Università degli Studi di Padova

Corso di Laurea in

BIOTECNOLOGIE

Piano di studi Farmaceutico

Anno Accademico 2024-2025

Immunologia Farmaceutica

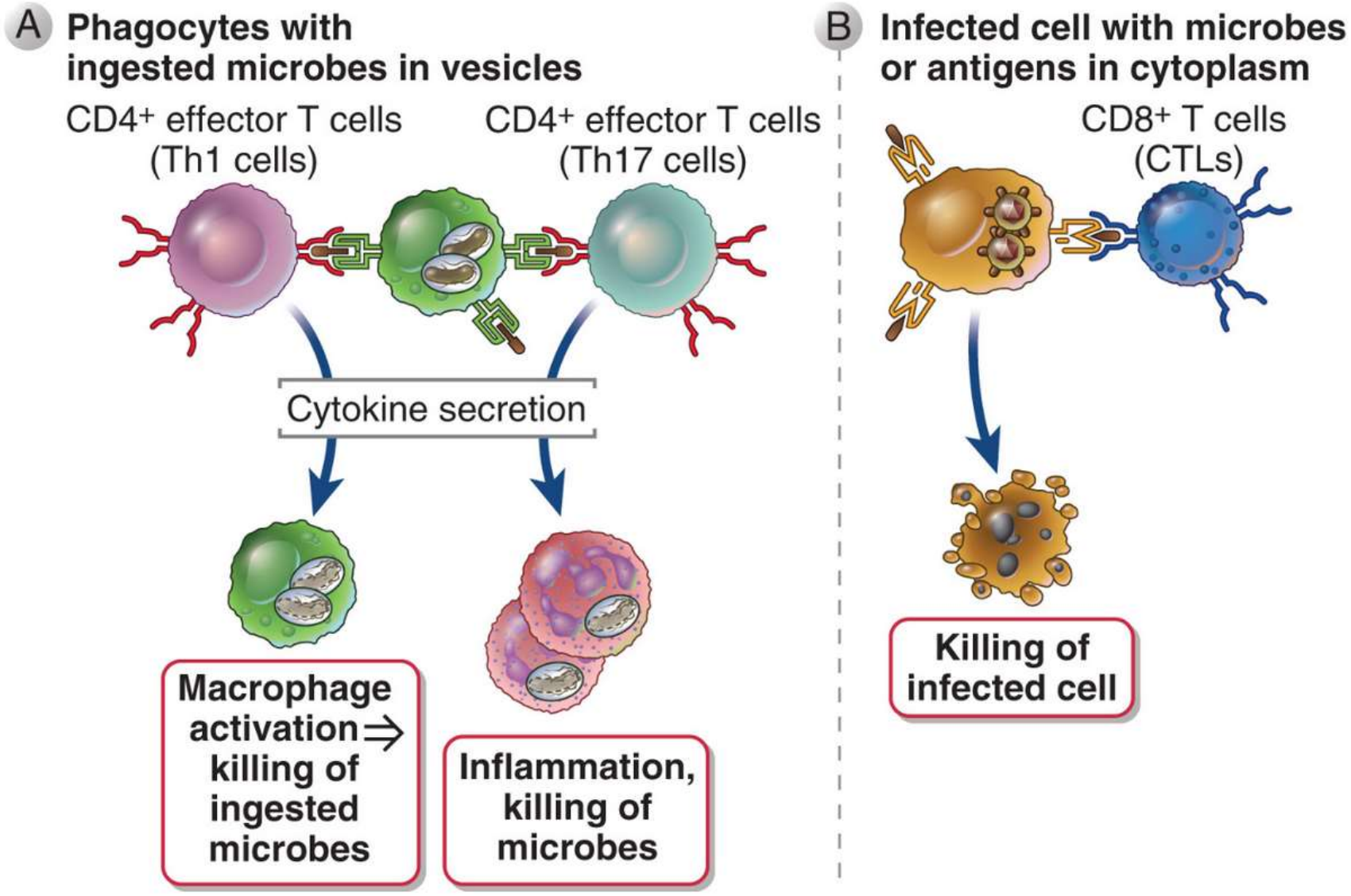
Differenziazione e funzioni dei linfociti T CD4^{+} effettori

T Cell-mediated Immunity (CMI)

La difesa contro I microrganismi da parte dei linfociti T è chiamata immunità cellulo-mediate (o immunità cellulare)

- T cells leave the thymus and circulate through the blood and lymphoid organs
- Naïve T cells have not reacted with their specific antigen
- When a naïve T cells appropriately interacts with antigen it gets activated (proliferation and differentiation).
- The result is that lots of antigen-specific cells acquire their **effector function**. That is, they become **armed effector T cells** that can act on **target cells.**

Ruolo dei linfociti T nell'eradicazione delle infezioni



Sottopopolazioni e funzioni dei linfociti T

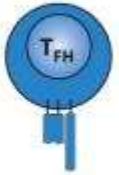
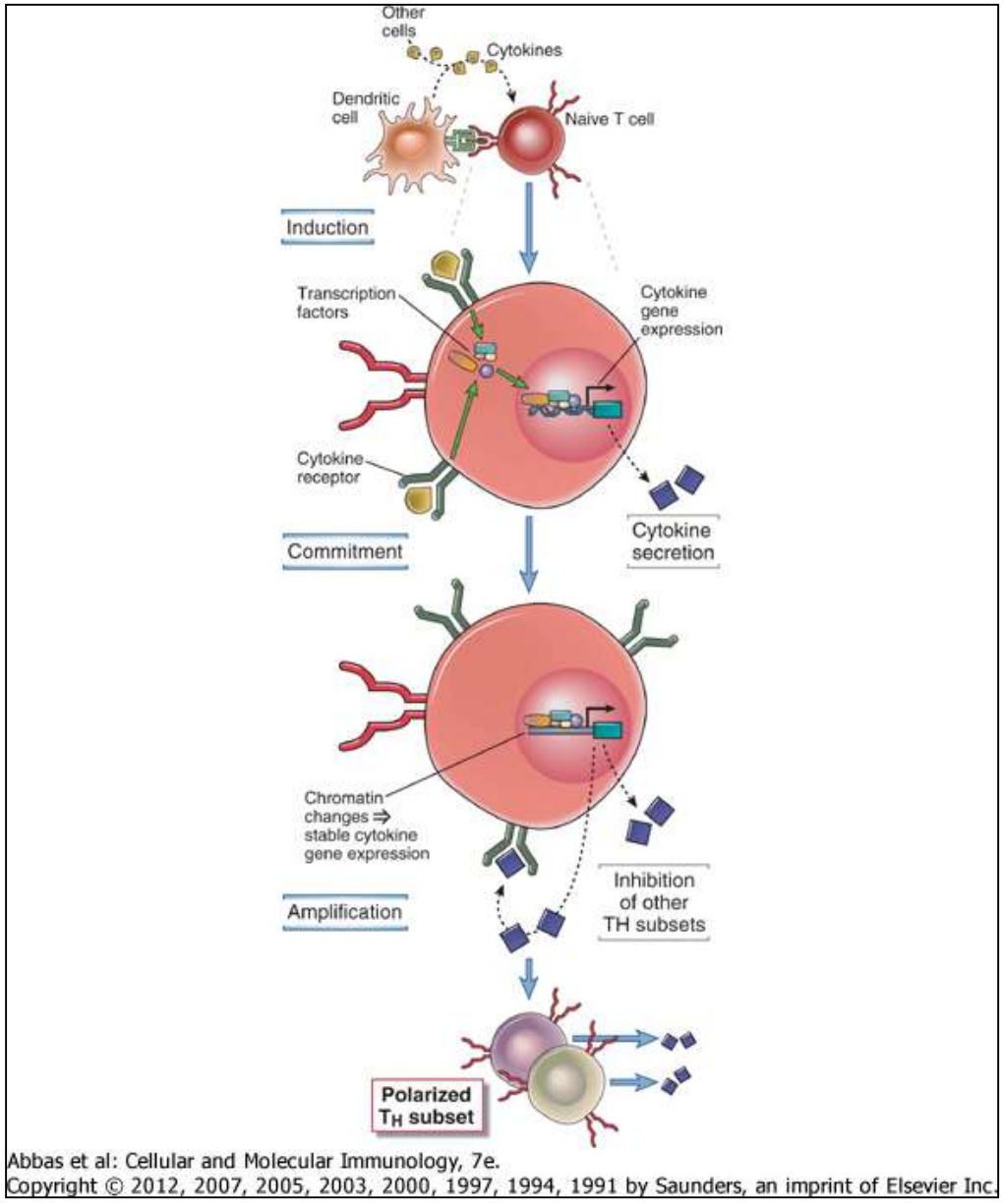
	CD8 cytotoxic T cells	CD4 T_H1 cells	CD4 T_H2 cells	CD4 T_H17 cells	T_{FH} cells	CD4 regulatory T cells (various types)
Types of effector T cell						
Main functions in adaptive immune response	Kill virus-infected cells	Activate infected macrophages Provide help to B cells for antibody production	Provide help to B cells for antibody production, especially switching to IgE	Enhance neutrophil response Promote barrier integrity (skin, intestine)	B-cell help Isotype switching Antibody production	Suppress T-cell responses
Pathogens targeted	Viruses (e.g. influenza, rabies, vaccinia) Some intracellular bacteria	Microbes that persist in macrophage vesicles (e.g. mycobacteria, *Listeria*, *Leishmania donovani*, *Pneumocystis carinii*) Extracellular bacteria	Helminth parasites	*Klebsiella pneumoniae* Fungi (*Candida albicans*)	All types	

Figure 9.1 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Development of T_H1, T_H2, and T_H17 subsets.



Cytokines produced early in the innate or adaptive immune response to microbes promote the differentiation of naive $CD4^+$ T cells into T_H1, T_H2, or T_H17 cells by activating transcription factors that stimulate production of the cytokines of each subset (the ***early induction step***). Progressive activation leads to stable changes in the expressed genes (***commitment***), and cytokines promote the development of each population and suppress the development of the other subsets (***amplification***).

These principles apply to all three major subsets of $CD4^+$ effector T cells.

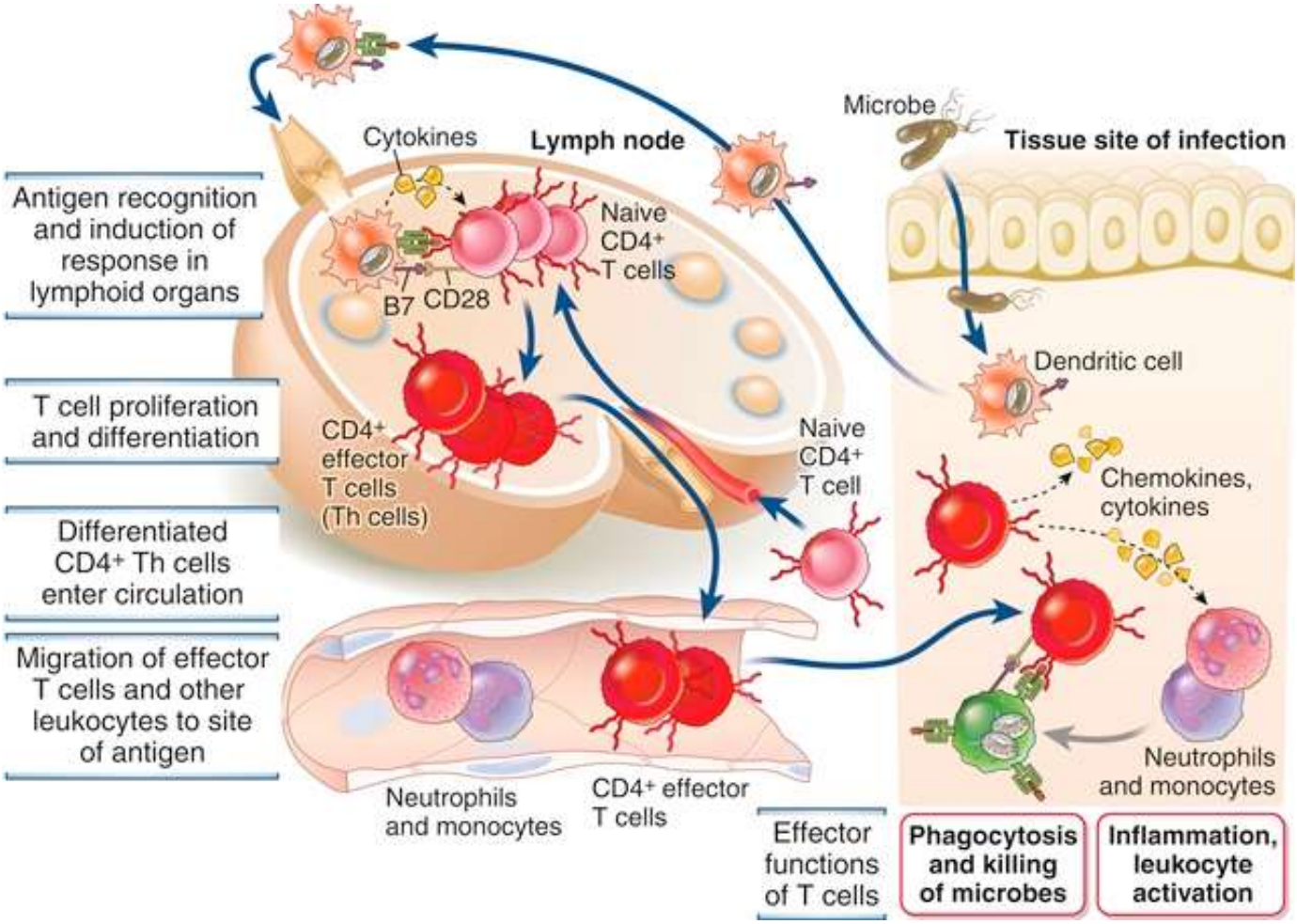
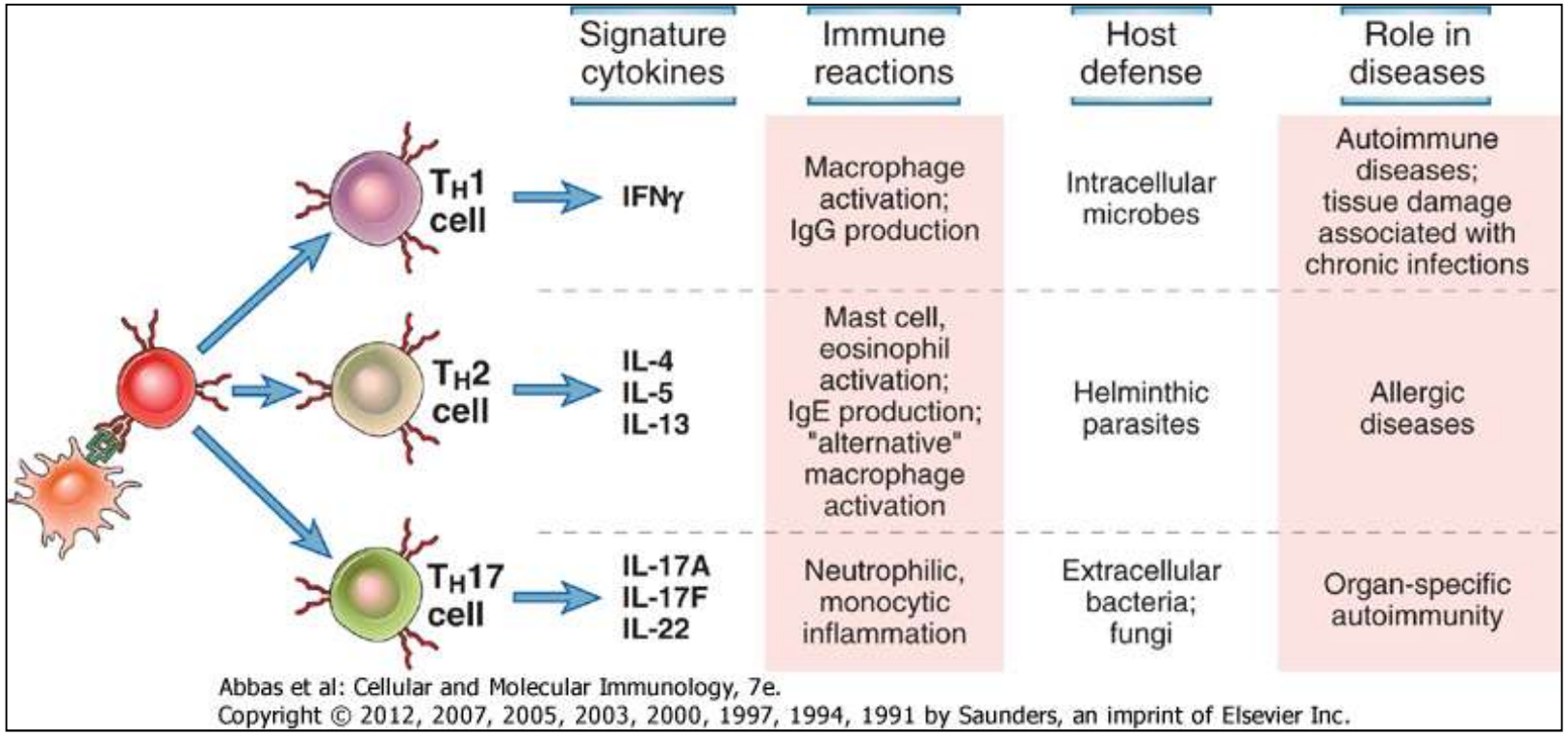


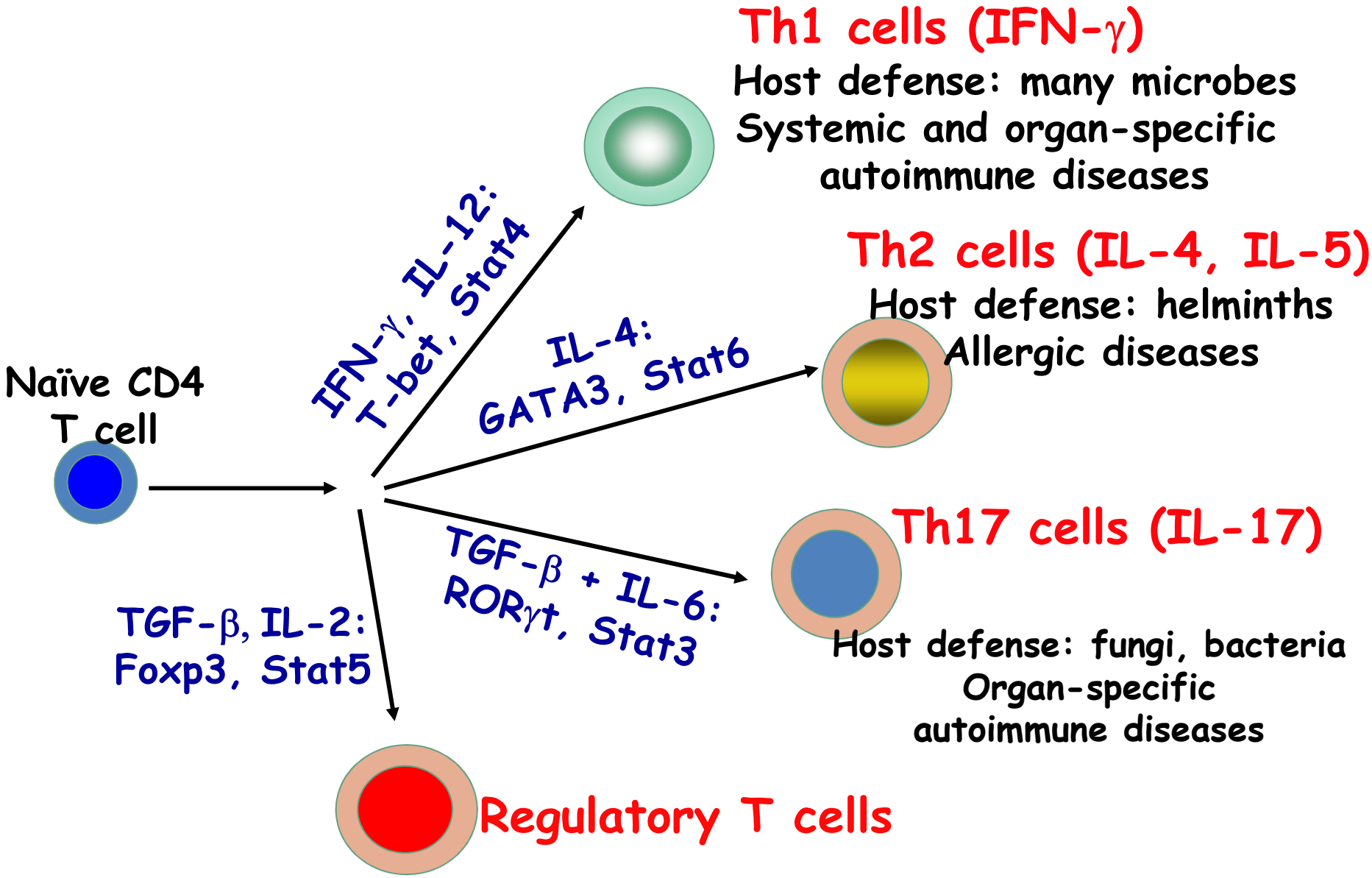
FIGURE 10.2 Steps in CD4⁺ T cell–mediated immune responses. CD4⁺ T cells recognize peptides that are derived from protein antigens and presented by dendritic cells in secondary lymphoid organs. The T lymphocytes are stimulated to proliferate and differentiate into effector (and memory) cells, which enter the circulation and migrate to sites of infection in peripheral tissues. In the tissues, effector T cells recognize the antigen and respond by secreting cytokines that recruit more leukocytes and activate phagocytes to eradicate the infection.

Development of Th1, Th2 and Th17 subsets



Differentiated T_H1, T_H2, and T_H17 cells all develop from naive $CD4^+$ T lymphocytes, mainly in response to cytokines present early during immune responses, and differentiation involves transcriptional activation and epigenetic modification of cytokine genes.

CD4 subsets: generation and function

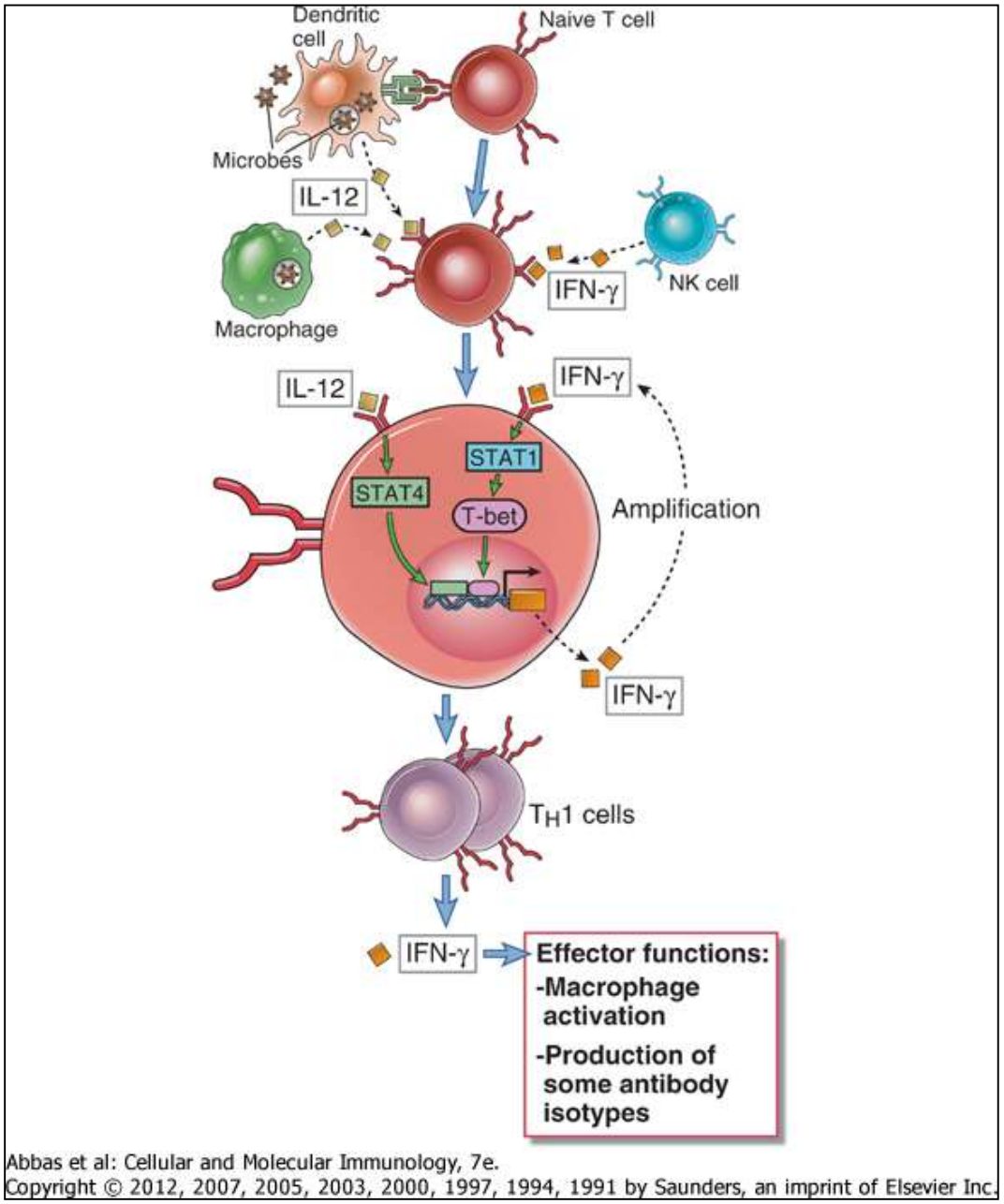


Development of T_H1 cells

T_H1 differentiation is driven mainly by the cytokines IL-12 and IFN-γ and occurs in response to microbes that activate dendritic cells, macrophages, and NK cells.

The differentiation of antigen-activated $CD4^+$ T cells to T_H1 effectors is stimulated by many intracellular bacteria, such as *Listeria* and mycobacteria, and by some parasites, such as *Leishmania,* all of which infect dendritic cells and macrophages.

A common feature of these infections and immunization conditions is that they elicit innate immune reactions that are associated with the production of certain cytokines, including IL-12, IL-18, and type I interferons. All these cytokines promote T_H1 development; of these, IL-12 is probably the most potent. Once T_H1 cells have developed, they secrete IFN-γ, which promotes more T_H1 differentiation and thus strongly amplifies the reaction. In addition, IFN-γ inhibits the differentiation of naive $CD4^+$ T cells to the T_H2 and T_H17 subsets, thus promoting the polarization of the immune response in one direction.



Abbas et al: Cellular and Molecular Immunology, 7e.

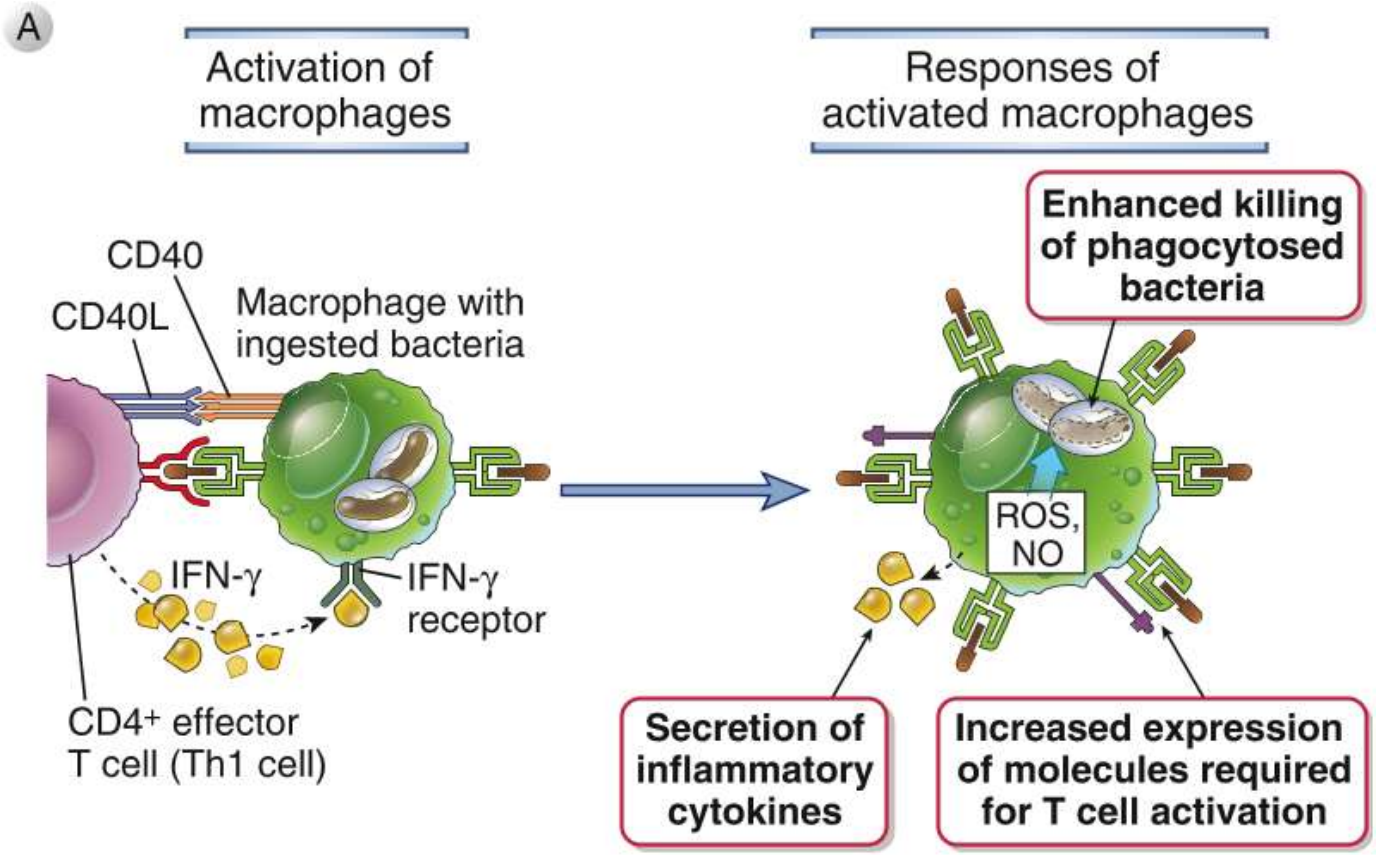


FIGURE 10.7 Macrophage activation by Th1 cells. A, Macrophages are activated by CD40LCD40 interactions and by interferon-γ *(IFN-γ)* expressed by Th1 cells and perform several functions that kill microbes, stimulate inflammation, and enhance the antigen-presenting capacity of the cells. **B,** The principal responses of macrophages activated by the classical activation pathway, and their roles in T cell–mediated host defense, are listed. Macrophages are also activated during innate immune reactions and perform similar functions (see Chapter 4). *IL,* Interferon; *MHC,* major histocompatibility; *NO,* nitric oxide; *ROS,* reactive oxygen species; *TNF,* tumor necrosis factor.

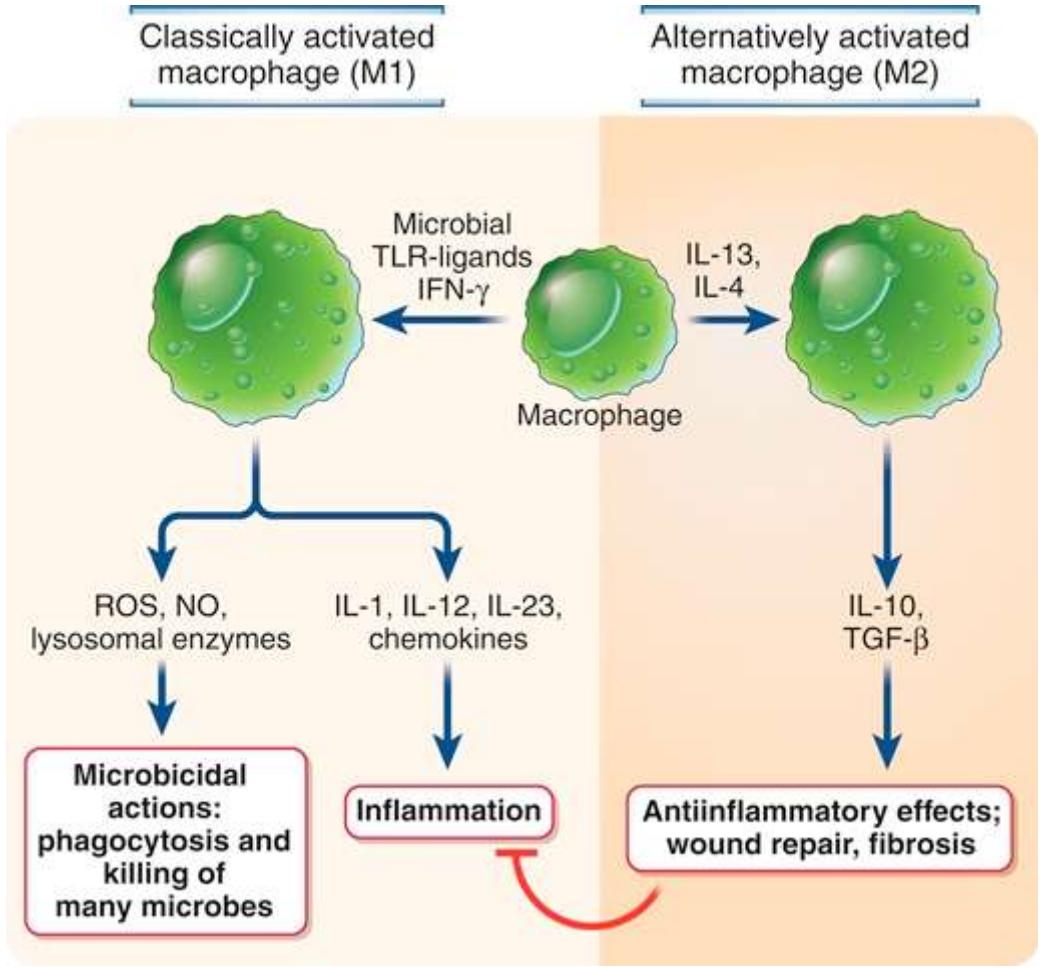


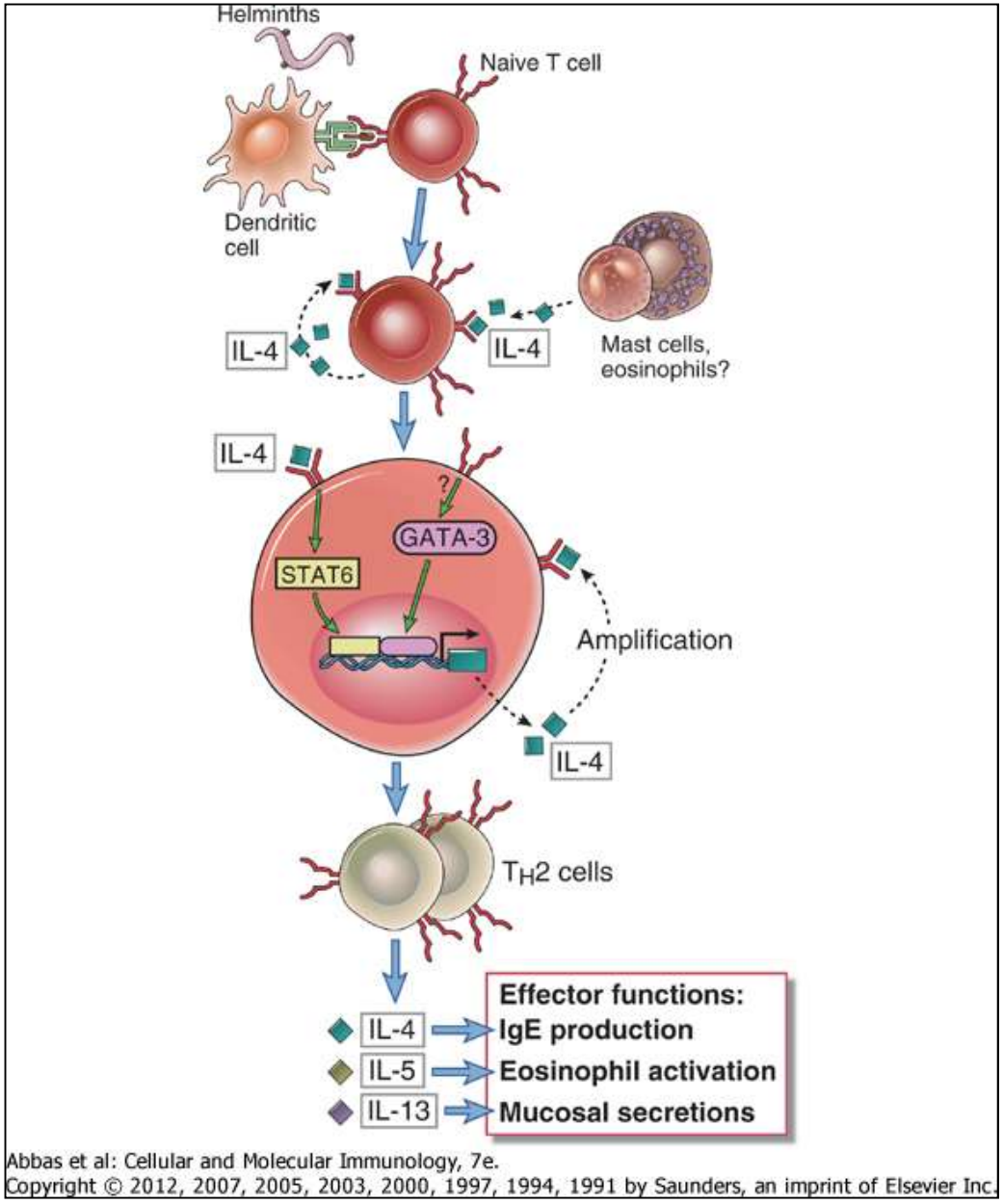
FIGURE 10.10 Classical and alternative macrophage activation. Different stimuli activate tissue macrophages to develop into functionally distinct populations. Classically activated macrophages are induced by microbial products and cytokines, particularly interferon-γ *(IFN-γ),* and are microbicidal and involved in potentially harmful inflammation. Alternatively activated macrophages are induced by interleukin-4 *(IL-4)* and IL-13 produced by Th2 cells and other leukocytes and function to control inflammation and to promote tissue repair and fibrosis. Some evidence suggests that M2 macrophages comprise subpopulations, some of which are mainly antiinflammatory and others are responsible for tissue repair. *NO,* Nitric oxide; *ROS,* reactive oxygen species; *TGF-β,* transforming growth factor-β; *TLR,* Toll-like receptor.

Th2 differentiation

T_H2 differentiation is stimulated by the cytokine IL-4 and occurs in response to helminths and allergens.

IL-4 stimulates T_H2 development by activating the transcription factor STAT6, and STAT6, together with TCR signals, induces expression of GATA-3. GATA-3 is a transcription factor that acts as a master regulator of T_H2 differentiation,

Furthermore, GATA-3 blocks T_H1 differentiation by inhibiting expression of the signaling chain of the IL-12 receptor. Knockout mice lacking IL-4, STAT6, or GATA-3 are deficient in T_H2 responses.



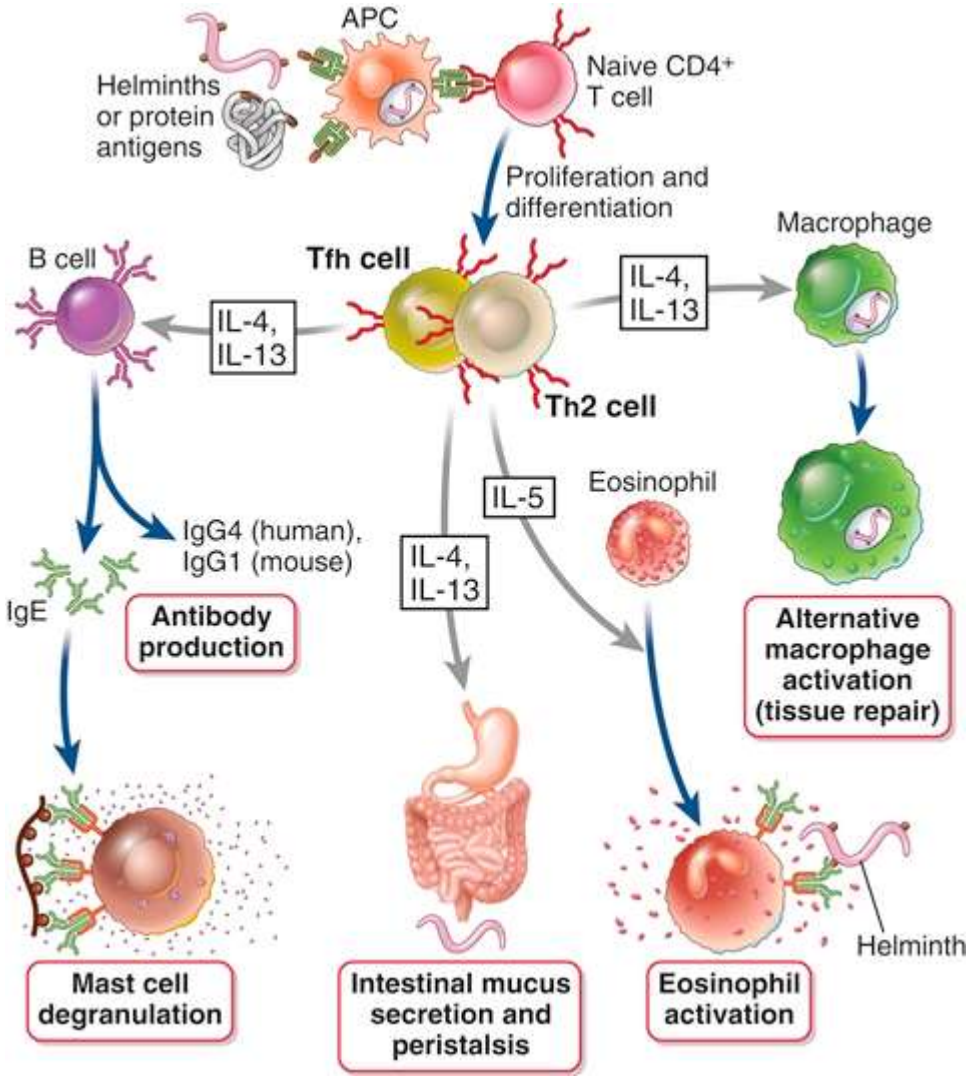


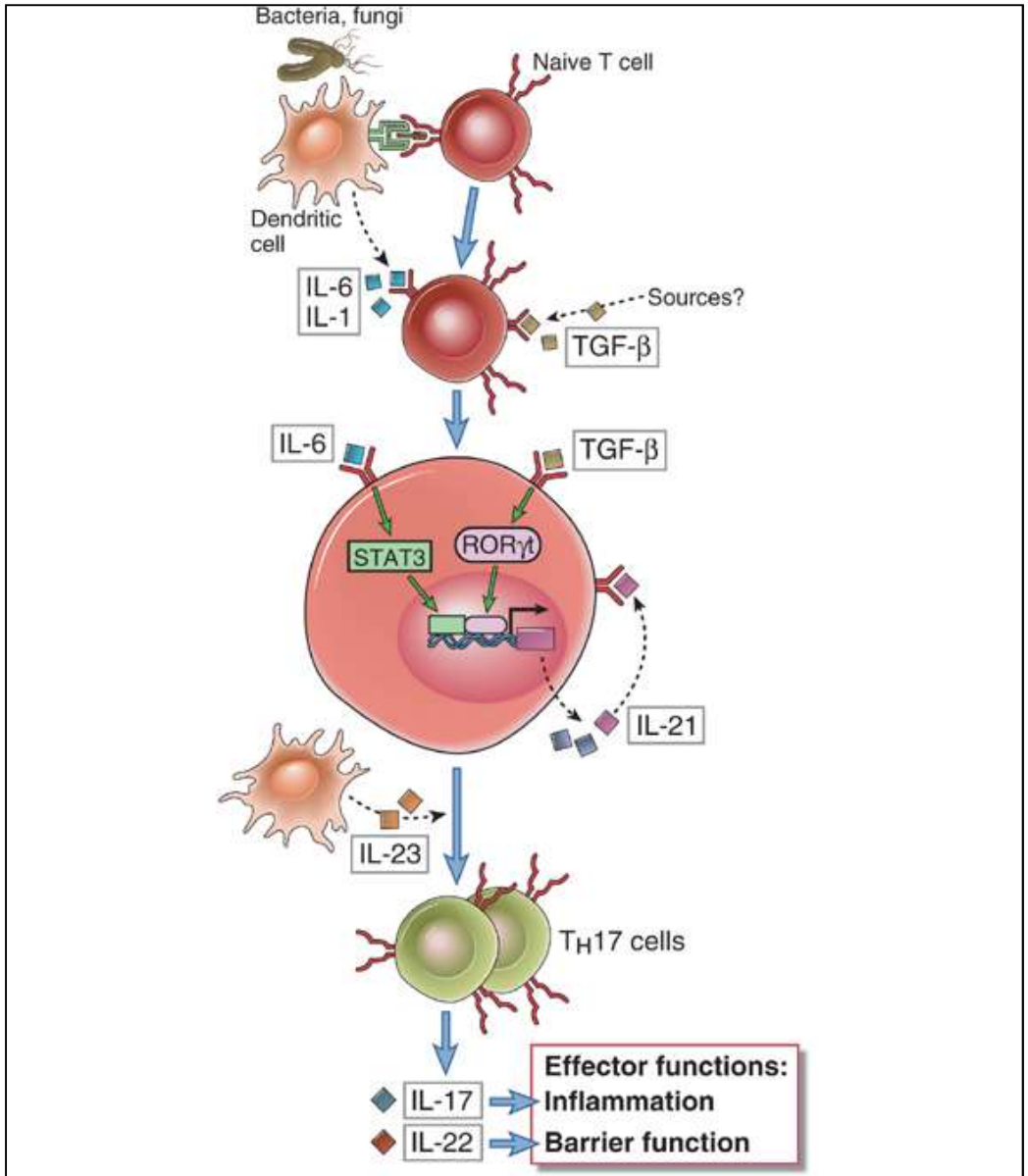
FIGURE 10.9 Functions of Th2 cells. $CD4^+$ T cells that differentiate into Th2 cells secrete IL-4, IL-5, and IL-13. IL-4 (and IL-13) act on B cells to stimulate production of antibodies that bind to mast cells and eosinophils, such as IgE. Help for antibody production may be provided by T follicular helper *(Tfh)* cells that produce Th2 cytokines and reside in lymphoid organs, and not by classical Th2 cells. IL-5 activates eosinophils, a response that is important for defense against helminthic infections. IL-4 and IL-13 are involved in immunity at mucosal barriers, induce an alternative pathway of macrophage activation, and inhibit classical Th1-mediated macrophage activation. *APC,* Antigen-presenting cell; *Ig,* immunoglobulin; *IL,* interleukin

Development of T_H17 cells

The development of T_H17 cells is stimulated by pro-inflammatory cytokines produced in response to bacteria and fungi.
The development of T_H17 cells is dependent on the transcription factors RORγt and STAT3.

TGF-β and the inflammatory cytokines, mainly IL-6 and IL-1, work cooperatively to induce the production of RORγt.

T_H17 cells appear to be especially abundant in mucosal tissues, particularly of the gastrointestinal tract, suggesting that the tissue environment influences the generation of this subset, perhaps by providing high local concentrations of TGF-β and other cytokines. This observation also suggests that T_H17 cells may be especially important in combating intestinal infections and in the development of intestinal inflammation.



Abbas et al: Cellular and Molecular Immunology, 7e.

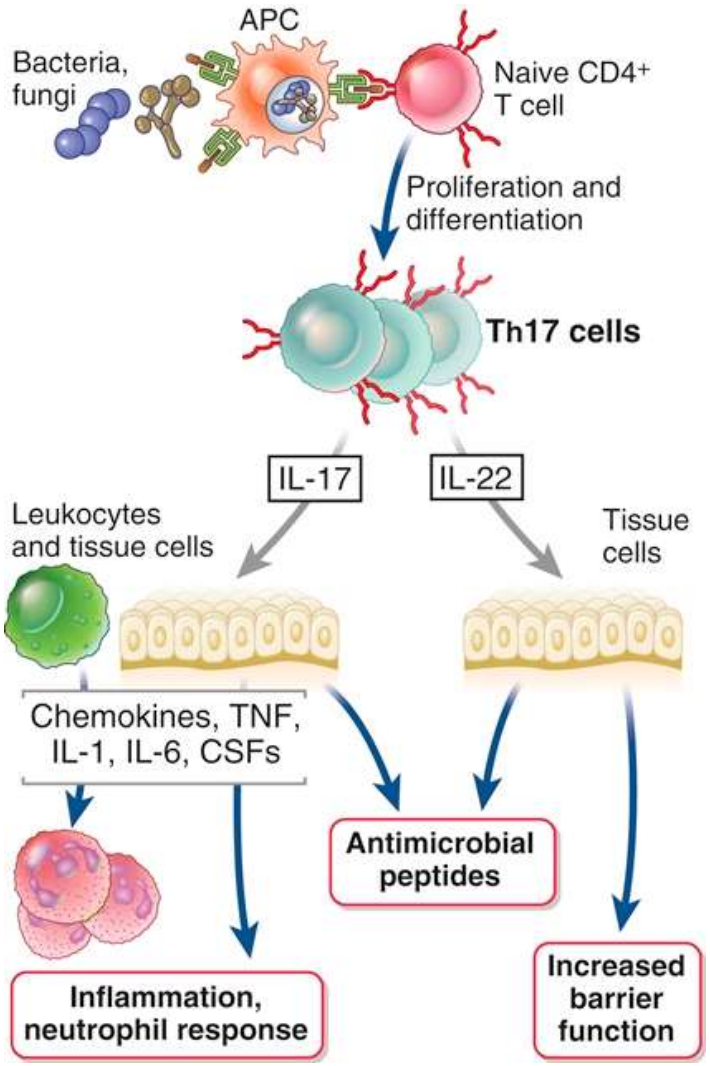
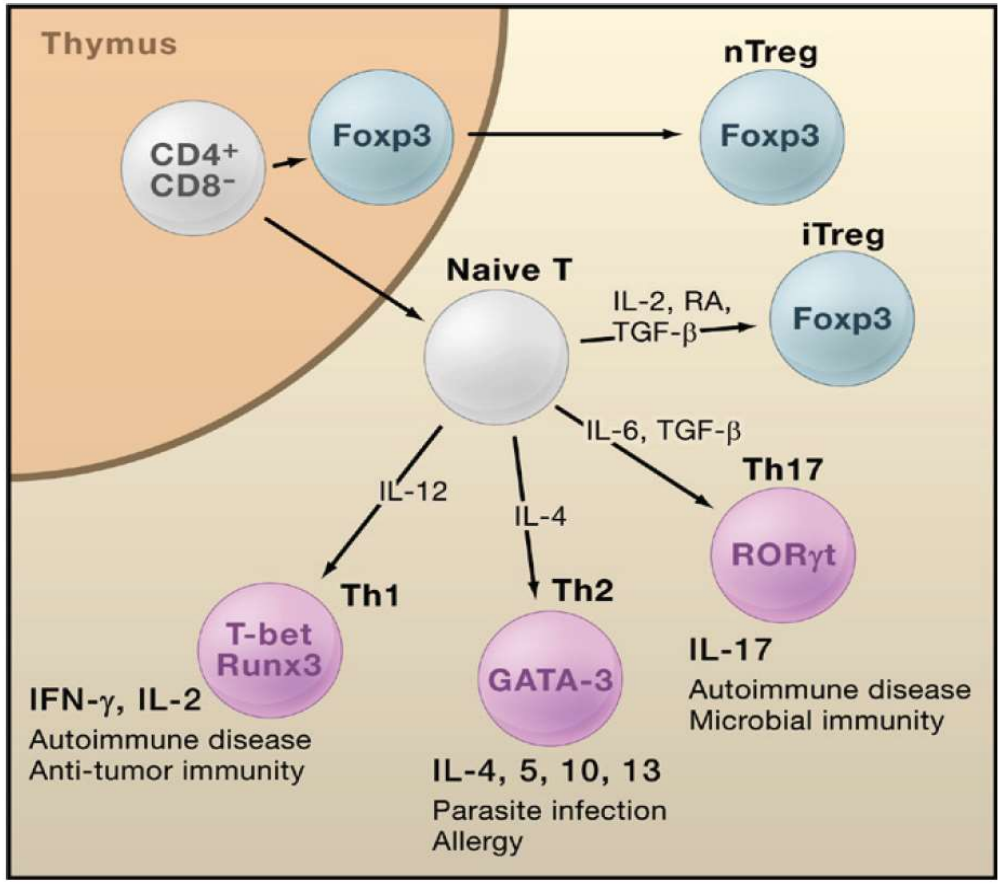


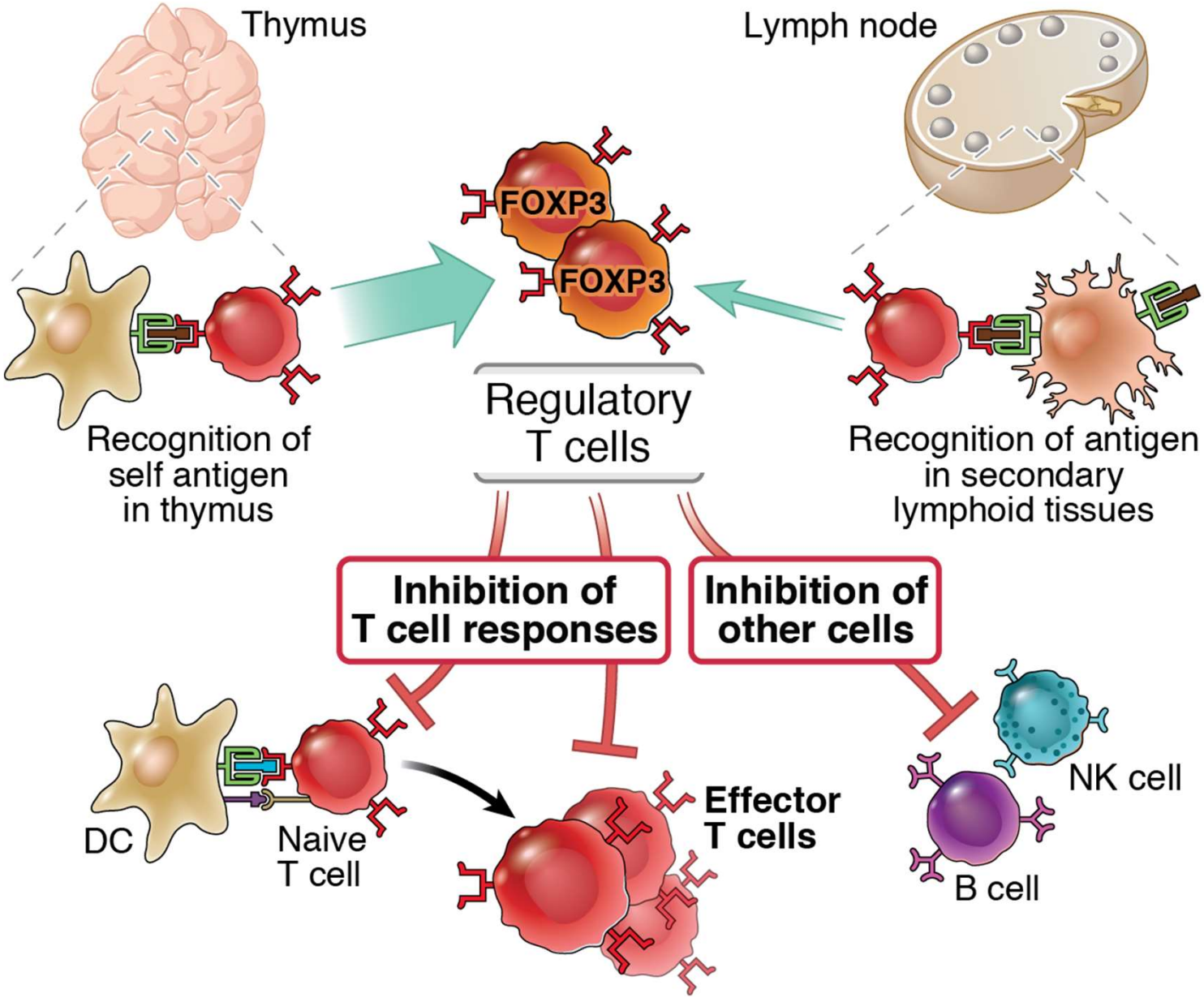
FIGURE 10.12 Functions of Th17 cells. Cytokines produced by Th17 cells stimulate local production of chemokines that recruit neutrophils and other leukocytes, increase production of antimicrobial peptides (defensins), and promote epithelial barrier functions. *APC,* Antigen-presenting cell; *CSF,* colony-stimulating factor; *TNF,* tumor necrosing factor.

T regulatory lymphocytes (T_{reg})



Sakaguchi S. et al., Cell, 2008

Regulatory T cells



Properties of regulatory T cells

- **Phenotype:** CD4+, high IL-2 receptor (CD25), Foxp3 transcription factor; other markers

- **Essential features** of stable Tregs:
  - Foxp3 expression: requires demethylated non-coding CNS2 sequence in promoter
  - CD25 (IL-2Rα) expression: IL-2 is a necessary survival factor
  - CTLA-4 expression: required for suppressive function of most Tregs

Take home messages

TABLE 14–1 Phenotypic Characteristics of Regulatory T Lymphocytes

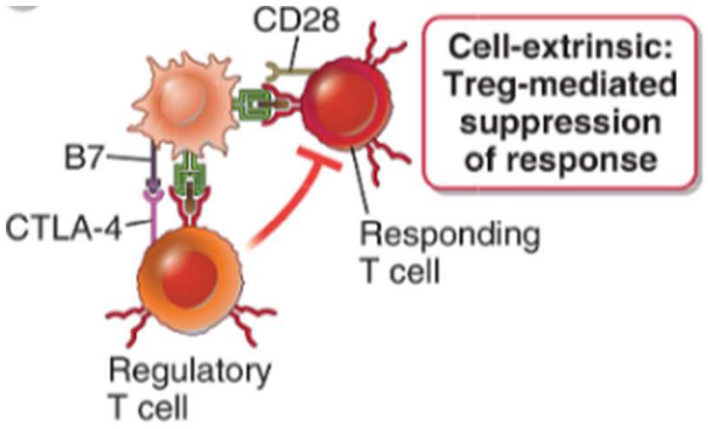
	Regulatory T Cells	Naive T Cells	Effector and Memory T Cells
Surface markers	CD25 high CTLA-4 GITR CD127 (IL-7R α chain) low	CD25 CD127 (IL-7Rα) high	CD25 high or medium CD127 (IL-7Rα) low on effector cells, high on memory cells
Cytokines produced on activation	TGF-β, IL-10	IL-2	Different subsets of effector and memory cells produce IFN-γ, IL-4 and IL-5, IL-17, others CXCR3, others
Chemokine receptors	CCR6	CCR7	CXCR3, others
Growth factor requirement	IL-2	IL-7	Effector cells: IL-2, IL-4 Memory cells: IL-7
Major transcription factors expressed	FoxP3, STAT5	KLF-2, absence of transcription factors specific for effector cells	In different effector cell subsets: T-bet, GATA-3, RORγt, and various STATs Some memory cells: BLIMP-1

Populations of Tregs

- Thymic (natural)
  - Induced by self antigen recognition during T cell maturation
- Peripheral (adaptive)
  - In response to antigen exposure in the periphery; contribution to preventing inflammatory disease?
- Induced (in vitro)
  - Culture with TGFβ + IL-2; therapeutic options
- There are no reliable markers for distinguishing these Tregs in a "bulk" population

Mechanisms of action of regulatory T cells

- Production of the immunosuppressive cytokines ***IL-10*** and ***TGF-beta***
- Reduced ability of APC to stimulate T cells



- Consumption of IL-2

Mechanisms of action of Foxp3+ Tregs

- CTLA-4 on Tregs removes B7 on APCs, reduces CD28 engagement and T cell activation
  - Genetic deletion of CTLA-4 in Foxp3+ cells results in severe systemic autoimmunity and lymphoproliferation
  - Commonly used assay for Treg function in humans bypasses the need for B7 and cannot measure this activity

FoxP3 transcription factor

- FoxP3 spontaneous mutations induces autoimmunity:
  - -IPEX in humans: *Immunodysregulation, polyendocrinopathy, enteropathy,X-linked syndrome*
  - -Scurfy in mice.

- FoxP3$^{-/-}$ develop spontaneous autoimmunity- defective Treg cells

- FoxP3 is preferentially expressed in $CD4^{+}CD25^{+}$ T cells

- FoxP3 Tg have ↑ cellular frequency of $CD4^{+}CD25^{+}$ Treg cells.

- FoxP3 Tg mice x CTLA-4$^{-/-}$ = resolved/delayed autoimmunity

- FoxP3 retroviral transduction in non-regulatory $CD4^{+}CD25^{-}$ T cells induces regulatory potential.
  - Phenotypically and functionally similar to naturally occuring lineage.

- Genes induced by FoxP3 remain unknown.

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Capitolo 9

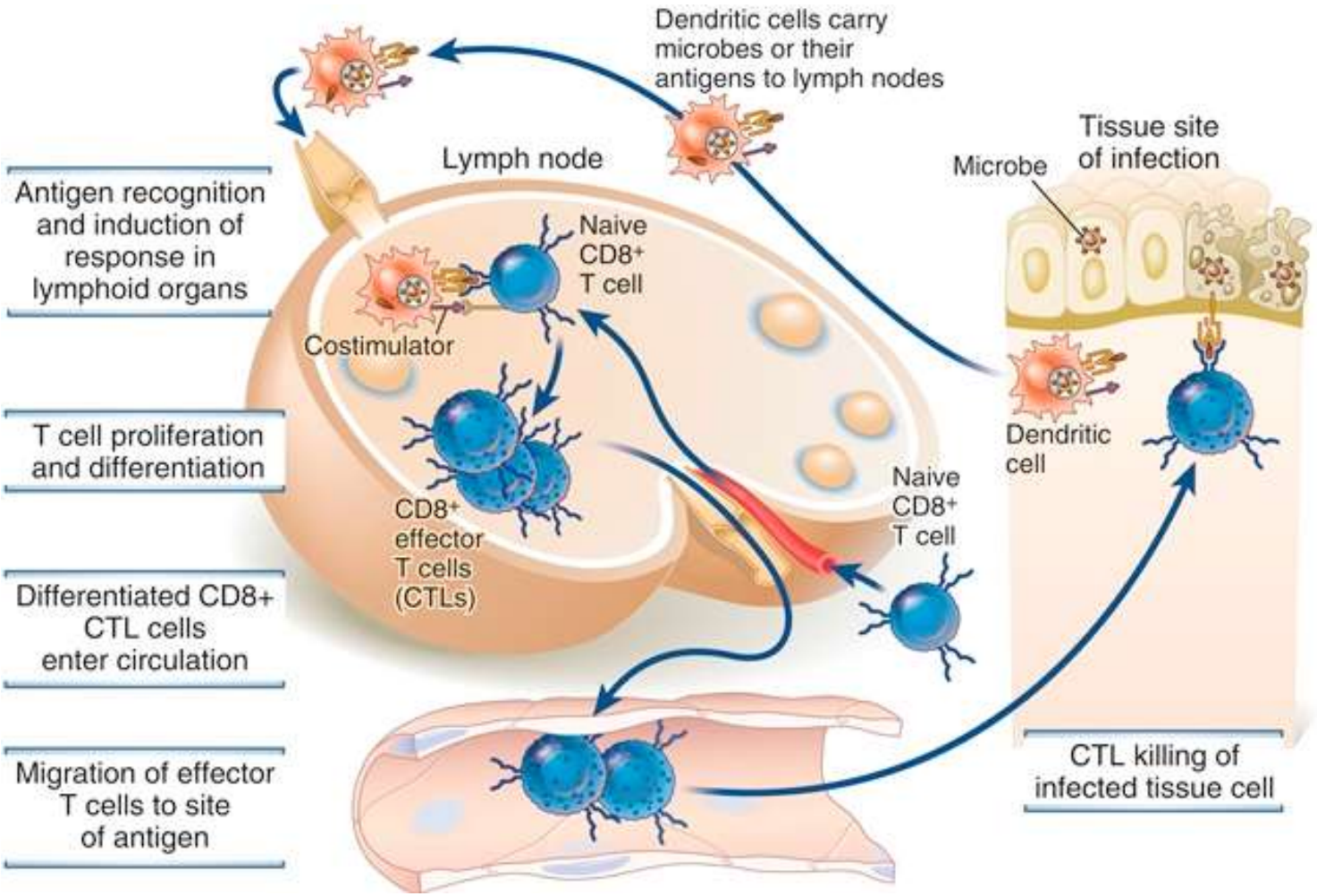
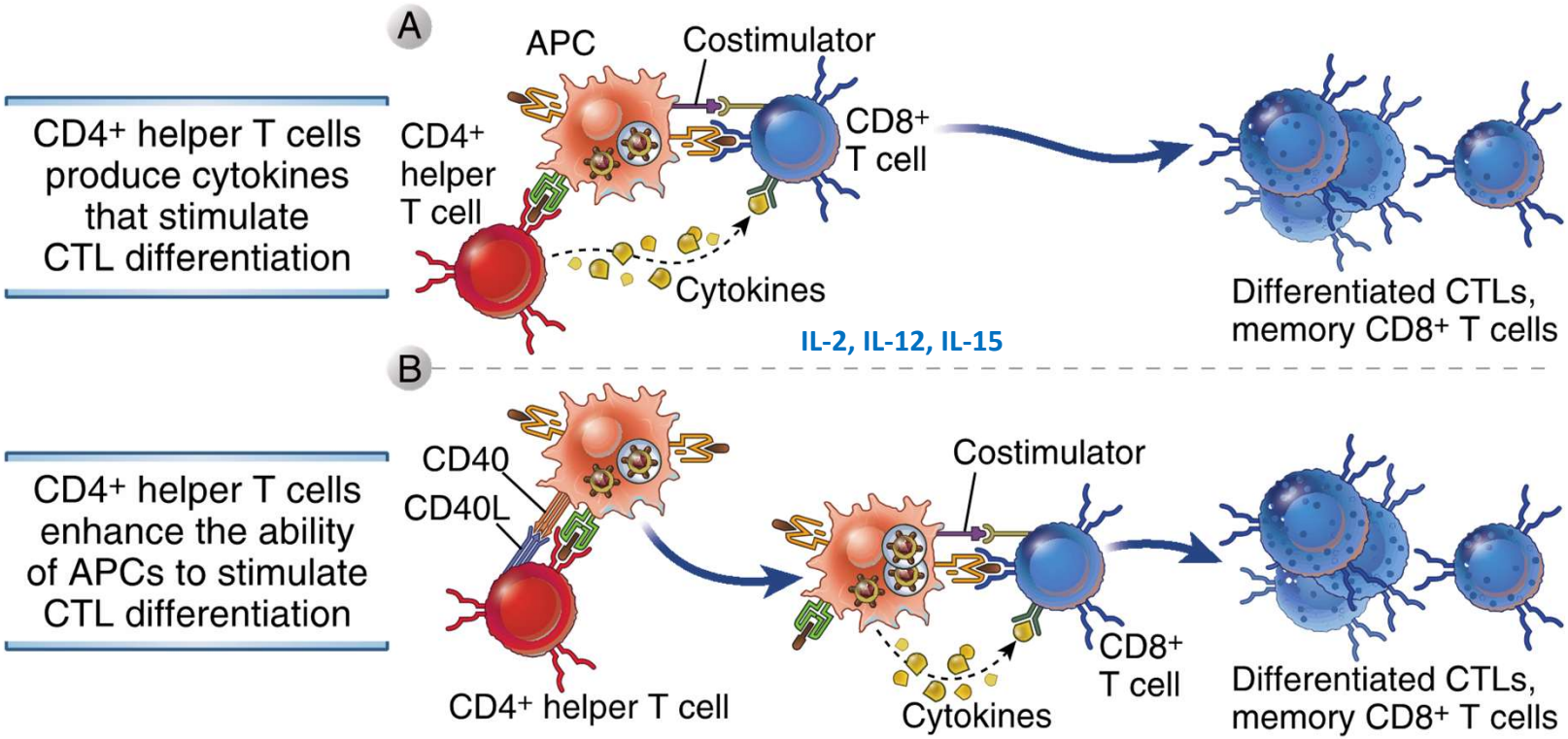


FIGURE 11.1 Induction and effector phases of CD8⁺ T cell responses. Naive CD8⁺ T cells recognize antigens presented by dendritic cells in secondary lymphoid organs and are stimulated to proliferate and differentiate into effector cells (cytotoxic T lymphocytes *[CTLs]*) and memory cells. The CTLs migrate to tissues at sites of infection, tumor growth, or graft rejection, where they recognize the antigen and respond by killing the cells where the antigen is produced.

Ruolo dei linfociti T helper nella differenziazione dei linfociti T CD8$^+$

In molte infezioni virali e in molti tumori e trapianti d'organo l'attivazione delle risposte innate è debole e non viene fornito il 2° segnale. In queste situazioni il 2° segnale deve essere fornito dai T helper, che possono promuovere l'attivazione dei linfociti T CD8+ in diversi modi:



In topo privi di cellule CD4$^+$ la capacità di eradicare le infezioni è compromessa dalla mancata generazione di CTL e di cellule CD8+ della memoria

CTL activation on APCs: need for T help

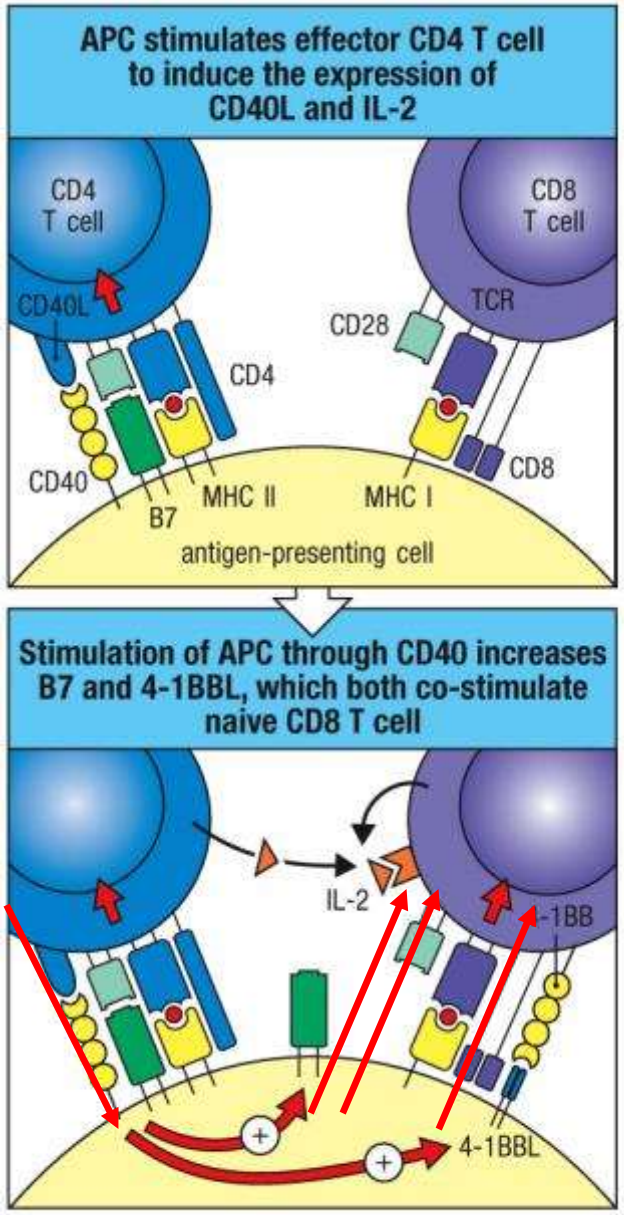


Figure 9.29 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

The $CD4^+$ cell activates the APC via CD40L. Then, the APC can activate the CTL (**the $CD4^+$ cell "licenses" the APC to activate CTLs**).

This is key when there are few bacterial products to activate the APC (e.g., viral infection) but is not necessary when bacterial products activate the APC.

Non-specific interactions mediated by adhesions molecule initiate CTL-target interactions but, by themselves are insufficient to initiate killing of the target. Killing requires TCR binding of the appropriate MHC-peptide complex

CTLs

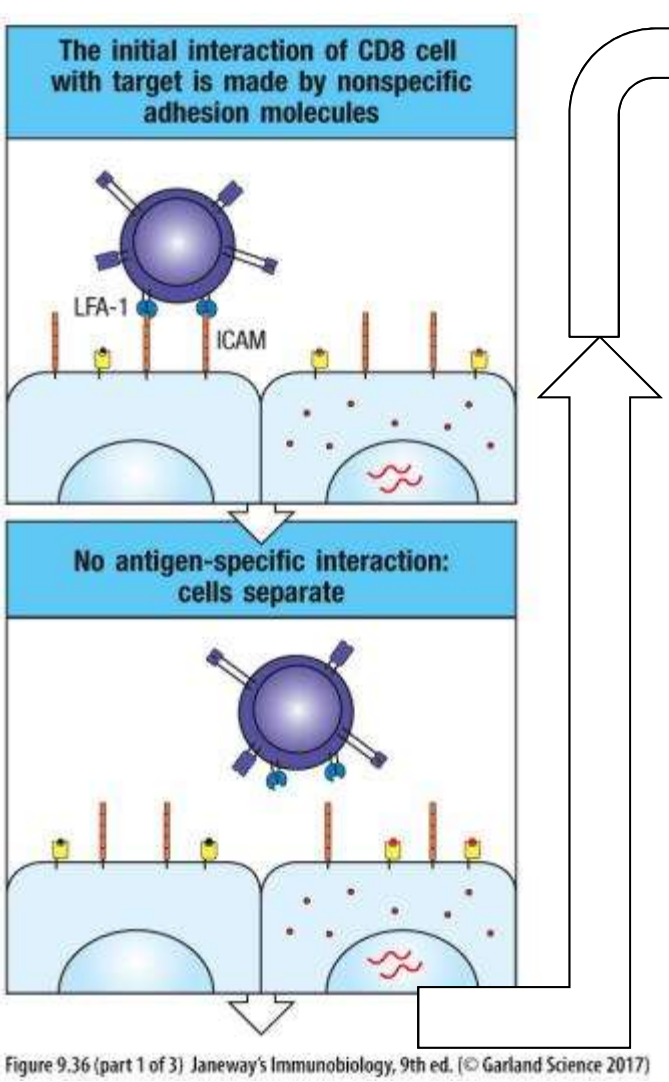


Figure 9.36 (part 1 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

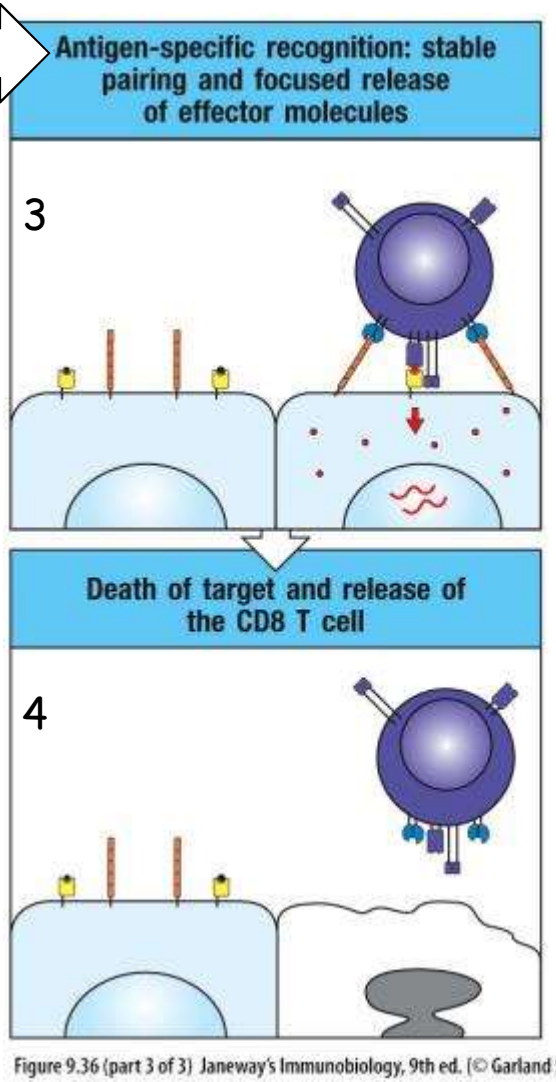
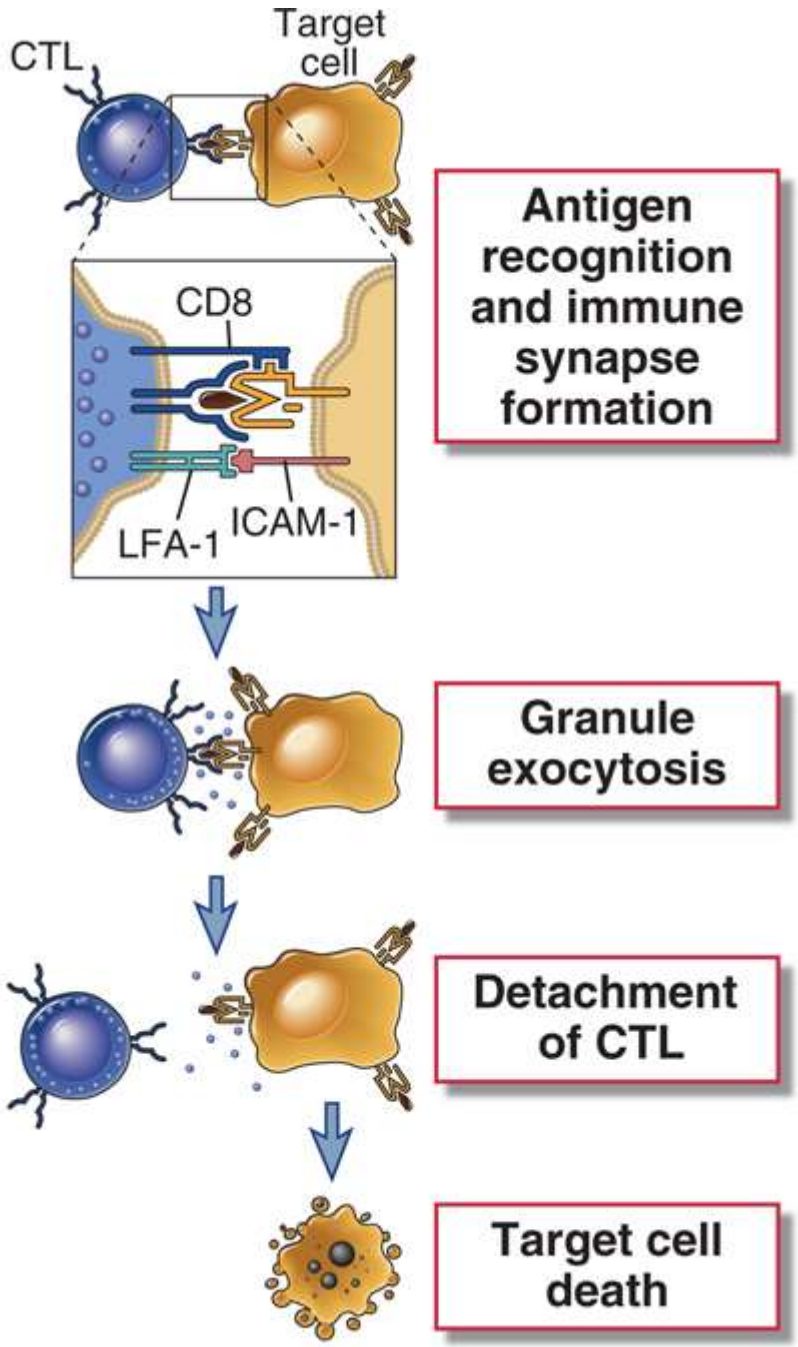
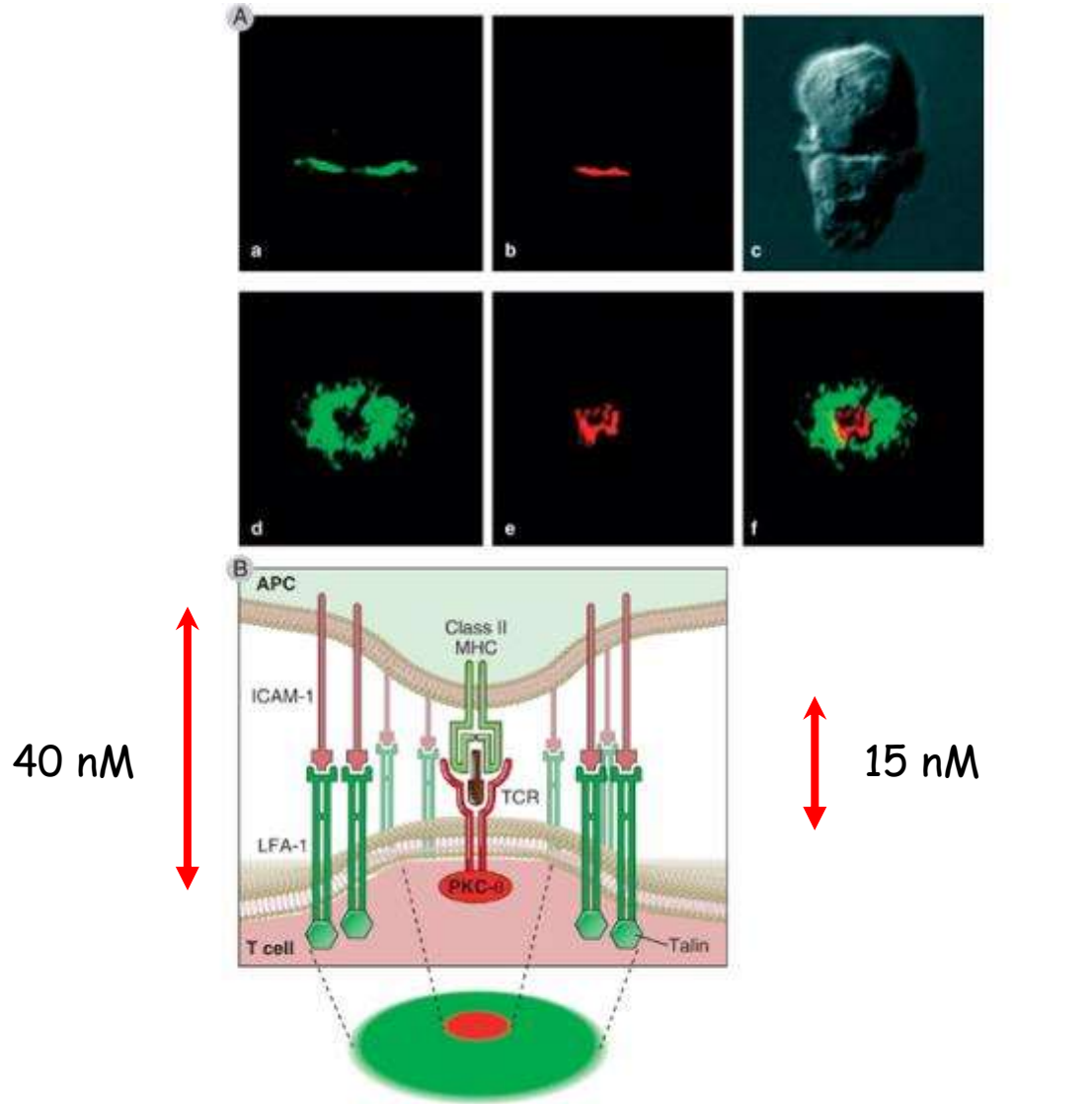


Figure 9.36 (part 3 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)



Formation of a specialized structure: the immunologic synapse (or supramolecular activation cluster=*SMAC*)



Not only signalling but also orientation of effector action

Introduction to CTL killing of target cells

CTLs

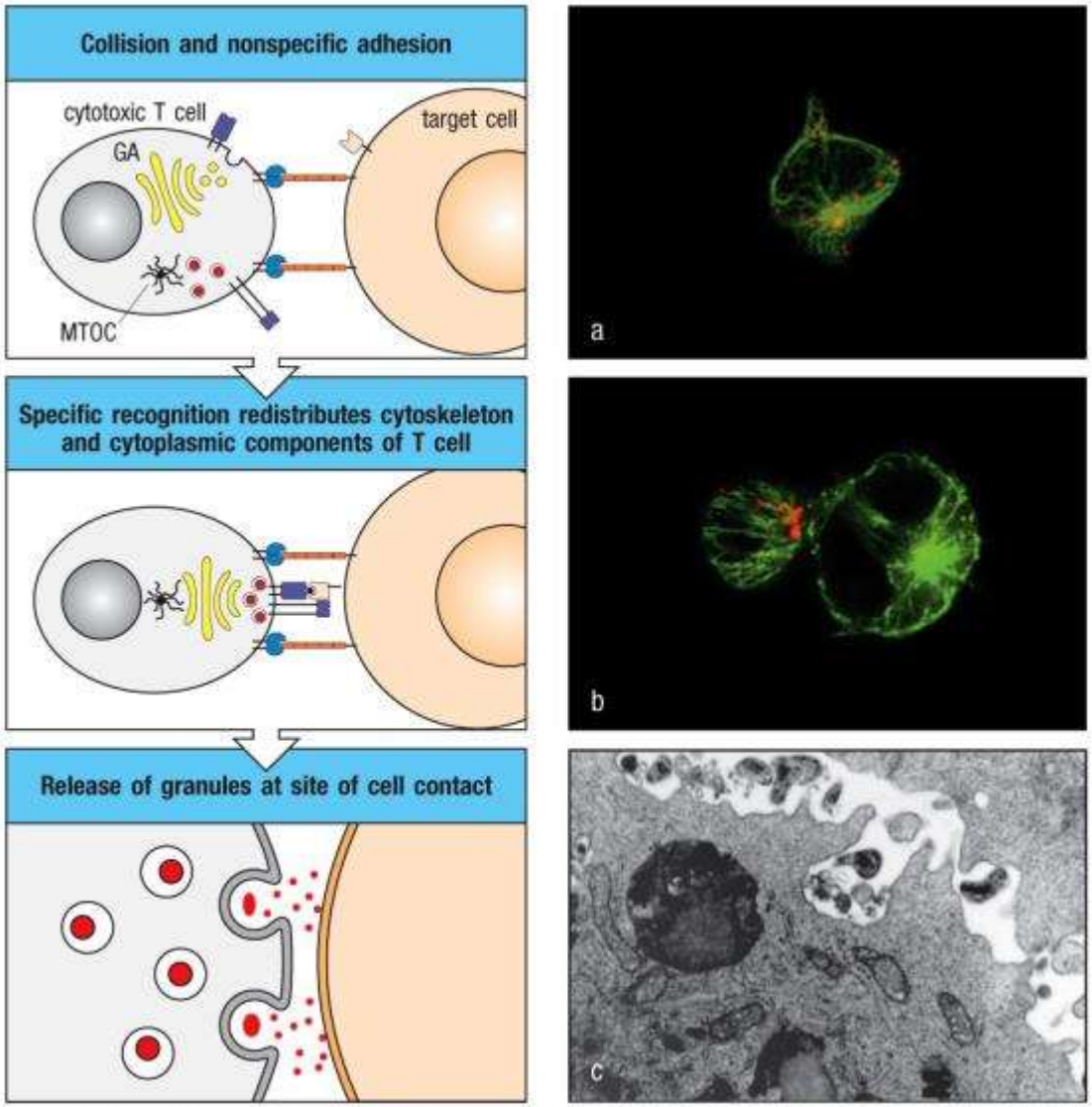


Figure 9.38 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

CTL alone

Red stained lytic granules

CTL attacking a target

CTLs are serial killers

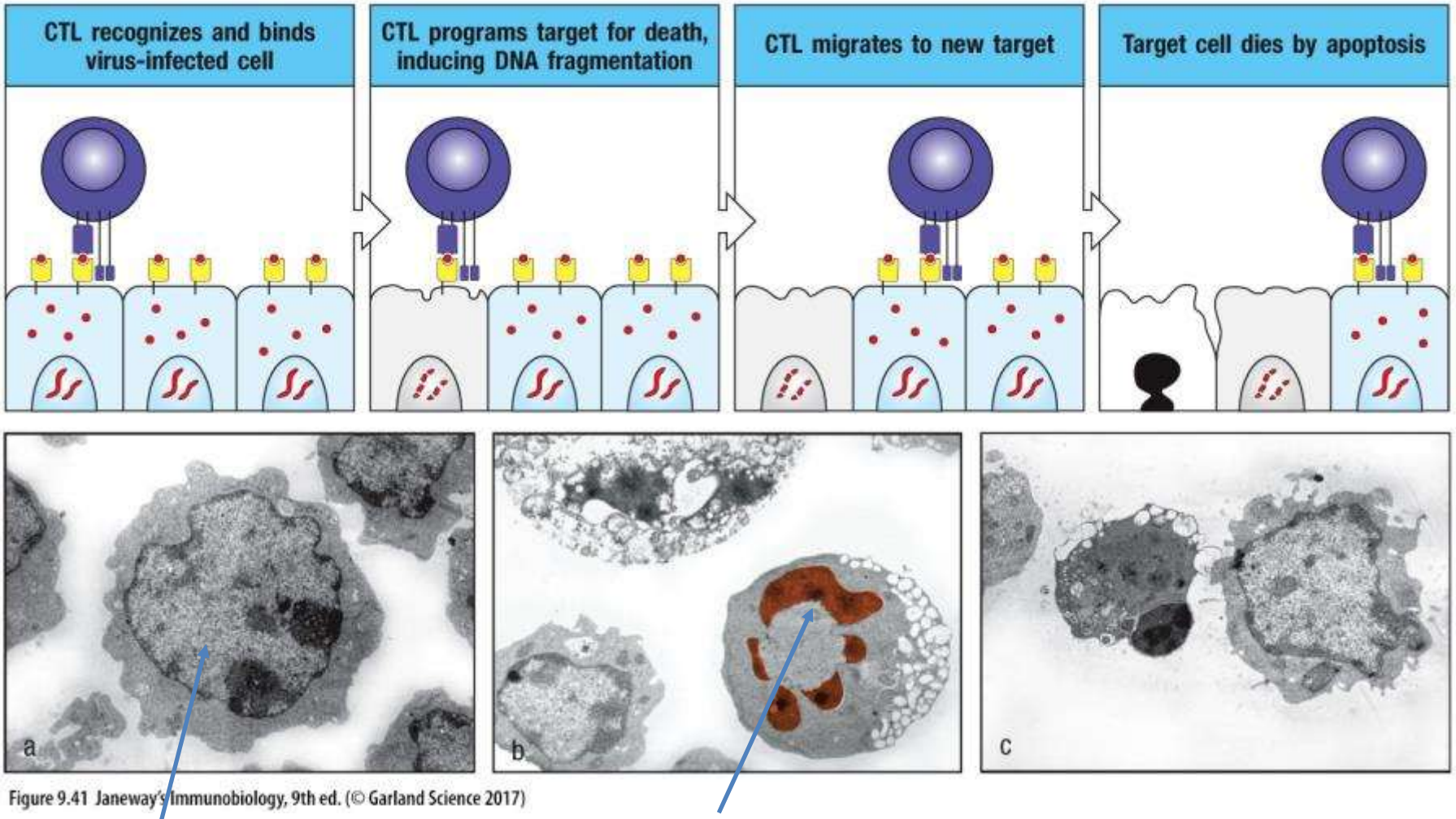


Figure 9.41 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Cellula sana

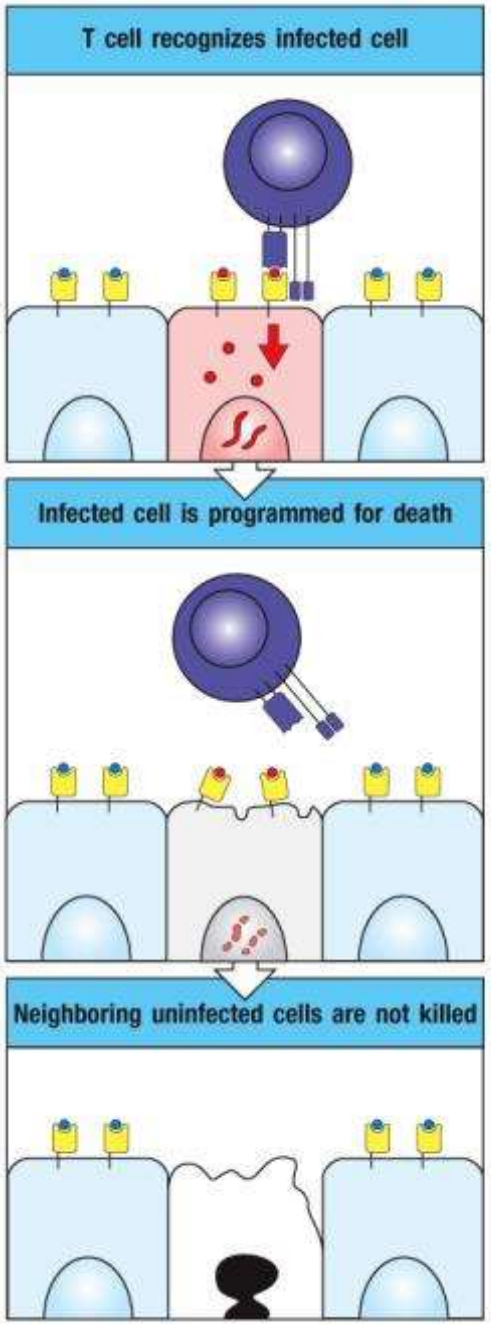
cromatina

Fase precoce di apoptosi

Fase tardiva di apoptosi

There is little bystander killing by CTLs

This is quite different from T_H1 activated macrophages which cause a lot of bystander killing



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CTL lytic mechanisms

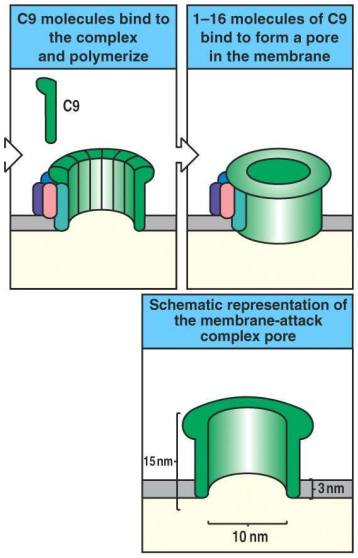
Protein in granules of cytotoxic T cells	Actions on target cells
Perforin	Aids in delivering contents of granules into the cytoplasm of target cell
Granzymes	Serine proteases, which activate apoptosis once in the cytoplasm of the target cell
Granulysin	Has antimicrobial actions and can induce apoptosis

Figure 8-37 Immunobiology, 7ed. (© Garland Science 2008)

Also, CTLs release IFN-γ to attract and activate macrophages and to inhibit viral replication.

Endonucleases that degraded host DNA during apoptosis may also degrade viral DNA

Perforin (a complement MAC-like molecule)



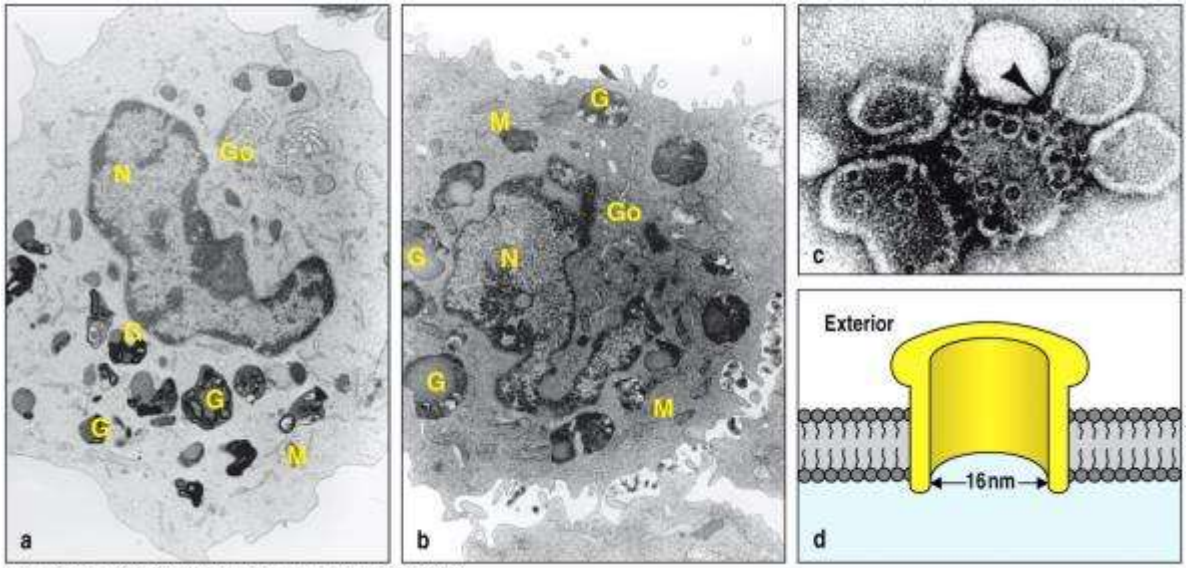
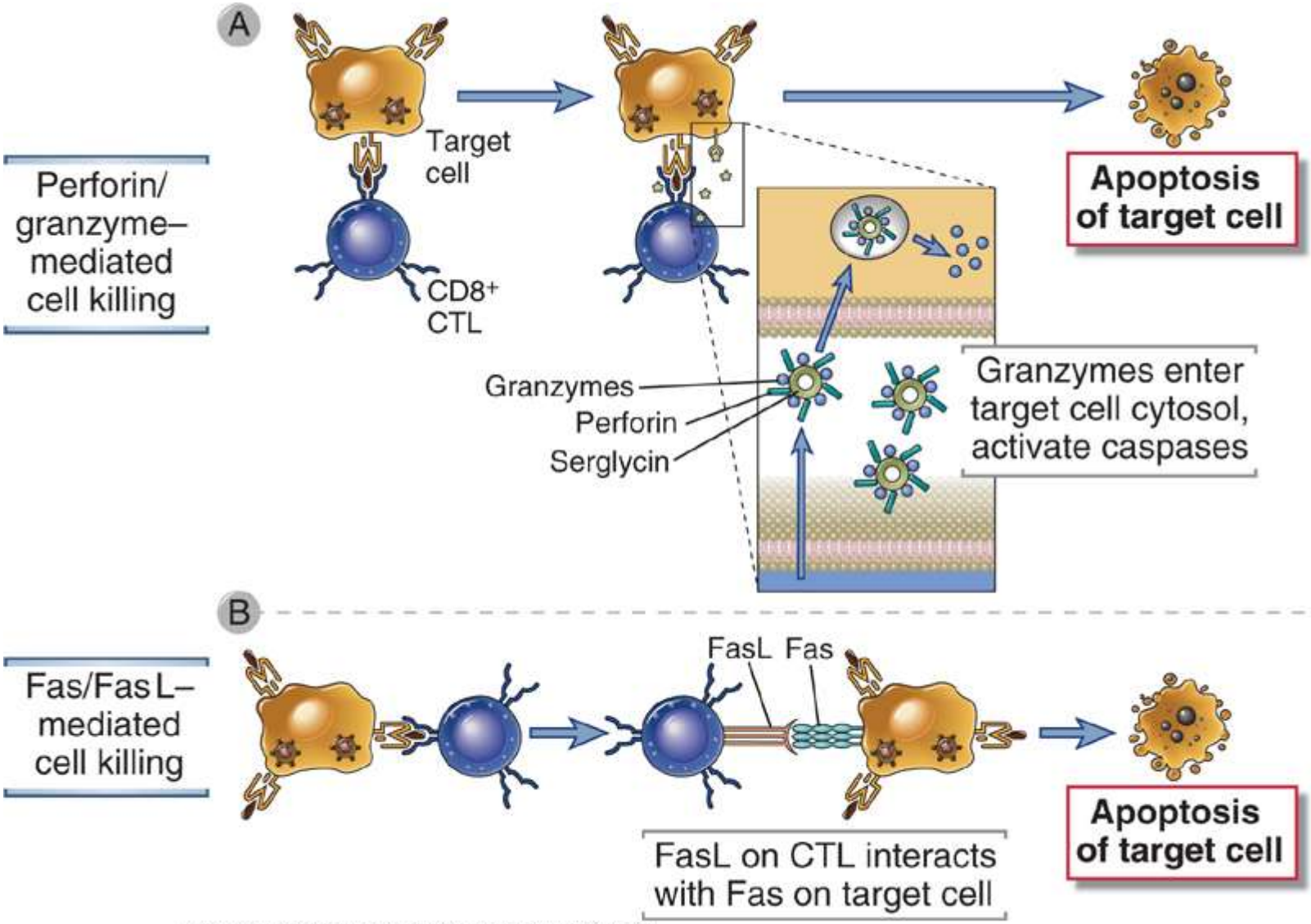
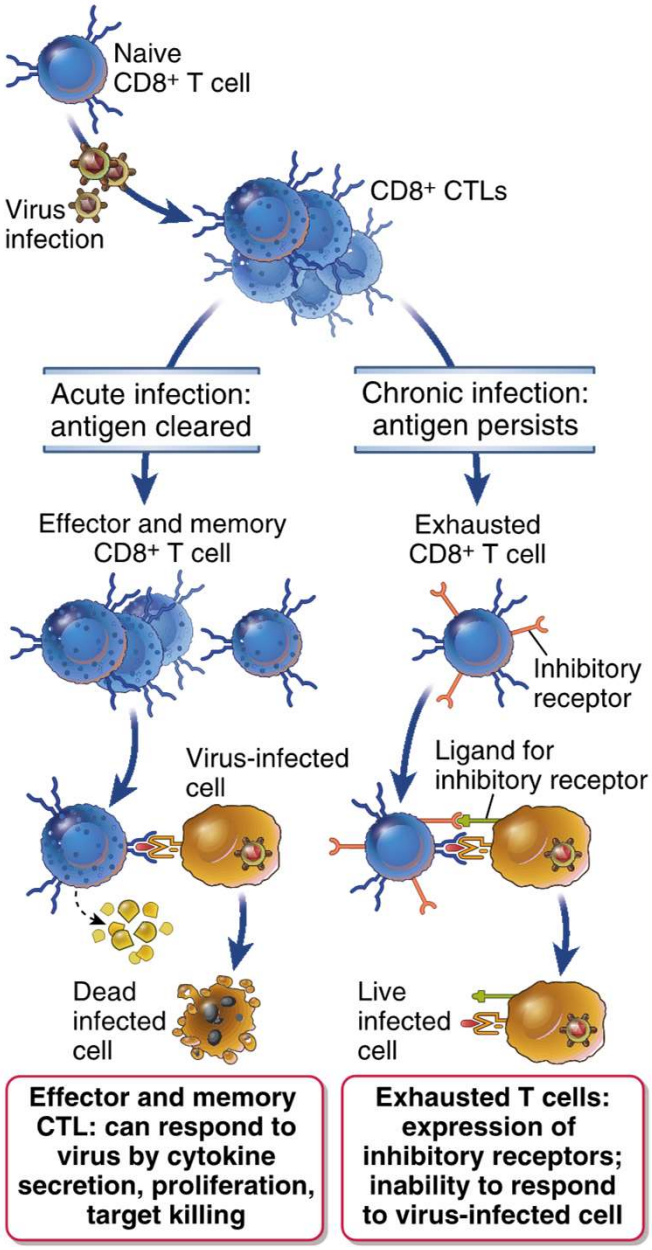


Figure 8-36 Immunobiology, 6/e. (© Garland Science 2005)



T cell exhaustion



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Capitolo 9

Different effectors deal with pathogens differently

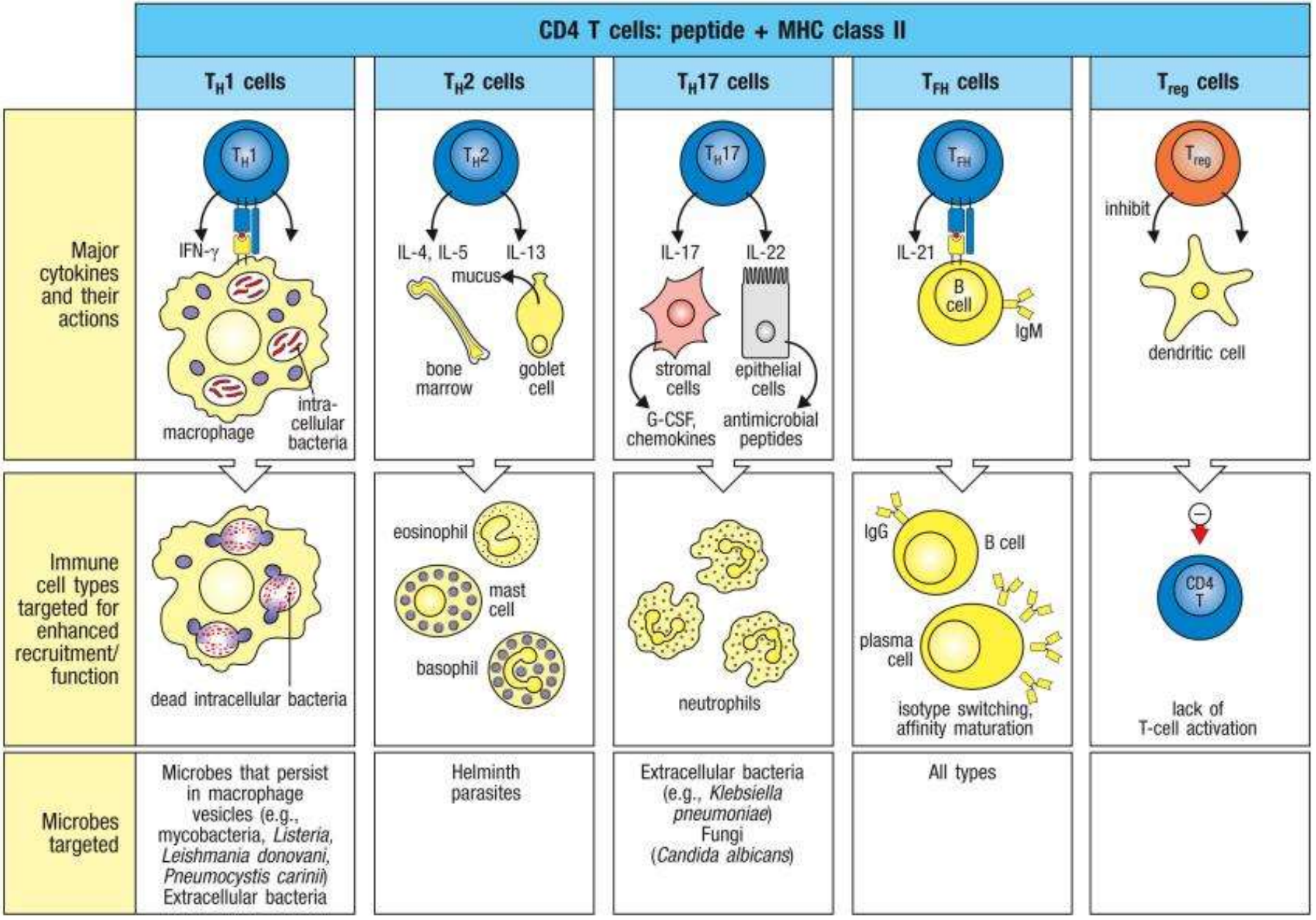


Figure 9.30 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Cytokines (signal 3) cause CD4$^+$ T cells to acquire one of several functions

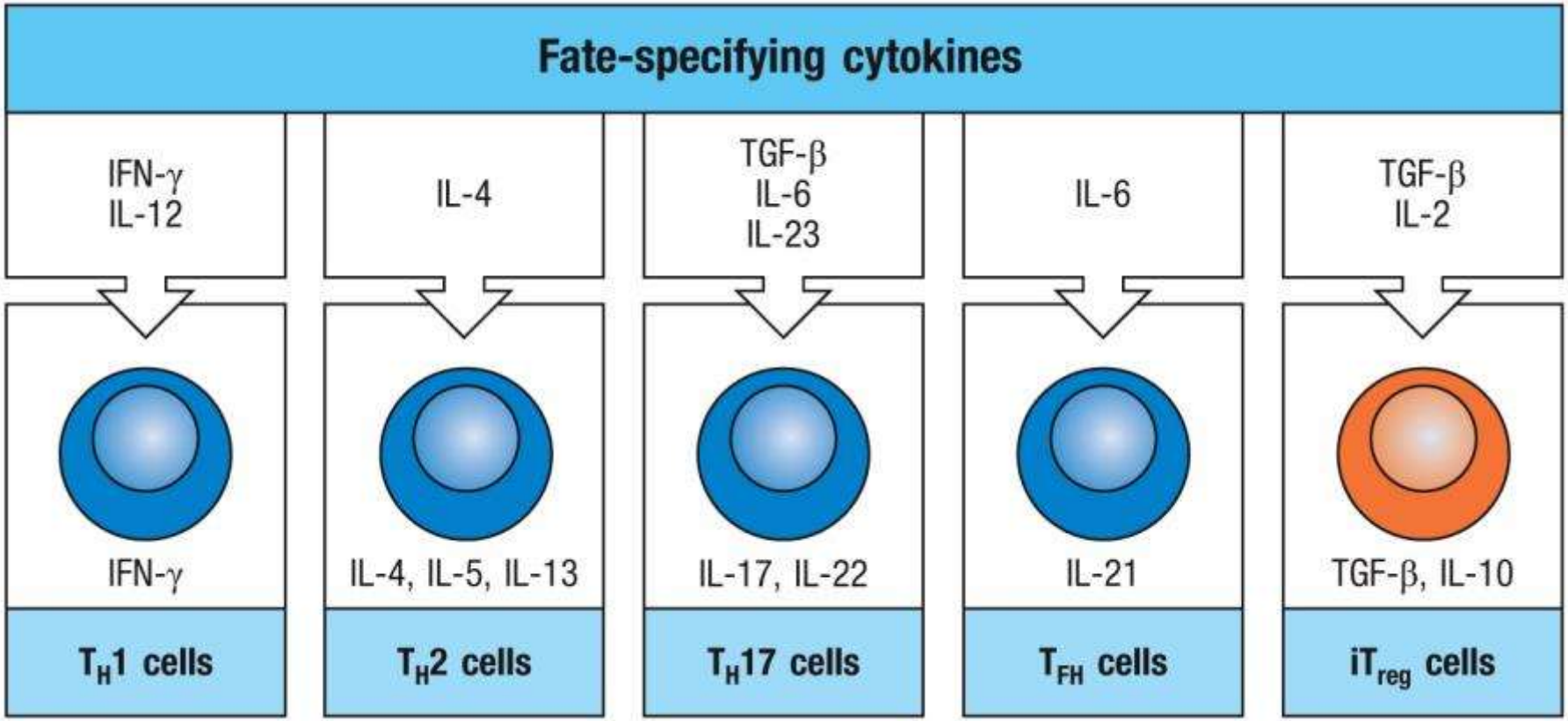


Figure 9.31 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Different members of the STAT family of transcription factors act immediately downstream of cytokines to determine T-cell subset development

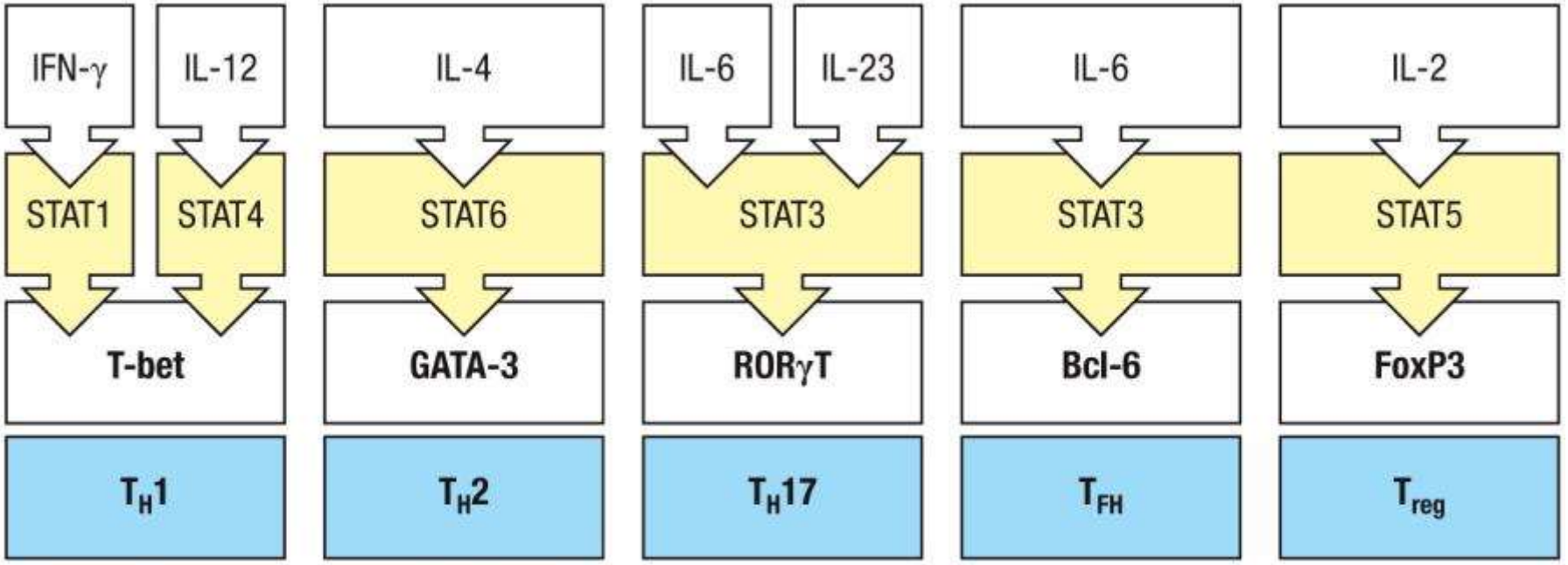


Figure 9.32 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Properties of the major subsets of $CD4^+$ helper T cells

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
Tfh	IL-21 (and IFN-γ or IL-4)	B cells	Antibody production	Extracellular pathogens	Autoimmunity (autoantibodies)

The cytokines that drive the development of $CD4^+$ T cell subsets are produced by APCs (primarily dendritic cells and macrophages) and other immune cells (such as NK cells, ILC and basophils or mast cells) present at the site of the immune response.

Stimuli other than cytokines may also influence the pattern of helper T cell differentiation (DC subsets; genetic background)

The distinct cytokine profiles of differentiated cell populations are controlled by particular transcription factors that activate cytokine gene transcription and by chromatin modifications affecting cytokine gene loci

Each subset of differentiated effector cells produces cytokines that promote its own development and may suppress the development of the other subsets

Differentiation of each subset is induced by the types of microbes which that subset is best able to combat (specialization of adaptive immunity – immune effector modules)

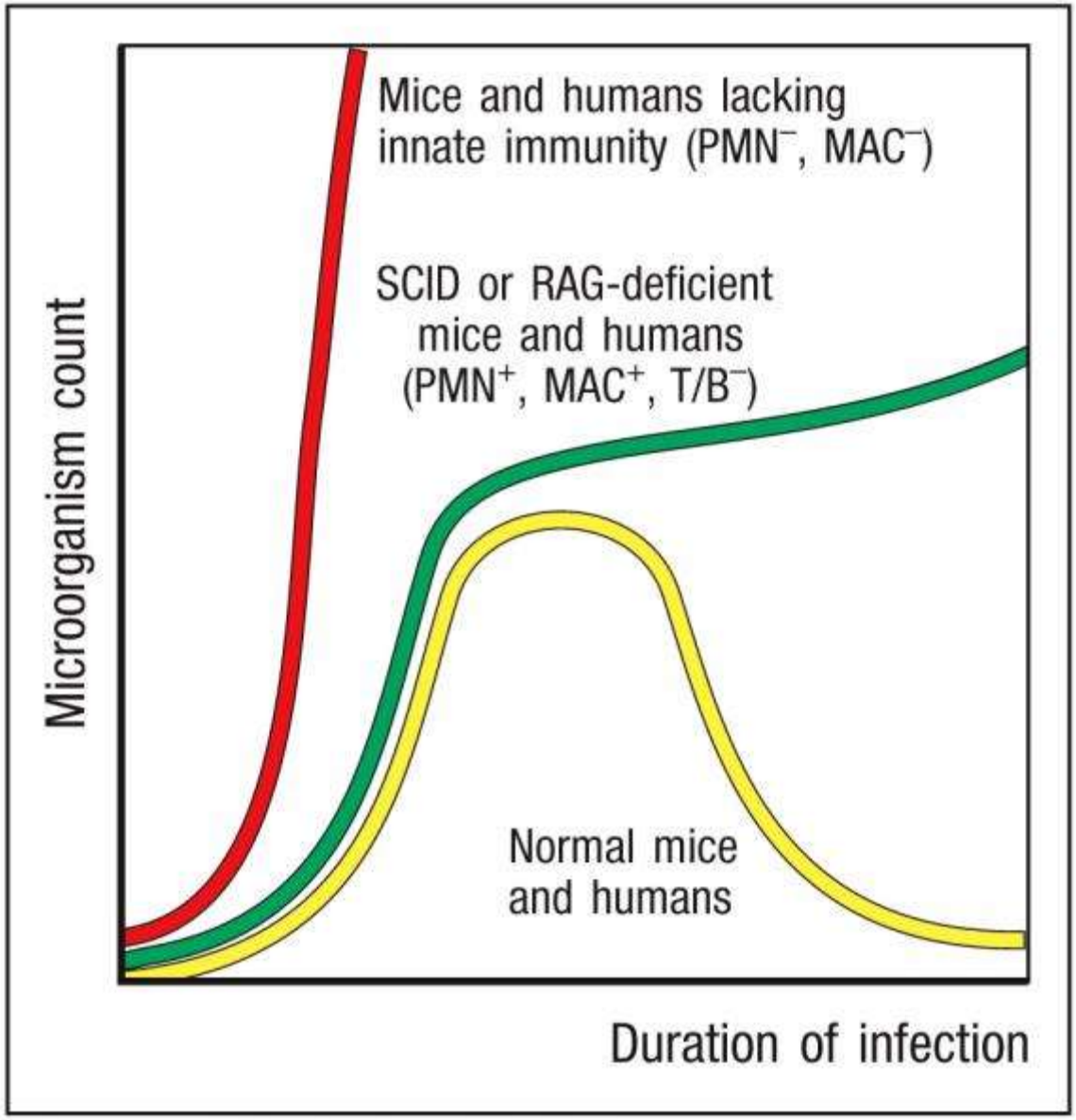


Figure 11.4 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

- Macrophages and neutrophils can kill <u>most bacteria</u> without the help of antibodies or T_H1 cells.
- Antibodies serve as opsonins to aid in phagocytosis
- T_H1 provide IFN-γ to activate macrophages
- Certain bacteria, such as *Mycobacterium tuberculosis* and *M. leprae* can live in macrophage vesicles. T_H1 helps lysosomes fuse with vesicles containing bacteria.

Macrophages activation by T_H1 requires IFN-γ and CD40L

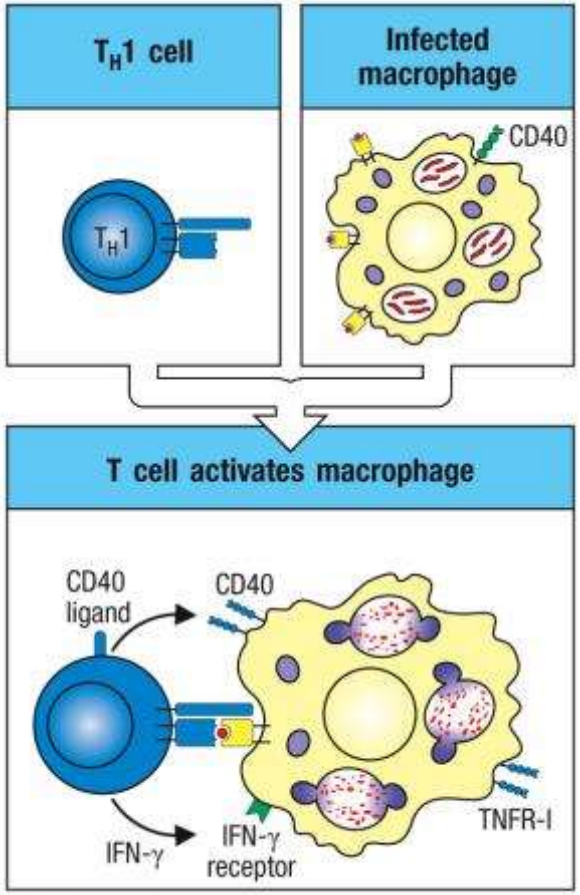


Figure 11.10 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

$O_2^{\bullet-}$ = superoxides and other reactive oxygen species

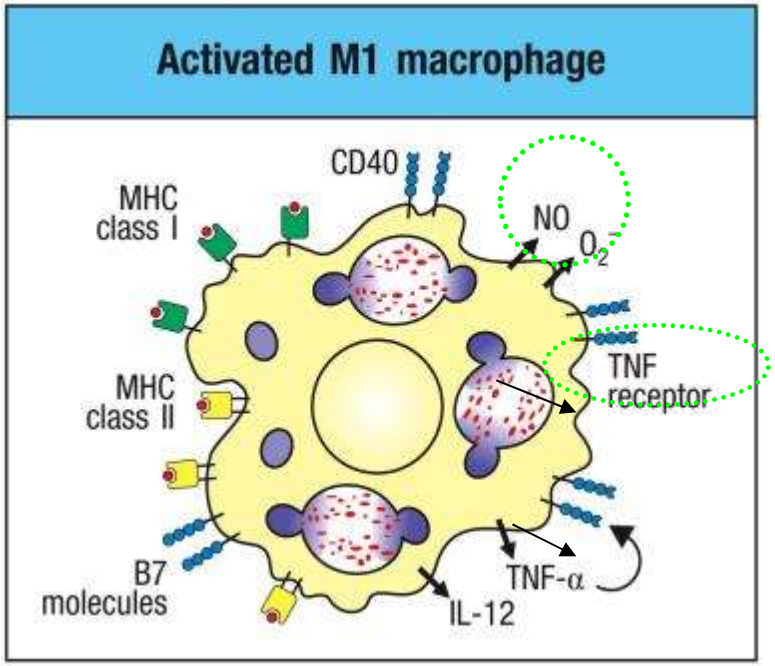
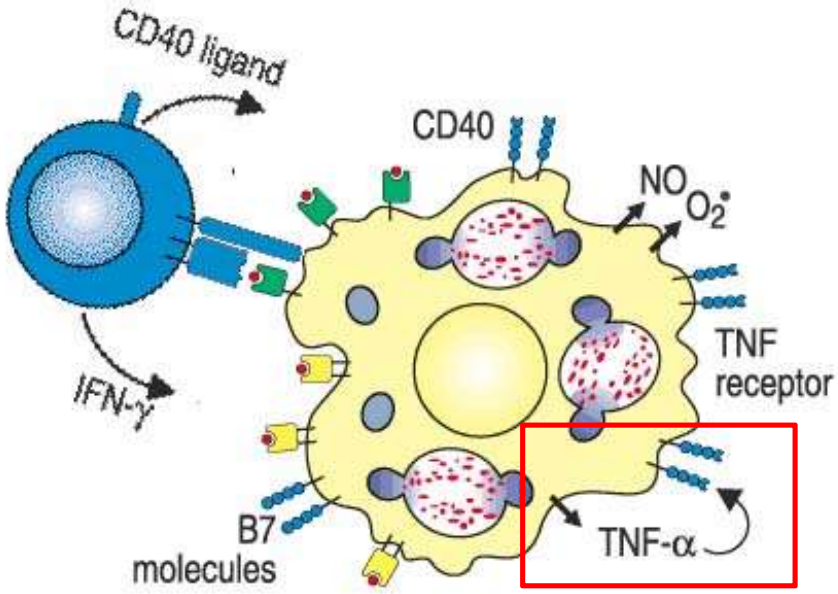


Figure 11.11 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Macrophage activation by T_H1 requires IFN-γ and CD40L

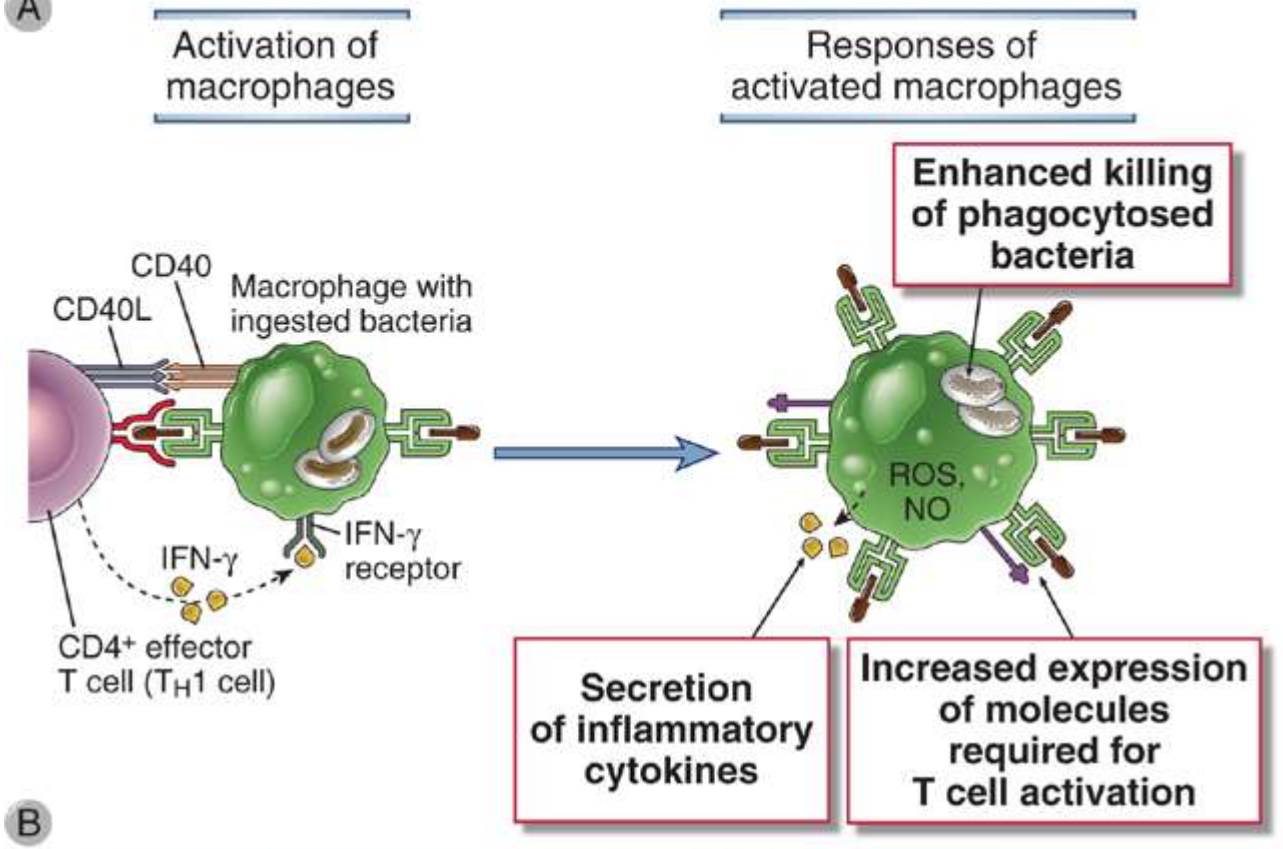


- IFN-γ from the T_H1 or from CTL. Therefore, **IFN-γ is characteristic of cell-mediated immunity (CMI)**
- IFN-γ increases macrophage expression of CD40
- LPS and other bacterial products make macrophage more responsive to IFN-γ
- TNF delivers survival signal

T_H1 does not store IFN-γ so it must synthesize it upon contact with macrophage!

It may take hours to make the IFN and to activate the macrophage. The T_H1 cell stays engaged with the macrophage for the entire process.
There is some bystander activation but mostly one T_H1 on one macrophage.

A



B

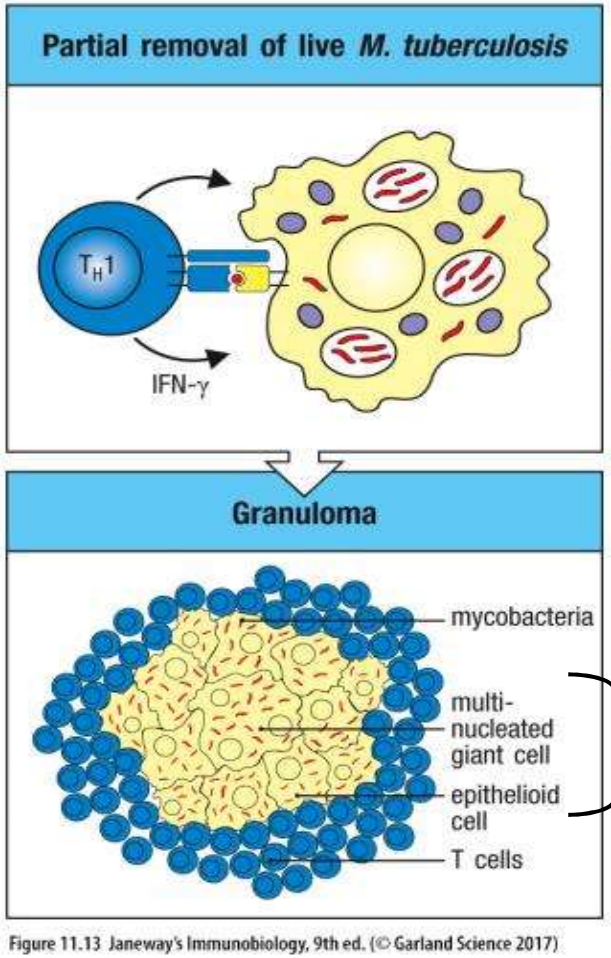
Macrophage response	Role in cell-mediated immunity
Production of reactive oxygen species, nitric oxide, increased lysosomal enzymes	Killing of microbes in phagolysosomes (effector function of macrophages)
Secretion of cytokines (TNF, IL-1, IL-12) and chemokines	TNF, IL-1, chemokines: leukocyte recruitment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

Abbas et al: Cellular and Molecular Immunology, 7e.

L'attivazione dei macrofagi da parte delle cellule CD4+ deve essere strettamente regolata per evitare un danno tissutale!

L'attivazione cronica dei macrofagi da parte dei Th1 media la formazione dei granulomi

When the macrophage cannot kill intracellular bacteria (or there is chronic inflammation from an agent that cannot be removed) a granuloma may form



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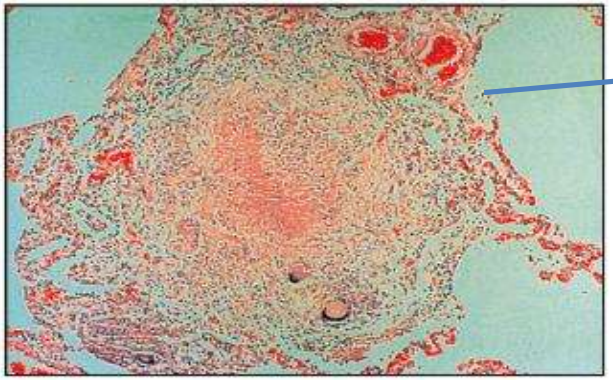

Micobatteri (in rosso)

Zona centrale di macrofagi circondata da linfociti attivati

Multinucleated giant cell (formati dalla fusione di diversi macrofagi