27 Interferons and Interleukins

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INTRODUCTION

PHARMACOTHERAPY INFORMATION

Further information on the applied pharmacotherapy with interferons and interleukins can be found in the following frequently used textbooks:

Applied Therapeutics: The Clinical Use of Drugs (Koda-Kimble, MA, et al., Eds.), 10th edition, Lippincott Williams & Wilkins, Baltimore 2013.

Pharmacotherapy: A Pathophysiologic Approach (DiPiro, JT, et al., Eds.), 9th edition, McGraw-Hill, New York 2014.

Textbook of Therapeutics: Drug and Disease Management (Helms, RA, et al., Eds.), 8th edition, Lippincott Williams & Wilkins, Baltimore 2006: Chapters 9, 33, 46, 49, 65, 93, 103.

In 1957 Alick Isaacs and Jean Lindenmann described a substance which was produced by virus-infected cell cultures and "interfered" with infection by other viruses; it was called interferon. Over the following decades it was realized that "interferon" comprises a family of related proteins with several additional properties. Starting in the 1960s various "factors" produced primarily by white blood cell (WBC) as well as otherv-cell supernatants were described which acted in various ways on other WBCs or somatic cells. They were usually given a descriptive name either associated with their cell of origin or their activity on other cells resulting in a myriad of names. The application of molecular biological techniques allowed us to determine that some cytokines had multiple activities and that different cytokines had similar overlapping activities. A classification system based on genetic structure and protein characterization is being used. The interactive networks and cascades of cytokines, interferons (IFN), interleukins (IL), growth factors (GF), chemokines (CK), their receptors (r or R) and signaling pathways are highly complex and will be further explored in this chapter.

Cytokine is a term coined in 1974 by Stanley Cohen in an attempt to develop a more systematic approach to

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the numerous regulatory proteins secreted by hematopoietic and non-hematopoietic cells. Cytokines play a critical role in modulating the innate and adaptive immune systems. They are multifunctional peptides that are now known to be produced by normal and neoplastic cells, as well as by cells of the immune system. These local messengers and signaling molecules are involved in the development of the immune system, cell growth and differentiation, repair mechanisms and the inflammatory cascade. Traditionally, interleukins can be classified as T-helper cells type 1 (Th1; pro-inflammatory), e.g. IL-2, IL-12, IL-18, IFNγ, or T-helper cells type 2 (Th2; anti-inflammatory) stimulating, e.g. IL-4, IL-10, IL-13, TGF-β. More recently a third category T-helper cells 17 (Th17) have been described which are associated with autoimmunity. A review of the Th1/Th2 and Th17 concept is provided by Steinman (2007).

(a) *Interferons*: proteins produced by eukaryotic cells in response to viral infections, tumors and other biological inducers. They promote an antiviral state in other, neighboring cells and also help to regulate the immune response. They exhibit a variety of activities and represent a wide family of proteins.
(b) *Interleukins*: a group of cytokines mainly secreted by leukocytes and primarily affecting growth and differentiation of hematopoietic and immune cells. They are also produced by other normal and malignant cells and are of central importance in the regulation of hematopoiesis, immunity, inflammation, tissue remodeling, and embryonic development.

"*Thus, all interleukins are cytokines however not all cytokines are interleukins*"

(c) *Growth factors*: proteins that activate cellular proliferation and/or differentiation. Many growth factors stimulate cellular division in numerous different cell types; others are specific to a particular cell type. They also promote proliferation of connective tissue, glial and smooth muscle cells, enhance normal wound healing and promote proliferation and differentiation of erythrocytes (erythropoietin). Hematopoietic growth factors are reviewed in Chap. 24. Some ILs have a function overlap with growth factors, e.g. IL-2, IL-3, IL-11 (see Table 27.1).
(d) *Chemokines*: (*chemo*tactic cyto*kines*) a large family of structurally related low molecular weight proteins with potent leukocyte activation and/or chemotactic activity. "CXC" (or α) and "C-C" (or β) chemokine subsets are based on presence or absence of an amino acid between the first two of four conserved cysteines. A third subset, "C", has only two cysteines and to date only one member, IL-16, has been identified. The fourth subgroup, the C-X3-C chemokine has three amino acid residues between the first two cysteines.
(e) *Others*, such as tumor necrosis factors (TNF) lymphotoxin alpha (LT)-α] and -β and transforming growth factor (TGF)-α and -β.

All cytokines including interferons and interleukins act by binding to specific transmembrane receptors. In general, these receptors have two main components: a low affinity ligand-binding domain that ensures ligand specificity and a high affinity effector domain activating target gene promoters via an intracellular signaling pathway. Because cytokines can bind to their receptors only where these are expressed on the cell membrane, a functional tissue or cell specificity is ensured.

Cytokine signaling is tightly controlled within the cell through the action of multiple different negative regulators. Members of the suppressors of cytokine signaling (SOCS) family specifically interfere with cytokine signaling by several different mechanisms including direct binding and inhibition of Janus activated kinase (JAK) proteins, competition with janus activated signal transducer and activator of transcription (STAT) for binding sites on the cytokine receptor (see below), and activation of proteosomal degradation of signaling components.

Their action is described as:

- *autocrine*, if the cytokine acts on the cell that secretes it,
- *paracrine*, if the action is restricted to the immediate vicinity of a cytokine's secretion, or
- *endocrine*, if the cytokine diffuses or is otherwise transported to distant regions of the body to affect different tissues.

They can act on many targets, can act in concert, or can antagonize one another:

- *synergy*—action together to induce a different response than either can induce alone
- *antagonism*—cytokines can counteract one another
- *pleiotropy*—action in a similar way on more than one "target" cell
- *redundancy*—more than one cytokine triggers identical or similar responses in a given "target" cell
- *pathway activation*—triggered sequential induction or "cascade"

INTERFERONS: NOMENCLATURE AND FUNCTIONS

Interferons are a family of naturally occurring proteins and glycoproteins with molecular weights of 16,776 to 22,093 Da produced and secreted by cells in

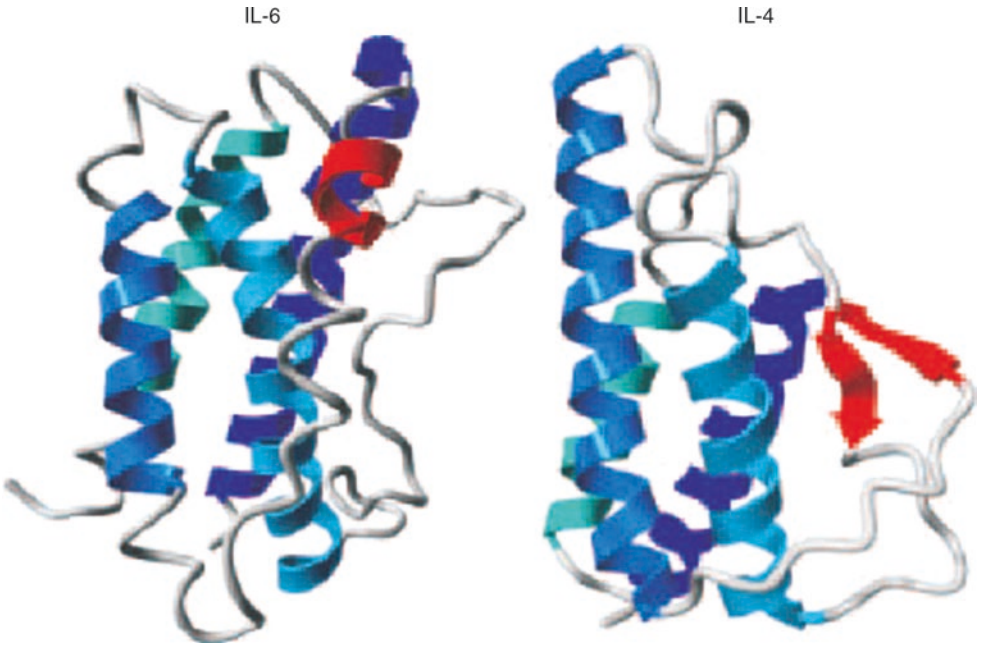


Figure 27.1 ■ Class-1 helical cytokines. Class-1 helical cytokines fold into a bundle of four tightly packed α-helices. On the basis of their helix length, class-I helical cytokines are characterized as (**a**) long chain, such as IL-6, or (**b**) short chain, such as IL-4. From: Journal of Endocrinology (2006) 189, 1–25, with permission

response to viral infections and to synthetic or biological inducers. By interacting with their specific heterodimeric receptors on the surface of cells, the interferons initiate a broad and varied array of signals that induce cellular antiviral states, modulate inflammatory responses, inhibit or stimulate cell growth, produce or inhibit apoptosis, and modulate many components of the immune system. Structurally, they are part of the helical cytokine family (Fig. 27.1). During the past 30 years, major research efforts have been undertaken to understand the signaling mechanisms through which these cytokines induce their effects. Figure 27.2 as a generic example, illustrates the JAK-STAT (Janus activated kinase, originally "just another kinase"—signal transducer and activator of transcription), the best characterized IFN signaling pathway. However, coordination and cooperation of multiple distinct signaling cascades, including the mitogen-activated protein kinase p38 cascade and the phosphatidylinositol 3-kinase cascade, are required for the generation of responses to interferons (Platanias 2005). For a review of the IFN signaling pathways see Journal of Interferon and Cytokine Research 2005; 25: 731–811, Special Issue: The Neoclassical Pathways of Interferon Signaling. Many of the symptoms of acute viral infections are the consequence of the high systemic IFNα response induced by the infecting viruses particularly during the viremic phase.

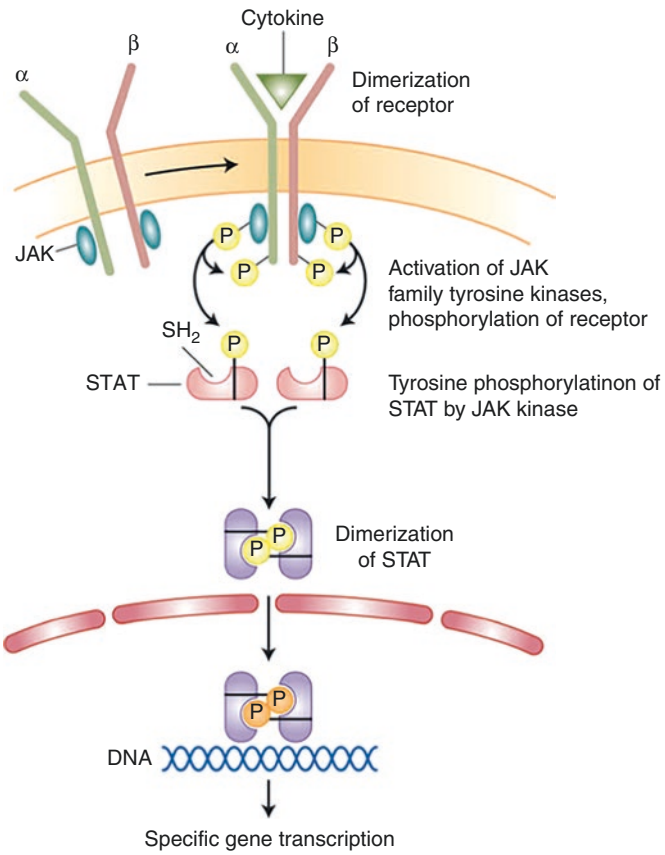


Figure 27.2 ■ Generic JAK-STAT signaling pathway mediated by most cytokine receptors

Human type I interferons comprise 13 different IFNα isoforms or subtypes with varying specificities, e.g. affinities to different cell types, and downstream activities. Although there are 13 human IFNα proteins, two of them (IFNα1 and IFNα13) are identical proteins so that the total number of type I IFNs often is listed as 12 (Pestka 1981a, 1981b, 1986). There is also one subtype each for IFNβ (beta), IFNε (epsilon), IFNκ (kappa) and IFNω (omega). Their ability to establish an "antiviral state" is the distinctive fundamental property of type I IFNs. They are produced by most cells. However, certain types seem to be more selectively expressed, e.g. IFNκ by keratinocytes.

Type II IFN consists of a single representative: IFNγ (gamma) (Pestka 1981a, 1981b, 1986). IFNγ or immune interferon plays an essential role in cell-mediated immune responses. It is produced by NK cells, dendritic cells, cytotoxic T cells, progenitor Th0 cells and Th1 cells. IFNα2, -β and -γ are the most extensively studied to date. All IFNs and IFN-like cytokines have been reviewed in Pestka et al. 2004a.

The names for the human IFNs presently approved by the Human Genome Nomenclature Committee (HGNC) are listed in Table 27.1. For an exhaustive review see Meager 2006.

Symbol	Name	Symbol	Name
IFNα1	Interferon, alpha 1	IFNβ1	Interferon, beta-1, fibroblast
IFNα2	Interferon, alpha 2	IFNε1	Interferon, epsilon-1
IFNα4	Interferon, alpha 4	IFNκ	Interferon, kappa
IFNα5	Interferon, alpha 5	IFNω1	Interferon, omega-1
IFNα6	Interferon, alpha 6	IFNγ	Interferon, gamma
IFNα7	Interferon, alpha 7	There are in addition a number of interferon pseudogenes (Ps) (non-functional and related to interferon genes) mentioned for completion's sake: IFNα22, IFNν (nu) 1, IFN-P 11, 12, 20, 23 and 24, IFNω-P 2, 4, 5, 9, 15, 18 and 19	
IFNα8	Interferon, alpha 8		
IFNα10	Interferon, alpha 10		
IFNα13	Interferon, alpha 13[a]		
IFNα14	Interferon, alpha 14		
IFNα16	Interferon, alpha 16		
IFNα17	Interferon, alpha 17		
IFNα21	Interferon, alpha 21		

P pseudogene
[a]IFNα13 sequence identical to IFNα1

Table 27.1 ■ HGNC approved interferons names (adapted from ExPASy and HGNC)

INTERLEUKINS: NOMENCLATURE AND FUNCTIONS

The first interleukins were identified in the 1970s. Initially it was believed that interleukins were made chiefly by leukocytes to act primarily on other leukocytes. For this reason they were named interleukins, meaning "between leukocytes." It was later realized that they are also produced by and interact with a host of cells not involved in immunity and are involved in many other physiological functions. The role that interleukins play in the body is much greater than was initially understood. They are, however, primarily a collection of immune cell growth, differentiation and maturation factors. Collectively they orchestrate a precise and efficient immune response to toxins and pathogens, including cancer cells, recognized as foreign. As is the case for IFNs, ILs bind to related specific cell surface receptors that activate similar intracellular signaling cascades. Many interleukins, mainly those with pro-inflammatory function, are intrinsically toxic either directly or indirectly, i.e. through induction of toxic gene products. Therefore, the human body has an elaborate system of checks and balances that, under (patho)-physiological conditions, regulates the magnitude and duration of an immune response. ILs are produced upon appropriate stimulation, have a short circulation time. Their production is regulated by positive and negative feedback loops. Furthermore, their effect is mostly localized, and in some cases soluble receptors or neutralizing antibodies limit their dissemination. Specific receptor antagonists can also control their activity.

Table 27.2 lists the ILs for which the protein and gene structure have been characterized. Their names and symbols have been approved by the HGNC.

Under patho-physiological conditions, the sequential concentrations of agonistic and antagonistic interleukins establish a delicate balance in driving pro- and anti-inflammatory phases. This process can be disturbed by various pathogenic agents or mechanisms:

- Infectious agents or toxins
- Allergens
- Malignant tumors
- Genetic variants

These pathogenic agents or mechanisms can result in a self-limited or protracted disequilibrium. Symptoms of disease are the consequence of an adequate immune response at the end of which the steady-state is reestablished. A brisk inflammatory response is the sign of a healthy immune reaction. In some instances, an inadequate response can manifest itself as relapsing remitting progressive disease, e.g. rheumatoid arthritis, asthma, psoriasis, chronic inflammatory

Symbol	Approved name	Previous symbol	Aliases
IL-1A	Interleukin-1, alpha	IL-1	IL-1 alpha, hematopoietin-1, interleukin -1 family member (IL-1F) 1
IL-1B	Interleukin-1, beta		IL-1 beta, IL-1F2, catabolin
IL-1F3	Interleukin-1 family, member 3		IL-1 delta, IL-1 receptor antagonist homolog 1, IL-1-related protein 3
IL-RN	Interleukin 1 receptor antagonist	IL1F3	IL1RA, ICIL-1RA, IRAP, MGC10430
IL-2	Interleukin-2		T-cell growth factor (TCGF) aldesleukin
IL-3	Interleukin-3		Multi-CSF
IL-4	Interleukin-4		BSF1
IL-5	Interleukin-5		TRF, EDF, BCDF 1
IL-6	Interleukin-6	IFNB2	BCSF2, HSF, HGF, CTL differentiation factor, MGI-2
IL-7	Interleukin-7		
IL-8	Interleukin-8		CXCL8 (chemokine), MDNCF, TCCF, NAP1, GCP1, MONAP, emoctakin
IL-9	Interleukin-9		TCGF P40, P40 cytokine
IL-10	Interleukin-10		CSIF, TGIF, IL-10A
IL-11	Interleukin-11		AGIF, oprelvekin
IL-12A	Interleukin-12A	NKSF1	CLMF p35, CLMF1, IL-12 p35
IL-12B	Interleukin-12B	NKSF2	CLMF p40, CLMF2, IL-12 p40
IL-13	Interleukin-13		
IL-15	Interleukin-15		
IL-16	Interleukin-16		LCF, proIL-16
IL-17A	Interleukin-17A	IL-17	CTLA-8
IL-17B	Interleukin-17B		Cytokine Zcyto7, neuronal interleukin-17-related factor, interleukin-20
IL-17C	Interleukin-17C		Cytokine CX2
IL-17D	Interleukin-17D		Interleukin-27
IL-17F	Interleukin-17F		Interleukin-24, cytokine ML-1
IL-18	Interleukin-18	IL-1F4	IFN-gamma-inducing factor, IL-1 gamma, iboctadekin
IL-19	Interleukin-19		Melanoma differentiation-associated protein-like protein, IL-10C
IL-20	Interleukin-20		Zcyto10
IL-21	Interleukin-21		Za11
IL-22	Interleukin-22		Zcyto18, IL-TIF
IL-23A	Interleukin-23A		IL-23 subunit p19, SGRF
IL-24	Interleukin-24		MDA-7, suppression of tumorigenicity 16 protein
IL-25	Interleukin-25	IL-17E	Interleukin-17E
IL-26	Interleukin-26		AK155 protein
IL-27	Interleukin-27	IL-30	IL-27A, p28
IL-28A	Interleukin-28A		IFN lambda-2, Zcyto20
IL-28B	Interleukin-28B		IFN lambda-3, IFN lambda-4 Zcyto22
IL-29	Interleukin-29		IFN lambda-1, Zcyto21
IL-31	Interleukin-31		

Table 27.2 ■ HGNC approved interleukins names (adapted from ExPASy and HGNC)

Symbol	Approved name	Previous symbol	Aliases
IL-32	Interleukin-32		NK cell protein 4, TAIF
IL-33	Interleukin-33	IL-1F11	NF-HEV
IL-34	Interleukin-34		
IL-35	Interleukin-35		
IL-36A	Interleukin-36 alpha	IL-1F6	FIL-1 epsilon
IL-36B	Interleukin-36 beta	IL-1F8	Interleukin-1 eta, interleukin-1 homolog 2
IL-36G	Interleukin-36 gamma	IL-1F9	Interleukin-1 epsilon, interleukin-1 homolog 1, IL-1-related protein 2
IL-36RN	Interleukin 36 receptor antagonist	IL-1F5	FIL1 delta, FIL1D, IL1HY1, IL1RP3, IL1L1, IL-1F5, IL36Ra, MGC29840
IL-37	Interleukin-37	IL-1F7	FIL-1 zeta, IL-1 zeta, IL-1 homolog 4, IL-1-related protein 1
IL-38	Interleukin 38	IL-1F10	Interleukin-1 receptor antagonist-like, FIL-1 theta, IL-1 theta, IL-1 HY2

The symbols IL-14 and IL-30 are no longer used as approved nomenclature

Table 27.2 ■ (continued)

bowel disease, multiple sclerosis, chronic hepatitis, or chronic insulitis leading to diabetes mellitus. All have in common that they need a genetic predisposition and an environmental trigger factor to become active, and are, at best, only partially understood. In many cases these diseases are caused by either insufficient production or overproduction of key interleukins. Thus, in principle, once the diagnosis is made these interleukins can be therapeutically supplemented or suppressed to restore proper balance (Ryff 1996).

Our current knowledge of the interleukins listed in Table 27.2 is briefly summarized below and each referenced article selected expands on the subject. Readers interested in the current knowledge about the protein, DNA, RNA, gene, chromosome location etc. for individual interferons or interleukins are referred to the following databases:

1. www.genatlas.org (with a links to other databases), or
2. http://au.expasy.org/sprot/, or

www.rcsb.org/pdb/ for the 3D models of individual IFNs or ILs. For a better understanding of interleukins and their wide ranging, overlapping, redundant and antagonistic functions we have grouped them into a working classification of various "families" according to gene clustering on chromosomes, gene sequence homologies, secondary and tertiary structure, use of related receptors and also according to their function. This classification should be considered as "work in progress" as it is impossible in many cases to assign a member to a specific "family" or "subfamily". Today structural analysis has become the principal approach for classification; it is therefore likely that these groups will expand as crystallisation data and structural analyses become available (for a recent review see Akdis et al. 2016).

■ Interleukin-1 Family

The IL-1 (Garlanda et al. 2013) family comprises 11 different members: IL-1α, IL-1β, IL-1 receptor antagonist (Ra), IL-18, IL-33, IL-36A, B, C, IL-36Ra, IL-37 and IL-38. All are thought to have arisen from a common ancestral gene that underwent multiple duplications. A tight regulation via receptor antagonists, decoy receptors and signaling inhibitors ensures that amplification of innate immunity does not degenerate into uncontrolled inflammation. All cells of the innate immune system express and/or are affected by IL-1 family members.

Interleukin-1

IL-1 (Towne et al. 2004) is generally used to describe IL-1α and IL-1β both of which have the same biological effects and play a primordial role in the innate and adaptive immune response. Although a prototypical pro-inflammatory cytokine, it also plays a key role in hematopoiesis, appetite control, and bone metabolism. IL-1 is released as part of the acute phase reaction of hepatocytes. The primary producers of IL-1 are macrophages, B-cells and neutrophils. IL-1α and IL-1β are synthesized as pro-peptides of approximately 30 kDa, and are then cleaved to produce products of 159 and 153 amino acids. Differences in glycosylation are responsible for the variation of reported molecular weights.

Interleukin-1Ra

IL-1Ra (Towne et al. 2004) is a naturally occurring IL-1 receptor antagonist (IL-1Ra), an inhibitor of IL-1. It has limited sequence similarity to either IL-1α or IL-1β, but does have the ability to bind to the IL-1 receptors. Lacking IL-1 activity, it acts as a useful blocker of the receptor. A recombinant IL-1Ra has been investigated for its potential use in sepsis; the clinical trials were inconclusive. Recombinant IL-1Ra

has, however, been used successfully for the treatment of rheumatoid arthritis and is marketed under the name of Kineret® (see section "Therapeutic Use of Recombinant Interleukins" below). The interleukin-1 receptor antagonist (IL-1Ra) contributes to tumor survival and progression in multiple cancer entities.

Interleukin-18

IL-18 (Liu et al. 2000) shares unique structural features with the IL-1 family, but it does not have the usual four-helix structure rather an all β-pleated sheet structure. It is produced by activated macrophages such as Kupffer cells of the liver and other resident macrophages from which, after cleavage of its precursor pro-IL-18, the mature protein is released. IL-18 is an early inducer of the Th1 response, co-stimulating with IL-12, the production of IFNγ, TNFα, granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-2. IL-18 is associated with the metabolic syndrome and coronary vascular disease (Trøseid et al. 2010).

Interleukin-33

IL-33 (Schmitz et al. 2005), unlike other members of the IL-1 family which are all pro-inflammatory, has a major role in the development of a Th2 type immune response by inducing IL-5 and IL-13. Human smooth muscle cells as well as epithelial cells forming bronchus and small airways show constitutive expression of IL-33 mRNA; in lung or dermal fibroblasts and keratinocytes IL-33 mRNA is induced after activation with TNFα and IL-1β. Activated dendritic cells and macrophages are the only hematopoietic cells showing low quantities of IL-33 mRNA. In addition, IL-33 and IL-18 are the only known IL-1 family member genes not located on chromosome 2. IL-33 is thought to play a key role in mediating an anaphylactic shock. This effect can be completely neutralized by anti-IL-33 antibodies in an experimental model. Thus IL-33 may be a potential target for the treatment of anaphylactic shock and prevention or treatment of atherosclerosis.

Interleukin-36A, B, and G

IL-36 A, B and G (or α, β, and γ) (Towne et al. 2011) previously classified as interleukin-1 family member (IL-1F) 6, IL-1F8, IL-1F9 respectively.

IL-36A (IL-36 alpha) is a member of the IL-1 family of proteins. Cells reported to express IL-36 alpha include monocytes, B cells, and T cells. Notably, IL-36 alpha is the only novel IL-1 family member expressed on T-cells. It is expressed in the immune system and fetal brain, but not in other tissues tested or in multiple hematopoietic cell lines.

IL-36B (IL-36 beta) is expressed at low levels in tonsils, bone marrow, heart, placenta, lung, testes and colon but not in any hematopoietic cell lines nor in adipose tissue. It is detected at higher levels in psoriatic plaques than in symptomless psoriatic skin or healthy control skin. Increased levels are not detected in inflamed joint tissue. It is induced by proinflammatory cytokines IL-1α, IL-1β and TNF in synovial fibroblasts and by IL-1α and TNF in keratinocytes. It is constitutively expressed in articular chondrocytes. IL-36-B stimulates the production of interleukin-6 and interleukin-8 in synovial fibrobasts, articular chondrocytes and mature adipocytes.

IL-36G (IL-36 gamma), is highly expressed in tissues containing epithelial cells: skin, lung, stomach and esophagus. In the skin it can only be detected in keratinocytes but not in fibroblasts, endothelial cells or melanocytes. TNF and IFNγ up-regulate IL-36G in the keratinocytes of psoriatic skin lesions.

Interleukin-36Ra

IL-36Ra (Towne et al. 2011) acts as an IL-36R antagonist controlling the activity of IL-36. Cells expressing IL-36Ra include monocytes, B cells, dendritic cells/Langerhans cells, keratinocytes, and gastric fundus parietal and chief cells. IL-36Ra is essential for normal skin maintenance. A variant of interleukin-36Ra shows impaired IL-36R affinity and dysregulated secretion of inflammatory cytokines leading to generalized pustular psoriasis (Marrakchi et al. 2011). Il-36Ra has also been documented to suppress inflammation of the brain by enhancing IL-4 response (Collison et al. 2008).

Interleukin-37

IL-37 (Nold et al. 2010) expressed in human monocytes and epithelial cells is a fundamental inhibitor of innate immunity. The overexpression of IL-37 in cells of monocytic or epithelial origin almost completely abolishes the production of pro-inflammatory cytokines.

Interleukin-38

IL-38 was formerly known and HGNC approved as IL-1F10 (Yuan et al. 2015) or IL-1HY2 and has been shown to be expressed in basal epithelia of in fetal skin, in the spleen and in proliferating B-cells of tonsil. This tissue specific expression pattern and the membership of the IL-1 family suggests a role in establishing a normal immune response and inflammatory pathophysiology.

As an efficient method to generate a relatively large quantity of IL-38 is lacking, its biology is largely unexplored. The association of inflammatory pathologies with IL-38 polymorphisms and structural similarities with IL-1Ra suggested an immune regulatory role.

■ Interleukin-2 Family

Interleukin-2 belongs to a family of cytokines, which also includes IL-4, IL-7, IL-9, IL-15 and IL-21 (Liao et al. 2011). These interleukins all share a common receptor γ chain (γc) and are also known as γc-family cytokines.

Interleukin-2

IL-2 (Malek and Castro 2010) originally described as T-cell growth factor (TCGF) is synthesized and secreted primarily by T-cells. IL-2 stimulates the growth, differentiation and activation of T-cells, B-cells, and NK-cells. The major physiological effect is to promote self-tolerance by suppressing T-cell response in vivo. IL-2 signals through a receptor complex consisting of IL-2 specific IL-2 receptor alpha, IL-2 receptor beta and a common gamma chain, which is shared by all members of this cytokine family. A soluble form of the IL-2R capable of binding IL-2, a truncated version of the α chain without cytoplasmic tail, has been found in human serum (soluble receptor or sR). High levels of IL-2sR have been found in patients with a wide variety of disorders, including chronic hepatitis C, HIV infection, cancer, solid organ transplant rejection, and arthritis. Soluble IL-2R can bind released IL-2 prior to its binding to cells to prevent overflow or over-stimulation. Several other cytokine and adhesion molecule receptors also have circulating forms. This is one manner in which the immunological cascade maintains its checks and balances.

Interleukin-4

IL-4, an anti-inflammatory cytokine (Gilmour and Lavender 2008) is produced by Th2 cells and by mast cells, basophils, and eosinophils and acts as an antagonist to interferon- (IFN) γ. It stimulates B-cell proliferation and activation, induces class switch to IgE and IgG_4 expression by B-cells, as well as class II major histocompatibility complex (MHC) expression. In addition, it induces the differentiation of eosinophils and activity of cytotoxic T-cells. IL-4 regulates the differentiation of helper T-cells to the Th2 type. These T-cells produce the cytokines IL-4, IL-5, IL-9, and IL-13, which can all participate in the allergic response. IL-4 regulates the production of IgE by B-lymphocytes. It also has the ability to stimulate chemokine production and mucus hypersecretion by epithelial cells. Overproduction of IL-4 is associated with allergy and asthma.

Interleukin-7

IL-7 (Fry and Mackall 2002) is an essentially tissue-derived cytokine. Its primary sources are stromal and epithelial cells in various locations including intestinal epithelium, liver and, to a lesser degree, dendritic cells. IL-7 acts primarily on pre-B-cells to stimulate their differentiation. It can also stimulate the development of human T-cells. IL-7 is classified as a type I short chain cytokine of the hematopoietin family which also includes IL-2, IL-3, IL-4, IL-5, GM-CSF, IL-9, IL-13, IL-15, macrophage-colony stimulating factor (M-CSF) and stem cell factor (SCF).

Interleukin-9

IL-9 (Noelle and Nowak 2010) is a Th2 cytokine originally characterized as a factor produced by activated T-cells and able to support the long-term growth of some T-helper clones. IL-9 activities extend to various cell types including mast cells, B-lymphocytes, hematopoietic progenitors, eosinophils, lung epithelial cells, neuronal precursors and T-lymphocytes. Increased IL-9 production has been implicated in major pathologies such as asthma supported by its effects on IgE production, mucus production, mast cell differentiation, eosinophil activation and bronchial hyper-responsiveness. IL-9 stimulates the growth of murine thymic lymphomas and an autocrine loop has been suggested in Hodgkin lymphoma. Finally, IL-9 is required for an efficient immune response against intestinal parasites. IL-9 exerts its effects through a receptor that belongs to the hematopoietic receptor superfamily and consists of two chains, also involved in IL-2, IL-4, IL-7, IL-15 and IL-21 signaling.

Interleukin-15

IL-15 (Waldmann 2015) shares the IL-2βγ receptor complex components IL-2Rβ and IL-2Rγ. However, specificity is conferred by a unique α-chain (IL-15Rα) completing the IL-15Rαβγ heterotrimeric high-affinity receptor complex. While the role of interleukin-2 is in the elimination of self-reactive T cells to prevent autoimmunity, interleukin-15 is dedicated to the prolonged maintenance of memory T-cell responses to invading pathogens. It does not stimulate T regulatory cells (Tregs) formerly suppressor T-cells. Thus, boosting IL-15 activity could enhance innate and specific immunity and fight tumors.

Interleukin-21

IL-21 (Yi et al. 2010) is the most recently discovered member of the IL-2 family of cytokines that utilize the common γ-chain receptor subunit for signal transduction. Structurally, it shows homology to the other interleukins of the IL-2 family. The heterodimeric IL-21R has an IL-21 specific subunit besides the γ-chain. IL-21 expression is restricted primarily to activated $CD4^+$ T-cells. IL-21 expression seems transient and stage specific during T-cell differentiation. It is required for normal humoral immunity and regulates antibody production in cooperation with IL-4. IL-21 also regulates cell-mediated immunity by inducing IFNγ, TNF-α and synthesis of perforin and granzyme B leading to cytolytic activity. It can cooperate with other cytokines to generate potent killer T-cells and thus has anti-tumor activity. Lastly, it also has inhibitory activity by inducing IL-10. Thus, altogether, it is responsible for the coordination of the initiation and cessation of an efficient immune response.

■ Interleukin-10 Family

The IL-10 family (Pestka et al. 2004b) includes, besides IL-10, the interleukines: IL-19, -20, -22, -24, -26, -28A, -28B and -29. They share a classical four-helix bundle, a signature element of all helical cytokines (Fig. 27.1) and all share the IL-10R2 or α chain of their dimeric receptor, while each has its own R1 or α chain.

Interleukin-10

IL-10 (Pestka et al. 2004b) is a major endogenous anti-inflammatory mediator, which acts by profoundly inhibiting the synthesis of proinflammatory molecules such as IFNγ, IL-2, IL-12 and TNFα. Macrophages are the major source of IL-10, a homodimer. Th2 cell subsets, monocytes and several other cells can also synthesize this imterleukin. A number of molecules produced under stress conditions including reactive oxygen species stimulate IL-10 synthesis. Recombinant human IL-10 has been tested in clinical trials in rheumatoid arthritis, inflammatory bowel disease, psoriasis, organ transplantation, and chronic hepatitis C. To date the results are mixed or disappointing. However, they give new insight into the immunobiology of IL-10.

Interleukin-19

IL-19 (Azuma et al. 2010) is a member of the IL-10 family. The induction of IL-19 in human monocytes is down-regulated by IFN-γ and up-regulated by IL-4. IL-19 influences the balance of Th1/Th2 cells in favour of Th2 cells by up-regulating IL-4 and down-regulating IFNγ. IL-19 is essential for the induction and maintenance of endotoxin tolerance and appears to play a key role in innate immunity. IL-19 together with IL-20 with whom it shares the same receptor complex, has been associated with psoriasis and is thought to be involved in regulating inflammatory response in various tissues and be of particular importance for proper skin development and function.

Interleukin-20

IL-20 (Xu 2004) was originally identified from a keratinocyte library, its mRNA isolated from skin and trachea. It is classified as a helical cytokine member of the IL-10 family. Keratinocytes and activated monocytes synthetize IL-20. IL-1β, TGF-α and epidermal growth factor (EGF), factors known to be involved with proliferative and pro-inflammatory signals in the skin, enhance the response to IL-20. It binds to two cell surface receptors: IL-20Rα and IL-20Rβ on keratinocytes and other epithelial cells. IL-20 mediates the hyperproliferation of keratinocytes associated with cutaneous inflammation and has a central role in inflammatory skin diseases such as psoriasis and eczema. It also promotes the expansion of pluripotential hematopoietic progenitor cells indicating a role beyond the response of epithelial cells to inflammation.

Interleukin-22

IL-22 (Kotenko et al. 2001a) also belongs to the family of cytokines structurally related to IL-10. In contrast to IL-10, it has proinflammatory activities: it upregulates the production of acute-phase proteins. The IL-22 receptor is composed of an IL-22-binding chain, IL-22R1 and the IL-10R2 subunit, which is shared with the IL-10R. IL-22 is produced by activated human T-helper cells and mast cells. A soluble IL-22-binding protein, IL22RBP, encoded by a distinct gene, has been identified. This soluble receptor, which has 34% amino acid identity to the extracellular domain of the IL-22R1, binds IL-22 and antagonizes its functional activities (Kotenko et al. 2001b). The skin is also a target for IL-22; high IL-22 expression has been detected in the skin of patients with T-cell-mediated dermatoses. Normal human epidermal keratinocytes express a functional receptor for IL-22 but not for IL-10. IL-22 plays a role in skin inflammatory processes and wound healing.

Interleukin-24

IL-24 (Wang and Liang 2005) is a member of the IL-10 family secreted by activated peripheral blood mononuclear cells and the ligand for two heterodimeric receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2. The latter is also a receptor chain for IL-20. Under physiological conditions, the major sources of IL-24 are activated monocytes and Th2 cells, whereas the major IL-24 target tissues, based on the receptor expression pattern, are non-hematopoietic in origin and include skin, lung and reproductive tissues. Structurally and functionally, IL-24 is highly conserved across species. It has shown anti-angiogenic activity and its gene is a tumor suppressor gene (Dent et al. 2010).

Interleukin-26

IL-26 (Donnelly et al. 2010) is part of the IL-10 family and produced by Th17 cells, to some extent in NK cells. It binds to a heterodimeric receptor composed of the IL-20R1 and IL-10R2 chains and is frequently co-expressed with IL17 and IL-22. Targeting epithelial cells that express IL-20R1, IL-26 is likely to play a role in local mechanisms of mucosal and cutaneous immunity. Furthermore, IL-26 appears to play a central role in autoimmune disease.

Interleukin 28A and B and Interleukin 29

Recently, the human genomic sequence for a family of three cytokines, designated IL-28A, IL-28B and IL-29 (Donnelly and Kotenko 2010), that are distantly related to type I IFNs (IFNλ1-3) (Pestka et al. 2004a, b) and the IL-10 family has been described. Like type I IFNs, IL-28 and IL-29 are induced by viral infection and have antiviral activity. However, IL-28 and IL-29 interact with a heterodimeric class II cytokine receptor that consists of the IL-10 receptor II (IL-10R2) and an orphan class 2 receptor chain, designated IL-28R1. This newly described cytokine family may serve as an alternative to type I IFNs in providing resistance to viral infection and antitumor activity.

■ Interleukin-12 Family

The IL-12 family (Collison et al. 2008) includes IL-12, IL-23, IL-27 and IL-35, consist of mediators of inflammation. Each member is a heterodimeric complex com-

posed of two subunits whose expression is regulated independently (see also IL-23, IL-27 and IL-35 below).

Interleukin-12

IL-12 (Trinchieri 2003) is a 70 kDa heterodimeric proinflammatory cytokine composed of two covalently linked glycosylated chains: p35 and p40. It is mainly produced by activated monocytes, macrophages and dendritic cells DCs), enhances proliferation and cytolytic activity of NK- and T-cells, and stimulates their IFNγ production, towards a Th1 response while it inhibits Th2 cells. Dysregulation of IL-12 production can have a major impact on the modulation of immune and allergic responses. Recombinant IL-12 has several potential therapeutic uses in infectious diseases, allergy, and cancer.

Interleukin-23

IL-23 (Aggarwal et al. 2003) is a heterodimeric cytokine comprising the IL-12 p40 subunit of IL-12 and an IL-23 specific p19 subunit. It is produced by activated dendritic cells and acts on memory CD4+ T-cells. IL-23 induces IL-17 and thus plays an early role in defense against Gram-negative infection. It is also pivotal for establishing and maintaining organ-specific inflammatory autoimmune disease. IL-23 and IL-27 both have potent antitumor activity even against poorly immunogenic tumors using different effector mechanisms.

Interleukin-27

IL-27 (Fabbi et al. 2017) is a pleiotropic two-chain cytokine, composed of EBI3 and IL-27p28 subunits, which is structurally related to both IL-12and IL-6 cytokine families. It acts through a heterodimer receptor consisting of IL-27Rα (WSX1) and gp130 chains. It was initially reported as an immune-enhancing cytokine (Th1-type) acting in concert with IL-12. However, later research outcomes showed that IL-27 displays complex immune-regulatory functions, which may result in either proinflammatory or anti-inflammatory effects. Several pieces of evidence, obtained in preclinical tumor models, indicated that IL-27 has a potent antitumor activity, related not only to the induction of tumor-specific Th1 and cytotoxic T lymphocyte (CTL) responses but also to direct inhibitory effects on tumor cell proliferation, survival, invasiveness, and angiogenic potential. In view of its dual roles, the effects of IL-27 on cancer may also have protumor effects.

Interleukin-35

IL-35 (Collison et al. 2008) is a member of the IL-12 cytokine family, which is linked to the IL-6 cytokine superfamily. The IL-12 family comprises IL-12, IL-23, IL-27 and IL-35. Unlike the other three family members, IL-35 is an anti-inflammatory cytokine produced by regulatory T-cells (T-reg), which are a critical subpopulation of $CD4^+$ T cells essential for maintaining self-tolerance and preventing autoimmunity. IL-35 is a hetero-dimeric protein composed of the IL-12α and IL-27β chains.

■ Interleukin-17 Family

IL-17 (Iwakura et al. 2011) a homodimeric glycoprotein more recently renamed IL-17A can also form a heterodimer with IL-17F to which it is the most closely related family member. Four additional members IL-17B to IL-17E have been discovered, whereby IL-17E has been renamed IL-25 (see below). IL-17A and IL-17F are predominantly produced upon stimulation of Th17 cells (CD^+ T-helper cells type 17) by IL-23 after induction of differentiation of naive T-cells by IL-6 and TGFβ. IL-17C has a very restricted expression pattern but has been detected in adult prostate and fetal kidney. Aside from their importance in modulating T-cell mediated inflammatory response and effective host defense against pathogen infections, IL-17 s also have a role in the homeostasis of tissues. The IL-17A/F pathway is implicated in the progression of autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and psoriasis. Although IL-17A and IL-17F share the highest amino acid sequence homology, they perform distinct functions. IL-17A is involved in the development of autoimmunity, inflammation, and tumors, and also plays important roles in the host defenses against bacterial and fungal infections, whereas IL-17F is mainly involved in mucosal host defense mechanisms. The functions of IL-17B, IL-17C, and IL-17D remain largely elusive. IL-17E (IL-25) is an amplifier of Th2 immune responses.

Interleukin-25

IL-25 (Fort et al. 2001) is a cytokine that shares sequence similarity with IL-17 and was previously called IL-17E. It is produced by Th2-cells, and its biological effects differ markedly from those of the other described IL-17 family members and have been implicated in the promotion of Th2 immunity. IL-25 induces IL-4, -5 and -13 and causes histological changes in the lungs and GI tract, including eosinophilic and mononuclear infiltrates, increased mucus production, and epithelial cell hyperplasia and hypertrophy. IL-25 appears to be a key cytokine for the development of Th2 associated pathologies such as asthma and other allergic reactions, as well as antiparasitic response.

■ Hematopoietin Family

Because many cytokines are multifunctional and have overlapping activities, several members of the hematopoietin family overlap with other classifications. The hematopoietins (Metcalf 2008) constitute a family of structurally related proteins that includes various interleukins IL-3, IL-4, IL-5, IL-6, IL-9, IL-11 and IL-13, growth factors including G-CSF, GM-CSF, M-CSF and

thrombopoietin, erythropoietin, SCF (stem cell factor), SCPF (stem cell proliferation factor) and other proteins identified initially by some biological activities not related to hematopoiesis such as IL-2, IL-12 (see Chap. 24: Haematopoietic Growth Factors).

Interleukin-3

IL-3 (Martinez-Moczygemba and Huston 2003) is produced by activated T cells, monocytes/macrophages and stroma cells. It is a multicolony stimulating, hematopoietic growth factor which stimulates the generation of hematopoietic progenitors of every lineage. Administration of IL-3 produces an increase in erythrocytes, neutrophils, eosinophils, monocytes and platelets. IL-3, however, is not involved in constitutive hematopoiesis but rather in inductive hematopoiesis upon exposure to immunological stress. IL-3 can act synergistically or additively with other hematopoietic growth factors such as GM-CSF, IL-5 and erythropoietin (EPO).

Interleukin-5

IL-5 (Greenfeder et al. 2001) acts as a homodimer originally known as T-cell replacement factor (TRF), eosinophil differentiation factor (EDF) and B-cell growth factor (BCGF) II. It is produced by Th2-helper and mast cells. It acts on the eosinophilic lineage, stimulating eosinophil expansion and chemotaxis and also affects basophils. In humans, IL-5 is a very selective cytokine as only eosinophils and basophils express IL-5 receptors. Interleukin-5 has been associated with the cause of several allergic diseases including allergic rhinitis and asthma and is therefore a target for the treatment of severe asthma.

Interleukin-6

IL-6 (Kamimura et al. 2003) is a proinflammatory cytokine that not only affects the immune system, but also acts in many physiological events in various organs that influence homeostatic processes. It is produced by lymphoid and non-lymphoid cells and was formerly known as interferon-β_2 for its weak antiviral activity. By stimulating hepatocytes to produce "acute phase proteins" it plays a central role in the "acute phase reaction". It is also responsible for the reactive thrombocytosis seen in acute inflammatory processes by stimulating thrombopoietin. Furthermore, IL-6 is associated with insulin resistance in type 2 diabetes mellitus (Kristiansen and Mandrup-Paulsen 2005). Together with IL-11 (below) and IL-27, IL-6 is also a member of the gp130 receptor cytokine family, which also includes other cytokines not classified as interleukins.

Interleukin-11

IL-11 (Du and Williams 1997) initially described as hematopoietic factor with thrombopoietic activity has subsequently been shown to be expressed and active in many other tissues including brain, spinal cord neurons, gut, and testes. IL-11 acts synergistically with other cytokines such as IL-3, -4, -7, -12, -13, SCF and GM-CSF to stimulate various stages and lineages of hematopoiesis. In particular with IL-3 and thrombopoietin (TPO) also termed megakaryocyte growth and development factor (MGDF), it works on various stages of megakaryocytopoiesis and thrombopoiesis. Treatment with IL-11 results in production, differentiation, and maturation of megakaryocytes. IL-11 also has a direct effect on erythroid progenitors and modulates the differentiation and maturation of myeloid progenitor cells. Alveolar and bronchial epithelial cells produce IL-11, which is upregulated by inflammatory cytokines and respiratory syncitial virus (RSV) suggesting that it plays a role in pulmonary inflammation. Moreover, IL-11 is an important regulator of bone metabolism. Evidence indicates that IL-11 together with transforming growth factor (TGF)β, IL-1 and -15 are crucial for successful human implantation and placentation.

■ Interleukin-13

IL-13 (Wills-Karp 2004) is a glycoprotein cloned from activated T-cells. IL-13 was first recognized for its effects on B cells and monocytes, where it upregulated MHC class II expression, promoted IgE class switching and inhibited inflammatory cytokine production. The functions of IL-13 overlap considerably with those of IL-4, especially with regard to changes induced on hematopoietic cells. IL-13 also has several unique effector functions that distinguish it from IL-4. Resistance to most gastrointestinal nematodes is mediated by type-2 cytokine responses, in which IL-13 plays a dominant role. By regulating cell-mediated immunity, IL-13 modulates resistance to intracellular organisms. In the lung, IL-13 is the central mediator of allergic asthma, where it regulates eosinophilic inflammation, mucus secretion, and airway hyperresponsiveness. IL-13 can also inhibit tumor immune-surveillance. Thus, inhibitors of IL-13 might be effective as cancer immunotherapeutics by boosting type-1-associated antitumor defenses. Investigations into the mechanisms that regulate IL-13 production and/or function have shown that IL-4, IL-9, IL-10, IL-12, IL-18, IL-25, IFN-γ, TGF-β, TNF-α, and the IL-4/IL-13 receptor complex are essential for these processes.

■ Others Not (Yet) Assigned to a Family

Interleukin-8

IL-8 (Remick 2005) is a 6–8 kDa CXC chemokine, a potent chemoattractant for neutrophils. It affects the pro-inflammatory effector side, including the stimulation of neutrophil degranulation and the enhancement of neutrophil adherence to endothelial cells. It is produced by monocytes, macrophages, fibroblasts, keratinocytes and endothelial cells. Elevated levels of IL-8

have been found in psoriatic arthritis, synovial fluid and synovium. IL-8 contributes to human cancer progression through its potential functions as a mitogenic, angiogenic, and motogenic factor. Because of the roles that IL-8 plays in favoring tumor progression, several therapeutic strategies are being developed to interfere with its functions (Alfaro et al. 2017).

Interleukin-16

IL-16 (Cruikshank and Little 2008) is a pro-inflammatory cytokine produced by a variety of immune (T-cells, eosinophils, dendritic cells [DCs]) and non-immune (fibroblasts, epithelial and neuronal) cells and induces chemotaxis of not only CD4+ T-cells but also monocyte/macrophages and eosinophils. It is synthesized as a precursor molecule (pro-IL-16), cleaved in the cell cytoplasm and secreted as mature IL-16. It regulates T-cell growth and primes $CD4^+$ T cells for IL-2 and IL-15. IL-16 has been shown to play a role in asthma, Crohn's Disease (CD) and systemic lupus erythematosus (SLE). IL-16 also inhibits human (HIV) and simian (SIV) immunodeficiency virus. A neuronal form of IL-16 detected in neurons of the cerebellum and hippocampus has been described.

Interleukin-31

IL-31 (Bilsborough et al. 2006) is a 4-helix bundle cytokine preferentially expressed by activated T-cells with a Th2 bias. Together with IL-4 and IL-13, IL-31 has been implicated in the pathogenesis of atopic dermatitis because they are produced by a subset of T-cells that home to the skin. IL-31 signals through a heterodimeric receptor constitutively expressed by epithelial cells including keratinocytes. IL-31 stimulated keratinocytes induce a whole array of inflammatory chemokines, which also facilitate the recruitment of lymphocytes, monocytes and polymorphonuclear cells to the epidermis.

Interleukin-32

IL-32 (Kim et al. 2005) is a polypeptide, which was described several years ago as natural killer cell transcript 4 (NK4) of activated T-cells and NK-cells and belongs to the pro-inflammatory cytokines. Subsequently it has been detected in higher concentration in patients with sepsis compared to healthy individuals. It was also found to induce the expression of various inflammatory cytokines including TNF-α, IL-6, IL-1β and macrophage inflammatory protein-2 (MIP-2), a chemokine, in different cells via the signal pathway of proinflammatory cytokines. IL-32 is involved in the pathogenesis and progression of inflammatory bowel disease (IBD), gastric inflammation and cancer, rheumatoid arthritis, and chronic obstructive pulmonary disease (COPD). Moreover, it regulates cell growth, metabolism and immune regulation and is therefore involved in the pathology of inflammatory diseases.

Interleukin-34

IL-34 (Lin et al. 2008) forms homodimers and promotes survival and differentiation of monocytes and macrophages. It elicits its activity by binding to the shared (macrophage) colony stimulating factor 1 receptor (CSF-1R). Messenger RNA (mRNA) expression of human IL-34 is found mostly in the spleen but occurs in several other tissues as well: thymus, liver, small intestine, colon, prostate gland, lung, heart, brain, kidney, testes, and ovary. IL 34 also plays an important role in the regulation of osteoclast proliferation and differentiation, and in the regulation of bone resorption (Baud'huin et al. 2010).

THERAPEUTIC USE OF RECOMBINANT INTERFERONS

■ IFNα Therapeutics

Together with recombinant human insulin and growth hormone, recombinant IFNα was one of the first rDNA-derived pharmaceuticals. The drive to produce recombinant interferon and other rDNA-derived pharmaceuticals developed from the need to obtain large amounts of a well defined, purified protein for large-scale therapeutic use (Pestka 1981a, 1981b, 1986). Availability of the necessary basic technologies (see Chaps. 1 and 2) made this possible. Starting in the early 1980s, a number of cytokines produced by recombinant gene technology were developed to become innovative therapeutic modalities called biologicals or biopharmaceuticals. Table 27.3 summarizes the recombinant IFNs approved for therapeutic use.

Interferon alfa-2 (a modified generic name for IFNα2) was developed independently by Hoffmann-LaRoche Ltd. (Interferon alfa-2a; Roferon®A) and Schering Plough Corporation (Interferon alfa-2b; Intron®A). Both were obtained by recombinant DNA technology in *E. coli*, consist of 165 amino acids with an approximate molecular weight of 19 kDa and differ by one amino acid in position 23: Lys for interferon alfa-2a and Arg for interferon alfa-2b (Pestka 1986). For all practical purposes there is no difference between these two products in terms of pharmacological properties or clinical application.

The metabolism of interferon alfa-2a is consistent with that of alfa interferons in general and is therefore used as example. Alfa interferons are totally filtered through the glomeruli and undergo rapid proteolytic degradation during tubular reabsorption (see Chap. 6). Liver metabolism and subsequent biliary excretion are considered minor pathways of elimination for alfa interferons. After intramuscular (IM) and subcutaneous (SC) administrations of 36 million international units (MIU), peak serum concentrations range from 1500 to 2580 picograms/milliliter (pg/mL) (mean 2020 pg/mL) at a mean time to peak of 3.8 h and from 1250 to 2320 pg/mL (mean 1730 pg/mL) at a mean time to peak of

Recombinant Interferons	Company	1st Indication	1st approval
Interferon-α			
IFN-α2a produced in *E.coli;* Roferon A®	Hoffmann–La Roche (Basel, Switzerland)	Hairy cell leukemia	1986 (EU and US)
IFN-α2b produced in *E. coli;* Intron® A; Viraferon®; Alfatronol®	Schering-Plough (Kenilworth NJ, USA)	Hairy cell leukemia	1986 (US and EU)
IFN-αcon1, synthetic type I IFN produced in *E. coli;* Infergen®	Amgen (Thousand Oaks, US), Yamanouchi Europe (Leiderdorp, The Netherlands, EU)	Chronic hepatitis C	2001 (US)
Interferon-β			
IFN-β1a produced in CHO cells; Rebif®	Serono (Geneva, Switzerland)	Relapsing/remitting multiple sclerosis	1998 (EU) 2002 (US)
IFN-β1a produced in CHO cells; Avonex®	Biogen (Cambridge, MA, USA)	Relapsing/remitting multiple sclerosis	1997 (EU), 1996 (US)
IFN-β1b Cys17 Ser substitution; produced in *E. coli*; Betaferon®	Schering AG (Berlin, Germany)	Relapsing/remitting multiple sclerosis	1995 (EU)
IFN-β1b, Cys17 Ser substitution; produced in *E. coli*; Betaseron®	Berlex Labs/Chiron (Richmond/Emeryville, CA, USA)	Relapsing/remitting multiple sclerosis	1993 (US)
Interferon-γ			
Actimmune® (IFN-γ1b; produced in *E. coli*)	Genentech (San Francisco CA, USA), InterMune (Palo Alto, CA, USA)	Chronic granulomatous disease	1990 (US)

Adapted from *Nature Biotechnology* 2006, 24: 769–776

Table 27.3 ■ Interferons approved as biopharmaceuticals approved in the United States and Europe

7.3 h, respectively. The apparent fraction of the dose absorbed after intramuscular injection is >80%. The pharmacokinetics of interferon alfa-2a after single intramuscular doses to patients with disseminated cancer are similar to those found in healthy volunteers. Dose proportional increases in serum concentrations are observed after single doses up to 198 MIU. There are no changes in the distribution or elimination of interferon alfa-2a during twice daily (0.5–36 MIU), once daily (1–54 MIU), or three times weekly (1–136 MIU) dosing regimens up to 28 days of dosing. At the higher doses multiple IM doses of interferon alfa-2a result in an accumulation of two to four times the serum concentrations seen after a single dose.

Roferon®A and Intron®A are approved for the following indications: chronic hepatitis B and C, Kaposi's sarcoma, renal cell carcinoma, malignant melanoma, carcinoid tumor, multiple myeloma, non-Hodgkin lymphoma, hairy cell leukemia, chronic myelogenous leukemia, thrombocytosis associated with chronic myelogenous leukemia and other myeloproliferative disorders. The approved indications vary depending on company and regulatory policies; for detailed information as well as for the recommended dosing the reader is referred to the respective product information current in their countries.

The adverse event profile for these IFNα products is the same; it is generally more or less well tolerated depending on the dose regimen used and subjectively consists primarily of the "influenza-like symptoms" named as such because they mimic the symptoms of early influenza. This, of course, should come as no surprise as these symptoms are caused by peaks of endogenous interferon stimulated by the influenza virus infection. For a detailed reporting of all adverse events, the reader is referred to the product information for each product.

Given the principle that the toxicity of a given medication may be defined by its peak concentration and by the time it is above a toxic threshold concentration and the efficacy by the time the substance is above the minimal therapeutic level, it would be desirable to obtain a therapeutic regimen that minimizes fluctuations in the range below the toxic and above the therapeutic threshold concentration. A constant therapeutic drug concentration would be an ideal goal. The first step towards that goal, as a proof of concept, was to model a long-acting interferon using an insulin pump to inject patients with chronic hepatitis C with interferon α-2a at predetermined rates per hour for 28 days. A similar study was performed in patients with renal cell carcinoma. These studies indicated that interferon α-2a at a constant dose was indeed better tolerated while showing activity when administered by continuous SC infusion (Carreño et al. 1992, Ludwig et al. 1990). The next step therefore was to develop a new longer acting molecule by attaching several polyethylene glycol (PEG) chains to the native interferon molecule (see section "Engineering IFNs and ILs: A Continuing Story - Pegylation", below.)

Pharmaceutical Formulations and Dosing for Interferon alfa Therapeutics

Roferon® A is supplied as pre-filled syringes containing 3 MIU, 4.5 MIU, 6 MIU or 9 MIU in 0.5 mL, or as cartridges containing 18 MIU per mL for SC injection only, or as vials each containing 3 MIU, 6 MIU, 9 MIU or 36 MIU in 1 mL, or multidose injectable solutions containing 9 MIU (each 0.3 mL contains 3 MIU) or 18 MIU of Interferon α-2a (each mL contains 6 MIU) for SC or IM injection. All presentations are human HSA (human serum albumin)-free liquid formulations with 7.21 mg sodium chloride, 0.2 mg polysorbate 80, 10 mg benzyl alcohol (as a preservative), 0.77 mg ammonium acetate and sterile water for injections.

IntronA® is supplied as vials containing 10 MIU, 15 MIU or 50 MIU as lyophilisate and a vial with 1 mL of diluent for reconstitution containing 20 mg glycine, 2.3 mg sodium phosphate dibasic, 0.55 mg sodium phosphate monobasic and 1.0 mg HSA, or as solution vials containing 10 MIU as single dose, 18 MIU or 25 MIU as multidose with 7.5 mg sodium chloride, 1.8 mg sodium phosphate dibasic, 3 mg sodium phosphate monobasic, 0.1 mg edetate disodium, 0.1 mg polysorbate 80, and 1.5 mg m-cresol as a preservative per mL for SC, IM or intralesional injection, or solution in multidose pens containing 6 doses of 3 MIU, 5 MIU or 10 MIU Interferon α-2b per 0.2 mL and excipents as above for SC injection.

Infergen® (interferon alfacon-1) is a synthetic "consensus" interferon consisting of 166 amino acids and not occurring in nature. It was genetically engineered in *E. coli* by Amgen. The amino acid sequence of the product is derived by comparison of the sequences of several natural interferon-α subtypes and assigning the most frequently observed amino acid in each corresponding position. Infergen® is supplied as single-dose, preservative-free vials containing either 9 μg (0.3 mL) or 15 μg (5 mL) of interferon alfacon-1 for SC injection.

■ IFNβ Therapeutics

Three IFNβ-products (Table 27.3) are marketed worldwide for the treatment of multiple sclerosis: the first was Berlex's Betaseron®, marketed by Schering AG as Betaferon® in Europe. It is Interferon β-1b with 165 amino acids and an approximate molecular weight of 18,500 Da, with a cysteine-17-serine substitution. It is produced in *E. coli*, which was then the standard method. It is non-glycosylated, as without further engineering glycosylation is not possible in the *E. coli* system (see Chap. 4). Independently, Biogen and Serono developed a glycosylated IFNβ-1a produced in Chinese hamster ovary cells. Thus, not only is the amino acid sequence of these IFNβs identical to that of natural fibroblast derived human interferon beta, but they are also glycosylated, each containing a single N-linked complex carbohydrate moiety. The two products are marketed as Avonex® and Rebif®, respectively. All three products are indicated for the treatment of multiple sclerosis.

Glycosylating proteins fundamentally alters their pharmacokinetic and pharmacodynamic properties. The non-glycosylated interferon β-1b ($IFN\beta_{ser17}$) has the expected short circulation time: time to peak concentration (C_{max}) between 1 and 8 h with a mean peak serum interferon concentration of 40 IU/mL after a single SC injection of 0.5 mg (16 MIU). Bioavailability is about 50%. Patients receiving single intravenous (IV) doses up to 2.0 mg (64 MIU) show an increase in serum concentrations, which is dose proportional. Mean terminal elimination half-life values ranged from 8.0 min to 4.3 h. Thrice weekly IV dosing for 2 weeks resulted in no accumulation of IFNβ-1b in sera of patients. Pharmacokinetic parameters after single and multiple IV doses were comparable. Following every other day SC administration of 0.25 mg (8 MIU) IFNβ-1b in healthy volunteers, biologic response marker levels (neopterin, β2-microglobulin, myxovirus resistance protein 1 [MxA protein] and IL-10) increased significantly above baseline for 6 to 12 h after the first dose. Biologic response marker levels peaked between 40 and 124 h and remained elevated above baseline throughout the 7-day (168-h) study.

Glycosylated IFNβ-1a such as Rebif®, on the other hand, is slower to reach C_{max}, with a median of 16 h and the serum elimination half-life is 69 ± 37 h (mean ± SD). In healthy volunteers a single SC injection of 60 μg (~18 MIU) of interferon β-1a resulted in a C_{max} of 5.1 ± 1.7 IU/mL. Following every other day SC injections in healthy volunteers, an increase in AUC of approximately 240% was observed, suggesting that accumulation of IFNβ-1a occurs after repeated administration. Biological response markers (e.g. 2′,5′-oligoadenylate synthetase [2,5′ OAS], neopterin and β_2-microglobulin) are induced by IFNβ-1a following a single SC administration of 60 μg. Intracellular 2′,5′-OAS peaked between 12 and 24 h and β_2-microglobulin and neopterin serum concentrations showed a maximum at approximately 24 to 48 h. All three markers remained elevated for up to 4 days. Administration of 22 μg (6 MIU) IFNβ-1a three times per week inhibited mitogen-induced release of pro-inflammatory cytokines (IFNγ, IL-1, IL-6, TNF-α and TNF-β) by peripheral blood mononuclear cells that, on average, was near double that observed with IFNβ-1a administered once per week at either a 22 (6 MIU) or 66 μg (12 MIU) dose.

Pharmaceutical Formulations and Dosing for Interferon Beta Therapeutics

Betaseron®/Betaferon® is formulated as a sterile powder with a 0.54% sodium chloride solution as diluent. Reconstituted it presents as 0.25 mg (8 MIU of antiviral activity) per mL. The recommended dose is 0.25 mg injected SC every other day.

Avonex® is formulated as a lyophilized powder for IM injection. After reconstitution with the supplied diluent (sterile water for injection) each vial contains

30 µg of IFNβ-1a, 15 mg human serum albumin (HSA), 5.8 mg sodium chloride, 5.7 mg dibasic sodium phosphate and 1.2 mg monobasic sodium phosphate in 1.0 mL at a pH of approximately 7.3, or as a prefilled syringe with a sterile solution for IM injection containing 0.5 mL with 30 µg of interferon β-1a, 0.79 mg sodium acetate trihydrate, 0.25 mg glacial acetic acid, 15.8 mg arginine hydrochloride and 0.025 mg polysorbate 20 in water for injection at a pH of approximately 4.8. The recommended dosage is 30 µg injected IM once a week.

Rebif® is supplied in pre-filled 0.5 mL syringes: each 0.5 mL contains either 22 µg (6 MIU) or 44 µg (12 MIU) of IFNβ-1a, 2 or 4 mg HSA, 27.3 mg mannitol, 0.4 mg sodium acetate, and water for injection. The recommended dosage is 22 micrograms (µg) (6 MIU) given 3 times per week by SC injection. This dose is effective in the majority of patients to delay progression of the disease. Patients with a higher degree of disability EDSS (Kurtzke 1983) of 4 or higher may require a dose of 44 µg (12 MIU) 3 times per week.

The adverse event profile for the three IFNβ is similar to IFNα. It is generally reasonably well tolerated and subjectively again consists primarily of the "influenza-like symptoms". For a detailed reporting of all adverse events, the reader is referred to the product information for each biopharmaceutical.

■ IFNγ Therapeutics

Actimmune® (recombinant interferon γ-1b; immune IFN) is a single-chain polypeptide containing 140 amino acids. It is produced by genetically engineered *E. coli* containing the DNA encoding the human protein. It is a highly purified sterile solution consisting of non-covalent dimers of two identical 16,465 Da monomers. Actimmune® is slowly absorbed; after IM injection of 100 µg/m^2, a C_{max} of 1.5 ng/mL is reached in approximately 4 h, and after SC injection a C_{max} of 0.6 ng/mL is reached in 7 h. The apparent fraction of dose absorbed is >89%. The mean half-life after IV administration was 38 min and after IM and SC dosing with 100 µg/m^2 were 2.9 and 5.9 h, respectively. Multiple-dose SC pharmacokinetics showed no accumulation of Actimmune® after 12 consecutive daily injections of 100 µg/m^2.

Pharmaceutical Formulations and Dosing for Interferon Gamma Therapeutics

Actimmune® is a solution filled in a single-dose vial for SC injection. Each 0.5 mL contains: 100 µg (2 million IU) of IFNγ-1b, formulated in 20 mg mannitol, 0.36 mg sodium succinate, 0.05 mg polysorbate 20 and sterile water for injection. The dosage for the treatment of patients with chronic granulomatous disease or severe, malignant osteopetrosis is 50 µg/m^2 (1 million IU/m^2) for patients with a body surface area greater than 0.5 m^2 and 1.5 mcg/kg/dose for patients with a body surface area equal to or less than 0.5 m^2.

The adverse event profile of IFNγ is similar to IFNα; it is generally well tolerated and subjectively consists primarily of the "influenza-like symptoms". For a detailed reporting of all adverse events, the reader is referred to the Actimmune® product information.

THERAPEUTIC USE OF RECOMBINANT INTERLEUKINS

In general, the approach to the development of interleukins as a therapeutic modality is even more challenging than for IFNs. Most interleukins are embedded in a regulatory network and so far, the therapeutic use of interleukins has been somewhat disappointing. This was largely due to our lack of understanding of the role of these molecules and of the best way to use them; they are less well studied than IFNs. IL-2, for example, was initially developed by oncologists in the days when "go in fast, hit them hard and get out" was the prevalent strategy. Terms such as maximal tolerated dose (which we called minimal poisonous dose) actually defined the dose at which a given drug was in most cases no longer tolerated. Thus, IL-2 got an undeserved bad reputation. Similar thinking nearly killed the development of IFNα for the treatment of chronic viral hepatitis and was ultimately the main reason for discontinuing the development of IL-2 in chronic hepatitis B (Pardo et al. 1997, Artillo et al. 1998) and IL-12 in chronic hepatitis B and C (Zeuzem et al. 1999, Carreño et al. 2000, Pockros et al. 2003). In spite of this progress has been made and our understanding of the complexities of such substances and their antagonists is growing. Interleukins currently approved as biopharmaceuticals worldwide are listed in (Table 27.4).

Recombinant interleukins	Company	1st Indication	1st approval
Proleukin® (aldesleukin; IL-2, lacking N-terminal alanine, C125 S substitution, produced in *E. coli*)	Chiron therapeutics (Emeryville, CA)	RCC (renal-cell carcinoma)	1992 (EU and US)
Neumega® (oprelvekin; IL-11, lacking N-terminal proline produced in *E. coli*.)	Genetics Institute (Cambridge, MA) now Pfizer Inc	Prevention of chemo-therapy induced thrombocytopenia	1997 (US)
Kineret® (anakinra; IL-1 receptor antagonist (produced in *E. coli*)	Amgen (Thousand Oaks, CA)	RA (rheumatoid arthritis)	2001 (US)

Adapted from *Nature Biotechnology* 2006, 24: 769–776

Table 27.4 ■ Interleukins approved as biopharmaceuticals worldwide

■ Aldesleukin

Proleukin® (aldesleukin), a non-glycosylated human recombinant interleukin-2 product, is a highly purified protein with a molecular weight of approximately 15 kDa. The chemical name is des-alanyl-1, serine-125 human interleukin-2. It is produced by recombinant DNA technology using a genetically engineered *E. coli* containing an analog of the human interleukin-2 gene. The modified human IL-2 gene encodes a modified human IL-2 differing from the native form: the molecule has no N-terminal alanine; the codon for this amino acid was deleted during the genetic engineering procedure; moreover, serine was substituted for cysteine at amino acid position 125. Aldesleukin exists as biologically active, non-covalently bound microaggregates with an average size of 27 recombinant interleukin-2 molecules. The pharmacokinetic profile of aldesleukin is characterized by high plasma concentrations following a short IV infusion, rapid distribution into the extravascular space and elimination from the body by metabolism in the kidneys with little or no bioactive protein excreted in the urine. Studies of aldesleukin administered IV indicate that upon completion of infusion, approximately 30% of the administered dose is detectable in plasma. Observed serum levels are dose proportional. The distribution and elimination half-life after a 5-min IV infusion are 13 and 85 min, respectively. In humans and animals, aldesleukin is cleared from the circulation by both glomerular filtration and peritubular extraction in the kidney. The rapid clearance of aldesleukin has led to dosage schedules characterized by frequent, short infusions. The adverse event profile of IL-2 is similar to that seen for IFNs and many ILs; it is generally reasonably well tolerated and subjectively consists primarily of the "influenza-like symptoms". For a detailed reporting of all adverse events, rarely severe, and pharmacological properties the reader is referred to the product information for Proleukin®.

Pharmaceutical Formulations and Dosing of Aldesleukin

Proleukin® is supplied as a sterile, lyophilized cake in single-use vials intended for IV injection. After reconstitution with 1.2 mL sterile water for injection, each mL contains 18 million IU (1.1 mg) aldesleukin, 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, without preservatives, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5. It is indicated for the treatment of adults with metastatic renal cell carcinoma or metastatic melanoma. Each treatment course consists of two 5-day treatment cycles: 600,000 IU/kg (0.037 mg/kg) are administered every 8 h by a 15-min IV infusion for a maximum of 14 doses. Following 9 days of rest, the schedule is repeated for another 14 doses, or a maximum of 28 doses per course, as tolerated.

■ Oprelvekin

Neumega® (oprelvekin) a non-glycosylated form of IL-11 is produced in *E. coli* by recombinant DNA technology and consists of a 177 amino acid sequence and a molecular mass of approximately 19 kDa. It differs from the 178 amino acid primary sequence of native IL-11 in lacking the amino-terminal proline residue. It is used as a thrombopoietic growth factor that directly stimulates the proliferation of hematopoietic stem cells and megakaryocyte progenitor cells and induces megakaryocyte maturation resulting in increased platelet production. Pharmacokinetics show a rapid clearance from the serum and distribution to highly perfused organs. The kidneys are the primary route of elimination and little intact product can be found in the urine (see Chap. 6). After injection the C_{max} of 17.4 ± 5.4 ng/mL is reached after 3.2 ± 2.4 hrs (T_{max}) with a half-life of 6.9 ± 1.7 hrs. The absolute bioavailability is >80%. There is no accumulation after multiple doses. Patients with severely impaired renal function show a marked decrease in clearance to 40% of that seen in subjects with normal renal function.

Pharmaceutical Formulations and Dosing of Oprelevkin

Neumega® is supplied as single use vials containing 5 mg of oprelvekin (specific activity approximately 8 x 10^6 U/mg) as a sterile lyophilized powder with 23 mg of glycine, 1.6 mg of dibasic sodium phosphate heptahydrate, and 0.55 mg monobasic sodium phosphate monohydrate. When reconstituted with 1 mL of sterile water for injection, the solution has a pH of 7.0. It is indicated for the prevention of severe thrombocytopenia following myelosuppressive chemotherapy. The recommended dose is 50 µg/kg given once daily by SC injection after a chemotherapy cycle in courses of 10 to 21 days. Platelet counts should be monitored to assess the optimal course of therapy. Treatment beyond 21 days is not recommended. Oprelvekin is generally well tolerated. Reported adverse events, mainly as a consequence of fluid retention, include edema, tachycardia/palpitations, dyspnea, and oral moniliasis. For a detailed reporting of all adverse events, rarely severe, the reader is referred to the product information for Neumega®.

■ Anakinra

Kineret® (anakinra) is a recombinant, non-glycosylated form of the human interleukin-1 receptor antagonist (IL-1Ra) produced using an *E coli* bacterial expression system. It consists of 153 amino acids, has a molecular weight of 17.3 kDa and differs from native human IL-1Ra in that it has a single methionine residue added to its amino terminus. The absolute bioavailability of Kineret® after a 70 mg SC bolus injection is 95%. C_{max} occurs 3 to 7 h after SC administration at clinically relevant doses (1 to 2 mg/kg) and the half-life ranges

from 4 to 6 h. There is no accumulation of Kineret® after daily SC doses for up to 24 weeks. The mean plasma clearance with mild and moderate (creatinine clearance 50-80 mL/min and 30-49 mL/min) renal insufficiency was reduced by 16% and 50%, respectively. In severe renal insufficiency and end stage renal disease (creatinine clearance <30 mL/min), mean plasma clearance declined by 70% and 75%, respectively. Less than 2.5% of the administered dose is removed by hemodialysis or continuous peritoneal dialysis. A dose schedule change should be considered for subjects with severe renal insufficiency or end stage renal disease.

Pharmaceutical Formulations and Dosing of Anakirna

Kineret® is supplied in single use prefilled glass syringes with 27-gauge needles as a sterile, clear, preservative-free solution for daily SC administration. Each prefilled glass syringe contains: 0.67 mL (100 mg) of anakinra in a solution (pH 6.5) containing 1.29 mg sodium citrate, 5.48 mg sodium chloride, 0.12 mg disodium EDTA, and 0.70 mg polysorbate 80 in water for injection. It is indicated for the reduction of signs and symptoms and slowing of the progression of structural damage in moderately to severely active rheumatoid arthritis and can be used alone or in combination with disease-modifying anti-rheumatic drugs (DMARD) other than TNF-blocking agents (see Chap. 26). The recommended dose for the treatment of patients with rheumatoid arthritis is 100 mg/day. Patients with severe renal insufficiency or end stage renal disease should receive 100 mg every other day. Anakinra is generally well tolerated; the most common adverse reaction is injection-site reactions, the most serious adverse reactions neutropenia, particularly when used in combination with TNF-blocking agents, and serious infections. For a detailed reporting of all adverse events, rarely severe, the reader is referred to the product information for Kineret®.

Because all therapeutic interleukins or interleukin-antagonists act by correcting an inadequate immune reaction they have been classified as BRMs.

ENGINEERING IFNS AND ILS: A CONTINUING STORY

■ Pegylation

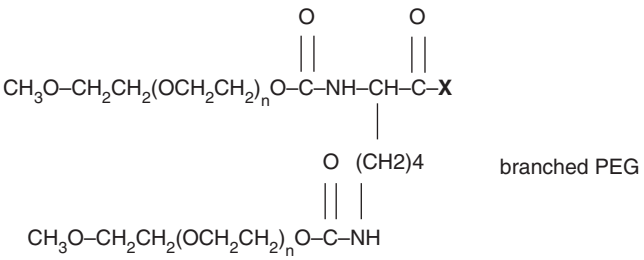
Since 1977 it has been known that polyethylene glycol (PEG) conjugated proteins are frequently more effective than their native parent molecule. Our understanding of PEG chemistry and how it affects the behavior of a biopharmaceutical has increased with the number of PEGylated proteins developed as therapeutic agents (Table 27.5 gives some examples). PEG is hydrophilic, inert, nontoxic, non-immunogenic and in its most common form either linear or branched terminated with hydroxyl groups that can be activated to couple to the desired target protein. It has been approved for human administration by mouth, injection, and topical application. Its general structure is:

$$HO-\left(CH_2CH_2O\right)_{\mathbf{n}}-CH_2CH_2-OH \text{ bifunctional linear PEG}\left(\text{diol}\right)$$

For polypeptide modification one hydroxyl group is usually inactivated by conversion to monomethoxy or mPEG, which is monofunctional, i.e., only one hydroxyl group is activated during the PEGylation process, thus avoiding the formation of interprotein (oligomerisation) or intraprotein bridges:

$$CH_3O-\left(CH_2CH_2O\right)_{\mathbf{n}}-CH_2CH_2-OH \text{ monofunctional linear mPEG}$$

To couple PEG to molecules such as polypeptides, polysaccharides, polynucleotides or small organic molecules it is necessary to chemically activate it. This is done by preparing a PEG derivative with a functional group chosen according to the desired profile for the final product. In addition to the linear PEGs, branched structures have proven useful for peptide and protein modifications:



Branched PEG or PEG2 have a number of advantages over linear structures:

- Attached to proteins they'act' much larger than a linear mPEG of the same molecular weight.
- Two PEG chains are added per attachment site, reducing the chance of protein inactivation.
- They are more effective in protecting proteins from proteolysis, reducing antigenicity and immunogenicity.

Depending on the desired use for the PEG-modified molecule different PEGylation strategies can be chosen, for example:

- Multiple shorter chain PEGylation if the biological activity should be preserved.
- A weak PEG-protein bond if a slow release effect is desired.

- A branched chain with high molecular weight and a strong bond if prolonged circulation and receptor saturation is the goal.

Table 27.5 lists the PEGylated interferons approved in the United States and Europe. For a more in-depth review of PEG chemistries and characteristics the interested reader is referred to Roberts et al. 2002 and Bailon et al. 2001.

The development of rhIFNα from the native, unmodified molecule to the PEGylated form with the desired pharmacological profile is an example of how the understanding of PEG chemistry progressed with experience (Zeuzem et al. 2003). Increasing the length of the PEG chain resulted in progressively longer circulating half-life due to protracted resorption and lower clearance, ultimately resulting in a near constant serum concentration over an entire week summarized in Fig. 27.3.

The first PEGylated interferon, IFN alfa-2a, used a linear, 5 kDa mPEG with a weak urethane PEG-IFN alfa-2a link. Clinical trials conducted with this compound were unsuccessful because the blood circulation half-life for the conjugate (Fig. 27.3b) was only slightly improved relative to that of the native protein (Fig. 27.3a) (Wills 1990). Development of the product was therefore halted at Phase II clinical trials (Zeuzem et al. 2003). The second compound was developed by Schering Plough, Kenilworth, NJ in collaboration with Enzon Pharmaceutical Inc., Bridgewater, NJ. It used of a longer (12 kDa), linear PEG with an urethane linkage

PEGylated recombinant interferons	*company*	*1st indication, 1st approval*	
Pegasys® (PEGylated IFN α-2a produced in *E. coli*)	Hoffman–La Roche (Basel, Switzerland)	Chronic hepatitis B and C	2002 (EU and US)
ViraferonPeg® (PEGylated IFN α-2b produced in *E. coli*)	Schering-Plough (Kenilworth NJ, USA)	Chronic hepatitis C	2000 (EU)
PegIntron® (PEGylated IFN α-2b produced in *E. coli)*	Schering-Plough (Kenilworth NJ, USA)	Chronic hepatitis C	2000 (EU) 2001 (US)

Adapted from *Nature Biotechnology* 2006, 24: 769–776

Table 27.5 ■ PEGylated interferons approved in the United States and Europe

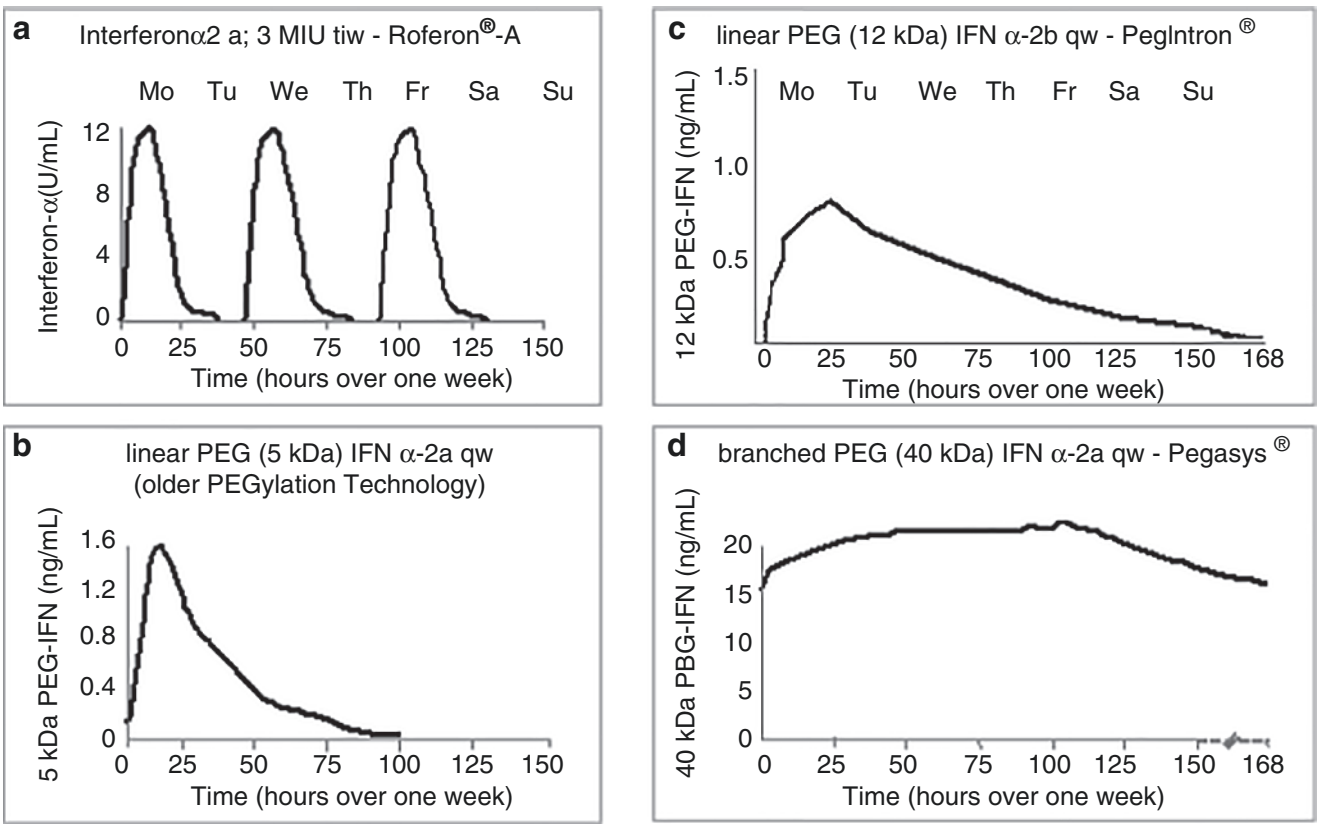


Figure 27.3 ■ Pharmacokinetic Profiles for IFN and PEG-IFN (repeated dosing)

to IFN alfa-2b. The chosen strategy was to combine the advantages of high specific activity with slower serum clearance resulting in PegIntron® (Wang et al. 2002) with markedly improved pharmacological properties allowing once a week administration (Fig. 27.3c) (Glue et al. 2000). PegIntron®, also marketed as Viraferon® in some countries, is approved worldwide for the treatment of chronic hepatitis C.

The development of the third PEGylated interferon, IFN alfa-2a, took a different approach. The strategic goal was to achieve lasting and constant serum concentrations over an entire week. In a collaboration of Roche with Shearwater Polymers in Huntsville, AL, now Nektar; San Carlos, CA, IFNα-2a was linked by a stable amide bond to four different PEG chains of various sizes, structures, and site-attachment numbers. The resulting products were tested for antiviral activity and a variety of pharmacokinetic parameters including half-life, absorption rate, and mean residence time:

- 20-kDa linear mono-PEGIFN alfa-2a,
- 40-kDa linear di-PEGIFN alfa-2a,
- 20-kDa branched mono-PEGIFN alfa-2a
- 40-kDa branched mono-PEGIFN alfa-2a

The 40-kDa, branched PEGylated molecule (later named Pegasys®) exhibited sustained absorption, decreased systemic clearance, and an approximate ten-fold increase in serum half-life over regular interferon. The biological activity was similarly prolonged resulting in an optimal pharmacological profile Fig. 27.3d, (Algranati et al. 1999). It was therefore chosen for further clinical development (Reddy et al. 2002) leading to its approval worldwide for the treatment of chronic hepatitis B and C.

The rapidly growing understanding of the potential of advanced PEGylation chemistry to improve the stability and pharmacological properties of biopharmaceuticals has fostered the development of an increasing number of PEG-biopharmaceuticals. Several of those have proven to offer significant advantages over their native counterparts and found their place in our therapeutic armamentarium. PEG is also used for a variety of other (non-bio) pharmaceutical applications. Table 27.6 lists several examples of different marketed products.

Further Cytokine Engineering

Based on the understanding of the function and limitations of a given therapeutic protein product (TPP) rational protein engineering allows the creation of a new product with improved and expanded activities. Having shown a degree of activity in the treatment of certain cancers, IL-2 is a good example to illustrate this line of thought. Systemic IL-2 (Aldesleukin) treatment has shown significant clinical benefit in a minority of renal cell and melanoma patients, with long term survival in some cases. However, a number of limiting factors have been identified. Its pharmacological properties, short half-life, and its adverse effects, mainly vascular leak syndrome (VLS) with different organ manifestations -a pathophysiological manifestation of acute inflammation- make it difficult to handle. Acute inflammation is a process typical of vascularized tissues whereby interstitial fluid and white blood cells accumulate at the site of injury. Thus, flooding the body with exogenously administered IL-2 can induce a dose dependent "vascular leak

Protein Name	PEGylation	Product name	Reference
IFN α-2a IFN α-2b	Branched, 40 kDa Linear, 12 kDa	Pegasys® PegIntron®	Reddy et al. (2002) Wang et al. (2002)
Interferon β	Linear, 20 kDa	Plegridy® (mPEGIFN β1a)	Biogen PI
Erythropoietin[a]	60 kDa	Mircera® (mPEG epoetinβ)	Schellekens (2006)
G-CSF[a]	Linear, 20 kDa	Neulasta® (pegfilgrastim)	Lyman (2005)
Adenosine deaminase	Linear, 5 kDa	Adagen® (pegademase)	FDA drug label
Arginine deiminase	Linear, 20 kDa	ADI-SS PEG20	Tsai et al. (2017)
Asparaginase	Linear, 5 kDa	Oncaspar®(pegaspargase)	Cao et al. (1990)
rhGH analog[b]	Linear, 5 kDa	Somavert® (pegvisomant)	Ross et al. (2001)
rhFactor VIII[c]	Linear, 40 kDa	Adynovate® (octocog alfa pegol)	Lieuw (2017)
RNA aptamer	40 kDa	Macugen® (pegaptanib)	Ng et al. (2006)

[a]See Chap. 24: hematopoietic growth factors
[b]See Chap. 20: growth hormones
[c]See Chap. 21: recombinant coagulation factors and thrombolytic agents

Table 27.6 ■ Examples of PEGylated (bio)-pharmaceuticals

syndrome" in any vascularized organ. So far, we have been "playing the piano with boxing gloves – now is the time to take off our boxing gloves". What is needed is a possibility to specifically target those cells we wish to impact and only those.

IL-2 has dual properties: one, the ability to expand and activate innate and adaptive effector cells which is the basis of its anticancer activity. Two, to coordinate an immunosuppressive microenvironment by recruiting regulatory T-cells (Tregs) and myeloid derived suppressor cells (MDSCs) as a regulatory mechanism that prevents excessive immune responses and autoimmunity. Unfortunately, the expansion of immune-suppressive Treg cells as well as other immune dysregulation limit or impede IL-2's anticancer activity (Setrerrahmane and Xu 2017).

While PEGylating IL-2 may have resolved the issue of short halflife and ensuing peak toxicity, we are still repeatedly flooding the whole organism with a TPP of known toxicity. With a better understanding of the factors limiting its mechanism of action as well as the structure-function relationship of proteins, rational design and engineering strategies allow adaptation of its beneficial or deleterious (toxic) activity or the creation of new activities.

Reengineering IL-2 by creating a recombinant fusion protein composed of a genetically engineered human monoclonal antibody directed against carcinoembryonic antigen (CEA), i.e. cergutuzumab, linked to an engineered, variant form of interleukin-2 (IL-2v): amunaleukin, with potential immunostimulating and antineoplastic activities (see Chaps. 1, 7, and 9). Upon administration of cergutuzumab amunaleukin (Fig. 27.4), the antibody moiety recognizes and binds to CEA, thereby specifically targeting IL-2v to CEA-expressing tumor tissue. Subsequently, the IL-2v moiety stimulates a local immune response, which activates both natural killer cells and cytotoxic T cells, and eventually leads to tumor cell killing. CEA is a cell surface protein that is expressed on a wide variety of cancer cells. The mutations found in IL-2v inhibit its binding to the IL-2 receptor-alpha (IL-2Rα), which prevents the activation of Treg. However, it can still bind to and induce signaling through the IL-2Rβγ, which allows the preferential expansion of NK cells and CD8-positive T cells. The Fc domain of cergutuzumab has been modified to prevent Fc-gamma binding and downstream cell activation (Klein et al. 2017).

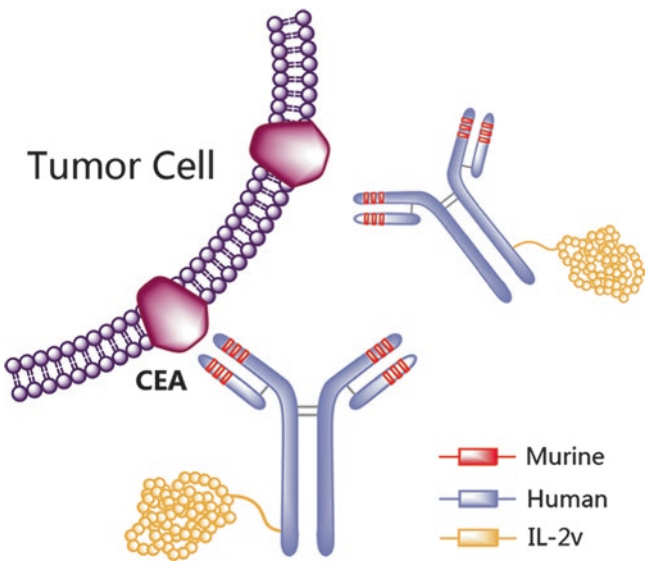


Figure 27.4 Graphic model of the cergutuzumab amunaleukin fusion protein

OUTLOOK AND CONCLUSIONS

There is a very precise and organized order in the intricate function of the immune system to make it work effectively and we are well on our way to map it. But, a lot of hard work still lies ahead. The fundamental approach to cytokine or cytokine antagonist therapy with biopharmaceuticals is to identify diseases caused by insufficient or excessive cytokine production. In the first case, e.g. with certain chronic viral diseases or cancers, appropriate cytokines are used pharmacologically to boost the immune response. Examples include IFNα with antiviral as well as immunomodulatory properties in chronic viral hepatitis, or IL-2 or IL-12 in renal cell cancer and malignant melanoma. For chronic inflammatory or atopic diseases caused by unchecked overproduction of interleukins, two options are available: one can either inhibit the interleukin or its receptor(s), e.g. with human(ized) monoclonal antibodies such as Remicade®, Cinqair®, Nucala®, Sylvant®, Taltz®, Cosentyx®, Humira®, Stelara® or Enbrel® (see Chap. 26) for various indications, or the IL-1R antagonist (Kineret®, see above) in rheumatoid arthritis. Another option would be to downregulate excessively produced interleukin using its antagonistic cytokine, e.g. PEGylated IL-12 in asthma or IL-10 in psoriasis. To date it appears that the first option is more successful than downregulation by anti-inflammatory cytokines, which has so far not resulted in any approved product.

Considering the great potential of cytokines and anti-cytokines, the success stories to-date may appear modest, but they do set the scene. In parallel with the exponential boost of basic knowledge initiated by mastering the tools of biotechnology our understanding of the complex systems we are dealing with has progressed. Diagnostic and therapeutic applications are following closely behind, as well as the capability to monitor the effect of our interventions accurately. As a consequence, an interesting paradigm shift in our approach to many diseases has taken place. Atherosclerosis, psoriasis, insulitis, insulin resistance

and asthma as examples for chronic inflammatory diseases in which interleukins and other cytokines play central roles have become therapeutic targets for treatment with biological response modifiers (BRMs). A huge amount of knowledge and experience is available. What is sometimes missing is an integrative view of the many islands of knowledge: "Join the dots to see the greater picture". The tools are there: polymerase chain reaction, genomics, sequencing, cloning, proteomics, microarrays (see Chaps. 1 and 9). Time is an essential factor as we need to learn to recognize potential or established disease in an early stage when intervention is often more effective. Time is, lastly, also a consideration when treating patients, as a beneficial response can require weeks, months, years or a life-time of therapy. There are still issues in need of solutions: how to manage toxicities of mainly the pro-inflammatory cytokines, particularly for their therapeutic use in cancer. Better understanding of the interaction with their receptors, where those receptors are expressed, the dynamics of that expression, and the actions of the cascade their interaction induces. Can we develop a computer model to visualize and help us understand the intricacies of the immune system better? How much can cell and animal models tell us? Can we predict individuals at risk for certain chronic inflammatory diseases or cancer due to allelic variants of interferon-, interleukin- or their receptor-genes? Will gene therapy ultimately displace pharmacological replacement or inhibition of cytokines? Most importantly, can biopharmaceuticals be targeted for better efficacy and less toxicity? New, engineered BRMs with cell specific affinity are a first step in the right direction. They are activated locally when they bind to their receptors and as a consequence can drastically reduce systemic toxicity.

It is no longer visionary to anticipate that proteins will be more extensively engineered in the future. These new "design TPPs" beside showing prolonged half-life, increased target specificity and decreased intrinsic toxicity, will carry neo-sequences not found in nature. The potential risks of immunogenicity will also increase and require immunogenicity risk assessment and mitigation (see also Chap. 7).

SELF-ASSESSMENT QUESTIONS

■ Questions

Decide whether each of the statements below is true or false. If you believe a statement is false explain why.

1. Interferons are defined:
   (a) by the cell type which produces them,
   (b) by their anti-inflammatory properties,
   (c) by their antiviral activity,
   (d) by their protein structure,
   (e) by their genetic structure.
2. Human interferon alpha:
   (a) is produced selectively by leukocytes
   (b) is a virucidal substance
   (c) triggers antiviral effects in cells expressing appropriate receptors
   (d) acts on the immune system to booster specific antiviral response
   (e) comprises twelve subtypes.
3. Interleukins are characterized by:
   (a) their action on target cells,
   (b) their protein structure,
   (c) their genetic structure,
   (d) pro- or anti-inflammatory effect,
   (e) their cell of origin.
4. The following interleukins are generally considered to be "pro-inflammatory" i.e. induce and/or be part of a Th1 response:
   (a) the IL-1 family, IL-2, -8, -12.
   (b) IL-3.
   (c) IL-4, -5, -9.
   (d) IL-10, -19, -20, -22, -24, -26, -28A, -28B and -29.
   (e) IL-15, -16, -17, -18, -22, -23, and -32.
5. Interleukins are:
   (a) secreted specifically by leukocytes to act on other leukocytes,
   (b) bound to a specific receptor complex to exert their effect,
   (c) a family of proteins which co-regulate the immune response,
   (d) non-toxic products of the body in response to pathogens and other potentially harmful agents,
   (e) long-acting immune modulators.
6. Interferons and interleukins can be toxic, several (patho-) physiological containment mechanisms exist to counteract excessive production:
   (a) soluble receptors,
   (b) binding to cell surface receptors,
   (c) neutralizing antibodies,
   (d) negative feedback mechanisms,
   (e) naturally occurring IL receptor antagonists.
7. The following interferons are used as approved therapy:
   (a) IFNα2,
   (b) IFNβ,
   (c) IFNγ,
   (d) IFNω,
   (e) IFNα8.

   Where appropriate, specify some of the indications they are used for.
8. The following interleukins are approved for therapeutic use:
   (a) IL-1
   (b) IL-2.
   (c) IL-10
   (d) IL-11
   (e) IL-12

Where appropriate, specify some of the indications they are used for.

9. Protein PEGylation:
   (a) prolongs circulation half-life of the PEGylated protein,
   (b) decreases antigenicity of the PEGylated protein,
   (c) protects the protein from proteolysis,
   (d) is difficult due to the toxicity of polyethylene glycol,
   (e) improves the therapeutic efficacy of the PEGylated protein.
10. The following PEGylated IFNs and ILs have been approved for therapeutic use:
   (a) Interferon α2,
   (b) Interferon β,
   (c) Interleukin-1,
   (d) Interleukin-2,
   (e) Interleukin-12.

■ Answers

1. Interferons are defined:
   a. false. Although IFNα used to be called "leukocyte interferon" and IFNβ "fibroblast interferon" because they were initially produced from buffy coats (leukocytes) infected with Sendai virus and human diploid fibroblasts stimulated with poly(I)-poly(C) or Newcastle disease virus (NDV) respectively, interferons and their Units (IU) are defined by their antiviral activity.
   b. false: while they can act as immune-modulators and on occasion have anti-inflammatory properties (e.g. IFNβ, for the treatment of multiple sclerosis), they will more often induce a Th1 or pro-inflammatory response. IFNγ is one of the classical pro-inflammatory markers.
   c. true.
   d. and e. false. The full protein and genetic sequences of the different interferons and their subtypes were only defined long after the initial crude IFN mixtures had been tested in the clinic initially against viral diseases and subsequently against cancers.

Today however the protein and genetic sequences are necessary to specify an interferon and its purity during the production by biotechnological techniques. Also new interferons or interleukins will be accepted as such by the Human Genome Nomenclature Committee (HGNC) based on their function and a previously unknown genetic sequence.

2. Human interferon alpha:
a. false. Interferon alpha is produced by many cell types, including T-cells and B-cells, macrophages, fibroblasts, endothelial cells and osteoblasts among others.
b. false. By interacting with their specific heterodimeric receptors on the surface of cells, the interferons initiate a broad and varied array of signals that induce antiviral state.
c. true.
d. true.
e. true. See Table 27.2. Each IFNα subtype has a distinct antiviral, antiproliferative, and stimulation of cytotoxic activities of NK and T-cells. To date only one recombinant subtype, IFNα2, has been predominantly used therapeutically.
3. Interleukins are classified according:
a. and e. false. ILs are characterised by their protein and gene structures registered in the HCGN database (and similar centralised databases). Their names and symbols must be approved by the HGNC.
b. true.
c. true.
d. false. While some ILs can be classified as pro- or anti-inflammatory this is not what basically defines them.
4. The following interleukins are generally considered to be "pro-inflammatory" i.e. induce and/or be part of a Th1 response:

a. true.
b. false. IL-3 is a multicolony stimulating, hematopoietic growth factor which stimulates the generation of hematopoietic progenitors of every lineage.
c. false. These three interleukins all play a role in the differentiation and activation of basophils and eosinophils leading to a Th2 response.
d. false. These interleukins are all part of the IL-10 family. However IL-10,-19, and -20 are "anti-inflammatory", IL-22, -24, -26, -28A, -28B and -29 are considered "pro-inflammatory".
e. true.
5. Interleukins are:
a. false. Interleukins are mainly secreted by leukocytes and primarily affecting growth and differentiation of hematopoietic and immune cells. They are also produced by other normal and malignant cells and are of central importance in the regulation of hematopoiesis, immunity, inflammation, tissue remodeling, and embryonic development.
b. true.
c. true.
d. false. Many interleukins, primarily those with pro-inflammatory function, are intrinsically toxic either directly or indirectly, i.e. through induction of toxic gene products.
e. false. Interleukins usually have a short circulation time, and their production is regulated by positive and negative feedback loops.
6. Interferons and interleukins can be toxic, several (patho-) physiological containment mechanisms exist to counteract excessive production.

a. true.
b. false. Binding to cell surface receptor is a physiological process and has negligible effect on "circulating" interferons or interleukins.
c. to e. are true.

7. The following interferons are used as approved therapy.
a. true. IFNα (Roferon® A, IntronA®, Infergen®) are indicated for the treatment of chronic hepatitis B and C, Kaposi's sarcoma, renal cell carcinoma, malignant melanoma, carcinoid tumor, multiple myeloma, non-Hodgkin lymphoma, hairy cell leukemia, chronic myelogenous leukemia, thrombocytosis associated with chronic myelogenous leukemia, and other myeloproliferative disorders.
b. true. IFNβ (Betaseron®, Betaferon®, Avonex®, Rebif®) are indicated for the treatment of multiple sclerosis.
c. true. IFNγ (Actimmune®) is indicated for the treatment of chronic granulomatous disease, severe, malignant osteopetrosis.
d. false. IFNω has only been studied in vitro and in the nude mouse model where it has shown anticancer activity against several tumor cell lines and transplants.
e. false. IFNα8 has only been studied in various cell lines where it has consistently shown the most powerful antiviral effect of the subtypes tested.

8. The following interleukins are approved for therapeutic use:
a. true. An IL-1 analog/antagonist (Kineret®) is indicated for the treatment of rheumatoid arthritis.
b. true. IL-2 (Proleukin®) is indicated for the treatment of adults with metastatic renal cell carcinoma or metastatic melanoma.
c. false. Clinical development IL-10 (Tenovil™) as an anti-inflammatory drug for several indications such as: psoriasis, Crohn's disease, rheumatoid arthritis was discontinued in phase III due to insufficient efficacy to warrant further development.
d. true. IL-11 (Neumega®) is indicated for the prevention of severe thrombocytopenia following myelosuppressive chemotherapy.
e. false. Early clinical trials have been performed in patients with chronic hepatitis C. The programme was, however, discontinued in early phase II due to toxicity.

9. Protein PEGylation:
a. true.
b. true.
c. true.
d. false. PEG is inert, nontoxic, non-immunogenic and in its most common form either linear or branched terminated with hydroxyl groups that can be activated to couple to the desired target protein.
e. true.

10. The following PEGylated IFNs and ILs have been approved for therapeutic use:
a. true: for chronic hepatitis C and B. Limited clinical trials have also been conducted in renal cell carcinoma, malignant melanoma and non-Hodgkin lymphoma.
b. c. d. and e. are false although early clinical trials have been conducted with PEGylated IL-2 in RCC and malignant melanoma and pharmacokinetic studies with PEGylated IFNβ in animal models.

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