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IMMUNE RESPONSE IN INFLAMMATION AND REPAIR

I. What is a cytokine?

A. Soluble proteins induced during an immune or inflammatory response.

B. Synthesized by, and act upon, a variety of cell types.

C. Induction of cytokine synthesis leads to an adaptive alteration in the physiology of these cells enabling them to resolve a microbiologic or physicochemical insult with minimal damage to the host.

D. However, dysregulation of cytokine production does occur, and is often a central feature in the pathogenesis of many diseases.

II. Classification of cytokines

A. Interleukins (**IL**) 1-26

B. Interferons (**IFN**)-α, β, and γ

C. Colony stimulating factors (**CSF**)
1. GM-CSF (granulocyte-macrophage-**CSF**)
2. M-CSF (macrophage-**CSF**)
3. G-CSF (granulocyte-**CSF**)

D. Tumor necrosis factor-alpha (**TNF**-α)

E. Lymphotoxin (**TNF**-β)

F. Transforming growth factor-beta (**TGF**-β)

G. Platelet-derived growth factor (**PDGF**).

III. Cell sources of cytokine production

Although low level cytokine production may occur constitutively (homeostatic processes) high-level induction occurs in response to an immunologic or inflammatory insult:

A. T cells

1. Both $CD4^+$ and $CD8^+$ T cells release cytokines upon interaction with antigen expressed on an antigen-presenting cell (APC) in association with MHC molecules.
2. APCs include B cells, macrophages, and dendritic cells.
3. Two types of $CD4^+$ and $CD8^+$ T cells are recognized based on their profile of secreted cytokines:
   a. Type 1-secrete **IL-2, IFN-γ**, and lymphotoxin
   b. Type 2-secrete **IL-4, IL-5, IL-6, IL-10**, and **IL-13**

B. Natural killer (NK) cells

1. NK cells release cytokines (esp. **IFN-γ**) upon stimulation with
   a. **IL-12** and **TNF-α** from activated macrophages
   b. bacteria and their products
   c. virus-infected cells
   d. tumor cells.

C. Macrophages

1. Macrophages release cytokines upon interaction with bacteria and their products (e.g. lipopolysaccharide [LPS]) and antigen-antibody complexes.
2. Important macrophage-derived cytokines include **IL-1, IL-6, TNF-α, IL-10, IL-12, IL-15,** and **TGF-β**.

D. Mast cells

1. Mast cells can release cytokines after activation by
   a. cross-linking of bound IgE by allergen
   b. complement components (i.e. C3a and C5a-anaphylatoxins)
2. Important mast cell-derived cytokines include the Type 2 cytokines such as **IL-4** and **IL-5.**

E. B cells

1. B cells may also release cytokines after interaction with antigen and T cells.
2. An important B cell-derived cytokine is **IL-6**.

IV. Role of cytokines in pathophysiologic processes

A. Acute inflammation in response to pyogenic bacteria

1. Pyogenic bacteria are rapidly dividing and can overwhelm a host within days; thus the host response must likewise be rapid.
2. Macrophages, NK cells, platelets, and certain types of T cells are important sources of cytokines during the acute response.
3. Macrophages, neutrophils, antibody, and the acute phase reactants are the major anti-bacterial effector mechanisms during the acute phase of the response.
4. Fibroblasts and macrophages are recruited later during the reparative phase of the response.
5. The following cytokines play important roles in orchestrating the acute inflammatory response to pyogenic bacteria:
   a. **IL-1**, **IL-6**, **TNF**-α-These cytokines, largely produced by macrophages mediate the acute phase response; IL-6 from macrophages and B cells also stimulates antibody production.

   b. **Interleukin-1 (IL-1)**

      *stimulate hepatic protein synthesis (acute phase proteins) These proteins have been highly conserved during evolution and hence are believed to play a fundamental role in the host response to acute injury:

      1) serum amyloid A (secondary amyloidosis)
      2) C-reactive protein (promote phagocytosis)
      3) complement factors
      4) clotting factors
      5) metalloprotein synthesis (bind serum iron and zinc necessary for bacterial cell growth
      6) $_1$ macroglobulin and ceruloplasmin (oxygen scavengers)

      *endothelial cells

      1) stimulate release of PGI_2 and PGE_2 (both potent vasodilators)
      2) adherence of neutrophils, monocytes, and lymphocytes (induction of membrane adhesion proteins)
      3) synthesis of platelet activating factor (PAF-aggregation of platelets and release of histamine and serotonin)
      4) increase in surface thrombogenicity.

      *bone marrow

1) release of neutrophils.

*CNS

1) fever-2^0 to hypothalamic PGE_2 synthesis-reduced replication of bacteria and viruses, augmented function of immunocompetent cells.
2) increased slow wave sleep-conservation of energy and metabolic resources;
3) decrease in appetite (minimize food seeking activity).

*pituitary

1) release of ACTH

*hemodynamic

2) shock

c. **Tumor necrosis factor-alpha (TNF-α", cachectin)**

* **TNF-** induces many of the same effects as **IL-1**; However, unlike **IL-1**, **TNF-α** is not an immunostimulant, whereas **IL-1**, in contrast to **TNF-** , is not cytotoxic for tumor cells
* **TNF-α** and **IL-1** are structurally distinct and have different receptors but share many properties, likely due to the fundamental importance of the acute phase response. **IL-1** and **TNF-α** may act synergistically.
* Chronic stimulation of **TNF-α** synthesis plays a key role in the cachexia or wasting syndrome seen in patients with malignant neoplasms.

d. **Interleukin-6 (IL-6)**

1) **IL-6** shares properties of **IL-1** and **TNF-α** for mediating acute phase response
2) a major role of **IL-6** is to stimulate the maturation of B cells into antibody-producing cells (plasma cells).

e. **IL-8-produced by macrophages;** an important neutrophil chemotactic and activation factor.

f. **IL-3, GM-CSF**, and **M-CSF**-Released by macrophages and/or T cells; recruit hematopoietic elements from the bone marrow and participate in their activation.

g. **IL-12** and **IFN-γ-IL-12** released by macrophages stimulates **NK** cells and T cells to release **IFN-γ IFN-γ** released by **NK** cells and T cells activates macrophages for enhanced bacterial killing.

h. **TGF-β and PDGF**-released by macrophages and platelets; stimulate fibroblasts for enhanced growth and collagen and matrix deposition during the reparative phase of the response.

i. **Tissue Repair**-During the process of eradication of the microorganism and removal of cellular debris, especially by neutrophils and macrophages, the process of repair evolves. In addition to reepitheliazation, repair involves the influx of granulation tissue (capillaries and activated fibroblasts) with attendant synthesis of collagen and other matrix proteins to replace permanently lost tissue. **This process is regulated by TGF-β and PDGF**

j. **Transforming growth factor-beta (TGF-β)**-primary mediator of fibrogenesis:

1) stimulates fibroblast chemotaxis
2) stimulates the synthesis of collagen and fibronectin by fibroblasts.
3) inhibits collagen degradation.

4) a potent inhibitor of proliferation and differentiation of many cell types, including macrophages, favoring the down-modulation of the inflammatory response during the process of repair.

k. **Platelet-derived growth factor (PDGF)**-Like **TGF**-β, **PDGF** plays a major role in fibrogenesis:
1) stimulate fibroblast proliferation
2) stimulate fibroblast chemotaxis
3) stimulate collagen synthesis by fibroblasts.
4) Also stimulates monocyte chemotaxis and proliferation and thus, unlike **TGF**-β, has pro-inflammatory properties.

B. Immediate hypersensitivity (including allergic disease) and the host response to nematode parasites-Nematode worms are large multicellular, slow-growing organisms, unlike the rapidly growing pyogenic bacteria or the intracellular pathogens (see "C" below). Hence, the strategy adopted for their control and/or eradication is distinct.

1. The primary effectors which mediate these related responses are
   a. antibodies of the **IgE** class
   b. mast cells
   c. eosinophils
   d. macrophages
2. The primary cytokines are **IL-3**, **IL-4**, **IL-5**, **IL-10** and **IL-13** (Type 2 cytokines). **IL-13**, has many of the same effects as **IL-4**.
3. IgE
   a. production dependent upon the synthesis of **IL-4**, but may also occur independently in response to **IL-13**.
   b. binds to high affinity IgE receptors (FcERII) on mast cells and basophils and, upon cross-linkage by antigen, mediates degranulation.
   c. binds to low affinity IgE receptors (FcERII) on eosinophils and macrophages thereby mediating antibody-dependent cellular cytotoxicity (ADCC) against parasites; FcERII expression up-regulated by **IL-4**.
4. Mast cell
   a. growth stimulated by **IL-3, IL-4**, and **IL-10**
   b. cross-linking of IgE on mast cell and basophil FcERII by antigen, induces degranulation with release of inflammatory mediators and synthesis of Type 2 cytokines (Mast cell degranulation also induced by C3a and C5a and physical trauma)
   c. Primary mast cell mediators:
      1) histamine: vasodilation, increased vascular permeability, and bronchial smooth muscle contraction.
      2) eosinophil chemotactic factor of anaphylaxis (ECF-A)
      3) neutrophil chemotactic factors
   d. *Secondary* mast cell mediators:
      1) leukotrienes C4 and D4-increased vascular permeability and bronchial smooth muscle contraction.
      2) leukotriene B4-chemotactic for neutrophils, eosinophils, and monocytes.
      3) prostaglandin D2-vasodilation and bronchial smooth muscle contraction, and increased mucus secretion.
      4) platelet activating factor (PAF)-platelet aggregation and release of histamine.
5. Eosinophil
   a. growth critically dependent upon the production of **IL-5**
   b. attracted to sites of tissue reaction by mast cell-derived eosinophil chemotactic factor (ECF-A); **IL-4** induces endothelial adhesion protein which selectively binds eosinophils and thus facilitates entry into tissue.

c. mediates IgE-dependent cellular cytotoxicity (via FcERII) against parasites

d. mediates damage to host epithelial cells, in part, through the release of major basic protein (MBP). Eosinophils appear to be largely responsible for the progressive tissue damage which may occur in chronic bronchial asthma.

6. IL-10- A major macrophage inhibition factor; may serve to control inflammation during allergic and anti-nematode responses.

C. <u>Cell-mediated immune response to intracellular pathogens</u>

1. Many non-pyogenic bacteria, protozoans, and viruses are intracellular pathogens.
2. The phagocytic macrophage is a primary intracellular target, although many parenchymal cell types may become infected. These infections evolve more slowly than those mediated by the pyogenic bacteria.
3. Macrophage activation for enhanced killing of phagocytosed organisms, and recruitment of cytotoxic cells ($CD8^+$ T cells and NK cells) for killing of pathogen-infected parenchymal cells are key elements of the immune response, whereas antibody production at best plays a secondary role; hence the term "cell-mediated" immunity.
4. **IL-12** and the Type 1 cytokines (**IL-2**, **IFN**-γ, and lymphotoxin) are the key cytokine mediators of this response.
5. Sequence of events:
   a. processing and presentation of microbial antigen by dendritic cells and macrophages, with subsequent release of **IL-12** and TNF- by activated macrophages and **IL-2** by T cells.
   b. **IL-12**, **TNF**- α and **IL-18** stimulates initial **IFN**-γ synthesis by **NK** cells; **IL-12** and **IL-18** stimulates **IFN**-γ synthesis by specialized T cells; **IL-2** stimulates **NK** and T cell proliferation.
   c. Presence of **IL-12, IL-18** and **IFN**-γ early during immune response directs later, more sustained production of Type 1, and <u>not</u> Type 2, cytokines by activated $CD4^+$ and $CD8^+$ T cells.
   d. **IFN**-γ activates macrophages for enhanced killing
   e. Lymphotoxin mediates direct killing of pathogen-infected host cells.
   f. <u>Conclusion</u>-Cell-mediated immunity thus occurs through combined action of activated macrophages, and activated and expanded NK cells and Type 1 $CD4^+$ and $CD8^+$ T cells.