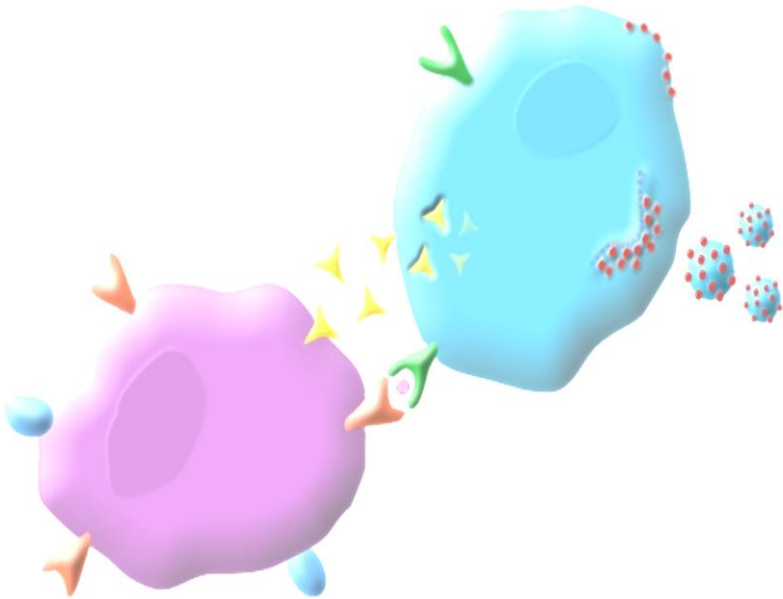
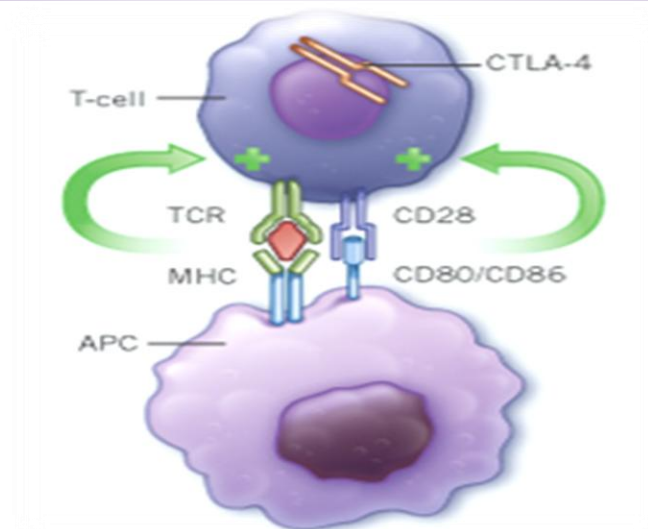


Teaching unit 05




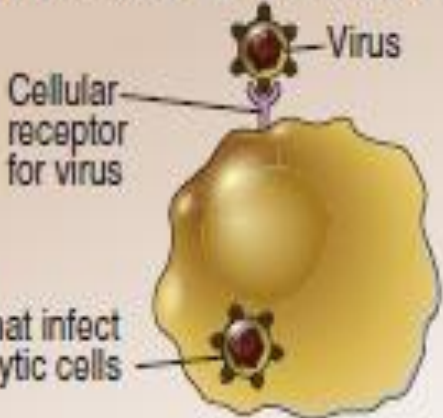
Cellular immune response

Cellular immune response



Activation of T lymphocytes by intracellular microorganisms

... let us remind ourselves

Intracellular microbes	Examples
<p>A Phagocyte</p>  <p>Phagocytosed microbes that survive within phagolysosomes</p> <p>Microbes that escape from phagolysosomes into cytoplasm</p>	<p>Intracellular bacteria: <i>Mycobacteria</i> <i>Listeria monocytogenes</i> <i>Legionella pneumophila</i></p> <p>Fungi: <i>Cryptococcus neoformans</i></p> <p>Protozoa: <i>Leishmania</i> <i>Trypanosoma cruzi</i></p>
<p>B Nonphagocytic cell (e.g., epithelial cell)</p>  <p>Virus</p> <p>Cellular receptor for virus</p> <p>Microbes that infect nonphagocytic cells</p>	<p>Viruses: All</p> <p>Rickettsiae: All</p> <p>Protozoa: <i>Plasmodium falciparum</i> <i>Cryptosporidium parvum</i></p>

Cellular immunity protects us from intracellular microorganisms

T lymphocytes play a major role in this type of acquired immunity

There are two types of intracellular infections

Phases of T-cell response

The response of T lymphocytes to antigens of intracellular microorganisms takes place in several successive stages.

During this response:

- ✓ The number of T lymphocytes **specific** for the given antigen increases.
- ✓ The transformation of **naive** into **effector** and **memory** T lymphocytes.

... let us remind ourselves

Naive T lymphocytes...

...recirculate constantly...

...before eliminating antigens, they must additionally
differentiate from naive to effector lymphocytes...

...that process begins with antigen recognition.

T lymphocytes **recognize** peptide fragments of protein antigens...

...and that as part of the products of the MHC on the APC that bring
processed antigens from the periphery to the secondary lymphatic
organs...

...the most effective in this process are dendritic cells because they provide
an additional (second) signal for activation.

... let us remind ourselves

...after activation of T lymphocytes (antigen-specific)
they begin to synthesize and secrete cytokines
... Cytokines also stimulate **clonal expansion**

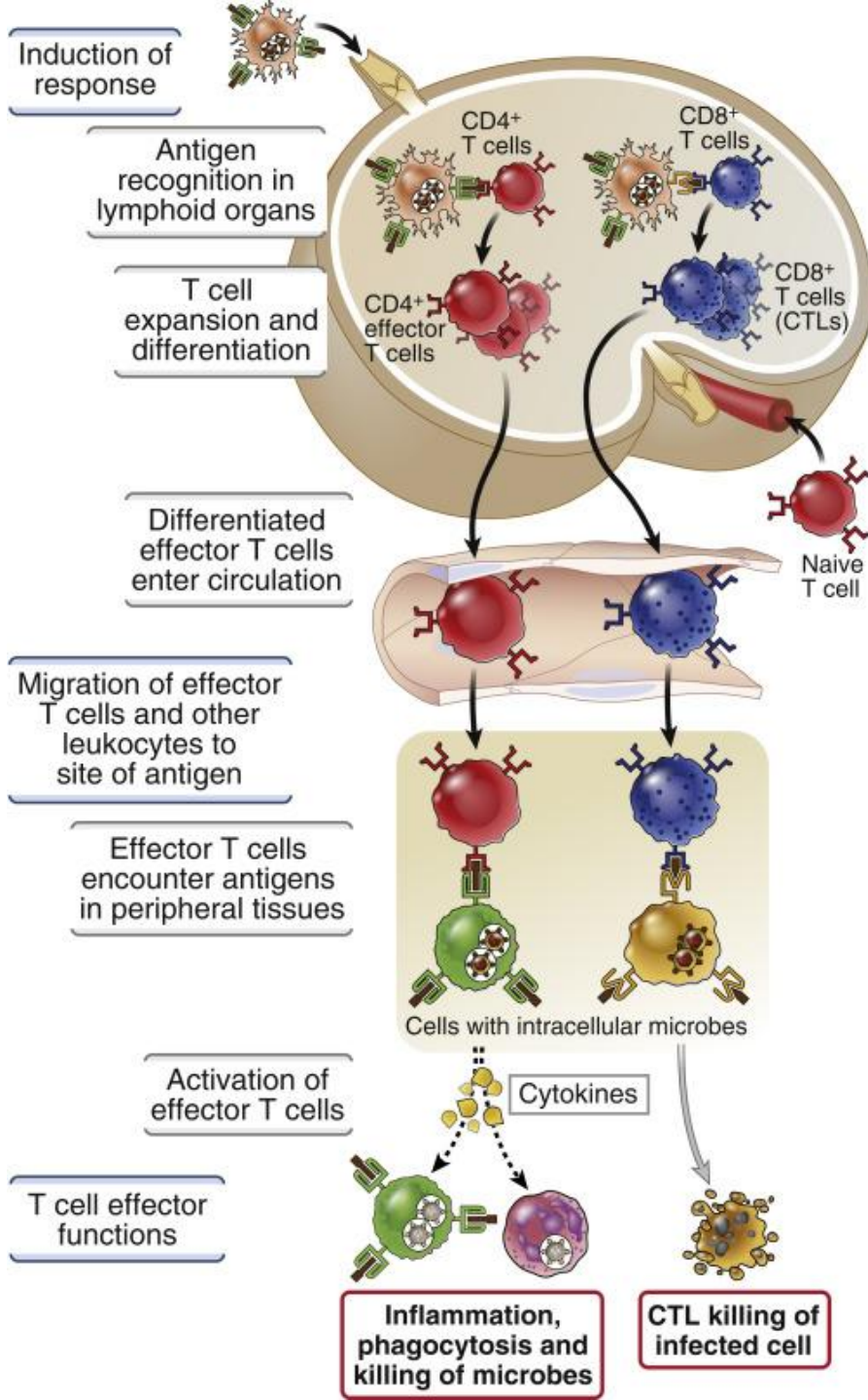
...lymphocytes activated in this way
further **differentiate** into effector and memory lymphocytes

...some of these cells remain in the lymph node
to participate in the elimination of the infected
cells and to help B lymphocytes

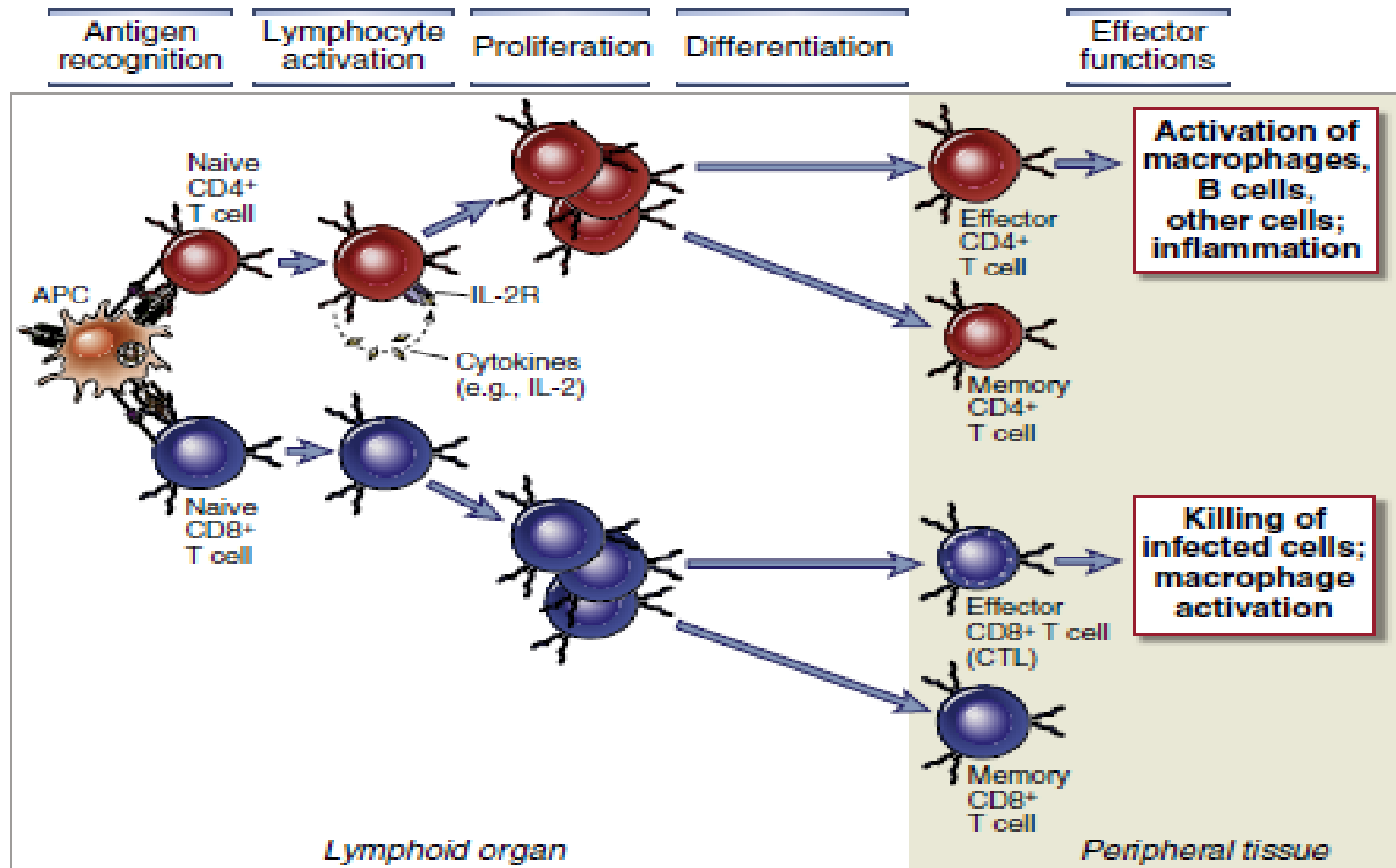
...most other effector T lymphocytes migrate to the site of infection...

...after antigen elimination, some of these lymphocytes become memory T
lymphocytes

Initiation and effector phases of cellular immunity

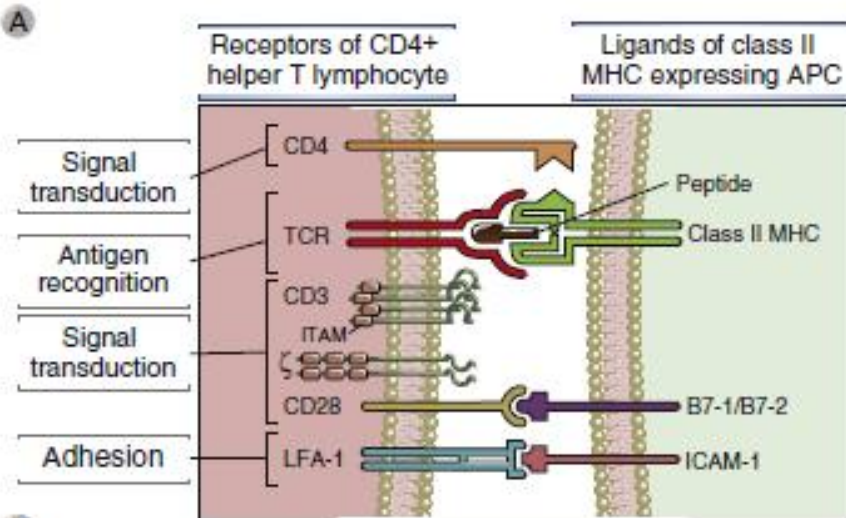






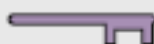
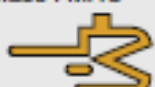







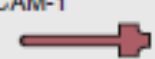
Phases of T lymphocyte activation: from naive to effector T lymphocytes



Antigen recognition and costimulation

Initiating a T-cell response requires multiple molecules on T lymphocytes to recognize the appropriate ligands on APC.



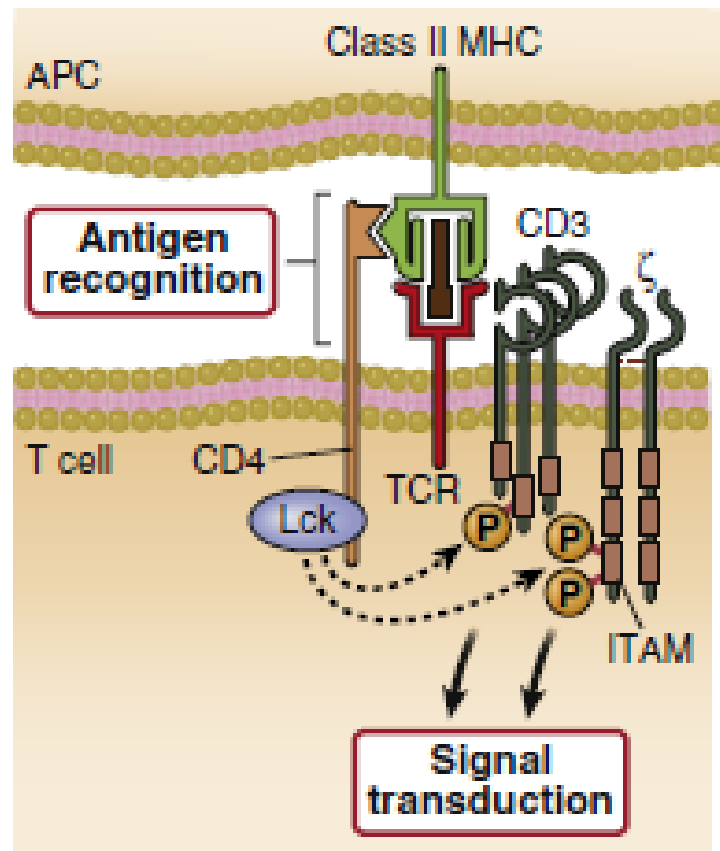
T cell accessory molecule	Function	Ligand	
		Name	Expressed on
CD3 	Signal transduction by TCR complex	None	
ζ 	Signal transduction by TCR complex	None	
CD4 	Signal transduction	Class II MHC 	Antigen presenting cells
CD8 	Signal transduction	Class I MHC 	All nucleated cells
CD28 	Signal transduction (costimulation)	B7-1/B7-2 	Antigen presenting cells
CTLA-4 	Signal transduction (negative regulation)	B7-1/B7-2 	Antigen presenting cells
PD-1 	Signal transduction (negative regulation)	PD-L1/PD-L2 	Antigen presenting cells, tissue cells, tumor cells
LFA-1 	Adhesion	ICAM-1 	Antigen presenting cells, endothelium

1. Recognition of peptides within the MHC molecule

This is the first signal for the activation of T lymphocytes. Receptor (TCR complex and coreceptors).

Coreceptors are **CD4** or **CD8** molecules.

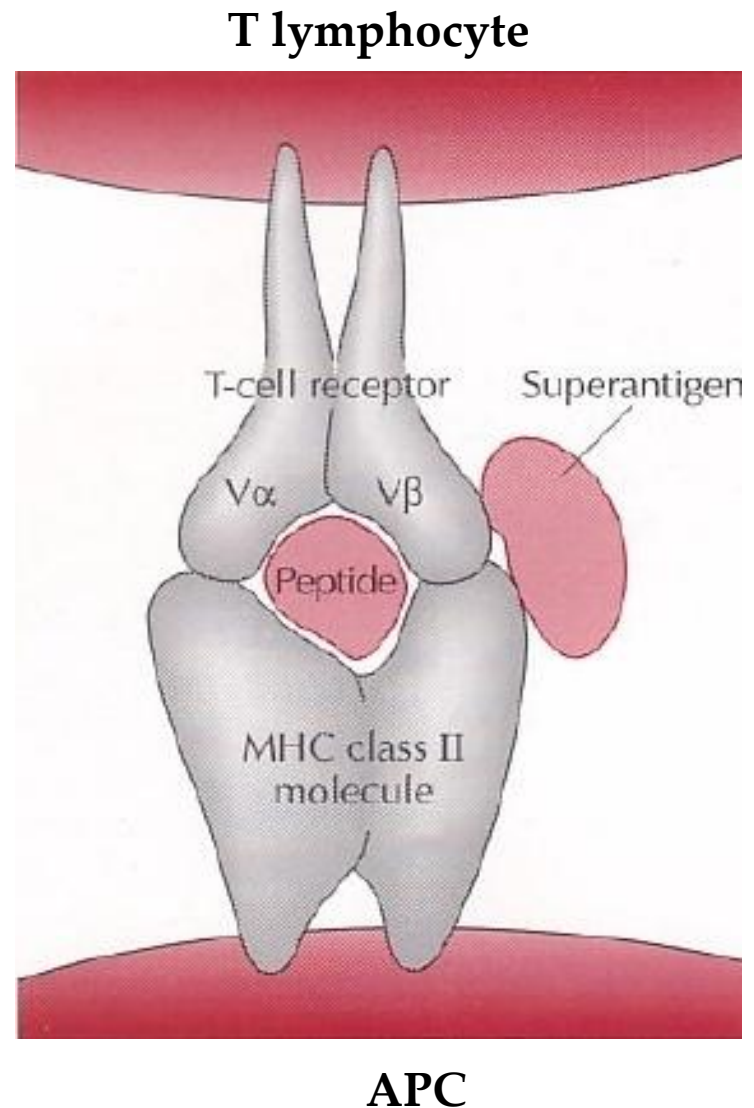
In the TCR complex, Recognition of antigens perform variable regions of α and β chains of TCR molecules, while the invariable function of signalling perform proteins CD3 and ζ .



Superantigens

Superantigens - some exotoxins of Gram-positive bacteria (*S. aureus* and *S. pyogenes*) stimulate a large number of CD4⁺ T lymphocytes by directly binding to class II MHC molecules on APC and to regions of V β TCR on T lymphocytes that are not part of active site.

By nonspecifically activating large numbers of CD4⁺ T lymphocytes, superantigens stimulate the production of large amounts of cytokines, resulting in a systemic reaction similar to septic shock.



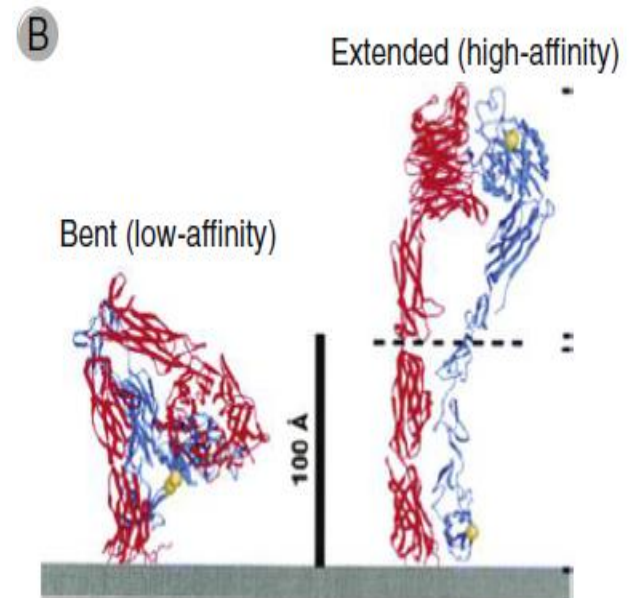
2. Adhesive molecules in T lymphocyte activation

Adhesive molecules are expressed on T lymphocytes, recognize their ligands on APC and thus stabilize the binding of T lymphocytes to APC.

The most important adhesive molecules belong to a family of heterodimeric proteins called integrins. The main integrin on T cells is **LFA-1** (Leukocyte Function Associated Antigen -1). Its ligand is ICAM-1 (Intercellular adhesion molecule-1).

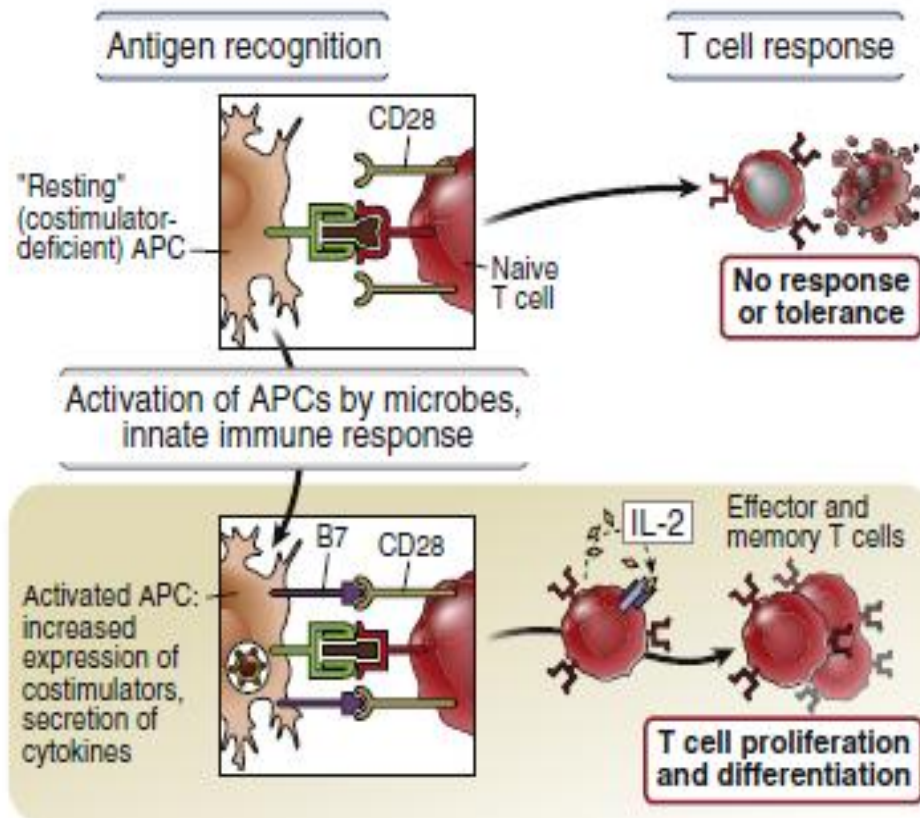
Adhesive molecules in T lymphocyte activation

- The integrins on blood leukocytes are normally in a low-affinity state.
- *An important feature of integrins is their ability to respond to intracellular signals by rapidly increasing their affinity for their ligands*
- Chemokine and antigen receptor engagement in cells lead to increased affinity of the integrins
- Integrins are also important in directing the migration of effector T lymphocytes to the site of infection.



3. Costimulators in the activation of T lymphocytes

Costimulators are molecules expressed on **APCs** and provide a **second signal**.



The best studied **B7-1(CD80)** and **B7-2(CD86)** are expressed on professional APC.

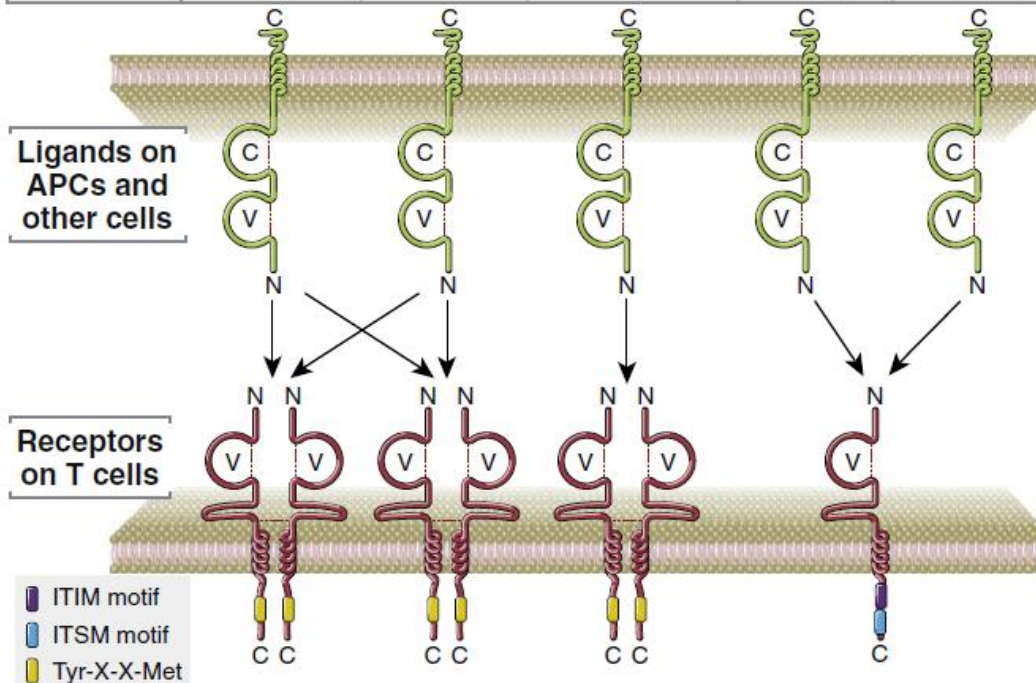
The expression of these molecules increases significantly when APC comes into contact with microorganisms.

The ligand for these molecules is **CD28** expressed on T lymphocytes.

In the absence of CD28 and B7 interaction, not only there is no lymphocyte activation, but the lymphocyte can be disabled for a long time.

Members of the B7 and CD28 protein families

Expression	DCs; macrophages, B cells		DCs; macrophages, B cells, other cells	DCs; macrophages, B cells; endothelial, epithelial and tumor cells (PD-L1 only)	
Name	B7-1 (CD80)	B7-2 (CD86)	ICOS-L (CD275)	PD-L1 (B7-H1, CD274)	PD-L2 (B7-DC, CD273)



Name	CD28	CTLA-4	ICOS	PD-1
Expression	T cells; constitutive	T cells; inducible	T cells; inducible	T cells, B cells, myeloid cells; inducible
Major function	Costimulation of naive T cells; generation of regulatory T cells	Negative regulation of immune responses; self-tolerance	Costimulation of effector and regulatory T cells; generation of follicular helper T cells	Negative regulation of T cells

Another group of costimulatory molecules consists of **CD40** on APC and its **CD40 ligand** (CD154) on T lymphocytes.

The contact of these molecules does not directly enhance the activation of T lymphocytes. Instead, this binding increases the expression of B7 molecules on APCs and prompts them to secrete IL-12, which stimulates T lymphocyte differentiation.

Protein antigens (e.g. those used in vaccines are inert and cannot induce a T cell immune response on their own, but it is necessary to give them substances that activate APS (dendritic cells, macrophages, and probably also B lymphocytes). These substances are **adjuvants**.

Adjuvants work by inducing the expression of costimulators on APCs and prompting them to secrete activating cytokines.

Different members of the CD28 family participate in the activation, but also in the inhibition of T lymphocytes.

To limit or end the immune response are important:

...**CTLA4** which also binds to B7 on APC, but transmits an inhibitory signal and prevents the immune response to some tumors.

...**PD-1** which binds to similar ligands and inhibits the response to infection allowing chronicity.

Receptor: TCR recognizes the peptide within the APC

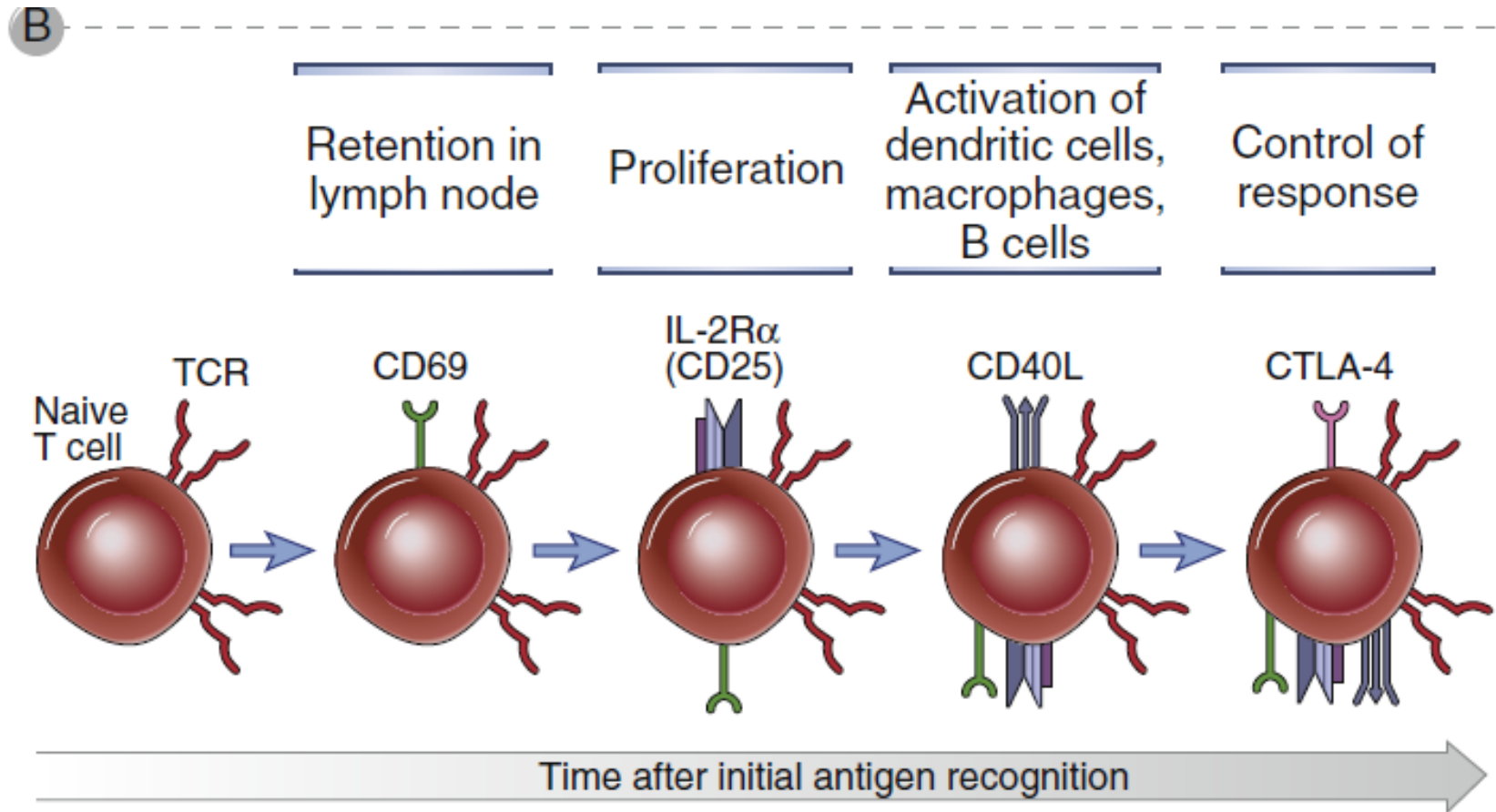
ACCESSORY MOLECULES :

CO-RECEPTORS (expressed on T lymphocytes): CD4 and CD8

ADHESIVE MOLECULES (expressed on T lymphocytes): LFA-1

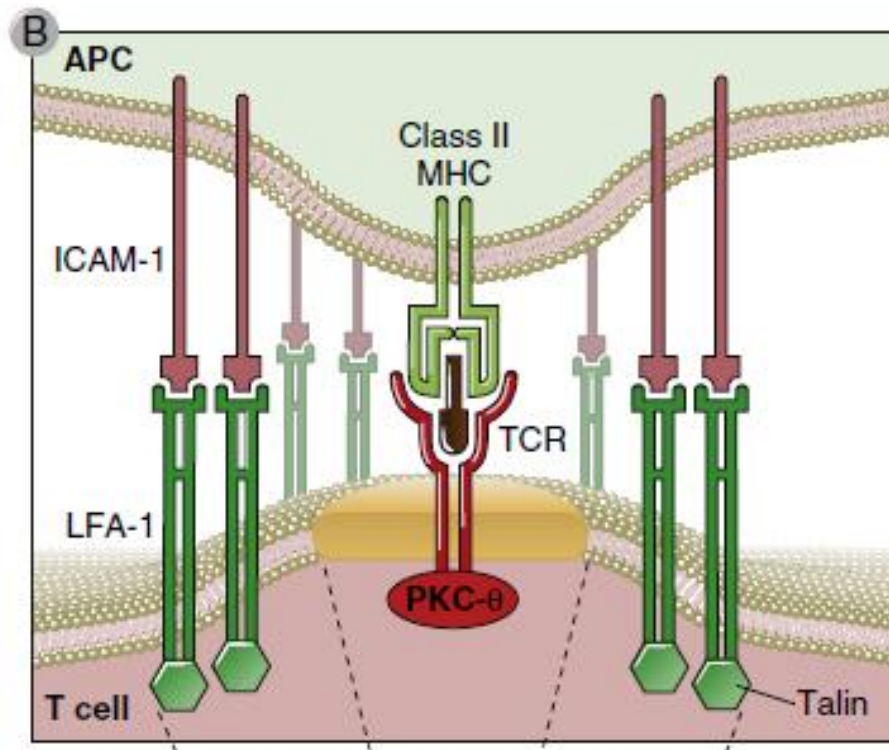
COSTIMULATORS (expressed on APC): B7-1, B7-2, CD40

Proteins produced by antigen-stimulated T lymphocytes



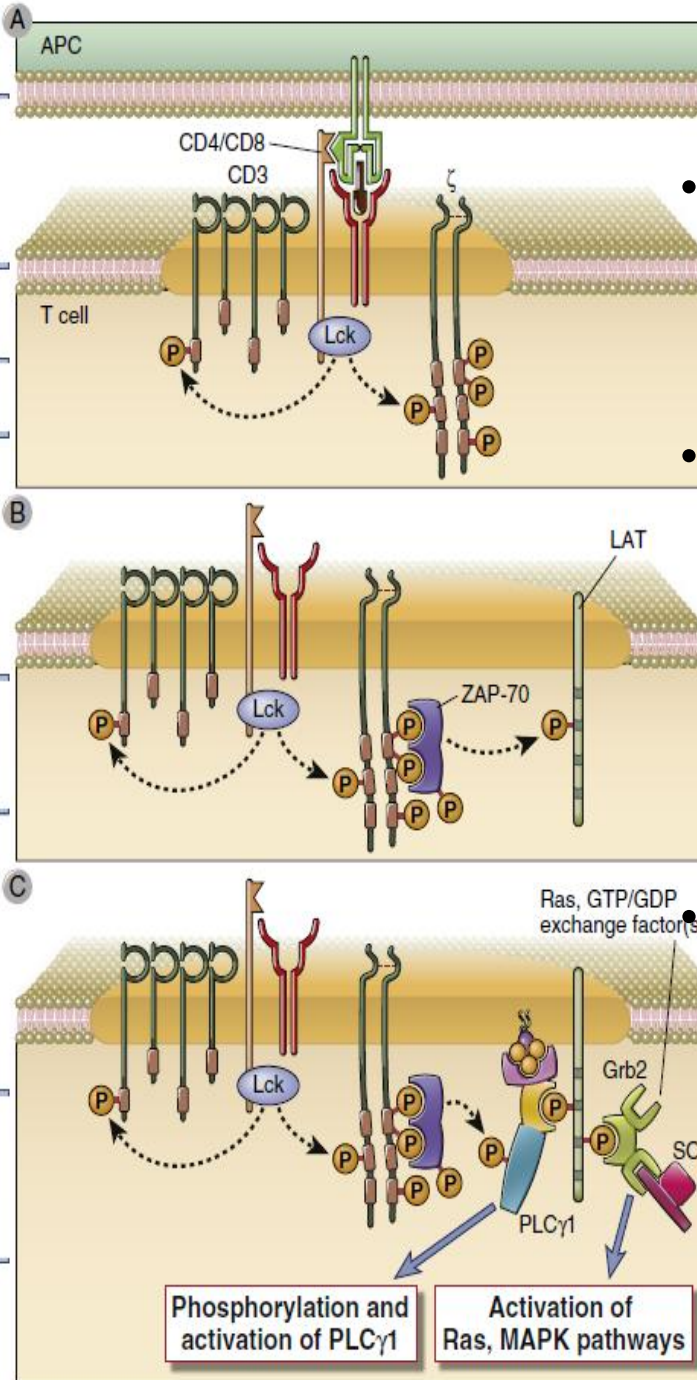
After the initiation of activation by antigen recognition and costimulator binding, there are characteristic changes in the expression of various surface molecules in T cells. These proteins are typically expressed at low levels in naive T cells and are induced by activating signals.

When the TCR complex recognizes MHC-associated peptides on an APC, several T cell surface proteins and intracellular signaling molecules are rapidly mobilized to the site of T cell–APC contact. This region of physical contact between the T cell and the APC forms structure that is called an **immunologic synapse**



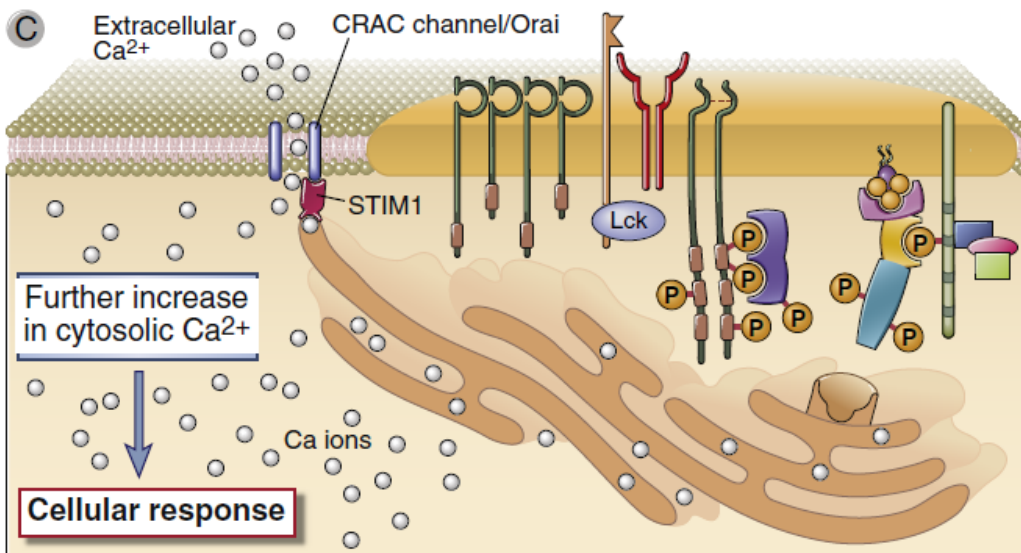
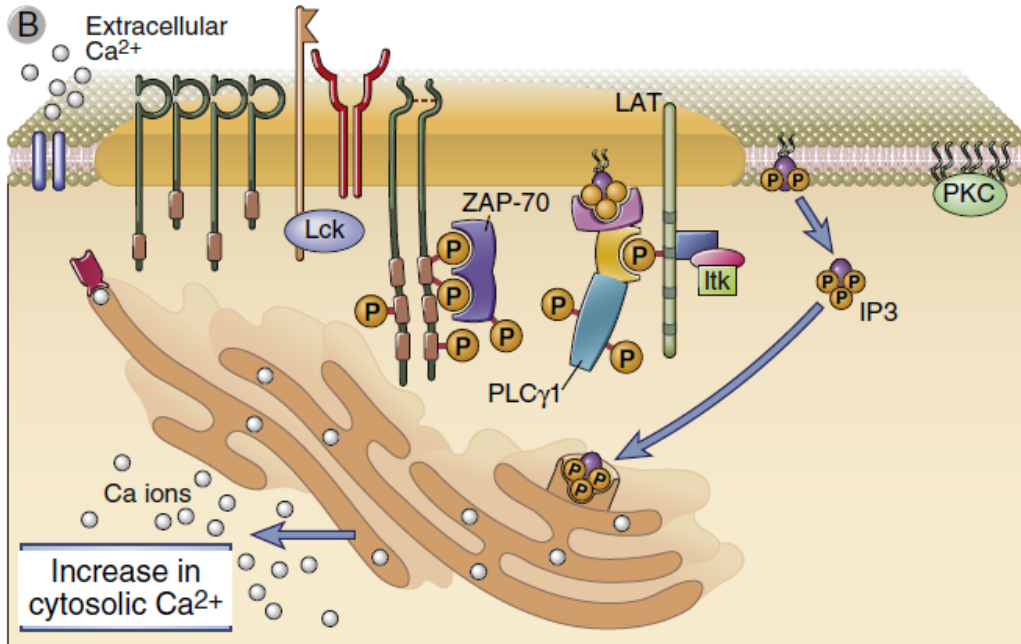
Early tyrosine phosphorylation events in T cell activation.

- *Phosphorylation of proteins and lipids plays a central role in the transduction of signals from the TCR complex and coreceptors*
- On antigen recognition, there is clustering of TCR complexes with coreceptors (CD4/CD8). Coreceptor-associated **Lck** becomes active and phosphorylates tyrosines in the ITAMs of **CD3** and **ζ** chains.



ZAP-70 binds to the phosphotyrosines of the ζ chains and is itself phosphorylated and activated. Active **ZAP-70** then **phosphorylates tyrosine's** on various adaptor molecules that results activating multiple signaling pathways.

1. NFAT cells signaling pathway



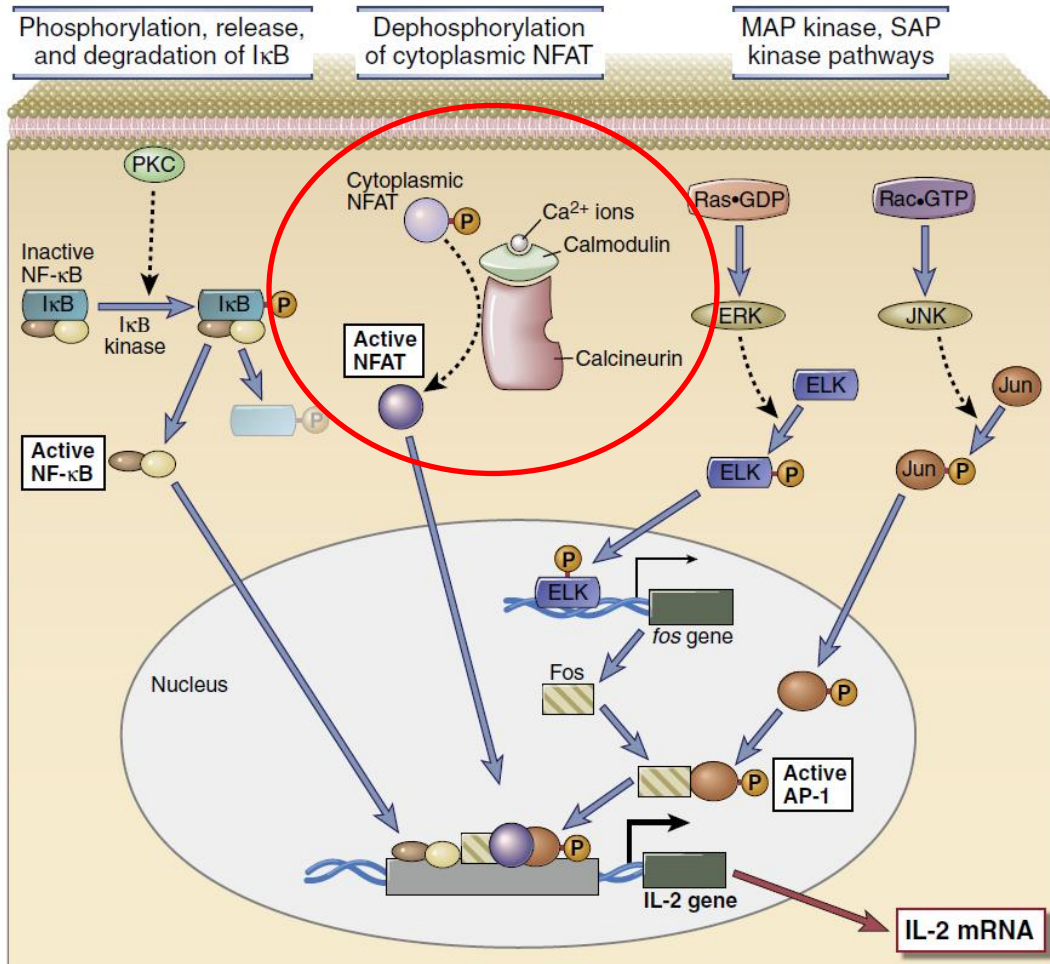
NFAT is a transcription factor required for the expression of genes encoding **IL-2**, **IL-4**, **TNF**, and other cytokines.

The LAT adaptor protein that is phosphorylated on T cell activation binds the cytosolic enzyme **PLC $\gamma 1$** , which is phosphorylated by ZAP-70 and activated.

Active **PLC $\gamma 1$** hydrolyzes membrane PIP_2 to generate **IP3**.

IP3 induces the opening of the CRAC channel that facilitates entry of extracellular calcium into the cytosol.

1. NFAT cells signaling pathway



NFAT is present in an **inactive phosphorylated** form in the cytoplasm of resting T lymphocytes.

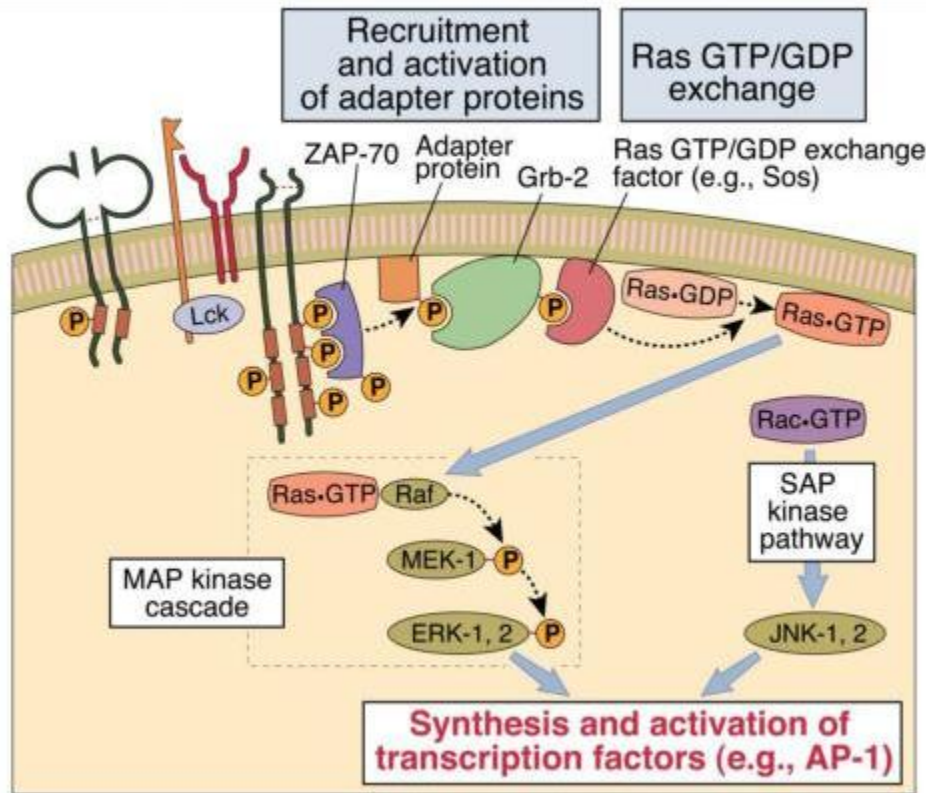
Ca⁺⁺ binds to the protein calmodulin, and this complex activates the **calcineurin phosphatase**, which removes phosphate groups from NFAT.

NFAT translocates into the nucleus.

In the nucleus, NFAT binds to the regulatory regions of IL-2 and other cytokine genes and initiates transcription of the IL-2 gene

2. The Ras-MAP kinase pathway

The Ras/MAP-kinase pathway in T cells



ZAP-70 phosphorylates membrane-associated adaptor proteins, which then bind another adaptor, Grb-2.

Grb-2 provides a docking site for the GTP/GDP exchange factor SOS.

SOS converts Ras·GDP to Ras·GTP. **Ras·GTP** activates a cascade of enzymes, which results in the activation of the MAP kinase

ERK.

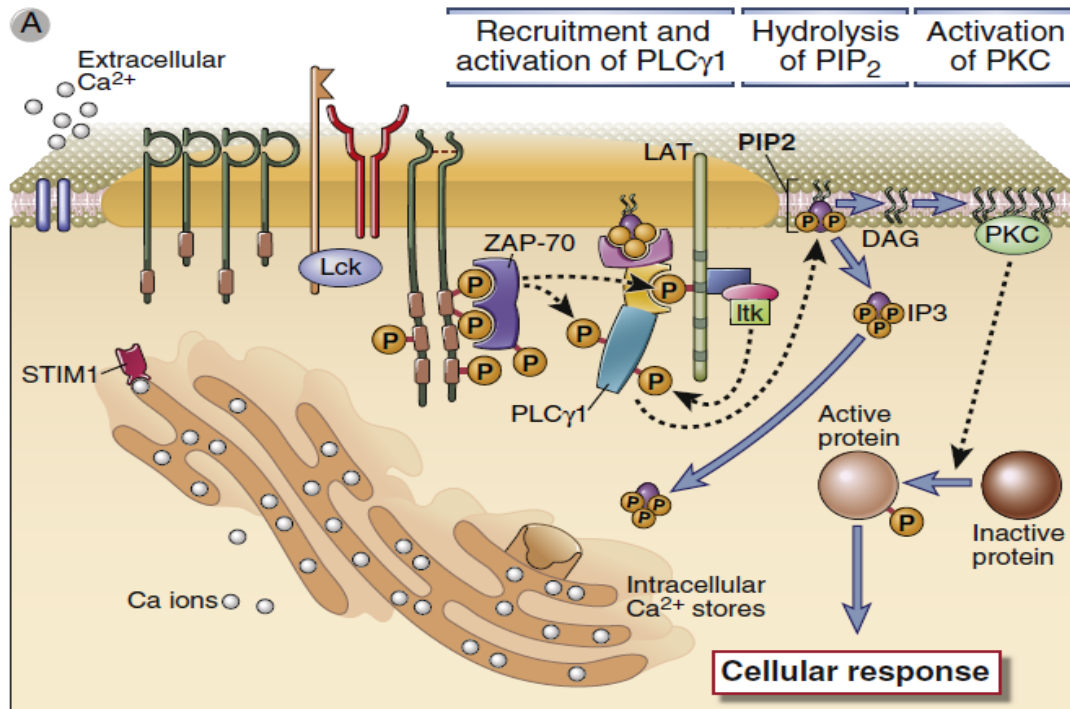
A parallel **Rac-dependent pathway** generates another active MAP kinase, JNK

These kinases stimulate:

the expression of the protein **c-Fos**

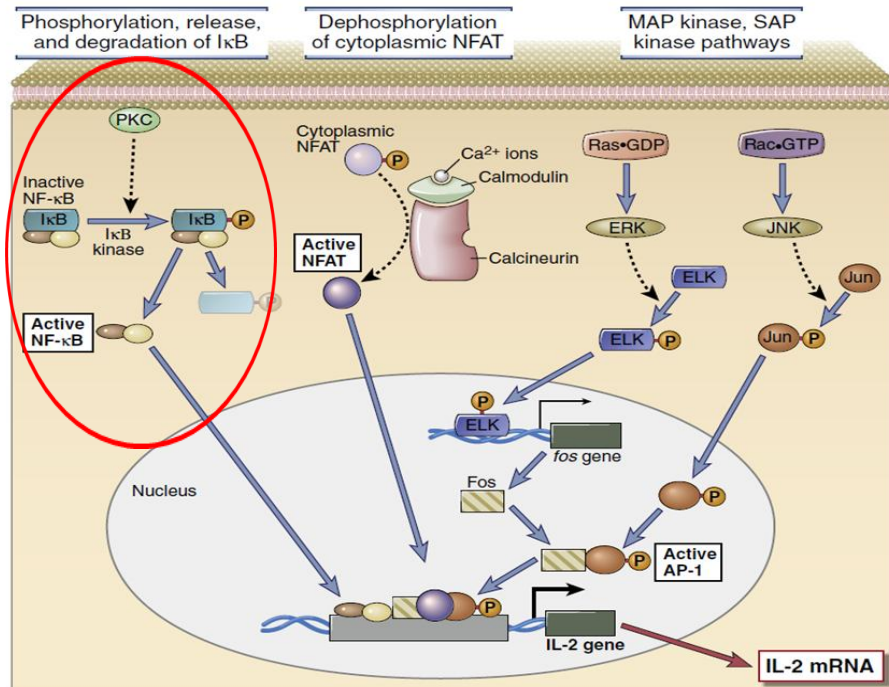
the phosphorylation of the protein **c-Jun**

c-Fos and c-Jun together form the transcription factor **AP-1** (activating protein – 1)



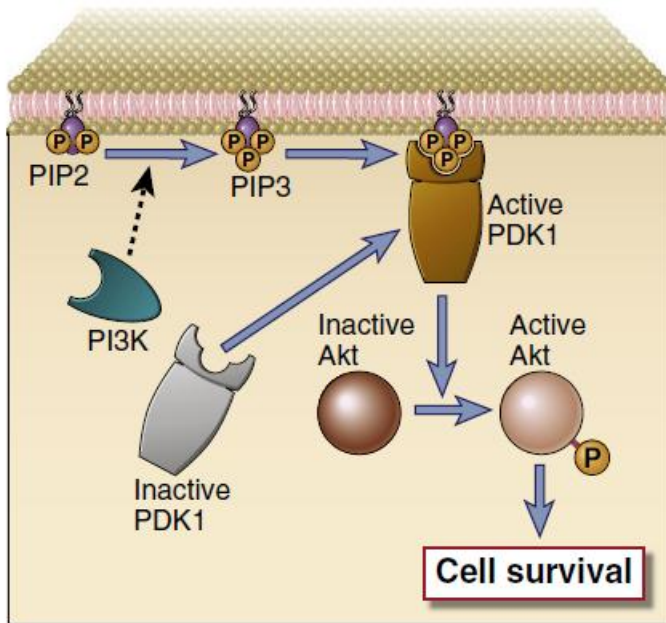
3. $\text{PKC}\theta$ -NF- κB signal pathway :

$\text{PLC}\gamma$ hydrolyzes the membrane inositol phospholipid to IP3 and diacylglycerol (DAG). DAG activates $\text{PKC}\theta$ (protein kinase C).



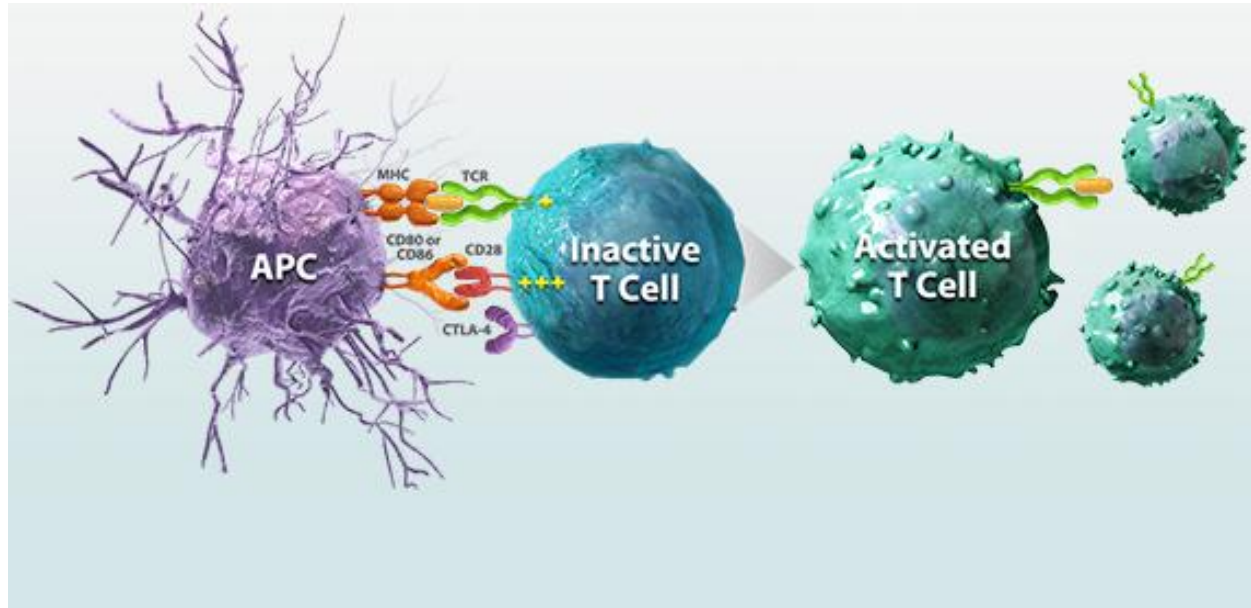
$\text{PKC}\theta$ activates NF- κB by phosphorylating its inhibitor $\text{I}\kappa\text{B}$, so the activated NF- κB moves into the nucleus where it stimulates the transcription of several genes.

4. PI3-kinase signaling pathway



- Phosphatidylinositol 3-kinase **PI3-kinase**, phosphorylates a specific phosphatidylinositol bisphosphate (PIP2) and generates phosphatidylinositol trisphosphate (**PIP3**).
- PIP3 activates **Akt** kinase.
- Activated **Akt** phosphorylates crucial targets and contributes to cell survival in a number of ways, including the inactivation of pro-apoptotic members of the Bcl-2 family.

After antigen recognition and costimulators bind to their ligands, gene transcription for cytokines, their receptors, cell cycle activators and effector molecules (eg CD40 ligand) begins.



The final result of the activation of T lymphocytes is the **proliferation** (expansion) of the antigen-specific clone and the **differentiation** of naive into effector lymphocytes.

Functional response of T lymphocytes to antigens and costimulation

1. Cytokine secretion and expression of their receptors

In a non-specific immune response, the main source of cytokines is the **macrophage**.

In the specific immune response
it is **CD4+ T lymphocyte**.

Cytokines secreted by helper CD4+ T lymphocytes

General properties of T cell cytokines

Property	Significance
Produced transiently in response to antigen	Provides cytokine only when needed
Usually acts on same cell that produces the cytokine (autocrine) or nearby cells (paracrine)	Systemic effects of cytokines usually reflect severe infections or autoimmunity
Pleiotropism: each cytokine has multiple biological actions	Provides diversity of actions but may limit clinical utility of cytokines because of unwanted effects
Redundancy: multiple cytokines may share the same or similar biological activities	Blocking any one cytokine may not achieve a desired effect

3) Biologic actions of selected T cell cytokines

Cytokine	Principal action	Cellular source(s)
IL-2	T cell proliferation; regulatory T cell survival	Activated T cells
Interferon- γ (IFN- γ)	Activation of macrophages (classical pathway)	CD4+ Th1 and CD8+ T cells, natural killer (NK) cells
IL-4	B cell switching to IgE; alternative macrophage activation	CD4+ Th2 T cells, mast cells
IL-5	Activation of eosinophils	CD4+ Th2 T cells, mast cells, innate lymphoid cells
IL-13	B cell switching to IgE; alternative macrophage activation	CD4+ Th2 T cells, mast cells, innate lymphoid cells
IL-17	Stimulation of acute inflammation	CD4+ Th17 T cells, other cells
IL-21	B cell activation; Tfh differentiation	CD4+ Tfh T cells
IL-22	Maintenance of epithelial barrier function	CD4+ Th17 T cells, NK cells, innate lymphoid cells

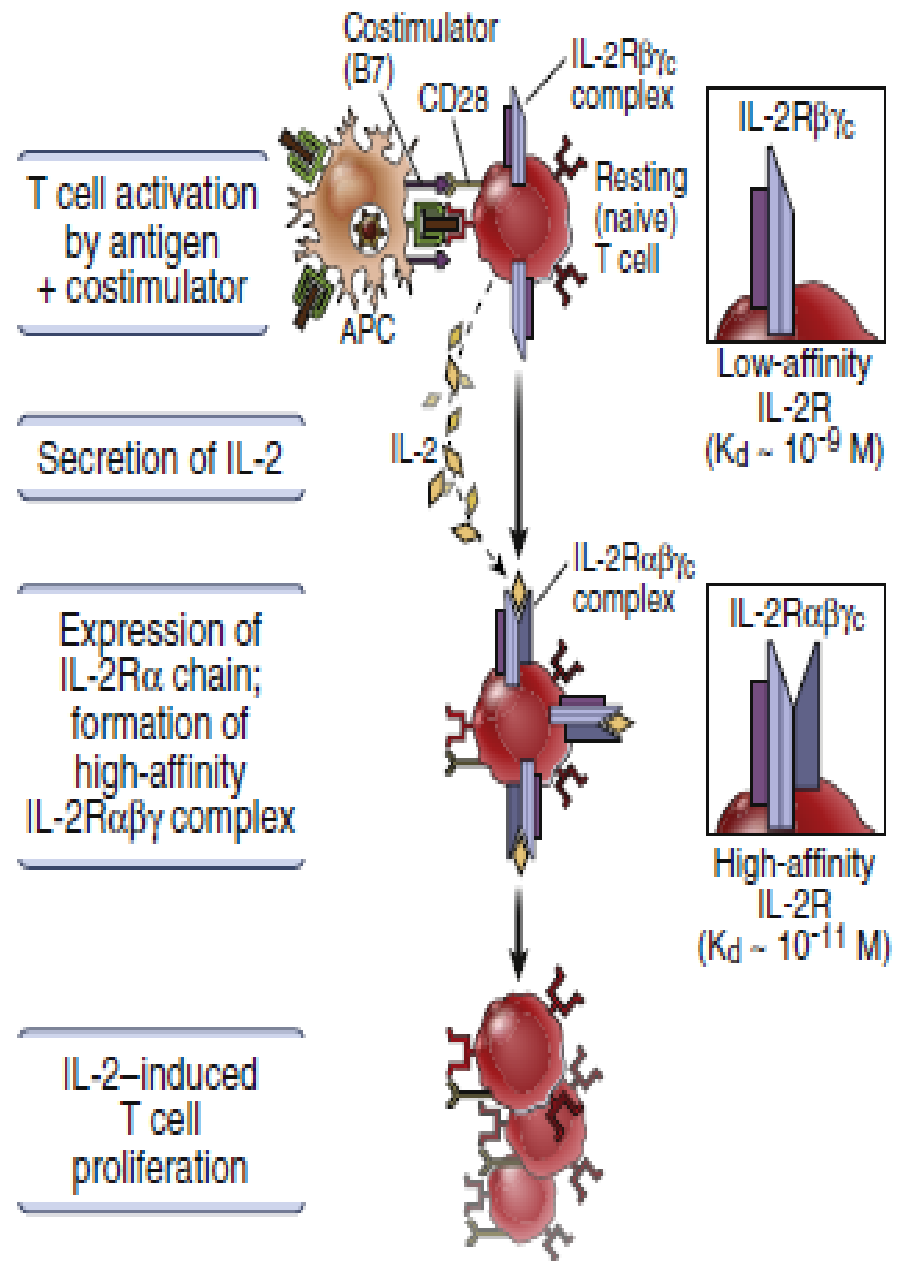
IL-2 is the first cytokine secreted immediately (one to two hours) after activation. Activation also stimulates **IL-2 receptor expression**.

The IL-2 receptor (IL-2R) consists of three non-covalently associated proteins

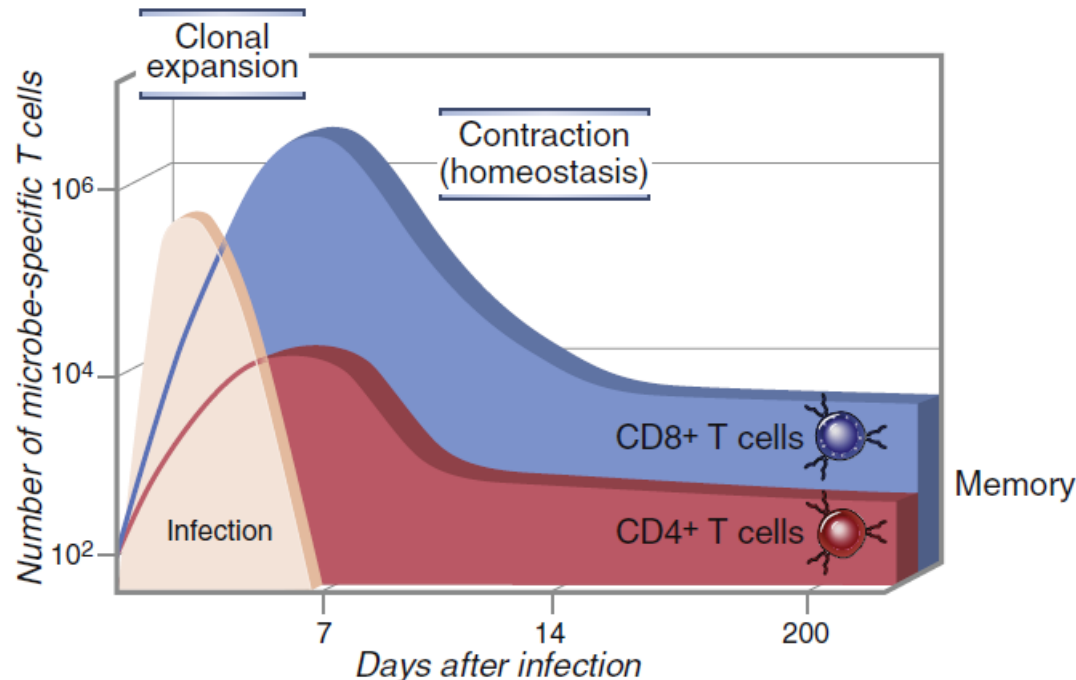
Resting (naive) T lymphocytes express the IL-2R $\beta\gamma$ c complex which has a low affinity for IL-2

Activation of the T cells and IL-2 itself leads to expression of the IL-2R α chain and increased levels of the high-affinity IL-2R $\alpha\beta\gamma$ complex.

IL-2 is a growth (proliferation) and survival factor of T lymphocytes.

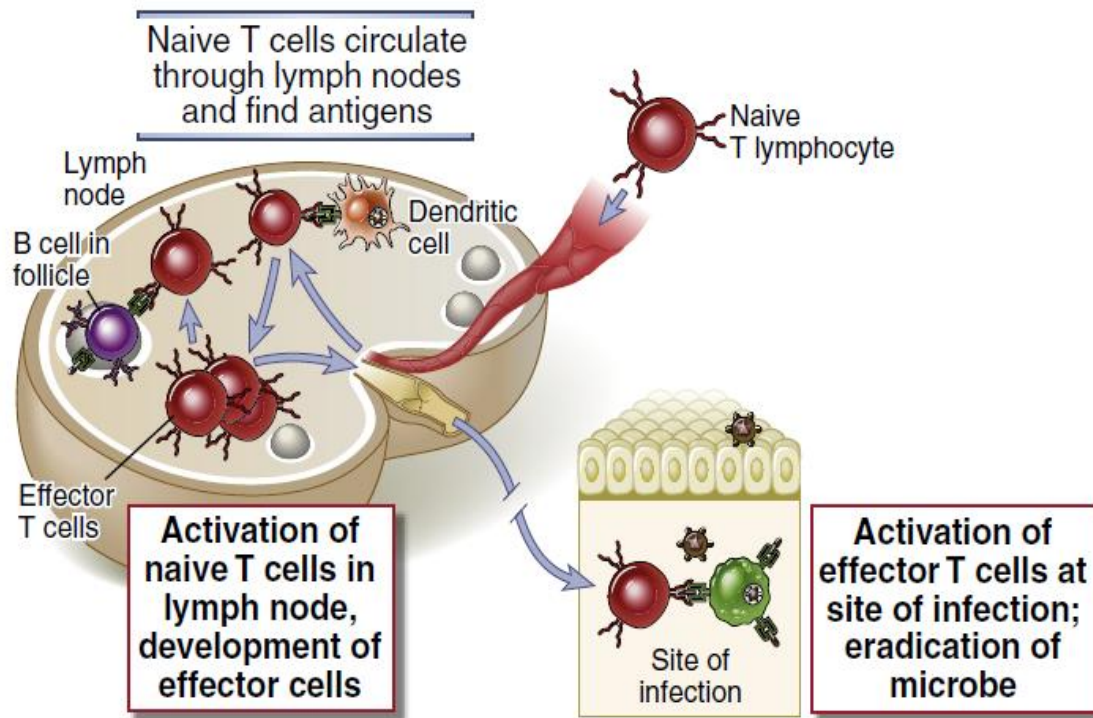


2. Clonal expansion



In response to some viruses, the number of antigen-specific T lymphocytes can increase more than 10,000-fold with an estimated doubling time of about 6 hours (especially for CD8+ T lymphocytes).

3. Differentiation of naive into effector T lymphocytes

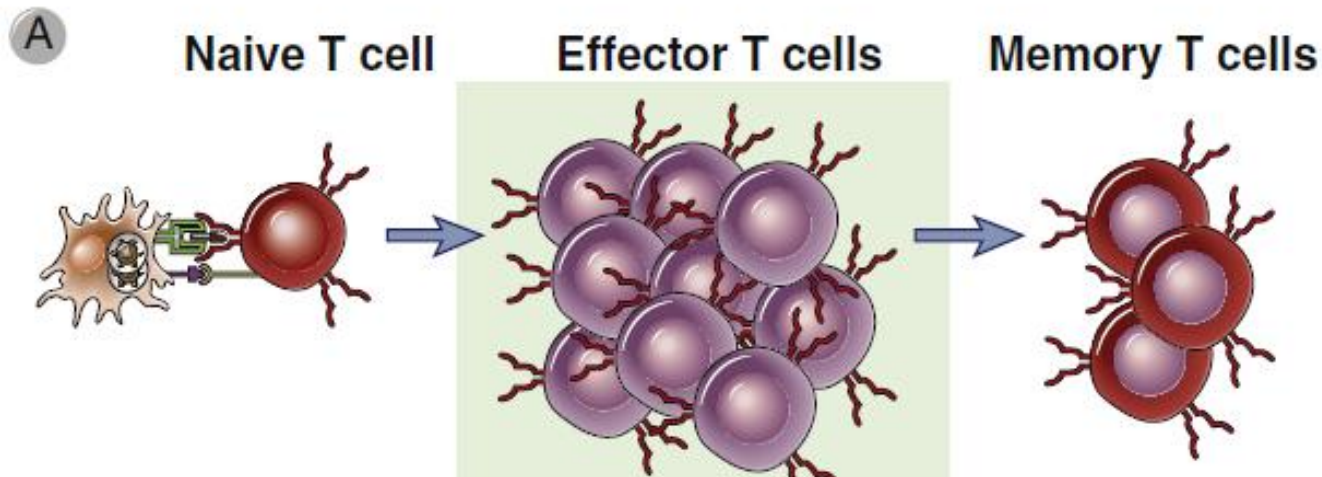


- After antigen recognition T cells activate and differentiate into effector cells, which may remain in the lymphoid organs to help B lymphocytes or migrate to sites of infection, macrophage activation.

These **effector** lymphocytes produce **membrane molecules** and **cytokines** in response to antigen.

These products mainly activate **macrophages** and **B lymphocytes**.

Development of memory T lymphocytes






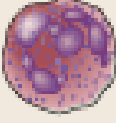


Some antigen-activated T lymphocytes differentiate into long-lived memory lymphocytes

IL-7 is important for the survival of memory T lymphocytes

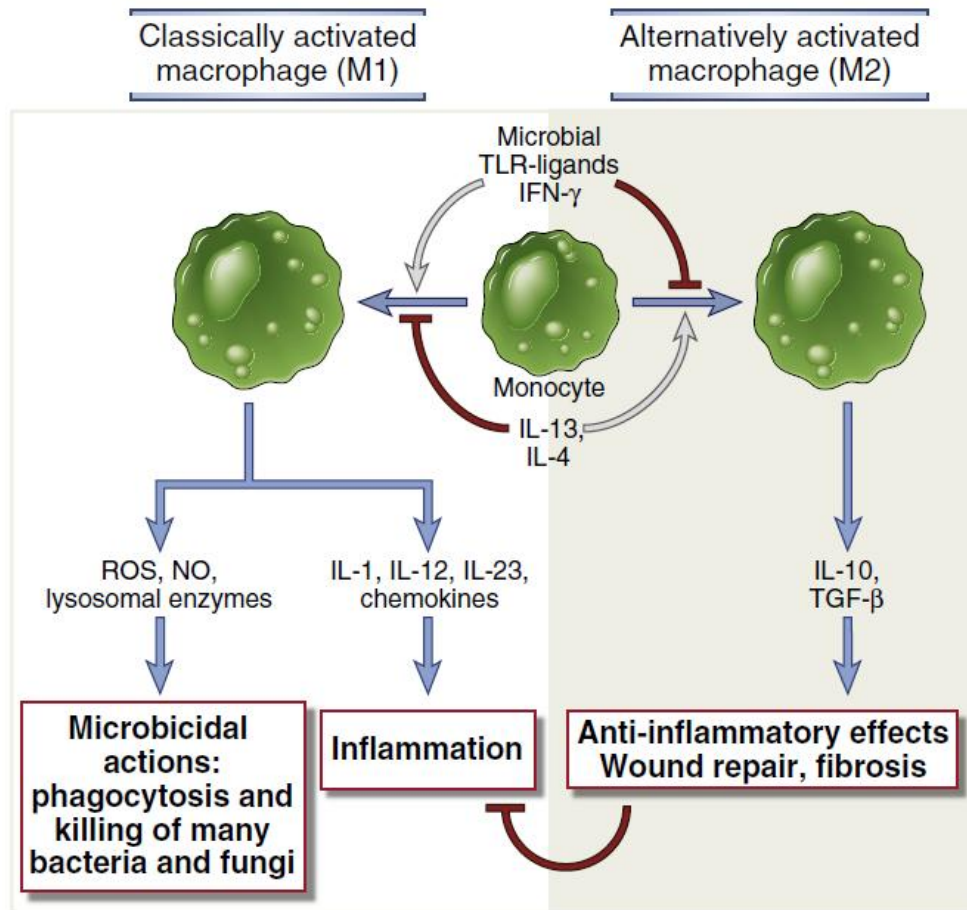
There are central and effector memory lymphocytes

Naive CD4⁺T lymphocytes differentiate into **different effector cells** that secrete **different sets of cytokines** and perform **different functions**

Effector T cells	Defining cytokines	Principal target cells	Major immune reactions	Host defense	Role in disease
Th1 	IFN- γ	Macrophages 	Macrophage activation	Intracellular pathogens	Autoimmunity; chronic inflammation
Th2 	IL-4 IL-5 IL-13	Eosinophils 	Eosinophil and mast cell activation; alternative macrophage activation	Helminths	Allergy
Th17 	IL-17 IL-22	Neutrophils 	Neutrophil recruitment and activation	Extracellular bacteria and fungi	Autoimmunity; inflammation

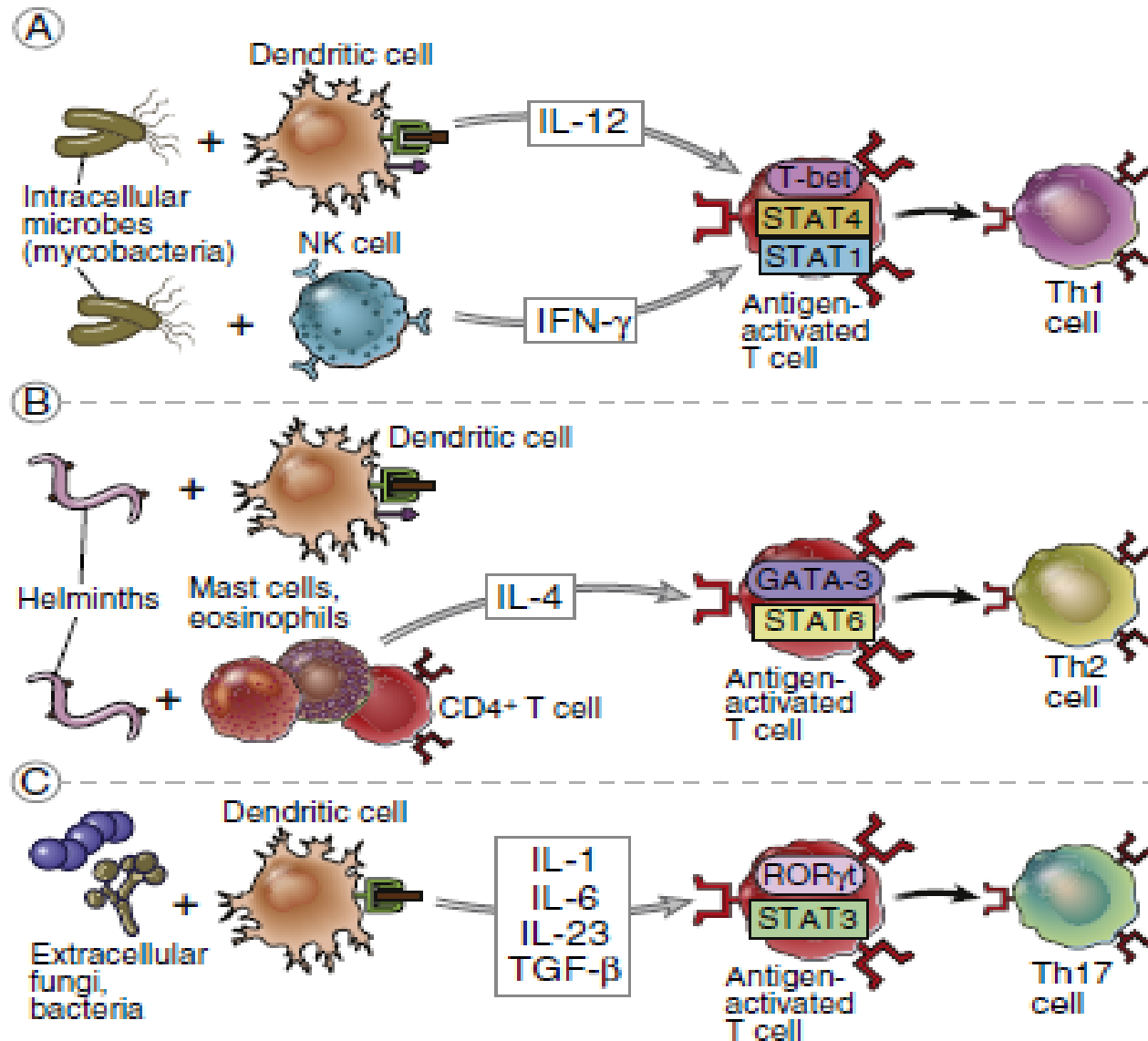
The emergence of effector Th1, Th2, Th17 from naive CD4+ T(Th0) lymphocytes is not a random process, but the direction of differentiation depends on the signals that arise after the contact of Th0 with the antigen. And the type of signal will depend on the characteristics of the pathogen, as well as on the genetic predisposition.

Classical and alternative macrophage activation



- Different stimuli activate monocytes-macrophages to develop into functionally distinct populations.
- Classically activated macrophages are induced by microbial products and cytokines (IFN- γ). They are microbicidal and involved in inflammation.
- Alternatively activated macrophages are induced by IL-4 and IL-13 produced by TH2 cells. They control inflammation and promote tissue repair and fibrosis.

The development of **Th1**, **Th2** and **Th17** effector lymphocytes



After activation, CD8+ T lymphocytes differentiate into **CTL**

