

Interleukin-10 Therapy—Review of a New Approach

K. ASADULLAH, W. STERRY, AND H. D. VOLK

Corporate Research Business Area Dermatology (K.A.), Schering AG, Berlin, Germany; and Departments of Dermatology and Allergology (W.S.) and Institute of Medical Immunology (H.D.V.), University Hospital Charité, Humboldt University, Berlin, Germany

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Address correspondence to: Dr. Khusru Asadullah, Head of Corporate Research Business Area Dermatology, Schering AG, D-13342 Berlin, Germany. E-mail: khusru.asadullah@schering.de

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Abstract—Interleukin (IL)-10 is an important immunoregulatory cytokine produced by many cell populations. Its main biological function seems to be the limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, natural killer cells, antigen-presenting cells, mast cells, and granulocytes. However, very recent data suggest IL-10 also mediates immunostimulatory properties that help to eliminate infectious and noninfectious particles with limited inflammation. Numerous investigations, including expression analyses in patients, in vitro and animal experiments suggest a major impact of IL-10 in inflammatory, malignant, and autoimmune diseases. So IL-10 overexpression was found in certain tumors as melanoma and several lymphomas and is

considered to promote further tumor development. Systemic IL-10 release is a powerful tool of the central nervous system to prevent hyperinflammatory processes by activation of the neuro-endocrine axis following acute stress reactions. In contrast, a relative IL-10 deficiency has been observed and is regarded to be of pathophysiological relevance in certain inflammatory disorders characterized by a type 1 cytokine pattern such as psoriasis. Recombinant human IL-10 has been produced and is currently being tested in clinical trials. This includes rheumatoid arthritis, inflammatory bowel disease, psoriasis, organ transplantation, and chronic hepatitis C. The results are heterogeneous. They give new insight into the immunobiology of IL-10 and suggest that the IL-10/IL-10 receptor system may become a new therapeutic target.

I. Introduction

Cytokines have been in the focus of scientific interest for more than a decade now. Analyzing their expression has enabled a better understanding of the pathogenesis of various diseases. Moreover, they are now far beyond the stage when they were of interest for pathophysiological

research; some cytokine therapies are already used as part of clinical practice, ranging from early exploratory trials to well established therapies that have already received approval (Asadullah et al., 2002a,b).

Mosmann and coworkers (Fiorentino et al., 1989) first described a cytokine that is produced by T helper 2 (Th2¹) cell clones and inhibits interferon (IFN)- γ synthesis in Th1 cell clones (Fiorentino et al., 1989). Today this “cytokine synthesis inhibiting factor (CSIF)” is known as interleukin (IL)-10, and although we also know that several immune cells produce IL-10, macrophages are the major source. Investigations during the last decade showed that this cytokine is of crucial importance for immunoregulation and led to its use in first clinical trials.

II. Interleukin-10

A. Interleukin-10 and Interleukin-10 Homologs

The human cytokine is a homodimer with a molecular mass of 37 kDa. Each monomer consists of 160 amino acids with a molecular mass of 18.5 kDa. Murine and human IL-10 exhibit a homology of about 80%. There are several viral IL-10 homologs: Epstein-Barr virus (BCRF1) (Hsu et al., 1990), herpes virus type 2 (Rode et al., 1994), cytomegalovirus (Kotenko et al., 2000; Spencer, 2002), and Orf virus (Fleming et al., 1997), with the EBV-derived BCRF1 being the most studied homolog.

¹Abbreviations: Th, T helper; APC, antigen-presenting cells; ATF-1, activating transcription factor-1; CBCL, cutaneous B cell lymphomas; CD, Crohn's disease; CMV, cytomegalovirus; CREB-1, cAMP response element binding protein-1; CRF, cytokine receptor family; CSIF, cytokine synthesis-inhibiting factor; CTCL, cutaneous T cell lymphoma; CTL, cytotoxic T cell lysis; DC, dendritic cells; EAE, experimental allergic encephalomyelitis; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; GM-CSF, granulocyte-macrophage colony-stimulating factor; HIV, human immunodeficiency virus; HO, heme oxygenase; IFN, interferon; Ig, immunoglobulin; IKK, I κ B kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; IP, inducible protein; kb, kilobase(s); KC, keratinocytes; LPS, lipopolysaccharide; mAb, monoclonal antibody; M ϕ , macrophages; MF, mycosis fungoides; MHC, major histocompatibility complex; MIG, monokine induced by IFN- γ ; MMP, matrix metalloproteinase; NF- κ B, nuclear factor- κ B; NHL, non-Hodgkin's lymphoma; NK cells, natural killer cells; PASI, psoriasis area and severity index; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SOCS, suppressor of cytokine synthesis; SSc, systemic sclerosis; STAT, signal transducer and activator of transcription; TAP, transporter associated with antigen processing; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor.

The structure of human IL-10 and BCRF1 (ebvIL-10) was studied by X-ray crystal-structure-analysis (Zdanov et al., 1995, 1997) (Fig. 1). Apart from marginal differences predominantly in the N-terminal part of the molecule, the structures of hIL-10 and ebvIL-10 are strikingly similar; the two identical intertwining polypeptide chains of 160 (hIL-10) or 145 (ebvIL-10) amino acids are rotated by 180° to each other, forming two domains oriented in a V-shaped structure. Each domain contains six helices, four (A–D) from one monomer and two (E + F) from the other (Spits et al., 1992; Moore et al., 2001). Such a topology has been described first for the interferon- γ homodimer (Ealick et al., 1991)—a cytokine with many biological properties antagonistic to those of IL-10.

The capability for IL-10 production has been demonstrated for various cell populations; in addition to certain T cell subsets (Th2, Tc2, Tr1), also monocytes, macrophages, and several other cells may synthesize IL-10 (Table 1). Whether human keratinocytes really produce IL-10 like their murine counterparts is subject to contrary discussions (Enk and Katz, 1992; Kang et al., 1994; Enk et al., 1995; Grewe et al., 1995; Teunissen et al., 1997). The major source of IL-10 in vivo seems to be macrophages.

Five new human molecules structurally related to IL-10 have been discovered (Jiang et al., 1995; Gallagher et al., 2000; Dumoutier et al., 2000a,b,c; Knappe et al., 2000; Blumberg et al., 2001). They are called IL-19, IL-20, IL-22, IL-24 (mda-7), and IL-26 (AK155). Similar to IL-10, they are α -helical proteins with similar cysteine localizations, whose amino acid sequences are about 30% identical. Interestingly, in the human ge-

Cell Population	Reference
T helper 2 cells	Fiorentino et al., 1989
Monocytes	De Waal Malefyt et al., 1991a
Macrophages	Spits and De Waal Malefyt, 1992
B cells	Pistoia, 1997
Eosinophils	Nakajima et al., 1996
Mast cells	Lin and Befus, 1997
Keratinocytes (?)	Enk and Katz, 1992; Teunissen et al., 1997

nome, the encoding genes are located in two clusters, one comprising the genes for IL-10, IL-19, IL-20, and IL-24 (mda-7) on chromosome 1q31-32, whereas the second cluster comprising the genes encoding IL-26 (AK155) and IL-22 is located on human chromosome 12q15 near the IFN- γ gene (12q14) (Dumoutier et al., 2000b; Blumberg et al., 2001) (Fig. 2). Taking into account the clear structural relation between the new IL-10 homologs and IL-10, all of these six molecules should be considered as (IL-10) family members.

In contrast to the extensively studied IL-10 (as described below and recently reviewed by Moore et al., 2001), the knowledge of the biology of the new IL-10 homologs is still fragmentary. The first functional data exists for IL-20, IL-22, and IL-24 (mda-7) (Fickenscher et al., 2002). Overexpression of IL-20 in transgenic mice induced neonatal lethality, psoriasis-like skin abnormalities, lack of adipose tissue, and elevated apoptosis of thymic lymphocytes (Blumberg et al., 2001). It has been suggested that IL-22 plays a role in inflammatory processes through the observation that it induces acute phase-reactant production in a hepatoma cell line and in

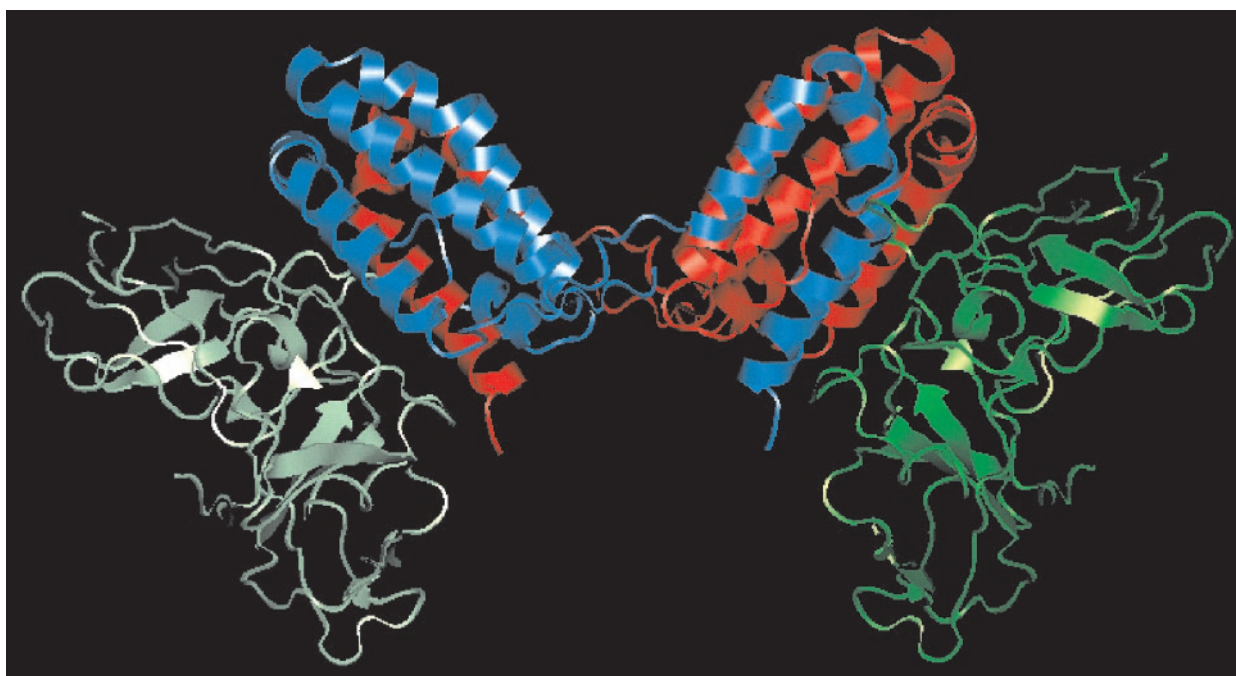


FIG. 1. Three-dimensional structure of hIL-10 (figure reproduced from a review by Asadullah et al., 2000a initially developed by Zdanov et al., 1995, 1997 and reprinted with permission from Ashley Publications Ltd., London).

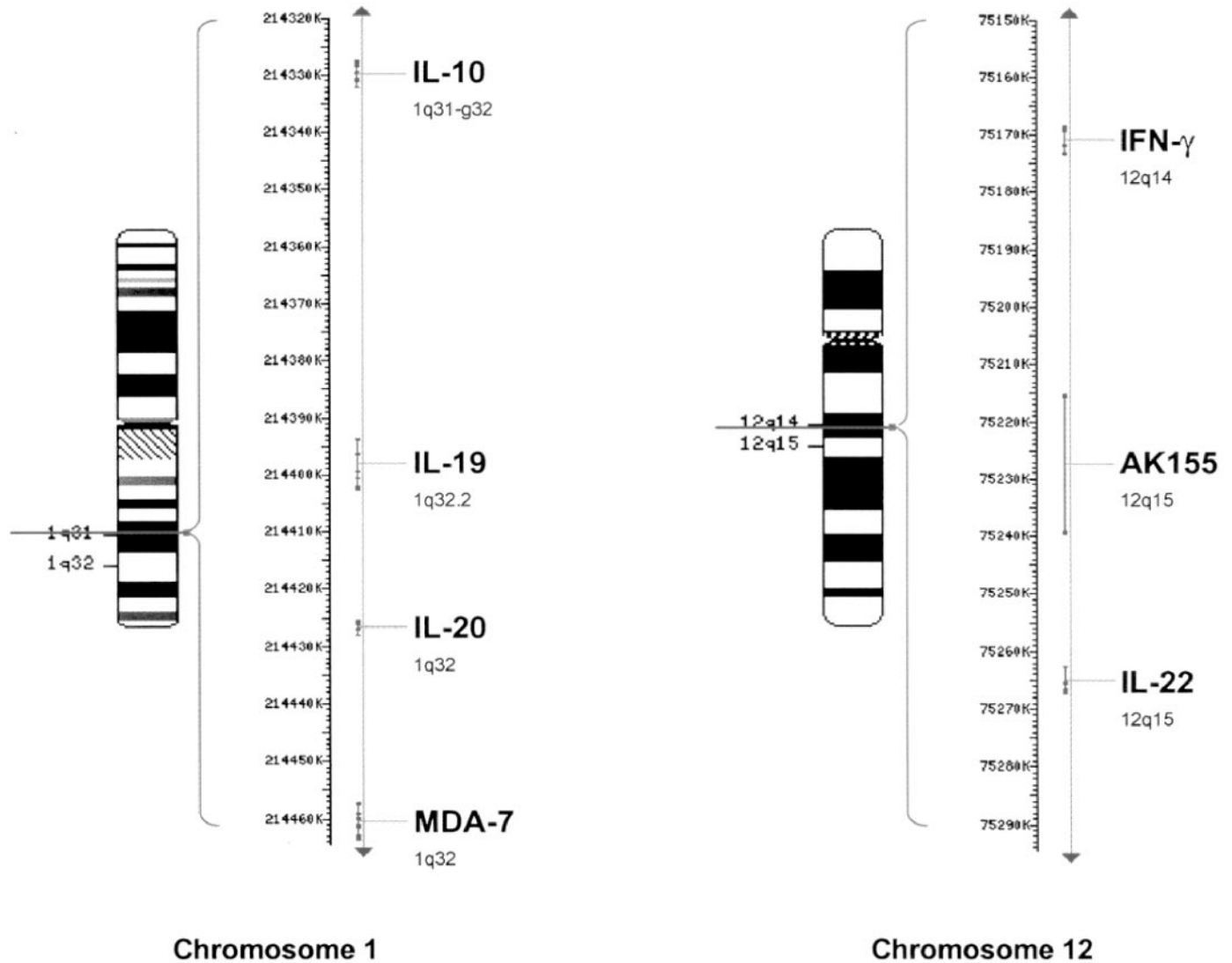


FIG. 2. Chromosomal location of IL-10 and homologous cytokines. The genomic localization of the IL-10 family genes. AK155, Andre Knappe, MDA-7, melanoma differentiation-associated gene-7 (figure modified from Volk et al., 2001).

vivo (Dumoutier et al., 2000b). Overexpression of *mda-7* via adenoviral gene transfer induced growth inhibition in various tumor types (Jiang et al., 1996). Interestingly, the IL-24 (*mda-7*) mouse counterpart, called FISP, was postulated to be a Th2-specific protein (Schaefer et al., 2001). No function is known for IL-19 and IL-26 (AK155) to date. Table 2 summarizes the most important properties known so far (Volk et al., 2001).

We recently investigated the expression of five new human IL-10-related molecules and their receptors in blood mononuclear cells (Wolk et al., 2002). IL-19 and IL-20 were found to be preferentially expressed in monocytes. IL-22 and IL-26 (AK155) expression was exclusively detected in NK and T cells, especially upon T1 polarization. IL-24 (*mda-7*) expression was restricted to monocytes and T cells. Secretion of these molecules by lymphocytes was predominantly linked to cellular activation. Regarding T cells, IL-26 was primarily produced by memory cells, and its expression was independent of

costimulation. This data suggests that immune cells are a major source of the new IL-10 family members (Wolk et al., 2002).

B. Interleukin-10 Promoter and Interleukin-10 Polymorphisms

The human IL-10 gene is located on chromosome 1 and encodes for 5 exons (5.1 kb) (Spits and De Waal Malefyt, 1992). The IL-10 promoter is highly polymorphic with two informative microsatellites, IL10.G and IL10.R, 1.2 kb and 4 kb upstream of the transcription start site (Eskdale and Gallagher, 1995; Eskdale et al., 1996) and three frequent point mutations $-1082(G/A)$, $-819(C/T)$, and $-592(C/A)$ (Eskdale et al., 1997a; Turner et al., 1997a; Hurme et al., 1998). Recently, several new single-nucleotide polymorphisms have been defined in the human IL-10 locus. A correlation of particular microsatellite polymorphisms with lipopolysaccharide (LPS)-induced IL-10 secretion by PBMC in vitro

TABLE 2
Properties of IL-10 homolog (Volk et al., 2001)

IL-10 Homologs	Cellular Source	Biological Effects		Receptor
		In Vivo	In Vitro	
IL-19	Activated monocytes (LPS or GM-CSF)	?	No effects on cytokine synthesis by PBMC	?
IL-20	LPS-stimulated PBMC monocytes (?)	Overexpression in mice causes retarding of growth and development, skin abnormalities, and neonatal lethality	Enhancement of IL-10-induced expression of inflammation-related genes (HaCaT)	IL-20R α IL-20R β
IL-22	Activated T cells (Th1 ?), IL-9-stimulated mast cells, mesangium cells	Application in adult mice induces acute phase response and basophilia in proximal renal tubules, autocrine factor for mesangium cells	Induction of acute phase response (hepatoma cell lines), inhibition of IL-4 production from Th2 cells, activates Stat3 in hepatocytes but not macrophages	IL-22R α IL-10R β
AK155	T cell lines (CD4+ and CD8+) herpesvirus saimiri-infected T cells, activated monocytes (?)	?	?	?
MDA-7	Melanoma cells, skin fibroblasts, activated PBMC (LPS or PHA)	Antitumor effects ?	Irreversible growth arrest of tumor (induction of apoptosis or differentiation) inhibition of angiogenesis induction of Th1 type cytokines in PBMC (?)	?

AK, Andrea Knappe; PHA, phytohemagglutinin.

(presumably mostly from monocytes) was reported (Eskdale et al., 1998); the -1082(G) allele was associated with higher ConA-induced IL-10 production (likely both T cells and monocytes) (Turner et al., 1997a).

C. Regulation of Interleukin-10 Secretion

The IL-10 promoter contains several transcription factor-responsive elements (Platzer et al., 1994). Thus macrophages, the major source of IL-10, are stimulated to produce IL-10 by several endogenous and exogenous factors such as endotoxin (via Toll-like receptor 4, NF- κ B dependent), tumor necrosis factor (TNF)- α (via TNF receptor p55, NF- κ B-dependent), catecholamines, and cAMP-elevating drugs (both via protein kinase A, CREB-1/ATF-1 dependent) (Platzer et al., 1995, 1999, 2000; Meisel et al., 1996; Woiciechowsky et al., 1998; Riese et al., 2000).

In particular, the stress axis plays a significant role in regulating IL-10 expression in vivo. Inflammation of the central nervous system (particularly local IL-1 release following trauma, neurosurgery, or increase of intracranial pressure) or indirect activation of the stress axis by endotoxemia/bacteremia triggers the release of catecholamines that up-regulate IL-10 production in macrophages, particularly in the liver (Barsig et al., 1995; Jilg et al., 1996; Woiciechowsky et al., 1998). Blocking the stress axis increases the susceptibility to endotoxemia-mediated shock. So the cross-talk between the central nervous system and (liver) macrophages controls systemic inflammation whereby the cAMP/protein kinase A/CREB-1/ATF-1 signaling pathway seems to play an essential role in inducing IL-10. On the other hand, systemic release of TNF- α also induces IL-10 via a negative feedback by using a NF- κ B-dependent pathway

(Barsig et al., 1995; Meisel et al., 1996). Recent data suggests that the p38 mitogen-activated kinase pathway also regulates the human IL-10 promoter via the activation of sp1 transcription factor (Ma et al., 2001).

III. Interleukin-10 Receptors and Signaling

A. Interleukin-10 Receptors and Other Cytokine Receptor Family Type 2 Members

IL-10 activity is mediated by its specific cell surface receptor complex, which is expressed on a variety of cells, in particular immune cells. Only a few copies of the IL-10R are expressed on the surface of the cells (Carson et al., 1995; Jurlander et al., 1997). The expression is variable, but so far only a few regulating factors are known. Endotoxin increases the expression of IL-10R on fibroblasts (Weber-Nordt et al., 1994). After T cell stimulation with anti-CD3-antibodies or phorbol ester, a decrease of IL-10R gene expression has been found (Liu et al., 1994). It has been demonstrated that dermatological therapeutic agents such as glucocorticoids, vitamin D₃, and calcipotriol significantly increase IL-10R expression (Michel et al., 1997a,b). The IL-10 receptor is composed of two different chains, α (Ho et al., 1993) and β (CRFB4) (Kotenko et al., 1997), both members of the class II cytokine receptor family. The interaction of hIL-10R with hIL-10 has been characterized recently and seems to be highly complex (Ho et al., 1993; Tan et al., 1993; Reineke et al., 1998, 1999). The IL-10R β chain is essential for IL-10-mediated effects and CRFB4-deficient mice display the same phenotype as IL-10 deficient mice (Spencer et al., 1998). Interestingly, for cells that only express IL-10R β , no IL-10/IL-10R complexes are formed suggesting that only IL-10/IL-10R α complexes interact

with the β -chain. Only in cells expressing both the IL-10R α and β chains is the characteristic STAT transcription factor activation pattern for IL-10 signaling observed (Kotenko et al., 1997; Spencer et al., 1998).

As for IL-10, all the receptors of the new molecules from the IL-10 family known so far belong to the cytokine receptor family type 2 (CRF2) (Kotenko and Pestka, 2000). They are generally transmembrane glycoproteins whose extracellular domains consist of about 210 amino acids comprising two tandem fibronectin type III domains and having several conserved amino acid positions important for the secondary structure. More recently, it has been discovered that some of the human IL-10 homologs share single receptor chains and even whole receptor complexes (Dumoutier et al., 2000c, 2001; Xie et al., 2000; Kotenko et al., 2001). One receptor from the family is soluble (Gruenberg et al., 2001). Overall, the interaction of IL-10 homologs with their receptors is quite complex (Fig. 3) and so far only partially understood. Although the predicted helical structure of these homodimeric molecules is conserved, certain receptor-binding residues are variable and define the interaction with specific heterodimers of different CRF2. This leads, through the activation of STAT factors, to diverse biological effects.

We recently investigated the expression of the receptors for the five new human IL-10-related molecules in blood mononuclear cells (Wolk et al., 2002). In contrast to the high expression of receptors for IL-10 homologs in different tissues and cell lines, immune cells (monocytes, NK, B, and T cells) showed only expression of IL-10R1, IL-10R2, and IL-20R2. In these cells, IL-20R2 might be part of a still unknown receptor complex. Immune cells, therefore, may represent a major source but a minor target of the new IL-10 family members (Fig. 3).

B. Interleukin-10 Receptor Polymorphisms

Polymorphisms within the human IL-10 receptor cDNA gene sequence have been described (Tanaka et al., 1997). However, their biological relevance is not clear so far.

C. Interleukin-10 Receptor Signaling

IL-10/IL-10R interaction in immune cells results in transcriptional activation of several hundred genes,

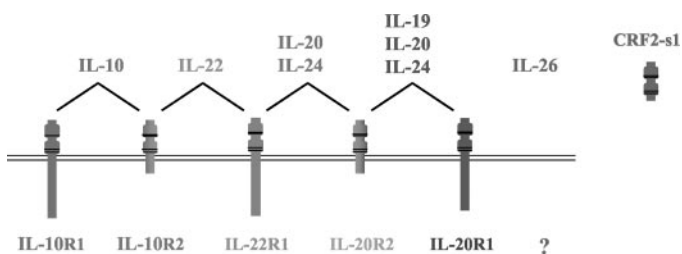


FIG. 3. Ligand/receptor binding of the IL-10 family molecules. All the receptors of the IL-10 family known to date belong to the CRF2. As shown, some of the human IL-10 homologs share single receptor chains and even whole receptor complexes.

some of them are more than 50-fold up-regulated. IL-10 down-regulates expression of far fewer genes (M. Jung, R. Sabat, J. Krätschmar, K. Wolk, C. Schönbein, S. Schütt, M. Freidrich, W. D. Asadullah, H. D. Volk, and G. Grütz, submitted). There is only limited knowledge, however, regarding the IL-10 intracellular signal transduction pathway to date. The IL-10/IL-10R interaction activates the tyrosine kinases Jak1 and Tyk2, which are associated with the IL-1R1 and IL-10R2, respectively (Moore et al., 2001). The receptor engagement and tyrosine phosphorylation activates the cytoplasmically localized inactive transcription factors STAT 1, 3, and 5, resulting in translocation and gene activation (Finbloom et al., 1995). The evidence for the key role of these signaling molecules for the inhibitory effects of IL-10 have been excellently reviewed recently (Moore et al., 2001).

How does IL-10 signaling result in the inhibition of immune functions? IL-10 controls inflammatory processes by suppressing the expression of proinflammatory cytokines, chemokines, adhesion molecules, as well as antigen-presenting and costimulatory molecules in monocytes/macrophages, neutrophils, and T cells (Moore et al., 2001). As all of these inflammatory proteins are transcriptionally controlled by NF- κ B it was suggested that IL-10 may exert a significant part of its anti-inflammatory properties by inhibiting this transcription factor. In fact, a number of studies were able to demonstrate that IL-10 blocks nuclear translocation of the classic NF- κ B p65/p50 heterodimer in monocytes/macrophages (Wang et al., 1995; Clarke et al., 1998). It has been recently shown that IL-10 inhibits NF- κ B activity through dual mechanisms: 1) it blocks NF- κ B nuclear translocation by inhibiting IKK activity; and 2) IL-10 blocks DNA-binding of NF- κ B already present in the nucleus (Fig. 4). Since the inhibition of nuclear NF- κ B could not be explained by an increase of nuclear levels of its inhibitor I κ B (Schottelius et al., 1999), the mechanisms underlying this observation needs to be further investigated. Our recent unpublished findings suggest that IL-10 exerts its anti-inflammatory activity, in part, by a selective induction of p50 nuclear translocation while blocking translocation of the classical p65-p50 heterodimer (F. Driessler, R. Sabat, K. Asadullah, and A. J. G. Schottelius, submitted).

Recent reports (Ito et al., 1999; Yamaoka et al., 1999; Moore et al., 2001) demonstrated that IL-10 inhibits IFN-induced gene transcription (e.g., IP-10, ISG-54), which correlated with the IL-10-mediated inhibition of IFN-induced STAT1 phosphorylation. Moreover, IL-10 inhibition can be overcome by increasing IFN concentrations suggesting competitive interaction between the two cytokine pathways. This interaction at the STAT1 activation level results in inhibition of IFN-mediated antiviral effects by IL-10 (Ichikawa et al., 2002).

IL-10 induces the suppressor of cytokine synthesis (SOCS)-3 probably via a STAT3-dependent pathway

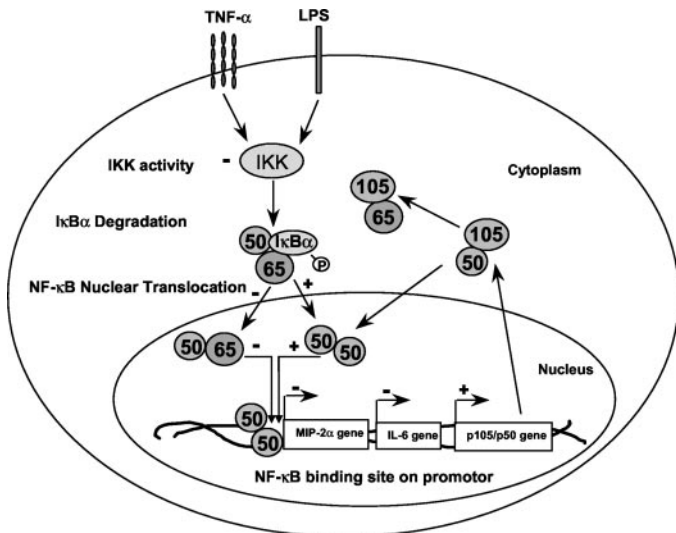


FIG. 4. Scheme representing the molecular mechanisms used by IL-10 to inhibit NF-κB activity. In the absence of an activating stimulus such as TNF-α, IL-10 specifically induces the nuclear translocation of repressive p50/p50 homodimers, which compete with proinflammatory p65/p50 heterodimers for DNA binding to NF-κB promoter sites on inflammatory genes such as IL-6 or MIP-2α. In the presence of a stimulus such as TNF-α, IL-10 can suppress nuclear translocation and DNA binding of p65/p50 heterodimers by inhibiting IKK activity and thus delaying degradation of IκBα. Conserved levels of IκBα will sequester p65 in the cytoplasm, whereas p105/p50 expression is up-regulated and p50 is free to translocate to the nucleus to form homodimers. Up-regulated p105 may also additionally sequester p65 in the cytoplasm. In the absence of the p105/p50 gene, IL-10 loses its ability to suppress constitutive NF-κB activity. Upon activation of p105/p50-deficient cells, p65 may recruit a different Rel protein to form transcriptionally active heterodimers, which can still be inhibited by IL-10 (modified from Schottelius et al., 1999).

(Cassatella et al., 1999; Ito et al., 1999; Moore et al., 2001). There is indirect (Donnelly et al., 1999) and more direct (Berlato et al., 2002) evidence that SOCS-3 plays a key role as mediator of the inhibitory effects of IL-10 on macrophage activation. Very recently, it was shown (Shen et al., 2000) that the IL-10-mediated attenuation of IFN-activated STAT1 is also dependent on SOCS-2 and SOCS-3.

Recent data suggest that IL-10 induces heme oxygenase-1 (HO-1), a heat-shock protein, in murine macrophages via a p38 mitogen-activated protein kinase-dependent pathway (Lee and Chau, 2002). This stress protein degrades heme to carbon monoxide, free iron (that induces ferritin), and biliverdin/bilirubin (Buelow et al., 2002) and plays an essential role in controlling tissue homeostasis in inflammation by inhibiting proinflammatory cytokine synthesis and inducing antiapoptotic processes. Blocking HO-1 by zinc protoporphyrin attenuated the IL-10-mediated protection against endotoxin-induced septic shock in mice, suggesting HO-1 might be an important downstream effector of IL-10 (Lee and Chau, 2002). Induction of HO-1 by cobalt protoporphyrin is associated with up-regulation of SOCS-3 and STAT3 supporting this connection (K. Kotsch, R. Buelow, U. Janssen, and H. D. Volk, submitted).

IV. Immunobiology of Interleukin-10

A. Effects of Interleukin-10 on Immune Cells in Vitro

Antigen-presenting cells and lymphocytes are the primary targets of IL-10. Direct effects on these populations explains the major immunological impact of this cytokine, including the regulation of the Th1/Th2 balance (Fig. 5). Th1 cells are known to be essential for effective cell-mediated immunity [cytotoxic T cell lysis (CTL), cell-mediated inflammation, complement/Fcγ-R binding antibodies] in particular against intracellular organisms, whereas a Th2 (or type 2) cytokine pattern is especially responsible for effective production of IgE, IgA, and noncomplement/Fc-R binding IgG in particular for mucosal immunity (Romagnani, 1995). IL-10 promotes the development of a type 2 cytokine pattern by inhibiting the IFN-γ production of T lymphocytes particularly via the suppression of IL-12 synthesis in accessory cells. According to this, IL-10 costimulates the proliferation and differentiation of B cells, which is important in the adequate defense against intestinal parasites, neutralization of bacterial toxins, and in local mucosa defense (Romagnani, 1995). Moreover, IL-10 suppresses proinflammatory cytokine production and the antigen-presenting capacity of monocytes/macrophages and dendritic cells (De Waal Malefyt et al., 1991a,b; Fiorentino et al., 1991a,b; Romagnani, 1995). Therefore, IL-10 represents a substantial suppressor of the cellular immunity (Spits and De Waal Malefyt, 1992). Important effects of IL-10 on immune cells are summarized in Table 3 and have recently been reviewed by Moore et al., 2001.

1. Effects on Myeloid Antigen-Presenting Cells. Peripheral blood monocytes are very sensitive to IL-10

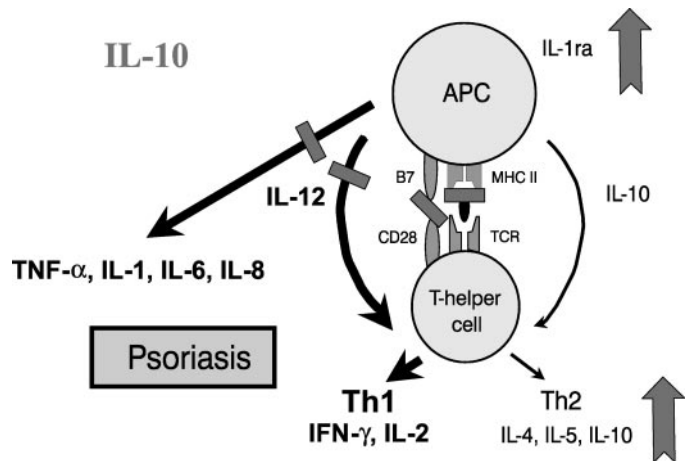


FIG. 5. Effects of IL-10 on the Th1/Th2 dysbalance. An immune deviation toward a type 1 cytokine pattern is a typical finding in several indications such as psoriasis, rheumatoid arthritis, inflammatory bowel disease, transplant rejection, and multiple sclerosis. IL-10 reverses the Th1 cytokine pattern present. It promotes the development of a type 2 cytokine pattern by inhibiting the IFN-γ production of T lymphocytes particularly via the suppression of IL-12 synthesis in accessory cells. Moreover, it inhibits MHC class II and costimulatory molecule expression (Asadullah et al., 2002a; reprinted with permission from Ashley Publications Ltd., London).

TABLE 3
Effect of IL-10 on immune cells (Asadullah et al., 1999b)

Cell Population	Suppression	Induction	Reference
Langerhans cells	Antigen presentation		Enk et al., 1993
Dermal dendritic cells	CD86 expression, antigen presentation		Mitra et al., 1995
Monocytes/macrophages	TNF- α , IL-1, 6, 8, 12 production, expression of MHC class II, CD86, CD54, CD40, antigen presentation	IL-1RA production Soluble TNF receptors	De Waal Malefyt et al., 1991a; Fiorentino et al., 1991b; D'Andrea et al., 1993; Jenkins et al., 1994; Hart et al., 1996
Eosinophils	IL-8, GM-CSF liberation		Takanashi et al., 1994; Nakajima et al., 1996
Neutrophils	TNF- α , IL-1, IL-8 production	IL-1RA production	Cassatella et al., 1994; Jenkins, 1994
Mast cells	TNF- α production	Growth Antigen-induced histamine liberation	Lin and Befus, 1997 Thompson-Snipes et al., 1991
T cells	IL-2 and IFN- γ production, mitogen-induced proliferation		Matsuda et al., 1994
NK cells		Cytotoxicity	Carson et al., 1995
B cells		Growth, IgE synthesis	Uejima et al., 1996; Pistoia, 1997
Keratinocytes	TNF- α , IL-6 secretion capacity (?)		Bécherel et al., 1995; Seifert et al., 2000, 2002
Endothelial cells	No effects	E-Selectin expression	Vora et al., 1996

presence. These cells are not a finely differentiated population. After their 24- to 48-h residence in the circulation, they migrate into the stromal tissues where, depending on the micromilieu, they develop into more specialized cell populations, into either macrophages ($M\phi$) or type 1 dendritic cells (DC1) (Randolph et al., 1998). IL-10 is able to prevent monocyte differentiation into DC1, which are the most important antigen-presenting cells (APC) especially for primary immune responses (Buelens et al., 1997; Allavena et al., 1998; Banchereau and Steinman, 1998). During DC1 development, the influence of IL-10 on these cells decreases. This is associated with a decrease of cellular IL-10R α expression (R. Sabat, unpublished). In contrast, IL-10 supports monocyte maturation to $M\phi$, and the sensitivity of $M\phi$ to IL-10 is comparable to that of monocytes (Allavena et al., 1998; R. Sabat, unpublished). The functions of monocytes and $M\phi$ that are regulated by IL-10 can be divided into three groups: 1) production of soluble immunomodulators regulating inflammation and tissue repair; 2) antigen presentation; and 3) phagocytosis. In general, IL-10 inhibits all those activities that favor the inflammatory or specific cellular immune response and enhances those activities that are associated with the induction of tolerance in adaptive immunity as well as with scavenger function. More concretely, IL-10 inhibits the production of proinflammatory mediators by monocytes and $M\phi$, such as endotoxin- and IFN- γ -induced release of IL-1 β , IL-6, IL-8, G-CSF, GM-CSF, and TNF- α (de Waal Malefyt et al., 1991a; Fiorentino et al., 1991a). In addition, it enhances the production of anti-inflammatory mediators such as IL-1RA and soluble TNF- α receptors (Jenkins et al., 1994; Joyce et al., 1994; Hart et al., 1996). IL-10 inhibits the capacity of monocytes and $M\phi$ to present antigen to T cells. This is realized by down-regulation of constitutive and IFN- γ -induced cell surface levels of MHC class II, of costimulatory molecules such as CD86 and of some adhesion molecules such as CD58 (de Waal Malefyt et al., 1991b; Willems et al.,

1994; Creery et al., 1996). Moreover, IL-10 inhibits the monocytic production of IL-12, an essential mediator for the development of specific cellular immune defense (D'Andrea et al., 1993). Beside these suppressive activities, IL-10 favors the phagocytic activity of monocytes and $M\phi$ (Buchwald et al., 1999). This is mediated via up-regulation of specific receptors that are essential for the uptake of opsonized and nonopsonized microorganisms. Indeed, IL-10-treated monocytes and $M\phi$ display an enhanced expression of IgG-Fc receptors (CD16, CD32, and CD64) as well as scavenger receptors (CD163 and CD14) (te Velde et al., 1992; Spittler et al., 1995; Calzada-Wack et al., 1996; Ritter et al., 1999). Interestingly, IL-10 simultaneously diminishes the killing of ingested microorganisms (Roilides et al., 1998). The up-regulated expression of scavenger receptors seems to be responsible for the observation that IL-10-treated monocytes and $M\phi$ more strongly ingest apoptotic cells (W. D. Döcke and R. Sabat, unpublished), whereas the chemotaxis of monocytes is only marginally impaired by IL-10 (Vicioso et al., 1998).

2. Effects on T Cells. Besides the dominating indirect impact via the APC (Fig. 5), IL-10 also exerts some direct effects on T cells. In particular, inhibitory effects have been described on CD4⁺ T cells. IL-10 inhibits the proliferation as well as the cytokine synthesis of these cells. Concerning the latter, it affects their IL-2 and IFN- γ as well as their IL-4 and IL-5 production, which has been induced by various stimuli (Del Prete et al., 1993; Groux et al., 1996). At least in the human system, IL-10, therefore, seems to inhibit both the Th1-type and the Th2-type responses, although the effect on Th1 cells appears to be stronger (Asadullah et al., 1998). Whereas naive CD4⁺ T cells are targeted by IL-10, activated and memory T cells seem to be rather insensitive toward this cytokine. This might be related to the down-regulation of IL-10R α upon T cell activation (Liu et al., 1994). However, we observed a similar SOCS-3 induction in activated and resting T cells following IL-10 incubation,

making a functional receptor down-regulation less likely (M. Schroeder, unpublished). The presence of IL-10 during the activation of CD4⁺ T cells results in the development of a regulatory phenotype of these cells (Groux et al., 1997; Zeller et al., 1999; Levings et al., 2001a,b). It is characterized by weak proliferation, absence of IL-2 production, and a specific cytokine profile (IL-10⁺, IFN- γ ⁺, IL-4⁻, IL-5⁻) after repeated stimulation. Typically these cells also have the capacity to transfer this phenotype to other T cells with the same antigen specificity. This transfer may not be dependent on soluble mediators but on cell surface molecules (Jonuleit et al., 2000). Whether the influence of IL-10 on CD4⁺ T cells or on APC is more important in vivo or the generation of such regulatory cells remains to be clarified. In vitro, both pathways have been demonstrated. IL-10 does not exert potent direct inhibitory effects on CD8⁺ T cells. It can even activate CD8⁺ T cells under certain conditions (Groux et al., 1998; Santin et al., 2000).

3. Effects on Natural Killer Cells. The effect of IL-10 on NK cells is mainly stimulatory. IL-10 favors the cytotoxic activity of these cells. It increases the IL-2-induced production of cytokines such as IFN- γ , GM-CSF, and TNF- α . Furthermore, it amplifies the IL-2-induced proliferation of the CD56-bright NK cell subpopulation (Carson et al., 1995). Moreover, IL-10 augments the ability of IL-18 to stimulate NK cells (Cai et al., 1999).

4. Effects on Other Immune Cells. IL-10 has various but weak stimulatory effects on B cells. It prevents apoptosis and enhances the proliferation and differentiation toward plasma cells as well as the IgM synthesis (Levy and Brouet, 1994; Rousset et al., 1995). It also plays a role in the Ig switch. In combination with IL-4, it induces IgG4 but inhibits IgE production; in combination with TGF- β , IL-10 induces IgA1 and IgA2 secretion (Defrance et al., 1992; Jeannin et al., 1998).

Very similar to monocytes and M ϕ , in granulocytes IL-10 inhibits the production of proinflammatory (TNF- α , IL-1 β) and induces the production of anti-inflammatory (IL-1RA) mediators. Moreover, it inhibits the release of various chemokines by neutrophils (Casatella et al., 1993; Kasama et al., 1994). The synthesis of cyclooxygenase-2 as well as the production of prostaglandin E2 is also inhibited by IL-10 (Niuro et al., 1997). Another effect of IL-10 is the inhibition of LPS-induced synthesis of proinflammatory mediators in eosinophils and mast cells (Takanaski et al., 1994; Arock et al., 1996). In combination with IL-3 and IL-4, however, IL-10 favors the growth of mast cells (Lin and Befus, 1997).

5. Effects on Epithelial Cells. It has been shown that several epithelial cells express the IL-10R. IL-10 exerts direct effects on these cells (Bourreille et al., 1999; Denning et al., 2000; Parry et al., 2001). The capability of IL-10 to target keratinocytes (KC) is still a matter of debate. When we analyzed the biological effects of IL-10 on KC in vitro, we did not find any evidence for IL-10

effects on KC proliferation, cytokine formation, and expression of surface molecules with impact on immunoregulation. No effects on unstimulated or stimulated KC were observed in primary human KC or on cultured HaCaT cells (Seifert et al., 2000). These results are in line with other observations (Chatelain et al., 1998) showing that IL-10 inhibits intercellular adhesion molecule-1 (ICAM-1) expression on Langerhans cells but not on KC, but do not support some earlier observations regarding a certain in vitro effect of IL-10 in KC. So it was reported that IL-10 inhibits the cytokine synthesis of TNF- α and IL-6 (Bécherel et al., 1995) as well as proliferation (Michel et al., 1997) of KC. This discrepancy might result from impure primary KC cultures (for example contamination with fibroblasts), other culture conditions, different IL-10 proteins used (LPS contamination?), or differences in the experimental proceedings, but overall the reasons remain unclear (Seifert et al., 2000). Recently, we investigated further the direct effects of IL-10 on keratinocytes and addressed the reason for potential IL-10 unresponsiveness using the keratinocyte-like cell line HaCaT as well as primary foreskin keratinocytes. Using real time reverse transcription-polymerase chain reaction, we demonstrated that IL-10 is neither able to induce its typical early gene product SOCS-3 nor to modulate the IFN- γ -induced expression of SOCS-1 and -3. Although flow cytometric analyses showed binding of biotin-labeled IL-10 to HaCaT cells, blocking experiments indicated that this resulted from unspecific binding, which may explain discrepancies to some earlier observations (Michel et al., 1997a,b). Moreover, scattered plot analyses excluded specific binding to primary KC and HaCaT cells. Finally, real-time mRNA analyses demonstrated that the absence of any specific binding results from the lack of IL-10R1 (α -chain) expression, whereas the IL-10R2 (β -chain) is constitutively expressed. This indicates that IL-10 unresponsiveness of keratinocytes could be explained by a lack of IL-10R1 expression and suggest that any IL-10 effects on these cells observed in vivo are indirectly mediated (Seifert et al., 2003).

There is some evidence that IL-10 regulates collagen and DNA synthesis in activated hepatic stellate cells (Mathurin et al., 2002). Taken together, IL-10 is a pluripotent cytokine with potent effects on numerous cell populations, in particular circulating and resident immune cells, as well as epithelial and some other parenchymal cells. Whereas initial data after its discovery suggested that IL-10 mainly mediates suppressive functions, more recent data showed stimulatory properties on certain cell populations, too. Recent data suggests that the effects of IL-10 are quite complex and still considering IL-10 just as immunosuppressive and anti-inflammatory (as it was done in the past) might be an oversimplification. Considering IL-10 as immunoregulatory instead of immunosuppressive is supported by recent in vivo data.

B. Effects of Interleukin-10 in Animals/Animal Models

The data from investigations of IL-10 effects on immune cells suggests that the major physiological importance of IL-10 seems to be the limitation of inflammation, the prevention of uncontrolled nonadequate immunologic reactions, as well as the support of the humoral (Th2) immune responses (De Waal Malefyt et al., 1991a,b; Romagnani, 1995). This hypothesis was confirmed by experimental research in animals, including analyses of IL-10 knockout mice as well as by the effects of IL-10 observed in several inflammatory, autoimmune, and tumor models.

1. *Interleukin-10 Knockout Mice.* IL-10-deficient mice develop lethal inflammation of the intestine, which can be stopped by application of IL-10 (Kuhn et al., 1993). Interestingly, IL-10^{-/-} mice kept under germ-free conditions do not develop enterocolitis, which suggests that in the absence of the immunomodulatory effects of IL-10, an unrestricted intestinal inflammatory response develops toward normal enteric antigens (Renick et al., 2000).

2. *Inflammation and Autoimmune Models.* The observations in the IL-10^{-/-} mice were the rationale for administering IL-10 in several animal models for colitis. The results of these studies clearly showed prevention of intestinal inflammation by IL-10, mainly by down-regulation of an intestinal proinflammatory Th1-like response. However, systemic IL-10 administration was successful only when administered before the initiation of colitis but was ineffective at reversing any established inflammation (Powrie et al., 1993; Herfarth et al., 1996, 1998; Barbara et al., 2000).

Effects of IL-10 application have been investigated in various other inflammatory animal models, too. It turned out that treatment with IL-10 is beneficial in models of experimental autoimmune encephalomyelitis (Rott et al., 1994), pancreatitis (Van Laethem et al., 1995), diabetes mellitus (Pennline et al., 1994), and experimental endotoxemia (Gerard et al., 1993). IL-10 was also effective in various animal models of arthritis, in reducing inflammation, in cellular infiltrates, and in joint destruction (Persson et al., 1996; Tanaka et al., 1996).

However, some of the data are conflicting. For examples, studies have shown both inhibition and exacerbation of experimental allergic encephalomyelitis (EAE) after systemic IL-10 administration. Different therapeutic outcomes are also dependent on the mode of delivery of IL-10 by gene therapeutic vectors (Broberg et al., 2001; Croxford et al., 2001; Cua et al., 2001). Thus the action of IL-10 may differ depending on the local microenvironment, the disease stage, and the IL-10 concentration.

The majority of experimental data suggests the IL-10 application might be beneficial in several inflammatory and organ-restricted autoimmune diseases. With regard

to systemic autoimmune diseases a different picture is emerging. For example, anti-IL-10 mAb treatment of SCID mice injected with PBMC from systemic lupus erythematosus (SLE) patients strongly inhibits autoantibody production in vivo (Llorente et al., 1995); also, treatment of New Zealand black/white mice (mice that spontaneously develop a severe autoimmune disease that closely resembles SLE) with anti-IL-10 mAb substantially delayed onset of autoimmunity (Ishida et al., 1994). This may indicate that neutralizing IL-10 might be a new therapeutic option here. In contrast to the organ-specific autoimmunopathies, SLE is thought to be more a "B cell disease". The different pathogenesis might explain the opposite effects of IL-10.

3. *Tumor Models.* Several animal experiments have been performed to analyze the role of IL-10 on tumor development. The data are complex, showing diverse effects regarding the influence of IL-10 on cancer. Dependent on the experimental model, IL-10 seems to favor or inhibit the existence and progression of tumors (Sabat and Asadullah, 2002).

IL-10 is able to favor tumor growth both directly by affecting the tumor cells and indirectly by inhibition of immune cells. IL-10 can convert tumor cells to a CTL-resistant phenotype. Kiessling and coauthors reported an approximately 50% reduction of MHC class I expression in human melanoma cells after IL-10 treatment. This pretreatment resulted in a dose-dependent, and up to 100% inhibition of autologous CTL-mediated, tumor-specific lysis (Matsuda et al., 1994). This effect is mediated by reduced expression of the so-called transporter associated with antigen processing (TAP)-1 and -2, which results in reduced translocation of peptides to the endoplasmic reticulum and, therefore, in diminished MHC class I peptide loading and cell surface levels (Salazar-Onfray et al., 1997). However, the down-regulation of MHC class I expression results in higher sensitivity of these cells toward NK cell activity (Salazar-Onfray et al., 1995; and see below). The consequence of IL-10 presence and thereby the resulting inhibition of the antitumor immune reaction might be the uncontrolled development of cancers. This has been demonstrated in transgenic mice expressing IL-10 under control of the IL-2 promoter. These animals are unable to limit the growth of immunogenic tumors. However, administration of anti-IL-10 antibodies restored the anticancer response (Hagenbaugh et al., 1997).

The direct negative effect of IL-10 on tumor survival has been described by the Fulton group. They observed that the IL-10 gene transfer in murine mammary tumor cells was associated with increased expression of the inducible isoform of nitric-oxide synthase (iNOS). The activity of this enzyme was elevated as well. This can result in elevated levels of nitric oxide in transfected tumor cells (Kundu et al., 1998). Nitric oxide is known to show potent antitumor activity.

IL-10 can inhibit the generation of new vessels within the tumor both directly by acting on the tumor cells and indirectly by influencing infiltrating immune cells. IL-10 induced the tissue inhibitor of metalloproteinase 2 (TIMP-2) in primary human prostate cancer cells. Simultaneously, it reduced the secretion of matrix metalloproteinase (MMP)-2 and MMP-9 from these cells. The consequence was the inhibition of microvessel formation (Stearns et al., 1999). Interestingly, TGF- β induced the expression of MMP-2, and this induction was prevented by IL-10. When primary human prostate cancer cells either expressing TGF- β or IL-10 were implanted in SCID mice, TGF- β -promoted tumor growth, angiogenesis, and metastasis. In contrast, IL-10 reduced growth rates, angiogenesis, and metastasis. More importantly, none of the mice bearing TGF- β -expressing tumor cells survived compared with 80% of those expressing IL-10 (Stearns et al., 1999). IL-10 can also inhibit the angiogenesis by inhibiting tumor-resident macrophages. Bar-Eli and coauthors (Huang et al., 1996, 1999) reported that the transplantation of human melanoma cells that had been transfected with the murine IL-10 cDNA into nude mice resulted in fewer lung metastases and significant inhibition of tumor growth. The authors suggested that this was due to inhibition of angiogenesis by IL-10. They referred to the fact that IL-10 down-regulated the production of vascular endothelial growth factor in the tumor-associated macrophages. Other factors involved in neovascularization such as IL-1 β , TNF- α , and IL-6 were also inhibited (Huang et al., 1996, 1999). Velu and coauthors (Gerard et al., 1996) demonstrated a loss of tumorigenicity of melanoma cells injected into syngeneic mice after the previous retroviral transfection of these cells with IL-10 cDNA. Host T cells and NK cells might be involved in the observed tumor eradication because IL-10-producing tumor cells grew in nude mice and in CD8⁺ T or NK cell-depleted mice (Gerard et al., 1996). Similar observations have been reported by the Fulton group. They described that injection of a murine mammalian tumor cell line in syngeneic mice resulted in progressive tumor growth and death from pulmonary metastases. In contrast, transfection of IL-10 cDNA in these cell lines resulted in complete inhibition of growth and metastatic disease. Interestingly, the antimetastatic activity of IL-10 is observed also in T cell-deficient mice but is lost when NK cell activity is suppressed (Kundu and Fulton, 1997).

4. Experimental Models of Infections. It is well recognized that IL-10 can inhibit protective immune response to infections (Moore et al., 2001). It has been shown that the trauma-, burn-, and major surgery-induced immunodepression, which predispose to infectious complications, is related to IL-10 overexpression (Ayala et al., 1994; Woiciechowsky, 1998; Kobayashi et al., 2001). Prolonged IL-10 expression increases the risk for infectious complications, whereas neutralizing IL-10 >12 h post

trauma reduces the immunodepression and infection-related mortality (Song et al., 1999).

Overexpression of IL-10 or other type 2 cytokines also modifies the immune response to intracellular bacteria and parasites as well as the susceptibility of mice to these infections (Yamakami et al., 2002).

On the other hand, IL-10 has also protective effects in infection because it prevents an uncontrolled inflammatory response to infectious triggers. IL-10-deficient mice show a prolonged inflammatory response to acute *Pseudomonas* challenge resulting in neutrophil accumulation in the lung. This observation suggests that IL-10 deficiency might contribute to prolonged inflammatory responses early in cystic fibrosis, a lung disease that is characterized by a neutrophilic infiltrate that is excessive relative to the burden of infection (Chmiel et al., 2002). Overexpression of IL-10 prevents mice from endotoxin or bacteria-induced septic shock whereas lack of IL-10 increases the susceptibility to toxin-related shock (Moore et al., 2001; Oberholzer et al., 2002). IL-10 also protects against experimental group B streptococcal arthritis (Puliti et al., 2002). Similar protective properties of IL-10 were observed for gastrointestinal helminth infections (Schopf et al., 2002).

C. Interleukin-10 and Interleukin-10 Receptor Expression in Diseases

Its considerable anti-inflammatory effects and ability to act as a main suppressor of cellular immunity (Spits and De Waal Malefyt, 1992) raises the question of the IL-10 expression under pathophysiological conditions. Both overexpression (e.g., in lymphoma) as well as IL-10 deficiency were found (e.g., in inflammatory bowel disease, psoriasis) and seems to have a pathophysiological significance (Schreiber et al., 1995). Numerous studies have investigated the expression and suggested the importance of IL-10 dysregulation in different entities.

1. Expression in Malignant Diseases.

a. Melanoma. Krüger-Kraskagakes et al. (1994) could demonstrate significant IL-10 mRNA expression in melanoma and melanoma metastases but not in healthy skin. Moreover, they found IL-10 mRNA and the biologically active protein in 3 of 13 melanoma cell lines. This suggests that melanoma cells themselves are contributing at least in part to the IL-10 overexpression in melanoma lesions. Similar results were reported by Dummer et al. (1996b) who also demonstrated IL-10 production by a high percentage of melanoma metastases and corresponding cell lines. This may be of particular pathogenetic importance, since IL-10 functions as autocrine growth factor for malignant melanoma and reduces the expression of HLA class I and II on melanoma cells (Yue et al., 1997).

b. Carcinoma. There are reports on the overexpression of IL-10 in basal cell and squamous cell carcinoma (Kim et al., 1995). Cytotoxic T cell lines recognizing these tumors proliferated in the presence of the tumor

cells only when IL-10 was neutralized by monoclonal antibodies. On the other hand, the intralesional injection of IFN- α resulted in a tumor regression that was associated with the down-regulation of IL-10 mRNA expression. IL-10, therefore, seems to be an important mediator in evading the T cell-mediated immune response in these cutaneous malignancies (Kim et al., 1995). The Strieter group described increased levels of IL-10 protein in tissue homogenates of human bronchogenic carcinomas compared with normal lung tissues. Staining of these tumors illustrated primary localization of IL-10 protein to cancer cells. Furthermore, IL-10 protein was present in supernatants of several unstimulated human bronchogenic cell lines (Smith et al., 1994).

Interestingly, a correlation between IL-10 and vascular endothelial growth factor expression in esophageal cancer was demonstrated suggesting a relation between IL-10 and tumor-promoting angiogenic factor gene expression (Nagata et al., 2002).

c. Lymphoma. Tumor cells from B, T, and NK cell lymphoma are able to produce biologically active IL-10 (Kitabayashi et al., 1995; Masood et al., 1995; Sjoberg et al., 1996; Beatty et al., 1997; Boulland et al., 1998; Jones et al., 1999). As early as 1993, Favrot and coauthors (Blay et al., 1993) investigated IL-10 serum levels using an ELISA, which detects both viral and human IL-10 in patients with active non-Hodgkin's lymphoma (NHL) and healthy volunteers. They described the detection of IL-10 in serum from about 50% of these patients but none of the control blood donors. IL-10 was detectable with a similar frequency in all subtypes of NHL and in all clinical stages, as well as in both EBV-seropositive and EBV-seronegative patients (Blay et al., 1993). One year later the Papa group (Stasi et al., 1994a) demonstrated similar results obtained in patients with aggressive non-Hodgkin's lymphoma. In the following years these observations were extended to Hodgkin's disease and other lymphoma species, and due to improved sensitivity of ELISA systems, it was possible to demonstrate that lymphoma patients had significantly higher serum levels of IL-10 than healthy volunteers (Cortes et al., 1995; Cortes and Kurzrock, 1997; Sarris et al., 1999; Bohlen et al., 2000; Vassilakopoulos et al., 2001; Fayad et al., 2001). An elevated local expression of IL-10 was detected in various cutaneous T cell lymphoma entities (CTCL). Dummer et al. (1996a) showed IL-10 production by malignant T cells in Sézary syndrome, a leukemic type of cutaneous T cell lymphoma. We demonstrated cutaneous IL-10 mRNA overexpression in mycosis fungoides (MF) lesions (Asadullah et al., 1996). Increasing IL-10 gene expression correlated with the tumor progression. An increased cutaneous IL-10 mRNA expression was also found in CD30⁺ pleomorphic T cell lymphomas (Asadullah et al., 1996; Yagi et al., 1996) and cutaneous B cell lymphomas (CBCL) (Asadullah et al., 2000b). The IL-10 overexpression in CTCL might contribute to a number of immunological abnormalities well

known in these patients. These include eosinophilia and elevated IgE and IgA levels (Edelson, 1980). We recently observed a stage-dependent decrease in T cell activation of antigen expression suggesting impairment of tumor surveillance in advanced MF stages (Asadullah et al., 1997a). Such findings might result from the IL-10 overexpression that also might be responsible for the development of a systemic type 2 cytokine pattern in CTCL (Dummer et al., 1993).

d. Prognostic Value of Interleukin-10 Overexpression. In different lymphomas, increased IL-10 production has been reported and a negative prognostic meaning of increased IL-10 plasma levels is being discussed (Blay et al., 1993; Stasi et al., 1994a,b; Cortes et al., 1995). Elevated IL-10 serum levels have been also described as a negative prognostic factor for responsiveness toward treatment, as well as the disease-free and overall survival by patients with melanoma and solid tumors, particularly with lung, gastrointestinal, and renal cell cancer. Several groups including ours reported on increased circulating IL-10 serum levels in gastric, colon, and renal-cell cancer patients (Ordemann et al., 2002). IL-10 serum levels commonly returned to normal in radically resected patients. Persistently elevated IL-10 serum levels after surgery predicted tumor recurrence (Galizia et al., 2002a,b; Uwatoko et al., 2002). Moreover, a further significant increase in IL-10 serum levels has been observed in nonresponders after chemotherapy (Wojciechowska-Lacka et al., 1996; De Vita et al., 1999, 2000a,b; Nemunaitis et al., 2001).

In summary, there are several lines of evidence that IL-10 overexpression in different malignancies might contribute to tumor development, in particular, by suppressing the antitumor immune response (Matsuda et al., 1994). Moreover, IL-10 might even be a tumor cell growth factor in certain tumors such as B cell lymphoma and melanoma.

2. Autoimmune and Inflammatory Diseases. IL-10 has been investigated in several immune disorders with identified autoantigen. This includes lupus erythematosus, systemic sclerosis, and bullous diseases. Overall enhanced IL-10 levels were observed and seem to be of pathophysiological relevance. Several investigations pointed to the major role of IL-10 in chronic inflammatory disorders characterized by the predominance of a type 1 cytokine pattern. These included psoriasis, inflammatory bowel disease such as Crohn's diseases, multiple sclerosis, rheumatoid arthritis, transplant rejection, and allergic contact dermatitis. So in contrast to several malignant and autoimmune diseases, a relative deficiency rather than an overexpression is considered to be of pathophysiological relevance here.

a. Systemic Lupus Erythematosus. IL-10 functions as a potent B cell stimulator that enhances activation, proliferation, and differentiation of B cells. With regard to B cell activation, IL-10 might play a critical role in SLE, since this systemic autoimmune disease is charac-

terized by high autoantibody production and by decreased cellular immune responses. In SLE, high levels of autoantibodies generate immune complexes causing tissue damage. Therefore, expression of this cytokine was investigated. Compared with healthy individuals, levels of IL-10 in SLE patients are significantly higher and there is a correlation of IL-10 levels with the clinical disease activity (Park et al., 1998). Depletion of IL-10 by anti-IL-10 mAb in vitro treatment of SLE patient-derived PBMC significantly decreased autoantibody production (Llorente et al., 1995). Together with the data from animal models described above, this data suggests that IL-10 is harmful in systemic lupus and that IL-10 antagonists may be beneficial in the treatment of human SLE (Llorente et al., 1999).

b. Systemic Sclerosis. Increased IL-10 serum levels (significant in diffuse SSc, not significant in limited SSc) in patients compared with levels in healthy controls has been found. Although the role of IL-10 in SSc is not known yet, it is suggested that IL-10 may contribute to the development of SSc (Hasegawa et al., 1997).

c. Bullous Pemphigoid. Titers of IL-10 in blister fluids of patients were significantly higher than in suction blisters from control probands (Schmidt et al., 1996; Giacalone et al., 1998). Apart from this correlation, the role of IL-10 in bullous pemphigoid is not understood yet.

d. Psoriasis. We have recently demonstrated that the cutaneous IL-10 mRNA expression in psoriasis was significantly lower than in atopic dermatitis or cutaneous T cell lymphoma. The level of IL-10 mRNA expression did not differ from healthy skin, even though numerous proinflammatory cytokines are overexpressed. This indicates a relative IL-10 deficiency in psoriasis (Asadullah et al., 1998). These results are supported by immunohistochemical findings of low cutaneous IL-10 protein expression (Nickoloff et al., 1994) and by quantification of IL-10 protein in blister fluids (Mussi et al., 1994). Accordingly, it has also been shown that T cells involved in psoriasis vulgaris belong to the Th1 subset (Schlaak et al., 1994).

e. Rheumatoid Arthritis. A number of published studies have shown a correlative relationship between IL-10 and RA. This includes studies carried out to detect IL-10 in the serum, synovial fluid, or synovial explants of patients (Jenkins et al., 1994; Cush et al., 1995; Al-Janadi et al., 1996). Several ex vivo studies show that IL-10 can effectively block the production of the proinflammatory cytokines TNF- α , IL-1, and IL-8 by snivel macrophages and synoviocytes. IL-10 has been correlated with an increased autoantibody production, serum factor, and B cell activation in RA patients (Chomarat et al., 1995; Hart et al., 1995, 1996). These findings indicate that it might be difficult to predict the response of a human patient population to IL-10, despite the promising findings in animal models of arthritis (Narula, 2000).

f. Allergic Contact Dermatitis and Other Non-Atopic Eczemas. Important clues for the role of IL-10 in contact dermatitis were demonstrated by in vivo experiments. Allergic contact dermatitis represents a classic type 1 cytokine-dominated immune reaction, suggesting only low levels of the type 2 cytokine IL-10. Kondo and Schwarz demonstrated that the application of IL-10 blocks the effector phase in allergic contact hypersensitivity reactions (epicutaneous application) (Kondo et al., 1994; Schwarz et al., 1994). Consequently, it might be speculated that therapeutic IL-10 application in allergic contact dermatitis would be beneficial.

The expression of IL-10 in other eczematous non-atopic skin diseases might represent an important counter-regulatory, i.e., protective mechanism in stopping overwhelming inflammatory reactions. This hypothesis is strengthened by the observation that therapeutic UV radiation, effective in treating several inflammatory dermatoses, enhances IL-10 production. Kang et al. (1994) showed that skin-infiltrating macrophages are the main source of epidermal IL-10 production after UV exposure. This IL-10 seems to be responsible for the fast disappearance (resolution) of UV-induced erythema within a couple of days seen for example in solar dermatitis (Kang et al., 1994).

g. Chronic Inflammatory Bowel Diseases. The gastrointestinal tract serves as a barrier between the host and several foreign antigens and pathogens that are contained within its lumen. The mucosa-associated immune system must balance two opposing functions: the development of an immune response to pathogens while maintaining tolerance to antigens derived from food and "natural" microbial flora. The regulation of this balance is very complex but cytokines seem to play a key role. Mice deficient in the immunoregulatory cytokines IL-10 or TGF- β develop an imbalance of this system resulting in severe inflammatory disease and death by uncontrolled inflammation. Local release of IL-10 by genetically modified bacteria, intestinal cells, or T cells prevents chronic ileocolitis in these mice (Kuhn et al., 1993; Lindsay and Hodgson, 2001).

Transfer of T cells depleted of CD4⁺25⁺ regulatory T cells into immunodeficient SCID mice induces chronic inflammatory bowel disease as well. Enriched IL-10-expressing regulatory T cells can prevent the disease (Lindsay and Hodgson, 2001).

h. Multiple Sclerosis. In several experimental models of EAE, a protective effect of IL-10 has been described. In vitro generation of IL-10 producing regulatory CD4⁺25⁺ T cells is induced by a combination of vitamin D and dexamethasone. The regulatory function of these cells that in vitro inhibit both Th1- and Th2-inducing cytokines was demonstrated in vivo by their ability to prevent EAE, when targeted to the site of inflammation, and this function was shown to be IL-10-dependent (Barrat et al., 2002).

Local delivery of IL-10 in the brain using gene transfer methods also improved the EAE disease, although the kind of delivery system seems to be important (Croxford et al., 2001; Cua et al., 2001). Intranasal or mucosal delivery of IL-10 with or without coapplication of autoantigen was also sufficient to prevent EAE (Massey et al., 2002)

In most models, however, IL-10 was active in preventing EAE whereas its efficiency in ongoing or established EAE was less clear (Link and Xiao, 2001).

Interestingly, IFN- β application, the best established therapeutic approach in multiple sclerosis in patients, modulates the IL-10/IL-12 cytokine circuit resulting in dominance of IL-10 (Tuohy et al., 2000).

i. Transplantation. The role of IL-10 in transplantation is poorly understood. Because of its anti-inflammatory properties and its association with regulatory T cells, IL-10 should have some benefit on graft survival. In fact, IL-10 inhibits ischemia/reperfusion injury (Deng et al., 2001), prolongs allograft survival (Feng et al., 1999; Zuo et al., 2001), and is essential for the action of regulatory T cells mediating tolerance at least in some transplant models (Hara et al., 2001). In addition, IL-10 prevents several side effects of OKT3 mAb-mediated cytokine release (Moore et al., 2001).

On the other hand, in several transplant models, particularly across strong MHC barriers, IL-10 did not improve graft survival (T. Ritter, unpublished). Moreover, intragraft up-regulation of IL-10 showed a strong correlation to acute and chronic rejection of human kidney transplant, much stronger than proinflammatory cytokines such as TNF- α , IFN- γ , IL-2, etc. (Ode-Hakim et al., 1996; Suthanthiran and Strom, 1998). Tacrolimus is much more potent in preventing chronic rejection than cyclosporin A. A major risk factor of chronic rejection is incomplete reversal of acute rejection. Ongoing acute allograft rejection was found to be rescued by tacrolimus but not by cyclosporin A at the equivalent dose. Tacrolimus but not cyclosporin A inhibited intragraft IL-10 and perforin expression as well as CD8⁺ T cell infiltration. However, both drugs inhibited other immune cells (CD4⁺, ED2⁺ macrophages) and cytokines (IL-1 β , IL-2, IL-4, IL-6, IL-12, IFN- γ , and TGF- β). The inability of cyclosporin A to overcome ongoing allograft rejection could be addressed by cotreating recipients with neutralizing anti-IL-10 antibody. These data suggest that intragraft IL-10 production might be a critical factor in persistent rejection resulting in chronic graft injury (Jiang et al., 2002).

The main problem in allogeneic bone marrow transplant patients is the development of graft-versus-host disease. Increased IL-10 production before allogeneic bone marrow transplant correlated with a subsequent low incidence of graft-versus-host disease and transplant related mortality as compared with low IL-10 production (Baker et al., 1999; Holler et al., 2000).

3. Expression in Atopic Disorders. IL-10 overexpression is considered to be responsible for the immune deviation into a type 2 direction a hallmark of atopic disorders such as atopic dermatitis and allergic asthma. Consequently, IL-10 is speculated to be harmful here and a potential target for neutralization. However, IL-10 with its obvious anti-inflammatory capacities and its overexpression might in fact also contribute to the limitation of inflammation (as counter-regulation). Indeed, it has therefore even been postulated that it might therapeutically be applied even in the treatment of allergic inflammation (Pretolani and Goldman, 1997), but IL-10 treatment of a pre-existing type 2 cytokine pattern may have only limited effect, or may even worsen the course of the disease.

a. Atopic Dermatitis. In atopic dermatitis, Ohmen et al. (1995) reported a marked overexpression of IL-10 mRNA, predominantly of monocytic origin but possibly also from skin-infiltrating Th2 cells. We found similar results in our atopic patients (Asadullah et al., 1996). The cutaneous overexpression of IL-10 reflects the general immunological imbalance in atopic dermatitis. According to current knowledge, type 2 cytokine (IL-4, IL-5, IL-10)-producing allergen-specific T cells are considered to be crucial in atopic dermatitis. In fact, such allergen-specific activated T cells were isolated (Renz et al., 1992; Van Reijssen et al., 1992). The type 2 cytokine pattern in atopic dermatitis (Bos et al., 1992; Chan et al., 1993; Romagnani, 1995) explains the atopy-associated eosinophilia and hyper-IgE phenomenon, findings considered to be of pathophysiological importance for this disease. IL-10, however, is of considerable importance in the regulation of the type 1/type 2 balance as outlined before. Moreover, the persistently elevated cutaneous IL-10 expression might also be responsible for the lasting suppression of the skin immune system, which might contribute to the increased incidence of cutaneous infections in patients with atopic eczema (Braun-Falco et al., 1995). Infiltrating macrophages also contribute to the high IL-10 expression in atopic dermatitis. It is possible, therefore, that IL-10 production in atopy also exerts counter-regulatory effects.

b. Allergic Asthma. In animal models of asthma, IL-10 was shown to be capable of inhibiting allergen-induced airway inflammation and nonspecific airway responsiveness (Tournoy et al., 2000). IL-10 gene transfer to the airway abrogated both the cellular and physiological recall response in vivo. However, IL-10 did not prevent expansion and activation of T cells (Stampfli et al., 1999). In addition, studies in gene-deficient mice suggest IL-10 is necessary for the expression of airway hyperresponsiveness but not pulmonary inflammation after allergic sensitization (Makela et al., 2000). These data suggest the complexity of IL-10 in the pathogenesis of asthma (Barnes, 2002).

A relative underproduction of IL-10 from alveolar macrophages and in sputum of atopic asthmatics has

been reported in patients (Takanashi et al., 1999; Chung, 2001). During classic specific immunotherapy that has been proved to be clinically effective, the Th2-dominated immune response is modified toward a Th1 response leading to a decline in allergen-specific IgE and an increase in allergen-specific IgG production. Most importantly, however, production of IL-10 is also induced leading to T cell anergy (Bellinghausen et al., 2001). A randomized, double-blind trial of the effect of glucocorticoid, antileukotriene, and β -agonist treatment in children with asthma demonstrated that triamcinolone and montelukast treatment increased the serum IL-10 levels but decreased eosinophil counts and that all clinical parameters improved; treatment with formoterol had no significant effects on these parameters. Thus, the positive effects of triamcinolone and montelukast on asthma might be related to increasing IL-10 serum levels (Stelmach et al., 2002).

4. Expression in Infection. Because IL-10 expresses potent immunomodulatory properties it can modulate the course of infections. The main target of IL-10 is macrophages, and these cells play a central role in infections, as a target for pathogens and in the activation of both specific and innate immune response. Certain viruses induce production of host IL-10 by macrophages, whereas other viruses encode their own viral IL-10 homologs. As already suggested (see *Section II.A.*), several DNA viruses such as herpes viruses (e.g., EBV, CMV, primate CMVs, equine herpesvirus 2, herpesvirus samiri) and pox viruses (e.g., parapoxvirus orf) encode for their own IL-10. Other viruses such as respiratory syncytial virus, human rhinovirus-14, and parainfluenza virus 3 have developed strategies to induce cellular IL-10 in host macrophages. The exact molecular mechanisms by which these viruses cause increased IL-10 secretion have not been determined. However, it is apparent that expression of viral IL-10 homologs or induction of cellular IL-10 by viruses that target macrophages leads to suppression of the localized and perhaps also generalized inflammatory response (Kotenko et al., 2000; Moore et al., 2001; Redpath et al., 2001; Spencer et al., 2002).

On the other hand, cellular IL-10 can activate several viruses by triggering promoters encoding essential viral proteins; e.g., the early promoter of human papilloma virus type 16 (Arany et al., 2002) and the immediate early promoter of human CMV (Kline et al., 1998; K. Pioch, C. Priemer, C. Liebenthal, D. H. Krüger, H. D. Volk, and S. Prösch, submitted). Additionally, specific intracellular bacteria, including several mycobacteria and *Listeria monocytogenes*, can replicate in macrophages while inducing cellular IL-10 (Redpath et al., 2001; Demangel et al., 2002).

As in experimental models, trauma, burn, and major surgery induces increased production of IL-10. Stress mediators seem to play an important role in regulating IL-10 (Woiciechowsky et al., 1998). Recent data from our

group shows that blocking sympathetic activation after major surgery by high epidural anesthesia significantly reduces the postoperative IL-10 release (Volk et al., 2003). Several studies report on an association between IL-10 release, immunodepression, and decreased resistance to infections (Lyons et al., 1997; van Dissel et al., 1998; Opal and Huber, 2000; Volk et al., 2000; Muehlstedt et al., 2002; Spiess et al., 2002).

D. Interleukin-10 and Interleukin-10 Receptor Polymorphisms and Diseases

Genetic markers in cytokine genes are becoming widely used in studies of immune-mediated disease, and it is becoming apparent that they can be markers of disease susceptibility as well as of disease severity (Bidwell et al., 1999). Since inadequate expression of IL-10 seems to be of pathophysiological relevance in several diseases and the expression levels seem to have a genetic background (as described above), linkage analyses of IL-10 promoter haplotypes to diseases have been performed. Possible linkage of IL-10 promoter haplotypes to disease susceptibility or severity has been reported (Moore et al., 2001).

The strongest association seems to be established for SLE, where it has been suggested that high IL-10 expression (Llorente et al., 1995, 1997), and the corresponding IL-10 alleles (Eskadale et al., 1997b), play a causative or aggravating role (Lazarus et al., 1997; Gonzalez-Amaro et al., 1998; Rood et al., 1999). The IL10.G microsatellite showed significant allele skewing in patients versus controls. This has been observed in patients from the United Kingdom (Eskdale et al., 1997a), Mexico (Mehrian et al., 1998), and Italy (D'Alfonso et al., 2000). Both in the UK and Italian study the same allele was associated with SLE (IL10.G13). To what extent high IL-10 expression actually contributes to or is just a consequence of the disease is not really understood. Healthy relatives of lupus erythematosus patients also exhibit elevated IL-10 expression (Llorente et al., 1997; Grondal et al., 1999), suggesting that high IL-10 levels may predispose to disease and precede onset. Another study also indicated a 40-fold increased risk for developing SLE in individuals who have particular alleles of both the IL-10 and bcl-2 genes (Mehrian et al., 1998). One of the lupus susceptibility loci in the New Zealand mouse is near IL-10 on chromosome 1 (Kono et al., 1994), and an IL-10 promoter polymorphism in this strain has been noted (Morse et al., 1999). By contrast, the IL-10 receptor genotype does not determine susceptibility to SLE (Nakashima et al., 1999).

When the two known polymorphic microsatellite markers in the human IL-10 promoter IL10.G and IL10.R were investigated in psoriasis patients, no difference was noted in comparison to the control group. Also, no difference in allele distribution was observed when the psoriasis patients were stratified according to age of onset. However, a clear differential distribution was re-

vealed when patients were grouped according to whether they had a positive family history of psoriasis. In this case, allele IL10.G13 was positively associated with familial psoriasis, and this was also true when younger patients were considered; patients with age-of-onset less than 40 were 3-fold more likely to have a psoriatic family background if they carried this allele. Thus, it appears that the IL-10 locus contributes to the genetic background in familial psoriasis (Asadullah et al., 2001a).

There is only some preliminary evidence for a relationship between genetic IL-10 secretion predisposition and infectious disease. Genetic predisposition to high IL-10 expression has been reported to be associated with a higher rate of mortality in meningococcal disease (Westendorp et al., 1997). Moreover, chronically infected hepatitis C patients who are genetically predisposed to high IL-10 production were reportedly less likely to benefit from IFN- α therapy (Edwards-Smith et al., 1999). An association between IL-10 polymorphism and recurrence of hepatitis C in liver transplant patients has also been reported (Tambur et al., 2001).

Some groups found an association between IL-10 polymorphism and severity of, but not susceptibility to, asthma (Zhang 2002). Several reports suggest that genetic factors in the IL-10 gene may modify the incidence and outcome of tumors (McCarron et al., 2002; Wu et al., 2002).

IL-10.G microsatellites mark promoter haplotypes associated with protection against the development of reactive arthritis (Kaluza et al., 2001). In female rheumatoid arthritis patients followed up for >12 years, the mean increase in radiographic damage score during the first 6 years was significantly lower in the IL-10 high responder group expressing the -1082 GG genotype (Huizinga et al., 2000). In addition, IL-10 microsatellite polymorphisms also influences the susceptibility to rheumatoid arthritis (Eskdale et al., 1998).

A significant association between IL-10 polymorphisms and the outcome of organ and bone marrow allografts has also been reported although different results were found between distinct transplant populations (Turner, 1997b; Takahashi, 2000; Asderakis et al., 2001; Hahn et al., 2001; McShane, 2002).

Although it has been shown that genetic relationships between cytokine alleles and secretion may vary in patient groups compared with normal groups, the possibility must be considered that the dysregulation of IL-10 seen in psoriasis (Asadullah et al., 1998) and other diseases and the association between an IL-10 locus gene marker and the disease investigated are complimentary rather than related. Of course, this allele may well be of importance in alternative pathways of IL-10 regulation, but if IL10.G13 is not associated with differential IL-10 secretion then it is likely that this allele is associated with both psoriasis and SLE, through linkage disequilibrium with a new functional element yet to be defined.

Recently, several new single-nucleotide polymorphisms have been defined in the human IL-10 locus, but none of these was specifically linked to IL10.G13. Furthermore, the complex haplotypic nature of the IL-10 locus (Eskdale et al., 1999) placed the IL10.G13 on at least two frequent haplotypes. Both these observations support the hypothesis that any functional element linked to this allele may in fact lie outside the IL-10 locus per se. If true, this would implicate IL-10 as a disease-modifying gene in psoriasis (and probably other immune diseases) rather than a disease-causing gene.

V. Interleukin-10 As a Therapeutic Agent

The powerful immunomodulatory properties of IL-10 and the promising results from IL-10 delivery on the course of several inflammatory diseases in experimental models induced the interest on clinical application of IL-10. To our knowledge, so far human recombinant IL-10 (ilodecakin/Tenovil; Schering-Plough Research, Kenilworth, NJ) has been tested in healthy volunteers, patients with Crohn's disease, rheumatoid arthritis, psoriasis, hepatitis C infection, HIV infection, and for the inhibition of therapy associated cytokine releases in organ transplantation and Jarisch-Herxheimer reaction.

A. Phase I Trials in Healthy Volunteers

In phase I clinical trials, safety, tolerance, pharmacokinetics, pharmacodynamics, immunological, and hematological effects of single or multiple doses of IL-10 administered by intravenous (i.v.) or subcutaneous (s.c.) route have been investigated in various settings on healthy volunteers (Chernoff et al., 1995; Huhn et al., 1996, 1997). The first administration of human recombinant IL-10 in human was performed in 1995 (Chernoff et al., 1995). Overall, these studies showed that IL-10 is well tolerated without serious side effects at doses up to 25 $\mu\text{g}/\text{kg}$; mild to moderate flu-like symptoms were observed in a fraction of recipients at doses up to 100 $\mu\text{g}/\text{kg}$ (Moore et al., 2001).

Single i.v. or s.c. doses of IL-10 resulted in transient dose-dependent changes in white blood cell populations, including increases in total white blood cells and neutrophils. A reduction was observed in the number of CD3⁺CD4⁺ and CD3⁺CD8⁺ lymphocytes accompanied by an increase in the percentage of CD14⁺ HLA-DR⁺ monocytes. Furthermore, transient decreases in expression levels of CD11a (LFA1) on CD3⁺ T cells, which may account for some of the observed changes in lymphocyte circulation, and a decrease in the expression levels of HLA-DR on CD14⁺ monocytes, but not on CD20⁺ B cells, were measured following a single i.v. dose of IL-10 in healthy volunteers (Fuchs et al., 1996; Huhn et al., 1999). In addition to transient neutrophilia, lymphocytopenia, and monocytosis, a delayed decrease in platelet

counts was observed following a single s.c. dose of IL-10 (Huhn et al., 1997).

Pharmacokinetic parameters of IL-10 were determined following i.v. or s.c. administration of doses ranging from 0.1 to 100 $\mu\text{g}/\text{kg}$ (recently reviewed by Moore et al., 2001). Following i.v. administration, IL-10 serum levels initially declined fairly rapidly but yielded a less steep terminal phase with a $t_{1/2}$ of 2 to 3 h. Mean exposure parameters (maximum serum concentration, C_{max} , and AUC) were linearly related to dosage, and IL-10 tended to remain in the vascular compartment. Because hIL-10 is nonglycosylated, it is cleared mainly through the kidney, as indicated by the increased $t_{1/2}$ and AUC of IL-10 in patients with moderate to severe renal insufficiencies. Administration of IL-10 did not produce adverse effects in this patient population (Andersen et al., 1999). Subcutaneous administration of IL-10 resulted in slow absorption from the IL-10 depot formed at the injection site, which reached C_{max} at 2 to 6.5 h post injection. The slower absorption of IL-10 following s.c. versus i.v. administration led to prolonged but lower AUC with a mean terminal $t_{1/2}$ of 2.7 to 4.5 h and so resulted in a prolonged immunosuppressive effect. Mean exposure parameters were also linearly related to dosage (Radwanski et al., 1998). Production of neutralizing antibodies was not observed in any of the studies. Co-administration of IL-10 and prednisolone did not result in pharmacokinetic alterations of either drug. In addition, IL-10 administration did not significantly alter cytochrome P450-mediated drug metabolism (Gorski et al., 2000).

In vivo administration of IL-10 inhibited the ex vivo LPS-induced production of IL-6, IL-1, and TNF in whole blood cell assays and decreased proliferate responses and IFN- γ production following phytohemagglutinin stimulation of PBMC, indicating that IL-10 retains immunomodulatory activity when administered in vivo. The doses required to effect 50% maximum inhibition (IC_{50}) of TNF and IL-1 β production and a maximum fraction of inhibition (I_{max}) indicated that IL-10 inhibited production of proinflammatory mediators in vivo at concentrations similar to those used in vitro experiments (Radwanski et al., 1998). Similarly, i.v. administration of 25 $\mu\text{g}/\text{kg}$ IL-10-inhibited LPS-induced rises in temperature and release of TNF, IL-6, IL-8, and IL-1RA in healthy human volunteers, when given 2 min before but not 1 h after endotoxin (Pajkrt et al., 1997a). Such "pretreatment" with IL-10 also reduced endotoxin-induced granulocyte accumulation in the lungs, granulocyte degranulation, cortisol levels, activation of the fibrinolytic system, inhibition of fibrinolysis, activation of the coagulation system, and inhibition of expression of the CC chemokines Mip1 α , Mip1 β , and MCP1 (Pajkrt et al., 1997b; Olszyna et al., 2000). Delay in administration of IL-10 for 1 h only reduced IL-6 and Mip1 β production, cortisol levels, inhibition of fibrinolysis, and activation of the coagulation system, indicating that timing of IL-10

administration is important for its full anti-inflammatory activity during experimental endotoxemia.

Taken together, IL-10 application induces a number of immunological changes and is well tolerated. Some clinical trials have been accompanied by a reversible decline in platelet counts and hemoglobin levels. Recently, it was confirmed in healthy probands that IL-10 decreases both parameters. No significant change was observed in the bone marrow cellularity or myeloid/erythroid ratio or in the number of megakaryocytes. A decrease was observed in the number of megakaryocyte colony-forming units after administration of IL-10 compared with those receiving placebo. No differences were observed in granulocyte-macrophage, mixed lineage or erythroid burst-forming colonies suggesting IL-10 selectively targets platelet production (Sosman et al., 2000). The mild anemia might be related to changes in iron homeostasis (see below; Tilg et al., 2002a).

B. Prevention of Cytokine Release in Transplant Patients and Jarisch-Herxheimer Reaction

The effects of IL-10 on systemic production of proinflammatory cytokines in organ transplant patients who received anti-T cell therapy as induction therapy were investigated. OKT3 monoclonal antibody is a powerful T cell-targeting immunosuppressive agent, but it stimulates a dramatic cytokine release by triggering almost all T cells. This results in severe side effects that are particularly related to systemic TNF- α , IL-1 β , IL-2, and IFN- γ release. Pretreatment with IL-10 reduced release of TNF induced by OKT3, but high IL-10 doses may have promoted early sensitization to OKT3 and exerted reversible adverse effects on graft acceptance (Wissing et al., 1997; Moore et al., 2001).

By contrast, IL-10 failed to alter proinflammatory cytokine production or physiological changes associated with the Jarisch-Herxheimer reaction, an acute systemic inflammatory response that follows antibiotic treatment of *Borrelia recurrentis* infection (Cooper et al., 2000).

C. Therapy of Crohn's Disease

Based on the successful experimental findings in animal models of intestinal inflammation, IL-10 therapy was introduced as a potential new anti-inflammatory therapy in Crohn's disease (CD). Indeed the first therapeutic administration was reported in patients successfully treated with IL-10 for steroid-refractory Crohn's disease (Van Deventer et al., 1997).

Several large multicenter trials were performed, testing multiple IL-10 dosages in patients with mild/moderate or therapy refractory CD, as well as in patients undergoing curative ileal or ileocolonic resection to prevent endoscopic postoperative occurrence by systemic administration (Fedorak et al., 2000; Schreiber et al., 2000; Colombel et al., 2001). Overall the data indicates that IL-10 therapy is safe and well tolerated. However,

IL-10 treatment did not result in significantly higher remission rates or clinical improvement compared with placebo treatment.

In another trial, IL-10 was not used for treatment of acute Crohn's disease but for prevention of postoperative recurrence of Crohn's disease. At 12 weeks after curative ileal or ileocolonic resection and primary anastomosis, the incidence of severe endoscopic recurrence was similar in the IL-10 and placebo-treated groups (Colombel et al., 2001). Thus the clinical results are overall unsatisfying.

Several explanations for the disappointment with this therapeutic strategy are discussed (Herfarth and Schölmerich, 2002).

1. With the administered dose of IL-10 in the clinical trials, the ultimate local IL-10 concentrations in the intestine could be too low to result in down-regulation of inflammation. Increasing the dose of systemically administered IL-10 is limited due to side effects (for example, anemia, headache).
2. IL-10 administration is only successful in preventing and not treating an established disease, as was suggested by the results of the animal experiments.
3. Administration of IL-10 alone fails to effectively suppress the dysregulation of the wide variety of proinflammatory mediators that are involved in the perpetuation of chronic intestinal inflammation.
4. The immunostimulatory properties of IL-10 on B cells and on IFN- γ production by CD4⁺, CD8⁺, and/or natural killer cells counterbalance its immunosuppressive properties.
5. Very recently, data has been presented which may explain, at least in part, the dilemma of IL-10 therapy in CD. Tilg et al. (2002a) have investigated the influence of subcutaneous administration of various doses of human recombinant IL-10 on lymphocytic IFN- γ production and LPS-induced TNF secretion by macrophages in whole blood assays as well as on serum neopterin and nitrite/nitrate levels. The study was conducted using samples from two multicenter therapeutic trials in patients with steroid-dependent chronic active CD and patients with mild to moderately active CD.

In patients treated with the highest dose of IL-10 (20 $\mu\text{g}/\text{kg}$), the study described a significant increase in neopterin, which is produced by human monocytes/macrophages in response to IFN- γ , as well as an increase in phytohemagglutinin-induced IFN- γ production compared with pretreatment levels. Furthermore, LPS-induced TNF- α production was dose dependently down-regulated by IL-10. Neither the elevation in neopterin or IFN- γ nor suppression of TNF correlated with the clinical response of the patients, which may also reflect the divergence of the clinical (Crohn's disease activity index) and immunological (for example, proinflammatory me-

diators) readouts in trials employing cytokine or anticytokine strategies.

The immunopotentiating effects of IL-10 found by Tilg et al. (2002a) are corroborated by a study in healthy volunteers subjected to experimental endotoxemia (Lauw et al., 2000). Systemic IL-10 treatment enhanced endotoxin (LPS)-induced IFN- γ release as well as the IFN- γ -dependent chemokines, IFN- γ -inducible protein 10 (IP-10), and monokine induced by IFN- γ (MIG). The stimulatory effects were most pronounced when IL-10 administration was performed 1 h after the LPS challenge.

Patients receiving higher doses of IL-10 developed anemia and presented with a dose-dependent increase of ferritin and soluble transferrin receptor levels, an indicator of iron restriction to erythroid progenitor cells. Hyperferritinemia may result from direct stimulation of ferritin translation by IL-10 in activated monocytic cells (Tilg et al., 2002b).

All in all the clinical results of IL-10 therapy in Crohn's disease have been unsatisfying. Therefore it is very unlikely that this cytokine will be approved for therapy in this inflammatory bowel disease.

D. Therapy of Rheumatoid Arthritis

Limited data regarding the effects of IL-10 in rheumatoid arthritis are available (Moore et al., 2001). Only a limited efficacy but a good safety profile was observed when IL-10 was administered for 28 days to RA patients (Keystone et al., 1998). A combination of IL-10 and methotrexate in a multicenter, placebo-controlled, dose-escalating study in RA patients was described where 8 $\mu\text{g}/\text{kg}$ q.i.d. or 8 $\mu\text{g}/\text{kg}$ 3 times per week produced an ACR20 response in 50% of patients compared with 63% at a dose of 20 $\mu\text{g}/\text{kg}$ 3 times per week and 10% with placebo. Similarly, ACR50 was achieved in 13% (8 $\mu\text{g}/\text{kg}$ q.i.d.) and in 25 and 13% (8 and 20 $\mu\text{g}/\text{kg}$ 3 times per week, respectively). A decrease in platelet count and hemoglobin were noted. Taken together, the clinical data from RA patients has been rather discouraging, showing only marginal activity of the drug (Narula, 2000).

Overall the role of IL-10 in rheumatoid arthritis remains unclear both from a clinical as well as from a theoretical point of view. On the one hand, there are the known anti-inflammatory properties of IL-10 and effects in various animal models of arthritis; on the other hand, there is the known correlation between IL-10 and an increased autoantibody production, serum factor, and B cell activation in RA patients (Narula, 2000). It has to be further determined whether IL-10 in combination with other therapies such as low dose steroid or therapeutic anti-TNF monoclonal antibodies (Maini and Taylor, 2000) may benefit a significant patient population (Moore et al., 2001). All in all, however, it seems unlikely that IL-10 will become an approved therapy in rheumatoid arthritis.

E. Therapy of Psoriasis

Therapeutic effects of recombinant human (rh)IL-10 (Schering Plough Research) in psoriatic patients has been studied in seven trials to date (Table 4). In our pilot trial starting in 1997, daily injections of 8 μg of rhIL-10/kg body weight directly under a psoriatic plaque over a 24-day period led to complete clearance of the plaque in one of two patients (Asadullah et al., 1998). Moreover, some systemic antipsoriatic effects were observed in all three patients treated in this pilot trial (subcutaneous injections under nonlesional skin in the third patient).

In a second trial (open-label phase II), ten psoriatic patients received subcutaneously rhIL-10 over a 7-week period in a dosage of 8 $\mu\text{g}/\text{kg}$ daily ($n = 5$) or 20 $\mu\text{g}/\text{kg}$ three times per week ($n = 5$), respectively (Asadullah et al., 1999a). Patients were followed up for an additional 7 weeks. The treatment was well tolerated. We found antipsoriatic effects in 9 of 10 patients resulting in a significant decrease of the psoriasis area and severity index (PASI) by $55.3 \pm 11.5\%$ (mean \pm S.E.M., $p < 0.02$). The antipsoriatic effect was confirmed by histological examination. Heterogeneity in the effectiveness was found among the patients but seemed to be independent of the dosage regime (Asadullah et al., 1999a) (Fig. 6). Similar clinical effectiveness of IL-10 application has recently been reported by Reich et al. (1998). In this open-label phase II trial, ten patients were treated subcutaneously with 4 $\mu\text{g}/\text{kg}$ rhIL-10 daily. The mean of the disease activity score PASI decreased by 67.9% after 6 weeks of treatment and was associated with improvement of his-

tological parameters (Reich et al., 2001). The clinical response was associated with a significant decrease of cutaneous cell infiltration and the lesional expression of type 1 cytokines (IFN- γ , TNF), IL-17, IL-8, and IL-8 receptor CXCR2. There was some evidence that genetic factors are involved in the response to IL-10 (Reich et al., 2001).

In a recent study (Kimball et al., 2002), 28 patients with moderate-to-severe psoriasis received rhIL-10 (20 $\mu\text{g}/\text{kg}$) or placebo subcutaneously three times weekly for 12 weeks in a randomized, double-blind manner. Remarkably, treatment with rhIL-10 resulted in only temporary clinical improvement after 6 and 8 weeks, despite sustained systemic decreases in proinflammatory and type 1 cytokine production.

The effect of IL-10 in psoriatic arthritis patients has been investigated by McInnes et al. (2001). IL-10 was given s.c. for 28 consecutive days in a double-blind, placebo-controlled study including 29 patients (0, 1, 5, or 10 $\mu\text{g}/\text{kg}$). Modest, but significant, clinical improvement in skin but not articular disease activity scores with only minor adverse effects was observed.

Recently, we investigated the effects of long-term IL-10 application on the immune system and duration of psoriasis remission (Friedrich et al., 2002). We performed a placebo-controlled, double-blind, phase II trial using IL-10 in patients with chronic plaque psoriasis in remission. Patients received subcutaneous injections with either IL-10 (10 $\mu\text{g}/\text{kg}$ body weight; $n = 7$) or placebo ($n = 10$) three times per week until relapse or

TABLE 4
IL-10 therapy in psoriasis: published results from clinical trials

Study	Design, patients, and interventions	Outcome and Remarks	Reference
Pilot study in exacerbated psoriasis vulgaris	Open label, not placebo controlled; three patients with moderate to severe psoriasis; 8 $\mu\text{g}/\text{kg}/\text{day}$, s.c. over a 24-day period	Safe, clinical, and histological confirmed improvement	Asadullah et al., 1998
Pilot study in exacerbated psoriasis vulgaris	Open label, not placebo controlled, ten patients with moderate to severe psoriasis; 4 $\mu\text{g}/\text{kg}/\text{day}$ s.c. over 42 days	Safe, good to moderate response, histological confirmed; significant mean decrease in PASI by 40% after 3 weeks and 68% after 6 weeks. These patients are a subgroup of the study by Reich et al. (2001).	Reich et al., 1998
Phase II in exacerbated psoriasis vulgaris	Open label, not placebo controlled, ten patients with moderate to severe psoriasis; 8 $\mu\text{g}/\text{kg}$ daily ($n = 5$) or 20 $\mu\text{g}/\text{kg}$ three times per week ($n = 5$) s.c. over 49 days	Safe, good to moderate response, histological confirmed, significant mean decrease in PASI by 55% after 7 weeks	Asadullah et al., 1999
Phase II in exacerbated psoriasis vulgaris	Open label, not placebo-controlled, 15 patients with moderate to severe disease; 4 $\mu\text{g}/\text{kg}/\text{day}$, s.c. over 42 days	Safe, good to moderate response, histological confirmed; 14 patients evaluated, mean decrease in PASI by 50% after 4 weeks and 59% after 6 weeks.	Reich et al., 2001
Phase II in psoriatic arthritis	Placebo-controlled, double-blind, 29 patients with psoriatic arthritis, dose escalating with 1, 5, or 10 $\mu\text{g}/\text{kg}$, s.c. daily for 28 days	Well tolerated, significant clinical improvement in skin (>30%) PASI reduction: 50% in IL-10 group (10 $\mu\text{g}/\text{kg}$) versus 10% in placebo after 4 weeks. No decrease in articular disease activity.	McInnes et al., 2001
Phase II in exacerbated psoriasis vulgaris	Randomized, double-blind, placebo-controlled, 28 patients with moderate to severe psoriasis; 20 $\mu\text{g}/\text{kg}$ three times per week for 12 weeks	Modest trend towards improvement after 6 and 8 weeks (mean decrease in PASI by 31% and 35% in IL-10 group versus 7% and 13% in placebo group, respectively), but no significant difference at week 12 (17% versus 13% in placebo group).	Kimball et al., 2002
Phase II in psoriasis vulgaris in remission	Placebo-controlled, double-blind, 17 patients with moderate to severe psoriasis in remission; 10 $\mu\text{g}/\text{kg}$; IL-10 ($n = 7$) or placebo ($n = 10$) three times per week for 4 months	Well tolerated; 90% relapse in the placebo group versus 28% in the IL-10 treated group; significant prolongation of relapse-free interval (101 days versus 66 in placebo).	Friedrich et al., 2002

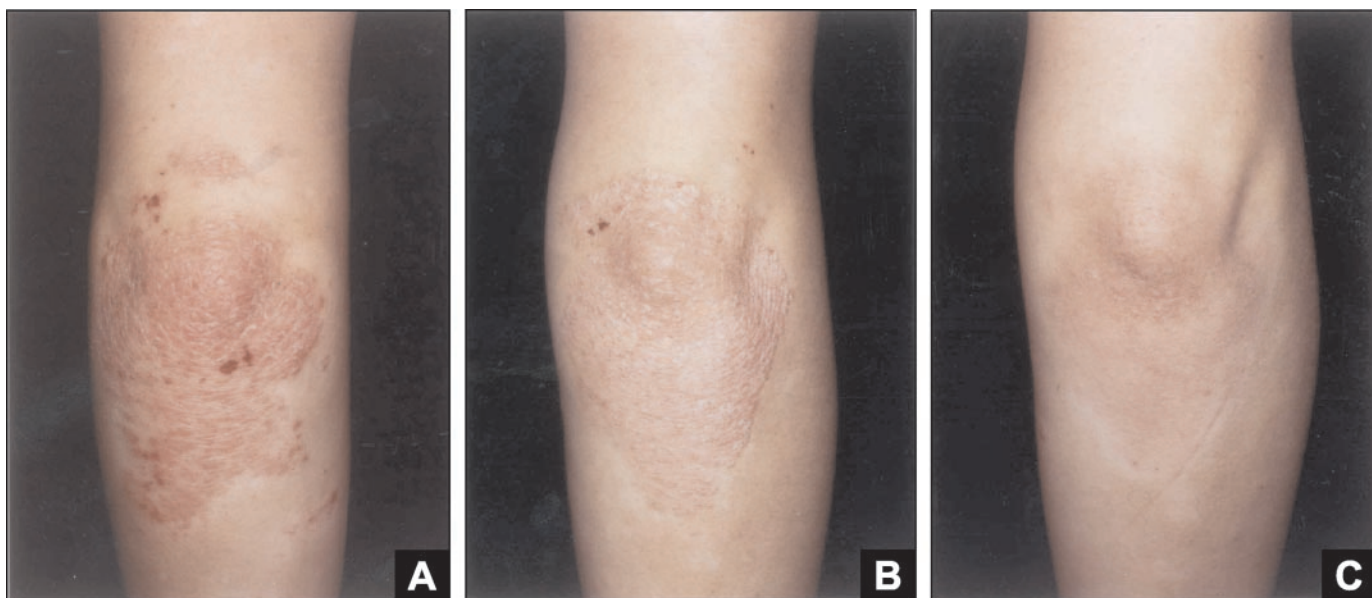


FIG. 6. Clinical effects of short course IL-10 therapy in established psoriasis. Example of a patient showing good response toward IL-10 therapy. Typical skin lesions before (A), during (B, day 15), and at the end (C, day 50) of therapy are shown. The patient received IL-10 injections in a dosage of 20 $\mu\text{g}/\text{kg}$ three times per week. (Copyright 1999, American Medical Association; Asadullah et al., 1999).

study termination after 4 months. The treatment was well tolerated. In the placebo group almost all patients (90%) showed a relapse during the observation period. In contrast to this, only two of seven patients (28.6%) relapsed in the IL-10-treated group. Kaplan-Meier analysis revealed a significantly lower relapse incidence in the IL-10 group than in the placebo group ($p = 0.02$). The mean relapse-free interval time was 101.6 ± 12.6 days in the IL-10 group compared with 66.4 ± 10.4 days in the placebo group (Fig. 7).

It is likely that IL-10 exerts its antipsoriatic activity by effects on different cell populations including T cells and APCs as well as their mutual interaction. Psoriasis is a T cell-dependent (auto)immune disease (Valdimarsson et al., 1985), probably initiated by presentation of so far unknown "psoriasis-related antigens" by specialized cutaneous APCs (Mitra et al., 1995; Weinstein, 1996; Norris et al., 1997). IL-10 is able to suppress the APC activity of monocytes/macrophages and the development of dendritic cells. In fact, depressed monocytic HLA-DR and CD86 expression as well as TNF- α and IL-12 secretion capacities were observed by us during IL-10 therapy. Moreover, IL-10 led to a lasting type 1/type 2 shift (increasing proportion of IL-4, IL-5, and IL-10 producing T cells, selective increase in IgE serum levels). A significant negative correlation was demonstrated between the IL-4 secretion capacity and PASI score that was found in our long-term trial. The physiological significance of these findings was reflected by the depressed DTH reaction to recall antigens during IL-10 therapy (Asadullah et al., 1999a). Interestingly, IL-10 therapy led to a decrease in cutaneous IL-8 and an increase in IL-4 expression, both of which might contribute to the antipsoriatic effect (Asadullah et al., 2001b; Reich et al.,

2001). Direct effects of IL-10 on keratinocytes are unlikely to have contributed to the clinical response, since the IL-10 unresponsiveness of keratinocytes has been demonstrated by us recently (see above).

Overall, IL-10 therapy seems to be well tolerated and immunologically effective in psoriasis. Determination of definitive clinical efficacy, however, awaits phase III studies. Moreover, the effects of IL-10 on the skin immune system has to be investigated in more detail.

F. Therapy of Viral Infections—Chronic Hepatitis C and Human Immunodeficiency Virus

It has been shown that TNF and other inflammatory mediators promote the replication of HIV. This was the rationale for investigating the effects of IL-10 on viral load and CD4 counts in HIV infected patients who did not fully respond to antiretroviral treatment. The lack of a demonstrable virological and immunological benefit with 4 weeks of IL-10 treatment does not support the development of this therapy for treatment of HIV infection (Angel et al., 2000).

Considerable progress has been made in the field of hepatitis C virus since its discovery about 10 years ago but a major effort needs to be made to control hepatitis C virus-related liver disease. Despite new therapeutic options (modified interferons, antiviral drugs) the long-term outcome of hepatitis C is still unsatisfactory (Boyer and Marcillan, 2000). There are several reports on an association between IL-10 polymorphisms and the course of hepatitis C infection (see above). Moreover, IL-10 is able to express antifibrotic properties in experimental models of liver cirrhosis (Boyer and Marcillan, 2000). Recently, it was shown that IL-10, although it has no apparent antiviral activity, normalizes serum ALT

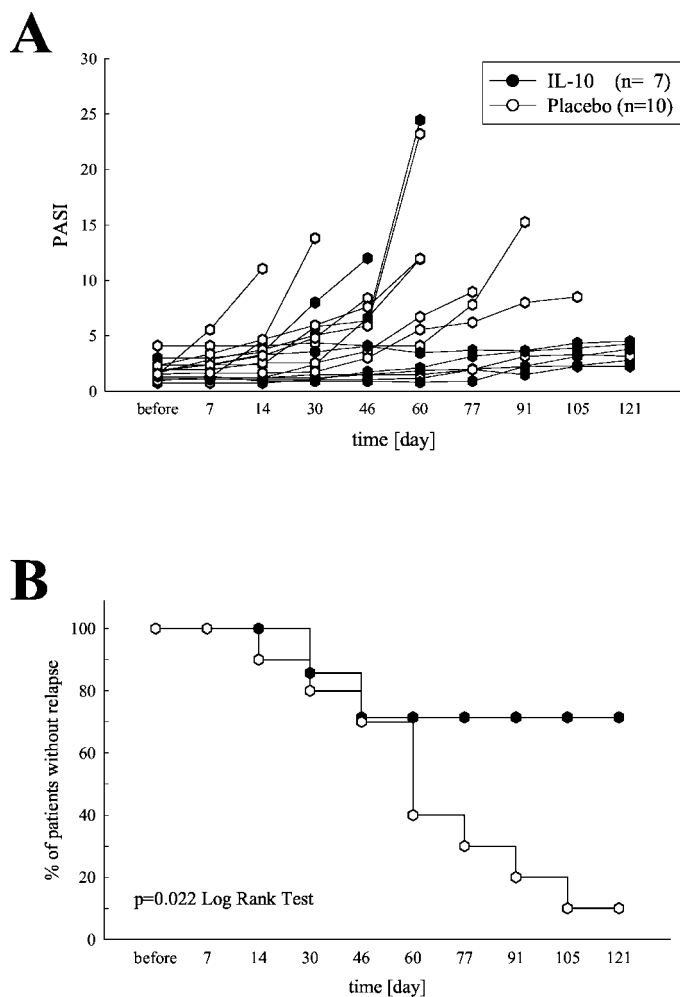


FIG. 7. Clinical effects of IL-10 therapy (Asadullah et al., 2002a). A, individual courses of psoriatic disease. The patients dropped out of the analysis when fulfilling the criteria for psoriasis relapse. B, Kaplan-Meier analysis of relapse-risk. Reprinted with permission from Blackwell Publishing, Oxford, UK.

levels, improves liver histology, and reduces liver fibrosis in a large proportion of patients (63–86%) receiving treatment (Dharancy et al., 2000; Nelson et al., 2000; Schuppan and Hahn, 2000).

VI. Prospects of Interleukin-10/Interleukin-10 Receptor As a Therapeutic Target

Application of IL-10 in humans seems to be safe and immunologically active. Indeed the majority of studies indicate that IL-10 seems to reverse a pre-existing Th1/Th2 imbalance (Fig. 5). Since such an immune deviation is based on the current pathophysiological understanding in several indications, characterized by inflammation with a type 1 cytokine pattern, IL-10 should be a promising approach there.

The clinical effects of recombinant IL-10, however, has been quite heterogeneous in different entities. Whereas almost no effect was seen in rheumatoid arthritis, significant response was observed in psoriasis. The clinical studies published to date clearly indicate some but over-

all no really relevant advantage of systemic IL-10 therapy compared with placebo in active and postoperative CD. Results of Tilg et al. (2002a) indicate that higher doses of systemically administered IL-10 (which were also used in the clinical trials) may be detrimental rather than helpful in CD. This may also be true for other indications. Nevertheless, the concept of rebalancing the intestinal immunological homeostasis with IL-10 is still very compelling and applying IL-10 locally in high concentrations may result in strong immunosuppression and circumvent the systemic side effects (Herfarth and Schölmerich, 2002). We do not know whether high IL-10 concentrations also have immunostimulatory properties, e.g., in the intestine or the skin. Furthermore IL-10 prevented intestinal inflammation in animal studies but could never completely cure an established disease, indicating that IL-10 therapy in CD would succeed in preventing relapses rather than abolishing acute or chronic inflammation. The recent results from IL-10 therapy in psoriasis (Friedrich et al., 2002) seems to support this hypothesis. So it might very well be that IL-10 is much more effective in a (primary or secondary) prophylactic than in a therapeutic approach. Theoretically, this can be explained by the observation that IL-10 is powerful in preventing proinflammatory effects of macrophages and differentiation of dendritic cells but less effective in targeting T cells, particularly memory/effector T cells, and mature dendritic cells. Additionally, IL-10 supports the generation of regulatory T cells (Levings et al., 2001a; Roncarolo et al., 2001). So IL-10 may be more effective in preventing undesired immune reactions than in treating them.

Another problem is the adequate delivery of IL-10 to the site of action. Taking into account the adverse effects of high-dose IL-10 and its low half-life in vivo, local delivery may probably be the more promising approach in several diseases. Gene therapy by using viral vectors has several disadvantages for application in humans. Recently, an approach of local IL-10 therapy, which could also be used for long-term therapy, has been described. Steidler et al. (2000) demonstrated that intragastric administration of a genetically engineered IL-10 secreting *Lactobacillus lactis* caused a significant reduction in colitis in two different mouse models. Therefore, with dietary supplementation it may be possible to deliver high concentrations of IL-10 within the gut, thus preventing the recurrence of CD. However, this study has just proved a therapeutic principle, and there is still a long way to go before such a concept can be evaluated in clinical studies for chronic inflammatory bowel disease (Herfarth and Schölmerich, 2002). Topical application at least from a theoretic point of view might also be possible in inflammatory skin disorders or asthma.

Even in the majority of clinical trials where IL-10 showed a clinical effect, patients with both good and no responses at all were found. The reason for this heterogeneous interindividual response is unclear. What must

be done, therefore, is to identify which patients might be suitable for IL-10 therapy. Preliminary data supports this concept (Reich et al., 2001).

If this can be done, an effective immunotherapy with few side effects might conceivably be tailored to suit individual patients. Analyzing the individual cytokine and cytokine promoter polymorphism might be helpful. Similar heterogeneity in response is observed for other new immunomodulatory treatments. For example, only 2/3 of rheumatoid arthritis patients respond to anti-TNF treatment. A similar response rate was found for psoriasis patients in response to several immunomodulatory antibodies and drugs (Asadullah et al., 2002b). Thus, it would be of interest to analyze: 1) whether the group of nonresponders is overlapping between different drugs; and 2) whether combinations might be useful to improve the results (e.g., therapy with vitamin D derivatives known to induce IL-10 receptor expression).

To predict the therapeutic effect of IL-10 in inflammatory disorders dominated by a type 2 cytokine pattern is difficult. Its anti-inflammatory activity may lead to an improvement although a further shift into the type 2 cytokine deviation might be harmful. Indeed preliminary data on IL-10 therapy in atopic dermatitis patients suggests unresponsiveness in these patients (Reich et al., personal communication). Similarly, it is hard to predict a therapeutic effect of IL-10 in malignancies, since both tumor-suppressive as well as tumor-promoting effects have been described in *in vitro* and *in vivo* models. Depending on the particular type of the tumor, it is likely that IL-10 application could be either effective (e.g., in tumors mainly controlled by NK cells) or fatal (e.g., in tumors mainly controlled by cytotoxic T cells and where IL-10 is a growth factor). Considering the immunosuppressive effects of IL-10 in general, caution in its application in tumor patients is recommended. Indeed it might very well be that targeting IL-10 by antibodies or IL-10 receptor constructs is much more appropriate in tumors, e.g., in certain lymphomas. Well defined clinical studies might be the only way to obtain the final answer.

IL-10 as a cytokine has therapeutically been used as a recombinant protein, i.e., a large molecule. Therefore it is quite expensive to produce, must be administered by injection, which is quite inconvenient for the patient, and the induction of neutralizing antibodies, which might limit their effect, has to be excluded for long-term application. Identification of molecules mediating the effects of this cytokine that are suitable for pharmacological intervention with small molecules will be, therefore, of increasing interest. Interference of complex protein-protein interaction with small molecules, however, is almost impossible. Therefore, it is unlikely that IL-10 receptor agonists suitable for oral application will be discovered. Molecules acting downstream of the cytokine receptors targeting for example certain kinases or phosphatases or signal transduction molecules might represent much better "drugable" approaches. The problem

here, however, is the specificity, since the known IL-10 signal transduction molecules are shared by different cytokines. Induction of cytokine production with low molecular weight compounds may represent novel approaches. In any case, further investigations on the regulation of IL-10 expression and signaling are of major importance and may lead to better therapeutic approaches.

Whereas IL-10 application/induction seems to represent an interesting therapeutic approach in some indications, its neutralization might be promising in others. Thus, apart from in certain tumors as outlined above, IL-10 seems to play a critical role, e.g., in SLE. This systemic autoimmune disease is characterized by high autoantibody production and by decreased cellular immune responses. In SLE, high levels of autoantibodies generate immune complexes causing tissue damage. Compared with healthy individuals, levels of IL-10 in SLE patients are significantly higher, and there is a correlation of IL-10 levels with the clinical disease activity (Park et al., 1998). In experimental studies, depletion of IL-10 resulted in the following effects: 1) anti-IL-10 mAb *in vitro* treatment of SLE patient-derived PBMC significantly decreased autoantibody production (Llorente et al., 1995); 2) anti-IL-10 mAb treatment of SCID mice injected with PBMC from SLE patients strongly inhibit autoantibody production *in vivo* (Llorente et al., 1995); 3) treatment of New Zealand black/white mice—mice that spontaneously develop a severe autoimmune disease that closely resembles SLE—with anti-IL-10 mAb substantially delayed onset of autoimmunity (Ishida et al., 1994). The data suggest that IL-10 is harmful in systemic lupus and that IL-10 antagonists may be beneficial in the treatment of human SLE. In fact, beneficial effects of application of a neutralizing IL-10 antibody has been reported in a preliminary study very recently (Llorente et al., 1999). Furthermore, more investigation is necessary to support this in SLE and perhaps other indications as B cell lymphomas.

VII. Conclusions

IL-10 is a pluripotent cytokine with potent effects on numerous cell populations, in particular circulating and resident immune cells as well as epithelial cells. This makes it an important broad effector molecule in immunoregulation/host defense. Whereas soon after its discovery initial data suggested that IL-10 mainly mediates suppressive functions, more recent data showed stimulatory properties on certain cell populations also. Overall, the effects of IL-10 seem to be quite complex, and still considering IL-10 just as immunosuppressive and anti-inflammatory (as was done in the past) might be oversimplifying. Considering IL-10 as immunoregulatory instead of immunosuppressive might be more appropriate. It stimulates functions of innate immunity

(NK cell activity, noninflammatory removal of particles, cells, and microbes by stimulating phagocytosis) and of Th2-related immunity but suppresses inflammation-associated immune response (Th1, cytokine proinflammatory secretion by macrophages, modulation of Th2) both directly and indirectly.

Inadequate IL-10 expression seems to have considerable pathophysiological impact. Both overexpression (e.g., in lymphoma, SLE, intensive care unit patients) as well as IL-10 deficiency (e.g., in inflammatory bowel disease, psoriasis) are likely to have a pathophysiological significance. Therefore, neutralization of the cytokine could be a promising approach to treat diseases from the first group, whereas application of IL-10 itself could be helpful for diseases from the second group.

Some effectiveness of IL-10 application for the therapy of established, exacerbated immune diseases such as psoriasis, inflammatory bowel diseases, and rheumatoid arthritis has been suggested by several early phase II trials. Other studies, however, showed disappointing results. Further investigations, in particular multicenter placebo-controlled double-blind trials, are therefore required to better determine the therapeutic potential of this cytokine. The picture is already emerging that the potency of this treatment overall seems to be below that of other promising approaches such as the anti-TNF- α strategies. Thus, a complete disease clearance is usually a rare event after IL-10 application, suggesting a relatively low anti-inflammatory potential for this therapy. Moreover, a recent report (Lauw et al., 2000) and our recent unpublished observations suggest proinflammatory properties of IL-10. In agreement with this, we observed an increase of the sIL-2R levels in IL-10 long-term-treated patients. Therefore, we speculate that IL-10 might not be the major "breakthrough" in anti-inflammatory therapy of exacerbated disease. In contrast, impressive clinical effects with regard to prevention of psoriasis relapse, were found in a single study, reflecting the long-term immunomodulatory rather than the anti-inflammatory properties of IL-10. It might be that not anti-inflammatory but complex immunomodulatory effects might be primarily responsible for the prolongation of the disease-free interval we observed in psoriasis. Such a hypothesis is supported by recent data from animal and in vitro experiments, demonstrating that IL-10 can induce the formation of regulatory T cells with major impact on the immunoregulation (Cottréz et al., 2000; Levings et al., 2001a). Taken together, both first clinical and immunological data suggest that IL-10 application seems to be even more attractive in a secondary prophylactic (prevention of relapse) than in a therapeutic approach in psoriasis. It might be speculated that IL-10 long-term therapy could be equally effective in other entities such as inflammatory bowel diseases or rheumatoid arthritis and transplantation.

Why certain patients show a good response whereas others do not respond at all may lead to the identifica-

tion of patients suitable for IL-10 therapy. A better understanding of the molecular mechanisms of IL-10-mediated effects may lead to the identification of new drug targets (either for mimicking or inhibition of IL-10 effects) that could be approached by small orally bioavailable compounds. It has to be determined whether the new IL-10 homologs might have better therapeutic potential than IL-10 itself.

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REFERENCES

- Al-Janadi M, al-Dalaan A, al-Balla S, al-Humaidi M, and Raziuddin S (1996) Interleukin-10 (IL-10) secretion in systemic lupus erythematosus and rheumatoid arthritis: IL-10-dependent CD4⁺CD45RO⁺ T cell-B cell antibody synthesis. *J Clin Immunol* **16**:198–207.
- Allavena P, Piemonti L, Longoni D, Bernasconi S, Stoppacciaro A, Ruco L, and Mantovani A (1998) IL-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation to macrophages. *Eur J Immunol* **28**:359–369.
- Andersen SR, Lambrecht LJ, Swan SK, Cutler DL, Radwanski E, Affrime MB, and Garaud JJ (1999) Disposition of recombinant human interleukin-10 in subjects with various degrees of renal function. *J Clin Pharmacol* **39**:1015–1020.
- Angel JB, Jacobsen MA, Skolnik PR, Giordano M, Shapiro L, LeBaut A, Greaves W, and Fuchs AC (2000) A multicenter, randomized, double-blind, placebo-controlled trial of recombinant human interleukin-10 in HIV-infected subjects. *AIDS* **14**:2503–2508.
- Arany I, Grattendick KG, and Tyring SK (2002) IL-10 induces transcription of the early promoter of human papillomavirus type 16 through the 5' segment of the upstream regulatory region. *Antiviral Res* **55**:331–339.
- Arock M, Zuany-Amorim C, Singer M, Benhamou M, and Pretolani M (1996) Interleukin-10 inhibits cytokine generation from mast cells. *Eur J Immunol* **26**:166–170.
- Asadullah K, Döcke WD, Ebeling M, Friedrich M, Belbe G, Audring H, Volk HD, and Sterry W (1999a) Interleukin-10 treatment of psoriasis—clinical results of a phase II trial. *Arch Dermatol* **135**:187–192.
- Asadullah K, Döcke WD, Haeußler A, Sterry W, and Volk HD (1996) Progression of mycosis fungoides is associated with increasing cutaneous expression of interleukin 10 mRNA. *J Invest Dermatol* **107**:833–837.
- Asadullah K, Döcke WD, Sabat R, Volk HD, and Sterry W (2000a) The treatment of psoriasis with interleukin-10: rationale and review of the first clinical trials. *Expert Opin Invest Drugs* **9**:95–102.
- Asadullah K, Eskdale J, Wiese A, Gallagher G, Friedrich M, and Sterry W (2001a) Interleukin-10 promoter polymorphism in psoriasis. *J Invest Dermatol* **116**:975–978.
- Asadullah K, Friedrich M, Döcke WD, Jahn S, Volk HD, and Sterry W (1997a) Enhanced expression of T-cell activation and natural killer cell antigens indicates systemic anti-tumor response in early primary cutaneous T-cell lymphoma (CTCL). *J Invest Dermatol* **108**:743–747.
- Asadullah K, Friedrich M, Hanneken S, Rohrbach C, Audring H, Döcke WD, Vergopoulos A, Ebeling M, Volk HD, and Sterry W (2001b) Effects of systemic interleukin-10 therapy on psoriatic skin lesions: histological, immunohistological and molecular biological findings. *J Invest Dermatol* **116**:721–727.
- Asadullah K, Gellrich S, Haeußler-Quade A, Friedrich M, Döcke WD, Jahn S, and Sterry W (2000b) Cytokine expression in primary cutaneous germinal center cell lymphomas. *Exp Dermatol* **9**:71–76.
- Asadullah K, Sabat R, Wiese A, Döcke WD, Volk HD, and Sterry W (1999b) Interleukin-10 in cutaneous disorders: implications for its pathophysiological importance and therapeutic use. *Arch Dermatol Res* **291**:628–636.
- Asadullah K, Sterry W, Stephanek K, Jusulaitis D, Leupold M, Audring H, Volk HD, and Döcke WD (1998) IL-10 is a key cytokine in psoriasis: proof of principle by IL-10 therapy—a new therapeutic approach. *J Clin Invest* **101**:783–794.
- Asadullah K, Sterry W, and Trefzer U (2002a) Cytokine therapy in dermatology. *Exp Dermatol* **11**:97–106.
- Asadullah K, Volk HD, and Sterry W (2002b) Novel immunotherapies for psoriasis. *Trends Immunol* **23**:47–53.
- Asderakis A, Sankaran D, Dyer P, Johnson RW, Pravica V, Sinnott PJ, Roberts I, and Hutchinson IV (2001) Association of polymorphisms in the human interferon-gamma and interleukin-10 gene with acute and chronic kidney transplant outcome: the cytokine effect on transplantation. *Transplantation* **71**:674–677.
- Ayala A, Lehmann DL, Herdon CD, and Chaudry IH (1994) Mechanism of enhanced susceptibility to sepsis following hemorrhage. IL-10 suppression of T cell response is mediated by eicosanoid-induced IL-4 release. *Arch Surg* **129**:1172–1178.
- Baker KS, Roncarolo MG, Peters C, Bigler M, DeFor T, and Blazar BR (1999) High spontaneous IL-10 production in unrelated bone marrow transplant recipients is associated with fewer transplant-related complications and early deaths. *Bone Marrow Transplant* **23**:1123–1129.
- Banchereau J and Steinman RM (1998) Dendritic cells and the control of immunity. *Nature (Lond)* **392**:245–252.
- Barbara G, Xing Z, Hogaboam CM, Gaudie J, and Collins SM (2000) Interleukin 10 gene transfer prevents experimental colitis in rats. *Gut* **46**:344–349.

- Barnes PJ (2002) Cytokine modulators as novel therapies for asthma. *Annu Rev Pharmacol Toxicol* **42**:81–98.
- Barrat FJ, Cua DJ, Boonstra A, Richards DF, Crain C, Savelkoul H, DeWaal Malefyt R, Coffman RL, Hawrylowicz CM, et al. (2002) In vitro generation of IL-10-producing regulatory CD4⁺ T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 and Th2 inducing cytokines. *J Exp Med* **195**:603–616.
- Barsig J, Kusters S, Vogt K, Volk HD, Tiegs G, and Wendel A (1995) Lipopolysaccharide-induced IL-10 in mice: role of endogenous TNF- α . *Eur J Immunol* **25**:2888–2893.
- Beatty PR, Krams SM, and Martinez OM (1997) Involvement of IL-10 in the autonomous growth of EBV-transformed B cell lines. *J Immunol* **158**:4045–4051.
- Bécherel PA, Le Goff L, Ktorza S, Ouaz F, Mencia-Huerta JM, Dugas B, Debré P, Mossalayi MD, and Arook M (1995) Interleukin-10 inhibits IgE-mediated nitric oxide synthase induction and cytokine synthesis in normal human keratinocytes. *Eur J Immunol* **25**:2992–2995.
- Bellinghausen I, Knop J, and Saloga J (2001) The role of IL-10 in the regulation of allergic immune response. *Int Arch Allergy Appl Immunol* **126**:97–101.
- Berlato C, Cassatella MA, Kinjyo I, Gatto L, Yoshimura A, and Bazzoni F (2002) Involvement of suppressor of cytokine signaling-3 as a mediator of the inhibitory effects of IL-10 on LPS-induced macrophage activation. *J Immunol* **168**:6404–6411.
- Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, et al. (1999) Cytokine gene polymorphism in human disease: on-line databases. *Genes Immun* **1**:3–19.
- Blay JY, Burdin N, Rousset F, Lenoir G, Biron P, Philip T, Banchereau J, and Favrot MC (1993) Serum interleukin-10 in non-Hodgkin's lymphoma: a prognostic factor. *Blood* **82**:2169–2174.
- Blumberg H, Conklin D, Xu WF, Grossmann A, Brender T, Carollo S, Eagan M, Foster D, Haldeman BA, Hammond A, et al. (2001) Interleukin 20: discovery, receptor identification and role in epidermal function. *Cell* **104**:9–19.
- Bohlen H, Kessler M, Sextro M, Diehl V, and Tesch H (2000) Poor clinical outcome of patients with Hodgkin's disease and elevated interleukin-10 serum levels. Clinical significance of interleukin-10 serum levels for Hodgkin's disease. *Ann Hematol* **79**:110–103.
- Bos JD, Wierenga EA, Sillevs-Smit JH, van der Heijden FL, and Kapsenberg ML (1992) Immune dysregulation in atopic eczema. *Arch Dermatol* **128**:1509–1512.
- Boulland ML, Meignin V, Leroy-Viard K, Copie-Bergman C, Briere J, Touitou R, Kanavaros P, and Gaulard P (1998) Human interleukin-10 expression in T/natural killer-cell lymphomas: association with anaplastic large cell lymphomas and nasal natural killer-cell lymphomas. *Am J Pathol* **153**:1229–1237.
- Bourreille A, Segain JP, Raingeard de la Bletiere D, Siavoshian S, Valette G, Galmiche JP, and Blottiere HM (1999) Lack of IL-10 regulation of antigen presentation-associated molecules expressed on colonic epithelial cells. *Eur J Clin Invest* **29**:48–55.
- Boyer N and Marcellin P (2000) Pathogenesis, diagnosis and management of hepatitis C. *J Hepatol* **32**:98–112.
- Braun-Falco O, Plewig G, and Wolf HH (1995) Atopie und atopisches Ekzem, in *Dermatologie und Venerologie* 4th ed. (Braun-Falco O, Plewig G, and Wolf HH eds) pp 445–460, Springer-Verlag, Berlin, Heidelberg, New York.
- Broberg E, Setälä N, Roytta M, Salmi A, Erälä J, He B, Roizma B, and Hukkanen V (2001) Expression of IL-4 but not of IL-10 from replicative herpes virus type 1 viral vector precludes experimental allergic encephalomyelitis. *Gene Ther* **8**:769–777.
- Buchwald UK, Geerdes-Fenge HF, Vockler J, Ziege S, and Lode H (1999) Interleukin-10: effects on phagocytosis and adhesion molecule expression of granulocytes and monocytes in a comparison with prednisolone. *Eur J Med Res* **4**:85–94.
- Buelens C, Verhasselt V, De Groote D, Thielemans K, Goldman M, and Willems F (1997) Interleukin-10 prevents the generation of dendritic cells from human peripheral blood mononuclear cells cultured with interleukin-4 and granulocyte/macrophage-colony-stimulating factor. *Eur J Immunol* **27**:756–762.
- Buelow R, Tullius SG, and Volk HD (2001) Protection of grafts by hemoxygenase-1 and its toxic product carbon monoxide. *Am J Transplant* **1**:313–315.
- Cai G, Kastelein RA, and Hunter CA (1999) IL-10 enhances NK cell proliferation, cytotoxicity and production of IFN- γ when combined with IL-18. *Eur J Immunol* **29**:2658–2665.
- Calzada-Wack JC, Frankenberger M, and Ziegler-Heitbrock HW (1996) Interleukin-10 drives human monocytes to CD16 positive macrophages. *J Inflamm* **46**:78–85.
- Carson WE, Lindemann MJ, Baiocchi R, Linett M, Tan JC, Chou CC, Narula S, and Caligiuri MA (1995) The functional characterization of interleukin-10 receptor expression on human natural killer cells. *Blood* **85**:3577–3585.
- Cassatella MA, Gasperini S, Bovolenta C, Calzetti F, Vollebregt M, Scapini P, Marchi M, Suzuki R, Suzuki A, and Yoshimura A (1999) Interleukin-10 (IL-10) selectively enhances CIS3/SOCS3 mRNA expression in human neutrophils: evidence for an IL-10-induced pathway that is independent of STAT protein activation. *Blood* **94**:2880–2889.
- Cassatella MA, Meda L, Bonora S, Ceska M, and Constantin G (1993) Interleukin 10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumor necrosis factor and IL-1 β in mediating the production of IL-8 triggered by lipopolysaccharide. *J Exp Med* **178**:2207–2211.
- Cassatella MA, Meda L, Gasperini S, Calzetti F, and Bonora S (1994) Interleukin-10 upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation. *J Exp Med* **179**:1695–1699.
- Chan SC, Li SH, and Hanifin JM (1993) Increased interleukin-4 production by atopic mononuclear leukocytes correlates with increased cyclic adenosine-monophosphate-phosphodiesterase activity and is reversible by phosphodiesterase inhibition. *J Invest Dermatol* **100**:681–684.
- Chatelain R, Wollenberg A, Martin C, Panhans-Gross A, Bieber T, Degitz K, and Heckmann M (1998) IL-10 inhibits ICAM-1 expression on human Langerhans cells but not on keratinocytes, dermal endothelial cells or fibroblasts. *Arch Dermatol Res* **290**:477–482.
- Chernoff AE, Granowitz EV, Shapiro L, Vannier E, Lonnemann G, Angel JB, Kennedy JS, Rabnow AR, Wolff SM, and Dinarello CA (1995) A randomized, controlled trial of IL-10 in humans. Inhibition of inflammatory cytokine production and immune responses. *J Immunol* **154**:5492–5499.
- Chmiel JF, Konstan MW, Saadane A, Krenicky JE, Lester Kirchner H, and Berger M (2002) Prolonged inflammatory response to acute Pseudomonas challenge in IL-10 knockout mice. *Am J Respir Crit Care Med* **165**:1176–1181.
- Chomarat P, Vannier E, Dechanet J, Risoan MC, Banchereau J, Dinarello CA, and Miossec P (1995) Balance of IL-1 receptor antagonist/IL-1 β in rheumatoid synovium and its regulation by IL-4 and IL-10. *J Immunol* **154**:1432–1439.
- Chung F (2001) Anti-inflammatory cytokines in asthma and allergy: IL-10, IL-12, interferon- γ . *Mediat Inflamm* **10**:51–59.
- Clarke CJP, Hales A, Hunt A, and Foxwell BMJ (1998) IL-10-mediated suppression of TNF- α production is independent of its ability to inhibit NF κ B activity. *Eur J Immunol* **28**:1719–1726.
- Colombel JF, Rutgeerts P, Malchow H, Jacyna M, Nielsen OH, Rask-Madsen J, Van Deventer S, Ferguson A, Desreumaux P, Forbes A, et al. (2001) Interleukin 10 (Tenovil) in the prevention of postoperative recurrence of Crohn's disease. *Gut* **49**:42–46.
- Cooper PJ, Fekade D, Remick DG, Grint P, Wherry J, and Griffin GE (2000) Recombinant human interleukin-10 fails to alter proinflammatory cytokine production or physiologic changes associated with the Jarisch-Herxheimer reaction. *J Infect Dis* **181**:203–209.
- Cortes J and Kurzrock R (1997) Interleukin-10 in non-Hodgkin's lymphoma. *Leuk Lymphoma* **26**:251–259.
- Cortes JE, Talpaz M, Cabanillas F, Seymour JF, and Kurzrock R (1995) Serum levels of interleukin-10 in patients with diffuse large cell lymphoma: lack of correlation with prognosis. *Blood* **85**:2516–2520.
- Cottrez F, Hurst SD, Coffman RL, and Groux H (2000) T regulatory cells 1 inhibit a Th2-specific response in vivo. *J Immunol* **165**:4848–4853.
- Creery WD, Diaz-Mitoma F, Filion L, and Kumar A (1996) Differential modulation of B7-1 and B7-2 isoform expression on human monocytes by cytokines which influence the development of T helper cell phenotype. *Eur J Immunol* **26**:1273–1277.
- Croxford JL, Feldmann M, Chernajovsky Y, and Baker D (2001) Different therapeutic outcomes in experimental allergic encephalomyelitis dependent upon the mode of delivery of IL-10. *J Immunol* **166**:4124–4130.
- Cua DJ, Hutchins B, LaFace DM, Stohlman SA, and Coffmann RL (2001) Central nervous system expression of IL-10 inhibits autoimmune encephalomyelitis. *J Immunol* **166**:602–608.
- Cush JJ, Splawski JB, Thomas R, McFarlin JE, Schulze-Koops H, Davis LS, Fujita K, and Lipsky PE (1995) Elevated interleukin-10 levels in patients with rheumatoid arthritis. *Arthritis Rheum* **38**:96–104.
- D'Alfonso S, Rampi M, Bocchio D, Colombo G, Scorza-Smeraldi R, and Momigliano-Richardi P (2000) Systemic lupus erythematosus candidate genes in the Italian population: evidence for a significant association with interleukin-10. *Arthritis Rheum* **43**:120–128.
- D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, and Trinchieri G (1993) Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med* **178**:1041–1048.
- Defrance T, Vanbervliet B, Briere F, Durand I, Rousset F, and Banchereau J (1992) Interleukin 10 and transforming growth factor beta cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *J Exp Med* **175**:671–682.
- Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, and Romagnani S (1993) Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J Immunol* **150**:353–360.
- Demangel C, Bertolino P, and Britton WJ (2002) Autocrine IL-10 impairs dendritic cell derived immune responses to mycobacterial infection by suppressing DC trafficking to draining lymph nodes and local IL-12 production. *Eur J Immunol* **32**:994–1002.
- Deng J, Kohda Y, Chiao H, Wang Y, Hu X, Hewitt SM, Miyaji T, McLeroy P, Nibhanupudy B, Li S, et al. (2001) IL-10 inhibits ischemic and cisplatin-induced acute renal injury. *Kidney Int* **60**:2118–2128.
- Denning TL, Campbell NA, Song F, Garofalo RP, Klimpel GR, Reye VE, and Ernst PB (2000) Expression of IL-10 receptors on epithelial cells from the murine small and large intestine. *Int Immunol* **12**:133–139.
- De Vita F, Orditura M, Galizia G, Romano C, Infusino S, Auriemma A, Lieto E, and Catalano G (1999) Serum interleukin-10 levels in patients with advanced gastrointestinal malignancies. *Cancer* **86**:1936–1943.
- De Vita F, Orditura M, Galizia G, Romano C, Lieto E, Iodice P, Tuccillo C, and Catalano G (2000b) Serum interleukin-10 is an independent prognostic factor in advanced solid tumors. *Oncol Rep* **7**:357–361.
- De Vita F, Orditura M, Galizia G, Romano C, Roscigno A, Lieto E, and Catalano G (2000a) Serum interleukin-10 levels as a prognostic factor in advanced non-small cell lung cancer patients. *Chest* **117**:365–373.
- De Waal Malefyt R, Abrams J, Bennett B, Figdor CG, and de Vries JE (1991a) Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* **174**:1209–1220.
- De Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, te Velde A, Figdor C, Johnson K, Kastelein R, Yssel H, and de Vries JE (1991b) Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J Exp Med* **174**:915–924.
- Dharancy S, Canva V, Gambiez L, Paris JC, and Desreumaux P (2000) Hepatic deficiency of IL-10 in chronic hepatitis C. *Gastroenterology* **119**:1411–1412.
- Donnelly RP, Dickensheets H, and Finbloom DS (1999) The IL-10 signal transduction

- pathway and regulation of gene expression in mononuclear phagocytes. *J Interferon Cytokine Res* **19**:563–573.
- Dummer R, Heald PW, Nestle FO, Ludwig E, Laine E, Hemmi S, and Burg G (1996a) Sézary syndrome T-cell clones display T-helper 2 cytokines and express the accessory factor-1 (interferon-gamma receptor beta-chain). *Blood* **88**:1383–1389.
- Dummer R, Kohl O, Gillessen J, Kägi M, and Burg G (1993) Peripheral blood mononuclear cells in patients with nonleukemic cutaneous T-cell lymphoma. *Arch Dermatol* **129**:433–436.
- Dummer W, Bastian BC, Ernst N, Schnalze C, Schwaaf A, and Brocker EB (1996b) Interleukin-10 production in malignant melanoma: preferential detection of IL-10-secreting tumor cells in metastatic lesions. *Int J Cancer* **66**:607–610.
- Dumoutier L, Leemans C, Lejeune D, Kotenko SV, and Renaud JC (2001) Cutting edge: STAT activation by IL-19, IL-20 and mda-7 through IL-20 receptor complexes of two types. *J Immunol* **167**:3545–3549.
- Dumoutier L, Louahed J, and Renaud JC (2000c) Cloning and characterization of IL-10-related T cell-derived inducible factor (IL-TIF), a novel cytokine structurally related to IL-10 and inducible by IL-9. *J Immunol* **164**:1814–1819.
- Dumoutier L, Van Roost E, Ameye G, Michaux L, and Renaud JC (2000b) IL-TIF/IL-22: genomic organization and mapping of the human and mouse genes. *Genes Immun* **1**:488–494.
- Dumoutier L, Van Roost E, Colau D, and Renaud JC (2000a) Human interleukin-10-related T cell-derived inducible factor: molecular cloning and functional characterization as a hepatocyte-stimulating factor. *Proc Natl Acad Sci USA* **97**:10144–10149.
- Ealick SE, Cook WJ, Vijay-Kumar S, Carson M, Nagabhushan TL, Trotta PP, and Bugg CE (1991) Three-dimensional structure of recombinant human interferon-gamma. *Science (Wash DC)* **252**:698–702.
- Edelson RL (1980) Cutaneous T cell lymphoma: mycosis fungoides, Sézary syndrome and other variants. *J Am Acad Dermatol* **2**:89–106.
- Edwards-Smith CJ, Jonsson JR, Purdie DM, Bansal A, Shorthouse C, and Powell EE (1999) Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis C to interferon alpha. *Hepatology* **30**:526–530.
- Enk AH, Angeloni VL, Udey MC, and Katz SI (1993) Inhibition of Langerhans cell antigen-presenting function by IL-10. A role for IL-10 in induction of tolerance. *J Immunol* **151**:2390–2398.
- Enk AH and Katz SI (1992) Identification and induction of keratinocyte-derived IL-10. *J Immunol* **149**:92–95.
- Enk CD, Sredni D, Blauvelt A, and Katz SI (1995) Induction of IL-10 gene expression in human keratinocytes by UVB exposure in vivo and in vitro. *J Immunol* **154**:4851–4856.
- Eskdale J and Gallagher G (1995) A polymorphic dinucleotide repeat in the human IL-10 promoter. *Immunogenetics* **42**:444–445.
- Eskdale J, Keijsers V, Huizinga T, and Gallagher G (1999) Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. *Genes Immun* **1**:151–155.
- Eskdale J, Kube D, and Gallagher G (1996) A second polymorphic dinucleotide repeat in the 5' flanking region of the human IL10 gene. *Immunogenetics* **45**:82–83.
- Eskdale J, Kube D, Tesch H, and Gallagher G (1997a) Mapping of the human IL10 gene and further characterization of the 5' flanking sequence. *Immunogenetics* **46**:120–128.
- Eskdale J, McNicholl J, Wordsworth P, Jonas B, Huizinga T, Field M, and Gallagher G (1998) Interleukin-10 microsatellite polymorphisms and IL-10 locus alleles in rheumatoid arthritis susceptibility (letter). *Lancet* **352**:1282–1283.
- Eskdale J, Wordsworth P, Bowman S, Field M, and Gallagher G (1997b) Association between polymorphisms at the human IL-10 locus and systemic lupus erythematosus [published erratum appears in *Tissue Antigens* **50**:699]. *Tissue Antigens* **49**:635–639.
- Fayad L, Keating MJ, Reuben JM, O'Brien S, Lee BN, Lerner S, and Kurczok R (2001) Interleukin-6 and interleukin-10 levels in chronic lymphocytic leukemia: correlation with phenotypic characteristics and outcome. *Blood* **97**:256–263.
- Fedorak RN, Gangl A, Elson CO, Rutgeerts P, Schreiber S, Wild G, Hanauer SB, Kilian A, Cohard M, LeBeaut A, et al. (2000) Recombinant human interleukin 10 in the treatment of patients with mild to moderately active Crohn's disease. The Interleukin 10 Inflammatory Bowel Disease Cooperative Study Group. *Gastroenterology* **119**:1473–1482.
- Feng X, Zheng XX, Yi S, Lehnert AM, Strom TB, and O'Connell PJ (1999) IL-10/Fc inhibits macrophage function and prolongs pancreatic islet xenograft survival. *Transplantation* **68**:1775–1783.
- Fickenscher H, Hor S, Kupers H, Knappe A, Wittmann S, and Sticht H (2002) The interleukin-10 family of cytokines. *Trends Immunol* **23**:89–96.
- Finbloom DS and Winestock KD (1995) IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. *J Immunol* **155**:1079–1090.
- Fiorentino DF, Bond MW, and Mosmann TR (1989) Two types of mouse T-helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* **170**:2081–2095.
- Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, and O'Garra A (1991a) IL-10 inhibits cytokine production by activated macrophages. *J Immunol* **147**:3815–3822.
- Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, and O'Garra A (1991b) IL-10 acts on antigen-presenting cell to inhibit cytokine production by Th1 cells. *J Immunol* **146**:3444–3451.
- Fleming SB, McCaughan CA, Andrews AE, Nash AD, and Mercer AA (1997) A homolog of interleukin-10 is encoded by the poxvirus orf virus. *J Virol* **71**:4857–4861.
- Friedrich M, Döcke WD, Klein A, Philipp S, Volk HD, Sterry W, and Asadullah K (2002) Immunomodulation by IL-10 therapy decreases the incidence of relapse and prolongs the relapse-free interval in psoriasis. *J Invest Dermatol* **118**:672–677.
- Fuchs AC, Granowitz EV, Shapiro L, Vannier E, Lonnemann G, Angel JB, Kennedy JS, Rabson AR, Radwanski E, Affrime MB, et al. (1996) Clinical, hematologic and immunologic effects of interleukin-10 in humans. *J Clin Immunol* **16**:291–303.
- Galizia G, Lieto E, de Vita F, Romano C, Orditura M, Castellano P, Imperatore V, Infusino S, Catalano G, and Pignatelli C (2002b) Circulating levels of IL-10 and IL-6 in gastric and colon cancer patients before and after surgery: relationship with radicality and outcome. *J Interferon Cytokine Res* **22**:473–482.
- Galizia G, Orditura M, Romano C, Lieto E, Castellano P, Pelosio L, Imperatore V, Catalano G, Pignatelli C, and de Vita F (2002) Prognostic significance of circulating IL-10 and IL-6 serum levels in colon cancer patients undergoing surgery. *Clin Immunol* **102**:169–178.
- Gallagher G, Dickensheets H, Eskdale J, Izotova LS, Mirochnitchenko OV, Peat JD, Vazquez N, Pestka S, Donnelly RP, and Kotenko SV (2000) Cloning, expression and initial characterization of interleukin-19 (IL-19), a novel homologue of human interleukin-10 (IL-10). *Genes Immun* **1**:442–450.
- Gerard C, Bruyns C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux A, Fiers W, Goldman M, and Velu T (1993) Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *J Exp Med* **177**:547–550.
- Gerard CM, Bruyns C, Delvaux A, Baudouin N, Dargent JL, Goldman M, and Velu T (1996) Loss of tumorigenicity and increased immunogenicity induced by interleukin-10 gene transfer in B16 melanoma cells. *Hum Gene Ther* **7**:23–31.
- Giacalone B, D'Auria L, Bonifati C, Ferraro C, Riccardi E, Musci A, D'Agosto G, Cordiali-Fei P, and Ameglio F (1998) Decreased interleukin-7 and transforming growth factor-beta1 levels in blister fluids as compared to the respective serum levels in patients with bullous pemphigoid. Opposite behavior of TNF-alpha, interleukin-4 and interleukin-10. *Exp Dermatol* **7**:157–161.
- Gonzalez-Amaro R, Portales-Perez D, Baranda L, Abud-Mendoza C, Llorente L, Richaud-Patin Y, Alcocer-Varela J, and Alarcon-Segovia D (1998) Role of IL-10 in the abnormalities of early cell activation events of lymphocytes from patients with systemic lupus erythematosus. *J Autoimmun* **11**:395–402.
- Gorski JC, Hall SD, Becker P, Affrime MB, Cutler DL, and Haehner-Daniels B (2000) In vivo effects of interleukin-10 on human cytochrome P450 activity. *Clin Pharmacol Ther* **67**:32–43.
- Grewe M, Gyufko K, and Krutmann J (1995) Interleukin-10 production by cultured human keratinocytes: regulation by ultraviolet B and ultraviolet A1 radiation. *J Invest Dermatol* **104**:3–6.
- Gronald G, Kristjansdottir H, Gunnlaugsdottir B, Arnason A, Lundberg I, Klareskog L, and Steinsson K (1999) Increased number of interleukin-10-producing cells in systemic lupus erythematosus patients and their first-degree relatives and spouses in Icelandic multicase families. *Arthritis Rheum* **42**:1649–1654.
- Groux H, Bigler M, de Vries JE, and Roncarolo MG (1996) Interleukin-10 induces a long-term antigen-specific anergic state in human CD4⁺ T cells. *J Exp Med* **184**:19–29.
- Groux H, Bigler M, de Vries JE, and Roncarolo MG (1998) Inhibitory and stimulatory effects of IL-10 on human CD8⁺ T cells. *J Immunol* **160**:3188–3193.
- Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, and Roncarolo MG (1997) A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature (Lond)* **389**:737–742.
- Gruenberg BH, Schoenemeyer A, Weiss B, Toschi L, Kunz S, Wolk K, Asadullah K, and Sabat R (2001) A novel, soluble homologue of the human IL-10 receptor with preferential expression in placenta. *Genes Immun* **2**:329–334.
- Hagenbaugh A, Sharma S, Dubinett SM, Wei SH, Aranda R, Cheroutre H, Fowell DJ, Binder S, Tsao B, Locksley RM, et al. (1997) Altered immune responses in interleukin 10 transgenic mice. *J Exp Med* **185**:2101–2110.
- Hahn AB, Kasten-Jolly JC, Constantino DM, Graffunder E, Singh TP, and Conti DJ (2001) TNF-alpha, IL-6, IFN-gamma and IL-10 gene expression polymorphisms and the IL-4 receptor alpha-chain variant Q576R: effects on renal allograft outcome. *Transplantation* **72**:660–665.
- Hara M, Kingsley CI, Niimi M, Read S, Turvey SE, Bushell AR, Morris PJ, Powrie F, and Wood KJ (2001) IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. *J Immunol* **166**:3789–3796.
- Hart PH, Ahern MJ, Smith MD, and Finlay-Jones JJ (1995) Comparison of the suppressive effects of interleukin-10 and interleukin-4 on synovial fluid macrophages and blood monocytes from patients with inflammatory arthritis. *Immunology* **84**:536–542.
- Hart PH, Hunt EK, Bonder CS, Watson CJ, and Finlay-Jones JJ (1996) Regulation of surface and soluble TNF receptor expression on human monocytes and synovial fluid macrophages by IL-4 and IL-10. *J Immunol* **157**:3672–3680.
- Hasegawa M, Fujimoto M, Kikuchi K, and Takehara K (1997) Elevated serum levels of interleukin 4 (IL-4), IL-10 and IL-13 in patients with systemic sclerosis. *J Rheumatol* **24**:328–332.
- Herfarth H, Mohanty S, Rath H, Tonkonogy S, and Sartor R (1996) Interleukin 10 suppresses experimental chronic, granulomatous inflammation induced by bacterial cell wall polymers. *Gut* **39**:836–845.
- Herfarth H and Schölmerich J (2002) IL-10 therapy in Crohn's disease: at the crossroads. *Gut* **50**:146–147.
- Ho AS, Liu Y, Khan TA, Hsu DH, Bazan JF, and Moore KW (1993) A receptor for interleukin 10 is related to interferon receptors. *Proc Natl Acad Sci USA* **90**:11267–11271.
- Holler E, Roncarolo MG, Hintermeier-Knabe R, Eissner G, Ertl B, Schulz U, Knabe H, Kolb HJ, Andreesen R, and Wilmanns W (2000) Prognostic significance of increased IL-10 production in patients prior to allogeneic bone marrow transplantation. *Bone Marrow Transplant* **25**:237–241.
- Hsu DH, De Waal Malefyt R, Fiorentino DF, Dang MN, Vieira P, De Vries J, Spits H, Mosmann TR, and Moore KW (1990) Expression of interleukin-10 activity by Epstein-Barr virus protein BCRF1. *Science (Wash DC)* **250**:830–832.
- Huang S, Ullrich SE, and Bar-Eli M (1999) Regulation of tumor growth and metastasis by interleukin-10: the melanoma experience. *J Interferon Cytokine Res* **19**:697–703.
- Huang S, Xie K, Bucana CD, Ullrich SE, and Bar-Eli M (1996) Interleukin 10

- suppresses tumor growth and metastasis of human melanoma cells: potential inhibition of angiogenesis. *Clin Cancer Res* **2**:1969–1979.
- Huhn RD, Pennline K, Radwanski E, Clarke L, Sabo R, and Cutler DL (1999) Effects of single intravenous doses of recombinant human interleukin-10 on subsets of circulating leukocytes in humans. *Immunopharmacology* **41**:109–117.
- Huhn RD, Radwanski E, Gallo J, Affrime MB, Sabo R, Gonyo G, Monge A, and Cutler DL (1997) Pharmacodynamics of subcutaneous recombinant human interleukin-10 in healthy volunteers. *Clin Pharmacol Ther* **62**:171–180.
- Huhn RD, Radwanski E, O'Connell SM, Sturgill MG, Clarke L, Cody RP, Affrime MB, and Cutler DL (1996) Pharmacokinetics and immunomodulatory properties of intravenously administered recombinant human interleukin-10 in healthy volunteers. *Blood* **87**:699–705.
- Huizinga TW, Keijsers V, Yanni G, Hall M, Ramage W, Lanchbury J, Pitzalis C, Drossaers-Bakker WK, Westendorp RG, Breedveld FC, et al. (2000) Are differences in IL-10 production associated with joint damage? *Rheumatology* **39**:1180–1188.
- Hurme M, Lahdenpohja N, and Santtila S (1998) Gene polymorphisms of interleukins 1 and 10 in infectious and autoimmune diseases. *Ann Med* **30**:469–473.
- Ichikawa T, Nakao K, Nakata K, Yamashita M, Hamasaki K, Shigen M, Abiru S, Ishikawa H, Ishii N, and Eguchi K (2002) Involvement of IL-1beta and IL-10 in IFN-alpha mediated antiviral gene induction in human hepatoma cells. *Biochem Biophys Res Commun* **294**:414–422.
- Ishida H, Muchamuel T, Sakaguchi S, Andrade S, Menon S, and Howard M (1994) Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. *J Exp Med* **179**:305–310.
- Ito S, Ansari P, Sakatsume M, Dickensheets H, Vazquez N, Donnelley RP, Larner AC, and Finbloom DS (1999) Interleukin-10 inhibits expression of both interferon alpha- and interferon gamma-induced genes by suppressing tyrosine phosphorylation of STAT1. *Blood* **93**:1456–1463.
- Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, and Bonnefoy JY (1998) IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* **160**:3555–3561.
- Jenkins JK, Malyak M, and Arend WP (1994) The effects of interleukin-10 on interleukin-1 receptor antagonist and interleukin-1 beta production in human monocytes and neutrophils. *Lymphokine Cytokine Res* **13**:47–54.
- Jiang H, Lin JJ, Su ZZ, Goldstein NI, and Fisher PB (1995) Subtraction hybridization identifies a novel melanoma differentiation associated gene, MDA-7, modulated during human melanoma differentiation, growth and progression. *Oncogene* **11**:2477–2486.
- Jiang H, Su ZZ, Lin JJ, Goldstein NI, Young CS, and Fisher PB (1996) The melanoma differentiation associated gene mda-7 suppresses cancer cell growth. *Proc Natl Acad Sci USA* **93**:9160–9165.
- Jiang H, Wynn C, Pan F, Ebbs A, Erickson LM, and Kobayashi M (2002) Tacrolimus and cyclosporin differ in their capacity to overcome ongoing allograft rejection as a result of their differential abilities to inhibit IL-10 production. *Transplantation* **73**:1808–1817.
- Jilg S, Barsig J, Leist M, Kusters S, Volk HD, and Wendle A (1996) Enhanced release of IL-10 and soluble TNF receptors as novel principles of methyloxanthine action in murine models of endotoxic shock. *J Pharmacol Exp Ther* **278**:421–431.
- Jones KD, Aoki Y, Chang Y, Moore PS, Yarchon R, and Tosato G (1999) Involvement of interleukin-10 (IL-10) and viral IL-6 in the spontaneous growth of Kaposi's sarcoma herpesvirus-associated infected primary effusion lymphoma cells. *Blood* **94**:2871–2879.
- Jonuleit H, Schmitt E, Schuler G, Knop J, and Enk AH (2000) Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* **192**:1213–1222.
- Joyce DA, Gibbons DP, Green P, Steer JH, Feldmann M, and Brennan FM (1994) Two inhibitors of pro-inflammatory cytokine release, interleukin-10 and interleukin-4, have contrasting effects on release of soluble p75 tumor necrosis factor receptor by cultured monocytes. *Eur J Immunol* **24**:2699–2705.
- Jurlander J, Lai CF, Tan J, Chou CC, Geisler CH, Schriber J, Blumenson LE, Narula SK, Baumann H, and Caligiuri MA (1997) Characterization of interleukin-10 receptor expression on B-cell chronic lymphocytic leukemia cells. *Blood* **89**:4146–4152.
- Kaluza W, Leirisalo-Repo M, Marker-Hermann E, Westman P, Reuss PE, Hug R, Mastrovic K, Stradmann-Bellinghausen B, Granfor K, Galle PR, et al. (2001) IL-10G microsatellites mark promoter haplotypes associated with protection against the development of reactive arthritis in Finnish patients. *Arthritis Rheum* **44**:1209–1214.
- Kang K, Hammerberg C, Meunier L, and Cooper KD (1994) CD11b+ macrophages that infiltrate human epidermis after in vivo ultraviolet exposure potentially produce IL-10 and represent the major secretory source of epidermal IL-10 protein. *J Immunol* **153**:5256–5264.
- Kasama T, Strieter RM, Lukacs NW, Burdick MD, and Kunkel SL (1994) Regulation of neutrophil-derived chemokine expression by IL-10. *J Immunol* **152**:3559–3569.
- Keystone E, Wherry J, and Grint P (1998) IL-10 as a therapeutic strategy in the treatment of rheumatoid arthritis. *Rheum Dis Clin N Am* **24**:629–639.
- Kim J, Modlin RL, Moy RL, Dubinett SM, McHugh T, Nickloff BJ, and Uyemura K (1995) IL-10 production in cutaneous basal and squamous cell carcinomas. A mechanism for evading the local T cell immune response. *J Immunol* **155**:2240–2247.
- Kimball AB, Kawamura T, Tejera K, Boss C, Hancox AR, Vogel JC, Steinberg SM, Turner ML, and Blauvelt A (2002) Clinical and immunologic assessment of patients with psoriasis in a randomized, double-blind, placebo-controlled trial using recombinant human interleukin 10. *Arch Dermatol* **138**:1341–1346.
- Kitabayashi A, Hirokawa M, and Miura AB (1995) The role of interleukin-10 (IL-10) in chronic B-lymphocytic leukemia: IL-10 prevents leukemic cells from apoptotic cell death. *Int J Hematol* **62**:99–106.
- Kline JN, Hunninghake GM, He B, Monick MM, and Hunninghake GW (1998) Synergistic activation of the human cytomegalovirus major immediate early promoter by prostaglandin E2 and cytokines *Exp Lung Res* **24**:3–14.
- Knappe A, Hor S, Wittmann S, and Fickenscher H (2000) Induction of a novel cellular homolog of interleukin-10, AK155, by transformation of T lymphocytes with herpesvirus saimiri. *J Virol* **74**:3881–3887.
- Kobayashi H, Kobayashi M, Herndon DN, Pollard RB, and Suzuki F (2001) Susceptibility of thermally injured mice to cytomegalovirus infection. *Burns* **27**:675–680.
- Kondo S, McKenzie RC, and Sauder DN (1994) Interleukin-10 inhibits the elicitation phase of allergic contact hypersensitivity. *J Invest Dermatol* **103**:811–814.
- Kono DH, Burlingame RW, Owens DG, Kuramochi A, Balderas RS, Balomenos D, and Theofilopoulos AN (1994) Lupus susceptibility loci in New Zealand mice. *Proc Natl Acad Sci USA* **91**:10168–10172.
- Kotenko SV, Izotova LS, Mirochnitchenko OV, Esterova E, Dickensheets H, Donnelly RP, and Pestka S (2001) Identification of the functional interleukin-22 (IL-22) receptor complex: the IL-10R2 chain (IL-10Rbeta) is a common chain of both the IL-10 and IL-22 (IL-10-related T cell-derived inducible factor, IL-TIF) receptor complexes. *J Biol Chem* **276**:2725–2732.
- Kotenko SV, Krause CD, Izotova LS, Pollack BP, Wu W, and Pestka S (1997) Identification and functional characterization of a second chain of the interleukin-10 receptor complex. *EMBO (Eur Mol Biol Organ) J* **16**:5894–5903.
- Kotenko SV and Pestka S (2000) Jak-Stat signal transduction pathway through the eyes of cytokine class II receptor complexes. *Oncogene* **19**:2557–2565.
- Kotenko SV, Saccani S, Izotova LS, Mirochnitchenko OV, and Pestka S (2000) Human cytomegalovirus harbors its own unique IL-10 homolog (cmv IL-10). *Proc Natl Acad Sci USA* **97**:1695–1700.
- Krüger-Krasagakes S, Krasagakis K, Garbe C, Schmitt E, Hüls C, Blankenstein T, and Diamantstein T (1994) Expression of interleukin 10 in human melanoma. *Br J Cancer* **70**:1182–1185.
- Kuhn R, Lohler J, Rennick D, Rajewsky K, and Muller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* **75**:263–274.
- Kundu N, Dorsey R, Jackson MJ, Guitierrez P, Wilson K, Fu S, Ramanujam K, Thomas E, and Fulton AM (1998) Interleukin-10 gene transfer inhibits murine mammary tumors and elevates nitric oxide. *Int J Cancer* **76**:713–719.
- Kundu N and Fulton AM (1997) Interleukin-10 inhibits tumor metastasis, down-regulates MHC class I and enhances NK lysis. *Cell Immunol* **180**:55–61.
- Lauw FN, Pajkrt D, Hack CE, Kurimoto M, van Deventer SJ, and van der Poll T (2000) Proinflammatory effects of IL-10 during human endotoxemia. *J Immunol* **165**:2783–2789.
- Lazarus M, Hajer AH, Turner D, Sinnott P, Worthington J, Ollier WE, and Hutchinson IV (1997) Genetic variation in the interleukin 10 gene promoter and systemic lupus erythematosus. *J Rheumatol* **24**:2314–2317.
- Lee TS and Chau LY (2002) Heme oxygenase-1 mediates the anti-inflammatory effect of IL-10 in mice. *Nat Med* **8**:240–246.
- Levings MK, Sangregorio R, Galbati F, Squadron S, DeWaal Malefyt R, and Roncarolo MG (2001a) IFN-alpha and IL-10 induce the differentiation of human type 1 T regulatory cells. *J Immunol* **166**:5530–5539.
- Levings MK, Sangregorio R, and Roncarolo MG (2001b) Human cd25(+)/cd4(+) t regulatory cells suppress naive and memory T cell proliferation and can be expanded in vitro without loss of function. *J Exp Med* **193**:1295–1302.
- Levy Y and Brouet JC (1994) Interleukin-10 prevents spontaneous death of germinal center B cells by induction of the bcl-2 protein. *J Clin Invest* **93**:424–428.
- Lin TJ and Befus AD (1997) Differential regulation of mast cell function by IL-10 and stem cell factor. *J Immunol* **159**:4015–4023.
- Lindsay JO and Hodgson HJ (2001) Review article: the immunoregulatory cytokine IL-10—a therapy for Crohn's disease? *Aliment Pharmacol Ther* **15**:1709–1716.
- Link H and Xiao BG (2001) Rat models as tool to develop new immunotherapies. *Immunol Rev* **184**:117–128.
- Liu Y, Wei SH, Ho AS, De Waal Malefyt R, and Moore KW (1994) Expression cloning and characterization of a human IL-10 receptor. *J Immunol* **152**:1821–1829.
- Llorente L, Garcia-Padilla C, Richaud-Patin Y, Wijdenes J, Cardiel M, Varela JA, Alarcon-Segovia D, Galanaud P, and Emille D (1999) Treatment of systemic lupus erythematosus with an anti-IL-10 monoclonal antibody: clinical and biological aspects. *Presentation at the 2nd International IL-10 Workshop*; 1999 May 27–29; Milano, Italy.
- Llorente L, Richaud-Patin Y, Couderc J, Alarcon-Segovia D, Ruiz-Soto R, Alcocer-Castillejos N, Alcocer-Varela J, Granados J, Bahena S, Galanaud P, et al. (1997) Dysregulation of interleukin-10 production in relatives of patients with systemic lupus erythematosus. *Arthritis Rheum* **40**:1429–1435.
- Llorente L, Zou W, Levy Y, Richard-Patin Y, Wijdenes J, Alcocer-Varela J, Morel-Fourier B, Brouet JC, Alarcon-Segovia D, Galanaud P, et al. (1995) Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J Exp Med* **181**:839–844.
- Lyons A, Kelly JL, Rodrick ML, Mannick JA, and Lederer JA (1997) Major injury induces increased production of IL-10 by cells of the immune system with a negative impact on resistance to infection. *Ann Surg* **226**:450–458.
- Ma W, Lim W, Gee K, Aucoin S, Nandan D, Kozlowski M, Diaz-Mitoma F, and Kumar A (2001) The p38 mitogen-activated kinase pathway regulates the human interleukin-10 promoter via the activation of Sp1 transcription factor in lipopolysaccharide-stimulated human macrophages. *J Biol Chem* **276**:13664–13674.
- Maini RN and Taylor PC (2000) Anti-cytokine therapy for rheumatoid arthritis. *Annu Rev Med* **51**:207–229.
- Makela MJ, Kanehiro A, Borish L, Dakhama A, Loader J, Joetham A, Xing Z, Jordana M, Larsen GL, and Gelfand EW (2000) IL-10 is necessary for the expression of airway hyperresponsiveness but not pulmonary inflammation after allergic sensitization. *Proc Natl Acad Sci USA* **97**:6007–6012.
- Masood R, Zhang Y, Bond MW, Scadden DF, Moudgil T, Law RE, Kaplan MH, Jung B, Espina BM, and Lunardi-Iskandar Y (1995) Interleukin-10 is an autocrine growth factor for acquired immunodeficiency syndrome-related B-cell lymphoma. *Blood* **85**:3423–3430.
- Massey EJ, Sundstedt A, Day MJ, Corfield G, Anderton S, and Wraith DC (2002) Intranasal peptide-induced peripheral tolerance: the role of IL-10 in regulatory T

- cell function within the context of experimental autoimmune encephalitis. *Vet Immunol Immunopathol* **87**:357–372.
- Mathurin P, Xiong S, Kharabanda KK, Veal N, Miyahara T, Motomura K, Ripper RA, Bachem MG, and Tsukamoto H (2002) IL-10 receptor and coreceptor expression in quiescent and activated stellate cells. *Am J Physiol Gastrointest Liver Physiol* **282**:981–990.
- Matsuda M, Salazar F, Petersson M, Masucci G, Hansson J, Pisa P, Zhang QJ, Masucci MG, and Kiessling R (1994) Interleukin 10 pretreatment protects target cells from tumor- and allo-specific cytotoxic T cells and downregulates HLA class I expression. *J Exp Med* **180**:2371–2376.
- McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A, Southgate C, Easton DF, Eeles RA, and Howell WM (2002) Influence of cytokine gene polymorphisms on the development of prostate cancer. *Cancer Res* **62**:3369–3372.
- McInnes IB, Illei GG, Danning CL, Yarboro CH, Crane M, Kuroiwa T, Schlimmer R, Lee E, Foster B, Flemming D, et al. (2001) IL-10 improves skin disease and modulates endothelial activation and leukocyte effector function in patients with psoriatic arthritis. *J Immunol* **167**:4075–4082.
- McShane P (2002) Association of polymorphisms in the human interferon-gamma and IL-10 gene with acute and chronic kidney transplant outcome. *Transplantation* **73**:1682.
- Mehrian R, Quismorio FP Jr, Strassmann G, Stimmler MM, Horwitz DA, Kitridou RC, Gauderman WJ, Morrison J, Brautbar C, and Jacob CO (1998) Synergistic effect between IL-10 and bcl-2 genotypes in determining susceptibility to systemic lupus erythematosus. *Arthritis Rheum* **41**:596–602.
- Meisel C, Vogt K, Platzer C, Randow F, Liebenthal C, and Volk HD (1996) Differential regulation of monocytic tumor necrosis factor-alpha and interleukin-10 expression. *Eur J Immunol* **26**:1580–1586.
- Michel G, Gailis A, Jarzubska-Deussen B, Muschen A, Mirmohammadsadegh A, and Ruzicka T (1997a) 1,25-(OH)₂-vitamin D₃ and calcipotriol induce IL-10 receptor gene expression in human epidermal cells. *Inflamm Res* **46**:32–34.
- Michel G, Mirmohammadsadegh A, Olasz E, Jarzubska-Deussen B, Muschen A, Kemeny L, Abts HF, and Ruzicka T (1997b) Demonstration and functional analysis of IL-10 receptors in human epidermal cells: decreased expression in psoriatic skin, down-modulation by IL-8 and up-regulation by an antipsoriatic glucocorticosteroid in normal cultured keratinocytes. *J Immunol* **159**:6291–6297.
- Mitra RS, Judge TA, Nestle FO, Turka LA, and Nickoloff BJ (1995) Psoriatic skin-derived dendritic cell function is inhibited by exogenous IL-10. Differential modulation of B7-1 (CD80) and B7-2 (CD86) expression. *J Immunol* **154**:2668–2677.
- Moore KW, de Waal Malefyt R, Coffman RL, and O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* **19**:683–765.
- Morse HR, Bidwell JL, and Raveche ES (1999) A poly(C) repeat polymorphism in the promoter of the IL-10 gene in NZB mice. *Eur J Immunogenet* **26**:377–378.
- Muehlstedt SG, Lyte M, and Rodriguez JL (2002) Increased IL-10 production and HLA-DR suppression in the lungs of injured patients precede the development of nosocomial pneumonia. *Shock* **17**:443–450.
- Mussi A, Bonifati C, Carducci M, Viola M, Tomaselli R, Sacerdoti G, Fazio M, and Ameglio F (1994) IL-10 levels are decreased in psoriatic lesional skin as compared to the psoriatic lesion-free and normal skin suction blister fluids. *J Biol Regul Homeostatic Agents* **8**:117–120.
- Nagata Y, Kijima H, Tokunaga T, Takagi A, Mine T, Yamazaki H, Nakamura M, and Ueyama Y (2002) Correlation between IL-10 and vascular endothelial growth factor expression in human esophageal cancer. *Int J Mol Med* **10**:169–172.
- Nakajima H, Gleich GJ, and Kita H (1996) Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. *J Immunol* **156**:4859–4866.
- Nakashima H, Akahoshi M, Tanaka Y, Yamaoka K, Ogami E, Nagano S, Arinobu Y, Niino H, Otsuka T, and Niho Y (1999) Polymorphisms within the IL-10 receptor cDNA gene in Japanese patients with systemic lupus erythematosus. *Rheumatology* **38**:1142–1144.
- Narula K (2000) Interleukin 10—A therapeutic cytokine for chronic inflammatory diseases. *Curr Opin Anti-inflammatory Immunomodulatory Investigational Drugs* **2**:307–313.
- Nelson DR, Lauwers GY, Lau JY, and Davis GL (2000) IL-10 treatment reduces fibrosis in patients with chronic hepatitis C: a pilot trial of interferon nonresponders. *Gastroenterology* **118**:655–660.
- Nemunaitis J, Fong T, Shabe P, Martineau D, and Ando D (2001) Comparison of serum interleukin-10 (IL-10) levels between normal volunteers and patients with advanced melanoma. *Cancer Invest* **19**:239–247.
- Nickoloff BJ, Fivenson DP, Kunkel SL, Strieter RM, and Turka LA (1994) Keratinocyte interleukin-10 expression is upregulated in tape-stripped skin, poison ivy dermatitis and Sezary syndrome, but not in psoriatic plaques. *Clin Immunol Immunopathol* **73**:63–68.
- Niino H, Otsuka T, Izuhara K, Yamaoka K, Ohshima K, Tanabe T, Hara S, Nemoto Y, Tanaka Y, Nakashima H, et al. (1997) Regulation by interleukin-10 and interleukin-4 of cyclooxygenase-2 expression in human neutrophils. *Blood* **89**:1621–1628.
- Norris DA, Travers JB, and Leung DY (1997) Lymphocyte activation in the pathogenesis of psoriasis. *J Invest Dermatol* **109**:1–4.
- Oberholzer A, Oberholzer C, Bahjat KS, Ungaro R, Tannahill CL, Murday M, Bahjat FR, Abouhamze Z, Tsai V, LaFace D, et al. (2002) Increased survival in sepsis by in vivo adenovirus-induced expression of IL-10 in dendritic cells. *J Immunol* **168**:3412–3418.
- Ode-Hakim S, Docke WD, Kern F, Emmrich F, Volk HD, and Reinke P (1996) Delayed type hypersensitivity mechanisms dominate late acute rejection episodes in renal allograft recipients. *Transplantation* **61**:1233–1240.
- Ohmen JD, Hanifin JM, Nickoloff BJ, Rea TH, Wyzkowski R, Kim J, Jullien D, McHugh T, Nassif AS, Chan SC, and Modlin RL (1995) Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed-type hypersensitivity reactions. *J Immunol* **154**:1956–1963.
- Olszyna DP, Pajkrt D, Lauw FN, van Deventer SJ, and van Der Poll T (2000) Interleukin 10 inhibits the release of CC chemokines during human endotoxemia. *J Infect Dis* **181**:613–620.
- Opal SM and Huber CE (2000) The role of IL-10 in critical illness. *Curr Opin Infect Dis* **13**:221–226.
- Ordemann J, Jacobi CA, Braumann C, Schwenk W, Volk HD, and Muller JM (2002) Immunomodulatory changes in patients with colorectal cancer. *Int J Colorectal Dis* **17**:37–41.
- Pajkrt D, Camoglio L, Tiel-van Buul MC, de Bruin K, Cutler DL, Affrime MB, Rikken G, van der Poll T, ten Cate JW, and van Deventer SJ (1997a) Attenuation of proinflammatory response by recombinant human IL-10 in human endotoxemia: effect of timing of recombinant human IL-10 administration. *J Immunol* **158**:3971–3977.
- Pajkrt D, van der Poll T, Levi M, Cutler DL, Affrime MB, van den Ende A, ten Cate JW, and van Deventer SJ (1997b) Interleukin-10 inhibits activation of coagulation and fibrinolysis during human endotoxemia. *Blood* **89**:2701–2705.
- Park YB, Lee SK, Kim DS, Lee J, Lee CH, and Song CH (1998) Elevated interleukin-10 levels correlated with disease activity in systemic lupus erythematosus. *Clin Exp Rheumatol* **16**:283–288.
- Parry RG, Gillespie KM, and Mathieson PW (2001) Effects of type 2 cytokines on glomerular epithelial cells. *Exp Nephrol* **9**:275–283.
- Pennline KJ, Roque-Gaffney E, and Monahan M (1994) Recombinant human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse. *Clin Immunol Immunopathol* **71**:169–175.
- Persson S, Mikulowska A, Narula S, O'Garra A, and Holmdahl R (1996) Interleukin-10 suppresses the development of collagen type II-induced arthritis and ameliorates sustained arthritis in rats. *Scand J Immunol* **44**:607–614.
- Pistoia V (1997) Production of cytokines by human B cells in health and disease. *Immunol Today* **18**:343–350.
- Platzer C, Docke WD, Volk HD, and Prosch S (2000) Catecholamines trigger IL-10 release in acute systemic stress reaction by direct stimulation of its promoter/enhancer activity in monocytic cells. *J Neuroimmunol* **105**:31–38.
- Platzer C, Fritsch E, Elstner T, Lehmann MH, Volk HD, and Prosch S (1999) Cyclic adenosine monophosphate-responsive elements are involved in the transcriptional activation of the human IL-10 gene in monocytic cells. *Eur J Immunol* **29**:3098–3104.
- Platzer C, Meisel C, Vogt K, Platzer M, and Volk HD (1995) Up-regulation of monocytic IL-10 by tumor necrosis factor-alpha and cAMP elevating drugs. *Int Immunol* **7**:517–523.
- Platzer C, Volk HD, and Platzer M (1994) 5' noncoding sequence of human IL-10 gene obtained by oligo-cassette PCR walking. *DNA Seq* **4**:399–401.
- Powrie F, Leach M, Mauze S, Caddle L, and Coffman R (1993) Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int Immunol* **5**:1461–1471.
- Pretolani M and Goldman M (1997) IL-10: a potential therapy for allergic inflammation? *Immunol Today* **18**:277–280.
- Puliti M, von Hunolstein C, Verwaerde C, Bistoni F, Orefici G, and Tissi L (2002) Regulatory role of IL-10 in experimental group B streptococcal arthritis. *Infect Immun* **70**:2862–2868.
- Radwanski E, Chakraborty A, Van Wart S, Huhn RD, Cutler DL, Affrime MB, and Jusko WJ (1998) Pharmacokinetics and leukocyte responses of recombinant human interleukin-10. *Pharm Res (NY)* **15**:1895–1901.
- Randolph GJ, Beaulieu S, Lebecque S, Steinman RM, and Muller WA (1998) Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science (Wash DC)* **282**:480–483.
- Redpath S, Ghazal P, and Gascoigne NRJ (2001) Hijacking and exploitation of IL-10 by intracellular pathogens. *Trends Microbiol* **9**:86–92.
- Reich K, Bruck M, Grafe A, Vente C, Neumann C, and Garbe C (1998) Treatment of psoriasis with interleukin-10 (letter). *J Invest Dermatol* **111**:1235–1236.
- Reich K, Garbe C, Blaschke V, Maurer C, Middel P, Westphal G, Lippert U, and Neumann C (2001) Response of psoriasis to interleukin-10 is associated with suppression of cutaneous type 1 inflammation, downregulation of the epidermal interleukin-8/CXCR2 pathway and normalization of keratinocyte maturation. *J Invest Dermatol* **116**:319–329.
- Reineke U, Sabat R, Volk HD, and Schneider-Mergener J (1998) Mapping of the interleukin-10/interleukin-10 receptor combining site. *Protein Sci* **7**:951–960.
- Reineke U, Schneider-Mergener J, Glaser RW, Stigler RD, Seifert M, Volk HD, and Sabat R (1999) Evidence for conformationally different states of interleukin-10: binding of a neutralizing antibody enhances accessibility of a hidden epitope. *J Mol Recognit* **12**:242–248.
- Rennick DM and Fort MM (2000) Lessons from genetically engineered animal models. XII. IL-10-deficient (IL-10^{-/-}) mice and intestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* **278**:G829–G833.
- Renz H, Jujo K, Bradley KL, Domenico J, Gelfand EW, and Leung DY (1992) Enhanced IL-4 production and IL-4 receptor expression in atopic dermatitis and their modulation by interferon-gamma. *J Invest Dermatol* **99**:403–408.
- Riese U, Brenner S, Docke WD, Prosch S, Reinke P, Oppert M, Volk HD, and Platzer C (2000) Catecholamines induce IL-10 release in patients suffering from acute myocardial infarction by transactivating its promoter in monocytic but not in T cells. *Mol Cell Biochem* **212**:45–50.
- Ritter M, Buechler C, Langmann T, Orso E, Klucken J, and Schmitz G (1999) The scavenger receptor CD163: regulation, promoter structure and genomic organization. *Pathobiology* **67**:257–261.
- Rode HJ, Bugert JJ, Handermann M, Schnitzler P, Kehm R, Janssen W, Delius H, and Darai G (1994) Molecular characterization and determination of the coding capacity of the genome of equine herpesvirus type 2 between the genome coordinates 0.235 and 0.258 (the EcoRI DNA fragment N; 4.2 kbp). *Virus Genes* **9**:61–75.
- Roilides E, Anastasiou-Katsiardani A, Dimitriadou-Georgiadou A, Kadiltsoglou I, Tsapidou S, Panteliadis C, and Walsh TJ (1998) Suppressive effects of interleukin-10 on human mononuclear phagocyte function against *Candida albicans* and *Staphylococcus aureus*. *J Infect Dis* **178**:1734–1742.

- Romagnani S (1995) Biology of human TH1 and TH2 cells. *J Clin Immunol* **15**:121-129.
- Roncarolo MG, Bacchetta R, Bordignon C, Narula S, and Levings MK (2001) *Immunol Rev* **182**:68-79.
- Rood MJ, Keijsers V, van der Linden MW, Tong TQ, Borggreve SE, Verweij CL, Breedveld FC, and Huizinga TW (1999) Neuropsychiatric systemic lupus erythematosus is associated with imbalance in interleukin 10 promoter haplotypes. *Ann Rheum Dis* **58**:85-89.
- Rott O, Fleischer B, and Cash E (1994) Interleukin-10 prevents experimental allergic encephalomyelitis in rats. *Eur J Immunol* **24**:1434-1440.
- Rousset F, Peyrol S, Garcia E, Vezzio N, Andujar M, Grimaud JA, and Banchereau J (1995) Long-term cultured CD40-activated B lymphocytes differentiate into plasma cells in response to IL-10 but not IL-4. *Int Immunol* **7**:2143-2153.
- Sabat R and Asadullah K (2002) Interleukin-10 in cancer immunity. In *Cancer Immune Therapy: Experiences and Future Strategies* (Stuhler G and Walden P eds), Wiley-VCH Verlag GmbH, Weinheim, Germany.
- Salazar-Onfray F, Charo J, Petersson M, Frelund S, Noffz G, Qin Z, Blankenstein T, Ljunggren HG, and Kiessling R (1997) Down-regulation of the expression and function of the transporter associated with antigen processing in murine tumor cell lines expressing IL-10. *J Immunol* **159**:3195-3202.
- Salazar-Onfray F, Petersson M, Franksson L, Matsuda M, Blankenstein T, Karre K, and Kiessling R (1995) IL-10 converts mouse lymphoma cells to a CTL-resistant, NK-sensitive phenotype with low but peptide-inducible MHC class I expression. *J Immunol* **154**:6291-6298.
- Santin AD, Hermonat PL, Ravaggi A, Bellone S, Pecorelli S, Roman JJ, Parham GP, and Cannon MJ (2000) Interleukin-10 increases Th1 cytokine production and cytotoxic potential in human papillomavirus-specific CD8(+) cytotoxic T lymphocytes. *J Virol* **74**:4729-4737.
- Sarris AH, Kliche KO, Pethambaram P, Preti A, Tucker S, Jackow C, Messina O, Pugh W, Hagemester FB, McLaughlin P, et al. (1999) Interleukin-10 levels are often elevated in serum of adults with Hodgkin's disease and are associated with inferior failure-free survival. *Ann Oncol* **10**:433-440.
- Schaefer G, Venkataraman C, and Schindler U (2001) Cutting edge: FISP (IL-4-induced secreted protein), a novel cytokine-like molecule secreted by Th2 cells. *J Immunol* **166**:5859-5863.
- Schlaak JF, Buslau M, Jochum W, Hermann E, Girndt M, Gallati H, Meyer zum Büschenfelde KH, and Fleischer B (1994) T cells involved in psoriasis vulgaris belong to the Th 1 subset. *J Invest Dermatol* **102**:145-149.
- Schmidt E, Bastian B, Dummer R, Tony HP, Bröcker EB, and Zillikens D (1996) Detection of elevated levels of IL-4, IL-5 and IL-10 in blister fluid of bullous pemphigoid. *Arch Dermatol Res* **228**:353-357.
- Schopf LR, Hoffmann KF, Cheever AW, Urban JF, and Wynn TA (2002) IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. *J Immunol* **168**:2383-2392.
- Schottelius AJ, Mayo MW, Sartor RB, and Baldwin AS Jr (1999) Interleukin-10 signaling blocks inhibitor of kappaB kinase activity and nuclear factor kappaB DNA binding. *J Biol Chem* **274**:31868-31874.
- Schreiber S, Fedorak RN, Nielsen OH, Wild G, Williams CN, Nikolaus S, Jacyna M, Lashner BA, Gangl A, Rutgeerts P, et al. (2000) Safety and efficacy of recombinant human interleukin 10 in chronic active Crohn's disease. Crohn's Disease IL-10 Cooperative Study Group. *Gastroenterology* **119**:1461-1472.
- Schreiber S, Heinig T, Thiele HG, and Raedler A (1995) Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. *Gastroenterology* **108**:1432-1444.
- Schuppan D and Hahn EG (2000) IL-10: the magic bullet for liver fibrosis? *Gastroenterology* **119**:1412-1414.
- Schwarz A, Grabbe S, Riemann H, Aragane Y, Simon M, Manon S, Andrade S, Luger TA, Zlotnik A, and Schwarz T (1994) In vivo effects of interleukin-10 on contact hypersensitivity and delayed type hypersensitivity reactions. *J Invest Dermatol* **103**:211-216.
- Seifert M, Gruenberg BH, Sabat R, Donner P, Gruetz G, Wolk K, and Asadullah K (2002) Keratinocyte unresponsiveness toward interleukin 10: lack of specific binding due to deficient IL-10 receptor 1 expression. *Exp Dermatol* **12**:137-144.
- Seifert M, Sterry W, Effenberger E, Rexin A, Friedrich M, Haeußler-Quade A, Volk HD, and Asadullah K (2000) The antipsoriatic activity of IL-10 is rather caused by effects on peripheral blood cells than by direct effect on human keratinocytes. *Arch Dermatol Res* **292**:164-172.
- Shen X, Hong F, Nguyen VA, and Gao B (2000) IL-10 attenuates IFN-alpha activated SSTAT1 in the liver: involvement of SOCS2 and SOCS3. *FEBS Lett* **480**:132-136.
- Sjoberg J, Aguilar-Santelises M, Sjogren AM, Pisa EK, Ljungdahl A, Björkholm M, Jondal M, Mellstedt H, and Pisa P (1996) Interleukin-10 mRNA expression in B-cell chronic lymphocytic leukaemia inversely correlates with progression of disease. *Br J Haematol* **92**:393-400.
- Smith DR, Kunkel SL, Burdick MD, Wilke CA, Orringer MB, Whyte RI, and Strieter RM (1994) Production of interleukin-10 by human bronchogenic carcinoma. *Am J Pathol* **145**:18-25.
- Song KY, Chung CS, Chaudry IH, and Ayala A (1999) What is the role of IL-10 in polymicrobial sepsis: anti-inflammatory agent or immunosuppressant? *Surgery* **126**:378-383.
- Sosman JA, Verma A, Moss S, Sorokin P, Blend M, Bradlow B, Chachlani N, Cutler D, Sabo R, Nelson M, et al. (2000) IL-10 induced thrombocytopenia in normal healthy volunteers: evidence for decreased platelet production. *Br J Haematol* **111**:104-111.
- Spencer JV, Lockridge KM, Barry PA, Lin G, Tsang M, Penfold ME, and Schall TJ (2002) Potent immunosuppressive activities of cytomegalovirus-encoded IL-10. *J Virol* **76**:1285-1292.
- Spencer SD, Di Marco F, Hooley J, Pitts-Meek S, Bauer M, Ryan AM, Sordat B, Gibbs VC, and Aguet M (1998) The orphan receptor CRF2-4 is an essential subunit of the interleukin 10 receptor. *J Exp Med* **187**:571-578.
- Spies CD, Kern H, Schroder T, Sander M, Sepold H, Lang P, Stangl K, Behrens S, Sinha P, Schaffartzik W, et al. (2002) Myocardial ischemia and cytokine response are associated with subsequent onset of infections after noncardiac surgery. *Anesth Analg* **95**:9-18.
- Spits H and De Waal Malefyt R (1992) Functional characterization of human IL-10. *Int Arch Allergy Appl Immunol* **99**:8-15.
- Spittler A, Schiller C, Willheim M, Tempfer C, Winkler S, and Boltz-Nitulescu G (1995) IL-10 augments CD23 expression on U937 cells and down-regulates IL-4-driven CD23 expression on cultured human blood monocytes: effects of IL-10 and other cytokines on cell phenotype and phagocytosis. *Immunology* **85**:311-317.
- Stampfli MR, Cwiartka M, Gajewska BU, Alvarez D, Ritz SA, Inna MD, Xing Z, and Jordana M (1999) IL-10 gene transfer to the airway regulates allergic mucosal sensitization in mice. *Am J Respir Cell Mol Biol* **5**:585-596.
- Stasi R, Zinzani L, Galieni P, Lauta VM, Damasio E, Dispensa E, Dammacco F, Papa G, and Tura S (1994b) Prognostic value of serum IL-10 and soluble IL-2 receptor levels in aggressive non-Hodgkin's lymphoma. *Br J Haematol* **88**:770-777.
- Stasi R, Zinzani L, Galieni P, Lauta VM, Damasio E, Dispensa E, Dammacco F, Tura S, and Papa G (1994a) Detection of soluble interleukin-2 receptor and interleukin-10 in the serum of patients with aggressive non-Hodgkin's lymphoma. Identification of a subset at high risk of treatment failure. *Cancer* **74**:1792-1800.
- Stearns ME, Garcia FU, Fudge K, Rhim J, and Wang M (1999) Role of interleukin 10 and transforming growth factor beta1 in the angiogenesis and metastasis of human prostate primary tumor lines from orthotopic implants in severe combined immunodeficiency mice. *Clin Cancer Res* **5**:711-720.
- Stearns ME, Rhim J, and Wang M (1999) Interleukin 10 (IL-10) inhibition of primary human prostate cell-induced angiogenesis: IL-10 stimulation of tissue inhibitor of metalloproteinase-1 and inhibition of matrix metalloproteinase (MMP)-2/MMP-9 secretion. *Clin Cancer Res* **5**:189-196.
- Steidler L, Hans W, Schotte L, Neiryneck S, Obermeier F, Falk W, Fiers W, and Remaut E (2000) Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Science (Wash DC)* **289**:1352-1355.
- Stelmach I, Jerzynska J, and Kuna P (2002) A randomized, double-blind trial of the effect of glucocorticoid, antileukotriene and beta-agonist treatment on IL-10 serum levels in children with asthma. *Clin Exp Allergy* **32**:264-269.
- Suthanthiran M and Strom TB (1998) Mechanisms and management of acute renal allograft rejection. *Surg Clin N Am* **78**:77-94.
- Takahashi H, Furukawa T, Hashimoto S, Suzuki N, Kuroha T, Yamazaki F, Inano K, Takahashi M, Aizawa Y, and Koike T (2000) Contribution of TNF-alpha and IL-10 gene polymorphisms to graft-versus-host disease following allo-hematopoietic stem cell transplantation. *Bone Marrow Transplant* **26**:1317-1323.
- Takanashi S, Hasegawa Y, Kanehira Y, Yamamoto K, Fujimoto K, Satoh K, and Okamura K (1999) IL-10 level in sputum is reduced in bronchial asthma, COPD and in smokers. *Eur Respir J* **14**:309-314.
- Takanashi S, Nonaka R, Xing Z, O'Byrne P, Dolovich J, and Jordana M (1994) Interleukin 10 inhibits lipopolysaccharide-induced survival and cytokine production by human peripheral blood eosinophils. *J Exp Med* **180**:711-715.
- Tambur AR, Ortel JW, Ben-Ari Z, Shabtai E, Klein T, Michowicz R, Tur-Kaspa R, and Mor E (2001) Role of cytokine gene polymorphism in hepatitis C recurrence and allograft rejection among liver transplant recipients. *Transplantation* **71**:1475-1480.
- Tan JC, Indelicato SR, Narula SK, Zavodny PJ, and Chou CC (1993) Characterization of interleukin-10 receptors on human and mouse cells. *J Biol Chem* **268**:21053-21059.
- Tanaka Y, Nakashima H, Nemoto Y, Niuro H, Yamaoka K, Ogami E, Arinobu Y, Tachida H, Imamura T, and Niho Y (1997) Detection of polymorphisms within the human IL-10 receptor cDNA gene sequence by RT-PCR RFLP. *Immunogenetics* **46**:439-441.
- Tanaka Y, Otsuka T, Hotokebuchi T, Miyahara H, Nakashima H, Kuga S, Nemoto Y, Niuro H, and Niho Y (1996) Effect of IL-10 on collagen-induced arthritis in mice. *Inflamm Res* **45**:283-288.
- Teunissen MB, Koomen CW, Jansen J, de Waal Malefyt R, Schmitt E, Van den Wijngaard RM, Das PK, and Bos JD (1997) In contrast to their murine counterparts, normal human keratinocytes and human epidermoid cell lines A431 and HaCaT fail to express IL-10 mRNA and protein. *Clin Exp Immunol* **107**:213-223.
- te Velde AA, de Waal Malefyt R, Huijbens RJ, de Vries JE, and Figdor CG (1992) IL-10 stimulates monocyte Fc gamma R surface expression and cytotoxic activity. Distinct regulation of antibody-dependent cellular cytotoxicity by IFN-gamma, IL-4 and IL-10. *J Immunol* **149**:4048-4052.
- Thompson-Snipes L, Dhar V, Bond MW, Mosmann TR, Moore KW, and Rennick DM (1991) Interleukin 10: a novel stimulatory factor for mast cells and their progenitors. *J Exp Med* **173**:507-510.
- Tilg H, Ulmer H, Kaser A, and Weiss G (2002b) Role of IL-10 for induction of anemia during inflammation. *J Immunol* **169**:2204-2209.
- Tilg H, van Montfrans C, van den Ende A, Kaser A, van Deventer SJ, Schreiber S, Gregor M, Ludwiczek O, Rutgeerts P, Gasche C, et al. (2002a) Treatment of Crohn's disease with recombinant human interleukin 10 induces the proinflammatory cytokine interferon gamma. *Gut* **50**:191-195.
- Tournay-KG, Kips JC, and Pauwels RA (2000) Endogenous IL-10 suppresses allergen-induced airway inflammation and non-specific airway responsiveness. *Clin Exp Allergy* **30**:775-783.
- Tuohy VK, Yu M, Yin L, Mathisen PM, Johnson JM, and Kawczak JA (2000) Modulation of the IL-10/IL-12 cytokine circuit by interferon-beta inhibits the development of epitope spreading and disease progression in murine autoimmune encephalomyelitis. *J Neuroimmunol* **111**:55-63.
- Turner DM, Grant SC, Yonan N, Sheldon S, Dyer PA, Sinnott PJ, and Hutchinson IV (1997) Cytokine gene polymorphism and heart transplant rejection. *Transplantation* **64**:776-779.
- Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, and Hutchinson IV (1997) An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* **24**:1-8.
- Uejima Y, Takahashi K, Komoriya K, Kurozumi S, and Ochs HD (1996) Effect of interleukin-10 on anti-CD40- and interleukin-4-induced immunoglobulin E production by human lymphocytes. *Int Arch Allergy Appl Immunol* **110**:225-232.

- Uwatoko N, Tokunaga T, Hatanaka H, Osada H, Kawakami T, Yamazaki H, Abe Y, Kijima H, Ueyama Y, and Nakamura M (2002) Expression of IL-10 is inversely correlated with distant metastasis of renal cell carcinoma. *Int J Oncol* **20**:729–733.
- Valdimarsson H, Baker BS, Johnsdottir I, and Fry L (1985) Psoriasis: a disease of abnormal keratinocyte proliferation induced by T lymphocytes. *Immunol Today* **7**:256–259.
- Van Deventer SJ, Elson CO, and Fedorak RN (1997) Multiple doses of intravenous interleukin-10 in steroid refractory Crohn's disease. Chron's Disease Study Group. *Gastroenterology* **113**:383–389.
- Van Dissel JT, van Langevelde P, Westendorp RG, Kwappenberg K, and Frolich M (1998) Anti-inflammatory cytokine profile and mortality in febrile patients. *Lancet* **351**:950–953.
- Van Laethem JL, Marchant A, Delvaux A, Goldman M, Robberecht P, Velu T, and Deviere J (1995) Interleukin 10 prevents necrosis in murine experimental acute pancreatitis. *Gastroenterology* **108**:1917–1922.
- Van Reijssen FC, Bruijnzeel Koomen CA, Kalthoff FS, Maggi E, Romagnani S, Westland JK, and Mudde GC (1992) Skin-derived aeroallergen-specific T-cell clones of Th2 phenotype in patients with atopic dermatitis. *J Allergy Clin Immunol* **90**:184–193.
- Vassilakopoulos TP, Nadali G, Angelopoulou MK, Siakantaris MP, Dimopoulou MN, Kontopidou FN, Rassidakis GZ, Doussis-Anagnostopoulou IA, Hatzioannou M, Vaiopoulos G, et al. (2001) Serum interleukin-10 levels are an independent prognostic factor for patients with Hodgkin's lymphoma. *Haematologia* **86**:274–281.
- Vicioso MA, Garaud JJ, Reglier-Poupet H, Lebeaut A, Gougerot-Pocidallo MA, and Chollet-Martin S (1998) Moderate inhibitory effect of interleukin-10 on human neutrophil and monocyte chemotaxis in vitro. *Eur Cytokine Netw* **9**:247–253.
- Volk HD, Asadullah K, Gallagher G, Sabat R, and Grütz G (2001) Interleukin-(IL)-10 and IL-10 homologs: an expanding family of important immune mediators and emerging immunotherapeutic agents. *Trends Immunol* **22**:414–417.
- Volk HD, Reinke P, and Docke WD (2000) Clinical aspects: from systemic inflammation to "immunoparalysis". *Chem Immunol* **74**:162–177.
- Volk T, Dopfner UR, Schmutzler M, Rimpau S, Schnitzler H, Konertz W, Doecke WD, Spies CD, Volk HD, and Kox WJ (2003) Influence of effective perioperative epidural block on IL-10 release. *Cytokine*, in press.
- Vora M, Romero LI, and Karasek MA (1996) Interleukin-10 induces E-selectin on small and large blood vessel endothelial cells. *J Exp Med* **184**:821–829.
- Wang P, Wu P, Siegel MI, Egan RW, and Billah MM (1995) Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J Biol Chem* **270**:9558–9563.
- Weber-Nordt RM, Meraz MA, and Schreiber RD (1994) Lipopolysaccharide-dependent induction of IL-10 receptor expression on murine fibroblasts. *J Immunol* **153**:3734–3744.
- Weinstein GD (1996) Can immunomodulatory molecules work topically for psoriasis? *J Invest Dermatol* **106**:589.
- Westendorp RG, Langermans JA, Huizinga TW, Elouali AH, Verweij CL, Boomsma DI, Vandenbroucke JP, and Vandenbroucke JP (1997) Genetic influence on cytokine production and fatal meningococcal disease. *Lancet* **349**:170–173.
- Willems F, Marchant A, Delville JP, Gerard C, Delvaux A, Velu T, de Boer M, and Goldman M (1994) Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. *Eur J Immunol* **24**:1007–1009.
- Wissing KM, Morelon E, Legendre C, De-Pauw L, LeBeaut A, Grint P, Maniscalchi M, Ickx B, Vereerstraeten P, Chatenoud L, et al. (1997) A pilot trial of recombinant human interleukin-10 in kidney transplant recipients receiving OKT3 induction therapy. *Transplantation* **64**:999–1006.
- Woiciechowsky C, Asadullah K, Nestler D, Eberhardt B, Platzer C, Schöning B, Glöckner F, Lanksch WR, Volk HD, and Docke WD (1998) Sympathetic activation triggers systemic interleukin-10 release in immunodepression induced by brain injury. *Nat Med* **4**:808–813.
- Wojciechowska-Lacka A, Matecka-Nowak M, Adamiak E, Lacki JK, and Cerkaska-Gluszak B (1996) Serum levels of interleukin-10 and interleukin-6 in patients with lung cancer. *Neoplasma (Bratisl)* **43**:155–158.
- Wolk K, Kunz S, Asadullah K, and Sabat R (2002) Cutting edge: immune cells as sources and targets of the IL-10 family members? *J Immunol* **168**:5397–5402.
- Wu MS, Huang SP, Chang YT, Shun CT, Chang MC, Lin MT, Wan HP, and Lin JT (2002) TNF-alpha and IL-10 promoter polymorphisms in Epstein-Barr virus-associated gastric carcinoma. *J Infect Dis* **185**:106–109.
- Xie MH, Aggarwal S, Ho WH, Foster J, Zhang Z, Stinson J, Wood WI, Goddard AD, and Gurney AL (2000) Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. *J Biol Chem* **275**:31335–31339.
- Yagi H, Tokura Y, Furukawa F, and Takigawa M (1996) Th2 cytokine mRNA expression in primary cutaneous CD30-positive lymphoproliferative disorders: successful treatment with recombinant interferon-gamma. *J Invest Dermatol* **107**:827–832.
- Yamakami K, Akao S, Tadakuma T, Nitta Y, Miyazaki J, and Yoshizawa N (2002) Administration of plasmids expressing IL-4 and IL-10 causes Balb/c mice to induce a T helper 2-type response despite the expected T helper 1-type response with low-dose infection of *Leishmania major*. *Immunology* **105**:515–523.
- Yamaoka K, Otsuka T, Niuro H, Nakashima H, Tanaka Y, Nagano S, Ogami E, Niho Y, Hamasaki N, and Izuahara K (1999) Selective DNA-binding activity of interleukin-10-stimulated STAT molecules in human monocytes. *J Interferon Cytokine Res* **19**:679–685.
- Yue FY, Dummer R, Geertsens R, Hofbauer G, Laine E, Manolio S, and Burg G (1997) Interleukin-10 is a growth factor for human melanoma cells and down-regulates HLA class I, HLA class II and ICAM-1 molecules. *Int J Cancer* **71**:630–637.
- Zdanov A, Schalk-Hihi C, Gustchina A, Tsang M, Weatherbee J, and Wlodawer A (1995) Crystal structure of interleukin-10 reveals the functional dimer with an unexpected topological similarity to interferon gamma. *Structure (Lond)* **3**:591–601.
- Zdanov A, Schalk-Hihi C, Menon S, Moore KW, and Wlodawer A (1997) Crystal structure of Epstein-Barr virus protein BCRF1, a homolog of cellular interleukin-10. *J Mol Biol* **268**:460–467.
- Zeller JC, Panoskaltis-Mortari A, Murphy WJ, Ruscetti FW, Narula S, Roncarolo MG, and Blazar BR (1999) Induction of CD4+ T cell alloantigen-specific hyporesponsiveness by IL-10 and TGF-beta. *J Immunol* **163**:3684–3691.
- Zhang J, Chen H, Hu L, Fu J, Zhang H, and Chen Y (2002) Correlation between polymorphism of IL-4 and IL-10 gene promoter and childhood asthma and their impact upon cytokine expression. *Zhonghua Yixue Zazhi* **82**:114–118.
- Zuo Z, Wang C, Carpenter D, Okada Y, Nicolaidou, Toyoda M, Trento A, and Jordan SC (2001) Prolongation of allograft survival with viral IL-10 transfection in a highly histoincompatible model of rat heart allograft rejection. *Transplantation* **71**:686–691.