ÉCOLE POLYTECHNIQUE
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HSeT
Health Sciences
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Foundation

V. T cell response

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Introduction

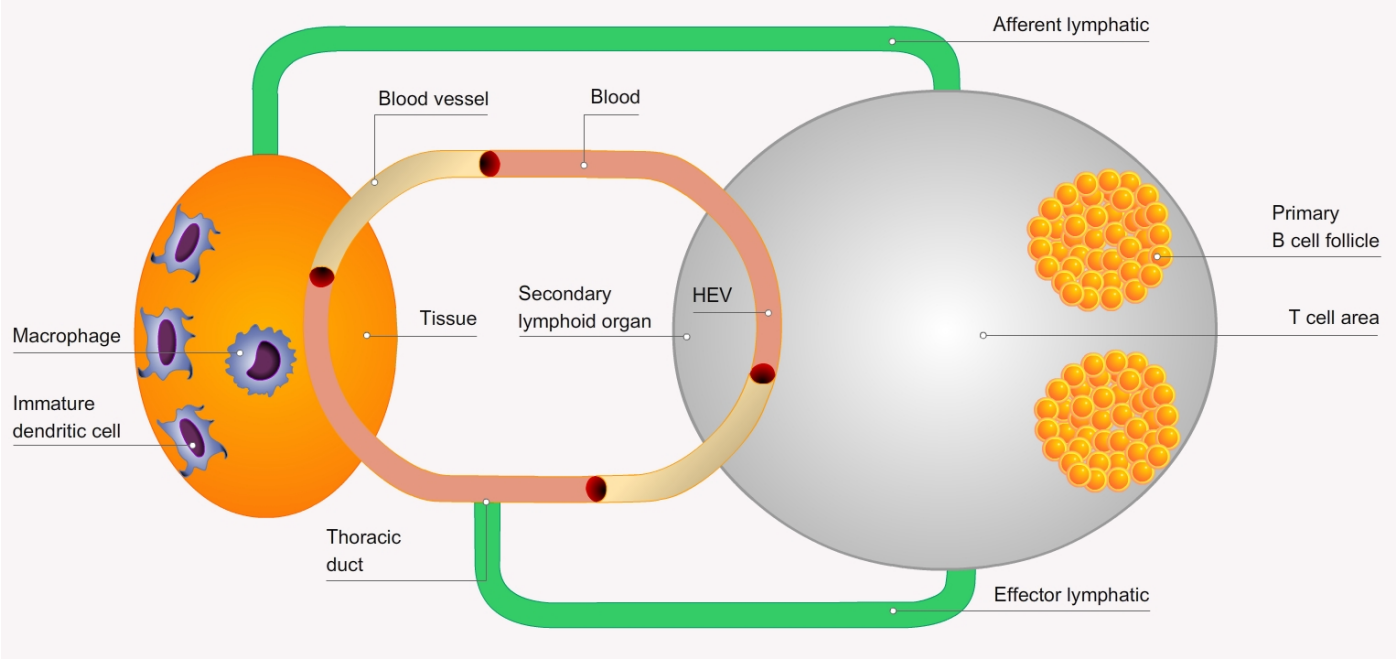
Questions

- Which **signals** activate T lymphocytes?

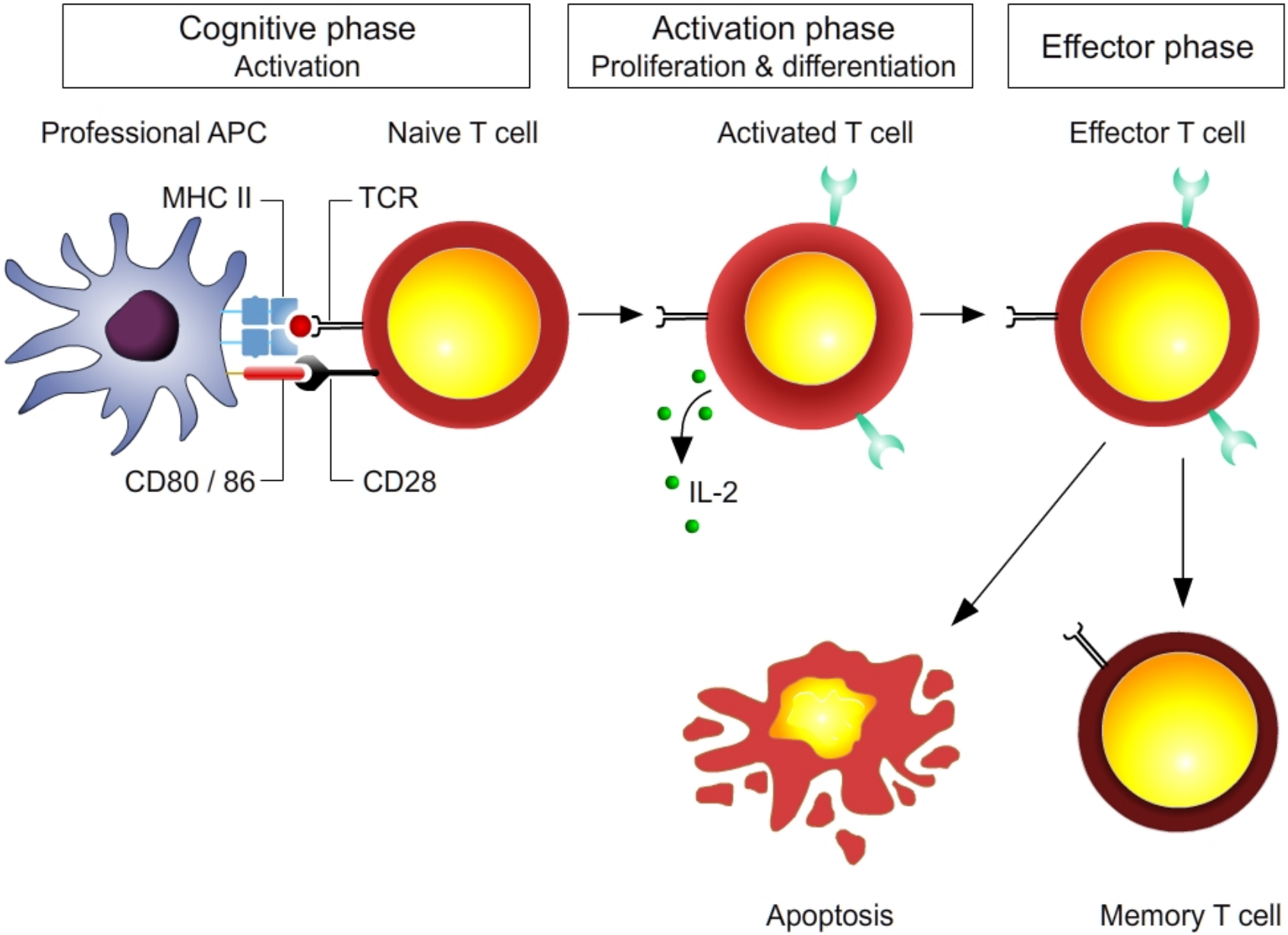
- How are the few **naïve T cells** specific for any microbe **converted** into the large number of **effector T cells** with specialized functions?

Role and Phases of T cell response

- T cell immune responses serve **three purposes:**
  - eliminate intracellular microbes, such as viruses, that infect and replicate inside various cell types, including non-phagocytic cells
  - defend against intracellular microbes that evolved to survive within phagocytes
  - assist the development of B cell responses, activate macrophages, cmast cells, and eosinophils
- T cell immune responses can be divided into **three phases:**
  - Induction phase
  - Effector phase
  - Contraction phase



Life of a mature T cell after contact with antigen



Outline

V-A T cell activation by APCs

V-B Consequences of T cell activation

V-C T cell differentiation (Th1 / Th2)

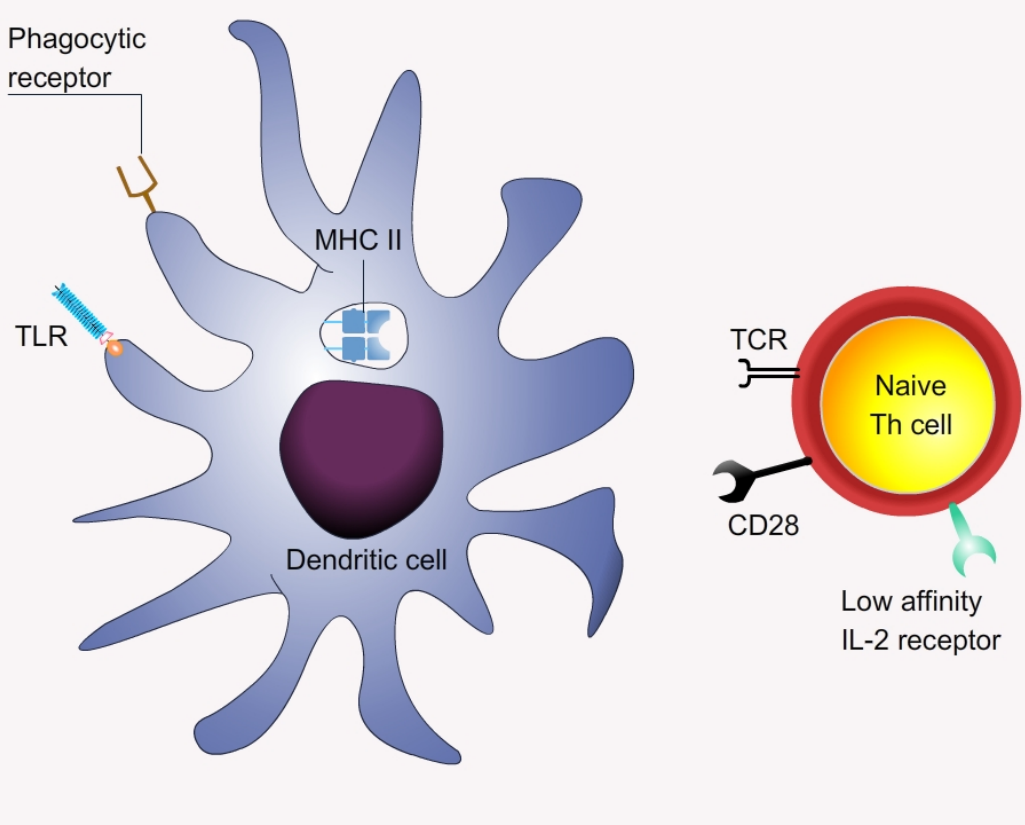
V-1
T cell activation by APCs

- The 2 signals
- The immunological synapse
- TCR activation by superantigens
- Role of co-stimulation and cell adhesion
- Specific features of CD8 T cell activation

The 2 signals

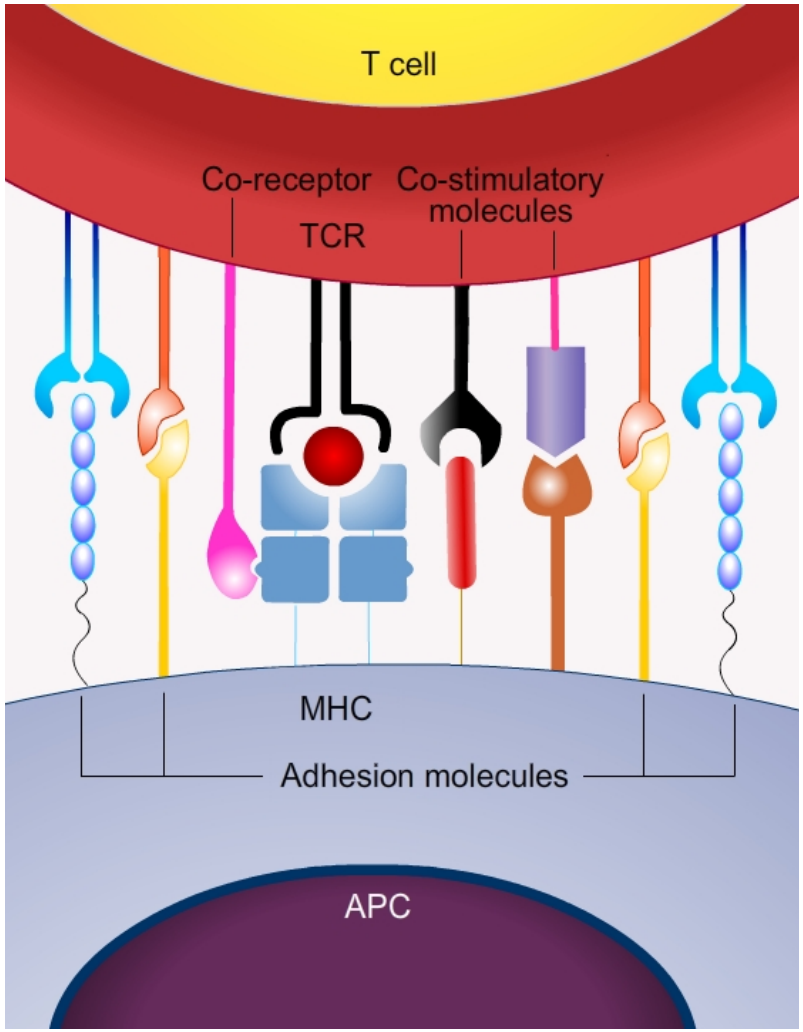
T-cell activation typically requires two events:

- **TCR engagement**: antigen recognition by T cell, a specific event that involves the ligation of the TCR/CD3 complex with antigen fragments bound to MHC molecules on APCs.
- **Co-stimulation**: antigen-nonspecific interactions between co-stimulatory molecules and their ligands on the APC and the T cell.



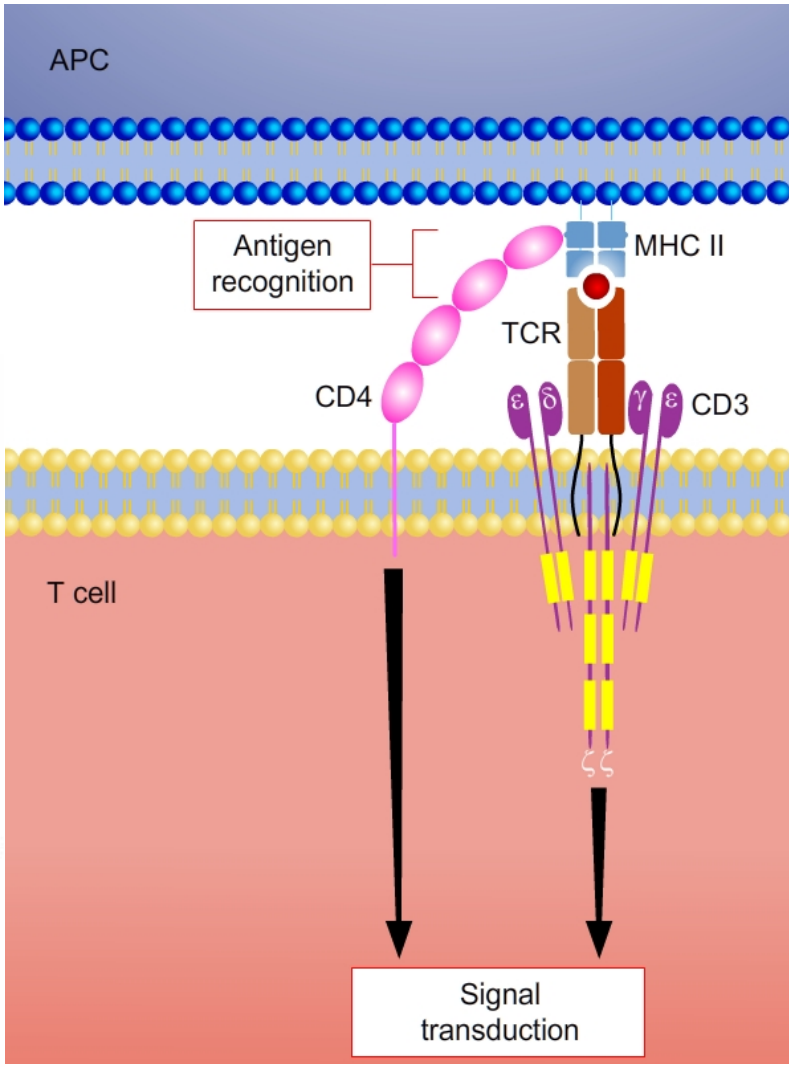
T cell / APC interactions

- **Initial contacts** between APCs and T cells are **antigen-independent** and mediated by cell-adhesion molecules (e.g. LFA1 on T cells and ICAM on APCs)
- **Upon antigen recognition**
  - interactions occur between the TCR and MHC
  - specialized junctions form at the cell surface, the **immunological synapses (IS)**
  - An IS contains
    - the TCR/MHC-peptide complex
    - the co-receptors (CD4/CD8)
    - the co-stimulatory molecules
    - peripheral cell adhesion molecules
  - An IS is maintained for up to 48 h enabling the prolonged signal transduction required for T cell activation



TCR / antigen interactions

- The primary activation signal is induced when specific TCRs on naïve T cells recognize antigenic peptides bound to the MHC/peptide complex on APCs.
- Each T cell needs to engage antigen for a long period, at least several minutes, or multiple times to generate enough signals to initiate a response.



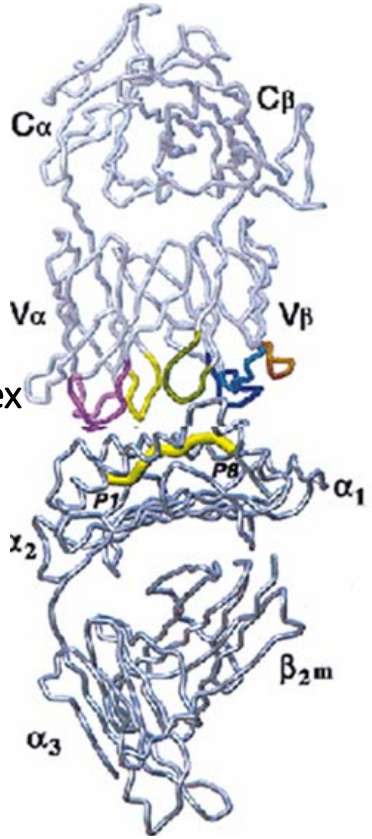
Structure of the TCR

The TCR

- α (or δ) chain
- β (or γ) chain

CD3: the signal transduction complex

- γ chain
- δ chain **heterodimers**
- ε chain
- ζ chain **homodimer**



TCR-activation by superantigens

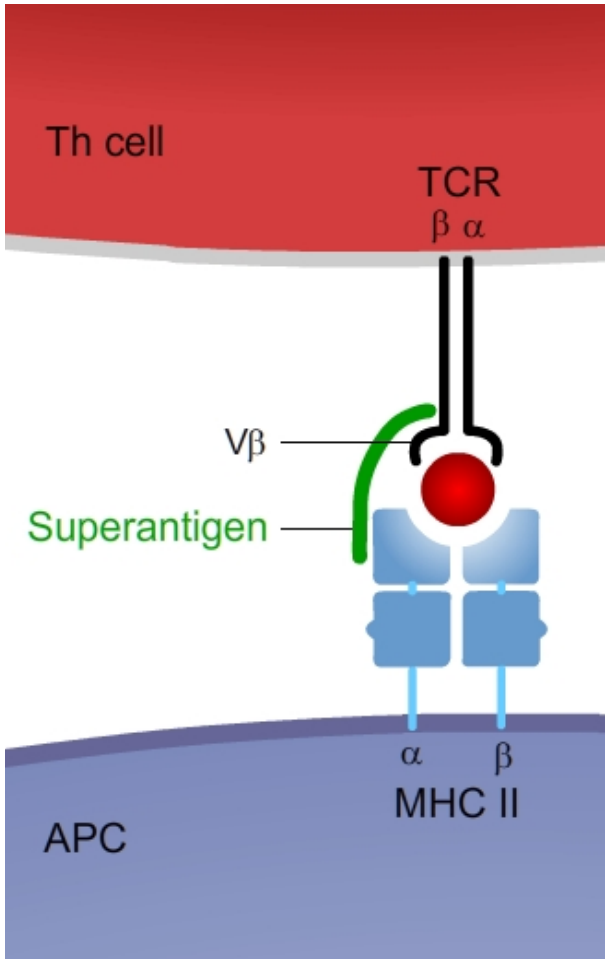
Superantigens

- viral or bacterial proteins that **bind simultaneously to** the **TCR V chain** and to the a chain of an **MHC class II** molecule. This induces crosslinking of TCR & MHCII resulting in T cell activation and proliferation.

e.g. staphylococcal enterotoxins (SEA, SEB, SEC, SED & SEE), Toxic shock syndrome toxin I (TSST-1)

- do not induce adaptive responses, but **trigger a massive burst of cytokines** that may cause fever, systemic toxicity & immune suppression

e.g. severe food poisoning, toxic shock syndrome



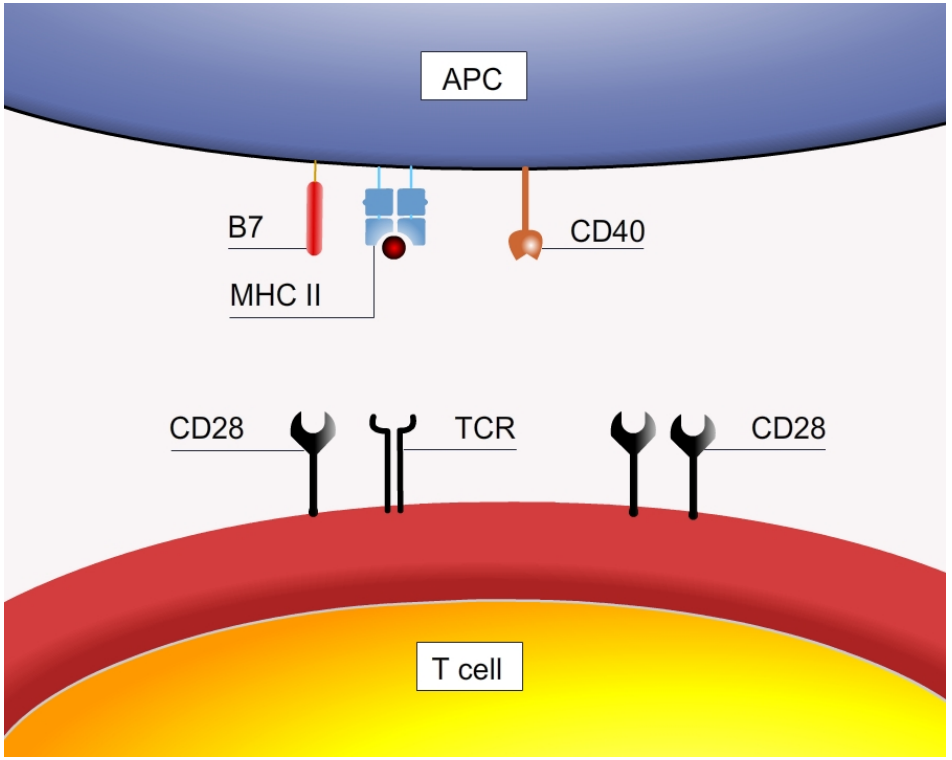
N.B. Polyclonal activators are often used as experimental tools to study T cell responses and in clinical settings to test for T cell function.

Co-stimulatory molecules

There are two major classes of co-stimulators:

CD80 (or B7-1) and CD86 (or B7-2)

- Belong to Ig superfamily
- interact with CD28 or CTLA4 on T cells
- provide the major co-stimulus to resting naïve T cells by reducing the threshold for TCR signalling

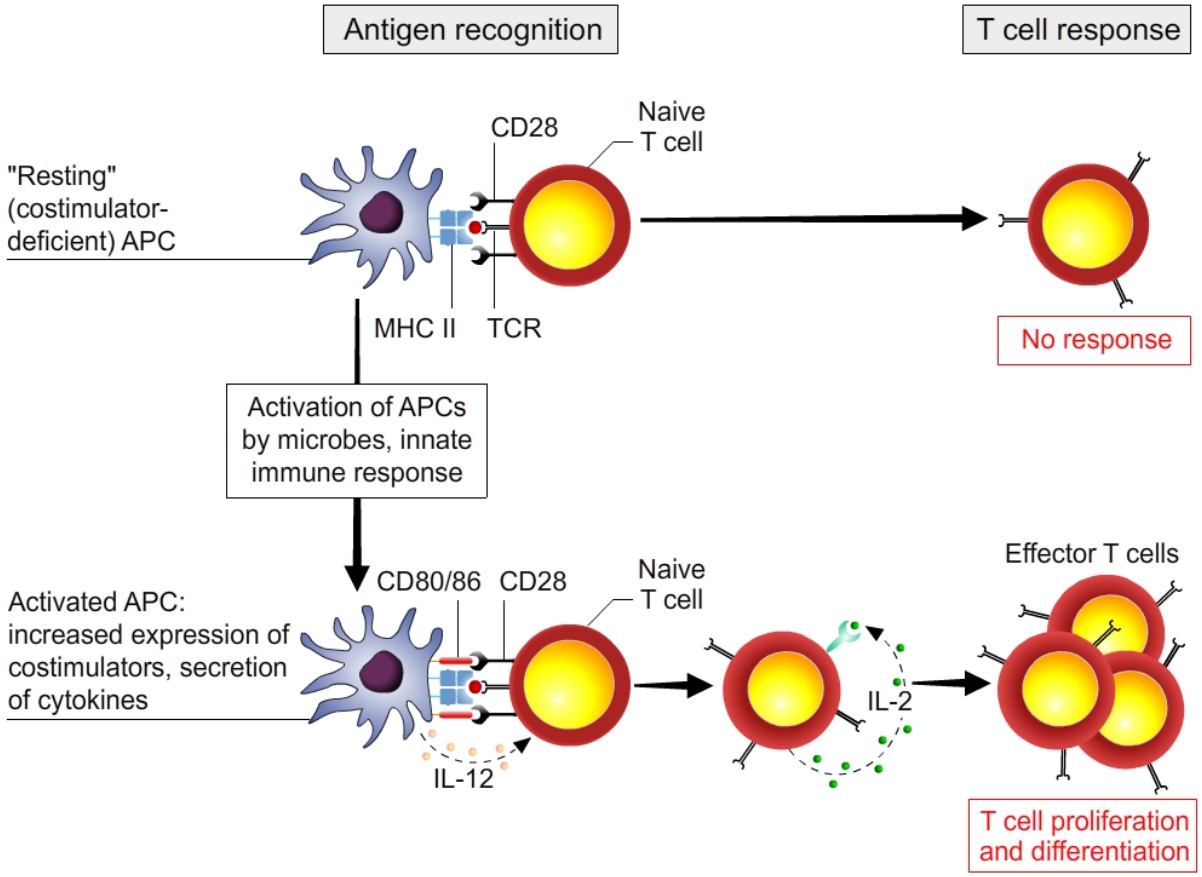


CD40/CD40L

- Belong to tumor necrosis factor receptor (TNF-R) superfamily
- CD40 (on APCs) induces APCs to produce more CD80/CD86 co-stimulators and IL-12
- CD40L (on T cells) determines the capacity of T cells to interact with other cells

The role of co-stimulation

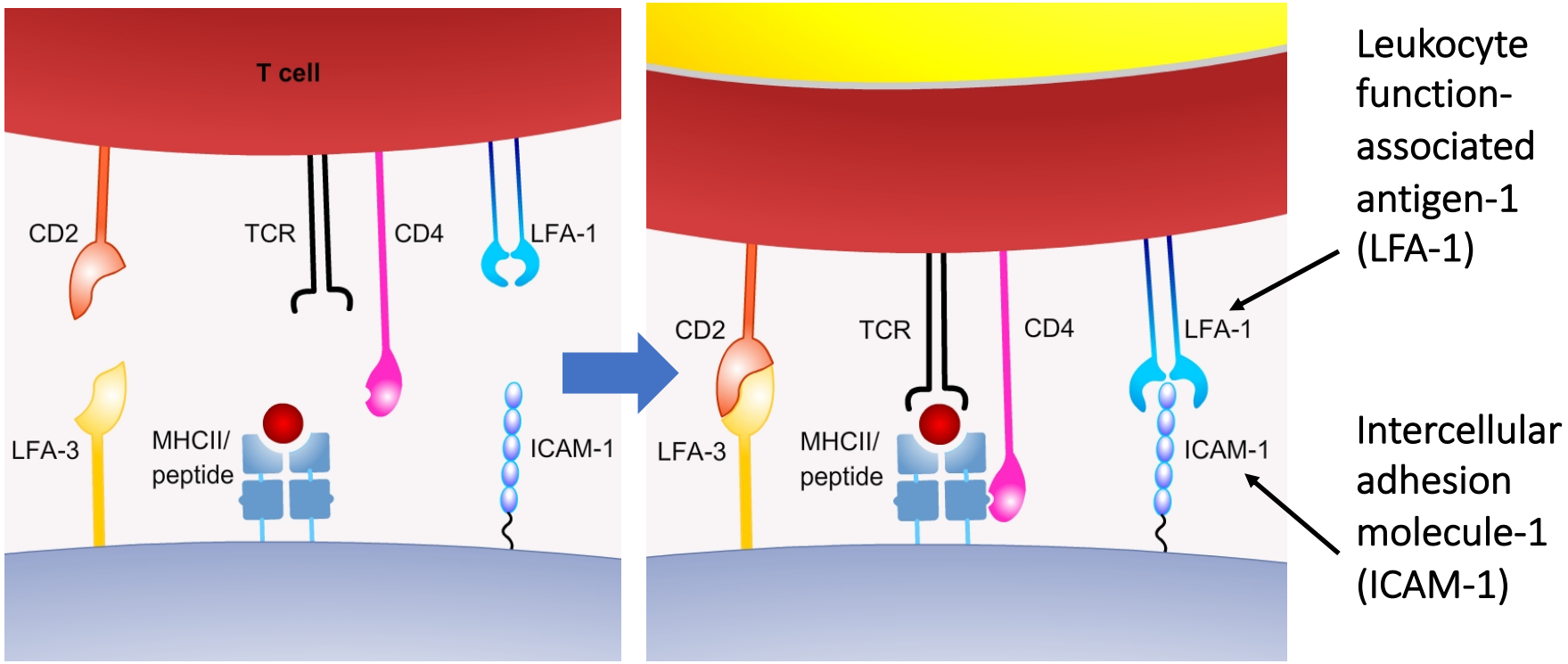
Signal 1 in the absence of signal 2 causes antigen-specific T cell unresponsiveness. The T cell is unable to produce IL-2 and therefore is unable to proliferate or be clonally selected.



The role of co-stimulation is illustrated by the effect of adjuvants (that activate co-stimulatory molecules). It ensures that T cells are activated in an infectious context. Understanding the biology of co-stimulators is essential in immunotherapies and vaccine development.

Adhesion molecules

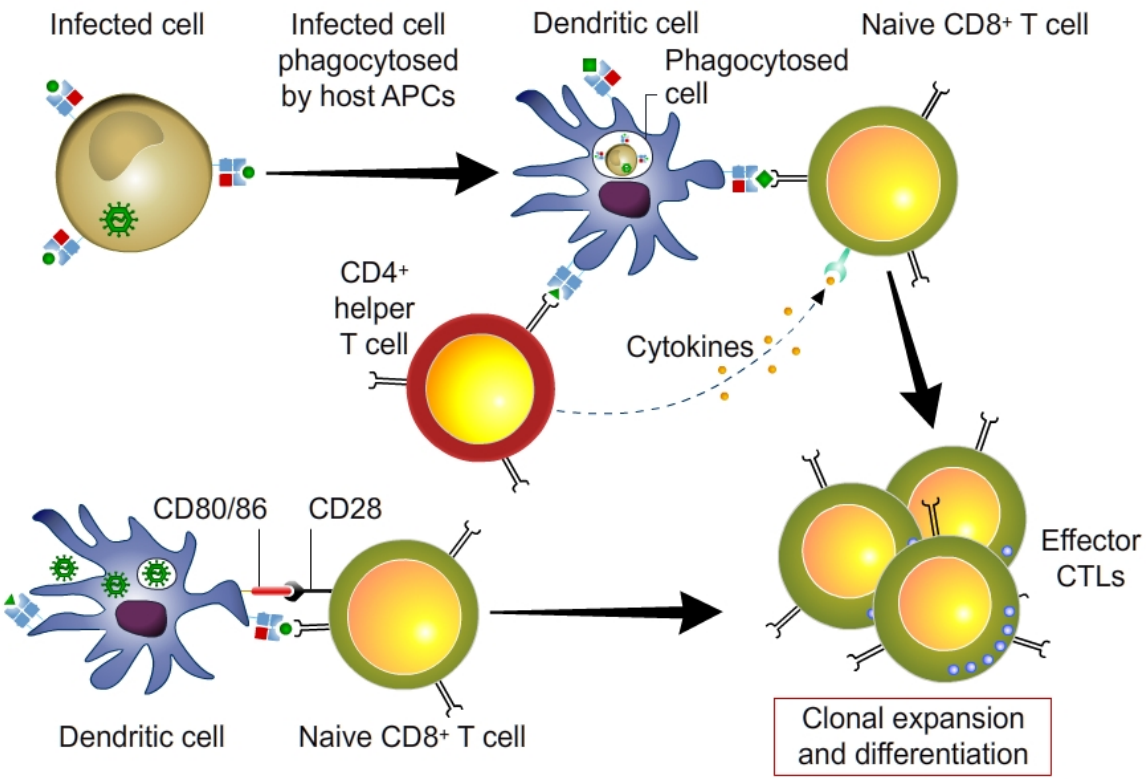
- To induce a productive response, the binding of T cells to APCs must be stabilized for a sufficiently long period to achieve the necessary signalling threshold. This is performed by adhesion molecules on the T cells whose ligands are expressed on APCs.
- The most important of these adhesion molecules belong to the family of heterodimeric (two-chain) proteins called integrins (e.g. LFA-1).
- Signals delivered by chemokines and antigen recognition increase integrin affinity and clustering.



Specific features of CD8+ T cell activation

1. An APC which has phagocytized an infected cell activates both CD8+ and CD4+ T cells

2. An infected APC can activate CD8+ T cells



Activation of CD8 T cells: two modes of antigen presentation.

1. **APCs ingest infected cells** and present microbial antigens to CD8+ T cells and to CD4+ helper T cells. The helper T cells then produce cytokines that stimulate the expansion and differentiation of the CD8+ T cells. This is mainly mediated trough a feedback activation from CD4 T cell to the DC that become more prone to stimulate the CD8 T cell.

2. If the **APC harbors a cytoplasmic microbe,** CD8+ T cells recognize class I MHC-associated peptides and receive costimulatory signals.

Specific features of CD8+ T cell activation

The activation of CD8+ T cells is stimulated by recognition of class I MHC-associated peptides and requires costimulation and/or helper T cells.

Specific features of CD8+ T cell activation:

i) **initiation** often requires that the **cytoplasmic antigen** from one cell be **cross-presented** by dendritic cells

ii) **differentiation into CTLs** may require the **concomitant activation of CD4+ helper T cells.**

When virus-infected cells are ingested by dendritic cells and the viral antigens are cross-presented by the APCs, the same APC presents antigens from the cytosol on class I MHC and from vesicles on class II MHC molecules. Thus, both CD8+ T cells and CD4+ T cells specific for viral antigens are activated together. The CD4+ T cells produce cytokines that activate the CD8+ T cells and stimulate the DC to produce more CD80/CD86.

Note: This requirement for helper T cells in CD8+ T cell responses is the likely explanation for the defective CTL responses to many viruses in patients infected with the human immunodeficiency virus (HIV), which kills CD4+ but not CD8+ T cells.

Conclusions | T cell activation

- T lymphocytes respond in sequential phases: recognition of cell-associated microbial proteins by naïve T cells, expansion of the antigen-specific clones by proliferation, and differentiation of some of the progeny into effector cells and memory cells.
- Dendritic cells and T cells interact by forming an **immunological synapse** involving
  - the TCR with its co-receptors (CD4/CD8)
  - co-stimulatory receptors
  - adhesion molecules
- Two types of **co-stimulation**
  - Expression of CD80/86 on DCs provides signal 2 (‘Danger’)
  - Expression of CD40L on T cells increases co-stimulatory signals on DCs (feedback)
- Two mechanisms of **activation of CD8 T cells by DCs**
  - An infected DC can activate CD8+ T cells in the absence of CD4+ T cells
  - A DC which has phagocytized an infected cell can activate CD4+ T cells and CD8+ T cells by cross-presentation. CD8+ T cell activation requires signals from CD4+ T cells through the production of cytokines and positive feedback on DCs (CD40L-CD40)

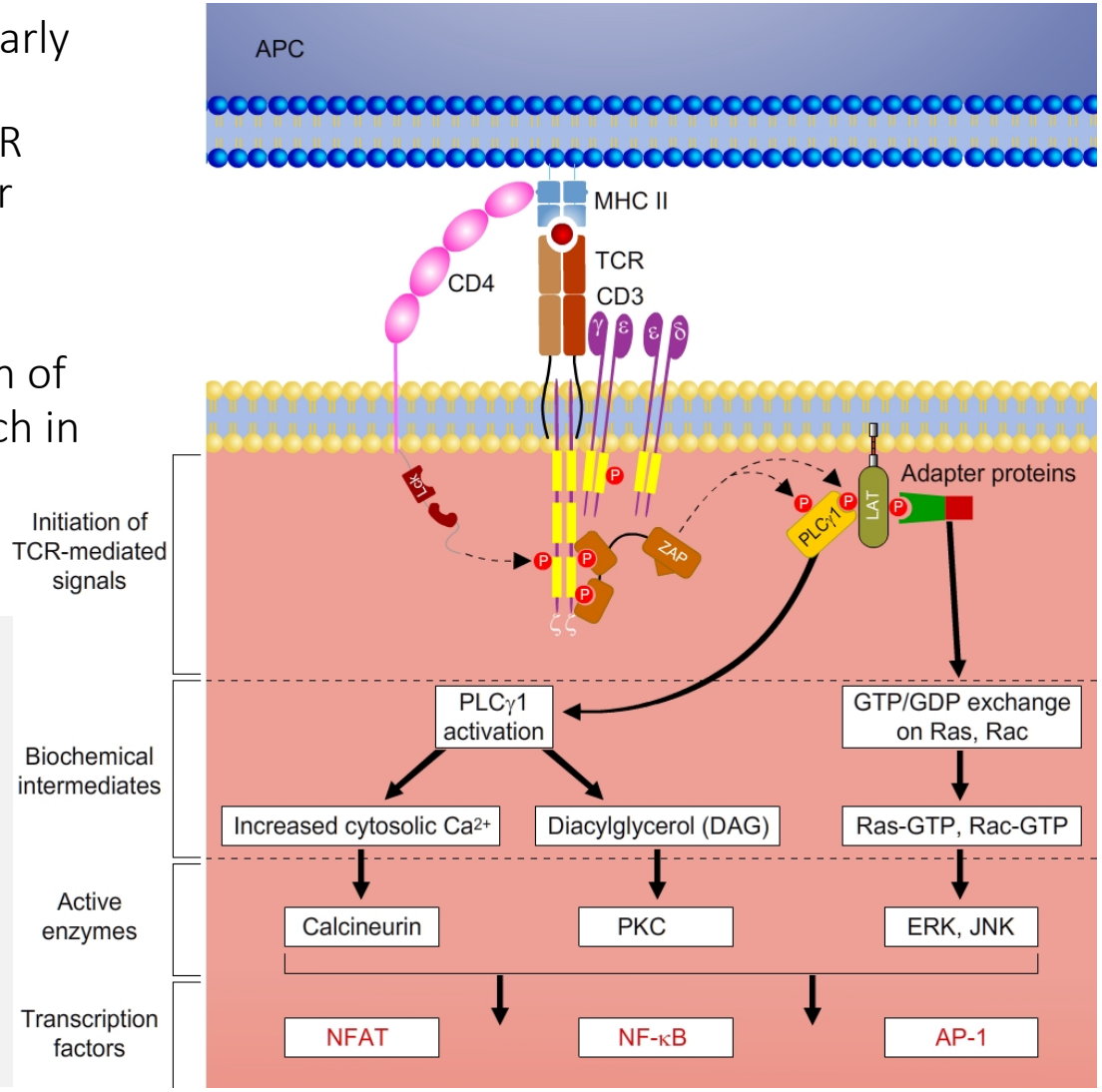
V-2
Consequences of T cell activation

- Signal transduction
- Cytokine production
- Clonal expansion

Signal transduction

Antigen recognition by T cells induces early signaling events, which include tyrosine phosphorylation of molecules of the TCR complex and the recruitment of adapter proteins to the site of T cell antigen recognition.

These early events lead to the activation of several biochemical intermediates, which in turn activate transcription factors.

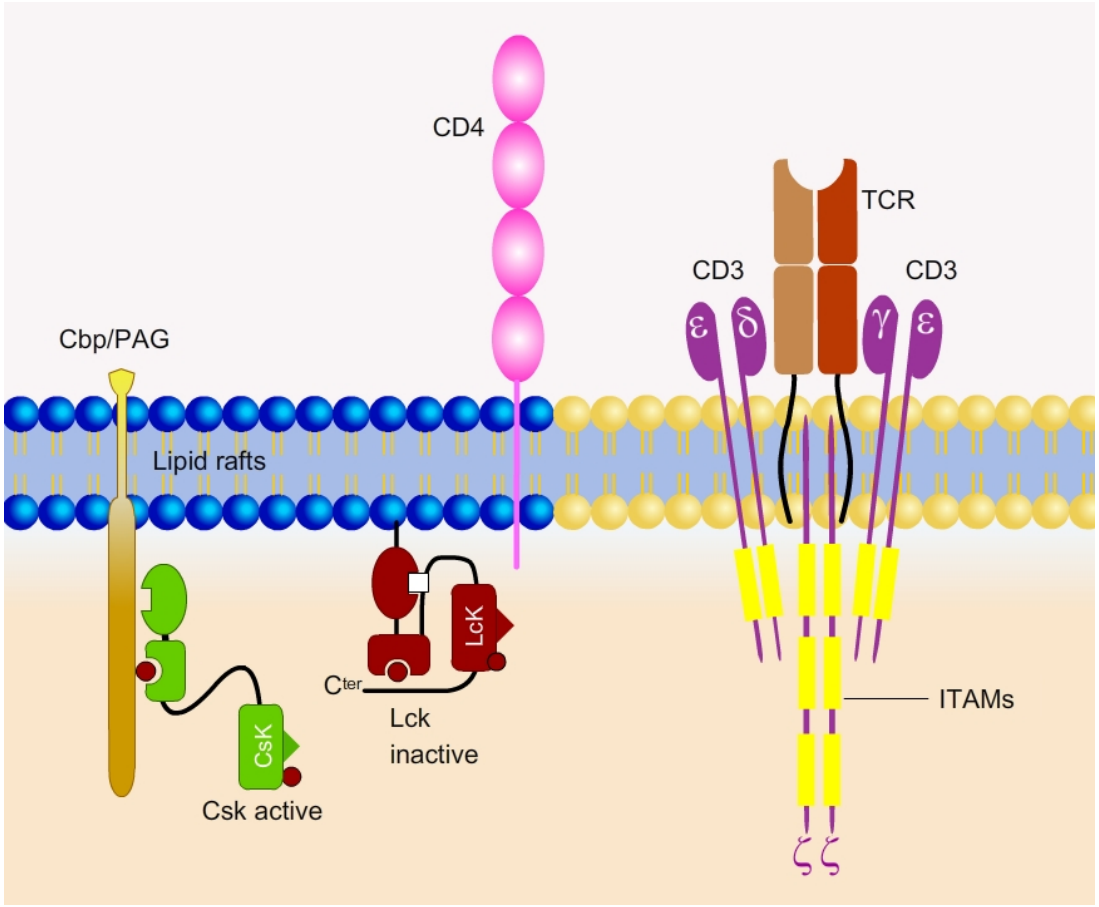


AP-1, activating protein-1
APC, antigen-presenting cell
GTP/GDP, guanosine triphosphate/diphosphate
ITAM, immunoreceptor tyrosine-based activation motif
NFAT, nuclear factor of activated T cells
PKC, protein kinase C
PLCγ1: γ1 isoform of phosphatidylinositol-specific phospholipase C
TCR, T cell receptor

Signal transduction: initial steps

Engagement of the TCR triggers 4 distinct transduction pathways leading to

- T cell proliferation
  - MAP kinase pathway
- T cell differentiation
  - NFAT pathway
  - NFκB pathways
- T cell motility
  - Rho/CDC42 pathway



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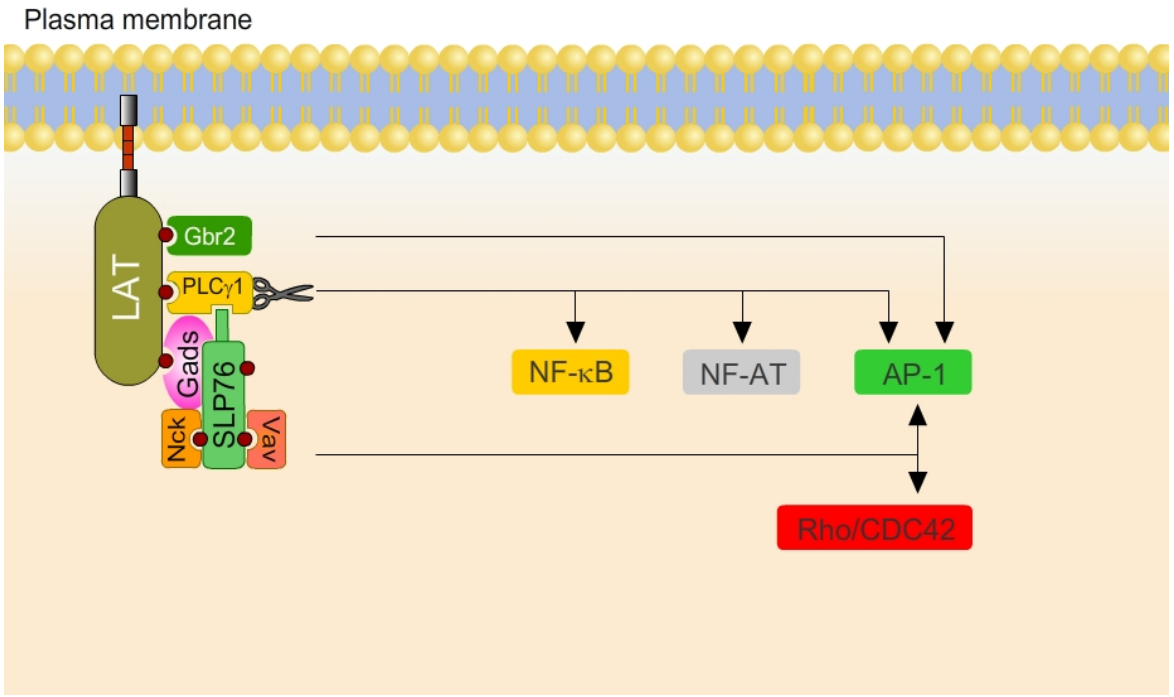

Signal transduction: the NFAT pathway

Engagement of the TCR triggers 4 distinct transduction pathways leading to

- T cell proliferation
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- T cell differentiation
  - NFAT pathway
  - NFκB pathways
- T cell motility
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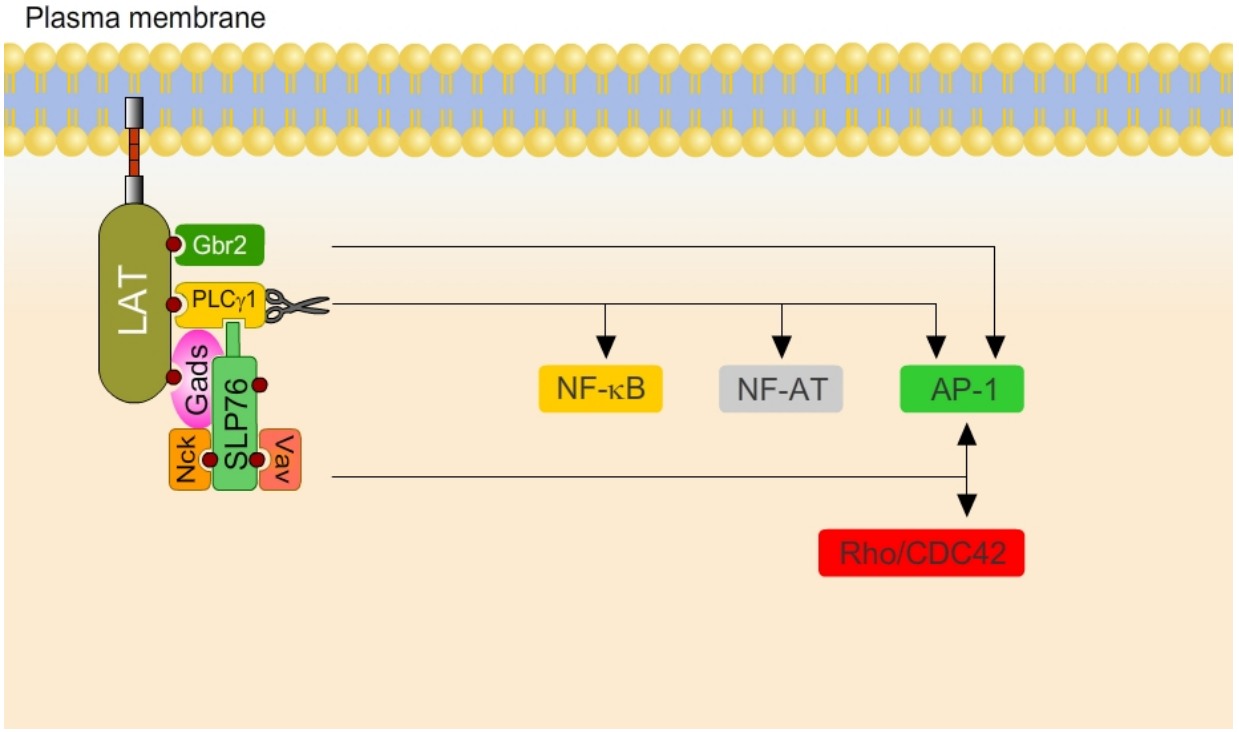


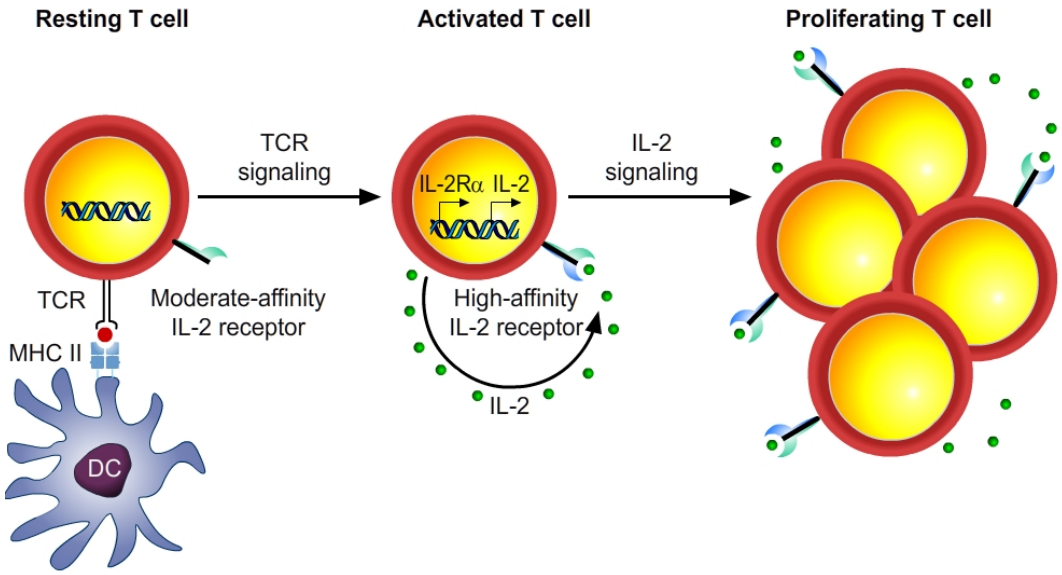
Cytokine production

In response to antigens and co-stimulators, T lymphocytes, especially CD4+ T cells, rapidly secrete several different cytokines that have diverse activities.

Cytokine	Source	Target	Effects
IL-2	CD4+ and CD8+ T cells	Effector and regulatory T cells	Survival, proliferation, differentiation
IL-4	CD4+ T cells, mast cells	B cells	Class switch to IgE
IL-5	CD4+ T cells, mast cells	Eosinophils	Activation
IFN-γ	CD4+ and CD8+ T cells, NK cells	Macrophages	Activation
TGF-β	CD4+ T cells, many other cell types	T cells	Inhibition, differentiation of regulatory T cells

IL, interleukin; IFN, Interferon; TGF, tissue growth factor

Clonal expansion (1)



T cell are in resting state (G0) when leaving the thymus.

- Naïve cells recirculate between blood and lymph system every 12-24 hours
- Activation of T cells results in primary response 2-7 days after activation:
  - Blast formation
  - Repeated rounds of cell division

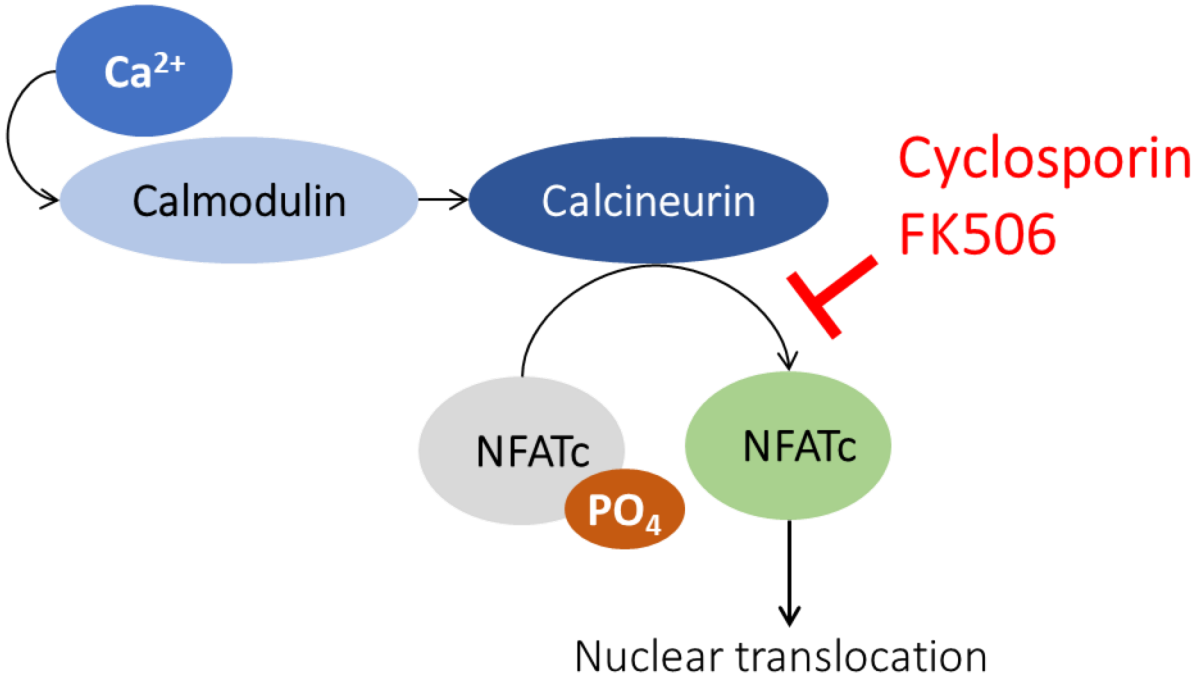
The key molecule that regulates T cell proliferation is the soluble cytokine IL-2.

- IL-2 produced by activated T cells induces expansion through interaction with its receptor (IL-2R)
  - autocrine pathway (promoting its own clonal expansion)
  - paracrine pathway (inducing proliferation of other IL-2R+ cells).

Importance of IL-2

Immunosuppressive drugs (e.g. used for organ transplantation) illustrate the importance of IL-2 in immune responses.

- Cyclosporin & FK506 inhibit IL-2 by disrupting TcR signalling by inhibiting the action of calcineurin.



Clonal expansion (2)

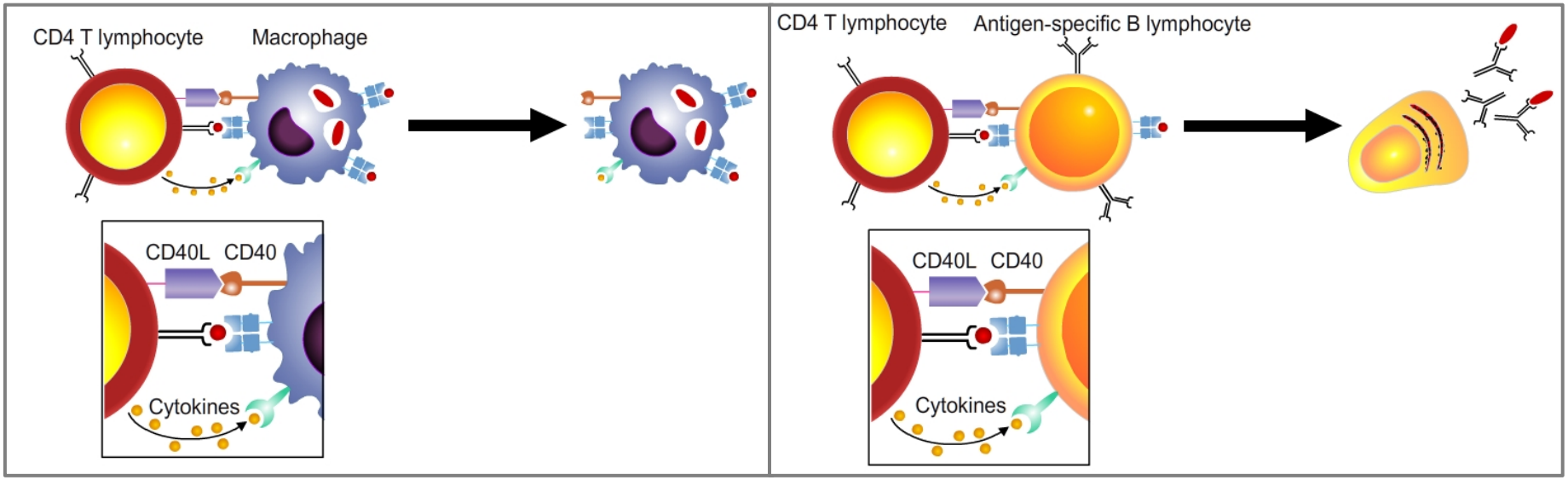
- **Magnitude of clonal expansion** differs between CD8+ and CD4+ T cells:
  - **CD8+ T cells** specific for a virus: **100,000-fold increase** (from $1/10^5$ - $1/10^6$ to 10% - 20% of all lymphocytes, with a doubling time of about 6 hours)
  - **CD4+ T cells**: **100- to 1000-fold increase**
- Difference in expansion reflects **difference in function:** CD8+ CTLs are effector cells that kill infected cells (direct action), while CD4+ effector cells secrete cytokines that activate many other effector cells (amplified action).
- **No detectable increase in "bystander" cells** that do not recognize the invading microbe
- **Majority** of the expanded clones are **specific for only a few (<5) immunodominant peptides** of the invading microbe

Role of CD40 ligand (CD40L)

CD4+ helper T cells differentiate into effector cells that respond to antigen by producing **surface molecules** and **cytokines** that function to activate phagocytes and B lymphocytes.

The most important cell surface protein of CD4+ T cells is **CD40 ligand (CD40L),** expressed in response to antigen recognition and costimulation.

CD40L binds to its **receptor CD40**, which is expressed **on macrophages, B cells, and dendritic cells**. Engagement of CD40 activates these cells, and thus CD40L is an important participant in the activation of macrophages and B lymphocytes by helper T cells



Conclusions | Consequences of T cell activation

- T cell activation by DCs starts complex signalling pathways inducing T cell proliferation and T cell differentiation.
- A key molecule that regulates T cell proliferation is the soluble cytokine IL-2.
- Immunosuppressive drugs used in transplantation target the NFAT pathway involved in IL-2 production.
- The CD40L gene expressed in differentiated CD4+ T allows T cells to interact with macrophages, B lymphocytes, and dendritic cells, which express CD40.
- During an adaptive immune response a few clones of T cells recognizing immunodominant peptides of the invading microbe expand massively.

V-C
T cell differentiation (Th1 / Th2)

- Functions of Th1 and Th2 cells
- Th1 / Th2 cell differentiation

T cell differentiation: Th1/Th2

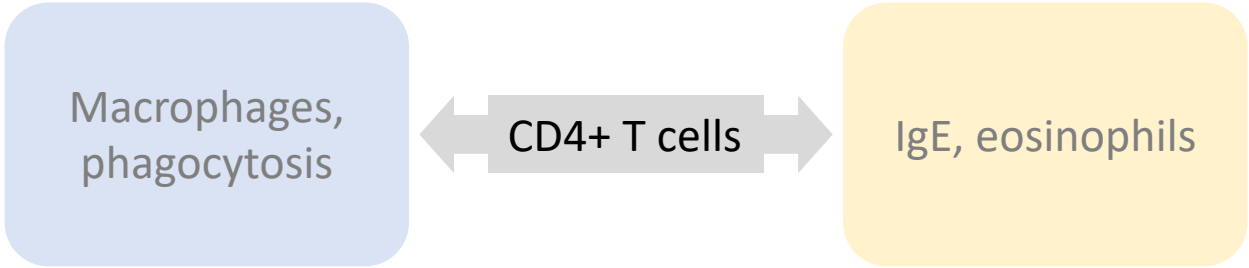
The immune system responds very differently to **different microbes.**

Examples:

- **Intracellular microbes** such as mycobacteria are ingested by phagocytes but resist intracellular killing. The adaptive immune response results in the activation of the **phagocytes** to kill the ingested microbes.

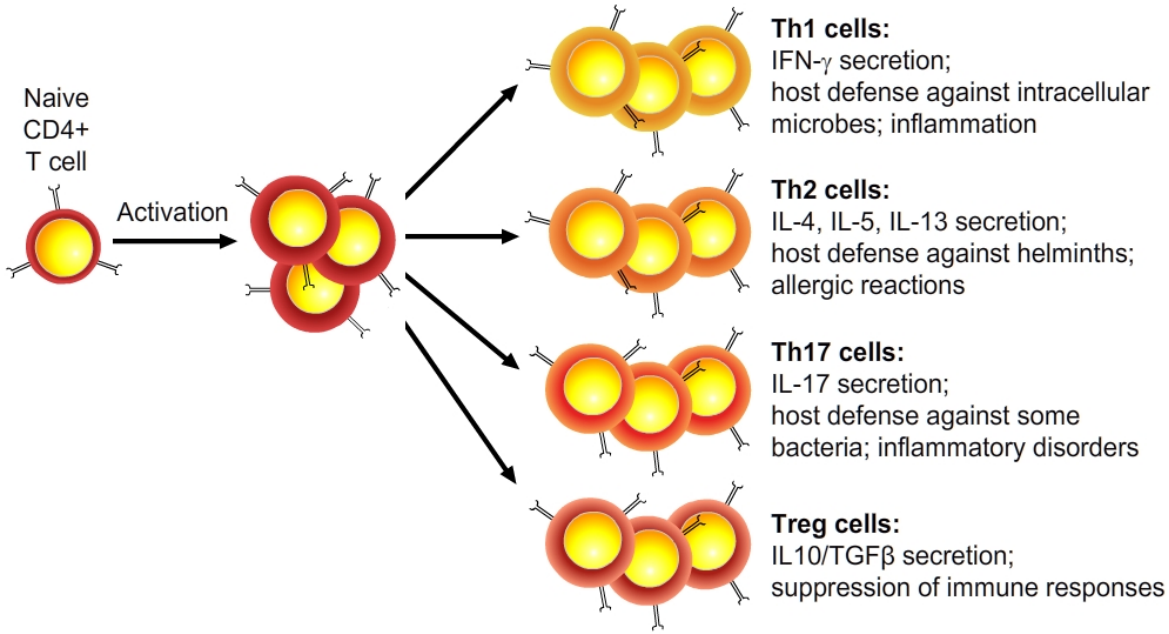
- **Helminthic parasites** are too large to be phagocytosed, and the immune response to helminths is dominated by the production of **immunoglobulin E (IgE) antibodies** and the activation of **eosinophils**.

T cell differentiation: Th1/Th2



- Both types of immune responses are dependent on CD4+ helper T cells, but for many years it was not clear how the CD4+ helper cells are able to stimulate such distinct immune effector mechanisms.
- This puzzle was solved by the discovery of functionally distinct **subpopulations of CD4** effector T cells that are distinguished by the **cytokines** they produce.
- The subsets that were defined first are called **Th1** cells and **Th2** cells (type 1 or type 2 helper T cells); more recently, a third population has been identified, called **Th17** cells because their signature cytokine is IL-17. Regulatory T (**T Reg**) cells are yet another subset of CD4 T cells; their role is to suppress immune responses.

Development and characteristics of subsets of CD4+ helper T lymphocytes



Characteristics	Th1	Th2
Cytokines		
IFN-γ, IL-2, TNF	+++	-
IL-4, IL-5, IL-13	-	+++
IL-10	+/-	++
IL-3, GM-CSF	++	++
Antibody isotypes stimulated	IgG2a (mouse)	IgE, IgG1 (mouse)/ IgG4 (humans)
Macrophage activation to kill microbes	+++	-

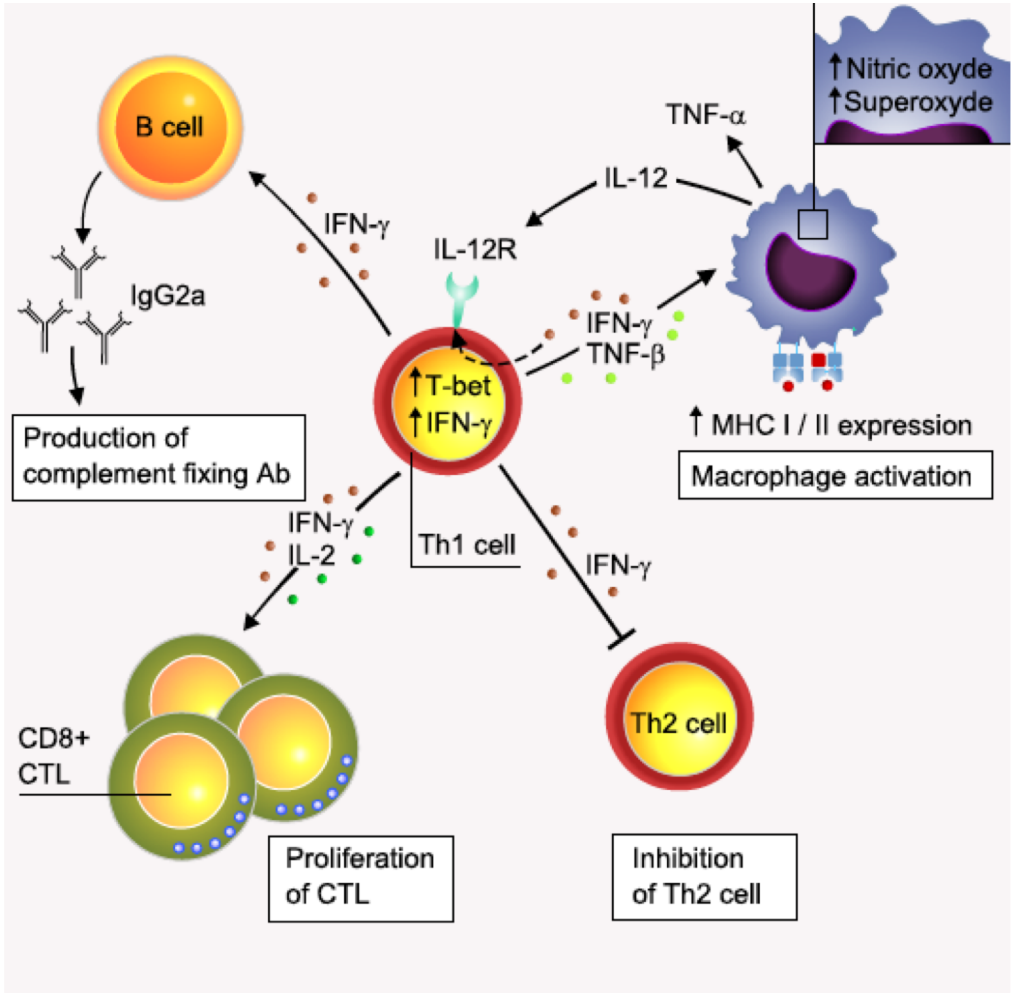
Note that many helper T cells are not readily classified into these distinct and polarized subsets.

GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; Ig, immunoglobulin; IL, interleukin; TNF, tumor necrosis factor.

Function of Th1 CD4 T Cells

TH1 cells produce the cytokine **interferon-γ (IFN-γ)**, which

- activates phagocytes to kill ingested microbes
- stimulates the production of antibody isotypes that promote the phagocytosis/ opsonization of microbes
- stimulates the expression of class II MHC molecules and B7 co-stimulators on macrophages and dendritic cells, to amplify T cell responses



Function of Th2 CD4 T Cells

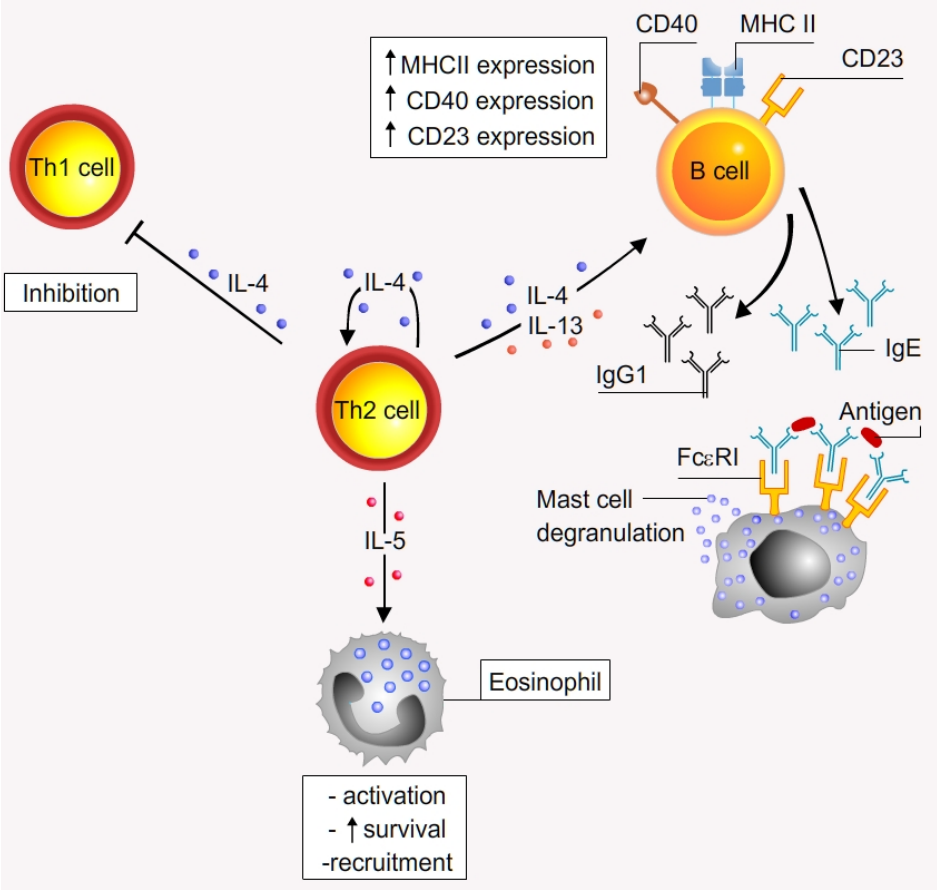
IL-4 produced by Th2 cells

- stimulates the production of IgE and IL-5, which activates eosinophils. IgE activates mast cells and binds to eosinophils, important in killing helminthic parasites.

IL-4 and IL-13 produced by Th2 cells

- promote expulsion of parasites from mucosal organs and inhibit the entry of microbes by stimulating mucus secretion.
  - IL-13 → mucus production
  - IL-4 → smooth muscle contraction
- inhibit the microbiocidal activities of macrophages and thus suppress Th1 cell-mediated immunity to balance the activation of Th1 and Th2 cells in response to that microbe.

Allergic reactions are Th2-like reactions in response to allergens that mimic foreign antigens.



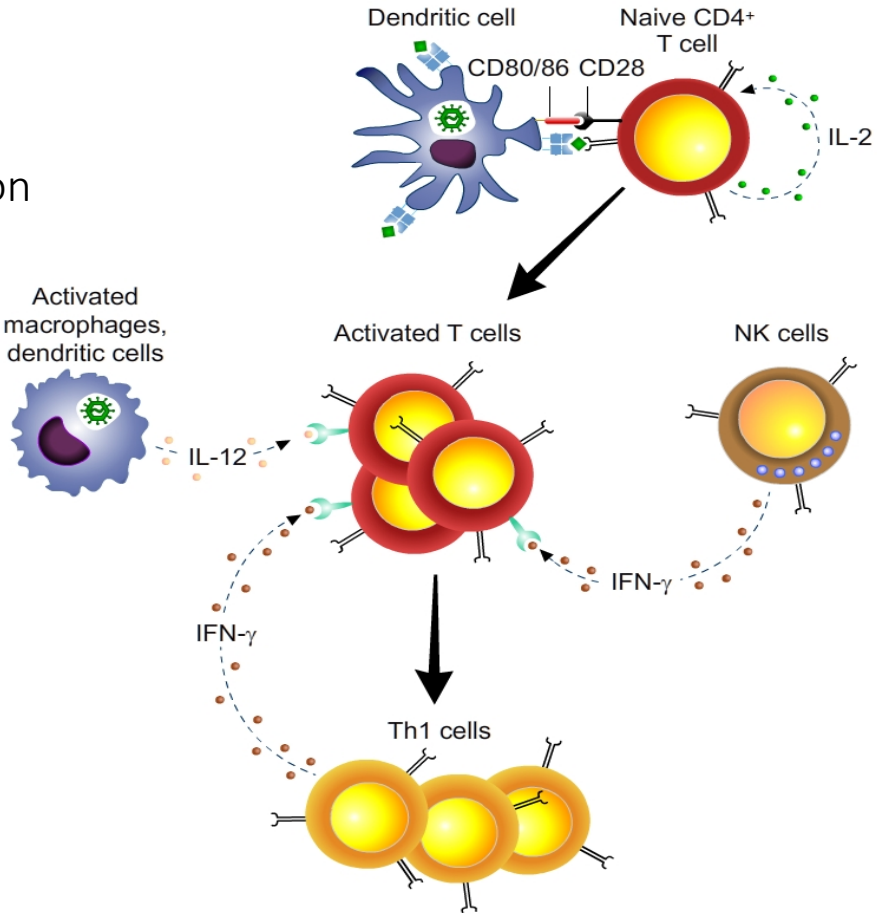
Development of the Th1 subset

Cytokines that induce Th1 development:

- **IL-12 (and IL-18)** produced by microbe-activated dendritic cells and macrophages
- **IFN-γ** made by NK cells or by the responding T cells themselves

Transcription factors involved in Th1 differentiation

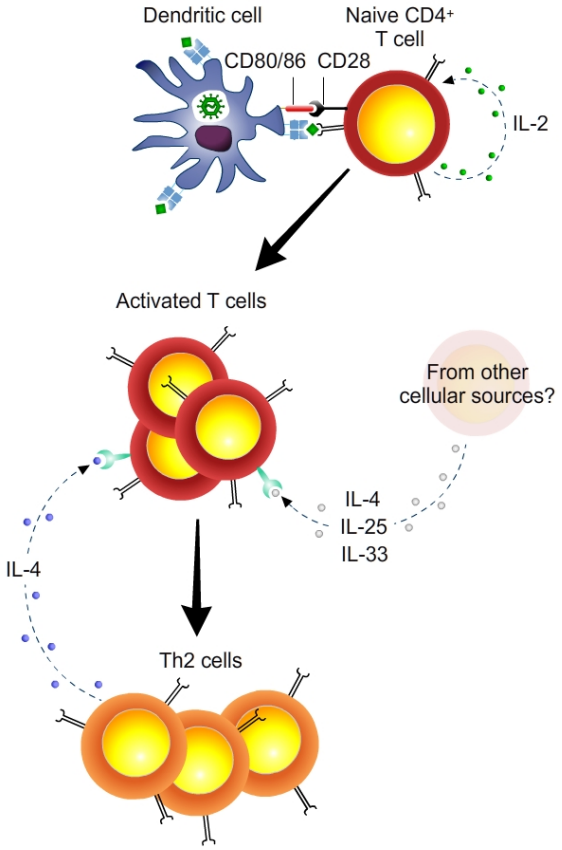
- **T-bet** (induced by IFN- γ)
- **Stat4** (induced by IL-12)



Development of the Th2 subset

The cytokines that induce Th2 cell differentiation:
- IL25 and IL33 produces by barrier epithelia
- IL-4 produced by the T cells themselves in the absence of IL-12

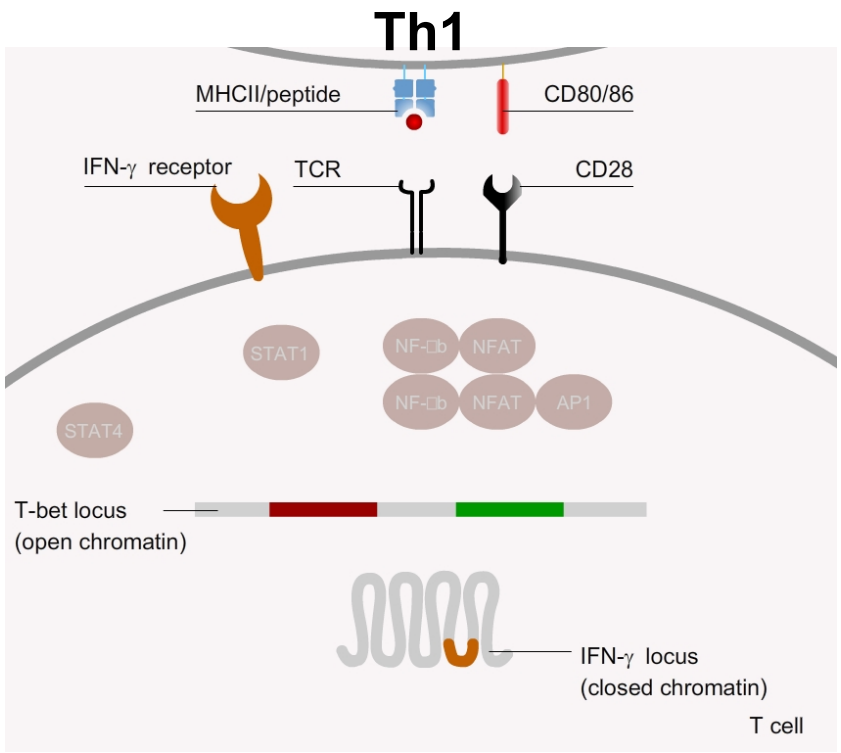
The transcription factors involved in Th2 development are GATA-3 and Stat6 (induced by IL-4).

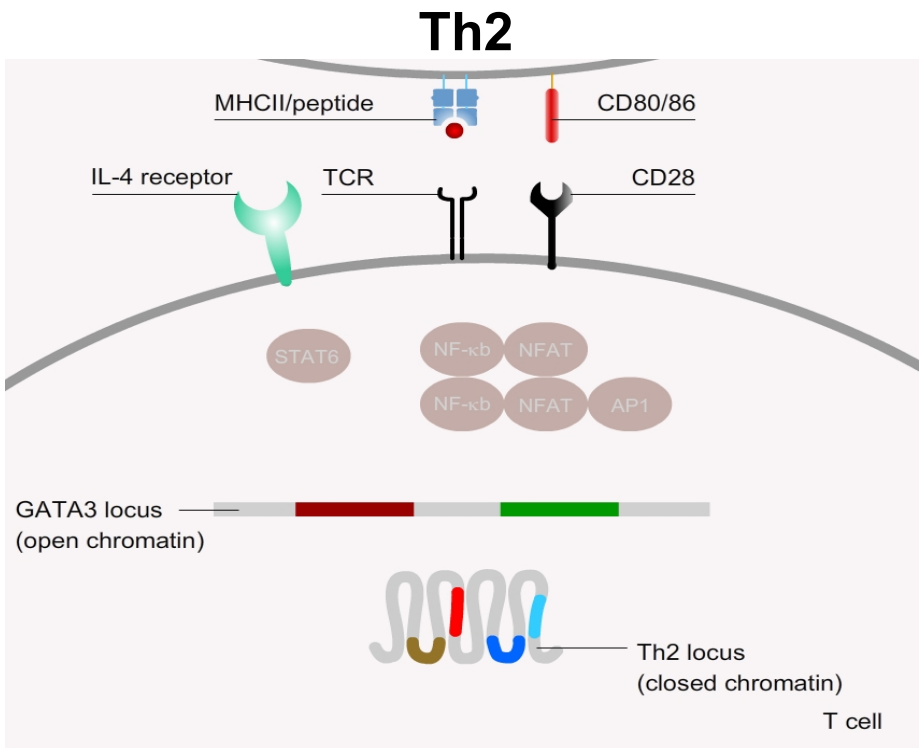


Th1/Th2 signalling pathways

Polarization into Th1 or Th2 phenotypes, which relies on different gene signalling pathways, depends on:

- the **cytokine microenvironment**
- interactions with DCs via **co-receptors**
- the nature and concentration of **antigen** presented.





Conclusions | T cell differentiation

- CD4 helper T cells differentiate into subsets of effector cells that produce restricted sets of cytokines and perform different functions.
- Th1 cells, which produce IFN-γ, activate phagocytes to eliminate ingested microbes, and stimulate the production of opsonizing and complement-binding antibodies.
- Th2 cells, which produce IL-4 and IL-5, stimulate IgE production and activate eosinophils, which function mainly in defense against helminths.
- Th17 cells, which produce IL-17, are implicated in several inflammatory diseases and may play a role in defense against bacterial infections.
- The polarization into various Th is mediated by cytokines and involves epigenetic changes of T cells populations.
- Th1 differentiation is induced by IL-12 and IFN-γ and involves the transcription factor T-Bet.
- Th2 differentiation is induced by IL-25, IL-33 and IL-4 and involves the transcription factor GATA-3.

Not discussed in the course: Innate lymphoid cells and their polarization