Chapter 3

Physiology and Pathology of Cytokine: Commercial Production and Medical Use

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Additional information is available at the end of the chapter

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Abstract

Cytokines are small, short-lived proteins secreted by many different cell types. As signaling molecules, cytokines provide communication between cells and play a crucial role in modulating innate and adaptive immune response. The family of cytokines includes interferons, interleukins, chemokines, mesenchymal growth factors, tumor necrosis factor family and adipokines. Interferons (IFNs) are a multigene family of inducible cytokines with antiviral, antiproliferative, and immunomodulatory function. Recombinant DNA technology can be useful in the production of human IFNs. This process includes fermentation, purification, and formation of the final product. Interleukins are classified in families based on sequence homology, receptor-binding properties, biological function, and cellular sources. TNF and IL-1 are considered to be key mediators of inflammatory response, while IL-6 plays a key role in the transition from acute to chronic inflammation. The inhibition of TNF includes administration of anti-TNF antibody and TNF receptor (TNFR). The reduction of IL-1 level can be achieved by the administration of anti-IL-1 antibody or IL-1 receptor antagonist (IL-1Ra), and the reduction of IL-6 level in the treatment of chronic inflammatory diseases can be achieved by the administration of anti-IL-6 antibody and anti-IL-6 receptor antibody. Recombinant cytokines and cytokine antagonists (antibodies and receptors) can be used in treating many different diseases.

Keywords: cytokines, interleukins, interferons, TNF

1. Introduction

Cytokines are low molecular weight proteins or glycoproteins secreted by a number of cell types. The term cytokine is made up of two parts: *cyto* (cell) and *kine* (movement) [1]. As

signaling molecules, cytokines provide communication between cells and play a crucial role in modulating of the innate and adaptive immune response (**Table 1**).

Nomenclature of cytokines was created either according to the type of cells which secrete them (in this case named interleukins and adipokines) or biological activity (in this case

Family	Members	Functions
IL-1 family		
IL-1 subfamily	**Agonist activity**	
	IL-1α (IL-1F1)	Induction of proinflammatory response
		Th17 cell differentiation
	IL-1β (IL-1F2)	Induction of proinflammatory response
		Th17 cell differentiation
	IL-33(IL-1F11)	Acts as an alarmin
		Induction of Th2 response
		Activation of ILC2 cells
		Th1 cell differentiation dependent on IL-12
		Induction of Treg cells
	Receptor antagonists	
	IL-1Ra (IL-1F3)	Antagonism of IL-1
IL-18 subfamily	**Agonist activity**	
	IL-18(IL-1F4)	Induction of IFN-γ in presence of IL-12
		Enhances NK cell cytotoxicity
		Promoting Th1 or Th2 cell responses depending
		cytokine milieu
		Activation and cytokines release from neutrophils
	Antiinflammatory activity	
	IL-37 (IL-1F7)	Suppression the production of proinflamatory cytokines
		Inhibition of dendritic cells (DCs) function (foremost through stimulation of TGF-β)
IL-36 subfamily	**Agonist activity**	
	IL-36α(IL-1F6)	Promoting Th1 and Th17 cell responses (skin, lungs, kidneys)
	IL-36β (IL-1F8)	Promoting Th1 and Th17 cell responses (joints)
	IL-36γ(IL-1F9)	Promoting Th1 and Th17 cell responses (lungs)
	Receptor antagonists	
	IL-36Ra(IL-1F5)	Antagonism of IL-36-α, IL-36-β and IL-36γ
		Antiinflammatory brain action via induction of IL-4 expression in glia cells

Family	Members	Functions
	IL-38(IL-1F10)	Antagonism of IL-36-α, IL-36-β and IL-36γ
		Inhibits the production of IL-17 and IL-22
		Inhibits the production of IL-8 induced by IL-36γ
Common γ chain cytokine family		
	IL-2	Proliferation and differentiation into effector and memory T cells
		Development of Treg cells
		Proliferation of B cells
		Proliferation and differentiation of NK cells
	IL-4	Th2 cell differentiation
		IgE class switching
		Antagonise the effects of IFN-γ
		Alternative activation of macrophages (M2 phenotype)
		Upregulation of class II MHC molecules expression on B cells and monocytes
		Upregulation of FcεRII (CD23) and IL-4R
		Survival factor for B and T cells
		Role in tissue adhesion and inflammation
	IL-7	Proliferation of early T and B cell progenitors
		Naive and memory T cell survival
		Development of γδT cells (VDJ recombination of TCRγ)
		Induction of CTLs and LAK cells
	IL-9	$CD4^+$ T cells and mast cells growth factor
		Proliferation of $CD8^+$ T cells and mast cells
		Inhibition of Th1 cytokines
		Promotes Th17 cell differentiation
		IgE production
		Chemokine and mucus production in bronchial epithelial cells
	IL-15	Induction of Th1 and Th17 responses
		Activation of T cell (decreased TCR activation threshold)
		Survival and proliferation of memory $CD8^+$ T cells
		Loss of Treg cells- and TGF-β immunoregulation
		Suppression of IL-2 induced AICD of T cells
		Differentiation of γδT cells
		Proliferation and activation of NK cells
		Homeostasis of NK and NKT cells
		Induction of LAK cells

Family	Members	Functions
	IL-21	Regulation of B cell proliferation, differentiation and apoptosis
		Antibody isotype balance (increased IgG and decreased IgE)
		Generation of long-lived plasma cells and T cell-dependent antigen responses and memory
		T cell and NK cell proliferation
		Increases cytotoxic activity of NK cells and CTL cells
		Induces the differentiation of T follicular helper (T_{FH}) cells
		Expansion of Th17 cells
		Inhibits DC activation and maturation
IL-6 family		
	IL-6	Synthesis of acute phase proteins in liver
		Inducing secretion of chemokines: CCL2, CCL8, CXCL5, CXCL6
		Induction of neutrophil apoptosis
		Switching from neutrophil to monocyte recruitment
		Transition from innate to acquired immunity
		Direct mediator of T cell migration
		T cell differentiation, activation and survival
		B cell differentiation and production of IgG, IgM, IgA
		Survival of hematopoietic stem cells and early progenitors
		Proliferation and differentiation of myeloid, erythroid, megakaryocyte progenitors
		Survival factor for neuronal cells
	IL-11	Synthesis of acute phase proteins in liver
		Growth factor for myeloid, erythroid, megakaryocyte progenitors
		Bone remodeling
		Protects epithelial cells and connective tissue
		Inhibition of macrophage activity
		Inhibition of adipogenesis
		Promotion of neuronal development
	IL-31	Induction of IL-1β, IL-6, IL-8, GRO-α (CXCL1), MCP-1 (CCL2) and DC-CK1 (CCL18) production in eosinophils
		Increased chemokines mRNA expression (GRO-α (CXCL1), TARC (CCL17), MIP-3β (CCL19), MDC (CCL22), MIP-3 (CCL23), MIP-1β (CCL4)) in keratinocytes
		Antiapoptotic effect on eosinophils
		Expression of growth factors and chemokines in epithelial cells
		Inhibition of proliferation and apoptosis in epithelial cells

Family	Members	Functions
	Leukemia inhibitory factor (LIF)	Self-renewal and block in differentiation embryonic stem cell
		Embryonic implantation (receptive state of endometrial, the interaction between endometrial and embryo, stromal decidualization, the invasion of blastocyst, blastocyst development, infiltration of uterine leukocytes, synthesis of prostaglandins)
		Anti-inflammatory effect
		Neuronal development
	Oncostatin M (OSM)	Maintenance of erythroid and megakaryocyte progenitor pools in BM by regulation hematopoietic cytokine production in stromal cells and direct effect on erythrocytic and megakaryocytic progenitors
		Tumor suppression
		Neuronal development
IL-10 family		
	IL-10	Immune suppression
		Inhibition of expression IL-12, costimulators , and class II MHC molecules on macrophages and DCs
		Inhibition of proliferation $CD4^+$ T cells and production of IL-2, IFN-γ, IL-4, IL-5 and TNF-α
		Enhancing Treg cells function (suppressing autoreactive T cells)
		Reduces immune suppression of autoreactive B cells
		and enhances antibody production
IL-20 subfamily	IL-19	Enhances the production of Th2 cytokines
		Induces IL-6, IL-8 and IL-10 production in monocytes
		Alternative activation of macrophages (M2 phenotype)
		Induction of angiogenesis
		Role in skin inflammation (psoriasis)
		Production of antimicrobial peptides (S100A7 (also known as psoriasin), S100A8, S100A9, and β-defensins) and barrier function increase
	IL-20	Role in skin inflammation (psoriasis)
		Development of hematopoietic cells
		Production of antimicrobial peptides (S100A7 (also known as psoriasin), S100A8, S100A9, and β-defensins) and barrier function increase
		Inhibition of neutrophil phagocytosis, granule exocytosis,and migration

Family	Members	Functions
	IL-22	Controlling the intestinal microbiota
		Production of antimicrobial peptides (S100A7 (also known as psoriasin), S100A8, S100A9, and β-defensins) and barrier function increase
		Wound healing
		Tissue regeneration (intestine, liver, thymus, pancreas and kidneys)
		Role in skin inflammation (psoriasis)
	IL-24	Tumor suppression (loss of proliferative capacity)
		Production of antimicrobial peptides (S100A7 (also known as psoriasin), S100A8, S100A9, and β-defensins) and barrier function increase
		Role in skin inflammation (psoriasis)
	IL-26	Production of proinflammatory cytokines (IL-1β, IL-6, TNF and CCL20)
		Th17 cell differentiation
		Direct killing of bacteria
See Type III interferons	IL-28A (IFN-λ2)	
	IL-28B (IFN-λ3)	
	IL-29 (IFN-λ1)	
IL-12 family		
	IL-12	Th1 cell differentiation
		Increases cytotoxic activity of NK cells and $CD8^+$ T cells and production of IFN-γ
		Antiangiogenic effect
	IL-23	Th17 cell expansion, maintaining activation and secretion of IL-17A, IL-17F, IL-22 and GM-CSF
		Dependent pathogenicity of Th17 cells
		Stimulation of macrophages to produce TNF, IL-1
	IL-27	Pro- and anti-inflammatory effects (induction of Th1 response and suppressive effect on $CD4^+$ T cell production of IL-2, inhibition of Th2, Th17 and iTreg cells)
		Limits the intensity and duration of innate and adaptive immune responses
	IL-35	Induction of Treg cells proliferation
		Inhibition of Th17 response

Family	Members	Functions
IL-17 family		
	IL-17A	Induction of proinflamatory cytokines, chemokines, and
	IL-17F	metalloproteases (controlling bacterial and fungal infection)
		Modulation of viral infection
		Recruitment of neutrophils
	IL-17B	Induction of proinflamatory cytokines, chemokines, and
	IL-17C	metalloproteases
	IL-17D	
	IL-25 (IL17E)	Induction of Th2 response
Th2 like cytokines		
	IL-5	Differentiation and function of myeloid cells
		Increment of chemotactic activity and adhesion capacity on eosinophils
		Remodeling and wound healing
	IL-13	Switching to IgE
		Antagonises the effects of IFN-γ
		Upregulation of FcεRII (CD23) and class II MHC molecules expression on B cells and monocytes
		Alternative activation of macrophages (M2 phenotype)
		Activation of eosinophils and mast cells
		Recruitment and survival of eosinophils
		Defense against parasite infections (mucus production)
Chemokine activities		
	IL-8	Chemoattractant for neutrophils, NK cells, T cells, basophils, eosinophils
		Induces phagocytosis
		Mobilisation of hematopoietic stem cells
		Angiogenesis
	IL-16	Chemoattractant for cells with CD4 molecule
		Modulation of T cell response
Non-classified		
	IL-3	Induces maturation of all hematopoietic lineages
		Activation of basophils
		Activation and survival of eosinophils
	IL-14	Induces growth and proliferation of B cells and inhibits antibody secretion

Family	Members	Functions
	IL-32	Induction of TNF-α, IL-6 and IL-8
		Induction of apoptosis
	IL-34	Differentiation and viability of monocytes and macrophages
Type I interferons		
	IFN-α	Antiviral state
	(13 subtypes)	Increases the expression of class I MHC molecules
		Activation of NK cells
	IFN-β	Antiviral state
		Increases the expression of class I MHC molecules
		Activation of NK cells
	IFN-κ	Antiviral response
	IFN-ω	
	IFN-ε	
	IFN-δ (pigs)	
	IFN-ζ	
	IFN-τ (ruminant)	
	IFN-ν	
Type II interferons		
	IFN-γ	Th1 cell differentiation
		Classical activation of macrophages (increased microbicidal functions)
		Promotes cytotoxic activity
		Isotype switching to opsonisation and complement-fixing IgG subclasses (established in mice)
		Upregulation of class I and class II MHC molecules
		Increases antigen processing and presentation to T cells
		Antiviral properties
		Inhibition of cell growth
		Proapoptotic effects
Type III interferons		
	IL-28A (IFN-λ2)	Antiviral response
	IL-28B (IFN-λ3)	Promotes cytotoxic activity
	IL-29 (IFN-λ1)	Antiviral response

Family	Members	Functions
	IFN-λ4	Antiviral response
		Impairs HCV antiviral program or clearance by impeding receptor binding of the other members of the IFN-λ family
TNF superfamily	TNF	Induction of inflammation (vasodilatation, edema, facilitation of the adhesion of leukocytes, production of reactive oxygen species)
		Induction of intravascular thrombosis
		Fever occurrence
		Induces secretion of IL-6 from leukocytes
		Loss of appetite, wasting of muscle and fat cells (cachexia)
		Induction of insulin resistance
		Inhibits myocardial contractility and vascular smooth muscle tone (low blood pressure)
		Stimulates capillary leak

Table 1. Characteristics of cytokines.

named interferons, chemokines, mesenchymal growth factors and tumor necrosis factor family). This nomenclature is still used [2]. There are several basic common properties of cytokines which are important in understanding their effect in the human body:

1. Synthesis of cytokine is mainly induced by various stimuli which act on cells. Also they can exist in preformed granules which are constitutively produced and secreted from cells.

2. Cytokines achieve their effects by binding with high affinity to specific membrane receptors on cells. Therefore, cells show a relatively small number of specific cytokine receptors (100–1000 per cell). In other words, very low concentrations of cytokines can trigger biological effects in cells. Cellular response to the effects of cytokines is well regulated and it is reflected in the changes of gene expression in target cells resulting in the expression of new functions.

3. Cytokines exert effects on different types of cells (the same cells express a variety of cytokine receptors), or one cytokine can exert many different biological effects. This cytokine action is called **pleiotropy**. Also, several cytokines share the same functional effects, and various cytokines can have the same or similar biological activity (various cytokines activate the same signaling pathways) which is called **redundancy** [3].

4. Cytokines affect the synthesis and the activity of other cytokines, acting antagonistically, additively or synergistically.

5. Cytokine activity can be autocrine (on the very cell that secretes it), paracrine (on surrounding cells) and endocrine (in distant cites from the production). Basic characteristics of cytokines suggest that their implementation achieves complex effects which are often accompanied by numerous side effects.

2. Interferons

Interferons (IFNs) consist of multigene family of inducible cytokines with predominantly antiviral activity [4]. Interferons were detected more than 50 years ago as a soluble substance which inhibits the replication of influenza virus. They were named after their ability to interfere (hinder) the replication in host cells protecting healthy cells from viral infection [5]. The family of interferons is now described as a key component of innate immune response and the first line of defense against viral infections. Interferons are proteins that synthesize and produce host cells in response to the presence of various pathogens: viruses, bacteria and parasites. As signaling molecules they provide communication between cells for the purpose of activating and directing the immune response in order to eliminate the pathogen in the most effective way. Due to the fact that interferons belong to the cytokine family, they influence the processes of proliferation, differentiation and apoptosis, and exhibit a number of immunomodulatory functions. According to the type of receptor complex via which they transmit the signal, all interferons are classified into three classes (Type I, II and III). This means that interferons can achieve their activity over three receptor complexes. Type I (IFN-α, IFN-β) and type III interferons have been identified as antiviral types, and type II (IFN-γ) is known as the immune interferon.

Antiviral and antiproliferative activity of interferons, as well as their ability to modulate immune and inflammatory responses, make them highly applicable in medical treatment [6]. There are many preparations of interferons which are approved for clinical use. Further clinical studies are conducted presently. It is expected that the obtained results will enable a wider application of interferons for medical purposes. Although it became clear in XX century that the possibilities for therapeutic application of interferons are huge, the following issues had to be resolved in order to find their wider application in medicine:

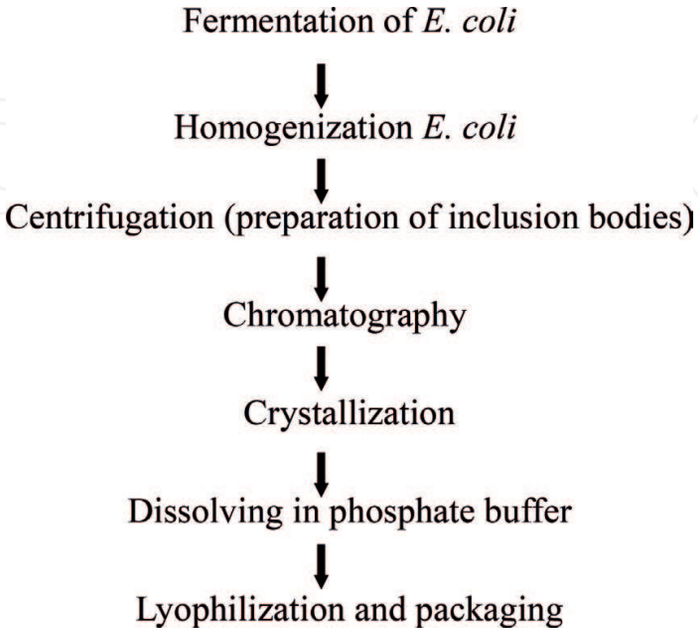
1. An extremely low level of interferons is produced in the human body.
2. Interferons show species specificity, which implies that only human interferons can be used for human clinical treatments.

Since interferons were grown from human leukocytes present in blood transfusion stocks until the 1970s of the last century, their massive production was virtually untenable. In fact, this method provided a mixture of various types and subtypes of interferons in different quantities. However, clinical studies conducted with modest amounts of insufficiently treated interferon preparations have yielded encouraging results. A significant production of interferons was achieved by mammalian cell lines used in the late 1970s. A variety of tumor cell lines was tested first and it was revealed that Namalwa cell line (a type of human

lymphoblastic cell) produced a large amount of interferons [7]. The exposure of these cells to certain viruses (typically Sendai virus) caused an increased production of IFN. Subsequent analysis showed that the final product contains thirteen subtypes of IFN-α, which is why it was necessary to introduce new production technologies. Nowadays, the recombinant DNA technology is used to obtain individual interferon (sub) types. The production of the desired interferon is achieved by insertion of specific genes using vectors for interferons (usually a virus) in mammalian cell lines (Chinese Hamster Ovary - CHO, monkey kidney, etc.), bacteria *Escherichia coli* (*E. coli*) or fungi. Although around 70% of pharmaceutical recombinant proteins currently used for medical purposes is produced in mammalian cells, interferons are produced mainly in *E. coli*. The purification of interferons aims to obtain a final product with 99% level purity, which is mainly achieved by using chromatographic techniques (metal-chelate affinity chromatography, exclusion chromatography (gel filtration), ion-exchange chromatography). Finally, the quality and quantity of interferons are checked by monoclonal antibodies and their effects are analyzed on biological material by different biological assays.

2.1. Production and medical use of IFN-α

Interferon production process includes the fermentation process, purification and the formation of the final product. Fermentation is conducted in specially designed vessels made of stainless steel. This process involves transfer and expression of genes in *E. coli* and the subsequent intracytoplasmic protein production of the inserted gene. Thus, recombinant proteins constitute intracytoplasmic accumulation of inclusion bodies. Homogenization *E. coli* and centrifugation, followed by a chromatographic purification process, need to be done in order to prepare inclusion bodies. The crystallization method is used for the final purification of interferons. The crystallized product is then dissolved in a phosphate buffer which contains glycine and human albumin (carriers of an active substance). Finally, lyophilization and packaging of the product is done.



There are several significant advantages achieved by the modification of interferons. Recombinant interferon modification is achieved by the process of "pegylation." This process was first described by Frank Davis and Abraham Abuchowski and his associates in 1977 [8]. In contrast to the modification process of drug formulation (using colloidal systems or osmotic pumps), the process of interferon pegylation involves covalently binding polyethylene glycol (PEG) polymer chain to the amino group of interferons, which significantly improves its pharmacokinetic and pharmacodynamic characteristics [9]. Many existing pharmacological limitations of interferons are overcome by the process pegylation. Native PEG is an inert, nontoxic polymer with two terminal hydroxyl groups. Chemically active form of PEG molecule is obtained by modifying the terminal hydroxyl group (substitution process of a hydroxyl group with reactive (functional) one). The incubation of purified interferon with methoxypolyethylene glycol (chemically active form of PEG) spontaneously forms covalent bonds [10]. There are different configurations of PEG molecules today, including linear and branched structures of various molecular weights. Formation of stable (covalent) bonds in the process of pegylation of interferon is necessary for a long-term preservation of incurred pharmacological changes. Resultant molecules of interferon are predominantly monopegylated with small impurities of di-, and non-pegylated molecules. Sodium phosphate, saccharose and polysorbate are used as carriers of the active substance for the pegylated product in lyophilized form.

After subcutaneous administration IFN-α is rapidly absorbed and the peak of serum concentration is reached 7–12 hours after which its concentration decreases rapidly. As half-life of IFN-α is 3–8 hours, its serum concentration falls below detection limit within 24 hours after administration [11]. Since the administration of IFN-α is conducted 3 times a week, the desired serum concentration is not maintained in most intervals between applications.

Different linear and branched structures of PEG molecules are used for the pegylation of IFN-α, so that the resulting preparations exhibit different pharmacokinetic and pharmacodynamic characteristics which are improved in comparison to standard IFN-α. PEG group protects IFN-α from enzymatic degradation, prolongs the absorption period at the administration site of subcutaneous injection and reduces its clearance from the body, so the period to reach maximum concentration is significantly prolonged (80 hours). Taking into consideration the above mentioned, it is clear that IFN-α concentration in blood is higher and more constant for a much longer period of time when compared to standard IFN-α. These pharmacokinetic properties enable once-a-week subcutaneous administration of pegylated IFN-α to achieve and maintain the concentration in blood that provides the desired effectiveness (desired effects are time and concentration dependent). Pegylation of IFN-α greatly improves its pharmacodynamic characteristics, and testing shows 100 times stronger antiviral activity and 20 times stronger antitumor activity. Nowadays, IFN-α is used in the treatment of hepatitis B and C, chronic myeloid leukemia, malignant melanoma, non-Hodgkin's lymphoma, Kaposi's sarcoma, and other diseases (**Table 2**).

2.2. Production and medical use of IFN-β

There are several preparations made of IFN-β used for therapeutic purposes. Some of them were made by recombinant DNA technology in *E. coli*, but nowadays CHO cell line is mostly

Generic name	Trade name	Treatment	Year of first FDA approval	Company
IFN-α – con-1	Infergen	Chronic hepatitis C	1997	Amgen
IFN-α – n3, leukocyte derived	Alferon-N	Condylomata acuminata	1989	Hemisperx Biopharma
IFN-α – 2a, pegylated	Pegasys	Chronic hepatitis C Chronic hepatitis B	2002	Roche
IFN-α – 2a, recombinant	Roferon-A	Chronic hepatitis C Hairy cell leukemia Kaposi's sarcoma Chronic myeloid leukemia	1986	Roche
IFN-α – 2b, pegylated	PEG-Intron	Chronic hepatitis C	2001	Schering-Plow
IFN-α – 2b, pegylated	Sylatron	Malignant melanoma	2011	Schering-Plow
IFN-α – 2b, recombinant	Intron-A	Hairy cell leukemia Kaposi's sarcoma Chronic hepatitis B/C Malignant melanoma Follicular lymphoma Condylomata acuminata	1997	Schering-Plow

Table 2. IFN-α approved by FDA for therapeutic use.

used in their production [12]. Recombinant human IFN-β produced in CHO cell line (rhIFN-β-1a) is glycosylated and it has the same amino acid sequence as natural IFN-β [13, 14]. Although *E. coli* does not generate glycosylation of IFN-β [15], this deficiency does not affect the efficiency of its therapeutic applications. IFN-β is presently used in the treatment of multiple sclerosis. However, in IFN-β glycans play an important role in the protein stabilization and thus enhance its biological activity [16] (**Table 3**).

Generic name	Trade name	Treatment	Year of first FDA approval	Company
IFN-β – 1a	Avonex	Relapsing multiple sclerosis High risk for MS	1996	Biogen IDEC
IFN-β – 1b	Betaseron	Relapsing multiple sclerosis High risk for MS	1993	Berlex
IFN-β – 1b	Extavia	Relapsing multiple sclerosis	2009	Novartis

Table 3. IFN-β approved by FDA for therapeutic use.

Generic name	Trade name	Treatment	Year of first FDA approval	Company
IFN-γ – 1b, bioengineered	Actimmune	Chronic granulomatous disease Malignant osteopetrosis	1990	Intermune Pharma

Table 4. IFN-γ approved by FDA for therapeutic use.

2.3. Production and medical use of IFN-γ

IFN-γ preparations are made by recombinant DNA technology in bacteria *E. coli*. Despite the fact that recombinant human IFN-γ is not glycosylated [17], its biological activity is not affected. It is used for the treatment of chronic granulomatous disease (**Table 4**).

2.4. Side effects of interferon application

Administration of interferons can cause many side effects [18].

Application of **IFN-α** causes increased temperature, chills and headache. These reactions are often manifested a few weeks after the application and paracetamol is simultaneously applied to alleviate them. Severe cases develop anorexia, insomnia, cardiovascular complications and autoimmune reactions, which requires immediate termination of its application.

Application of **IFN-β** causes increased temperature, chills and headache. Severe cases develop hypersensitivity reactions, depression and menstrual disorders.

Application of **IFN-γ** causes increased temperature, chills and headache. Severe cases develop heart failure, metabolic disorders and disorientation.

3. Interleukins

More than 40 interleukins with different properties are known today. Interleukins are classified in families based on sequence homology, receptor-binding properties, biological function and cellular sources.

There are 38 interleukins which are designated by the abbreviation IL (from **I**nterleukin) and Arabic numbers [19]. Interleukins are produced by various types of body cells, wherein the specific interleukins (IL-1) can be secreted by up to 20 different cell types. Most cells capable of synthesizing one interleukin are capable of synthesizing several different. Today, the recombinant DNA technology is used for interleukin production, enabling quantities sufficient to meet demanding medical needs.

3.1. Production and medical use of interleukin 1 (IL-1)

IL-1 is a proinflammatory cytokine which stimulates the synthesis of substances involved in the induction of inflammation. Activated mononuclear phagocytes are the main cellular source

of IL-1. When secreted in small quantities, IL-1 acts as a paracrine mediator of local inflammation, while in larger amounts the endocrine effect can induce body temperature increase, synthesis of acute-phase proteins in the liver and the production of neutrophils and platelets in the bone marrow. In diseases with the elevated level of IL-1 it is important to decrease IL-1 level due to its effect in the induction of inflammation [20]. It has been shown that preparations which reduce the level of IL-1 are therapeutically useful when administered alone or, more preferably, in the combination with low doses of other therapeutic agents. In accordance with the above, the reduction of IL-1 level can be achieved by the administration of:

1. Anti-IL-1 antibody
2. The IL-1 receptor antagonist (IL-1Ra)

3.1.1. Anti-IL-1 antibody

Canakinumab is a human anti-IL-1ß monoclonal antibody which is administered subcutaneously for the treatment of syndromes associated with periodic cryopyrin (CAPS—**c**ryopyrin **a**ssociated **p**eriodic **s**yndromes). The main sign of CAPS is urticaria with neutrophilia, accompanied by high fever, headache and arthralgia.

3.1.2. The IL-1 receptor antagonist (IL-1Ra)

Anakinra/Kineret is a recombinant, nonglycosylated form of human interleukin-1 receptor antagonist (IL-1Ra). Anakinra is different from native human IL-1Ra as it is non-glycosylated and may contain an additional N-terminal methionine residue at its amino-terminus, as the result of its production by recombinant DNA technology in prokaryotic system (*E. coli*). In people suffering from rheumatoid arthritis an elevated level of IL-1 is present in the synovial fluid of joints affected. IL-1 shows negative effects on the joints and bones, which include degradation of cartilage and stimulates bone resorption. Therefore, in patients suffering from rheumatoid arthritis the subcutaneous injection of 100 mg (0.67 ml) of Anakinra is administered daily. The following substances are present as carriers of active substance in the final product: sodium citrate (1.29 mg), ethylenediaminetetraacetic acid (EDTA) (0.12 mg), sodium chloride (5.48 mg) and polysorbate 80 (0.70 mg). It is also approved to be applied in the treatment of Neonatal-Onset Multisystem Inflammatory Disease (severe form Cryopyrin-Associated Periodic Syndromes).

3.2. Production and medical use of interleukin 2 (IL-2)

As it was the case with most other cytokines, the use of IL-2 for medical purposes was initially impractical because of small production quantities. Some transformed cell lines, particularly cell line Jurkat (T cell leukemia), produced IL-2 in larger quantities [21]. The largest amounts of IL-2 used in initial studies were obtained from this source. The production of significant amounts of IL-2 is possible by the development of recombinant DNA technology. Today, complementary DNA (cDNA) to the IL-2 gene is expressed in many cell lines, while *E. coli* was used at the beginning (absence of glycosylation of the recombinant product does not alter biological activity of IL-2).

Basiliximab (anti-IL-2Rα (CD25); chimeric monoclonal antibody) is produced in a cell line Sp2/0 and is administered intravenously to patients after kidney transplantation. Basiliximab and cyclosporine are administered in the treatment of kidney transplant [22]. It is important to note that studies have shown that basiliximab slows cyclosporine elimination from the body (it is believed that cytochrome P450 plays an important role in this process).

Daclizumab (anti-IL-2Rα (CD25); humanized monoclonal antibody) was administered intravenously to patients after kidney transplantation. It was withdrawn from the market of the European Union and the United States of America in 2009.

Recombinant IL-2.

Proleukin (Aldesleukin) is indicated for the treatment of adults with metastatic renal cell carcinoma (year of first approval-1992) and metastatic melanoma (year of first approval-1998). Proleukin helps an increased production of several different components of the immune system found in the blood, including T lymphocytes and natural killer cells. Proleukin should be restricted to patients with healthy heart and lung function. Proleukin can cause capillary leak syndrome and the treatment is associated with a reduced neutrophil chemotaxis and with an increased risk of disseminated infection, including sepsis.

3.3. Production and medical use of interleukin 6 (IL-6)

IL-6 has pro- and anti-inflammatory properties and plays a crucial role during the transition from innate to acquired immunity. It has the ability to stimulate neutrophil production, promote expansion and activation of T cells, the differentiation of B cells, and the regulation of the acute-phase response [23]. Proteolytic shedding of IL-6Rα from invading neutrophils subsequently drives IL-6 *trans-signaling* in resident tissue cells, leading to a switch from neutrophil to monocyte recruitment by suppressing mainly neutrophil-attracting (CXCL1, CXCL8 and CX3CL1) and enhancing mainly monocyte-attracting chemokines (CCL2, CCL8, CXCL5 and CXCL6), and cellular adhesion controlled by the lymph node–homing receptor CD62L, and modulates expression of adhesion molecules ICAM-1 and VCAM-1. Besides its role in attracting monocytes, IL-6 *trans-signaling* has been shown to skew monocyte differentiation toward macrophages by upregulating M-CSF receptor expression. The finding that IL-6 induces neutrophil apoptosis contributes to the resolution of acute neutrophil infiltration. Increased IL-6 level is often a better predictor of disease activity in the context of infection, autoimmunity or cancer than C-reactive protein [24, 25]. Consistent with the early description of IL-6 as a lymphocyte-stimulating factor, IL-6 deficiency leads to impaired innate and adaptive immunity to viral, parasitic and bacterial infection. Indeed, children with inhibitory autoantibodies to IL-6 develop recurrent staphylococcal cellulitis and subcutaneous abscesses. While IL-6 has a protective role in many infections, the same activity can be key to the maintenance of chronic inflammation that includes rheumatoid arthritis and multicentric Castleman's disease. The reduction of IL-6 level (**Table 5**) as the treatment of chronic inflammatory diseases can be achieved by the administration of:

1. Anti-IL-6 antibody
2. Anti-IL-6 receptor antibody

International nonproprietary name (INN)	Target	Type	Year of first EMA approval	Year of first FDA approval	Cell line	Therapeutic indication
Tocilizumab	IL-6R	Humanized IgG1	2009	2010	CHO	Rheumatoid arthritis Juvenile idiopathic arthritis
Siltuximab	IL-6	Chimeric IgG1	2014	2014	CHO	Multicentric Castleman's disease
Sarilumab	IL-6R	Human IgG1	2017	2017	CHO	Rheumatoid arthritis

Table 5. Monoclonal antibodies target at IL-6/IL-6R approved by FDA and EMA for therapeutic use.

3.3.1. Anti-IL-6 antibody

Siltuximab is a chimeric immunoglobulin (IgG)1 monoclonal antibody that binds IL-6. IL-6 antagonist is indicated for the treatment of patients with multicentric Castleman's disease (MCD) who are human immunodeficiency virus (HIV) negative and human herpesvirus-8 (HHV-8) negative.

3.3.2. Anti-IL-6 receptor antibody

Sarilumab is a human immunoglobulin (IgG)1 monoclonal antibody which binds specifically to both soluble and membrane-bound IL-6 receptors (sIL-6Rα and mIL-6Rα) and inhibits IL-6-mediated signaling. It is administered subcutaneously in a dose of 200 mg once every 2 weeks for the treatment of rheumatoid arthritis. By binding to IL-6Rα, sarilumab prevents the formation of high-affinity complex of IL-6 with IL-6Rα and thus blocks IL-6 signaling. As sarilumab blocks both mIL-6Rα and sIL-6Rα, it has the potential to inhibit both intra-articular and systemic IL-6 signaling. Patients treated with Sarilumab are at increased risk for developing serious infections.

3.4. Production and medical use of tumor necrosis factor (TNF)

Tumor **n**ecrosis **f**actor (TNF) was originally described as a circulating protein which causes tumor necrosis (discovered in 1975 as endotoxin-induced glycoprotein which causes necrosis of sarcoma in mice). Human TNF was first produced in 1985 by recombinant DNA technology in *E. coli* [26]. Activated mononuclear phagocytes are the most important cellular source of TNF. TNF exerts a variety of effects on vascular endothelial cells and leukocytes. In response to TNF endothelial cells enhance the expression of different combinations of leukocyte adhesion molecules (including E-selectin, ICAM-1 and VCAM-1), which in combination with the secretion of chemokines (IL-8, including, MCP-1 and IP-10) from peripheral blood leukocytes and endothelial cells induces chemotaxis of different populations of leukocytes to the site of inflammation apart from the recognition of the antigen. In addition, TNF stimulates microbicidal activity of neutrophils and monocytes in order to eliminate the cause easier. Secreted in small amounts, TNF acts locally (autocrine and paracrine), primarily on vascular endothelial

cells and leukocytes. The effect of TNF on the endothelium and leukocytes is most likely crucial for a successful local inflammatory response to microorganisms. TNF is produced in large quantities in severe infections. It enters the bloodstream and affects distant cites (endocrine), causing pathological and clinical effects on the body. Systemic effects caused due to excessive TNF production or exogenous administration in high doses can induce increased body temperature, synthesis of acute-phase proteins in the liver, production of neutrophils and platelets in the bone marrow. TNF is presently considered a key mediator of inflammatory response which has a wide range of effects in inflammation, infection and response to tumors. In this respect, several approaches can be useful, and this includes the administration of:

1. Anti-TNF antibody
2. TNF receptor (TNFR)
3. TNF

3.4.1. Anti-TNF antibody

Infliximab is an anti-TNF-α antibody which binds TNF-α and blocks the inflammation induced by cytokine. Today it is successfully used in the treatment of rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, etc.

Adalimumab and **Golimumab** are human anti-TNF-α antibodies which bind to TNF-α by blocking its activity. As TNF is a primary mediator of inflammation, these antibodies are very powerful anti-inflammatory agents.

Adalimumab has been successfully used in the treatment of rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, Crohn's disease, ulcerative colitis, etc. It is administered in the treatment of rheumatoid arthritis and ankylosing spondylitis as a subcutaneous injection in a dose of 40 mg twice a month (in severe conditions once a week). In the treatment of Crohn's disease starting dose is 80 mg, then 40 mg every other week (in severe conditions starting dose is 160 mg).

Golimumab has been successfully used in the treatment of rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis.

Certolizumab pegol is a humanized Fab fragment of a pegylated anti-TNF antibody produced by recombinant DNA technology in *E. coli*. It is used in the therapy of Crohn's disease and rheumatoid arthritis. Pegylation of Fab fragments significantly improves distribution in tissues and prolongs the half-elimination to 14 days. The lack of Fc fragment in cells prevents the antibody binding to the protective FcRn receptor (Neonatal Fc receptor).

3.4.2. TNF receptor (TNFR)

Etanercept/Enbrel is a recombinant human hybrid protein in which extracellular domain of TNFR2 fuses with Fc fragment of IgG (Soluble p75 TNF receptor-Fc fusion). It is produced by

recombinant DNA technology in a CHO cell line. After the purification and addition of the carrier (mannitol, saccharose, and trometamol), the product is lyophilized. It is used for the treatment of rheumatoid arthritis (RA) by subcutaneous injection of 25 mg dose twice a week or 50 mg once a week. It is also applied in the treatment of juvenile idiopathic arthritis, ankylozing spondylitis, psoriatic arthritis, plaque psoriasis and plaque psoriasis in children ages 4–17. Etanercept functions as a competitive inhibitor of TNF, because it prevents its binding to receptors present on cell surface. Side effects of etanercept include the development of infections and tumors, allergic reaction (hives, swelling of the face, etc.), headache and heart failure.

3.4.3. TNF

Tasonermin is a human TNF-α-1a produced by recombinant DNA technology in *E. coli*. The purified product is packed in tube vials (1 mg of active substance per bottle) as a lyophilizate. The carriers of active substance in the final product are sodium chloride, phosphate buffer and serum albumin. It is used in the treatment of soft tissue sarcoma on the limbs in order to prevent or postpone the amputation. Tasonermin is dissolved in physiological saline to a concentration of 0.2 mg/ml after which the tissue perfusion of the affected limb is done with 3–4 mg of the substance for a period of 90 minutes. Side effects of tasonermin could be local (edem, nerve damage) and systemic (arrhythmia, nausea, liver damage) if it enters the systemic circulation.

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