

# Lecture 18 and 19

## 14 Sept 2023



## Alternative pathway

- Four components: **C3, factor B, factor D, properdin**
- Triggering substances may be pathogens or non-pathogens
  - ✓ bacterial cell wall components,
  - ✓ fungi, viruses, parasites
  - ✓ immune complexes, RBCs, polymers



| <b>TABLE 7-1</b>  | <b>Initiators of the alternative pathway of complement activation</b> |
|---|---|
| <b>PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN</b>  |   |
| <b>Many strains of gram-negative bacteria</b>   |   |
| <b>Lipopolysaccharides from gram-negative bacteria</b>  |   |
| <b>Many strains of gram-positive bacteria</b>   |   |
| <b>Teichoic acid from gram-positive cell walls</b>  |   |
| <b>Fungal and yeast cell walls (zymosan)</b>  |   |
| <b>Some viruses and virus-infected cells</b>  |   |
| <b>Some tumor cells (Raji)</b>  |   |
| <b>Parasites (trypanosomes)</b>   |   |
| <b>NONPATHOGENS</b>   |   |
| <b>Human IgG, IgA, and IgE in complexes</b>   |   |
| <b>Rabbit and guinea pig IgG in complexes</b>   |   |
| <b>Cobra venom factor</b>   |   |
| <b>Heterologous erythrocytes (rabbit, mouse, chicken)</b>   |   |
| <b>Anionic polymers (dextran sulfate)</b>   |   |
| <b>Pure carbohydrates (agarose, inulin)</b>   |   |
| <p><b>SOURCE:</b> Adapted from M. K. Pangburn, 1986, in <i>Immunobiology of the Complement System</i>, G. Ross, ed., Academic Press, Orlando.</p> |   |

## Alternative pathway

1

C3 hydrolyzes spontaneously; C3b fragment attaches to foreign surface.

2

C3b  
Factor B binds C3a, exposes site acted on by factor D. Cleavage generates C3bBb, which has C3 convertase activity.

3

Binding of properdin stabilizes convertase.

4

Convertase generates C3b; some binds to C3 convertase, activating C5' convertase. C5b binds to antigenic surface.

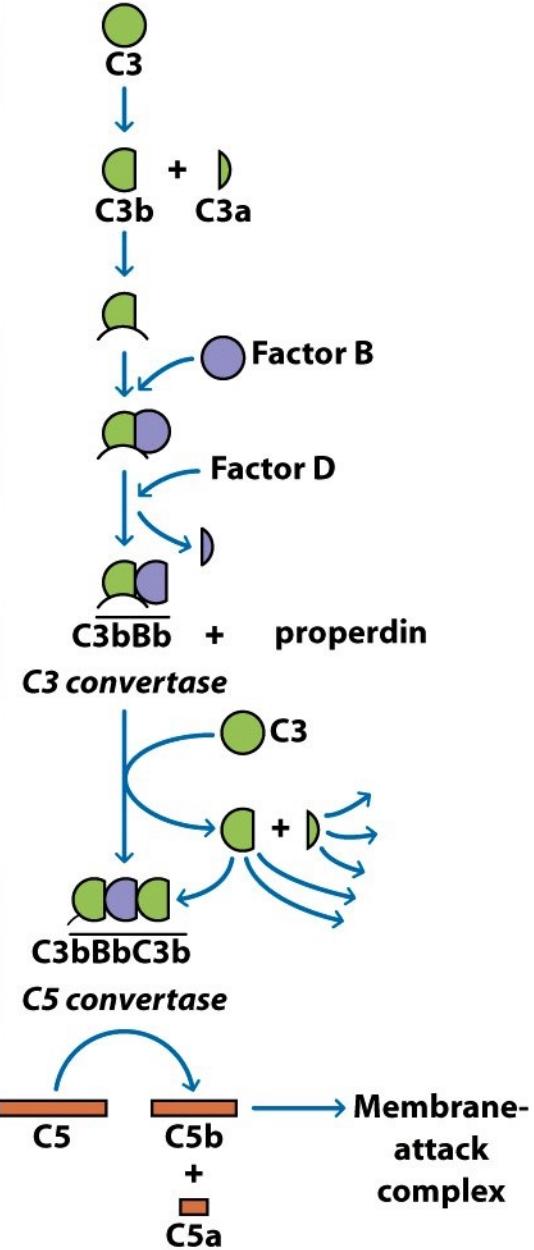


Figure 7-7

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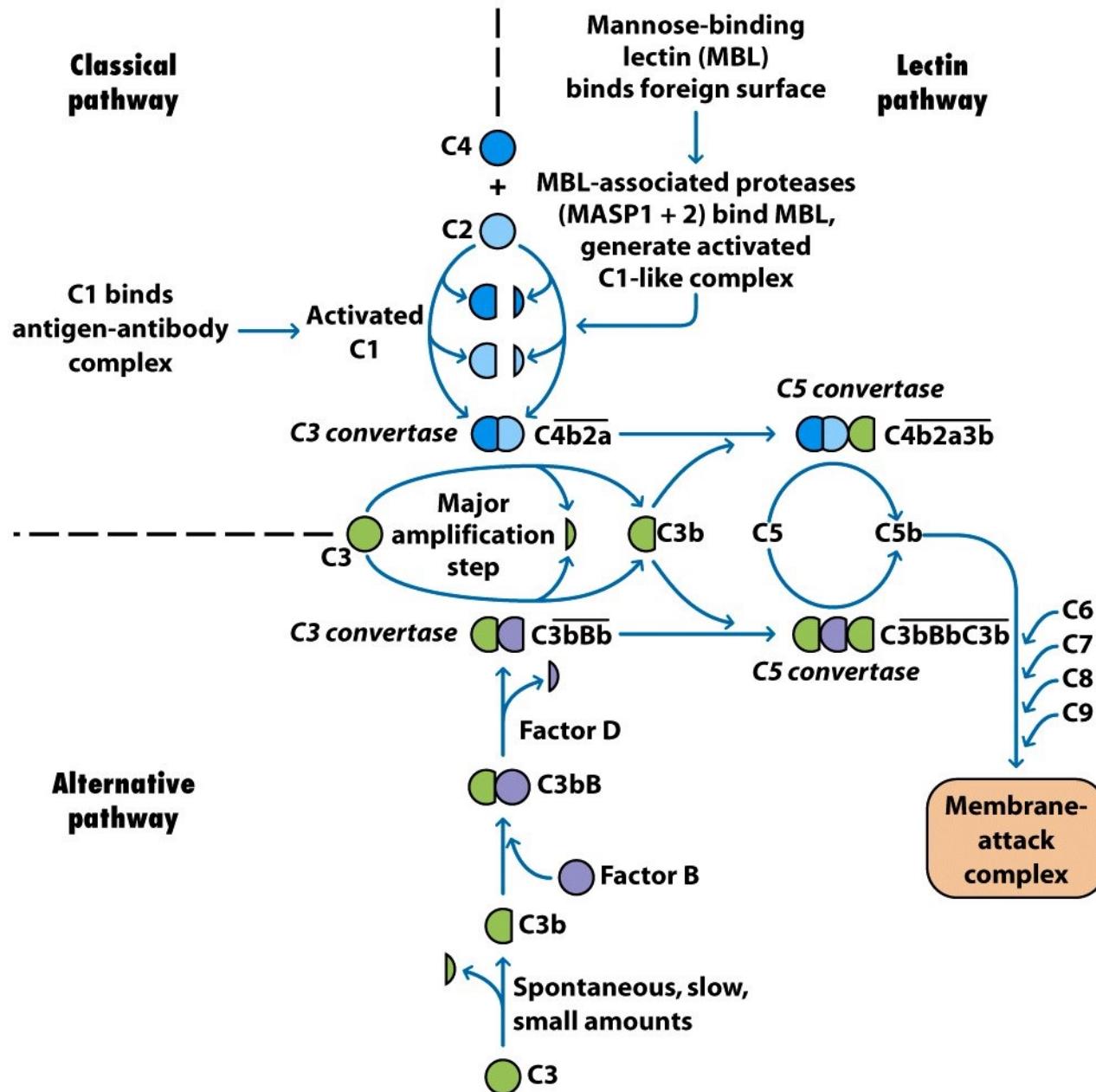
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## Lectin pathway

- Lectin is a protein that binds to carbohydrate
- MBL (mannose-binding lectin) binds to mannose on many bacterial cells
- MBL : produced by liver in acute-phase inflammatory reactions

Once MBL binds to target cell, 2 serine proteases (MASP-1, MASP-2) bind Acts like C1





p. 176

**Figure 7-9**  
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## Regulation of complement system

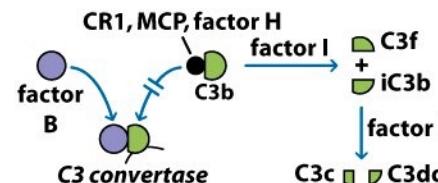
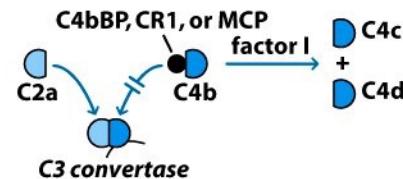
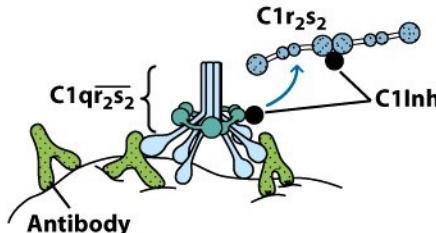
- Because it is nonspecific, several regulatory mechanisms are involved (otherwise there would be a lot of “collateral damage”)
- Many components are very labile
- Many regulatory proteins block activity through binding to target (p. 177)



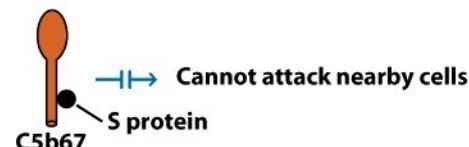
## Regulation of the Complement System

### (a) Before assembly of convertase activity

- 1 C1 inhibitor (C1Inh) binds C1<sub>r2s<sub>2</sub></sub>, causing dissociation from C1q.
- 2 Association of C4b and C2a is blocked by binding C4b-binding protein (C4bBP), complement receptor type I, or membrane cofactor protein (MCP).
- 3 Inhibitor-bound C4b is cleaved by factor I.
- 4 In alternative pathway, CR1, MCP, or factor H prevents binding of C3b and factor B.
- 5 Inhibitor-bound C3b is cleaved by factor I.



C4bBP, CR1, factor H, DAF      Dissociation of convertase; remaining C4b or C3b cleaved by factor I.

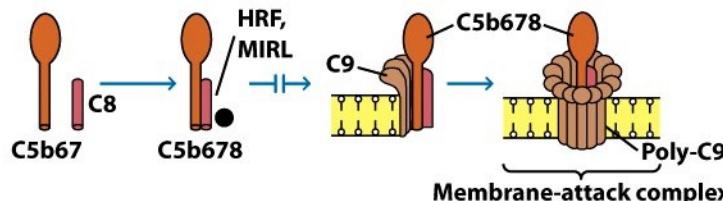


### (b) After assembly of convertase

C3 convertases are dissociated by C4bBP, CR1, factor H, and decay-accelerating factor (DAF).

### (c) Regulation at assembly of membrane-attack complex (MAC)

- 1 S protein prevents insertion of C5b67 MAC component into the membrane.
- 2 Homologous restriction factor (HRF) or membrane inhibitor of reactive lysis (MIRL or CD59) bind C5b678, preventing assembly of poly-C9 and blocking formation of MAC.



p. 178

Figure 7-10

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## Regulation of the Complement System

### Before assembly of convertase activity

- 1 C1 inhibitor (C1Inh) binds C1<sub>r2s<sub>2</sub></sub>, causing dissociation from C1q.
- 2 Association of C4b and C2a is blocked by binding C4b-binding protein (C4bBP), complement receptor type I, or membrane cofactor protein (MCP).
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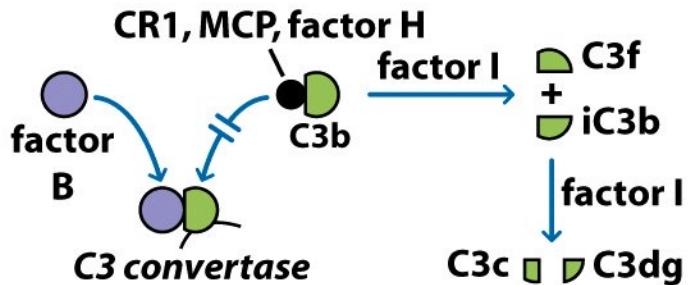
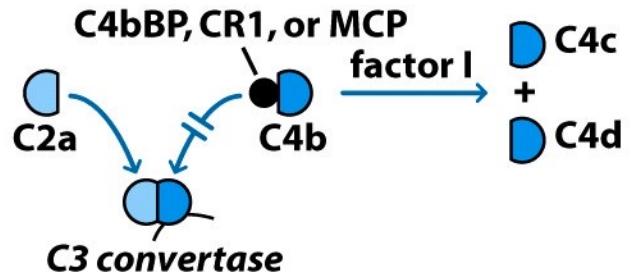
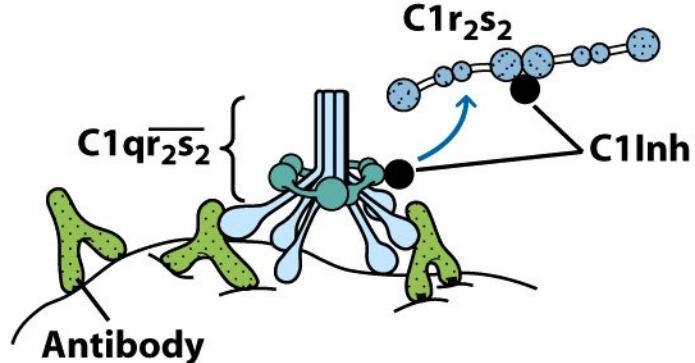
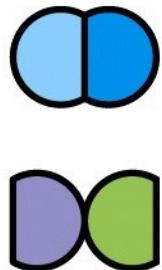


Figure 7-10a  
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# After assembly of convertase

C3 convertases are dissociated by C4bBP, CR1, factor H, and decay-accelerating factor (DAF).



**C4bBP, CR1,  
factor H, DAF**      **Dissociation of convertase;  
remaining C4b or C3b  
cleaved by factor I.**

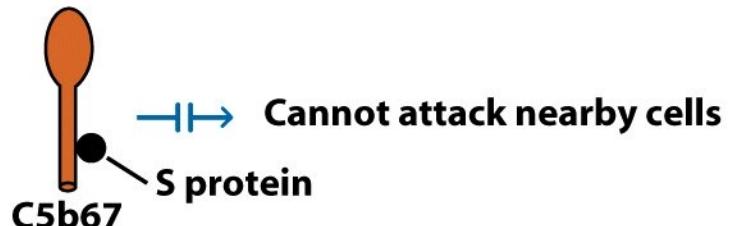
Figure 7-10b  
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## Regulation at assembly of membrane-attack complex (MAC)

1

S protein prevents insertion of C5b67 MAC component into the membrane.



2

Homologous restriction factor (HRF) or membrane inhibitor of reactive lysis (MIRL or CD59) bind C5b678, preventing assembly of poly-C9 and blocking formation of MAC.

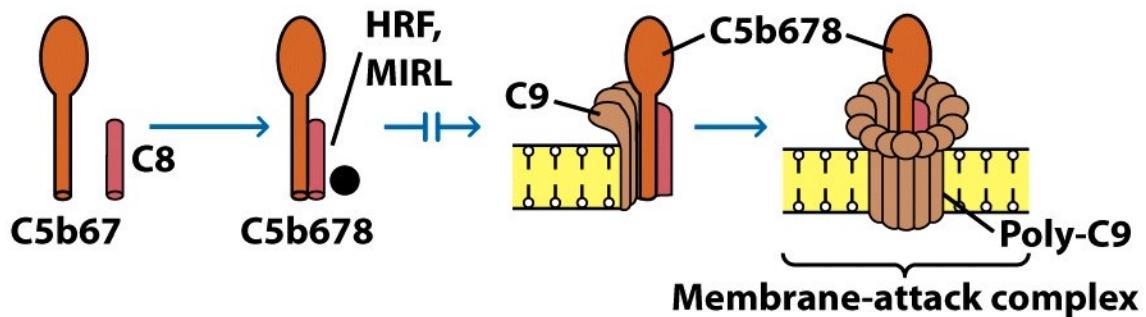


Figure 7-10c

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## Biological effects of complement activation

Complement fragments must bind to complement receptors expressed by various cells

**TABLE 7-3** Summary of biological effects mediated by complement products

| Effect   | Complement product mediating*            |
|--|--|
| Cell lysis   | C5b–9, the membrane-attack complex (MAC) |
| Inflammatory response  |  |
| Degranulation of mast cells and basophils <sup>†</sup>                                 | C3a,C4a, and C5a (anaphylatoxins)        |
| Degranulation of eosinophils   | C3a, <b>C5a</b>                          |
| Extravasation and chemotaxis of leukocytes at inflammatory site                        | C3a, <b>C5a</b> , C5b67                  |
| Aggregation of platelets   | C3a, C5a                                 |
| Inhibition of monocyte/macrophage migration and induction of their spreading           | Bb                                       |
| Release of neutrophils from bone marrow  | C3c                                      |
| Release of hydrolytic enzymes from neutrophils   | C5a                                      |
| Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils | C5a                                      |
| Opsonization of particulate antigens, increasing their phagocytosis                    | <b>C3b</b> , C4b, iC3b                   |
| Viral neutralization   | C3b, C5b–9 (MAC)                         |
| Solubilization and clearance of immune complexes                                       | C3b                                      |

\*Boldfaced component is most important in mediating indicated effect.

<sup>†</sup>Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.

**TABLE 7-4** Complement-binding receptors

| Receptor                         | Major ligands    | Activity   | Cellular distribution   |
|----------------------------------|------------------|--|---|
| CR1 (CD35)                       | C3b, C4b         | Blocks formation of C3 convertase; binds immune complexes to cells   | Erythrocytes, neutrophils, monocytes, macrophages, eosinophils, follicular dendritic cells, B cells, some T cells |
| CR2 (CD21)                       | C3d, C3dg,* iC3b | Part of B-cell coreceptor; binds Epstein-Barr virus  | B cells, follicular dendritic cells, some T cells   |
| CR3 (CD11b/18)<br>CR4 (CD11c/18) | iC3b             | Bind cell adhesion molecules on neutrophils, facilitating their extravasation; bind immune complexes, enhancing their phagocytosis | Monocytes, macrophages, neutrophils, natural killer cells, some T cells   |
| C3a/C4a receptor                 | C3a, C4a         | Induces degranulation of mast cells and basophils  | Mast cells, basophils, granulocytes   |
| C5a receptor                     | C5a              | Induces degranulation of mast cells and basophils  | Mast cells, basophils, granulocytes, monocytes, macrophages, platelets, endothelial cells                         |

\*Cleavage of C3dg by serum proteases generates C3d and C3g.

**Table 7-4**

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## □ Some bacteria can resist lysis

- Gram-positive bacteria

Some microbes produce inactivating enzymes

## □ Nucleated cells are harder to lyse

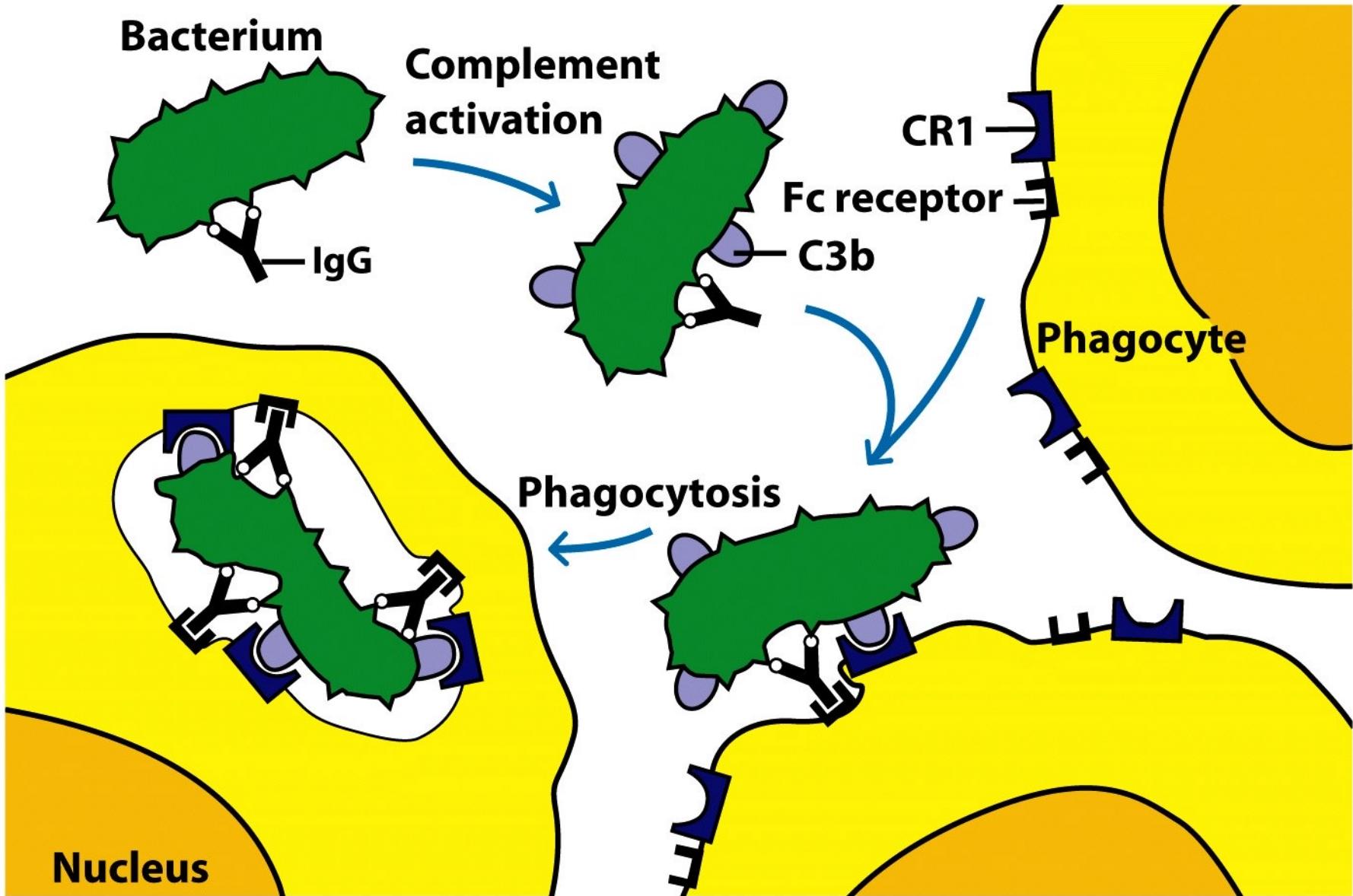
Not particularly effective against tumor cells  
(they can endocytose MAC and repair damage)



# Inflammation

- many of the released fragments help develop an inflammatory response
  - C3a, C4a, C5a- anaphylotoxins bind to receptors on mast cells and basophils; degranulation (smooth muscle contraction; capillary dilation; fluid influx)
- ✓ also play a role in blood cell chemotaxis





**Figure 7-13a**  
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# Summary

- The complement system comprises a group of serum proteins which, when activated, plays an important role in antigen clearance.
- The classical, alternative and lectin pathways have been described.
- Elaborate regulatory mechanisms are required to prevent damage to normal cells.



# Cytokines



# Chemical Structure

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- ▶ Cytokines are low molecular weight proteins,  
 $< 30 \text{ kD}$  (kilo Dalton)
- ▶ Cytokines have high affinity for receptors
- ▶ Cytokines are active in picomole amounts



# Chain of Cytokine Action

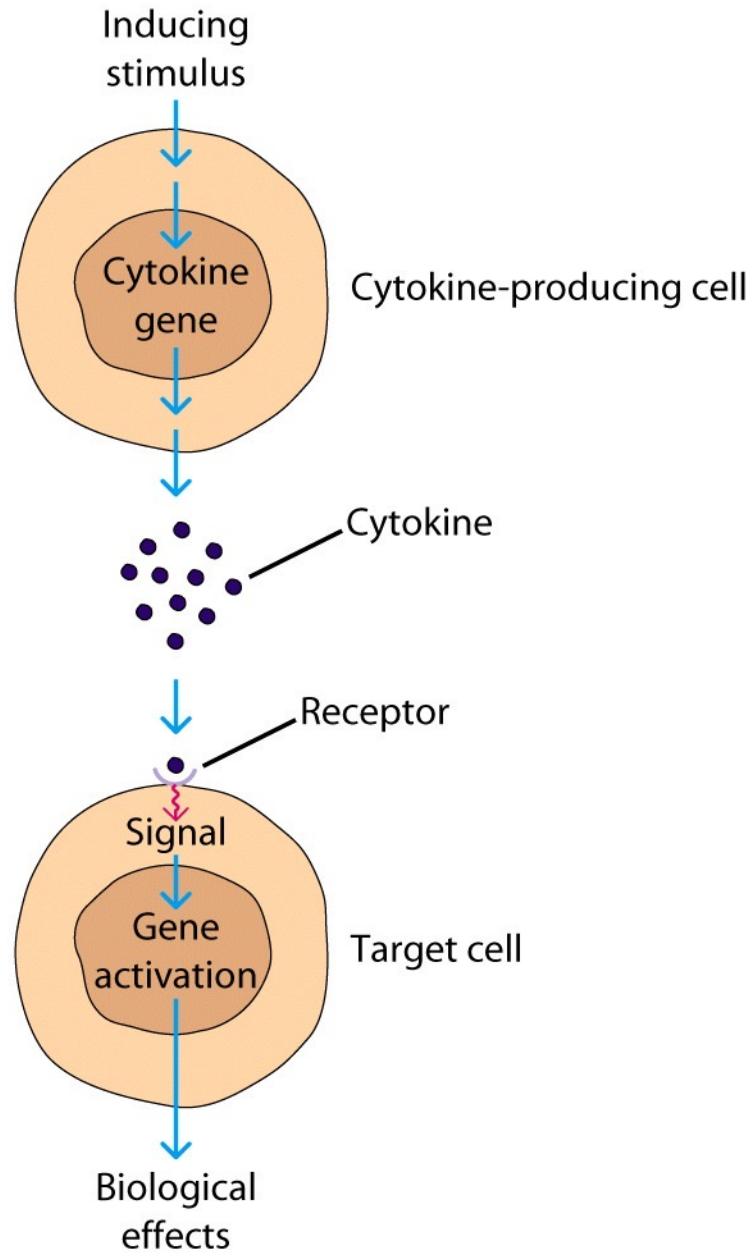
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The release and action of cytokines can be summarized in the following order:

- At first a stimulus is provided to the cytokine producing cells by engagement of PAMP and PRR and finally the biological effect may be activation/proliferation/differentiation of immune cells
- ▶ Stimulus > Cytokine-producing cell > Cytokine > Target cell > Receptor > Biological effect(s)



Stimulus > Cytokine-producing cell > Cytokine > Target cell > Receptor > Biological effect(s)



# Names of Cytokines

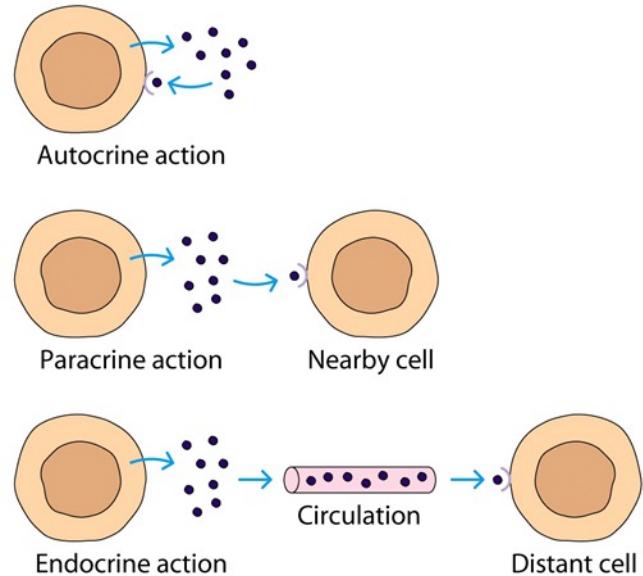
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- ▶ Source
  - ▶ e.g., Lymphokines
- ▶ Function
  - ▶ e.g., Chemokines
- ▶ Intercellular action
  - ▶ e.g., Interleukins



# Action of Cytokines

- ▶ Autocrine
  - ▶ Affects the generating cell (self)
- ▶ Paracrine
  - ▶ Affects cells in the immediate vicinity
- ▶ Endocrine
  - ▶ Affects cells remote from the secreting cell



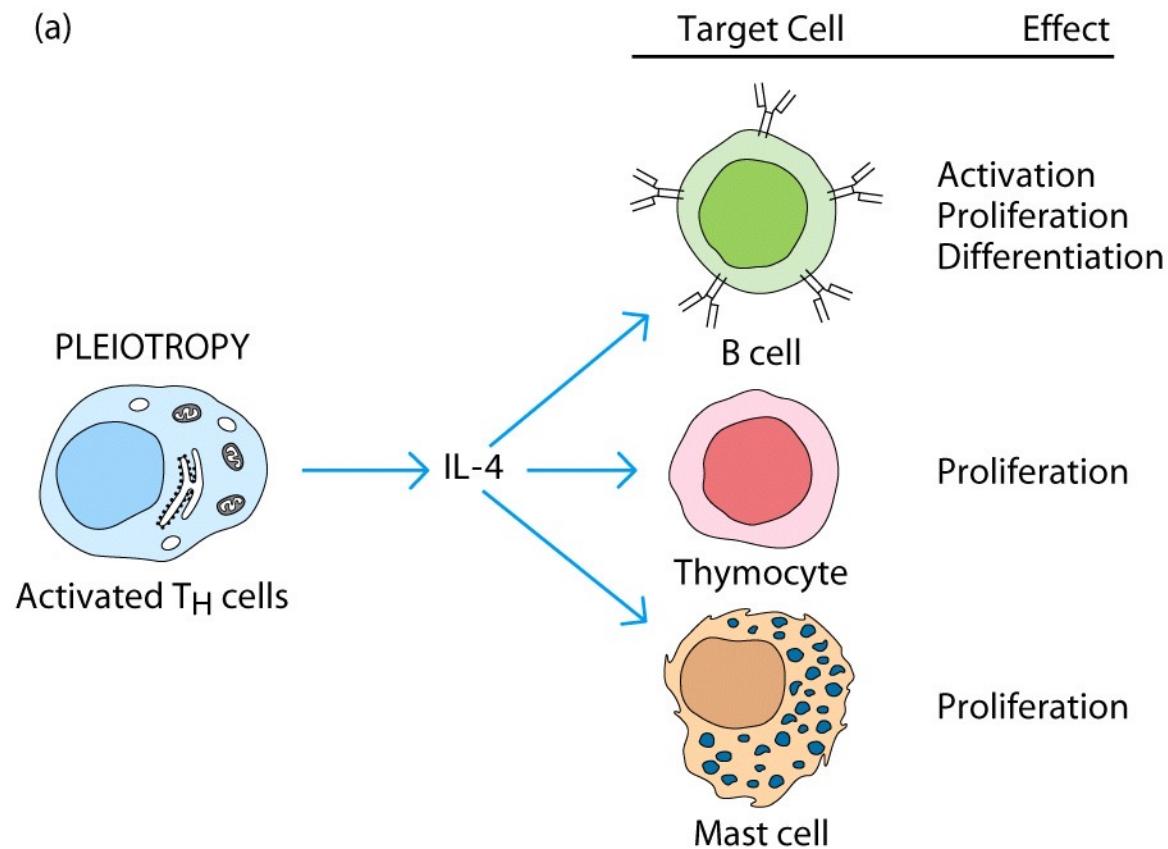
# Action of Cytokines

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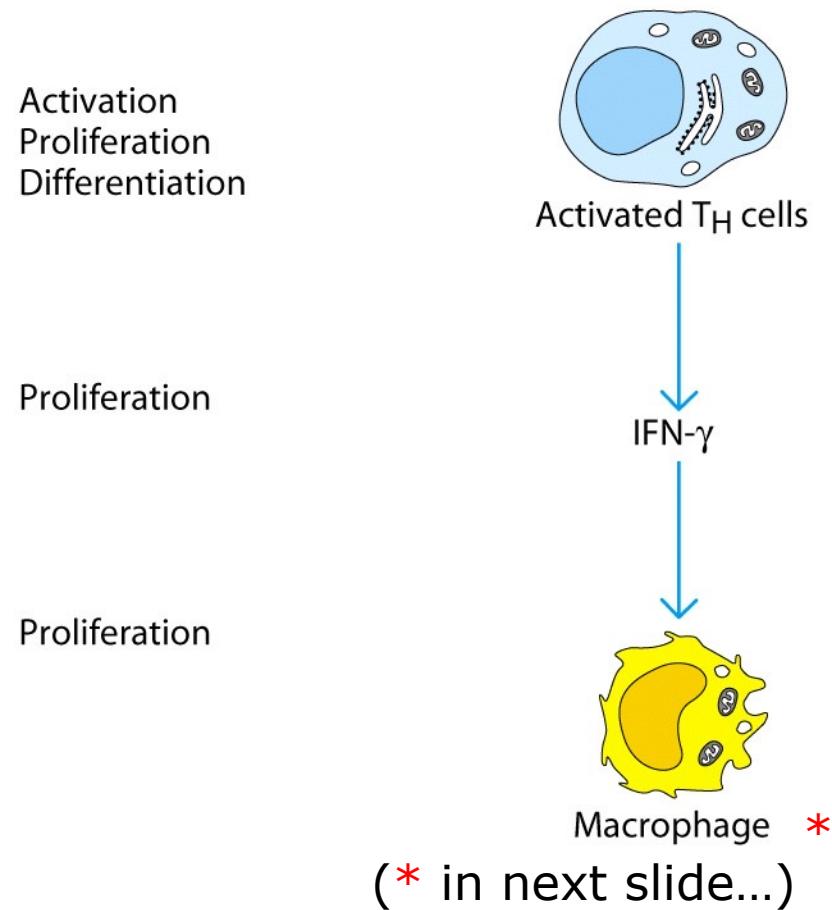
- ▶ **Pleiotropy**
  - ▶ Affects multiple cell types
- ▶ **Redundancy**
  - ▶ Multiple cytokines affects cells of the same type
- ▶ **Synergy**
  - ▶ Cytokines acting in concert on the same cell
- ▶ **Antagonism**
  - ▶ Competing actions
- ▶ **Cascading**
  - ▶ Cytokines acting sequentially



(a)

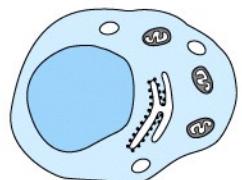


(b) CASCADE INDUCTION

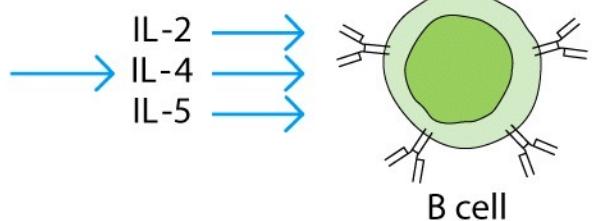


(\* in previous slide...)

REDUNDANT

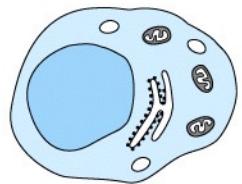


Activated T<sub>H</sub> cells

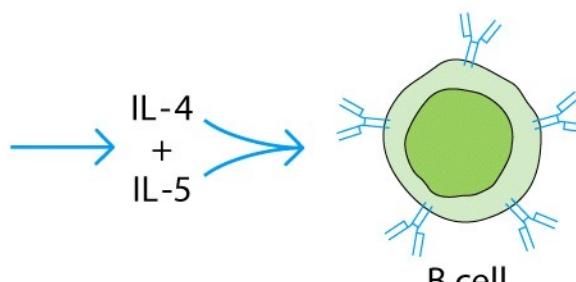


Proliferation

SYNERGY

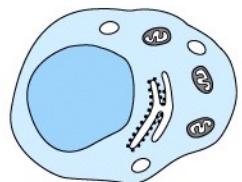


Activated T<sub>H</sub> cells

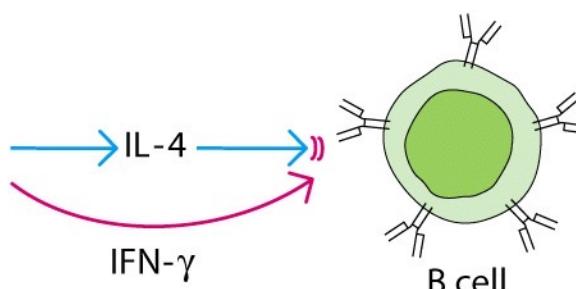


Induces class switch to IgE

ANTAGONISM



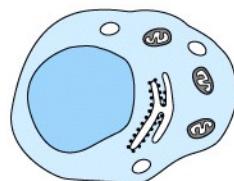
Activated T<sub>H</sub> cells



Blocks class switch to IgE induced by IL-4

Macrophage

IL-12



Activated T<sub>H</sub> cells

↓

IFN- $\gamma$ , TNF, IL-2, and other cytokines



# Cytokine-generating Cells

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- ▶ Innate immunity
  - ▶ Macrophages
  - ▶ Endothelial cells
  - ▶ Fibroblasts
- ▶ Adaptive immunity
  - ▶ T lymphocytes
  - ▶ Macrophages
  - ▶ NK cells



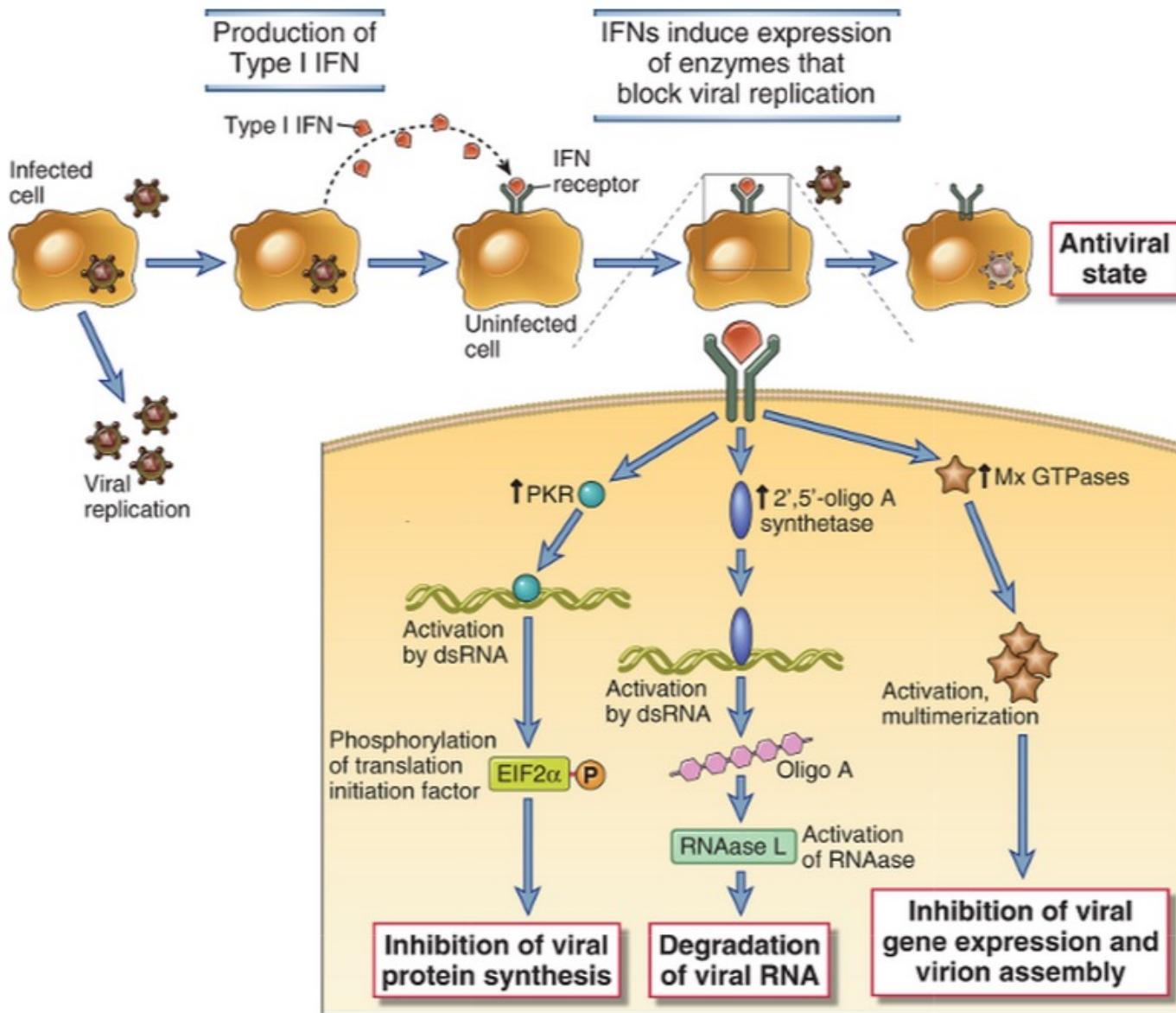
**TABLE 12-1** Functional groups of selected cytokines<sup>1</sup>

| Cytokine*   | Secreted by**   | Targets and effects  |
|---|---|--|
| SOME CYTOKINES OF INNATE IMMUNITY                                       |   |  |
| Interleukin 1 (IL-1)  | Monocytes, macrophages, endothelial cells, epithelial cells | Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins)  |
| Tumor necrosis factor- $\alpha$ (TNF- $\alpha$ )                        | Macrophages   | Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation |
| Interleukin 12 (IL-12)  | Macrophages, dendritic cells                                | NK cells; influences adaptive immunity (promotes T <sub>H</sub> 1 subset)  |
| Interleukin 6 (IL-6)  | Macrophages, endothelial cells                              | Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage)  |
| Interferon $\alpha$ (IFN- $\alpha$ )<br>(this is a family of molecules) | Macrophages   | Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells   |
| Interferon $\beta$ (IFN- $\beta$ )                                      | Fibroblasts   | Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells   |
| SOME CYTOKINES OF ADAPTIVE IMMUNITY                                     |   |  |
| Interleukin 2 (IL-2)  | T cells   | T-cell proliferation; can promote AICD. NK cell activation and proliferation; B-cell proliferation   |
| Interleukin 4 (IL-4)  | T <sub>H</sub> 2 cells; mast cells                          | Promotes T <sub>H</sub> 2 differentiation; isotype switch to IgE   |
| Interleukin 5 (IL-5)  | T <sub>H</sub> 2 cells                                      | Eosinophil activation and generation   |
| Interleukin 25 (IL-25)  | Unknown   | Induces secretion of T <sub>H</sub> 2 cytokine profile   |
| Transforming growth factor $\beta$ (TGF- $\beta$ )                      | T cells, macrophages, other cell types                      | Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgE; inhibits macrophages                                |
| Interferon $\gamma$ (IFN- $\gamma$ )                                    | T <sub>H</sub> 1 cells; CD8 $^{+}$ cells; NK cells          | Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation   |

<sup>1</sup>Many cytokines play roles in more than one functional category.

<sup>2</sup>Only the major cell types providing cytokines for the indicated activity are listed; other cell types may also have the capacity to synthesize the given cytokine.

<sup>3</sup>Also note that activated cells generally secrete greater amounts of cytokine than unactivated cells.



Type I interferons (IFN- $\alpha$ , IFN- $\beta$ ) are produced by virus-infected cells in response to intracellular TLR signaling and other sensors of viral RNA. Type I interferons bind to receptors on neighboring uninfected cells and activate JAK-STAT signaling pathways, which induce expression of genes whose products interfere with viral replication. Type I interferons also bind to receptors on infected cells and induce expression of genes whose products enhance the cell's susceptibility to CTL-mediated killing. PKR, double stranded RNA-activated protein kinase.

**Figure 11-14** Biologic actions of IFN- $\gamma$ .

IFN- $\gamma$  activates phagocytes and APCs and induces B cell switching to some immunoglobulin isotypes (that often bind complement and Fc receptors on phagocytes and are distinct from the isotypes induced by IL-4). The T<sub>H</sub>1-inducing effect of IFN- $\gamma$  may be indirect, mediated by increased IL-12 production and receptor expression.

