Basic Immunology for Vaccine

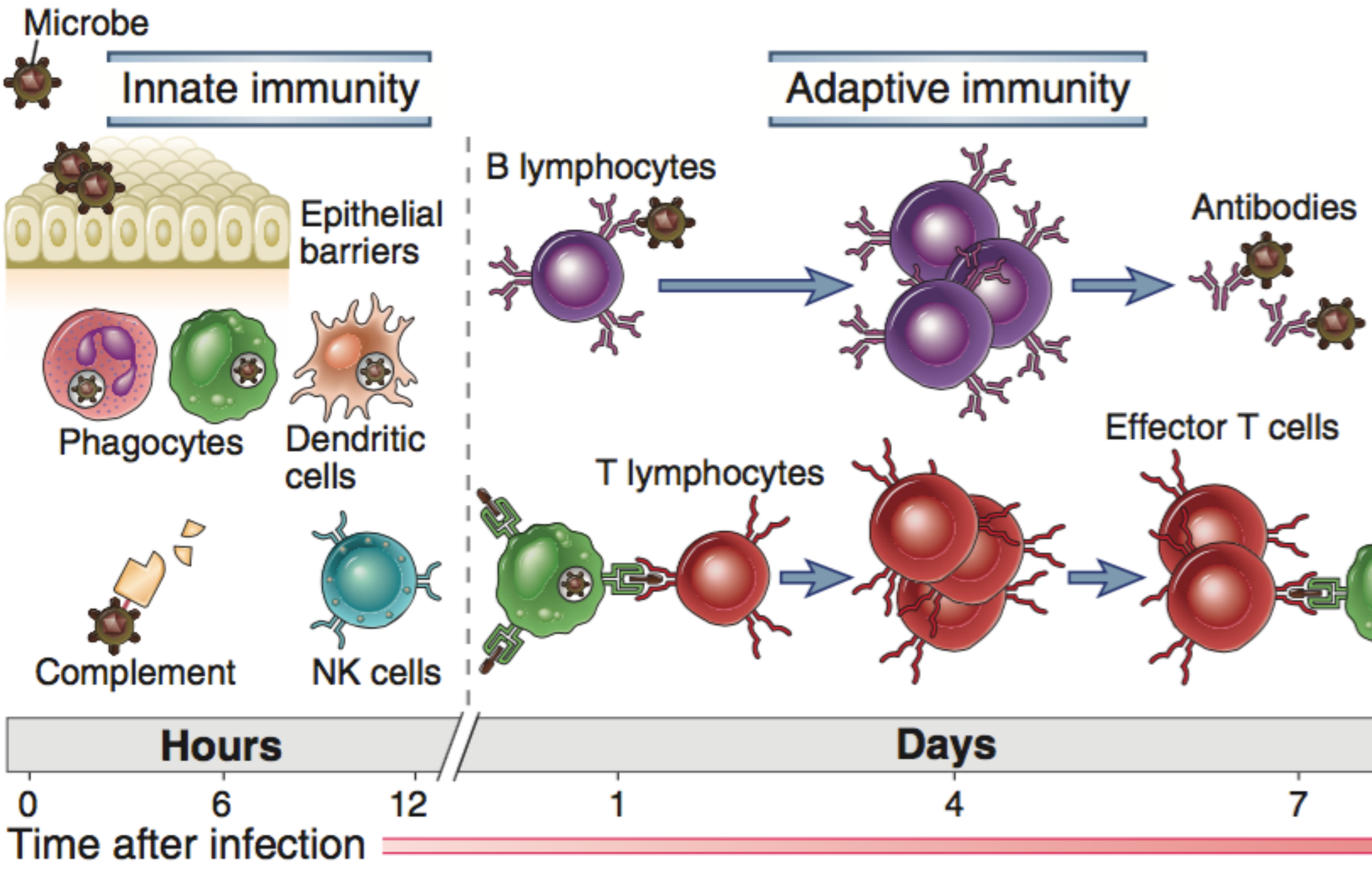
Surasak Wongratanacheewin, Ph.D
Dean, Graduate School, KKU
Microbiology, Faculty of Medicine, KKU
sura_wng@kku.ac.th

การอบรมหลักสูตรประกาศนียบัตรการขึ้นทะเบียนชีววัตถุ วันที่ 7-11 มีนาคม 2559 ณ โรงแรมดิเอมเมอรัล กรุงเทพมหานคร

Immunity

- **Innate (Natural) Immunity:**
  First line of defense, pattern recognition

- **Adaptive (Acquired) Immunity:**
  Need stimulation or infection, Memory and specific



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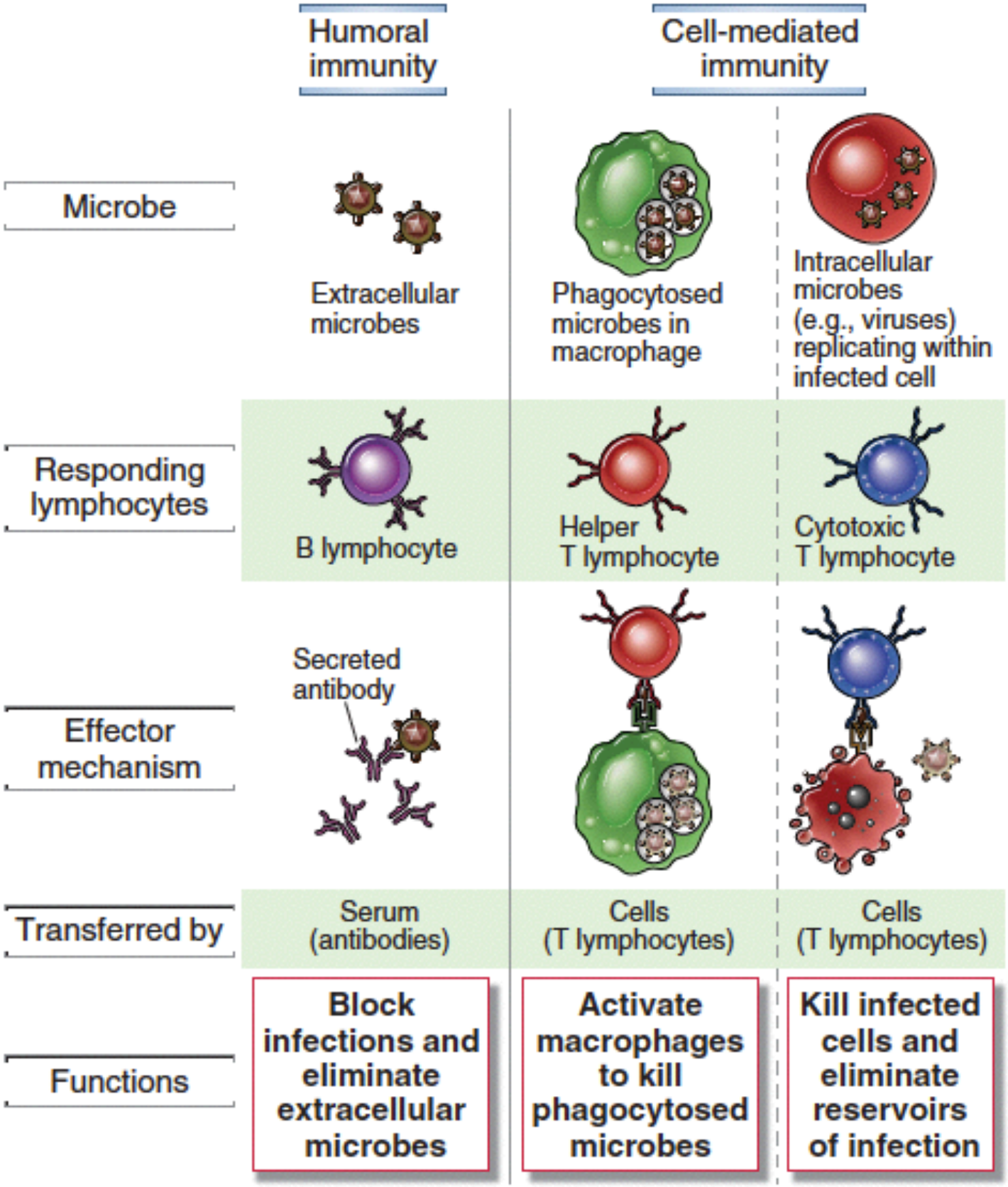
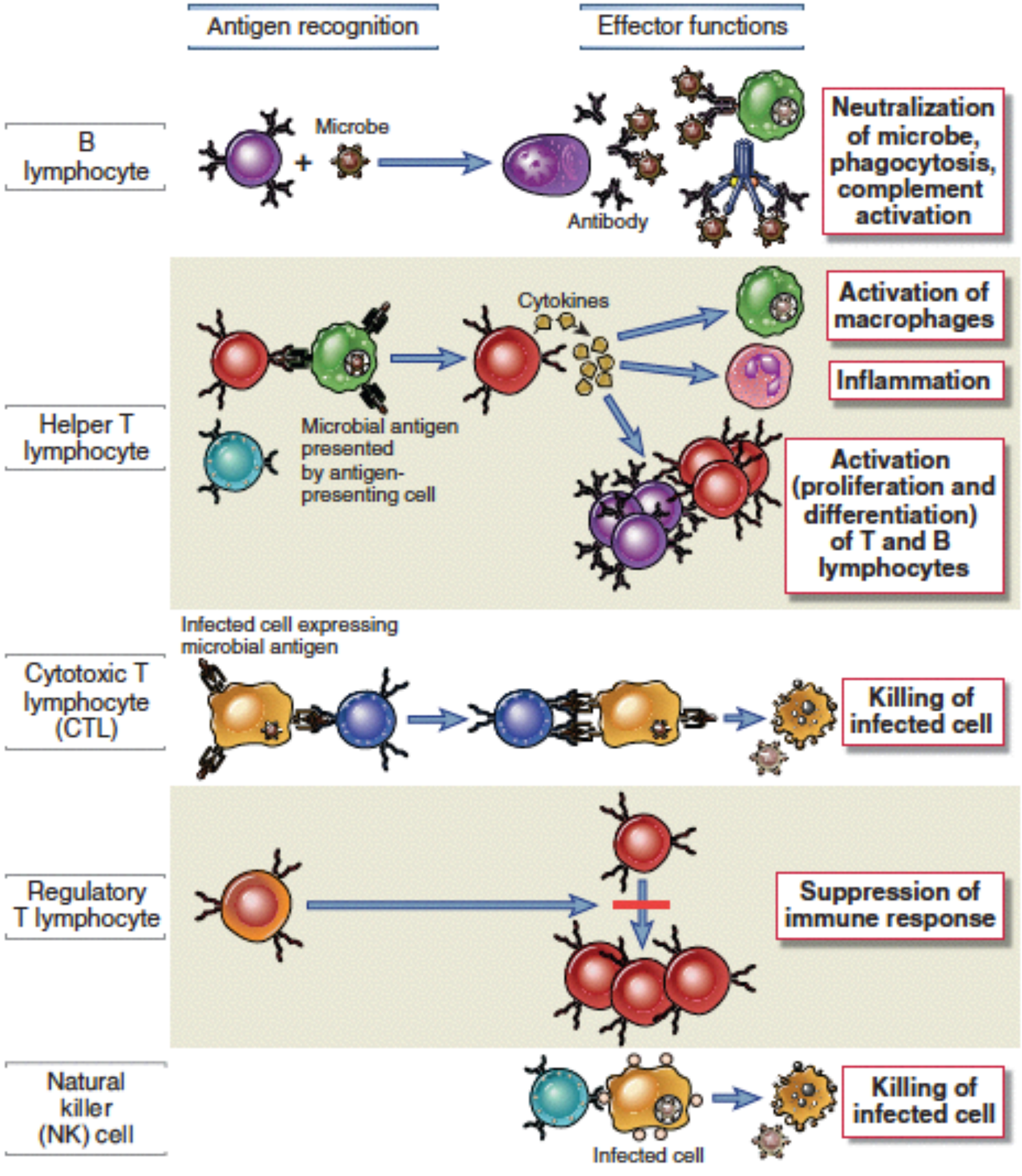


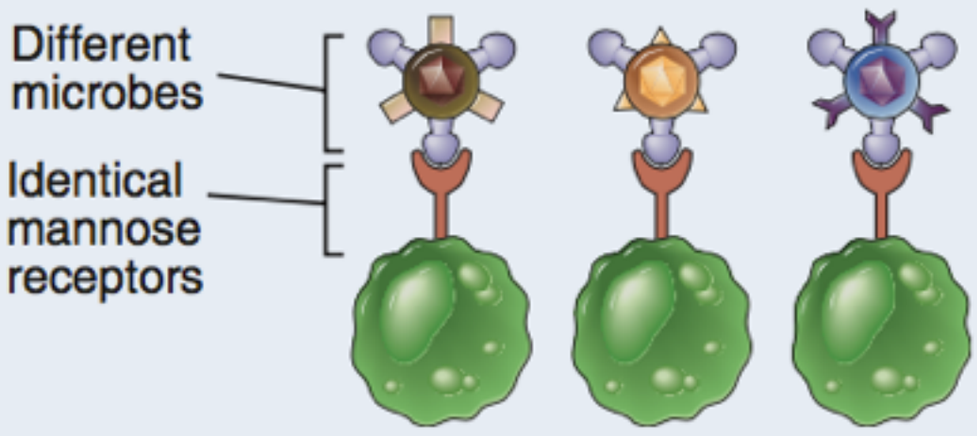
Innate immunity

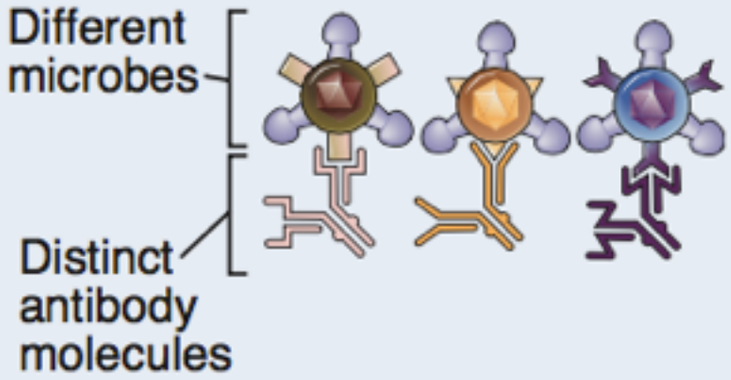
Adaptive immunity against infections

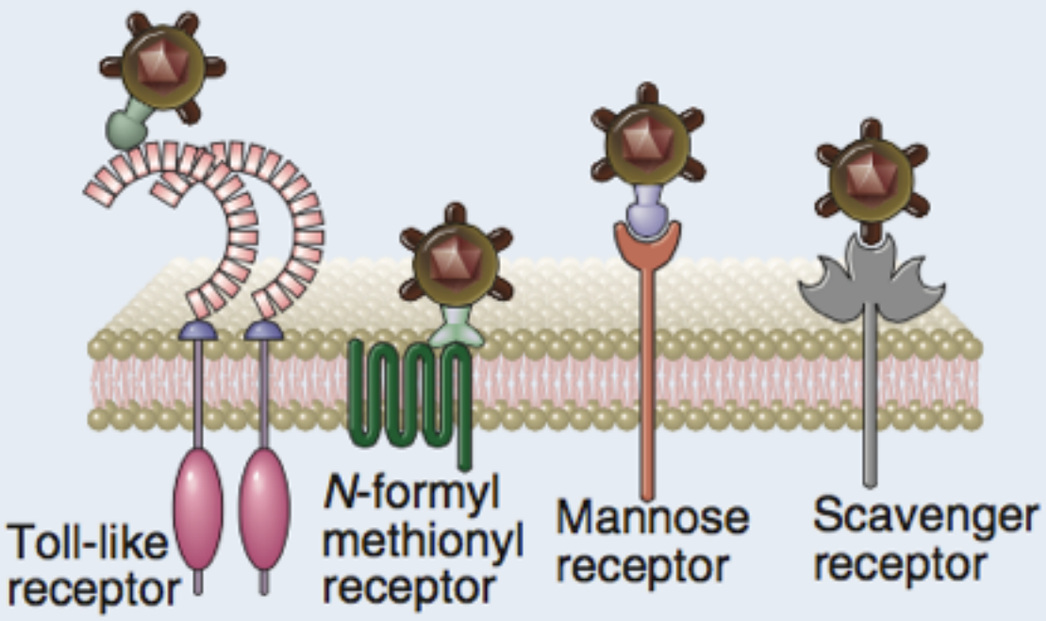

Antigen recognition
Effector functions
B lymphocyte
Microbe
+
Antibody
Neutralization of microbe, phagocytosis, complement activation
Helper T lymphocyte
Cytokines
Microbial antigen presented by antigen-presenting cell
Activation of macrophages
Inflammation
Activation (proliferation and differentiation) of T and B lymphocytes
Cytotoxic T lymphocyte (CTL)
Infected cell expressing microbial antigen
Killing of infected cell
Regulatory T lymphocyte
Suppression of immune response
Natural killer (NK) cell
Infected cell
Killing of infected cell

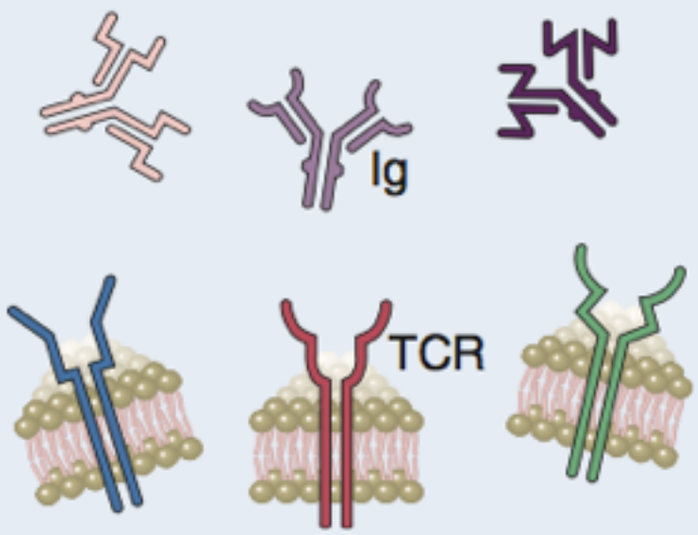
Innate-adaptive Immunity

	Innate Immunity	Adaptive Immunity
Specificity	For structures shared by classes of microbes (pathogen-associated molecular patterns)	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens
Receptors	Encoded in germline; limited diversity (pattern recognition receptors)	Encoded by genes produced by somatic recombination of gene segments; greater diversity









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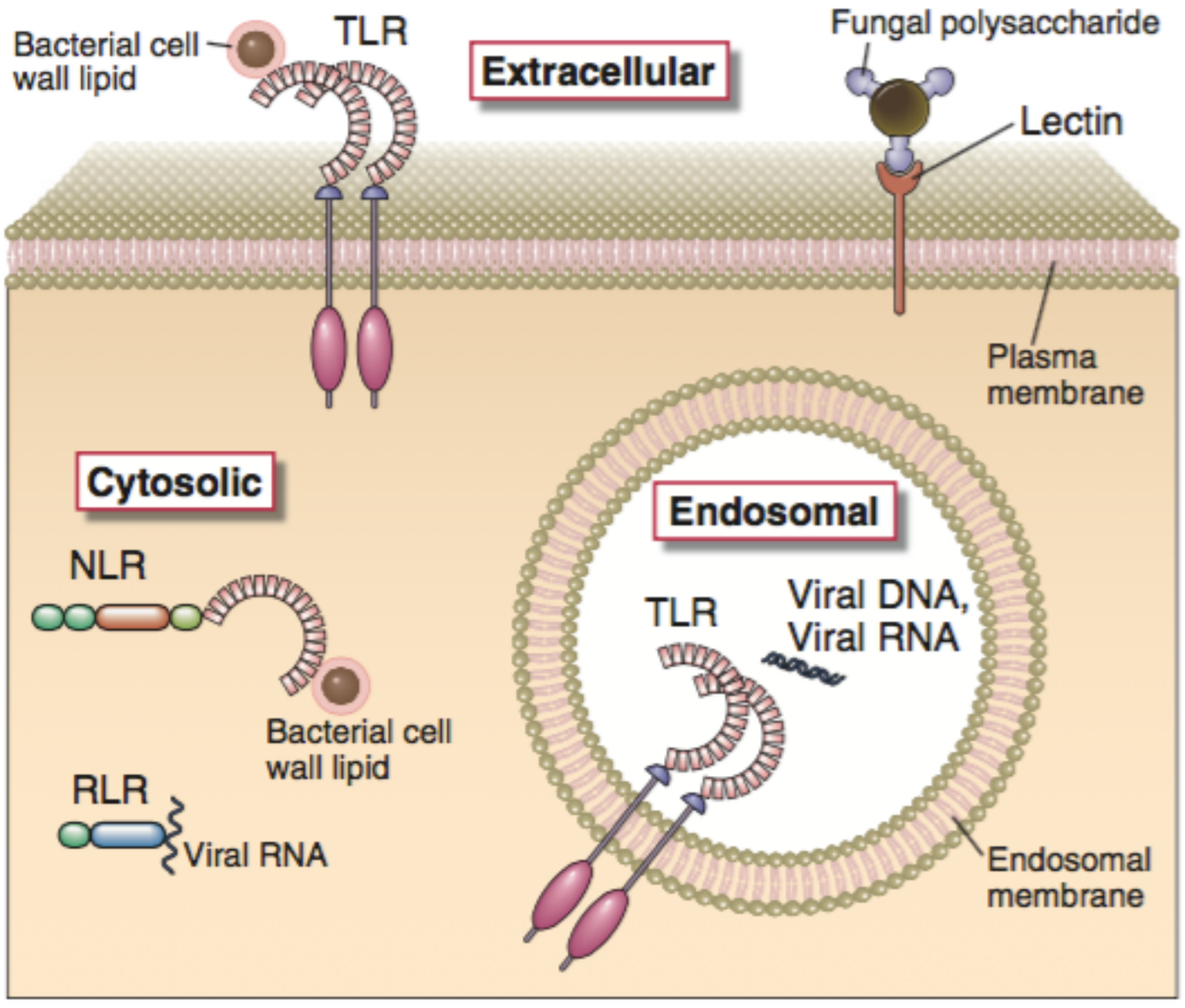
Ligands or microbes

TABLE 4–2 Examples of PAMPs and DAMPs

Pathogen-Associated Molecular Patterns		Microbe Type
Nucleic acids	ssRNA	Virus
	dsRNA	Virus
	CpG	Virus, bacteria
Proteins	Pilin	Bacteria
	Flagellin	Bacteria
Cell wall lipids	LPS	Gram-negative bacteria
	Lipoteichoic acid	Gram-positive bacteria
Carbohydrates	Mannan	Fungi, bacteria
	Dectin glucans	Fungi
Damage-Associated Molecular Patterns		
Stress-induced proteins	HSPs	
Crystals	Monosodium urate	
Nuclear proteins	HMGB1	

CpG, cytidine-guanine dinucleotide; dsRNA, double-stranded RNA; HMGB1, high-mobility group box 1; HSPs, heat shock proteins; LPS, lipopolysaccharide; ssRNA, single-stranded RNA.



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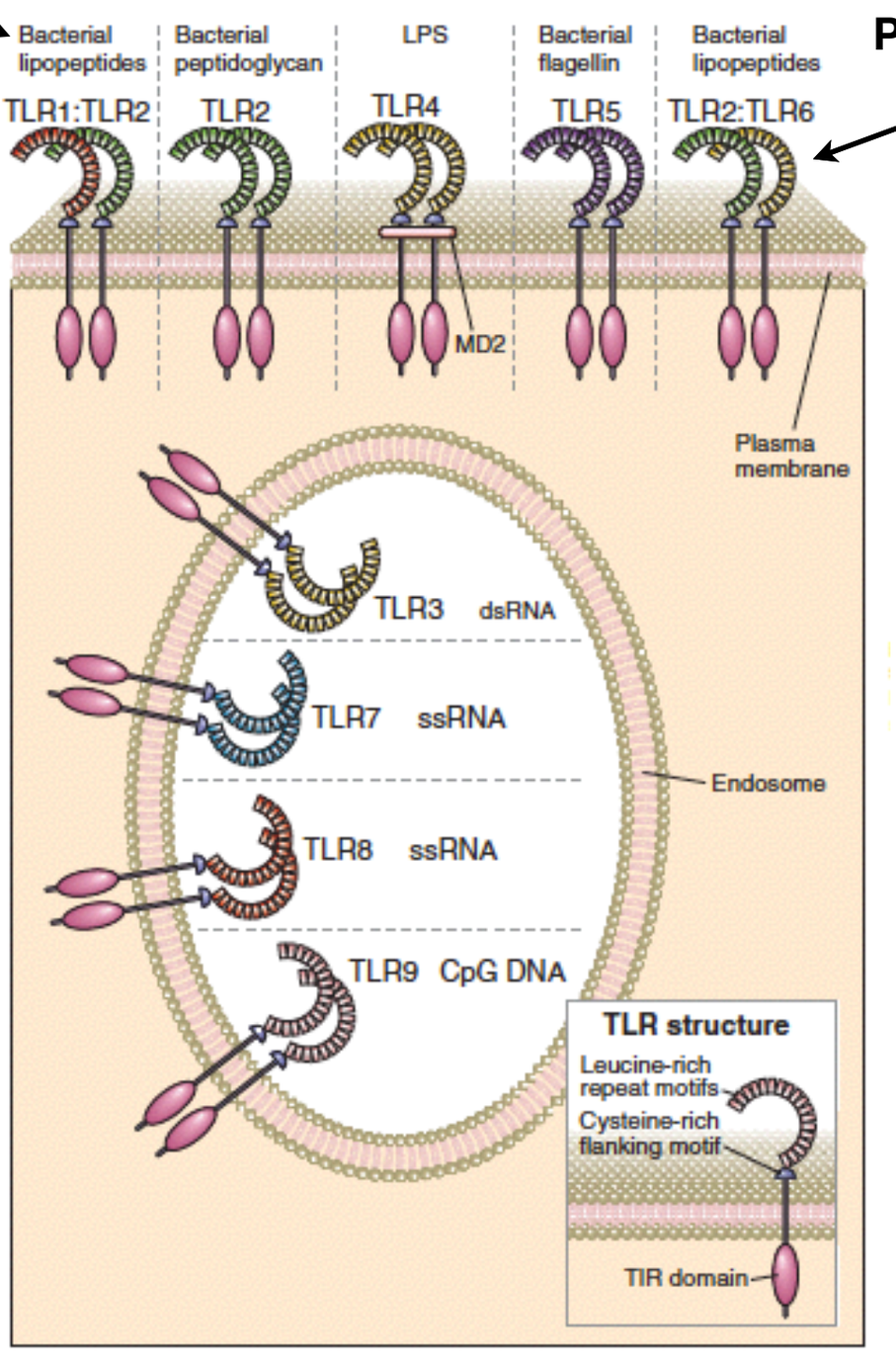
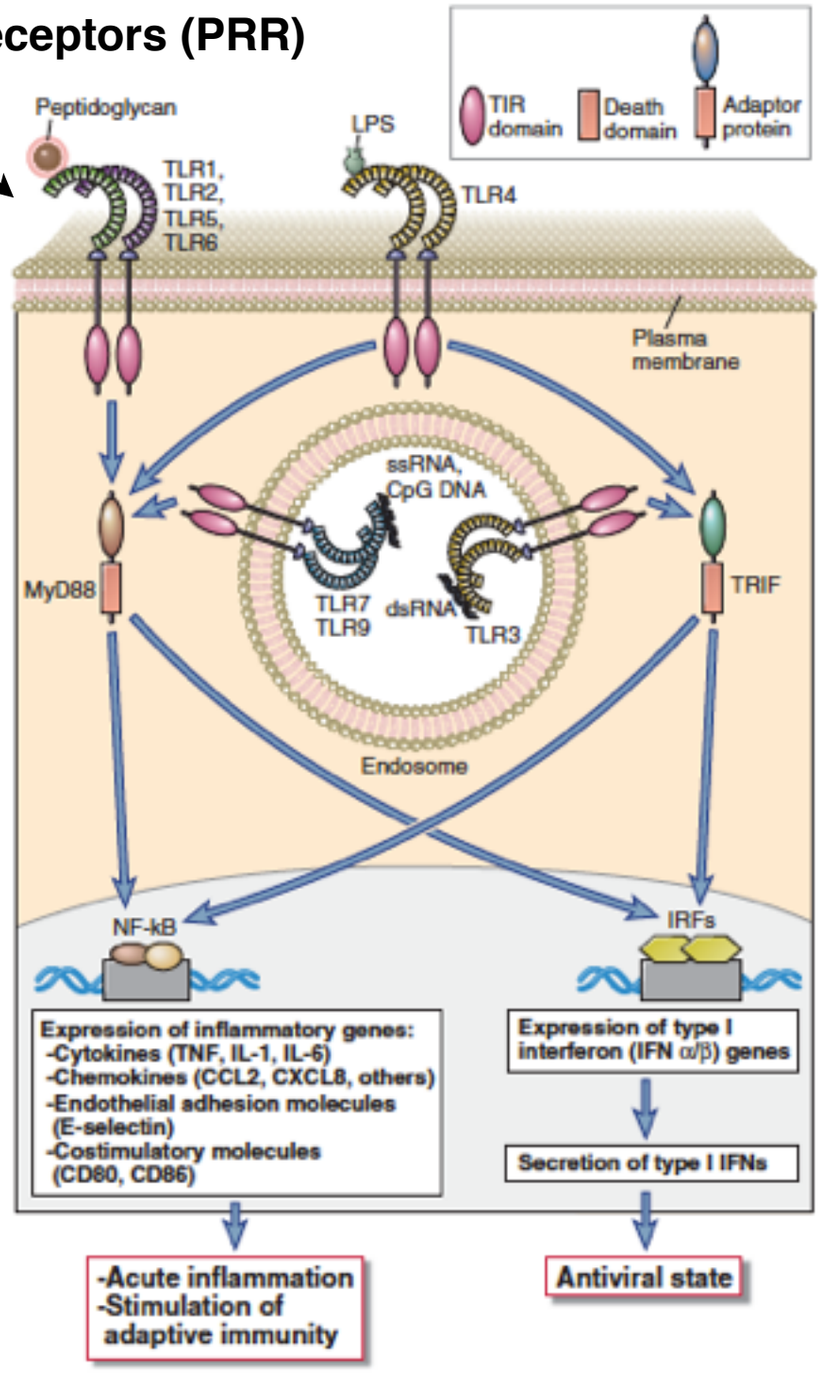
Different ligands bind to different receptors

PAMPs

Pattern recognition receptors (PRR)





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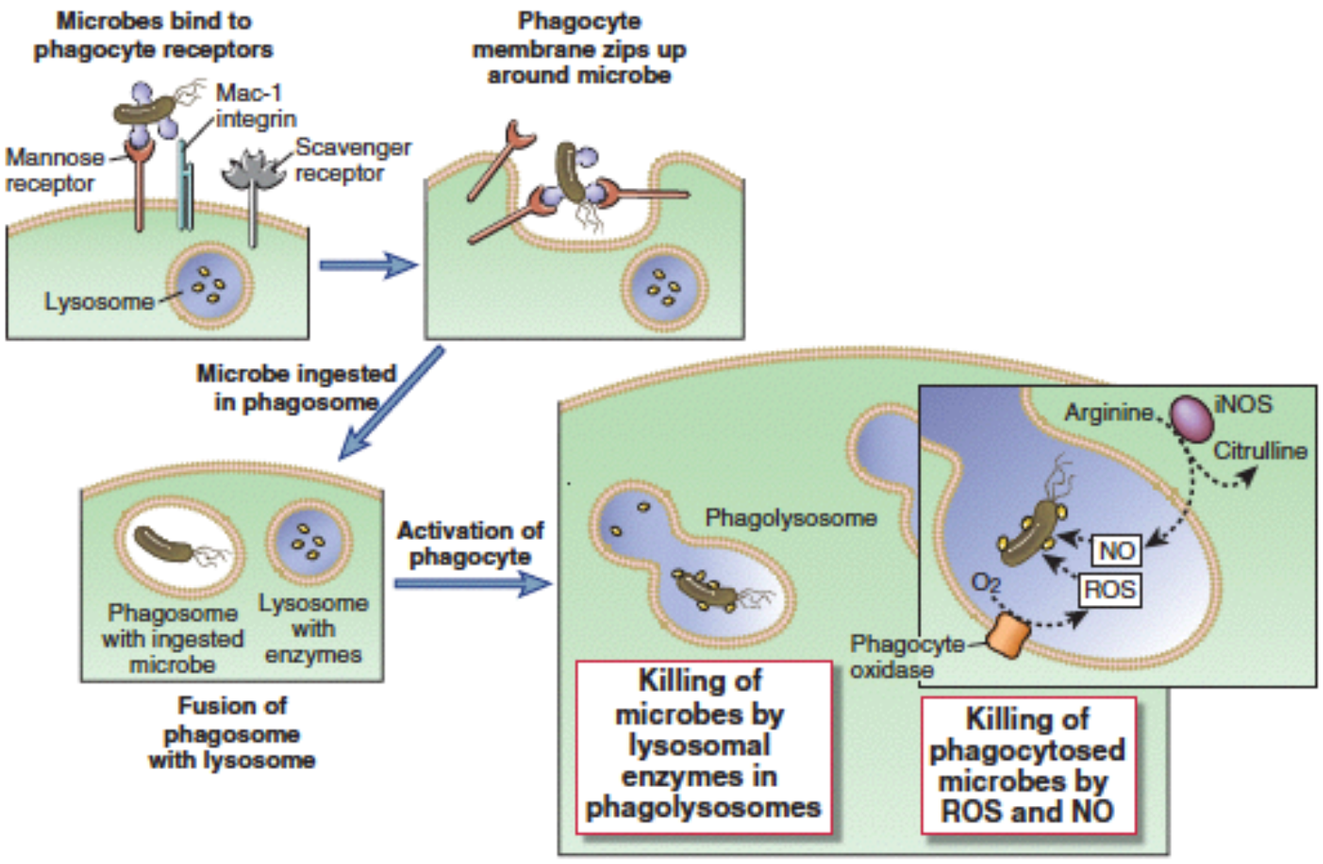
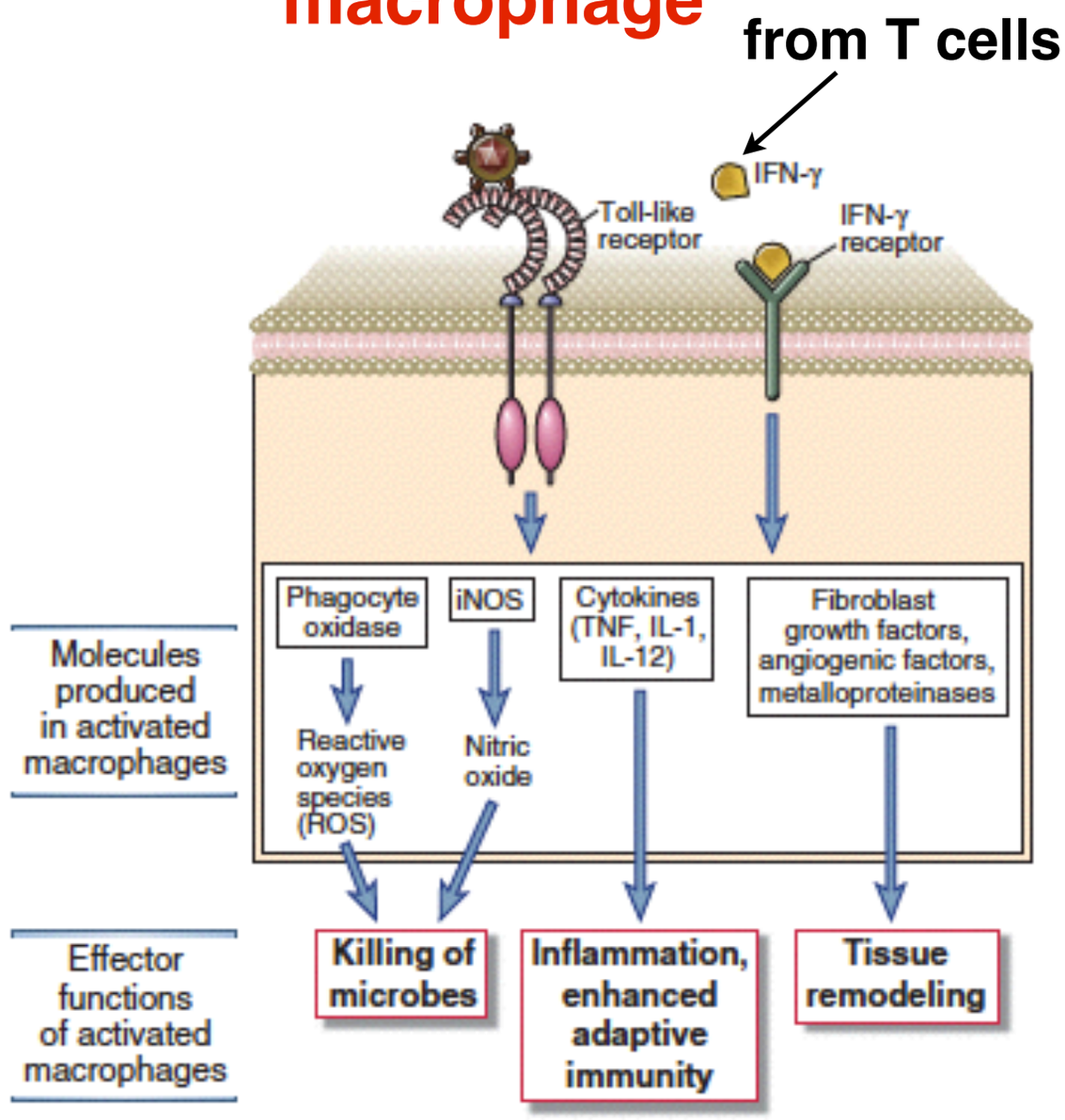
Killing mechanisms of innate cells

Killing by phagocytosis



Killing by activation of macrophage



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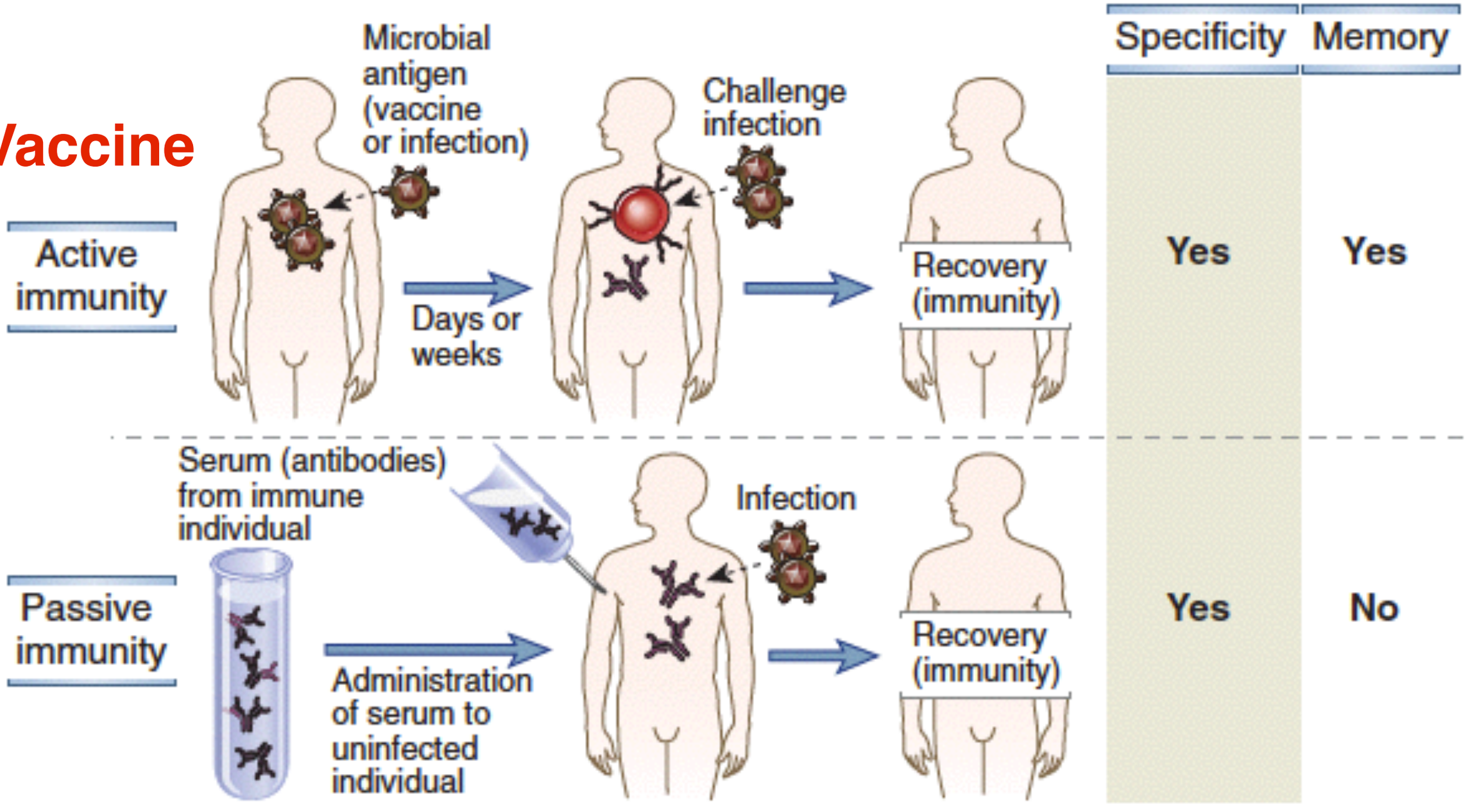
Active and passive immunity



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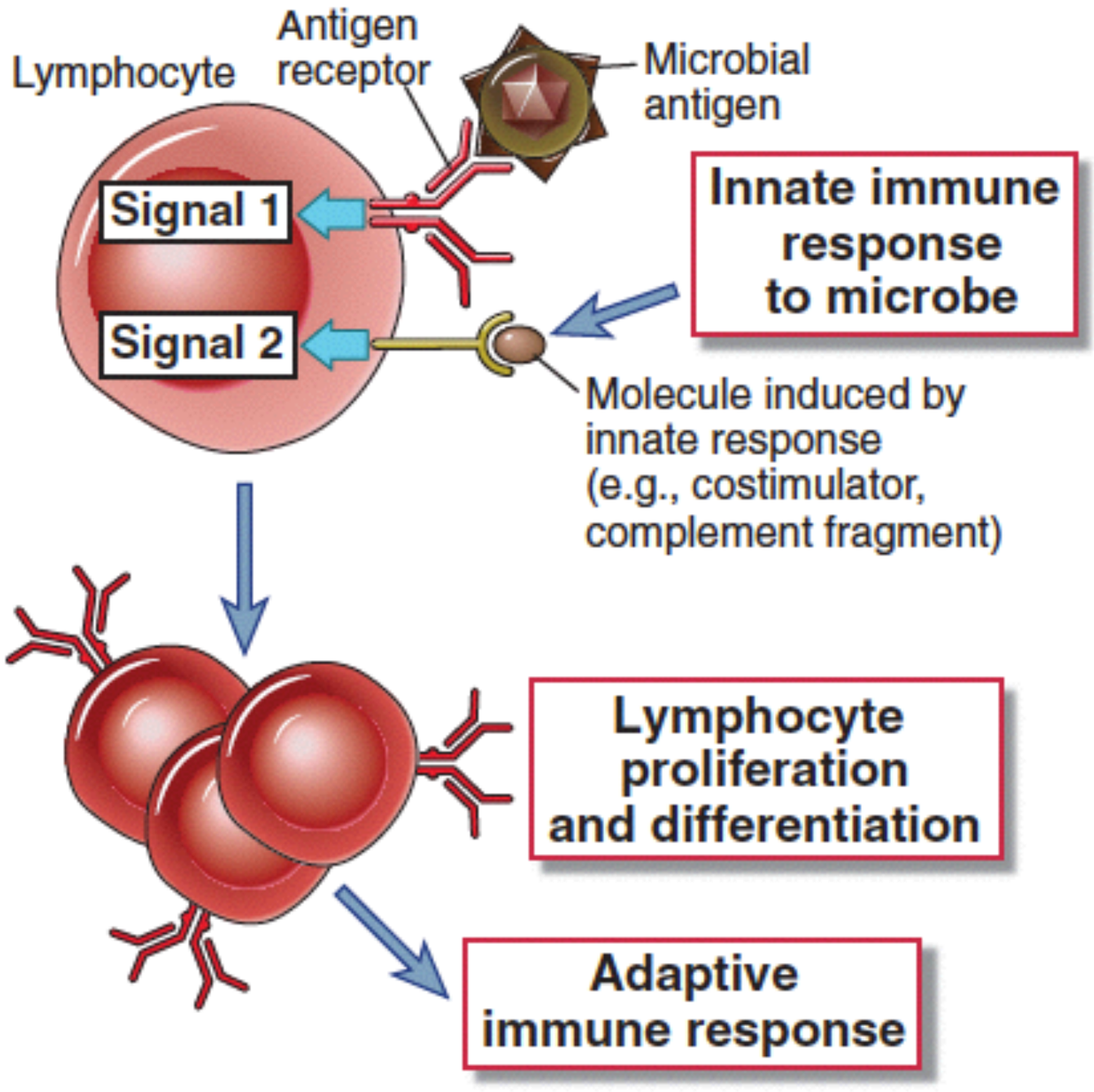
Adaptive immunity

Need activation via at least 2 signals:

antigens + costimulators, complements or innate responses



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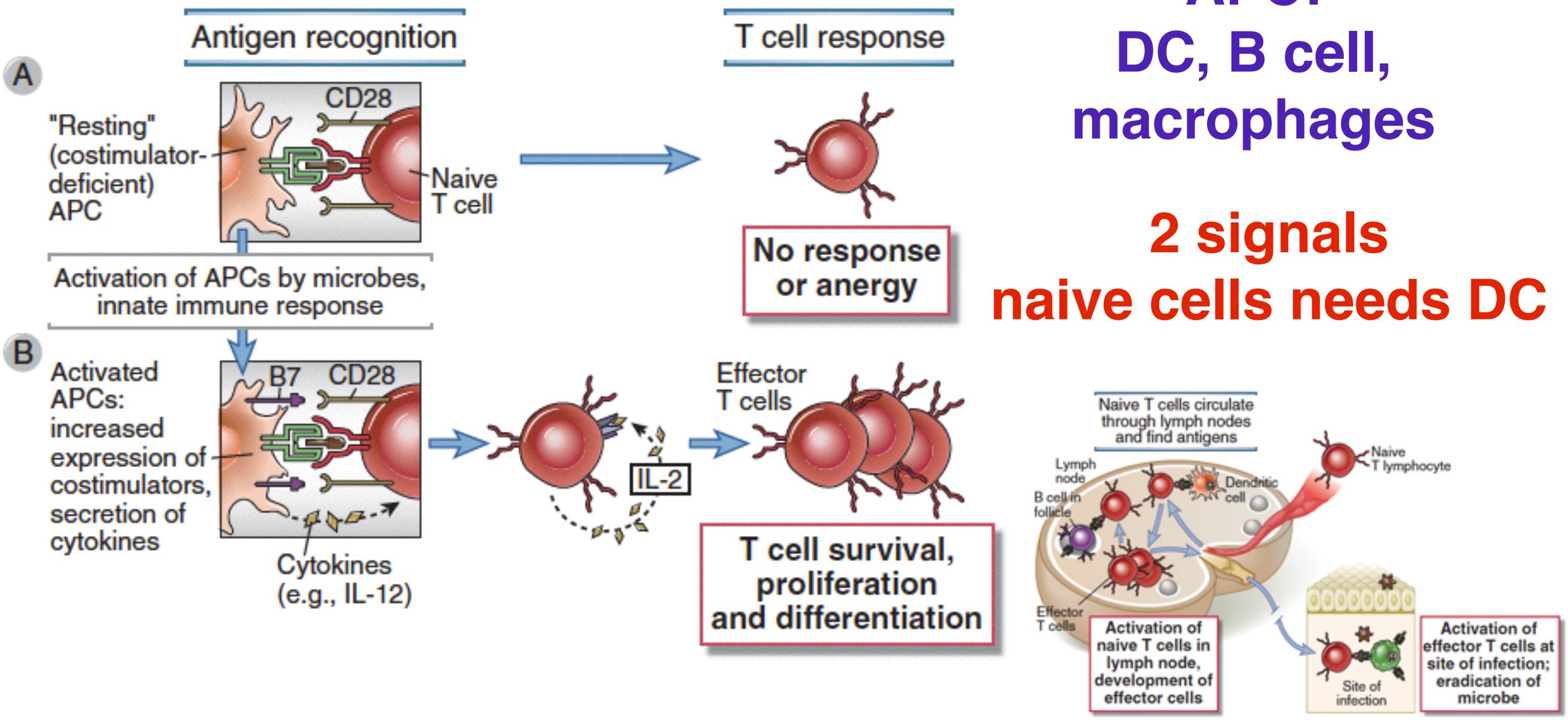
T cell activation in adaptive immunity



APC:
DC, B cell, macrophages

2 signals
naive cells needs DC

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CD28 and CD40L are essential for T cell activation

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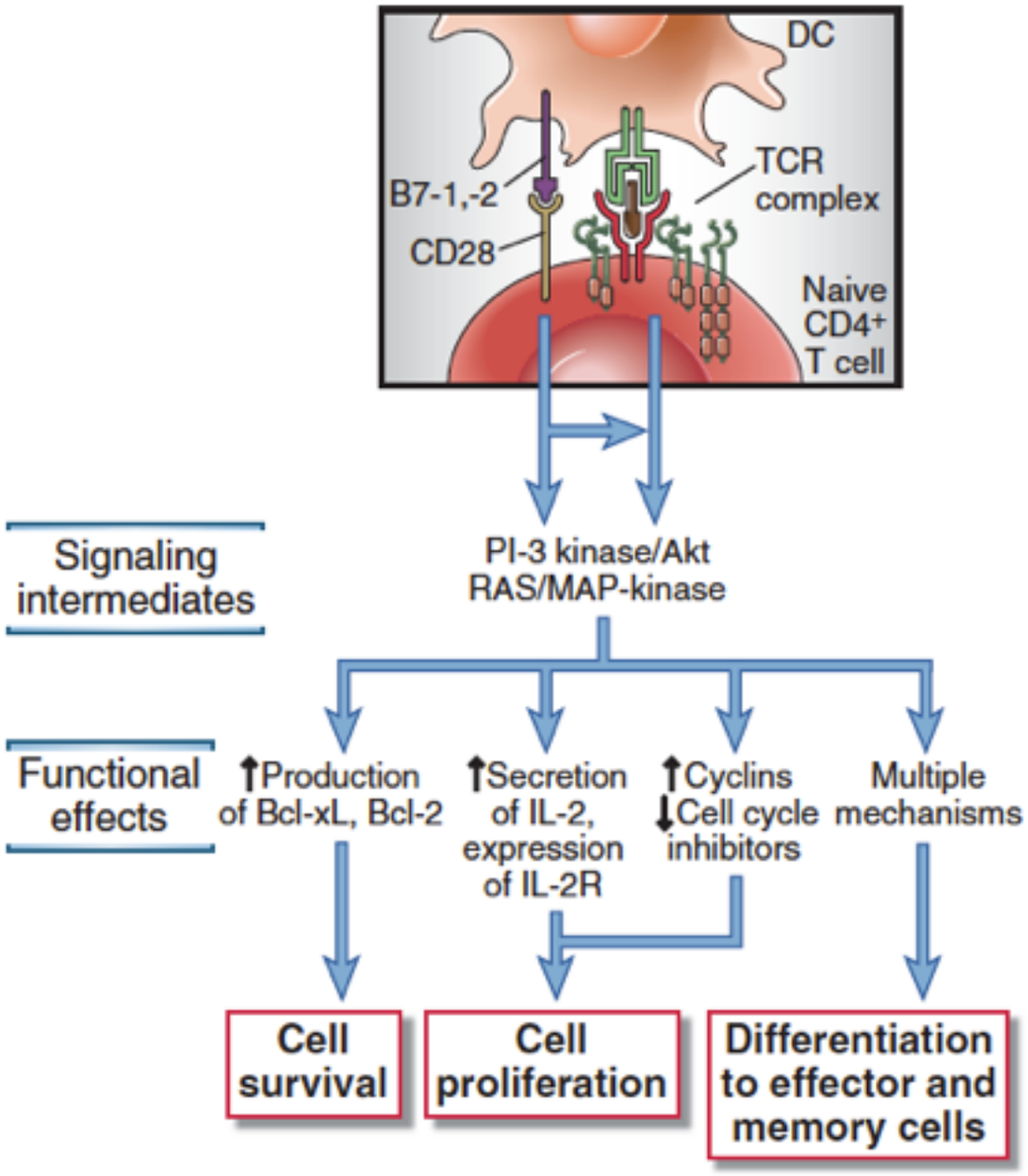
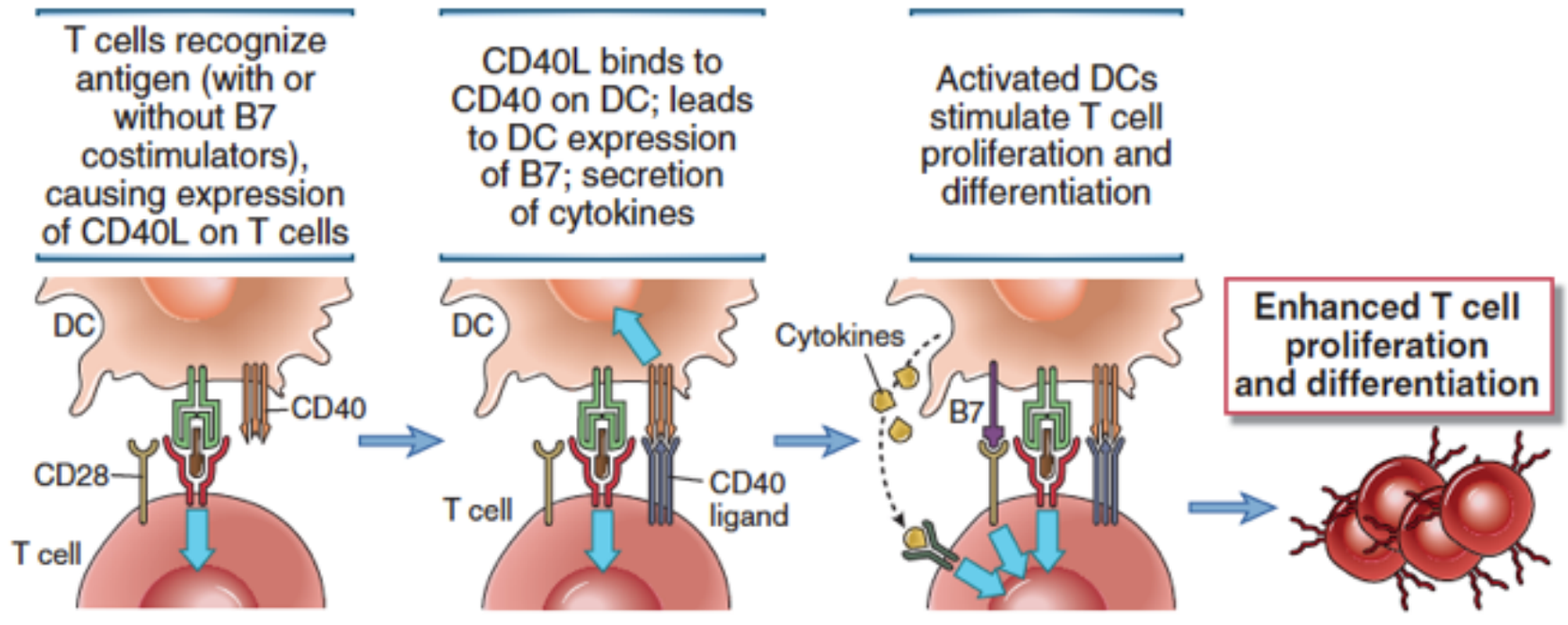
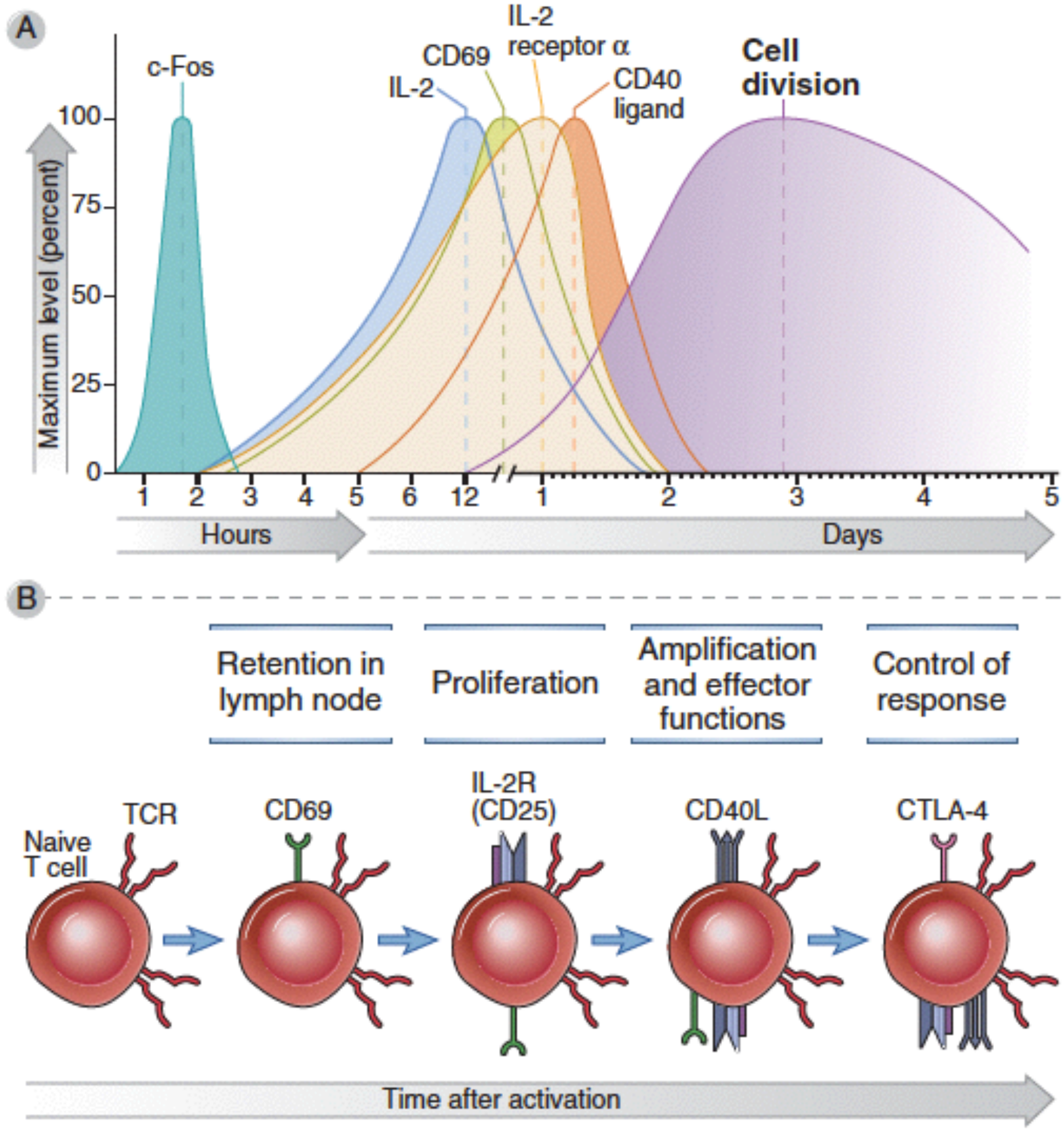
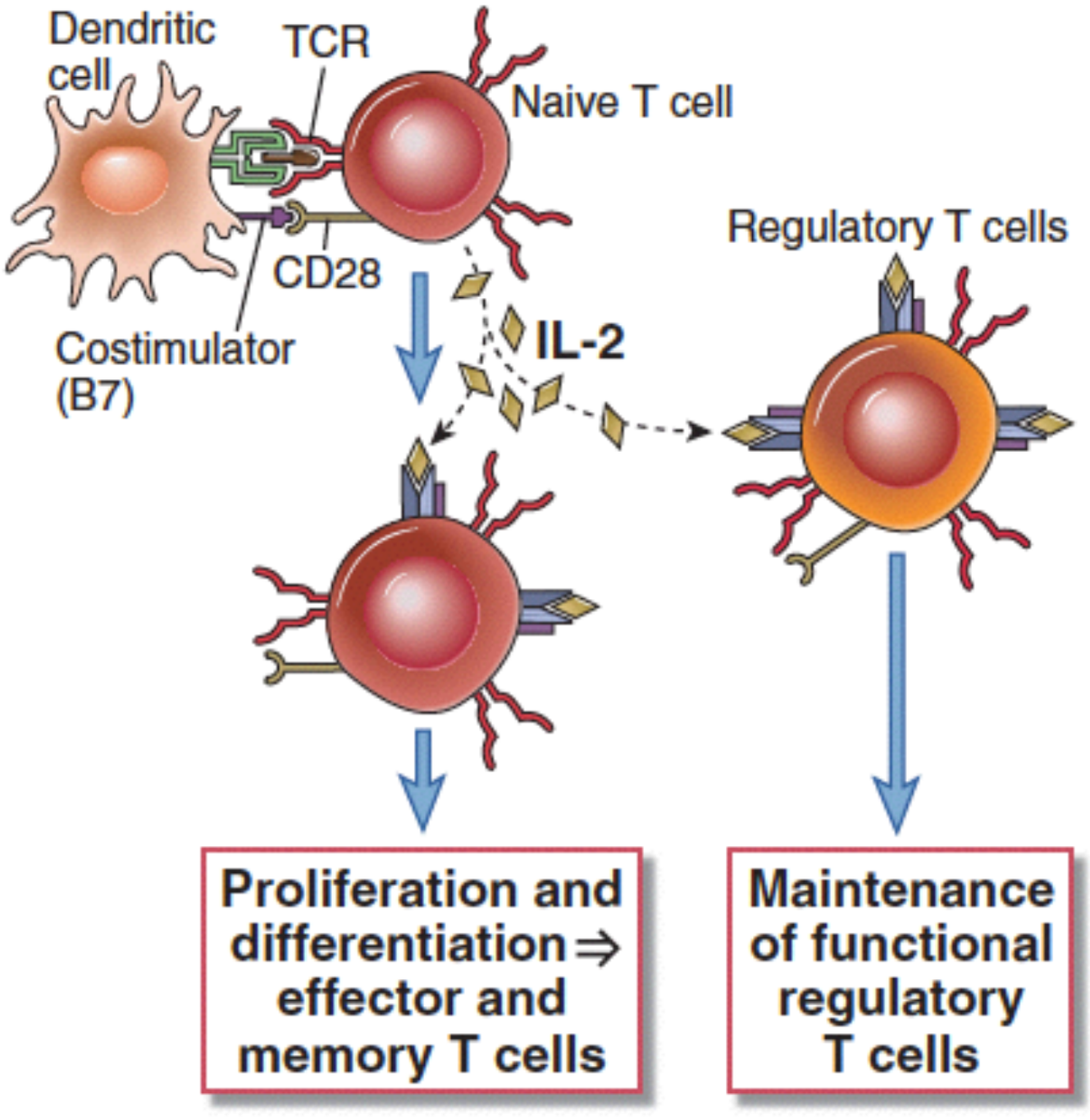




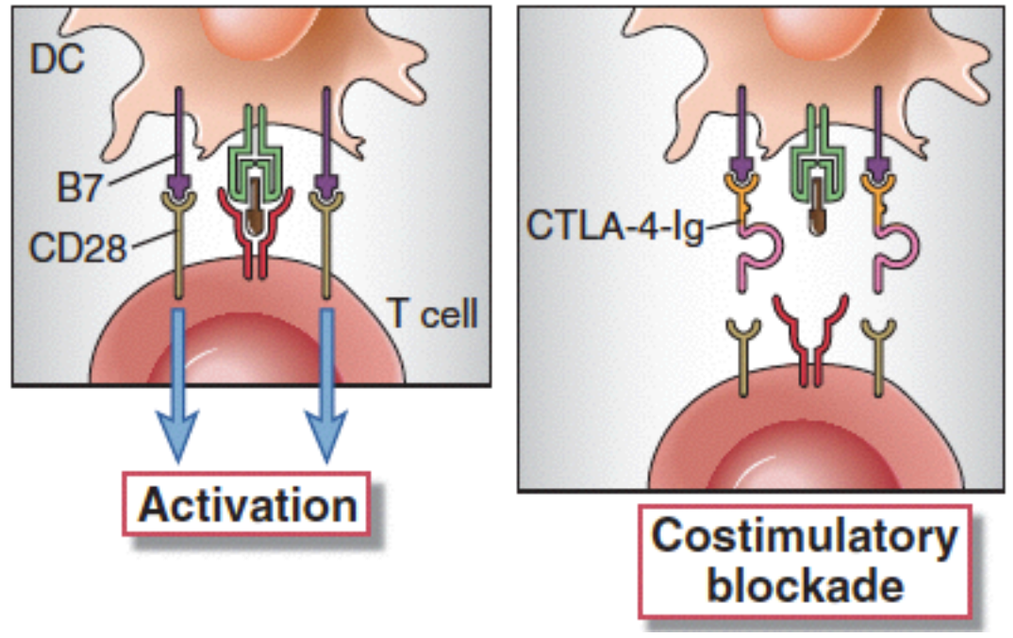
State and markers for T cell activation



Activation of T cells



T cell activation leads to differentiation of T cell subsets



Control of stimulation can be blocked by costimulatory blockade

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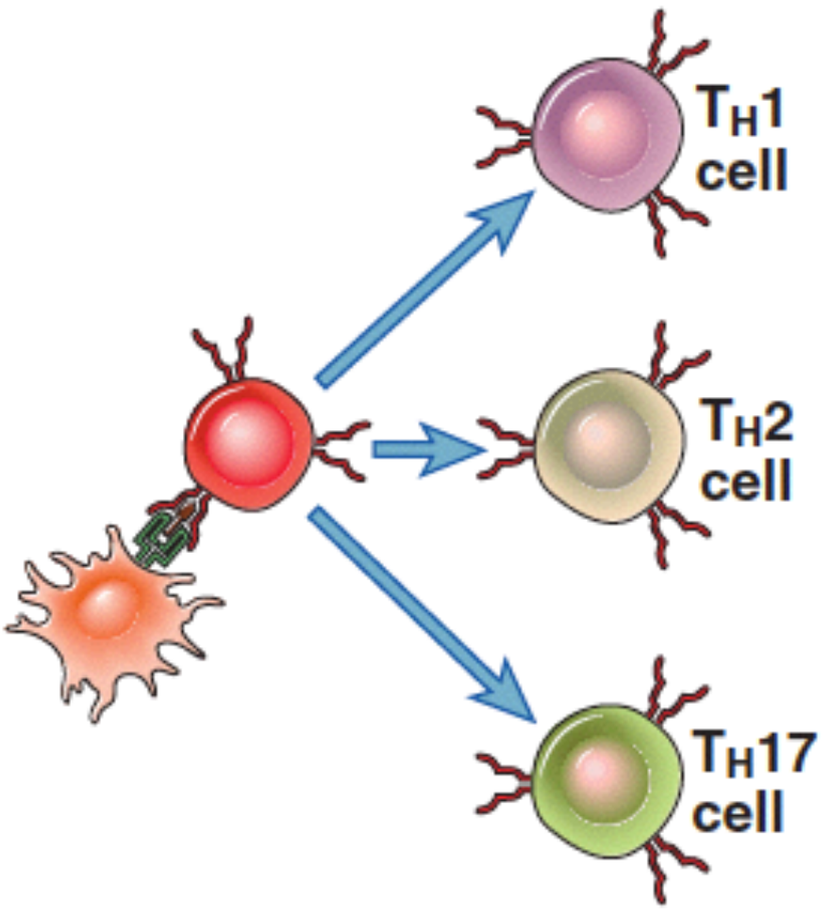
Type of T cell responses



Signature cytokines	Immune reactions	Host defense	Role in diseases
IFNγ	Macrophage activation; IgG production	Intracellular microbes	Autoimmune diseases; tissue damage associated with chronic infections
IL-4 IL-5 IL-13	Mast cell, eosinophil activation; IgE production; "alternative" macrophage activation	Helminthic parasites	Allergic diseases
IL-17A IL-17F IL-22	Neutrophilic, monocytic inflammation	Extracellular bacteria; fungi	Organ-specific autoimmunity

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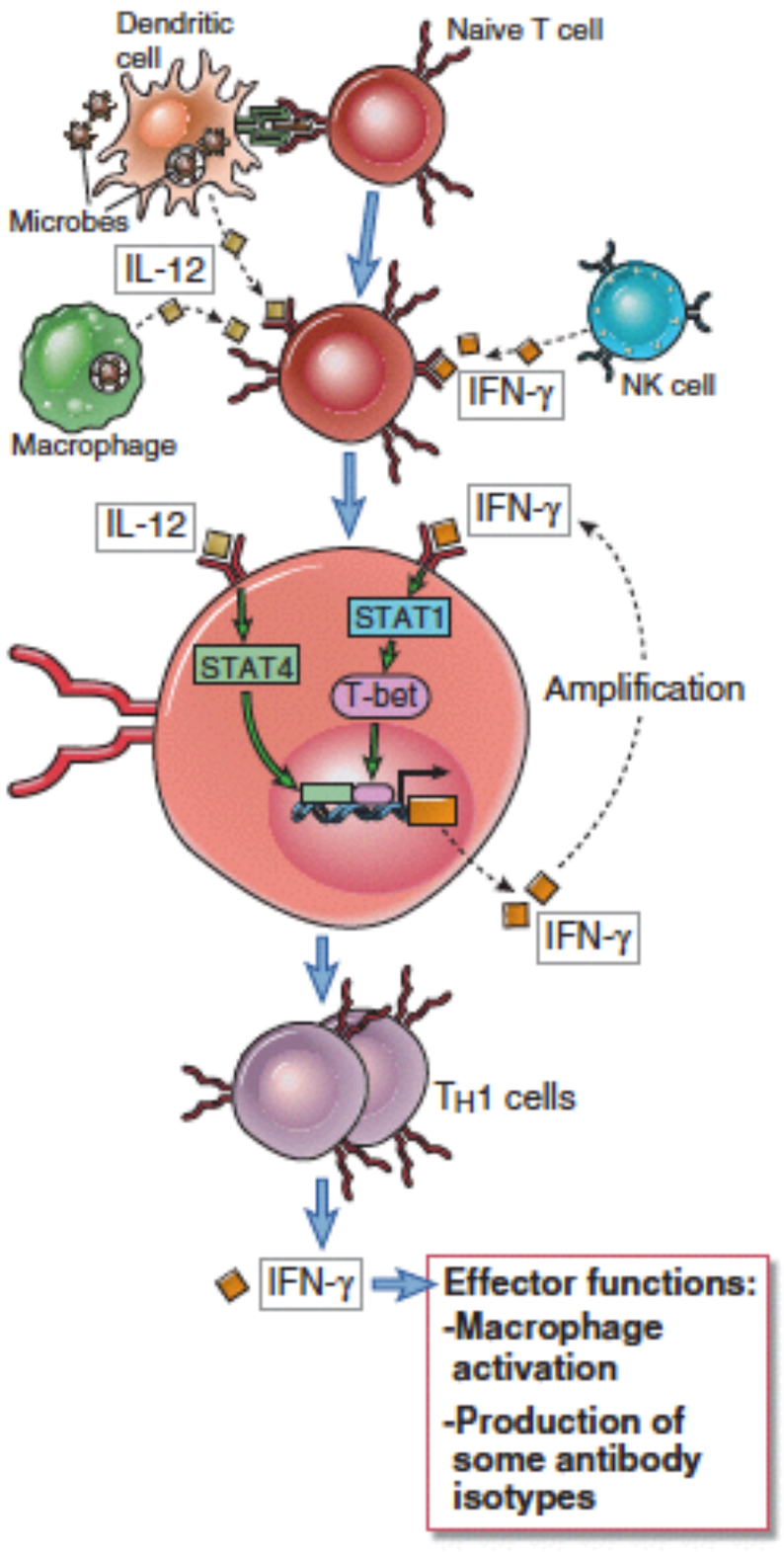

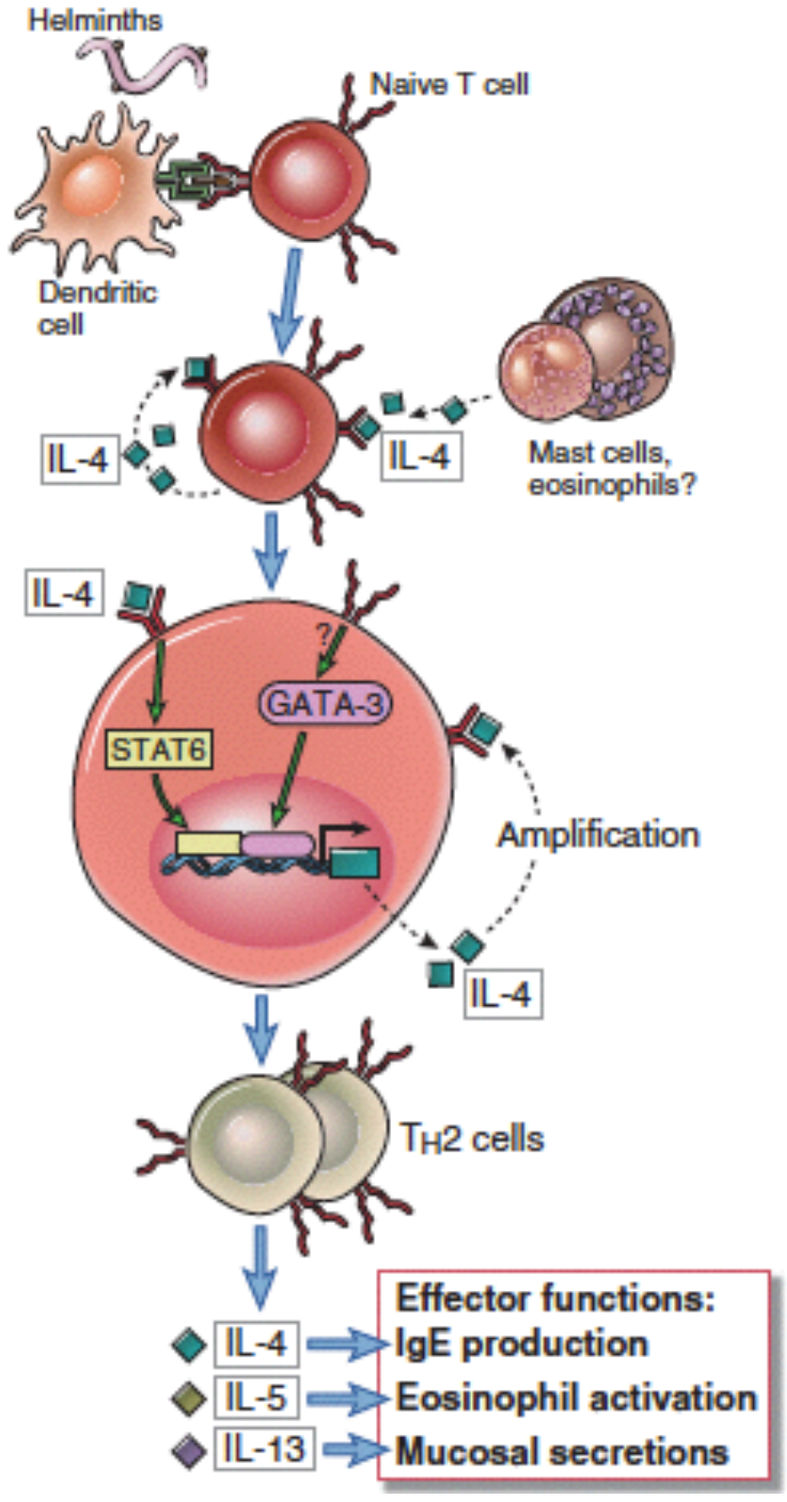

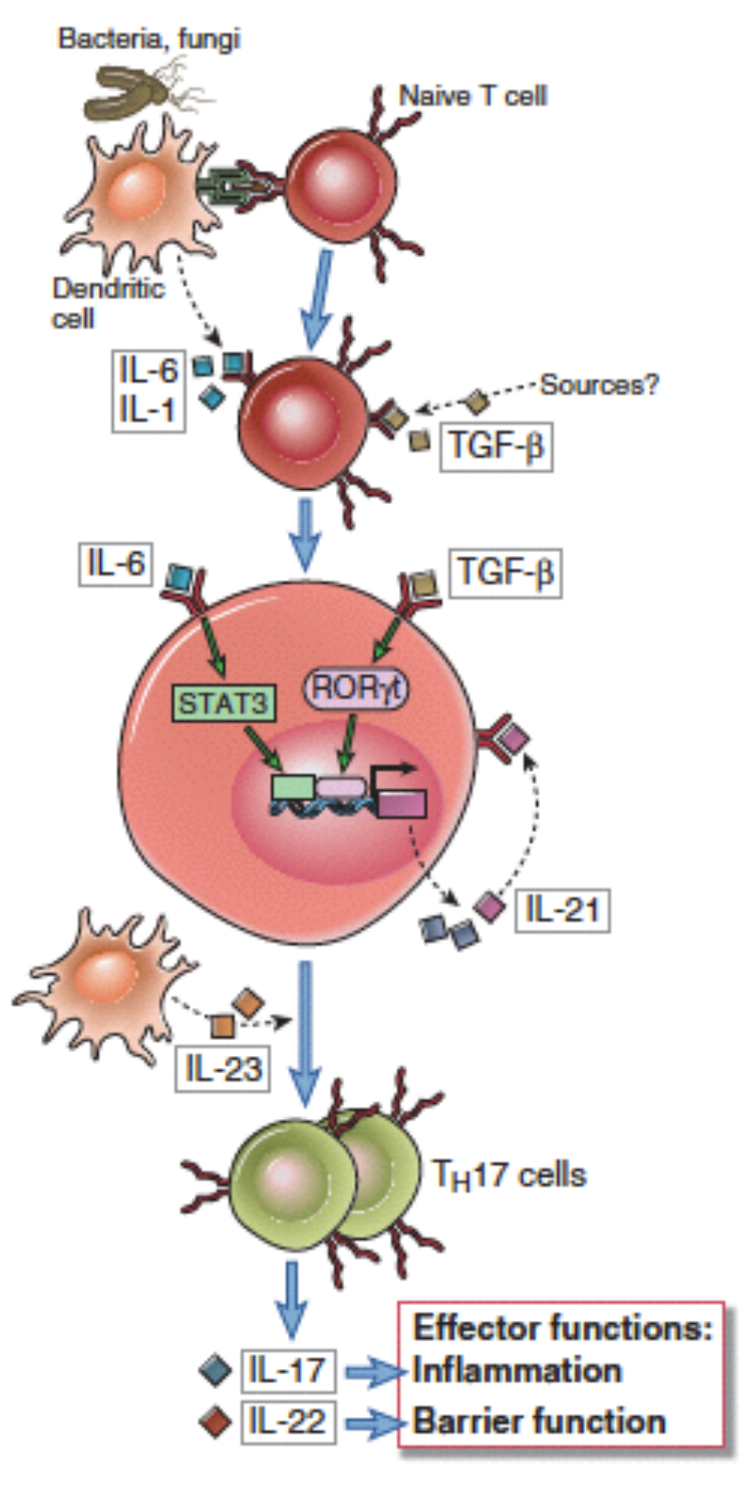

Th1 **Th2** **Th17**

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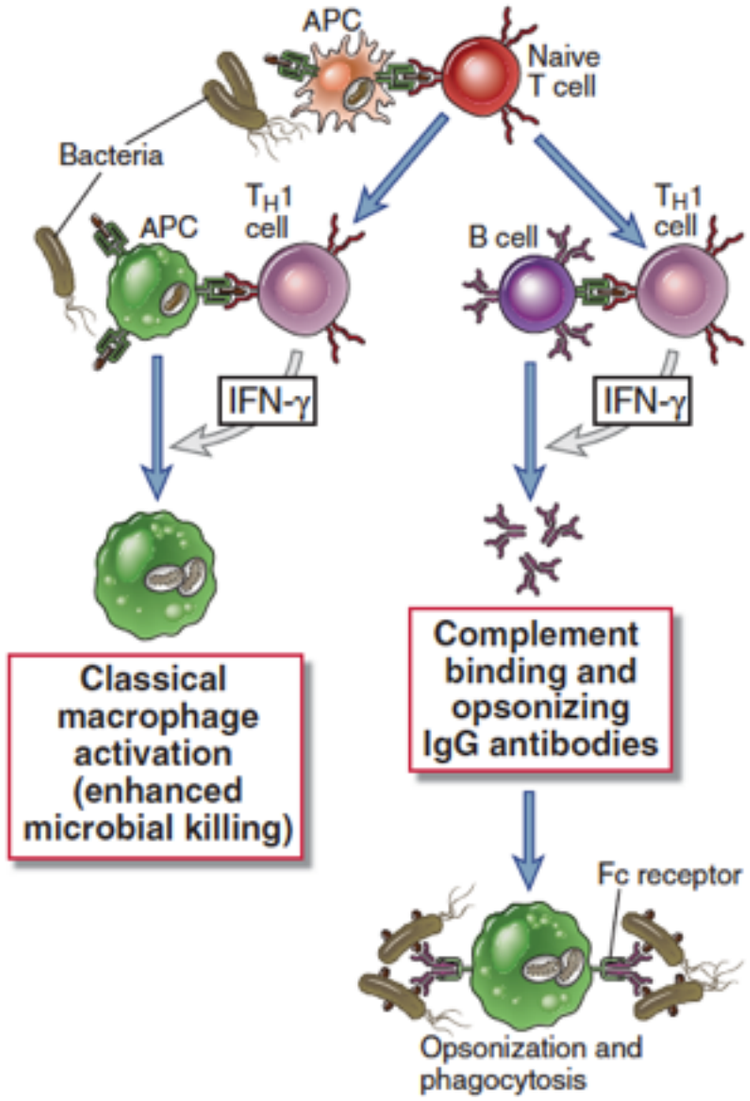
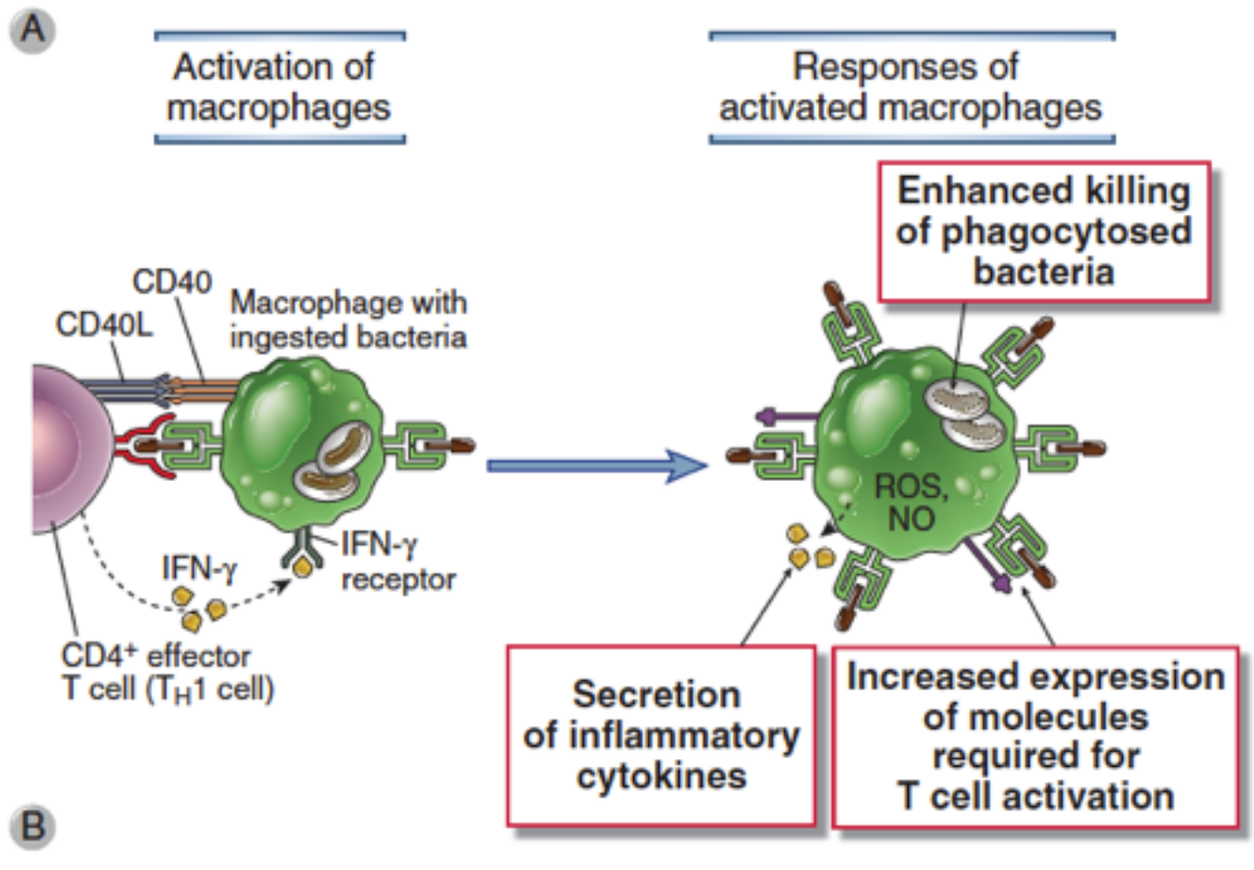
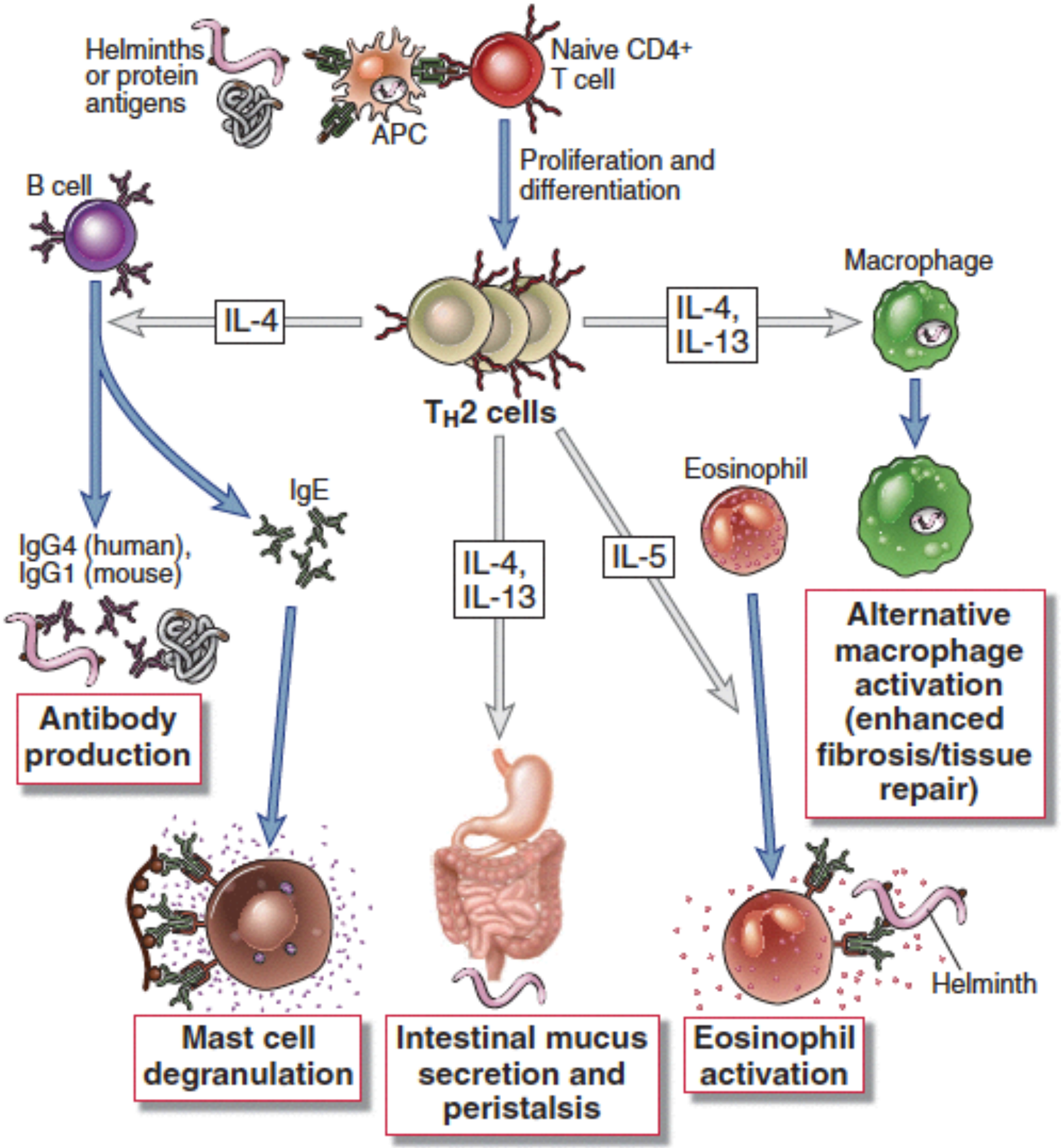


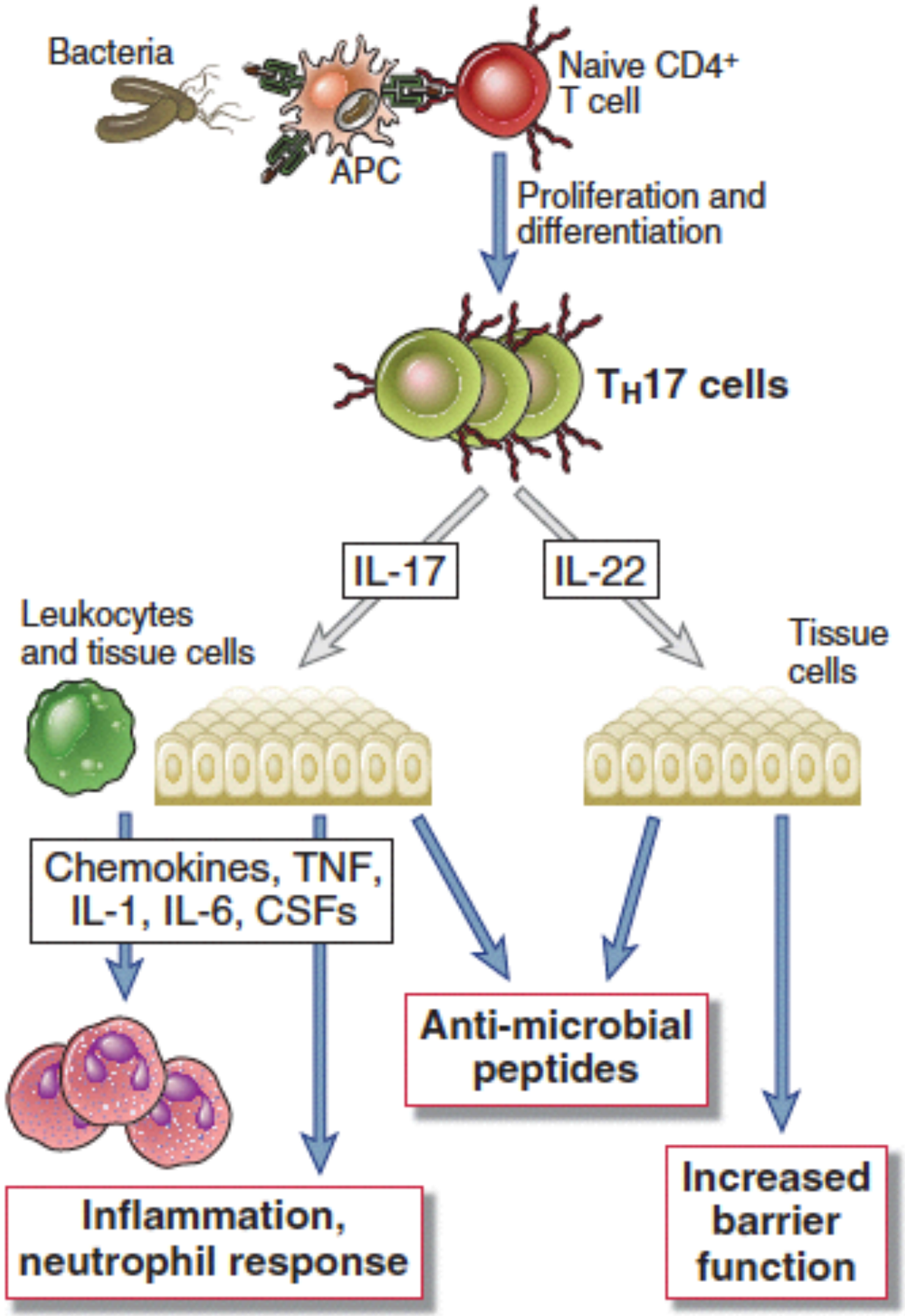


Macrophage response	Role in cell-mediated immunity
Production of reactive oxygen species, nitric oxide, increased lysosomal enzymes	Killing of microbes in phagolysosomes (effector function of macrophages)
Secretion of cytokines (TNF, IL-1, IL-12) and chemokines	TNF, IL-1, chemokines: leukocyte recruitment (inflammation) IL-12: T_H1 differentiation, IFN-γ production
Increased expression of B7 costimulators, MHC molecules	Increased T cell activation (amplification of T cell response)

Th1 help innate to clear intracellular pathogens



Th2 plays role in allergy and helminthic infections



Th17 cells are very important in inflammation and extracellular pathogens

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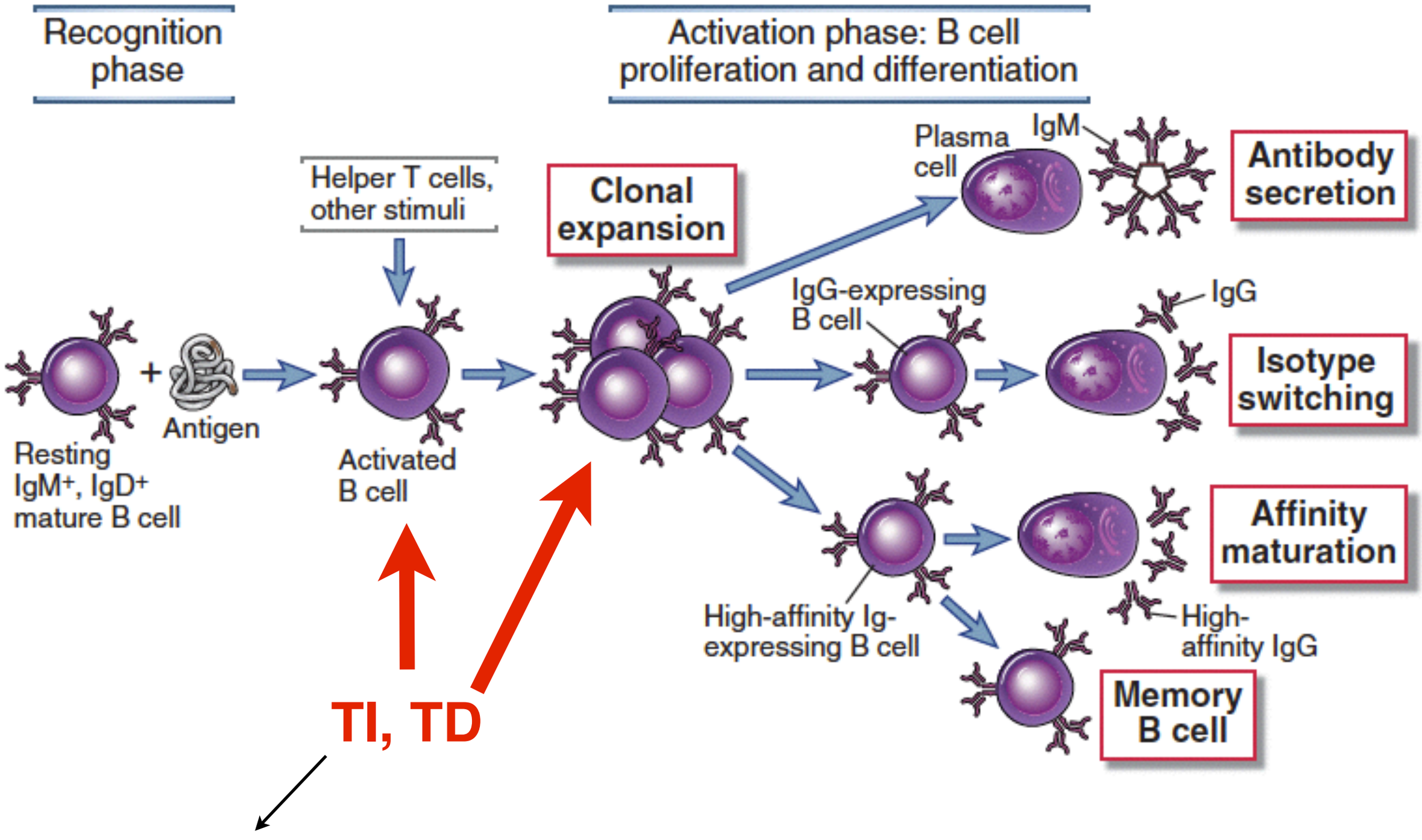
B cell activation



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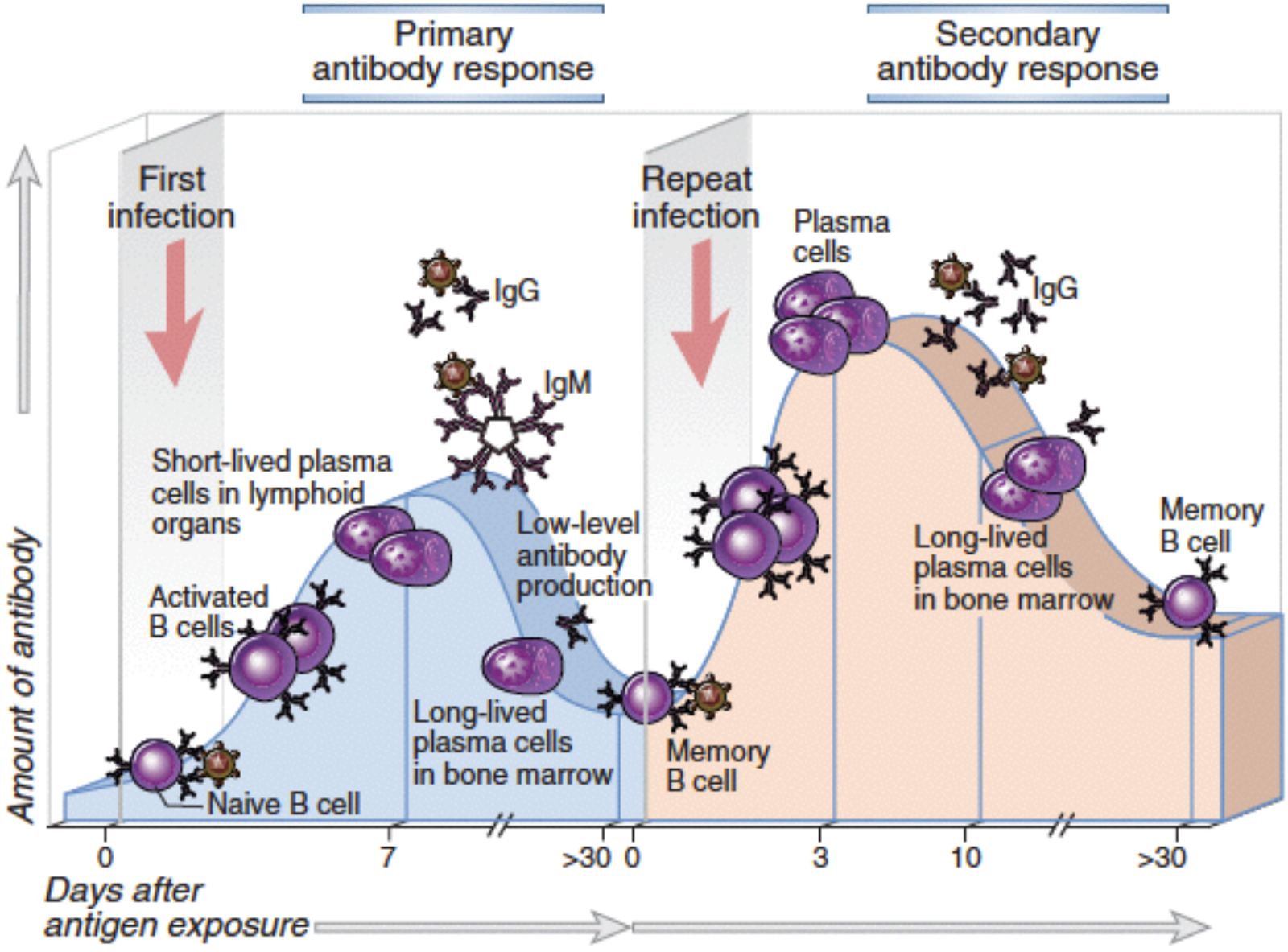


Feature	Primary response	Secondary response
Peak response	Smaller	Larger
Antibody isotype	Usually IgM > IgG	Relative increase in IgG and, under certain situations, in IgA or IgE
Antibody affinity	Lower average affinity, more variable	Higher average affinity (affinity maturation)
Induced by	All immunogens	Only protein antigens

Activation of memory cells:important to generate vaccines

Spleen, other lymphoid organs

IgD
IgM
Follicular B cells
Protein antigen + helper T cell
B
T
Germinal center reaction
T-dependent, isotype-switched, high-affinity antibodies; long-lived plasma cells
IgG
IgA
IgE

IgM
Marginal zone B cells
Polysaccharides, lipids, etc.
IgM
T-independent, mainly IgM; short-lived plasma cells

Mucosal tissues, peritoneal cavity

IgM
CD5
B-1 B cells
Polysaccharides, lipids, etc.
IgM
T-independent, mainly IgM; short-lived plasma cells

Helper T cell
Dendritic cell
T cell activation
Effector T cells
Initial T-B interaction
B cell activation
Follicle
Long-lived plasma cells, memory B cells
Follicular dendritic cell
Follicular helper T cell
Short-lived plasma cells
Extrafollicular focus
Extrafollicular helper T cell
Germinal center reaction

Vaccinology

Surasak Wongratanacheewin, Ph.D
Dean, Graduate School, KKU
Microbiology, Faculty of Medicine, KKU
sura_wng@kku.ac.th

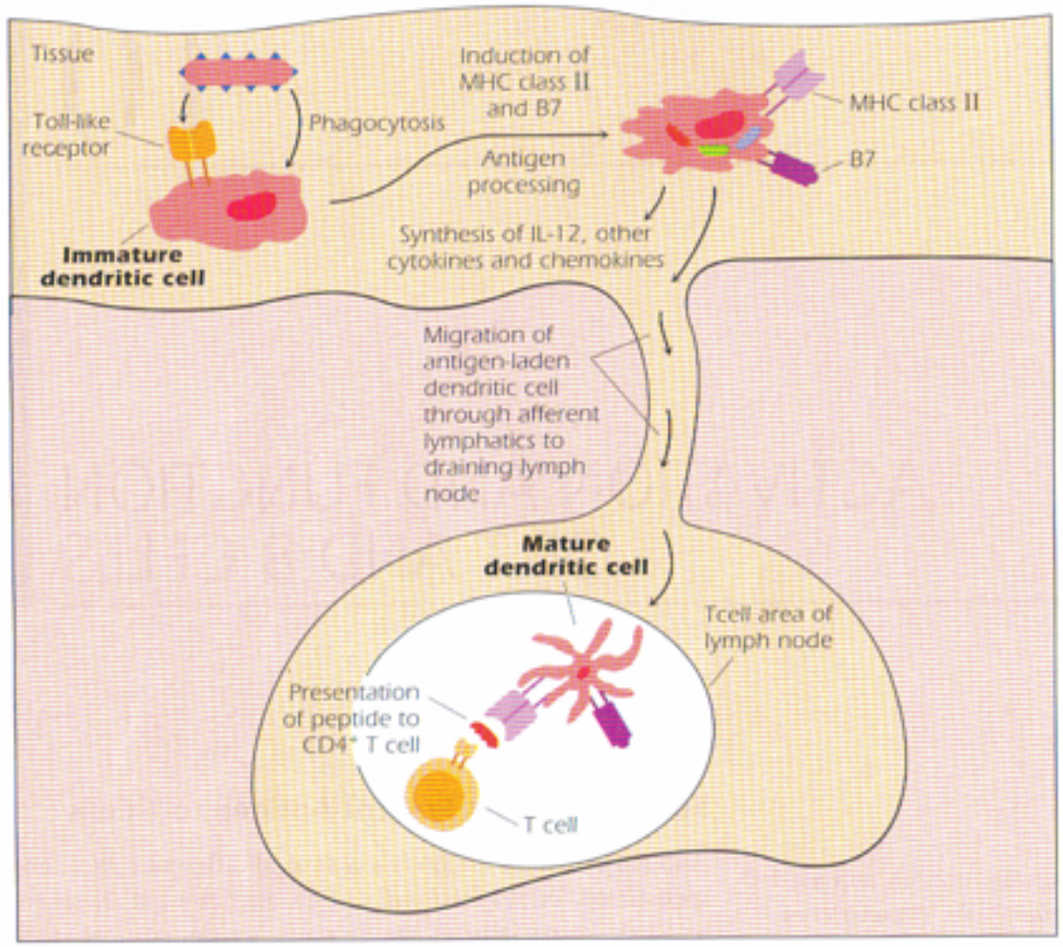
What is Vaccine?

- A vaccine is a non-pathogenic or attenuated antigen that mimics a particular pathogen in order to elicit an immune response.
- The goal of a vaccine is to establish immunity against that particular pathogen.

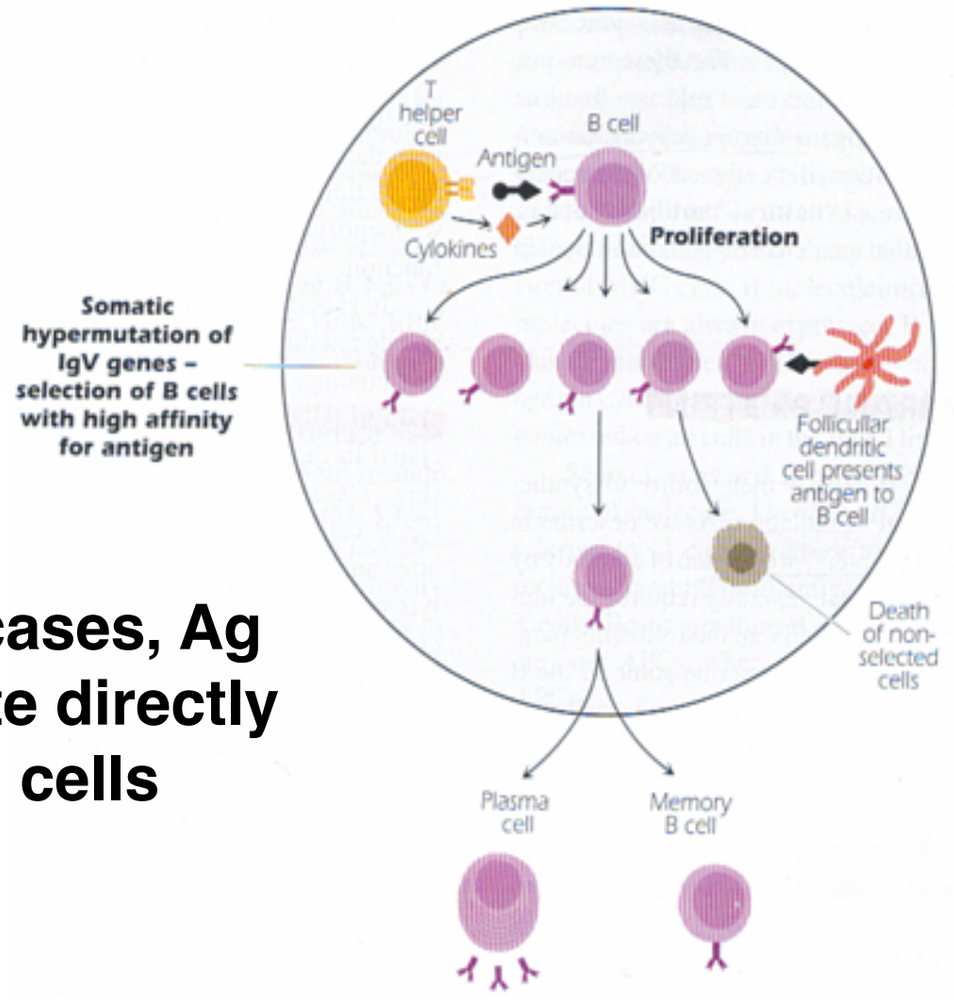
Principles of vaccination strategies

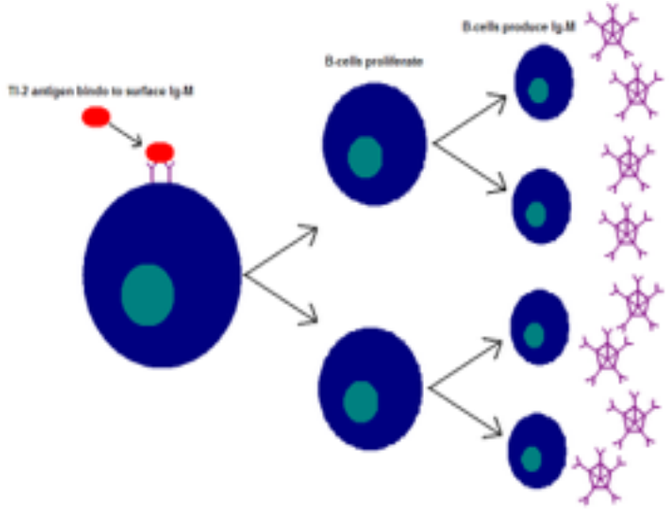
- **Purified antigens --> protective antibody**
  - **Not effective against microbes that mutate antigenic proteins or hide inside infected cells**
- **Attenuated microbes, viral vectors for antigens --> antibodies + CMI**
  - **Safety concerns**
- **Difficult to induce effective CTL responses with purified protein antigens**
  - **Potential of plasmid DNA vaccines**

- **Clinically usable adjuvants**



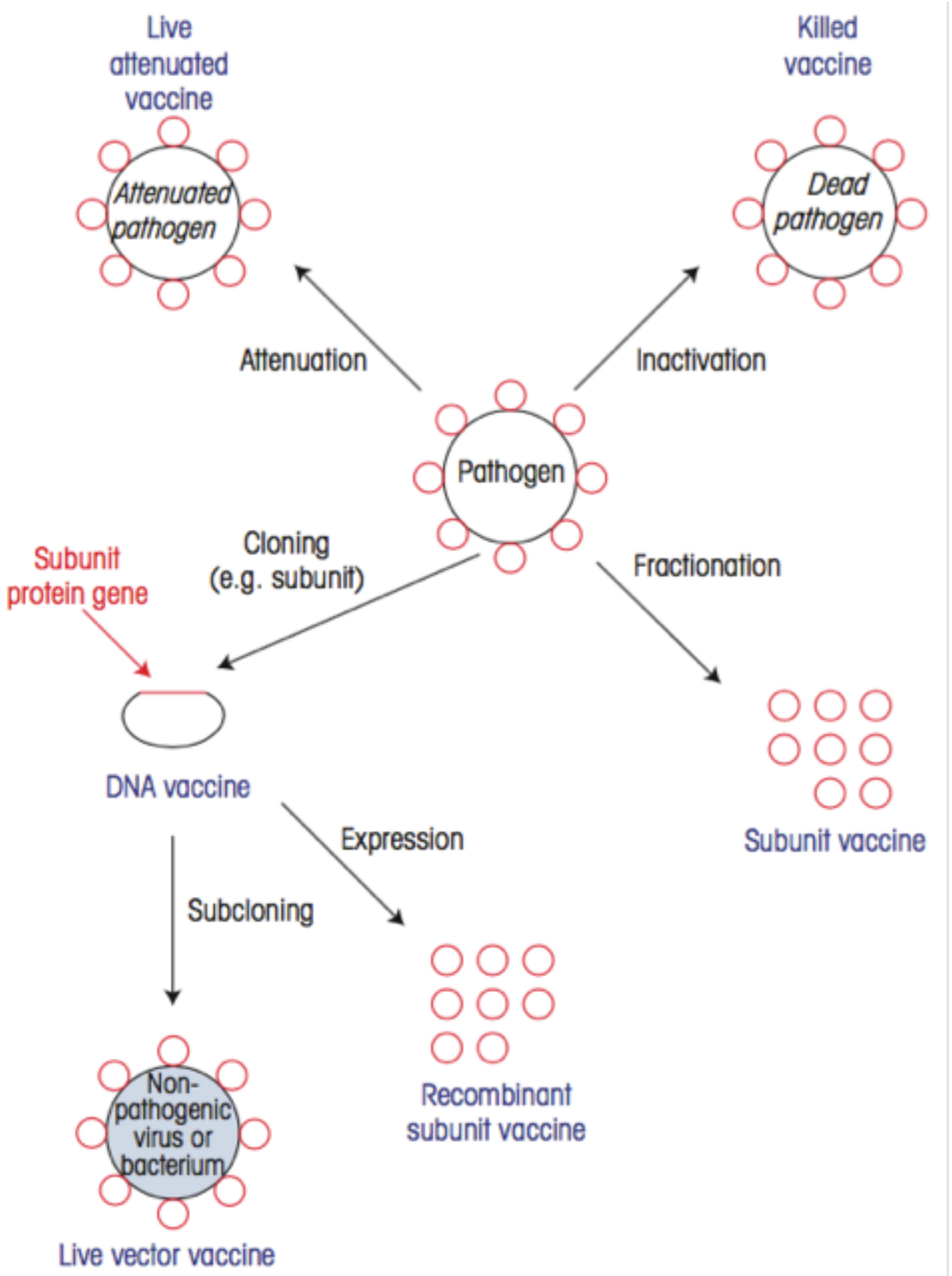
Antigens
↓
APC: DC
↓
T cells—> B cells





Some cases, Ag stimulate directly to B cells

Vaccine: How it works



Approaches for vaccines

Peter J. Delves, Seamus J. Martin, Dennis R. Burton and Ivan M. Roitt, Essential for Immunology. 12 ed. 2011

Table 13.2. Factors required for a successful vaccine.

Factor	Requirements
Effectiveness	Must evoke protective levels of immunity: at the appropriate site of relevant nature (Ab, Tc, Th1, Th2) of adequate duration
Availability	Readily cultured in bulk or accessible source of subunit
Stability	Stable under extreme climatic conditions, preferably not requiring refrigeration
Cheapness	What is cheap in the West may be expensive in developing countries but the Bill and Melinda Gates Foundation and governments help
Safety	Eliminate any pathogenicity

Peter J. Delves, Seamus J. Martin, Dennis R. Burton and Ivan M. Roitt, Essential for Immunology. 12 ed. 2011

Vaccine Types

1. Killed whole organisms

In crude approach, the vaccine is made from the entire organism, killed to make it harmless. The typhoid vaccine is an example.

2. Attenuated organisms

Here, the organism has been cultured so as to reduce its pathogenicity, but still retain some of the antigens of the virulent form. The Bacillus Calmette-Gu?rin (BCG) is a weakened version of the bacterium that causes tuberculosis in cows. BCG is used as a vaccine against tuberculosis in many European countries but is rarely used in the U. S.

3. Toxoids

In some diseases, **diphtheria** and **tetanus** are notorious examples, it is not the growth of the bacterium that is dangerous, but the protein toxin that is liberated by it. Treating the toxin with, for example, formaldehyde, denatures the protein so that it is no longer dangerous, but retains some epitopes on the molecule that will elicit protective antibodies.

4. Surface molecules

Antibodies are most likely to be protective if they bind to the surface of the invading pathogen triggering its destruction. Several vaccines employ purified surface molecules.

5. Inactivated virus

Like killed bacterial vaccines, these vaccines contain whole virus particles that have been treated (again, often with formaldehyde) so that they cannot infect the host's cells but still retain some unaltered epitopes. The Salk vaccine for polio (IPV) is an example.

Vaccine Types

6. Attenuated virus

In these vaccines, the virus can still infect but has been so weakened that it is no longer dangerous. The measles, mumps, and rubella ("German measles") vaccines are examples. The Sabin oral polio vaccine (OPV) is another example. 6. Attenuated virus

7. DNA Vaccine

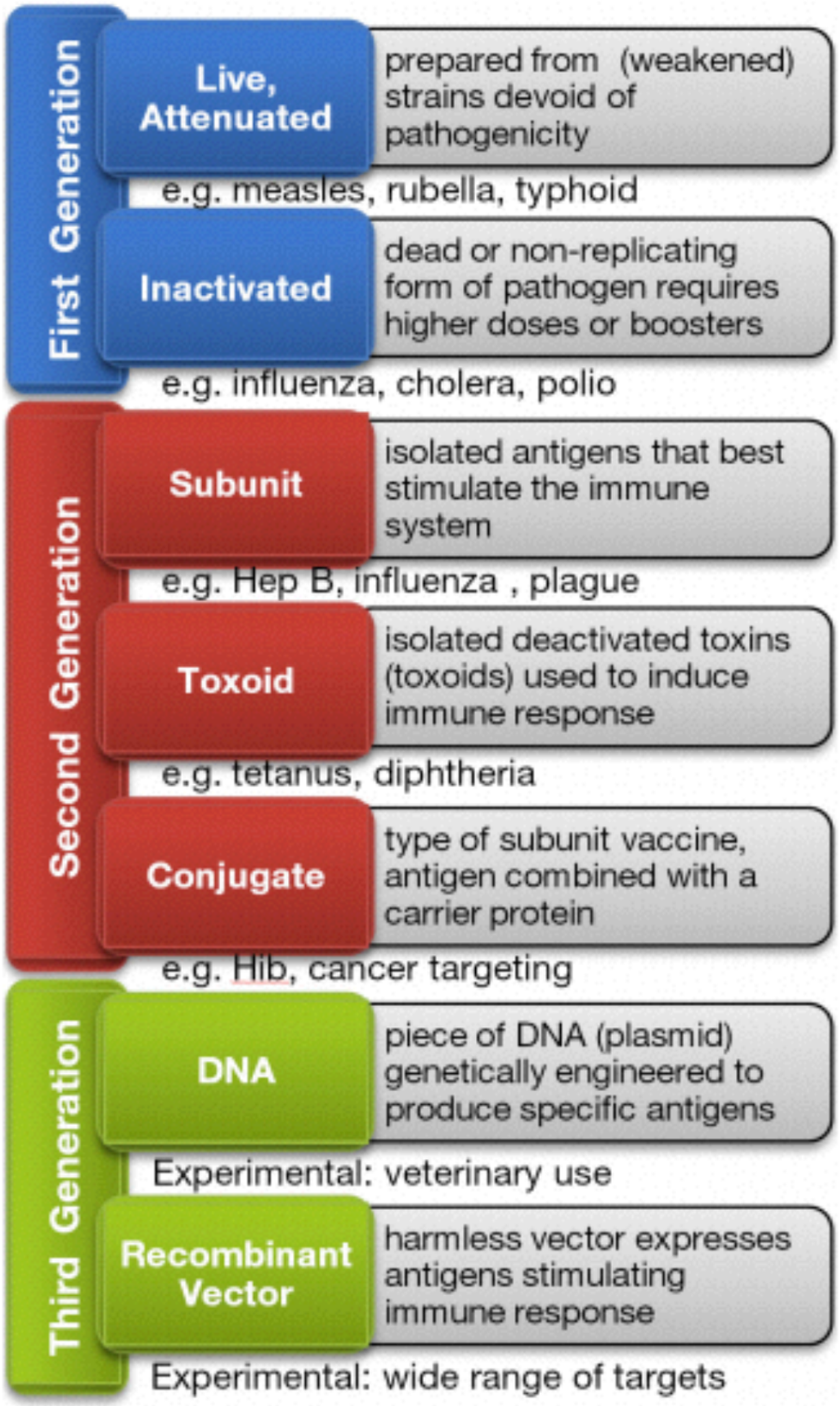
With DNA vaccines, the subject is not injected with the antigen but with DNA encoding the antigen. The DNA is incorporated in a plasmid containing

DNA sequences encoding one or more protein antigens or, often, simply epitopes of the complete antigen(s); DNA sequences incorporating a promoter that will enable the DNA to be efficiently transcribed in the human cells. Sometimes DNA sequences encoding costimulatory molecules sequences that target the expressed protein to specific intracellular locations (e.g., endoplasmic reticulum) are included as well.

The DNA vaccine can be injected into a muscle just as conventional vaccines are.

In contrast to conventional vaccines, DNA vaccines elicit cell-mediated — as well as antibody-mediated — immune responses.

Type of vaccines



Close to Nature. Virus: easy, Bacterial: difficult

Killed, Easy, safe and stable.

Only epitopes or antigens those recognized by T or B cells. Low side effects.

Inactivate toxins by treating them with formalin, a solution of formaldehyde and sterilized water.

Carbohydrate antigens with proteins

Naked DNA (or with particles) contained genes stimulate immune responses. (Herpes, West Nile, Influenza)

Use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. "Vector"

From: Ilchmann et al, Harvard Sussex Program project examining the role of S&T reviews within the BWC with modifications.

Vaccine concepts

- Extracellular bacteria or toxin
  - –Antibodies or B cells
  - –Blocking antibodies
  - –Complements
  - –Need T cells for class switching.
  - –Need conformational epitopes (B cells)
- Intracellular bacteria or virus
  - –CMI or T cells
  - –CTL
  - –Activated macrophages
  - –Cytokines

How to find the candidate antigens?

- Conventional approaches
  - –Antigens selected from specific criteria (surface molecules and accessible).
  - –Derived from basic investigations.
- Post genomic approaches
  - –Reverse vaccinology

Antigen selections (1)

- Accessible to immune cells
  - –Toxin, surface antigens
- Possess T and B cell epitopes
- Immunogenic in human (present or not present in nature)
- Important for survival and diseases causation of pathogens.
  - –present in all/most of disease isolates.
  - –loss/alteration of disease survival

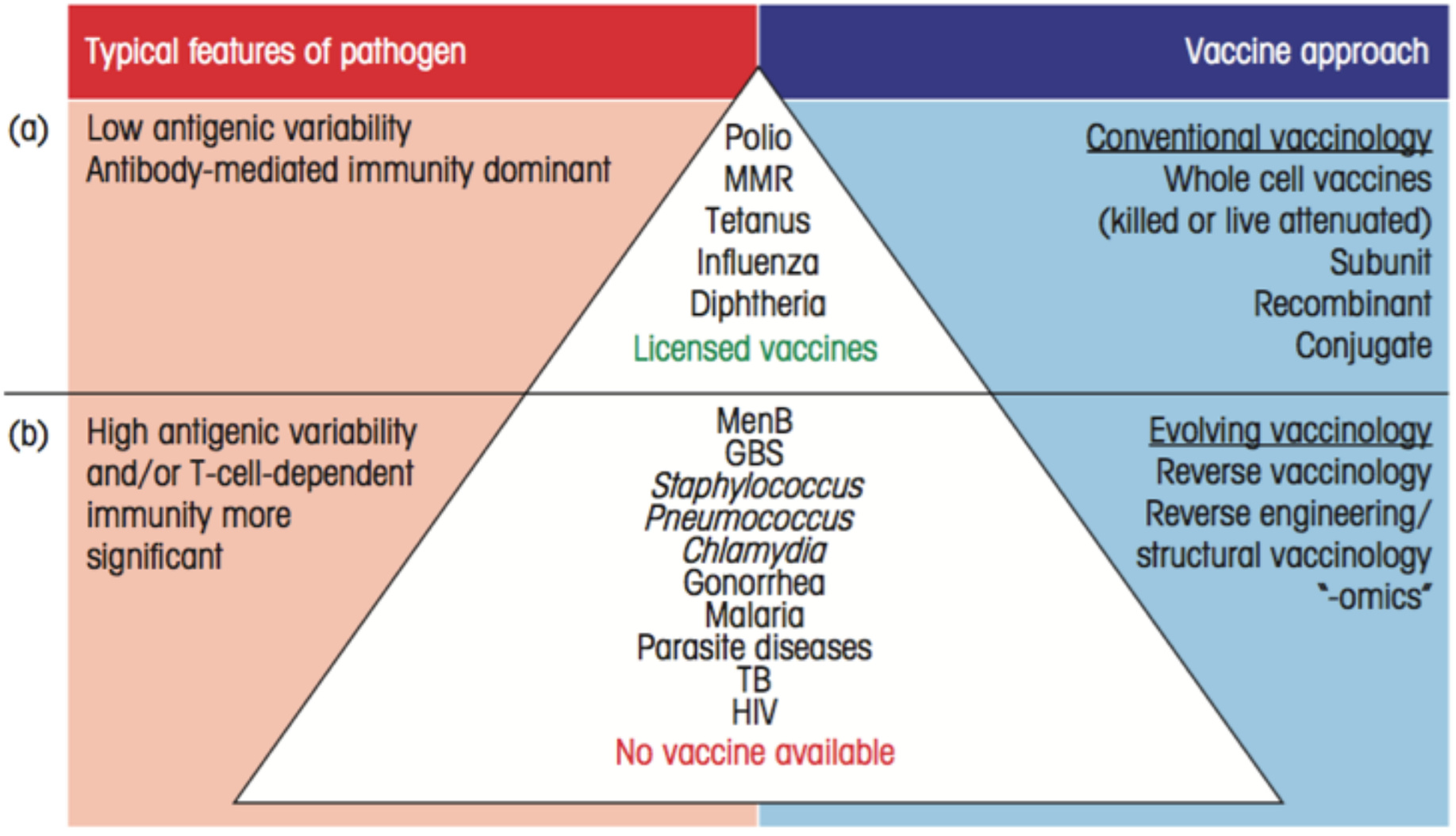
Antigen selections (2)

- Contain epitopes common to all/most isolates
- Target selection should based on
  –Pathogenesis of diseases

Efficacy of vaccines

- **Vaccines have been useful for generating protective antibodies, but so far, not for generating effective cell-mediated immunity**

- **Vaccines work best against microbes that:**
  - **Do not vary their antigens**
  - **Do not have animal reservoirs**
  - **Do not establish latent infection within host cells**
  - **Do not interfere with the host immune response**



Schematic view of conventional vaccinology and evolving vaccinology in the post-genome era.

Peter J. Delves, Seamus J. Martin, Dennis R. Burton and Ivan M. Roitt, Essential for Immunology. 12 ed. 2011

Subunit vaccines

- Whole organisms have a multiplicity of antigens, some of which are not protective, may induce hypersensitivity or might even be immunosuppressive.
- It makes particular sense in these cases to use purified components or those made recombinantly.
- Toxoids, inactivated toxins, are effective as vaccines in preventing illness due to some bacterial agents.
- The hepatitis B surface antigen particle is a classic example of an effective subunit viral vaccine.
- Many successful bacterial vaccines target glycans on the surface of the organism using glycoconjugate preparations.
- DNA encoding the proteins from a pathogen can be injected directly into muscle injected directly into muscle to generate the proteins *in situ* and produce immune responses. The advantages are stability, ease of production and cheapness. The method has not been as effective in humans as in mice but newer developments such as a DNA prime with a protein or vector boost are promising.

Killed organisms as vaccines

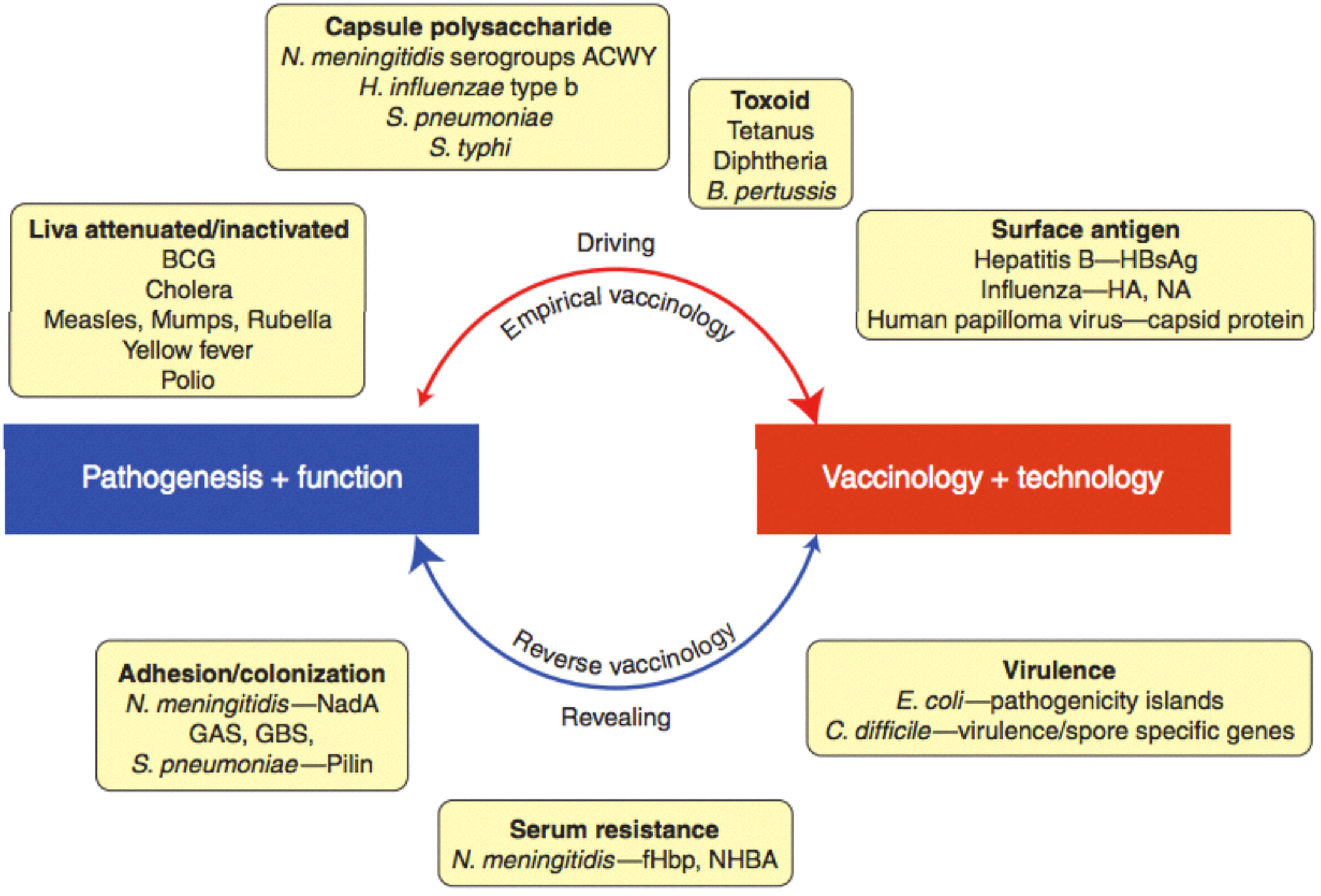
- Killed bacteria and viruses have been widely used as effective vaccines.

Live attenuated organisms

- The advantages include the larger antigen dose typically provided by a replicating organism, the tendency to elicit better cellular immunity and the generation of an immune response at the site of the natural infection.
- Nonpathogenic vectors such as adenovirus, attenuated fowlpox and modified vaccinia Ankara virus can serve as Trojan horses for genes from pathogenic organisms that are difficult to attenuate.
- BCG is a good vehicle for antigens requiring CD4 T-cell immunity and salmonella constructs may give oral and systemic immunity. Intranasal immunization is gaining popularity.
- The risk with live attenuated organisms is reversion to the virulent form and danger to immunocompromised individuals.

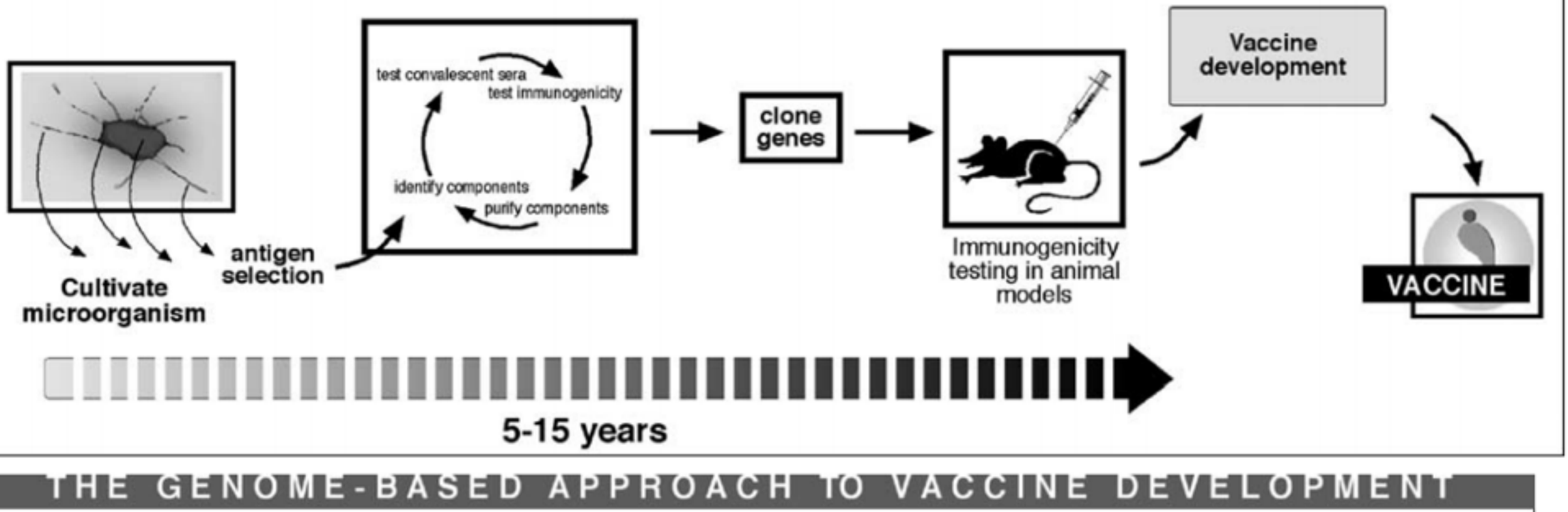
Newer approaches to vaccines

- The rise of genomics has been crucial in allowing a rational approach to the identification of many more bacterial vaccine targets. “Reverse vaccinology” has been successfully applied to the development of a MenB vaccine.
- Highly variable pathogens such as HIV and HCV present particular problems to vaccine design in that they require the elicitation of broadly protective immune responses. Here molecular approaches are being adopted to describe how broadly neutralizing antibodies interact with their targets and use the information to rationally design vaccine candidates.

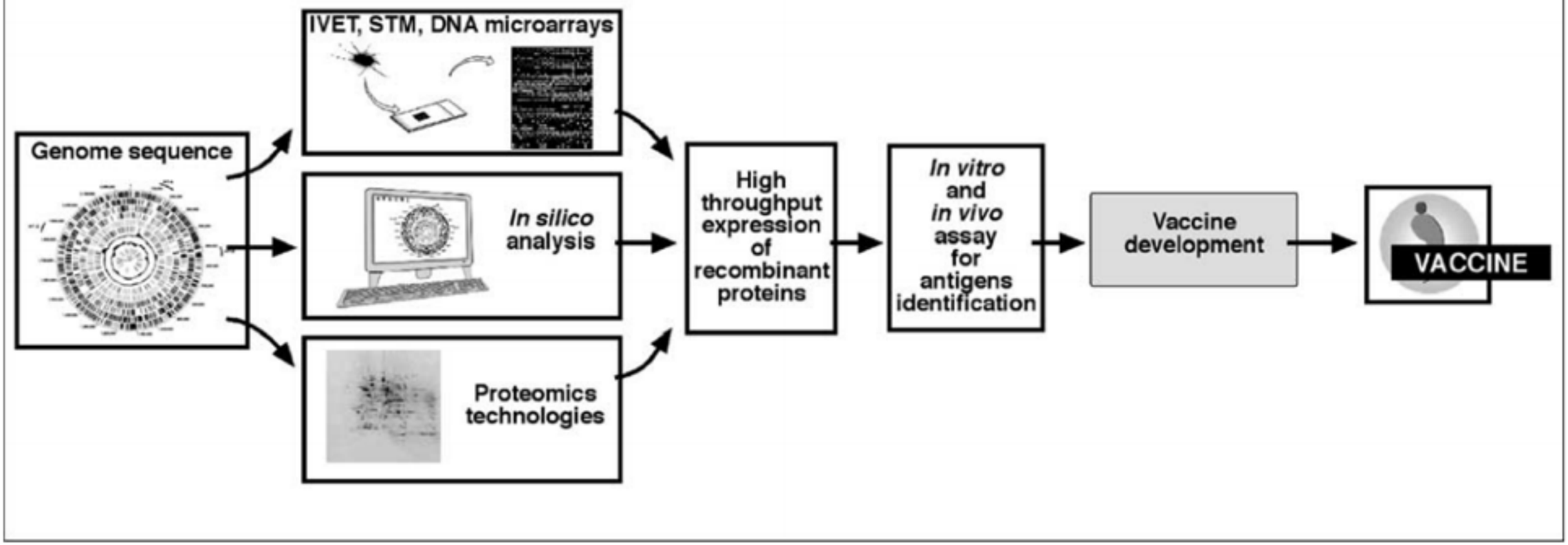


Delany, et al, *Spring Harb Perspect Med 2013.*

CONVENTIONAL VACCINOLOGY



THE GENOME-BASED APPROACH TO VACCINE DEVELOPMENT



J. Adu-Bobie et al. / Vaccine 21 (2003)

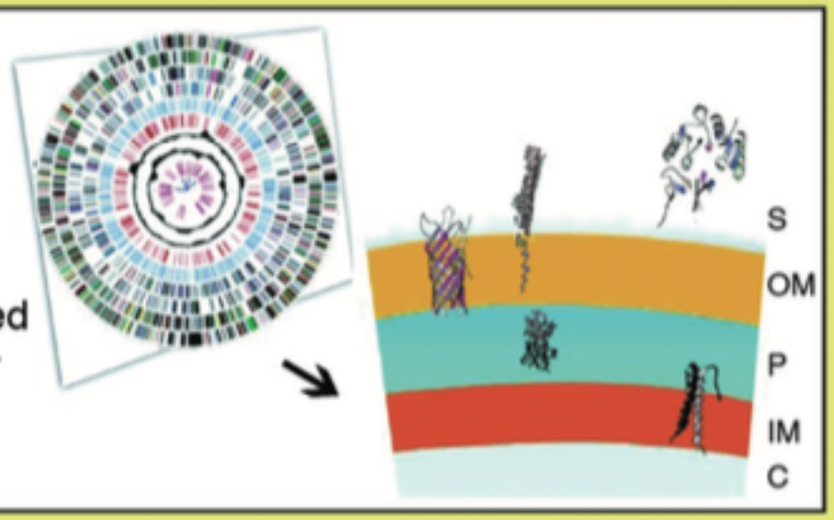
MenB vaccine development

Preclinical reverse vaccinology

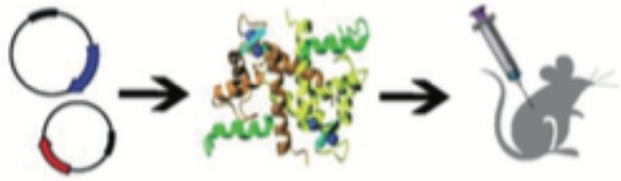
1998

2158 ORFs identified in the MenB MC58 genome

570 ORFs predicted to encode surface-expressed or secreted proteins



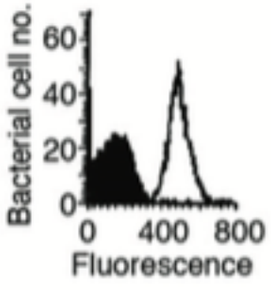
350 proteins expressed in *E. coli*, purified and used to immunize mice



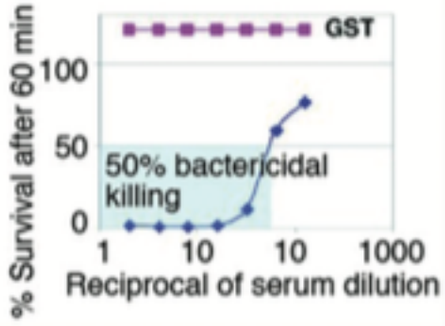
91 novel surface-exposed proteins identified

28 novel proteins induced bactericidal antibodies

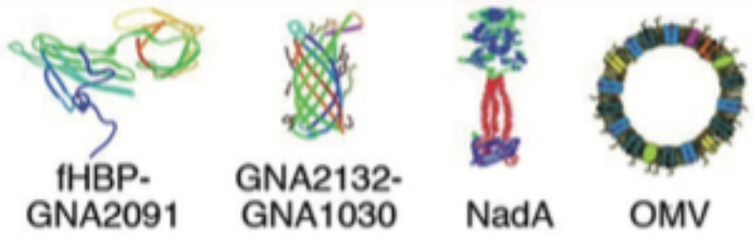
FACS

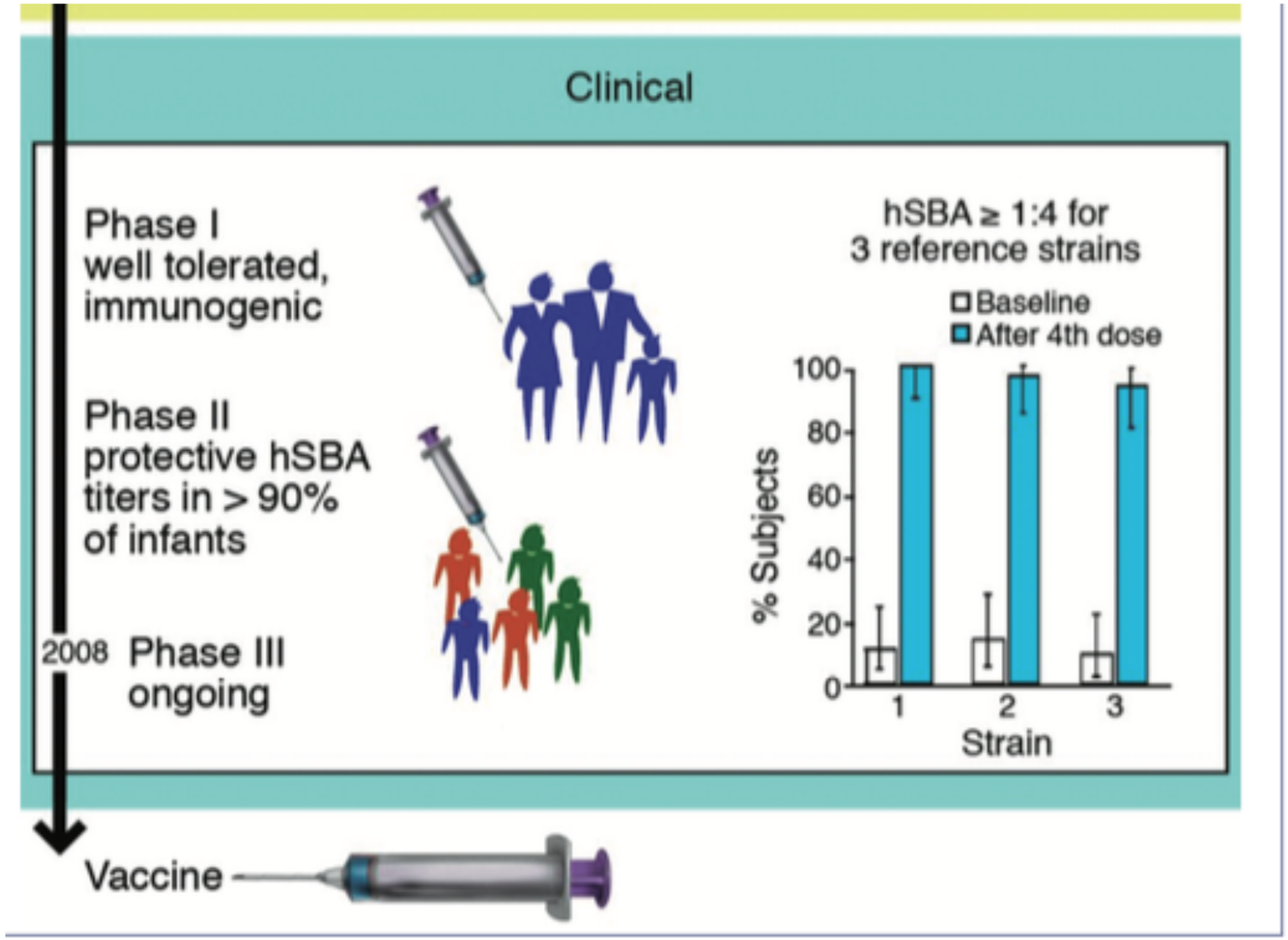


Serum bactericidal activity



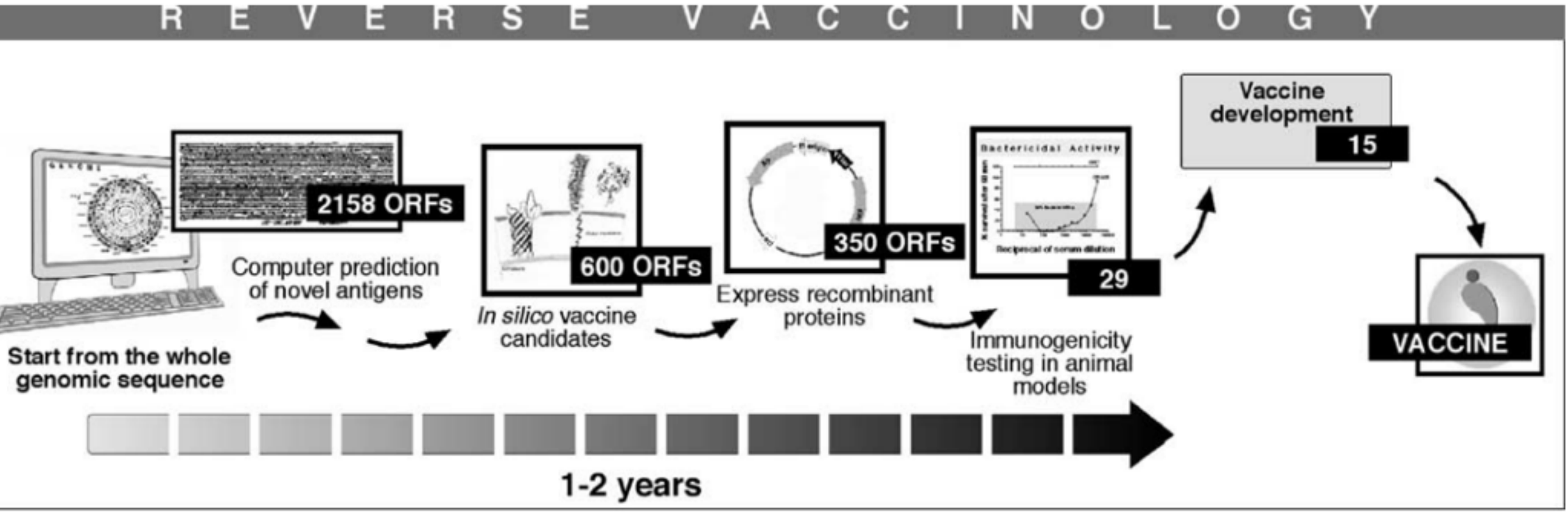
5 proteins selected for use in a four-component vaccine formulation





Peter J. Delves, Seamus J. Martin, Dennis R. Burton and Ivan M. Roitt, Essential for Immunology. 12 ed. 2011

Neisseria meningitidis serogroup B



J. Adu-Bobie et al. / Vaccine 21 (2003)

Comparison of conventional and genomic approaches to vaccine development

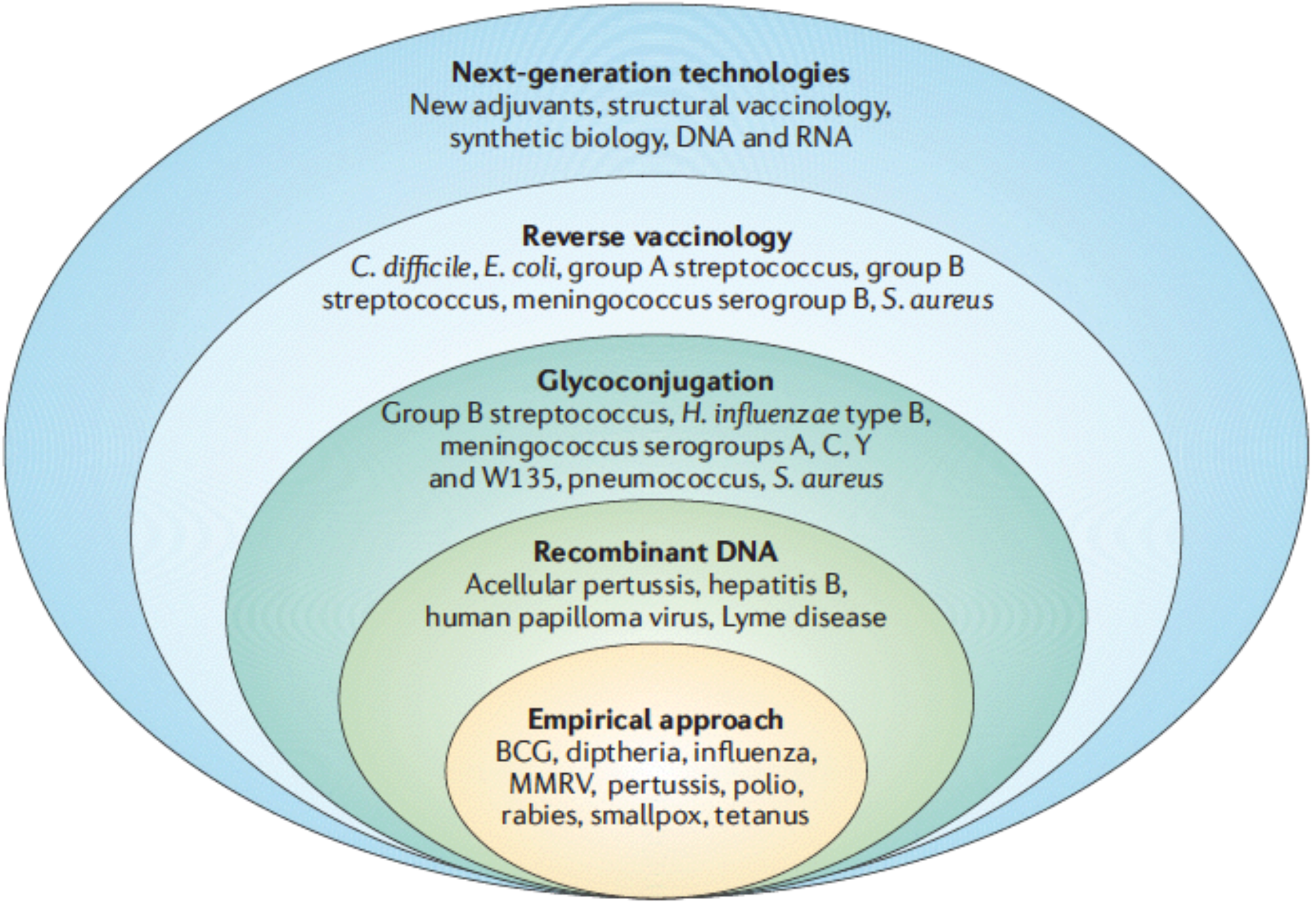
Conventional vaccinology	Reverse vaccinology
Most abundant antigens during disease	All antigens immunogenic during disease
Antigens expressed in vitro	Antigens expressed in vitro and in vivo
Cultivable microorganism	Antigens even in non-cultivable microorganisms
Animal models essential	Animal models essential
Correlates of protection useful	Correlates of protection essential
Structural components of microorganism	Non-structural components, including early proteins of viruses
	Correct folding in recombinant expression important
	High throughput expression/analysis important
Polysaccharides may be used as antigens	Non-proteic antigens cannot be used

J. Adu-Bobie et al. / Vaccine 21 (2003)

Problems with antigen selections

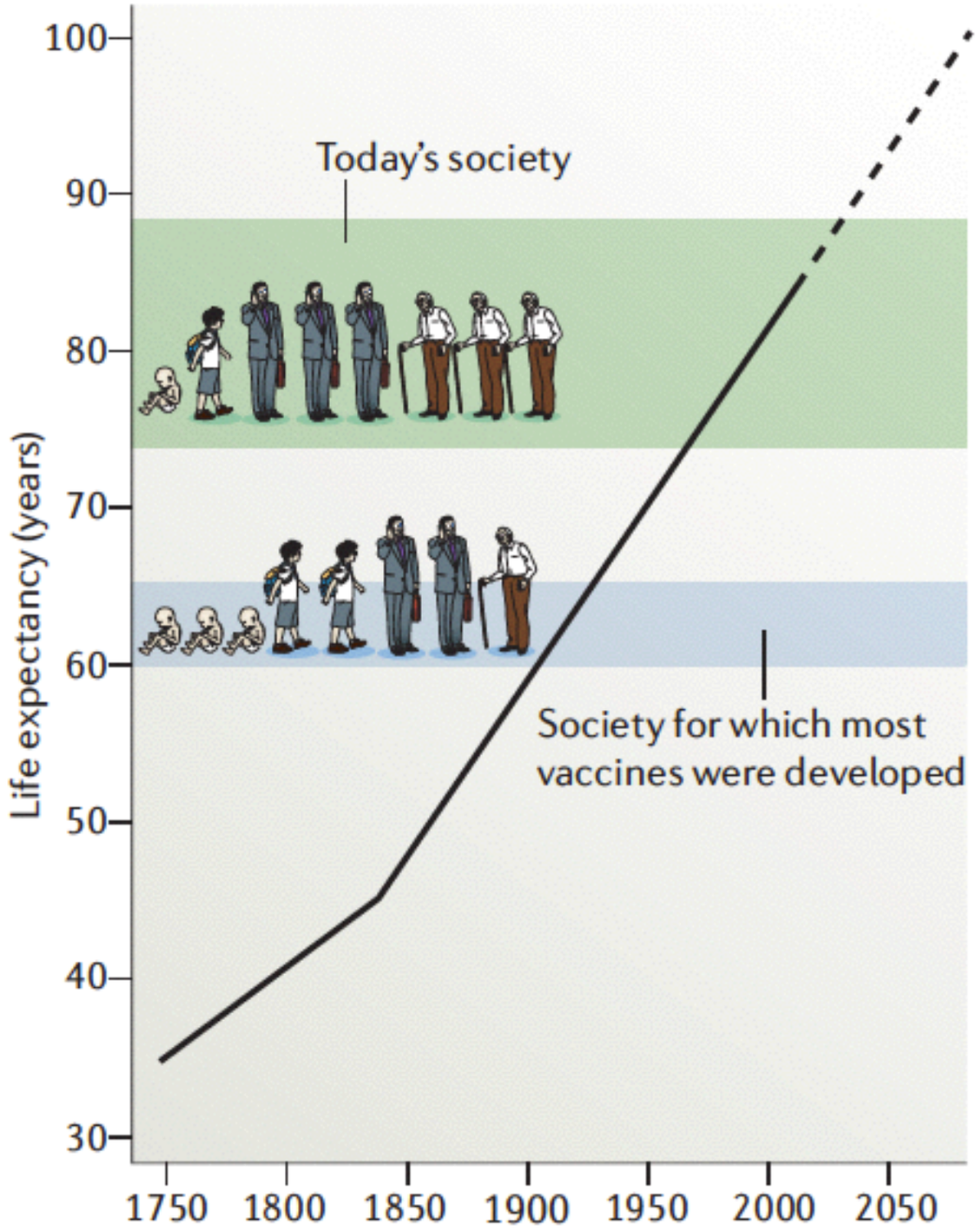
- **No real comparison between different antigens.**
- **Limitation in ability to predict efficacy.**
- **Lack of adequate infection models.**
- **Function assay do not reflect in vivo conditions.**
- **Do not know the antigen variation or loss of antigens.**

Technology for Vaccines



Rappuoli R. et al, Nature Rev Immunol, 2011

Vaccines in the 21st century



increase life expectancy

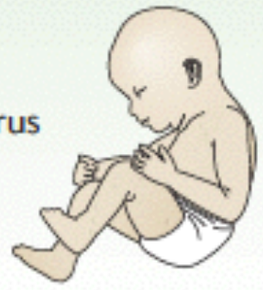
Rappuoli R. et al, Nature Rev Immunol, 2011

Different age groups need different vaccinations

a Age groups

Pre-birth
- Cytomegalovirus
- Group B streptococcus
- Hepatitis B virus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Pertussis
- Respiratory syncytial virus
- Tetanus

Infants and children
- Diphtheria
- Group A streptococcus
- *H. influenzae* type b
- *Helicobacter pylori*
- Hepatitis A virus
- Hepatitis B virus
- Inactivated poliovirus vaccine
- Influenza virus
- Measles
- Meningococcus serogroups A, B, C, Y and W135
- Mumps
- Pertussis
- Pneumococcus
- Respiratory syncytial virus
- Rotavirus
- Rubella
- Tetanus
- Varicella zoster virus



Adolescents
- Cytomegalovirus
- Diphtheria, tetanus acellular pertussis
- Epstein–Barr virus
- Herpes simplex virus
- Human papilloma virus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Parvovirus B19

Adults
- Diphtheria
- Hepatitis B virus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Pertussis
- Respiratory syncytial virus
- Tetanus

Elderly

Recurrent infections:
- Group B streptococcus
- Influenza virus
- Meningococcus serogroups A, B, C, Y and W135
- Pneumococcus
- Respiratory syncytial virus
- Varicella zoster virus

Antibiotic resistance:
- *Acinetobacter baumannii*
- *C. difficile*
- *Candida* spp.
- Enterotoxigenic *E. coli*
- *Klebsiella pneumoniae*
- *P. aeruginosa*
- *S. aureus*

Cancer:
- Breast cancer
- Colorectal cancer
- Prostate cancer

Rappuoli R. et al, Nature Rev Immunol, 2011

Some characteristics of an ideal vaccine

- Shows an impeccable safety profile in all populations, including young infants, the elderly and immunocompromised subjects (such as HIV–positive subjects)
- Elicits a high level of long-lived efficacy, including in young infants and the elderly
- Requires only a single dose (or at most two doses spaced fairly close together) to confer protection
- Stimulates protection within 2 weeks of administration
- Administrable without a needle and syringe; that is, orally, nasally or transcutaneously or with a needle-free injection device
- Administrable in combination with (in the same formulation) or concomitantly (coadministered) with other vaccines
- Can be manufactured in large scale and with quality control by relatively uncomplicated and economical processes
- Amenable to production in formulations that are resistant to high and low temperatures and therefore free from strict storage requirements

Myron M Levine & Marcelo B Sztein. Nature Immunology, 2004

Adjuvants

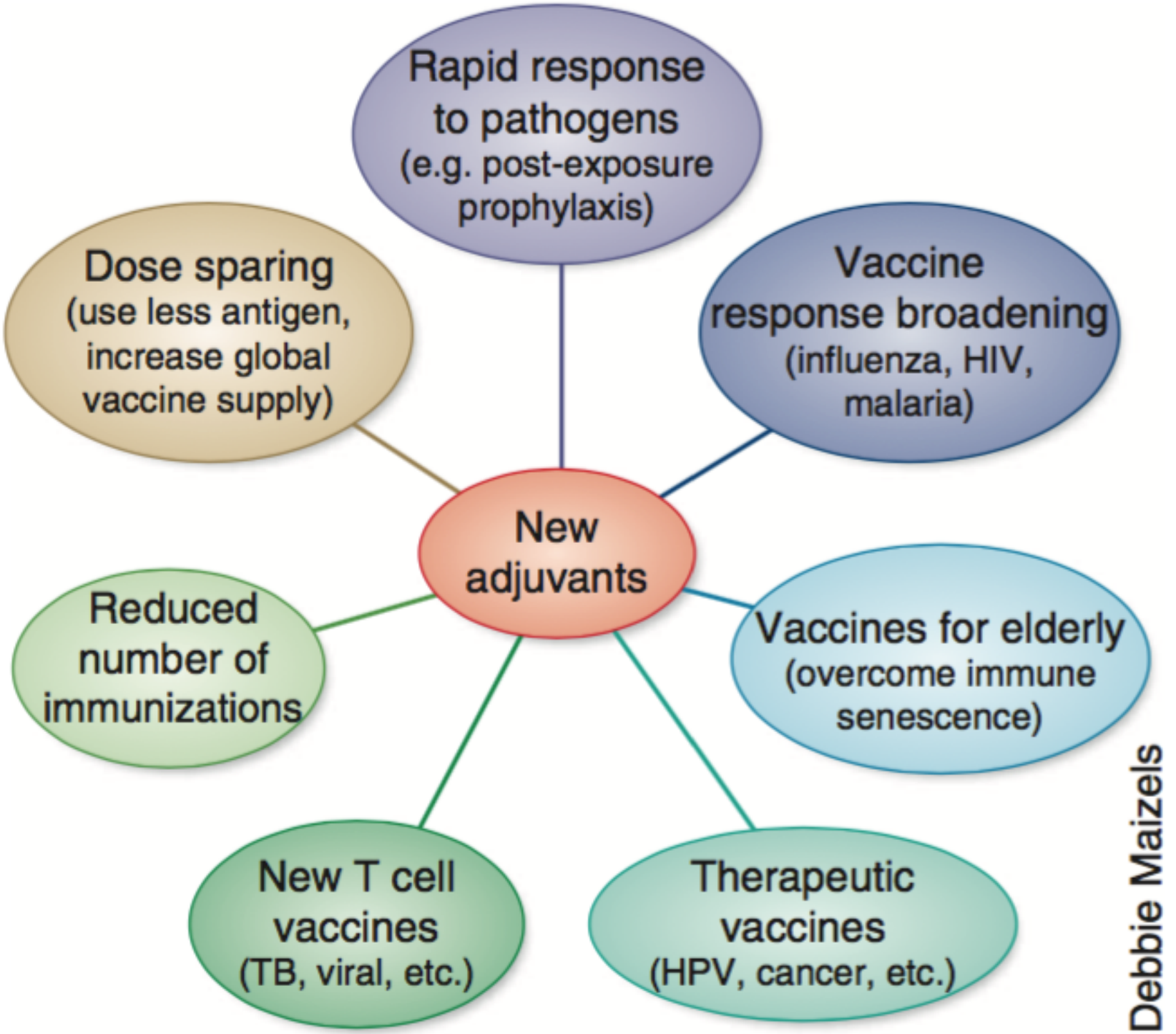
Surasak Wongratanacheewin, Ph.D
Dean, Graduate School, KKU
Microbiology, Faculty of Medicine, KKU
sura_wng@kku.ac.th

What is an adjuvant?

- **Adjuvants are the substances that essential for enhancing and directing the adaptive immune response to vaccine antigens.**

- **They enhance the either innate or adaptive immune responses.**

- **This response is mediated by two main types of lymphocytes, B and T cells.**



Steven G Reed, Mark T Orr & Christopher B Fox Nature Medicine, 2013.

Year	Vaccine	Adjuvant and mechanism	Scientific findings
1885	Rabies	ssRNA TLRs 7 and 8	
1886			Briegen describes endotoxin
1889			Coley shows tumor necrosis with bacterial extracts
1911	Typhoid	LPS, DNA TLRs 1, 2, 4, 5, 6 and 9	
1916		Lipovaccine	More durable immune response to typhoid vaccine
1921	BCG for TB	DNA, lipoprotein TLRs 1, 2, 6 and 9	
1926		Aluminum salts	Enhanced antibody responses to diphtheria vaccine
1937		Incomplete Freund's adjuvant (IFA) (water-in-oil emulsion)	Enhanced cellular and antibody responses to TB
1942	Diphtheria, pertussis and tetanus	LPS, DNA TLRs 1, 2, 4, 5, 6 and 9	
1949	Whole-cell influenza	ssRNA TLRs 7 and 8	
1955	Inactivated polio vaccine	ssRNA TLRs 7 and 8	
1966			LPS structure determined
1979			Ribi makes detoxified endotoxin MPL
1991	Hepatitis A		MPL tested in clinic

Steven G Reed, Mark T Orr & Christopher B Fox Nature Medicine, 2013.

1996			TLRs discovered
1997	Fluad	MF59 (oil-in-water emulsion)	
1997	Epaxal (for hepatitis A) Inflexal (for influenza)	Virosome	
1998			LPS shown to be TLR ligand
2004	Invivac (for influenza; Europe)	Virosome	
2005	Fendrix (for hepatitis B; Europe)	MPL Defined TLR4	
2007–2009	Pandemic influenza vaccines (Europe)	MF59, AS03 (oil-in-water emulsion)	
2009	Cervarix (for HPV16 and HPV18; USA)	MPL Defined TLR4	

Steven G Reed, Mark T Orr & Christopher B Fox Nature Medicine, 2013.

Mechanism of Adjuvants

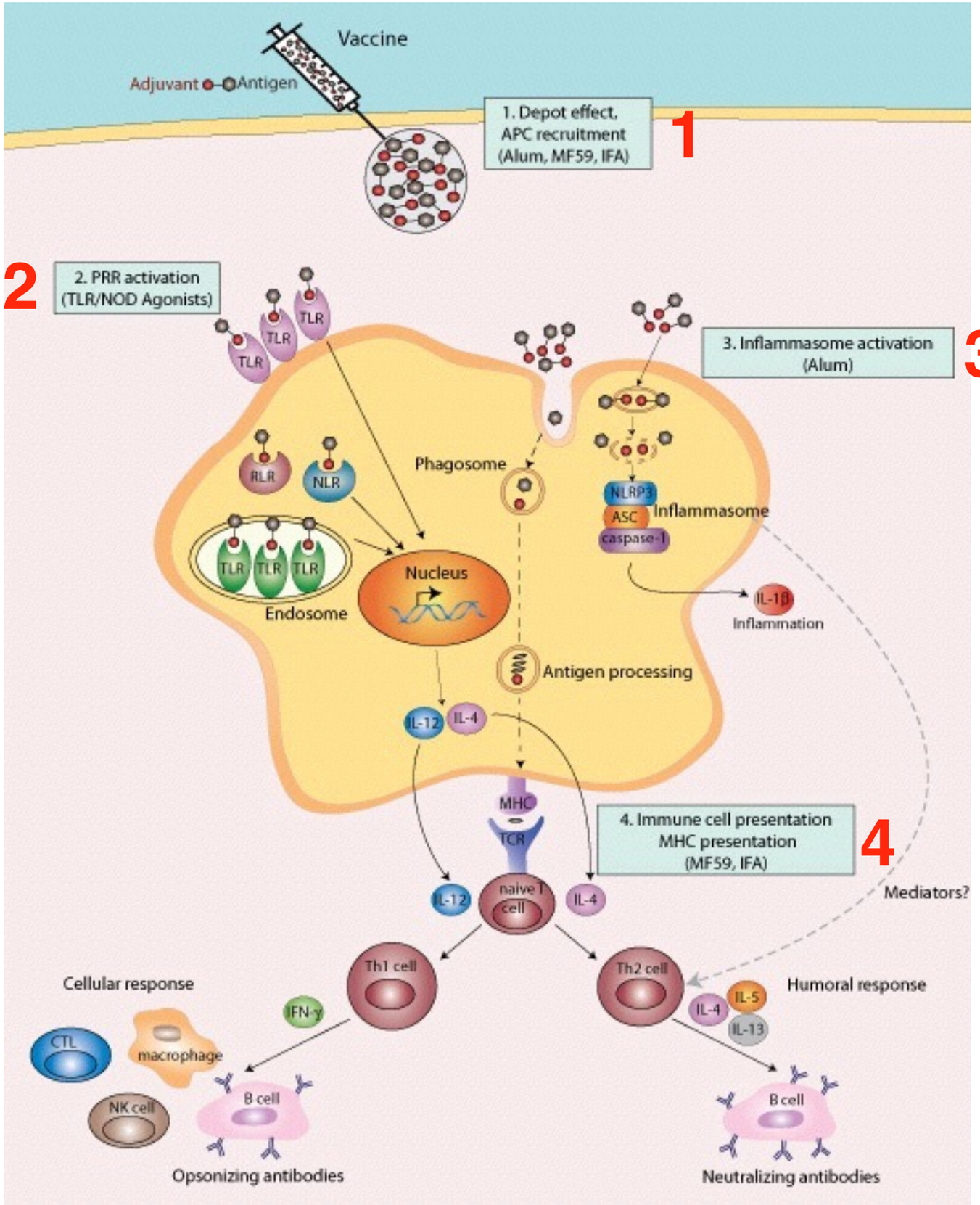
1. **Adjuvants may exert their effects through different mechanisms.**
2. **Some adjuvants, such as alum and emulsions (e.g. MF59®), function as delivery systems.**
3. **Sone providing slow release in order to continue the stimulation of the immune system.**
4. **Some enhance the antigen persistence at the injection site and increase recruitment and activation of antigen presenting cells (APCs). Some adjuvants are also capable of directing antigen presentation by the major histocompatibility complexes (MHC) [1].**
5. **Other adjuvants, essentially ligands for pattern recognition receptors (PRR), act by inducing the innate immunity, predominantly targeting the APCs and consequently influencing the adaptative immune response.**

- **Toll-like receptors (TLRs),**
- **NOD-like receptors (NLRs),**
- **RIG-I-like receptors (RLRs) and**
- **C-type lectin receptors (CLRs).**
- **They signal through pathways that involve distinct adaptor molecules leading to the activation of different transcription factors. These transcription factors (NF-κB, IRF3)**
- **Activation of some members of the NLR family, such as NLRP3 and NLRC4,**

Table I. Selective List of Different Classes of Adjuvants That Have Been Evaluated for Enhancing Immune Responses to Vaccines

Mineral salts	Aluminum hydroxide*
Aluminum phosphate*	
Calcium phosphate*	
Immunostimulatory adjuvants	Cytokines e.g., IL-2, IL-12, GM-CSF
Saponins e.g., QS21	
MDP derivatives	
Bacterial DNA (CpG oligos)	
LPS	
MPL and synthetic derivatives	
Lipopeptides	
Lipid particles	Emulsions e.g., Freund's, SAF, MF59*
Liposomes	
Virosomes*	
Iscoms	
Cochleates	
Particulate adjuvants	PLG microparticles
Poloxamer particles	
Virus-like particles	
Mucosal adjuvants	Heat labile enterotoxin (LT)
Cholera toxin (CT)	
Mutant toxins e.g., LTK63 and LTR72	
Microparticles	
Polymerized liposomes	
Chitosan	

Manmohan Singh and Derek T. O'Hagan,2002



Mechanisms of Adjuvants

Adjuvants

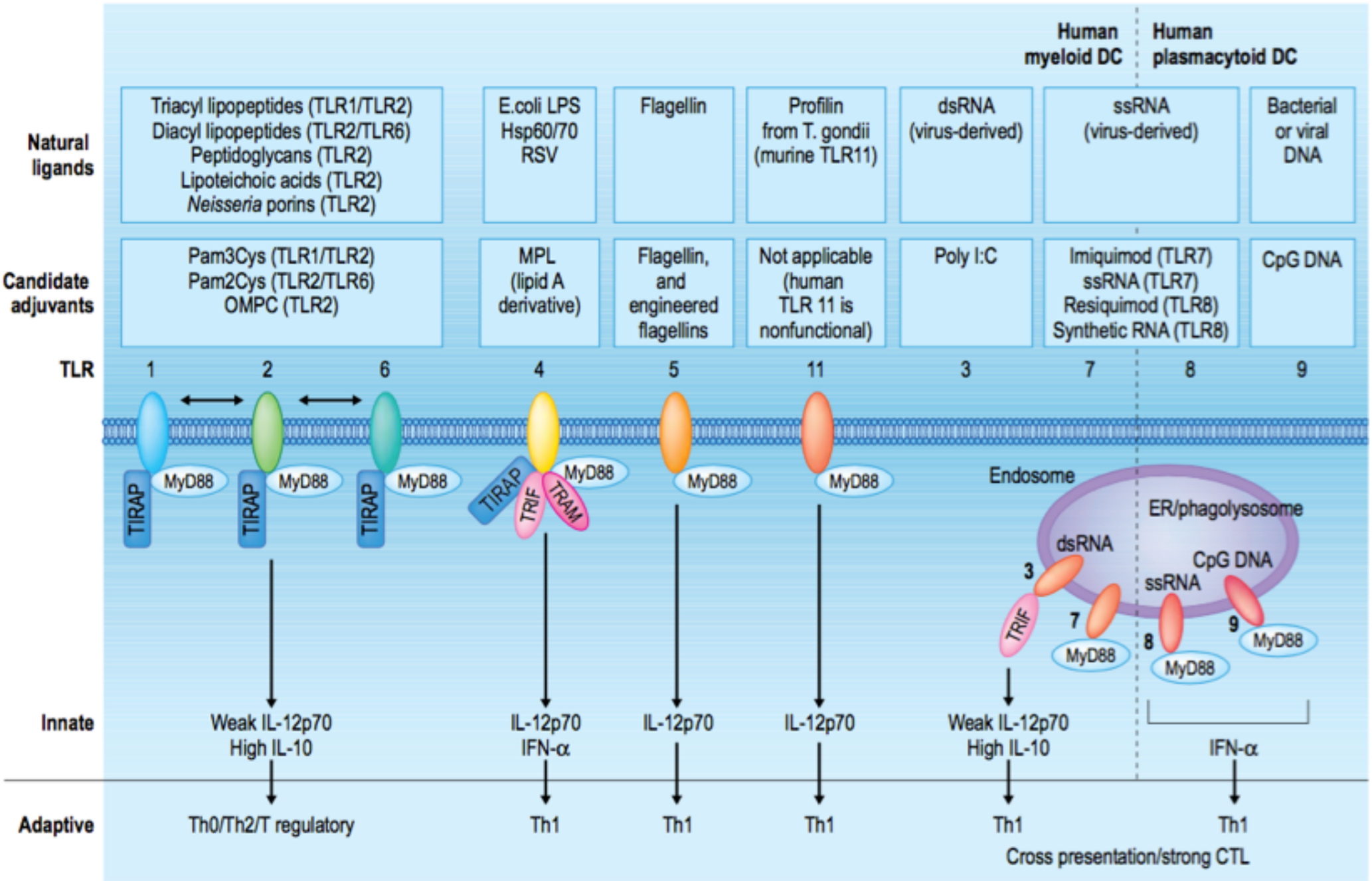
Adjuvant name (year licensed)	Adjuvant class	Components	Vaccines (disease)
Adjuvants licensed for use in human vaccines			
Alum* (1924)	Mineral salts	Aluminium phosphate or aluminium hydroxide	Various
MF59 (Novartis; 1997)	Oil-in-water emulsion	Squalene, polysorbate 80 (Tween 80; ICI Americas), sorbitan trioleate (Span 85; Croda International)	Fluad (seasonal influenza), Focetria (pandemic influenza), Aflunov (pre-pandemic influenza)
AS03 (GlaxoSmithKline; 2009)	Oil-in-water emulsion	Squalene, Tween 80, α-tocopherol	Pandremix (pandemic influenza), Prepandrix (pre-pandemic influenza)
Virosomes (Berna Biotech; 2000)	Liposomes	Lipids, hemagglutinin	Inflexal (seasonal influenza), Epaxal (hepatitis A)
AS04* (GlaxoSmithKline; 2005)	Alum-absorbed TLR4 agonist	Aluminium hydroxide, MPL	Fendrix (hepatitis B), Cervarix (human papilloma virus)
Vaccine adjuvants tested in humans but not licensed for use			
CpG 7909, CpG 1018	TLR9 agonist	CpG oligonucleotides alone or combined with alum/emulsions	–
Imidazoquinolines	TLR7 and TLR8 agonists	Small molecules	–
Poly I:C	TLR3 agonist	Double-stranded RNA analogues	–
Pam3Cys	TLR2 agonist	Lipopeptide	–
Flagellin	TLR5 agonist	Bacterial protein linked to antigen	–
Iscomatrix	Combination	Saponin, cholesterol, dipalmitoylphosphatidylcholine	–
AS01	Combination	Liposome, MPL, saponin (QS21)	–
AS02	Combination	Oil-in-water emulsion, MPL, saponin (QS21)	–
AF03	Oil-in-water emulsion	Squalene, Montane 80, Eumulgin B1 PH	–
CAF01	Combination	Liposome, DDA, TDB	–
IC31	Combination	Oligonucleotide, cationic peptides	–

Adjuvants in development for human vaccines

Adjuvants	Formulation	In pre-clinical or clinical trials
Montanides	Water-in-oil emulsions	Malaria (Phase I), HIV, cancer (Phase I/II)
Saponins (QS-21)	Aqueous	Cancer (Phase II), herpes (Phase I), HIV (Phase I)
SAF	Oil-in-water emulsion conteining squalene, Tween™ 80, Pluronic™ L121	HIV (Phase I – Chiron)
AS03	Oil-in-water emulsion conteining α-tocopherol, squalene, Tween™ 80	Pandemic flu (GSK)
MTP-PtdEtn	Oil-in-water emulsion	HSV
Exotoxins	*P. aeruginosa* E. coli heat-labile enterotoxin LT	*P. aeruginosa*, cystic fibrosis (AERUGEN – Crucell/Berna) ETEC (Phase II – Iornai Corp.)
ISCOMS	Phospholipids, cholesterol, QS-21	Influenza, HSV, HIV, HBV, malaria, cancer
TLR ligands		
MPL®-SE	Oil-in-water emulsion	*Leishmania* (Phase I/II – IDRI)
Synthetic Lipid A	Oil-in-water emulsion	Various indications (Avanti/IDRI)
MPL®-AF	Aqueous	Allergy (ATL); cancer (Biomira)
AS01	Liposomal	HIV (Phase I), malaria (ASO1, Phase III, GSK) cancer (Phase II/III, Biomira/MerckKGaA)
AS02	Oil-in-water emulsion containing MPL® and QS-21	HPV (Cervarix), HIV, tuberculosis, malaria (Phase III), herpes (GSK)
AS04	Alum + aqueous MPL®	HPV, HAV (GSK)
AS15	AS01 + CpG	Cancer therapy (GSK)
RC529	Aqueous	HBV, pneumovax
TLR-9	n/a	Cancer (ProMune – Coley/Pfizer)
(CpG)		HCV (ACTILON Coley)
TLR-9 ISS series	n/a	HIV, HBV, HSV, anthrax (VaxImmune Coley/GSK/Chiron) HBV (HEPLISAV, Phase III – Dynavax) Cancer (Phase II, Dynavax)
TLR-9 IMO series	n/a	Cancer (IMOxine, Phase I, Hybridon Inc.)
(YpG, CpR motif)	n/a	Cancer (IMO-2055, Phase II, Idera Pharm.) HIV (Remune, Phase I, Idera/IMNR)
TLR-9 agonist (MIDGE®)	n/a	Cancer (Phase I, Mologen AG)
TLR-7/8 (Imiquimod)	n/a	Melanoma (3M Pharmaceutical) HIV (prelinical), leishmaniasis

Reed, S.G et al, 2008

Adjuvants: as innate stimulators



Alan R. Shaw, Mark B. Feinberg , 2009

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