverage Combination vaccine and Co-administration Pregnancy
HPV[10]				2 doses (females)		Target 9-14 year old girls; Multi-age cohort vaccination; Pregnancy Older age groups ≥ 15 years 3 doses HIV and immunocompromised

Refer to http://www.who.int/immunization/documents/positionpapers/ for most recent version of this table and position papers.

- WHO Recommendation, 2017

Kötelező védőoltások	
Oltás neve	Beadás időpontja
BCG	0-4 hetesen
DTPa+IPV+Hib	2 hónaposan
PCV-13	2 hónaposan
DTPa+IPV+Hib	3 hónaposan
DTPa+IPV+Hib	4 hónaposan
PCV-13	4 hónaposan
MMR	15 hónaposan
PCV-13	15 hónaposan
DTPa+IPV+Hib	18 hónaposan
DTPa+IPV	6 éves korban

Kampányoltások	
MMR revakcináció	11év, általános iskola 6. osztályában szeptember
dTAp emlékeztető oltás	11év, általános iskola 6. osztályában október
Hepatitis B	13 év, általános iskola 7. osztályában, szeptember

Hungarian vaccination schedule, EMMI 2017

BCG: vaccine against TB (tuberculosis)
DTPa: Diphteria, Tetanus: lockjaw, Pertussis: wooping cough
IPV: Inaktivated poliovirus vaccine. Poliomyelitis= infantile paralysis
Hib: vaccine against Haemophilus influenzae b, bacterial meningitis
PCV 13: Pneumococcus bacteria may cause inflammation of the lungs, brain and middle ear
MMR = Morbilli: measles, Mumps: parotitis epidemica, Rubeola: roseola
dTap = diphteria-tetanus-pertussis components containing vaccine as booster vaccination
Hepatitis B: vaccine against Hepatitis, the contagious inflammation of the liver

Immunization Coverage - Fact sheet

- During 2016, about 86% of infants worldwide (116.5 million infants) received 3 doses of diphtheria-tetanus-pertussis (DTP3) vaccine, protecting them against infectious diseases that can cause serious illness and disability or be fatal.
- Immunization averts an estimated 2 to 3 million deaths every year from diphtheria, tetanus, pertussis (whooping cough), and measles; however, an additional 1.5 million deaths could be avoided if global vaccination coverage improves.
- An estimated 19.5 million infants worldwide are still missing out on basic vaccines.
- Uptake of new and underused vaccines is increasing.

http://www.who.int/mediacentre/factsheets/fs378/en/

History of the Immunization Schedule - I

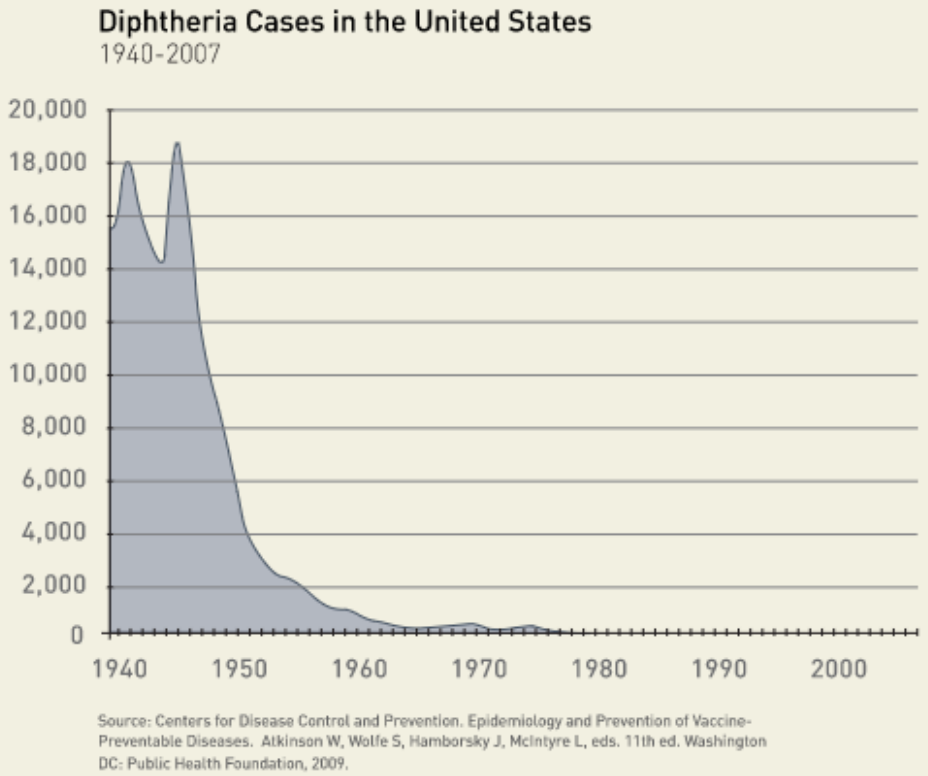
Recommended Vaccines

- Smallpox
- DTP NEW

The United States Public Service Act of 1944 mandated that the federal government issue licenses for biological products, including vaccines. This helped lead the way for a standardized system of vaccine regulation.

Smallpox vaccine had been available since the early 1800s. By the mid-1900s, it was generally recommended at around age 1.

The vaccine combining the whole-cell **pertussis** vaccine with **diphtheria** and **tetanus** toxoids was licensed in 1948. Combination vaccines have several advantages, including a reduced number of injections (and thereby reduced doctor's office visits) and lower costs of shipping and stocking vaccine doses.



History of the Immunization Schedule - II

Recommended Vaccines

- Smallpox
- DTP
- **Polio** NEW

An inactivated **poliovirus** vaccine (IPV) was licensed on April 12, 1955, following results that showed Jonas Salk's poliovirus vaccine to be 80-90% effective in preventing paralytic polio. The vaccine quickly reached widespread use.

Poliomyelitis Cases Reported in the United States
1952-62

Year	Total	Paralytic	Nonparalytic	Unspecified
1952	52,879	21,269	12,802	23,808
1953	35,592	15,648	12,144	7,800
1954	38,476	18,308	13,221	6,947
1955	28,985	13,850	12,453	2,682
1956	15,140	7,911	6,555	674
1957	5,485	2,499	2,826	160
1958	5,787	3,697	1,941	149
1959	8,425	6,289	2,045	91
1960	3,190	2,525	626	39
1961	1,312	988	305	19
1962	886	707	125	54

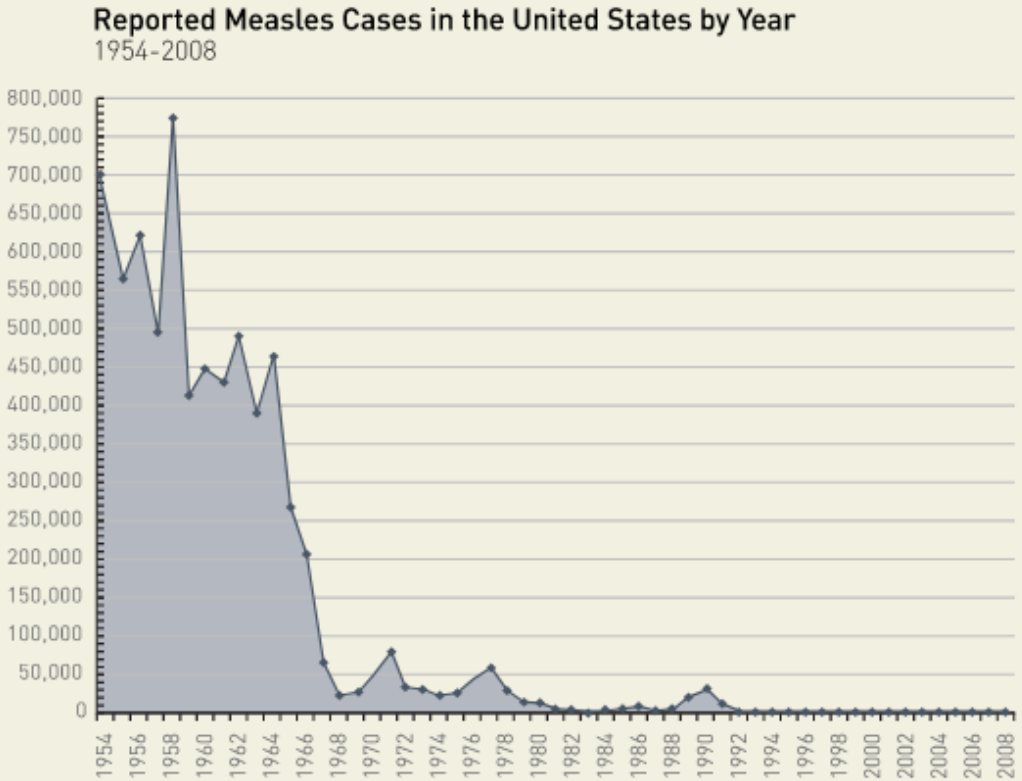
History of the Immunization Schedule - III

Recommended Vaccines

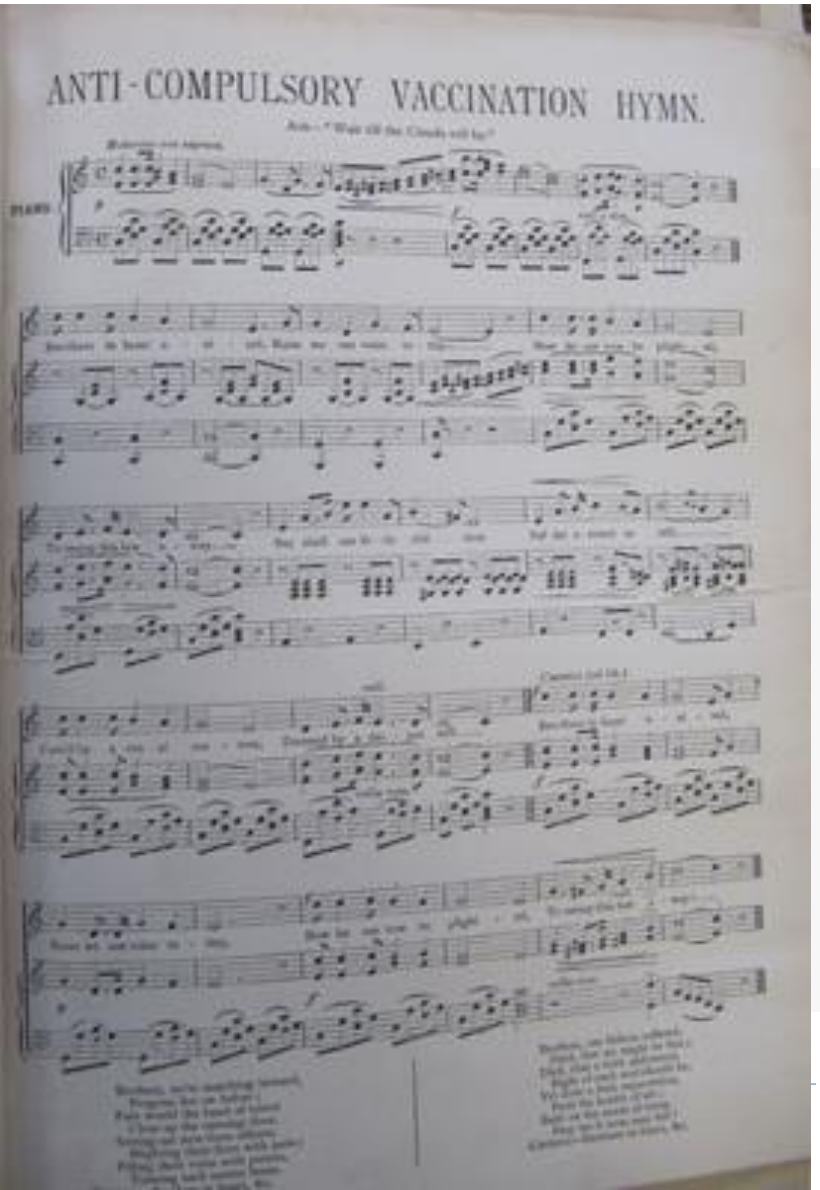
- DTP
- Polio (OPV)
- **MMR** NEW

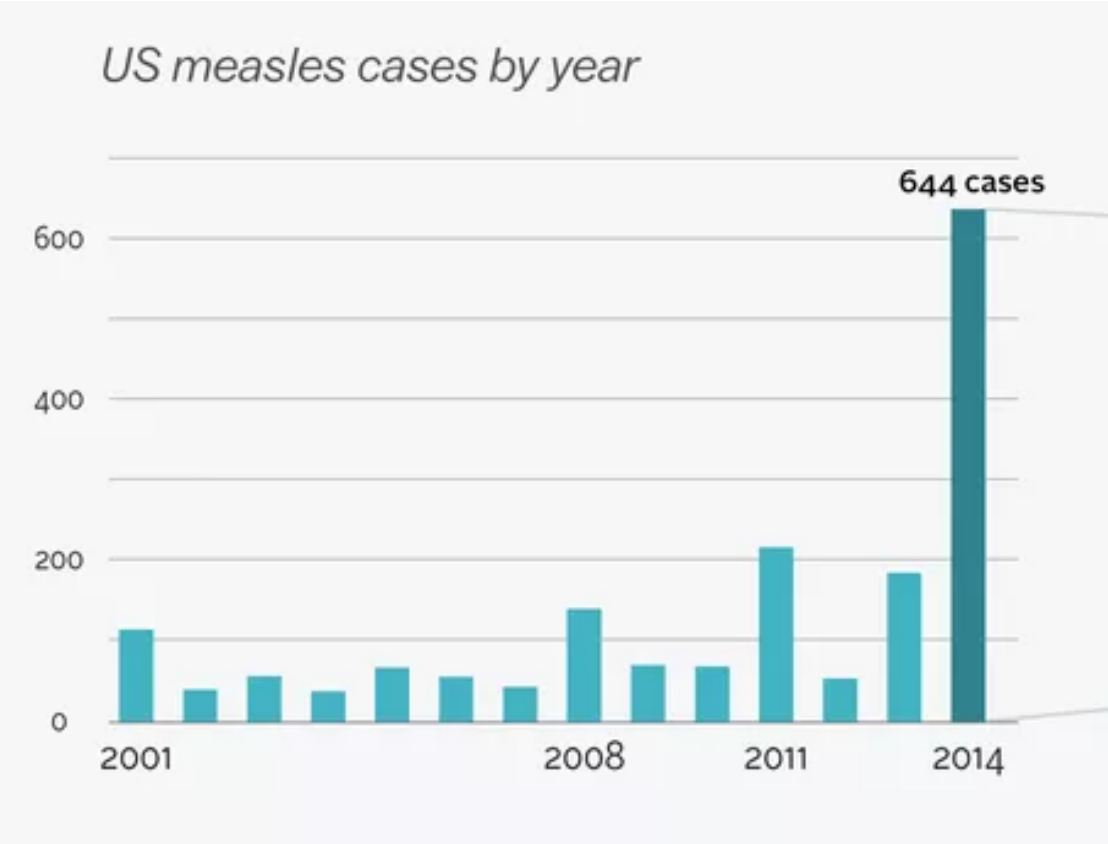
The **measles**, **mumps,** and **rubella** vaccine began to be more widely used after mumps vaccination was recommended for all children in 1977.

In 1977, 57,345 cases of measles were reported in the United States. By 1984, the number of cases had fallen by 95%, to 2,587.

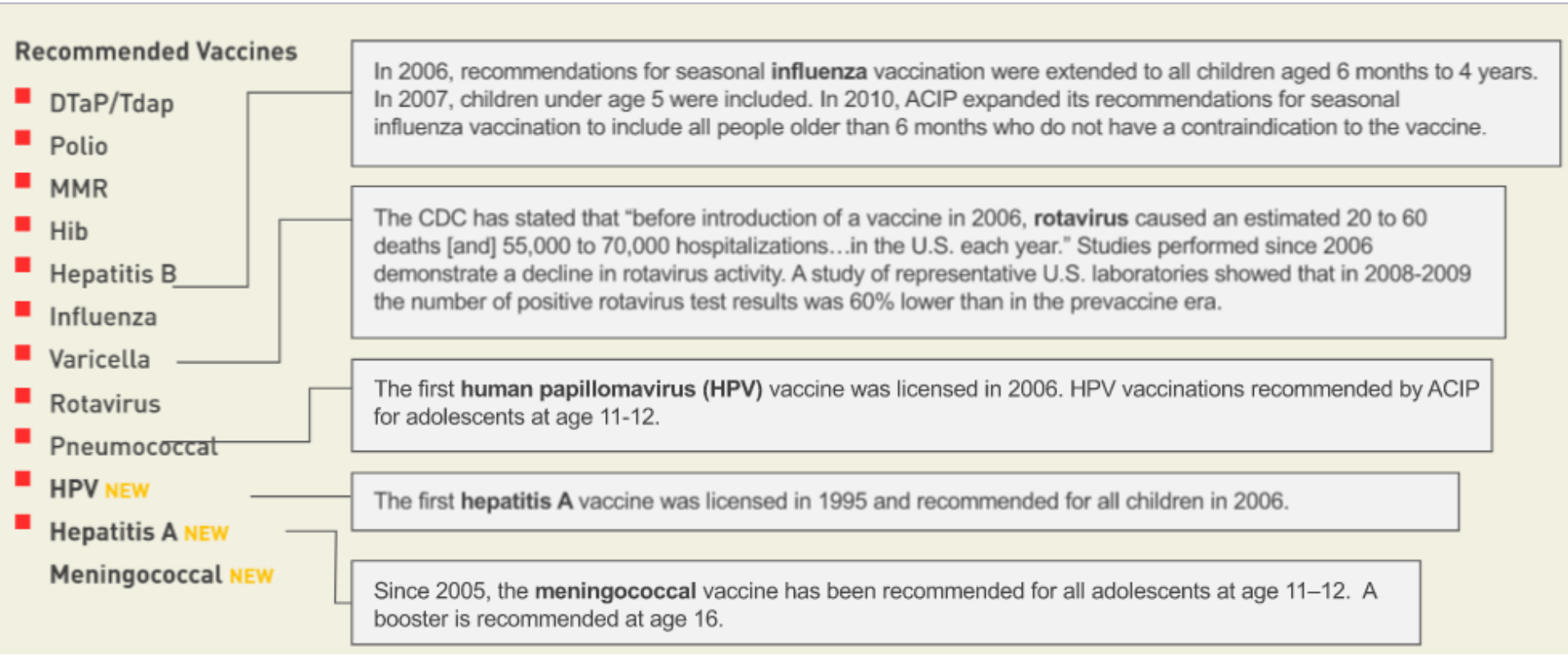


Measles Cases and Outbreaks





History of the Immunization Schedule - IV



Still Need: HIV, malaria, Ebola, hepatitis C virus, tuberculosis…

CHALLENGES:

1. **The genetic diversity of the target pathogen**
   - RNA viruses (HIV, HCV): the error prone RNA dependent polymerase generates quasispecies
   - Influenza vaccines need to be reformulated annually, due to antigenic drift.
   - Malaria: polymorphisms
   - Antigenic diversity of the organism in different geographic regions has major implications for vaccine efficacy
2. **The discrepancy between immunogenicity and protection**
   - immune subversion and immunosuppression
   - immuncompromised patients (HIV, cancer),
3. **The discrepancy between local and systemic responses**
4. **Infant vaccination: how much do we know?**
   - The innate immune system does not reach full capacity until the teenage years, and the neonatal and infant immune responses to many vaccines are suboptimal

Shuo Li et al. Why Vaccines to HIV, HCV, and Malaria Have So Far Failed—Challenges to Developing Vaccines Against Immunoregulating Pathogens Front Microbiol. 2015; 6: 1318.

Type of Vaccination

I. **Passive imunization**

II. **Active immunization**

1. Live, attenuated

2. Inactivated/Killed

3. Subunit/conjugate

4. Toxoid (inactivated toxin)

5. Recombinant vaccines

I - Passive immunization

Transfer of active humoral immunity (antibody-mediated immunity)

Antibodies are introduced into the person's body, the "loaned" antibodies help prevent or fight certain infectious diseases. The protection offered by passive immunization is short-lived, usually lasting only a few weeks or months. It is quick acting, producing an immune response within hours or days, faster than a vaccine. Additionally, passive immunization can override a deficient immune system, which is especially helpful in someone who does not respond to immunization.

In certain cases, passive and active immunity may be used together.

Today, patients may be treated with antibodies when they are ill with diphtheria or cytomegalovirus. Or, antibody treatment may be used as a preventive measure after exposure to a pathogen to try to stop illness from developing (such as with respiratory syncytial virus [RSV], measles, tetanus, hepatitis A, hepatitis B, rabies, or chickenpox).

Natural: Infants benefit from passive immunity acquired when their mothers' antibodies and T-cells cross the placenta in the third trimester, during birth and breastfeeding

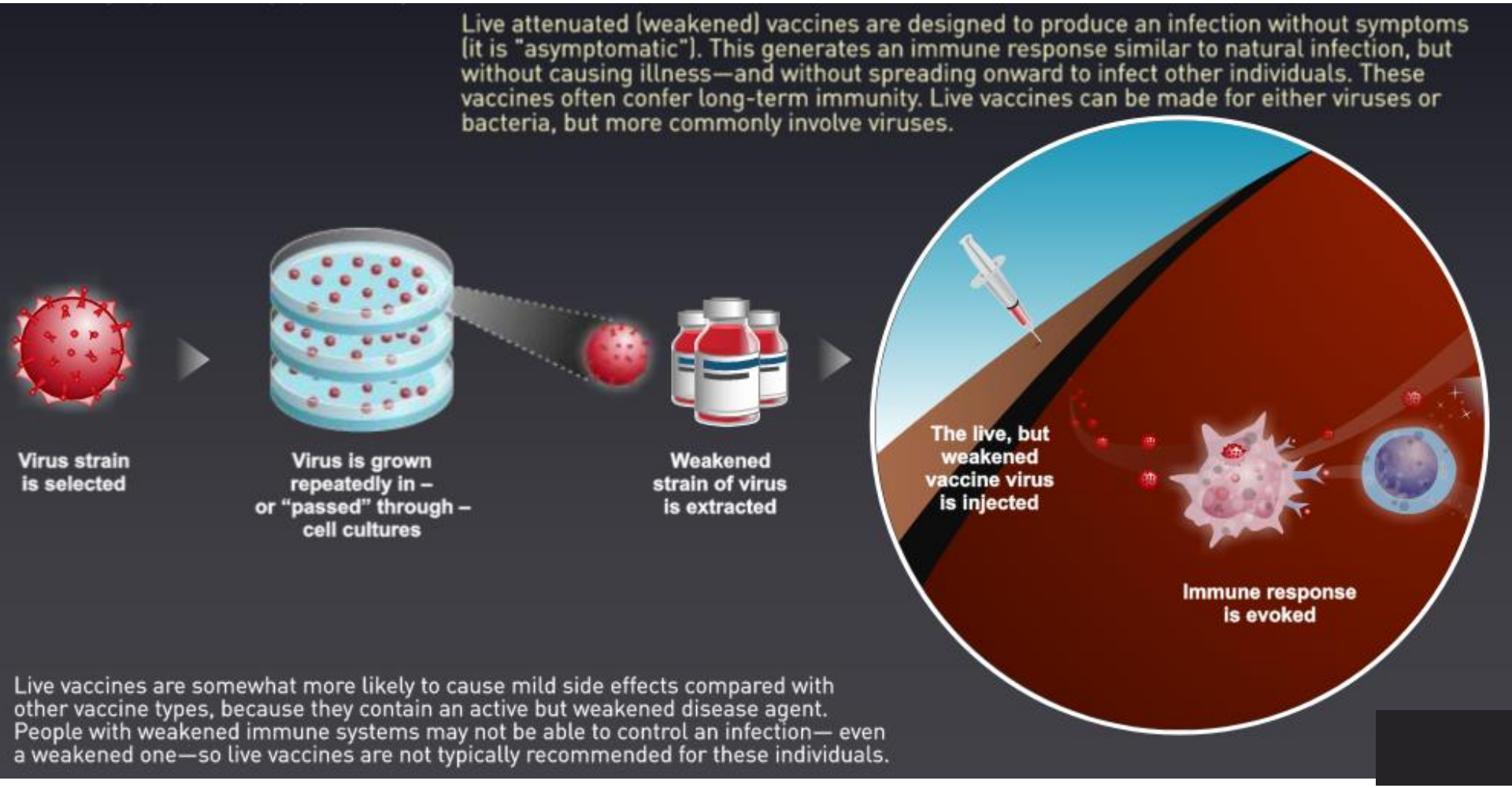
Keller, M.A., Stiehm, E.R. Passive immunity in prevention and treatment of infectious diseases. *Clinical Microbiology Reviews.* October 2000, pp. 602-614, vol. 13, no. 4.

II - Active immunization

Vaccine type	Examples of this type
Live, attenuated	Measles, mumps, rubella (MMR combined vaccine) Varicella (chickenpox) Influenza (nasal spray) Rotavirus
Inactivated/Killed	Polio (IPV) Hepatitis A
Subunit/conjugate	Hepatitis B Influenza (injection) Haemophilus influenza type b (Hib) Pertussis (part of DTaP combined immunization) Pneumococcal Meningococcal
Toxoid (inactivated toxin)	Diphtheria, tetanus (part of DTaP combined immunization)
Recombinant vaccines	HPV

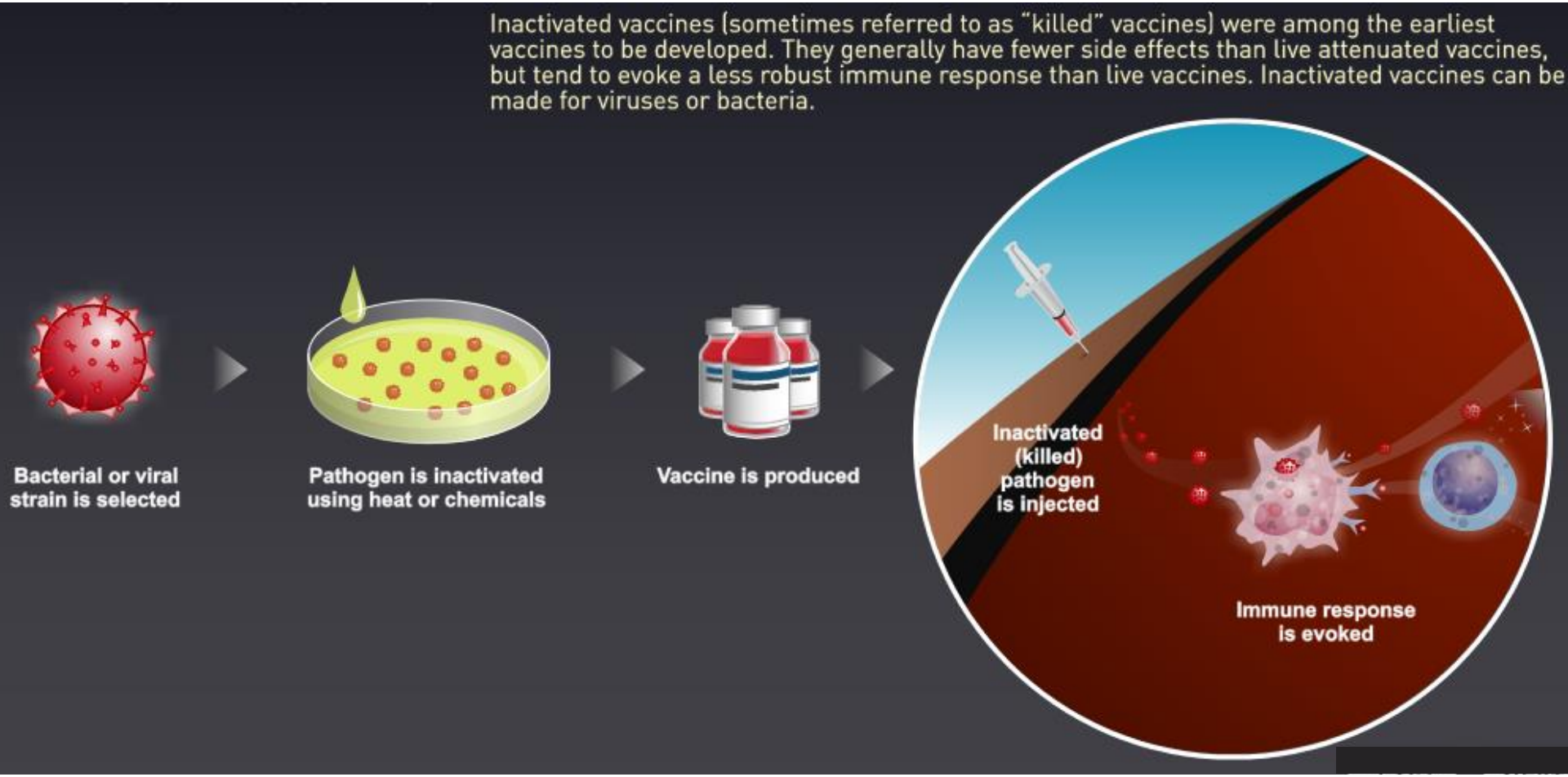
II/1 - Live, Attenuated

Advantages	Disadvantages
A single dose of this type of vaccine is more potent as infectious agent can replicate in host.	May cause disease itself.
Multiple doses may not be required.	Since vaccine is composed of live organism, storage is very critical.
Since micro-organism itself is used, immune response against all antigens is generated.	Cannot be given to immunosuppressed individuals.



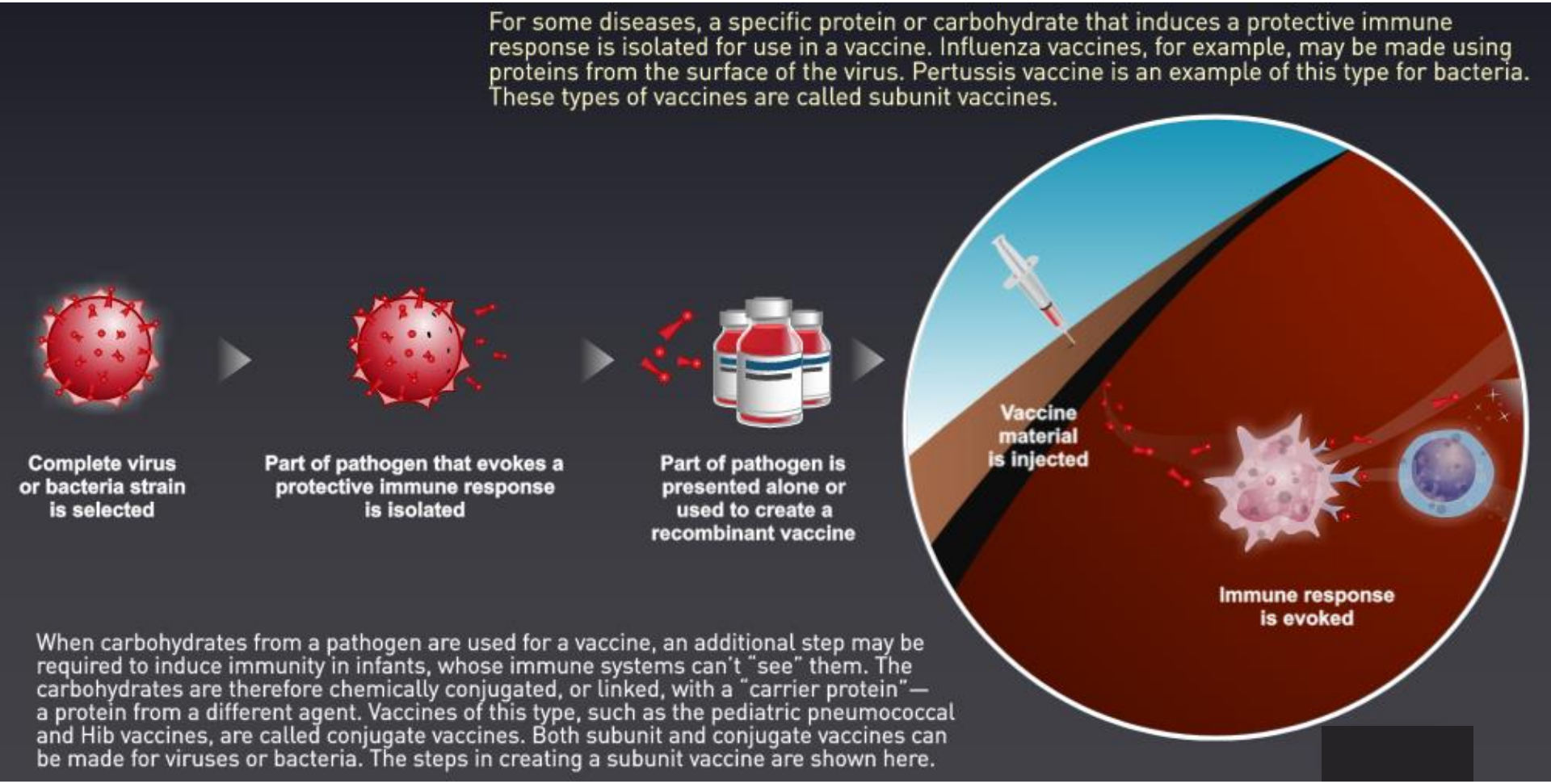
II/2 - Inactivated/Killed

Safe to use in immunosuppressed patients.	Less immunogenic than live attenuated vaccines.
Can't cause disease state.	May require more booster doses to achieve desired immunity.
Storage conditions are not critical compare to live attenuated vaccines.	



II/3 - Subunit/Conjugate

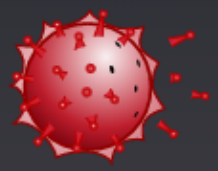
Safe to use in immunosuppressed patients.	Less immunogenic than live attenuated vaccines.
Cannot cause disease state.	Particular antigen or antigens should be identified causing the disease.
Because of the purified antigenic component, less chances of side-effects.	



Safe to use in immunosuppressed patients.	Less immunogenic than live attenuated vaccines.
Cannot cause disease state.	Particular antigen or antigens should be identified causing the disease.
Because of the purified antigenic component, less chances of side-effects.	

II/4 - Toxoid (inactivate

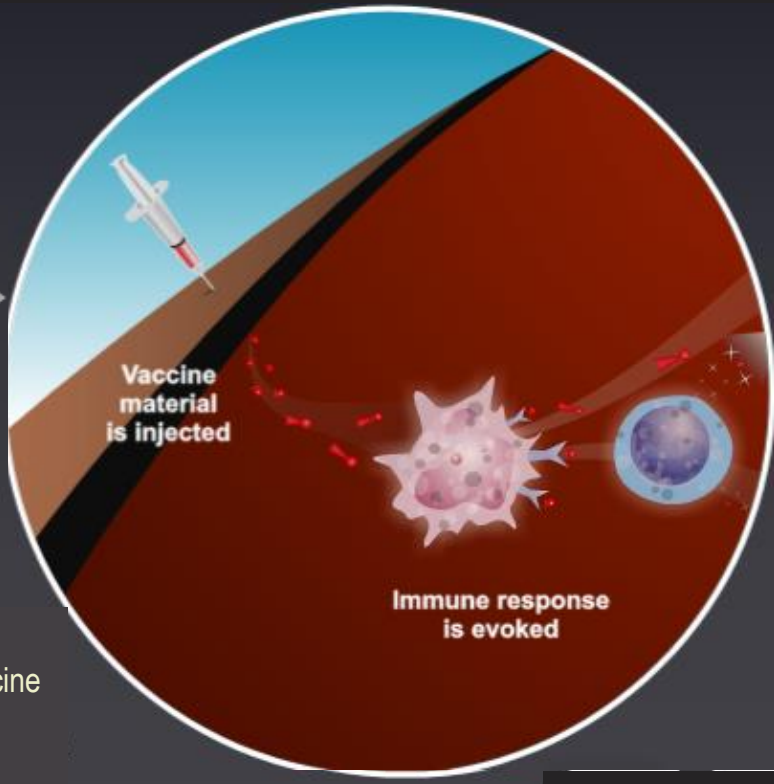
A vaccine made from a toxin that has been made harmless but that elicits an immune response against the toxin. They are based on the toxin produced by certain bacteria (e.g. tetanus or diphtheria).



Bacteria that produce toxins (ie. *Clostridium tetani*)

Harmless toxins are inactivated by heat or chemicals (ie. Formalin)

Vaccine is produced



The toxin invades the bloodstream and is largely responsible for the symptoms of the disease. The protein-based toxin is rendered harmless by heat inactivation used in vaccine production.) and used as the antigen in the vaccine to elicit immunity.

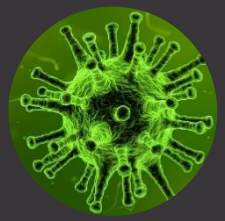
Better stability compare to traditional vaccines.	High production cost compare to other vaccine types.
Storage conditions not critical.	Mutation in host DNA is possible in case of DNA vaccines.
Better control on vaccine design as desired gene can be added or deleted.	

II/5 – Recombinant/DNA

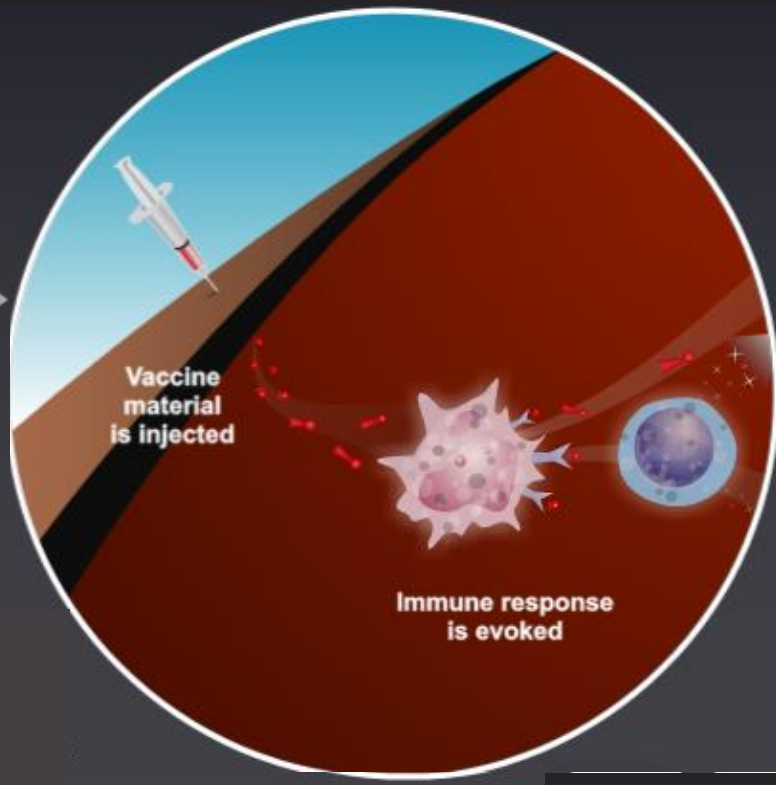
The gene segment for a protein from the disease-causing organism that is known to stimulate a protective immune response (protein of interest) is inserted into the gene of another cell, such as a yeast cell. When the cell replicates it has the same shape as the protein of interest.

Antigenic protein encoding gene and viral molecular plasmid

Recombinant viral mlecular plasmid



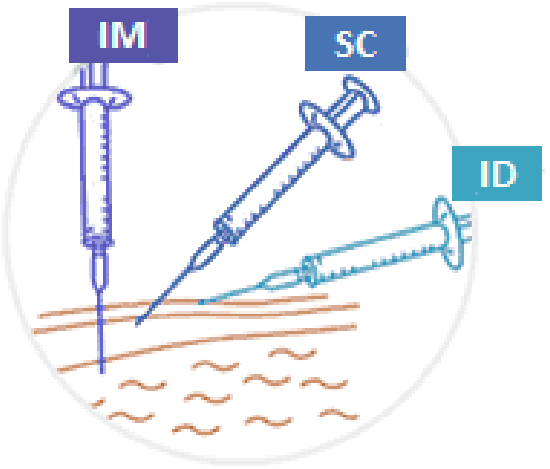
Virus vector vaccine



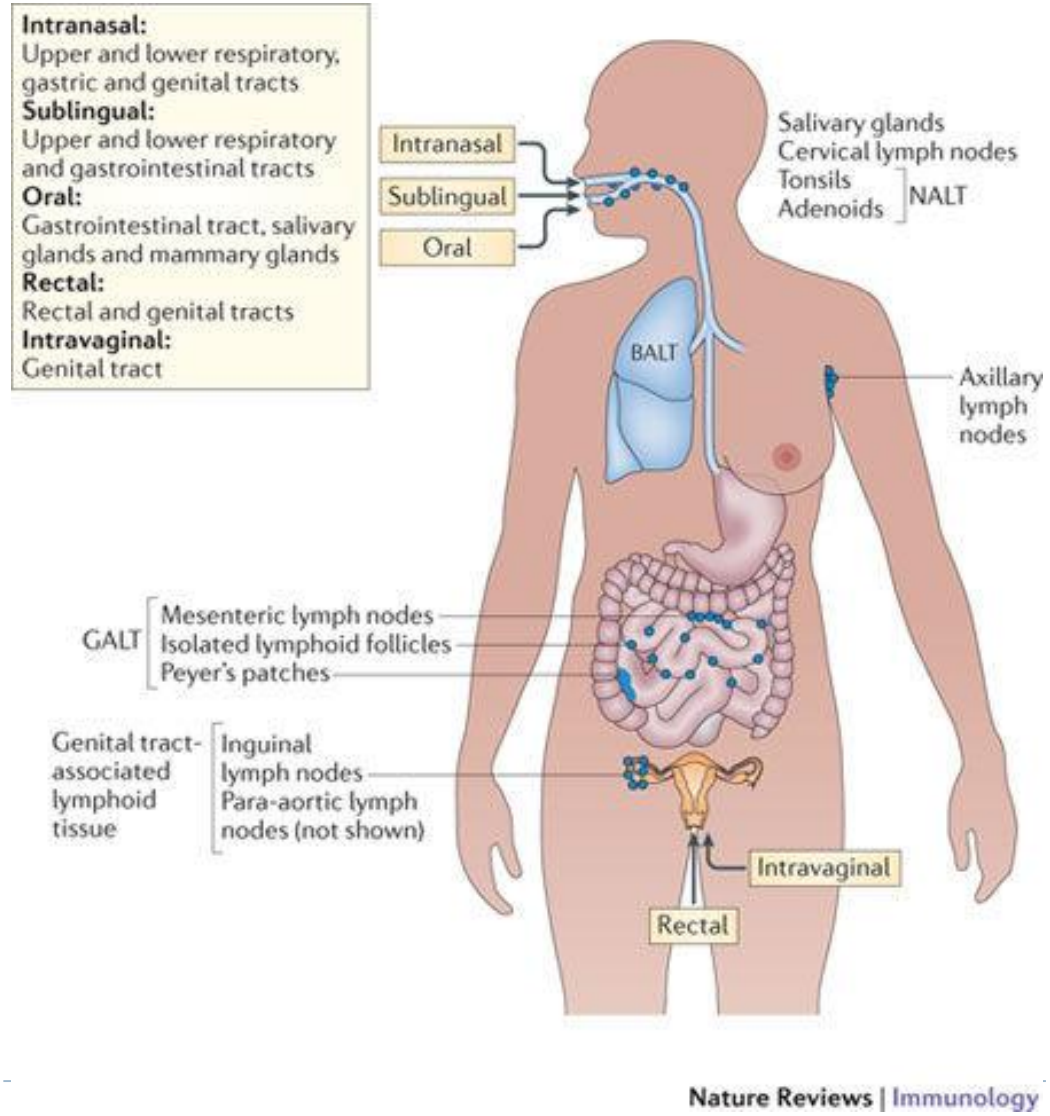
Much progress has been made towards the development of novel vaccines and vaccination approaches. Viral vectors have been studied as potential tools to deliver vaccines as they present advantages over traditional vaccines in that they stimulate a broad range of immune responses including cell mediated immunity.

Route of Administration

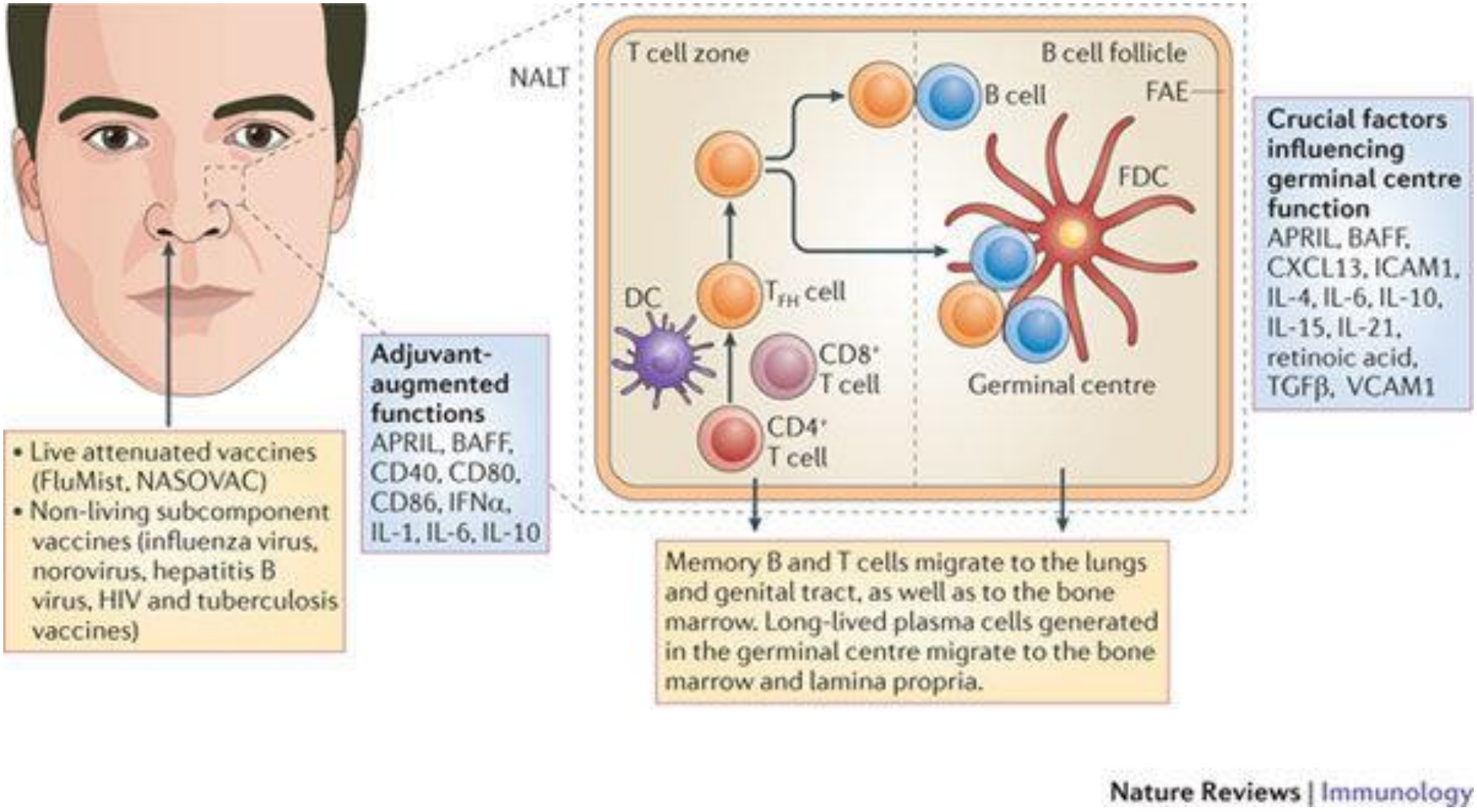
- **Oral**
  - OPV
  - Rotavirus
- **Intramuscular (IM)**
  - DTwP, DTaP, DT, Td, TT
  - Hepatitis B
  - IPV
  - Hib
  - PCV-7
- **Subcutaneous (SC)**
  - Measles
  - Yellow fever
- **Intradermal (ID)**
  - BCG



Recent Developments in Vaccine Administration



Intranasal Immunization



- For most microbes, the nasal mucosa is the first barrier which must be conquered.
  Advantages: ease of self administration and induction of mucosal as well as systemic immunity

Vaccine Ingredients

Each ingredient in a vaccine serves a specific purpose:

- Help provide immunity (protection) against a specific disease
- Help your immune system respond more strongly to a vaccine
- Help keep the vaccine safe and long lasting
- Be used during the production of the vaccine

Vaccine Ingredients

1. **Antigens** are very small amounts of weak or dead germs that can cause diseases. (whole pathogen, proteins, peptides, cell wall components, DNA, etc.)

2. **Adjuvants**, which are substances that help your immune system respond more strongly to a vaccine (ie. Alumn and Freund adjuvant)

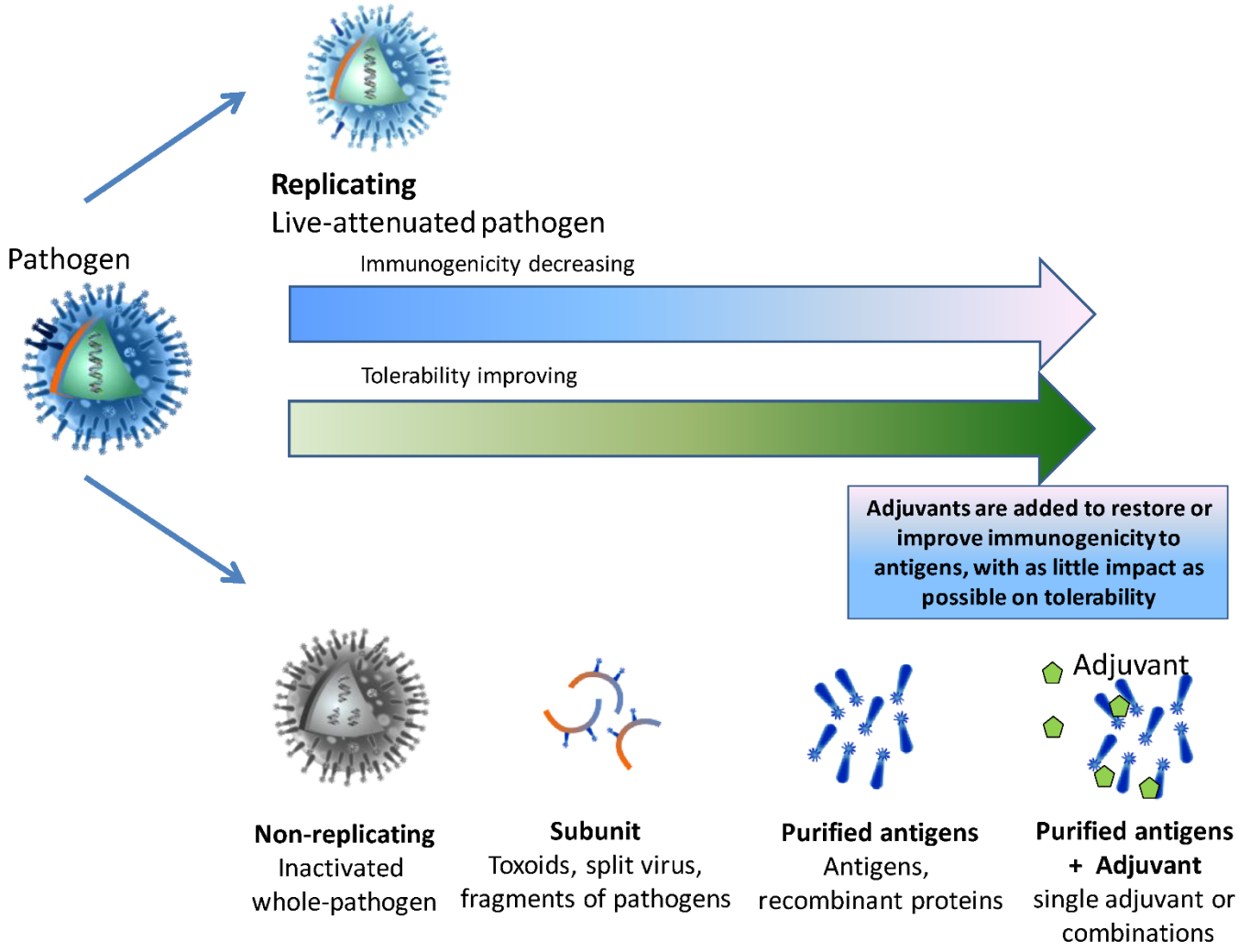
3. **Ingredients keep vaccines safe and long lasting:**

Preservatives, like thimerosal, protect the vaccine from outside bacteria or fungus.

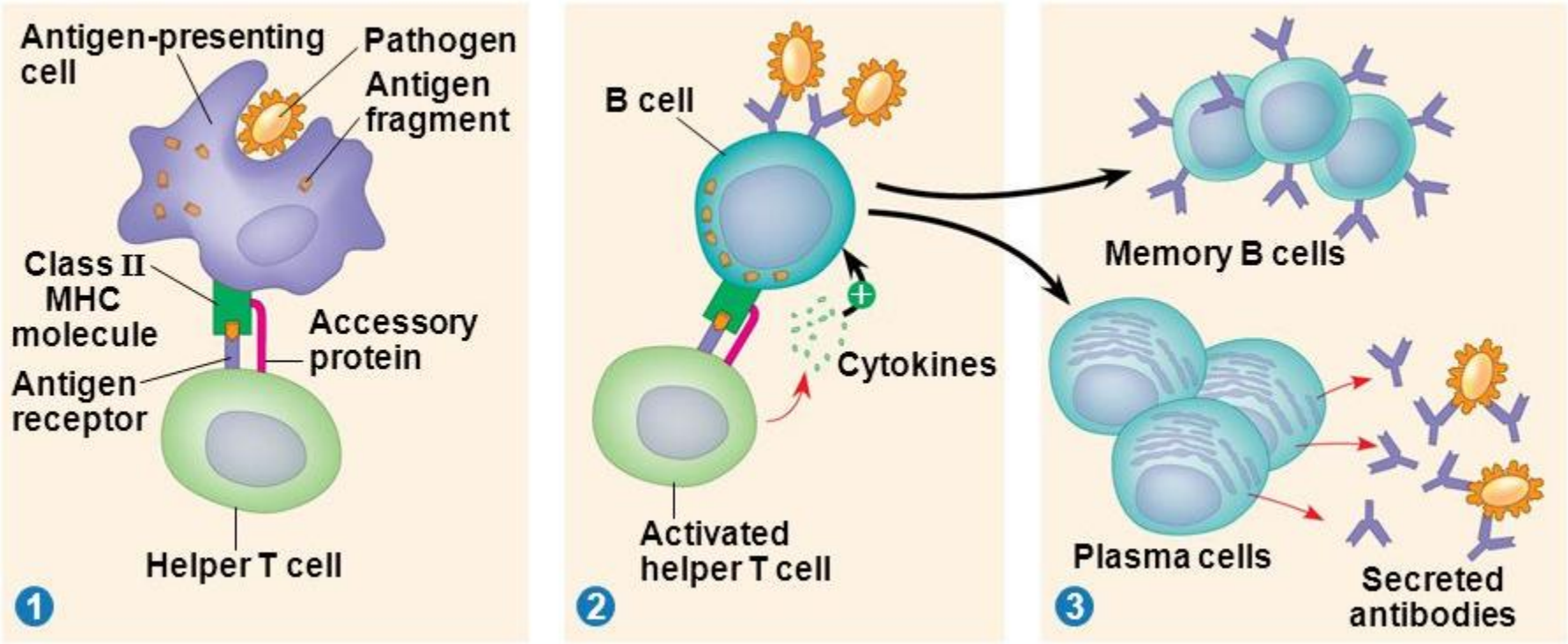
Stabilizers, like sugar or gelatin, help the active ingredients in vaccines continue to work while the vaccine is made, stored, and moved.

4. Formulation depends mainly on the route of administration (emulsion, liposomes, virus-like particles, etc)

Antigens

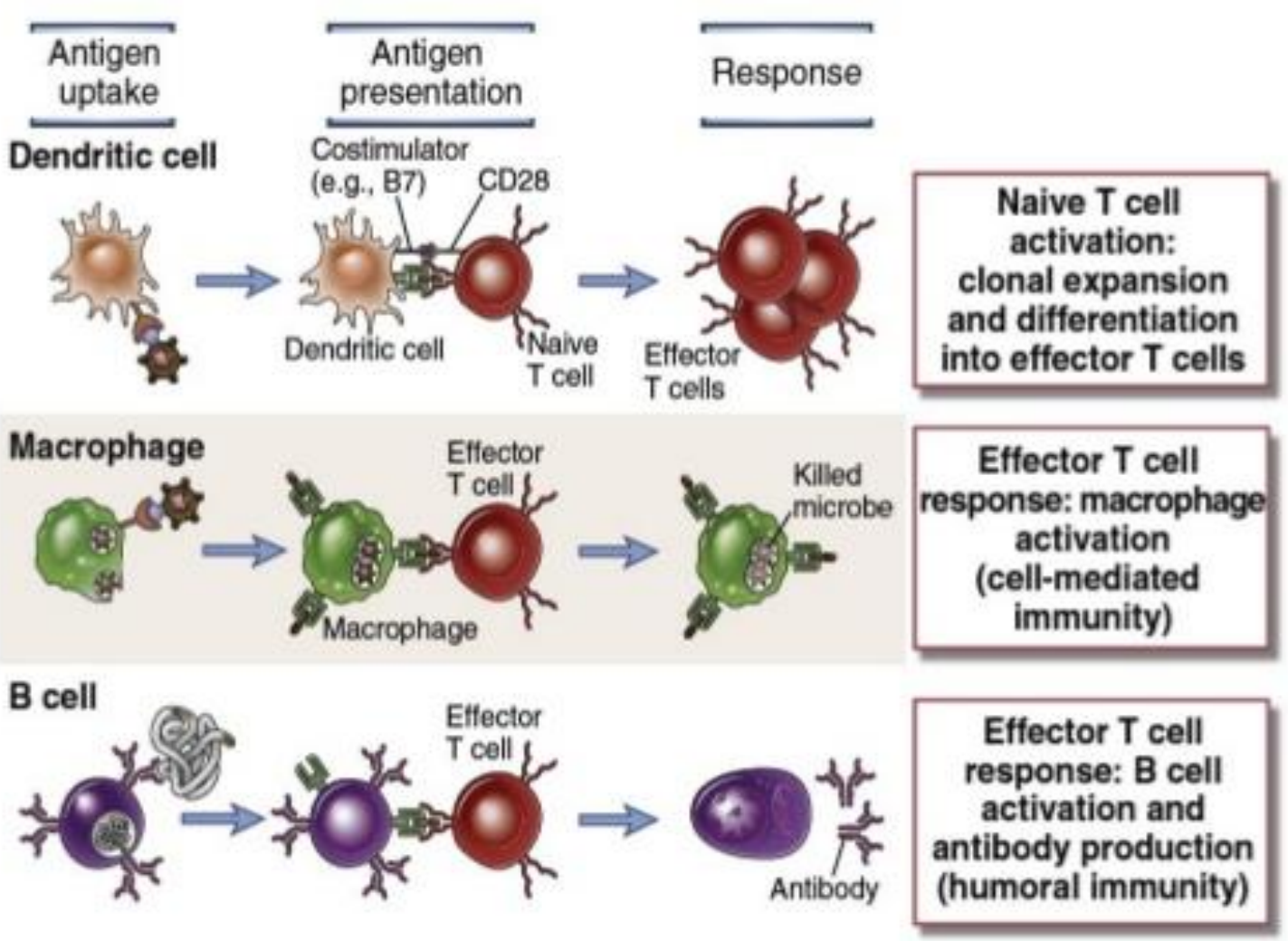

Pathogen
Replicating
Live-attenuated pathogen
Immunogenicity decreasing
Tolerability improving
Adjuvants are added to restore or improve immunogenicity to antigens, with as little impact as possible on tolerability
Adjuvant
Non-replicating
Inactivated whole-pathogen
Subunit
Toxoids, split virus, fragments of pathogens
Purified antigens
Antigens, recombinant proteins
Purified antigens + Adjuvant
single adjuvant or combinations

Immune Response to an Infectious Agent (or to Active Immunization)

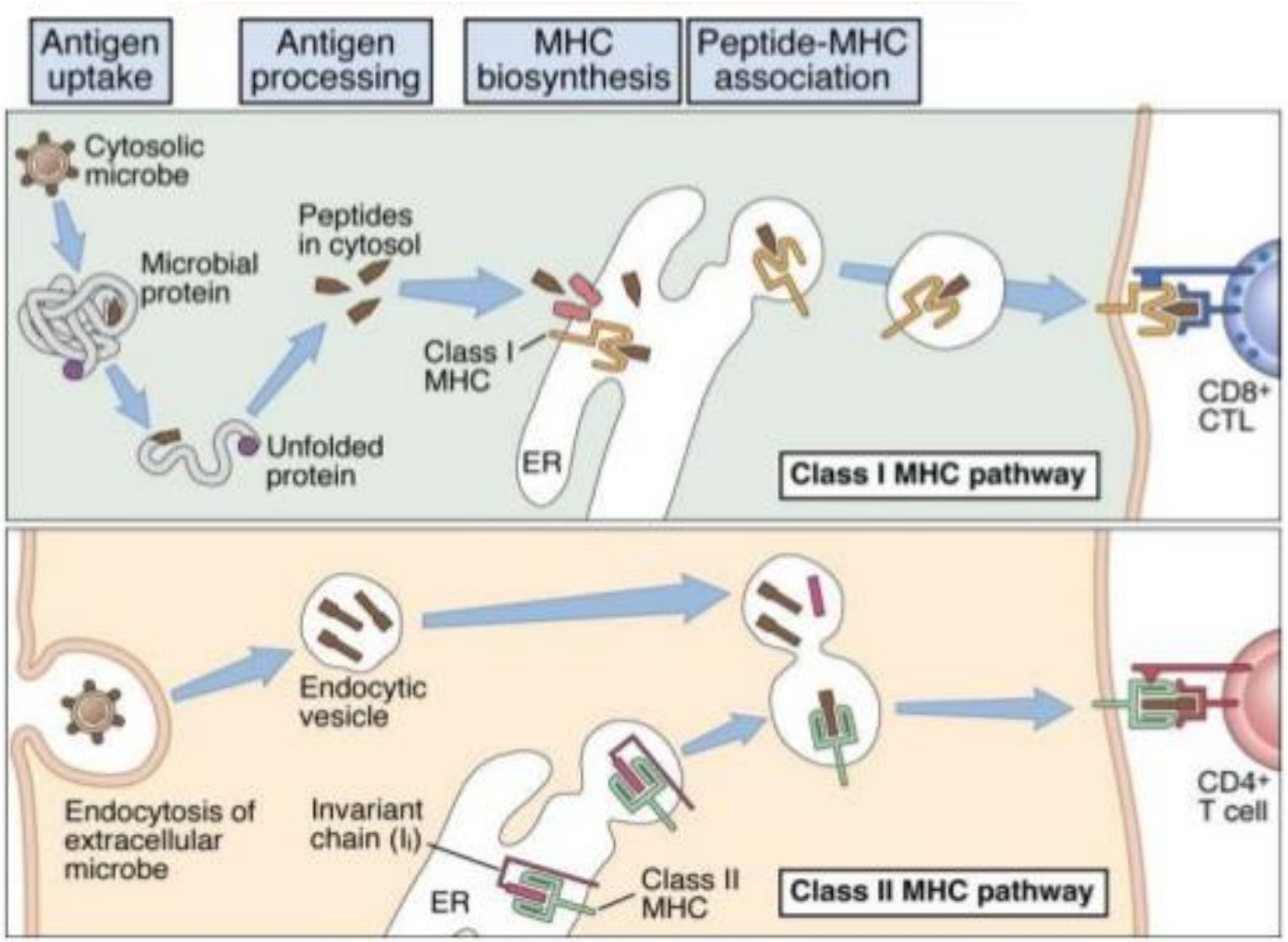


- When exposure to foreign matter occurs, cellular effectors of the innate immune response, such as macrophages, monocytes, neutrophils and dendritic cells, are able to recognize specific surface patterns that classify the agent as a threat or as benign

Functions of Different Antigen Presenting Cells



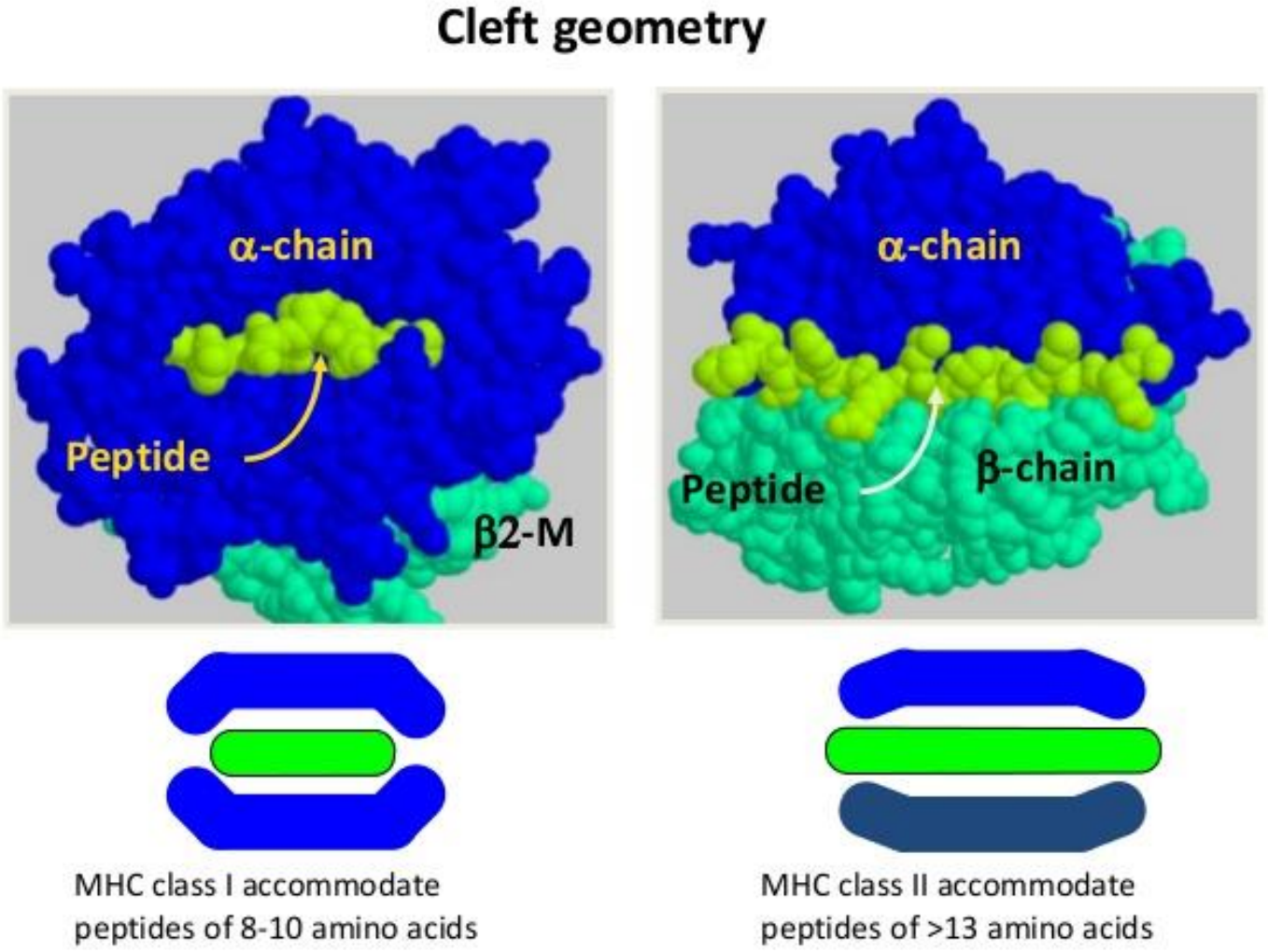
Pathways of Antigen Processing



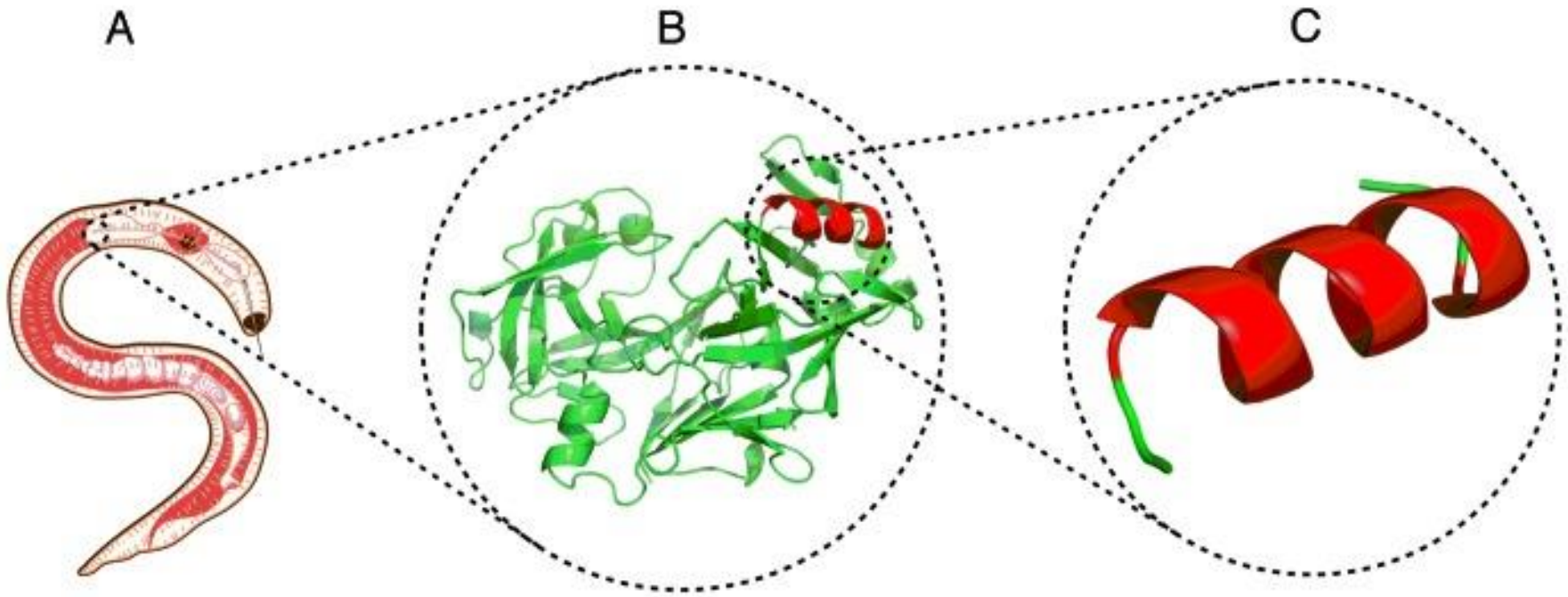
Protein antigen in cytosol (cytoplasm) --> class I MHC pathway

Protein antigen in vesicles --> class II MHC pathway

Peptides Bind to MHC I and II Molecules



The Evolution of Vaccine Antigens

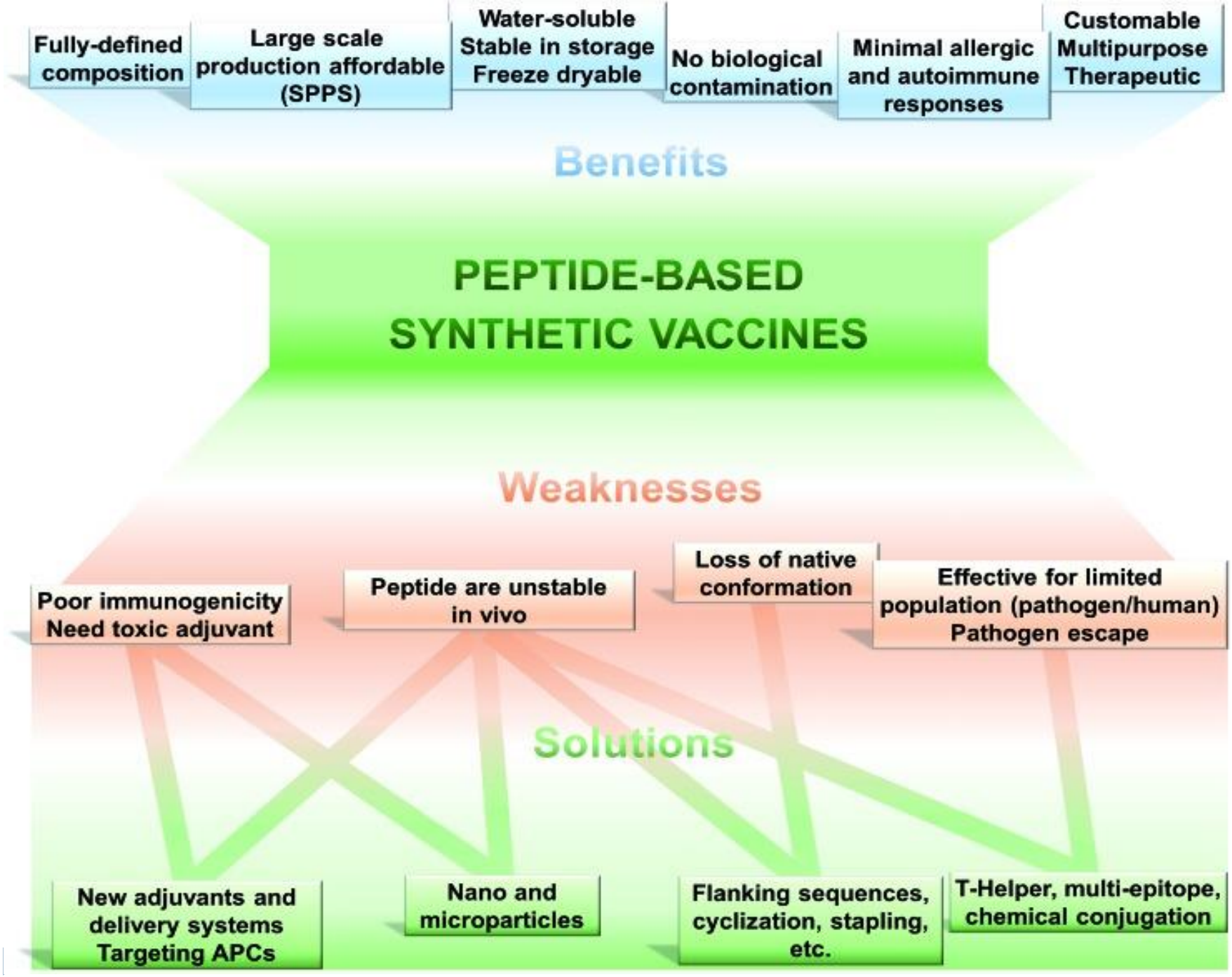


Traditional vaccine utilizing a whole pathogen

Protein-based subunit vaccine

Peptide-based subunit vaccine

Hum Vaccin Immunother. 2014 Mar 1; 10(3): 778–796.

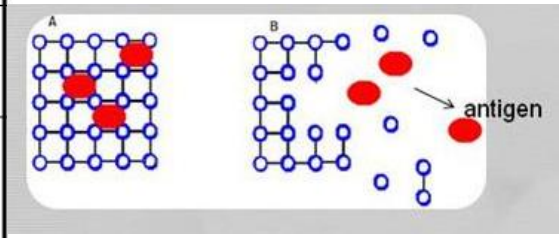


Chem Sci. 2016 Feb 1; 7(2): 842–854.

Adjuvants
Goal: Induction of Strong Aspecific Immune Response

1. Classical adjuvants: alum-based adjuvants, Freund adjuvant

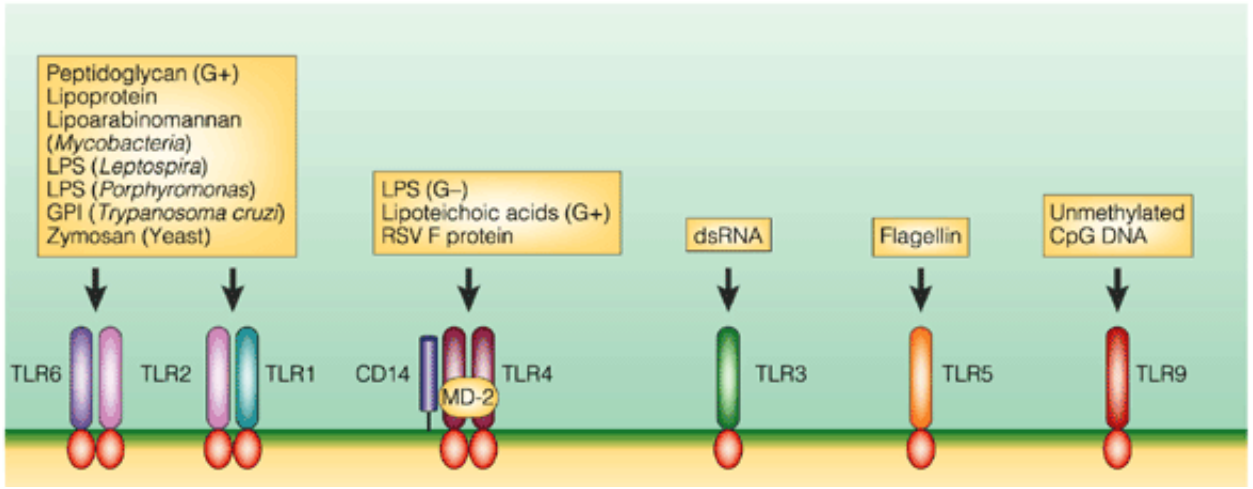
Adjuvant name	Compositions	Mechanism of action
Freund's incomplete adjuvant	Oil-in- emulsion	Delayed release of antigen, Enhanced uptake by macrophages
Freund's complete adjuvant	Oil-in- water with dead Mycobacteria	Delayed release of antigen, Enhanced uptake by macrophages Induction of co-stimulators in macrophages
Freund's adjuvant With MDP	Oil-in- water with Muramyldipeptid	Delayed release of antigen, Enhanced uptake by macrophages Induction of co-stimulators in macrophages
Alum	Aluminum Hidroxide gel	Delayed release of antigen, Enhanced uptake by macrophages
Alum+B.pertussis	Aluminum Hidroxide gel with Killed B.pertussis	Delayed release of antigen, Enhanced uptake by macrophages Induction of co-stimulators in macrophages



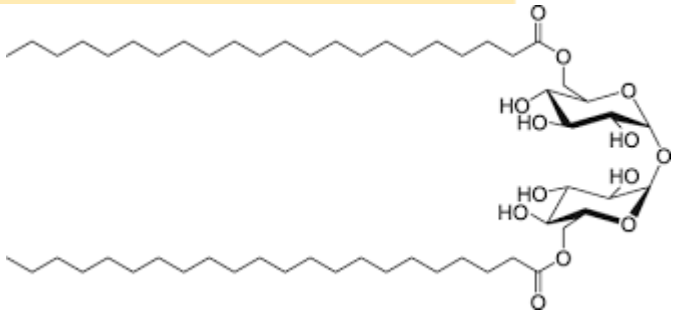
Adjuvants
Goal: Induction of Strong Aspecific Immune Response

2. Oils (mineral oils), emulsions, liposomes, virosomes, polysacchride-based adjuvants, saponin

3. Ligands of Toll-like receptors and pattern-recognition receptors
(IC31, AS01, GLA, stb.) [1]

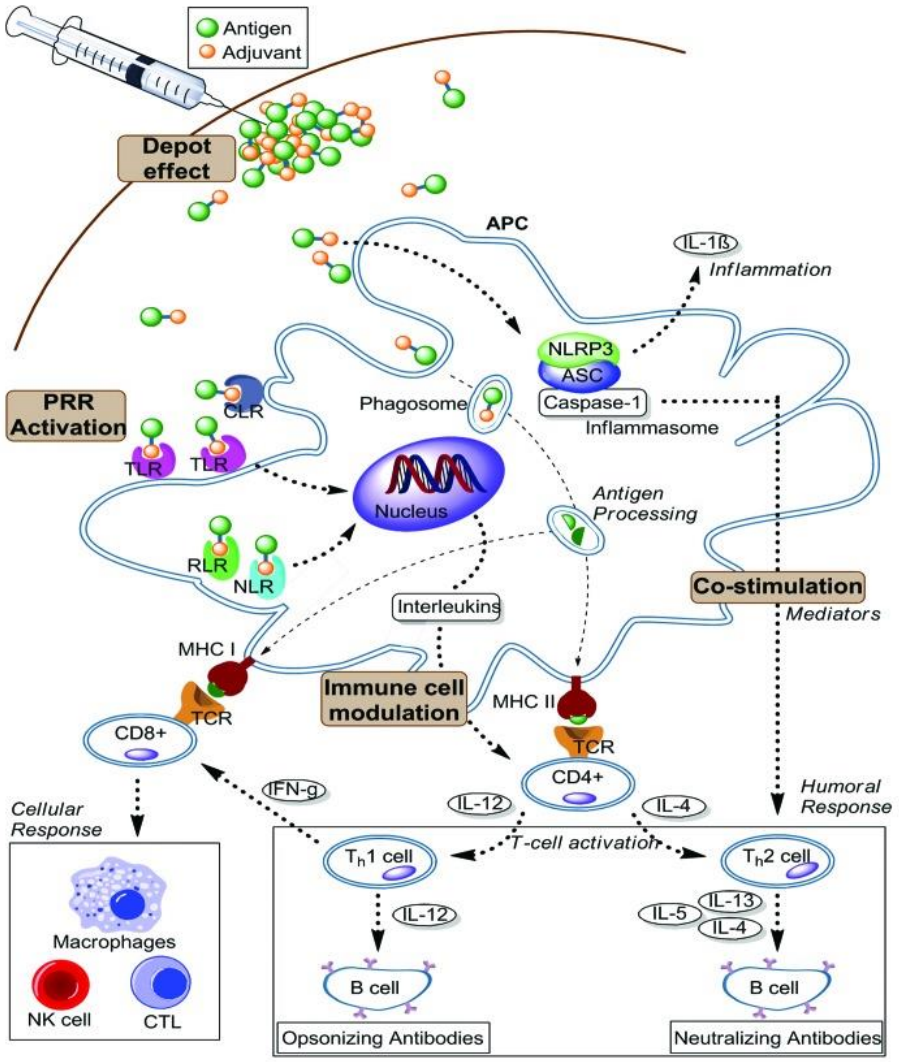


4. Bacterial cell wall components [2]
Synthetic derivative of *Mtb* cord factor (TDM) ->
trehalose-6,6-dibehenenate (TDB)



[1] Reed, S. G. et al. New horizons in adjuvants for vaccine development. ***Trends Iimmunol*** 2009**,** *30* (1), 23-32.
[2] Werninghaus, K et al Adjuvanticity of a synthetic cord factor analogue for subunit *Mycobacterium tuberculosis* vaccination requires FcRgamma-Syk-Card9-dependent innate immune activation. ***J Exp Med*** 2009, *206* (1), 89-97.

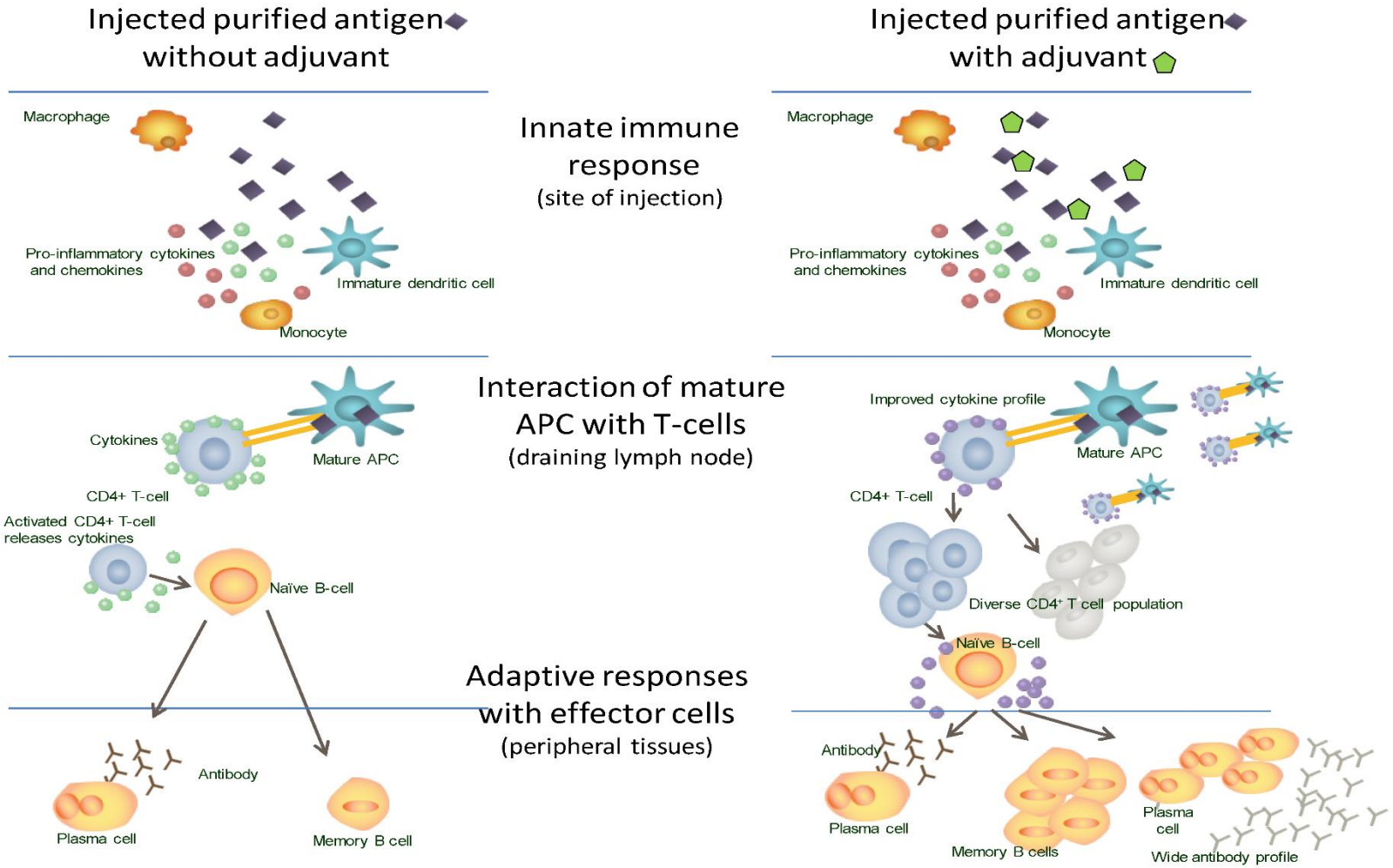
The Immunological Cascade Induced by Adjuvants



APC: Antigen Presenting Cell
CTL: Cytotoxic T Lymphocyte
NK cell: Natural killer cell
PRR: Pattern recognition receptor
TLR: Toll-like receptor
RLR: retinoic acid-inducible gene I -like receptor
NLR: NOD-like receptor
MHC: Major histocompatibility complex
NLRP3: NOD-like receptor family
ASC: The inflammasome adaptor
TCR: T cell receptor
CLR: C-type lectin receptors)

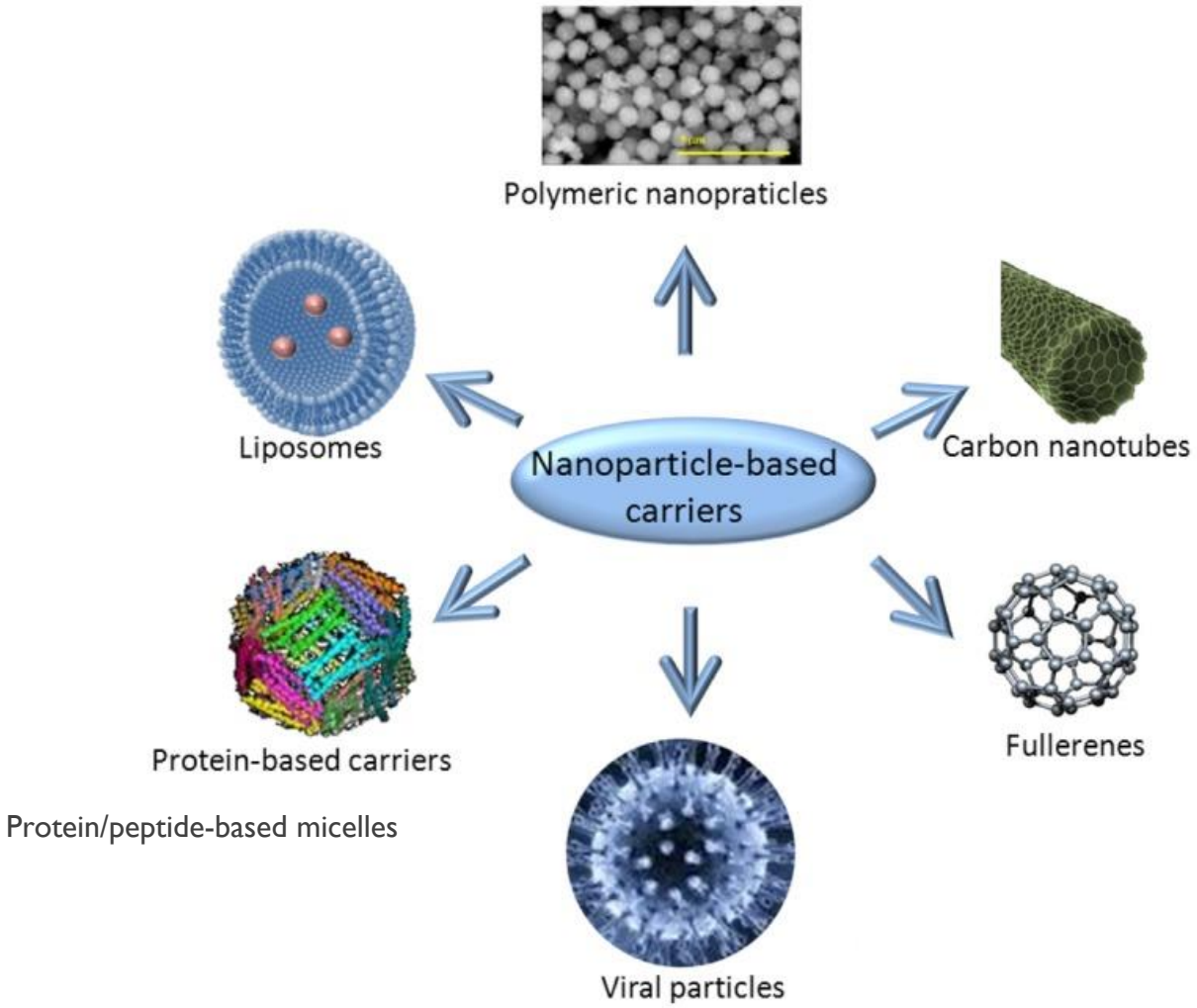
These immunological events are essential for enhancing and directing the adaptive immune response against vaccine antigens. The responses are primarily mediated by two main types of lymphocytes, T and B cells

Immune Response to Vaccination with and without Adjuvant



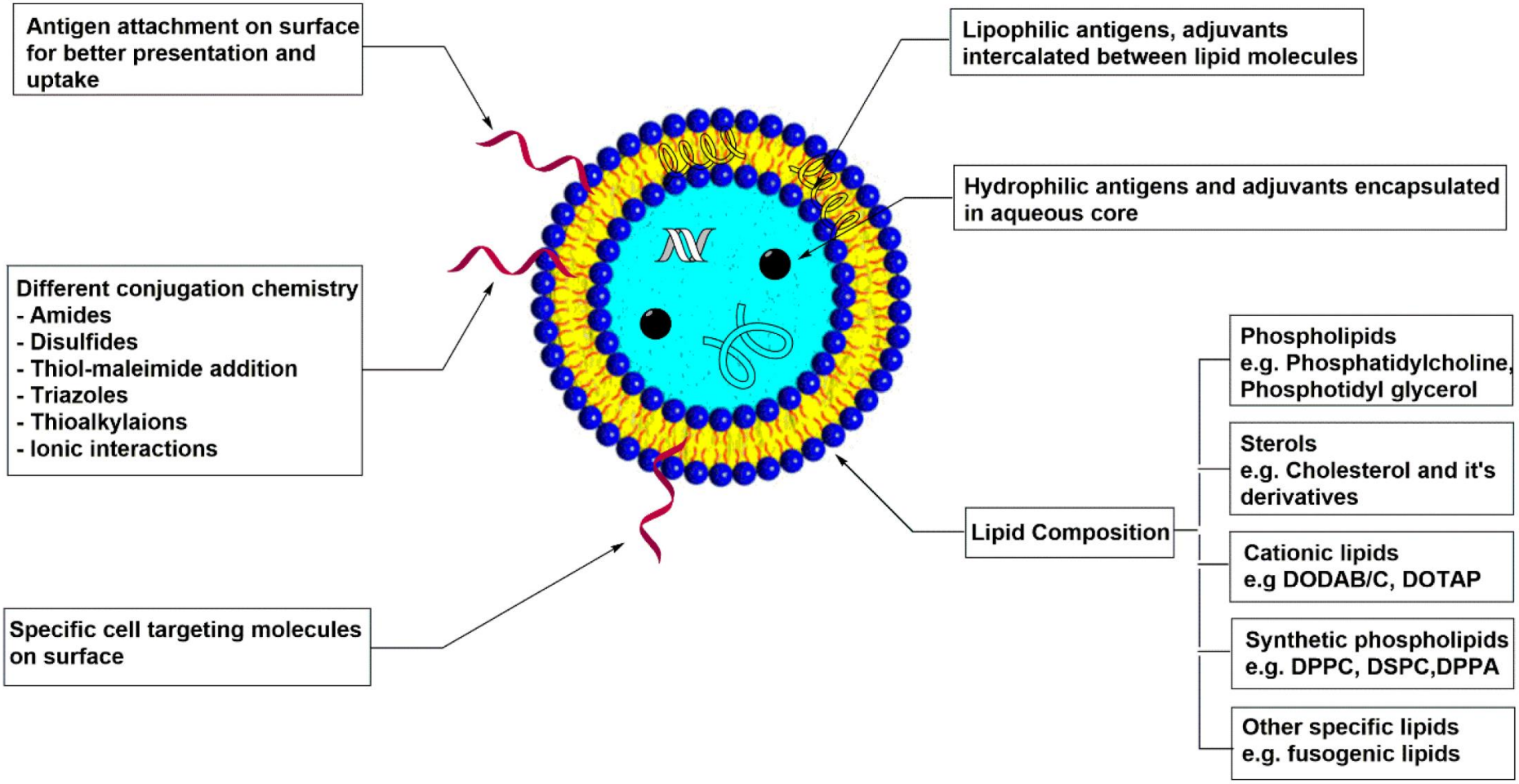
- Lack of PAMP (pathogen-associated molecular patterns) -> initial innate immune response is not activated
  Adjuvants can act like PAMPs, triggering the innate immune response through a variety of mechanisms, to identify the vaccine components as a "threat", with activation and maturation of APCs and initiation of downstream adaptive immune activities

Vaccine Formulation & Delivery

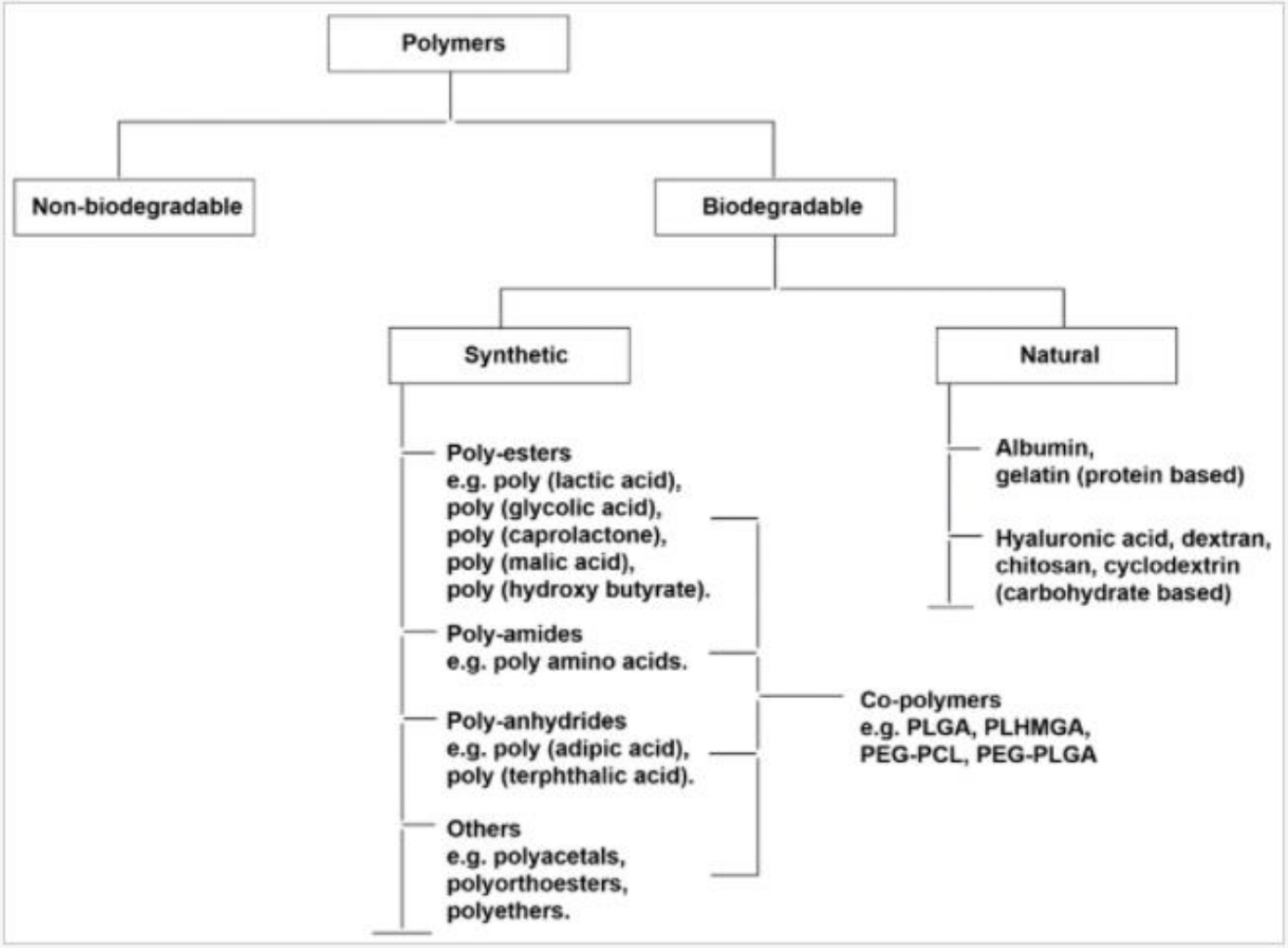


- Influences vaccine retention and distribution in the body

Liposomal Antigen Delivery

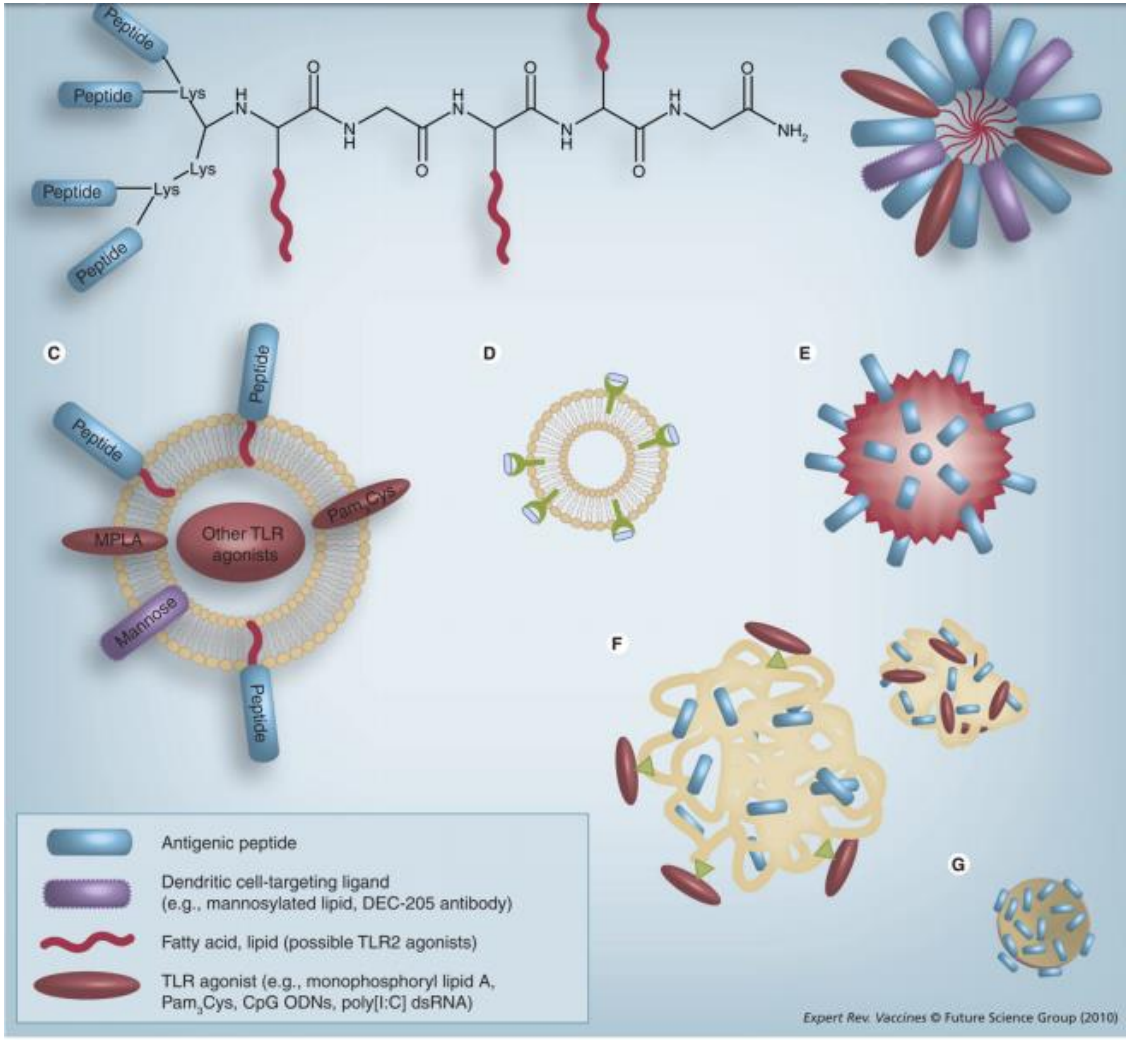


Polymers Used in Vaccine Delivery



Vartak A, Sucheck SJ. Recent Advances in Subunit Vaccine Carriers. Vaccines (Basel). 2016 r 19;4(2)

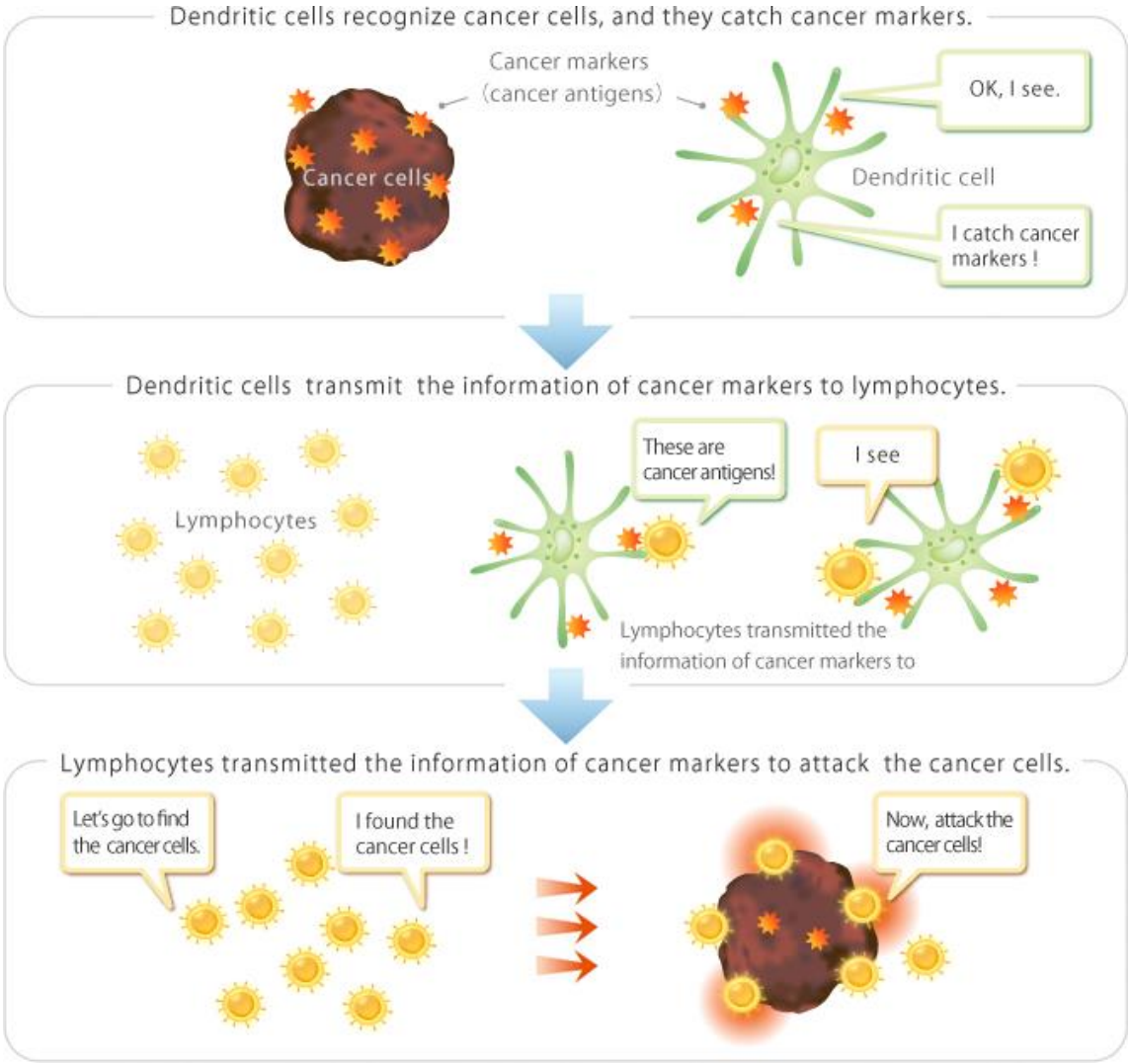
Peptide Antigen Constructs



(A) lipid–core peptides
(B) peptide amphiphiles self-assembled into mixed micelles (protein analogous micelles; lipid-based carriers including
(C) synthetic, multifunctional vesicles
(D) endogenous exosomes containing peptide loaded MHC molecules;
(E) noninfectious virus-like particles displaying recombinant peptide antigens
(F) microparticles and nanoparticles made from many types of polymers with encapsulated or surface-conjugated peptides
(G) solid-core nanobeads with conjugated peptides.
MPLA: Monophosphoryl lipid A; ODN: Oligodinucleotide; Pam3Cys: Tripalmitoyl-Sglyceryl cysteine; TLR: Toll-like receptor.

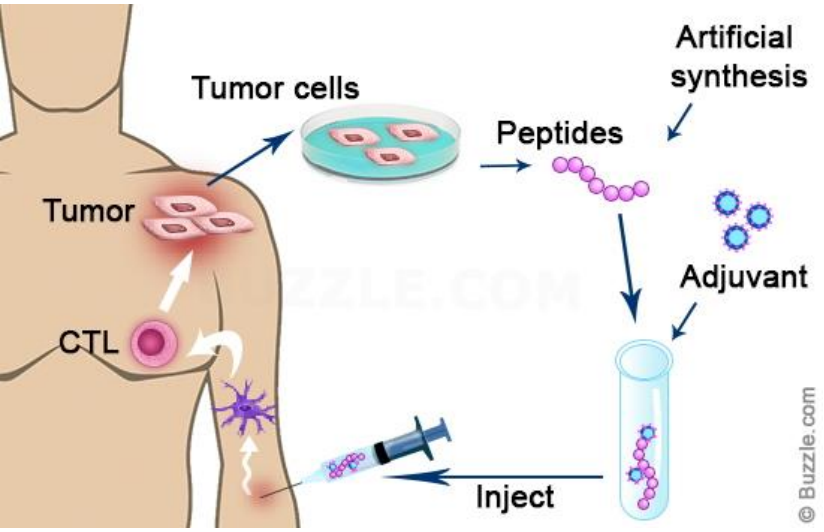
Black M et al. Advances in the design and delivery of peptide subunit vaccines with a focus on toll-like receptor agonists. Expert Rev Vaccines. 2010 Feb;9(2):157-73.

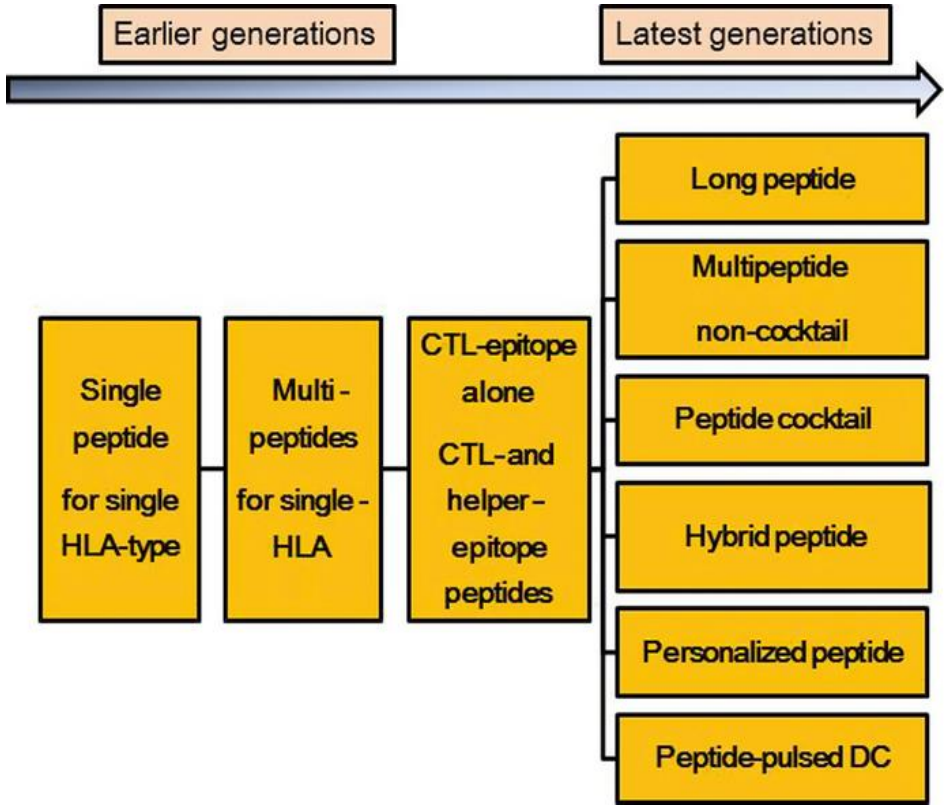
Relationship between Cancer and Immunity



As many as 5,000 to 6,000 cancer cells are suggested to develop every day even in the body of healthy people. Deterioration of immune system function results in its inability to inhibit cancer cell growth and ultimately cancer development.

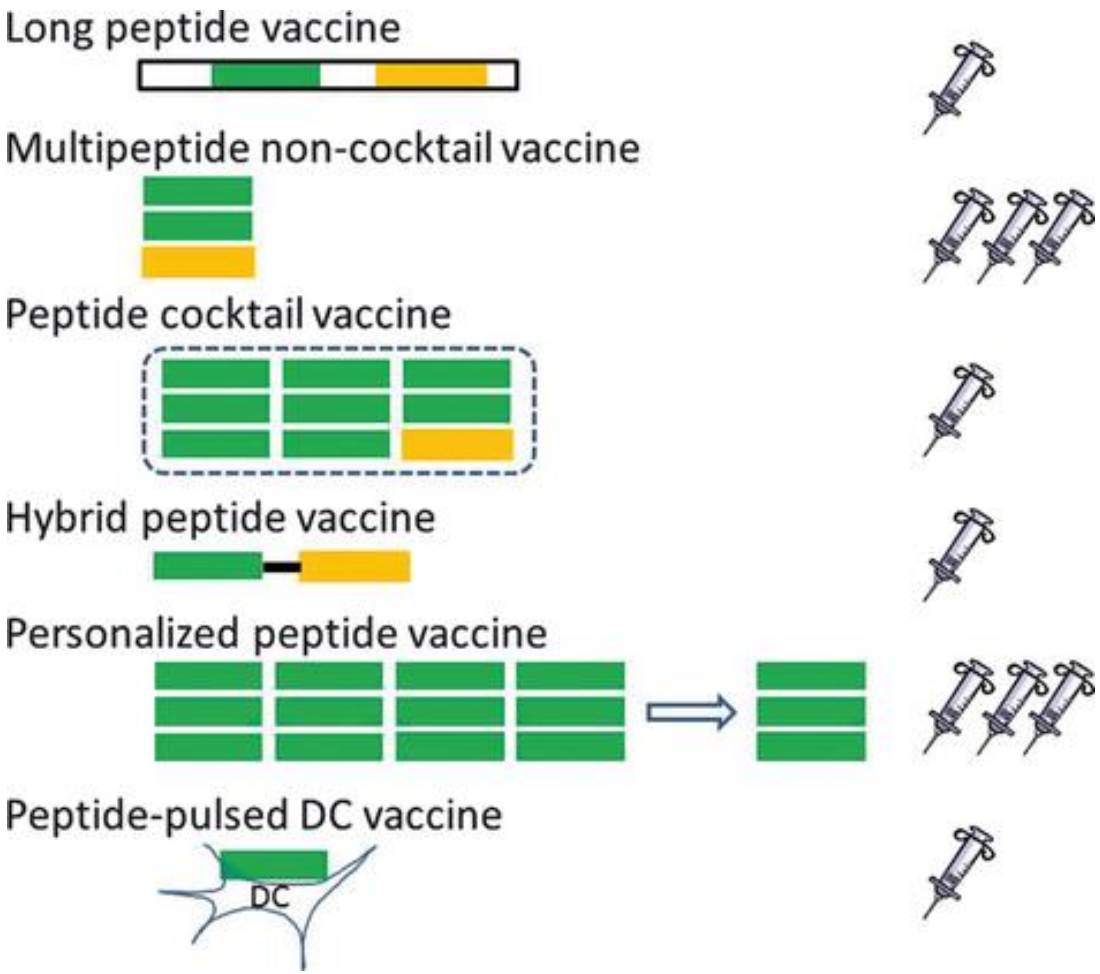
Peptide Vaccine Therapy



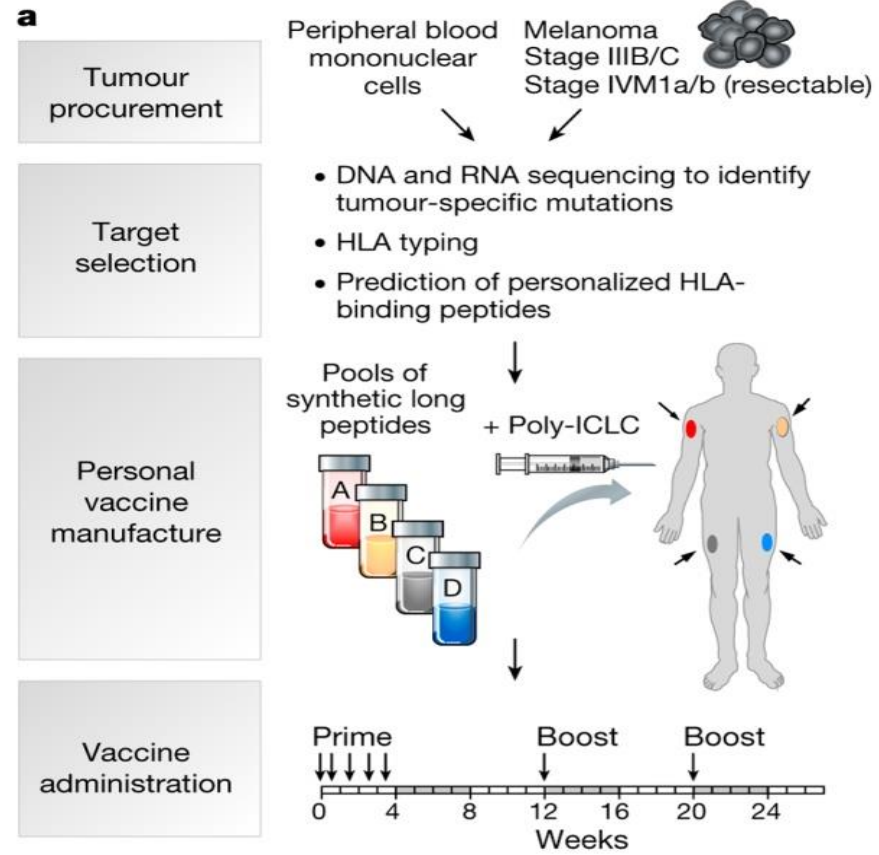


- **Next-generation peptide vaccines for advanced cancer, Volume: 104, Issue: 1, Pages: 15-21,**

Next-generation Peptide Vaccines for Advanced Cancer

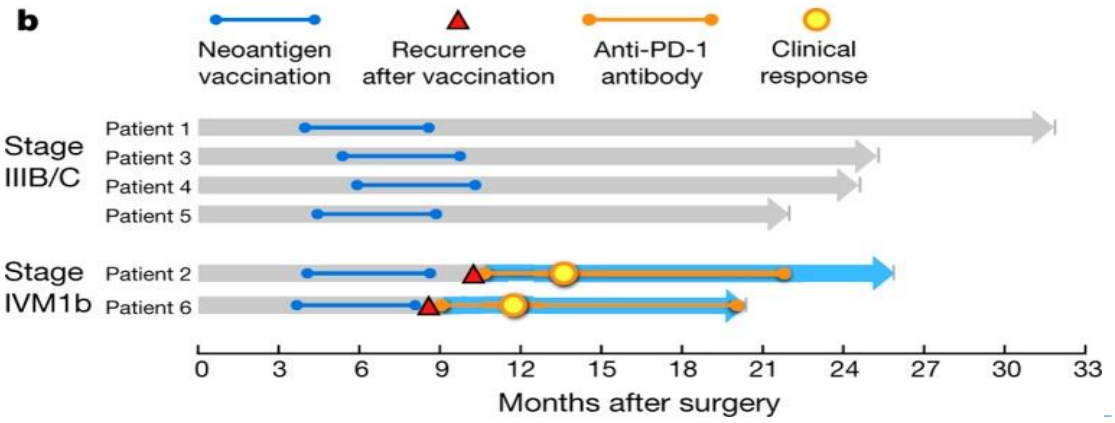


Generation of a personal, multi-peptide vaccine for patients with high-risk melanoma



whole-exome sequencing (WES) of matched tumour- and normal-cell DNA

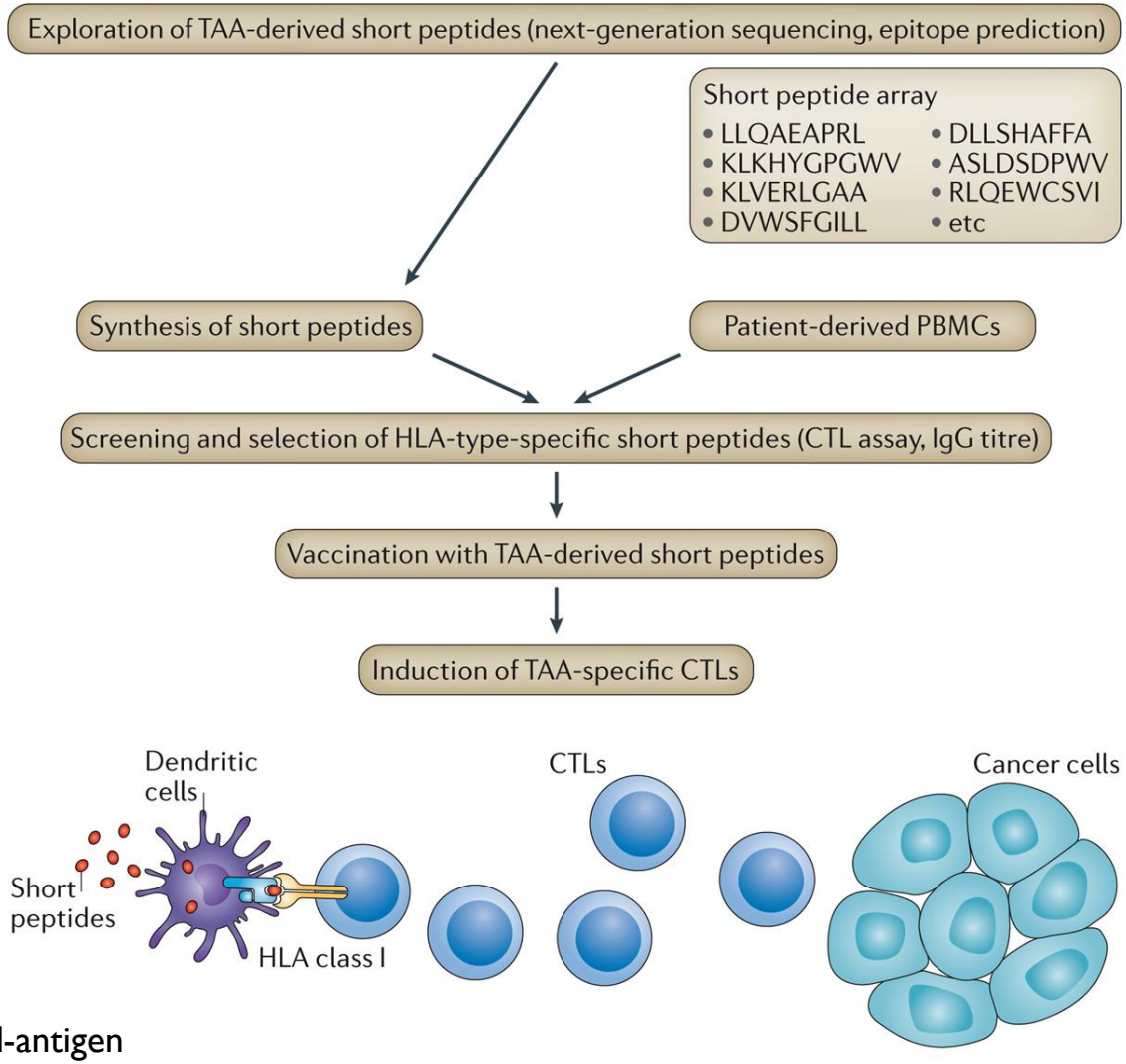
20 long-peptides in 4 pools
Adjuvant: aTLR3 ligand



Of six vaccinated patients, four had no recurrence at 25 months after vaccination, while two with recurrent disease were subsequently treated with anti-PD-1 (anti-programmed cell death-1) therapy and experienced complete tumour regression, with expansion of the repertoire of neoantigen-specific T cells.

P A Ott *et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature* 1–5 (2017) doi:10.1038/nature22991

Peptide Vaccination Using TAA-Derived Short Peptides

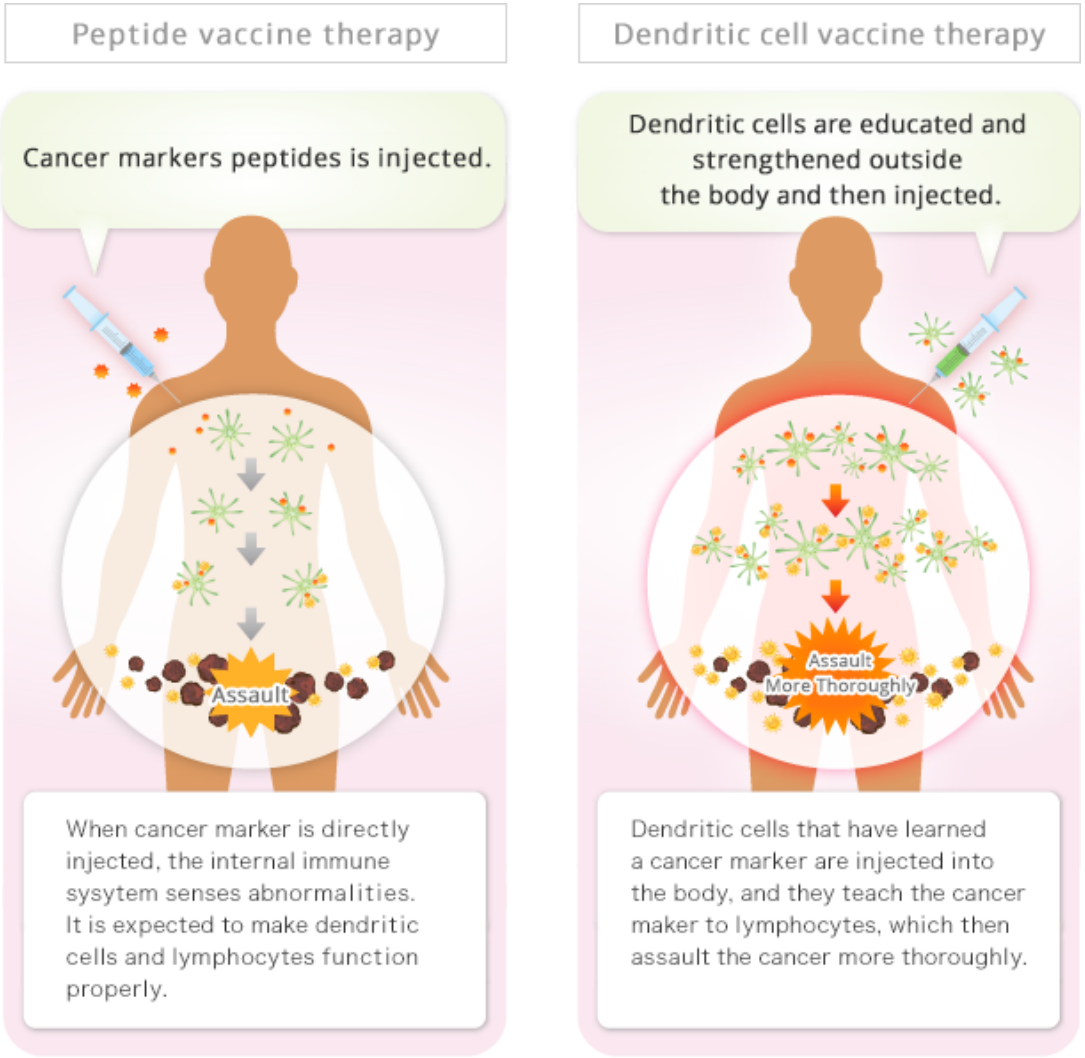


TAA: Tumour-associated-antigen

Nature Reviews | Urology

Kimura, T. *et al.* (2017) Personalized peptide vaccines and their relation to other therapies in urological cancer *Nat. Rev. Urol.* doi:10.1038/nrurol.2017.77

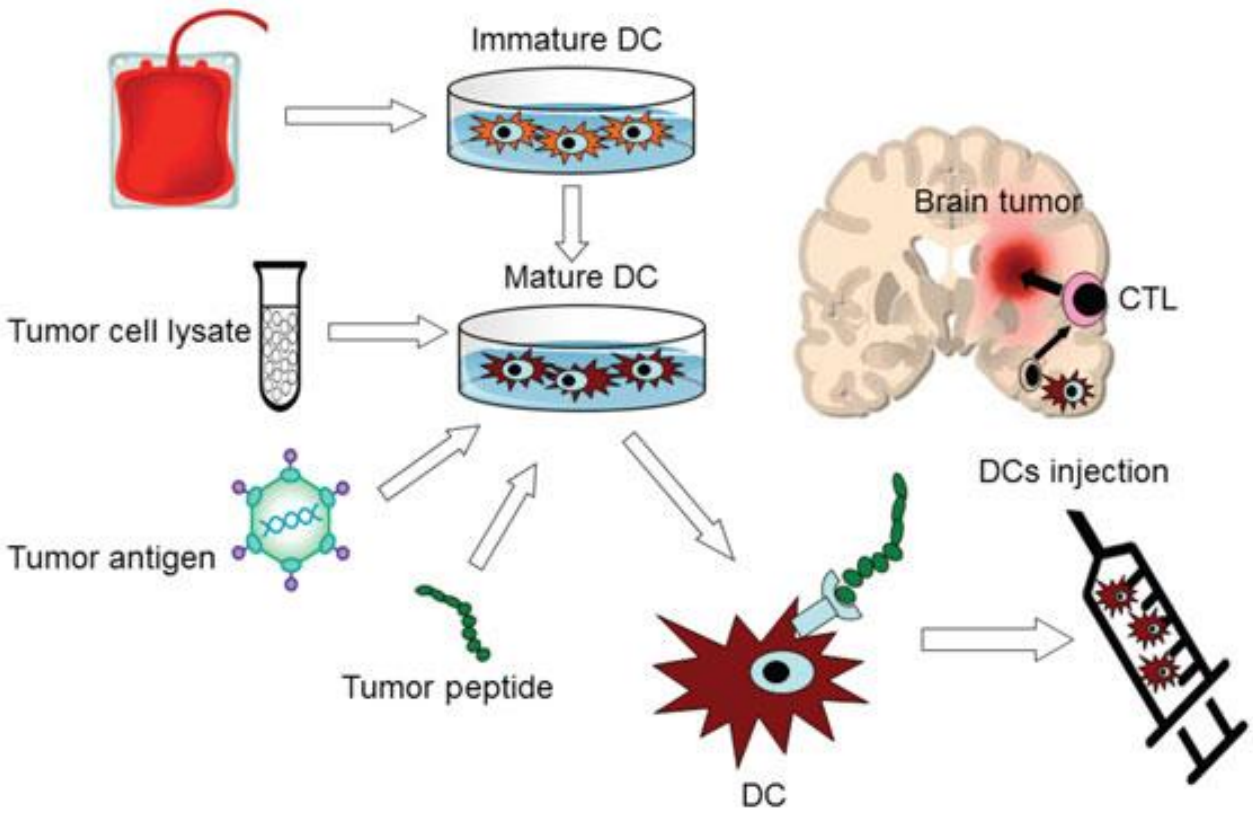
Peptide Vaccines vs. Dendritic Cell Vaccines



inadequate in immunocompromised patients

more effective since the dendritic cells, will instruct lymphocytes to activate the immune system

Dendritic Cell-Based Immunotherapy Treatment for Glioblastoma



Dendritic cells (DCs) obtained from differentiated monocytes in peripheral blood (red icon) are matured and then pulsed with tumor cell lysates, antigens, or peptides. The loaded cells are expanded and injected intradermally into patients, where the vaccine stimulates antitumor cytotoxic T lymphocytes (CTLs).

- Garg AD et al. Dendritic cell vaccines based on immunogenic cell death elicit danger signals and T cell-driven rejection of high-grade glioma. Sci Transl Med. 2016 2;8(328):328ra27.