Blood: 4-10,000 WBC per 1 μL

Lymphocytes - 10-15 %
(T-, B- & NK cells)

Granulocytes - 35-80 %
PMNs 35-80 %
Eos 0-8 %
Basos 0-2 %

Monocytes - 0-15 %
(Macs & DCs)

How did they get there?
Where are they going?
What regulates them?

Think Cytokines, Chemokines & Growth Factors !!
What are cytokines and chemokines?

- Small (10-30 kDa), usually secreted and usually glycosylated peptides.
- They bind specific, high affinity (e.g., $K_d$ of $10^{-10}$-$10^{-12}$ M) receptors found on target cells.
- Expression of cytokines and their receptors is usually tightly regulated (i.e., temporally/transiently and geographically).
- Cytokine receptors define the specific type of biological response cytokines stimulate.
- Other more anachronistic terms include monokines and lymphokines. The term interleukin (IL) is now commonly used (e.g., IL-1, IL-2, …).

What do cytokines, chemokines and growth factors do?

- They direct the development, maturation, localization, interactions, activation and life span of immune cells.
- Thus they play an essential role in regulating both immunity adaptive and innate.
How many flavors regulate immunity?

- Growth Factors (e.g., CSF-1, SCF, RANKL, Flt3L)
- IL-1 Family (e.g., IL-1, IL-18 & “Toll-like”)
- TNF Family (e.g., TNF-α, CD40L, FasL, LT-β, BAFF)
- TGF-β Family (e.g., TGF-β)
- Chemokines (e.g., CC and CXC families)
- Type I & II Cytokines (a.k.a. Hematopoietins or Four Helix Bundle (e.g., IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-15, GM-CSF, IFN-γ, IFN-α/β)
- Also steroid hormones and prostaglandins

Cytokines & Chemokines can be grouped into functionally related Families

- There are significant functional similarities within each receptor family. The same is true for corresponding ligands (see summary).
- There are important functional differences between receptor families (see summary).
**Cytokine Receptor Classes**

**Table 11-2. Signal Transduction Mechanisms of Cytokine Receptors**

<table>
<thead>
<tr>
<th>Signal transduction pathway</th>
<th>Cytokine receptors using this pathway</th>
<th>Signaling mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK/STAT pathway</td>
<td>Type I and type II cytokine receptors</td>
<td>JAK-mediated phosphorylation and activation of STAT transcription factors (see Box 11-2)</td>
</tr>
<tr>
<td>TNF receptor signaling by TRAFs</td>
<td>TNF receptor family: TNFR-I, CD40</td>
<td>Blending of adaptor proteins, activation of transcription factors (see Box 11-1)</td>
</tr>
<tr>
<td>TNF receptor signaling by death domains</td>
<td>TNF receptor family: TNFR-II, Fas</td>
<td>Blending of adaptor proteins, caspase activation (see Box 11-1)</td>
</tr>
<tr>
<td>Receptor-associated tyrosine kinases</td>
<td>M-CSF receptor, stem cell factor receptor</td>
<td>Intrinsic tyrosine kinase activity in receptor</td>
</tr>
<tr>
<td>G protein signaling</td>
<td>Chemokine receptors</td>
<td>GTP exchange and dissociation of Gox: GTP from Gp120: Gox: GTP activates various cellular enzymes</td>
</tr>
</tbody>
</table>

Consistent with their significant functional differences, IL-4 & TNF-α, and their corresponding receptors are structurally quite distinct.
Localized release of IL-4 in the cleft between T cell and APC ("The immunological synapse")

Cytokine receptors consist of at least two chains, the cytoplasmic domains of which bind Janus kinases (JAKs).

Cytokine binding dimerizes the receptor, bringing together the cytoplasmic JAKs, which activate each other and phosphorylate the receptor.

Transcription factors (STATs) bind to the phosphorylated receptors, and are in turn phosphorylated by the activated JAKs.

Phosphorylated STATs form dimers that translocate into the nucleus to initiate new gene transcription.
**General functional properties of Cytokines and Chemokines**

- Usually stimulate transient responses.
- Function at three ranges:
  - Autocrine - “self”
  - Paracrine - adjacent cells
  - Endocrine - through circulatory system
- **Pleitropism** - one ligand activate numerous types of responses (e.g., differentiation, growth & activation).
- **Redundancy** - two or more ligands exhibit functional overlap.
- **Synergy** - two or more ligands synergize to mount a single response.
- **Antagonism** - two or more cytokines mediating opposite responses to either limit a response or achieve balance (e.g. Feedback loops).

**Properties of Cytokines**

- **Pleitropism:**
  - Activated CD4+ helper T cell
  - IL-4
  - IgE production
- **Redundancy:**
  - B cell
  - IL-2
  - IL-4
  - IL-5
  - Proliferation
- **Synergy:**
  - IFN-γ
  - Increased expression of class I MHC molecules on many cell types
- **Antagonism:**
  - Macrophage activation
  - IL-10
  - Inhibition of macrophage activation
How do we protect ourselves from microbes?
The antiviral response
For adaptive CD8 immunity

An innate response
Type I & II Cytokines mediate their biological response through the induction of genes

The JAK-STAT Signaling Paradigm

Cytokines (IFN-γ, IFN-β)
Chemokines (CXCL9, CXCL10)
Transcription (IRF1, CIITA) factors
Enzymes (iNOS)
Ag presentation (MHC-I, MHC-II, TAP)

What about during a bacterial infection, how do macrophage and DC sentries sense and respond?

Local vs Systemic Response
Local vs. systemic infection: The response to LPS

Infection vs. Sepsis
Macrophages critical in response to LPS

Biological actions of TNF

Note, this is one of the few times you can meaningfully measure serum cytokine levels!!
Cytokines and the Th1-Th2 paradigm

Dendritic Cells pick up antigen, get activated and migrate to lymph nodes

Figure 8-15 Immunobiology 6/e © Garland Science 2005
Naïve CD4 cells kiss APCs to sample them for right antigen (1<10^4-10^6)

Signal #1 (TCR) and Signal #2 (coreceptors) direct activation of naïve T-cells.
Signal 2, a.k.a co-receptor, is required for the activation of naïve T-cells. This represents an important regulatory hurdle in immune activation.

The important IL-2 “autocrine loop”
The IL-2 autocrine loop

TCR stimulation leads to induction of IL-2 and IL-2 receptor α-chain to generate high affinity receptor, culminating in potent T-cell proliferation.

Now let's digress to review how TCR signaling directs cytokine production. This is an important drug target!
TCR-mediated Signal Transduction: A Tyrosine Kinase Cascade

Cyclosporin A (CyA) & Tacrolimus (FK506) are two important drugs that block calcineurin activation → NFAT activation → IL-2 production! They are therefore potent immunosuppressive drugs.
Th1 and Th2 cells each secrete signature cytokines & chemokines that define their effector functions.

**Important Th1 effector cytokines**

- **IFN-γ** and **CD40 ligand**
  - Activates macrophage to destroy engulfed bacteria

- **Fas ligand or TNF-α**
  - Kills chronically infected cells, releasing bacteria to be destroyed by fresh macrophages

- **IL-2**
  - Induces T-cell proliferation, increasing numbers of effector cells

- **IL-3 + GM-CSF**
  - Induces macrophage differentiation in the bone marrow

- **TNF-α + TNF-β**
  - Activates endothelium to induce macrophage binding and exit from blood vessel at site of infection

- **CCL2**
  - Causes macrophages to accumulate at site of infection

**IFN-γ is the Th1 signature cytokine**
Important Th2 effector cytokines
IL-4, IL-5 & IL-6 promote humoral immunity

IL-4, the signature Th2 effector cytokine regulates B-cells, . . and IL-10 & TGF-β potently antagonize cellular immunity (think regulatory T-cells).
Polarization of CD4+ T-cells into functional Th1 and Th2 subsets

**Th1**
- IFN-γ
- IL-2
- TNF

**Th2**
- IL-4
- IL-5
- IL-6

"The Allergic Limb" "The Autoimmune Limb"

Other factors that influence the decision to become Th1 vs. Th2 include co-stimulators and nature of peptide:MHC interaction.

Failure to balance Th1 and Th2

- Defense against virus & intra-cellular pathogens
- Anti-tumor immunity
- DTH

- Defense against parasites
- Ab production & class switch

RA: Rheumatoid arthritis  MS: Multiple sclerosis  GVH: Graft-vs-Host disease

RA: Type I DM  Allergy  Asthma

DM: Diabes mellitus  GVH: Delayed-type hypersensitivity

DTH: Delayed-type hypersensitivity
Chemokines

Chemokines signal through G-protein coupled receptors making them desirable drug targets

Figure 2-40 Immunobiology, 6th. ed. (Garland Science 2005)
**Chemokine Redundancy**

<table>
<thead>
<tr>
<th>Chemokine receptor</th>
<th>Ligand (chemokine)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCR1</td>
<td>CCL5</td>
<td>Macrophage recruitment</td>
</tr>
<tr>
<td>CXCR2</td>
<td>CCL2</td>
<td>Macrophage recruitment</td>
</tr>
<tr>
<td>CXCR3</td>
<td>CCL8</td>
<td>Macrophage recruitment</td>
</tr>
<tr>
<td>CXCR4</td>
<td>CCL12</td>
<td>Macrophage recruitment</td>
</tr>
<tr>
<td>CXCR5</td>
<td>CCL16</td>
<td>Macrophage recruitment</td>
</tr>
<tr>
<td>CXCR6</td>
<td>CCL21</td>
<td>Macrophage recruitment</td>
</tr>
<tr>
<td>CXCR7</td>
<td>CXCL10</td>
<td>Macrophage recruitment</td>
</tr>
<tr>
<td>CXCR8</td>
<td>CCL23</td>
<td>Macrophage recruitment</td>
</tr>
<tr>
<td>CXCR9</td>
<td>CCL25</td>
<td>Macrophage recruitment</td>
</tr>
<tr>
<td>CXCR10</td>
<td>CXCL13</td>
<td>Macrophage recruitment</td>
</tr>
<tr>
<td>CXCR11</td>
<td>CXCL15</td>
<td>Macrophage recruitment</td>
</tr>
</tbody>
</table>

- **Secreted by Macrophage**
- **Secreted by Th1 cells**
- **Induced by IFN-γ**
- **Secreted by Th2 cells**

*Inflammatory*  
*Homeostatic*

**Leukocytes express unique sets of chemokines receptor signatures allowing them to be targeted to the appropriate tissues either homeostatically or drive an inflammatory response.**
Homeostatic targeting of lymphocytes and APCs in the spleen

<table>
<thead>
<tr>
<th>Cell</th>
<th>Chemokine receptor</th>
<th>Chemokine sensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>CCR7</td>
<td>ELC, SLC</td>
</tr>
<tr>
<td>naïve T</td>
<td>CCR7</td>
<td>ELC, SLC</td>
</tr>
<tr>
<td>naïve B</td>
<td>CXCL5</td>
<td>BLC</td>
</tr>
</tbody>
</table>

Chemokines are much more than just chemo-attractants

CXCL12

CXCR4

QuickTime™ and a GIF decompressor are needed to see this picture.
Of Note . . . . .

- Two chemokine receptors serve as co-receptors for HIV infection (CXCR4 and CCR5)

Cytokines you should know

<table>
<thead>
<tr>
<th>Type I &amp; II Cytokine Receptors (JAK-STAT)</th>
<th>IL-2 - Th1 cytokine ⇒ T-cell proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4 - Th2 cytokine ⇒ B-cell proliferation; Th2 polarization</td>
<td></td>
</tr>
<tr>
<td>IL-6 - Th2 cytokine ⇒ B-cell proliferation; Plasma cell growth</td>
<td></td>
</tr>
<tr>
<td>IL-10 - Th2 cytokine ⇒ antagonizes cellular immunity</td>
<td></td>
</tr>
<tr>
<td>IL-12 - DC cytokine ⇒ drives Th1 polarization</td>
<td></td>
</tr>
<tr>
<td>IFN-γ - Th1 cytokine ⇒ drives inflammation; Mac. Activation; DTH</td>
<td></td>
</tr>
<tr>
<td>IFN-α - All cells make this antiviral cytokine</td>
<td></td>
</tr>
<tr>
<td>IL-1 - Potent activator of inflammation &amp; innate immunity</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Toll (TLR)/IL-1 Receptors (NFκB)</th>
<th>IL-1 - Potent activator of inflammation &amp; innate immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α - All cells make this antiviral cytokine</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TNF Related Receptors (NFκB vs. Caspases)</th>
<th>TNF - Potent activator of inflammation &amp; innate immunity (arthritis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD40L - T-cell help (survival/proliferation) to B-cells</td>
<td></td>
</tr>
<tr>
<td>FasL - Induces cell death: to achieve negative selection; to terminate an immune response</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TGF-β Receptors</th>
<th>TGF-β - Antagonizes cellular immunity and promotes wound healing</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Chemokine Receptors (GPCRs*)</th>
<th>Chemokines (see Fig. 11.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory (e.g., CCL11, CCL17, CXCL2, CXCL8/9/10)</td>
<td></td>
</tr>
<tr>
<td>Non-inflammatory (i.e. homeostatic; e.g., CCL19, CCL21, CXCL12, CXCL13, S-1P)</td>
<td></td>
</tr>
</tbody>
</table>

*G-Protein Coupled Receptors - Good drug targets