Eukaryotic Gene Expression: Basics & Benefits

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Lecture 20

Regulation of gene expression by cytokines
How cytokines regulate gene expression?
What are cytokines?

Cytokines are proteins produced by diverse cell types and they primarily interact with cells of the immune system in order to regulate the body's response to disease and infection.

Cytokines also regulate several normal cellular processes in the body.

Types of Cytokines

Cytokines are a diverse group of molecules:

• colony stimulating factors (stimulate production of blood cells)

• growth and differentiation factors (function primarily in development)

• immunoregulatory and proinflammatory cytokines (interferons, interleukins and TNF-α that function in the immune system)
How Cytokines Work

Cytokines are released by various cell types into the circulation or directly into tissue.

The cytokines locate target immune cells and bind to specific cell surface receptors on the target immune cells.

The cytokine-cytokine receptor interaction activates specific signal transduction pathways leading to the modulation of activity of a number of proteins including transcription factors ultimately leading to distinct physiological responses.
Cytokines and their functions

**IL-1** activates Antigen Presenting Cell and CD4+ lymphocytes; affect the differentiation of the B-Cells and T-Cells and other immunocompetent cells and takes part in the regulation of productions of other cytokines and GMCSF (Granulocyte-Macrophage Colony-Stimulating Factor)

**IL-2** stimulates the proliferation and activation of B-Cells and T-Cells. IL-4 plays a role in the differentiation of TH2 (T Helper Type-2), in allergic responses, and in the switching of antibody types.

**IL-5** stimulates the production and maturation of eosinophils during inflammation.

**IL-8** acts as a chemotactic factor that attracts neutrophils, basophils and T-Cells to sites of inflammation.

**IL-12** is a critical linker between the innate immunity and adaptive immunity, capable of TH1 (T Helper Type-1) differentiation and IFN-Gamma release by T-Cells and NK cells.
Cytokines and their functions

**IL-10** acts to repress secretion of pro-inflammatory cytokines.

**IL-3** is a powerful activator of the hemopoietic cells. It stimulates NK-Cells and acts as a synergist with IL-4 during the induction of CD4+ lymphocyte activation process.

**IL-7** is known as the growth factor of the immature B-Cells and T-Cells. It induces apoptosis of tumor cells and causes differentiation of cells from a subgroup of acute myeloblastic leukemia.

**IL-9** stimulates the excretion of IL-2, IL-4, IL-6, IL-11, and takes part in a stimulation of cytotoxicity of T-killers and NK-Cells, inducing apoptosis.

**IL-11** is a pro-inflammatory factor, which regulates the functions of B-Cells and T-Cells. It also takes part in the induction of various killer cells' activities and acts as an autocrine factor for the proliferation of megacaryocytes.
Cytokines and their functions

The secretion of **TNF-Alpha and TNF-Beta** by TH1 cells activates macrophages, inhibits apoptosis of neutrophils and eosinophils, and induces vascular endothelial cells at the sites of infection to change the adhesion molecules they express so phagocytes circulating in the blood can bind to them.

**IFN-Alpha, IFN-Beta and IFN-Gamma** are produced in the area of infection during the early phase of immune response.

**IFN-Alpha and IFN-Beta** induce proliferation of NK-Cells and stimulate innate and adaptive immune responses that are specifically targeted to virus infections.

Upon activation, NK cells release **IFN-Gamma**, which activates macrophages to secrete cytokines that help to activate macrophages to secrete cytokines that help to activate T-Cells and promote the initiation of T-Cell responses.
Cytokines are key regulators of immune functions

They alter gene expression and promote cell growth

They either boost (IL-2) or suppress (i.e., IL-10, and TGFβ1) immunity

Type I IFN and TNFα are mediators of natural/innate immunity

IL-2, IL-4, IL-5, IL-12, IL-1 are regulators of lymphocytic growth, activation and differentiation

IFN γ is an activator of inflammatory cells

IL-3, GM-CSF, IL-7 stimulate hematopoiesis
Overproduction of Cytokines

Overproduction or inappropriate production of certain cytokines by the body can lead to diseases.

For example, in case of rheumatoid arthritis, cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha) are produced in excess and these leads to inflammation and tissue destruction.

IL-4 causes allergic diseases

IL-17 causes autoimmune diseases
A number of drugs have been developed which act by inhibiting the action of cytokines

Kineret (anakinra)

Used in the treatment of rheumatoid arthritis. It works by inhibiting IL-1 binding to its receptor.

Enbrel (etanercept)
Remicade (infliximab)
Humira (adalimumab)

These are TNF-alpha inhibitors (a.k.a. TNF blockers)

They bind TNF and prevent TNF from binding to cell surface receptors.
How do these cytokines act?

The first step in Cytokine action is their binding to cytokine receptors

CYTOKINE RECEPTORS
Cytokine Receptors

- Type I
- Type II
- Soluble
<table>
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<tr>
<th>Type I cytokine receptors</th>
<th>Type II cytokine receptors</th>
<th>Immunoglobulin superfamily</th>
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<tbody>
<tr>
<td>Type 1 interleukin receptors</td>
<td>Type II interleukin receptors</td>
<td>Interleukin 1 receptor</td>
</tr>
<tr>
<td>Erythropoietin receptor</td>
<td>Interferon-alpha/beta receptor</td>
<td>CSF1</td>
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<tr>
<td>GM-CSF receptor</td>
<td>Interferon-gamma receptor</td>
<td>C-kit receptor</td>
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<tr>
<td>G-CSF receptor</td>
<td></td>
<td>Interleukin-18 receptor</td>
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<tr>
<td>Growth hormone receptor</td>
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<tr>
<td>prolactin receptor</td>
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<tr>
<td>Oncostatin M receptor</td>
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<tr>
<td>Leukemia inhibitory factor receptor</td>
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</table>
Tumor necrosis factor receptor family
- CD27
- CD30
- CD40
- CD120
- Lymphotoxin beta receptor

Chemokine receptors
- Interleukin-8 receptor
- CCR1
- CXCR4
- MCAF receptor
- NAP-2 receptor

TGF beta receptors
- TGF beta receptor 1
- TGF beta receptor 2
Type I cytokine receptors

These are transmembrane receptors expressed on the surface of cells that recognize and respond to cytokines with four α-helical strands.

They share a common amino acid motif (WSXWS) in the extracellular portion adjacent to the cell membrane.

Members of the type I cytokine receptor family comprise different chains, some of which are involved in ligand/cytokine interaction and others that are involved in signal transduction.
Type II cytokine receptors

These are transmembrane proteins that are expressed on the surface of certain cells, which bind and respond to a select group of cytokines.

These receptors are similar to type I cytokine receptors except they do not possess the signature sequence WSXWS that is characteristic of type I receptors.

The intracellular domain of type II cytokine receptors is typically associated with a tyrosine kinase belonging to the Janus kinase (JAK) family.
**Type I cytokine receptors**

Interleukin receptors
- Interleukin-2 receptor
- Interleukin-3 receptor
- Interleukin-4 receptor
- Interleukin-5 receptor
- Interleukin-6 receptor
- Interleukin-7 receptor
- Interleukin-9 receptor
- Interleukin-11 receptor
- Interleukin-12 receptor
- Interleukin-13 receptor
- Interleukin-15 receptor
- Interleukin-21 receptor
- Interleukin-23 receptor
- Interleukin-27 receptor

Colony stimulating factor receptors
- Erythropoietin receptor
- GM-CSF receptor
- G-CSF receptor

Others
- growth hormone receptor
- prolactin receptor
- Oncostatin M receptor
- Leukemia inhibitory factor receptor

**Type II cytokine receptors**

Interferon receptors
- interferon-alpha/beta receptor
- interferon-gamma receptor

Interleukin receptors
- Interleukin-10 receptor
- Interleukin-20 receptor
- Interleukin-22 receptor
- Interleukin-28 receptor
Once the cytokine binds to its receptor
what happens?

Cytokine signal transduction pathways
THE JAK-STAT PATHWAY

The cytokine receptors lack catalytic domains (unlike RTKs).

On binding to cytokines, they interact with members of a unique family of cytoplasmic protein tyrosine kinases known as the Janus kinases (JAKs).

JAKs physically associate with the membrane-proximal region of the ligand-bound receptor, leading to their tyrosine phosphorylation and activation.

The activated JAKs phosphorylate the receptors as well as cytoplasmic proteins belonging to a family of transcription factors called the signal transducers and activators of transcription (STATs), providing a novel signaling pathway that is shared by all members of the cytokine receptor superfamily.

Adapted from: http://en.wikipedia.org/wiki/File:Jakstat_pathway.svg
Growth factor signalling (RTKs)

- Hormone
- Receptor
- Tyrosine autophosphorylation sites
- Receptor becomes tyrosine kinase

Cytokine signalling (JAK-STAT)

- Hormone
- Receptor
- Recruitment sites
- Receptor becomes docking site for tyrosine kinases
JANUS KINASES

JAK belongs to a family of non-receptor protein tyrosine kinases of approximately 130 kDa, comprising of JAK1, JAK2, JAK3 and TYK2 (non-receptor Protein Tyrosine Kinase-2).

STATs

There are seven STAT proteins: STAT1 to 6, including STAT5a and STAT5b, which are encoded by distinct genes.
<table>
<thead>
<tr>
<th>Cytokine receptor</th>
<th>Janus kinases</th>
<th>STATs</th>
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<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>Stat6</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>
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</tbody>
</table>
Interferon alpha signalling
Interferon alpha receptors
Activation of Jak1-Tyk2
STAT1-STAT2 heterodimerization
Transcriptional activation

Interferon gamma signalling
Interferon gamma receptors
Activation of Jak1- Jak2
STAT1-STAT1 homodimerization
Transcriptional activation
STAT binding sites:

**ISRE (STAT1-2)**
Interferon Stimulated Response Element
AGTTTNCNTTTCC

**GAS (STAT1-5)**
*Interferon*-Gamma Activated Sequence
TTCN3GAA

**STAT6**
TTCN4GAA
Each of the seven STATs has separate *in vivo* functions, as revealed by knockout experiments.

- STAT1 knockout mice have an impaired interferon signalling
- STAT4 knockouts impaired IL-12 signalling,
- STAT5a knockouts impaired prolactin signalling,
- STAT5b knockouts impaired growth hormone signalling, and
- STAT6 knockout impaired IL-4 and IL-13 signalling.
Growth hormone

JAK2

STAT5

STAT3

Nml, SOCS, BCL-XL, p21, Myc, Nos2, Gene Expression
The IL-2 receptor forms homo/heterodimers

Low affinity
$10^{-8}$,
expressed by activated T cells,
No function

Intermediate affinity
$10^{-9}$, resting T cells.

High affinity
$10^{-11}$, activated T cells
Termination of Jak/STAT signaling

1. Phosphotase

Receptor-Shp-1 (HCP, SH-PTP1, and PTP1c), Shp-2 (Syp and PTP1D), and SHIP

Nuclear phosphotase

2. Suppressors of Cytokine Signaling

(SOCS). SOCS1-7.

3. Degradation of Stats through

ubiquitination/proteasome pathway.
The PIAS family

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<th>DRUGS</th>
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<tr>
<td>ENBREL</td>
<td>MAb AGAINST TNFa RECEPTOR</td>
<td>RHEUMATOID ARTHRITIS, CROHN’S DISEASE</td>
</tr>
<tr>
<td>INTRON A</td>
<td>INTERFERON A-2B</td>
<td>HEPATITIS C MELANOMA</td>
</tr>
<tr>
<td>EPOGEN</td>
<td>ERYTHROPOIETIN</td>
<td>STIMULATES RBC PRODUCTION</td>
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## DRUGS

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<tr>
<th>ACTIMMUNE</th>
<th>INTERFERON $\gamma 1\beta$</th>
<th>CHROMINC GRANULOMATOUS DISEASE</th>
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<tr>
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<td>OSTEROPOROSIS</td>
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<tr>
<th>NEUPOGEN</th>
<th>G-CSF</th>
<th>STIMULATES PRODUCTION OF NEUTROPHILS</th>
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<tr>
<td></td>
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<td>REDUCTION OF INFECTION IN CANCER PATIENTS UNDERGOING CHEMOTHERAPY</td>
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**DRUGS**

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<tr>
<th>LEUKINE</th>
<th>GM-CSF</th>
<th>STIMULATES PRODUCTION OF MYELOID CELLS AFTER BONE MARROW TRANSPLANTATION</th>
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<tr>
<td>NEUMAGA /</td>
<td>INTERLEUKIN 11</td>
<td>STIMULATES PRODUCTION OF PLATELETS</td>
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REFERENCES


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