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AGE-ASSOCIATED INCREASED INTERLEUKIN-6 GENE EXPRESSION, LATE-LIFE DISEASES, AND FRAILTY

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■ **Abstract** Interleukin-6 (IL-6) is a proinflammatory cytokine that is normally tightly regulated and expressed at low levels, except during infection, trauma, or other stress. Among several factors that down-regulate IL-6 gene expression are estrogen and testosterone. After menopause or andropause, IL-6 levels are elevated, even in the absence of infection, trauma, or stress. IL-6 is a potent mediator of inflammatory processes, and it has been proposed that the age-associated increase in IL-6 accounts for certain of the phenotypic changes of advanced age, particularly those that resemble chronic inflammatory disease [decreased lean body mass, osteopenia, low-grade anemia, decreased serum albumin and cholesterol, and increased inflammatory proteins such as C-reactive protein (CRP) and serum amyloid A]. Furthermore, the age-associated rise in IL-6 has been linked to lymphoproliferative disorders, multiple myeloma, osteoporosis, and Alzheimer's disease. This overview discusses the data relating IL-6 to age-associated diseases and to frailty. Like the syndrome of inappropriate antidiuretic hormone, it is possible that certain clinically important late-life changes are due to an inappropriate presence of IL-6.

INTRODUCTION

Interleukin-6 (IL-6) is a multifunctional, proinflammatory cytokine that has been implicated in the pathogenesis of several chronic diseases associated with aging, including osteoporosis, Alzheimer's disease (AD), and neoplasia. IL-6 is tightly regulated and is normally not detected in the serum of healthy young individuals unless there is trauma, infection, or other stress. Under these circumstances, IL-6 is rapidly expressed, and it contributes to a cascade of events typical of inflammation. These include leukocytosis, thrombocytosis, lymphocyte activation,

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acute-phase protein synthesis [including fibrinogen, C-reactive protein (CRP), and amyloid precursor protein], and a general catabolic shift in metabolic pathways.

The regulation of the IL-6 gene has been intensely investigated in recent years. It appears that gene expression can be stimulated by a variety of transcription activators, many of which are commonly associated with acute inflammatory or proliferative states (e.g. NF-κB, NF-IL-6, and c-*fos*). It is also apparent that, in the quiescent state, gene expression is kept in check by a complex network that involves the secondary sex steroids, including estrogen and testosterone (see below). Recently, it has also been discovered that IL-6 levels become detectable in the serum of older individuals, particularly after menopause or andropause, even without signs of illness or inflammation. It has been postulated that this increase relates to the development of frailty and the predisposition to certain diseases (1).

BIOLOGY OF INTERLEUKIN-6

The biochemical characterization of IL-6 came about a decade ago from several different laboratories including those investigating hepatic regeneration, B-cell differentiation and proliferation, myeloma (hybridoma) growth, and T-cell activation [reviewed by Van Snick (2)]. IL-6 is a 26-kDa protein produced by a wide variety of cells. Like IL-1 and tumor necrosis factor (TNF), it is a critical factor in the acute-phase inflammatory response.

The long list of synonyms (Table 1) by which IL-6 has been identified is indicative of its pleiotropic biological activity (3). The molecule was first identified during an effort to isolate and characterize the virus-induced protein inter-

TABLE 1 Synonyms for interleukin-6

B-cell stimulatory factor 2
B-cell differentiation factor
T-cell–replacing factor
Cytotoxic T-cell differentiation factor
Interferon-β_2
Interleukin hybridoma plasmacytoma factor 1
Hybridoma growth factor
26-kDa protein
Plasmacytoma growth factor
Hepatocyte-stimulating factor
Thrombopoietin 2
Macrophage granulocyte-inducing factor

feron-β from cultured human fibroblasts (4). IL-6 is now well recognized for its role in inflammation, characterized by production of a variety of hepatic proteins termed acute-phase proteins (e.g. CRP, serum amyloid A, fibrinogen, complement, and α_1-antitrypsin) (5). In addition to its role in the acute-phase response, IL-6 is important for the development of specific immunologic responses. It induces differentiation of activated, but not resting, B cells (6–8), culminating in production of immunoglobulin (9, 10). In addition to differentiating B cells, IL-6 stimulates proliferation of thymic and peripheral T cells (11, 12), and, with IL-1 (13), induces T-cell differentiation to cytolytic T cells (14, 15) and activates natural killer cells (16). These observations emphasize the importance of IL-6 in both nonspecific and specific immune responses.

In addition to its immunologic/inflammatory role, IL-6 appears to play an important role in bone metabolism by inducing osteoclastogenesis and osteoclast activity (17, 18). Estrogen inhibits IL-6 gene expression (see below), and, in rodent models, in the absence of estrogen, IL-6 activity and bone resorption increase (19–22). The importance of this estrogen–IL-6 interaction is supported by the observation that IL-6 gene knockout mice are protected from cancellous bone loss associated with ovariectomy (23).

IL-6 also influences a number of other important cell types. For example, IL-6 stimulates the endometrial vasculature during the menstrual cycle (24, 25) and promotes spermatogenesis (26). Furthermore, IL-6 stimulates epidermal proliferation (27–29), hemostasis through induction of megakaryocytopoiesis (30–32), and neural cell differentiation and proliferation (33, 34).

THE INTERLEUKIN-6 GENE

The human IL-6 gene has been localized to chromosome 7p21 (35) and consists of 5 exons and 4 introns (36, 37). The human IL-6 molecule contains 212 amino acids, including a hydrophobic signal sequence of 28 amino acids. It includes two potential N-glycosylation sites. Murine IL-6 (chromosome 5) consists of 211 amino acids with a typical signal sequence of 24 residues. It includes no N-glycosylation site but several potential O-glycosylation sites (38).

Interleukin-6 Gene Regulation

Mechanisms of IL-6 gene regulation are incompletely understood. It was demonstrated several years ago that both protein kinase C and cAMP-dependent signal transduction pathways could trigger IL-6 gene expression (39–41). To further explain the wide range of functions of IL-6, the presence of multiple initiation sites and the preferential use of a specific initiation site in a variety of cells (37) suggest that different regulatory mechanisms may be responsible for IL-6 gene expression in different tissues [Table 2 (7, 42–55)]. In fact, sequences similar to several consensus sequences involved in gene activation are present in the 5′

TABLE 2 Factors that induce interleukin-6 promoter activity[a]

Stimulus	Cell type	Transcription factors	Reference
LAM or LPS	Monocyte	NF-IL-6, NF-κB	42
HTLV-I TAX	HTLV-I–infected T cell	NF-kB	7, 43, 44
Hypoxia	Endothelial cells	NF-IL-6	45
PGE_1, cAMP	PU5-1.8 monocyte	AP-1, NF-IL-6, NF-κB	46
LPS	Monocyte	NF-κB	46
HIV-I TAT	B-lymphoblastoid, HeLa (cervical carcinoma)	NF-IL-6, NF-κB	47
Ionizing radiation	Fibroblast	AP-1, NF-κB	48
Mutant p53	HeLa	NF-IL-6	49
Jun, TNF-α, PKC, IL-1, db-cAMP, PMA	HepG2 (hepatocyte), HeLa	Not characterized	50
LTB4	Monocyte	NF-IL-6, NF-κB	51
LIF	Monocyte	NF-κB	52
IL-1α, LPS, cAMP	OCI-LY3 (lymphoma)	Not characterized	53
IL-1α, TNF-α	HeLa, glioblastoma, fibroblasts	NF-κB	54, 55
Forskolin	Fibroblasts	Not NF-κB	54

[a]Abbreviations: cAMP, cyclic AMP; db-cAMP, dibutyryl cAMP; HIV-I, human immunodeficiency virus I; HTLV-I, human T-lymphotropic virus I; IL-1α, interleukin-1α; LAM, lipoarabinomannan; LIF, leukemia inhibitory factor; LPS, lipopolysaccharide; LTB4,leukotriene B4; PGE_1, prostaglandin E_1; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; TNF-α, tumor necrosis factor-α.

flanking region, such as the c-*fos* serum response element, the CCAAT enhancer binding protein (C/EBPβ) domain, and the NF-κB domain (Figure 1), all of which are conserved between human and mouse genes (56). The presence of this highly conserved but complex regulatory region has been interpreted to indicate the importance of IL-6 gene regulation and its varied responses in different tissues subjected to different stimuli.

Glucocorticoids and Interleukin-6 Expression

Glucocorticoids have been shown to inhibit IL-6 production from a variety of tissues (57–59). Initial investigation into the mechanism of glucocorticoid inhibition of IL-6 expression demonstrated that dexamethasone inhibited IL-1–induced transcriptional activation of the proximal 225 base pairs of the IL-6 promoter (60). This finding prompted the examination of the interaction of the glucocorticoid receptor (GR) and the IL-6 promoter (60). GR was found to weakly bind the IL-6 promoter at several important *cis*-acting sites. A DNA-binding

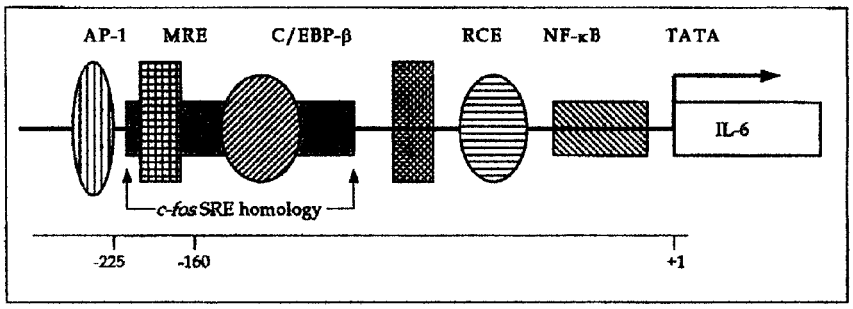

Figure 1 The proximal interleukin-6 promoter. Numbers indicate distance (in base pairs) from the transcription initiation site. MRE, multiple response element; C/EBP-β, CCAAT/enhancer binding protein; RCE, retinoblastoma control element; NF-κB, nuclear factor–κB; SRE, serum response element.

immunoprecipitation assay using HeLa cells indicated that transfected wild-type GR cDNA was capable of binding to the –225 fragment of the IL-6 promoter (61). Additionally, mutation of the GR DNA-binding domain resulted in the loss of GR inhibition of transcriptional activation (61). Thus, it appears that corticosteroid interacts with its receptor, which then binds the IL-6 promoter and inhibits IL-6 gene expression.

However, the interaction between GR and the IL-6 promoter is weak. Thus, alternative mechanisms have been sought to account for GR inhibition of the IL-6 promoter. Ray & Prefontaine (62) questioned whether GR interacted with two transcription factors known to stimulate the IL-6 promoter, NF-κB and NF-IL6. Using murine F9 embryonal carcinoma cells, which are devoid of endogenous NF-IL6 and NF-κB activities, they demonstrated that dexamethasone inhibited IL-6 promoter transactivation induced by transfected p65 (a component of the NF-κB complex) and NF-IL6 (62). In cross-precipitation assays, it was demonstrated that GR bound to p65, but not to NF-IL6. Thus, GR mediates inhibition of p65-induced activation of the IL-6 promoter through protein-protein interactions. This mechanism may occur in combination with the promoter-occlusion mechanism described above.

Still another mechanism of GR inhibition of IL-6 has recently been discovered (64, 65). Dexamethasone was shown to induce the expression of the cytoplasmic inhibitor of NF-κB, IκBα, and this was associated with inhibition of nuclear translocation of NF-κB p65. Thus, it follows that GR-induced IκBα protein synthesis promotes cytoplasmic sequestration of NF-κB, culminating in decreased activation of the IL-6 promoter.

IL-6 induced by stress, infection, or trauma stimulates release of corticotrophin-releasing factor from the hypothalamus, which results in elevated systemic levels of corticosteroids (66, 67). This observation suggests the presence of a CNS-immune-endocrine negative-feedback loop.

Estrogen and Interleukin-6 Expression

The ability of estrogen to repress IL-6 expression was first recognized in human endometrial stromal cells (68). Additional evidence came from the observations that menopause or ovariectomy resulted in increased IL-6 serum levels (69), increased IL-6 mRNA levels in bone cells (70), and increased IL-6 secretion by mononuclear cells (71–73). Direct evidence for estrogen's ability to repress IL-6 expression is derived from studies demonstrating that estradiol inhibited bone marrow stromal-cell and osteoblast IL-6 protein and mRNA production in vitro (20, 74) and that estradiol suppressed osteoclastogenesis in murine bone marrow cultures (20) or ovariectomized mice (21) as effectively as IL-6–neutralizing antibody. These data are somewhat controversial because, in a few studies using human bone-derived cells, estrogen did not inhibit IL-6 expression (75–78). Perhaps species-specific differences account for these conflicting data. The majority of the data available implicate an estrogen-mediated repression of IL-6 expression in a variety of systems.

To define the mechanism of estrogen-induced transrepression of the IL-6 promoter, Pottratz et al (79) and Ray et al (80) performed transient transfection assays using an IL-6 promoter linked to a reporter plasmid. They found that 17β-estradiol inhibited phorbol (an inflammatory stimulus), IL-1, or TNF-induced promoter activation in a murine bone marrow stromal-cell line, which constitutively expresses the estrogen receptor (ER). This was also observed in HeLa cells, which have very little or no endogenous ER. However, in these cells, the transfection of ER DNA was required to observe suppression of the IL-6 promoter by estradiol. Thus, 17β-estradiol inhibits IL-6 gene transcriptional activation by an ER-dependent mechanism.

Because there is no consensus ER element within the IL-6 promoter (as indicated by gene bank analysis), it stands to reason that ER inhibits the IL-6 promoter by a mechanism other than direct binding of ER to the IL-6 promoter (80). Rather, it appears that ER directly inhibits transcription factors and thus negatively influences the IL-6 promoter. For example, Stein & Yang (82) found that ER bound both NF-κB p65 and NF-IL-6. Furthermore, they demonstrated that the ER–transcription factor complex (whether ER-NF-κB or ER-NF-IL-6) inhibited unbound transcription factor enhancer activity through a mechanism that did not include the induction of IκBα. We have documented that estradiol inhibits degradation of IκBα (83). This observation suggests that estradiol promotes sequestration of NF-κB in the cytoplasm. Adding strength to this hypothesis is the recent observation that the NF-IL6–binding site (C/EBPβ) is required for estrogen-mediated repression (84).

Androgens and Interleukin-6 Expression

The first demonstration of the inhibitory action of androgens on IL-6 protein expression was made in a murine bone marrow stromal-cell line that had been stimulated with IL-1 and TNF (85). These findings were extended in HeLa cells,

in which it was demonstrated that dihydrotestosterone (DHT) inhibited PMA-induced activation , albeit to a lesser extent than did estrogen (79). Several adrenal androgens that are not known to bind the androgen receptor (i.e. androstenedione, androstenediol, and dehydroepiandrosterone sulfate) mediate repression of the IL-6 promoter in HeLa cells transfected with the androgen receptor (85). These experiments suggest that even weakly binding androgens can mediate transrepression of IL-6 promoter activation.

We have demonstrated that DHT requires the androgen receptor to inhibit IL-6 promoter (86). DHT inhibited PMA-induced activation of the IL-6 promoter by inhibiting nuclear translocation of NF-κB in a prostate cancer cell line, LNCaP. This was associated with maintenance of cytoplasmic IκBα levels in the presence of PMA. In the absence of androgen, IκBα levels in the cytoplasm decline after PMA stimulation, and the maintenance of IκBα levels in the presence of androgen could be caused by decreased phosphorylation (degradation) or increased production.

Orchiectomy induces bone marrow IL-6 protein and mRNA expression (87). The relevance of the above observations was pointed out by Bellido et al (85) based on an orchiectomized mouse model. Although serum or bone marrow IL-6 levels were not reported, these authors found that orchiectomy resulted in increased replication of bone marrow osteoclast progenitors, which was prevented by administration of IL-6–neutralizing antibody or implantation of a slow-release form of testosterone. However, because testosterone is converted to estradiol, these results are not conclusive evidence of the action of androgen. In fact, the observation of decreased bone density in a male patient who had normal androgen levels but had a mutation resulting in a nonfunctional ER suggests that estrogen's effects on bone in men may be as important as androgen's (88). This hypothesis is supported by the observation that estradiol can inhibit the bone loss that was observed in men treated by orchiectomy for prostate cancer (89). However, the ability of estrogen to inhibit bone loss may be mediated by its transrepression of the IL-6 promoter, and thus, these observations are still consistent with increased IL-6 activity from androgen loss.

THE INTERLEUKIN-6 RECEPTOR

The human IL-6 receptor (IL-6R; also known as gp80 or the IL-6Rα subunit) was first cloned from a human natural-killer–like cell line, YT (89), followed by a cloning from a human hepatoma cell line, HepG2 (90). IL-6R is an 80-kDa protein consisting of 467 amino acids. Located on chromosome 1 band q21 (91), the IL-6R gene encodes for a 5-kb mRNA containing a coding region of 1401 base pairs (89).

The structure of IL-6R has been deduced by comparative sequence analysis. A hydropathy plot revealed two major hydrophobic regions: one that encodes for the signal peptide between residues 1 and 20, and another that encodes for the

transmembrane domain in the region of residues 359–386 (89). The latter region is followed by a putative transmembrane anchoring stop codon consisting of several positively charged residues. Thus, IL-6R has a 339–amino-acid extracellular region, a 28–amino-acid transmembrane region, and an 82–amino-acid intracellular region. Intriguingly, the intracellular region does not contain any kinase domains, making it likely that this molecule is not capable of signaling activity (89).

A homology search indicated that the extracellular component of IL-6R contains a domain that shares extensive homology with the immunoglobulin superfamily (89) and two tandem fibronectin type-III motifs (92) present in a 200–amino-acid region. This region defines a domain that is found in a variety of other cytokine and growth factor receptors. It contains highly conserved components consisting of four cysteine residues in its amino-terminal region and a tryptophan-serine-X-tryptophan-serine (WSXWS) motif penultimate to the transmembrane region (92, 93). Fibronectin type-III domains are observed in cell adhesion molecules, and their presence has been interpreted to suggest that cytokine receptors evolved from an ancestral adhesive molecule (94).

As the protein structure suggests, IL-6R is not capable of inducing signal transduction directly. It is now understood that, in order to mediate signal, IL-6 first binds to gp-80, forming a low-affinity receptor complex. This complex then associates with the non-ligand-binding transmembrane glycoprotein gp-130 (95). Homodimerization of gp-130 is required for IL-6 signal transduction (96). Although it was originally believed that one unit of IL-6 and IL-6R bound to a gp-130 homodimer (96), the complex now appears to be hexameric, consisting of two molecules each of IL-6, IL-6R gp-80, and gp-130 (97). This complex provides high-affinity binding of IL-6, as opposed to the low-affinity binding of IL-6 and IL-6R gp-80 in the absence of gp-130.

IL-6R has an isoform that was first identified in human urine, the soluble IL-6R (sIL-6R) (98). In contrast to other soluble cytokine receptors (e.g. sIL-2R), which inhibit cytokine-induced signaling, sIL-6R forms a fully active hexameric IL-6:sIL-6R:gp-130 complex capable of inducing signal transduction. How sIL-6R is generated is not currently established, but both alternative splicing, resulting in loss of the transmembrane domain (99), and proteolysis of the mature cell surface IL-6R (100, 101) have been proposed.

Expression of the Interleukin-6 Receptor

IL-6R is expressed in a variety of cells including B-lymphocytes, hepatocytes, prostate cells, and osteoblasts. In general it is expressed at ~100–2000 sites/cell (102). However, up to 29,000 sites/cell have been identified in myeloma lines and Epstein-Barr virus-transformed cells (102). Except for the effects of dexamethasone, modulation of IL-6R expression by various factors has not been consistently demonstrated. Snyers et al (102) found that A23187 (a calcium

ionophore), lipopolysaccharide, prostaglandin E_1, IL-1, tumor necrosis factor (TNF), and muramyl dipeptide did not significantly alter IL-6R expression in a variety of cell lines. However, other investigators have found that IL-1 modulated IL-6R expression in various cell lines and tissues (102). Perhaps cell-specific differences in response account for these discordant results.

In contrast to these inconclusive results, dexamethasone has been repeatedly demonstrated to increase IL-6R in several tissue types, including liver primary cells and cell lines, monocyte primary cultures, myeloma cell lines, and an amniotic cell line (100). In addition to these in vitro observations, IL-6R mRNA expression has been documented to increase in murine bone marrow stroma postovariectomy (103).

Despite the great variety of cells that express IL-6R and its importance in many facets of physiology, the molecular mechanisms that regulate transcriptional control of the IL-6R gene have not been defined to date. Cloning and analysis of the IL-6R promoter will help in this analysis.

INTERLEUKIN-6 AND "NORMAL" AGING

A wealth of data indicate that IL-6 gene expression, as well as tissue and serum levels, increases with age [Table 3 (69, 71, 104–115)]. Early observations (104) demonstrate an age-associated rise in IL-6 in autoimmune-prone mice. This increase is considered an antecedent to the development of the lupus erythematosus-like syndrome characteristic of that strain. Subsequently, other laboratories demonstrated a similar age-associated increase in IL-6 in "normal" (i.e. non–disease-prone) strains of mice and also in rats, monkeys, and humans (Table 2).

The increase in IL-6 has not been fully explained, but it may result at least partially from coincident age-associated diseases in which elevated IL-6 has been described (see below). However, increases in circulating IL-6 have been demonstrated in apparently healthy older individuals. One proposed mechanism is the reduced influence of the normally inhibiting sex steroids on endogenous IL-6 expression (as described above). Accordingly, at the time of menopause or andropause, IL-6 gene expression is not as tightly regulated, inappropriate expression occurs (at least in some tissues), and serum levels rise. The biological importance of this rise in IL-6 has yet to be fully established but, at least for certain age-associated diseases (such as osteoporosis, neoplasia, and AD), preliminary investigations have suggested an important role. Thus, it is possible that the age-associated rise in IL-6 is of physiological consequence, rendering an individual susceptible to the myriad of processes induced by this proinflammatory signal, including lymphoproliferation, osteoclast stimulation, and a metabolic orientation towards catabolic processes.

TABLE 3 Interleukin-6 and age[a]

Group (reference)	Species	Finding(s)
Tang et al (104)	MRL/lpr mice	Serum levels increase with age
Suzuki et al (105)	MRL/lpr mice	Serum soluble IL-6 receptors are elevated with age
Effros et al (106)	C3B10F_1 mice	LPS-stimulated peritoneal macrophage IL-6 levels rose with age
Zhou et al (107)	C57BL/6J mice	Increased production of IL-6 by spleen cells from old mice
Foster et al (108)	Fisher 344 rats	LPS-stimulated peripheral MNC IL-6 production increased with age
Wei et al (109)	Humans	Serum IL-6 levels elevated in old people but no age differences in IL-1 levels
Goya et al (110)	Mice	Growth-hormone–treated old mice had higher IL-6 levels than similarly treated middle-aged or young mice
Daynes et al (71)	BALB/c and 3H/HeN mice and humans	Serum IL-6 levels rise through the lifespan
Ershler et al (111)	Rhesus monkeys	Serum IL-6 levels rise through the lifespan
	Humans	Serum IL-6 levels elevated in healthy old people
Sindermann et al (112)	Humans	IL-6 (but not interferon or IL-2) synthesis in PHA-stimulated lymphocyte cultures was greater from old compared with young healthy volunteers
McKane et al (113)	Humans	Serum IL-6 levels rise with age in healthy women
Fagiolo et al (114)	Humans	Increased IL-6 in vitro production by cells from healthy old people
Liao et al (115)	Humans	Urine IL-6 levels increase with age
Kania et al (69)	Humans	IL-6 levels increase post-menopause

[a]LPS, lipopolysaccharide; MNC, mononuclear cell; PHA, phytohemagglutinin.

INTERLEUKIN-6 AND CERTAIN AGE-RELATED DISEASES

Lymphoproliferation and Lymphoproliferative Disorders

IL-6 stimulates B-cell maturation and proliferation, and its overproduction has been demonstrated in a variety of malignancies of B-cell lineage (including multiple myeloma (116–118), non-Hodgkin's lymphoma (119, 120), and chronic

lymphocytic leukemia (121). In addition, other lymphoproliferative diseases, including Castleman's disease (122, 123), Hodgkin's disease (120, 124), angioimmunoblastic lymphadenopathy with dysproteinemia (125), and certain non–B-cell lymphomas (126), are typically associated with measurably high circulating levels of this cytokine. Although this may be an important clue to the pathogenesis of these disorders, the high IL-6 level may be of additional clinical importance because it has been shown to correlate with the presence of prominent constitutional symptoms (night sweats, fever, weight loss, etc) (120), stage of disease (119, 127, 128), and survival (128).

Both experimental data and clinical observations suggest that IL-6 may be involved in the pathogenesis of some or all of these diseases. For example, it has been demonstrated in certain disorders that the neoplastic cells in culture were stimulated to proliferate in the presence of recombinant IL-6 (129). Furthermore, lymphoma and myeloma cells have been shown, both in vivo and in vitro, to produce IL-6 (130–133), and the level of IL-6 production by these neoplastic cells correlates with serum levels (133) and with survival (132).

The potential importance of IL-6 in the development of B-cell malignancies has also been demonstrated in animals, e.g. C57Bl/6 mice (Figure 2), over 50% of which will develop lymphoma by 24 months of age (134). We showed that

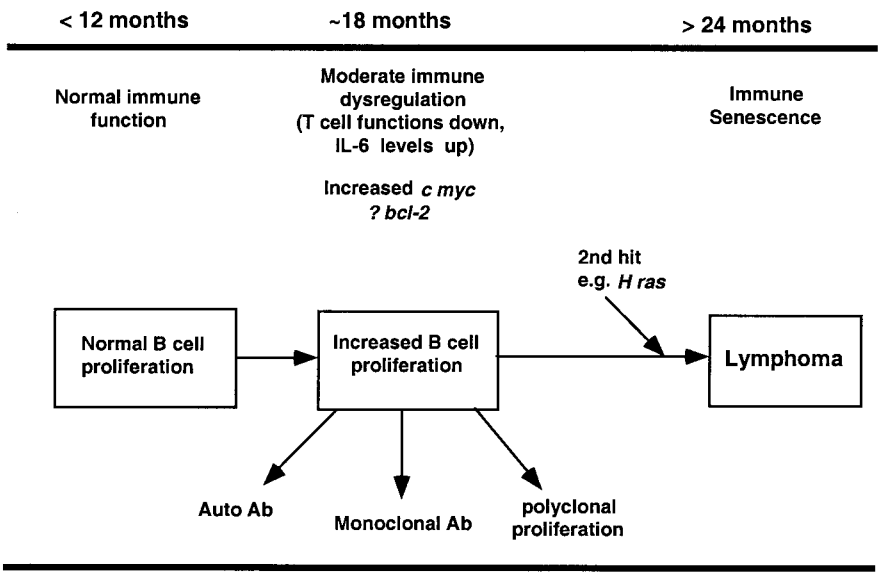

Figure 2 Interleukin-6 and lymphoma development. This model proposes that the rise in IL-6 level that occurs in middle-aged mice is the antecedent to a polyclonal lymphoproliferative disorder and that a second oncogenic event, such as mutation and activation of the c-*myc* oncogene, is required for lymphoma development. Ab, antibody(ies).

the appearance of IL-6 with advancing age correlated with increased lymphoid proliferation, c-*myc* expression, and ultimately lymphoma development in this strain of mice (135). Lymphomas occurred only in mice known to have prior IL-6 elevations, and mice on controlled caloric restriction [which is known to markedly reduce lymphomagenesis and prolong maximal lifespan in rodents (136)] had both lower IL-6 levels and less lymphoma development. Similarly, splenocytes from AKR mice destined to develop B-cell lymphomas have markedly elevated levels of IL-6 (and IL-1) before developing overt disease, and lymphoma occurrence can be significantly inhibited by in vivo administration of monoclonal antibody to IL-6 (137). Finally, plasma cell tumors that can readily be induced in normal mice could not be induced in IL-6–deficient mice (IL-6 knockouts generated by introduction of a germline-encoded null mutation in the IL-6 gene) (138). In those experiments, mice that were homozygous (+/+) or heterozygous (+/−) for the wild-type IL-6 allele yielded the typical high incidence of plasmacytoma, but mice homozygous for the IL-6 null allele (−/−) were completely resistant.

The importance of IL-6 in the pathogenesis of multiple myeloma in humans has been suspected for many years, perhaps because it was initially identified as a necessary factor for myeloma cell growth in vitro (117, 139). IL-6 levels are high in patients with multiple myeloma, and myeloma cells themselves have high levels of IL-6 receptors (140). Furthermore, serum IL-6 levels have been correlated with myeloma tumor cell mass (141) and disease severity (142), although there is some controversy about this (143).

A recent report sheds light on the importance of IL-6 dysregulation in multiple myeloma. The cell surface molecule, CD44, on plasma (and myeloma) cells mediates binding of tumor cells to stroma and regulates IL-6 production. Variants of this molecule are produced by alternative splicing of the nuclear RNA, and these variants express additional protein-binding domains, primarily in the extracellular region of the molecule. In one series, expression of specific variants (determined by immunohistochemical staining) was observed in more than one third of myeloma patients (144), and this was associated with advanced, more rapidly progressive disease and shorter survival.

Overwhelming evidence has indicated that IL-6 stimulates proliferation of tumor cells of the B-cell lineage. This conclusion has led to the development of IL-6 inhibitors as a treatment strategy. Klein et al (145, 146) treated nine multiple-myeloma patients with anti–IL-6 monoclonal antibody and found dramatic blockage of myeloma cell proliferation as well as reduction of toxicity related to overproduction of IL-6 in vivo (fever, hypercalcemia, and acute-phase proteins). The same group treated a plasma-cell leukemia patient with anti–IL-6 monoclonal antibody, again finding blockage of myeloma cell proliferation and reduction in serum calcium, serum monoclonal immunoglobulin G, and serum C-reactive protein (CRP), as well as slight improvement in performance status (147). It has also been demonstrated that other agents (125, 148), including corticosteroids (149), are capable of inhibiting IL-6 production in vitro. It has been hypothesized that

the efficacy of corticosteroids in treating patients with multiple myeloma could, at least in part, be due to their ability to inhibit IL-6 production. It is possible that other agents will be found to specifically inhibit IL-6 production or IL-6 receptor function (150), and these may also have efficacy in the treatment of multiple myeloma.

Osteoporosis

It is now clear that several cytokines and growth factors contribute to the bone-remodeling process, including IL-1, IL-6, IL-11, and TNF. IL-6 has received special attention because it is the only one of these cytokines that has consistently been demonstrated to be elevated with age. Furthermore, both IL-6 and IL-6R have been recognized as important normal contributors to the process of bone remodeling (151–153).

It is now well accepted that IL-6 stimulates bone-resorbing osteoclasts, but its effect on osteoblasts remains unclear. For example, some investigators have demonstrated that IL-6 has a moderate effect on osteoblast proliferation (154) and differentiation (155, 156), whereas others have not observed these effects (157, 158). Accordingly, it is likely that the most important effect on bone is enhanced osteoclast activity.

IL-6 and IL-6R enhance osteoclastogenesis. Parathyroid hormone, a well-recognized inducer of bone resorption, stimulates IL-6 production in bone (59, 152, 159, 160). In addition to parathyroid hormone, other substances that stimulate bone remodeling [e.g. bradykinin (161) and bone morphogenic protein-2 (162)] also stimulate IL-6 production by human osteoblast-like cells. Although occasional reports suggest only a limited role for IL-6 in bone resorption (163, 164), the majority of published findings conclude the opposite. For example, IL-6 induces differentiation of bone marrow mononuclear cells to osteoclasts in long-term human marrow cultures (17) and in cocultures of murine bone marrow and primary osteoblastic cells (18). The addition of IL-6R was required to observe this effect in vitro (18). Moreover, conditioned media from marrow cultures obtained from patients with Paget's disease (characterized by increased osteoclastogenesis) stimulated osteoclast-like cell formation in normal human marrow cultures. This was reversed by the addition of neutralizing antibody to IL-6 (165). IL-6–neutralizing antibody also blocks bone resorption induced by a variety of agents including TNF (20, 166). Along with increasing osteoclast numbers, IL-6 has been shown to stimulate bone resorption in fetal mouse metacarpi (160), calivaria (156), and bone resorption pit assays (165, 167).

The importance of IL-6 dysregulation in the development of osteoporosis is highlighted by Bellido et al (85), who demonstrated that orchiectomy increased osteoclastogenesis in mice but that this increase was inhibited by androgen replacement or IL-6–neutralizing antibody. Furthermore, osteoclastogenesis was not observed in orchiectomized mice deficient in IL-6 production (IL-6 knockouts). This study must be interpreted with caution because one must assume that

the bone in IL-6–deficient mice is not representative of normal bone. However, the study provides strong evidence for the role of androgens on IL-6–mediated osteoclastogenesis. The findings also gain significance in conjunction with the earlier observation (23) that ovariectomized IL-6–knockout mice maintain bone density, unlike control mice.

Taken together, these observations provide substantial support for the hypothesis that certain proinflammatory cytokines (especially IL-1 and IL-6) are important both in the normal bone-remodeling process and in the pathogenesis of postmenopausal osteoporosis. The interesting pattern of increased late-life expression of IL-6 lends further support. It is now clear that estrogens and androgens are important inhibitors of IL-6 gene expression, and it is likely that hormone replacement therapy mediates bone preservation by effecting this inhibition. Future strategies to specifically regulate menopausally dysregulated IL-6 are the focus of great interest and research at this time.

Alzheimer's Disease

IL-6 contributes to the pathogenesis of AD. Proinflammatory cytokines (including IL-1 and IL-6) may also contribute to the development of AD (165). One theory on the pathogenesis of AD postulates that IL-6 mediates neurodegeneration by potentiating chronic inflammation in the brain (168–170). This is supported by the observation that IL-6 and certain acute-phase proteins induced by IL-6 [e.g. α2-macroglobulin, CRP, and antichymotrypsin (171)] are found in either amyloid deposits or brain tissue extract of AD patients (172–175). Yet, with the exception of one report (176), IL-6 levels have not been found to be increased in the cerebrospinal fluid (173, 177, 178) or serum (178, 179) of AD patients, raising the likelihood that IL-6 production and activity are localized to the relevant microenvironment.

IL-6 secreted by microglial and/or neuronal cells (180, 181) may induce synthesis of certain acute-phase proteins, including amyloid precursor protein (182). Amyloid precursor protein itself can induce the expression of IL-1, but not of IL-6, by neuronal cells (181). However, IL-6 has been found in the early stage of plaque formation (diffuse plaques) (183, 184), whereas none of the other cytokines tested (including IL-1 and IL-2) were detected (175, 181, 184, 185). In fact, IL-1, which can induce IL-6 expression, was found not to be elevated in brain from AD patients, whereas IL-6 was elevated (175).

There may be some evidence that IL-6 is specifically involved in AD dementia, distinct from other forms of dementia. IL-6 secretion by peripheral blood mononuclear cells was increased in patients with AD as opposed to normal control subjects or those suffering from probable vascular dementia (186, 187), and IL-6 was found in plaques of AD patients who suffered from dementia as opposed to those who did not (184). In addition to these observations, the overexpression of IL-6 in the brain in transgenic mice has been associated with a variety of neuropathologic findings, including gliosis and selective disruption of cholinergic

transmission in the hippocampus (188–193). This latter finding is of particular interest in light of the cholinergic model of AD.

Thus, there is strong evidence that IL-6 overexpression plays an important role in the development of AD. Whether the mechanism for the IL-6 overexpression in the brain of AD patients differs from the overexpression found with advancing age is unknown (183). However, it is clear that determining this mechanism will improve understanding of the pathogenesis of AD and may lead to new treatment strategies. In this regard, it is tempting to postulate that the mechanism of the positive effects of hormone replacement therapy (194) and nonsteroidal anti-inflammatory drugs (195) is their inhibition of IL-6 expression.

INTERLEUKIN-6 AND FRAILTY

In late life, IL-6 levels rise, presumably because of a loss of the influence of the inhibitory secondary sex steroids. The predicted musculoskeletal and metabolic consequences include a pattern of changes attributed to chronic inflammatory disease or frailty. Features of frailty that may be related to increased IL-6 include decreased lean body mass, anemia, thrombocytosis, decreased bone mineral density, decreased serum cholesterol and albumin, and dementia. That such changes could be produced by IL-6 may be supported by a study of young and middle-aged rhesus monkeys that received relatively low doses of IL-6 daily for 1 month. These animals lost 10% of their body weight (primarily lean body weight as determined by dual energy X-ray absorptiometry), became osteopenic and anemic, and had significant drops in serum albumin and cholesterol and corresponding rises in CRP and alkaline phosphatase (196).

In a large series of healthy humans across the lifespan, increasing IL-6 with age was associated with functional declines typical of frailty (197). Thus, the age-associated rise in IL-6 may well explain the "chronic inflammation" appearance of some elderly individuals, even in the absence of an inflammatory focus.

SUMMARY

IL-6 is one of several proinflammatory cytokines. Under normal circumstances in young individuals, its expression is tightly regulated by the interplay of several transcription factors and hormonal factors including the secondary sex steroids and glucocorticoids. Unlike the expression of IL-1 and TNF, which are in the same class of proinflammatory cytokines, IL-6 expression increases in late life. This increase is thought to result from loss of the normally inhibiting sex steroids. It has been proposed that many of the features of frailty (particularly those that resemble chronic inflammatory disease) are the result of this age-associated dysregulation of IL-6. Prospective long-term clinical trials testing the effects of inhib-

itors of IL-6, such as estrogens, androgens, and nonsteroidal anti-inflammatory drugs, on the development of frailty will be of great interest.

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Annual Review of Medicine
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CONTENTS