Chapter 13: Cytokines

Definition: secreted, low-molecular-weight proteins that regulate the nature, intensity and duration of the immune response by exerting a variety of effects on lymphocytes and/or other cells.

- Cytokines bind to specific receptors on target cells.

- Originally were called lymphokines because they were initially thought to be produced only by lymphocytes. Then monokines because they were secreted by monocytes and macrophages. Then interleukin because they are produced by some leukocytes and affect other leukocytes. The term “cytokine” is now used more widely and covers all of the above.

- Don’t forget chemokines, they are also considered cytokines.

Cytokines can act in an:

- Autocrine (same cell),
- Paracrine (close proximity),
- Endocrine (long distance)

1. Cytokines are pleiotropic ... one cytokine can have different effects on different cells.

2. Cytokines can be redundant ... different cytokines can have the same effects.

3. Cytokines can synergize with each other.
4. Cytokines can **antagonize** each other.

<table>
<thead>
<tr>
<th>ANTAGONISM</th>
<th>Target Cell</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated T cells</td>
<td>IFN-γ</td>
<td>B cell blocks class switch to IgG1 induced by IL-4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgG2a or IgG3</td>
</tr>
</tbody>
</table>

5. **Cascade effect**, cytokines can stimulate the production of other cytokines.

6. Cytokines can influence the expression of **cytokine receptors**.

<table>
<thead>
<tr>
<th>Receptor transduction</th>
<th>Up regulation</th>
<th>Down regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive IL-2 RECEPTOR</td>
<td>IL-4 α β IL-5</td>
<td>Negative IL-2 RECEPTOR</td>
</tr>
</tbody>
</table>

7. Cytokines play key roles in regulating hematopoiesis, innate immunity and acquired immunity.

SO...cytokines can have many effects, depending on:

- The target cell
- The state of differentiation/activation of the target cell
- The presence or absence of other cytokines

**Sandwich ELISA**

Cytokine levels in serum or in tissue culture supernatants can be measured with a Sandwich ELISA assay.
There are many cytokines, including...

- IL-1
- IL-2
- IL-3
- IL-4
- IL-5
- IL-6
- IL-7
- IL-8
- IL-9
- IL-10
- IL-11
- IL-12
- IL-13
- IL-15
- IL-16
- IL-17
- IL-18
- IL-19
- IL-20
- IL-21
- IL-22
- IL-23
- IFN-α
- IFN-β
- IFN-γ
- TNF-α
- TNF-β
- TGF-β1
- M-CSF
- G-CSF
- GM-CSF

Best way to learn about cytokines... is by their action!!!

Four Structural Families:

- Hematopoietin Family (IL-2, IL-4)
- Interferon Family (IFN-α, β, γ)
- Chemokine Family
- Tumor necrosis family

Based on structural homology, there are five major cytokine receptor families:

- Ig superfamily receptors
- Class I receptors (Hematopoietin receptor family)
- Class II receptors (Interferon receptor family)
- TNF receptor family
- Chemokine receptors
- TGF receptor family
Three subfamilies of the class I cytokine receptor family (hematopoietin)

LIGANDS

- IL-2
- IL-21
- IL-3
- IL-23
- IL-4
- IL-27
- IL-5
- GM-CSF
- IL-6
- G-CSF
- IL-7
- OSM
- IL-9
- LIF
- IL-11
- CNTF
- IL-12
- Growth hormone
- IL-13
- Prolactin
- IL-15
Cytokine receptors

- Sharing of signal transducing molecules explains the **redundancy** and **antagonism** exhibited by some cytokines!
**IL-2 Receptor**

- Composed of 3 subunits: α, β, and γ chains
- IL-2 receptor is present in 3 forms: low, medium, and high affinity
- The low affinity (monomeric, IL-2Rα), medium affinity (dimeric, IL-2Rαβ), and high affinity (trimeric, IL-2Rαβγ)
- Binding component: α chains
- Transducing components: β and γ chains.

*A* Boy in the Bubble → the absence of a functional IL-2R-α chain protein.

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**Competition of ligand-binding chains of different receptors for a common subunit.**

This can result in antagonistic effects between cytokines.

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**A number of cytokine receptors signal via the JAK/STAT pathway.**

These include the receptors for IL-2, IL-3, IL-4, IL-6, IL-10, IL-12 and IFN-γ.

**Cytokine receptor subunits are associated with JAK kinases.**

1. Binding of cytokine causes dimerization of receptors and activation of JAK kinases.

2. Activated JAK kinases phosphorylate receptor sites and create docking sites for STAT molecules.

**JAK = Janus Kinase OR Just Another Kinase**

**STAT = Signal Transducers and Activators of Transcription**

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**Different receptors associate with different JAK/STAT combinations.**

**TABLE 12-2 STAT AND JAK INTERACTION WITH SELECTED CYTOKINE RECEPTORS DURING SIGNAL TRANSDUCTION**

<table>
<thead>
<tr>
<th>Cytokine receptor</th>
<th>JAK</th>
<th>STAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>JAK1 and JAK2</td>
<td>Stat1*</td>
</tr>
<tr>
<td>IFN-α/β</td>
<td>JAK1 and Tyk-2</td>
<td>Stat2</td>
</tr>
<tr>
<td>IL-2</td>
<td>JAK1 and JAK3</td>
<td>Stat5</td>
</tr>
<tr>
<td>IL-3</td>
<td>JAK2</td>
<td>Stat5</td>
</tr>
<tr>
<td>IL-4</td>
<td>JAK1 and JAK3</td>
<td>Stat5*</td>
</tr>
<tr>
<td>IL-6</td>
<td>JAK1 (and sometimes others)</td>
<td>Stat3</td>
</tr>
<tr>
<td>IL-10</td>
<td>JAK1 and Tyk-2*</td>
<td>Stat3</td>
</tr>
<tr>
<td>IL-12</td>
<td>JAK2 and Tyk-2*</td>
<td>Stat4*</td>
</tr>
</tbody>
</table>

*Despite its name, Tyk-2 is also a Janus kinase.

Other pathways may also be involved in cytokine signaling.

**Cytokine Antagonists**

**Action:**
1) Blocking the receptor (IL-1Ra), and
2) Binding to the cytokine (IL-2, IFN-γ)

### Table 12-3: Viral mimics of cytokines and cytokine receptors

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leporipoxivirus (a myxoma virus)</td>
<td>Soluble IFN-γ receptor</td>
</tr>
<tr>
<td>Several poxviruses</td>
<td>Soluble IFN-γ receptor</td>
</tr>
<tr>
<td>Vaccinia, smallpox virus</td>
<td>Soluble IL-1β receptor</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>IL-10 homolog</td>
</tr>
<tr>
<td>Human herpesvirus-8</td>
<td>IL-6 homolog; also homologs of the chemokines MIP-1 and MIP-2</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Three different chemokine receptor homologs, one of which binds three different soluble chemokines (RANTES, MCP-1, and MIP-1α)</td>
</tr>
</tbody>
</table>

**Helper T cells**

Helper T cells can be divided into two main types - Th1 and Th2 - with distinct patterns of cytokine secretion.

**Table 12-4: Cytokine secretion and principal subsets of Th1 and Th2 cytokines.**

<table>
<thead>
<tr>
<th>Cytokine secretion</th>
<th>Th1</th>
<th>Th2</th>
</tr>
</thead>
<tbody>
<tr>
<td>cytokines</td>
<td>IFN-γ γ γ γ</td>
<td>IL-4, IL-5, IL-6, IL-10, IL-13, IL-15, GM-CSF, IL-13</td>
</tr>
<tr>
<td>Functions</td>
<td>IL-1 R, IFN-γ</td>
<td>IL-4, IL-5, IL-6, IL-10, IL-13, IL-15, GM-CSF, IL-13</td>
</tr>
</tbody>
</table>

**Th1/Th2 differentiation**

- IL-4 promotes Th2 differentiation.
- IFN-γ and IL-12 promotes Th1 differentiation.

**T1 cells**

- IL-12R = β1, β2

- Th1 cells
  - IL-12R = β1, β2
  - IFN-γ → T-Bet
  - IL-2 → GATA-3
  - GM-CSF → neutrophils, eosinophils, macrophages, DCs

- Th2 cells
  - IL-4 → GATA-3
  - IL-13 → Th2

**Note:**
- T-Bet is a T-cell-specific transcription factor that promotes Th1 differentiation.
- GATA-3 is a transcription factor that promotes Th2 differentiation.
- GM-CSF (granulocyte-macrophage colony-stimulating factor) is a cytokine that stimulates the proliferation and differentiation of granulocytes, macrophages, and monocytes.
Cytokine cross-regulation

- IFN-γ (Th-1) inhibits proliferation of Th-2
- IL-4 and IL-10 (Th-2) inhibits proliferation of Th-1 by decreasing IL-12 production
- INF-γ (Th-1) promotes IgG2a production and decreases IgG1 and IgE by B cells
- IL-4 (Th-2) promotes production of IgE and IgG1 by B cells and decreases IgG2a.

Transcriptional Regulation of Cytokines

Transcription factors can be used to characterize Th lineage

Cytokine & Diseases

- **Bacterial Septic Shock**
  - Due to several Gram (-) bacteria
  - Stimulation of Macrophages & DCs by LPS → ↑ TNF-α, IL-1β
  - Drop in blood pressure, fever, diarrhea, systemic blood clotting in various organs, increased respiratory rate, capillary leakage, etc

- **Bacterial Toxic Shock**
  - Caused by superantigens (wide variety of toxins)
  - Activation of T cells → ↑ cytokines from T cells and activated MØ (↑ TNF-α, IL-1β)

- **Infectious Diseases**
  - Leprosy, Chagas Disease (↓ IL-2Rα).

Relative predominance of Th1 vs Th2 helper T cells can influence the course of infectious disease (Mycobacterium leprae)

<table>
<thead>
<tr>
<th>Th1 activity</th>
<th>Th2 activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculoid</td>
<td>Lepromatous</td>
</tr>
<tr>
<td>IL-2</td>
<td>IL-4</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>IL-5</td>
</tr>
<tr>
<td>TNF-β</td>
<td>IL-10</td>
</tr>
</tbody>
</table>

Tuberculoid: ↑ CMI (granulomas) - No RIP
Lepromatous: ↑ HI (dissemination) - RIP

Neuroendocrine regulation

IL-1, IL-6 and TNF-α can induce production of glucocorticoids by acting on the hypothalamic-pituitary-adrenal (HPA) axis.
Glucocorticoid hormones can influence ongoing immune responses - particularly suppressing inflammatory responses.

Sex hormones also influence immune responses - e.g. females tend to be more prone to autoimmune disorders than males.

Stress may suppress Th1 immune responses.

- Outcome of CD4 T cells
  - Th0 will give rise to at least 3 Th populations
  - Th populations differentiated based on cytokine production
  - Treg

Macrophage activation by Th1 Cells:
- Cytotoxicity
- \( \uparrow \) Phagocytosis
- \( \uparrow \) opsonizing & complement fixing Abs
EFFECTOR ROLE OF Th2 CELLS:
1) IgE production
2) IgA production
3) Eosinophil recruitment
4) Basophil & Mast cell recruitment

Similar JAK/STAT signaling in the IL-4 receptor.