# Vaccine Adjuvants

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*Journées inter-DES sur la vaccination*
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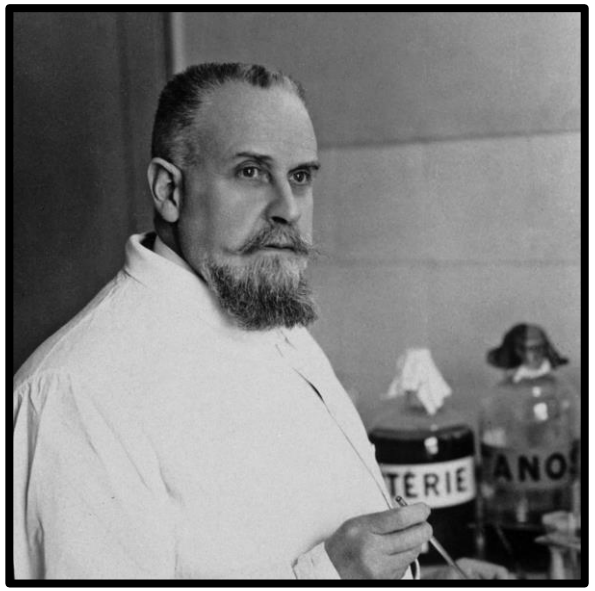
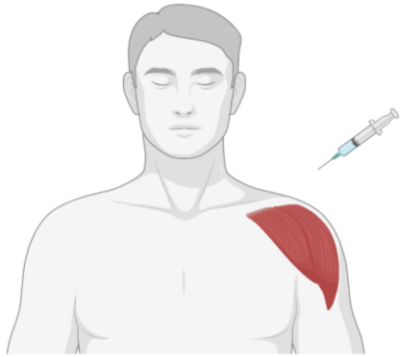
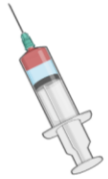
# How were adjuvants discovered ?

**1926**

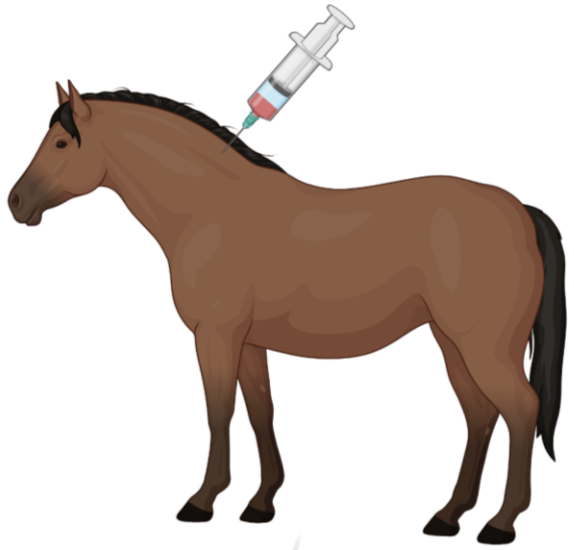


Gaston Ramon, French veterinarian and biologist





Anti-toxin serum

Production of anti-toxin against tetanus and diphtheria

https://pmc.ncbi.nlm.nih.gov/articles/PMC10819242/

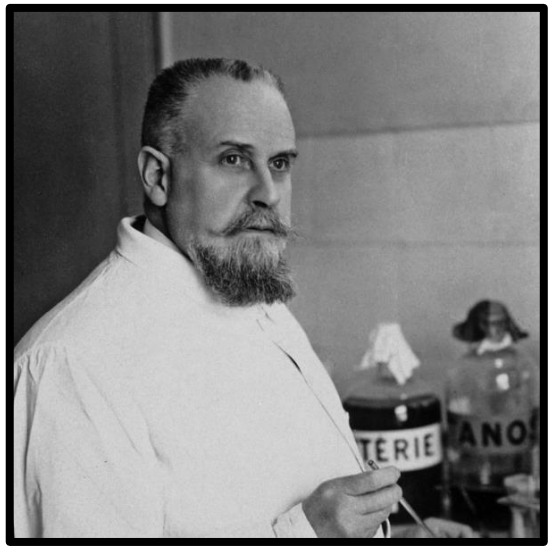
# Discovery of adjuvants

Observation: Ramon observed abscess formation at the site of antigen injection and a higher yield of anti-toxin in horses with a strong local inflammatory reaction.

Inflammation at the injection site → Increase anti-toxin production

“A specifically inert substance which, when injected in association with the specific vaccine antigen, increases to a greater or lesser extent the immunity that the latter is capable of developing” (1926)

Ramon tested different substances: pus, germs from abscess, calcium chloride, gelatin, agar, glycerin etc



Gaston Ramon, French veterinarian and biologist

They adsorbed the antigen

Bread Crumbs

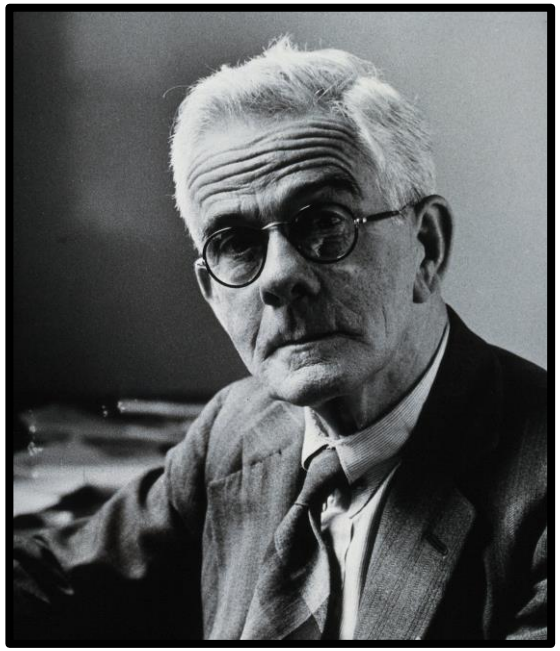

Starch

https://pmc.ncbi.nlm.nih.gov/articles/PMC10819242/

# Discovery of adjuvanticity of Aluminum salts

1926: In order to concentrate diphtheria toxoids (attenuated toxins) to produce a vaccine, Glenny and his colleagues added potassium aluminum sulfate (alum) to the production. They observed precipitates of antigen and higher antibody responses in guinea pigs immunized with this formulation compared to animals immunized with soluble toxoids alone.

1930's and 1940's: several studies reported that aluminum hydroxide and aluminum phosphate were able to absorb diphtheria antigen from an aqueous solution and due to their better manufacturing reproducibly, aluminum hydroxide commercialized as alhydrogel, and aluminum phosphate replaced alum for commercial vaccines.



Alexander Glenny, British Immunologist


Alum-Based Adjuvants

This material stood as the only approved adjuvant in vaccines for 70 years.

# What is an adjuvant ?

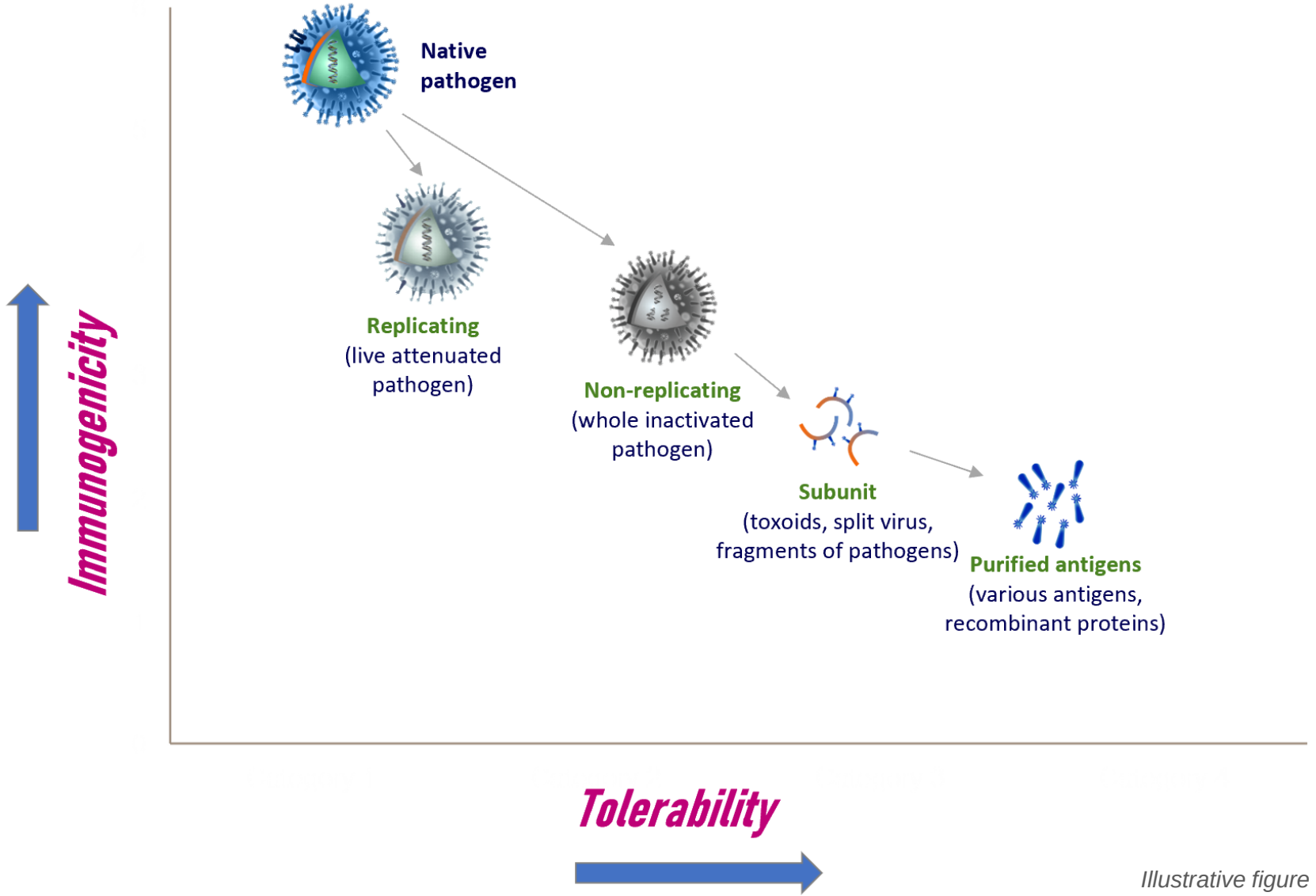
## “The immunologist’s dirty little secret”

Charles Janeway ‘Approaching the asymptote? Evolution and revolution in immunology’, Cold Spring Harb Symp Quant Biol, 54, 1989

## “An adjuvant is any substance (or a mixture of substances) that enhances the immune response to an antigen with which it is mixed”

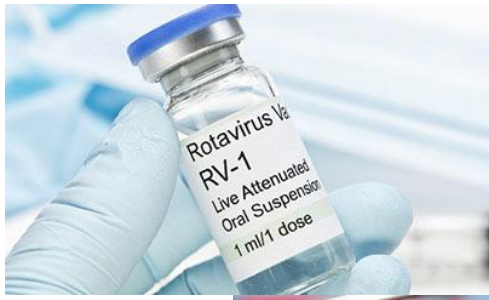
Janeway, Immunobiology, 7th Edition, 2008

# Why do we need Adjuvants ?




*Illustrative figure based on concepts from Garçon et al. 2011*

# Why do we need Adjuvants ?



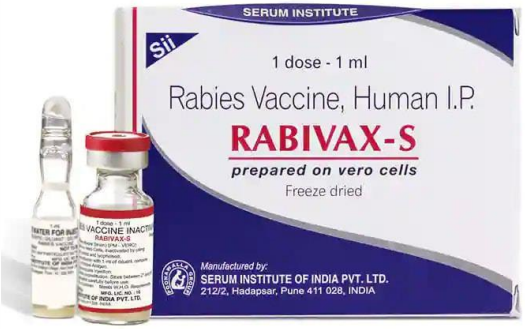

Live Attenuated Vaccines
- MMR
- Rotavirus
- Smallpox
- Chickenpox
- Yellow fever
- Oral Polio

Inactivated Vaccines
- Hepatitis A
- Polio (shot only)
- Rabies

→

Hghly Immunogenic
Tolerability Concerns
Safety Concerns



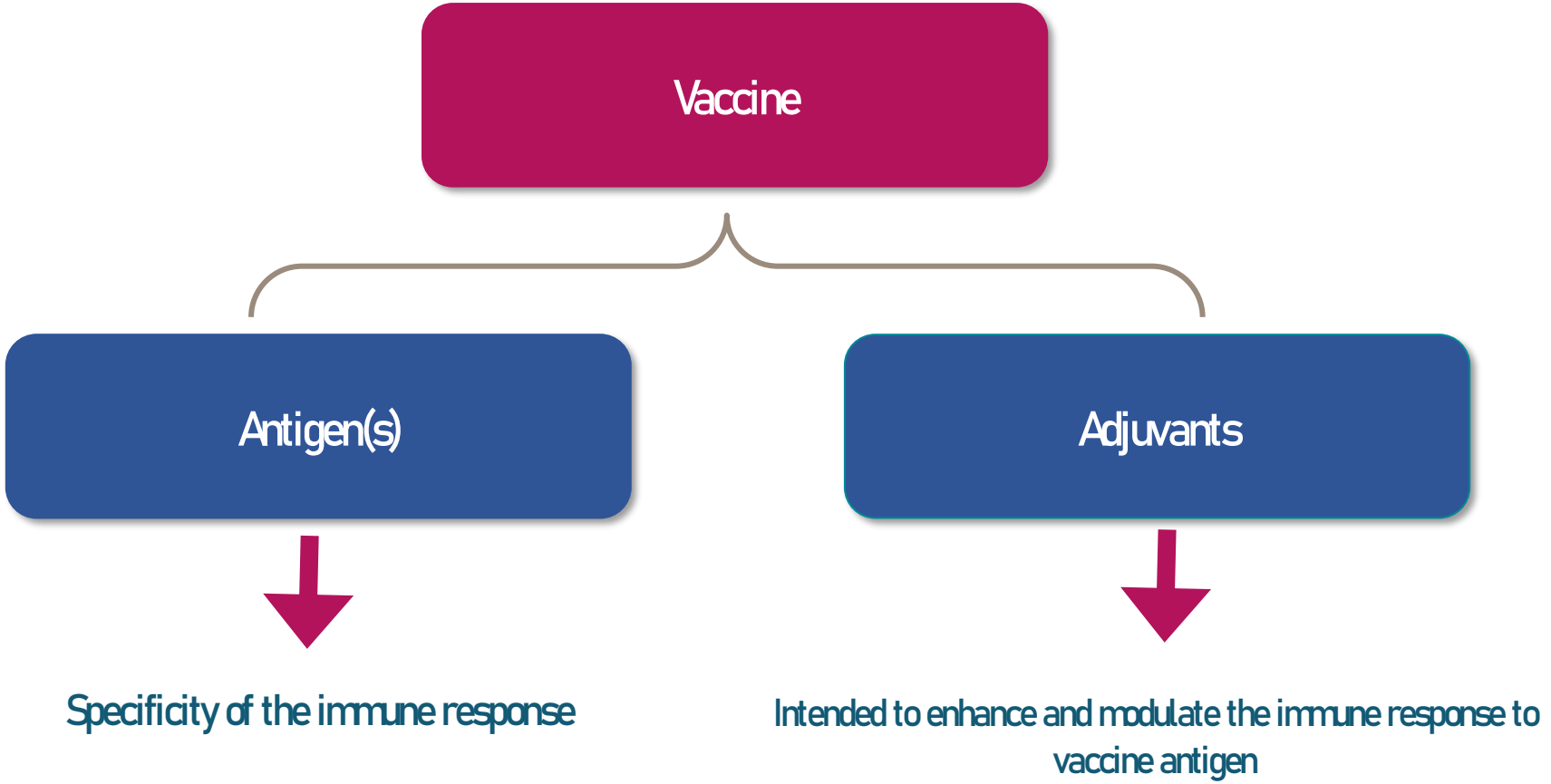



# Why Do We Need Adjuvants?

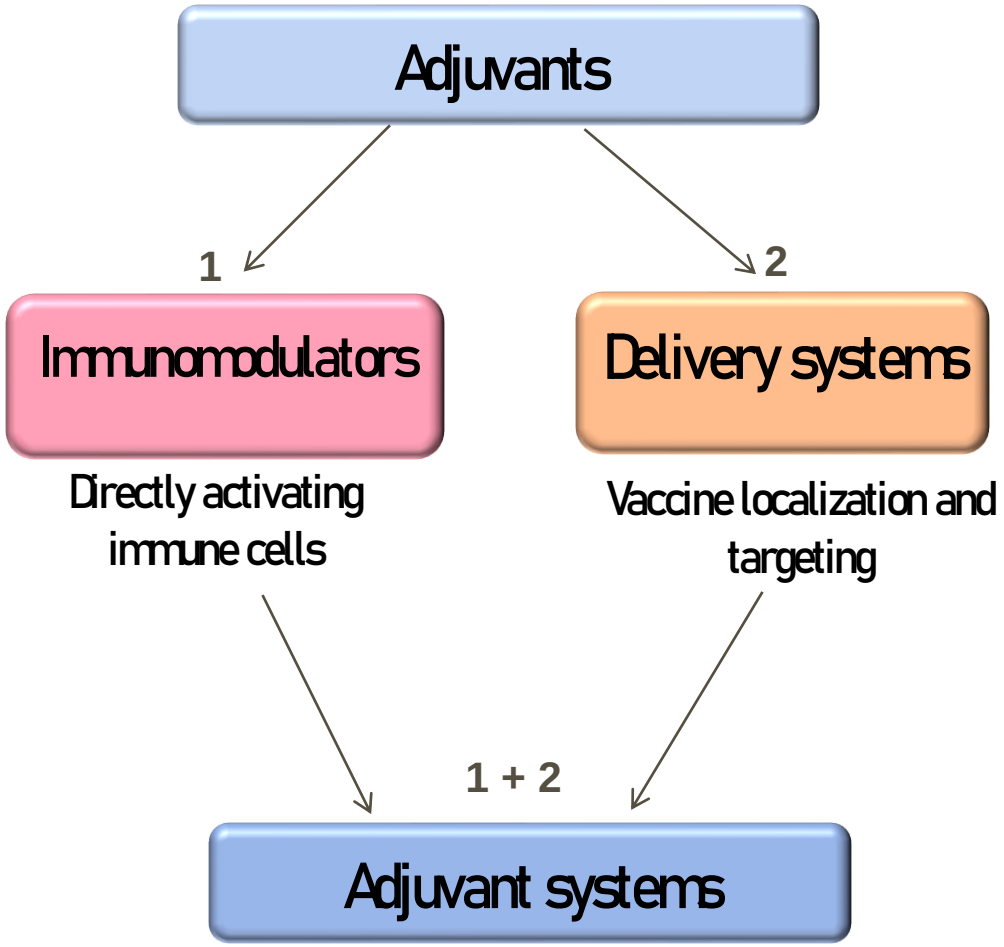
Subunit vaccines
Viral vector vaccines
Toxoid Vaccines
Conjugate vaccines
VLPs
OMV

Less immunogenic with Antigen alone
Hgh tolerability

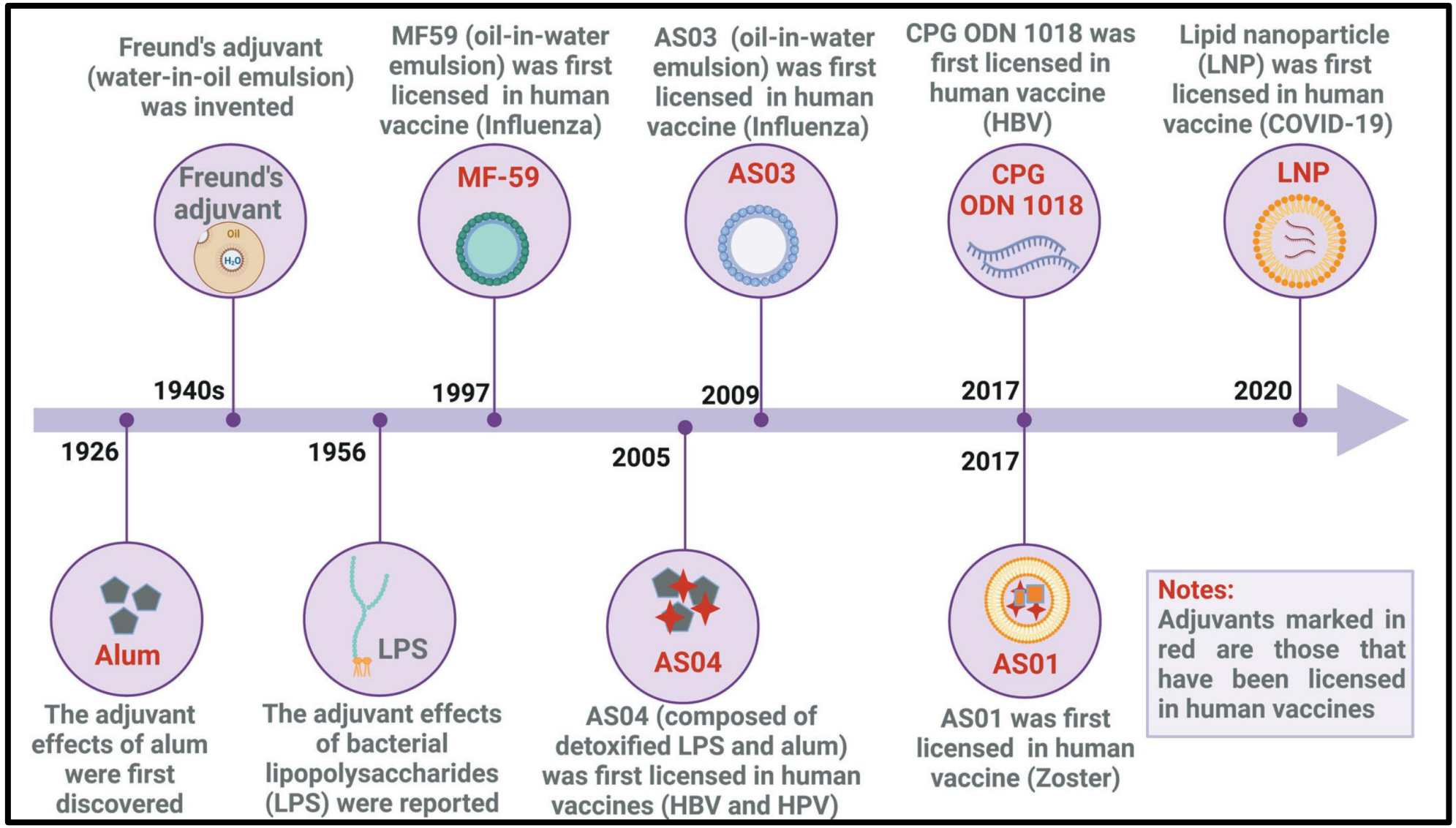
# Key components of Vaccines



# What are Adjuvant Systems?



# Timeline - Adjuvants




Source: Vaccine adjuvants: mechanisms and platforms, Tingmei Zhao et al, Signal Transduction and Targeted Therapy, Nature, 2023, https://doi.org/10.1038/s41392-023-01557-7

# Licenced adjuvants for human use

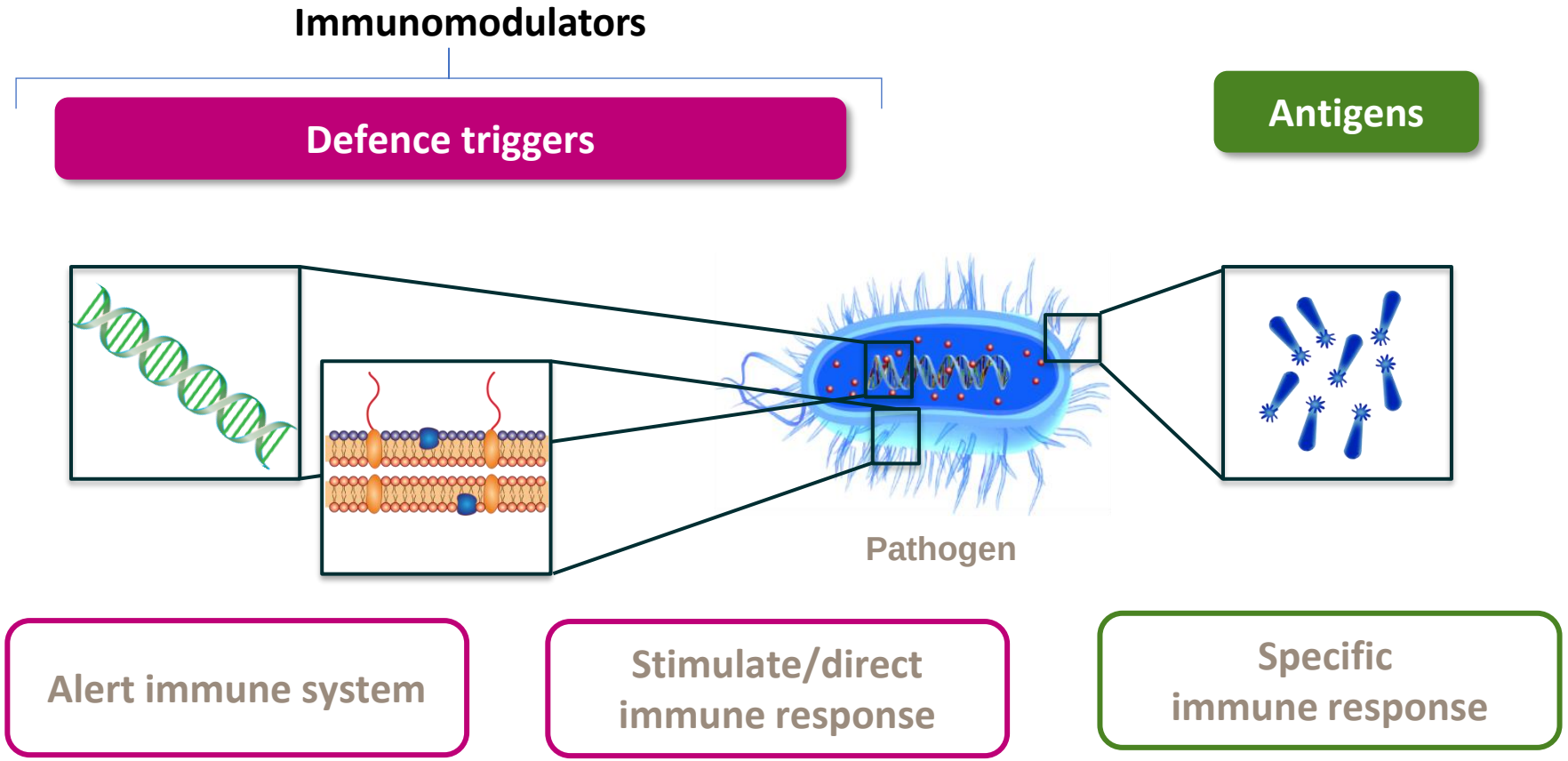
| Adjuvant | Classification | Components | Proposed mode of action | Component of (licensed vaccines) |
|---|---|---|---|---|
| Alum | aluminum-salt-based | ≥1 of the following:•amorphous aluminum hydroxyphosphate sulfate•aluminum hydroxide•aluminum phosphate•potassium aluminum sulfate | •alarmin/DAMP release•inflammasome activation | •BioThrax•Twinrix•Daptacel/Infanrix•Quadracel/ Pentacel•Gardasil•Ixiaro•Prevnar•TicoVac•and others |
| AS04 | combination | •alum•monophosphoryl lipid A (MPL/MPLA) Derived LPS | •as above for alum•TLR4 signaling | •Cervarix•Fendrix |
| MF59 | oil-in-water emulsion | Detergents •squalene oil•Span85•polysorbate 80 Animal source (*Squalus acanthias*) | •cell recruitment•ER/mitochondrial stress•DAMP release•lymph node draining | •Fluad•Focetria/Celtura |
| AS03 | oil-in-water emulsion | •squalene oil•α-tocopherol•polysorbate 80 Derived Vit E | •as above for MF59•IFN-I signaling | •Arepanrix•Pandemrix•Prepandrix |
| AS01 | combination | •liposomes•MPL•QS-21 saponin Purified plant extract (*Quillaja saponaria*) | •early IFN-I/IL-12/IL-18•TLR4 signaling•improved uptake•lysosomal disruption | Shingrix |
| Matrix-M | nanoparticle | •saponins•cholesterol•phospholipids | •lymph node trafficking•IFN-I signaling | •Nuvaxovid/Covovax•Mosquirix |
| LNPs | nanoparticulate delivery system | •ionizable cationic lipid cholesterol•PEGylated lipid•distearoylphosphatidylcholine (DSPC) | •lymph node trafficking•enhanced uptake•cell recruitment•MDA5/IFN-I/IL-6 | •Comirnaty•Spikevax |
| Alhydroxiquim-II | combination | •alum•imidazoquinolin gallamide | •as above for alum•TLR7/8 signaling | Covaxin |
| CpG-ODN 1018 | TLR agonist | 22-mer oligonucleotide sequence | TLR9 signaling | Heplisav-B |

# How does the adjuvanticity principle work ?



Microbial structures contain:

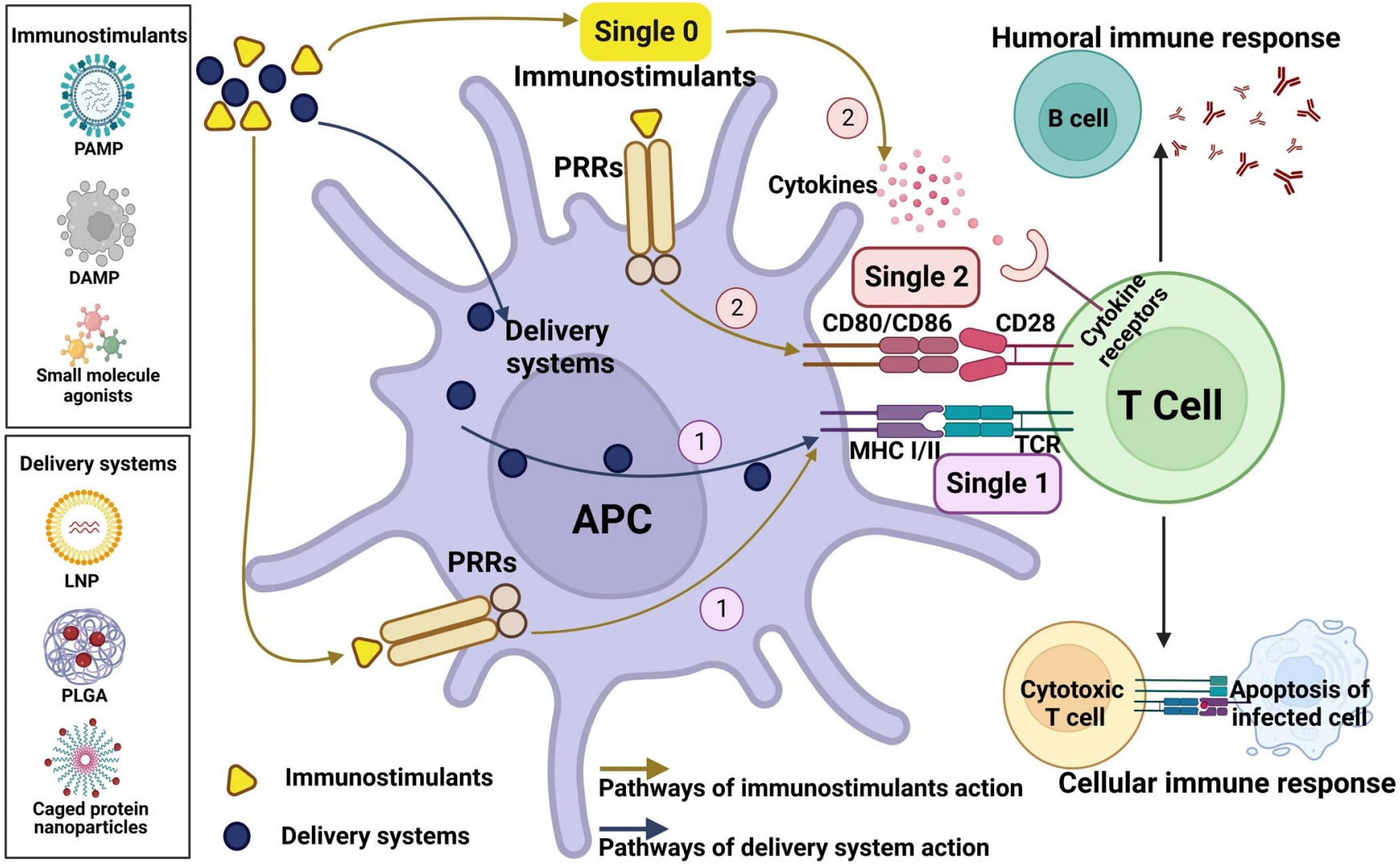
- Antigens
- Defence triggers e.g. PAMPs act as intrinsic immune-triggers




PAMPs = pathogen-associated molecular patterns

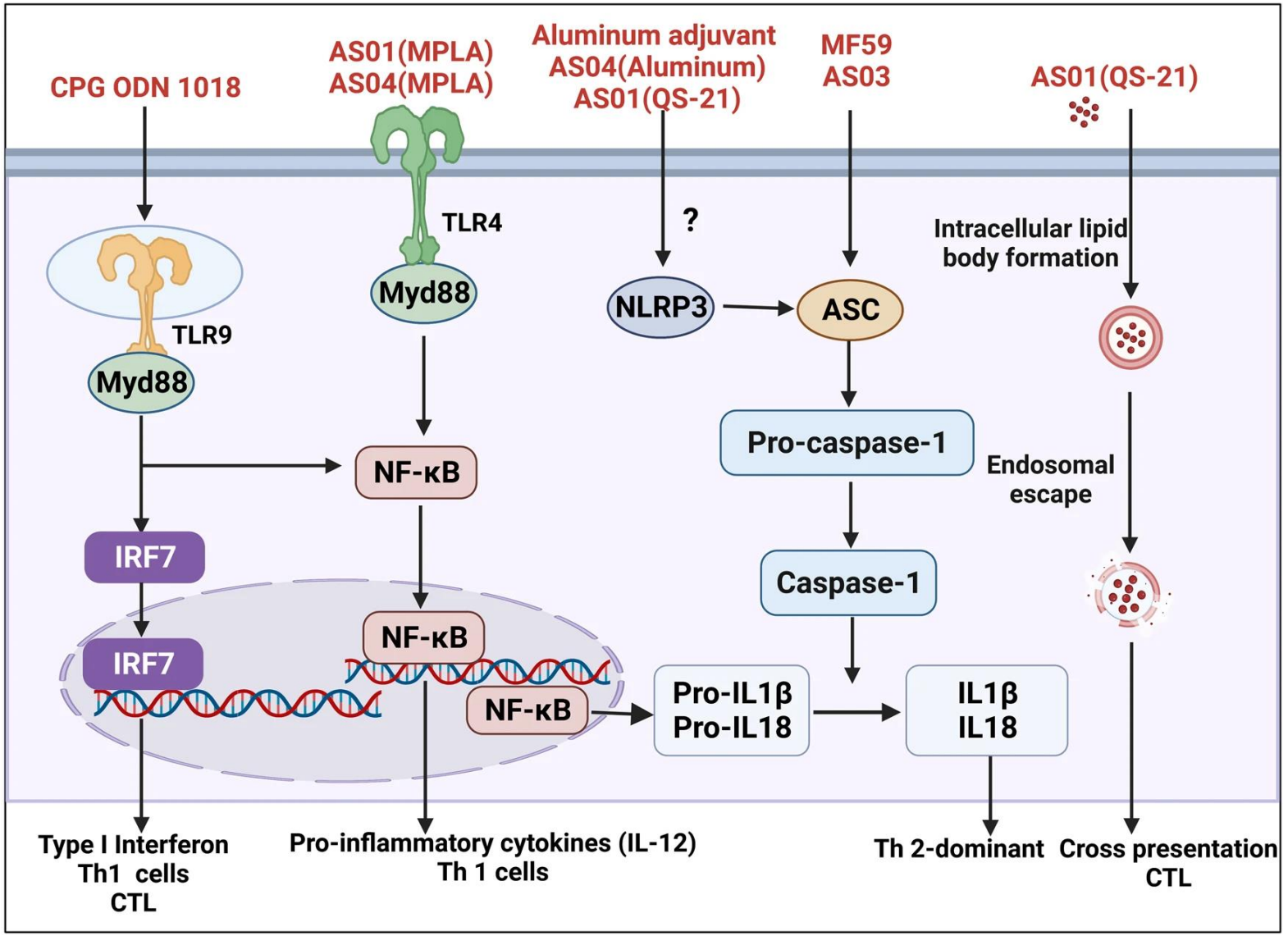
Dougan G & Hormaeche C. *Vaccine* 2006;24S2:S2/13–9; O'Hagan DT & Valiente NM. *Nat Rev Drug Discov* 2003;2:727–35; Garçon N *et al.* Chapter 4 in: Garçon *et al.* Understanding Modern Vaccines, Perspectives in Vaccinology, Vol 1, Amsterdam, Elsevier, 2011

# How does the adjuvanticity principle work ?



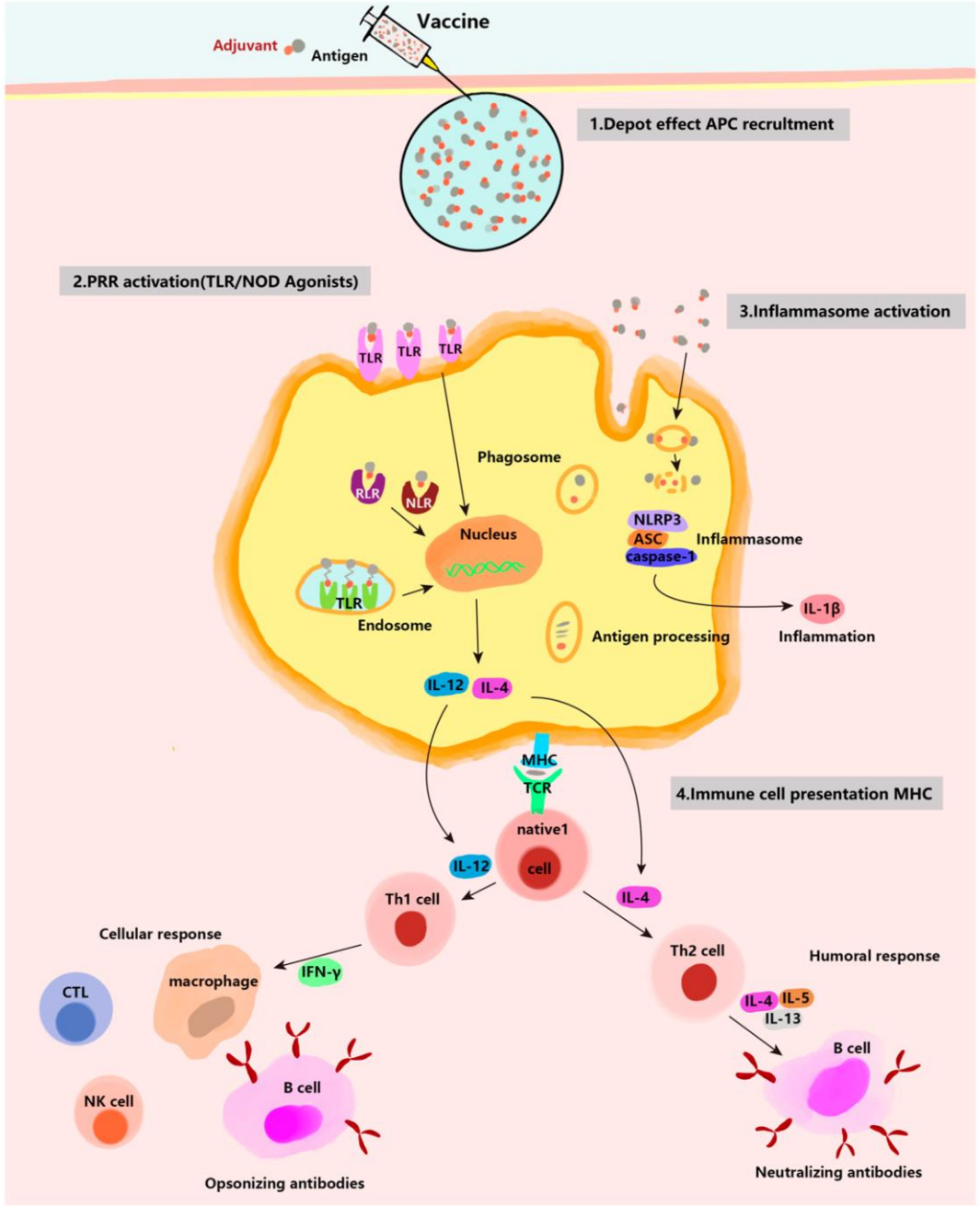

Source: Vaccine adjuvants: mechanisms and platforms, Tingmei Zhao et al, Signal Transduction and Targeted Therapy, Nature, 2023, https://doi.org/10.1038/s41392-023-01557-7

# Which are signaling pathways triggered by adjuvants ?




Source: Vaccine adjuvants: mechanisms and platforms, Tingmei Zhao et al, Signal Transduction and Targeted Therapy, Nature, 2023, https://doi.org/10.1038/s41392-023-01557-7

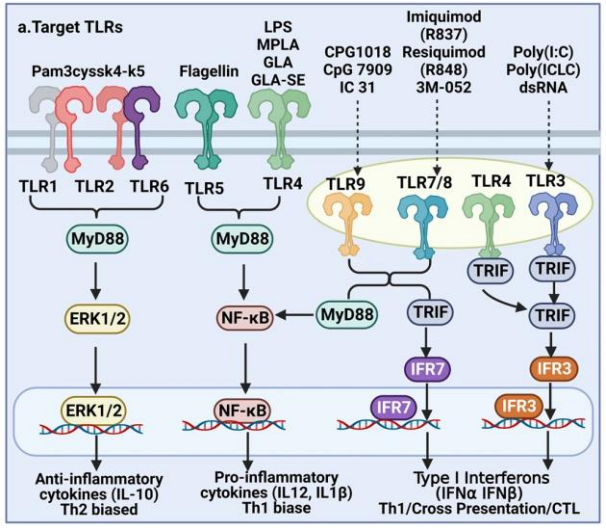
# Mechanisms of action of Alum salts



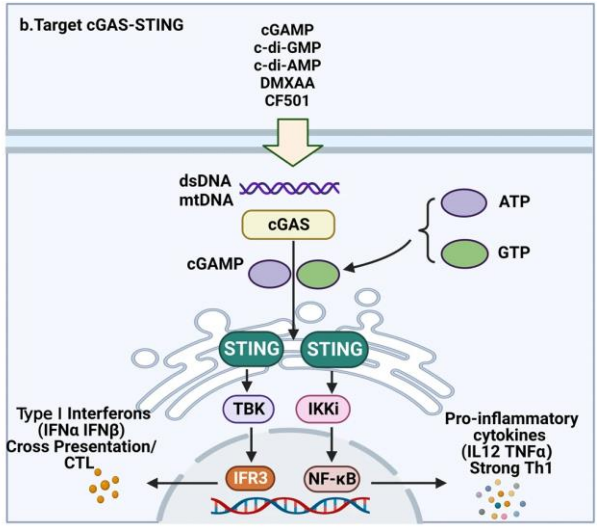

Lan et al, 2024, Vaccines

# Adjuvant research: other immunomodulatory components to activate other signaling pathways

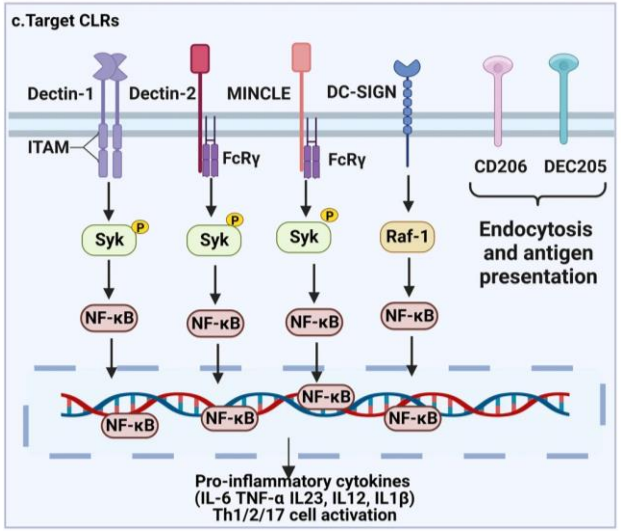
Nucleotides



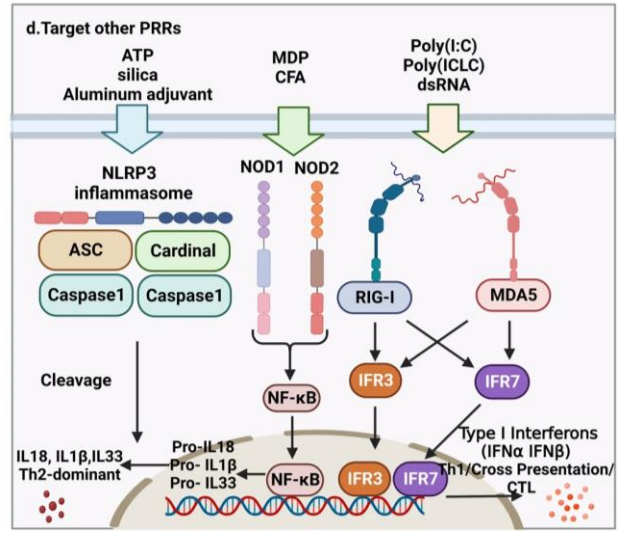

Toll-like receptors




cGAS-STING

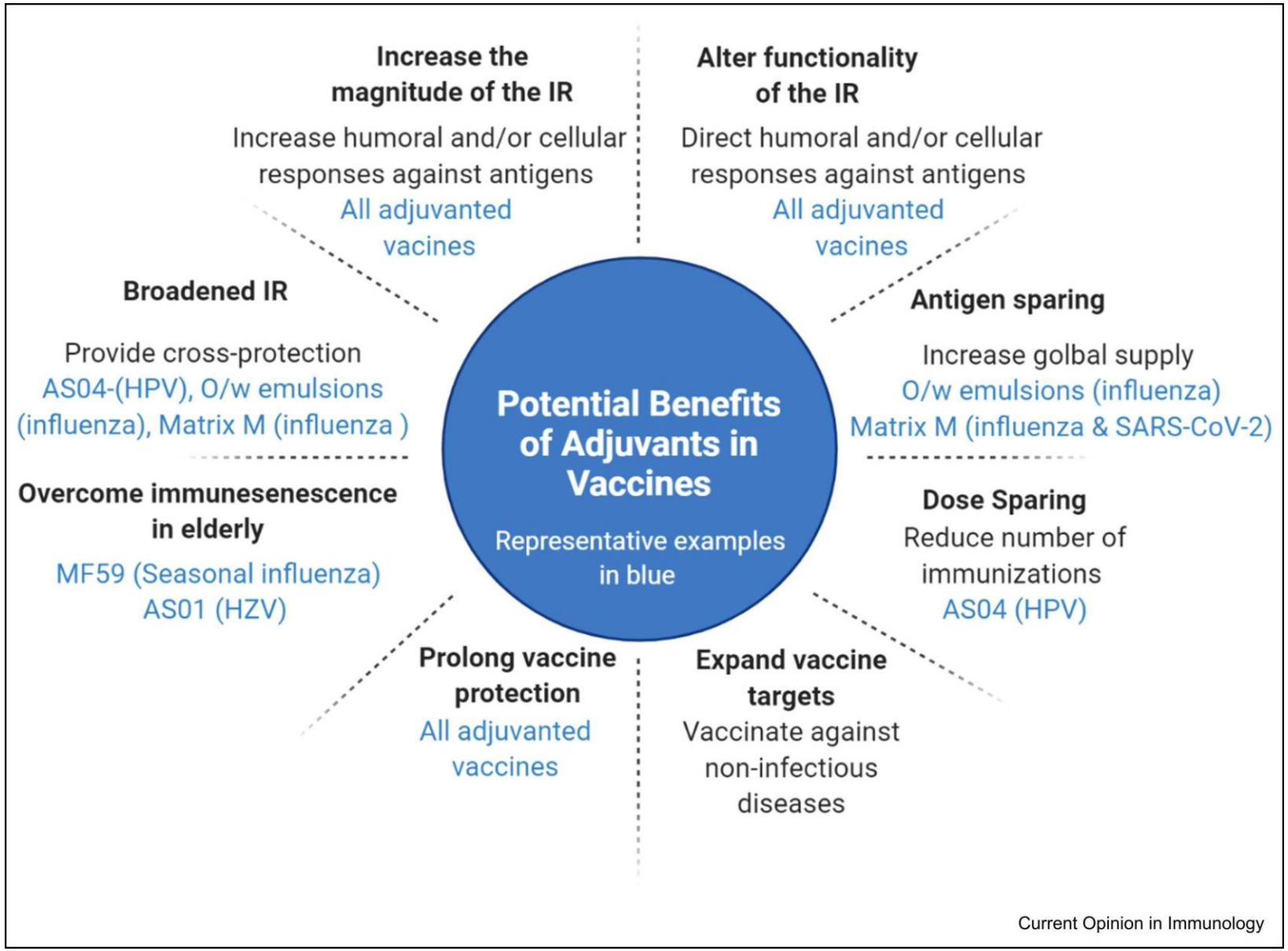



C-type lectin receptors



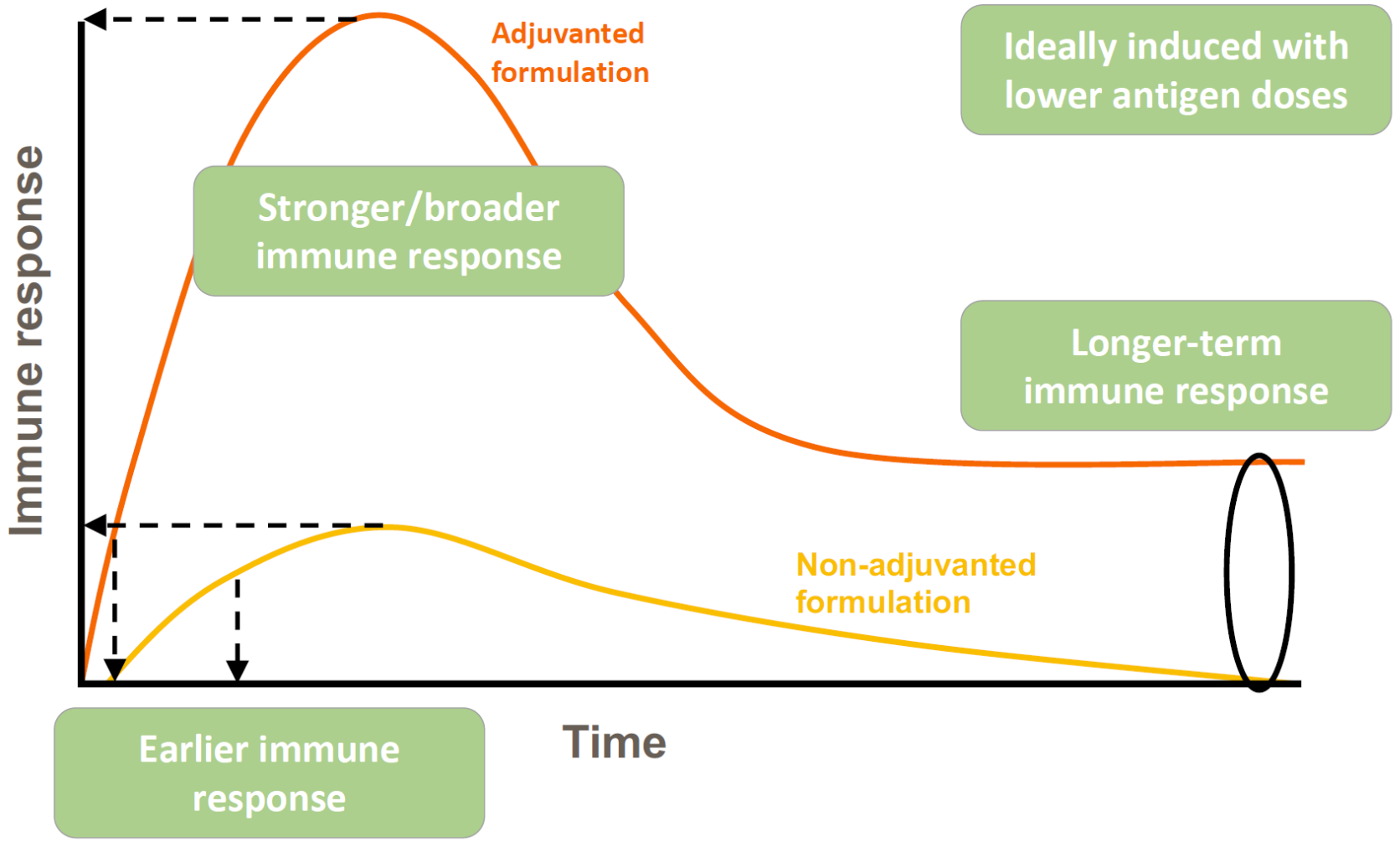

Other Pathogen recognition receptors

# Roles of novel adjuvants




Turley and Lavelle, 2022, Current Opinion in Immunology

# Roles of novel adjuvants




Modified from Pulendran B & Ahmed R. *Cell* 2006; 124:849–863.

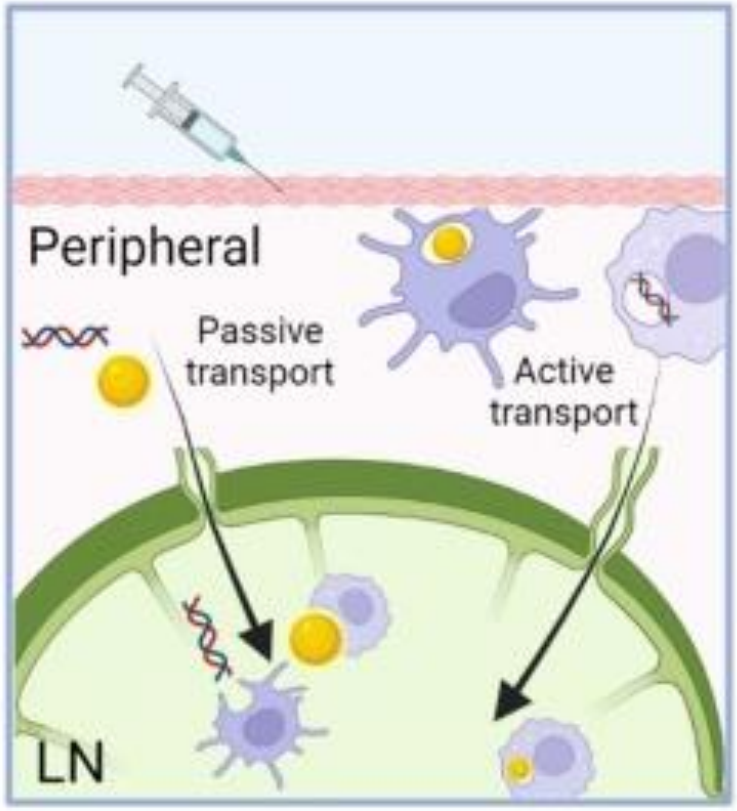
# Remaining questions linked to adjuvants

Mechanisms of action - trafficking

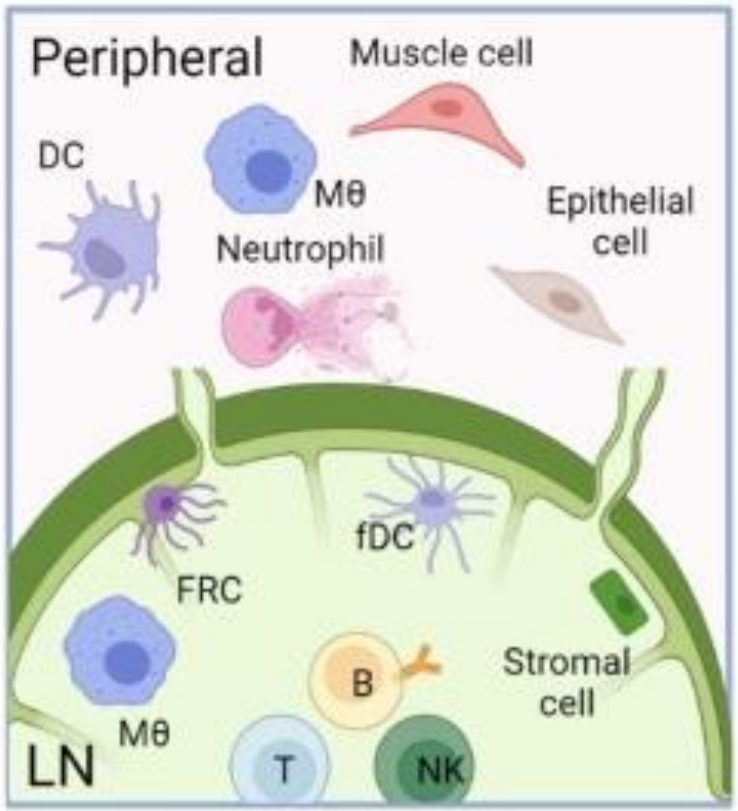
Source

Site of administration

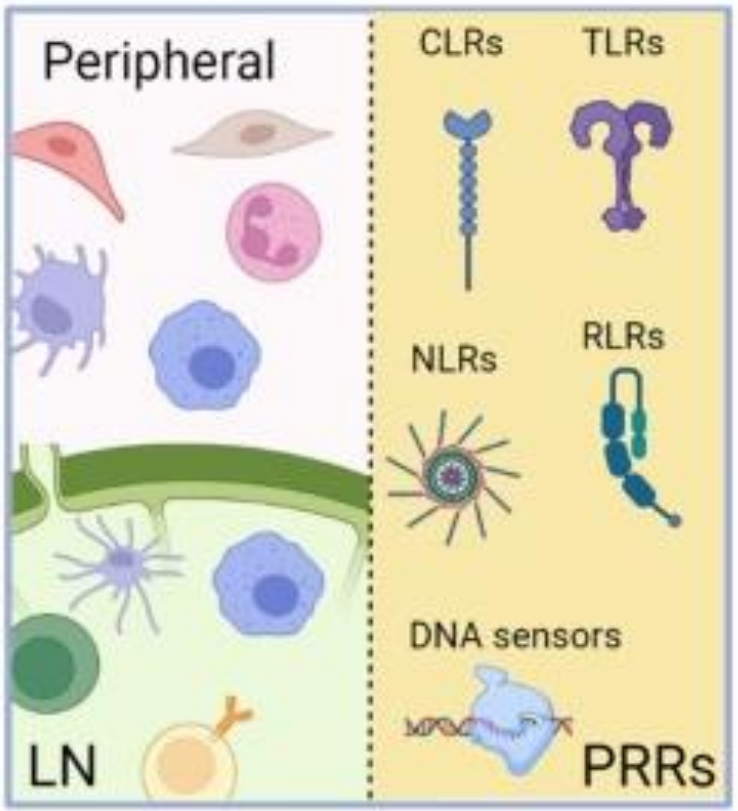
**1. How does the adjuvant traffic to the draining lymph node upon injection?**




**2. If the adjuvant is not a purified PAMP, what is the tissue and cellular source of the adjuvant-induced DAMP?**




**3. What PRR(s), cell(s) and tissue(s) does the PAMP or adjuvant induced-DAMP engage?**



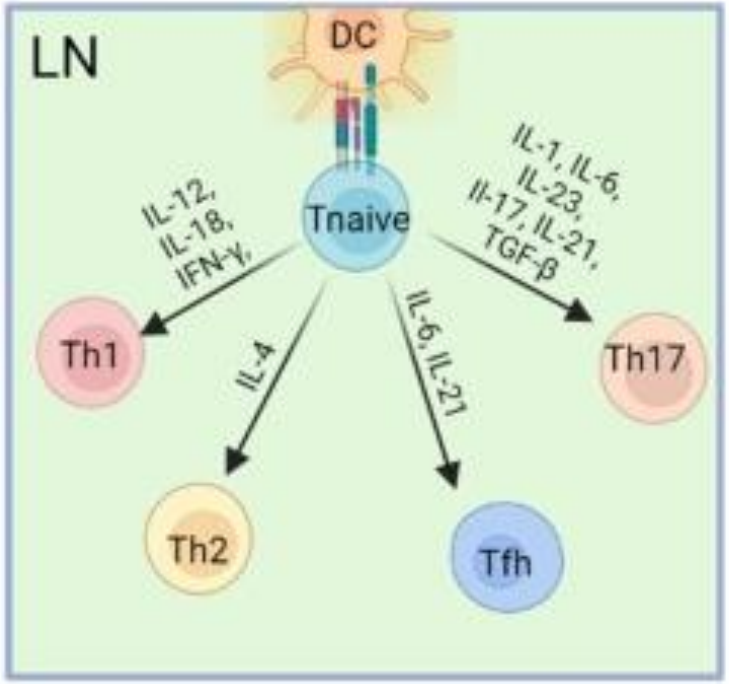
# Remaining questions linked to Adjuvants

Activated signaling pathway

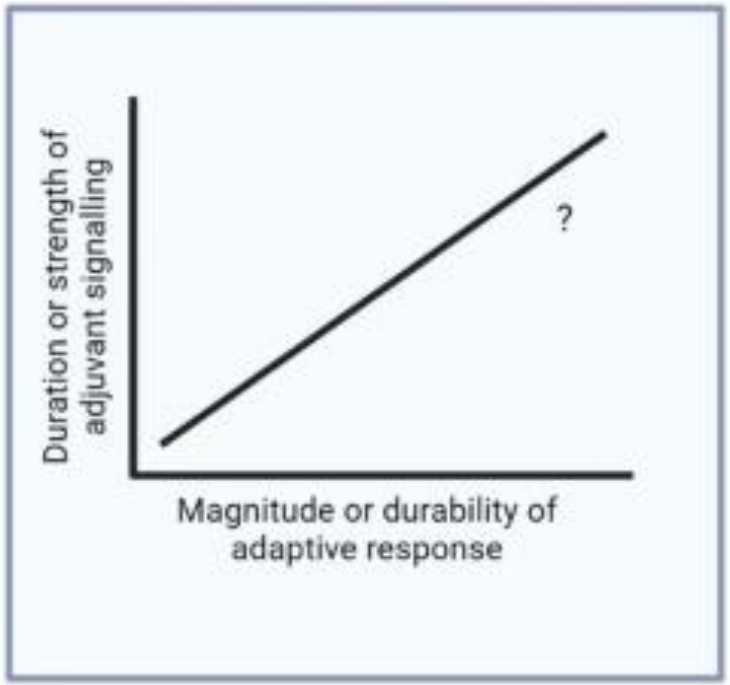
Kinetics

Dose

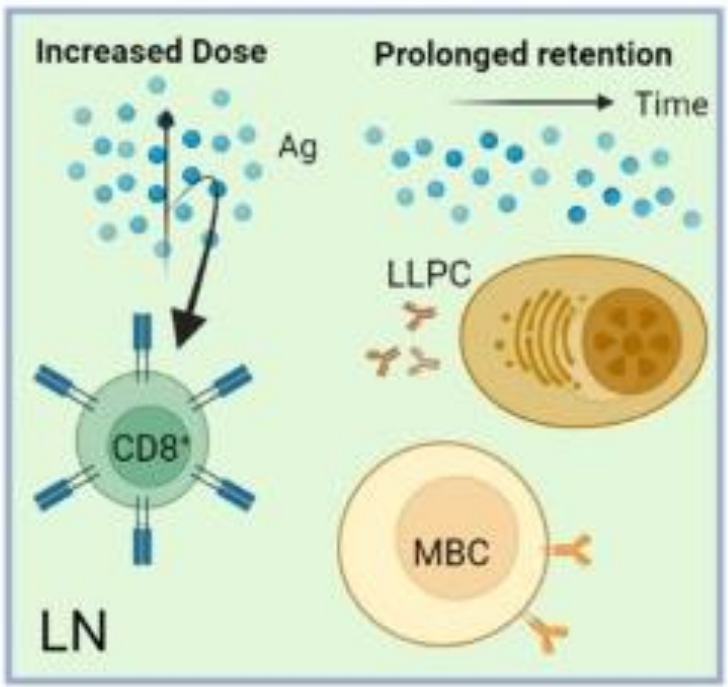
**4. What T cell response(s) do the adjuvant induced PRR-dependent cytokines promote?**




**5. What are the kinetics of the adjuvant-induced innate response and how do they impact the adaptive response?**



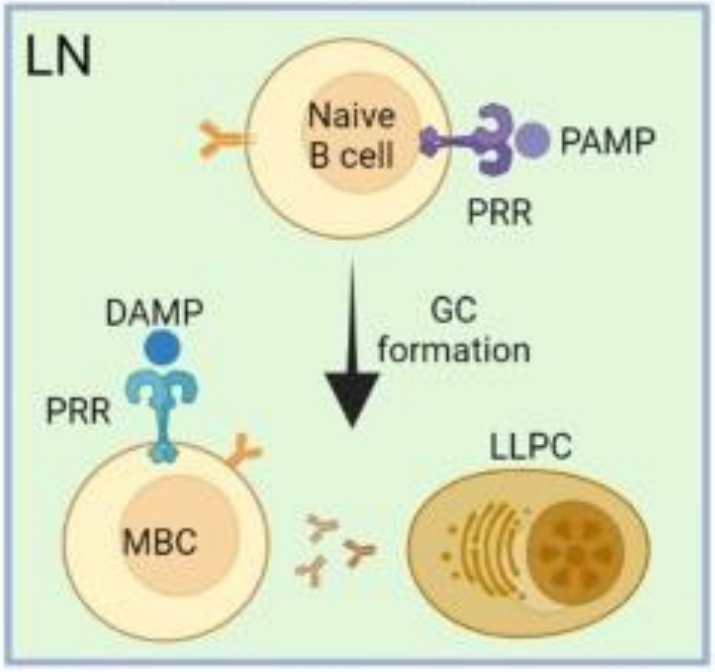

**6. Does increased antigen dose or retention alter adjuvant-induced adaptive response?**



# Remaining questions linked to Adjuvants

Targeting specific immune cells

**7. Can the PAMP or adjuvant-induced DAMP engage B cells directly to promote high avidity Abs?**



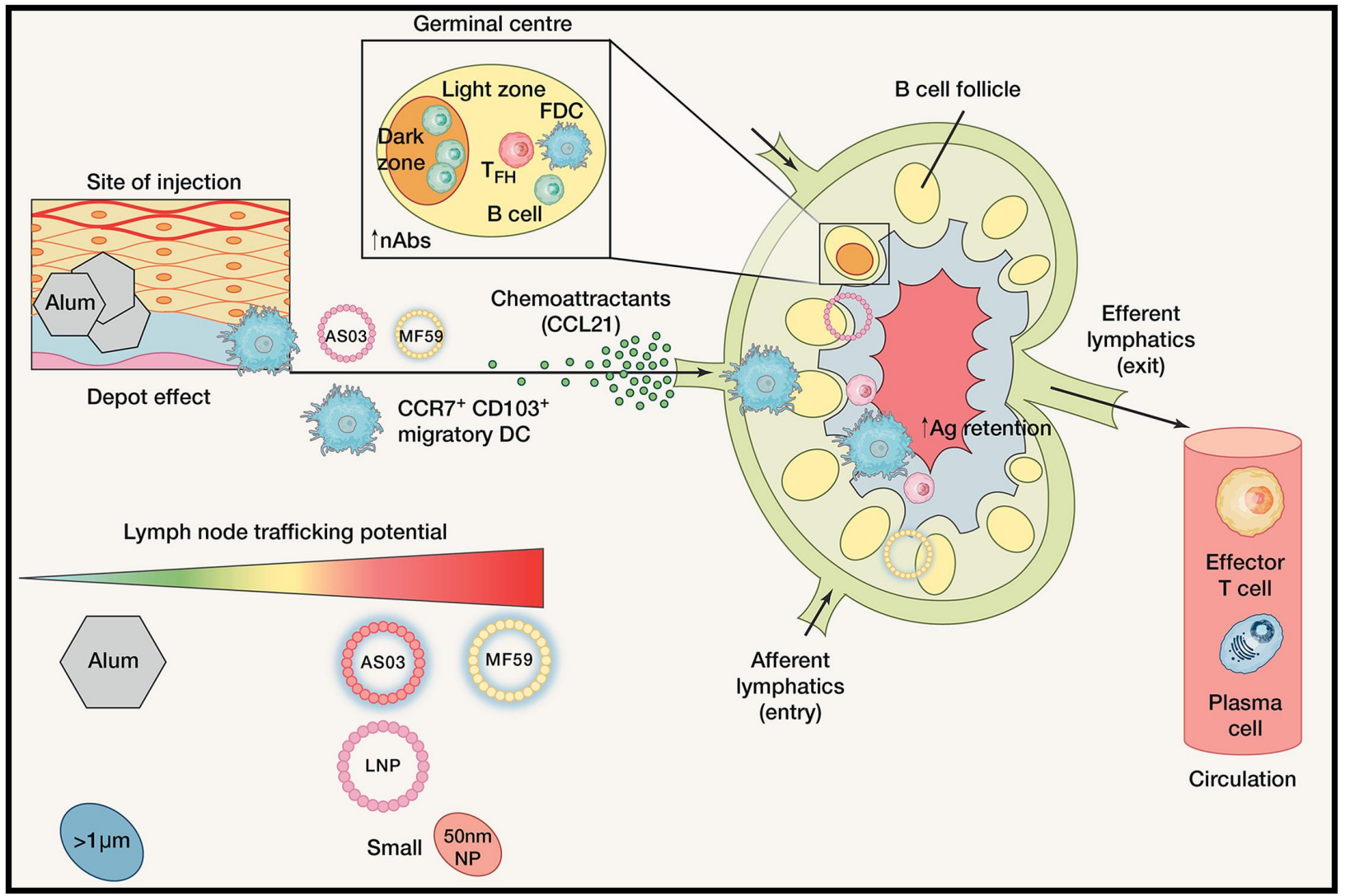

Species-dependant

**8. Does the adjuvant function similarly across species**

Population-dependant

**9. Does the adjuvant function similarly across diverse human populations**

# Lymph node trafficking potential of adjuvants

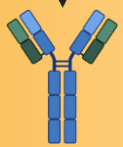



Lavelle, McEntee et al 2024, Cell

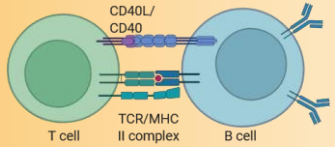
## Durable Humoral Immunity

Long Lived Plasma Cells

Memory B-cells

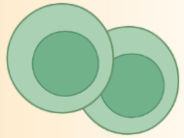


High Affinity Antibodies




Germinal center reactivation

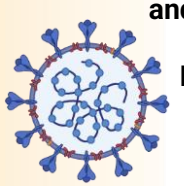
## Durable Cellular Immunity



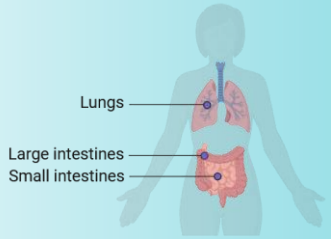
Memory and Resident Memory T-cells

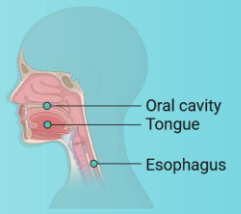
CD4+
CD8+
Th17

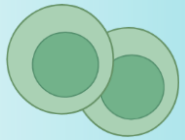
Increases Depth and Breadth of Immune Response



## Durable Mucosal Immunity



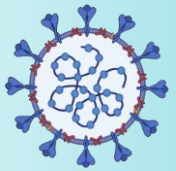



Memory and Resident Memory T-cells

CD4+
CD8+
Th17

Increase Protection Right From Pathogen Entry Sites



TLR Agonist

3M-052
R848 and MPL
AS03

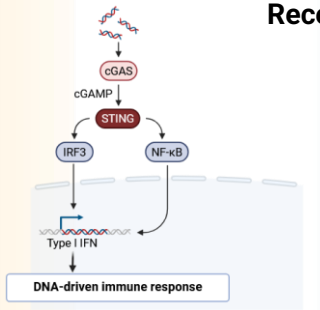
Adjuvant Formulations

Hydrogels
Saponin
Nanoparticles

TLR Agonist

Poly (I:C)
CpG

STING Agonist




Piscrasidine S

C-Type Lectin Receptor Ligands

CAF10b
CAF01

Mast cell activators

Mastroparan-7 (M7) and Compound C48/80

TLR Agonist

Poly (I:C)
NexaVant
IV209
CpG

# How is an Adjuvant selected?

Understand:

- Host-pathogen interaction
- Antigen selection and production
- Optimised immunological tools

Identify need for adjuvant

In case classical aluminium not sufficient, consider:

Immune response desired?

Stability over time?

Compatibility with antigen?

Safety and reactogenicity?

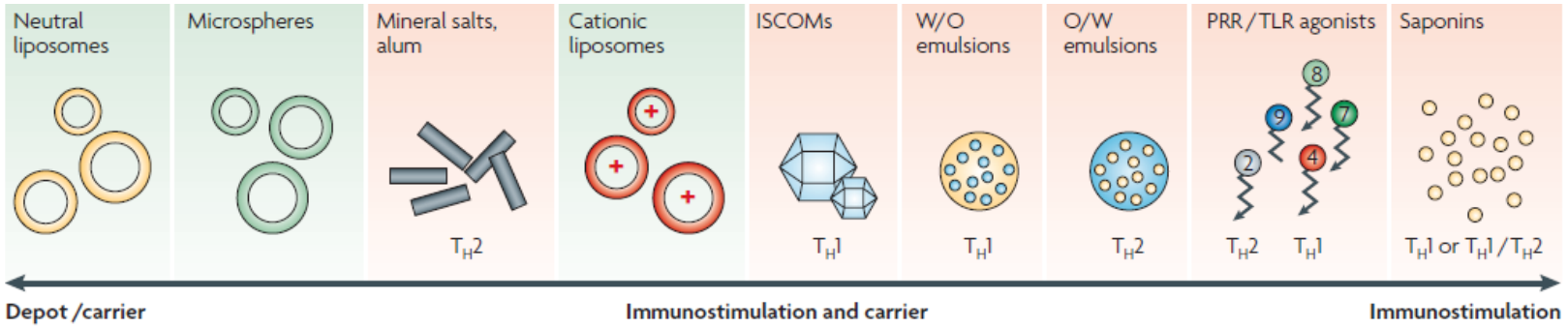
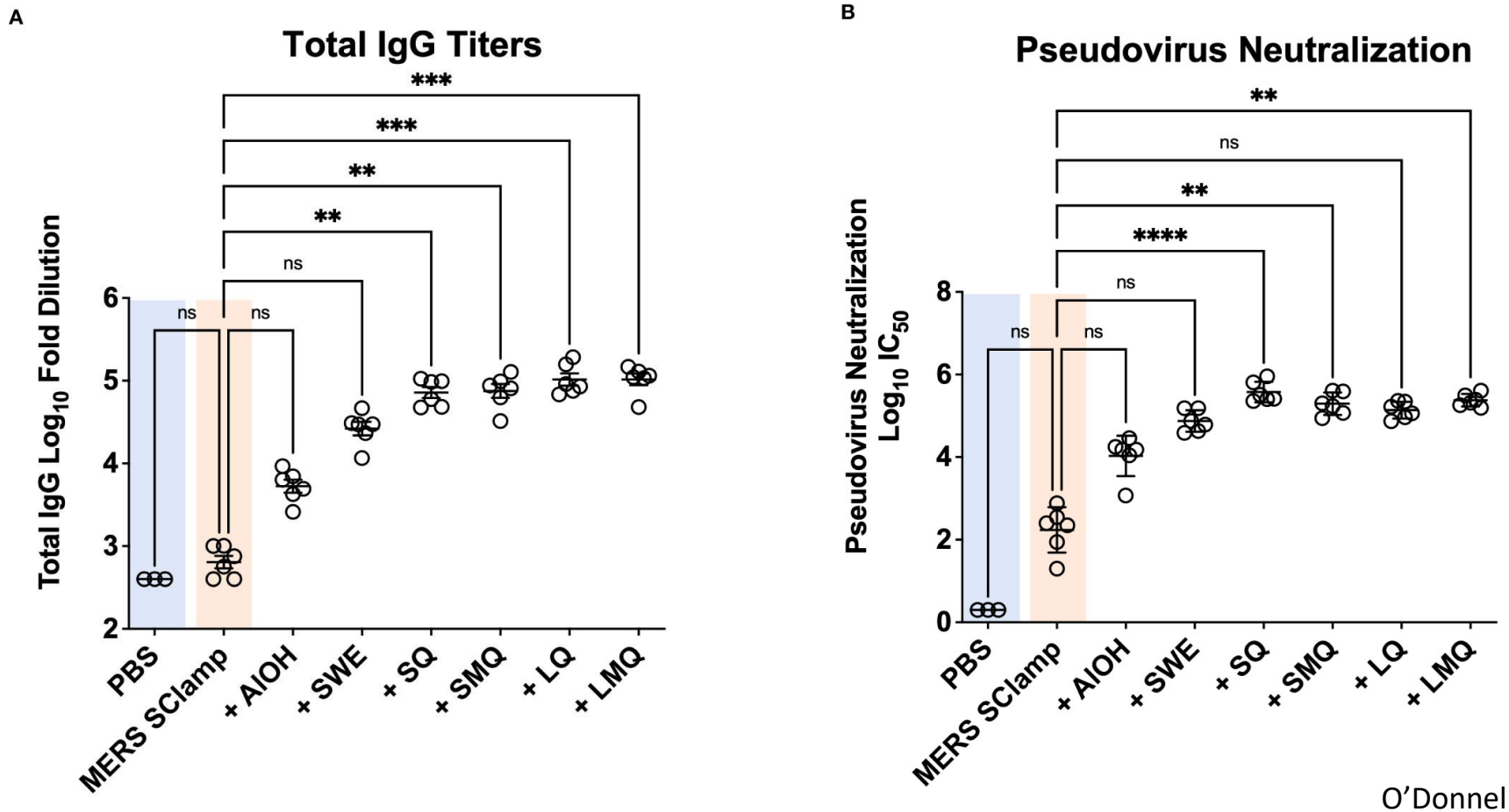



*Figure reproduced from B Guy, Nature Reviews Microbiology 5, 505-517 (July 2007)*

*Strugnell et al, chapter 3 pp. 61–88; Garçon N et al, chapter 4 pp. 89–113, in Understanding Modern Vaccines, Perspectives in Vaccinology, Vol 1, Amsterdam, Elsevier, 2011*

# Current evaluation in systemic adjuvanted vaccine in mouse model

MERS clamp with or without adjuvants in mouse model – intramuscular route (Prime-boost) ELISA -serum

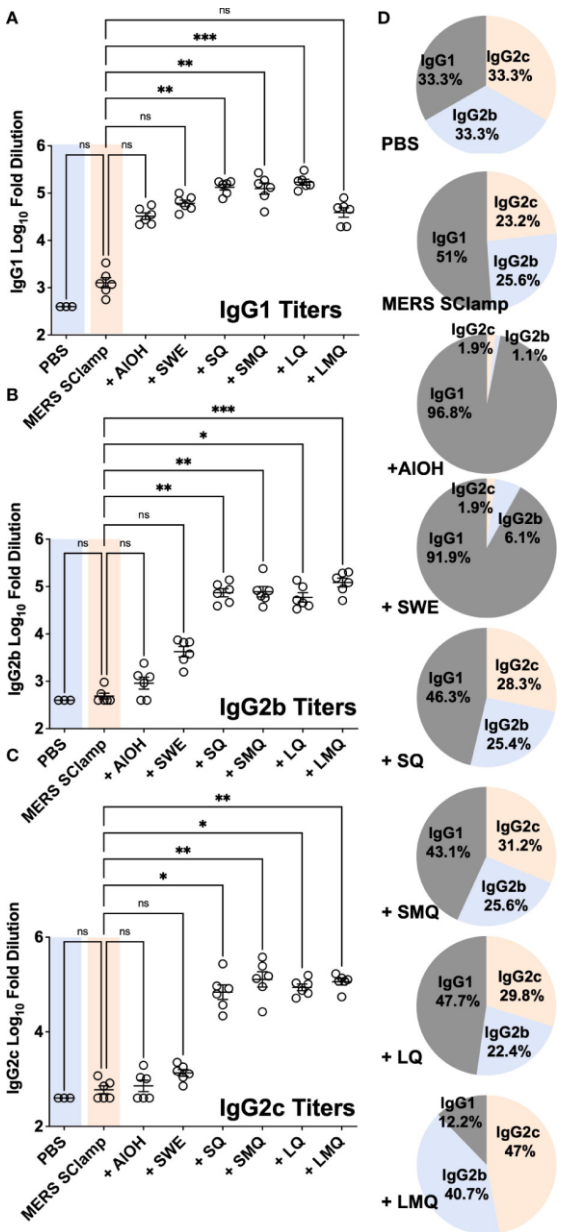



O'Donnell et al 2022, Front in Immunol

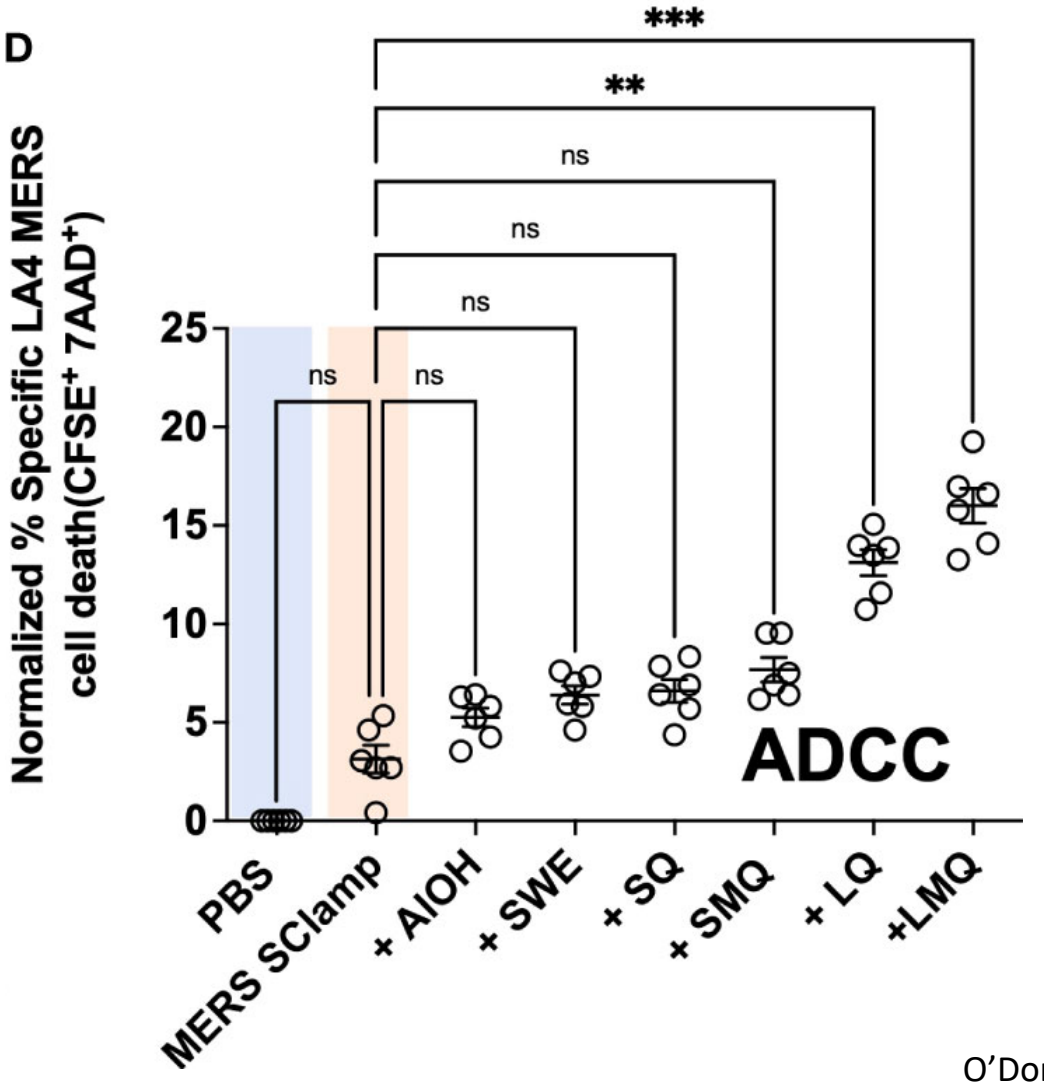
(i) aluminium hydroxide, (ii) SWE, a squalene-in-water emulsion, (iii) SQ, a squalene-in-water emulsion containing QS21 saponin, (iv) SMQ, a squalene-in-water emulsion containing QS21 and a synthetic toll-like receptor 4 (TLR4) agonist 3D-6-acyl Phosphorylated HexaAcyl Disaccharide (3D6AP); (v) LQ, neutral liposomes containing cholesterol, 1.2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and QS21, (vi) or LMQ, neutral liposomes containing cholesterol, DOPC, QS21, and 3D6AP

ELISA

# Functionality of antibodies




Antibody-dependent cell-mediated cytotoxicity

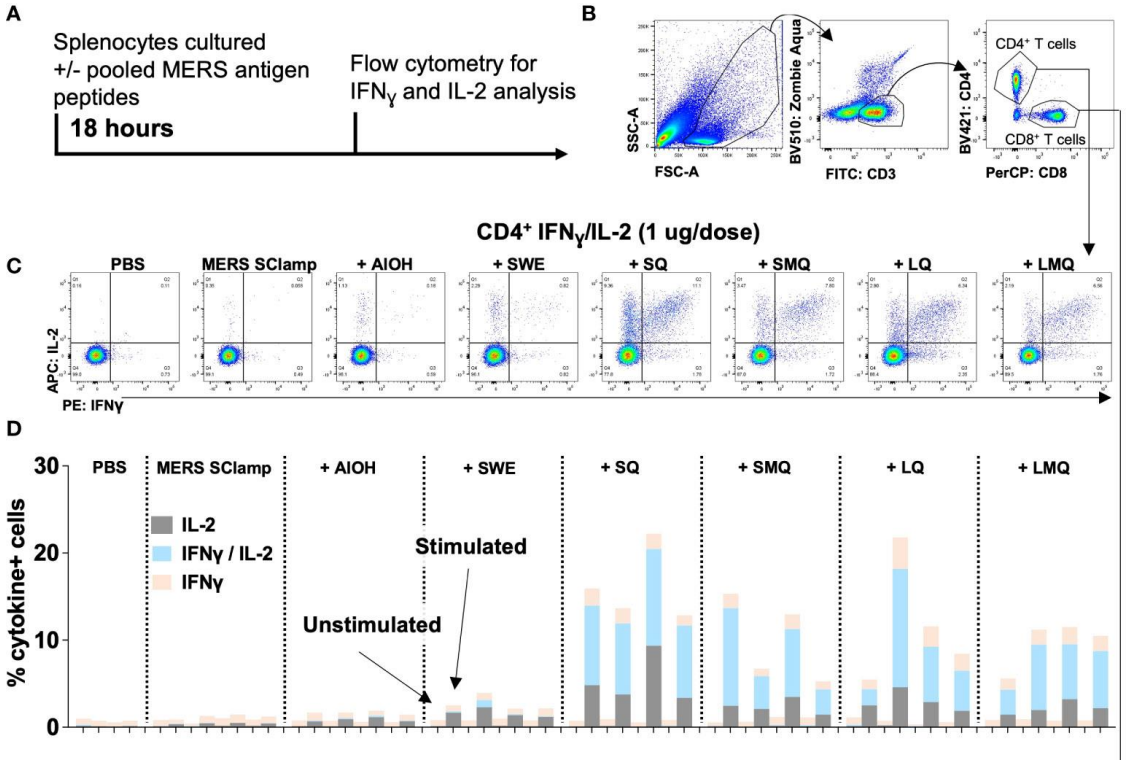



O'Donnell et al 2022, Front in Immunol

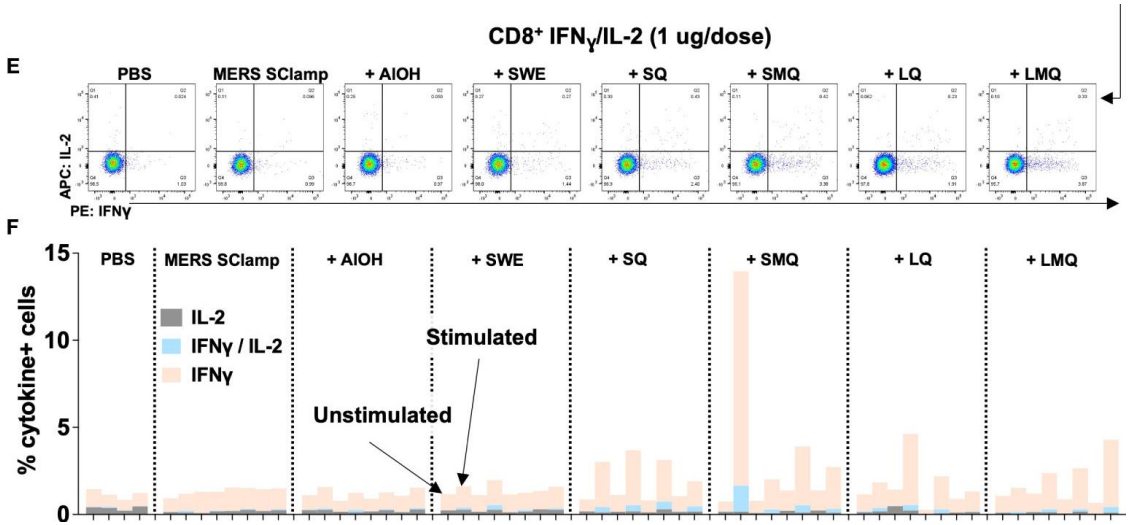
# T cell responses

## Intracellular cytokine staining (flow cytometry)

CD4



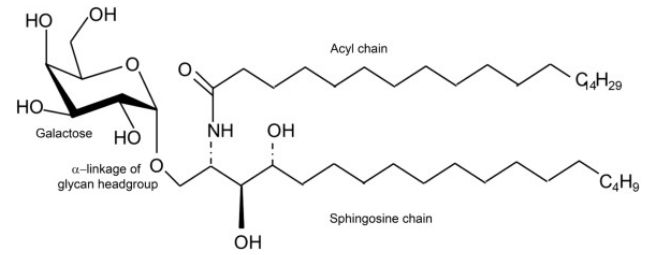

CD8




Others: ELISpot

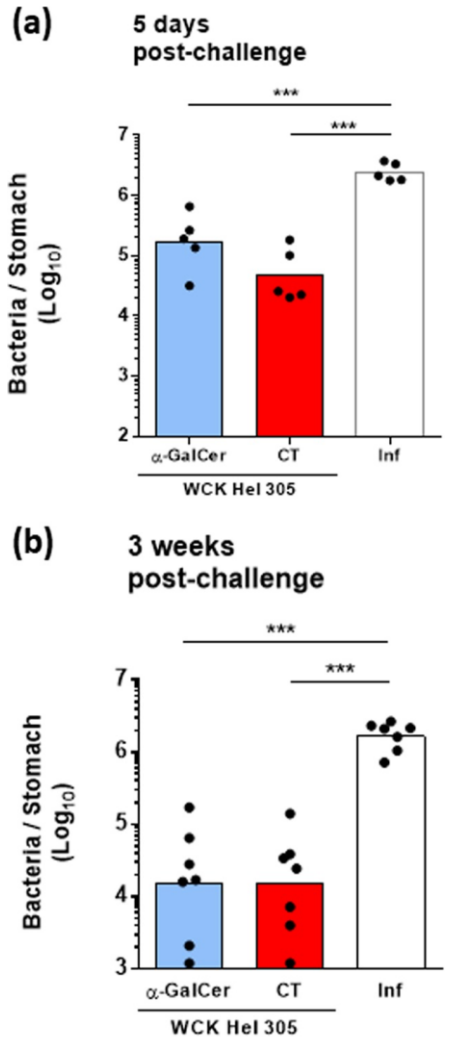
O'Donnell et al 2022, Front in Immunol

# Current evaluation of mucosal adjuvanted vaccine in mouse model

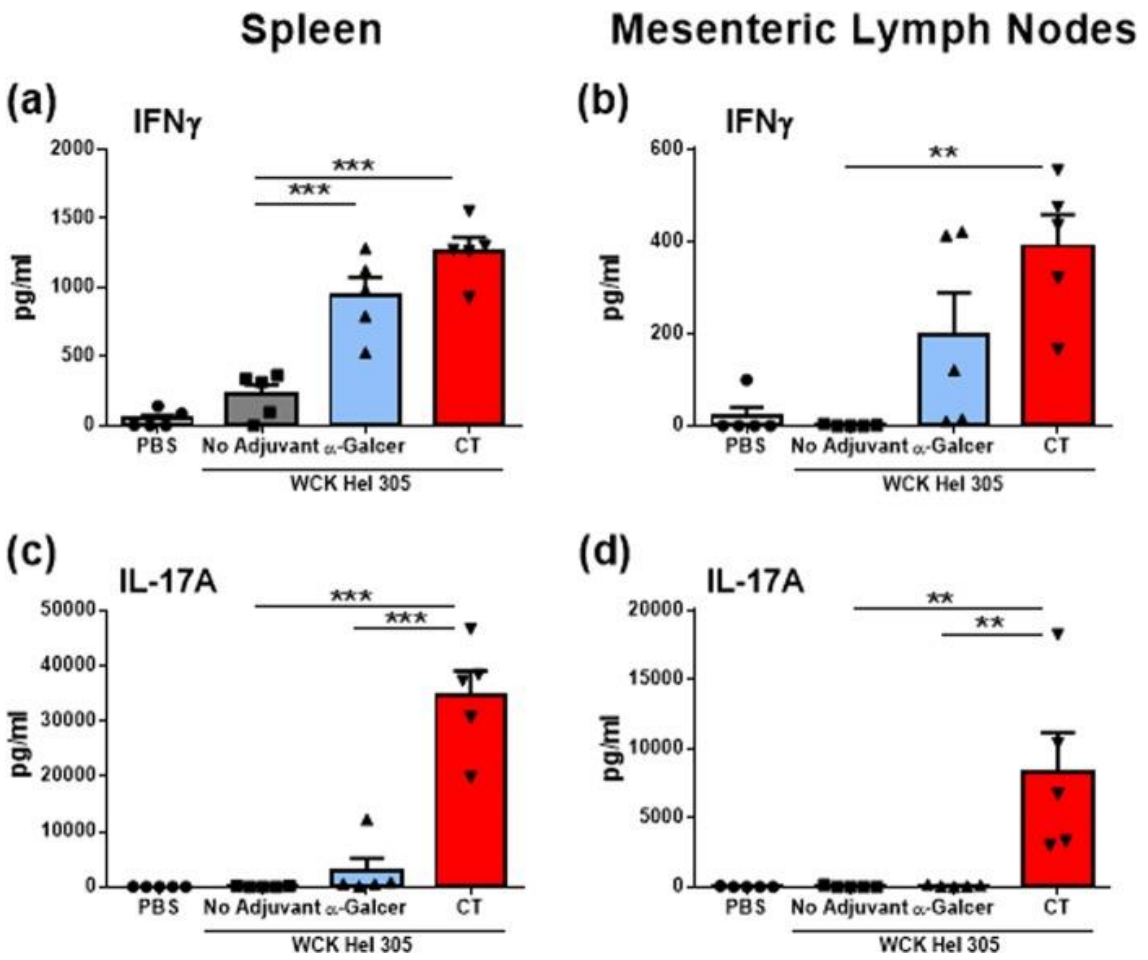



Oral vaccination with whole-cell killed helicobacter pylori with or without iNKT cell activator alpha-Galactosylceramide in mice

Challenge

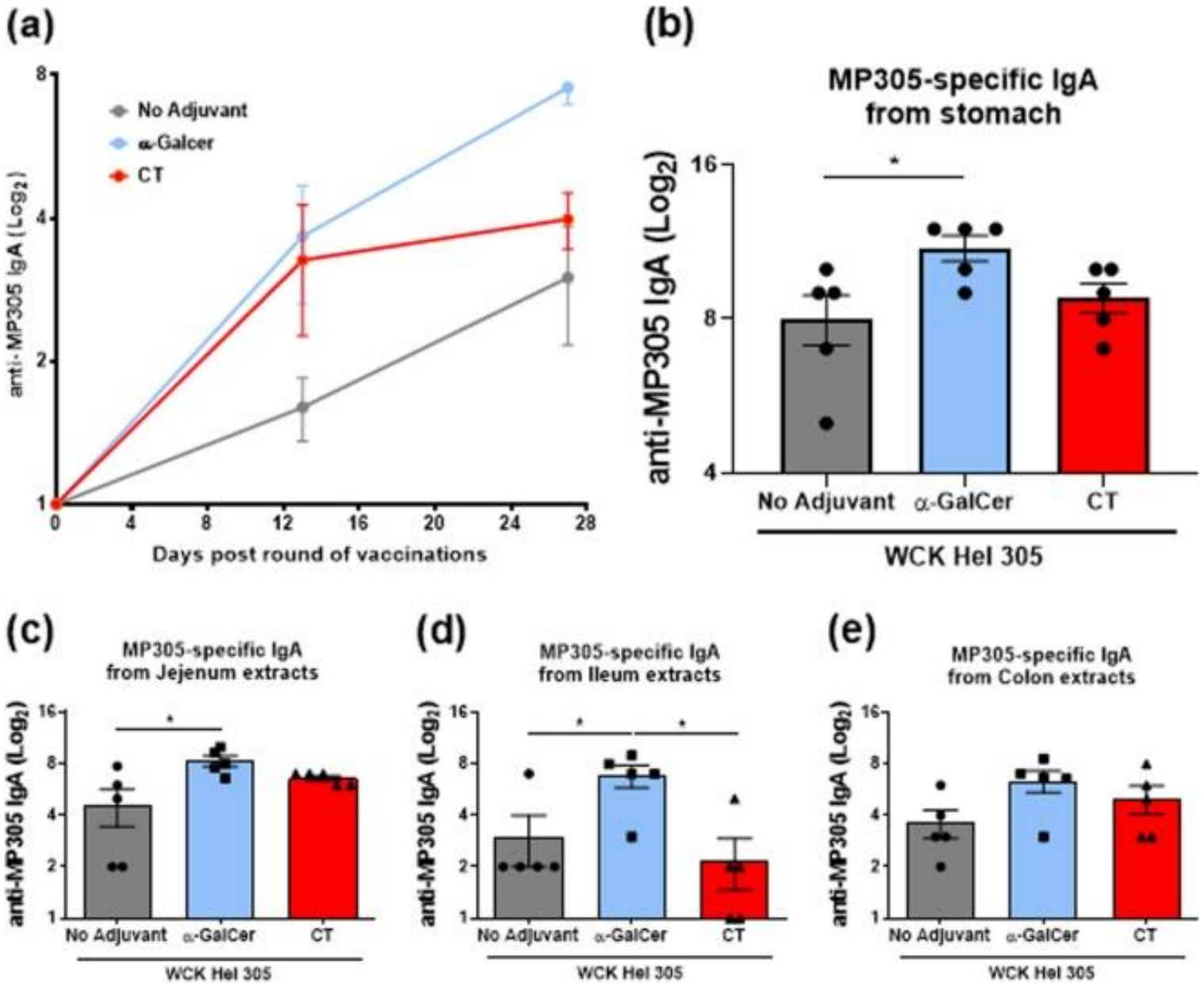



Th1 and Th17 responses – restimulation of cells




Longet et al 2019, NPJ Vaccines

# IgA responses in faeces and intestinal tissues – ELISA

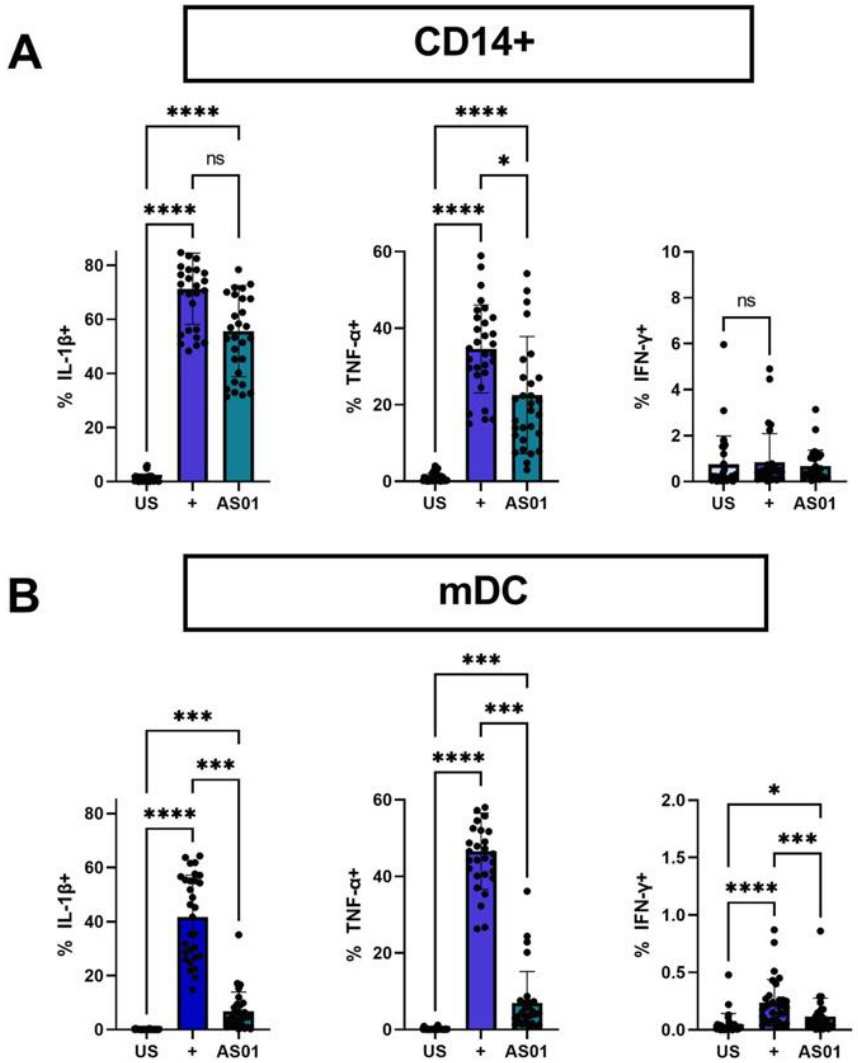


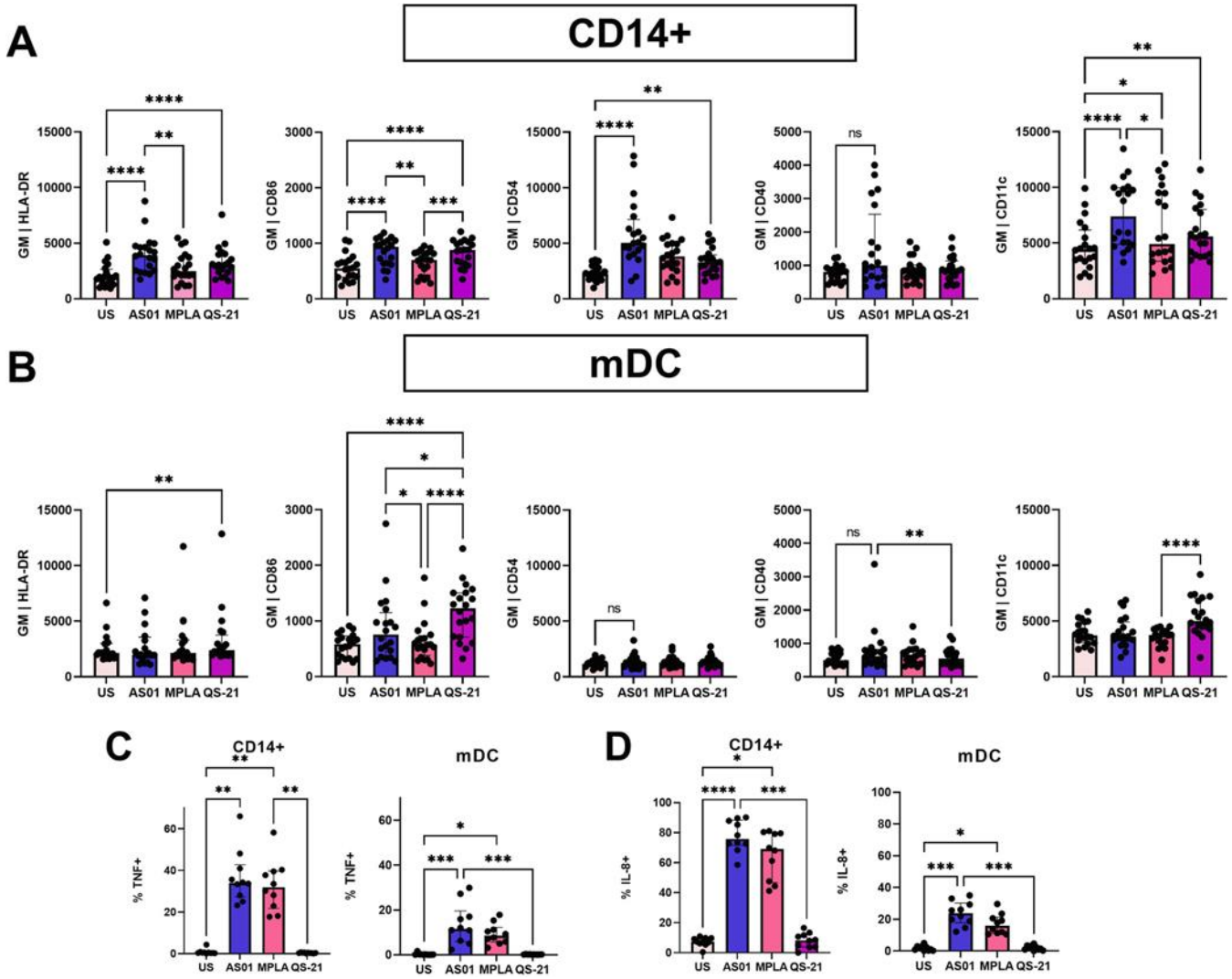

Longet et al 2019, NPJ Vaccines

# Current evaluation of adjuvant in human PMBC model

Restimulation of human PBMCs with AS01 for 18h or LPS/Influenza vaccine as positive control

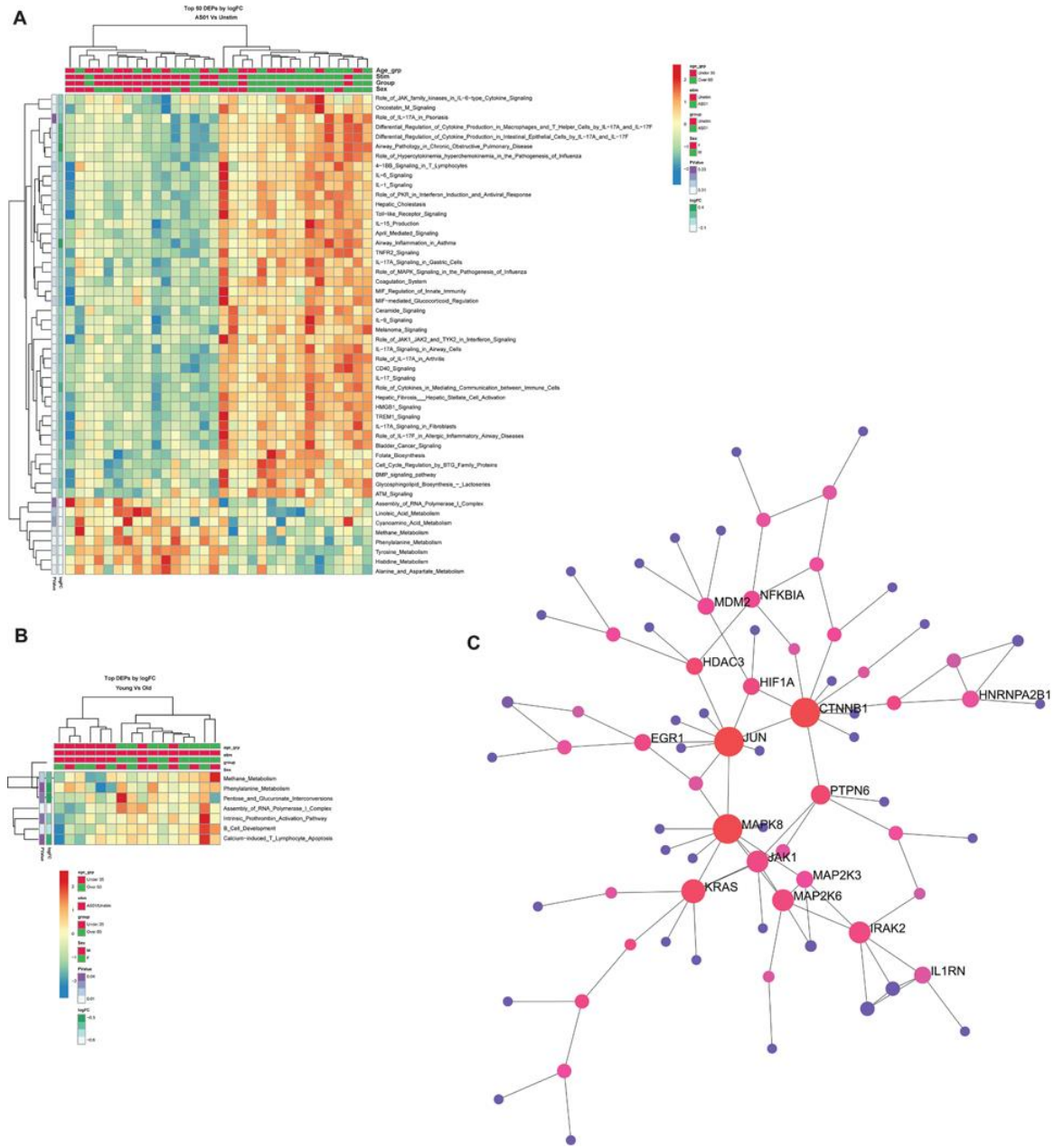
**Intracellular cytokine staining  Flow cytometry**




Smith et al 2023, Vaccine

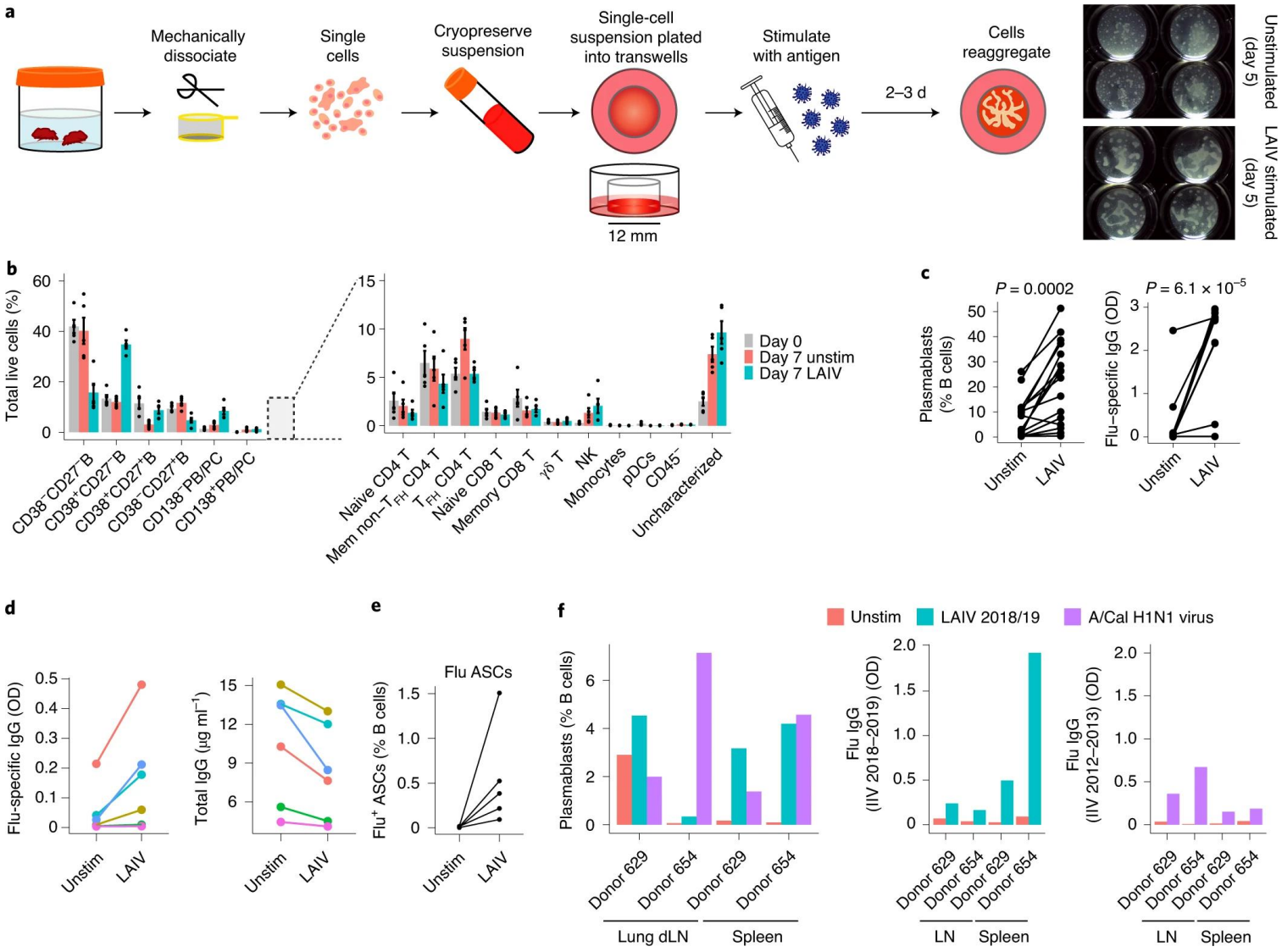
# Transcriptomics in human monocytes




Smith et al 2023, Vaccine

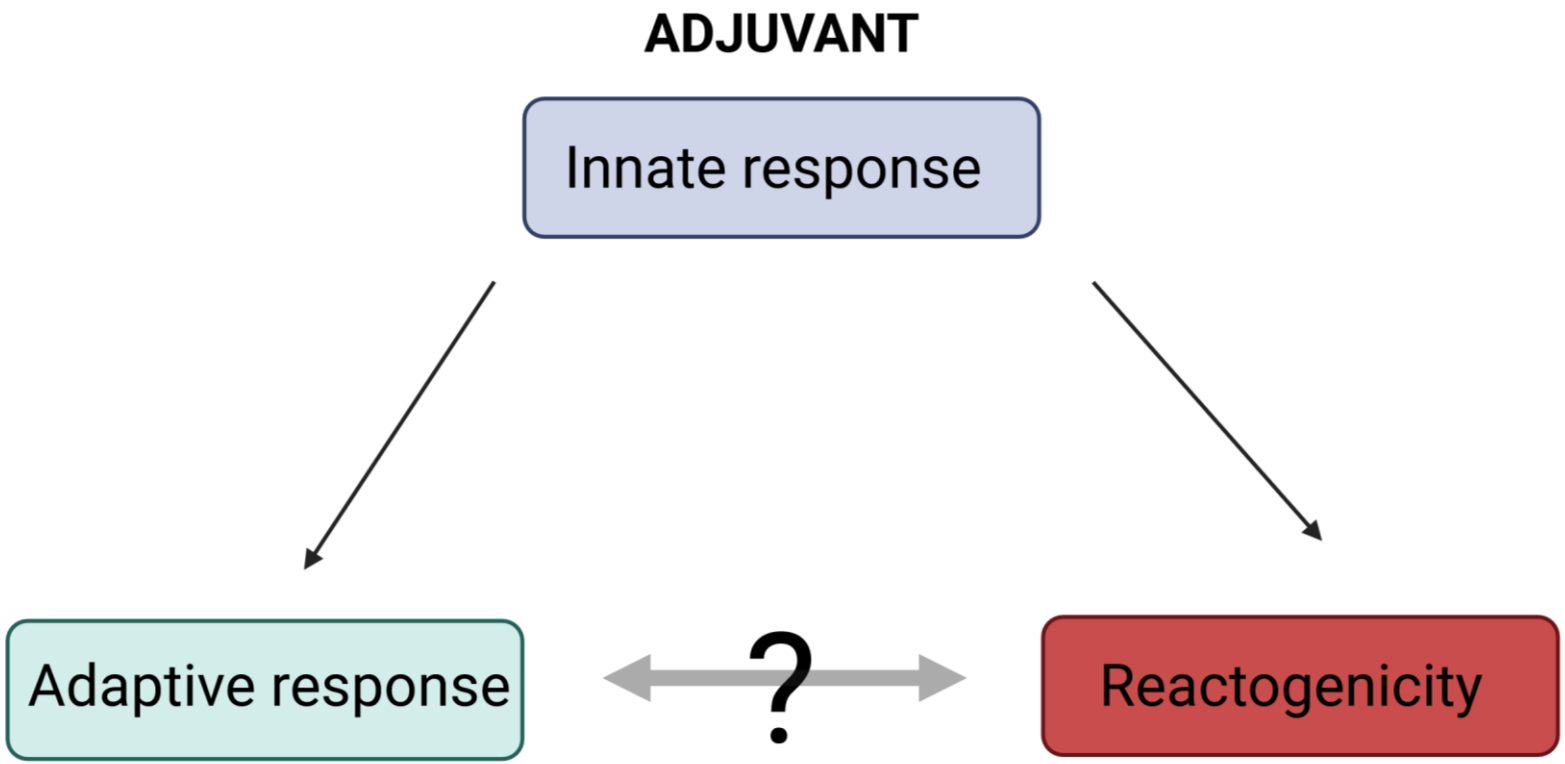
# Gap between preclinical models and clinical trials

Novel complex human models. E.g. human tonsil-based model to test nasal adjuvants



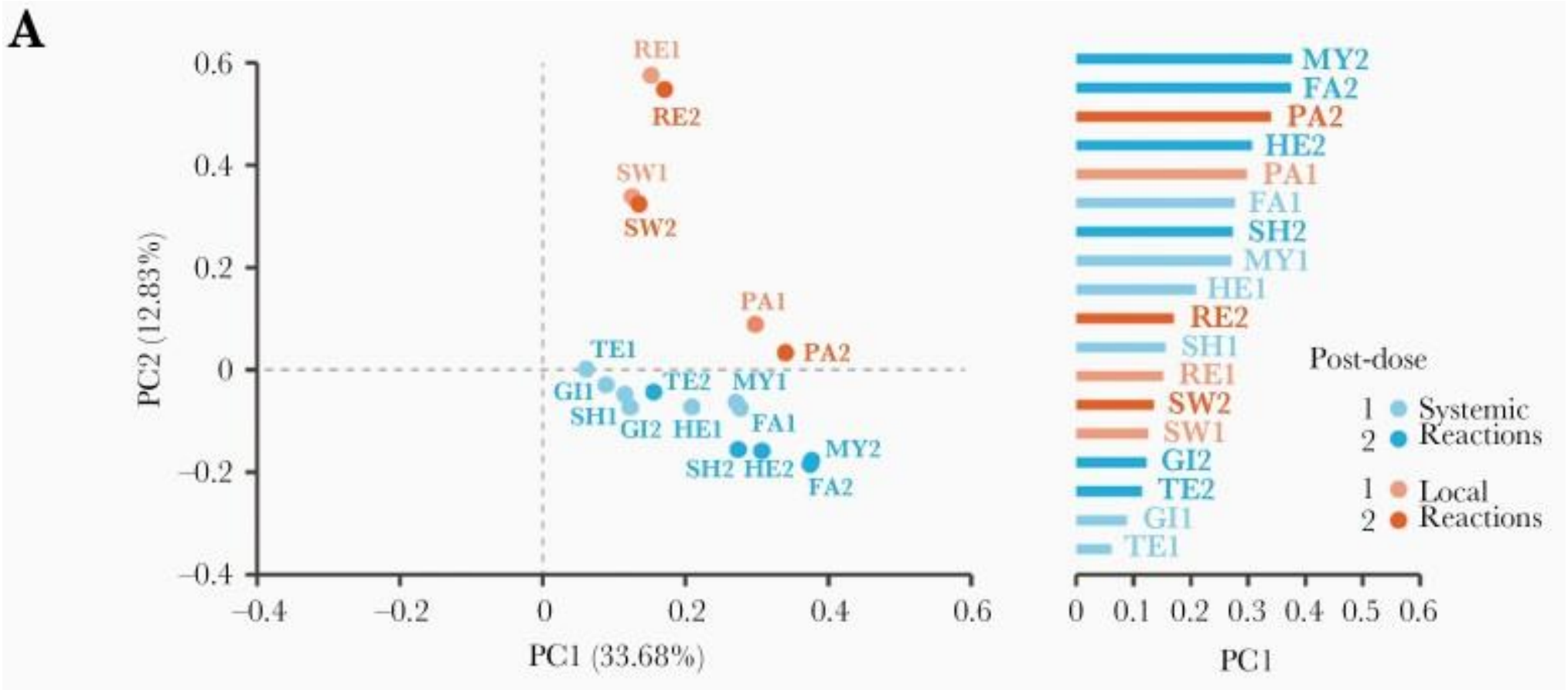

Wagar et al 2024, Nat Med

# Reactogenicity



# Reactogenicity

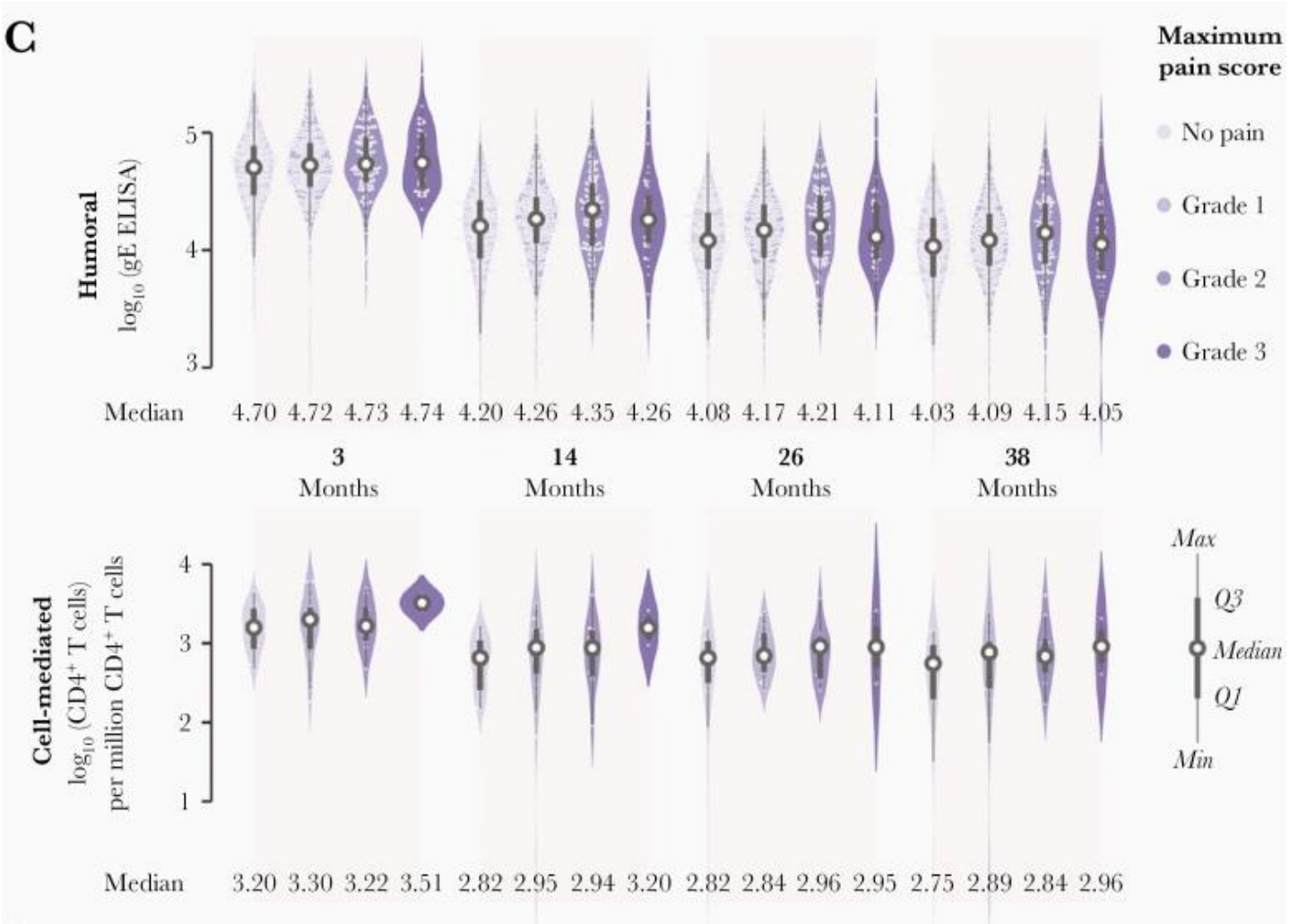
Adjuvanted recombinant herpes zoster vaccine (RZV: Shingrix, GSK)




Callegaro et al 2021, J Infect Dis

# Reactogenicity cannot be used to evaluate vaccine-induced adaptive immune responses

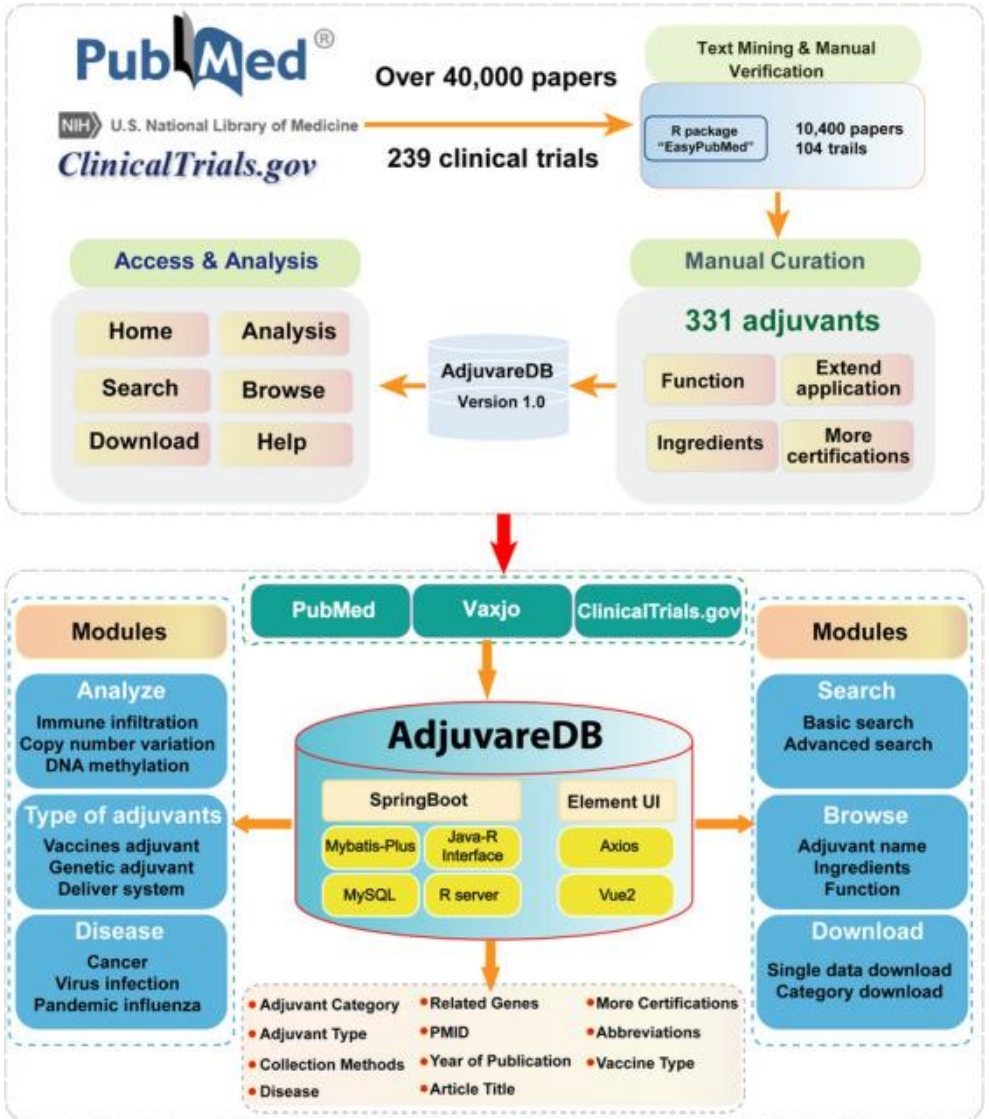
"The impact on the immune response is so small, and the immune response in individuals without pain already sufficient, that pain cannot be a surrogate marker for an appropriate immune response."

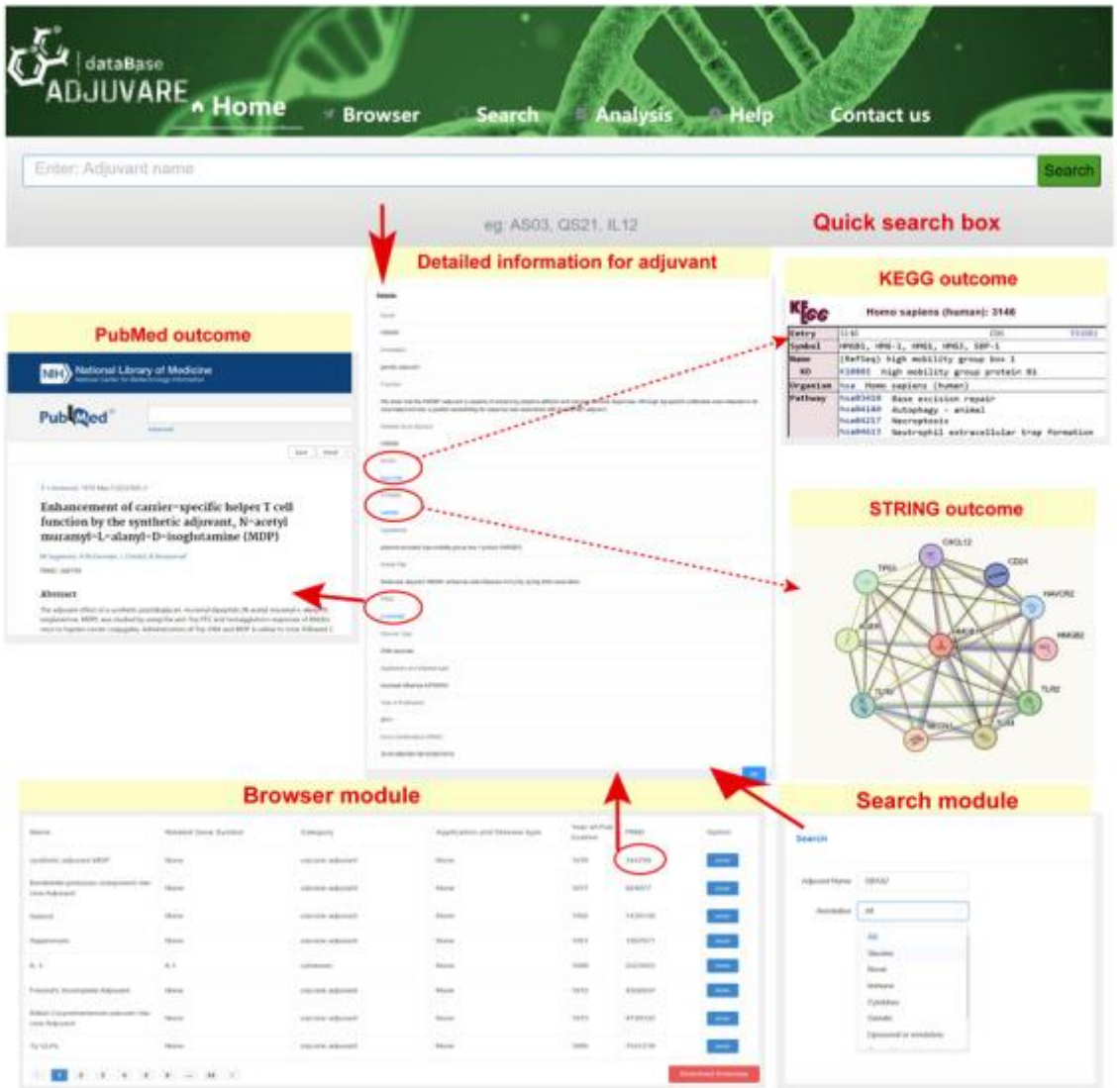



Callegaro et al 2021, J Infect Dis

# Future of adjuvant research

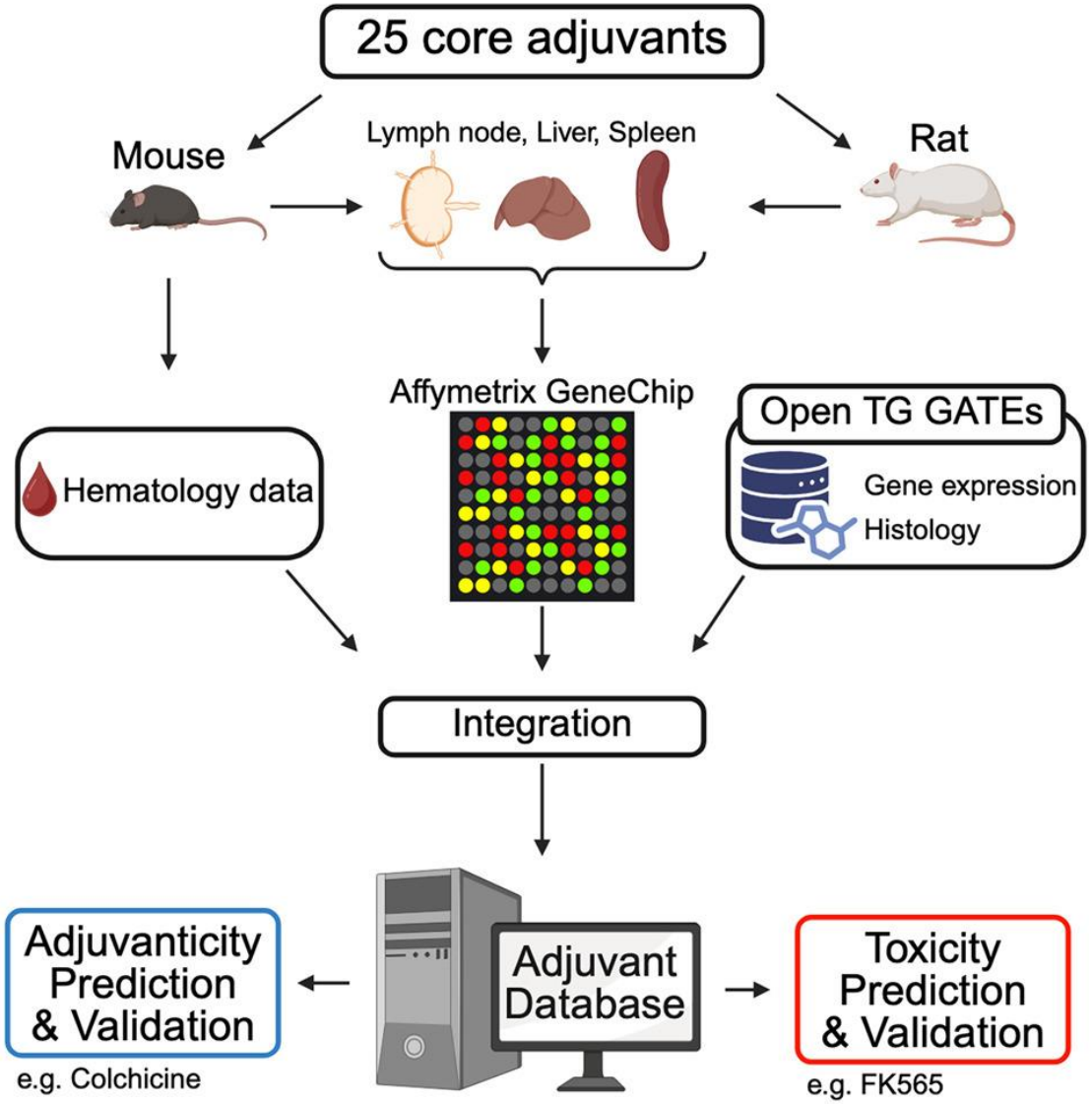
Adjuvant databases:




Ren et al 2024, Clin Transl Med

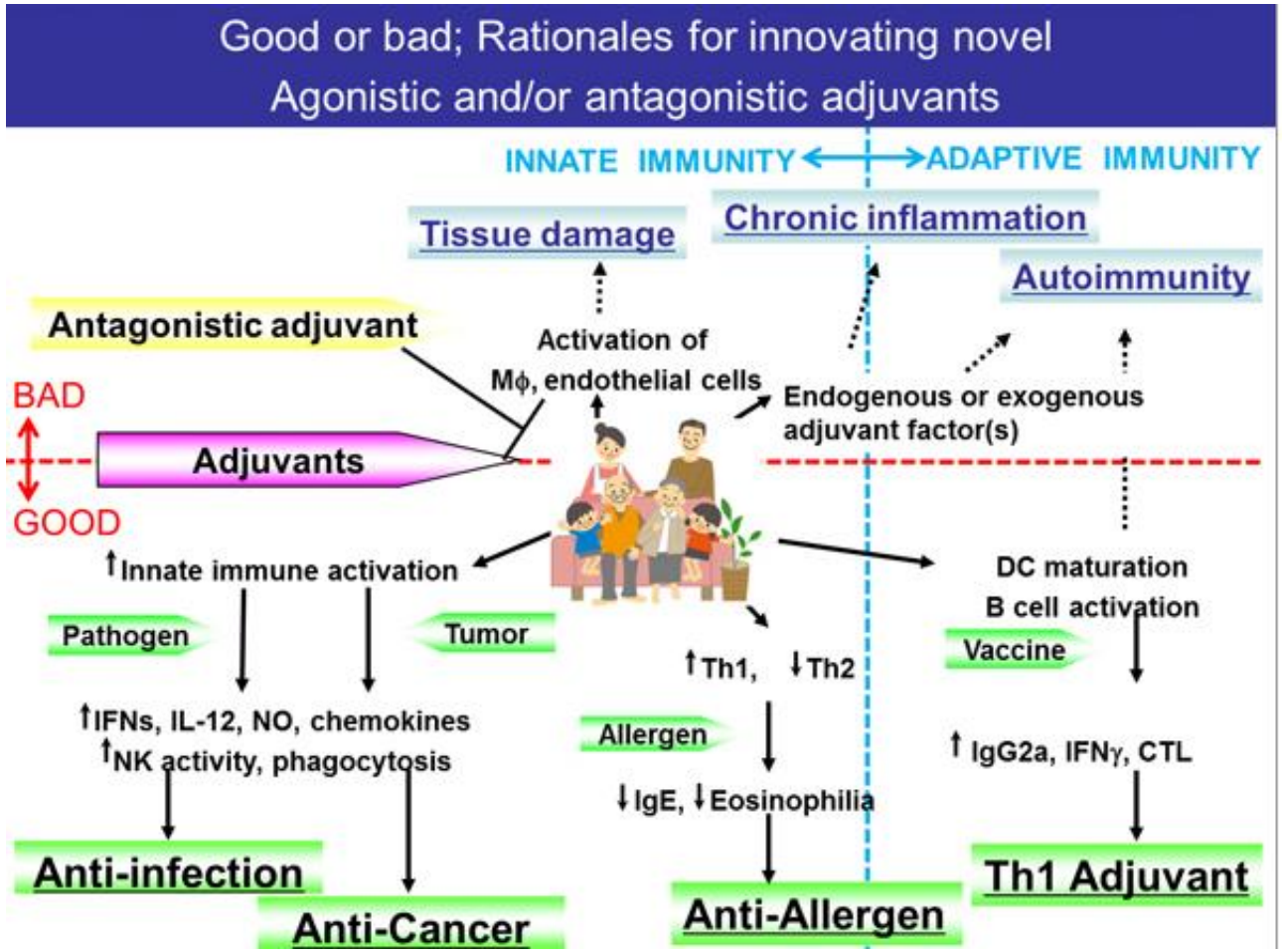
# Future of adjuvant research




Natsume-Kitatani et al 2025, Cell Chem Biol

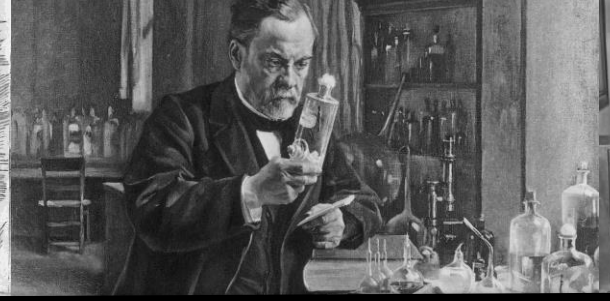
# Future of adjuvant research

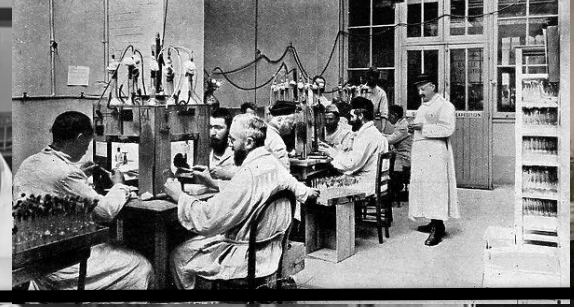
Adjuvant database -> ML/AI => determine which adjuvant is the best candidate for a particular purpose



# Vaccine Adjuvants

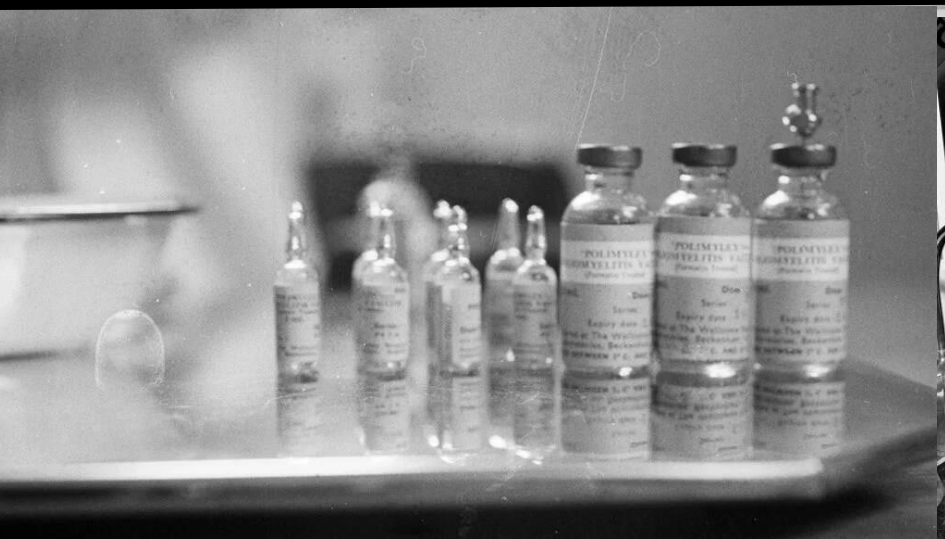
1. What Are Adjuvants?
2. Rationale Behind Adjuvant Use
3. Adjuvant Discovery
4. Some Commonly Used Adjuvants
5. Mechanism of Action

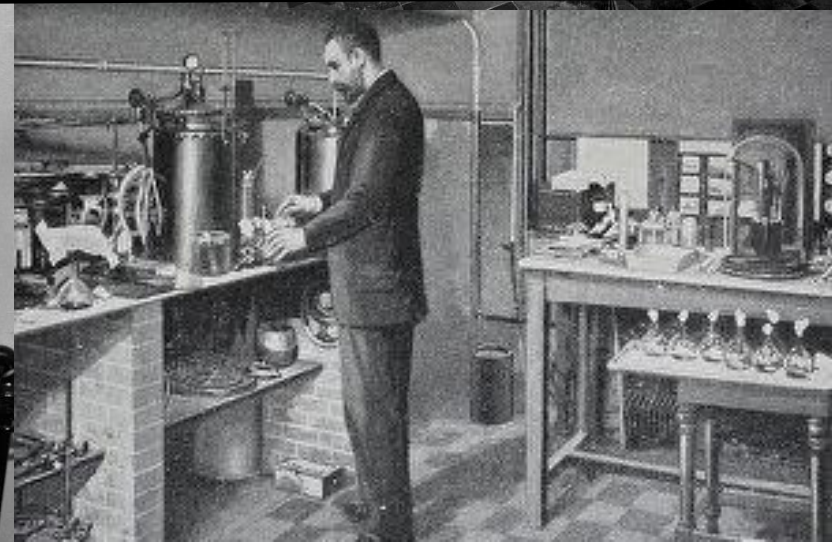



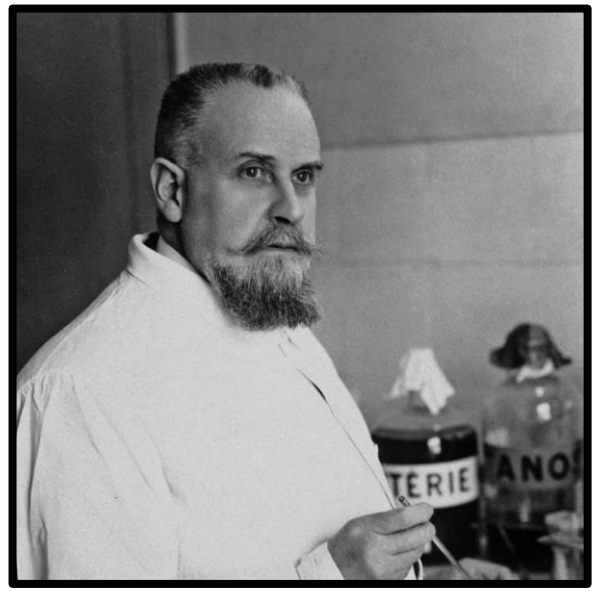




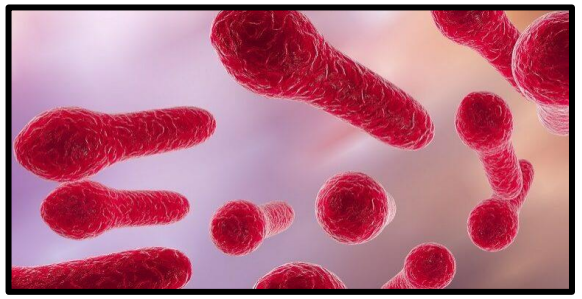




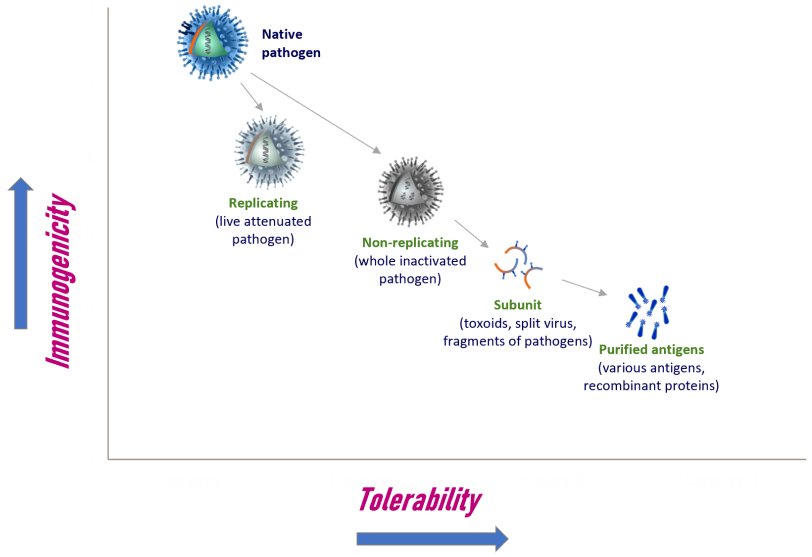


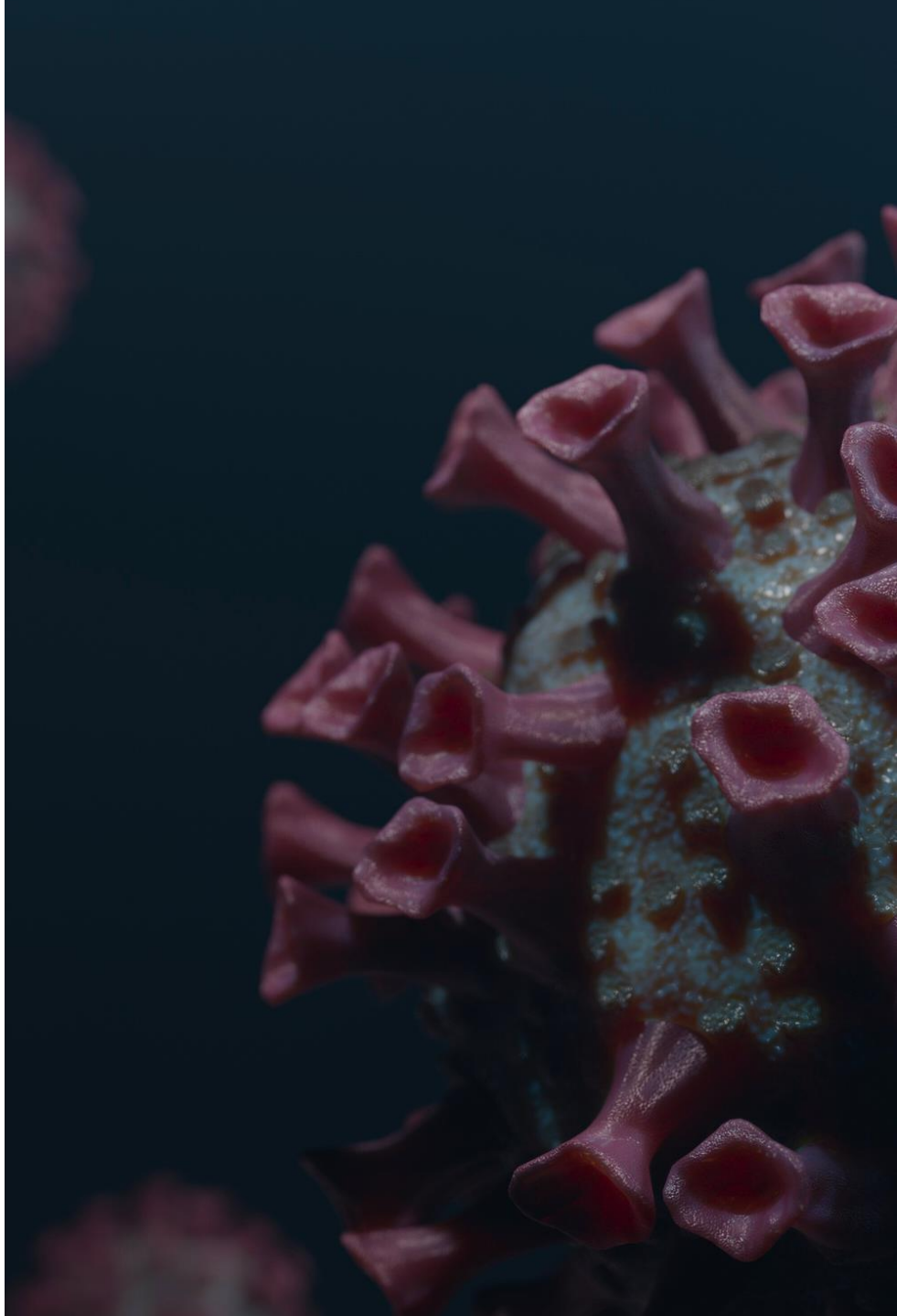

Gaston Ramon, French veterinarian and biologist



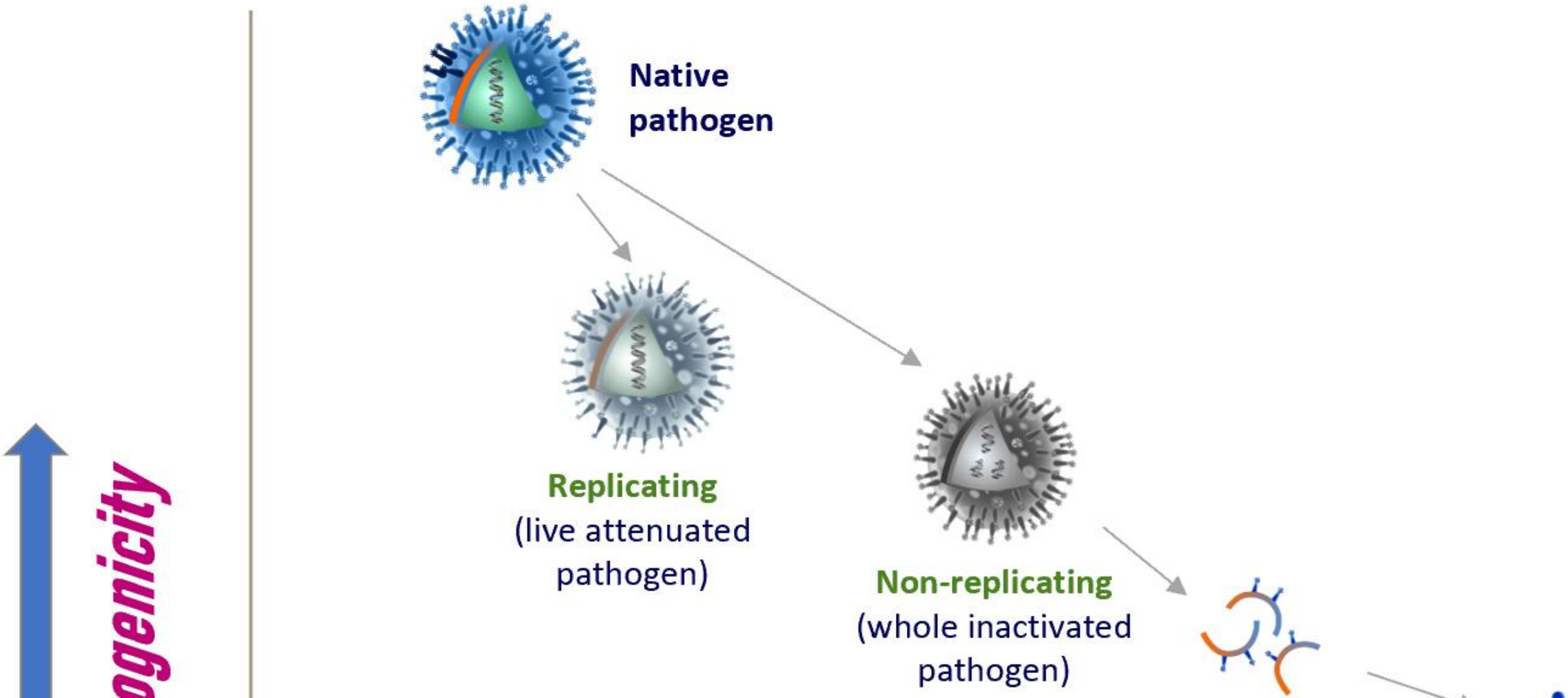
Tetanus Bacteria

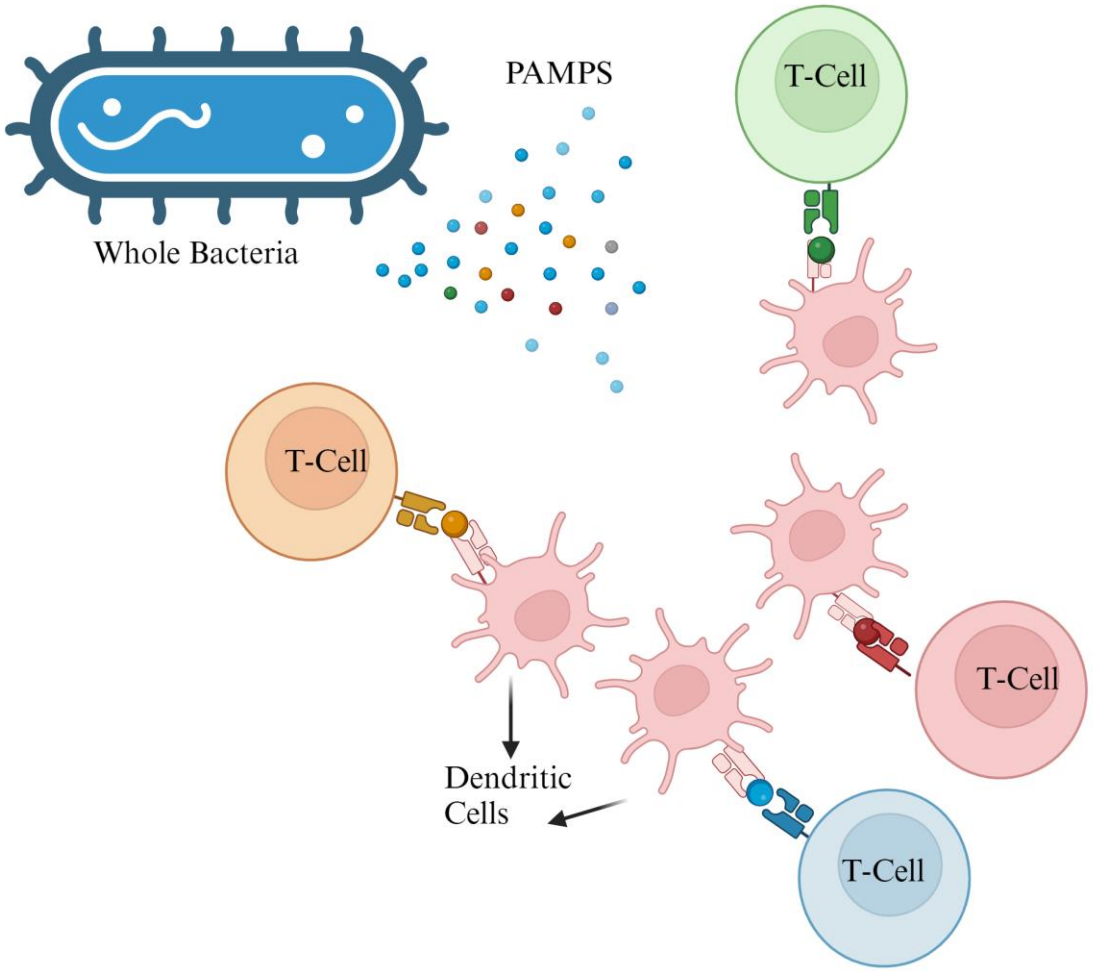
# Why Do We Need Adjuvants?



# Why Do We Need Adjuvants?

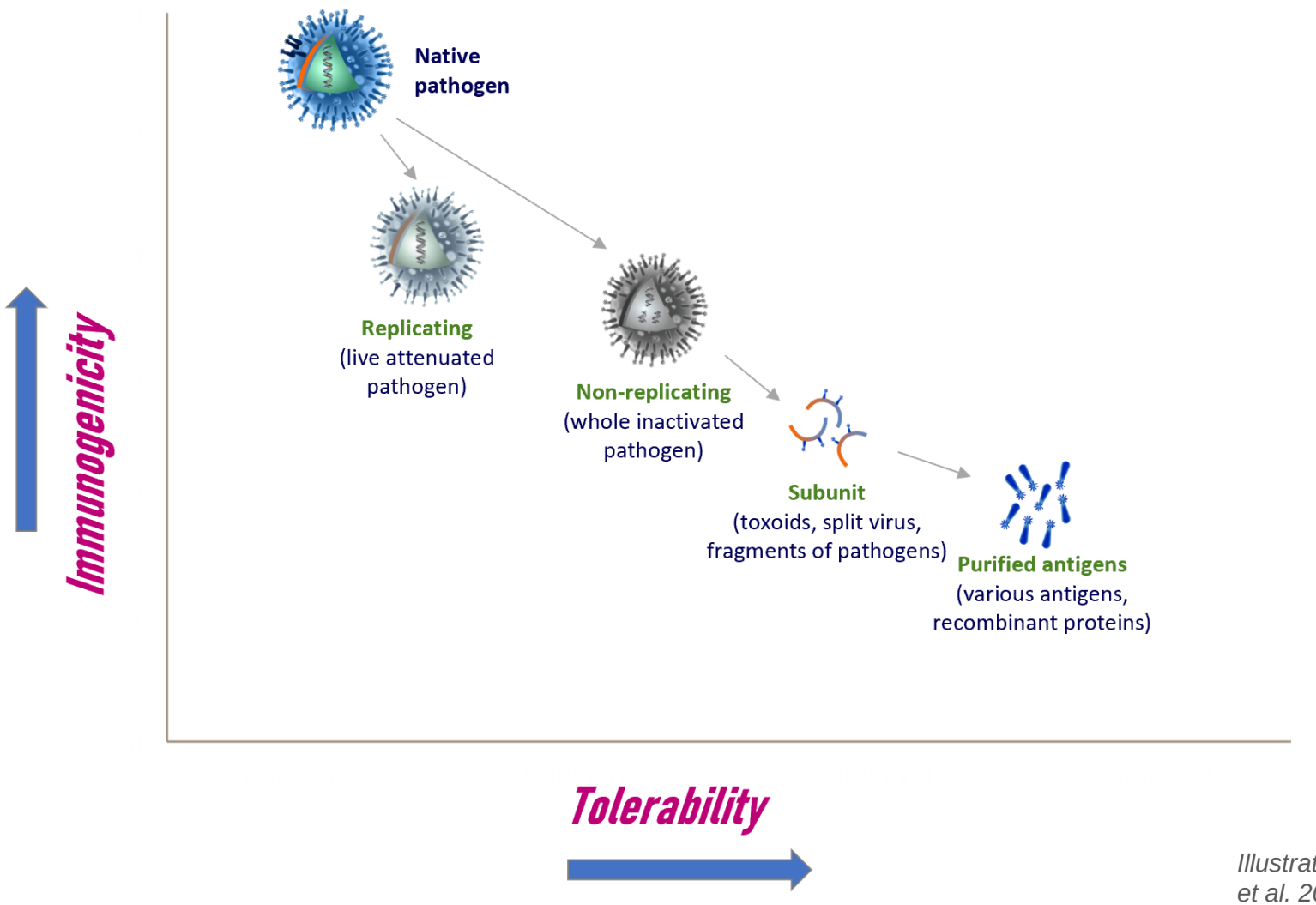





Hgher Antigen diversity in Whole Pathogens

Hgher Immune cell activation via dendritic cells

# Why Do We Need Adjuvants?




*Illustrative figure based on concepts from Garçon et al. 2011*

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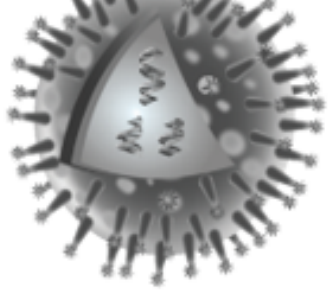
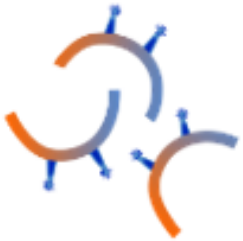


**Non-replicating**
(whole inactivated pathogen)

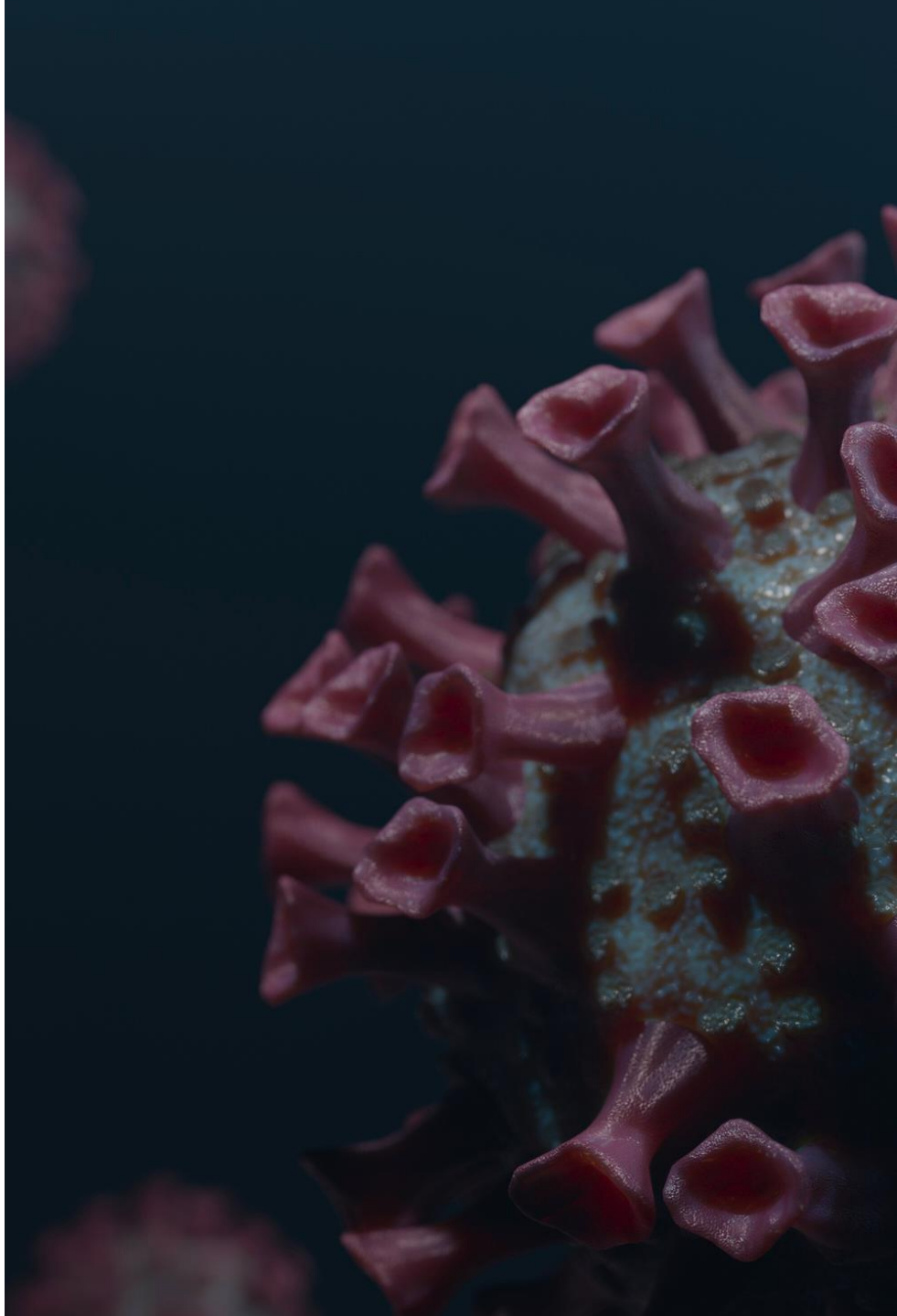


**Subunit**
(toxoids, split virus, fragments of pathogens)

**Purified antigens**
(various antigens, recombinant proteins)

*Illustrative figure based on concepts from Garçon et al. 2011*

# Adjuvants act as substitutes for natural immune-defence triggers



# Advantages Of Adjuvants

1

Stronger immune Immune:

- Faster immune response
- Elevated immune response
- Broader, cross-protective immunity against genotypes or drift variants different from vaccine antigen(s)
- Longer lasting immune response, fewer boosters needed

2

Antigen sparing (use of smaller amounts of the antigen) particularly relevant for pandemic events

3

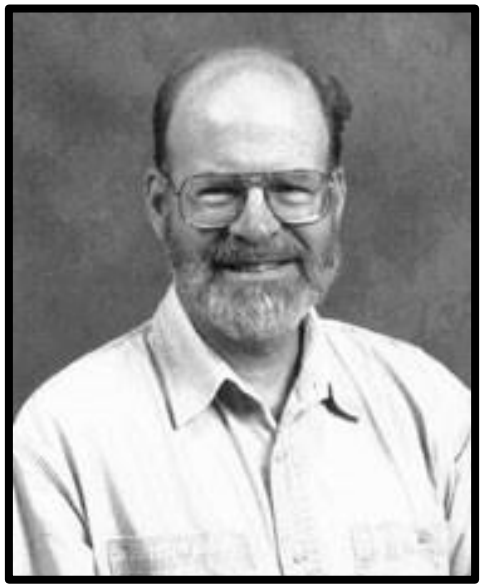
Enhanced and effective immune responses in specific low-responding populations, such as the elderly or immunocompromised patients

4

Acceptable safety profile

# But How do Adjuvants Works?

## Approaching the Asymptote: Revolution and Evolution in Immunology



Charles Janeway, immunologist

Toward a Modern Synthesis of Immunity: Charles A. Janeway Jr. and the Immunologist's Dirty Little Secret

Peter M. Gayed

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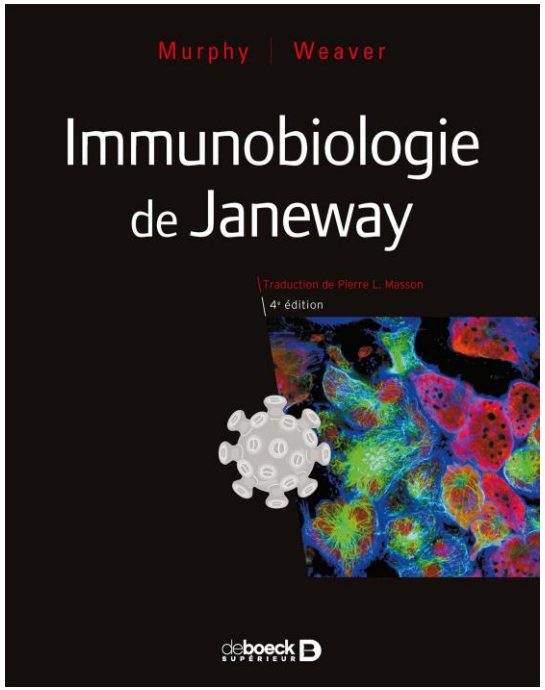
Abstract Go to: ▸

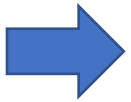
This essay chronicles the major theoretical and experimental contributions made by Charles A. Janeway, Jr. (1943-2003), Howard Hughes Medical Institute investigator and Yale Professor of Immunology, who established the fundamental role of the innate immune system in the induction of the adaptive arm.



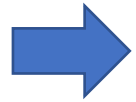
# Janeway's Pattern Recognition' Theory

The costimulatory signal required for lymphocyte activation was inducible

The costimulatory signal was suggested to be inducible by conserved microbial products

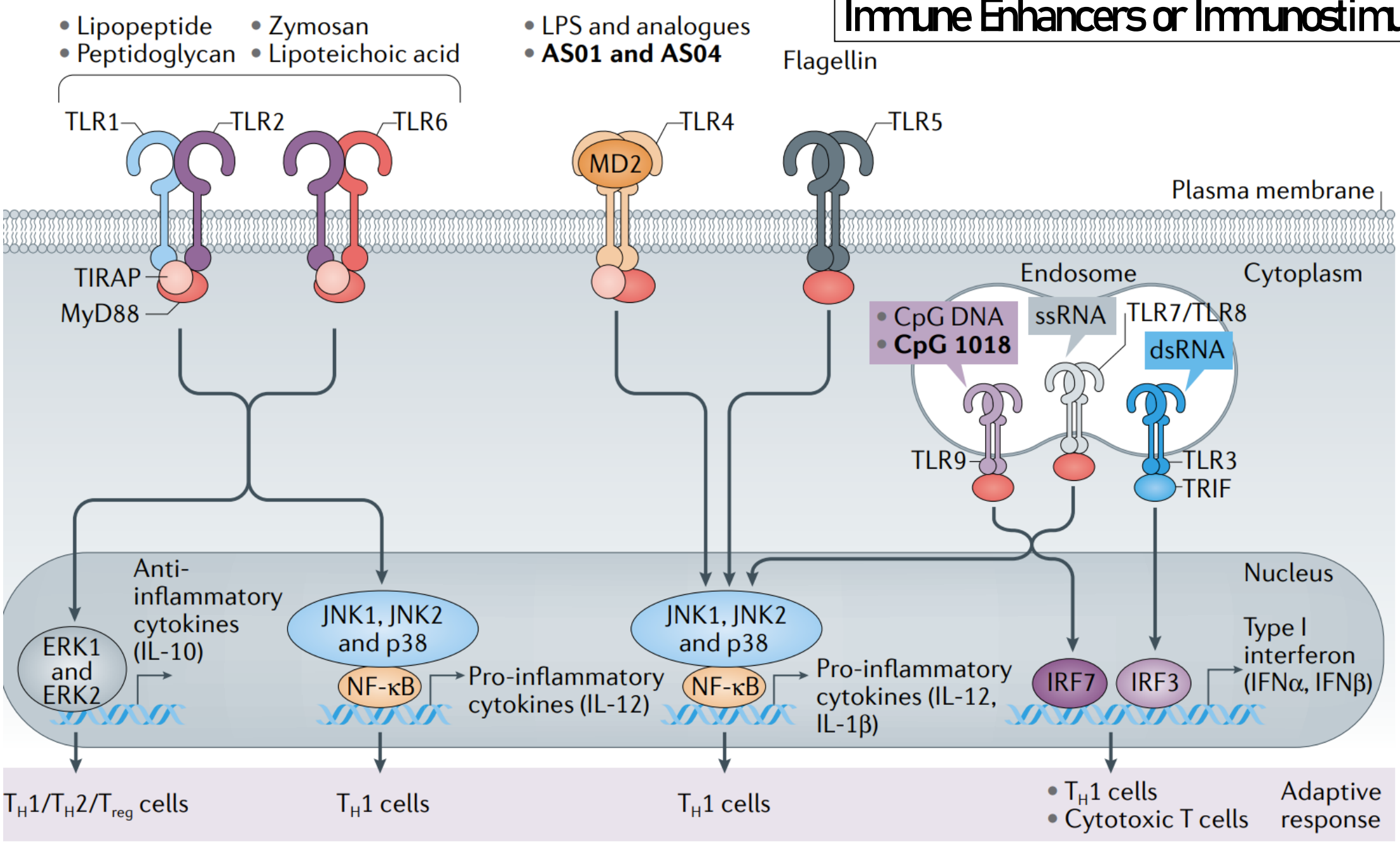


Thus, the activation of adaptive immunity is under the control of pathogen-sensing mechanisms.

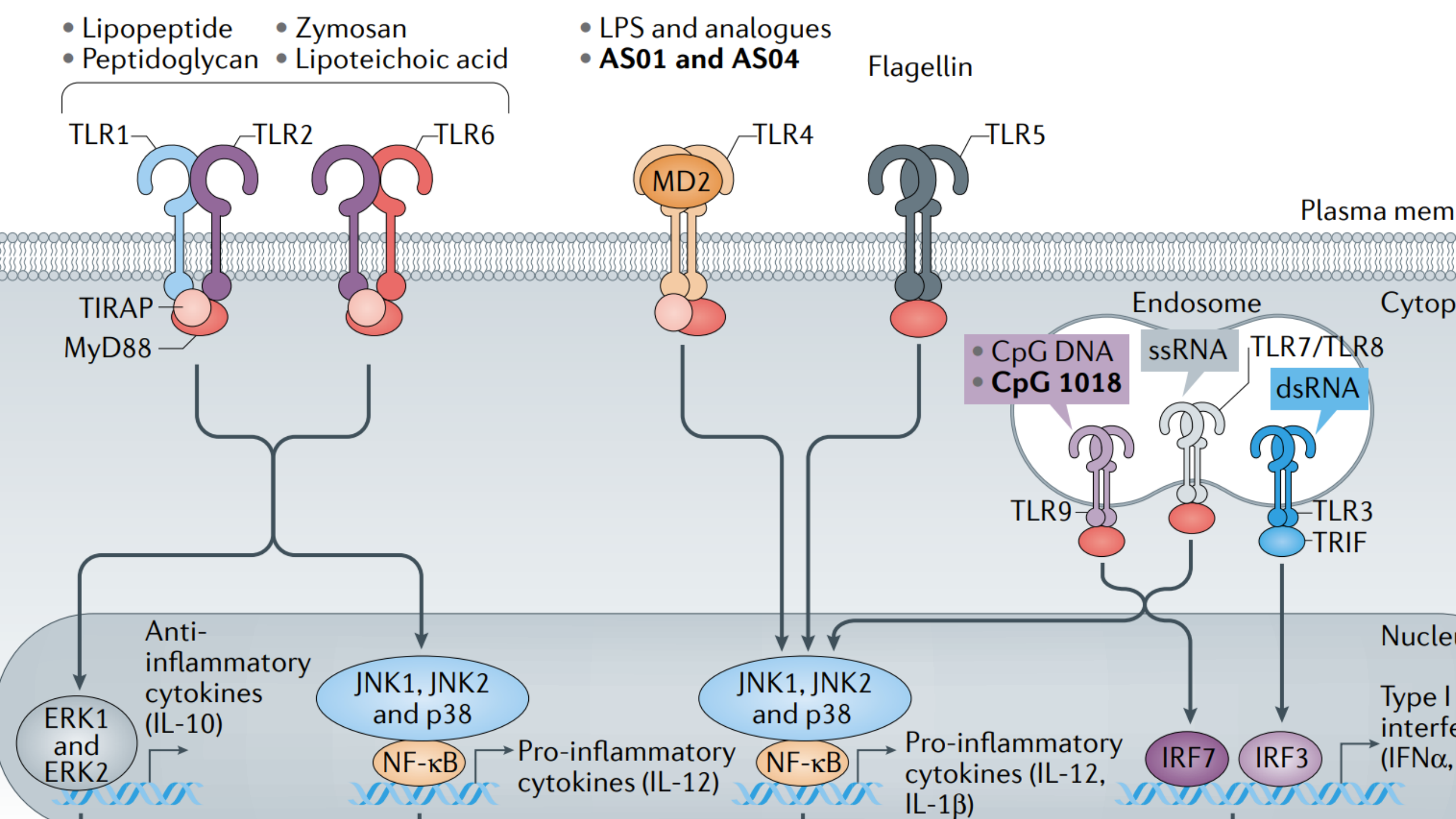


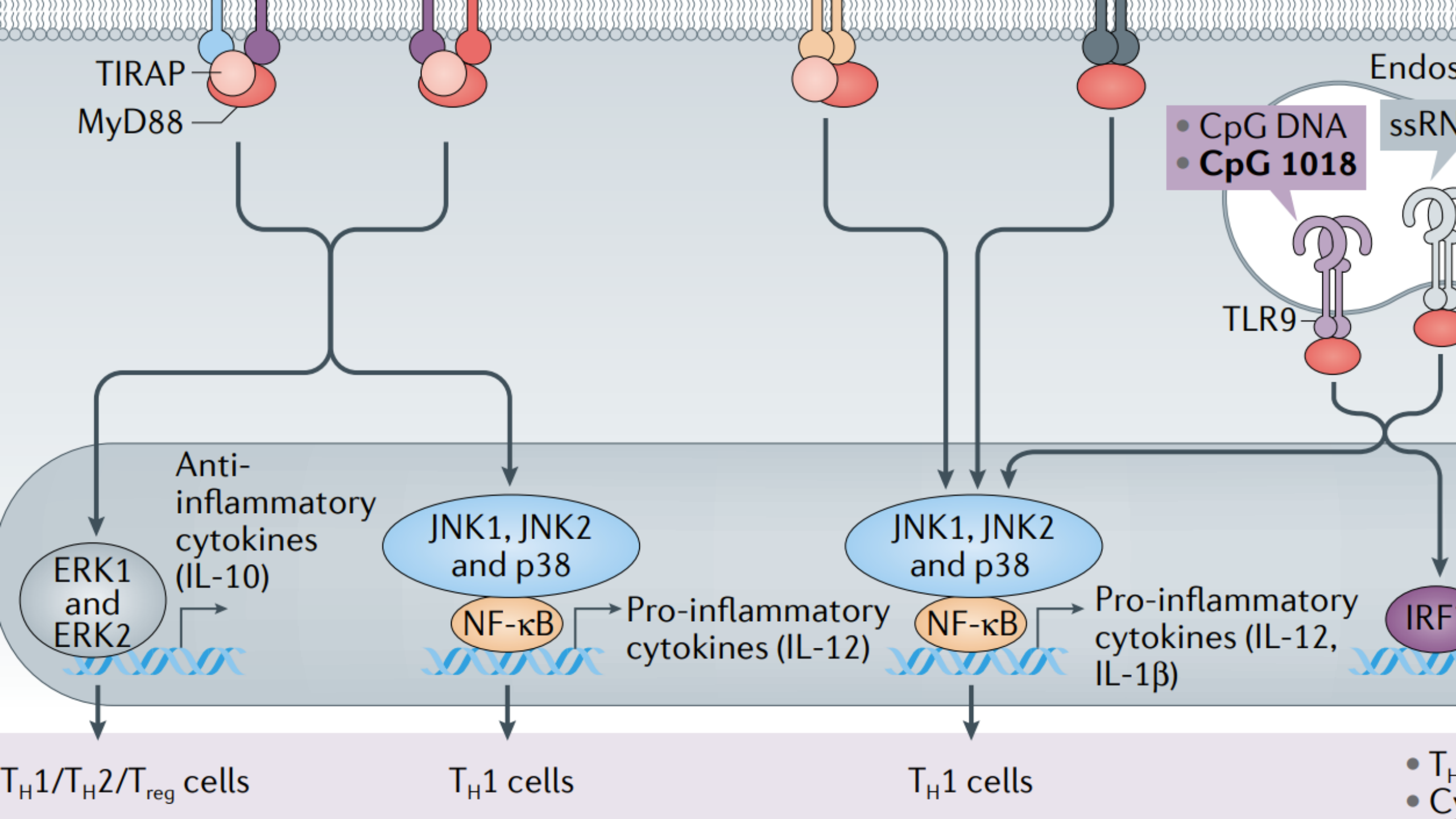
This also explains the adjuvant properties of certain microbial stimuli

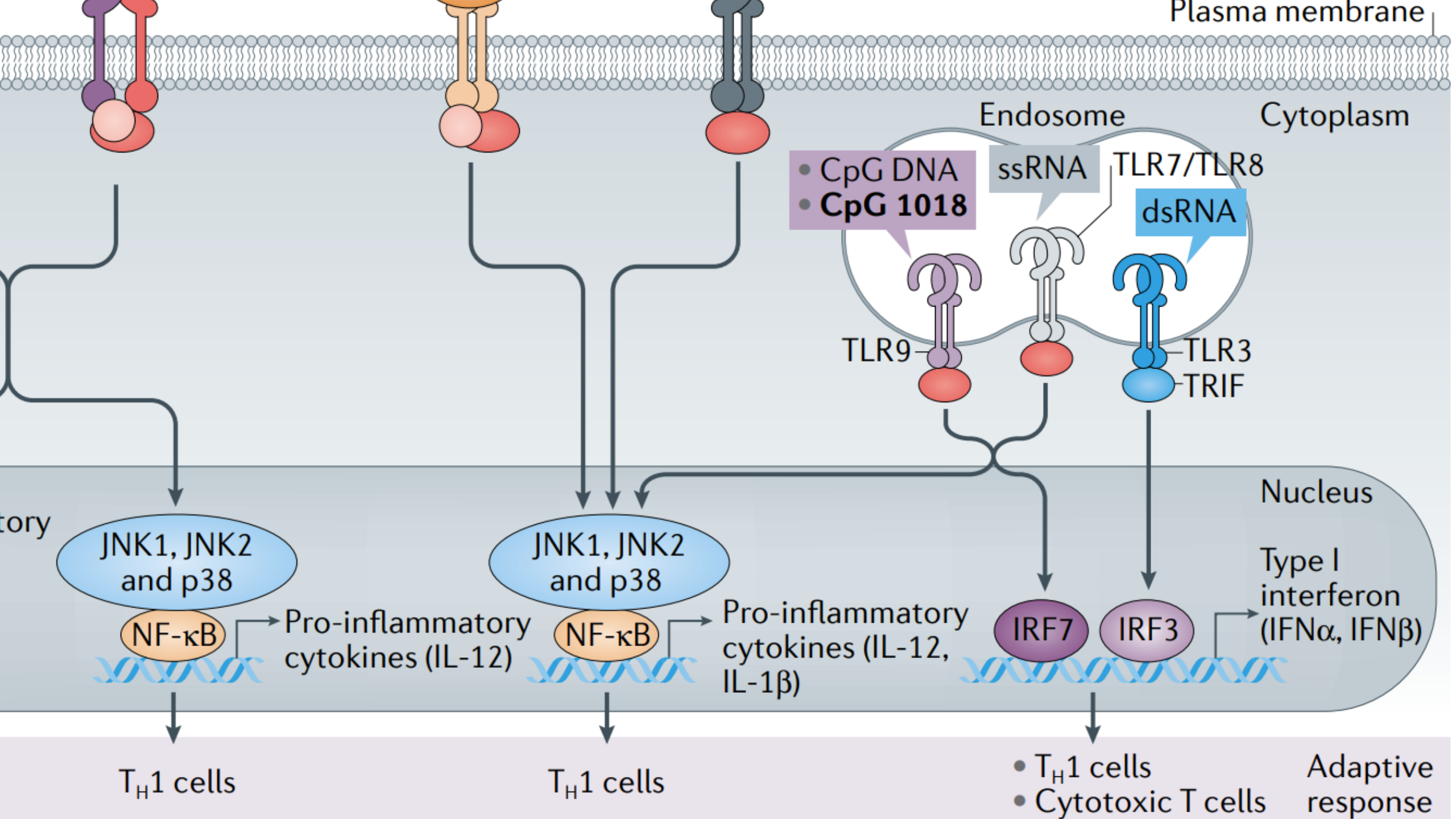
# Immune Enhancers or Immunostimulants



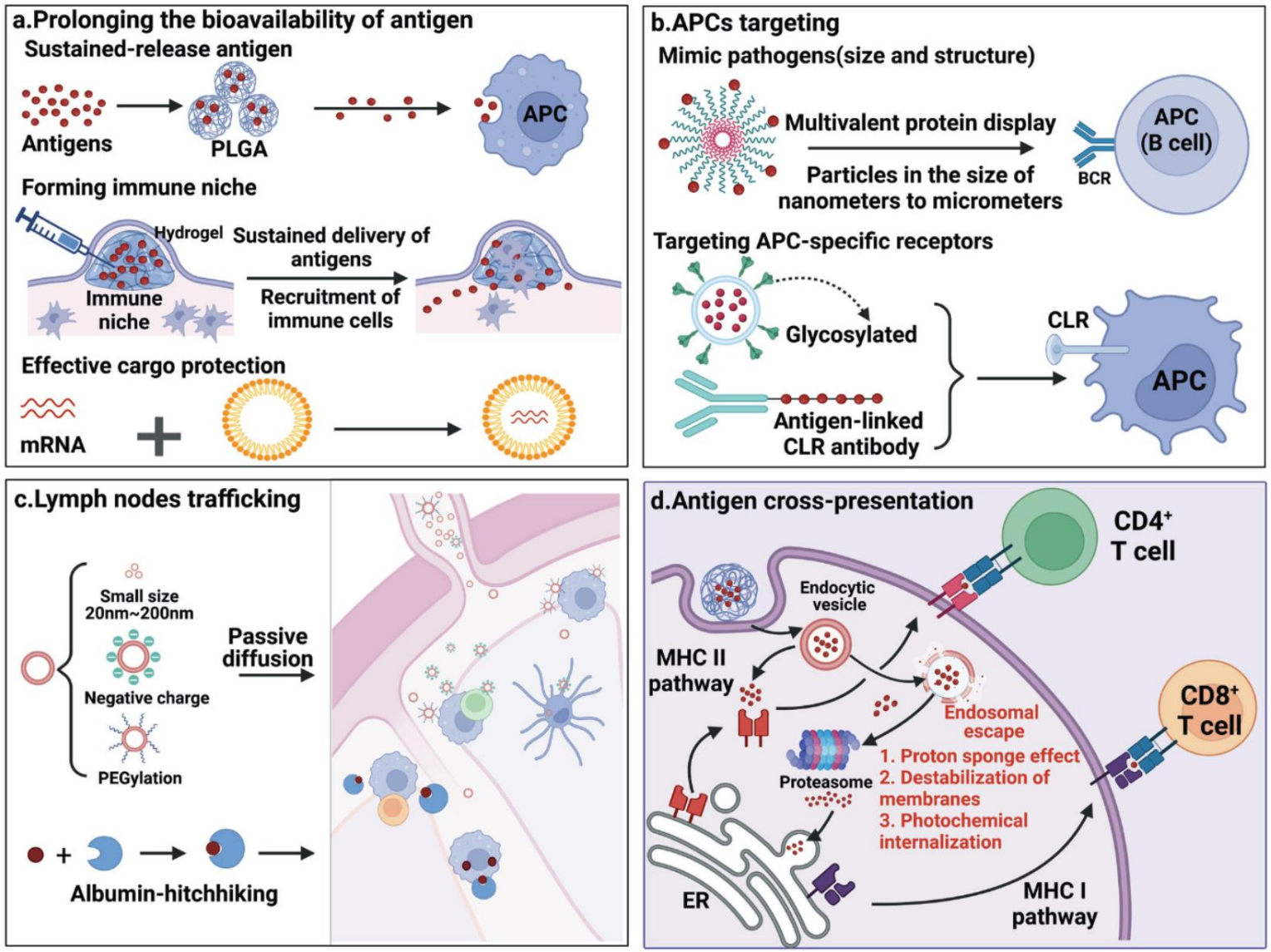

Emerging concepts in the science of vaccine adjuvants Pulendran et al. Nature Reviews, Drug Discovery, Volume 20, June 2021, https://doi.org/10.1038/s41573-021-00163-y


• Lipopeptide
• Peptidoglycan
• Zymosan
• Lipoteichoic acid
• LPS and analogues
• **AS01 and AS04**
Flagellin
TLR1
TLR2
TLR6
TLR4
TLR5
MD2
Plasma mem
TIRAP
MyD88
Endosome
Cytop
• CpG DNA
• **CpG 1018**
ssRNA
TLR7/TLR8
dsRNA
TLR9
TLR3
TRIF
Anti-inflammatory cytokines (IL-10)
ERK1 and ERK2
JNK1, JNK2 and p38
NF-κB
Pro-inflammatory cytokines (IL-12)
JNK1, JNK2 and p38
NF-κB
Pro-inflammatory cytokines (IL-12, IL-1β)
IRF7
IRF3
Nucle
Type I interfe
(IFNα,

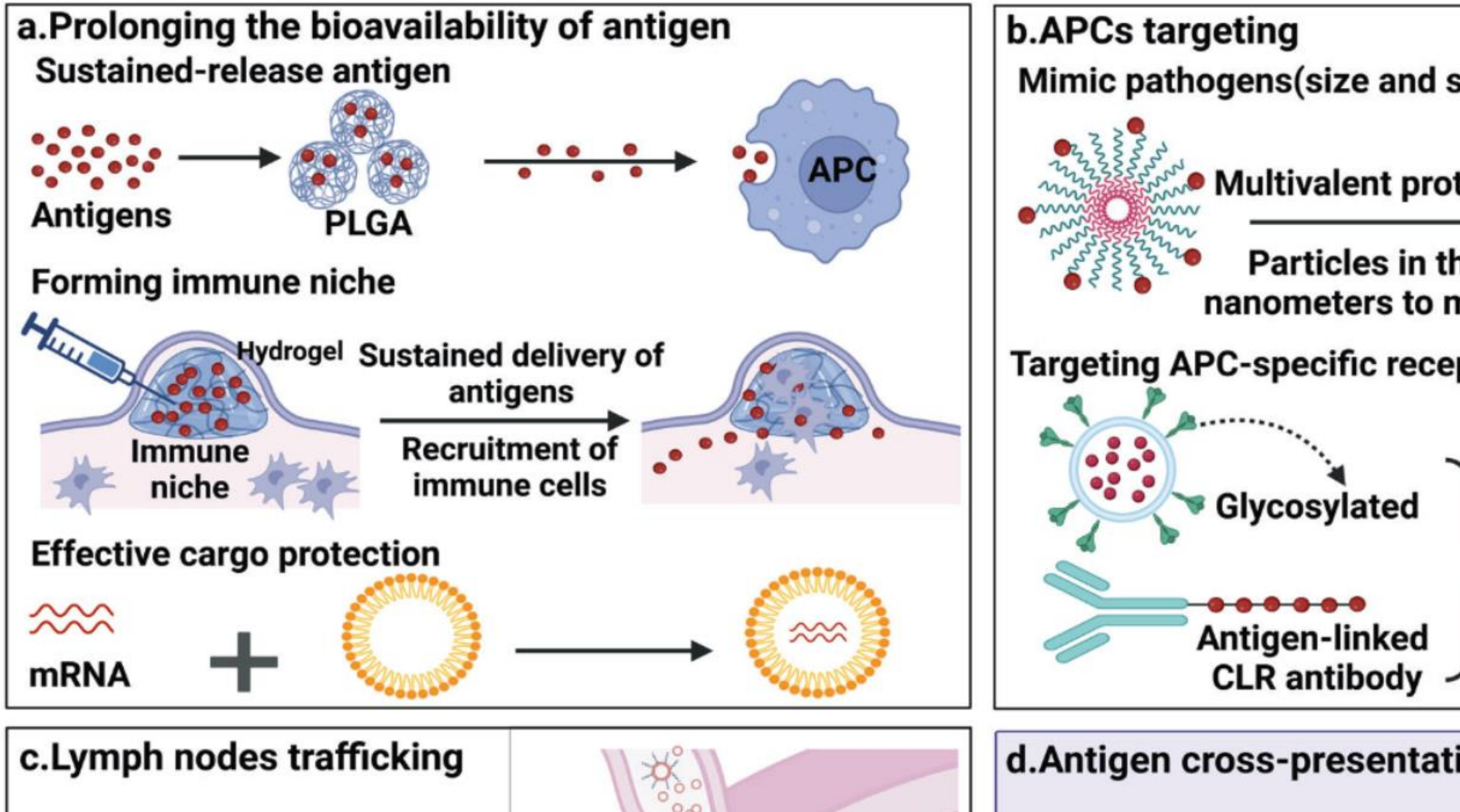

TIRAP
MyD88
Endos
• CpG DNA
• CpG 1018
ssRN
TLR9
Anti-inflammatory cytokines (IL-10)
ERK1 and ERK2
JNK1, JNK2 and p38
NF-κB
Pro-inflammatory cytokines (IL-12)
JNK1, JNK2 and p38
NF-κB
Pro-inflammatory cytokines (IL-12, IL-1β)
IRF
$T_H1/T_H2/T_{reg}$ cells
$T_H1$ cells
$T_H1$ cells
• $T_H$
• C

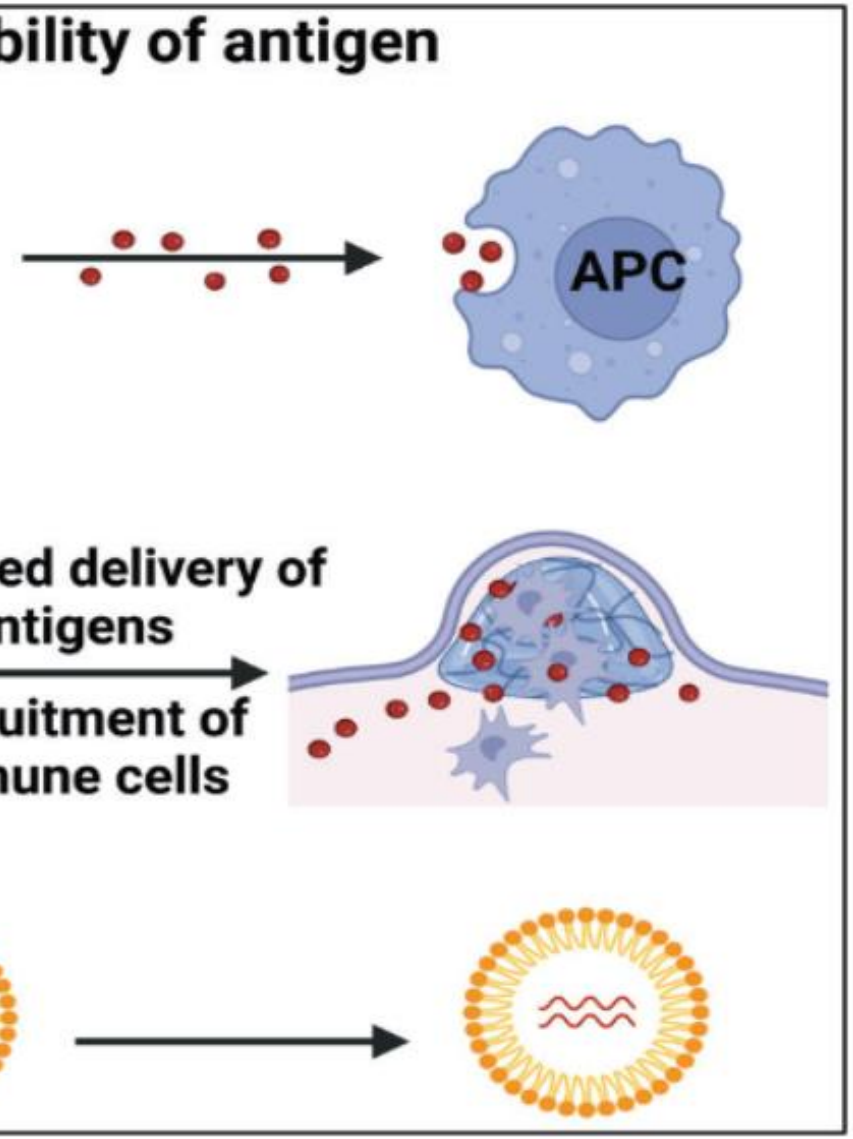

Plasma membrane
Endosome
Cytoplasm
CpG DNA
CpG 1018
ssRNA
TLR7/TLR8
dsRNA
TLR9
TLR3
TRIF
Nucleus
JNK1, JNK2 and p38
NF-κB
Pro-inflammatory cytokines (IL-12)
JNK1, JNK2 and p38
NF-κB
Pro-inflammatory cytokines (IL-12, IL-1β)
IRF7
IRF3
Type I interferon (IFNα, IFNβ)
$T_H1$ cells
$T_H1$ cells
$T_H1$ cells
Cytotoxic T cells
Adaptive response

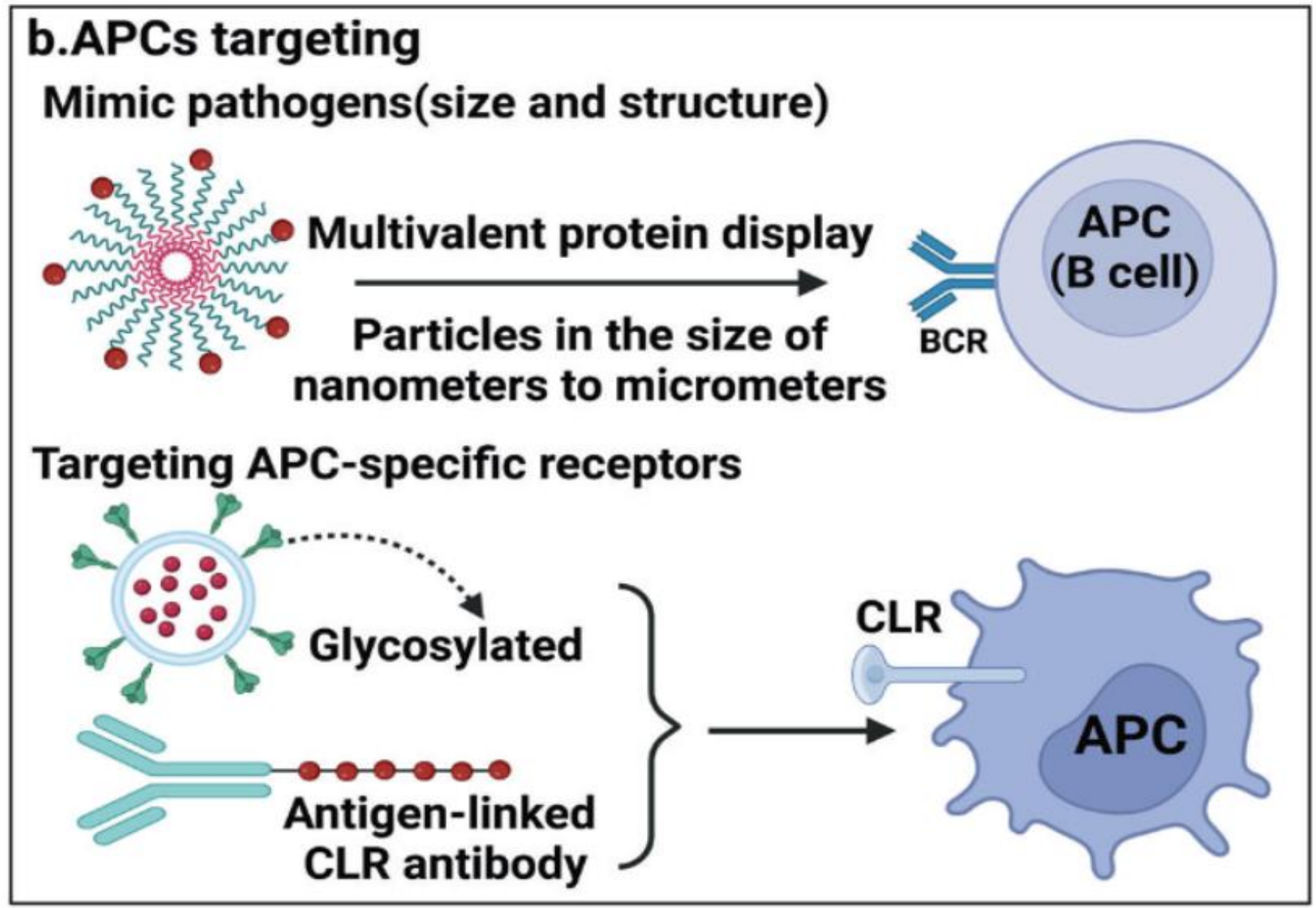
# Delivery Systems



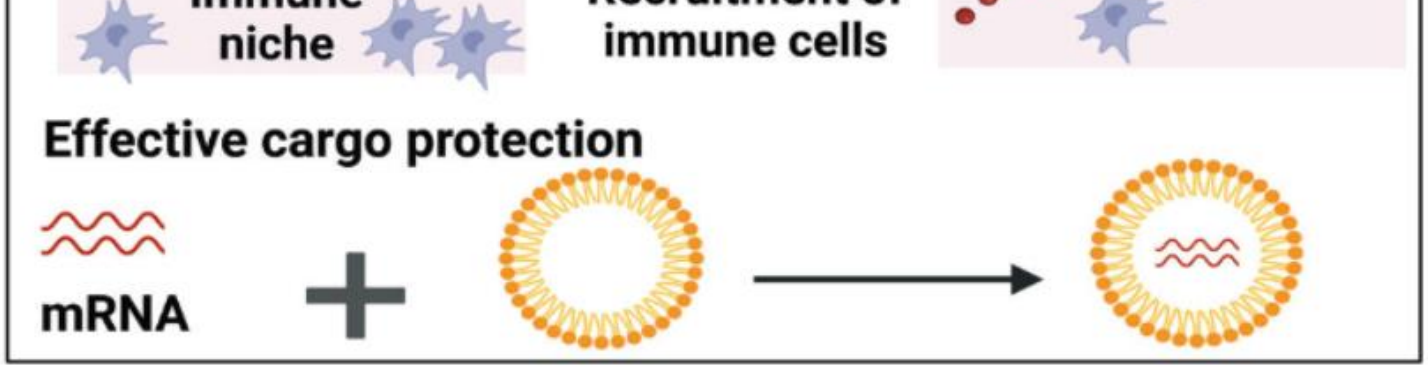

Vaccine adjuvants: mechanisms and platforms, Zhao et al, Signal Transduction and Targeted Therapy, Nature, 2023, https://doi.org/10.1038/s41392-023-01557-7

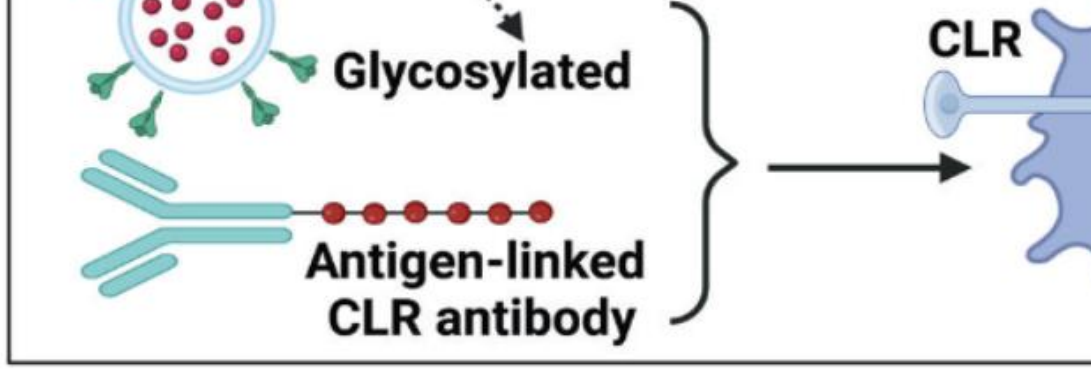

a.Prolonging the bioavailability of antigen
Sustained-release antigen
Antigens
PLGA
APC
Forming immune niche
Hydrogel
Immune niche
Sustained delivery of antigens
Recruitment of immune cells
Effective cargo protection
mRNA
b.APCs targeting
Mimic pathogens(size and s
Multivalent prot
Particles in th
nanometers to m
Targeting APC-specific recep
Glycosylated
Antigen-linked
CLR antibody
c.Lymph nodes trafficking
d.Antigen cross-presentati

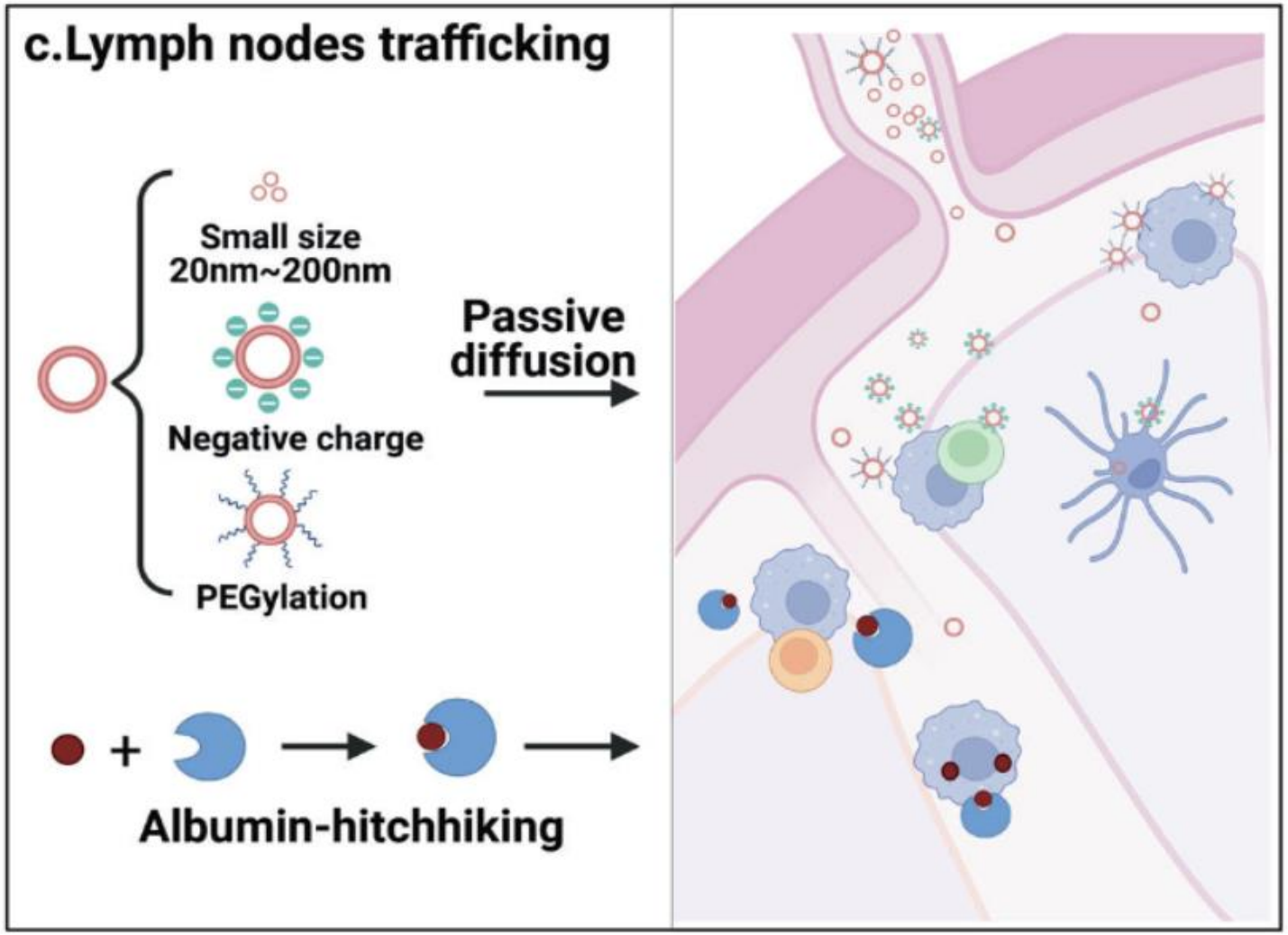

bility of antigen
APC
ed delivery of
ntigens
uitment of
nune cells

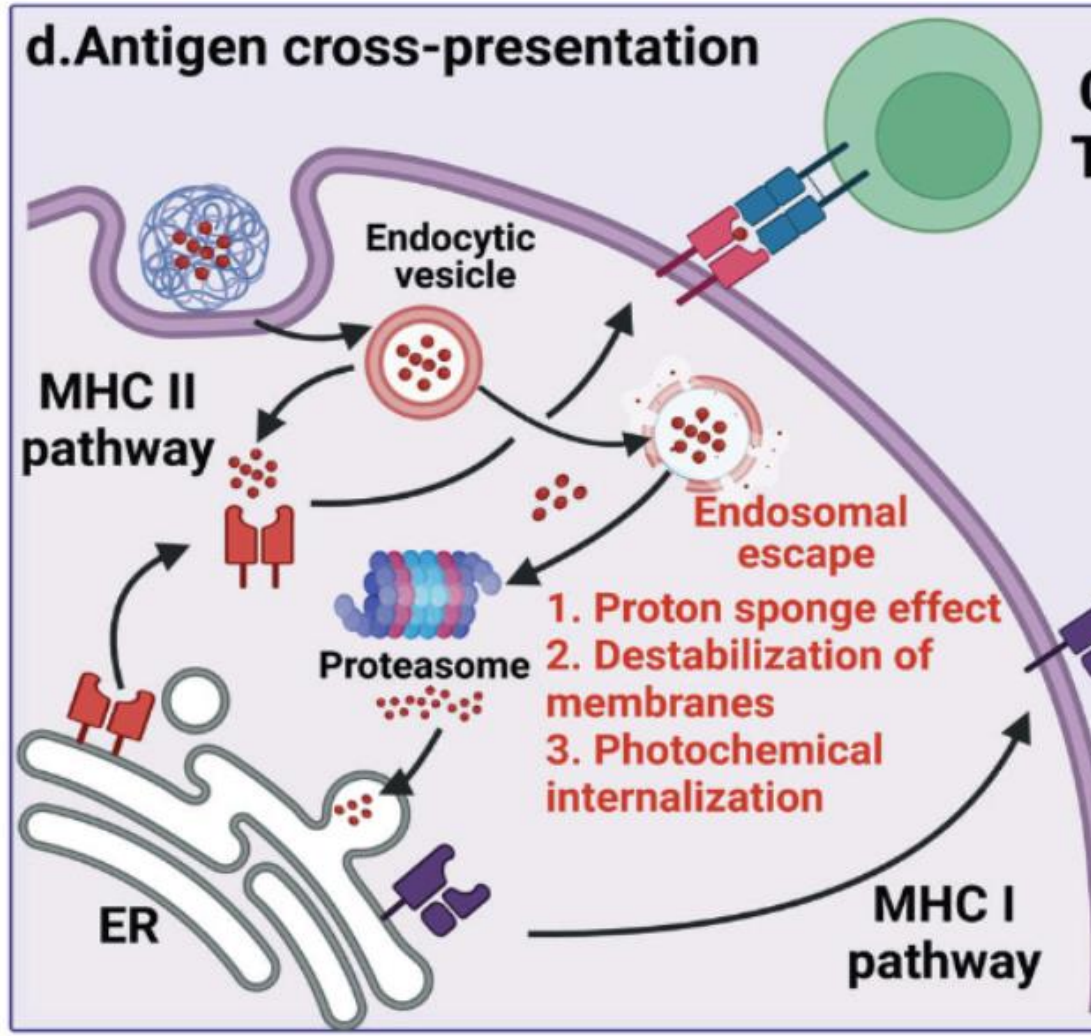

b.APCs targeting
Mimic pathogens(size and structure)
Multivalent protein display
Particles in the size of
nanometers to micrometers
APC
(B cell)
BCR
Targeting APC-specific receptors
Glycosylated
Antigen-linked
CLR antibody
CLR
APC

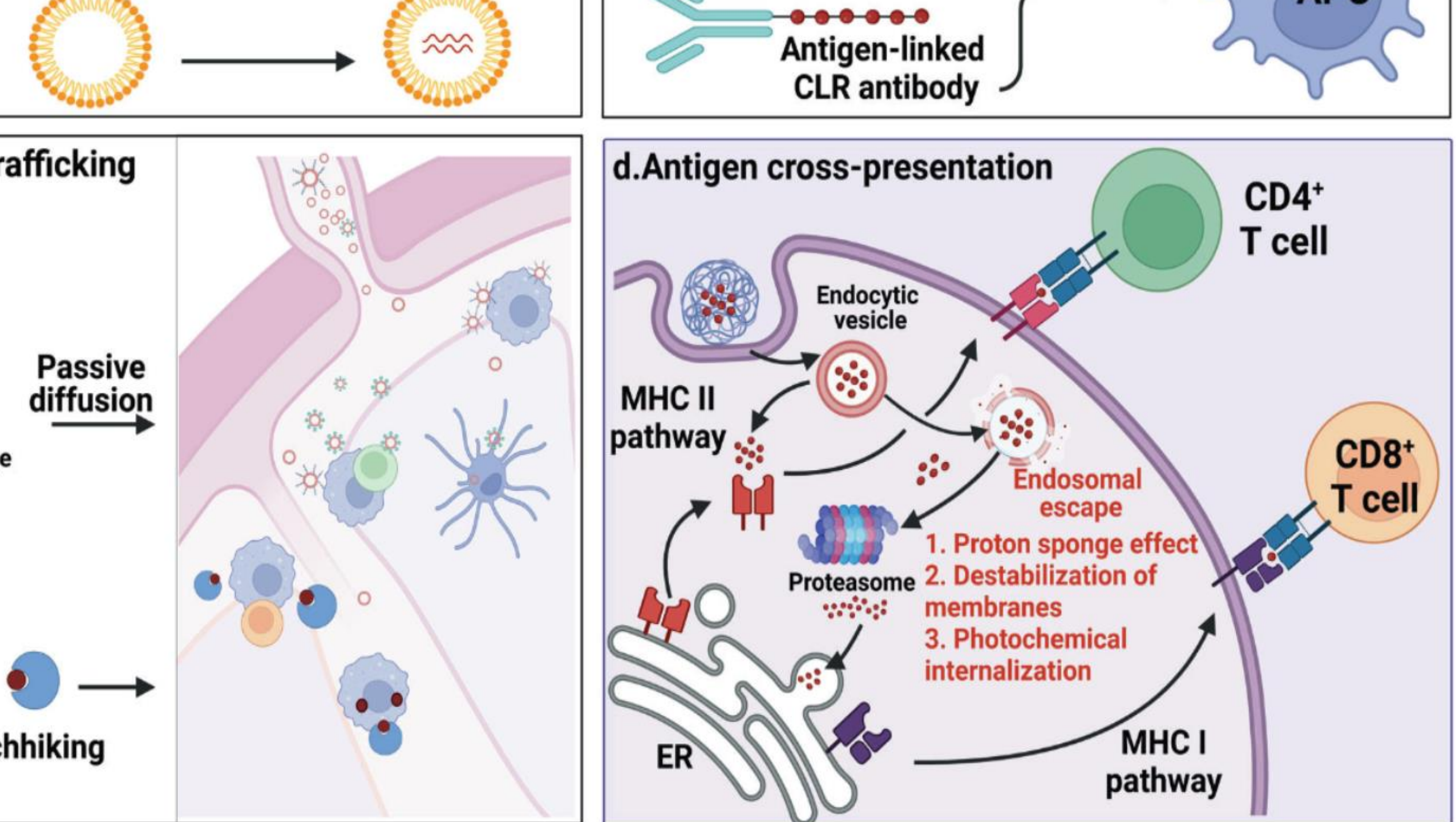

d.Antigen cross-presentation
CD4+
T cell


niche
immune cells
Effective cargo protection
mRNA


Glycosylated
CLR
Antigen-linked
CLR antibody


c.Lymph nodes trafficking
Small size
20nm~200nm
Passive
diffusion
Negative charge
PEGylation
Albumin-hitchhiking


d.Antigen cross-presentation
Endocytic
vesicle
MHC II
pathway
Endosomal
escape
1. Proton sponge effect
2. Destabilization of
membranes
3. Photochemical
internalization
Proteasome
ER
MHC I
pathway


Antigen-linked
CLR antibody
rafficking
Passive
diffusion
e
hhiking
d.Antigen cross-presentation
CD4+
T cell
Endocytic
vesicle
MHC II
pathway
Endosomal
escape
1. Proton sponge effect
2. Destabilization of
membranes
3. Photochemical
internalization
CD8+
T cell
Proteasome
ER
MHC I
pathway

1940s

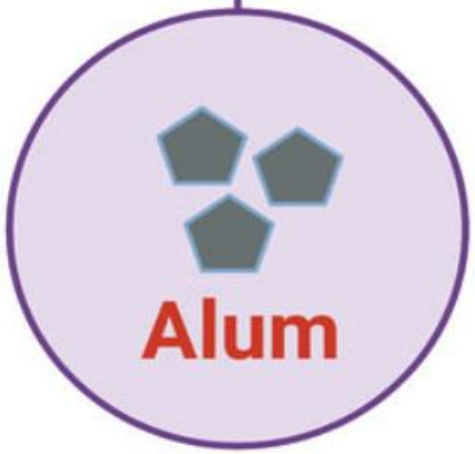
1997

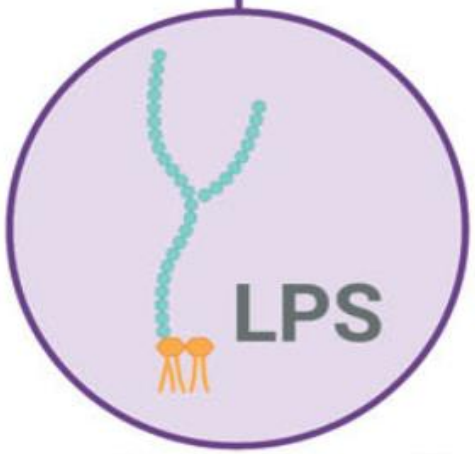
2009

1926

1956

2005





The adjuvant effects of alum were first discovered

The adjuvant effects of bacterial lipopolysaccharides (LPS) were reported

AS04 (composed of detoxified LPS and alum) was first licensed in human vaccines (HBV and HPV)

# Adjuvant: Alum Salts

## Forms: Aluminium hydroxide
## Aluminium phosphate

Mechanism of action still discussed:

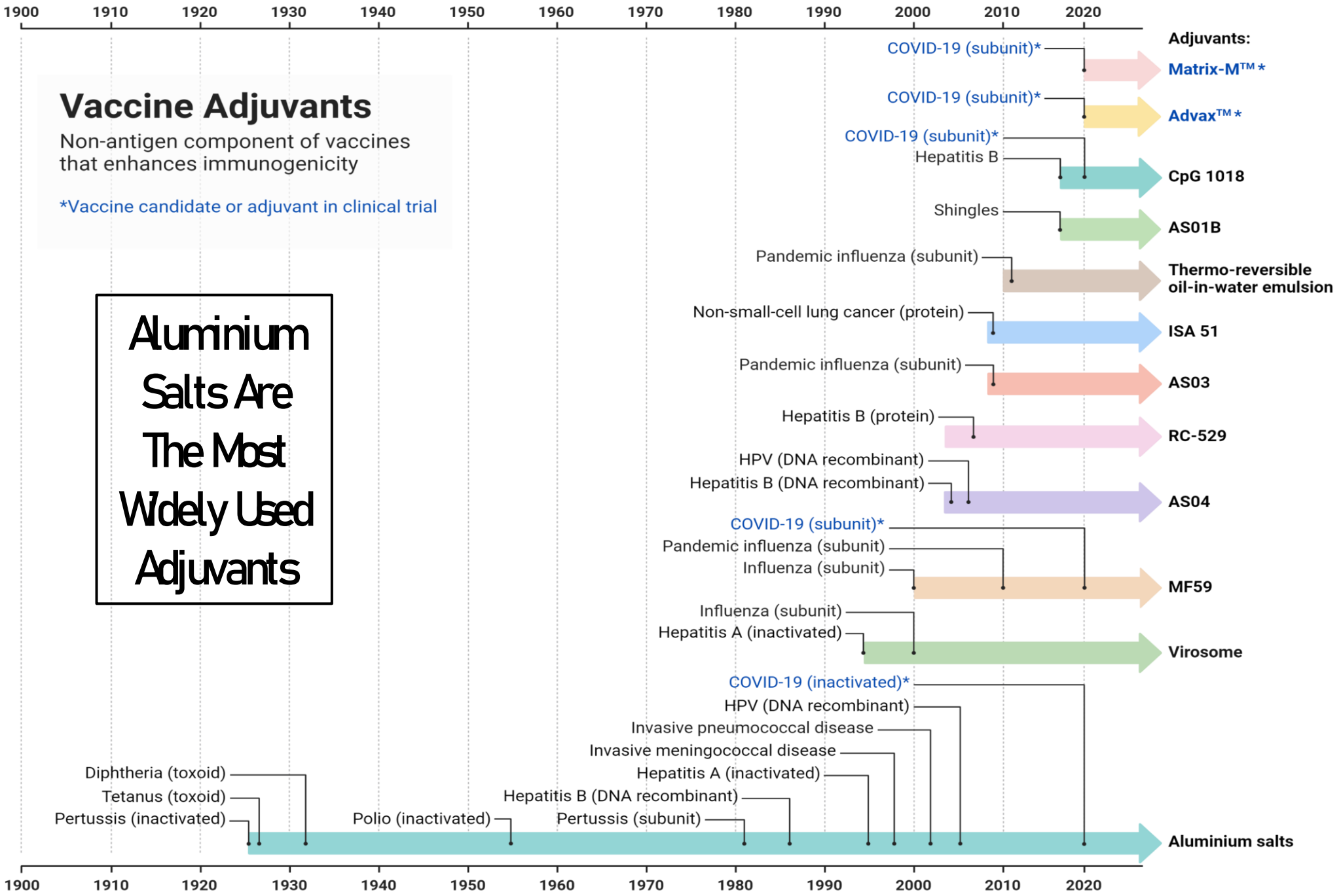
- ❖ Depot effect (persistence of antigen)
- ❖ Local irritation/inflammation: inflammasome
- ❖ Better uptake of antigen by APC

Adsorption makes antigen appear as particles

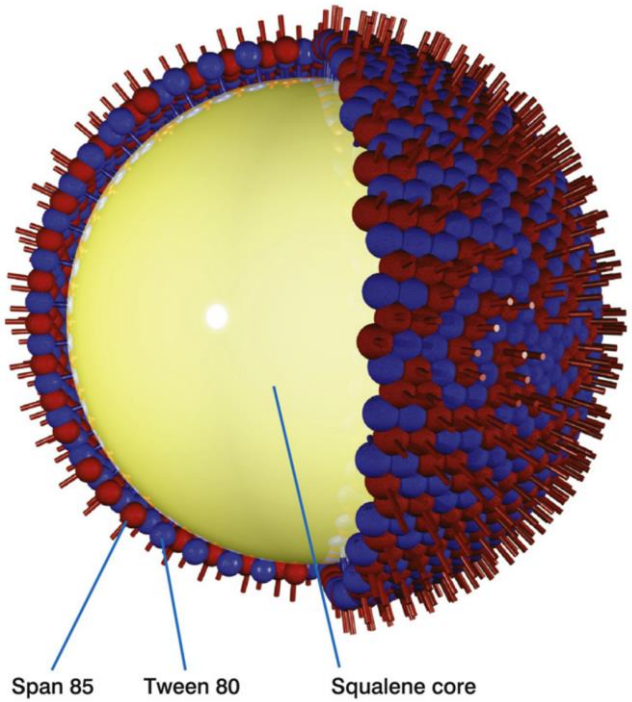
Most widely used adjuvants for > 80 years

Well-established safety profile

Indirect benefit on antigen stability


Vaccine Adjuvants
Non-antigen component of vaccines that enhances immunogenicity
*Vaccine candidate or adjuvant in clinical trial
Aluminium Salts Are The Most Widely Used Adjuvants
1900
1910
1920
1930
1940
1950
1960
1970
1980
1990
2000
2010
2020
Adjuvants:
COVID-19 (subunit)*
Matrix-M™*
COVID-19 (subunit)*
Advax™*
COVID-19 (subunit)*
Hepatitis B
CpG 1018
Shingles
AS01B
Pandemic influenza (subunit)
Thermo-reversible oil-in-water emulsion
Non-small-cell lung cancer (protein)
ISA 51
Pandemic influenza (subunit)
AS03
Hepatitis B (protein)
RC-529
HPV (DNA recombinant)
Hepatitis B (DNA recombinant)
AS04
COVID-19 (subunit)*
Pandemic influenza (subunit)
Influenza (subunit)
MF59
Influenza (subunit)
Hepatitis A (inactivated)
Virosome
COVID-19 (inactivated)*
HPV (DNA recombinant)
Invasive pneumococcal disease
Invasive meningococcal disease
Hepatitis A (inactivated)
Hepatitis B (DNA recombinant)
Pertussis (subunit)
Polio (inactivated)
Diphtheria (toxoid)
Tetanus (toxoid)
Pertussis (inactivated)
Aluminium salts

# Adjuvant: MF59: o/w emulsion




Optimized Oil in Water Emulsion Formulation

Surfactants Polysorbate 80 and Span 85 allow the production of small, stable emulsion droplets that can be sterile filtered

Shown to invoke inflammatory responses:

acts on macrophages (site of injection)

↑ chemokine release

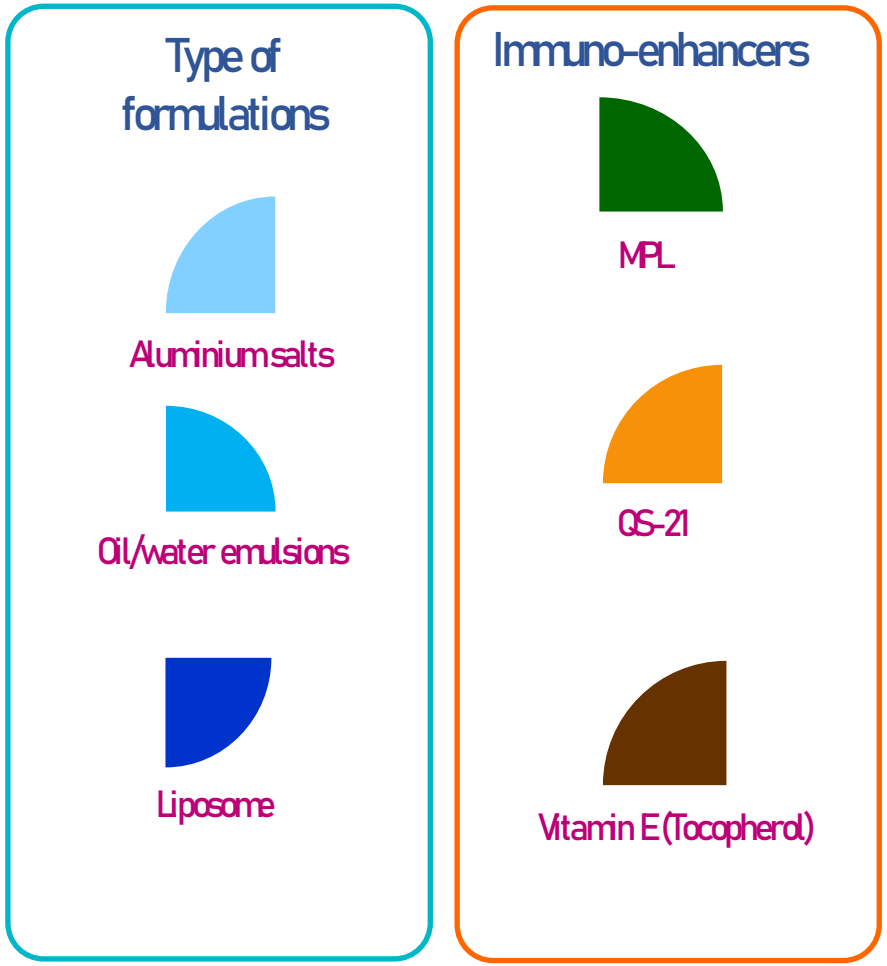
↑ immune cells recruitment (mainly monocytes)

induces recruitment and activation of APCs with ↑ antigen load and promotes migration to the lymph node

Approved for use in : licensed influenza vaccine in Europe since 1997 and has now been administered to more than 100 million people in more than 30 countries.

O'Hagan *et al*. *Expert Rev Vaccines 2013;*12(1):13–30; Seubert *et al. J Immunol* 2008;180:5402-5412; Garçon *et al.* Chapter 4 in: Garçon *et al.* Understanding Modern Vaccines, Perspectives in vaccinology, Vol 1, Amsterdam. Elsevier 2011;p89–113
Image: Emerging concepts in the science of vaccine adjuvants Pulendran et al. Nature Reviews, Drug Discovery, Volume 20, June 2021, https://doi.org/10.1038/s41573-021-00163-y

# GSK Propriety Adjuvants




**AS04**[1,2]

- HBV (in (pre)-haemodialysis)**
- HPV***

**AS03**[3]

- Pandemic influenza*
- Pandemic COVID-19

**AS01**[1,2,4]

- Malaria
- TB, HV, Zoster

HBV = hepatitis B virus; HPV = human papilloma virus; MPL = 3-O-desacyl-4'-monophosphoryl lipid A; QS = Quillaja saponaria; TB = tuberculosis; HV = human immunodeficiency virus

1. Garçon N et al. Expert Rev Vaccines 2007;6:723–39; 2. Garçon N et al. Expert Rev Vaccines 2011;10:471–86;
3. Garçon N et al. Expert Rev Vaccines 2012;11:349–66; 4. Lal H et al. Hum Vaccin Immunother 2013;9:1425–9

* H1Nv/AS03 license expired (August 2015); H5N1/AS03 licensed in Europe, US and international countries;
** Licensed only in Europe in >15 years of age; *** Licensed in Europe, Japan, US and international countries

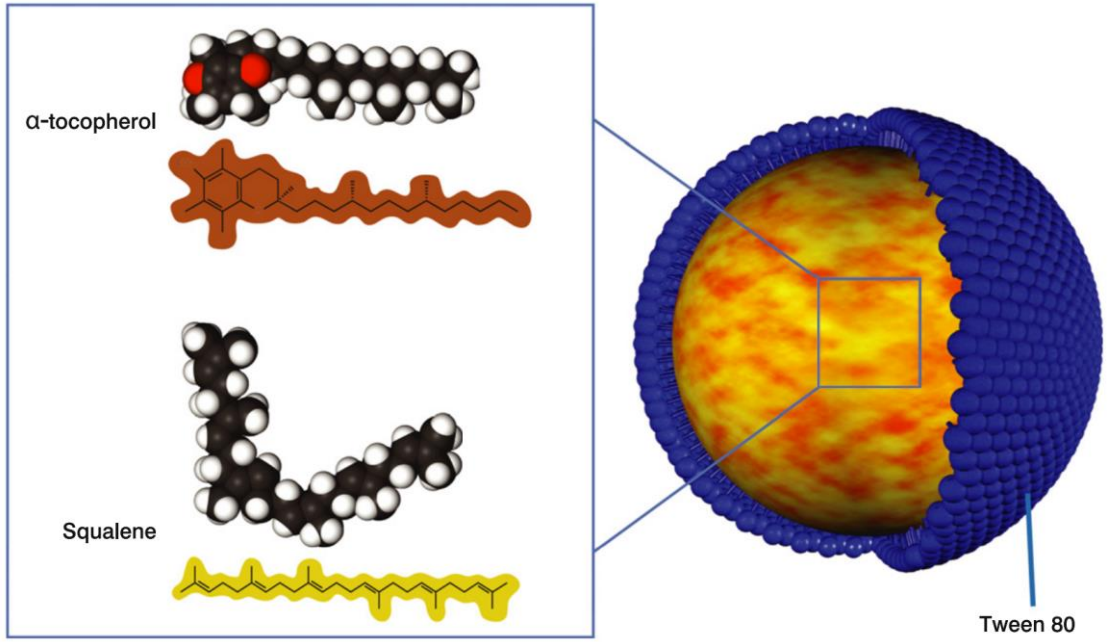
# Adjuvant: AS03

## GSK Propriety Adjuvants

AS03 is licensed in combination with pandemic influenza vaccine.

AS03 enhances the magnitude and breadth of antibody responses and CD4+ T cell responses, leading to enhanced protection against flu compared with non-adjuvanted vaccines

In humans, within 24h of vaccination with AS03-adjuvanted H5N1 avian influenza vaccine→ increased serum levels of IL-6 and IP10 (also known as CXCL10) as well as transcriptional signatures of interferon signaling and antigen processing and presentation in DCs, monocytes and neutrophils




AS03 is a squalene oil-in-water emulsion adjuvant that is similar to MF59, but also contains α-tocopherol (vitamin E) as an additional immune-enhancing component.

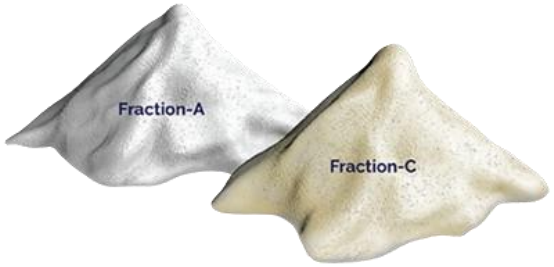
Image and Information: Emerging concepts in the science of vaccine adjuvants Pulendran et al. Nature Reviews, Drug Discovery, Volume 20, June 2021, https://doi.org/10.1038/s41573-021-00163-y

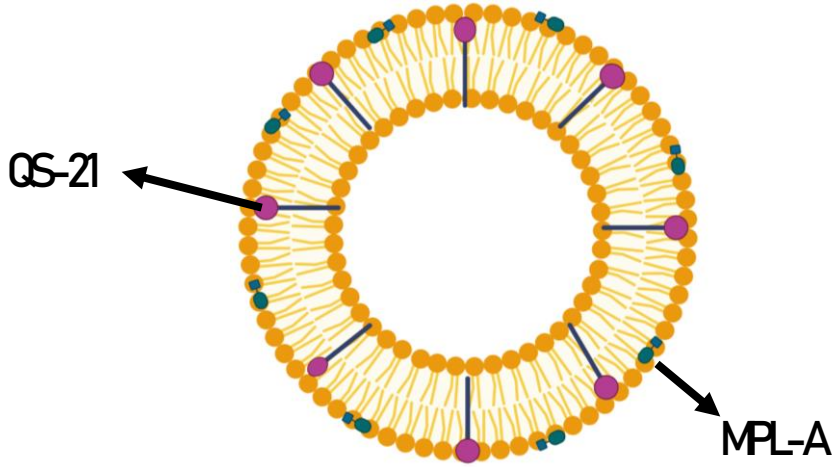
# Adjuvant: AS01

## GSK Propriety Adjuvants

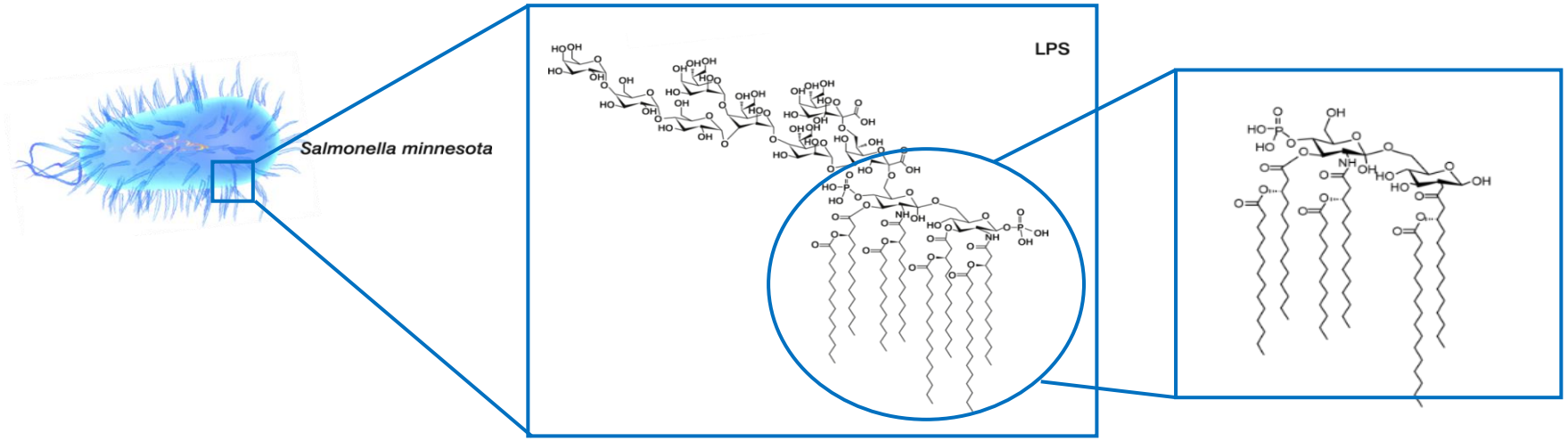
QS-21 is a triterpene glycoside purified from the bark extracts of the tree Quillaja saponaria Molina

Saponin Bark from *Quillaja saponaria*




AS01 Liposome– TLR4 ligand MPL and an saponin fraction, QS-21




Emerging concepts in the science of vaccine adjuvants Pulendran et al. Nature Reviews, Drug Discovery, Volume 20, June 2021, https://doi.org/10.1038/s41573-021-00163-y

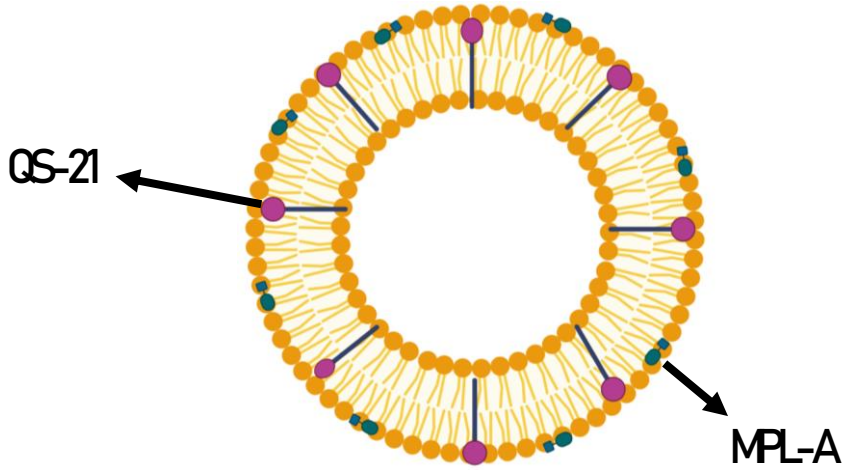
# Adjuvant: AS01

## GSK Propriety Adjuvants

AS01 is included in a licensed vaccine against varicella zoster (Shingrix), approved for use in older adults (50 years and older), with high efficacy (97.2%)

QS-21 → Significant concerns about tolerability profile when used alone.

In AS01→ MPL and QS-21 → Formulated together in liposomes → With Cholesterol→ binds QS-21 into the liposome → quench its reactogenicity.




AS01 Liposome– TLR4 ligand MPL and an saponin fraction, QS-21

Emerging concepts in the science of vaccine adjuvants Pulendran et al. Nature Reviews, Drug Discovery, Volume 20, June 2021, https://doi.org/10.1038/s41573-021-00163-y

# Adjuvant: AS04

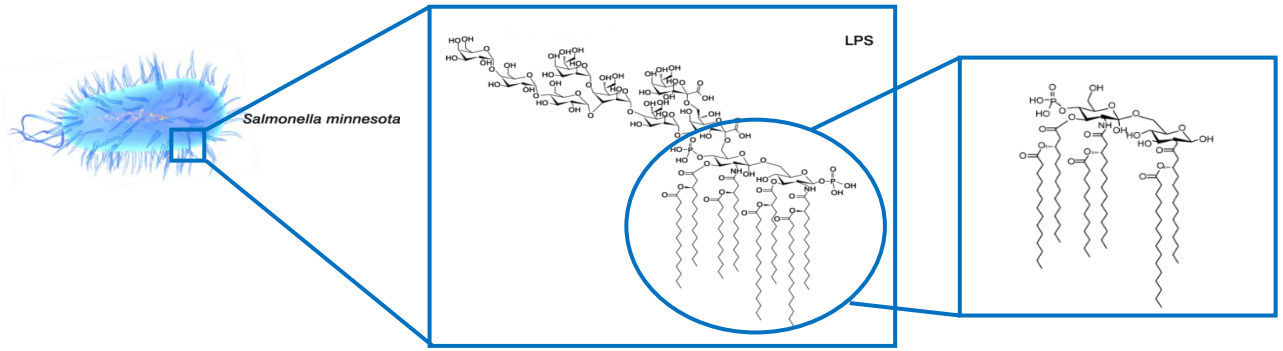
## GSK Propriety Adjuvants

AS04 consists of MPL, a detoxified form of lipopolysaccharide (LPS) extracted from Salmonella minnesota, which is adsorbed on aluminum salts

Addition of AS04 to the hepatitis B virus (HBV) and the human papillomavirus (HPV) vaccines → Higher levels of antibodies in comparison with vaccine adjuvanted with just alum, demonstrating the added value of the TLR4 agonist MPL-A in humans

The higher immunogenicity also translated into a high and long-lasting efficacy of the HPV-16/18 vaccine.

MPL-A




+

Aluminum Salts

Emerging concepts in the science of vaccine adjuvants Pulendran et al. Nature Reviews, Drug Discovery, Volume 20, June 2021, https://doi.org/10.1038/s41573-021-00163-y

# Adjuvant: CPG ODN1018

## Dynavax's Propriety adjuvant

Adjuvant in Heplisav-B, an improved HBV vaccine licensed for use in adults (>18 years).

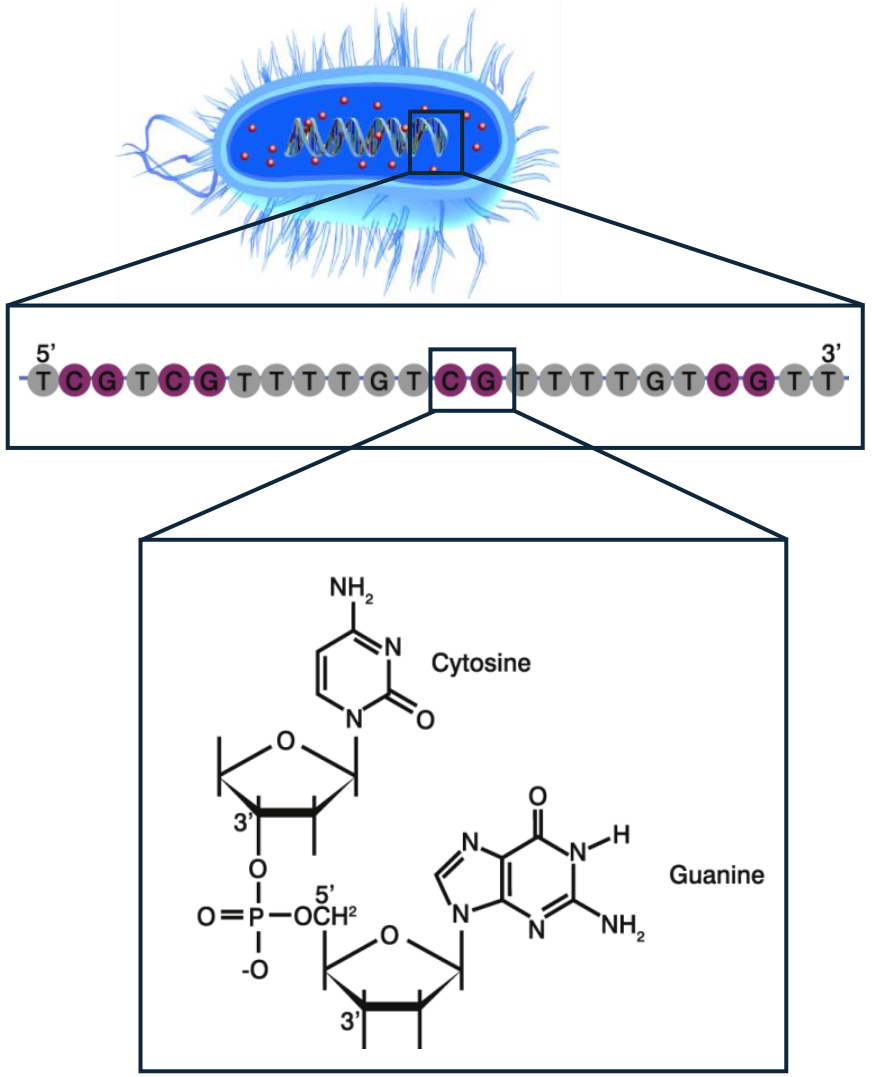
Offers a two dose regimen as opposed to typical 3 dose HBV vaccines

-CpG1018, a TLR9 agonist, increases antibody concentrations, stimulates helper (CD4+) and cytotoxic (CD8+) T cell populations.

-Generates robust T and B cell memory responses

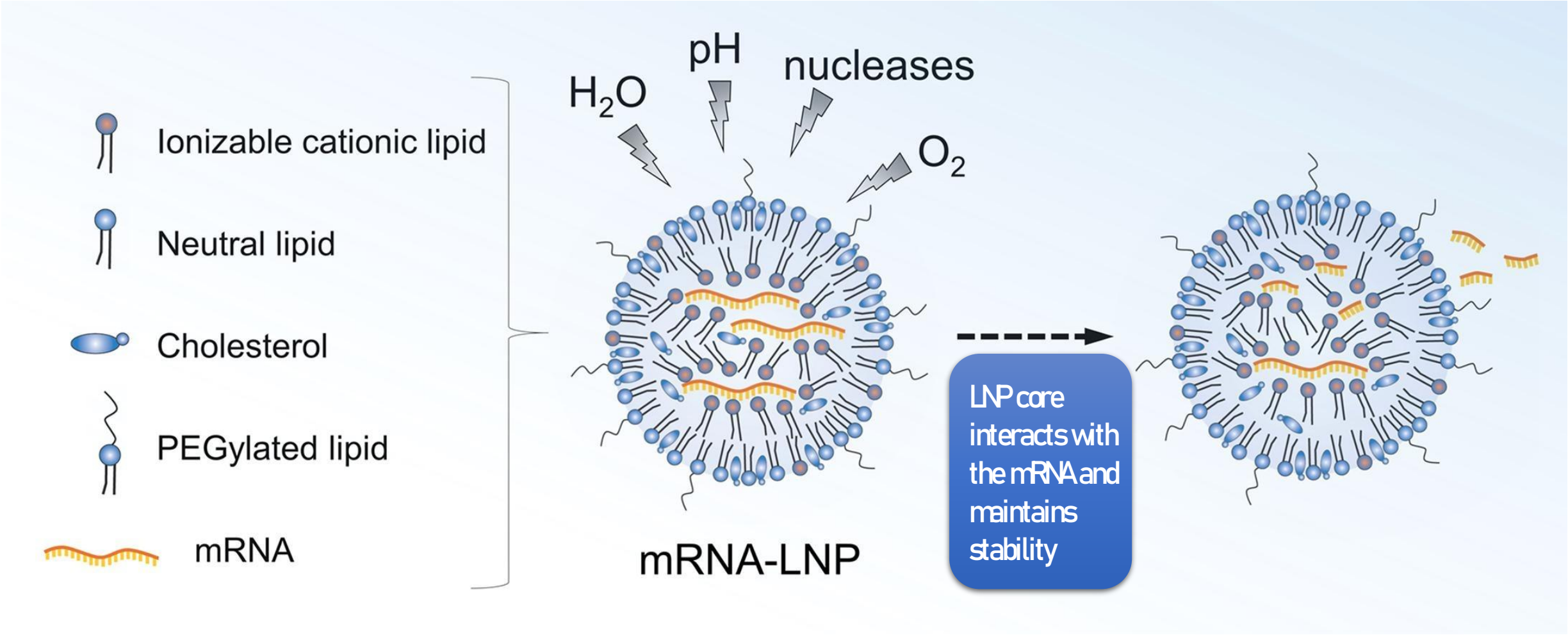
-Potent TH1 subset inducer

-CpG1018 is currently being evaluated in clinical trials as a potential vaccine adjuvant for COVID-19 vaccines




Development of the CpG Adjuvant 1018: A Case Study John D Campbell, Methods Mol Biol. 10.1007/978-1-4939-6445-1_2

# Adjuvant: Lipid Nanoparticles



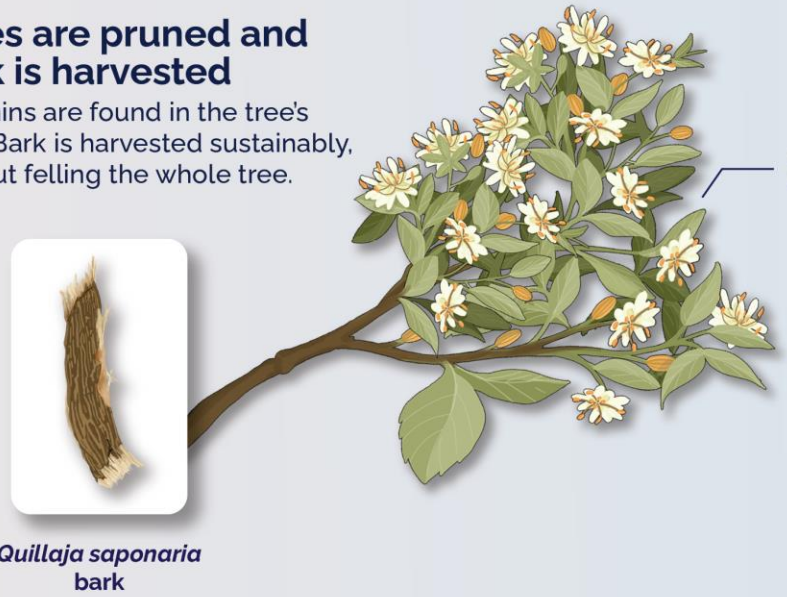

Source: Schoenmaker, Linde, et al. "MRNA-lipid Nanoparticle COVID-19 Vaccines: Structure and Stability." International Journal of Pharmaceutics, vol. 601, 2021, p. 120586, https://doi.org/10.1016/j.ijpharm.2021.120586. Accessed 4 Jan. 2024.

# Matrix-M™ adjuvant production process

## Saponins, from the *Quillaja saponaria* tree, help generate an enhanced immune response

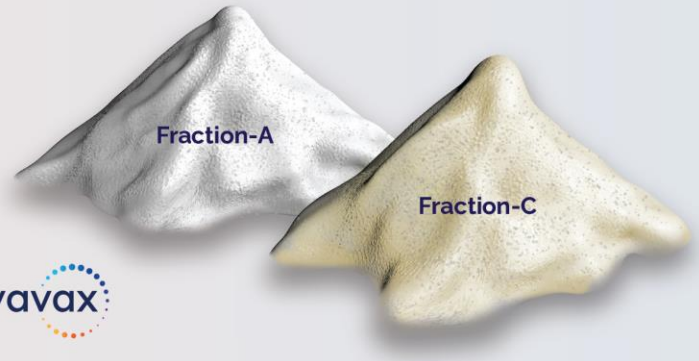
### 1 Trees are pruned and bark is harvested

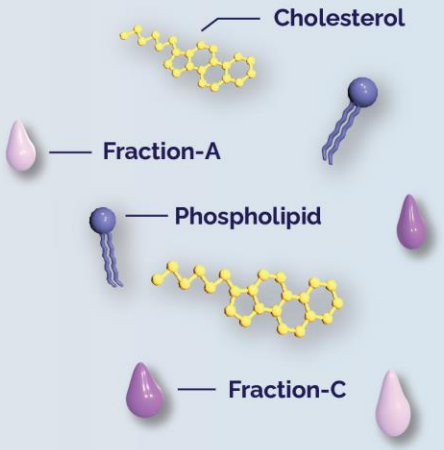
Saponins are found in the tree's bark. Bark is harvested sustainably, without felling the whole tree.




### 2 Bark is processed

Bark extract is processed into Fraction-A and Fraction-C, then freeze-dried (lyophilized). These powders contain "raw" saponin molecules.



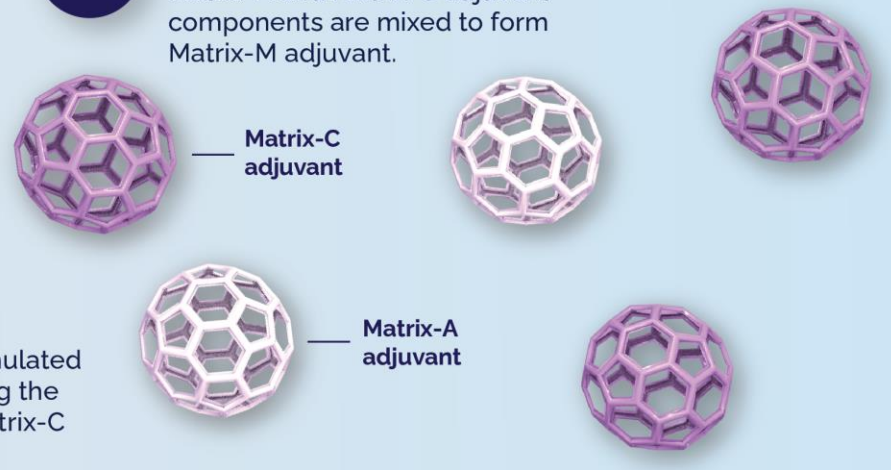




### 3 Liquid formulation prepared

Fraction-A and Fraction-C, as liquids, are formulated with phospholipids and cholesterol, producing the distinctive nanostructures of Matrix-A and Matrix-C adjuvants, respectively.

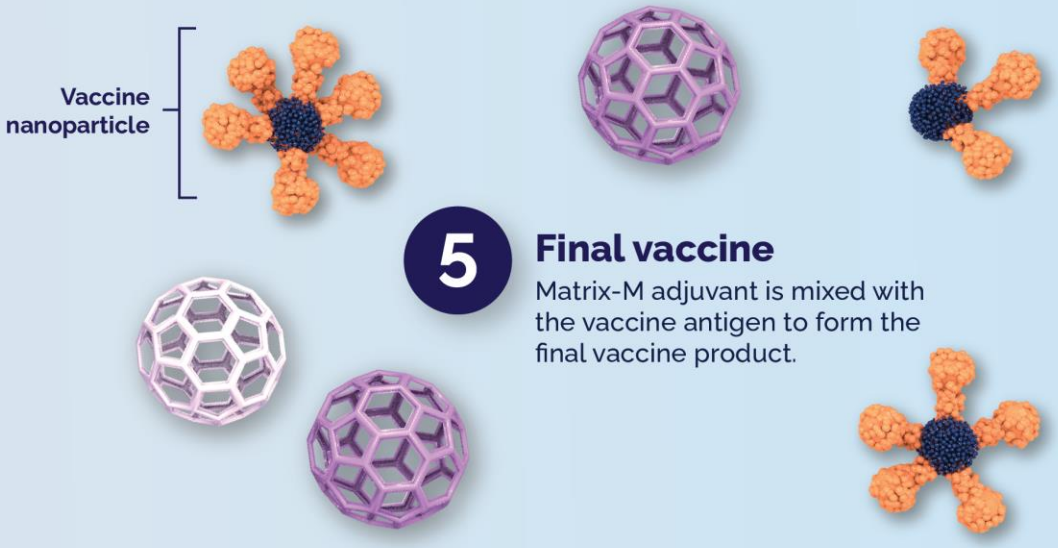
### 4 Matrix-M adjuvant formation

Matrix-A and Matrix-C adjuvant components are mixed to form Matrix-M adjuvant.




### 5 Final vaccine

Matrix-M adjuvant is mixed with the vaccine antigen to form the final vaccine product.




novavax



COR-GL-ALL-0022 09/2022

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