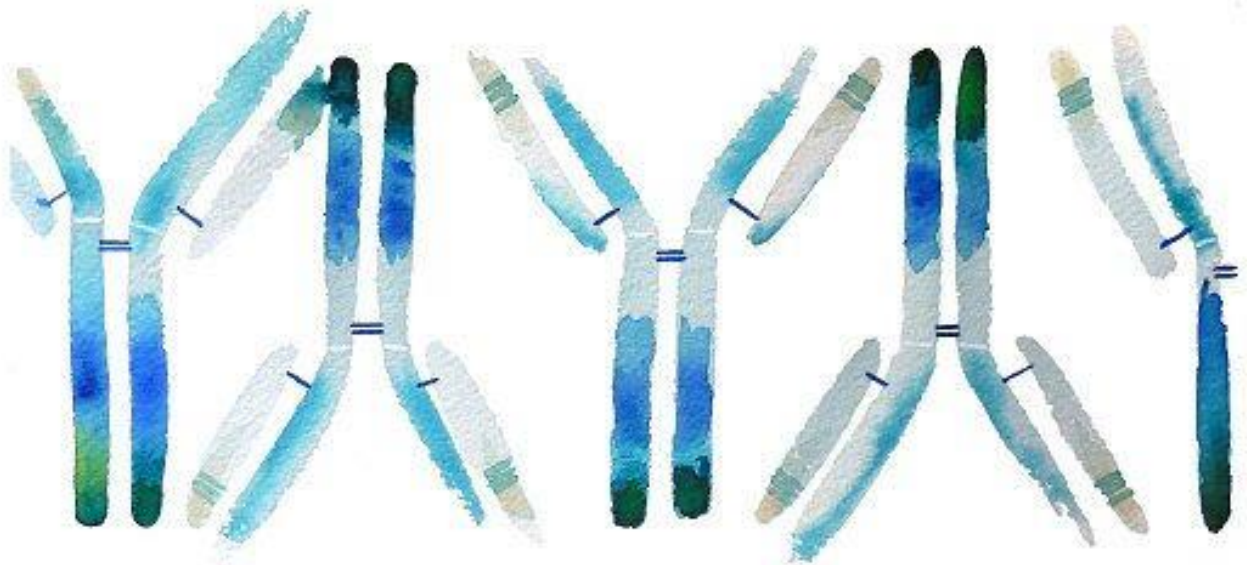
Medical Immunology



Anas Abu-Humaidan
M.D. Ph.D.

Lecture 9

- In this lecture we will discuss

- Main topics:

Immune responses to extracellular and intracellular pathogens

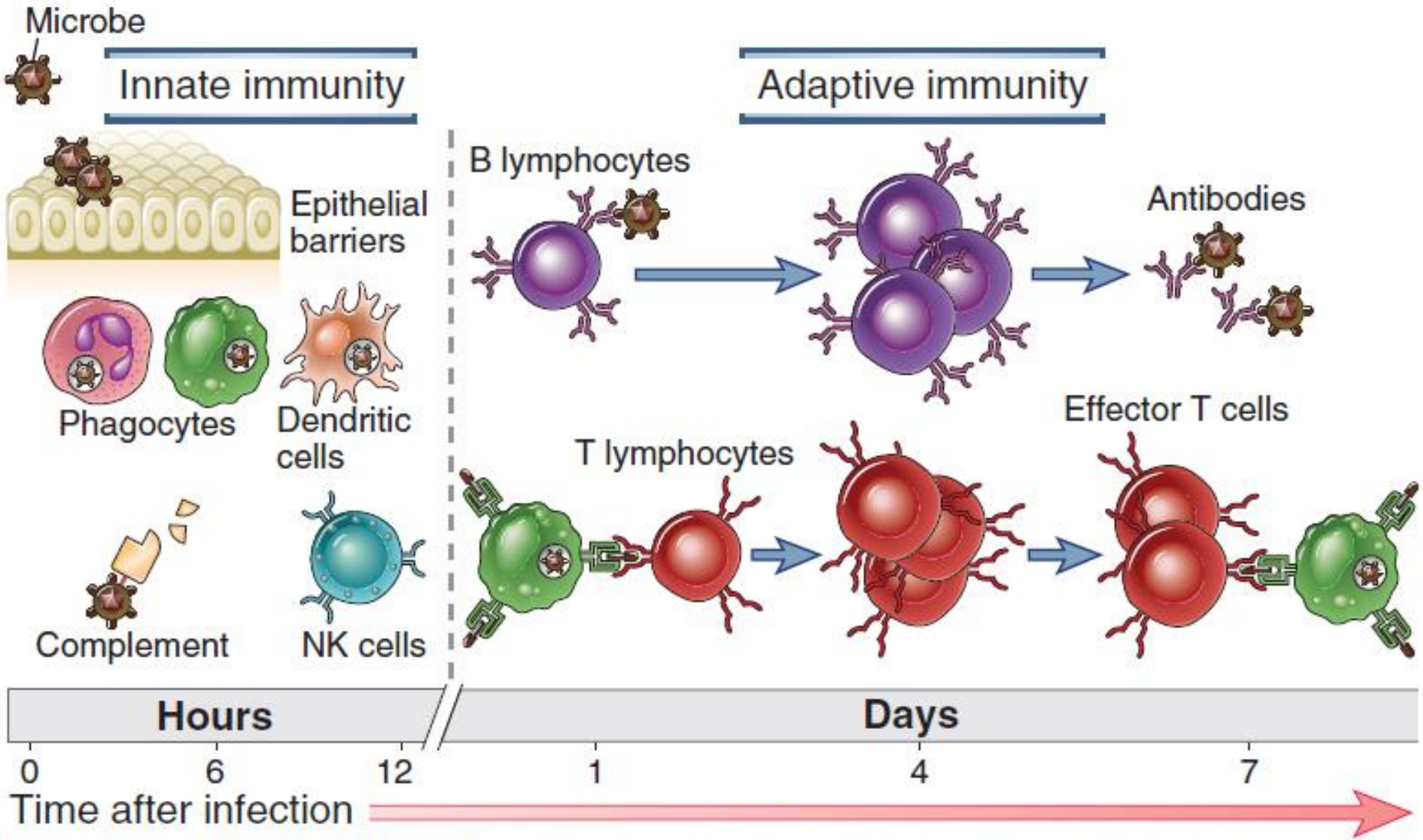


FIGURE 1–1 Innate and adaptive immunity. The mechanisms of innate immunity provide the initial defense against infections. Adaptive immune responses develop later and consist of activation of lymphocytes. The kinetics of the innate and adaptive immune responses are approximations and may vary in different infections.

GENERAL FEATURES OF IMMUNE RESPONSES TO MICROBES

- Defense against microbes is mediated by the effector mechanisms of innate and adaptive immunity
- The immune system responds in distinct and specialized ways to different types of microbes to most effectively combat these infectious agents.
- The survival and pathogenicity of microbes in a host are critically influenced by the ability of the microbes to evade or resist the effector mechanisms of immunity.
- Many microbes establish latent, or persistent, infections in which the immune response controls but does not eliminate the microbe and the microbe survives without propagating the infection.
- In many infections, tissue injury and disease may becaused by the host response to the microbe and its products rather than by the microbe itself.

Immunity to Extracellular Bacteria

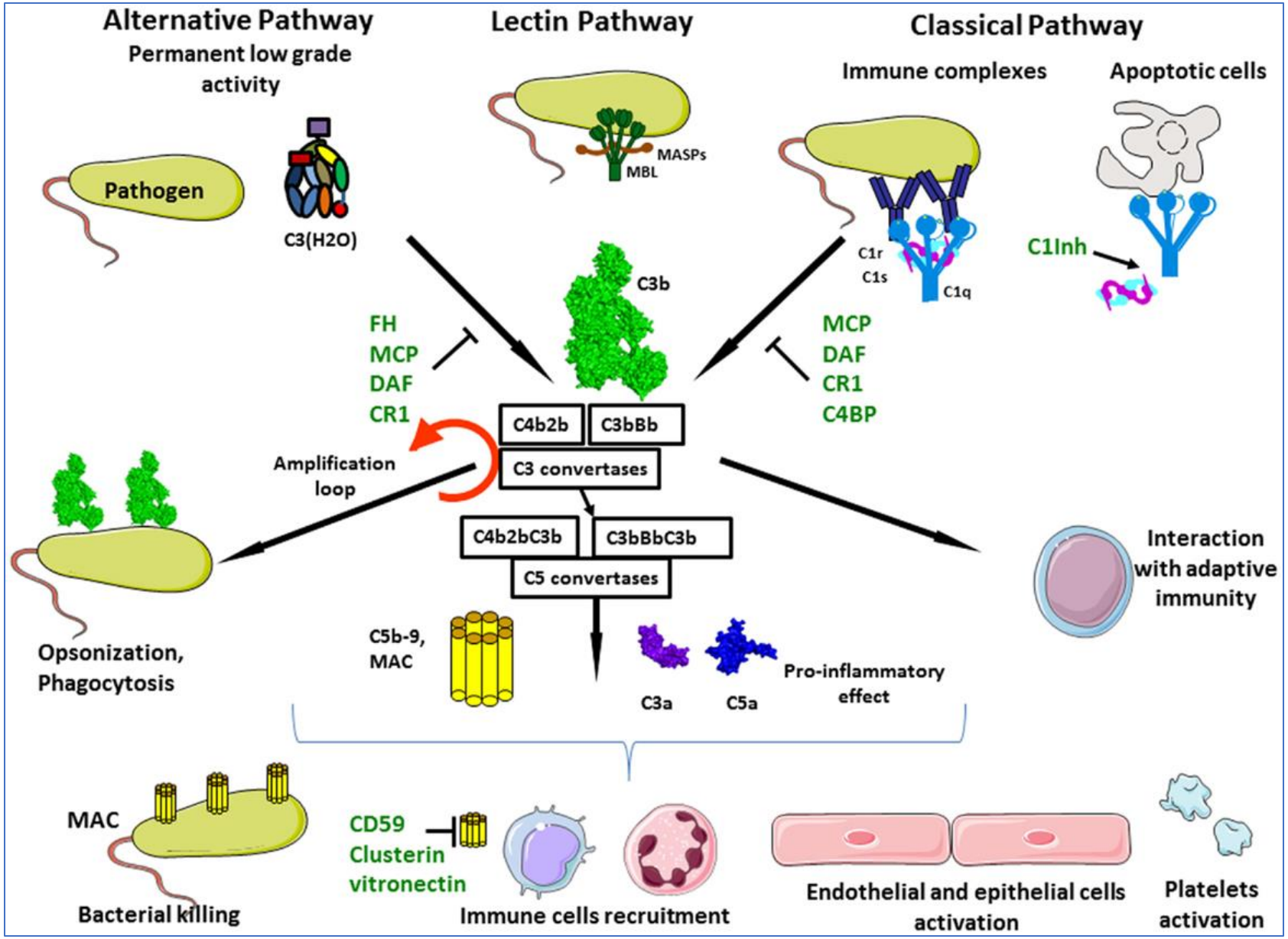
- The principal mechanisms of innate immunity to extracellular bacteria are :

- **complement activation**.

- **Phagocytosis**: Phagocytes use various surface receptors, including **mannose receptors** and **scavenger receptors**, to recognize extracellular bacteria, and they use **Fc receptors** and **complement receptors** to recognize bacteria **opsonized** with antibodies and complement proteins, respectively. As well as TLRs and other PRR.

- **The inflammatory response**: dendritic cells and phagocytes that are activated by the microbes secrete cytokines, which induce leukocyte infiltration, and initiates and propogates adaptive immune responses.

Immunity to Extracellular Bacteria / complement activation



Immunity to Extracellular Bacteria / professional phagocytes

- Cells that have specialized phagocytic functions, primarily macrophages and neutrophils, are the **first line of defense against microbes** that breach epithelial barriers.

- They serve several functions: 1) **Internalize and kill microbes**. Neutrophils macrophages are particularly good at this function. 2) Phagocytes respond to microbes by **producing various cytokines** that promote inflammation. Macrophages are particularly good at this.

- The essential role that phagocytes play in innate immune defense against microbes is demonstrated by the **high rate of lethal bacterial and fungal infections** in patients with **low blood neutrophil counts** caused by bone marrow cancers or cancer therapy, or inherited deficiencies.

- IgG subtypes that bind best to Fc receptors (IgG1 and IgG3) are the most efficient opsonins for promoting phagocytosis. Binding of **FcγRI** receptors on phagocytes to multivalent antibody-coated particles leads to **engulfment** of the particles and the **activation** of phagocytes.

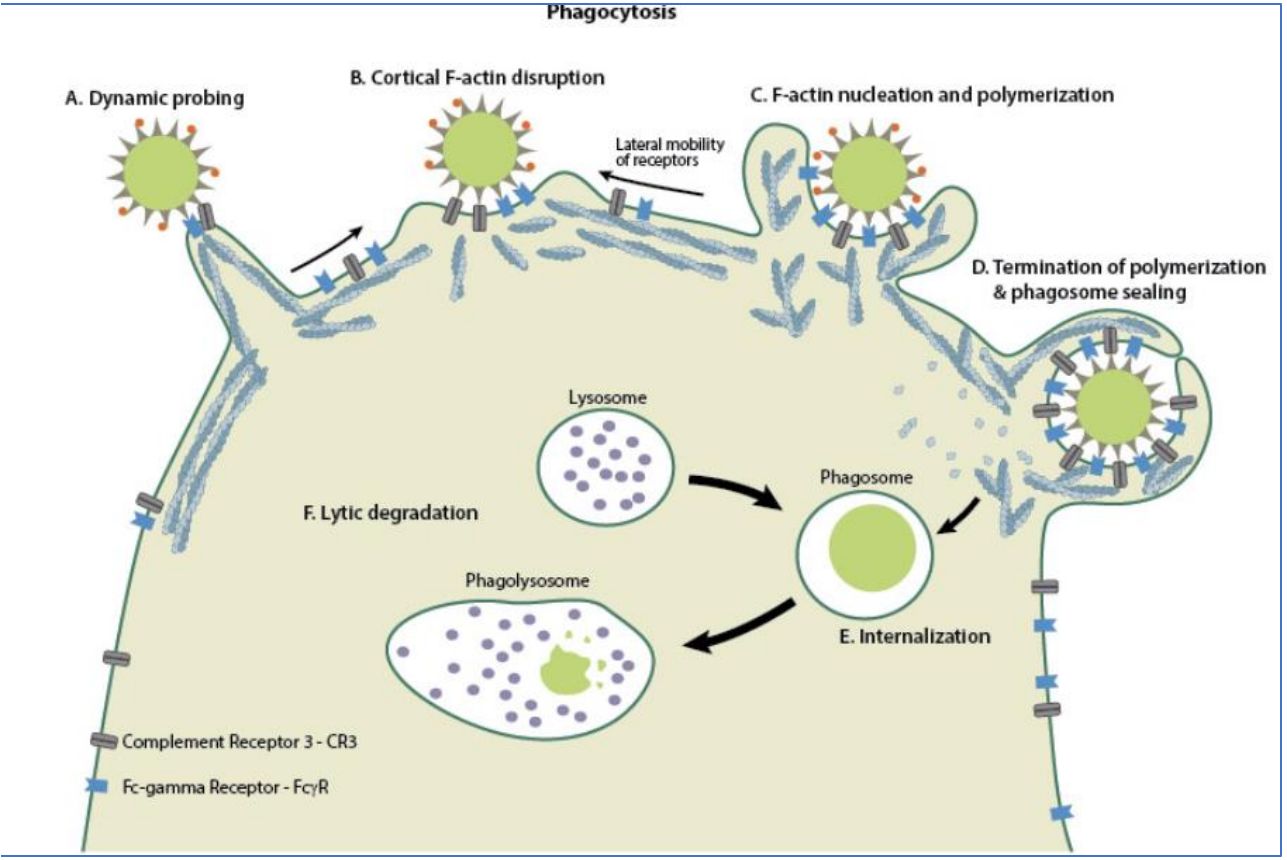
- Activation leads to:

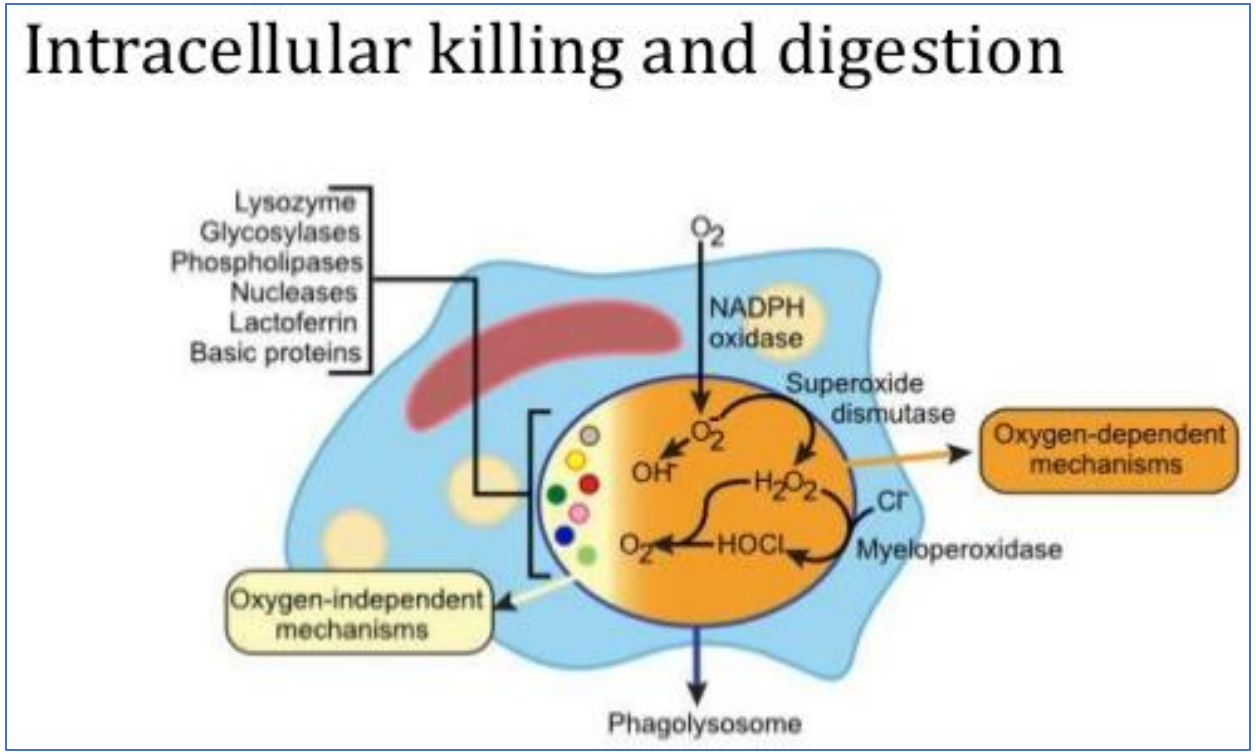
➢ Production of the enzyme **phagocyte oxidase**, which catalyzes the intracellular generation of **reactive oxygen species** that are cytotoxic for phagocytosed microbes. This process is called the **respiratory burst**.

➢ Activation of an enzyme called **inducible nitric oxide synthase** (iNOS), which triggers the production of **nitric oxide** that also contributes to the killing of pathogens.

➢ Secretion of **hydrolytic enzymes** and reactive oxygen intermediates into the external milieu that are capable of killing extracellular microbes too large to be phagocytosed. The same toxic products may **damage tissues.**

Immunity to Extracellular Bacteria / professional phagocytes





Binding of **Fc receptors** causes an increase in **oxygen uptake** by the phagocyte called the **respiratory burst**. This influx of oxygen is used in a variety of mechanisms to cause damage to microbes inside the phagolysosome, but the common theme is the creation of **highly reactive small molecules** that damage the biomolecules of the pathogen.

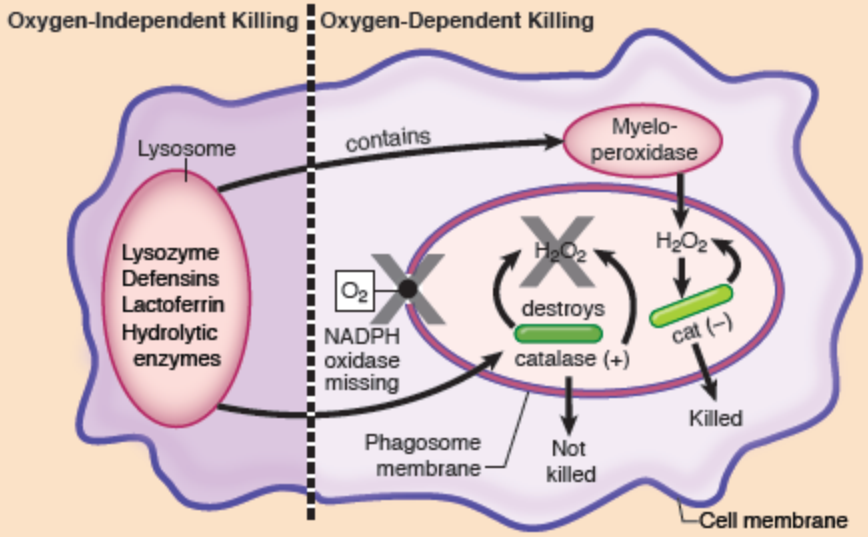
Immunity to Extracellular Bacteria / professional phagocytes

Clinical Correlate

When defects prevent phagocytes from performing their critical functions as first responders and intracellular destroyers of invading antigens, clinically important pathologic processes ensue. Such defects tend to make the patient susceptible to severe infections with **extracellular bacteria** and **fungi**.

Chronic granulomatous disease (CGD) is an inherited deficiency in the production of one of several subunits of **NADPH oxidase**. This defect eliminates the phagocyte's ability to produce many critical oxygen-dependent intracellular metabolites ($\cdot O_2^-$, $\cdot OH$, $^1O_2$, and H_2O_2). The 2 other intracellular killing mechanisms remain intact (myeloperoxidase + $H_2O_2 \rightarrow$ HOCl and lysosomal contents).

- If the patient is infected with a catalase-negative organism, the H_2O_2 waste product produced by the bacterium can be used as a substrate for myeloperoxidase, and the bacterium is killed.
- If the patient is infected with a catalase-positive organism (e.g., *Staphylococcus*, *Klebsiella*, *Serratia*, *Aspergillus*), the myeloperoxidase system lacks its substrate (because these organisms destroy H_2O_2), and the patient is left with the oxygen-independent lysosomal mechanisms that prove inadequate to control rampant infections. Thus, CGD patients suffer from chronic, recurrent infections with catalase-positive organisms.



Intracellular Killing in CGD

Effector mechanisms of humoral immunity

TABLE 12–3 Fc Receptors

FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγRI (CD64)	High ($K_d < 10^{-9}$ M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
FcγRIIA (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils; eosinophils, platelets	Phagocytosis; cell activation (inefficient)
FcγRIIB (CD32)	Low ($K_d > 10^{-7}$ M)	B lymphocytes	Feedback inhibition of B cells
FcγRIIC (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils, NK cells	Phagocytosis, cell activation
FcγRIIIA (CD16)	Low ($K_d > 10^{-6}$ M)	NK cells	Antibody-dependent cell-mediated cytotoxicity
FcγRIIIB (CD16)	Low ($K_d > 10^{-6}$ M); GPI-linked protein	Neutrophils	Phagocytosis (inefficient)
FcεRI	High ($K_d > 10^{-10}$ M); binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
FcεRII (CD23)	Low ($K_d > 10^{-7}$ M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
FcαR (CD89)	Low ($K_d > 10^{-6}$ M)	Neutrophils, eosinophils, monocytes	Cell activation?

GPI, glycophosphatidylinositol; NK, natural killer.

- Macrophages and dendritic cells function as antigen-presenting cells (APCs). They present peptide antigens derived from digested bacteria on the major histocompatibility complex class II and activate acquired immunity by activating helper T cells.

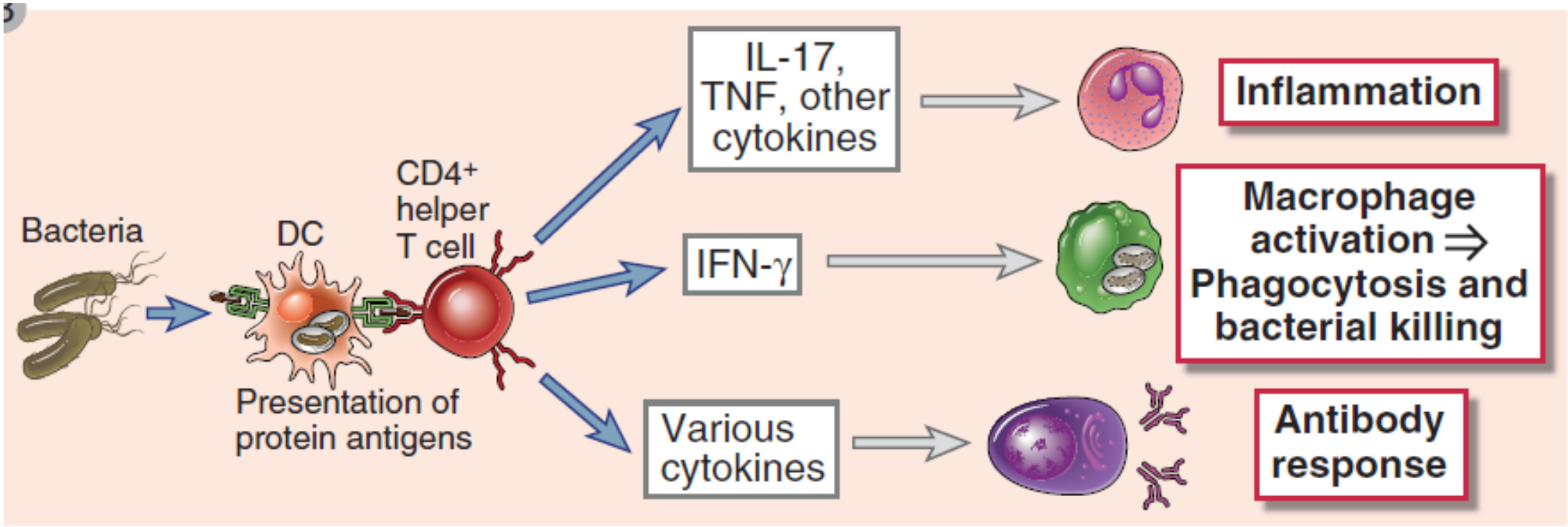
- While macrophages present antigens within tissues, dendritic cells present antigens in the lymph node. **Only dendritic cells can activate naïve T cells to become effector T cells, and are the most powerful APCs**

- Dendritic cells are a heterogeneous family of bone marrow–derived cells with long dendrite-like cytoplasmic processes are constitutively present in epithelia and most tissues of the body.

- Most versatile sensors of PAMPs and DAMPs among all cell types in the body.

- TLR signaling induces dendritic cell expression of molecules, including **costimulatory molecules and cytokines**, that are needed, in addition to antigen, **for the activation of the naive T cells.** Activation into **effector T cell** subtypes depends on the nature of the pathogen.

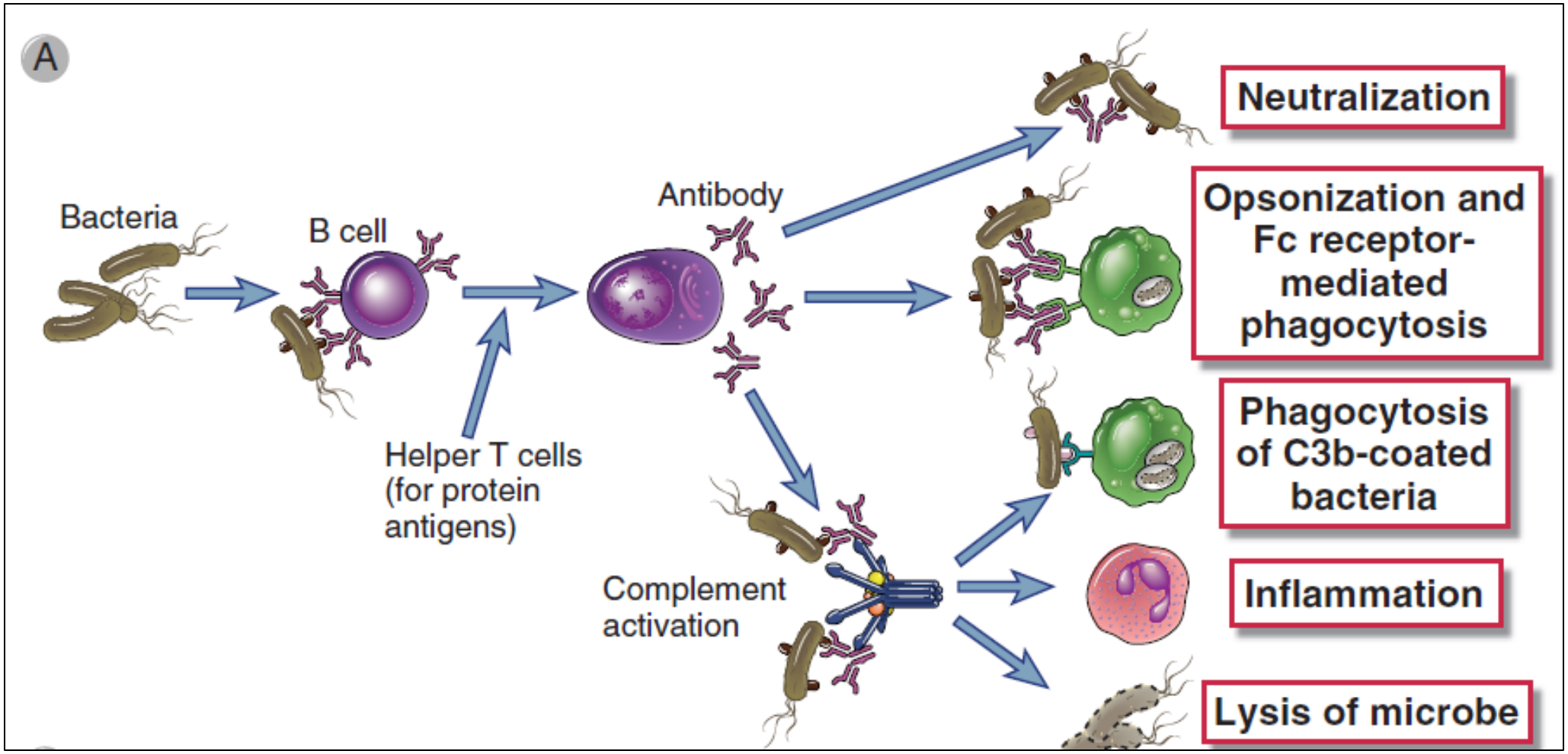
Adaptive Immunity to Extracellular Bacteria



- The protein antigens of extracellular bacteria also **activate CD4+ helper T cells**, which **produce cytokines that induce local inflammation**, **enhance the phagocytic and microbicidal activities of macrophages and neutrophils**, and **stimulate antibody production**

Adaptive Immunity to Extracellular Bacteria

- **Humoral immunity** is a major protective immune response against extracellular bacteria, and it functions to block infection, to eliminate the microbes, and to neutralize their toxins.



Injurious effects following immunity to extracellular

- Inflammatory reactions are usually self-limited and controlled. Cytokines secreted by leukocytes in response to bacterial products also stimulate the production of acute-phase proteins and cause the systemic manifestations of the infection. **Sepsis** is a severe pathologic consequence of disseminated infection by some gram-negative and gram-positive bacteria.

- A late complication of the humoral immune response to bacterial infection may be the **generation of disease-producing antibodies**. The best-defined examples are two rare sequelae of streptococcal infections of the throat or skin. Infection leads to the production of antibodies against a bacterial cell wall protein (M protein). Some of these antibodies cross-react with self antigens.

Injurious effects following immunity to extracellular

- Certain bacterial toxins stimulate all the T cells in an individual that express a particular family of Vβ T cell receptor (TCR) genes. Such toxins are called **superantigens** because they resemble antigens in that they bind to TCRs and to class II MHC molecules (although not to the peptide-binding clefts) but **activate many more T cells than do conventional peptide antigens**.

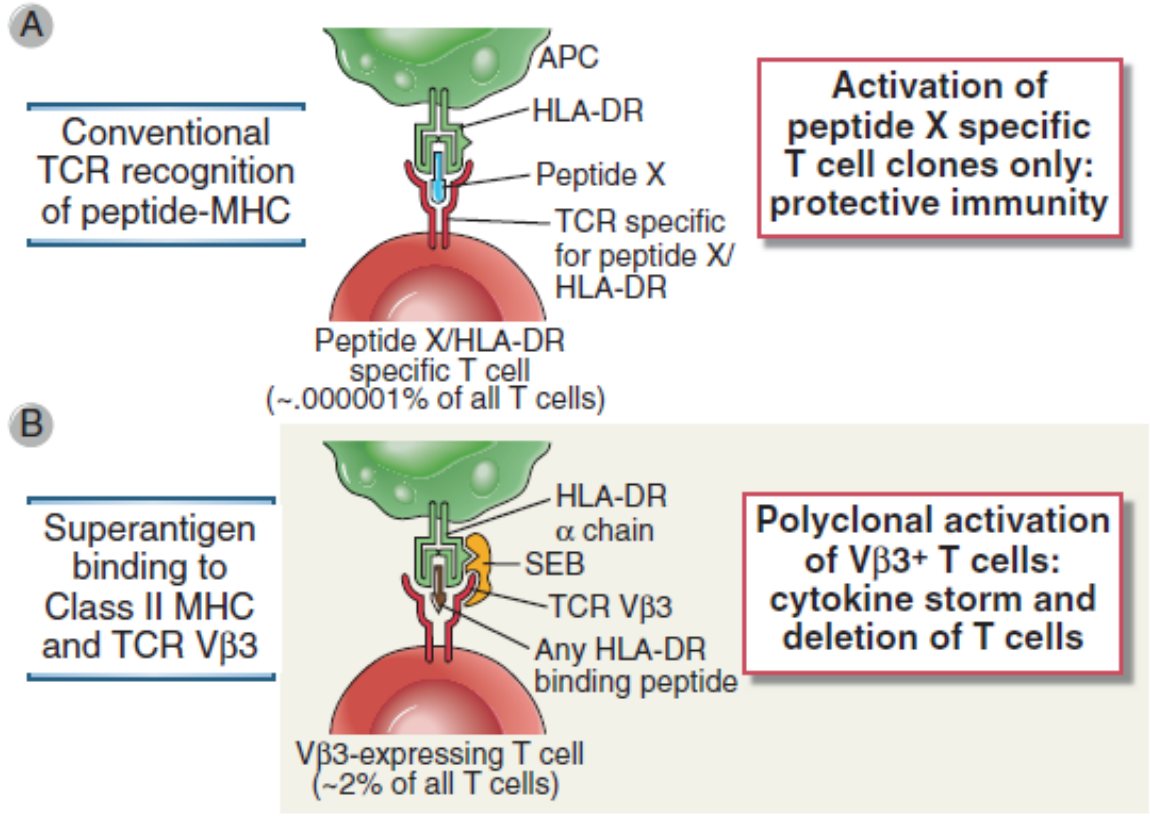


FIGURE 15–2 Polyclonal activation of T cells by bacterial superantigens. A, Conventional microbial T cell antigens, composed of a peptide bound to the peptide-binding groove of an MHC molecule, are recognized by a very small fraction of T cells in any one individual, and only these T cells are activated to become effector T cells that protect against the microbe. **B,** In contrast, a superantigen binds to class II MHC molecules outside the peptide-binding groove and simultaneously binds to the variable region of any TCR β chain, as long as it belongs to a particular V_β family, regardless of the peptide-MHC specificity of the TCR. In this way, superantigens activate T cells to secrete cytokines and also induce apoptosis of T cells. Different superantigens bind to TCRs of different V_β families. Because thousands of clones of T cells will express a TCR β chain from a particular V_β family, superantigens can induce massive cytokine release (cytokine storm) and cause deletion of many T cells. In the example shown, staphylococcal enterotoxin B (SEB) is the superantigen, which binds mainly to HLA-DR and the V_β segments of TCRs belonging to the $V_\beta 3$ family. APC, antigen-presenting cell.

Immunity to Intracellular bacteria

- The innate immune response to **intracellular bacteria** is mediated mainly by phagocytes and natural killer (NK) cells

- Phagocytes, initially neutrophils and later macrophages, ingest and attempt to destroy these microbes, but pathogenic intracellular bacteria are resistant to degradation within phagocytes.

- Products of these bacteria are recognized by TLRs and cytoplasmic proteins of the NOD like **receptor (NLR) family**, resulting in activation of the phagocytes.

- Intracellular bacteria activate NK cells by **inducing expression of NK cell–activating ligands on infected cells** and by **stimulating dendritic cell and macrophage production of IL-12 and IL-15**, both of which are NK cell–activating cytokines.

- The major protective immune response against intracellular bacteria is **T cell–mediated immunity.**

Immunity to Intracellular pathogens / Natural killer (NK) cells

- NK are lymphocytes important in innate immunity. The term natural killer derives from the fact that these cells are capable of performing their killing function without a need for clonal expansion and differentiation.

- NK cells distinguish infected and stressed cells from healthy cells, and NK cell activation is regulated **by a balance between signals** that are generated from activating receptors and inhibitory receptors.

- In general, the **activating receptors** recognize ligands on **infected and injured cells**, and the inhibitory receptors recognize healthy normal cells. Most NK cells express **inhibitory receptors** that recognize **class I major histocompatibility complex** (MHC) molecules.

- This ability of NK cells to become activated by host cells that lack class I MHC has been called **recognition of missing self**.

Immunity to Intracellular pathogens / Natural killer (NK) cells

- Antibodies that bind to antigens can be recognised by **FcϒRIII** (CD16) receptors expressed on NK cells, resulting in NK activation, release of cytolytic granules and consequent cell apoptosis. This allows NK cells to **target cells against which a humoral response** has been gone through and to lyse cells through antibody-dependant cytotoxicity (**ADCC**).

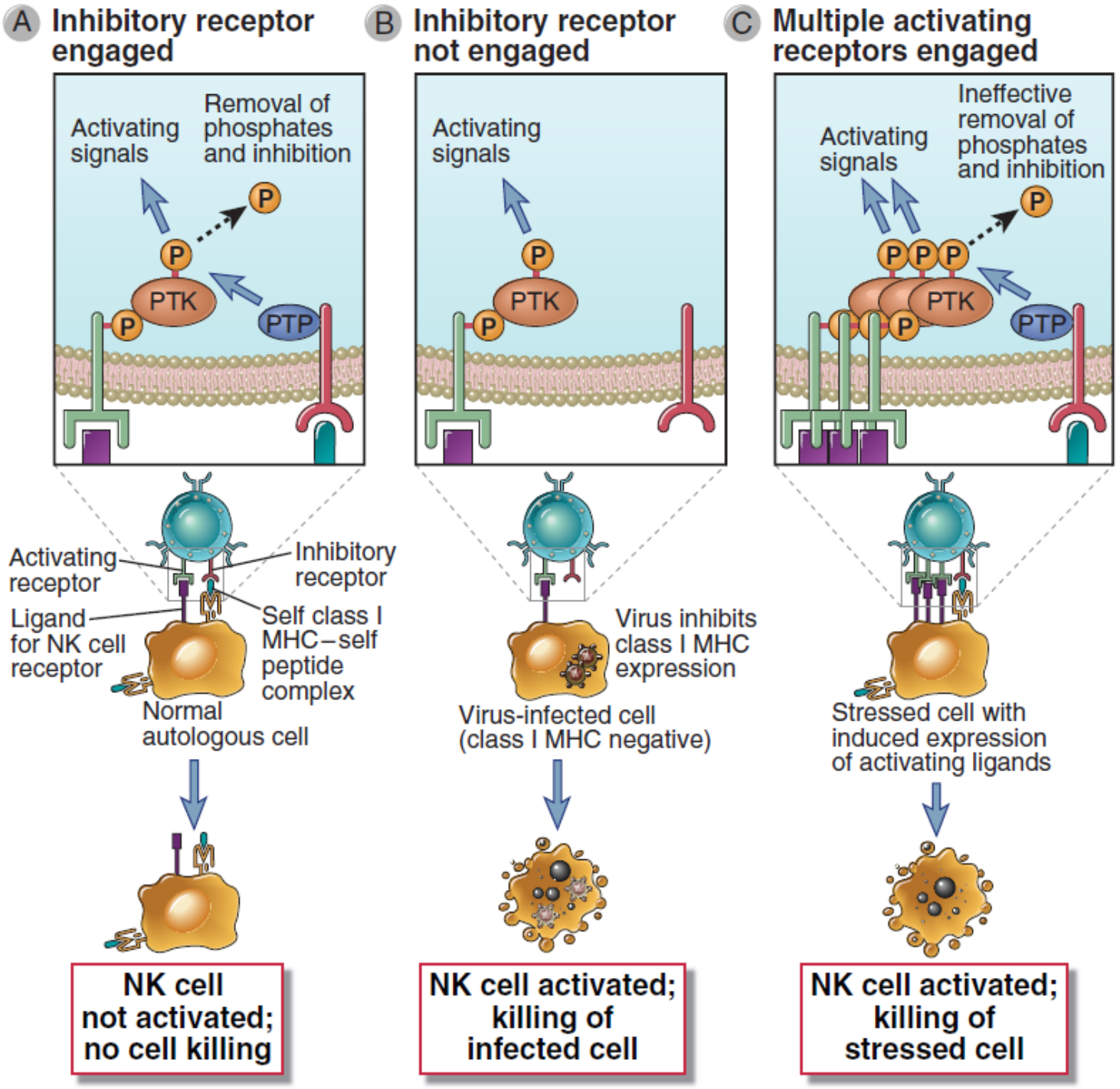
- NK cells work to **control viral infections** by secreting **IFNγ and TNFα**. IFNγ activates macrophages for phagocytosis and lysis, and TNFα acts to promote direct NK tumor cell killing.

Immunity to Intracellular pathogens / Natural k

A, Activating receptors of NK cells recognize ligands on target cells and activate protein tyrosine kinase (PTK), whose activity is inhibited by inhibitory receptors that recognize class I MHC molecules and activate protein tyrosine phosphatases (PTP). NK cells do not efficiently kill class I MHC–expressing healthy cells.

B, If a virus infection or other stress inhibits class I MHC expression on infected cells and induces expression of additional activating ligands, the NK cell inhibitory receptor is not engaged and the activating receptor functions unopposed to trigger responses of NK cells, such as killing of target cells and cytokine secretion.

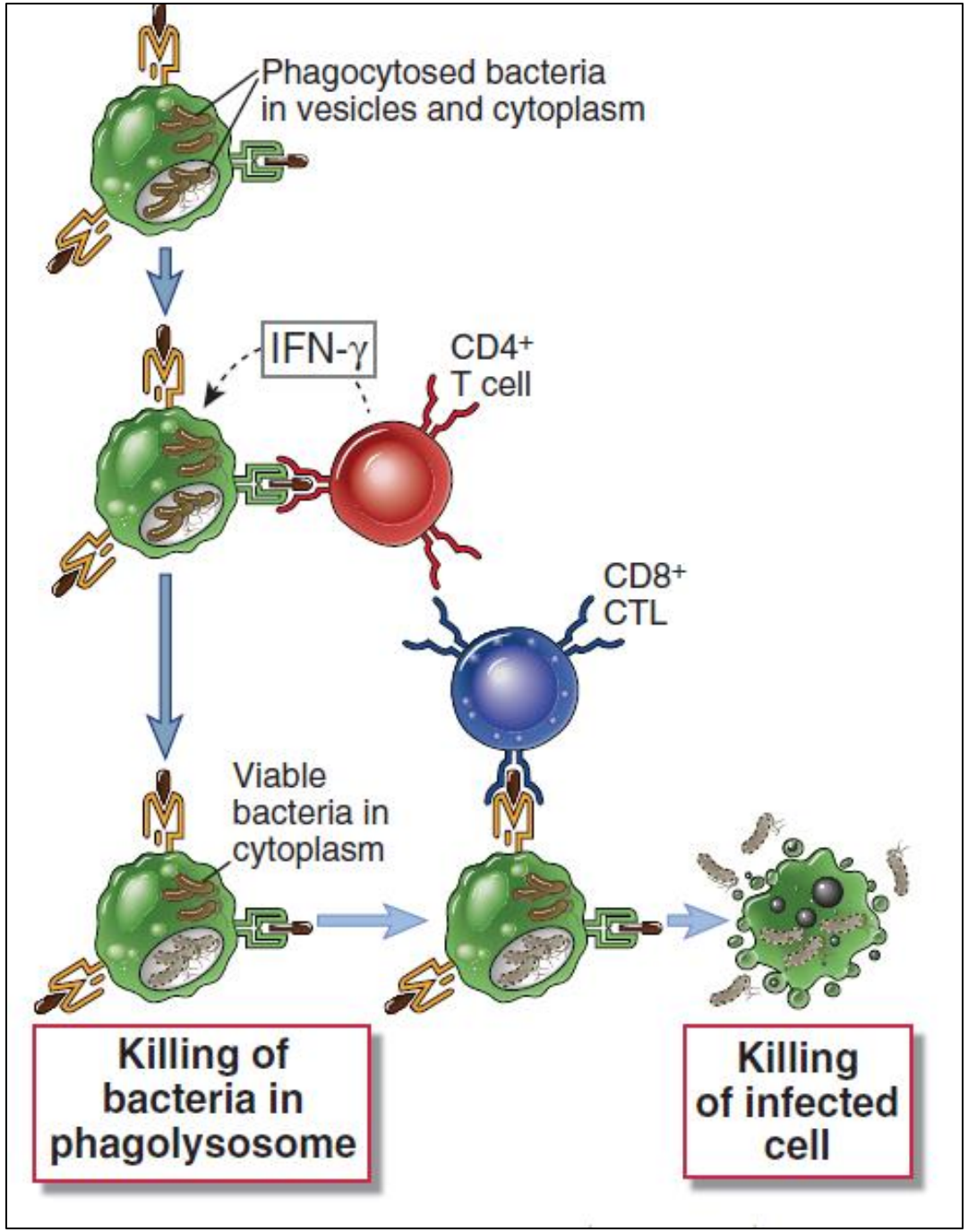
C. Cells stressed by infection or neoplastic transformation may express increased amounts of activating ligands, which bind NK cell activating receptors and induce more tyrosine phosphorylation than can be removed by inhibitory receptor associated phosphatases, resulting in killing of the stressed cells



Immunity to Intracellular bacteria / Adaptive immunity

- Cooperation of CD4+ and CD8+ T cells in defense against intracellular microbes. Intracellular bacteria such as L. monocytogenes are phagocytosed by macrophages and may survive in phagosomes and escape into the cytoplasm.

- CD4+ T cells respond to class II MHC–associated peptide antigens derived from the intravesicular bacteria. These T cells produce IFN-γ, which activates macrophages to destroy the microbes in phagosomes. CD8+ T cells respond to class I–associated peptides derived from cytosolic antigens and kill the infected cells.



Immunity to Intracellular bacteria

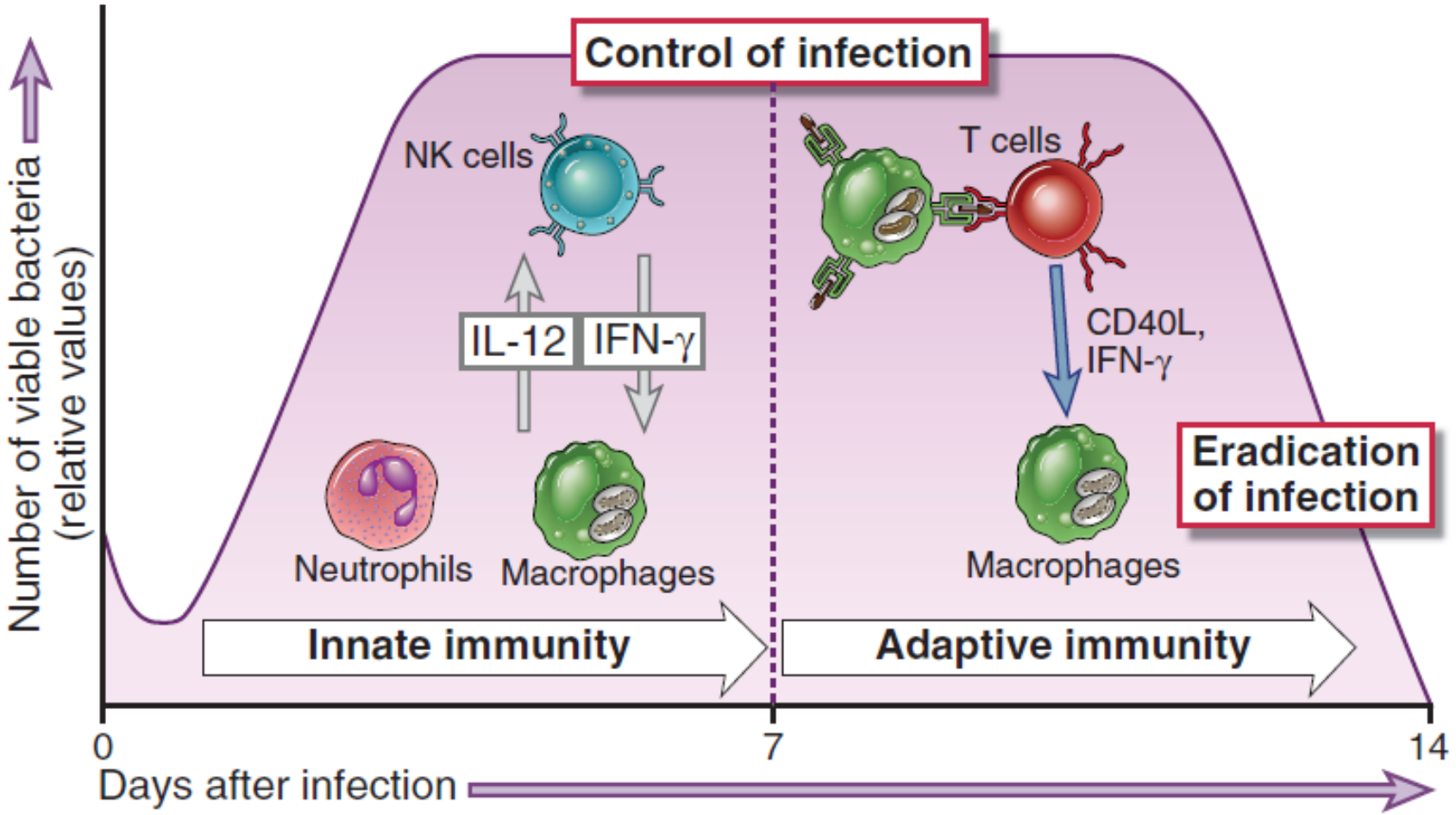


FIGURE 15–3 Innate and adaptive immunity to intracellular bacteria. The innate immune response to intracellular bacteria consists of phagocytes and NK cells, interactions among which are mediated by cytokines (IL-12 and IFN-γ). The typical adaptive immune response to these microbes is cell-mediated immunity, in which T cells activate phagocytes to eliminate the microbes. Innate immunity may control bacterial growth, but elimination of the bacteria requires adaptive immunity. These principles are based largely on analysis of *Listeria monocytogenes* infection in mice; the numbers of viable bacteria shown on the y-axis are relative values of bacterial colonies that can be grown from the tissues of infected mice. *(Data from Unanue ER. Studies in listeriosis show the strong symbiosis between the innate cellular system and the T-cell response. Immunological Reviews 158: 11-25, 1997.)*

- The major way by which the innate immune system deals with **viral infections** is to induce the expression of type I interferons. Type I interferons are a large family of structurally related cytokines that mediate the **early innate immune response to viral infections**.

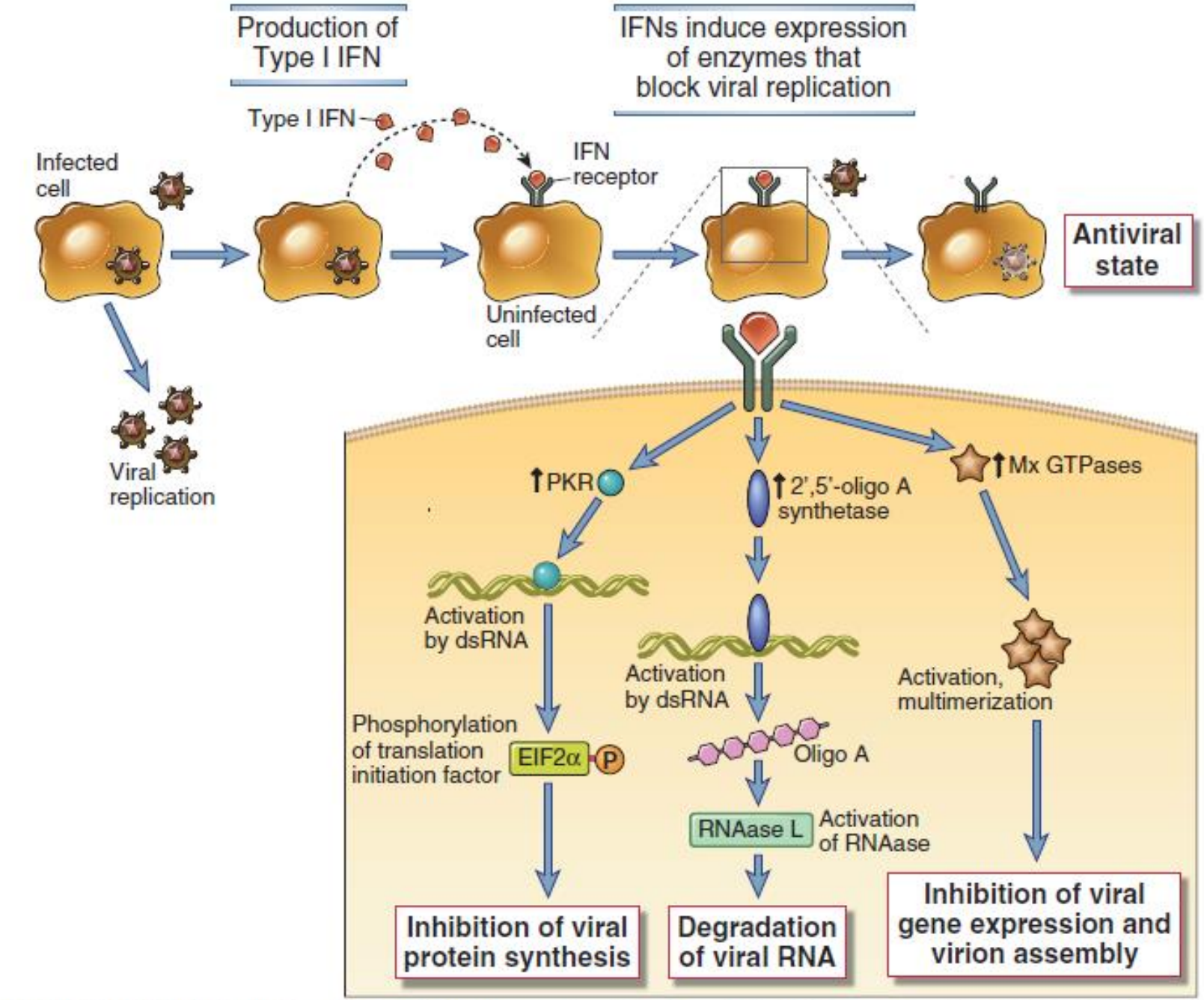
- Type I interferons, signaling through the type I interferon receptor, activate transcription of several genes that confer on the cells a resistance to viral infection, called an **antiviral state**.

- Type I interferons cause **sequestration of lymphocytes in lymph nodes**, thus maximizing the opportunity for encounter with microbial antigens.

- Type I interferons **increase the cytotoxicity of NK cells** and **CD8+ CTLs**

- **Upregulate expression of class I MHC** molecules and thereby increase the probability that virally infected cells will be recognized and killed by CD8+ CTLs.

Immunity to Intracellular pathogens / Interferons



Clinical Correlate

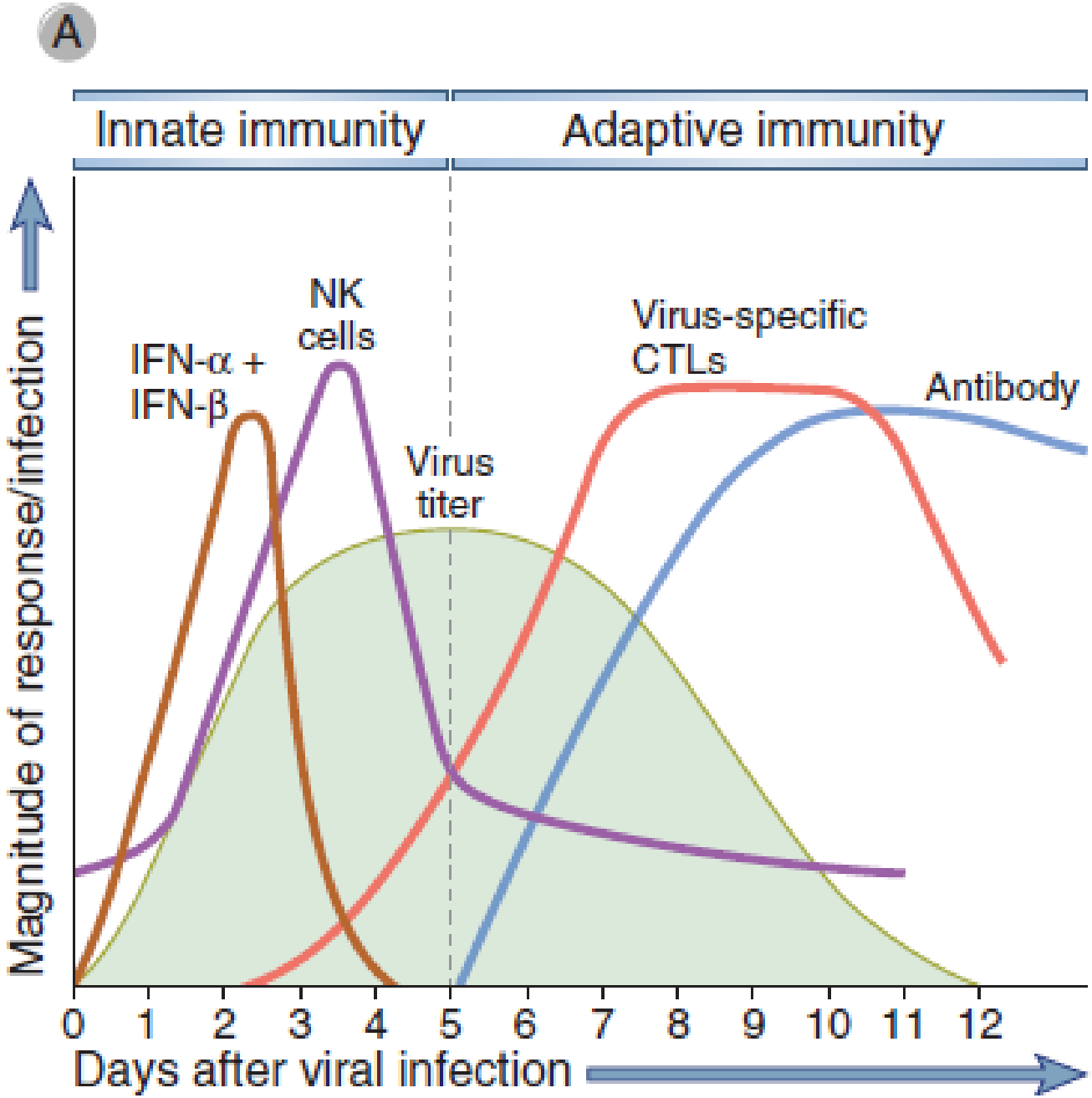
Therapeutic Use of Interferons

Since the first description of interferons (IFN) almost 50 years ago, a multitude of dramatic immunomodulatory roles have been discovered for this group of proteins. As a group, IFNs induce increases in the expression of class I and II MHC molecules and augment NK cell activity. They increase the efficiency of presentation of antigens to both cytotoxic and helper cell populations. Cloning of the genes that encode IFNs α, β, and γ has made it possible to produce sufficient amounts to make their use clinically practical.

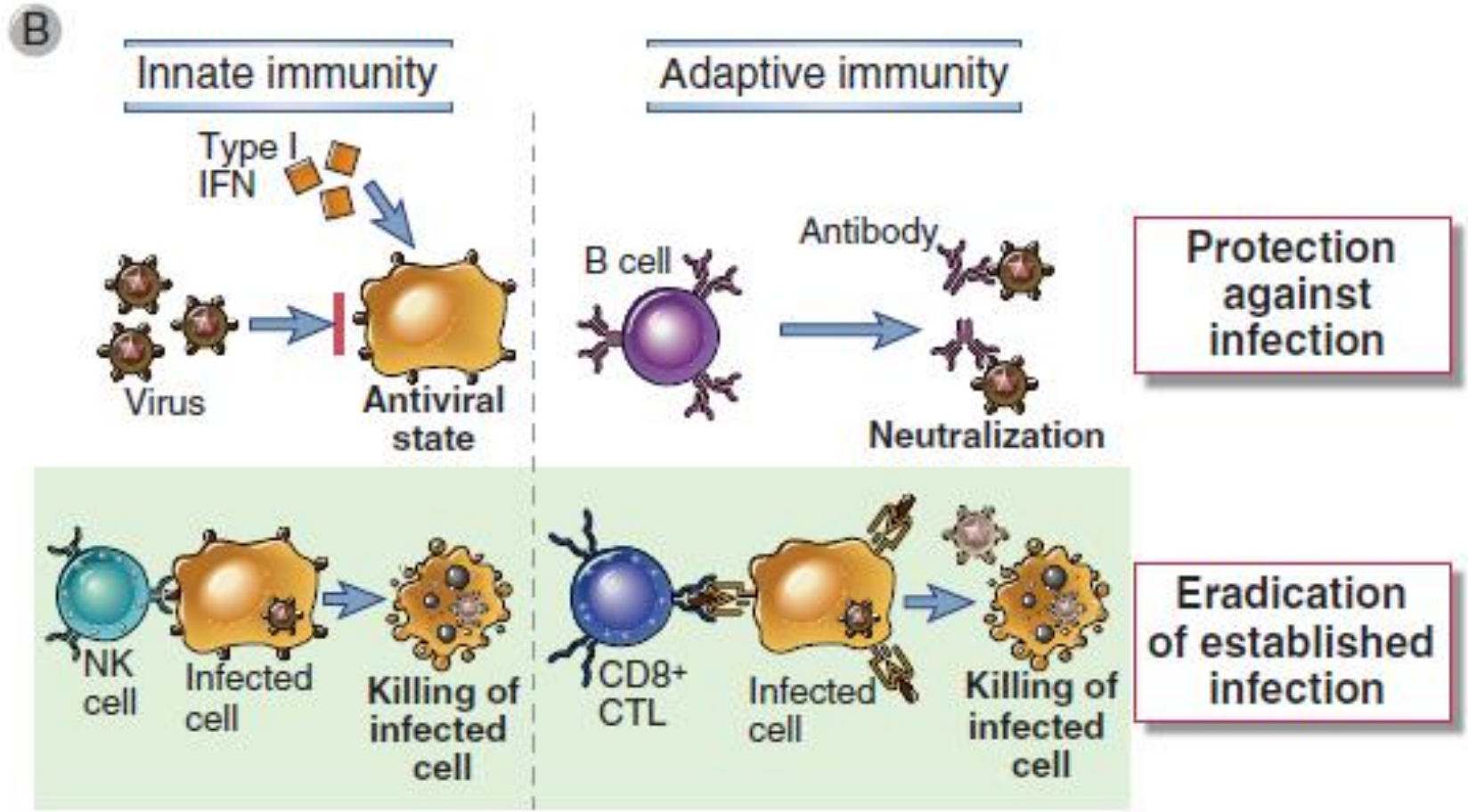
- **Interferon-α** has well-known antiviral activity and has been used in the treatment of hepatitis B and C infections. Within cancer therapy, IFN-α has shown promise in treatment of hairy B-cell leukemia, chronic myelogenous leukemia, and Kaposi sarcoma.
- **Interferon-β** was the first drug shown to have a positive effect on young adults with multiple sclerosis. Patients treated with IFN-β enjoy longer periods of remission and reduced severity of relapses.
- **Interferon-γ** is being used in the treatment of chronic granulomatous disease (CGD). This molecule is a potent inducer of macrophage activation and a promoter of inflammatory responses. Its application appears to significantly reverse the CGD patient's inability to generate toxic oxygen metabolites inside phagocytic cells.

The side effects of IFN therapy are fortunately mild and can be managed with acetaminophen. They include headache, fever, chills, and fatigue, and they diminish with continued treatment.

Immunity to Intracellular pathogens / Interferons



Innate immunity/ Innate Immunity to Intracellular Pathogens



Immunity to Fungi

- Fungal infections, also called mycoses, are important causes of morbidity and mortality in humans. Some fungal infections are endemic, and these infections are usually caused by fungi that are present in the environment and whose spores enter humans

- The principal mediators of innate immunity against fungi are neutrophils and macrophages. Patients with neutropenia are extremely susceptible to opportunistic fungal infections.

- less is known about antifungal immunity than about immunity against bacteria and viruses. This lack of knowledge is partly due to the paucity of animal models for mycoses and partly due to the fact that these infections typically occur in individuals who are incapable of mounting effective immune responses.

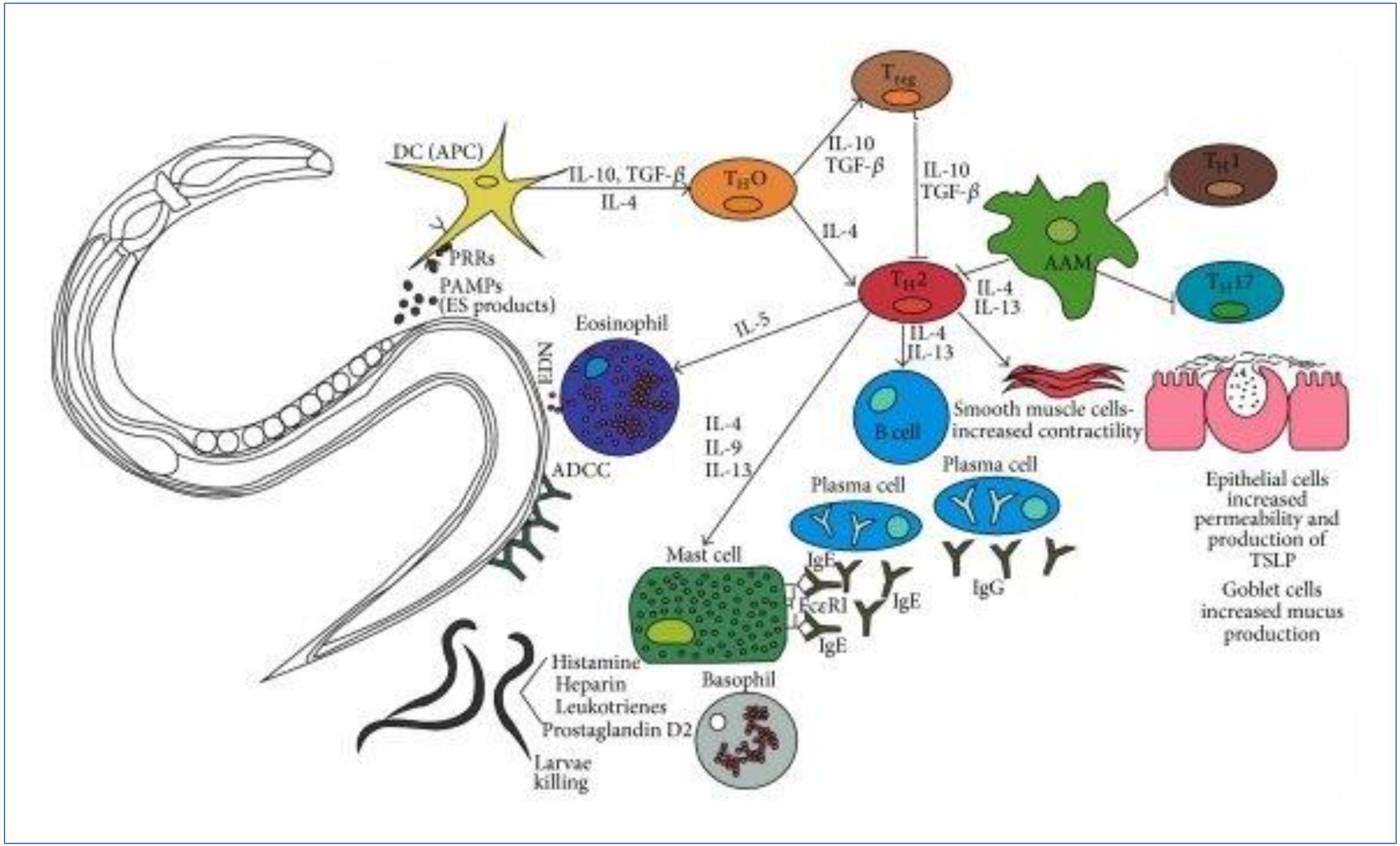
Immunity to Helminths

- **Antibodies**, **mast cells**, and **eosinophils** function with antibodies to mediate the expulsion and killing of some helminthic parasites. Helminths (worms) are too large to be engulfed by phagocytes, and their integuments are relatively resistant to the microbicidal products of neutrophils and macrophages.

- **IgE, IgG, and IgA** antibodies that coat helminths can bind to Fc receptors on eosinophils and cause the **degranulation of these cells**, releasing the **major basic protein**, a **toxic cationic protein**, present in the granules of eosinophils. Other eosinophil granule contents also aid in killing the parasites.

- **IgE antibodies** that recognize antigens on the surface of the helminths may initiate local **mast cell degranulation** through the **high-affinity IgE receptor**. Mast cell mediators may induce **bronchoconstriction and increased local motility**, contributing to the **expulsion** of worms.

Immunity to Helminths



Effector mechanisms of humoral immunity/ Antibody-Mediated Clearance of Helminths

Review Article

Harnessing the Helminth Secretome for Therapeutic Immunomodulators

Dana Ditgen,[1] Emmanuela M. Anandarajah,[1] Kamila A. Meissner,[2] Norbert Brattig,[3] Carsten Wrenger,[2] and Eva Liebau[1]

[1] *Department of Molecular Physiology, Westfälische Wilhelms-University Münster, Schlossplatz 8, 48143 Münster, Germany*
[2] *Unit for Drug Discovery, Department of Parasitology, Institute of Biomedical Science, University of São Paulo, 1374 Prof. Lineu Prestes Avenue, 05508-000 São Paulo, SP, Brazil*
[3] *Bernhard-Nocht-Institute, Bernhard-Nocht-Straße 74, 20259 Hamburg, Germany*

Correspondence should be addressed to Carsten Wrenger; cwrenger@icb.usp.br and Eva Liebau; liebaue@uni-muenster.de

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Helminths are the largest and most complex pathogens to invade and live within the human body. Since they are not able to outpace the immune system by rapid antigen variation or faster cell division or retreat into protective niches not accessible to immune effector mechanisms, their long-term survival depends on influencing and regulating the immune responses away from the mode of action most damaging to them. Immunologists have focused on the excretory and secretory products that are released by the helminths, since they can change the host environment by modulating the immune system. Here we give a brief overview of the helminth-associated immune response and the currently available helminth secretome data. We introduce some major secretome-derived immunomodulatory molecules and describe their potential mode of action. Finally, the applicability of helminth-derived therapeutic proteins in the treatment of allergic and autoimmune inflammatory disease is discussed.

Further reading:

- Cellular and Molecular Immunology. 7th Edition..

Chapter 4. Innate immunity

Chapter 15. Immunity to microbes