ImmunoScience Academy

Partnering for Education & Optimizing Treatment in ImmunoScience

www.immunoscienceacademy.be

Understanding immunoscience

A guide for specialists working with immunotherapies

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Module 1. Basic immunology

Click on a chapter below to start learning

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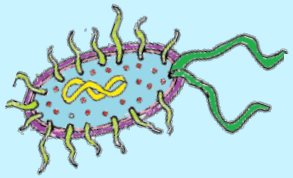
Introduction to the immune system

Module 1. Basic immunology

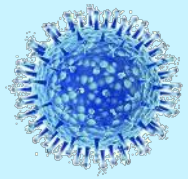
What is the immune system?

- A set of mechanisms that evolve to protect our organism against:

Pathogens



Bacteria

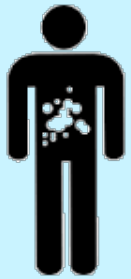


Viruses

Parasites

Harmful physical or chemical stimuli

Body's own cells during illness



- It comprises numerous sensors and effectors grouped into **innate** and **adaptive** immunity

PubMed Health 2019;4:11–22. Available from: https://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0072548/. Accessed October 2022.

Innate vs adaptive immunity

Feature[1–4]	Innate immunity	Adaptive immunity
Specificity	Broad, not fully specific to invading pathogen	Highly specific to the pathogen or threat
Memory	None	Yes, after exposure
Timing of response	Fast, acts within minutes	Slow, requires several days before becoming effective
Activation	Constitutionally active: present at birth, prior to any contact with antigen	Activated in each individual in response to pathogen presentation or antigen contact
Development	Fully functional at birth	Adapts over time, after contact with antigen

Effectors	- Physical barriers
	- Complement
	- Inflammation
	- Cells
	  - Granulocytes (neutrophils, basophils, eosinophils)
	  - Mast cells
	  - Natural killer cells
	  - Macrophages
	  - Dendritic cells
		- B lymphocytes, antibodies
		- T lymphocytes

1. Dranoff. Nat Rev Cancer 2004;4:11–22. 2. Janeway et al. Immunobiology, 5th edn, 2001. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27090/. Accessed October 2022 3. Moser & Leo. Vaccine 2010;28 Suppl. 3:C2–13. 4. Litman et al. Nat Rev Immunol 2010;10:543–53.

Interactions between innate and adaptive immunity

Chapter homepage

Innate immunity

Adaptive immunity

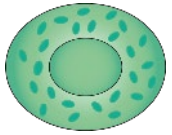
Complement

IgM or IgG antibodies bound to their antigen can activate complement

Antibodies

Learn more about antibodies

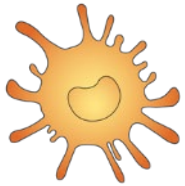
Mast cells



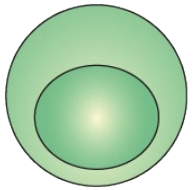
IgE antibodies bind to FcRs on mast cells; antigen recognition causes mast cell degranulation

Antibodies

Dendritic cells



Antigen presentation by DCs is required for T-cell priming (first activation)

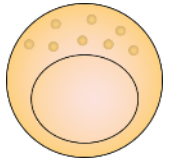


T lymphocytes

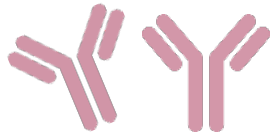
Learn more about dendritic cells

Learn more about T lymphocytes

Natural killer cells



Through their FcγRs, NK cells kill target cells recognized by IgG antibodies



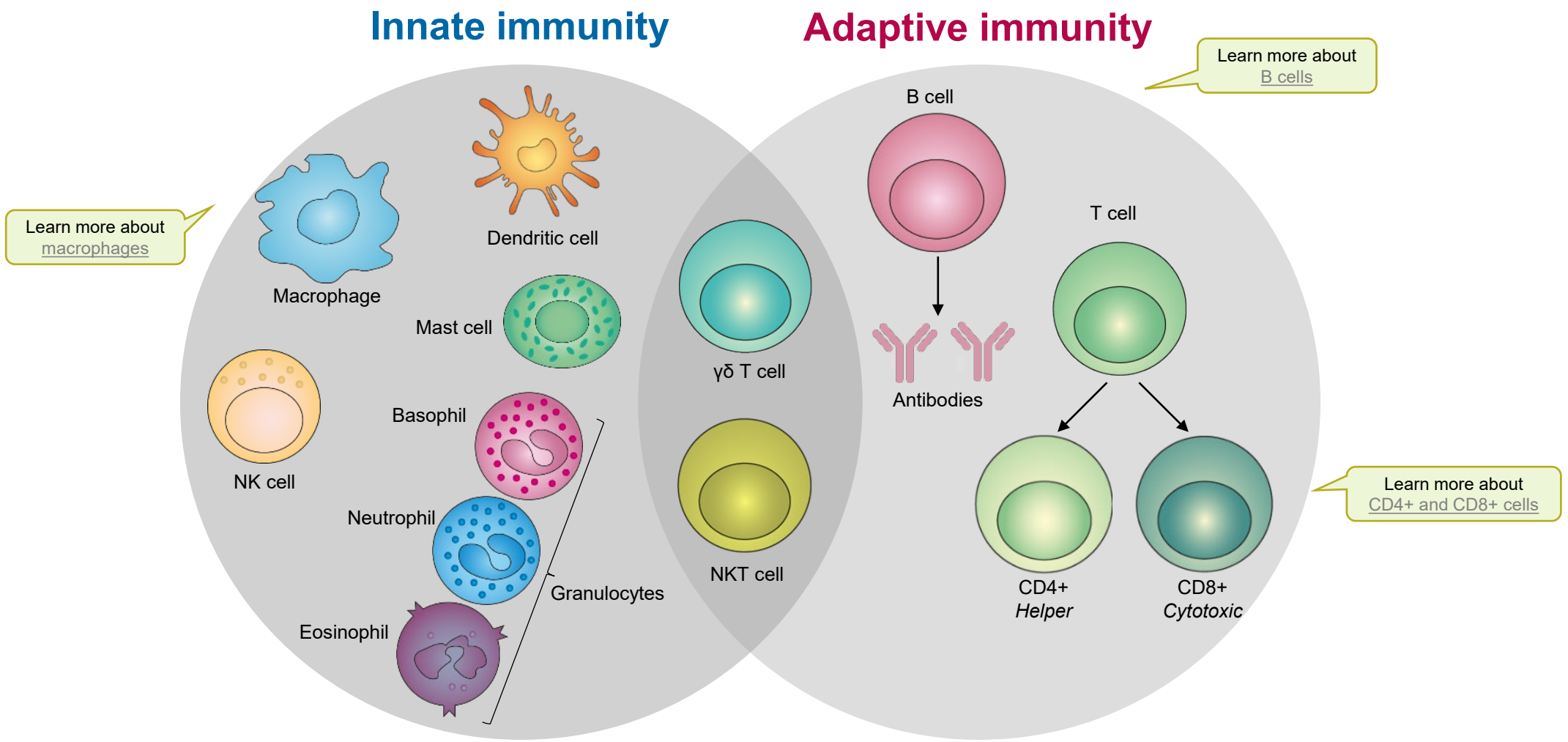
Antibodies

Learn more about NK cells

DC, dendritic cell; FcR, Fc receptor; FcγR, Fc receptor for IgG; Ig, immunoglobulin; NK, natural killer.
Professor P. Coulie, personal communication

Chapter homepage

Cells involved in innate and adaptive immunity



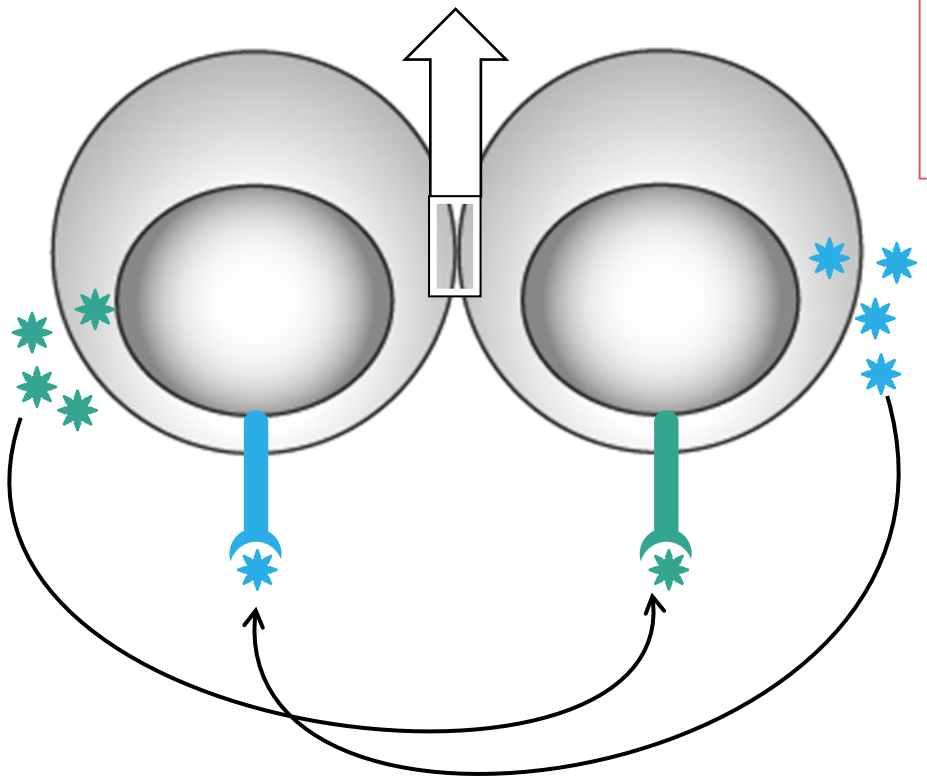
NK, natural killer; NKT, natural killer T.
Adapted from Dranoff. Nat Reviews Cancer 2004;4:11–22.

Communications between immune cells
and between immune and non-immune cells

Chapter homepage

Contact dependent

- Adhesion molecules (e.g. integrins)
- T-cell receptor > HLA-peptide
- Stimulatory coreceptors
- Inhibitory coreceptors
- Gap junctions

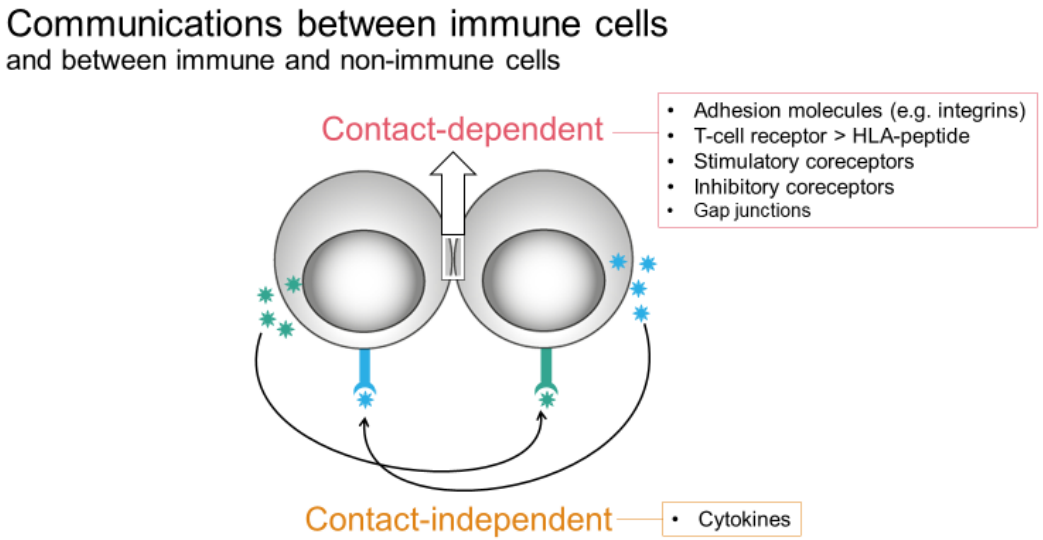


Contact independent

- Cytokines

HLA, human leukocyte antigen.
Professor P Coulie, personal communication 2017.

Clinical relevance
Communications between immune cells



- **Monoclonal antibodies that block integrin function on T cells are used therapeutically**
- **Natalizumab**
  - A humanized monoclonal anti-α4-integrin antibody (α4-integrin is a cell adhesion molecule)
  - It is indicated for the treatment of multiple sclerosis
  - Warning: natalizumab is associated with the rare neurological condition progressive multifocal leukoencephalopathy
- **Vedolizumab**
  - Humanized IgG1 monoclonal antibody that binds to the human $\alpha_4\beta_7$ integrin
  - It is indicated for the treatment of Crohn's disease and ulcerative colitis

HLA, human leukocyte antigen; IgG, immunoglobulin G; SmPC, summary of product characteristics.
Always refer to the SmPC. All product information listed is available in SmPCs. All SmPCs are available from http://www.ema.europa.eu/ema/.

Cytokines

- A group of over 100 small (5–20 kD) glycoproteins that are important in cell signaling[1]
- Bind to high-affinity receptors
- Act mostly on the cells that secrete them (autocrine effects) or on nearby cells (paracrine effects)
  - The IL-1 family are endocrine pyrogens[2]
- A single cytokine can have multiple biological actions (pleiotropy)[2]
- Similar functions can be stimulated by different cytokines (redundancy)[2]

Main types of cytokines[1]

Interleukins (n = 39)	Interferons	TNF family (n = 18)	Chemokines (n = 44)	TGF-β superfamily	Colony-stimulating factors
	• Type I: α, β, λ • Type II: IFN-γ	• TNFα • TNFβ (lymphotoxin α) • CD40L • FasL • CD70 • …etc.		• TGF-β1 • TGF-α • BMPs • GDNFs • …etc.	• Erythropoietin • Thrombopoietin • CSF1 (M-CSF) • CSF2 (GM-CSF) • CSF3 (G-CSF)

BMP, bone morphogenetic protein; CD40L, CD40 ligand; CSF, colony-stimulating factor; G-CSF, granulocyte CSF; GDNF, glial cell-derived neurotrophic factor; GM-CSF, granulocyte macrophage-CSF; IFN, interferon; IL, interleukin; M-CSF, macrophage CSF; TGF, transforming growth factor; TNF, tumor necrosis factor.

1. Professor P Coulie, personal communication. 2. Zhang & An. Int Anesthesiol Clin 2007;45:27–37.

Clinical relevance
Cytokines as therapeutic agents

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Zhang & An. Int Anesthesiol Clin 2007;45:27–37.

► Cytokines as therapeutic drugs

Therapeutic agent	Indications
IL-2 (aldesleukin)	Metastatic renal cell carcinoma
IL-11 (oprelvekin)	Severe thrombocytopenia prevention
G-CSF (e.g. filgrastim) and GM-CSF	Immunoreconstitution
IFNβ1α	Multiple sclerosis
IFNγ-1b	Chronic granulomatous disease and osteopetrosis
Epoetin-α	Anemia

BMP, bone morphogenetic protein; CD40L, CD40 ligand; CSF, colony-stimulating factor; G-CSF, granulocyte CSF; GDNF, glial cell-derived neurotrophic factor; G-CSF, granulocyte CSF; GM-CSF, granulocyte macrophage-CSF; IFN, interferon; IL, interleukin; M-CSF, macrophage CSF; SmPC, summary of product characteristics; TGF, transforming growth factor; TNF, tumor necrosis factor.
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Clinical relevance

Cytokines: blocking the effects with monoclonal antibodies

Chapter homepage

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Zhang & An. Int Anesthesiol Clin 2007;45:27–37.

Monoclonal antibodies that inhibit cytokine effects

Target	Drug	Licensed indications
Anti-TNFα agents	Infliximab, adalimumab	Severe inflammatory conditions, e.g. rheumatoid arthritis and Crohn's disease
TNF receptor inhibitor	Etanercept	Severe inflammatory conditions, e.g. rheumatoid arthritis, psoriasis, ankylosing spondylitis
Anti-IL-1β	Canakinumab	Autoinflammatory periodic fever syndromes, Still's disease
IL-1 receptor antagonist	Anakinra	Rheumatoid arthritis, periodic fever syndromes, autoinflammatory diseases, Still's disease, COVID-19
Anti-IL-6 receptor	Tocilizumab	Rheumatoid arthritis, systemic juvenile idiopathic arthritis, cytokine release syndrome
Anti-IL-17A	Secukinumab, Ixekizumab	Plaque psoriasis, psoriatic arthritis, juvenile idiopathic arthritis, ankylosing spondylitis
Anti-IL-17RA	Brodalumab	Plaque psoriasis
Anti-IL-12/23	Ustekinumab	Crohn's disease, ulcerative colitis

BMP, bone morphogenetic protein; CD40L, CD40 ligand; CSF, colony-stimulating factor; G-CSF, granulocyte CSF; GDNF, glial cell-derived neurotrophic factor; G-CSF, granulocyte CSF; GM-CSF, granulocyte macrophage-CSF; IFN, interferon; IL, interleukin; M-CSF, macrophage CSF; SmPC, summary of product characteristics; TGF, transforming growth factor; TNF, tumor necrosis factor.
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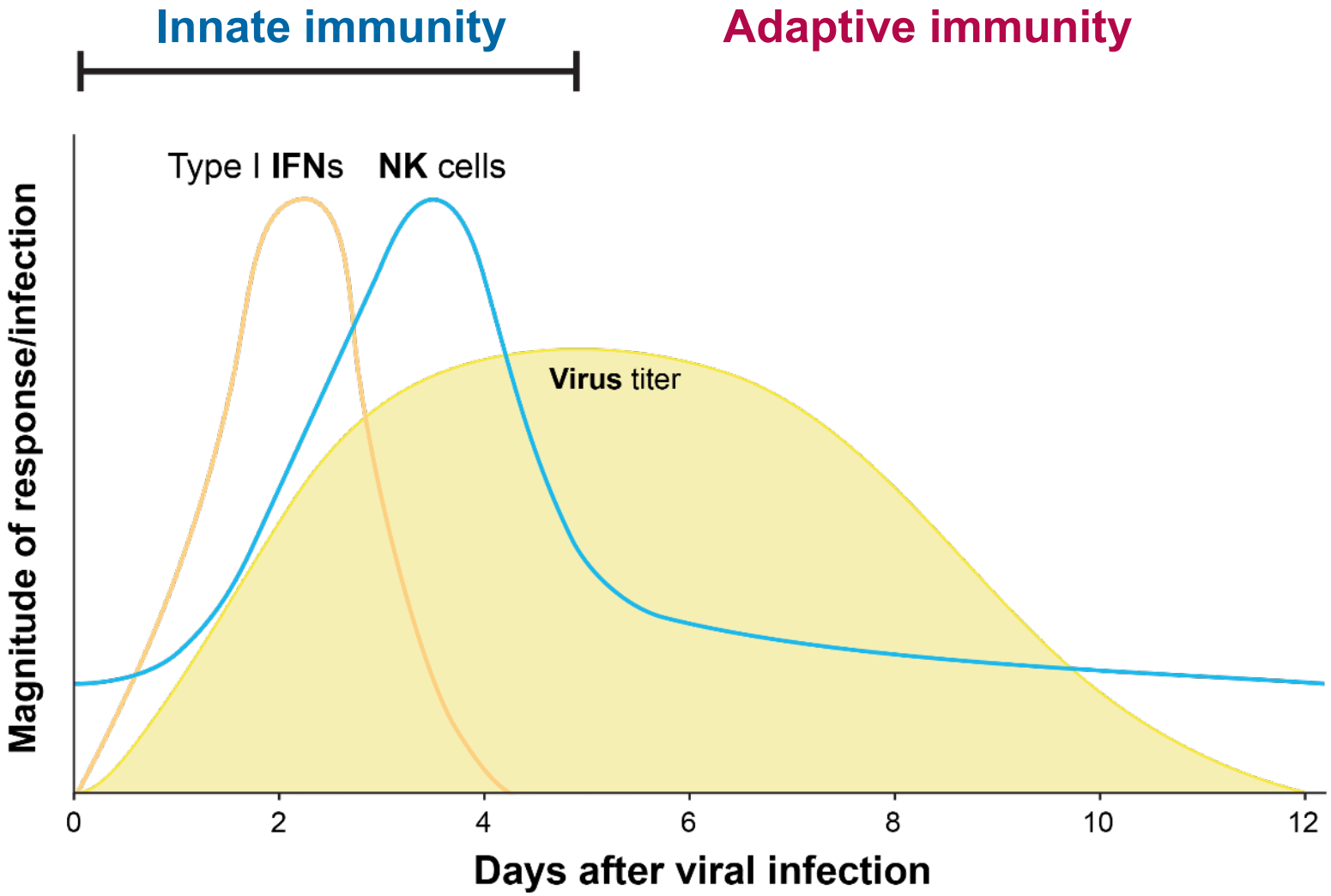
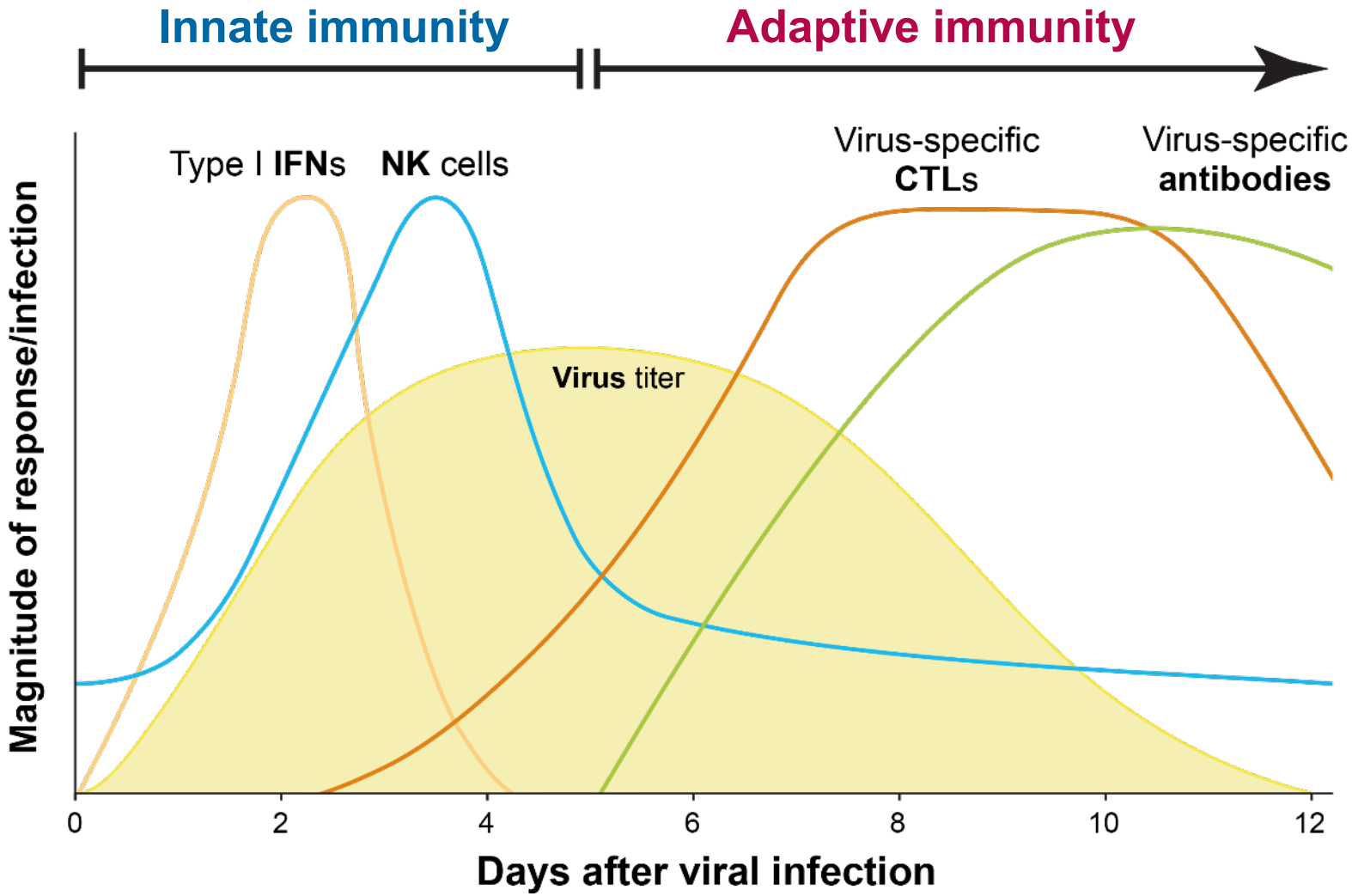
Timeline of a normal immune response to a virus



IFN, interferon; NK, natural killer.
Abbas et al. Cellular and Molecular Immunology, 8th edn, 2015.

Timeline of a normal immune response to a virus

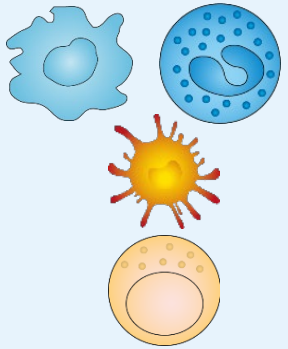


CTL, cytotoxic T lymphocyte; IFN, interferon; NK, natural killer.
Abbas et al. Cellular and Molecular Immunology, 8th edn, 2015.

Innate immunity

Module 1. Basic immunology

The innate immune response

Overview of the key players	
Physical and chemical barriers epithelia, mucus, defensins...	
Cells phagocytic cells (macrophages, neutrophils) dendritic cells natural killer cells	
Sensors on cells, inside cells and in blood, to detect microbes or 'danger'	
Blood proteins complement cytokines inflammatory mediators	

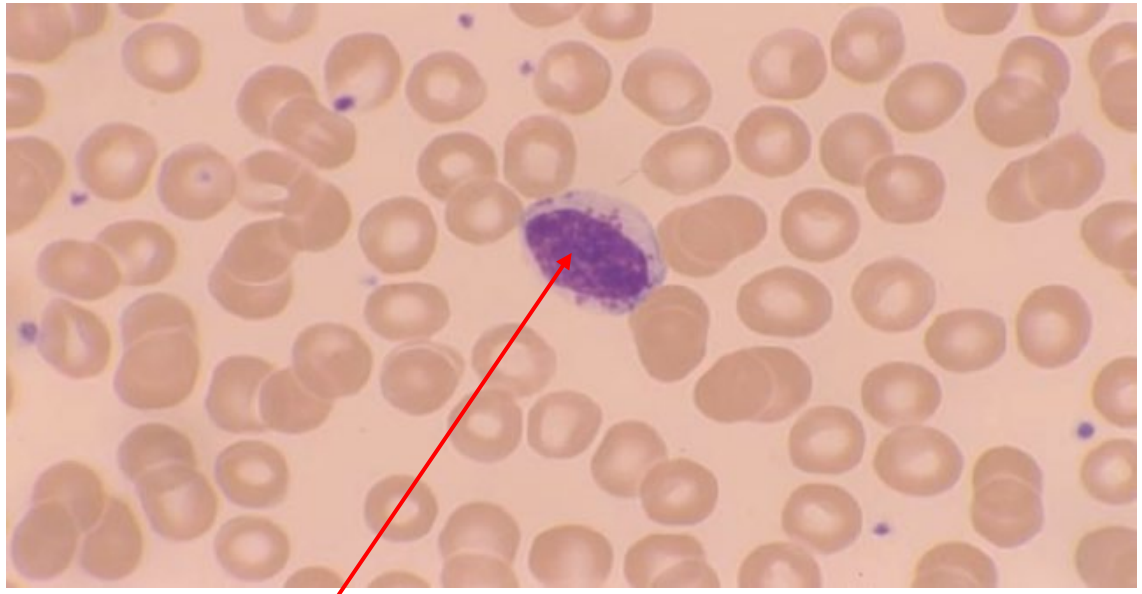
Main form of defense

1. Adapted from Dranoff. Nat Reviews Cancer 2004;4:11–22.Lowell 2. Lowell presentation Available from: https://immunox.ucsf.edu/sites/immunox.ucsf.edu/files/pdf/Innate%20Immunity%20%231.2_2018.pdf Accessed October 2022. 3. Rathinam and Fitzgerald. Virology 2011;411:153–162. 4. Mogensen et al. Retrovirology 2010;7:54.

The innate immune response: natural killer cells

Chapter homepage
Back to introduction

- 10% of peripheral blood mononuclear cells are NK cells[1]
- NK cells can be activated by target cells, which they lyse[1]
- NK cell activation depends on an array of activating and inhibitory receptors[1]
- KIR-HLA has an important role in the development and activity of NK cells[2]



Large granular lymphocyte = NK cell

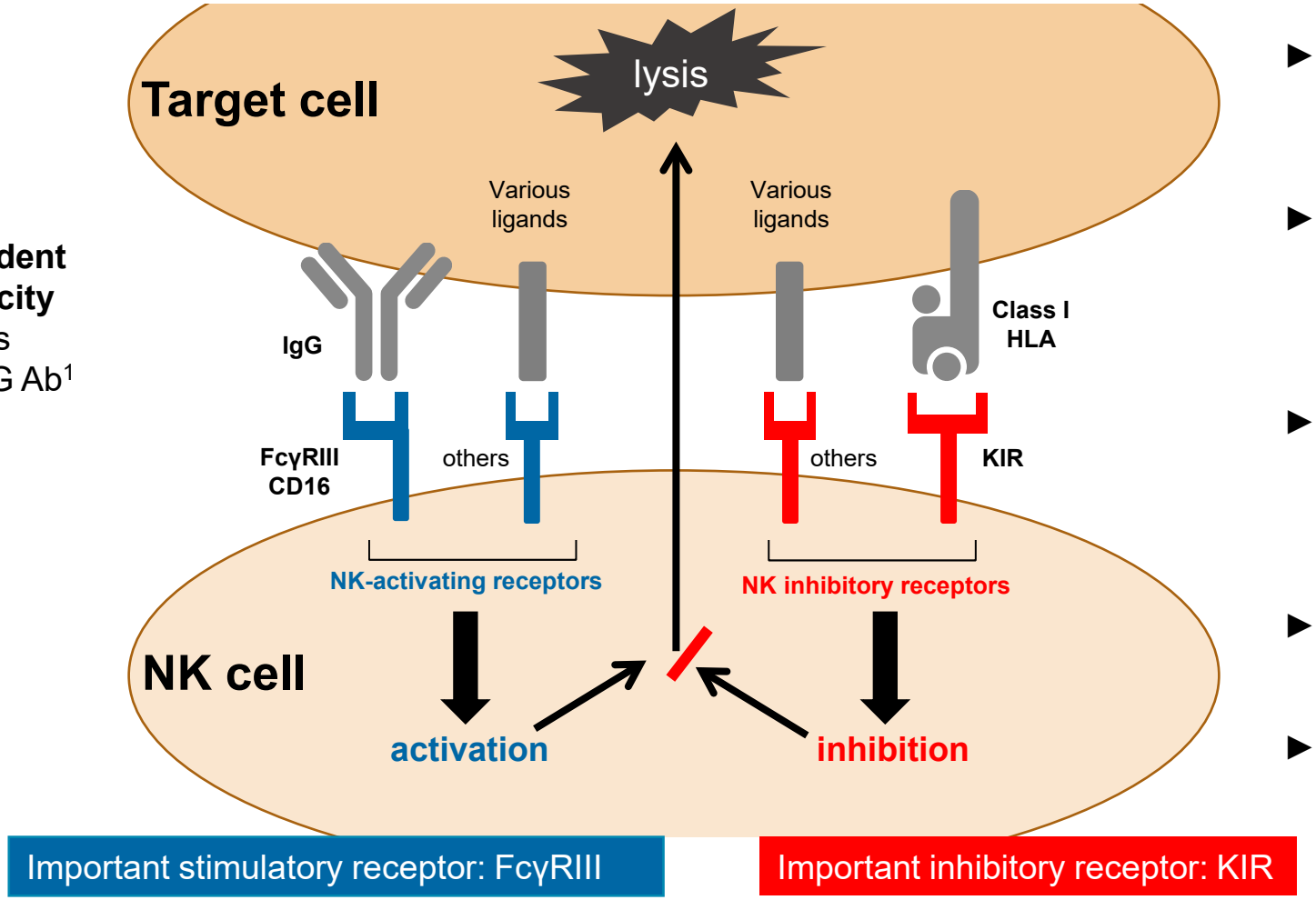
HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; NK, natural killer.

1. Mandal & Viswanathan. Hematol Oncol Stem Cell Ther 2015;8:47–55. 2. de Smith AJ et al. Blood 2014;123:2494–503. Large granular lymphocyte image from: Kern. PDQ Hematology, 2002:7.

The innate immune response: controlling NK cell activation

Chapter homepage

- **Antibody-dependent cellular cytotoxicity**
- NK cells lyse cells recognized by IgG Ab[1]



- NK cells and CTLs have complementary roles in immune responses
- CTLs recognize peptides that are derived from **tumor** and **virus** antigens presented by HLA class I molecules
- In tumor and microbe-infected cells, **expression of HLA class I** on the cell surface is often **downregulated**
- These cells are thereby able to **escape CTL killing**
- However, NK cells are able to recognize and kill these cells[2]

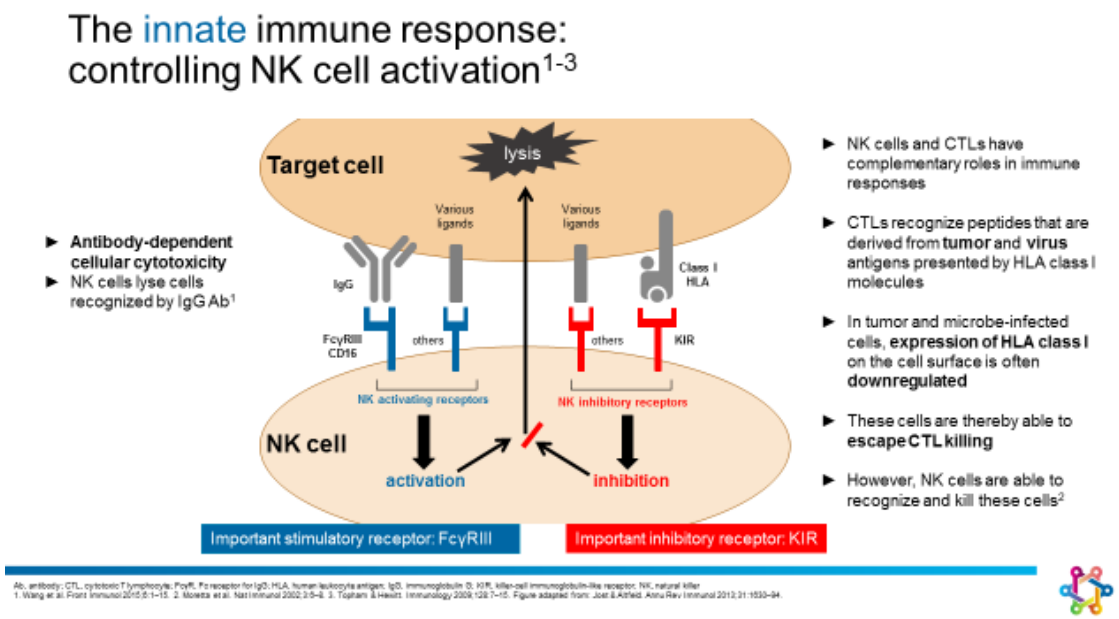
Ab, antibody; CTL, cytotoxic T lymphocyte; FcγR, Fc receptor for IgG; HLA, human leukocyte antigen; IgG, immunoglobulin G; KIR, killer-cell immunoglobulin-like receptor; NK, natural killer

1. Wang et al. Front Immunol 2015;6:1–15. 2. Moretta et al. Nat Immunol 2002;3:6–8. 3. Topham & Hewitt. Immunology 2009;128:7–15. Figure adapted from: Jost & Altfeld. Annu Rev Immunol 2013;31:1630–94.

Clinical relevance

Chapter homepage

The innate immune response: controlling NK cell activation



- **Monoclonal antibodies that promote cell lysis are used therapeutically**
- **Rituximab**
  - A humanized monoclonal anti-CD20 antibody (IgG1); CD20 is a B-cell-specific surface molecule
  - It is indicated for the treatment of B-cell malignancies (CLL and NHL), rheumatoid arthritis, granulomatosis with polyangiitis (Wegener's), microscopic polyangiitis and pemphigus vulgaris
  - It has several mechanisms of action, including ADCC, CD20-mediated signaling and cell death, complement activation and ADCP[1]
  - FcγRIII polymorphisms (158V instead of 158F) with higher affinity for IgG1 are associated with better clinical responses[2]
- **Monoclonal antibodies that block KIRs and are expected to increase NK cell lytic activity against tumor cells are being evaluated in patients with cancer**

ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CLL, chronic lymphocytic leukemia; FcγR, Fc receptor for immunoglobulin G; KIR, killer-cell immunoglobulin-like receptor; NHL, Non-Hodgkin's lymphoma; NK, natural killer; SmPC, summary of product characteristics.

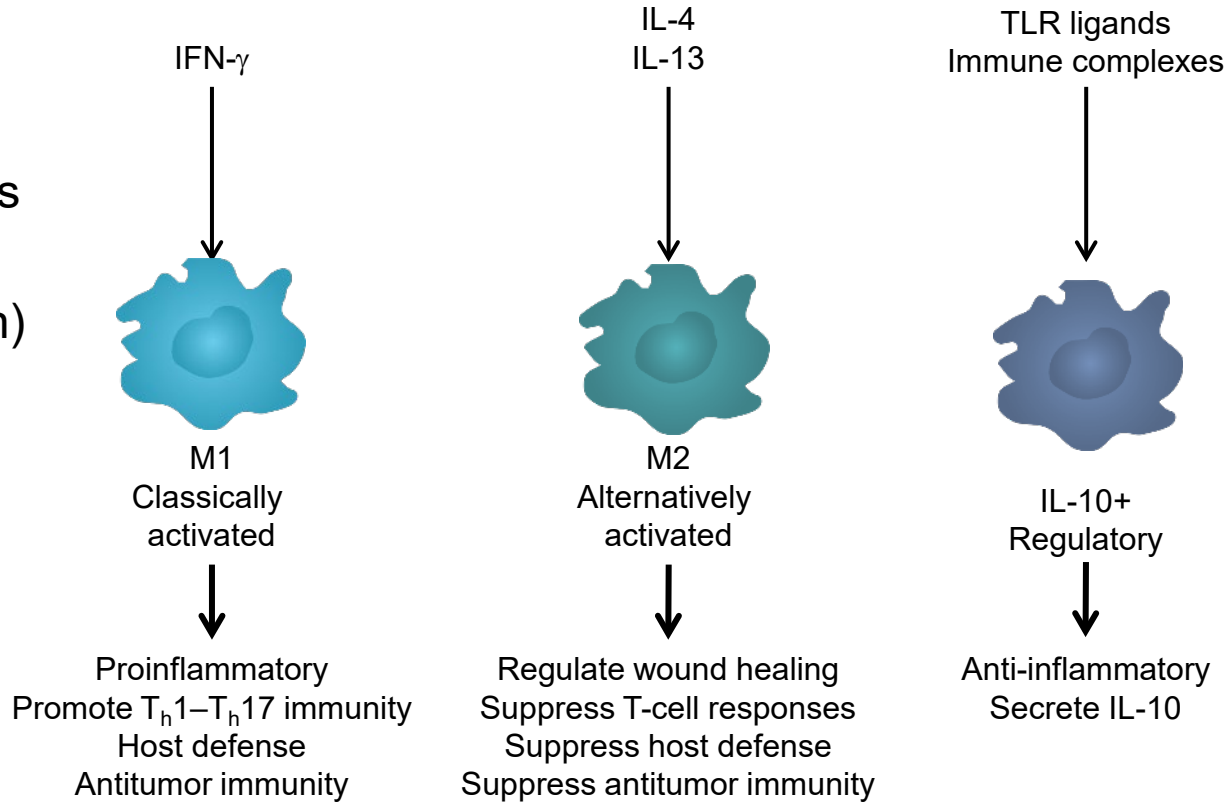
1. Dalakas. Nat Clin Pract Neurol 2008;4:557–67. 2. Wang et al. Front Immunol 2015;6:1–15. Always refer to the SmPC. All SmPCs available from http://www.ema.europa.eu/ema/

The innate immune response: macrophages

Chapter homepage
Back to introduction

- Macrophages are phagocytic cells found in all tissues[1]
- Macrophages are involved in antiviral responses via[1,2]
  - Phagocytosis and destruction of pathogens
  - Destruction of infected cells
  - Production of soluble factors (inflammation)
  - Presentation of microbial antigens to T and B lymphocytes as part of the adaptive immune response

Macrophages have multiple activation phenotypes, driven by environmental signals



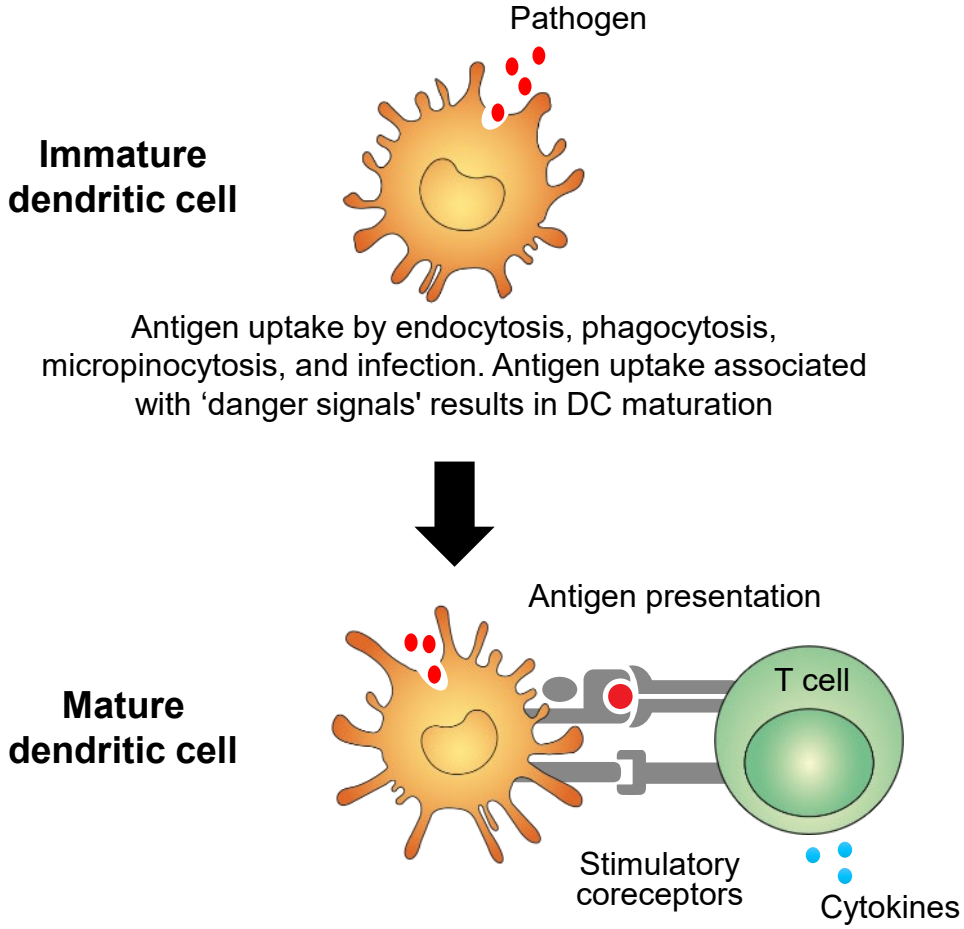
IFN, interferon; IL, interleukin; T_h, T helper; TLR, Toll-like receptor.

1. Elhelu. J Natl Med Assoc 1983;75:314–7. 2. Klimpel. In: Medical Microbiology, 4th edn, 1996. Available from: http://www.ncbi.nlm.nih.gov/books/NBK8423/. Accessed October 2022. Figure adapted from Galli et al. Nature Immunol 2011;12:1035–44.

The innate immune response: dendritic cells

Chapter homepage
Back to introduction

- Dendritic cells are typically located in tissues exposed to external environments, e.g. the respiratory system and gastrointestinal mucosae[1]
- They are recruited to sites of infection by chemokines[1]
- Pathogen recognition via Toll-like receptors triggers antiviral responses[1,2]
  - Phagocytosis
  - Secretion of inflammatory cytokines and interferons
  - Migration to lymph nodes (attracted by chemokines)
  - Processing of antigenic peptides and presentation to CD4+ and CD8+ T cells (DCs can 'prime' T cells)



Antigen presentation and activation of T cells

DC, dendritic cell.

1. Wen et al. J Leukocyte Biol 2008;83:439–46. 2. Martín-Fontecha et al. Dendritic Cells. In: Handbook of Experimental Pharmacology 2009:188. Figure adapted from Szatmari et al. EMBO J 2008;27:2353–62.

Sensors in innate immunity: pattern recognition receptors

Chapter homepage

- PRRs are a collection of receptors that can be present[1,2]
  - on cells
  - inside cells (cytoplasm, endosomes)
  - in plasma
- They recognize two classes of molecules, which are absent from 'normal' cells:

PAMPs

Pathogen-associated molecular patterns

- These are molecules associated with classes of microbes
  - Examples include lipopolysaccharide from Gram-negative bacteria and dsRNA from viruses

DAMPs

Danger-associated molecular patterns

- These are molecules present or released after cell damage (e.g. through UV, irradiation, heat) or death
  - Examples include HMGb1, heat shock proteins, and purine metabolites, such as ATP12 and uric acid13

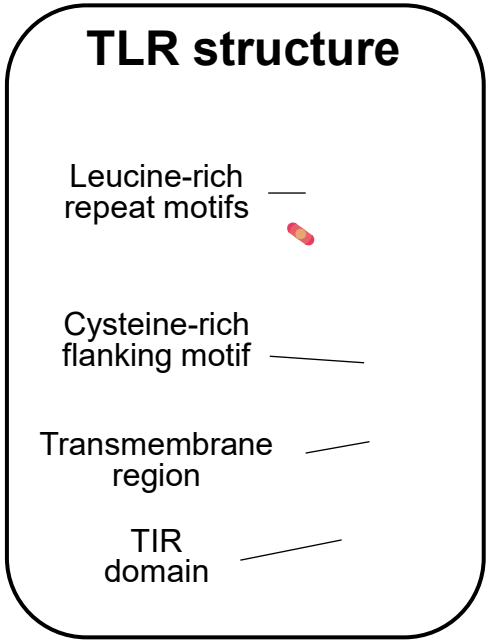
- Signaling PRRs induce **inflammation**; endocytic PRRs promote **phagocytosis**

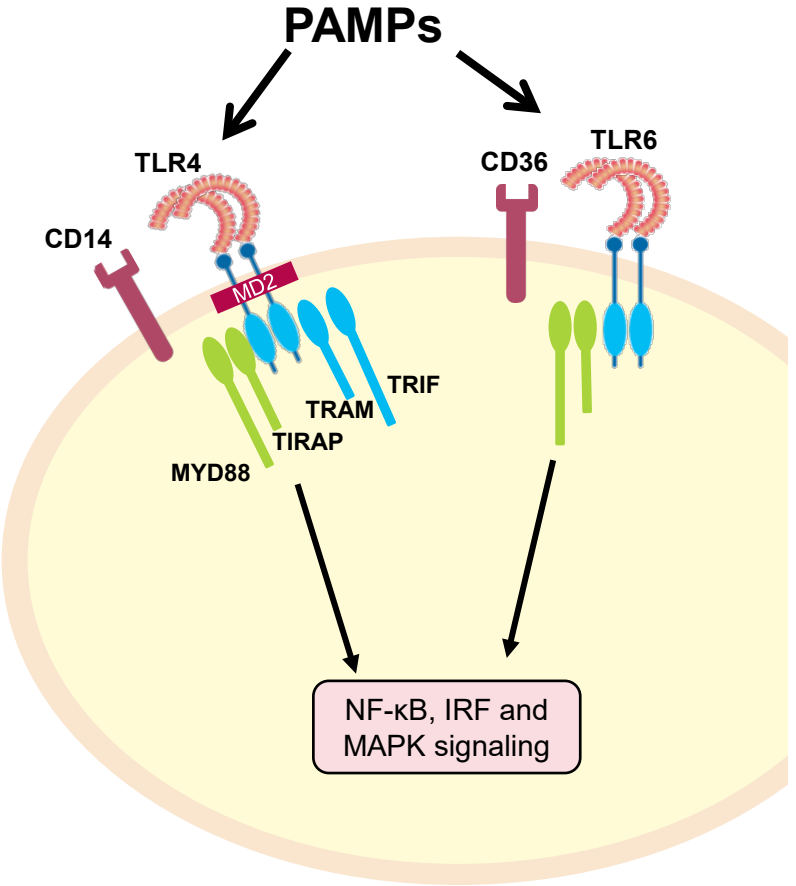
DAMP, danger-associated molecular pattern; dsRNA, double-stranded RNA; HMGB1, high mobility group box 1 protein; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; TAP, transporter associated with antigen processing; UV, ultraviolet.

Seong & Matzinger. Nat Rev Immunol 2004;4:469–78. 2. Chen & Nunez. Nat Rev Immunol 2010;10:826–37.

Toll-like receptors: a family of PRRs

- TLRs are a family of dimeric transmembrane receptors[1] (some TLRs need coreceptors)
- TLRs are present on many cell types, including sentinel cells of the immune system[2] and in endosomes within such cells[3]
- They recognize specific PAMPs on pathogens and initiate a cell signaling cascade via NF-κB, IRF and MAPK[2,3]
- Different TLRs bind to different ligands[4,5]

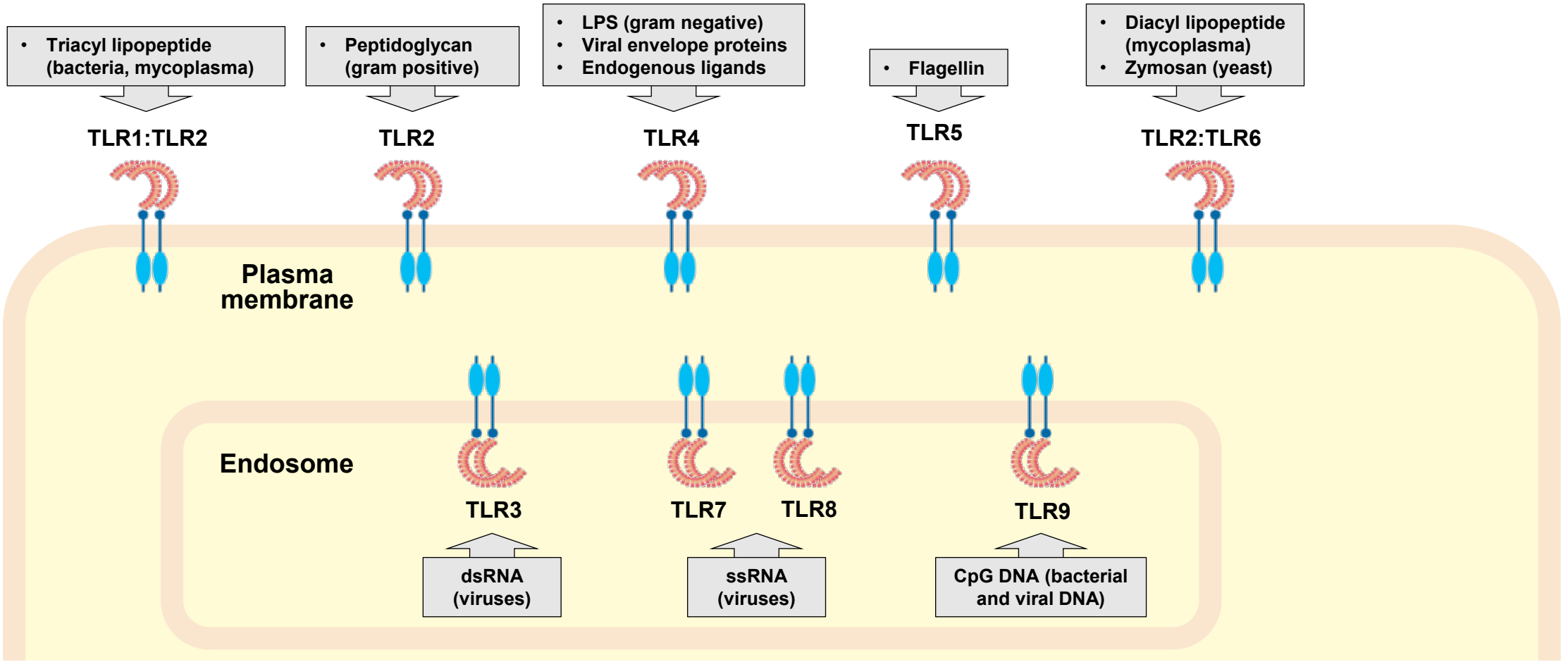




IRF, interferon regulatory factor; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; PAMP, pathogen-associated molecular pattern; TIR, Toll/interleukin-1 receptor; TIRAP, TIR domain-containing adaptor protein; TLR, Toll-like receptor; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adaptor protein inducing interferon beta.

1. Armant & Fenton. Genome Biol 2002;3:3011. 2. Netea et al. Nature Immunol 2012;13:535–42. 3. Mogensen et al. Retrovirology 2010;7:54. 4. Abbas et al. Cellular and Molecular Immunology, 7th edn, 2011. 5. Medzhitov. Nat Rev Immunol 2001;1:135–45. Figure adapted from references 4 and 5.

Different TLRs bind to different PAMPs

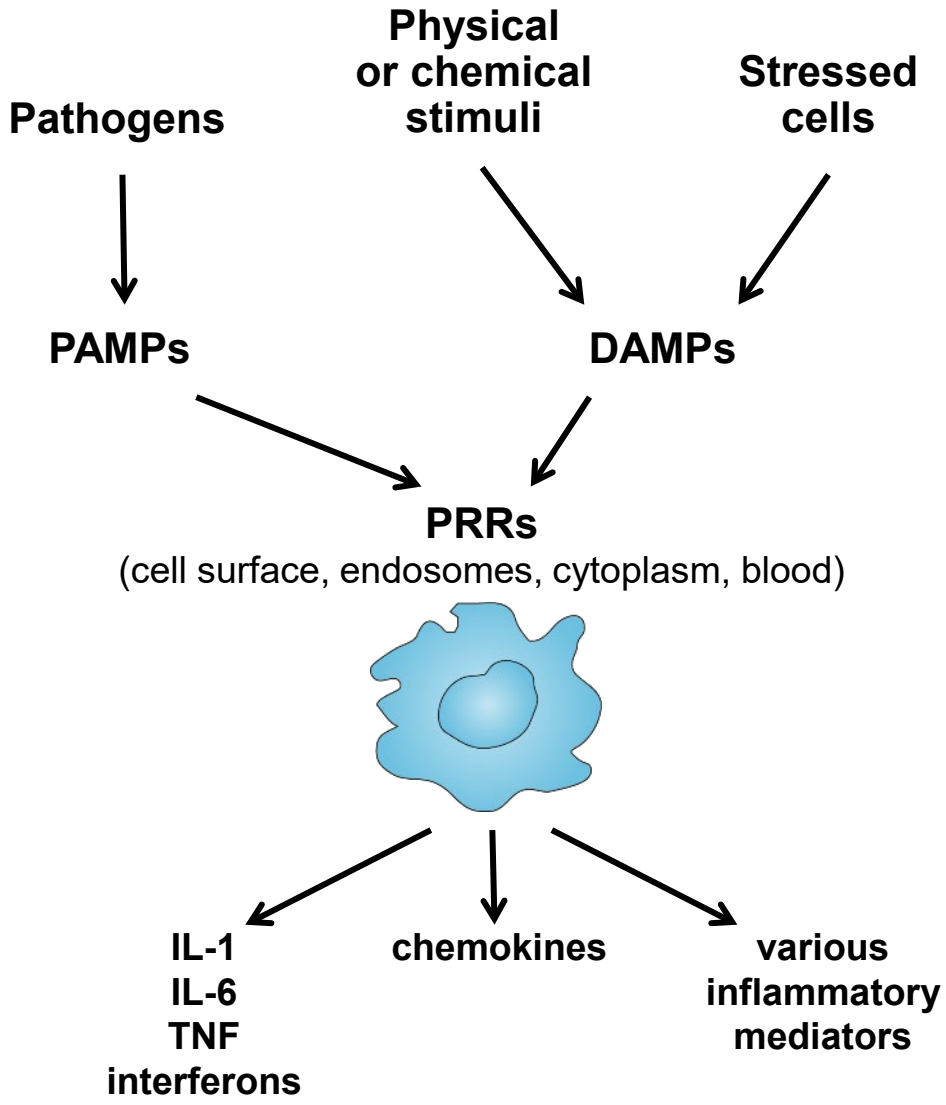


dsRNA, double-stranded RNA; LPS, lipopolysaccharide; PAMP, pathogen-associated molecular pattern; ssRNA, single-stranded RNA; TLR, Toll-like receptor.

1. Netea et al. Nat Immunol 2012;13:535–42. 2. Armant & Fenton, Genome Biol 2002;3:3011. 3. Mogensen et al. Retrovirology 2010;7:54. Figure adapted from 4. Abbas et al. Cellular and Molecular Immunology, 7th edn. 5. Leulier F and Lemaitre B, Nat Rev Gen 2008:9:165-178

The innate immune response: inflammation

- Inflammation is a biological response of the immune system to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation[1]
- Recognition of PAMPs by PPRs (e.g. on macrophages, dendritic cells) triggers signaling cascades that culminate in the production of cytokines, including chemokines and interferons, and other inflammatory mediators[2]
- This cascade of signals leads to the recruitment of inflammatory cells (phagocytic and immune cells) and tissue and wound repair, and participates in the induction of adaptive immune responses[3]



DAMP, danger-associated molecular pattern; IL, interleukin; PAMP, pathogen-associated molecular pattern; PPR, pathogen recognition receptor; TNF, tumor necrosis factor.

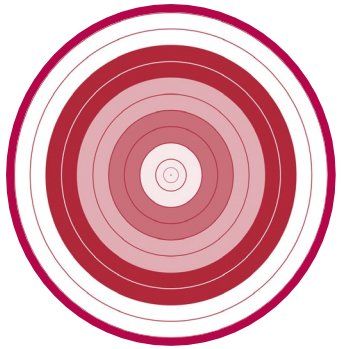
1 Chen et al. Oncotarget 2018;9:7204–7218. 2. Liu & Cao. Cell Mol Immunol 2016;13:711–14. 3. Chen et al. Nat Rev Immunol 2010;10:826–37. Image adapted from Chen et al. Nat Rev Immunol 2010;10:826–37.

Adaptive immunity

Module 1. Basic immunology

The adaptive immune response: hallmarks of adaptive immunity

Chapter homepage



Specificity

- B and T lymphocytes have diverse surface receptors (immunoglobulins and TCRs, respectively) that recognize antigens
- These receptors are very specific to each antigen

Memory

- Immune memory: a better (faster, stronger) B or T cell response compared with first contact with antigen
- Result of the long-term persistence of a fraction of antigen-specific B or T cells

TCR, T-cell receptor

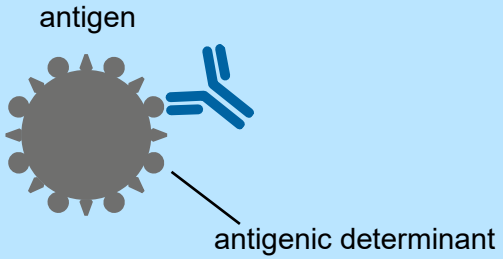
Janeway et al. In: Immunobiology: The Immune System in Health and Disease, 5th edn, 2001. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27090/ and https://www.ncbi.nlm.nih.gov/books/NBK27092/. Accessed October 2022.

The adaptive immune response: antigens

Chapter homepage

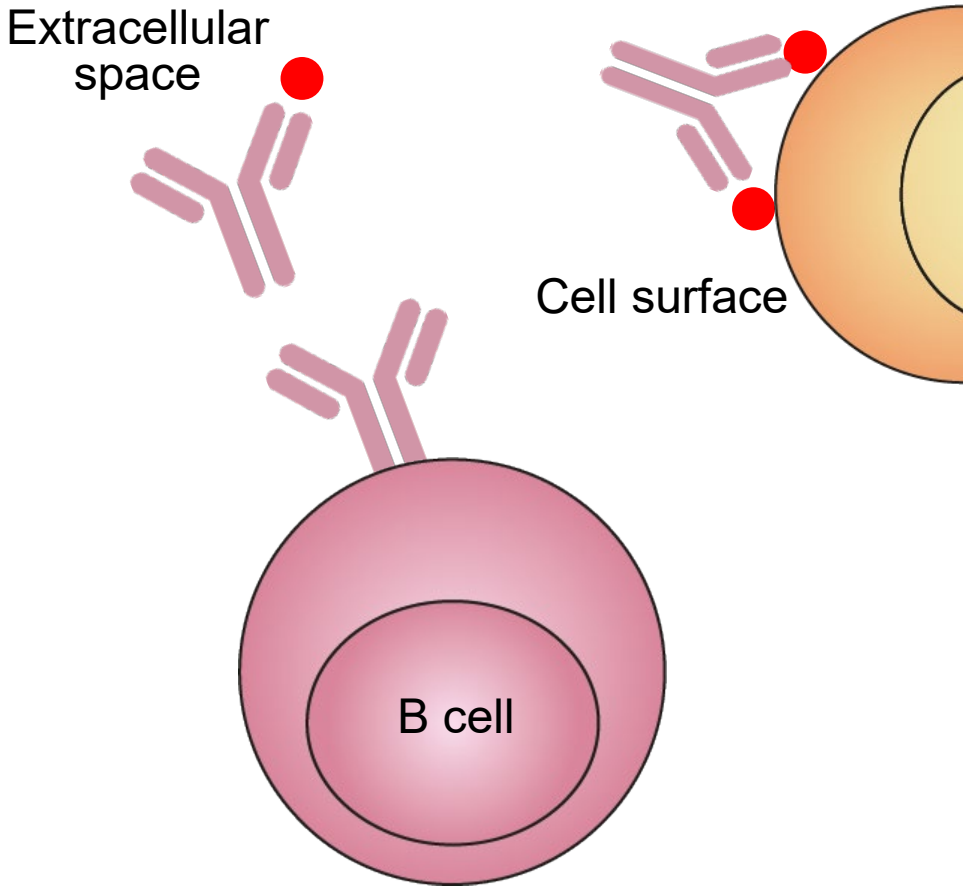
- An antigen is a substance, usually from the external environment of an organism (= 'non-self'), that can be specifically recognized by either antibodies or T lymphocytes
- An antigen does not necessarily induce a specific immune response; when it does so, the antigen is an immunogen (all immunogens are antigens, but all antigens are not immunogens)
- Antigens may have various sizes

Cell:	10,000 nm
Bacterium:	1000 nm
Virus:	50 nm
Protein:	5 nm
Drug:	< 1 nm

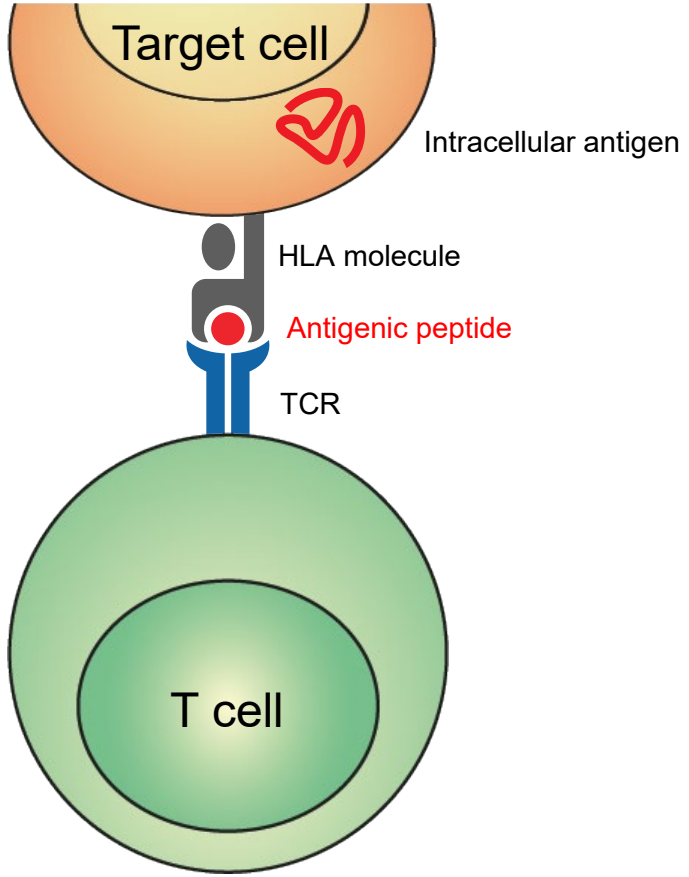


- The part of the antigen that is actually recognized is the antigenic determinant (epitope)
- Most common antigens have many antigenic determinants

Antigen recognition in adaptive immunity



Direct recognition of **extracellular** antigens by antibodies[1]



Recognition of **intracellular** antigens by TCRs via the HLA-peptide complex[2]

HLA, human leukocyte antigen; TCR, T-cell receptor.

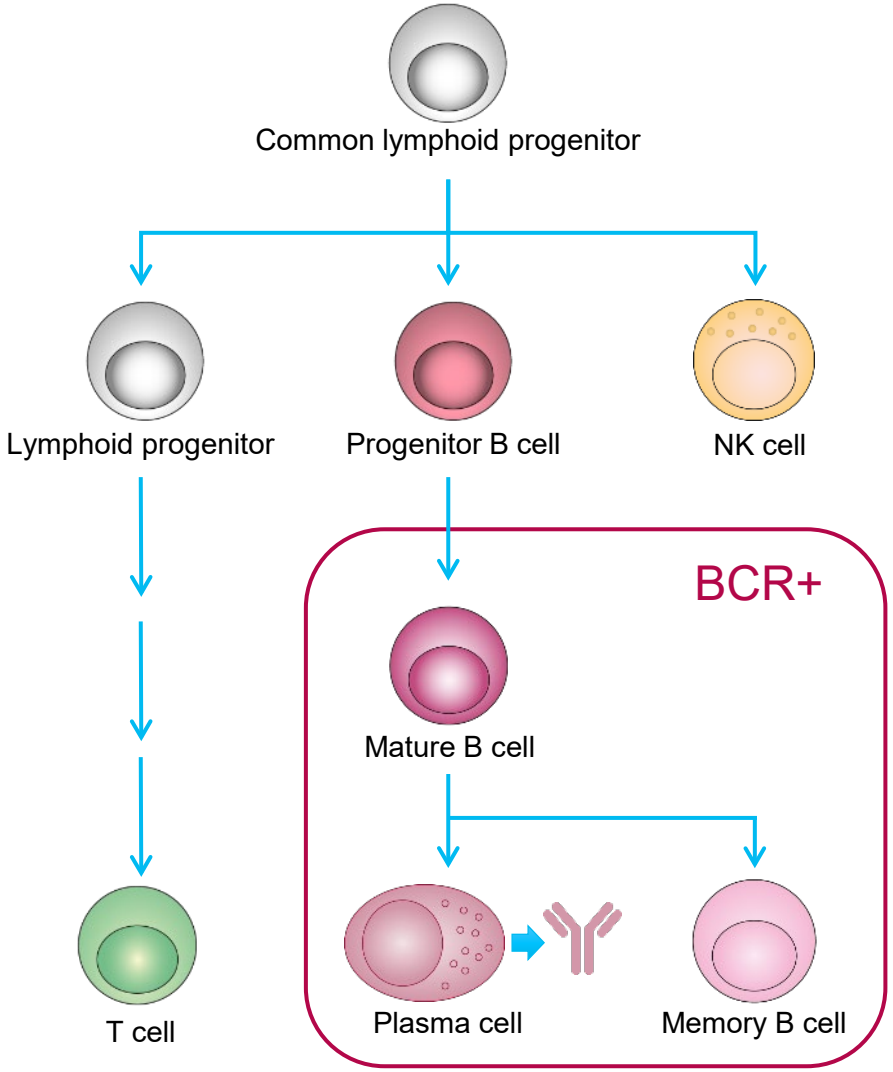
1. Alberts et al. Molecular Biology of the Cell, 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26884/. Accessed October 2022. 2. Heath & Carbone. Nat Rev Immunol 2001;1:126–35.

Adaptive immunity: B cells

Module 1. Basic immunology

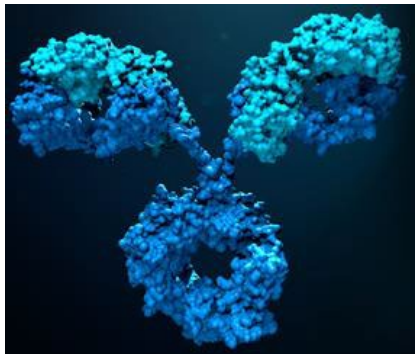
The adaptive immune response: B lymphocytes and antibodies

- B lymphocytes, or B cells, originate from the same lymphoid precursor as T cells
- Immature B cells are formed in the bone marrow, whereas mature B cells circulate in the blood and lymphatic systems
- B cells can be distinguished from other lymphocytes by the presence of an antigen-binding BCR (antibody) on the cell surface
- Only plasma cells secrete antibodies

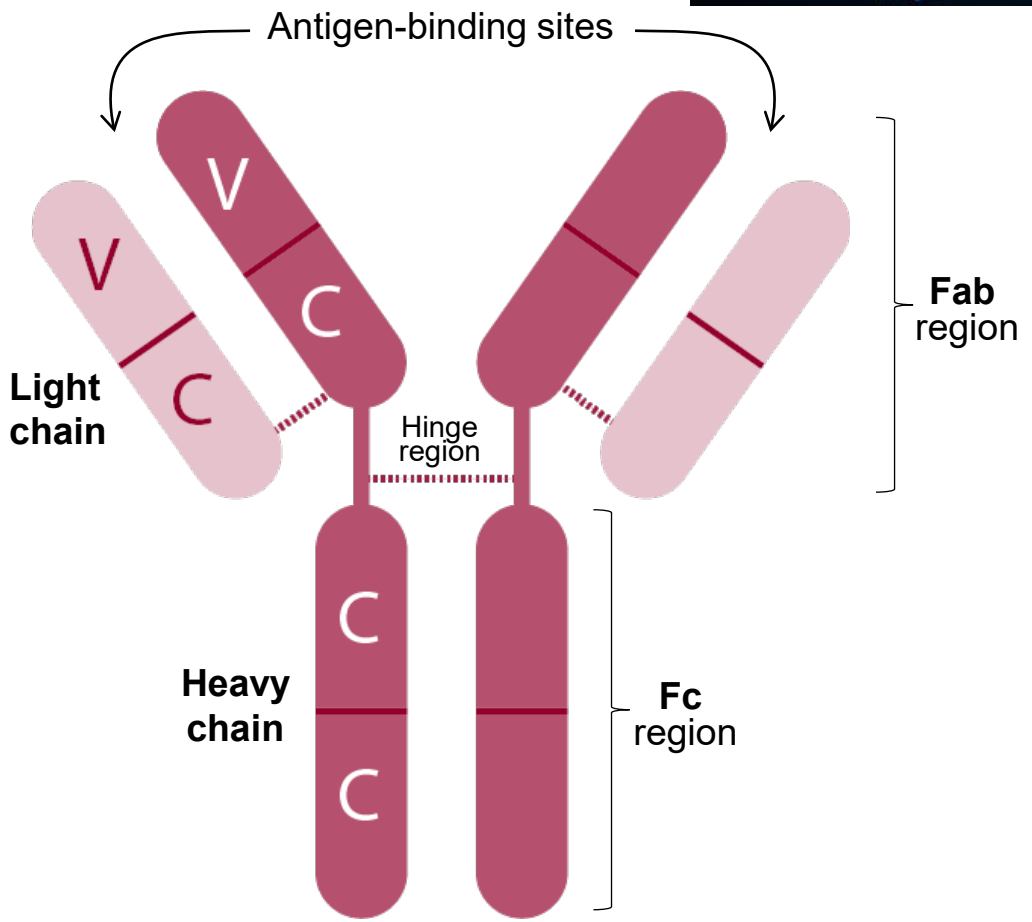


BCR, B-cell receptor; NK, natural killer.
Male et al. Immunology, 7th edn, 2001.

Antibody (immunoglobulin) structure



- All antibodies all are built from the same basic units
- Heavy and light chains
  - Antibodies comprise two identical light chains (approx. 25 kD) and two identical heavy chains (approx. 50 kD)
  - Heavy and light chains linked by disulfide bonds
- Variable (V) and constant (C) regions
  - Both heavy and light chains can be divided into two regions based on variability in amino acid sequences
- Hinge region
  - The region at which the arms of the molecule forms a Y-shape
- The antibody molecule is folded (see inset) into globular regions called immunoglobulin domains
  - Light chain: two domains
  - Heavy chain: four (or five) domains



C, constant; Fab, antigen-binding fragment; Fc, crystallizable fragment; V, variable.
Janeway et al. Immunobiology, 5th edn, 2001. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27144/. Accessed October 2022.

Antibody effector functions

Antibodies perform different functions in different regions of their structure[1–3]

Through Fab region

- **Neutralization:** antibodies prevent pathogens from binding their receptors
- **B-cell activation:** antibodies serve as B-cell surface antigen receptors

Through Fc region

- **Complement-dependent cytotoxicity:** activates complement system (IgM, IgG)

Functions that occur through Fc receptors

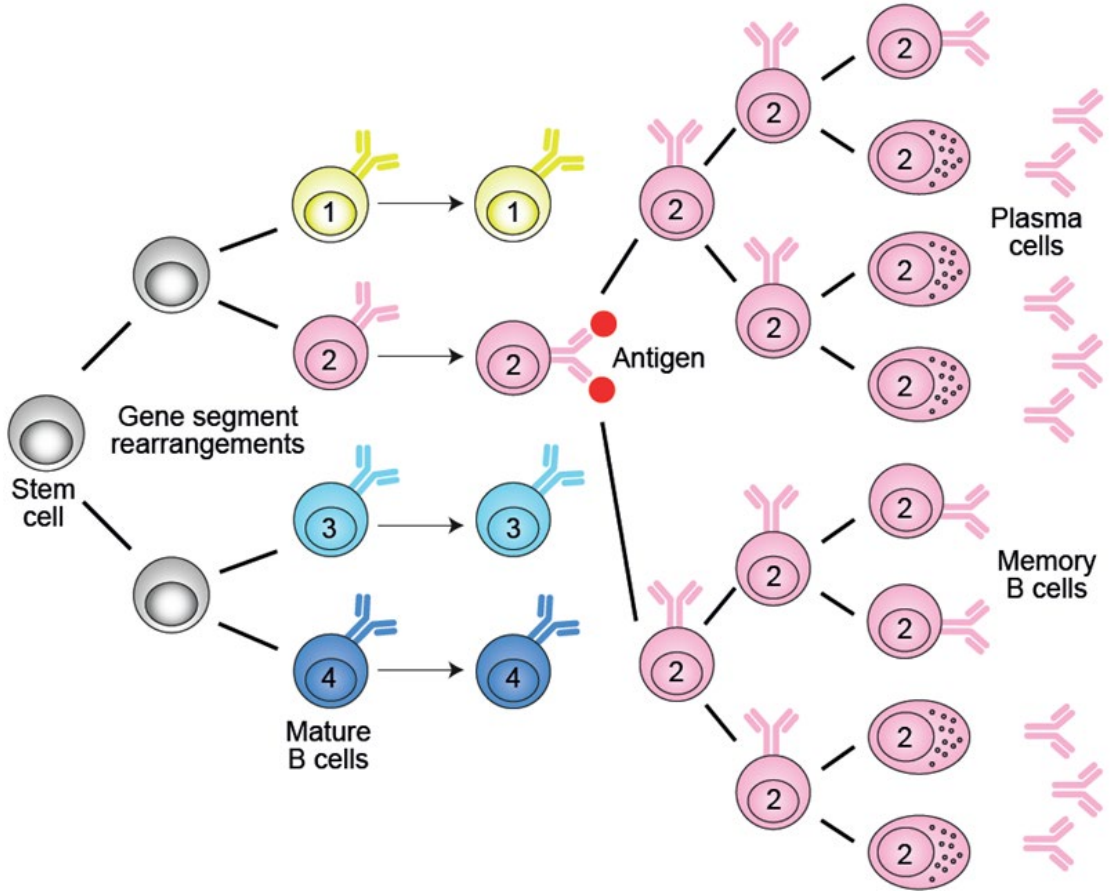
- **Opsonization:** antibody-tagged pathogen is consumed by phagocytes (IgG, IgA)
- **Degranulation:** of mast cells or basophils (IgE)
- **ADCC:** antibody-coated cells lysed by NK cells (IgG)
- **Tissue distribution:** occurs according to classes
  e.g. IgG → fetus via FcRn; IgA → mucosae via pIgR

ADCC, antibody-dependent cellular cytotoxicity; Fab, antigen-binding fragment; Fc, crystallizable fragment; FcRn, neonatal Fc receptor; Ig, immunoglobulin; NK, natural killer.

1. Boundless Anatomy and Physiology. Available from https://courses.lumenlearning.com/boundless-ap/chapter/humoral-immune-response/. Accessed October 2022. 2. Absolute antibody. Available from: http://absoluteantibody.com/antibody-resources/antibody-overview/antibody-effector-functions/. Accessed October 2022. 3. Alberts et al. Molecular Biology of the Cell, 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26884/. Accessed October 2022.

The adaptive immune response: clonal expansion of activated B cells

- Activated B cells are driven to divide and differentiate into plasma and memory cells
  - Plasma cells produce antibodies for neutralizing pathogens or labeling them for destruction
  - Memory cells have long lifespans and respond quickly upon reinfection with the same pathogen
- These cells have the same antigen specificity (same BCR) as the original B cell



Maturation into B cells that are antigenetically committed

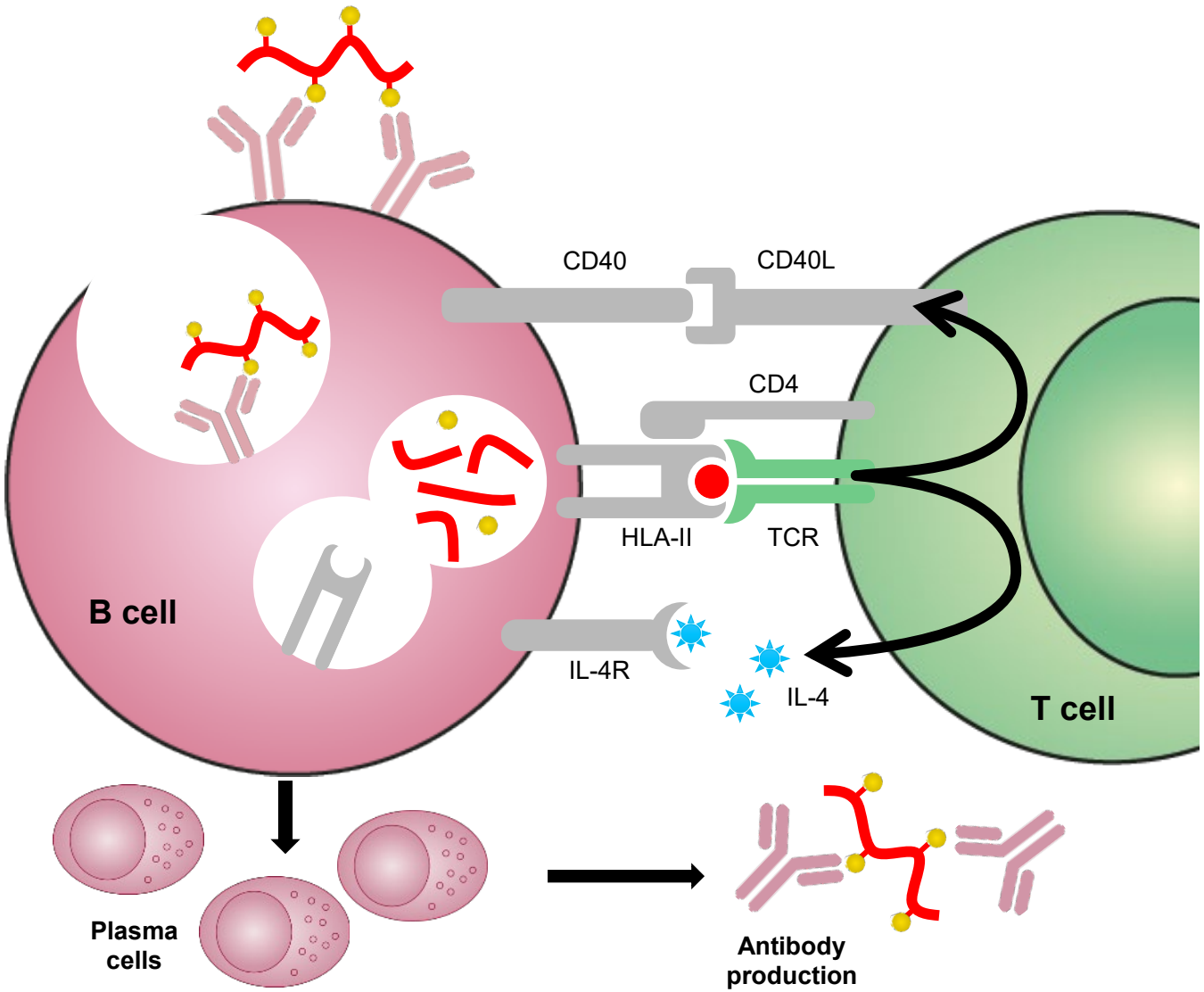
Antigen-dependent proliferation and differentiation into plasma and memory cells

BCR, B-cell receptor.

Male et al. Immunology, 7th edn, 2001. Image adapted from: Owen & Punt. Kuby Immunology, 7th edn, 2012.

T-cell-dependent B-cell activation (T–B collaboration)[1,2]

- The surface immunoglobulin that serves as the BCR has two roles in B-cell activation:
  - BCR binds antigen (a hapten-carrier complex), leading directly to the intracellular signaling cascade[1,2]
  - BCR delivers the antigen to intracellular sites where it is degraded and returned to the B-cell surface as peptides bound to HLA class II molecules[1]
- The peptide:HLA class II complex is recognized by helper T cells, stimulating them to express CD40L and secrete IL-4, which stimulates B-cell proliferation and differentiation into Ab-secreting cells[1]



Ab, antibody; BCR, B-cell antigen receptor; CD40L, CD40 ligand; HLA, human leukocyte antigen; IL-4R, interleukin-4 receptor; TCR, T-cell receptor.

1. Janeway et al. Immunobiology: The Immune System in Health and Disease, 5th edn, 2001. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27142/. Accessed October 2022. 2. Alberts et al. Molecular Biology of the Cell. 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21054/. Accessed October 2022.

Structure, cellular distribution and affinities of human activating and inhibitory Fcγ receptors

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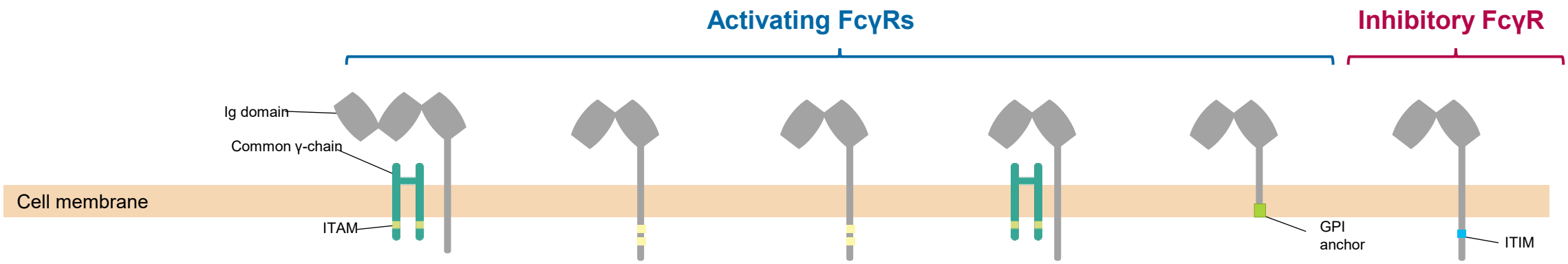
Human FcγRs differ in function, affinity for the Fc fragment of antibody and cellular distribution[1]



		FcγRI CD64	FcγRIIA CD32a	FcγRIIC CD32c	FcγRIIIA CD16	FcγRIIIB CD16	FcγRIIB CD32b
Expression	**Lymphoid**	–	–	NK cell	NK cell	–	B cell, plasma cell
	Myeloid	Monocyte, DC and macrophage	Monocyte, DC and macrophage	–	Monocyte, DC and macrophage	–	Monocyte, DC and macrophage
	Granulocyte	Neutrophil and eosinophil	Neutrophil	–	–	Neutrophil, mast cell and eosinophil	Neutrophil, basophil and mast cell
IgG subclasses binding affinity		1 >>> 2, 3 and 4	1 and 3 >> 2 > 4	1 > 3 >> 4 > 2	1 >> 2 and 3 > 4	1 and 3 >> 2 and 4	1 > 3 >> 4 > 2

DC, dendritic cell; Fc, crystallizable fragment; FcγR, Fc receptor for IgG; GPI, glycosylphosphatidylinositol; IgG, immunoglobulin G; ITAM, immunoreceptor tyrosine-based activating motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; NK, natural killer; (–) not expressed.

Adapted from 1. Smith & Clatworthy. Nat Rev Immunol 2010;10:328–43. 2. Nimmerjahn et al. Nat Rev Immunol 2008;8:34–47.

Adaptive immunity: T cells

Module 1. Basic immunology

The adaptive immune response: T lymphocytes

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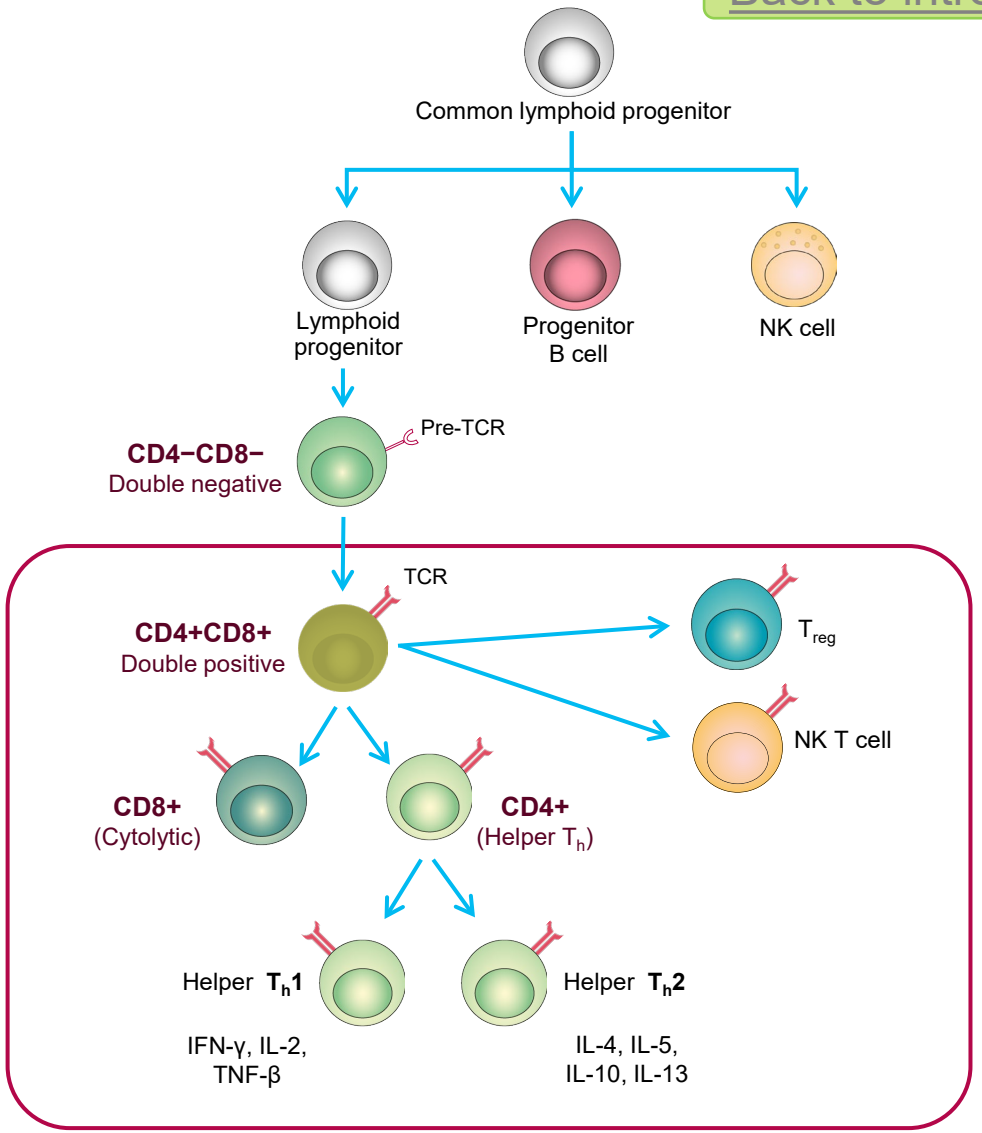
- T cells originate from lymphoid precursors in the bone marrow and develop in the thymus[1]
- T cells can be distinguished from other lymphocytes (e.g. B cells and NK cells) by the presence of an antigen-binding TCR on the cell surface[1]
- T cells differentiate into a number of subtypes
  - Cytolytic T cells (CD8+)
  - Helper T cells (CD4+: T_h1, T_h2 and T_h17)
  - T_{regs} (CD4+)
  - NK T cells

The main function of T lymphocytes is to recognize intracellular microbes that are inaccessible to antibodies[2]



IFN, interferon; IL, interleukin; NK, natural killer; TCR, T-cell receptor; T_h, T helper; TNF, tumor necrosis factor; T_{reg}, regulatory T cell.

1. Andersen. J Invest Dermatol 092006;126:32–41. 2. Janeway et al. Immunobiology: The Immune System in Health and Disease, 5th edn, 2001. Available from: http://www.ncbi.nlm.nih.gov/books/NBK27092/ and https://www.ncbi.nlm.nih.gov/books/NBK26827/. Accessed October 2022. Image adapted from Janeway et al. Immunobiology: The Immune System in Health and Disease, 5th edn, 2001 and Germain RN. Nat Rev Immuno 2002;2:3-322

Distinct functions of CD4 vs CD8 and HLA class I versus class II molecules

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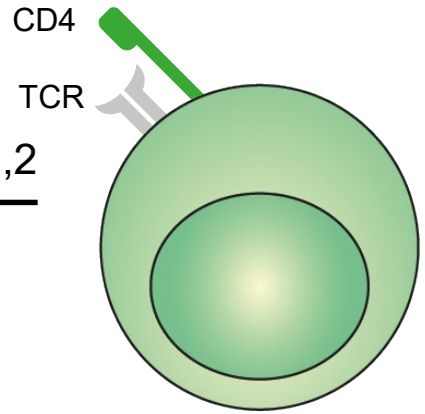
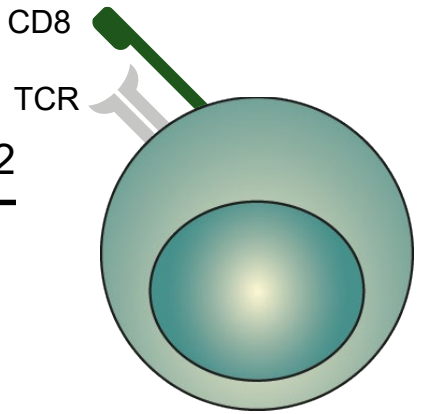
CD4+ T lymphocytes[1,2]



- T **helper** (T_h)
- Recognition of peptides derived from **extracellular** proteins (phagocytosis, endocytosis)
- Recognition of peptides presented by HLA **class II** molecules only
- These T cells are 'HLA class II-restricted'

CD8+ T lymphocytes[1,2]

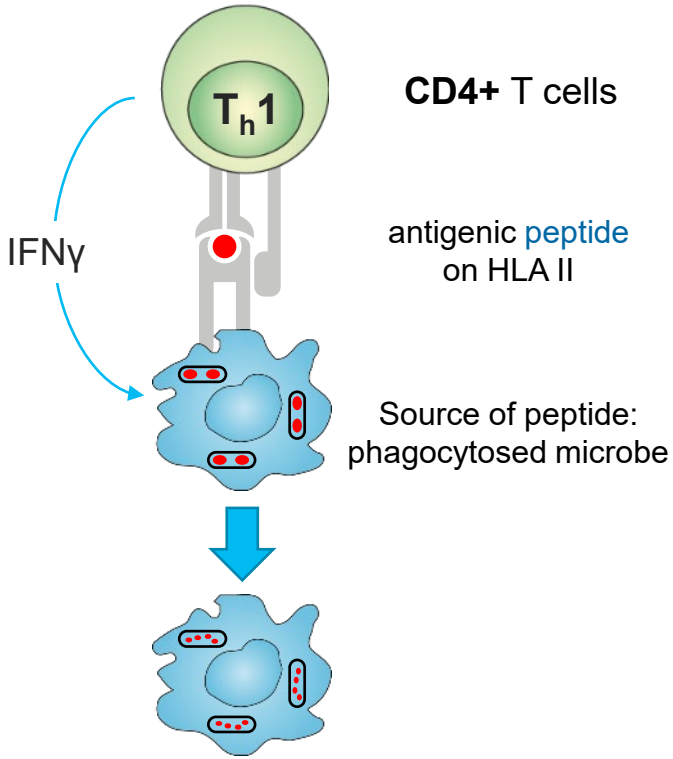


- **CTL: cytolytic**
- Recognition of peptides derived from **intracellular** proteins (i.e. those produced within the cells)
- Recognition of peptides presented by HLA **class I** molecules only
- These T cells are 'HLA class I-restricted'

CTL, cytotoxic T lymphocyte. HLA, human leukocyte antigen; TCR, T-cell receptor.

1. Blum et al. Annu Rev Immunol 2013;31:443–73. 2. Woodworth & Behar. Crit Rev Immunol 2006;26:317–52

Major T-cell functions against pathogens[1-5]



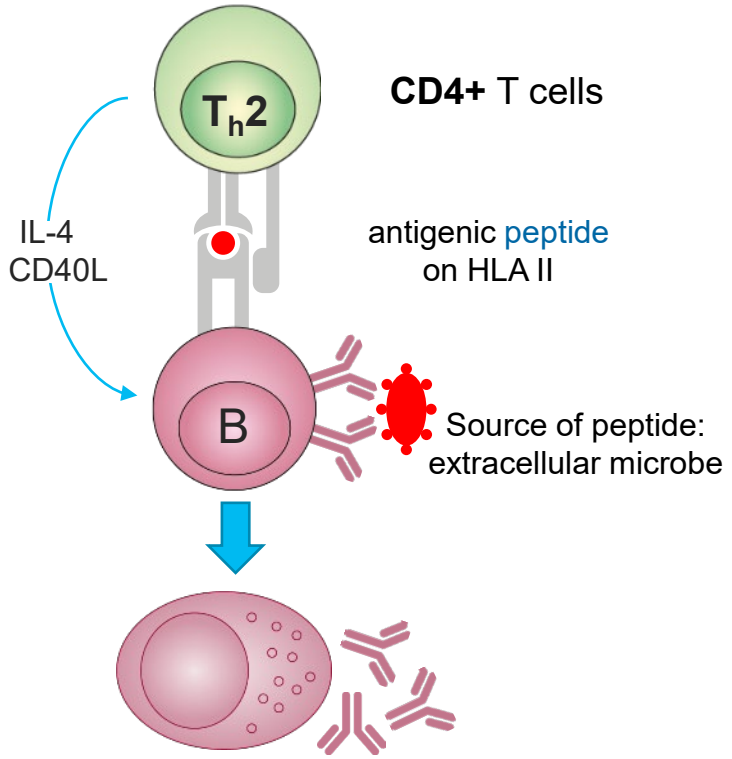
CD4+ T cells

antigenic peptide on HLA II

Source of peptide: phagocytosed microbe

T_h1 cells produce high amounts of IFNγ, which activates macrophages to kill phagocytosed bacteria

Function: macrophage activation



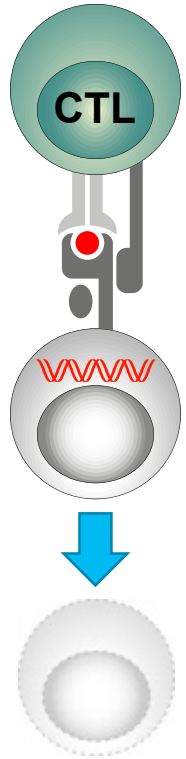
CD4+ T cells

antigenic peptide on HLA II

Source of peptide: extracellular microbe

B cells activated by antigen and T cells produce antibodies of high affinities and of IgG, IgA or IgE isotypes (instead of IgM)

Function: B-cell differentiation



CD8+ T cells

antigenic peptide on HLA I

Source of peptide: DNA/RNA of intracellular microbe

An activated CTL can kill infected cells through
- Production and release of **cytotoxic granules**
- **FasL** (on CTL)/Fas (on target) interactions
- Secretion of cytokines such as **TNF-α**

Function: target cell apoptosis

CD40L, CD40 ligand; CTL, cytotoxic T lymphocyte; FasL, Fas ligand; HLA, human leukocyte antigen; IFN, interferon; Ig, immunoglobulin; IL-4, interleukin 4; Th, T helper; TNF, tumor necrosis factor.

1. Janeway et al. Immunobiology: The Immune System in Health and Disease, 5th edn, 2001. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27149/. Accessed October 2022. 2. Bell. Available from: https://www.immunology.org/public-information/bitesized-immunology/cells/cd4-t-cells. Accessed October 2022. 3. Wissinger. Available from: https://www.immunology.org/public-information/bitesized-immunology/cells/cd8-t-cells. Accessed October 2022. 4. Andersen. J Invest Dermatol 2006;126:32–41. 5. Mosser & Zhang. Curr Protoc Immunol 2008;Chapter 14:Unit 14.2.

Major histocompatibility complex

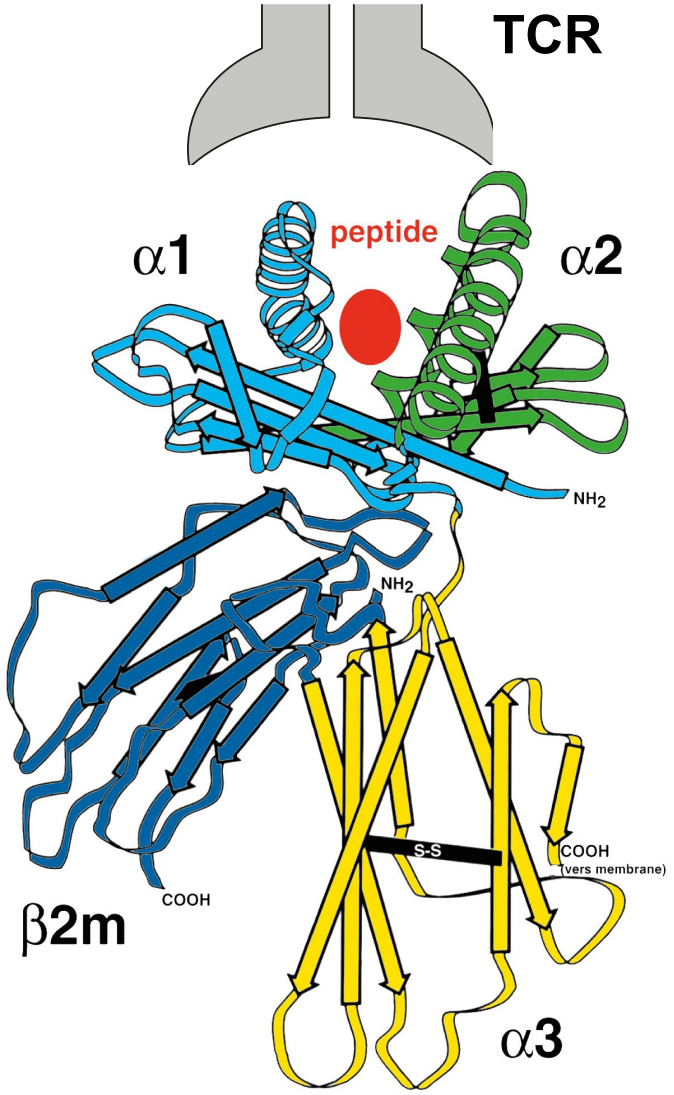
- The MHC is a set of genes identified in mice that determines graft rejection/ acceptance (histocompatibility)
- The MHC genes code for the MHC molecule
- In humans, the MHC genes/molecules were discovered on white blood cells and are therefore named the human leukocyte antigen (HLA) genes/molecules
  - Three genes encode the HLA class I molecules: *HLA-A*, *HLA-B*, *HLA-C*
  - Six genes encode the HLA class II molecules: *HLA-DR*, *HLA-DP*, *HLA-DQ* (two chains)
- The HLA genes are highly polymorphic (many alleles)
- The function of the HLA molecules is presentation of antigenic peptides to T lymphocytes

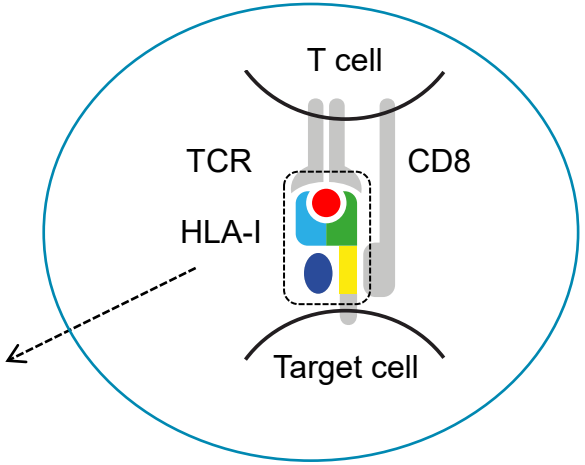
HLA, human leukocyte antigen; MHC, major histocompatibility complex.

Janeway et al. Immunobiology: The Immune System in Health and Disease, 5th edn, 2001. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27156/. Accessed October 2022.

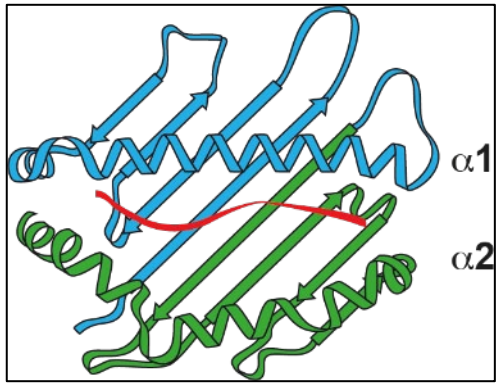
Crystal structure of HLA class I molecules

- The TCR recognizes a complex between a class I or class II HLA molecule and an antigenic peptide
- The HLA class I molecule is a heterodimer with a heavy chain, containing the α_1, α_2 and α_3 domains, and $ß_2$microglobulin
- The TCR interacts with the antigenic peptide presented in a groove on top of the HLA molecule, between the α_1 and α_2 domains
- The TCR contacts both the antigenic peptide itself and residues of the α_1 and α_2 domains of the presenting HLA molecule





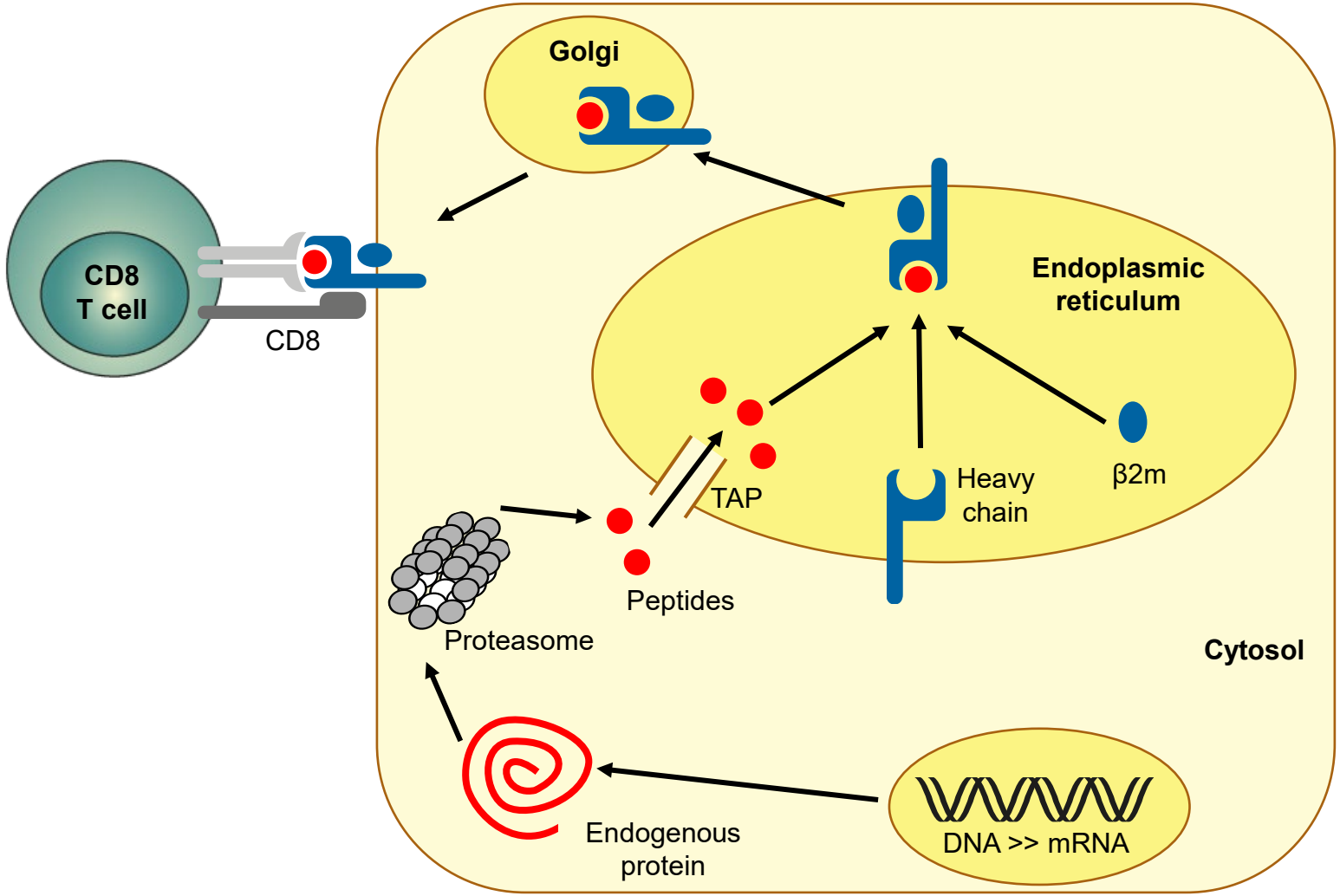
Top view



ß2m, beta$_2$ microglobulin; HLA, human leukocyte antigen; TCR, T-cell receptor.
Bjorkman et al. J Immunol 2005;174:13–9.

Canonical HLA class I antigen processing pathway

- Proteins are degraded by the proteasome
- Next, the resultant peptides are translocated by TAP into the ER lumen and loaded onto HLA class I molecules
- The peptide–HLA class I complexes are then released from the ER and transported via the Golgi to the plasma membrane
- The antigenic peptide is presented to CD8+ T cells



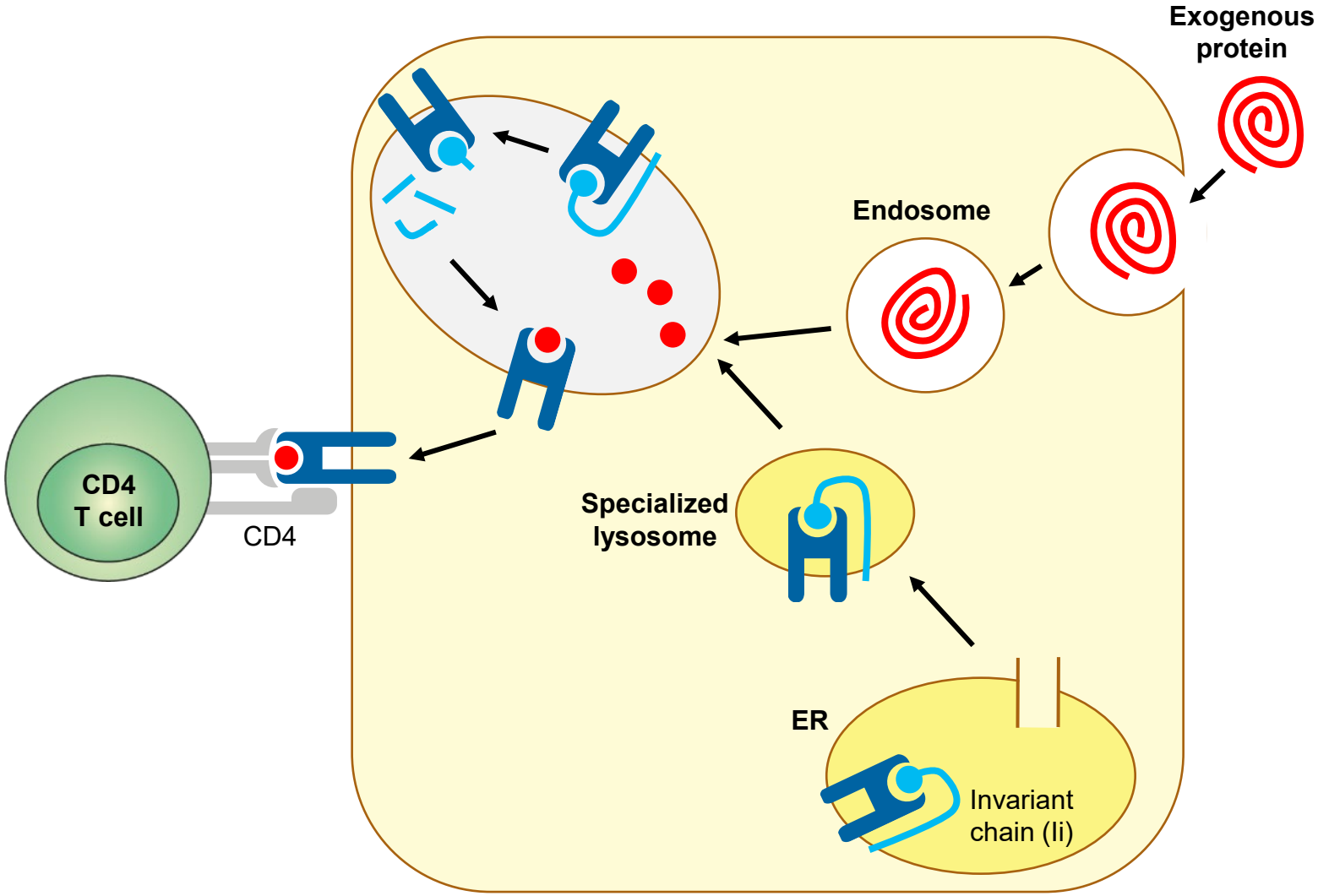
ß2m, beta$_2$ microglobulin; ER, endoplasmic reticulum; HLA, human leukocyte antigen; TAP, transporter associated with antigen presentation.

1. Neefjes et al. Nat Rev Immunol 2011;11:823–36.

Canonical HLA class II antigen processing pathway

Chapter homepage

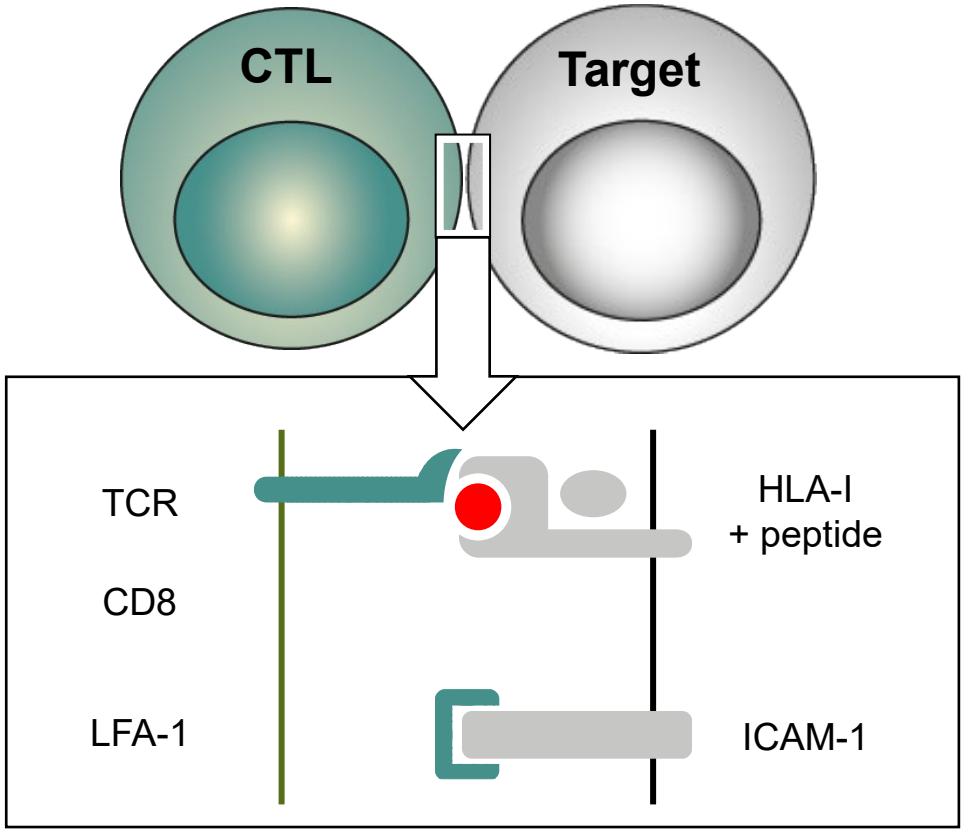
- HLA class II α- and β-chains assemble in the ER and form a complex with the invariant chain
- The heterotrimer is transported through the Golgi to the HLA class II compartment
- Endocytosed proteins and Ii are degraded by resident proteases
- The Ii fragment in the peptide-binding groove is exchanged for an antigenic peptide
- HLA class II molecules are transported to the plasma membrane to present antigenic peptides to CD4+ T cells



ER, endoplasmic reticulum; HLA, human leukocyte antigen; Ii, invariant chain; TAP, transporter associated with antigen presentation.
Neefjes et al. Nat Rev Immunol 2011;11:823–36.

Interactions that regulate the immune response occur in the nanoscale gap between T cells and APCs[1]

- The specificity of the interaction between a T cell and an APC depends on the TCR and HLA-peptide complexes
- ICAM-1 is an adhesion molecule that forms a link to LFA-1, an integrin that mediates adhesion between T cells and APCs[1,2]
- Adhesion molecules are needed to allow T cells to bind to APCs long enough for them to become activated[2]
- Once the TCR has been triggered, it can further enhance the activity of LFA-1 and promote formation of an immunological synapse[3]



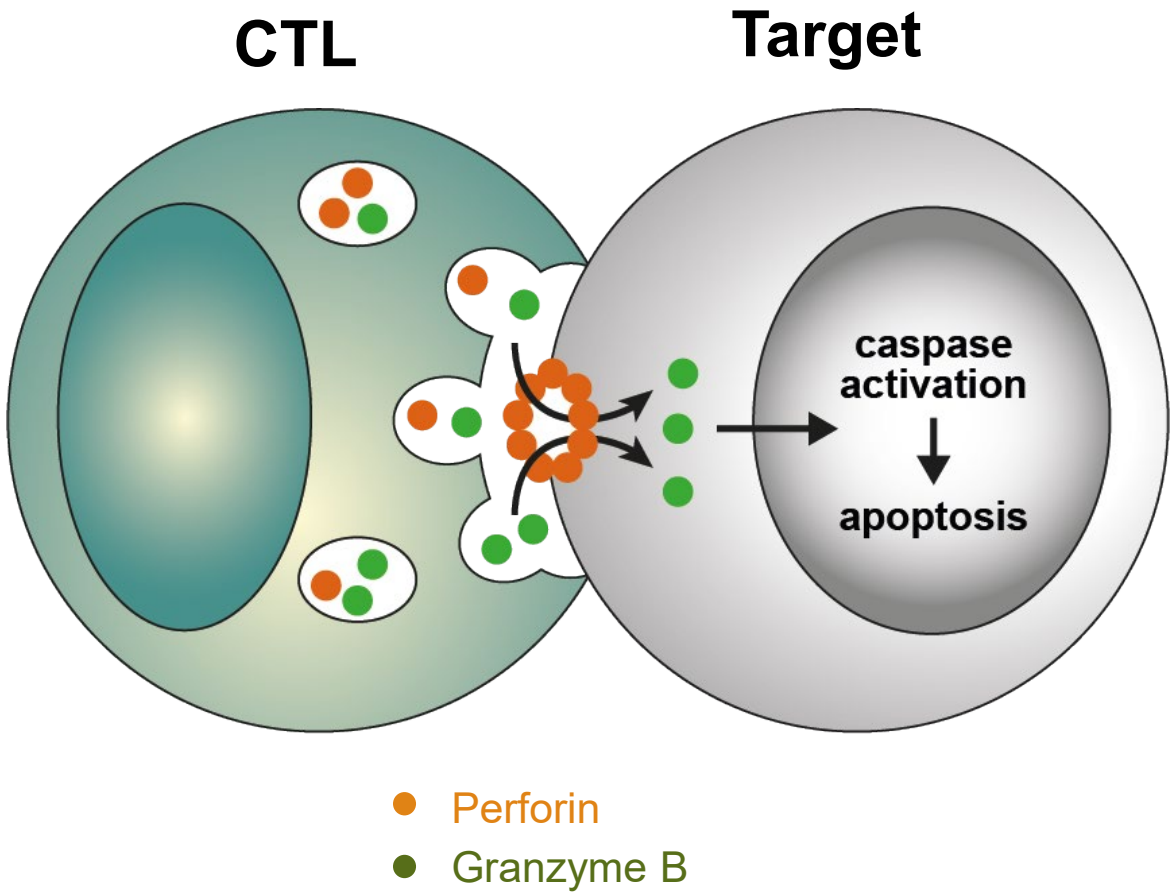
'Immunological synapse'
antigen recognition + adherence molecules

APC, antigen-presenting cell; HLA, human leukocyte antigen; ICAM, intercellular adhesion molecule; LFA, leukocyte function-associated antigen; TCR, T-cell receptor.

1. Shimizu. Nat Immunol 2003;4:1052–4. 2. Alberts et al. Molecular Biology of the Cell, 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26827/. Accessed October 2022. 3. Dustin. Cancer Immunol Res 2014;11:1023–33.

Granule-mediated cytolysis by CTLs

- Once bound to its target cell, a CTL can use different strategies to kill the target cell
- By killing the infected cell, the CTL can release **perforin**
- **Perforin** is stored in CTLs within secretory vesicles, which also contain serine proteases such as **granzyme B**
- **Perforin**, a pore-forming protein, polymerizes in the plasma membrane of the target cell, forming transmembrane channels
- **Granzyme B** cleaves and activates members of the caspase family that mediate apoptosis
- NK cells use the same lytic machinery as CTLs



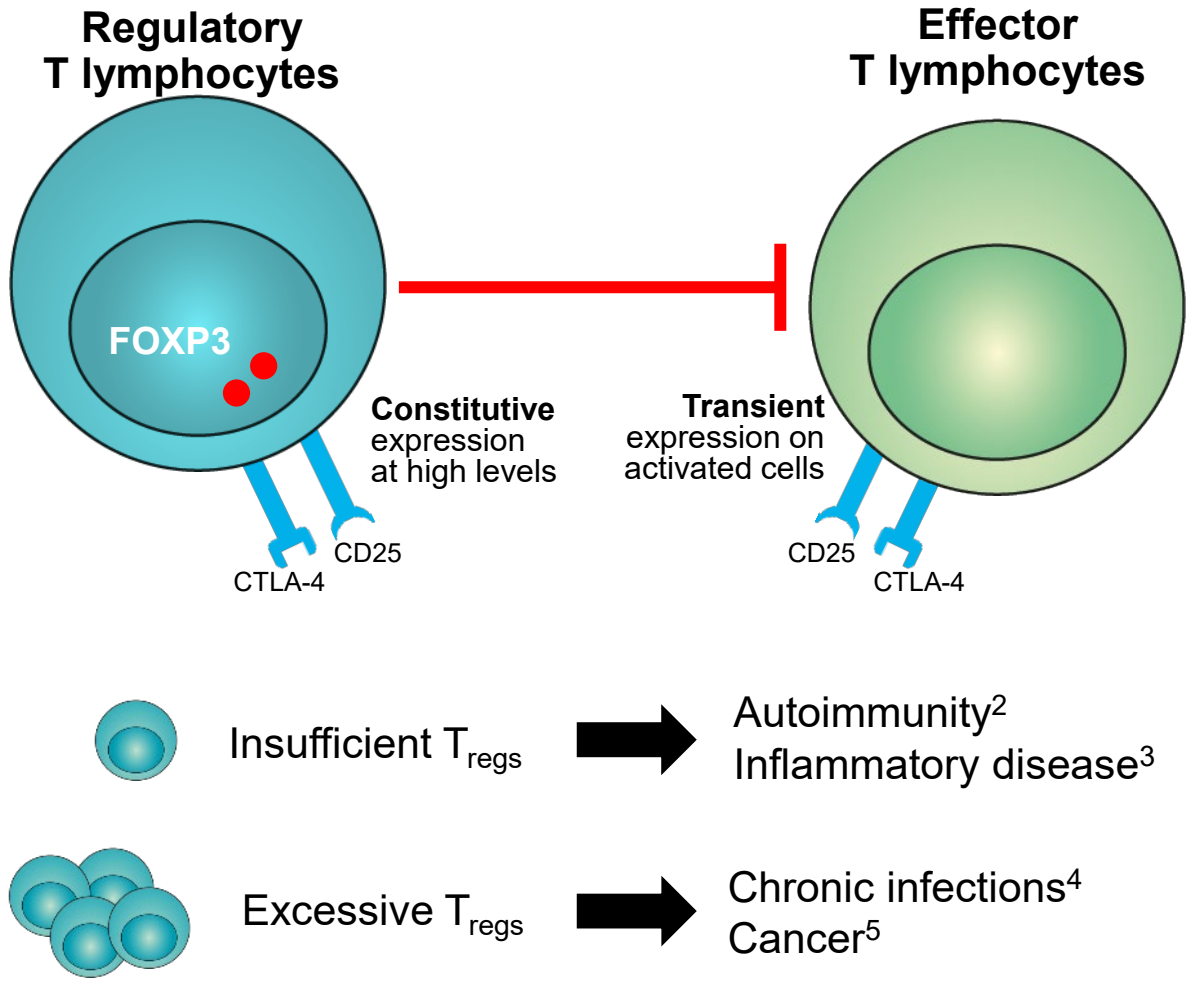
CTL, cytotoxic T lymphocyte; NK, natural killer.

Alberts et al. Molecular Biology of the Cell, 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26926/. Accessed October 2022.

Regulatory T cells are vital to immune homeostasis

Chapter homepage

- T_{reg} differentiation and immunosuppressive activity depend on transcription factor FOXP3 (*Foxp3*$^{-/-}$ mice die from autoimmunity at an early age)
- T_{regs} maintain tolerance to self-antigens and prevent autoimmune disease
- Human T_{regs} do not bear a unique surface marker. They constitutively express high levels of CD25 and CTLA-4
- T_{regs} are immunosuppressive through various mechanisms and generally suppress or downregulate induction and proliferation of effector T cells[1]



CTLA-4, cytotoxic T lymphocyte-associated protein 4; FOXP3, forkhead box P3; T_{reg}, regulatory T cell.

1. Chevalier et al. J Immunol 2014;193:4845–58. 2. Komatsu et al. Nat Med 2014;20:62–70. 3. Thorburn & Hansbro. Am J Respir Cell Mol Biol 2010;43:511–9. 4. Sanchez & Yang. Immunol Res 2011;49:124–34. 5. Smigiel et al. Immunol Rev 2014;259:40–59.

T-cell regulation: stimulatory and inhibitory coreceptors

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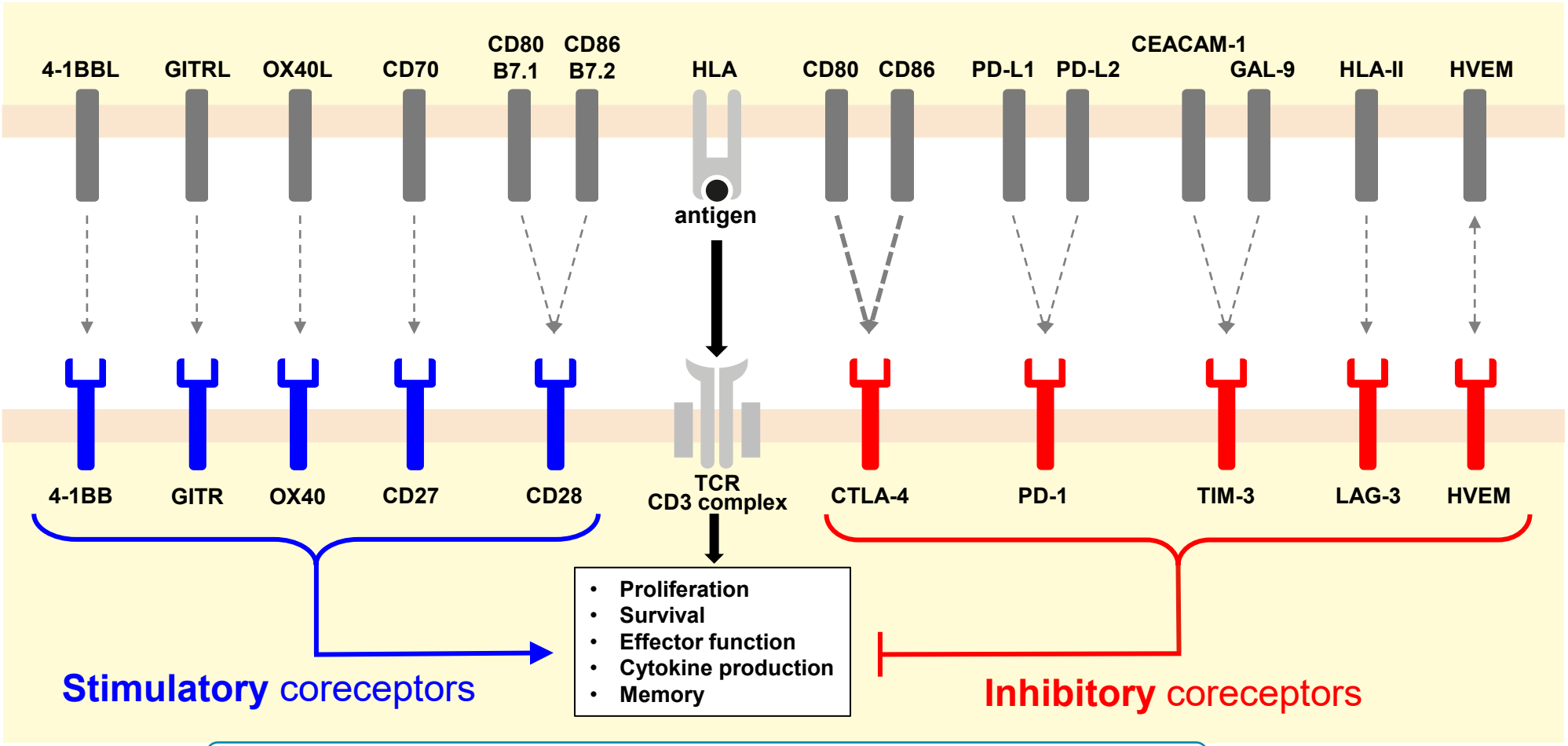

Function: fine-tuning of T-cell activation in time and space

APC, antigen-presenting cell; HLA, human leukocyte antigen; TCR, T-cell receptor.

Adapted from Kershaw et al. Nat Rev Cancer 2013;13:525–41. doi:10.1038/nrc3565 2. Dustin. Cancer Immunol Res 2014;11:1023–33. 3. Le Mercier et al. Front Immunol 2015; 6:1-15.

Stimulatory and inhibitory coreceptors fine tune T-cell activity

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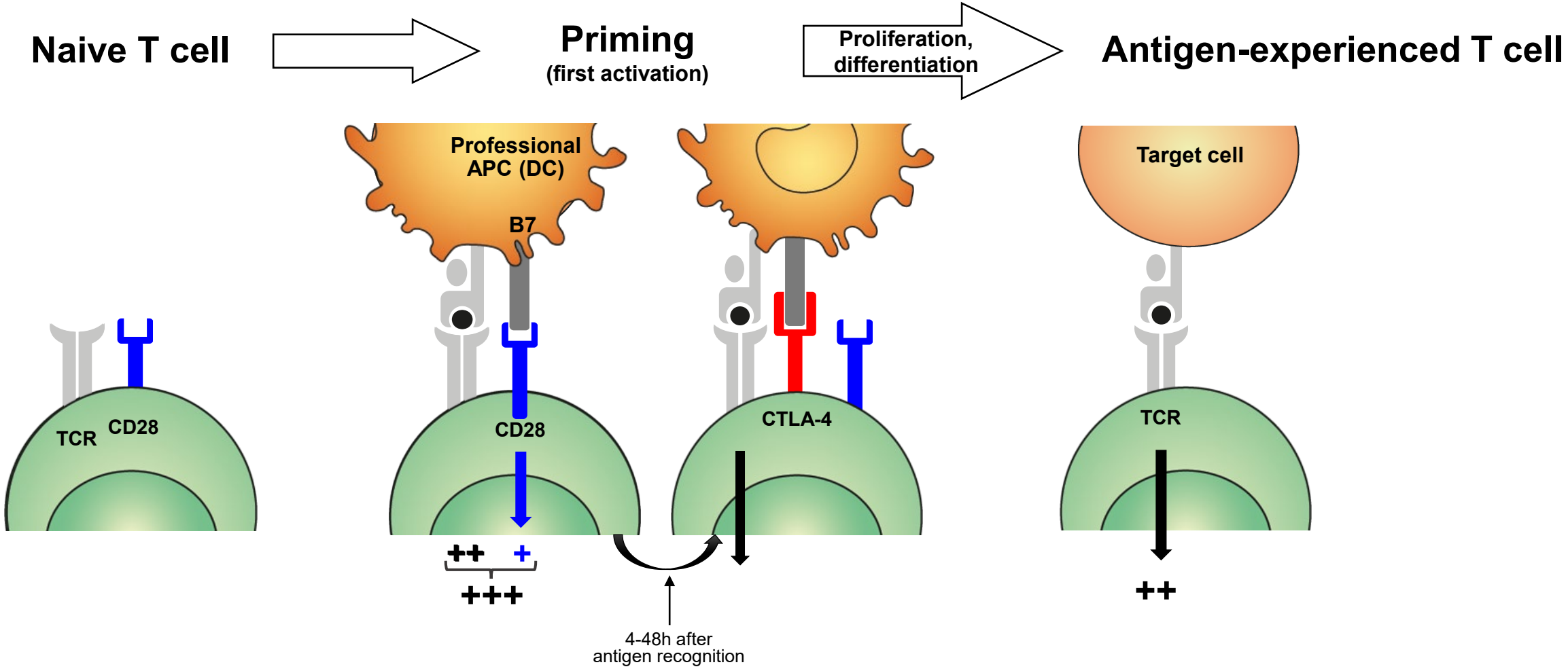
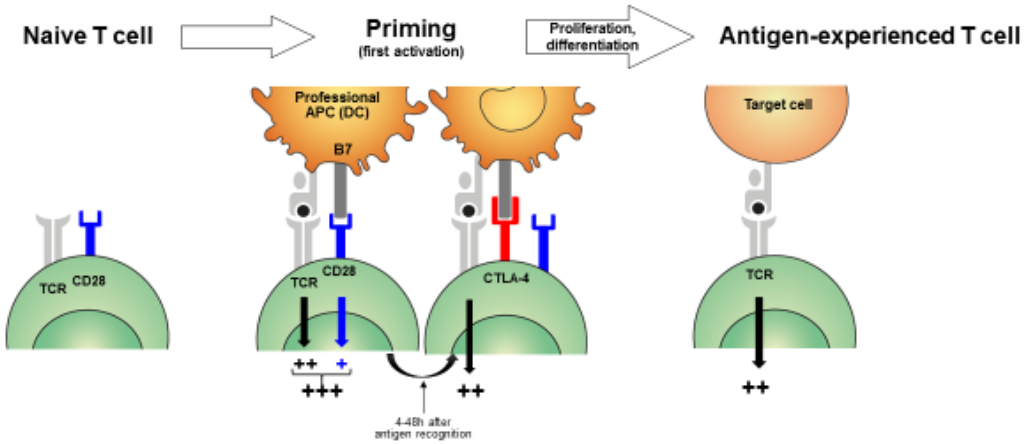


APC, antigen-presenting cell; CTLA-4, cytolytic T-lymphocyte-associated protein 4; DC, dendritic cell; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; TCR, T-cell receptor.

Alberts et al. Molecular Biology of the Cell, 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26827/. Accessed October2022.

Clinical relevance
Modulation of T-cell activity (1)

Stimulatory and inhibitory coreceptors fine tune T-cell activity



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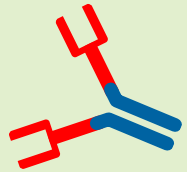
Alberts et al. Molecular Biology of the Cell: T Cells and MHC Proteins. 4th edn. 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26827/. Accessed May 2017.

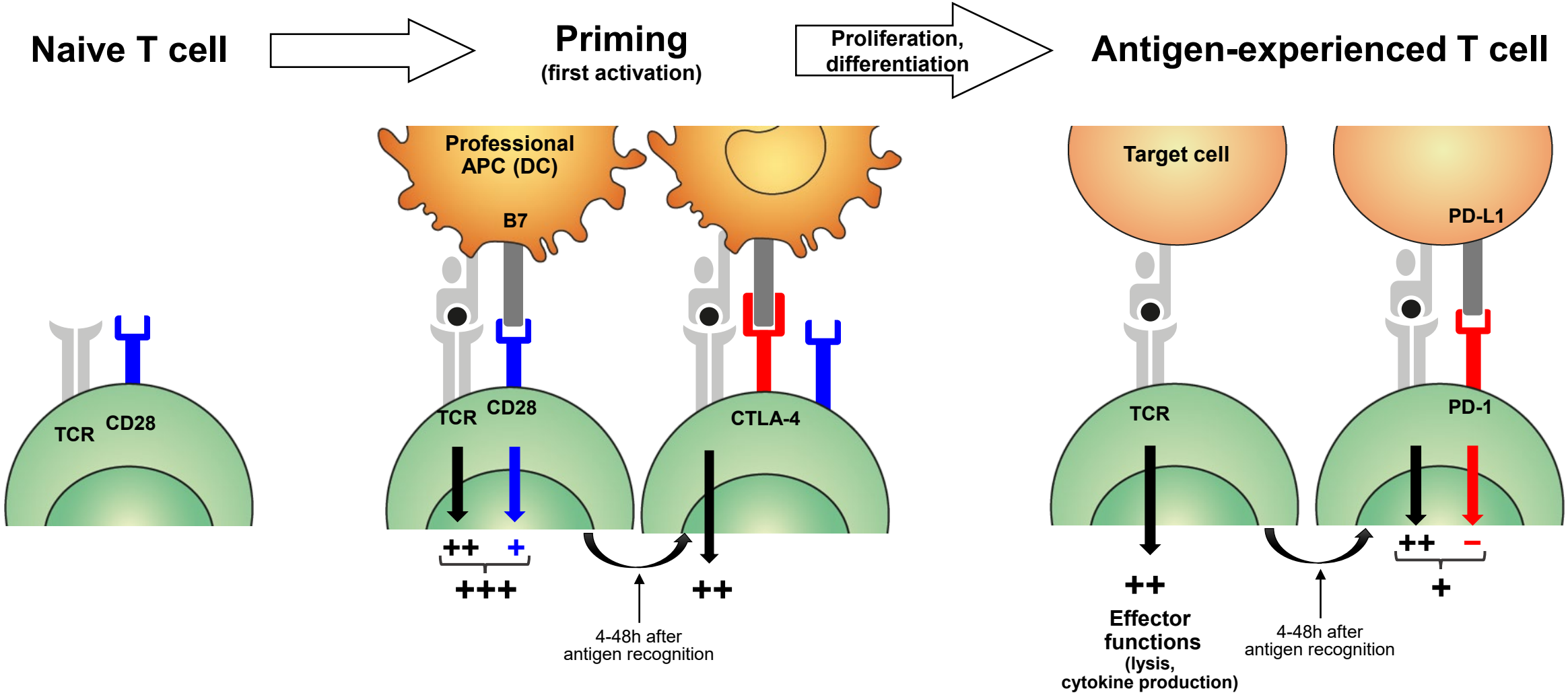
- **Agents that target T-cell coreceptors to modulate T-cell activity are used therapeutically**

	Abatacept	Belatacept
Structure	A fusion protein that consists of the extracellular domain of human CTLA-4 linked to a modified Fc portion of human IgG1 	
Mechanism of action	Binds to B7 (CD80/CD86) ligands and prevents T-cell costimulation by CD28	
Therapeutic indications	Rheumatoid arthritis, psoriatic arthritis, polyarticular juvenile idiopathic arthritis	Prophylaxis of graft rejection in adult recipients of a renal transplant

APC, antigen-presenting cell; CTLA-4, cytolytic T-lymphocyte-associated protein 4; DC, dendritic cell; Fc, crystallizable fragment; IgG, immunoglobulin G; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; SmPC, summary of product characteristics; TCR, T-cell receptor.
Always refer to the SmPC. All SmPCs are available from http://www.ema.europa.eu/ema/.

Stimulatory and inhibitory coreceptors fine tune T-cell activity

Chapter homepage

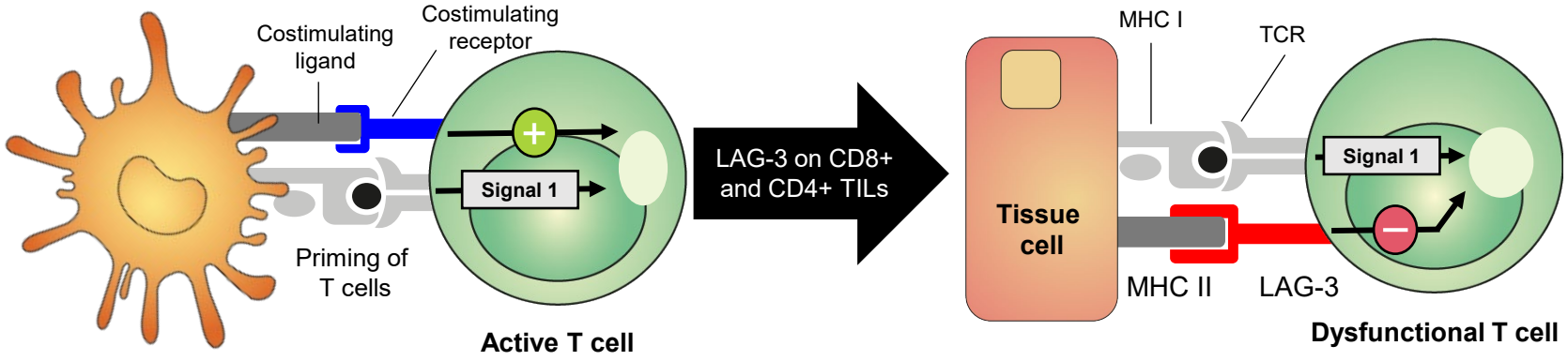


APC, antigen-presenting cell; CTLA-4, cytolytic T-lymphocyte-associated protein 4; DC, dendritic cell; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; TCR, T-cell receptor.
Adapted from 1. Alberts et al. Molecular Biology of the Cell, 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26827/. Accessed October 2022. and 2. Riley. Immunol Rev. 2009; 229:114–125.

Stimulatory and inhibitory coreceptors fine tune T-cell activity

- LAG-3 is a cell-surface molecule expressed on T cells and other immune cells that has diverse inhibitory biologic effects on the function of T cells[1,2]

LAG-3



Increased expression of LAG-3 on CD8+ and CD4+ TILs, especially in the context of PD-1 expression, inhibits effector T-cell function[1,3-5]

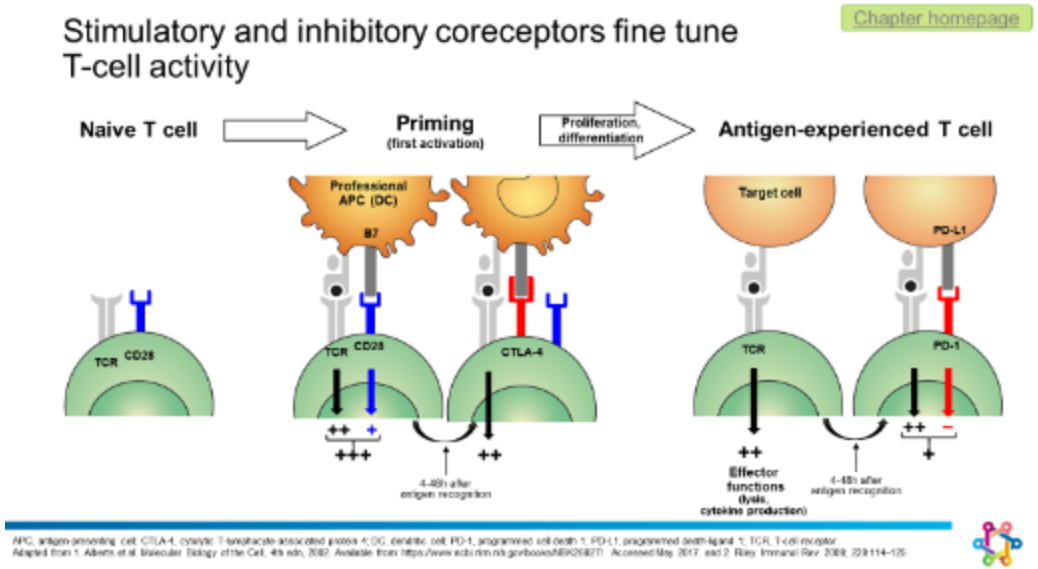
Interaction of LAG-3 with its ligands triggers inhibitory activity that reduces the function of effector T cells, leading to an impaired ability to fight tumor cells and an increased potential for tumor growth[1,5]

Ligands for LAG-3 are distinct from the ligands of other inhibitory immune checkpoints such as PD-1 or CTLA-4. Among its ligands, the most well established is MCH II; others are emerging, including FGL1[1,5–7]

APC, antigen-presenting cell; CTLA-4, cytotoxic T lymphocyte antigen 4; FGL1, fibrinogen-like protein 1; LAG-3, lymphocyte-activation gene 3; PD-1, programmed death-1; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

1. Long L, et al. Genes Cancer 2018;9:176-189; 2. Grosso JF, et al. J Clin Invest 2007;117:3383-3392; 3. Workman CJ, et al. J Immunol 2004;172:5450-5455; 4. Woo SR, et al. Cancer Res 2012;74:917-927;
5. Wang J, et al. Cell 2019;176:334-347.e12.; 6. Huang R-Y, et al. Oncoimmunology 2017;6:e1249561; 7. Maçon-lemaître L, Triebel F. Immunology 2005;115:170-178.

Clinical relevance
Modulation of T-cell activity (2)



Checkpoint inhibitors

Monoclonal antibodies that block T-cell inhibitory coreceptors to increase T-cell activity and are used in oncology

- **Anti-CTLA-4 antibodies**
  - Ipilimumab, indicated for melanoma, RCC, NSCLC, CRC, MPM and ESCC
- **Anti-PD-1 antibodies**
  - Nivolumab, indicated for melanoma, NSCLC, MPM, RCC, cHL, SCCHN, CRC, ESCC, gastric adenocarcinoma and urothelial carcinoma
  - Pembrolizumab, indicated for melanoma, NSCLC, cHL, urothelial carcinoma, SCCHN, ESCC, CRC, breast cancer, cervical cancer and RCC
- **PD-L1 antibodies**
  - Atezolizumab, indicated for urothelial carcinoma, NSCLC, SCLC, HCC and breast cancer
  - Durvalumab, indicated for urothelial carcinoma (FDA), NSCLC and SCLC
- **Anti-LAG-3 antibodies**
  - Relatlimab, indicated for melanoma (in combination with nivolumab; FDA)

cHL, classical Hodgkin lymphoma; CRC, colorectal cancer; CTLA-4, cytolytic T-lymphocyte-associated protein 4; HCC, hepatocellular carcinoma; MPM, malignant pleural mesothelioma; NSCLC, non-small-cell lung cancer; ESCC, esophageal squamous cell carcinoma; PD-1, programmed cell death 1; PD-L1, programmed death ligand 1; PI, prescribing information. RCC, renal cell carcinoma; SCCHN, squamous cell cancer of the head and neck; SCLC, small-cell lung cancer. Always refer to the SmPC or PI. All SmPCs are available from http://www.ema.europa.eu/ema/. PIs are available from https://www.fda.gov/drugs/. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/home. Accessed October 2022.

Module 1: Summary and key takeaways

- The immune system is a vital source of protection against pathogens, harmful substances and the body's own cells during illness
- While the innate immune system is broad, the adaptive immune system is highly specific to the pathogen or threat
- In innate immunity, key players include macrophages, which are important in antibacterial responses, and NK cells, which can kill HLA class I-deficient cells not detected by CTLs
- In adaptive immunity, T and B cells have vital roles:
  - B cells can be activated by T-cell-dependent pathways, leading to the production of antibodies, which are involved in pathogen elimination
  - The main function of T lymphocytes is to recognize intracellular microbes that are inaccessible to antibodies, along with other specialized functions
- Changes to the balance of the immune system are associated with various diseases, which can be targeted with immunotherapy

CTL, cytotoxic T lymphocyte; HLA, human leukocyte antigen; NK, natural killer.

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