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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Bristol-Myers Squibb

# Acknowledgments


- This slide deck has been developed and validated by the ImmunoScience Academy Steering Committee:

  - Prof. Dr Pierre Coulie (Chair), *de Duve Institute, UCL*
  - Prof. Dr Ahmad Awada, *Jules Bordet Institute*
  - Prof. Dr Veronique del Marmol, *Hôpital Erasme*
  - Prof. Dr Guy Jerusalem, *CHU de Liège*
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  - Prof. Dr Bernard Lauwerys, *Cliniques Universitaires Saint Luc Bruxelles*
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  - Prof. Dr Rik Schots, *UZ-VUB*
  - Prof. Dr Eric Van Cutsem, *UZ-KULeuven*
  - Prof. Dr Johan Vansteenkiste, *UZ-KULeuven*
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# Module 1. Basic immunology

Click on a chapter below to start learning

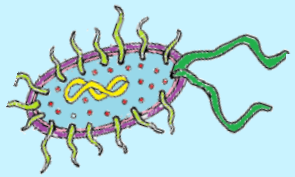
# Introduction to the immune system

Module 1. Basic immunology

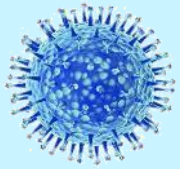
# What is the immune system?

- 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 2016;4:11–22. Available from: https://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0072548/. Accessed May 2017.

# 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<br>- Complement<br>- Inflammation<br>- Cells<br>  - Granulocytes (neutrophils, basophils, eosinophils)<br>  - Mast cells<br>  - Natural killer cells<br>  - Macrophages<br>  - Dendritic cells | - B lymphocytes, antibodies<br>- T lymphocytes |

1. Dranoff. Nat Rev Cancer 2004;4:11-22. 2. Janeway et al. Immunobiology, 6th edn, 2004. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27090/. Accessed August 2017. 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

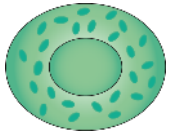
**Complement**

IgM or IgG antibodies bound to their antigen can activate complement

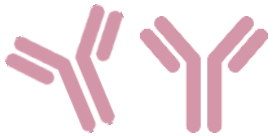
**Antibodies**

Learn more about antibodies

**Mast cells**

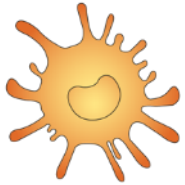


IgE antibodies bind to FcRs on mast cells; antigen recognition causes mast cell degranulation

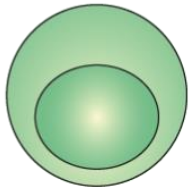


**Antibodies**

**Dendritic cells**



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

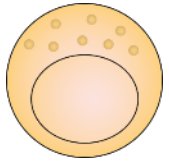


**T lymphocytes**

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

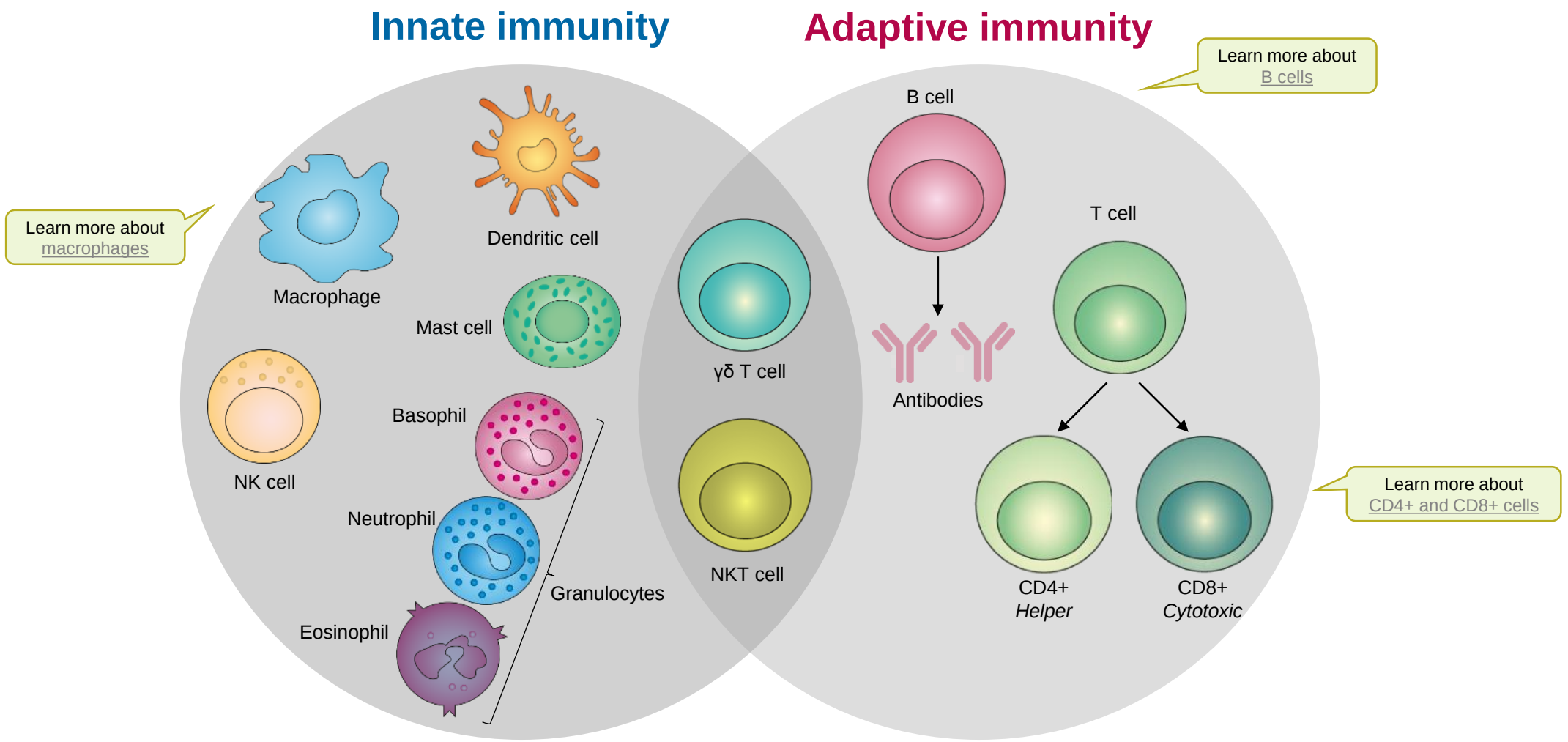
**Antibodies**

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

# Cells involved in innate and adaptive immunity

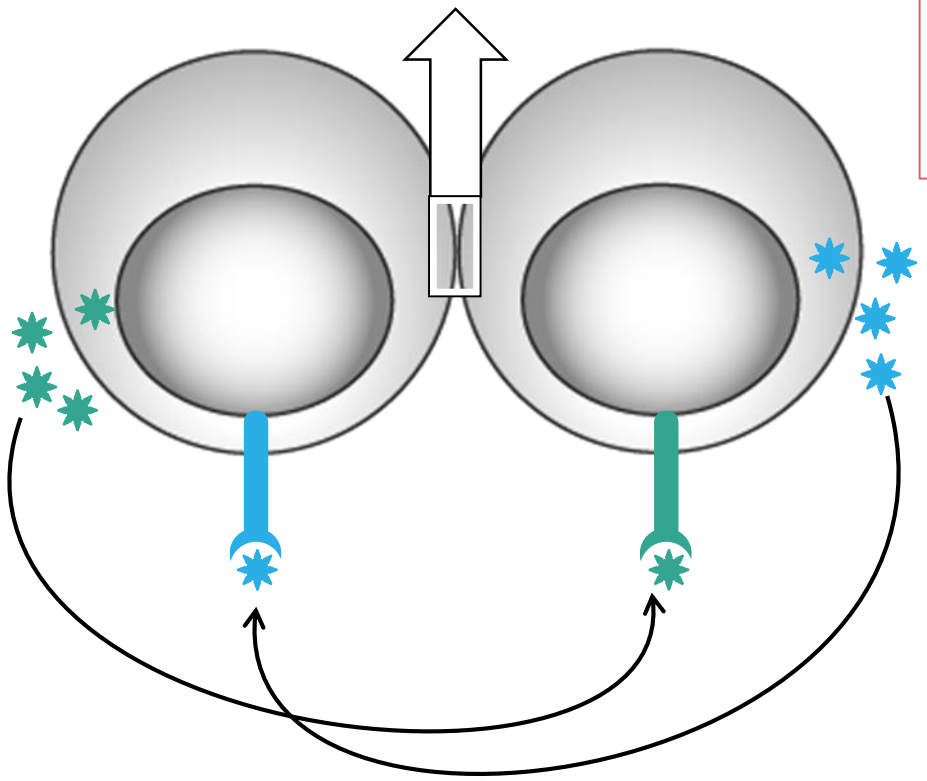



NK, natural killer; NKT, natural killer T.
Adapted from Dranoff. Nat Reviews Cancer 2004;4:11-22.

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

### Contact dependent

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

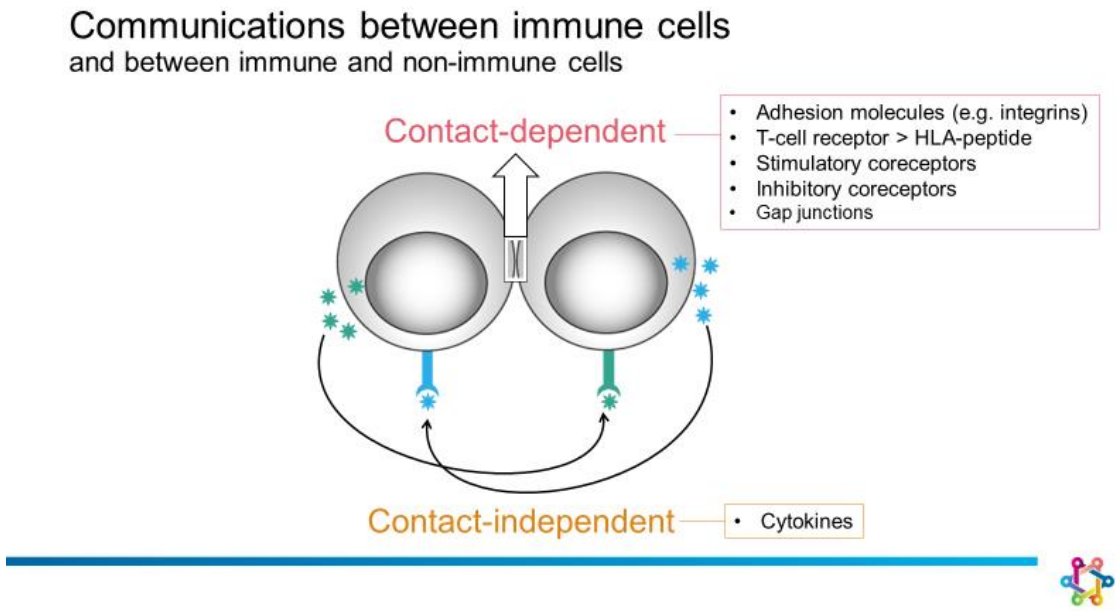


### Contact independent

- 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<br>• Type II: IFN-γ | • TNFα<br>• TNFβ (lymphotoxin α)<br>• CD40L<br>• FasL<br>• CD70<br>• …etc. | | • TGF-β1<br>• TGF-α<br>• BMPs<br>• GDNFs<br>• …etc. | • Erythropoietin<br>• Thrombopoietin<br>• CSF1 (M-CSF)<br>• CSF2 (GM-CSF)<br>• 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

### Cytokines

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

- **Cytokines as therapeutic drugs**
  - IL-2 (aldesleukin) for renal cell carcinoma
  - IL-11 (oprelvekin) for severe thrombocytopenia
  - G-CSF (e.g. filgrastim) and GM-CSF for immunoreconstitution
  - IFNβ1α for multiple sclerosis
  - IFNγ for chronic granulomatous disease and osteopetrosis
  - Epoetin-α for anemia

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# Clinical relevance

## Cytokines: blocking the effects with monoclonal antibodies

Chapter homepage

### Cytokines

- A group of over 100 small (5–20 kD) glycoproteins that are important in cell signaling
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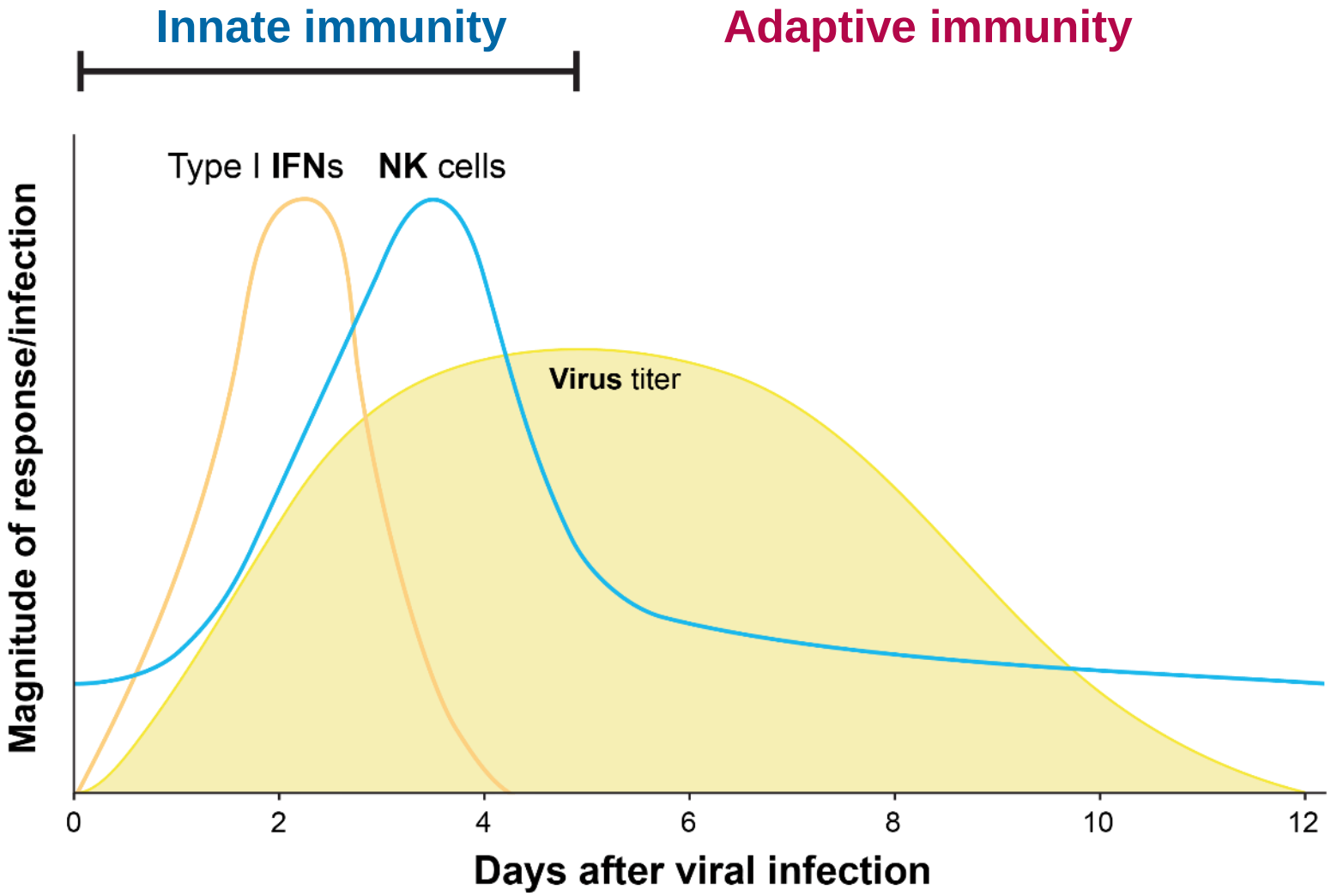
Zhang & An. Int Anesthesiol Clin 2007;45:27–37.

### 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, autoinflammatory diseases |
| **Anti-IL-2Rα (CD25)** | Daclizumab | Multiple sclerosis |
| **Anti-IL-6 receptor** | Tocilizumab | Rheumatoid arthritis, systemic juvenile idiopathic arthritis |
| **Anti-IL-17A** | Secukinumab, Ixekizumab | Plaque psoriasis, psoriatic arthritis, ankylosing spondylitis |
| **Anti-IL-17RA** | Brodalumab | Psoriasis |
| **Anti-IL-12/23** | Ustekinumab | Crohn's disease |

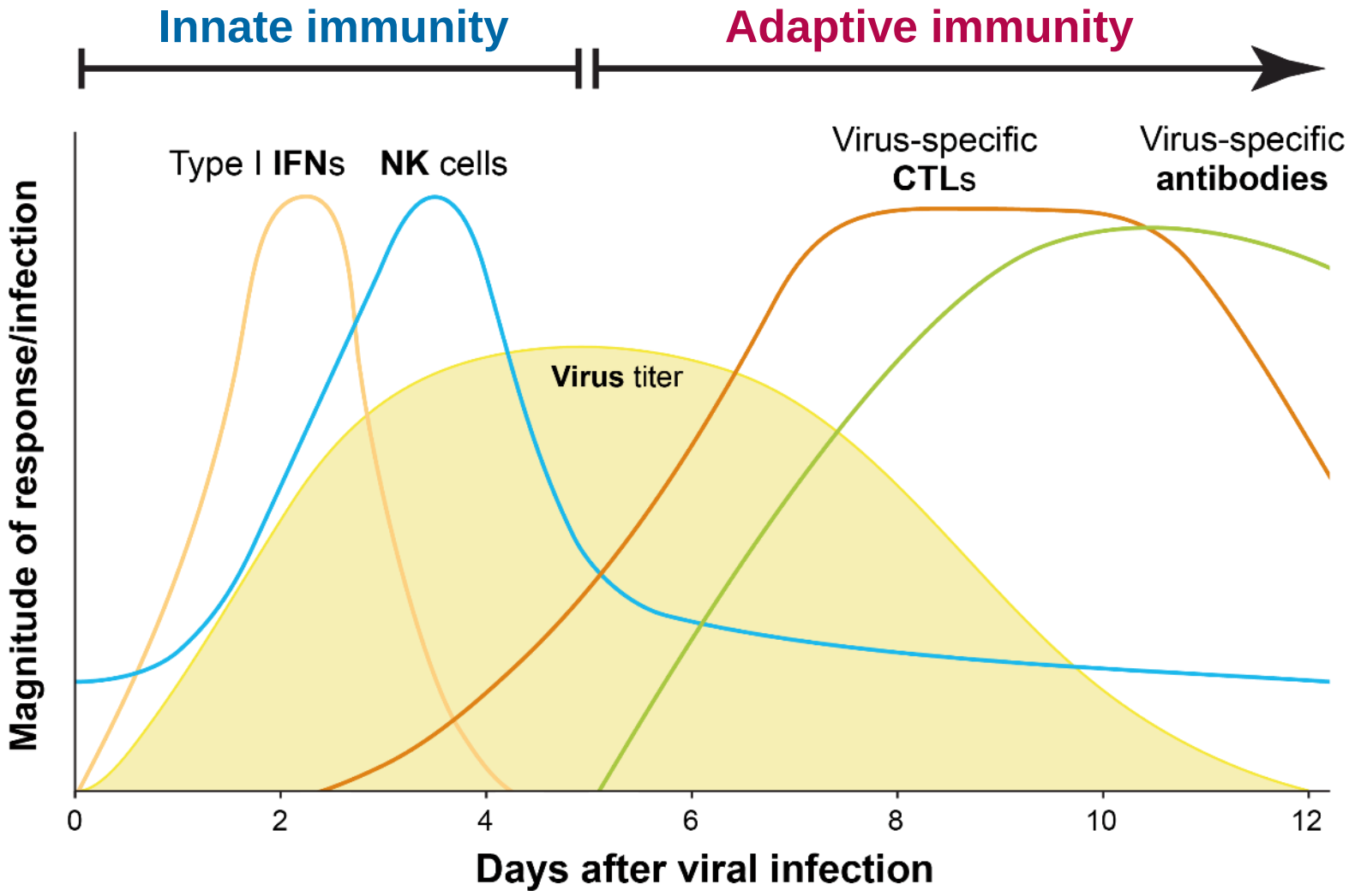
BMP, bone morphogenetic protein; CD40L, CD40 ligand; CSF, colony-stimulating factor; G-CSF, granulocyte CSF; GDNF, glial cell-derived neurotrophic factor; G-CSF, granulocyte CSF; GM-CSF, granulocyte macrophage-CSF; IFN, interferon; IL, interleukin; M-CSF, macrophage CSF; SmPC, summary of product characteristics; TGF, transforming growth factor; TNF, tumor necrosis factor.
Always refer to the SmPC. All SmPCs are available from http://www.ema.europa.eu/ema/.

# 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

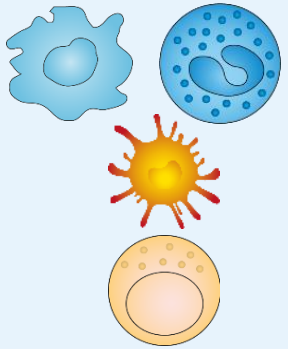



CTL, cytotoxic T lymphocyte; IFN, interferon; NK, natural killer.
Abbas et al. Cellular and Molecular Immunology, 8th edn, 2015.

# Innate immunity

Module 1. Basic immunology

# The innate immune response

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

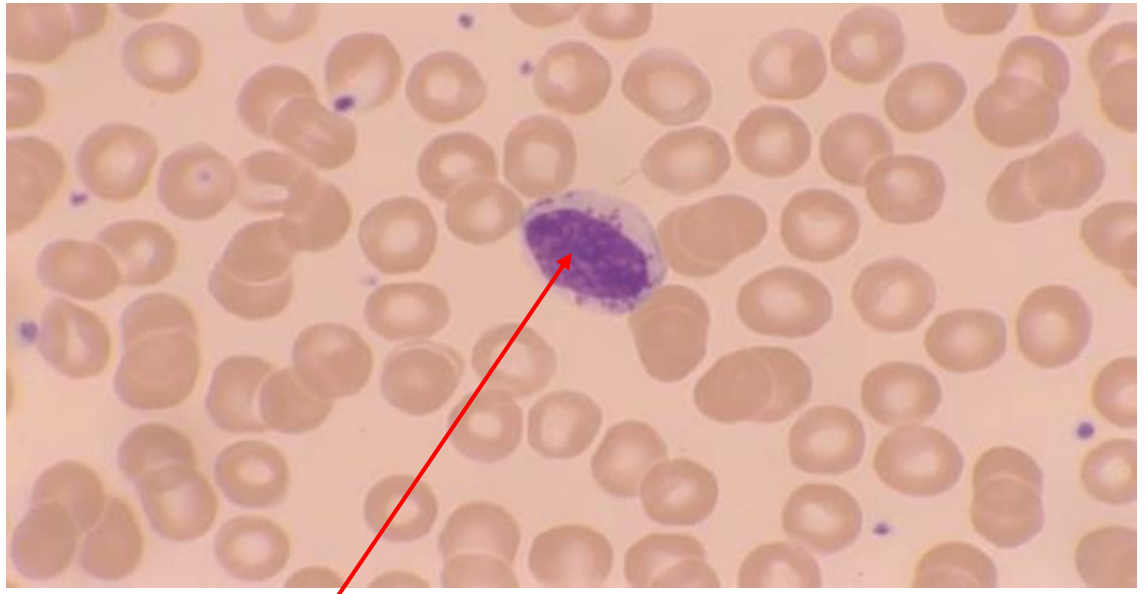
Main form of defense

Adapted from 1.Fisher. Available from: http://missinglink.ucsf.edu/lm/immunology_module/prologue/objectives/obj02.html. Accessed June 2017. 2. Rathinam and Fitzgerald. Virology 2011;411:153–162. 3. 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
- NK cells can be activated by target cells, which they lyse
- 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

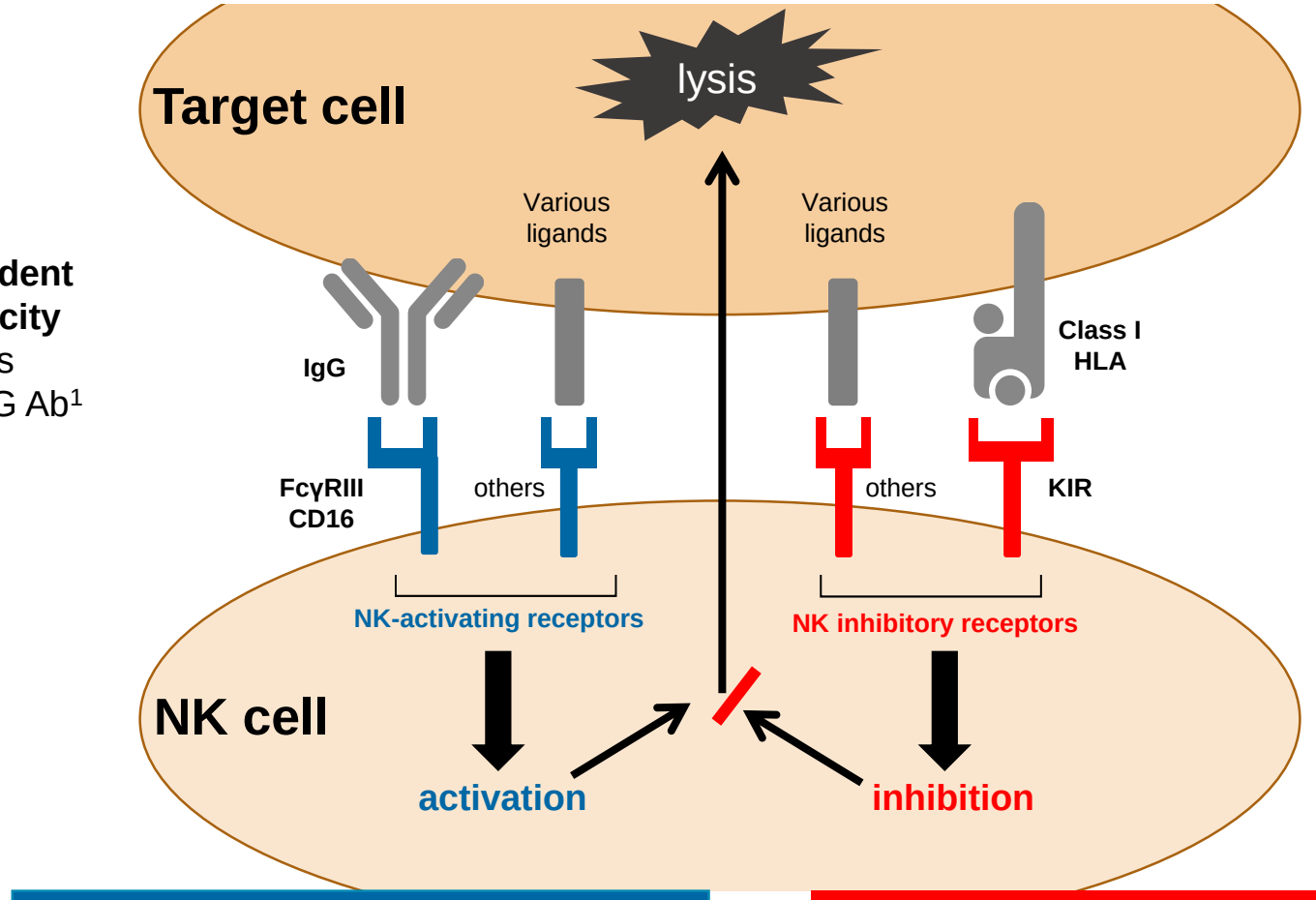
HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; NK, natural killer.

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

# The innate immune response: controlling NK cell activation[1-3]

Chapter homepage

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




Important stimulatory receptor: FcγRIII

Important inhibitory receptor: KIR

- 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]

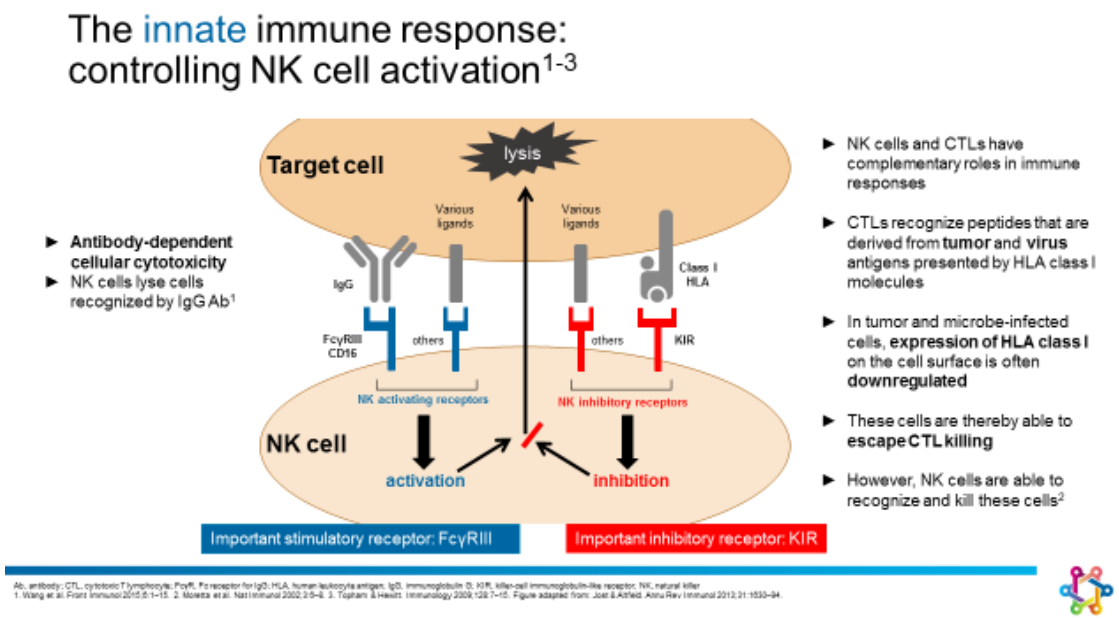
Ab, antibody; CTL, cytotoxic T lymphocyte; FcγR, Fc receptor for IgG; HLA, human leukocyte antigen; IgG, immunoglobulin G; KIR, killer-cell immunoglobulin-like receptor; NK, natural killer

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

## The innate immune response: controlling NK cell activation

Chapter homepage




- **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) and rheumatoid arthritis
  - 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.

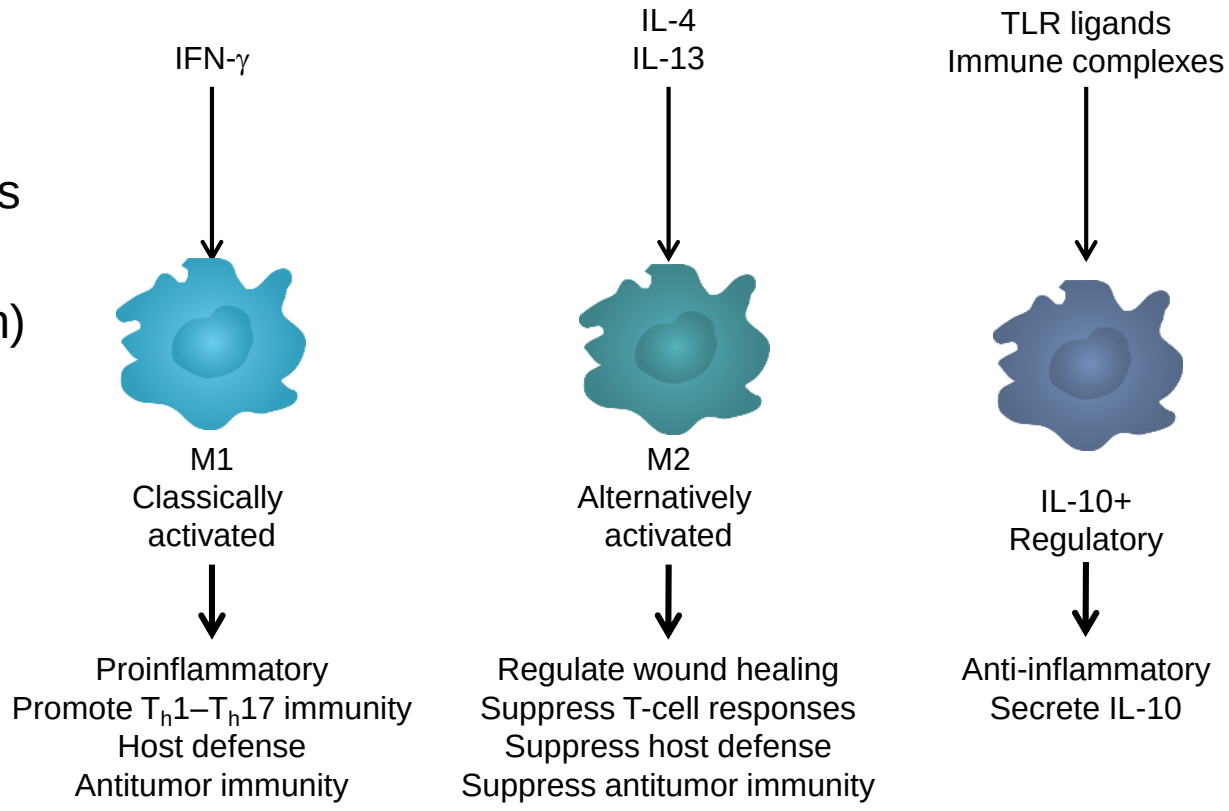
1. Dalakas. Nat Clin Pract Neurol 2008;4:557–67. 2. Wang et al. Front Immunol 2015;6:1–15. Always refer to the SmPC. All SmPCs available from http://www.ema.europa.eu/ema/

# 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**



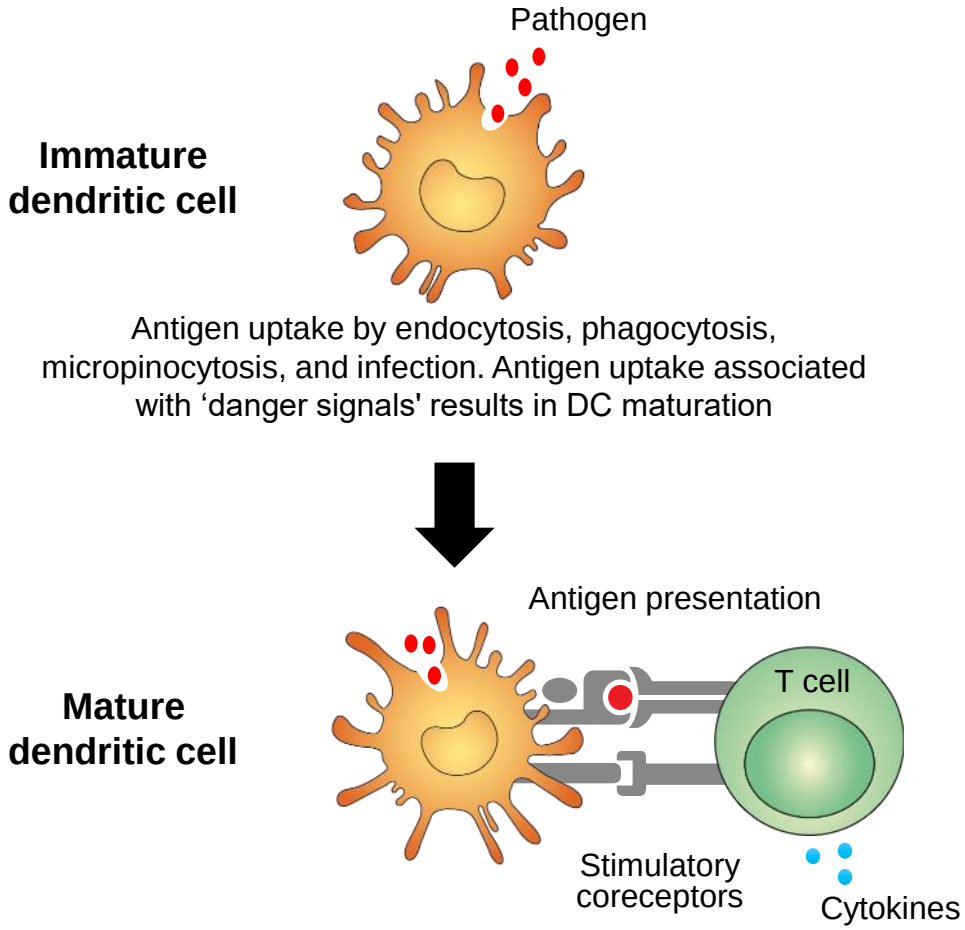

IFN, interferon; IL, interleukin; $T_h$, T helper; TLR, Toll-like receptor.

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 December 2015. 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

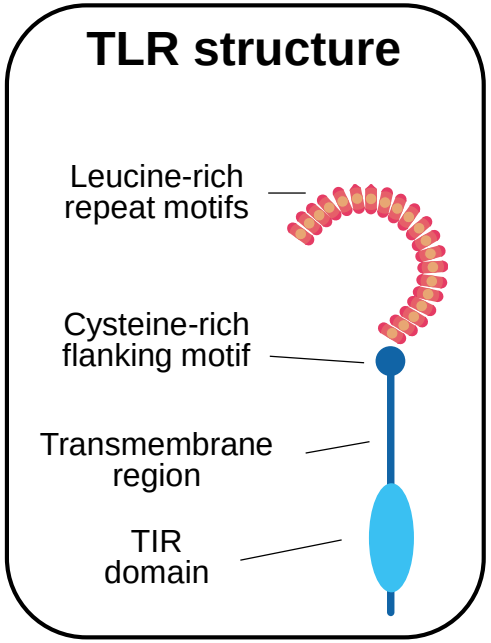
- Signaling PRRs induce **inflammation**; endocytic PRRs promote **phagocytosis**

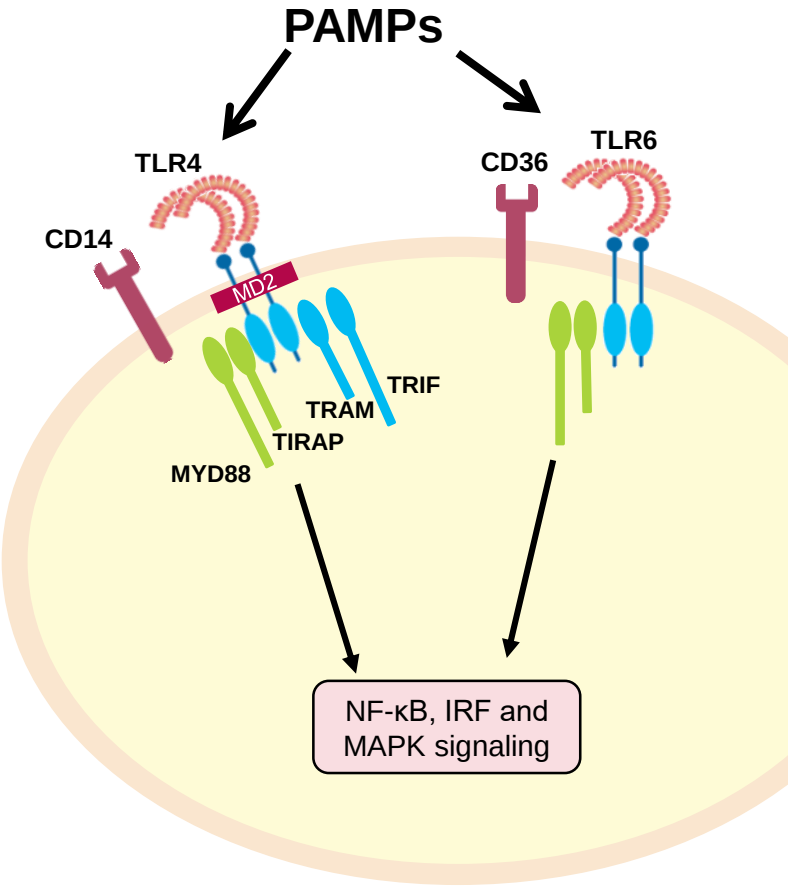
DAMP, danger-associated molecular pattern; dsRNA, double-stranded RNA; HMGB1, high mobility group box 1 protein; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; TAP, transporter associated with antigen processing; UV, ultraviolet.

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]

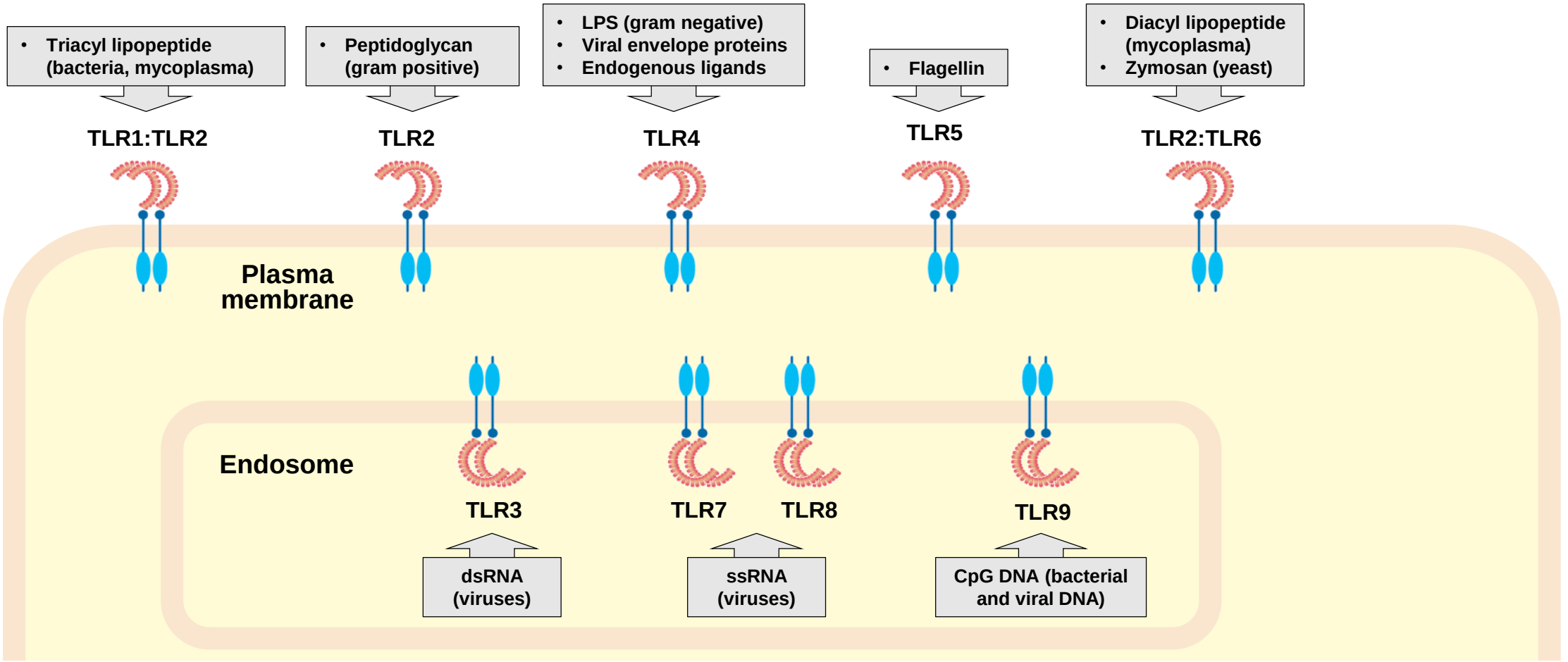



IRF, interferon regulatory factor; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; PAMP, pathogen-associated molecular pattern; TIR, Toll/interleukin-1 receptor; TIRAP, TIR domain-containing adaptor protein; TLR, Toll-like receptor; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing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



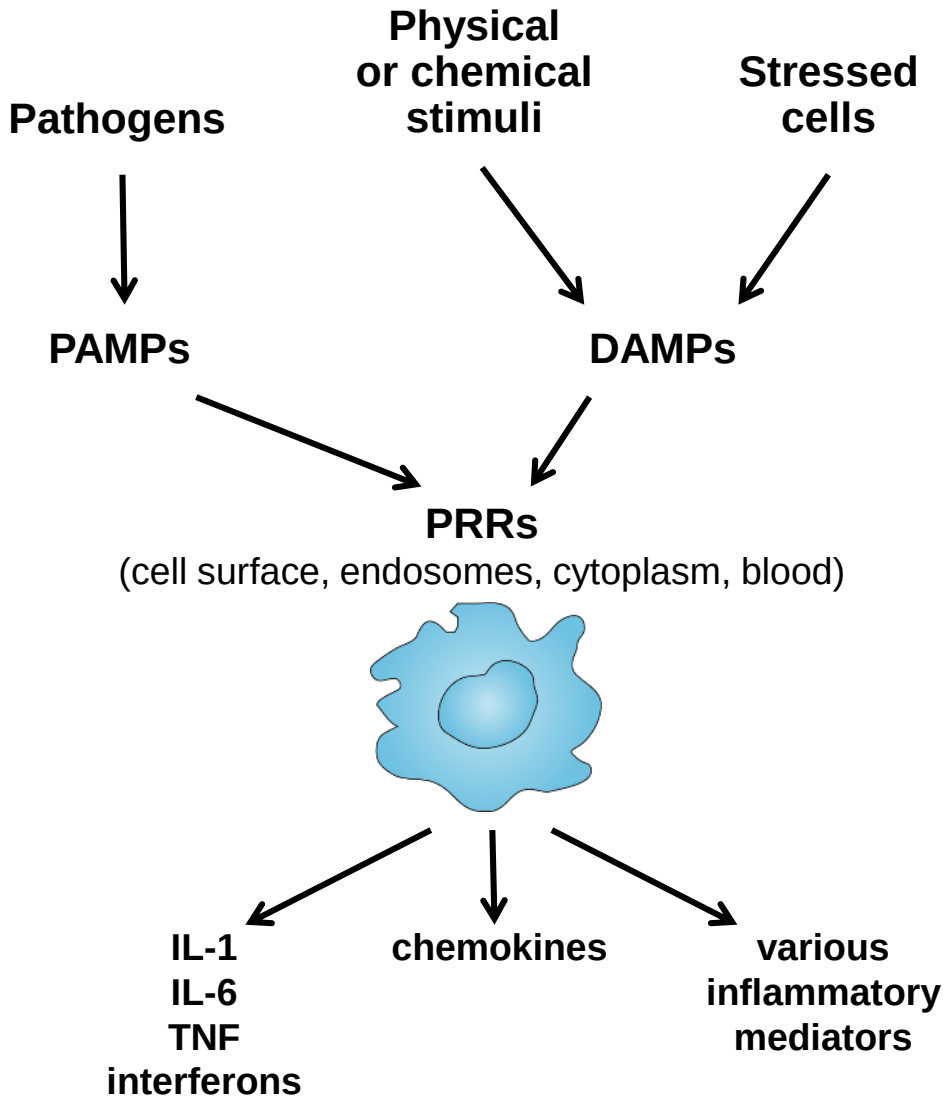

dsRNA, double-stranded RNA; LPS, lipopolysaccharide; PAMP, pathogen-associated molecular pattern; ssRNA, single-stranded RNA; TLR, Toll-like receptor.

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

Chapter homepage

- Acute inflammation is the local physiological response to injury or microbial invasion[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]



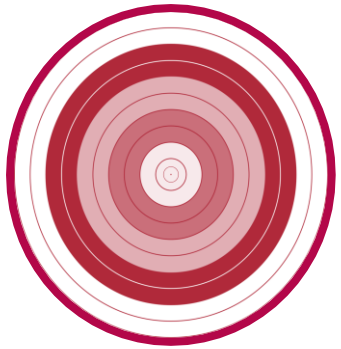

DAMP, danger-associated molecular pattern; IL, interleukin; PAMP, pathogen-associated molecular pattern; PPR, pathogen recognition receptor; TNF, tumor necrosis factor.

1. Brown. Available from: http://www.judithbrowncpd.co.uk/inflammation.pdf. 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



## 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

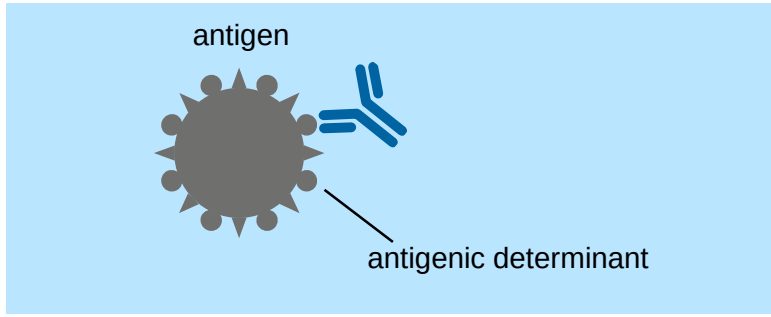
Janeway et al. In: Immunobiology: The Immune System in Health and Disease, 5th edn, 2001. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27090/ and https://www.ncbi.nlm.nih.gov/books/NBK27092/. Accessed August 2017.

# The adaptive immune response: antigens

Chapter homepage

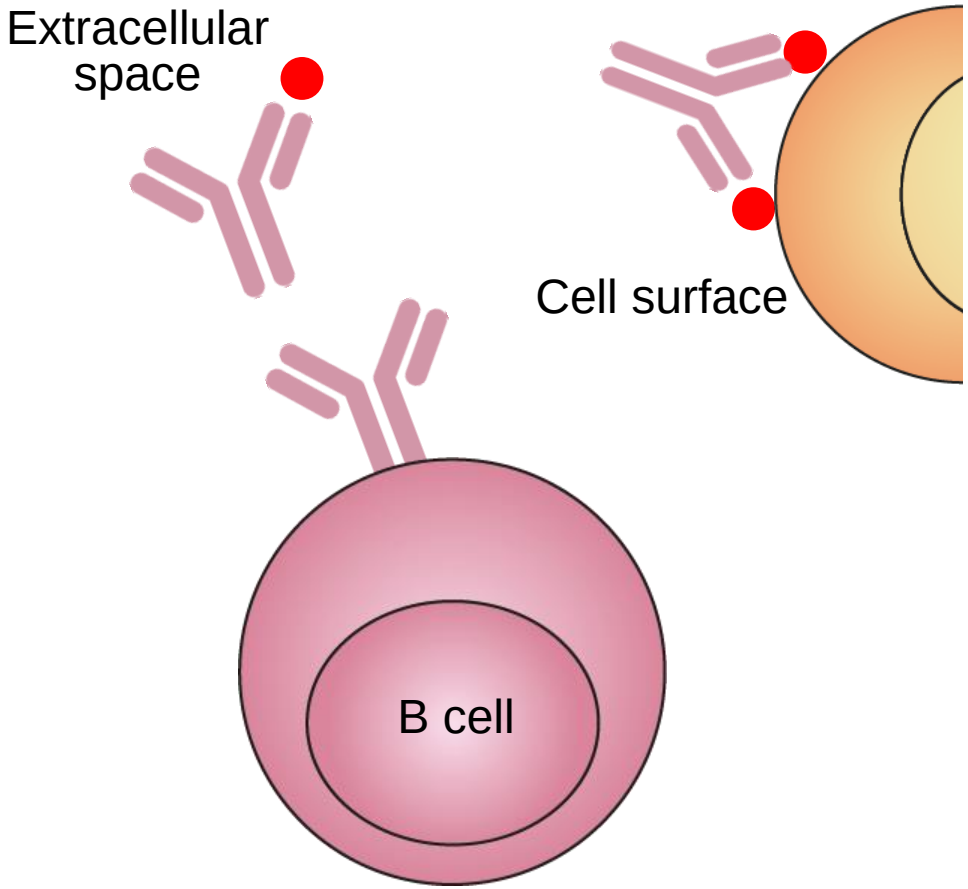
- An antigen is a substance, usually from the external environment of an organism (= 'non-self'), that can be specifically recognized by either antibodies or T lymphocytes
- An antigen does not necessarily induce a specific immune response; when it does so, the antigen is an immunogen (all immunogens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

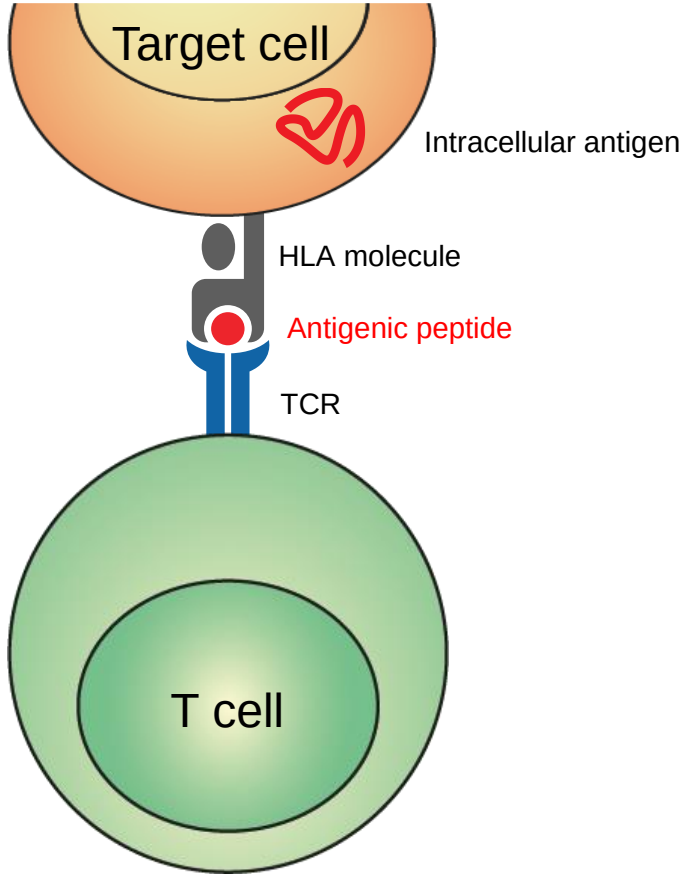



- The part of the antigen that is actually recognized is the antigenic determinant (epitope)
- Most common antigens have many antigenic determinants

# 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.

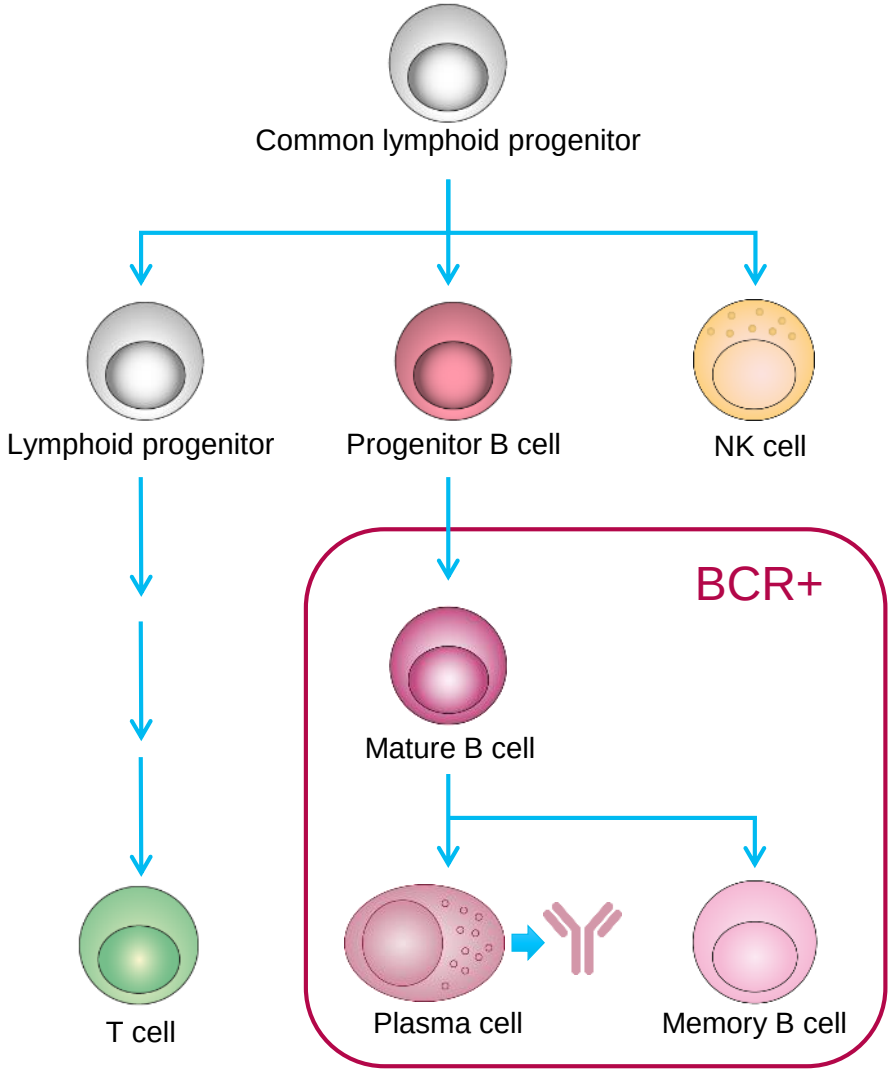
1. Alberts et al. Molecular Biology of the Cell, 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26884/. Accessed May 2017. 2. Heath & Carbone. Nat Rev Immunol 2001;1:126–35.

# Adaptive immunity: B cells

Module 1. Basic immunology

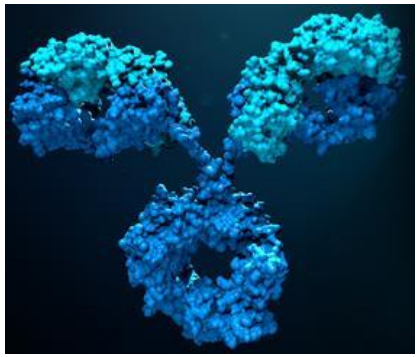
# The adaptive immune response: B lymphocytes and antibodies

- 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

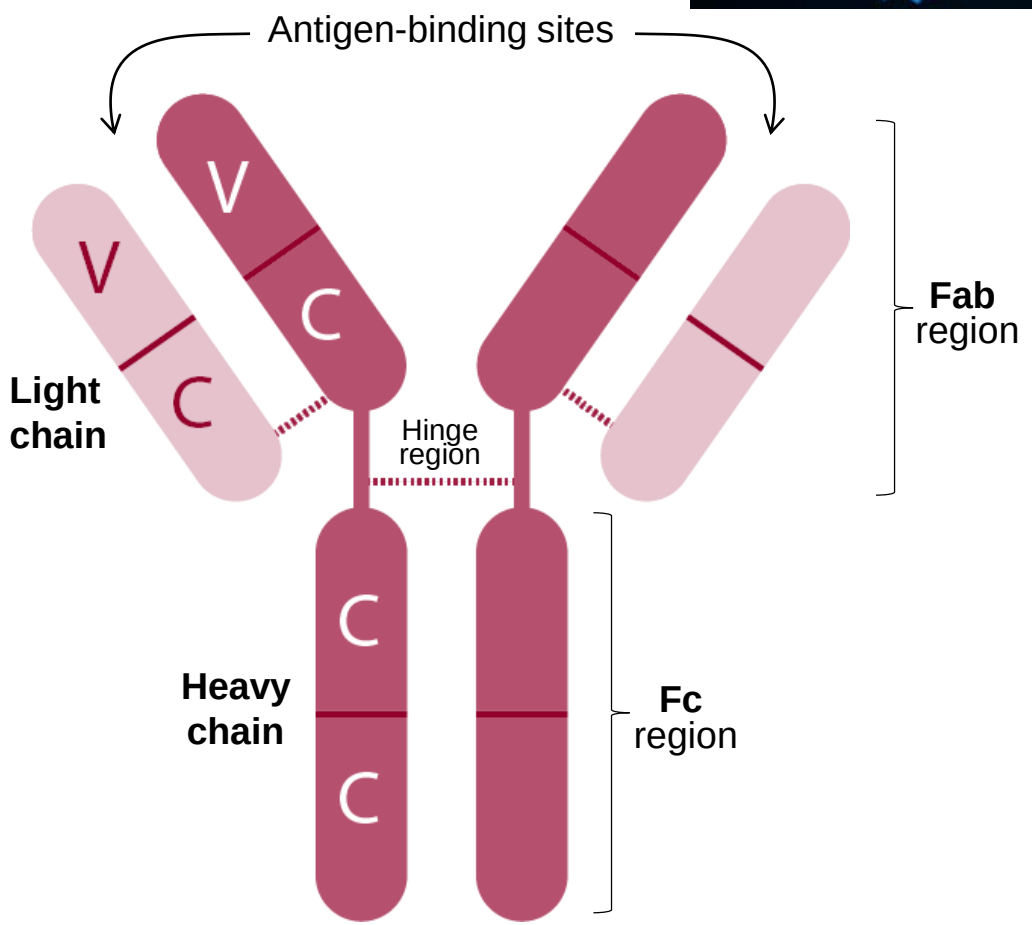



BCR, B-cell receptor; NK, natural killer.
Male et al. Immunology, 7th edn, 2001.

# Antibody (immunoglobulin) structure



- All antibodies all are built from the same basic units
- Heavy and light chains
  - Antibodies comprise two identical light chains (23 kD) and two identical heavy chains (50–70 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.
Microbiology And Immunology Mobile. Available from: http://www.microbiologybook.org/mobile/m.immuno-4.htm. Accessed May 2017.

# 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

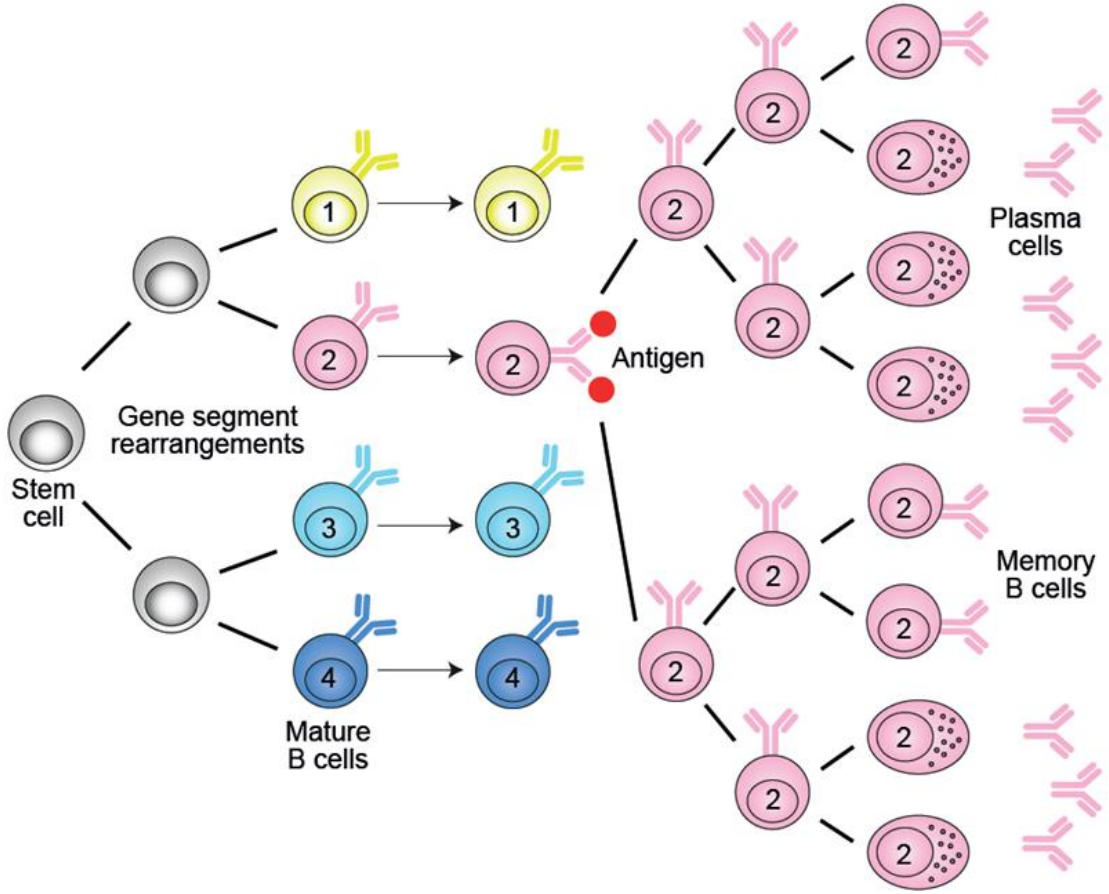
- **Opsonization:** antibody-tagged pathogen is consumed by phagocytes (IgG, IgA)
- **Degranulation:** of mast cells or basophils (IgE)
- **ADCC:** antibody-coated cells lysed by NK cells (IgG)
- **Tissue distribution:** occurs according to classes
  e.g. IgG → fetus via FcRn; IgA → mucosae via pIgR

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://www.boundless.com/physiology/textbooks/boundless-anatomy-and-physiology-textbook/immune-system-21/humoral-immune-response-201/structure-and-function-of-antibodies-985-10108/. Accessed May 2017. 2. Absolute antibody. Available from: http://absoluteantibody.com/antibody-resources/antibody-overview/antibody-effector-functions/. Accessed May 2017. 3. Alberts et al. Molecular Biology of the Cell, 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26884/. Accessed May 2017.

# 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

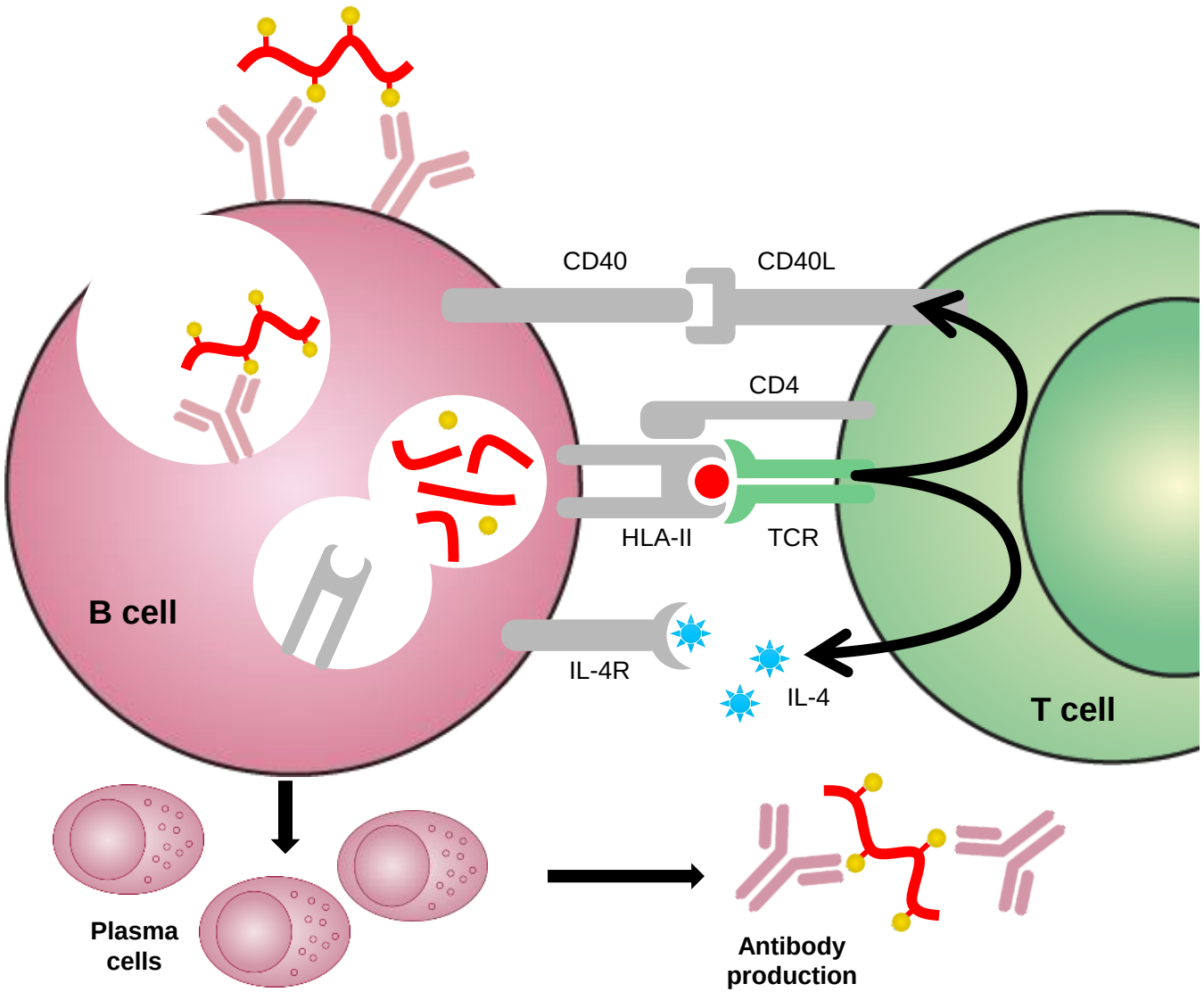
Antigen-dependent proliferation and differentiation into plasma and memory cells

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]



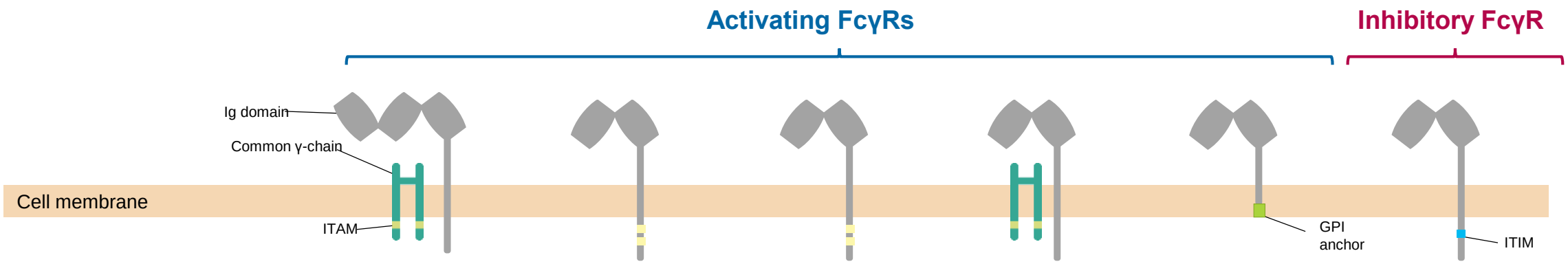

Ab, antibody; BCR, B-cell antigen receptor; CD40L, CD40 ligand; HLA, human leukocyte antigen; IL-4R, interleukin-4 receptor; TCR, T-cell receptor.

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 August 2017. 2. Alberts et al. Molecular Biology of the Cell. 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26827/. Accessed May 2017.

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

Chapter homepage

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.

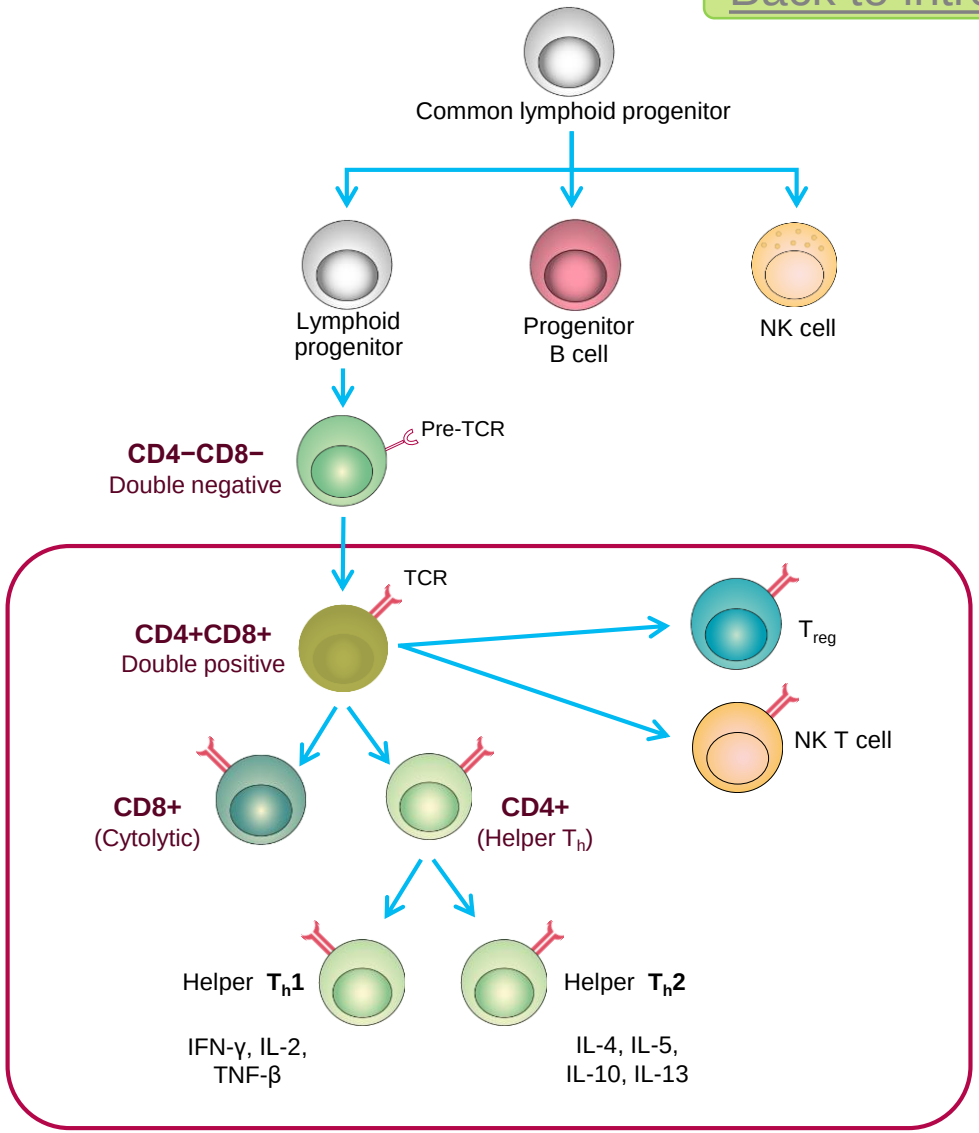
# Adaptive immunity: T cells

Module 1. Basic immunology

# The adaptive immune response: T lymphocytes

- 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]



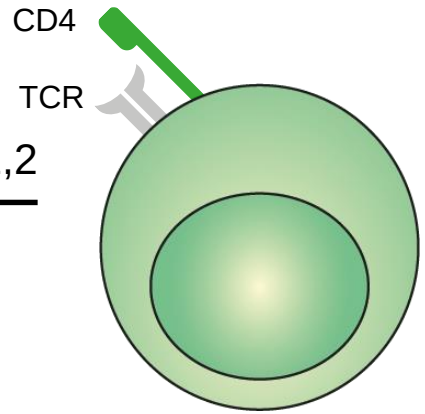

IFN, interferon; IL, interleukin; NK, natural killer; TCR, T-cell receptor; $T_h$, T helper; TNF, tumor necrosis factor; $T_{reg}$, regulatory T cell.

1. Andersen. J Invest Dermatol 2006;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 August 2017. Image adapted from Janeway et al. Immunobiology: The Immune System in Health and Disease, 5th edn, 2001 and Germain RN. Nat Rev Immuno 2002;2:309-322

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

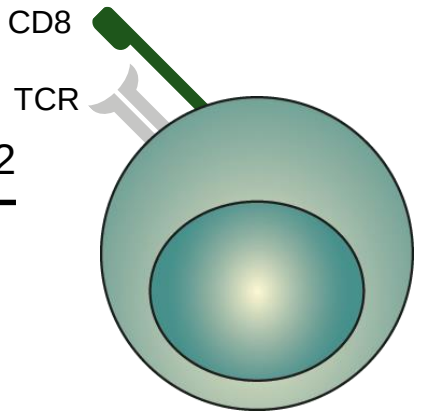
Chapter homepage
Back to introduction

## 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]

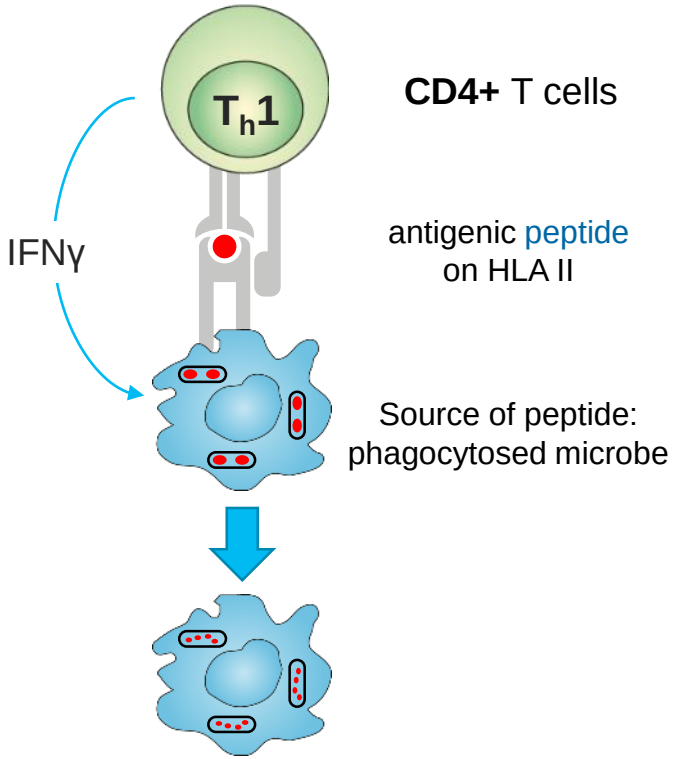



- **CTL: cytolytic**
- Recognition of peptides derived from **intracellular** proteins (i.e. those produced within the cells)
- Recognition of peptides presented by HLA **class I** molecules only
- These T cells are 'HLA class I-restricted'

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]



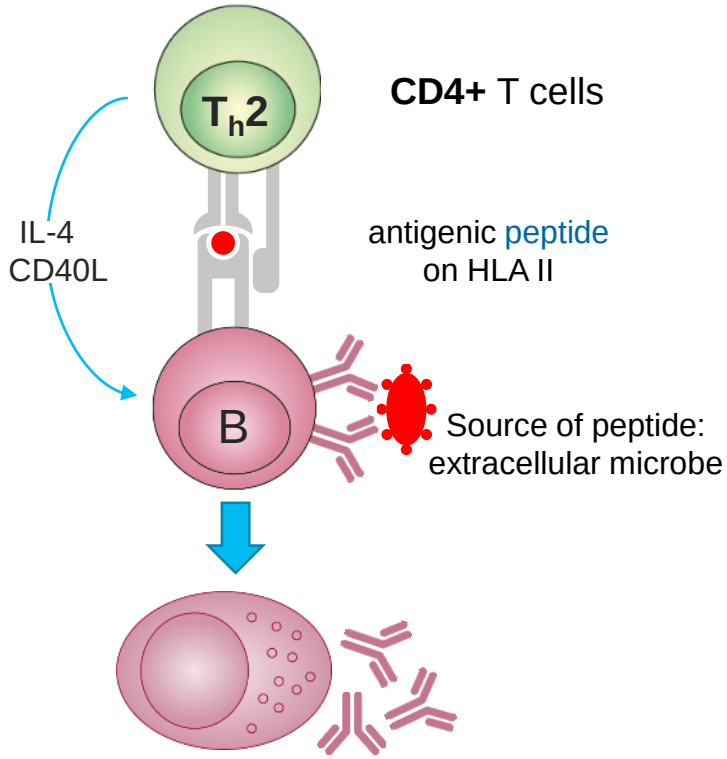

**CD4+** T cells

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**



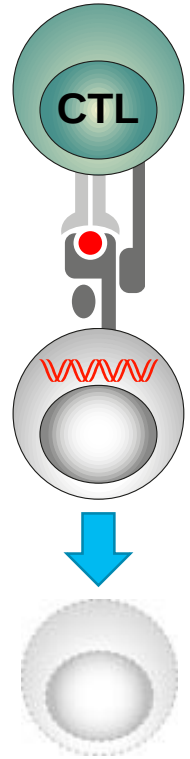

**CD4+** T cells

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 August 2017. 2. Bell. Available from: https://www.immunology.org/public-information/bitesized-immunology/cells/cd4-t-cells. Accessed May 2017. 3. Wissinger. Available from: https://www.immunology.org/public-information/bitesized-immunology/cells/cd8-t-cells. Accessed May 2017. 4. Andersen. J Invest Dermatol 2006;126:32–41. 5. Mosser & Zhang. Curr Protoc Immunol 2008;Chapter 14:Unit 14.2.

# Major histocompatibility complex

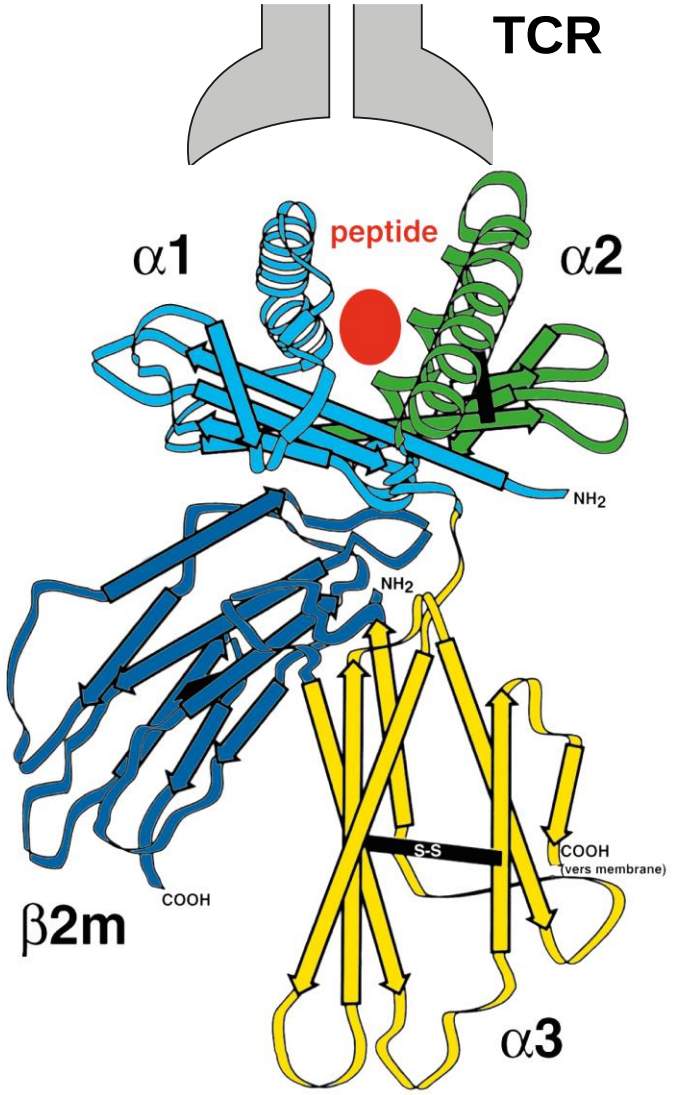
- The MHC is a set of genes identified in mice that determines graft rejection/acceptance (histocompatibility)
- The MHC genes code for the MHC molecule
- In humans, the MHC genes/molecules were discovered on white blood cells and are therefore named the human leukocyte antigen (HLA) genes/molecules
  - 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

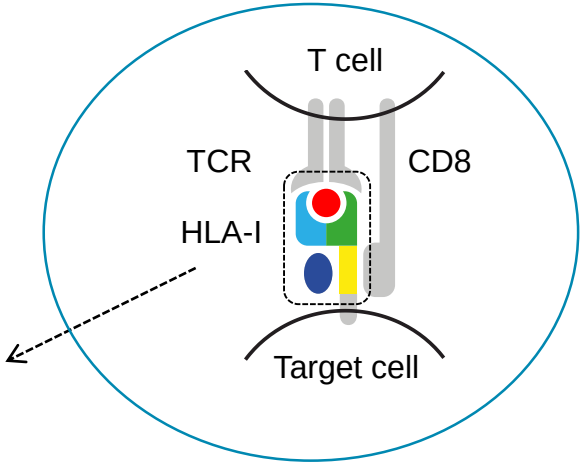
HLA, human leukocyte antigen; MHC, major histocompatibility complex.

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 August 2017.

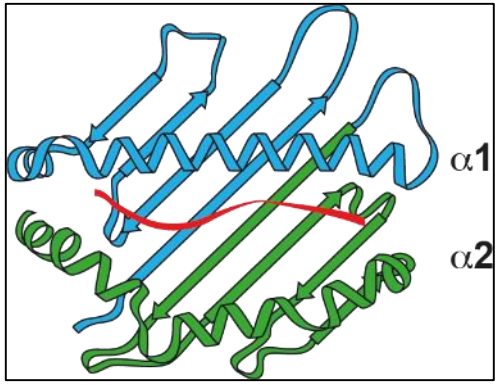
# Crystal structure of HLA class I molecules

- 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 $\alpha_1$, $\alpha_2$ and $\alpha_3$ domains, and $ß_2$microglobulin
- The TCR interacts with the antigenic peptide presented in a groove on top of the HLA molecule, between the $\alpha_1$ and $\alpha_2$ domains
- The TCR contacts both the antigenic peptide itself and residues of the $\alpha_1$ and $\alpha_2$ domains of the presenting HLA molecule




***Top view***

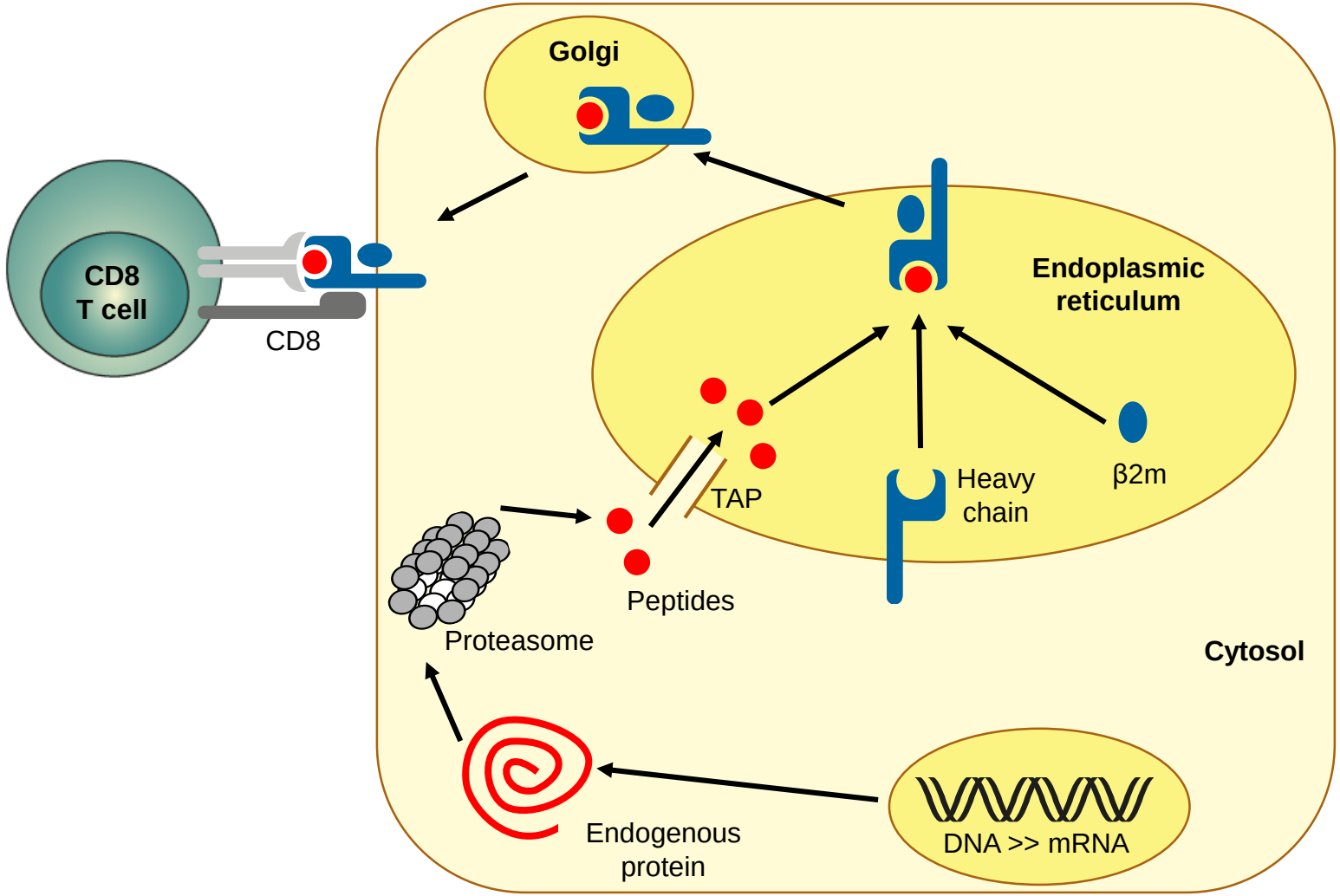



ß2m, beta$_2$ microglobulin; HLA, human leukocyte antigen; TCR, T-cell receptor.
Bjorkman et al. J Immunol 2005;174:13–9.

# Canonical HLA class I antigen processing pathway

Chapter homepage

- 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



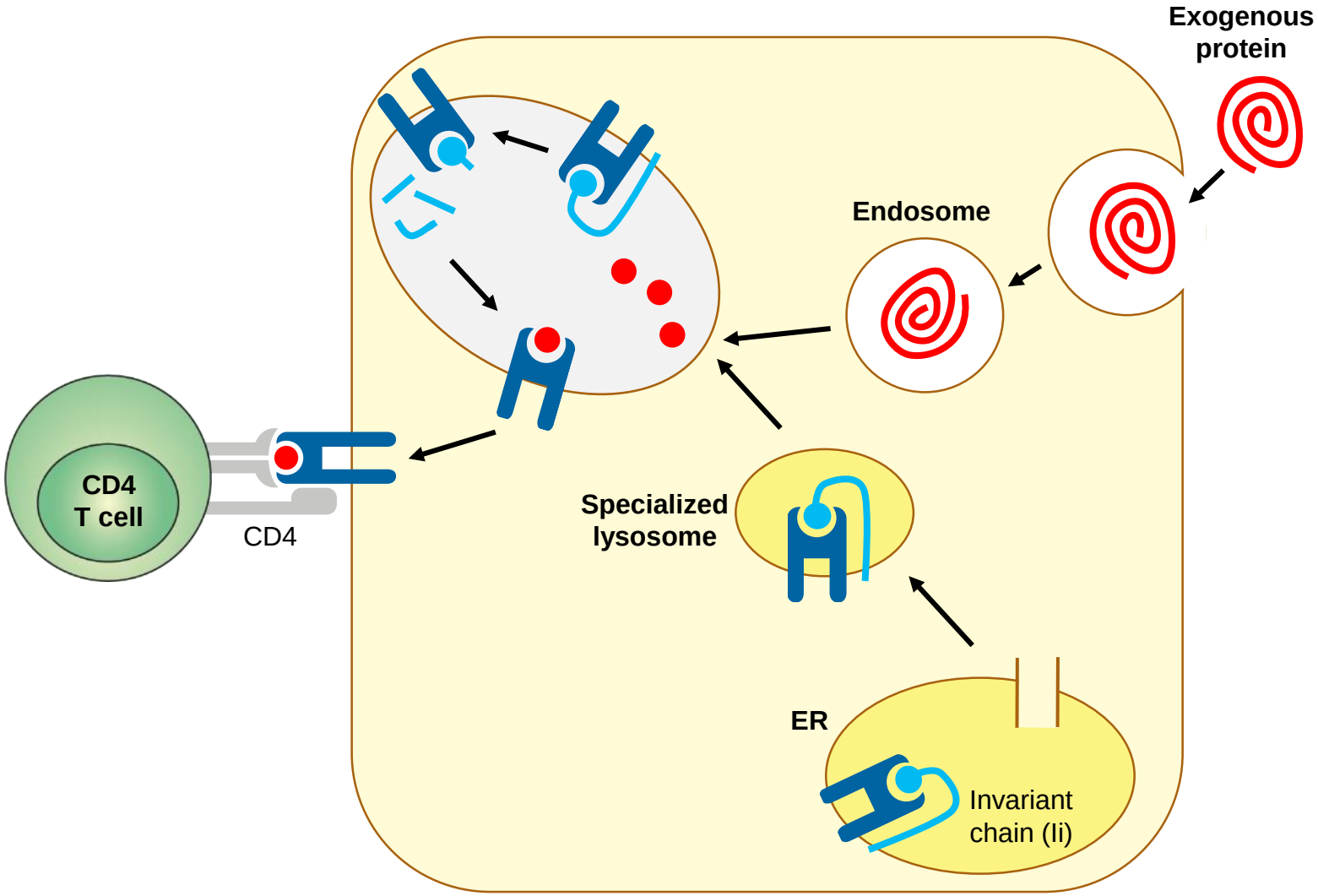

ß2m, beta$_2$ microglobulin; ER, endoplasmic reticulum; HLA, human leukocyte antigen; TAP, transporter associated with antigen presentation.

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

# Canonical HLA class II antigen processing pathway

Chapter homepage

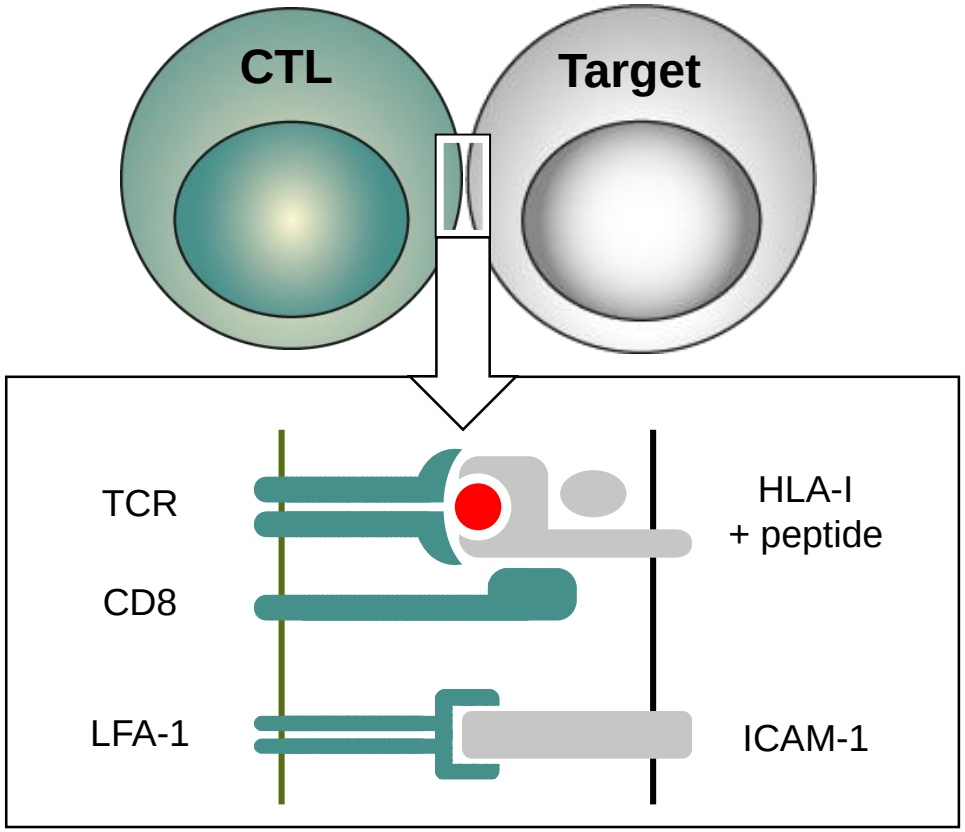
- HLA class II α- and β-chains assemble in the ER and form a complex with the invariant chain
- The heterotrimer is transported through the Golgi to the HLA class II compartment
- Endocytosed proteins and Ii are degraded by resident proteases
- The Ii fragment in the peptide-binding groove is exchanged 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]



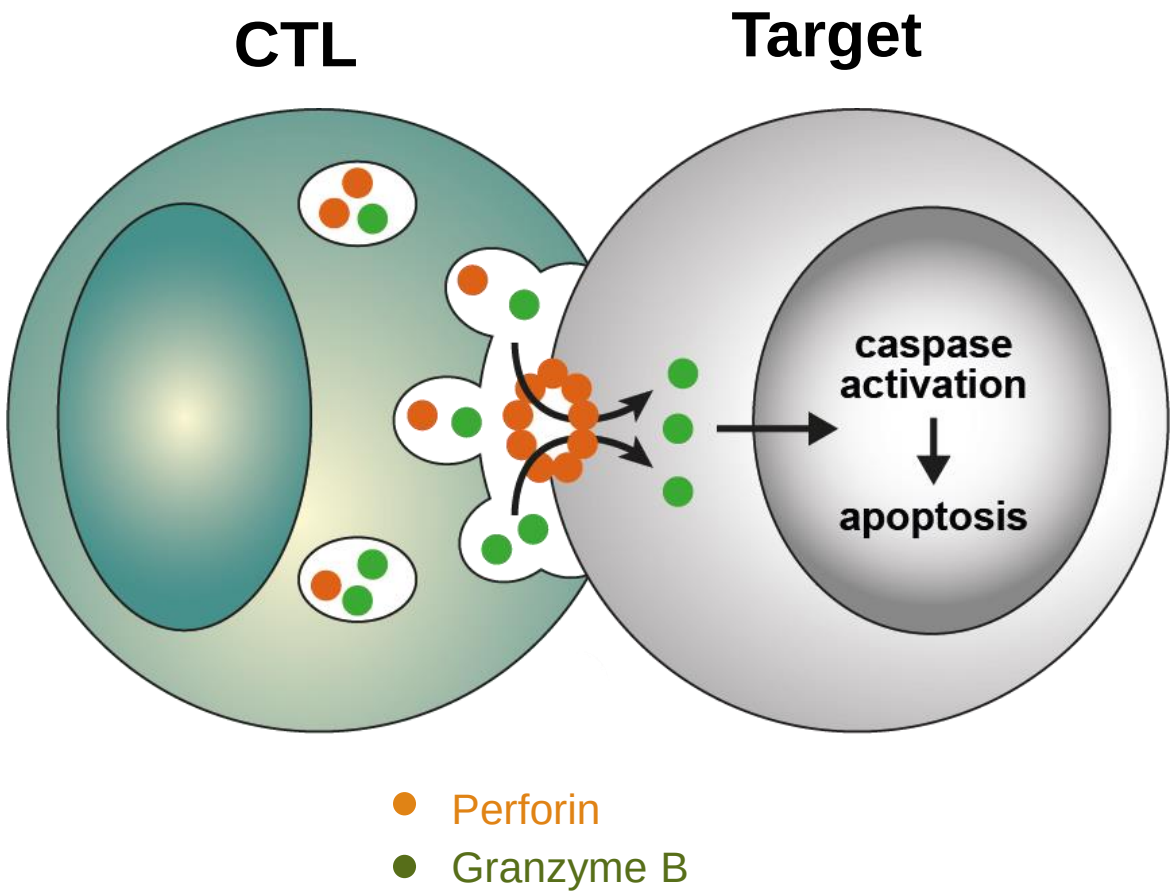

**'Immunological synapse'**
**antigen recognition + adherence molecules**

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 May 2017. 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



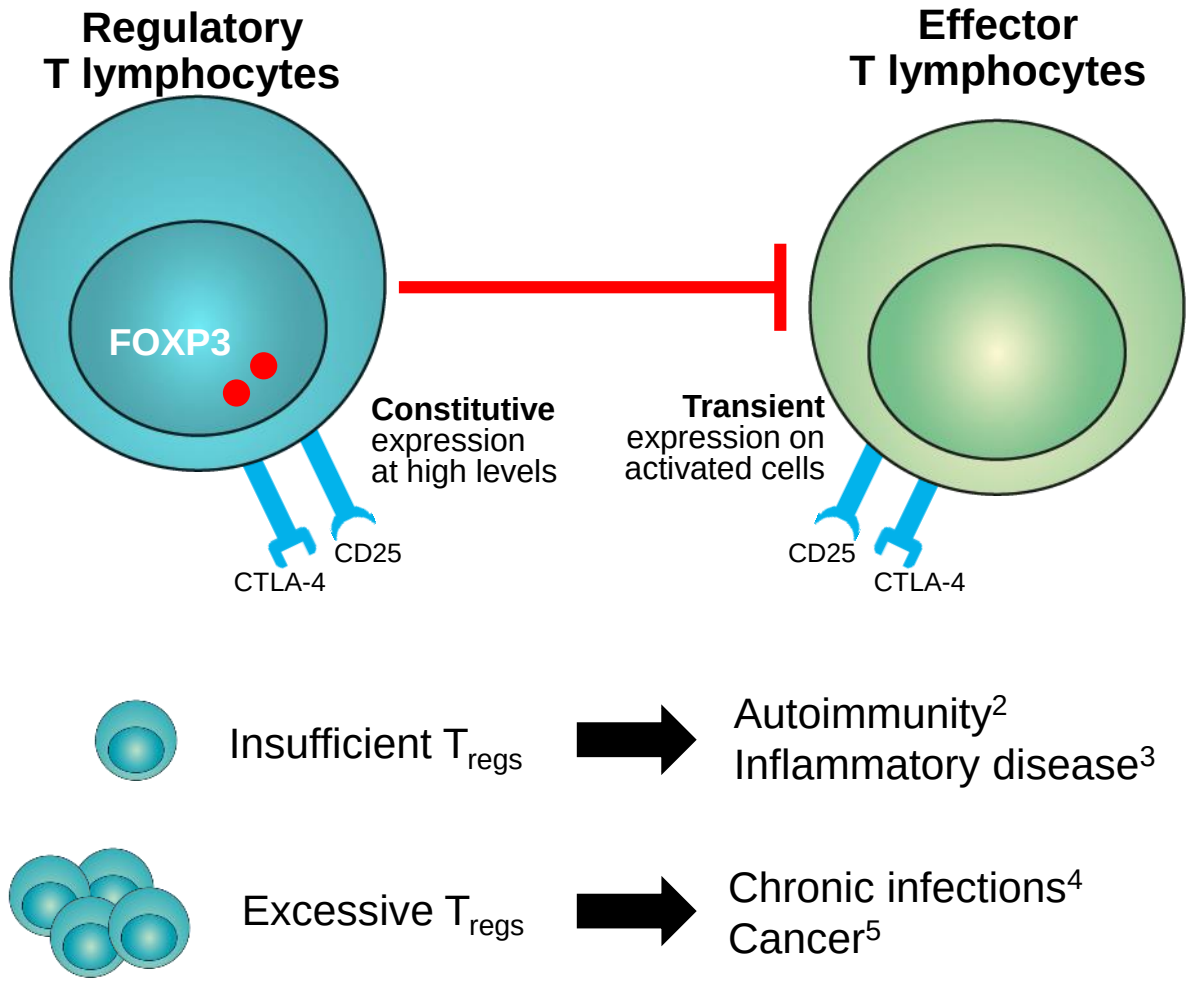

CTL, cytotoxic T lymphocyte; NK, natural killer.

Alberts et al. Molecular Biology of the Cell, 4th edn, 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26926/. Accessed May 2017.

# 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]

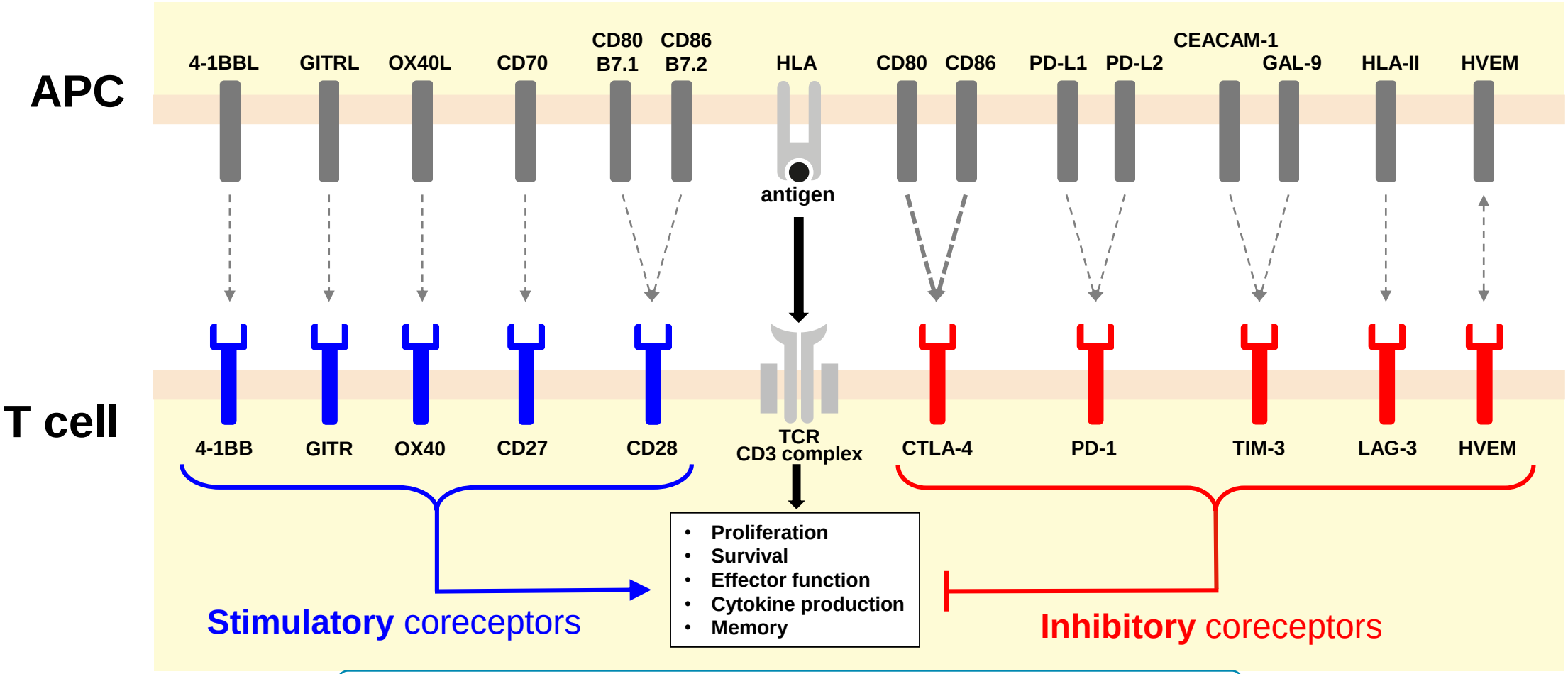



CTLA-4, cytotoxic T lymphocyte-associated protein 4; FOXP3, forkhead box P3; $T_{reg}$, regulatory T cell.

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

Chapter homepage



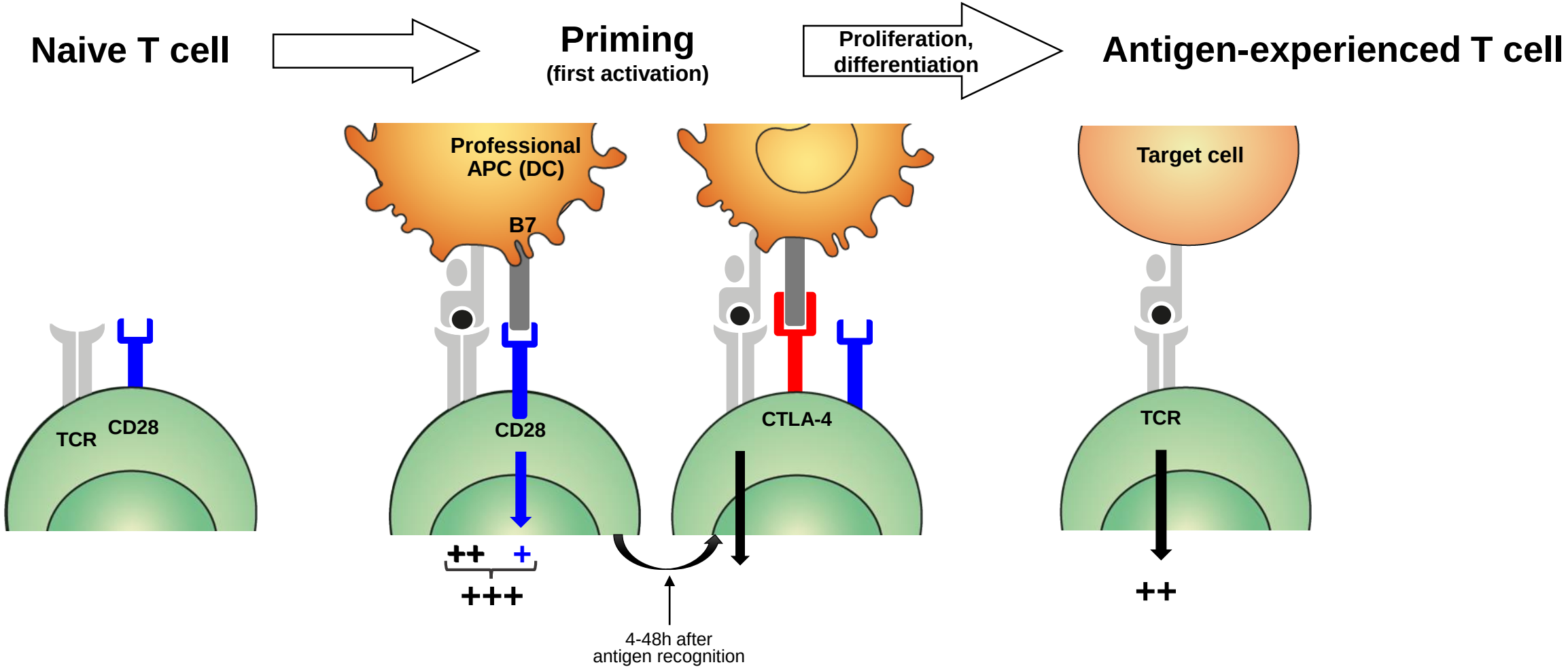

Function: fine-tuning of T-cell activation in time and space

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

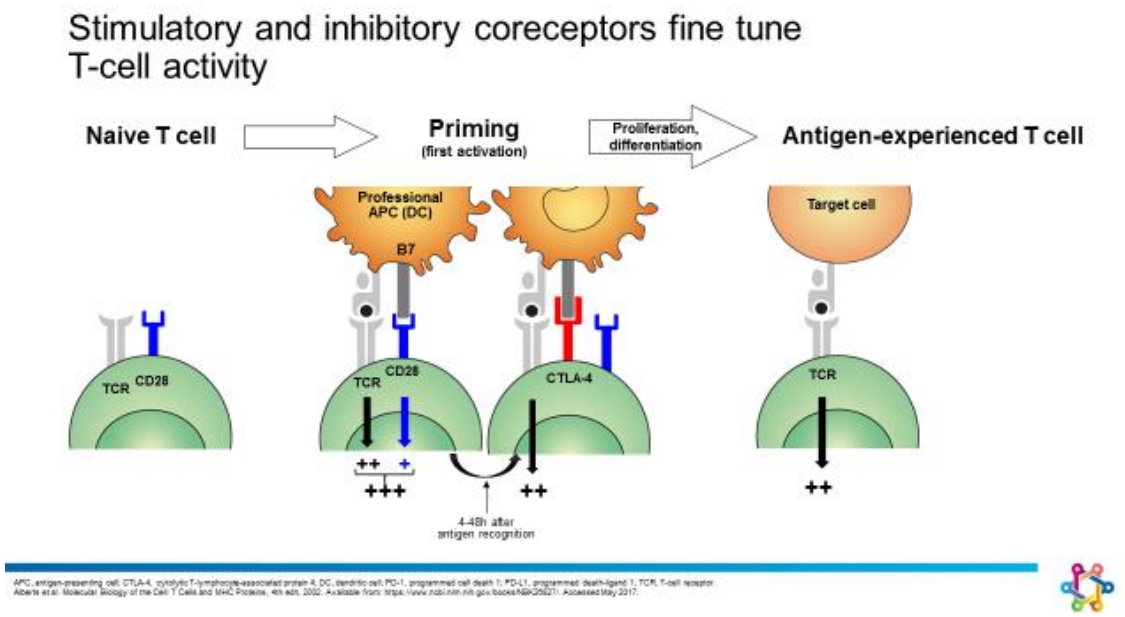
Chapter homepage




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.

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

# Clinical relevance
## Modulation of T-cell activity (1)



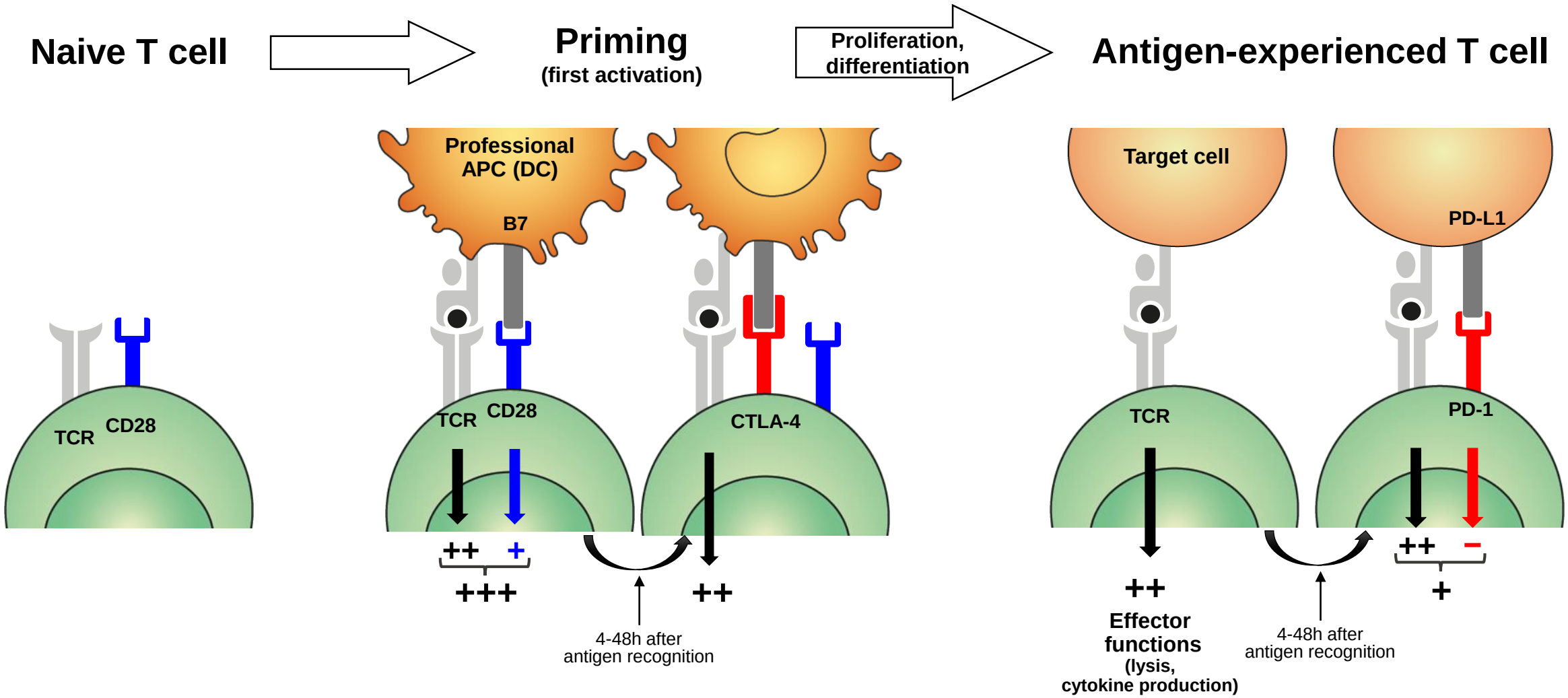

- **Agents that target T-cell coreceptors to modulate T-cell activity are used therapeutically**
- **Abatacept**
  - A fusion protein that consists of the extracellular domain of human CTLA-4 linked to a modified Fc portion of human IgG1

  - It binds to B7 and prevents T-cell costimulation by CD28
  - It is indicated for rheumatoid arthritis and psoriatic arthritis

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

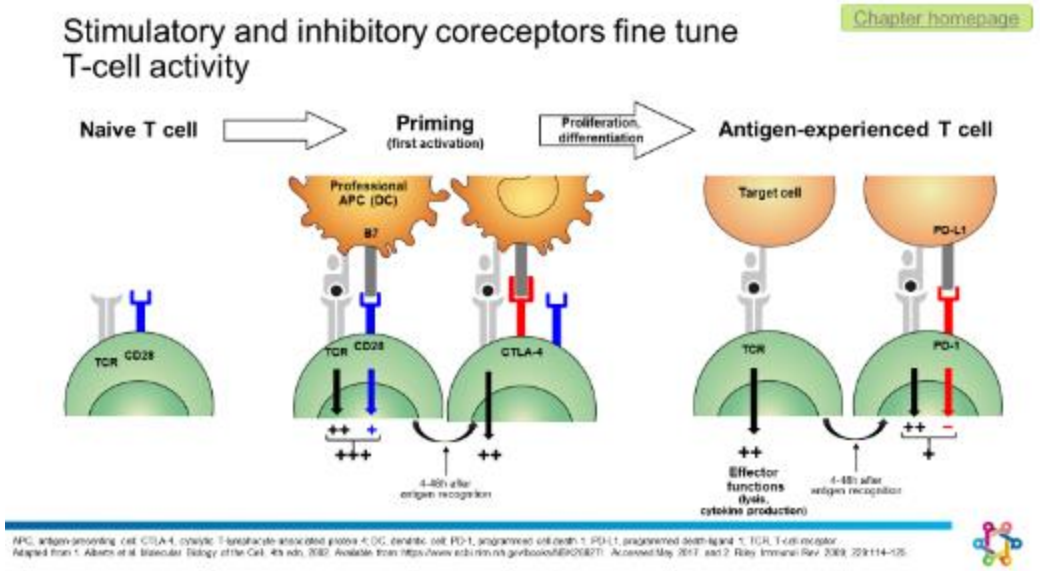
Chapter homepage




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 May 2017. and 2. Riley. Immunol Rev. 2009; 229:114–125.

Chapter homepage

# 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
- **Anti-PD-1 antibodies**
  - Nivolumab, indicated for melanoma, NSCLC, RCC, cHL SCCHN and urothelial carcinoma
  - Pembrolizumab, indicated for melanoma, NSCLC and cHL
- **PD-L1 antibodies**
  - Atezolizumab, indicated for urothelial carcinoma and NSCLC (US only)
  - Durvalumab, indicated for urothelial carcinoma (US only)

cHL, classical Hodgkin lymphoma; CTLA-4, cytolytic T-lymphocyte-associated protein 4; NSCLC, non-small-cell lung cancer; 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.
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/

# 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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