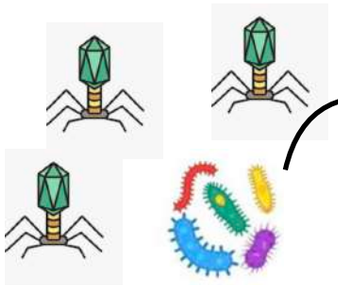
Intro 2 Immune System

Microscale interactions and molecular recognitions have system scale consequences for human health

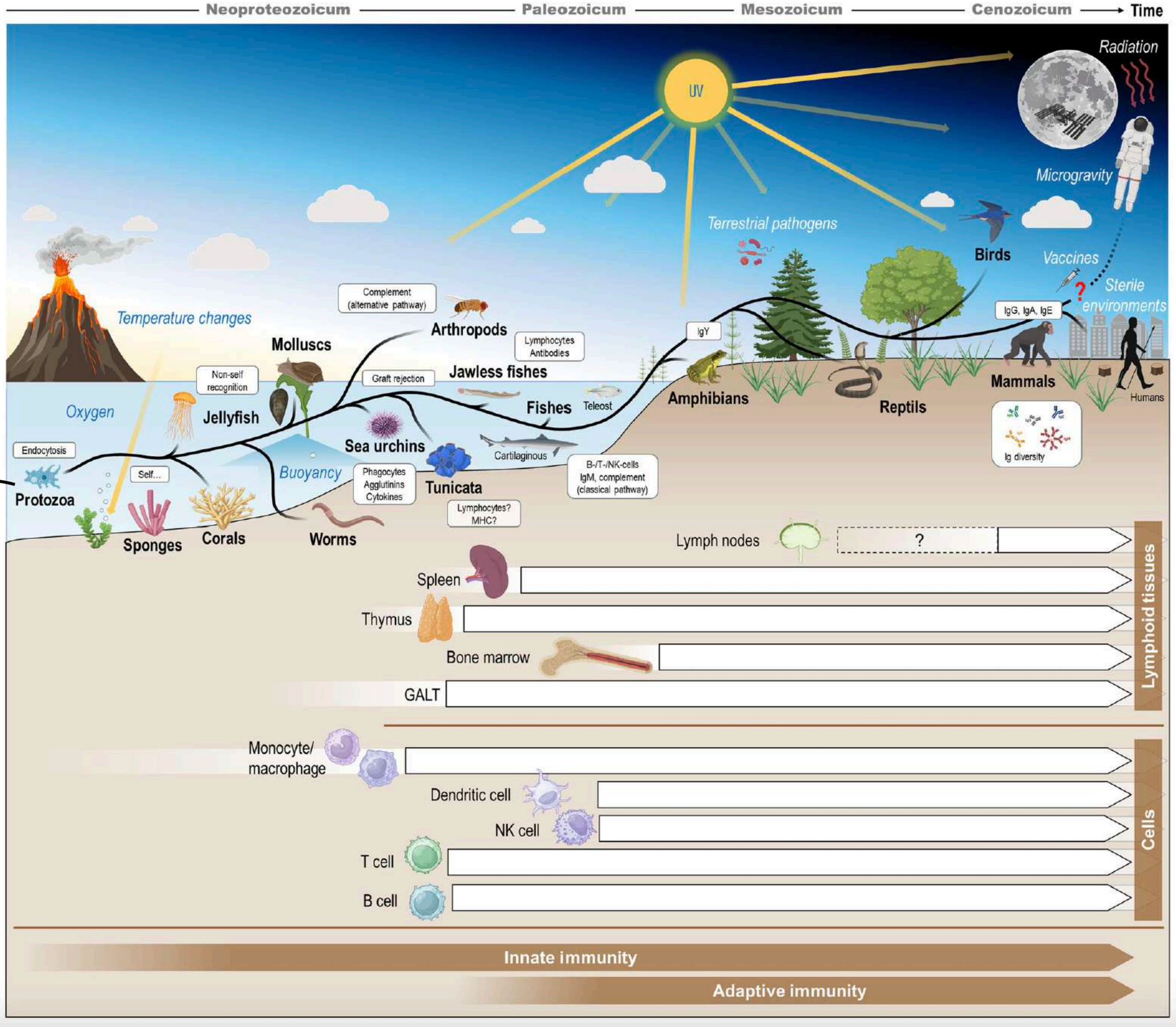
Microbes *vs.* Immune system cells and molecules

The immune system evolution



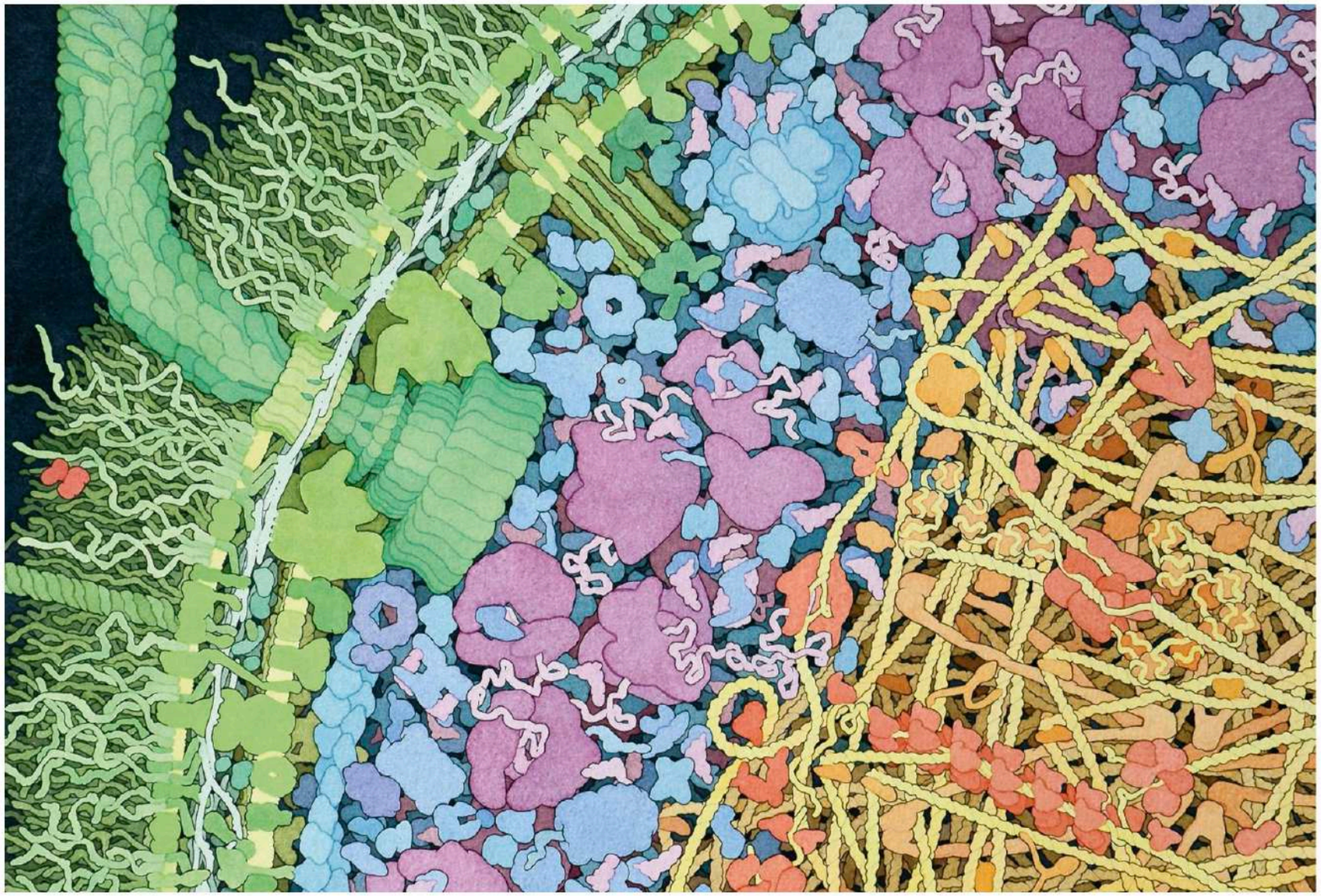
Prokaryotes

CRISPR-Cas



Adapted from Marchal et al., 2024

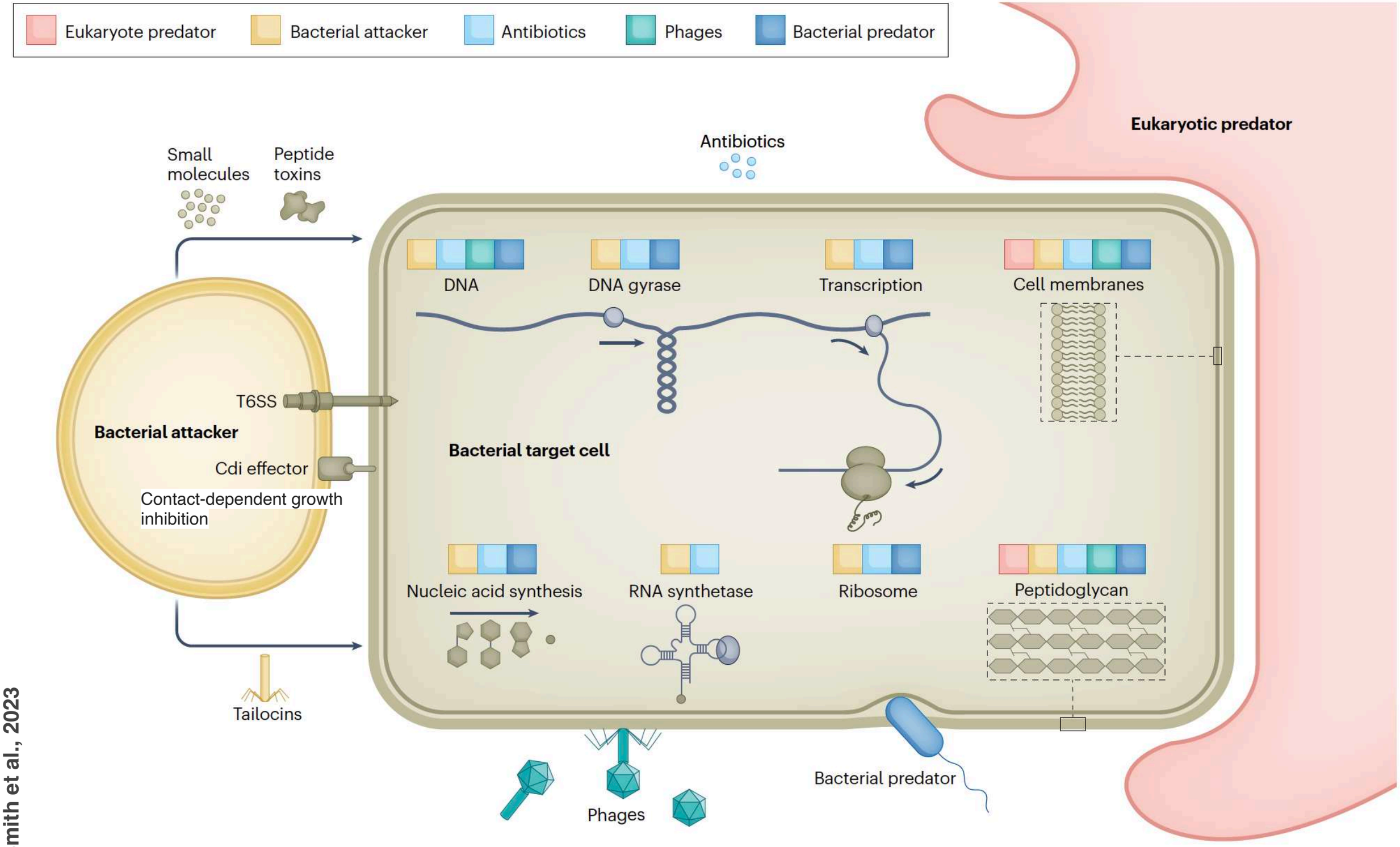
How to identify a microbe?



David S. Goodsell

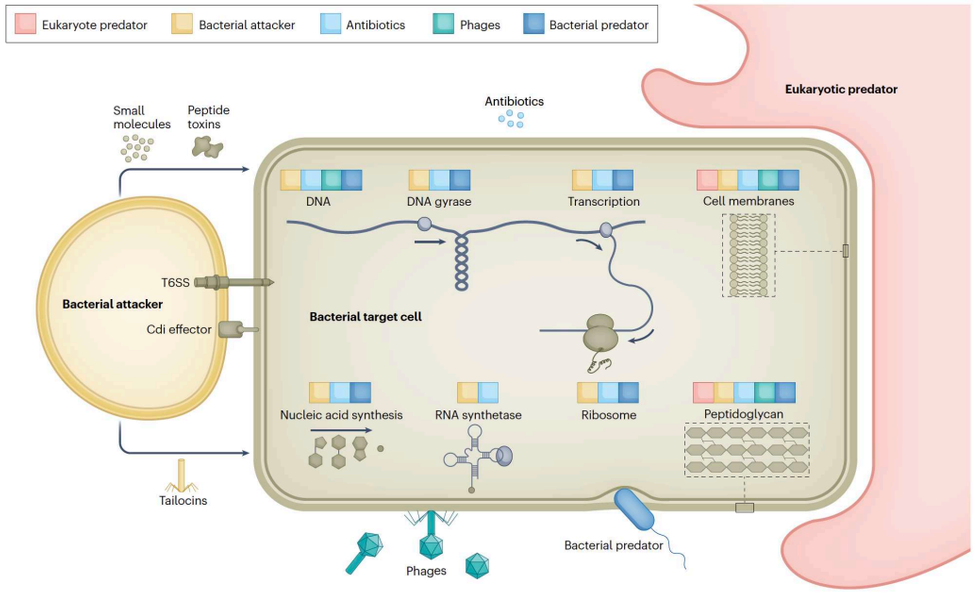
Cell surface structure
Metabolism —> molecules
Behaviour

The diverse microbial threats



Smith et al., 2023

Smith et al., 2023



- Most attacks target **core cellular processes and functions** of the microbial cell

- Microbial competitors antagonize a target bacterium via diverse mechanisms, including both **contact-dependent** weaponry (the type VI secretion system (T6SS); **Cdi effectors**) and **diffusible weaponry** (small molecules, antibiotics, peptide toxins and tailocins)

- The majority of clinical antibiotics are also derived from bacteria and other microorganisms

- Following infection of a bacterial cell, phages attack cell walls and membranes to release their progeny via cell lysis

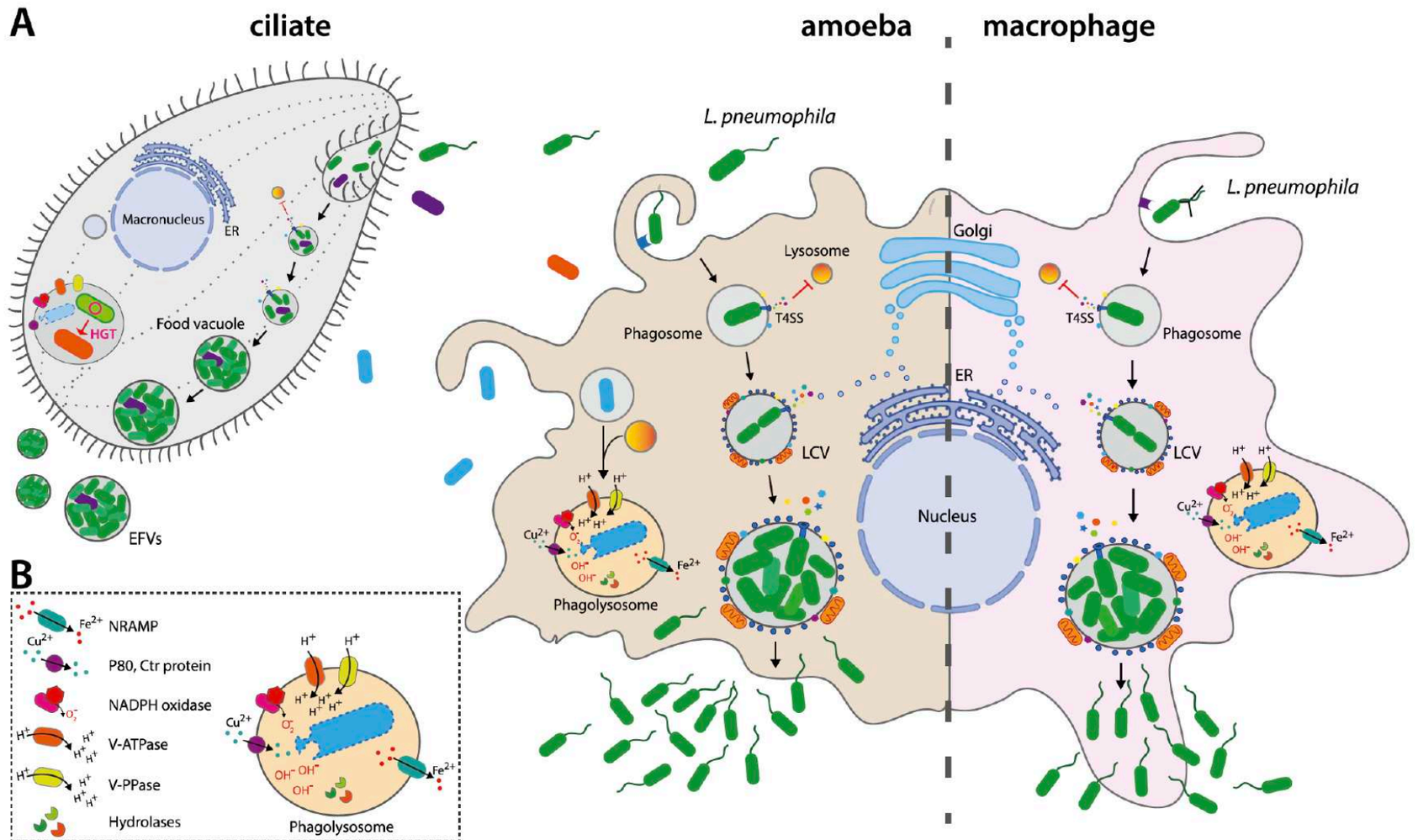
- Some bacterial predators, such as *Bdellovibrio* species and similar organisms, invade the host cell periplasm, injecting toxins that digest various cytoplasmic components

- Many eukaryotic predators **engulf and digest target bacteria whole in phagosome compartments**

Phagotrophy

Bacterium/Archaeon is trapped and engulfed in the phagosome
Fusion between phagosome and lysosome:

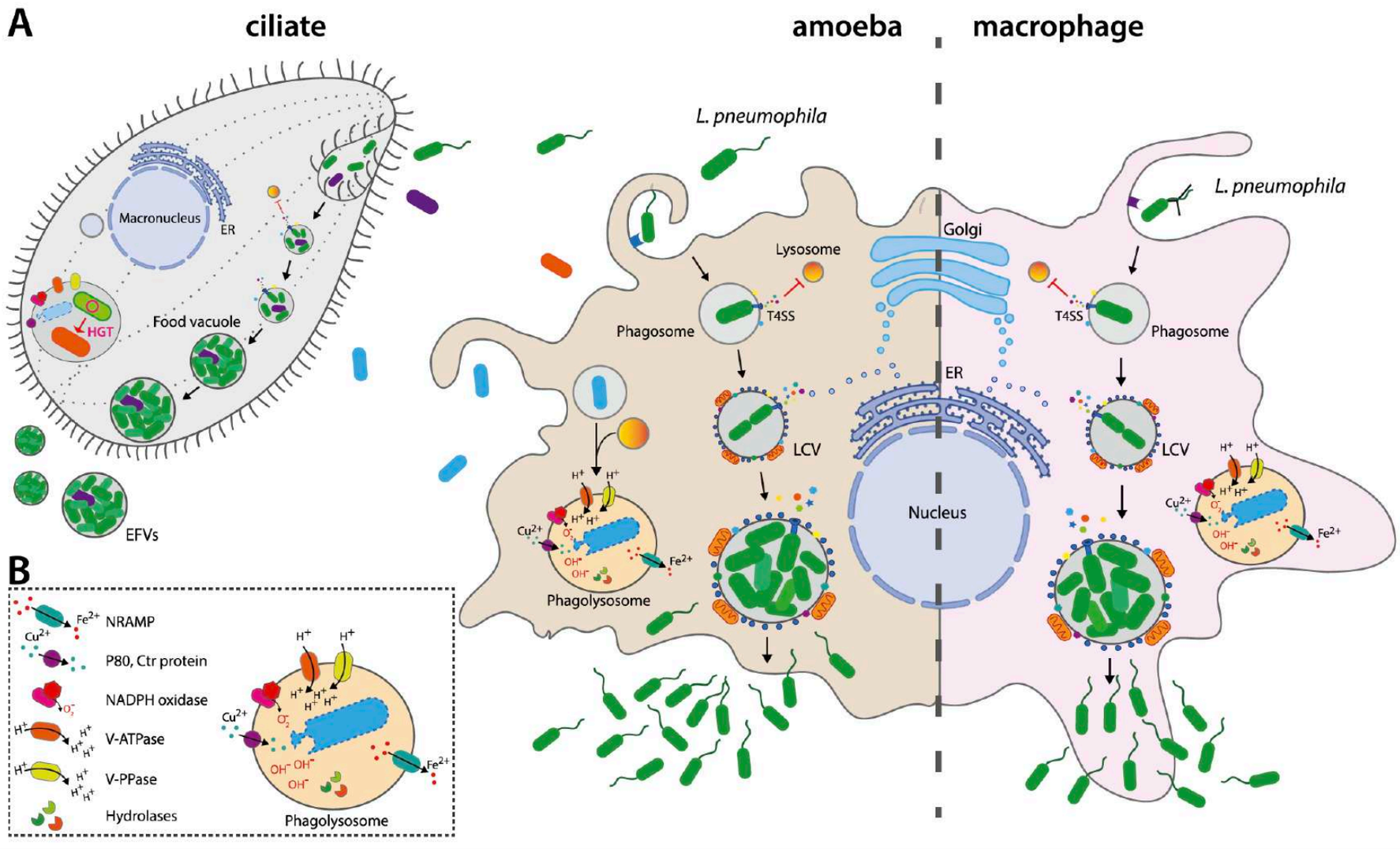
- ★ Enzymatic digestion
- ★ Phagosomal acidification
- ★ Oxidative burst
- ★ Fe2+ and Mn2+ depletion from the phagosome with efflux systems
- ★ Metal poisoning with Cu2+ and Zn2+



Amaro and Martin-Gonzalez, 2021

Escape strategy from phagolysosome

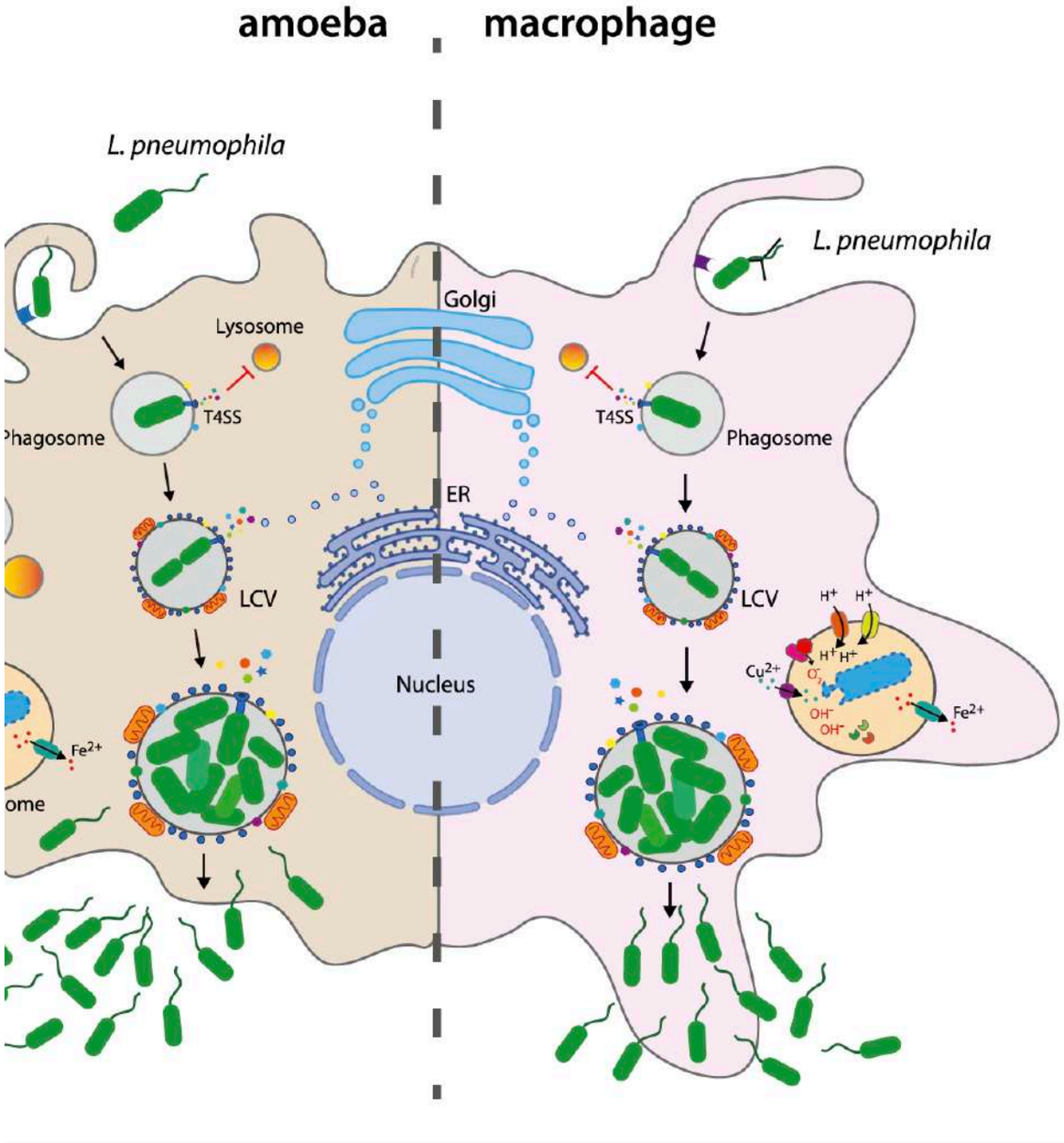
Bacteria and Archaea that **resist lysosomal digestion** in protozoa can be released into the environment freely after host cell lysis or packaged into **expelled food vacuoles (EFVs)** that serve as vectors for microbial dissemination



Amaro and Martin-Gonzalez, 2021

From a microbial point of view a macrophage is not different than a protist in the environment

- *Legionella* is enclosed in a phagosome that neither acidifies nor fuses with the lysosome
- *Legionella* remodels it into a replicative compartment called Legionella containing vacuole (LCV)
- LCV is decorated with recruited mitochondria, RER, and ER-to-Golgi complex-derived vesicles
- After several rounds of replication, *Legionella* breaks out the LCV membrane into the cytosol before lysing the host cell



Amaro and Martin-Gonzalez, 2021

MICROBIAL BATTLEFIELD

- An infection can be seen as a battle between the invading pathogens and the host
- Human bodies are equipped to fight off invading microbes that may cause disease
- **The immune response has to be tightly controlled to ensure a clearance of the microbes but also to prevent tissue damage and necrosis as result of sepsis**

- **Human natural defences are:**

1. **Aspecific defense: chemical and physical barriers**
2. **Costitutive / innate**
3. **Adaptive / inducible**

The Immune System

Why do we care about the Immune system?

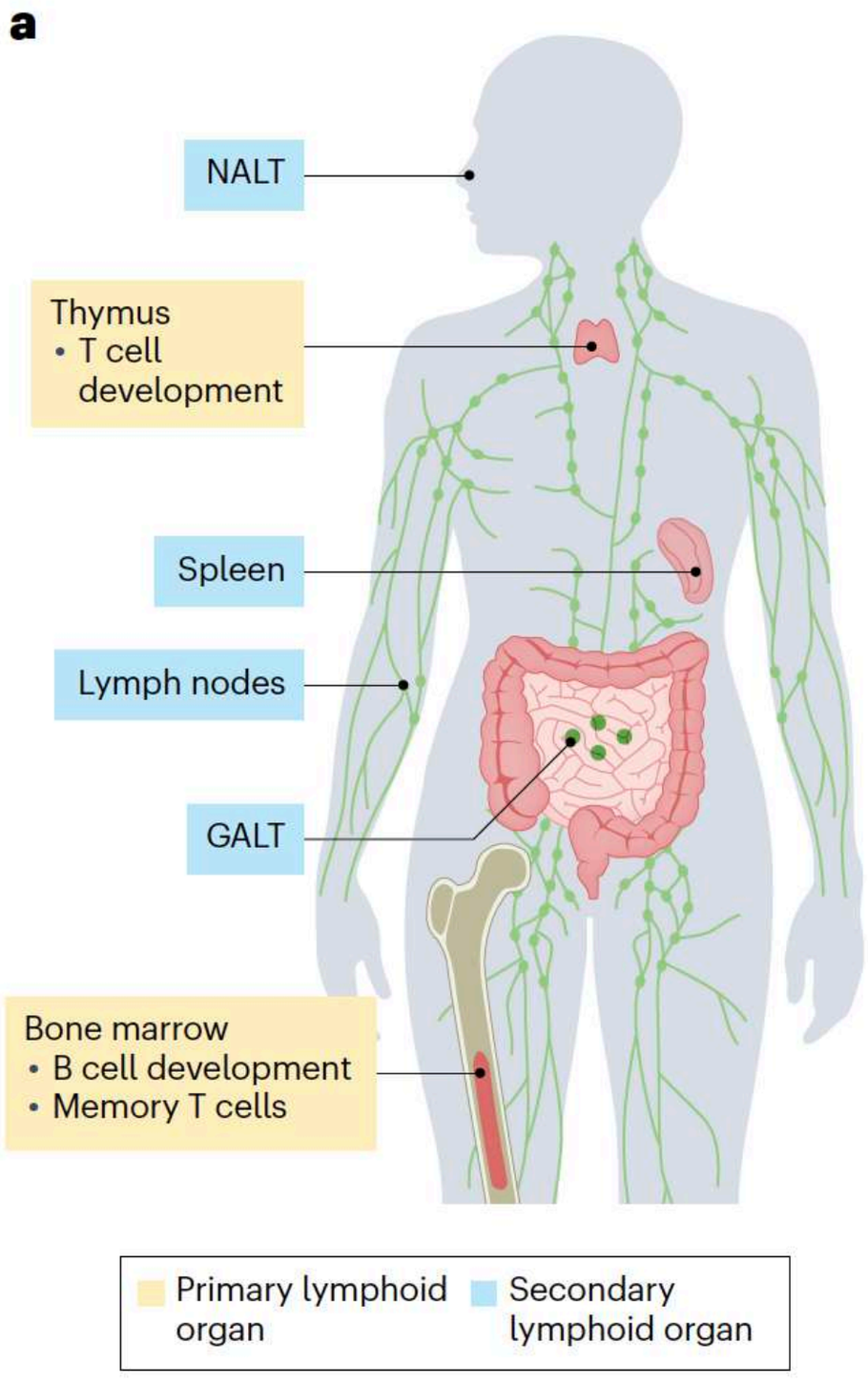
Peaceful coexistence, while avoiding microbial breach and takeover as well as over-exuberant immune responses, is essential for the functioning of the human ecosystem

Dead cell and non-self clearance

The immune system consists of well-defined regional control centres (lymphoid organs), important tissue-resident cell populations (especially at barrier tissues such as mucosal surfaces) and mobile cell populations that constantly recirculate through blood (and specialised free molecules) and tissues

Two systems in one: innate (general) and adaptive (specific/ tailored)

Systemic architecture



Yousefpour et al., 2023

GALT, gut-associated lymphoid tissue; NALT, nasal-associated lymphoid tissue

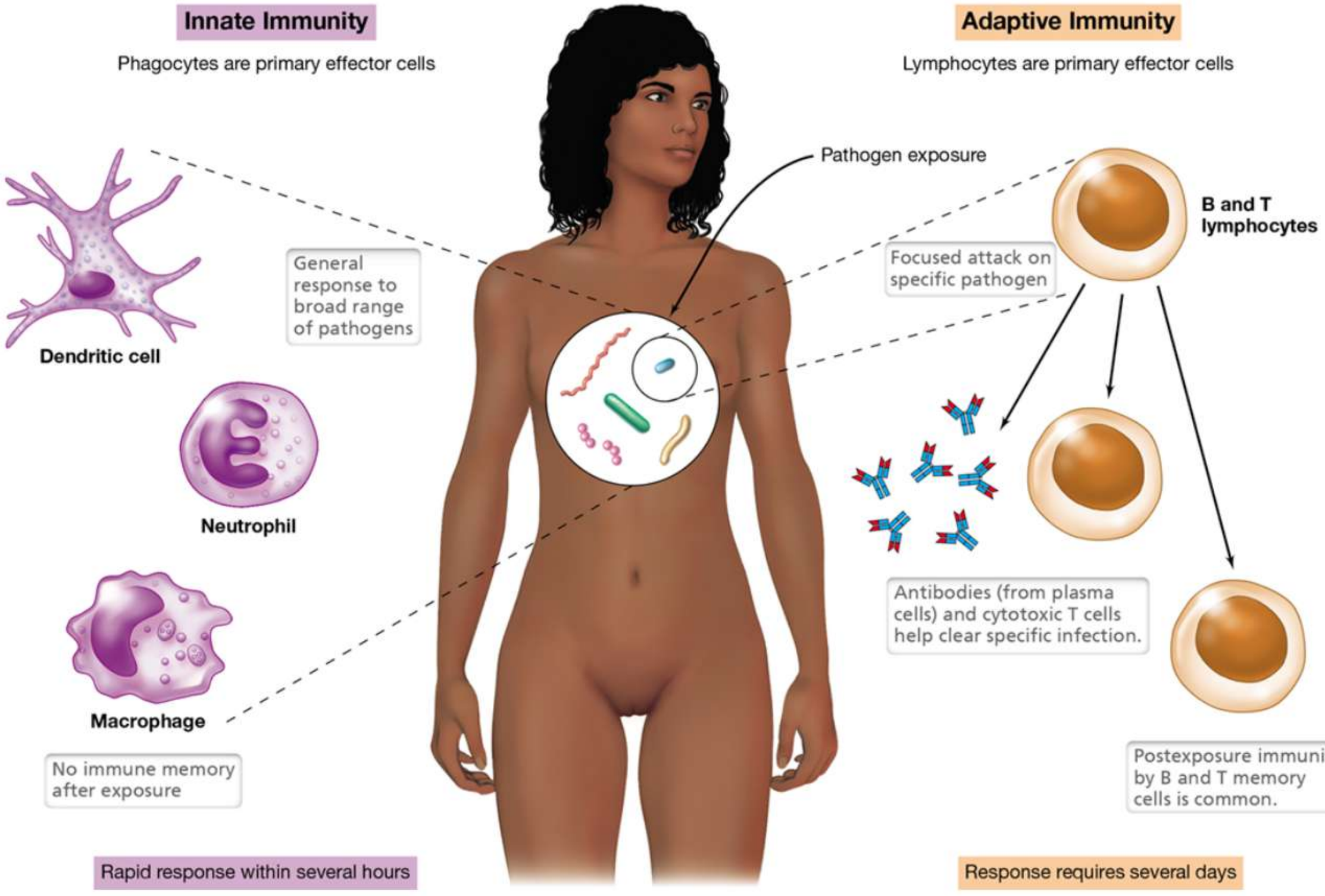
Lymphoid organs coordinate the **maturation** and **migration** of immune cells while **organizing** and **regulating** immune **responses**

Primary lymphoid organs in adults include the **bone marrow** and **thymus**, which serve as niches for **lymphocyte development**

Secondary lymphoid organs — which include 600–800 **lymph nodes** distributed across the body, the **spleen** and the **mucosa-associated lymphoid tissue** — **house and organize T cells, B cells and antigen-presenting cells (APCs)**

These organs serve as command centres of adaptive immunity where activation of naive B and T lymphocytes occurs and are thus natural targets for vaccines and immunotherapies

Innate/Constitutive *vs* Adaptive/Inducible immunity cell populations



Madigan, 2020

Lexicon I

Epitope, portion of a foreign protein, or antigen, that is capable of stimulating an immune response. An epitope is the part of the antigen that binds to a specific antigen receptor

Major histocompatibility complex, MHC

- MHC class I and class II molecules are similar in function: they present peptides at the cell surface to CD8+ and CD4+ T cells
- MHC are ubiquitous present in all nucleated cells MHC class II molecules are primarily expressed by professional APCs, such as DCs, macrophages and B cells CD4+ T cells

Natural killer (NK) cells are effector lymphocytes of the innate immune system that control several types of tumors and microbial infections by limiting their spread and subsequent tissue damage

NK are classified as group I Innate Lymphocytes (ILCs) and respond quickly to a wide variety of pathological challenges. NK cells are best known for killing virally infected cells, and detecting and controlling early signs of cancer

Lexicon II

T cells originate in the bone marrow (like **B cells**) and mature in the thymus. In the thymus, T cells multiply and differentiate into helper, regulatory, or cytotoxic T cells or memory T cells.

T CD4+ cells are necessary as **helpers** to promote B cell antibody production and are often required for the generation of **cytotoxic** and memory CD8+ T cell populations

Antibodies are secreted **immunoglobulin** molecules produced mainly by **plasma cells**. The antigen-binding site of the antibody has a unique structure that allows it to bind antigen in a highly specific manner

Lexicon III

Antibody is produced by rare populations of terminally differentiated B cells — known as plasmablasts (short lived) and plasma cells (long lived):

IgG: Provides long-term immunity and is the most abundant in blood and extracellular fluid.
IgA: Protects mucosal surfaces (e.g., in the respiratory and gastrointestinal tracts).
IgM: The first antibody produced during an initial infection; efficient in forming antigen-antibody complexes.
IgE: Involved in allergic responses and defense against parasitic infections.
IgD: Plays a role in the activation and regulation of B cells.

Lexicon IV: The Complement

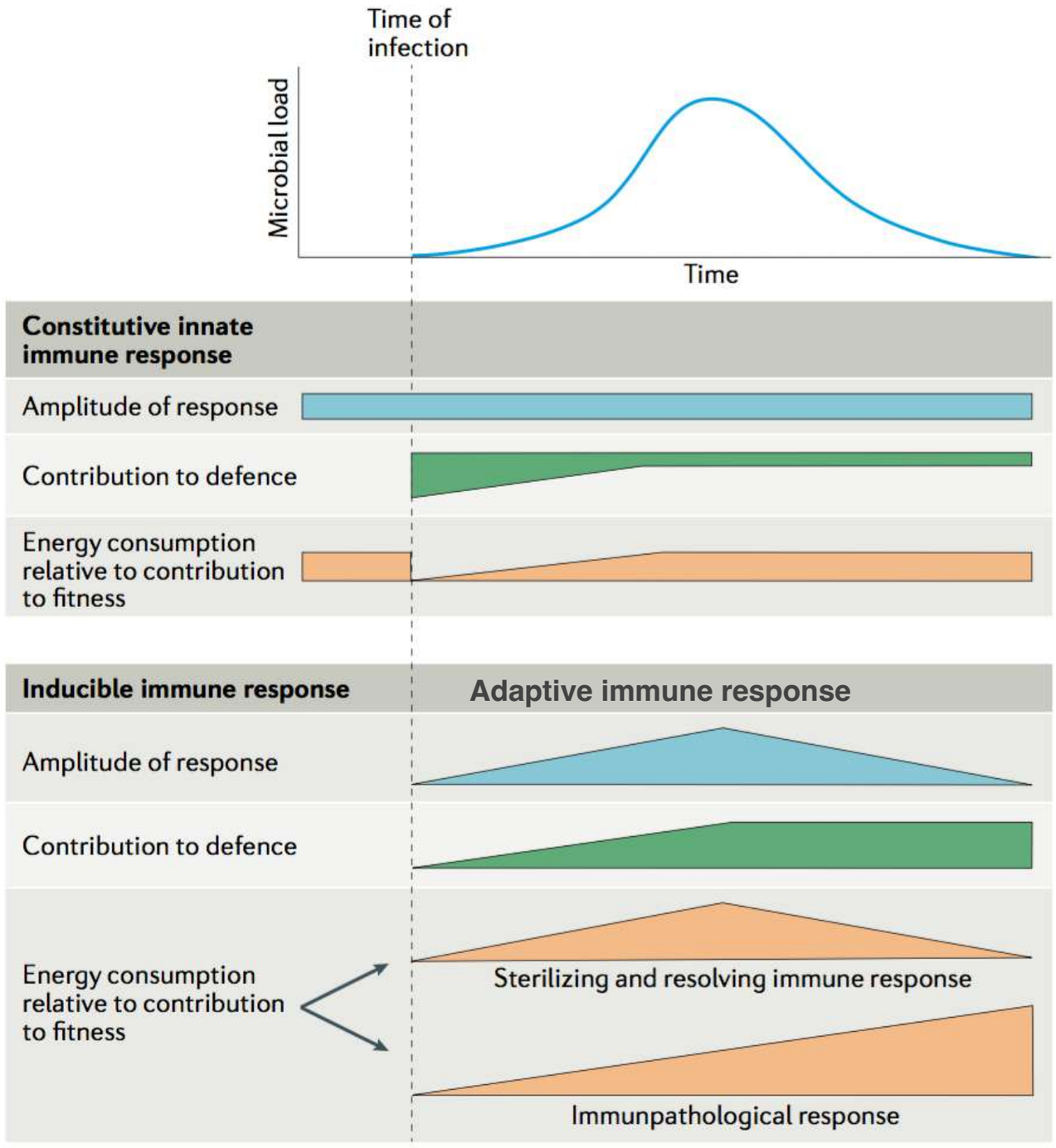
*The complement system was discovered over a century ago by Jules Bordet as a serum-operative key arm of innate immunity that '**complemented' the activity of antibodies during the detection and removal of blood borne pathogens***

Complement is traditionally known as a **serum-effective system**, whereby the **liver expresses and secretes most complement components**, which participate in the **detection** of blood borne pathogens and **drive an inflammatory reaction** to safely remove the microbial or antigenic threat (*e.g.*, opsonisation: bacteria are embellished by proteins that favour phagocytosis or induces direct lytic killing)

The complement system comprises more than **50 soluble or membrane-bound glycoproteins that engage in multi-tiered protein–protein interactions**, resulting in the **assembly and activation of enzymatic complexes** and the generation of bioactive fragments that initiate **diverse cellular responses** through binding to complement receptors and regulators

Complement function is compartmentalized and operates systemically, locally in the extracellular space, and intracellularly within sub-cellular compartments and organelles

Innate/Constitutive immune responses *versus* Inducible/Adaptive immune responses



- **Amplitude of response**
- **Contribution to defense**
- **Energy consumption**

a. Sterilizing and resolving immune response, the additional energy consumption required by the inducible immune response is balanced by the re-establishment of **homeostasis**

b. **Immunopathological** response, the energy that is consumed to mount an inducible response does not benefit the host and instead leads to tissue damage and disruption of homeostasis

Paludan et al., 2021

Innate/Constitutive immune system

An innate immune system must be specific and must:

1. **Recognize** pathogens, potentially through dedicated receptors
2. **Integrate** that information via signaling pathways
3. **Launch** a response that targets the pathogens
4. **Deal** with pathogens of various natures that can infect the host *via* **different** routes

This ability requires complex **crosstalk between local and systemic** immune responses

Rare *versus* recurrent infections across an organism's life stages require different types of reactions, engaging immune responses that can be constitutive or inducible, and can have long-term memory-like effects

An immune system must **avoid pathological autoimmunity** and must regulate and keep a balanced microbiota

Trained immunity is a functional state of the innate immune system that is characterized by long-term epigenetic and metabolic reprogramming of cells associated with potent immune responses

SELF or NON-SELF

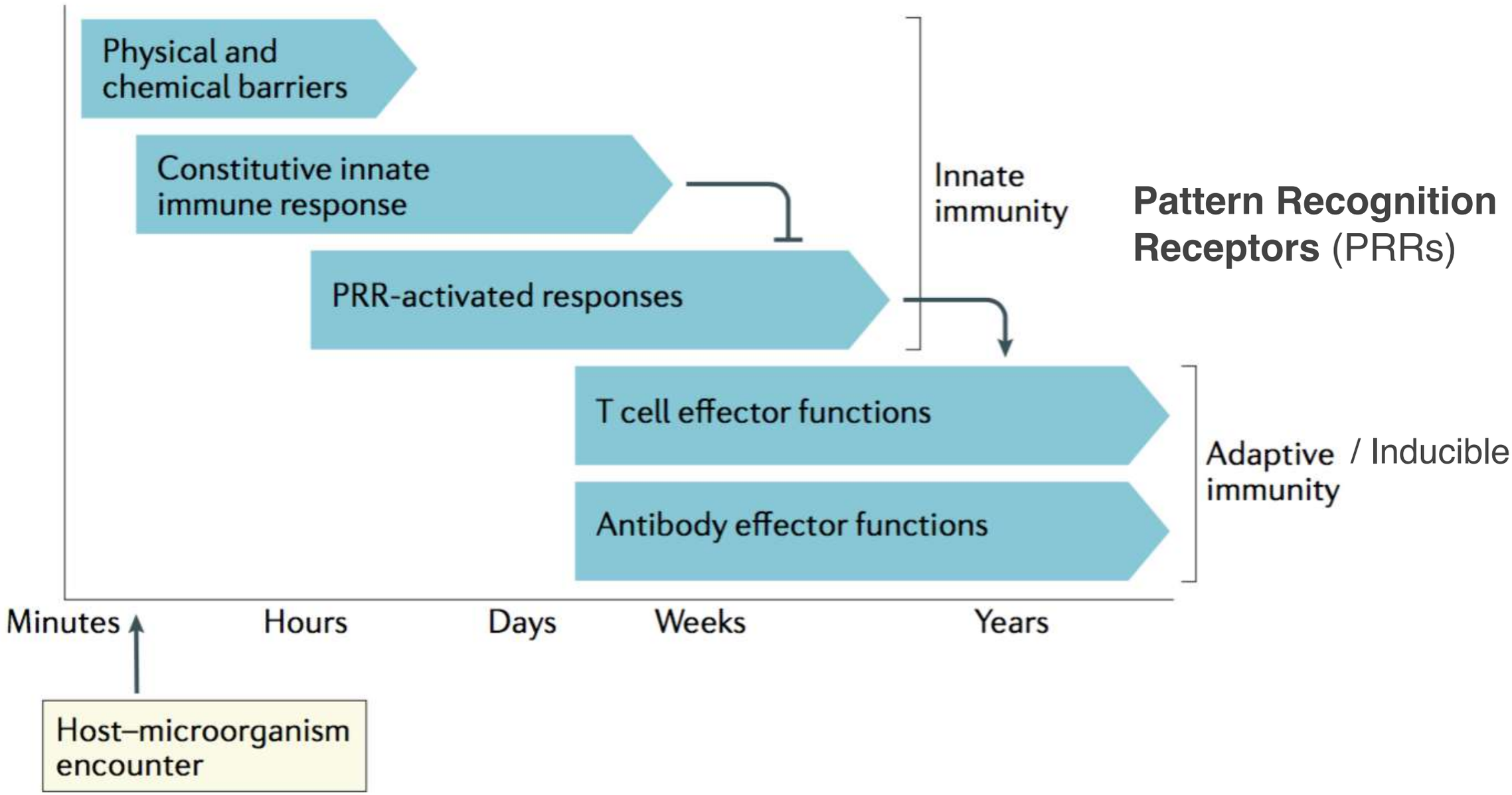
The innate immune system has the capacity to detect '**non-self**' molecules derived from pathogens, known as **pathogen/microbe-associated molecular patterns, via pattern recognition receptors**

The self–non-self theory was first formulated by Frank Macfarlane Burnet in **1959** and was refined in 1989, when Charles Janeway proposed the '**pattern recognition**' theory

It postulated that innate immune cells express distinct germ-line-encoded **pattern recognition receptors (PRRs) that recognize conserved pathogen-associated molecular patterns (PAMPs)/ microbe-associated molecular patterns (MAMPs, bacterial lipopolysaccharide, flagellin, EF-Tu, DNA, lipoproteins, peptidoglycans, and fungal chitin) unique to microbes**

The recognition of **DAMPs,** which are produced or released by **damaged and dying cells, promotes sterile inflammation**, which is important for **tissue repair and regeneration**, but can also lead to the development of numerous inflammatory diseases, such as metabolic disorders, neurodegenerative diseases, autoimmune diseases and cancer

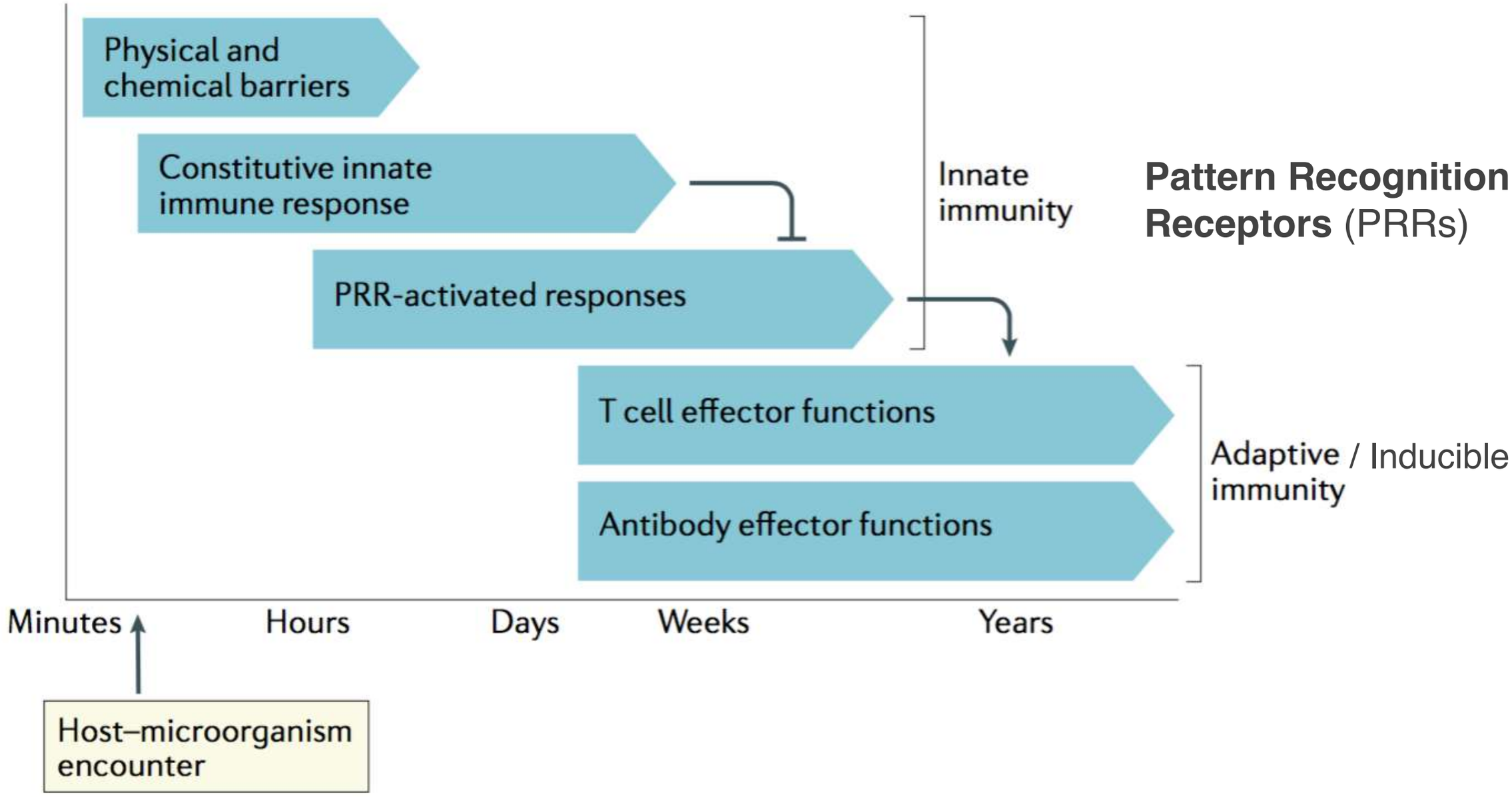
Time relationship among the different layers of the immune response, I



Paludan et al., 2021

- A first layer of defence is exerted by **physical and chemical barriers**
- Constitutive **innate immune mechanisms** function as soon as a danger **signal is detected** and eliminate harmful microorganisms and host molecules **by specific non-inflammatory mechanisms** that **operate independently of PRRs**

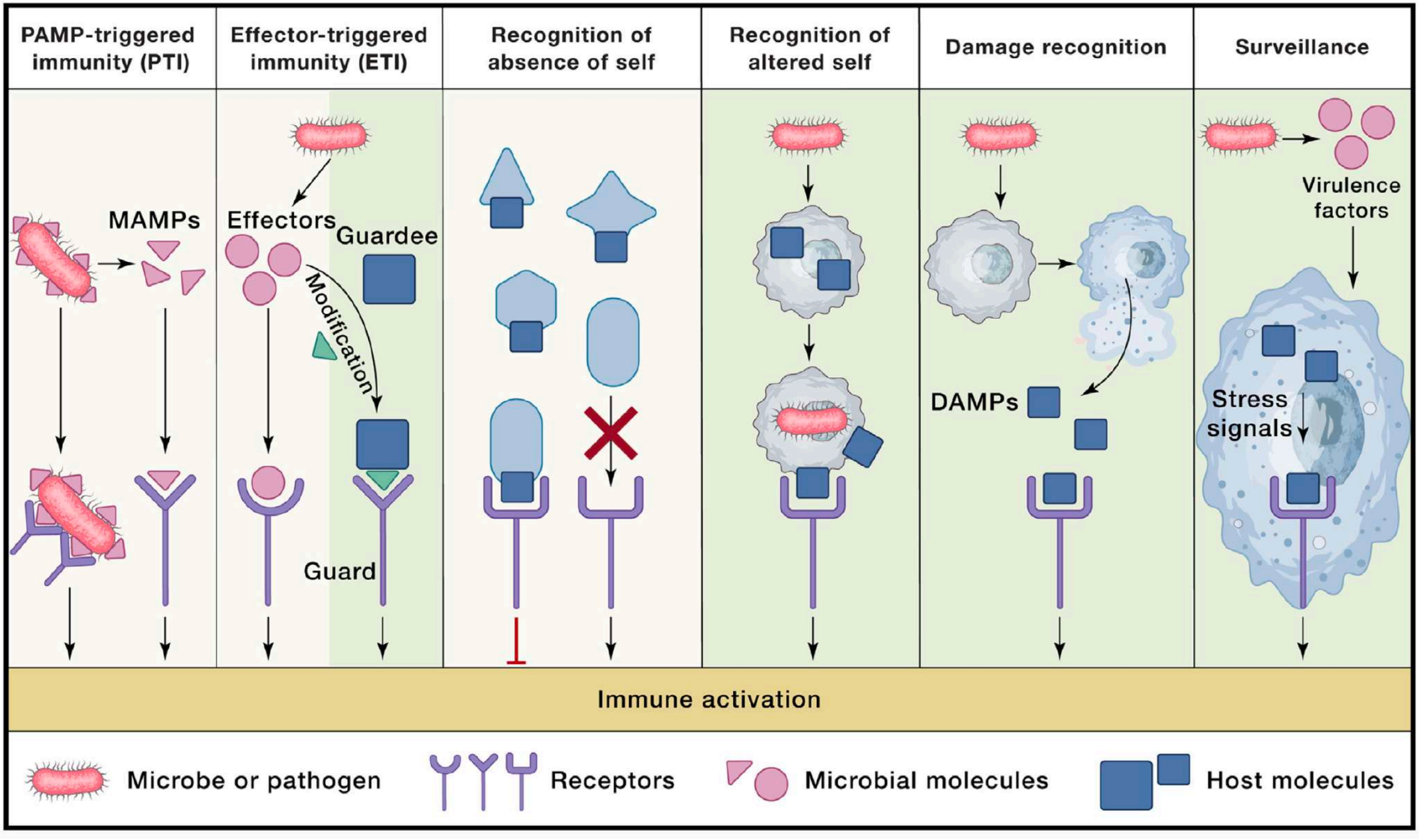
Time relationship among the different layers of the immune response, II



Paludan et al., 2021

- **Constitutive innate immune** response inhibit establishment of the infection and accumulation of **PAMP**s (**Pathogen-Associated-Molecular Pattern**) and **DAMP**s (**Damage-Associated-Molecular Pattern**), thus **limiting the activation of PRR-based** inducible innate immune responses
- **If PRR-based immunity is activated**, owing to the level of PAMPs exceeding a certain threshold, this leads to inflammation and **promotes activation of the adaptive/inducible immune response** mediated by T cells and antibodies

Six overlapping mechanisms of innate sensing



Pradeu et al., 2024

Pattern Recognition Receptors

Pattern-recognition receptors (PRRs) are evolutionarily conserved structurally different receptors, that detect pathogen/microbe-associated molecular patterns (PAMPs/ MAMPs)

Toll-like receptors (TLRs): Ten TLRs have been identified in humans. TLRs are type I **transmembrane glycoproteins that localize to either the plasma membrane** (in the case of TLR1–TLR6, TLR10 and TLR11) or the **endosomal membrane** (in the case of TLR3, TLR7 and TLR9, for example). Ligands for TLRs include bacterial **lipoproteins and lipopeptides** (for TLR2), **double-stranded RNA** (for TLR3), **lipopolysaccharide** (for TLR4), **flagellin** (for TLR5), **single-stranded RNA** (for TLR7), CpG **DNA** (for TLR9)

NOD-like receptors (NLRs) NLRs constitute a large family of cytosolic proteins: The first family members to be discovered — nucleotide-binding oligomerization domain protein 1 (NOD1) and NOD2 — recognize **bacterial peptidoglycan** fragments and activate nuclear factor-κB (NF-κB) signalling

RIG-I-like receptors (RLRs): There are three known RLRs: retinoic acid-inducible gene I (RIG-I), melanoma differentiation associated gene 5 (MDA5) and LGP2. RLRs are expressed in the **cytosol and sense nucleic acids, such as viral RNA**

C-type lectin receptors (CLRs): The CLRs are a large family of proteins that possess one or more C-type lectin domains and one or more immunoreceptor tyrosine-based activation motifs (ITAMs). They recognize a wide range of **carbohydrate ligands** (and probably also non-carbohydrate ligands)

Common principles of innate immune effector mechanisms

Immune effectors involved in innate immunity rely on a limited number of mechanisms that revolve around a few principles:

(A) Destruction of the radically different (*e.g.*, anti-microbial peptides recognizing negatively charged membrane of bacteria)

(B) Anti-virulence

(C) PRR-assisted elimination (*e.g.*, complement activation guided by C3b binding to pathogen or phagocytosis of opsonized microbe)

(D) Suicide of the infected cells

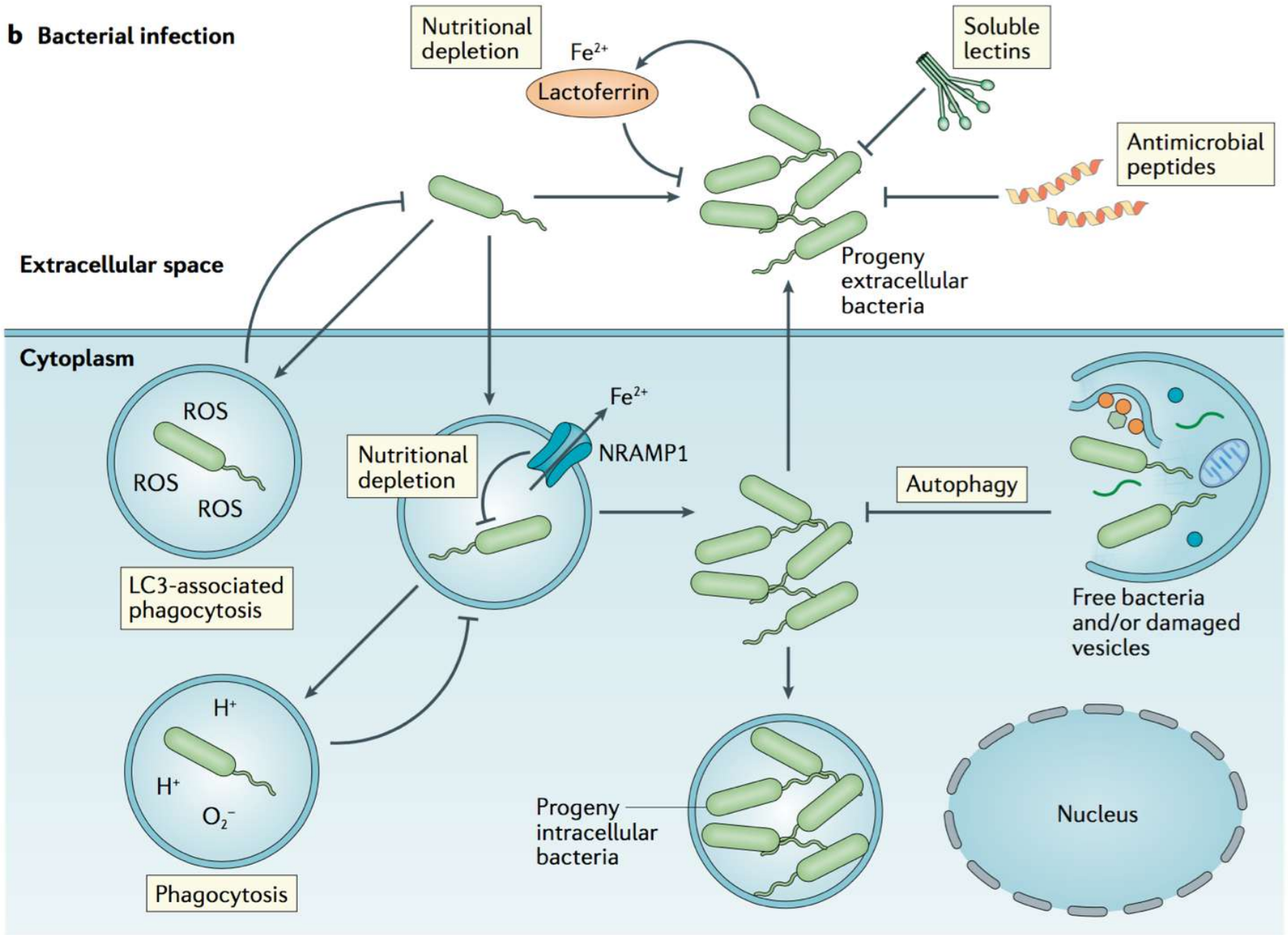
(E) Nutritional immunity

First encounter

Pathogen recognition by extracellular or endosomal receptors

Constitutive innate immune response

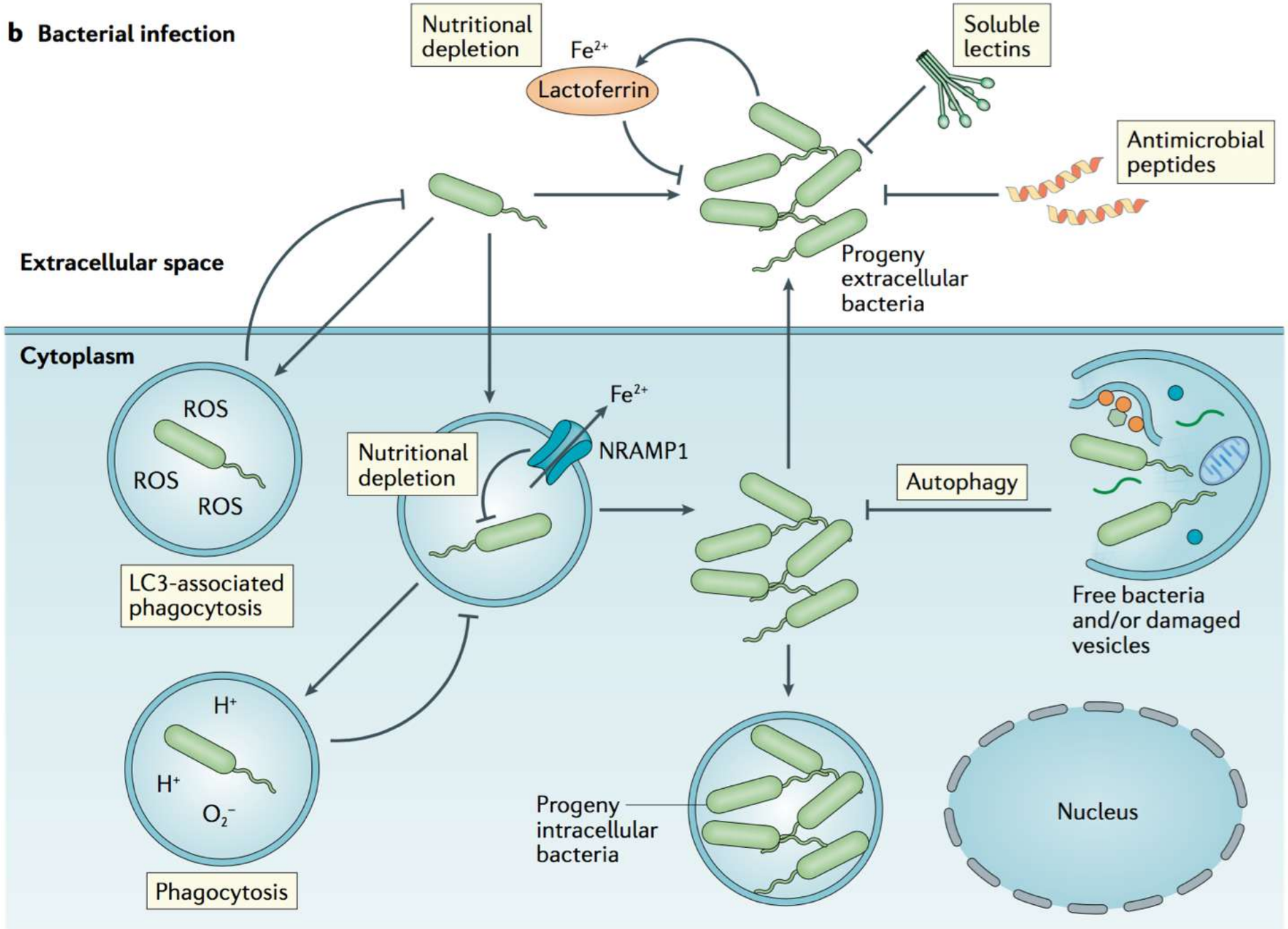
- **Host cell uses bacterial compartmentalization**, **oxidative and nutrient stress**, **antimicrobial peptides**, lysosome-mediate degradation, autophagy, inflammasome activation and pyroptosis to kill the pathogens
- Some intracellular pathogens can control the signalling pathways activated by host receptors, **interact with endocytic pathway, escape** from the phagosome, **inhibit fusion with lysosomes, manipulate vesicular trafficking and avoid autophagosome degradation and inflammasome activation**



Paludan et al., 2021

Targeting microbial replication

Direct inhibition of microbial replication is executed by molecules that interfere with specific steps in the replication cycle of a given microorganism. There are at least six mechanisms of action in this category: restriction factors that directly block a **specific replication step**; **restriction factors that deplete molecules essential for replication; RNA interference (RNAi); antimicrobial peptides; soluble lectins; and metabolite-mediated inhibition of microbial replication**



Paludan et al., 2021

Degenerative mechanisms

The second class of constitutive innate immune mechanisms functions through the **degradation of danger molecules and elimination of unwanted cells**. This class of mechanisms includes **autophagy, phagocytosis, proteasomal degradation and nucleases**. Collectively, degenerative programmes function to continually limit danger signals, allowing for the rapid elimination of unwanted molecules without the activation of energy-consuming amplificative induced immune responses

Transition from Innate to Adaptive Immune response

Innate immune responses help initiate and shape adaptive immune responses mediated by T and B cells

In a simplified three-signal model:

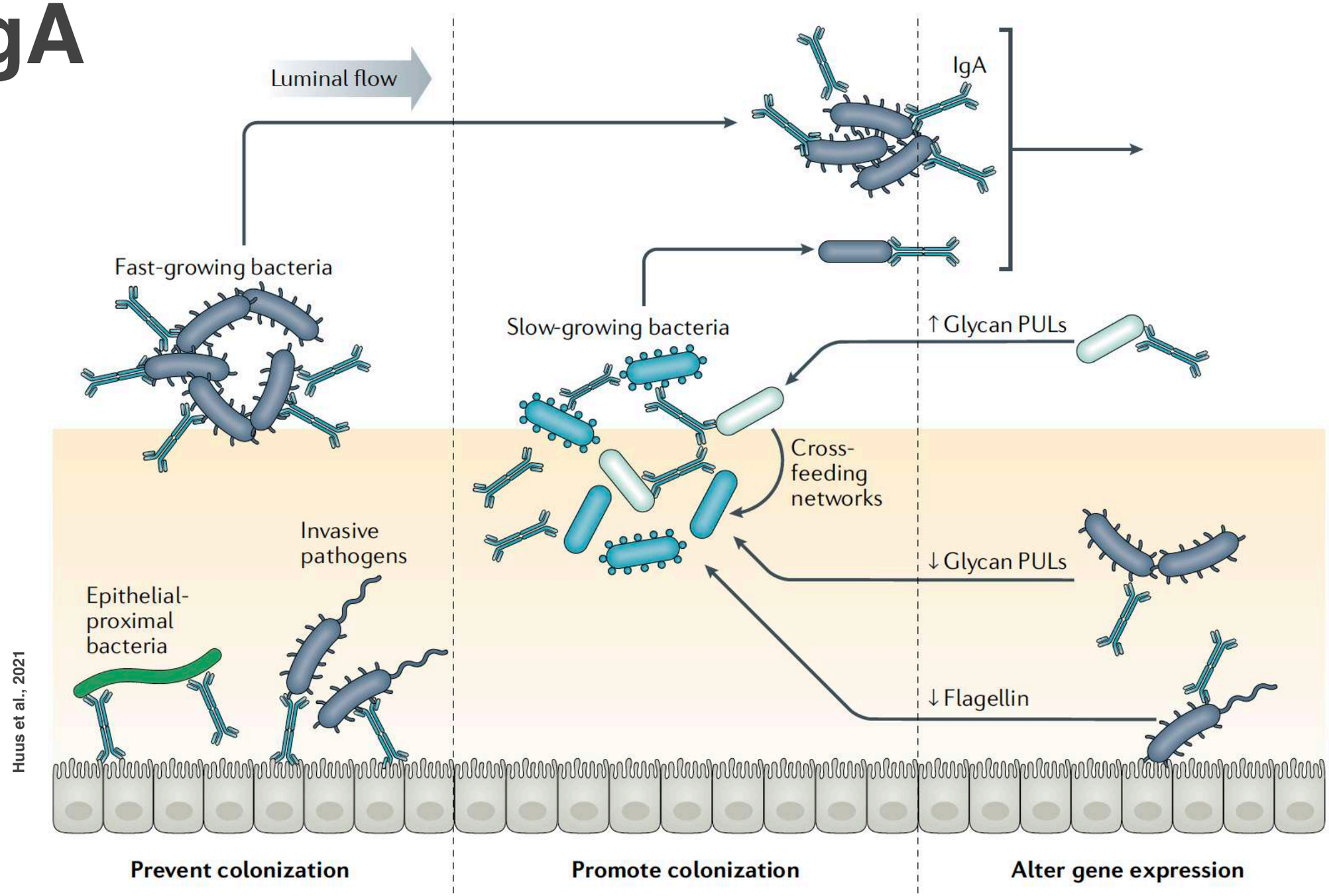
A. The first signal to activate T cells is provided by **T cell antigen receptor recognition of antigen**

B. The second signal is **costimulation** provided by the **antigen presenting cell (APC)**

C. The third signal is provided by **inflammatory cytokines** derived from innate immune activation, which may act directly on the T cell and/or indirectly by increasing costimulatory molecules on the APC

D. **B cells are activated by antigen** *via T cell-dependent or -independent mechanisms*

IgA



- IgA mediates microbial homeostasis at the intestinal mucosa
- IgA acts in a context- dependent manner to shape the colonization and function of the intestinal microbiota
- PULs, polysaccharide utilization loci

Microbes and Humans

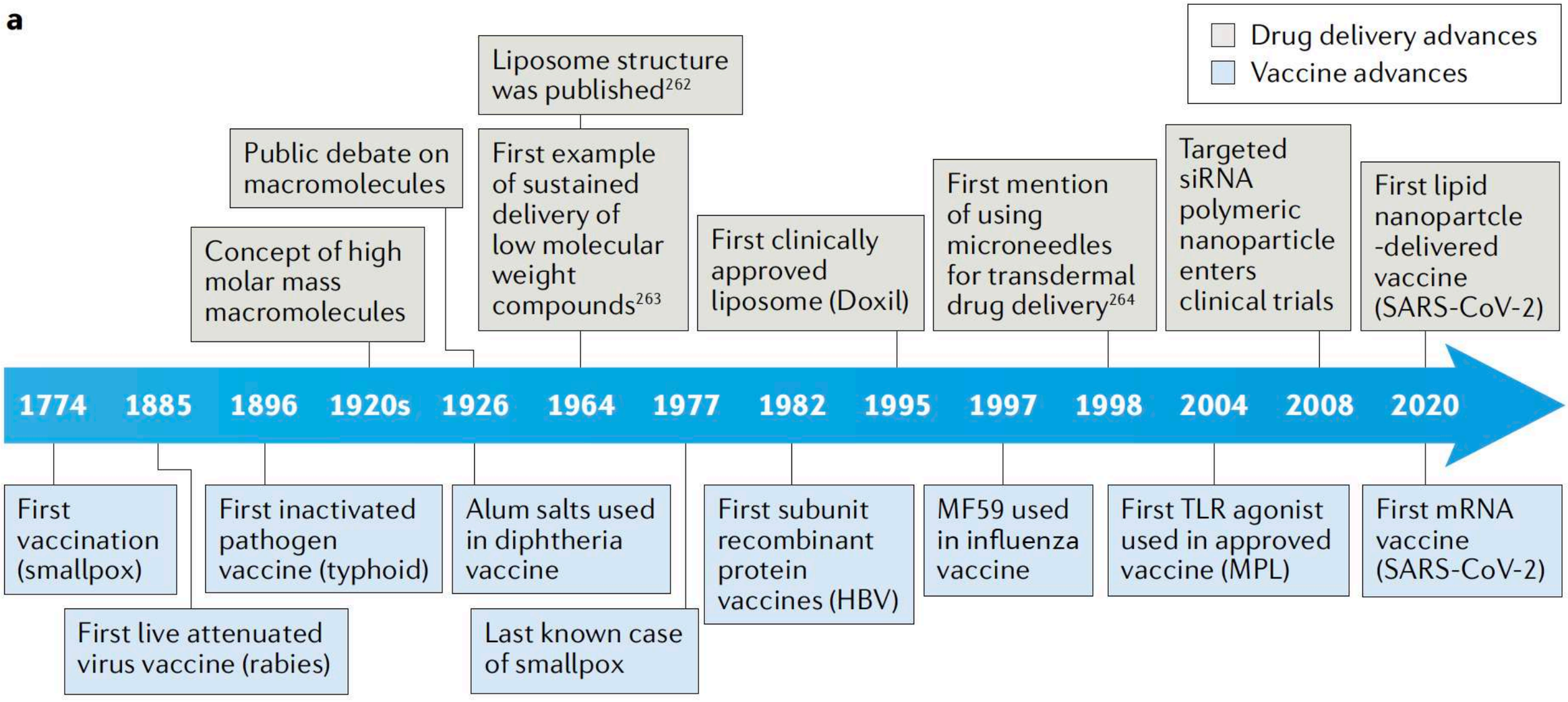
- Providing nutrients
- Fighting off microbial pathogens
- Maintaining the Human ecosystem functioning = healthy homeostasis (—> interaction with immune system)
- Training immune system to recognise the commensals from the pathogens (failure—> sepsis and microbial invasion/disease)
- Training immune system to recognise self from non self (failure—> autoimmune and allergic diseases)

Vaccine

A vaccine is a biological product that can be used to safely induce an immune response that confers protection against infection and/or disease on subsequent exposure to a pathogen

To achieve this, the vaccine must contain antigens that are either derived from the pathogen or produced synthetically to represent components of the pathogen

Vaccine timeline



Roth et al., 2022

Vaccine/Pathogen

Tissues at the interface with the outside world (for example, skin, lungs and mucosal sites) are the primary locations of infections, and therefore contain tissue resident immune cells and are constantly patrolled by migratory immune cells.

Lymph nodes downstream of the location of pathogen or vaccine exposure are called draining lymph nodes, and are key sites from the beginning of the immune response throughout the development of mature effector B cells and T cells.

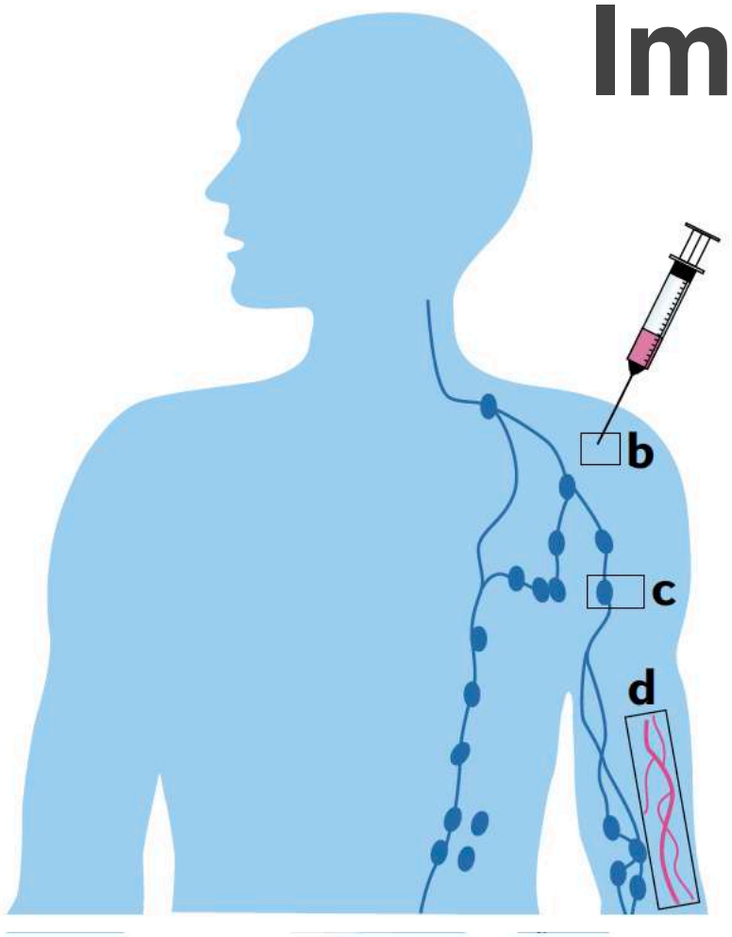
The blood provides an important route for innate immune cells to quickly infiltrate the site of vaccination or infection in the early immune response.

After the immune response is mounted, the blood enables antibodies and memory T cells to reach infected tissue and protect the entire body.

Activation of the innate immune system and migration of key cells and vaccine components to lymph nodes occurs within hours, followed by B cell and T cell maturation within days and weeks.

The long- term memory response remains for months to years following vaccination, providing protection against future infection.

Immune response to vaccine



- The vaccine immune response occurs in multiple locations
  — peripheral tissues, lymph nodes and systemic circulation
  — each of which has its own cell composition and function.

- This coordinated action of immune cells requires precise spatial and temporal cues.

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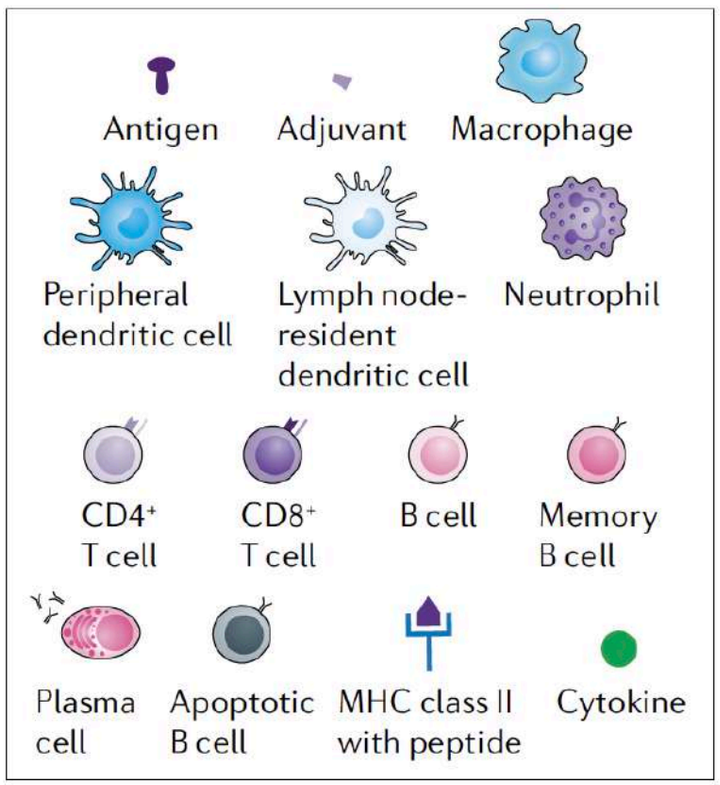
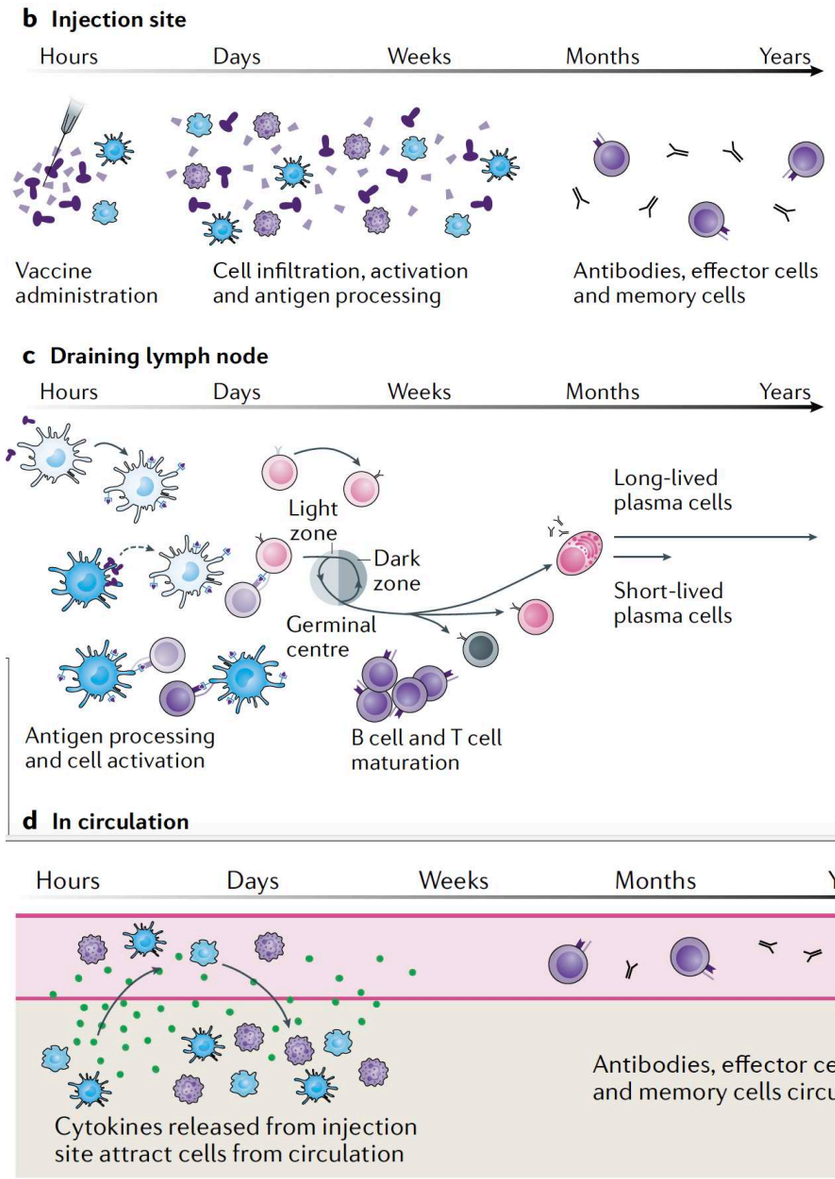
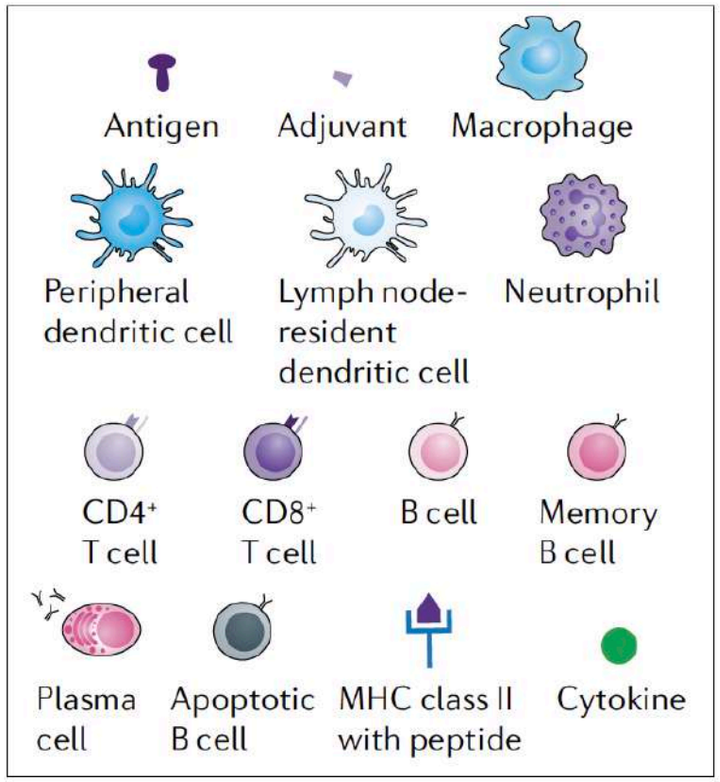
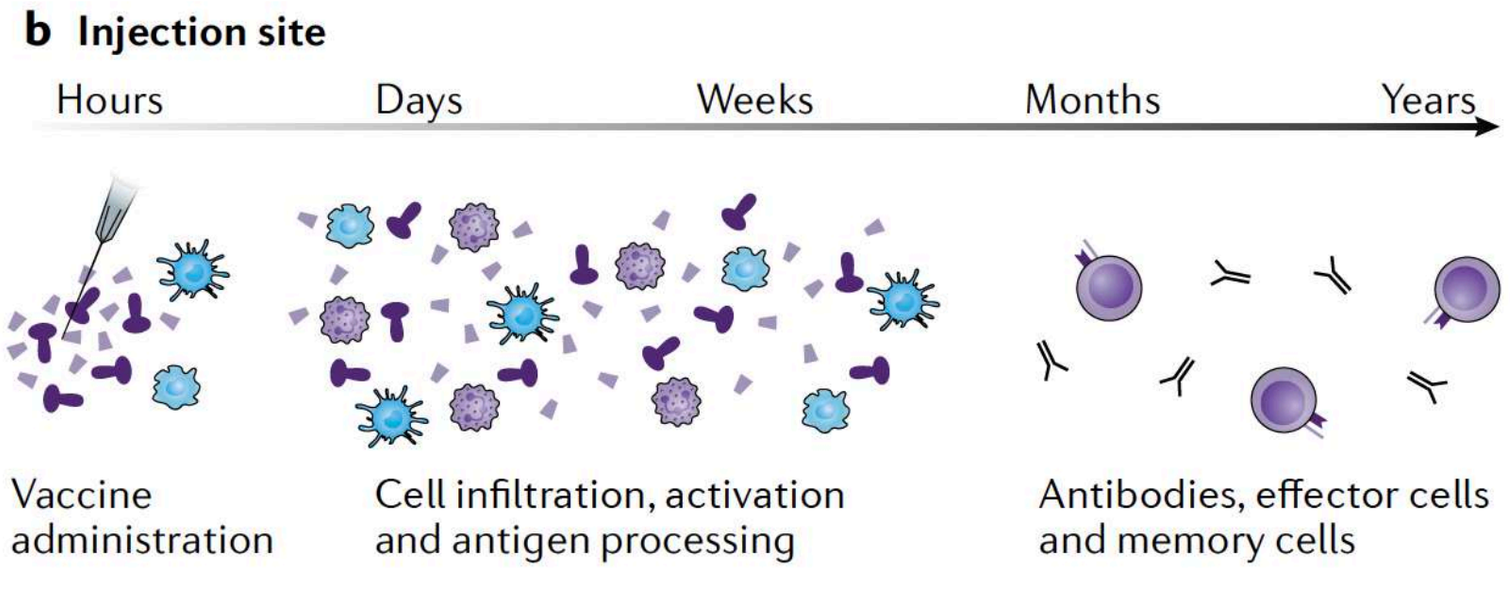


Roth et al., 2022



Immune response to vaccine

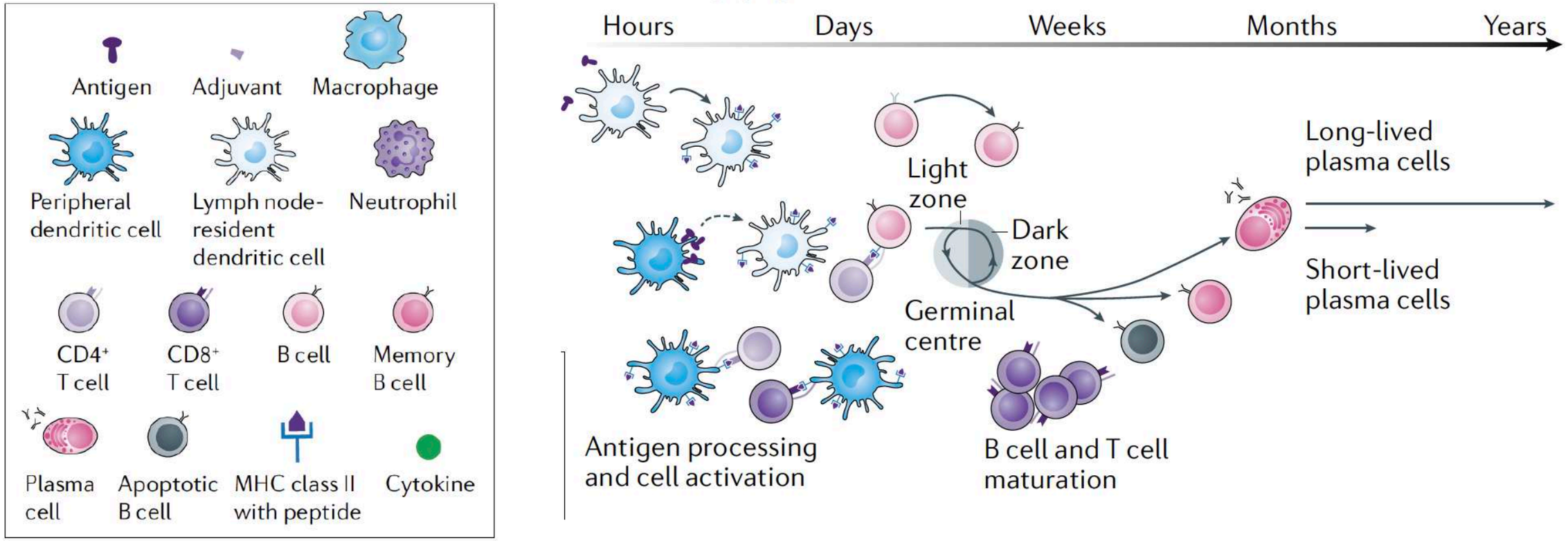




Roth et al., 2022

- At the site of administration, innate immune cells, such as neutrophils and antigen- presenting cells (APCs), first encounter the antigen and adjuvant
- The antigen component of the vaccine is endocytosed and broken down by APCs before being presented on the APC surface major histocompatibility complex (MHC) molecules.
- As innate immune cells become activated, they release cytokines that attract other immune cells from the bloodstream to the site of administration.
- Soluble vaccine components and activated cells enter the lymphatics and travel to local lymph nodes.

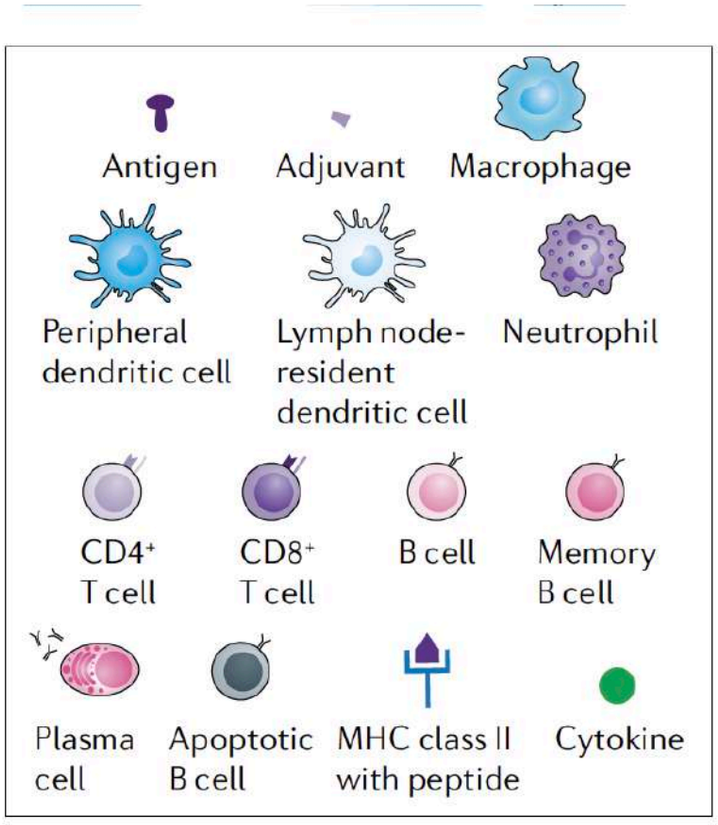
Immune response to vaccine

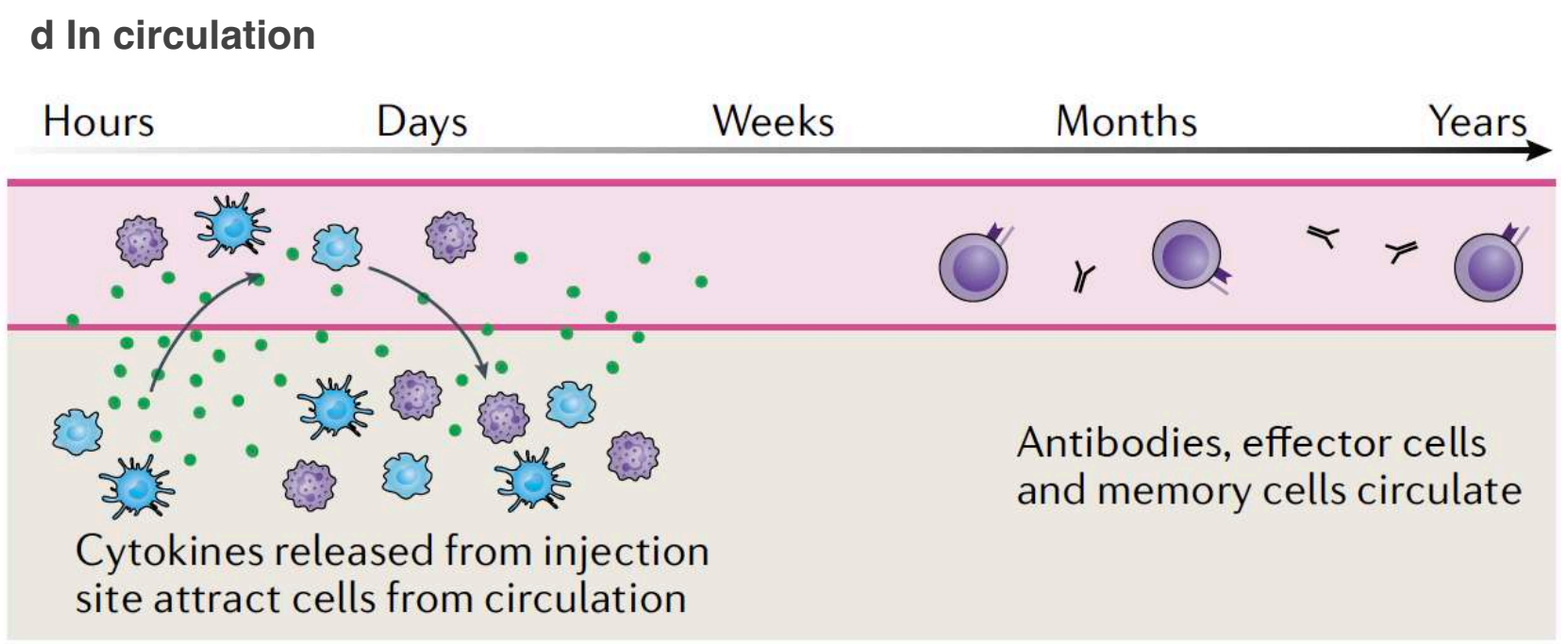


Roth et al., 2022

- Maturation and development of a potent adaptive response continues in lymph nodes downstream of the vaccination site (draining lymph nodes).
- Early in the vaccine response, lymph node- resident phagocytic cells and migratory innate cells arriving from peripheral tissues present antigen and produce inflammatory signals to activate T cells.
- As the immune response develops, sites of B cell development, called germinal centres, form in the B cell zones of the lymph nodes.

Immune response to vaccine

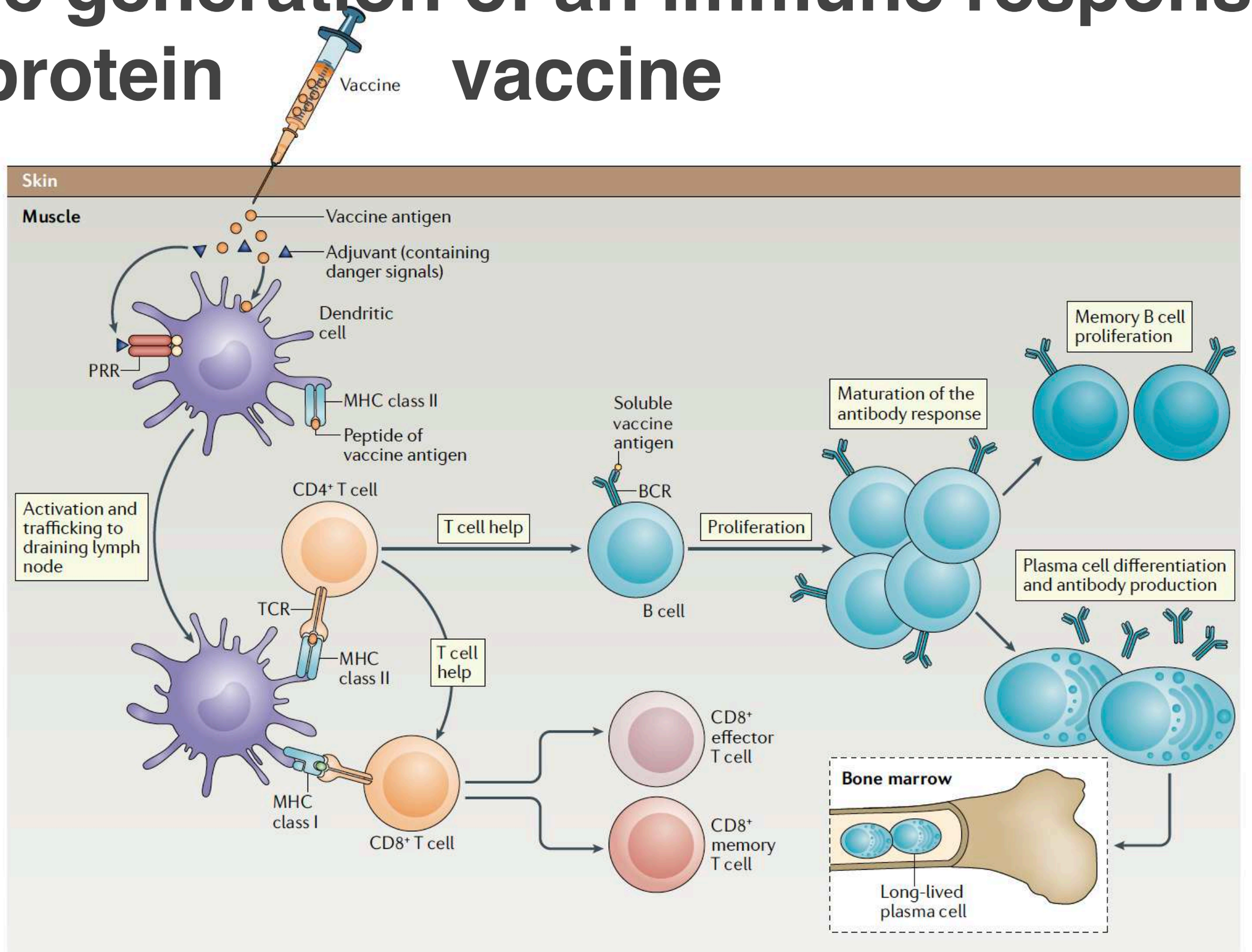




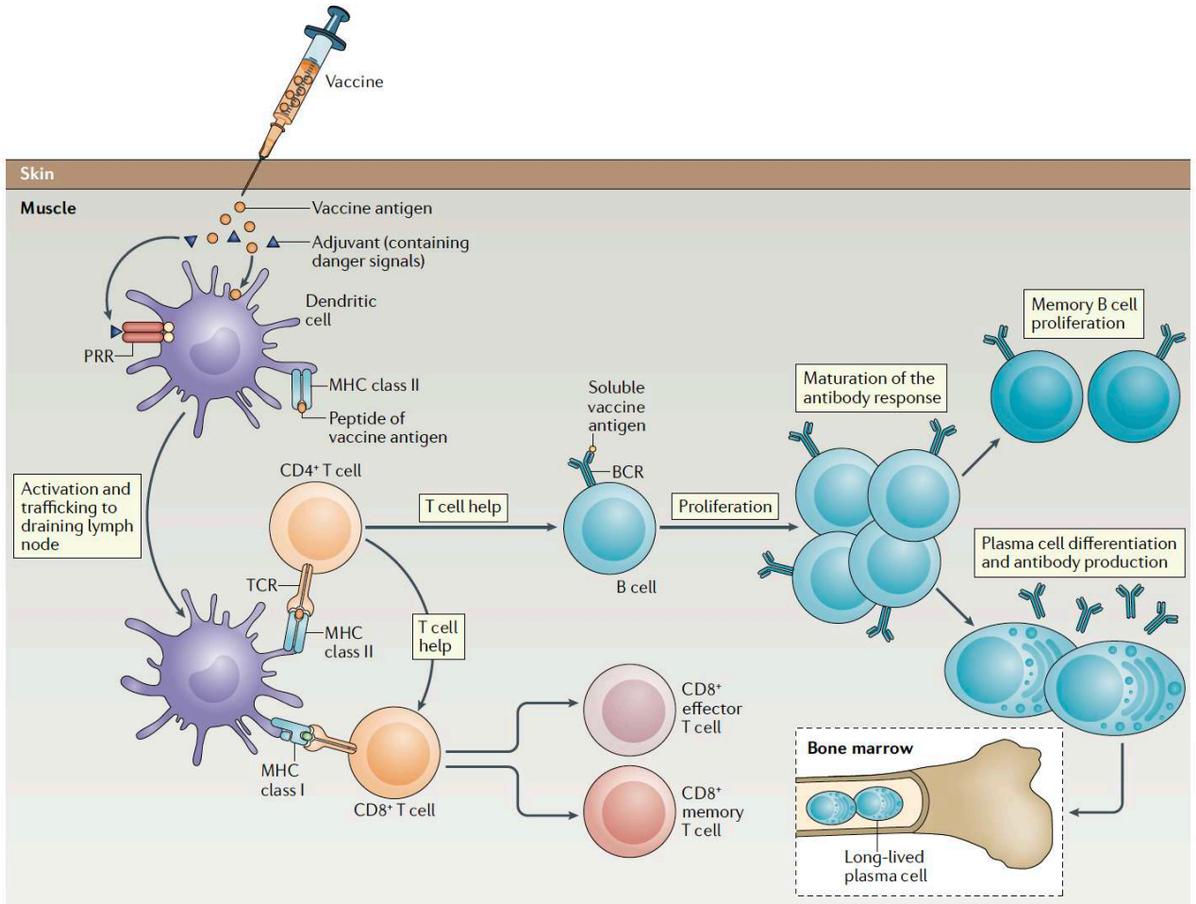
Roth et al., 2022

- Immediately following vaccine administration, local innate cells release cytokines into the circulation to enable a coordinated response thus triggering cell infiltration to the injection site.
- Following vaccination, plasma cells secrete antigen- specific antibodies, which travel through the circulatory system to tissues, where they respond immediately upon pathogen exposure.
- Memory T cells also use the circulatory system to inspect the body for foreign invaders.

The generation of an immune response to a protein vaccine



Pollard & Bijker, 2021



Pollard & Bijker, 2021

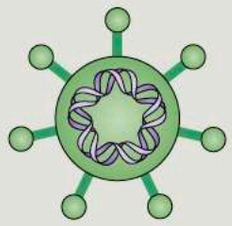
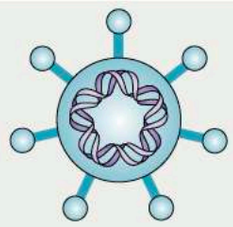
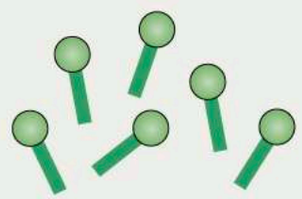
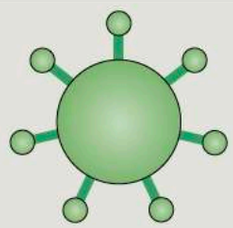
The vaccine is injected into muscle and the protein antigen is taken up by dendritic cells, which are activated through pattern recognition receptors (PRRs) by danger signals in the adjuvant, and then trafficked to the draining lymph node

Here, the presentation of peptides of the vaccine protein antigen by MHC molecules on the dendritic cell activates T cells through their T cell receptor (TCR)

In combination with signalling (by soluble antigen) through the B cell receptor (BCR), the T cells drive B cell development in the lymph node. Here, the T cell-dependent B cell development results in maturation of the antibody response to increase antibody affinity and induce different antibody isotypes

The production of short-lived plasma cells, which actively secrete antibodies specific for the vaccine protein, produces a rapid rise in serum antibody levels over the next 2 weeks

Memory B cells are also produced, which mediate immune memory. Long-lived plasma cells that can continue to produce antibodies for decades travel to reside in bone marrow niches. CD8+ memory T cells can proliferate rapidly when they encounter a pathogen, and CD8+ effector T cells are important for the elimination of infected cells.

Type of vaccine		Licensed vaccines using this technology	First introduced
Live attenuated (weakened or inactivated)		Measles, mumps, rubella, yellow fever, influenza, oral polio, typhoid, Japanese encephalitis, rotavirus, BCG, varicella zoster	1798 (smallpox)
Killed whole organism		Whole-cell pertussis, polio, influenza, Japanese encephalitis, hepatitis A, rabies	1896 (typhoid)
Toxoid		Diphtheria, tetanus	1923 (diphtheria)
Subunit (purified protein, recombinant protein, polysaccharide, peptide)		Pertussis, influenza, hepatitis B, meningococcal, pneumococcal, typhoid, hepatitis A	1970 (anthrax)
Virus-like particle		Human papillomavirus	1986 (hepatitis B)

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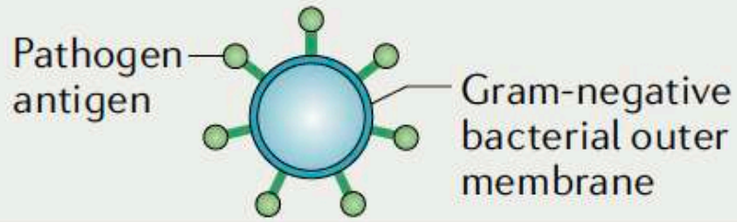
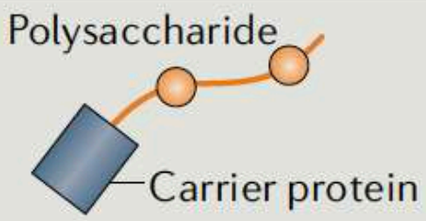
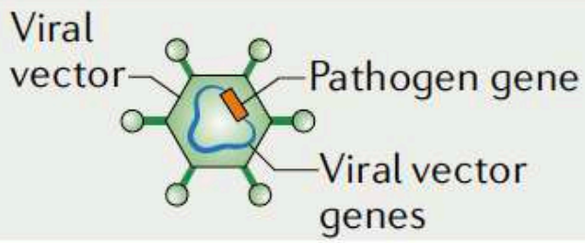
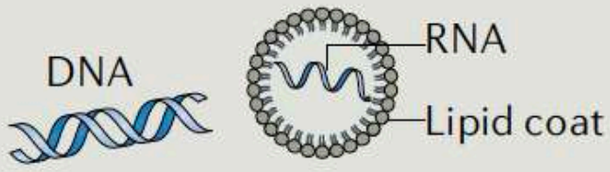
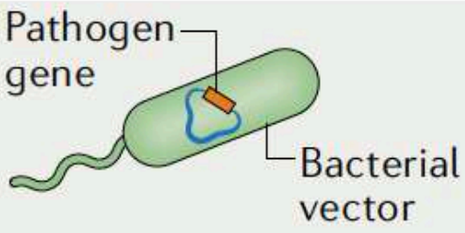
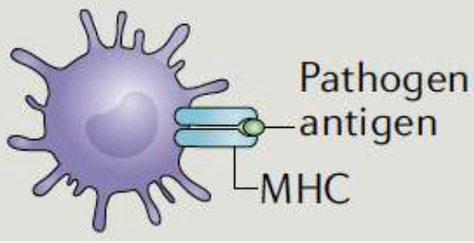
BCG, Mycobacterium bovis bacillus Calmette–Guérin.

Outer membrane vesicle		Group B meningococcal	1987 (group B meningococcal)
Protein–polysaccharide conjugate		*Haemophilus influenzae* type B, pneumococcal, meningococcal, typhoid	1987 (*H. influenzae* type b)
Viral vectored		Ebola	2019 (Ebola)
Nucleic acid vaccine		SARS-CoV-2	2020 (SARS-CoV-2)
Bacterial vectored		Experimental	–
Antigen-presenting cell		Experimental	–

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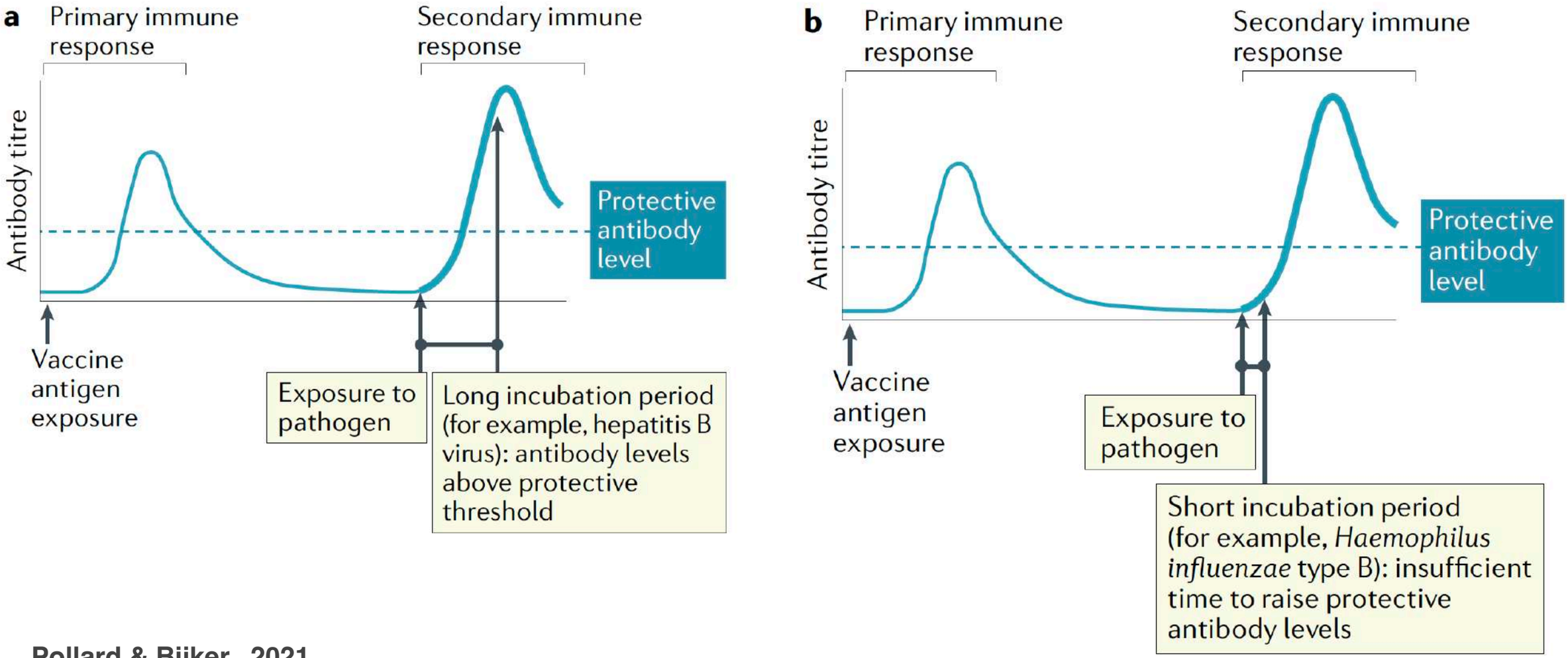
Immune memory is an important feature of vaccine-induced protection, I



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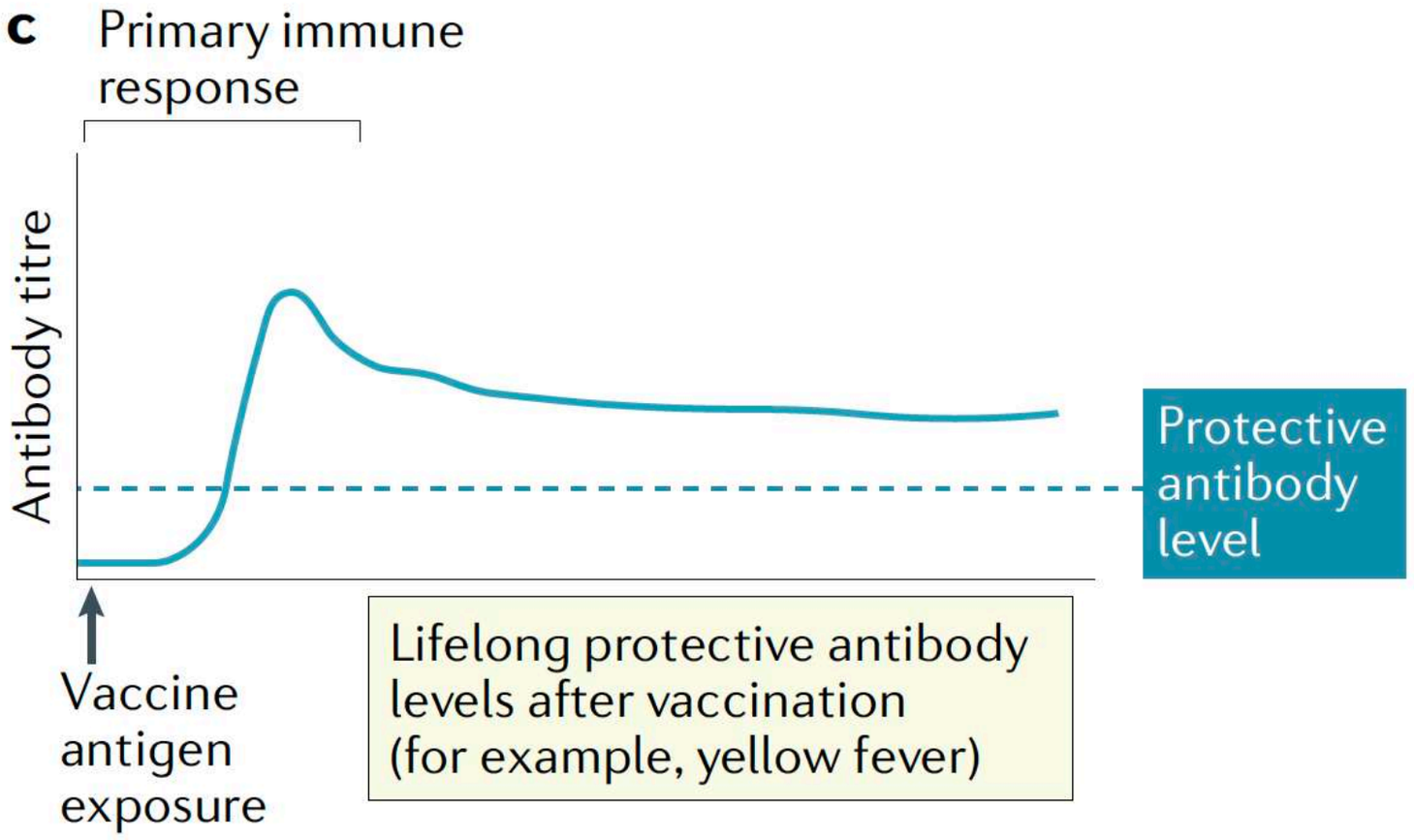
Antibody levels in the circulation wane after primary vaccination, often to a level below that required for protection

Whether immune memory can protect against a future pathogen encounter depends on the incubation time of the infection, the quality of the memory response and the level of antibodies induced by memory B cells

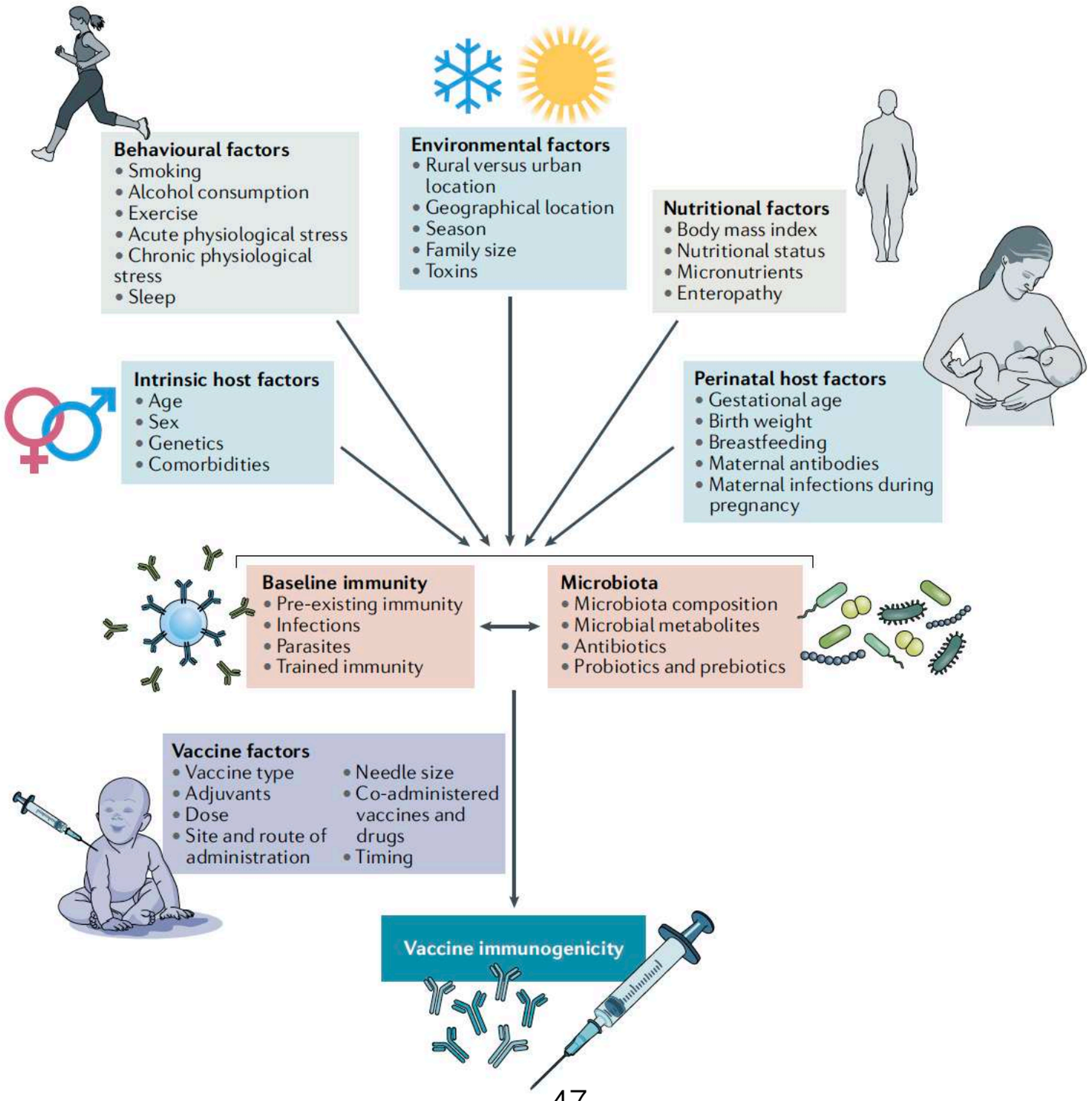
Immune memory is an important feature of vaccine-induced protection, II



Pollard & Bijker, 2021

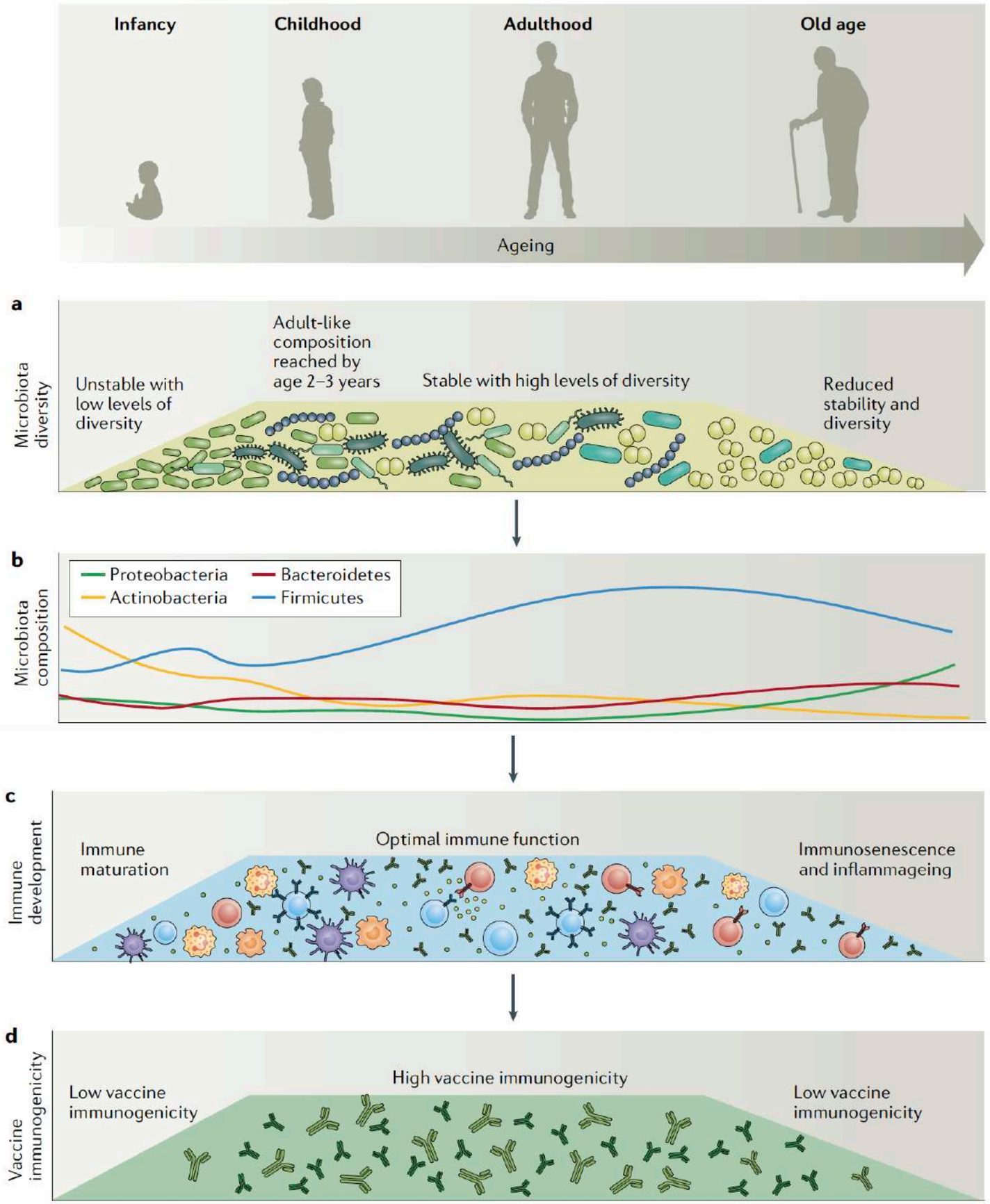
Life long immunity

Factors with the potential to influence vaccine immunogenicity and/or efficacy



Lynn et al., 2022

Differences in the gut microbiota of infants and the elderly compared with that of young adults correlate with altered immune status and suboptimal vaccine immunogenicity



Lynn et al., 2022