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MOLECULAR AND CELLULAR BIOLOGY OF INTERLEUKIN-6 AND ITS RECEPTOR

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TABLE OF CONTENTS

1. ABSTRACT

Interleukin-6 (IL-6) is a member of the family of cytokines collectively termed "the interleukin-6 type cytokines." Among its many functions, IL-6 plays an active role in immunology, bone metabolism, reproduction, arthritis, neoplasia, and aging. IL-6 expression is regulated by a variety of factors, including steroidal hormones, at both the transcriptional and post-transcriptional levels. IL-6 achieves its effects through the ligand-specific IL-6 receptor (IL-6R). Unlike most other cytokine receptors, the IL-6R is active in both membrane bound and soluble forms. Defining mechanisms to control IL-6 or IL-6R expression may prove useful for therapy of the many clinical disorders in IL-6 plays a role.

2. INTRODUCTION

Interleukin-6 (IL-6) contributes to a myriad of physiologic and pathophysiologic processes. Because of the large scope of its effects, the cellular and molecular biology of IL-6 has been explored by a variety of investigators representing a great number of basic biological and medical fields. In this review, we will describe the cellular and molecular biology of IL-6 and its receptor, delineate sources and targets of IL-6 and the IL-6 receptor (IL-6R), and correlate the basic biology of IL-6 with its role in pathophysiology.

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3. AN OVERVIEW OF INTERLEUKIN-6

3.1. Physiology and pathophysiology of interleukin-6

IL-6 is involved in a myriad of biologic processes, perhaps explaining its long list of synonyms (B-cell stimulatory factor-2, B cell differentiation factor, T cell-replacing factor, interferon-β_2, 26-kDa protein, hybridoma growth factor, interleukin hybridoma plasmacytoma factor 1, plasmacytoma growth factor, hepatocyte-stimulating factor, macrophage granulocyte-inducing factor 2, cytotoxic T cell differentiation factor, thrombopoietin) (1). Though not an exclusive representation, the several biologic activities of IL-6 are depicted in Figure 1.

Interleukin-6, termed at the time interferon-β_2, was first cloned during an effort to isolate and characterize the viral-induced protein interferon-β. This strategy included treating cultured human fibroblasts with the double-stranded RNA, poly(I)·poly(C), which mimics viral activity (2). IL-6

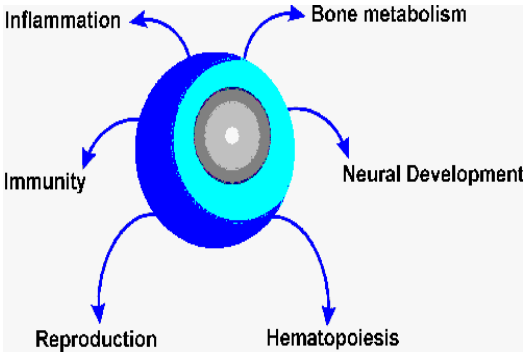

Figure 1: Roles of Interleukin-6 in Physiology

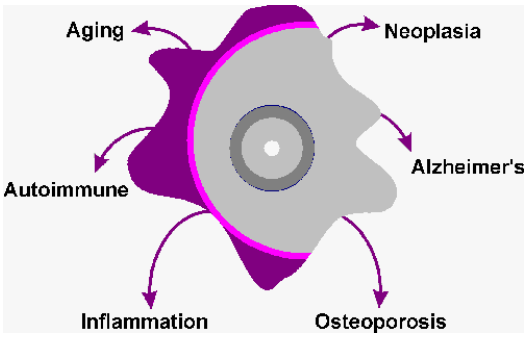

Figure 2: Roles of Interleukin-6 in Pathophysiology

is now well recognized for its role in the acute phase inflammatory response which is characterized by production of a variety of hepatic proteins termed acute phase proteins (*e.g.*, C-reactive protein, serum amyloid A, fibrinogen, complement, α_1-antitrypsin) (reviewed in (3)). In addition to its role in the acute phase response, IL-6 is important for the development of specific immunologic responses. IL-6 induces differentiation of activated, but not resting, B cells (4-6) culminating in production of immunoglobulin (7, 8). Along with B cell differentiation, IL-6 stimulates proliferation of thymic and peripheral T cells (9, 10) and in cooperation with IL-1 (11), induces T cell differentiation to cytolytic-T cells (12, 13) and activates natural killer cells (14). These observations emphasize the importance of IL-6 in both non-specific and specific immune responses.

In addition to its immunologic/inflammatory role, IL-6 appears to play an important role in bone metabolism through induction of osteoclastogenesis and osteoclast activity (15, 16). In rodents, inhibition of IL-6 gene expression is in part responsible for estrogen's ability to inhibit osteoclast activation (17-20). These findings are further supported by the observation that IL-6 gene knockout mice are protected from cancellous bone loss associated with ovariectomy (21).

In addition to the activities described above, IL-6 functions in a wide variety of other systems including the reproductive system by participating in menses (22, 23) and spermatogenesis (24), skin proliferation (25-27), megakaryocytopoiesis (28-30), macrophage differentiation (31-33), and neural cell differentiaion and proliferation (34, 35).

Because of its multidimensional and complex actions, dysregulation of IL-6 results in a myriad of disorders (summarized in Fig. 2) including a variety of neoplastic processes. For example, it may affect cancer progression by its actions on cell adhesion and motility (36), thrombopoiesis (30, 37), tumor specific antigen expression (38) and cancer cell proliferation. Depending on the cell type and the presence or absence of IL-6R, IL-6 can either inhibit (39-41) or stimulate (42) cancer cell proliferation. A great variety of tumor types are stimulated by IL-6, including melanoma (43), renal cell carcinoma (44, 45), prostate carcinoma (46), Kaposi's sarcoma (47), ovarian carcinoma (48), lymphoma and leukemia (49-51), and multiple myeloma (52-59). In many of these tumors, IL-6R have been detected and a direct proliferative signal has been proposed. Yet, when tumor cells are devoid of IL-6R, a tumor inhibiting effect of IL-6 has been demonstrated, presumably because of its immune enhancing properties.

Recently, IL-6 like other cytokine and growth factors (*e.g.*, IL1-α, IL1-β, and TNF-α) has been shown to contribute to the bone remodeling process (for review see references (60, 61)). IL-6 exerts its effect on bone by stimulating osteoclast progenitor cell differentiation and osteoclast proliferation as mentioned above. 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 and this was reversed by addition of neutralizing antibody to IL-6 (62). IL-6 neutralizing antibody also blocks bone resorption induced by a variety of agents including TNF (18, 63). In addition to increasing osteoclast numbers, IL-6 has been shown to stimulate bone resorption in rat long bones (64) and fetal mouse metacarpi (65) , calvaria (66), and bone resorption pit assays (62, 67). Although it is not clear that IL-6 alone is sufficient to mediate these activities (68), these data demonstrate the importance of IL-6 in enhancing osteoclastic activity thus providing a mechanism for IL-6 promoting osteoporosis.

Although a normal physiologic process, aging is accompanied by a variety of disorders (reviewed in (69)) including, Alzheimer's disease, arteriosclerosis, and thyroiditis. Because IL-6 levels are directly correlated with aging in a variety of species (reviewed in (70)), it may play an important role in the aging process. Intriguingly, dietary

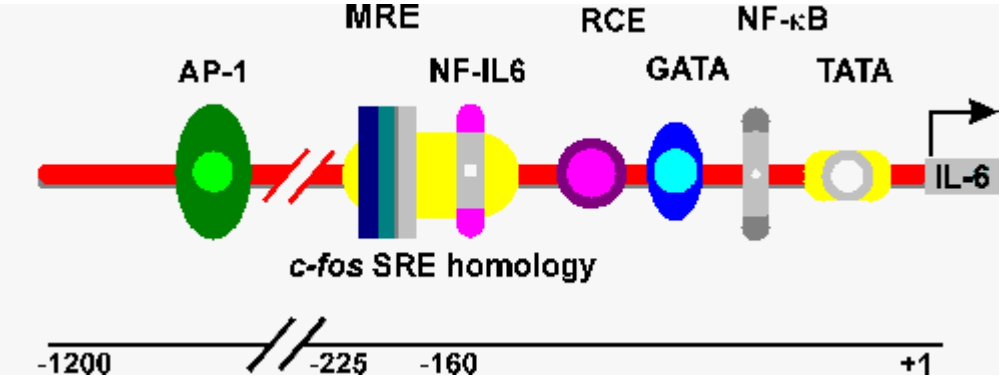

Figure 3: Schematic representation of the IL-6 promoter. See text for abbreviation definitions.

restriction, the only experimental intervention that reproducibly prolongs maximum lifespan in mammals (71) can restore to the young phenotype a variety of physiologic parameters, including IL-6 secretion and serum levels.(72, 73). Similarly, DHEA, currently thought to influence various aging processes (74), also has been shown to diminish the age-associated rise in serum IL-6 (75).

IL-6 may be an important mediator of several infectious and autoimmune diseases. These include human immunodeficiency virus (76, 77), rheumatoid arthritis (78), Castleman's disease (79, 80), and the paraneoplastic symptoms associated with cardiac myxoma (81-83). Furthermore, elevated serum and cerebrospinal fluid levels of IL-6 can be found in sepsis (84, 85). Inflammatory joint disease, particularly rheumatoid arthritis (78), is associated with increased synovial fluid levels of IL-6 (86).

In spite of the great variety of health consequences associated with IL-6, it manifests its activity by binding to a specific receptor, the IL-6R, which is described below.

3.2. Interleukin-6 structure and function

Human IL-6 has a molecular weight of between 21 to 28 Kd depending on post-translational processing such as glycosylation and phosphorylation (87, 88). The IL-6 peptide contains 212 amino acids (aa) of which a 28 aa hydrophobic signal peptide is cleaved off resulting in a mature protein of 184 aa. Even though the homology between human and mouse IL-6 is 65% at the nucleic acid level and only 42% at the amino acid level (89), human IL-6 can

Table 1. Factors which induce IL-6 promoter activity.

Stimulus	Cell type	Transcription factors	Reference
LAM* or LPS	Monocyte	NF-IL6, NF-κB	(100)
HTLV-I TAX	HTLV-I infected T cell	NF-κB	(101-103)
Hypoxia	Endothelial cells	NF-IL6	(104)
PGE^1, cAMP	PU5-1.8 monocyte	AP-1, NF-IL6, NF-κB	(105)
LPS	Monocyte	NF-κB	(105)
HIV-I TAT	B-lymphoblastoid, HeLa	NF-IL6, NF-κB	(76)
Ionizing radiation	Fibroblast	AP-1, NF-κB	(106)
Mutant p53	HeLa	NF-IL6	(107)
Jun, TNF-α, PKC, IL-1, db-cAMP, PMA	HepG2, HeLa	Not characterized	(108)
LTB4	Monocyte	NF-IL6, NF-κB	(109)
LIF	Monocyte	NF-κB	(110)
IL-1α, LPS, cAMP	OCI-LY3 (lymphoma)	Not characterized	(111)
IL-1α, TNF-α	HeLa, glioblastoma, fibroblasts	NF-κB	(112, 113)
Forskolin	Fibroblasts	Not NF-κB	(112)

* Abbreviations: cAMP, cyclic AMP; db-cAMP, dibutyryl cyclic AMP; HIVI, human immunodeficiency virus I; HTLV-I, human T-lymphotropic virus I; L-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.

Table 2. Characterization of factors which repress IL-6 promoter activity.

Repressor	Induction	Cell type	Transcription factors	Reference
Wildtype p53	Serum, IL-1α, PRV	HeLa	NF-IL6	(107, 114)
Mutant p53	Serum, IL-1α, PRV	HeLa	NF-IL6	(114)
Adenovirus E1A	TNFα, IL-1α, PMA, db-cAMP, PKA, Jun	HepG2, HeLa	NF-κB	(108)
c-Fos	Serum, IL-1α	HeLa	Fos	(115)
Rb	Serum	HeLa	Rb	(114)
Estradiol	PMA	HeLa, bone stromal cells	ER	(116, 117)
DHT	PMA, Rel family proteins	HeLa	AR through NF-κB	(117, 118)
Glucocorticoids	TNFα, forskolin, TPA, IL-1α, PRV	HeLa, F9, CCL-202 Lung fibroblast	GR through NF-κB	(119-121)

* Abbreviations:: db-cAMP, dibutyryl cyclic AMP; IL-1α, interleukin-1α; PKA, protein kinase A; PMA, phorbol 12-myristate 13-acetate; PRV, pseudorabies virus; TNFα, tumor necrosis factor α.

stimulate murine IL-6 responsive cells. This may be due to the highly conserved central region (57% homology at the amino acid level) of the molecule which contains four cysteine residues that can be perfectly aligned between mouse and human IL-6 (90). Additionally, the carboxy-terminus appears to be critical for IL-6 activity (91, 92). When just four aa were deleted from the carboxy-terminus, IL-6 activity was completely lost (93). In contrast, deletion of 28 aa from the amino-terminus did not affect IL-6 activity (94).

The human IL-6 gene, located on chromosome 7p21 (95-97), is approximately 5 Kb (compared to 7 Kb for the mouse (98)) and consists of four introns and five exons (99). The human IL-6 gene contains three transcriptional initiation sites which correspond with three TATA-like sequences (99).

3.3. Control of interleukin-6 promoter activity

Characterization of the IL-6 gene 5' flanking region has revealed a very complex control region. The importance of this region is underscored by the observation that the proximal 300 bp of the human and murine IL-6 gene 5'-flanking region share approximately 80% homology (98). Figure 3 summarizes the regulatory elements in the IL-6 promoter. Tables 1 and 2 summarize factors which induce or repress the IL-6 promoter, respectively.

Briefly, several *cis*-acting response elements mediate activation of the IL-6 promoter including those for AP-1, nuclear factor IL-6 (NF-IL6), NF-κB, and the multiple response element (MRE), The MRE and NF-IL6 response element are components of the serum response element (SRE). The SRE was first identified in *c-fos* and induces gene transcription when serum-starved cells are exposed to serum (122, 123). The MRE confers induction of the IL-6 promoter to TPA, serum, forskolin, IL-1α, and TNF (115). Repression of the IL-6 promoter can be mediated by various combinations of *trans*-acting factors and *cis*-acting elements including Fos binding to the SRE, retinoblastoma protein binding to the retinoblastoma control element (RCE), and a variety of steroids (described below)

4. STEROIDS AND REGULATION OF INTERLEUKIN-6 EXPRESSION

4.1. Glucocorticoid and interleukin-6 expression

Glucocorticoids repress expression of a variety of genes including proliferin, pro-opiomelanocortin, prolactin, and the a-subunit of glycoprotein hormone. Similarly, glucocorticoids inhibit IL-6 expression. During times of stress or inflammation IL-6 levels are increased. IL-6, in turn, can induce release of corticotrophin-releasing factor (124, 125), which results in elevated systemic levels of corticosteroids. These findings along with the observations that natural and synthetic corticosteroids inhibit IL-6 production from a variety of tissues (126-129), provide a mechanism for a negative-feedback loop. It was these observations along with the availability of the IL-6 promoter which were the impetus to analyze how glucocorticoid mediates repression of IL-6 expression at the molecular level. The initial studies demonstrated that dexamethasone could inhibit IL-1-induced transcriptional activation of the proximal 225 bp of the IL-6 promoter (130). Additionally, it was observed that dexamethasone abrogated the activity of the thymidine kinase (TK) minimal promoter fused downstream of either MRE I and MRE II when induced by IL-1, phorbol ester, or forskolin. These findings taken together with the observation that the glucocorticoid receptor (GR) could inhibit pseudorabies virus-induced activation of the proximal 110 bp fragment of the IL-6 promoter, in which the MRE had been deleted, prompted the

investigators to examine for interaction of GR and the IL-6 promoter by DNAse I footprinting (130). They found that GR protected the MRE, the TATA box, the major transcription initiation site (similar to the initiator (Inr) (131)), and an as yet functionally uncharacterized region between -201 to -210. These findings were further supported by results from a DNA-binding immunoprecipitation assay which showed that cell extract from HeLa cells transfected with wildtype GR cDNA was capable of binding to the -225 fragment of the IL-6 promoter (albeit not as strongly as to the GRE in the MMTV promoter) (119). Additionally, mutation of the DNA binding domain resulted in loss of GR's ability to repress transcriptional activation (119). These data are compatible with a model in which corticosteroid activates GR which then occludes the IL-6 promoter at these activation sites, thus blocking the binding of positive-acting and basal transcription factors to the IL-6 promoter.

Because the above studies demonstrated that GR bound weakly to the IL-6 promoter and it had been previously documented that GR was capable of protein:protein interactions with c-Jun (132, 133), Ray *et al.* examined the possibility that GR interacted with NF-kB and NF-IL6; transcription factors known to stimulate the IL-6 promoter (120). Using murine F9 embryonal carcinoma cells, which are devoid of endogenous NF-IL6, AP-1, and Rel-like activities, Ray *et al.* demonstrated that expression plasmids encoding NF-IL6, or p65 alone could not stimulate the IL-6 promoter, whereas when used together, the IL-6 promoter was stimulated. Furthermore, dexamethasone could inhibit this activation (120). In contrast, transfection of HeLa cells with either plasmid alone, resulted in activation of the IL-6 promoter, suggesting that the transgenic protein interacted with the endogenous co-activating protein. Regardless, in HeLa cells, dexamethasone was capable of inhibiting NF-IL6 and p65-induced IL-6 promoter activity (120). Finally, in cross precipitation assays, it was demonstrated that GR bound to p65, but not NF-IL6. These results suggest that 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 earlier.

Yet, additional clues on the action of GR on the IL-6 promoter may be gleaned from studies by Scheinman et. al. and Auphan et. al. (134, 135). Though not evaluated on the IL-6 promoter itself, these groups demonstrated that dexamethasone induces IκBα protein and mRNA expression. Auphan *et al* further demonstrated that dexamethasone could inhibit TNF-α-stimulated nuclear translocation of p65 (135). These data suggest that GR induces IκBα protein synthesis which results in cytoplasmic sequestration of NFκB culminating in decreased activation of the target promoter. This mechanism does not preclude the previously described mechanisms of promoter occlusion and GR:p65 protein interactions.

4.2. Estrogen and interleukin-6 expression

Estrogen's ability to repress IL-6 expression was first recognized in human endometrial stromal cells (23). Additional clues came from the observations that menopause or ovariectomy resulted in increased IL-6 serum levels (136) , increased IL-6 mRNA levels in bone cells (137), and increased IL-6 secretion by mononuclear cells (75, 138, 139). Further evidence for estrogen's ability to repress IL-6 expression is derived from studies which demonstrated that estradiol inhibits bone marrow stromal cell and osteoblastic cell IL-6 protein and mRNA production *in vitro* (18, 140) and that estradiol was as effective as neutralizing antibody to IL-6 to suppress osteoclast development in murine bone cell cultures (18) or in ovariectomized mice (19). Taken together, these data provide strong evidence for the occurrence of estrogen-mediated repression of IL-6 expression.

To explore estrogen's effect on the IL-6 promoter, Pottratz *et al.* (117) and Ray *et al.* (116) performed transient transfection assays using either a 1.2 Kb fragment of the promoter or a 225 bp fragment of the promoter. They found that basal IL-6 promoter activity was very low when used to drive a chloramphenicol acetyltransferase (CAT) gene in both HeLa, which does not express the estrogen receptor (ER), and the murine bone marrow stromal cell line MBA 13.2, which constitutively expresses the ER. However, phorbol-13-myristate acetate (PMA), IL-1, or TNF stimulated the promoter and 17β-estradiol inhibited this activity in both cell lines. Transfection of the HeLa cells with ER was required to observe the suppression. These results suggest that 17β-estradiol inhibits IL-6 gene transcriptional activation by an ER-dependent mechanism.

To investigate whether the ER-mediated repression was due to direct interaction between the ER and the IL-6 promoter, Pottratz *et al.* performed competition assays which assessed for the 225 bp IL-6 promoter fragment's ability to compete for binding of ER to a labeled estrogen response element (ERE) (117). However, even though ER bound to the labeled ERE, the 225 bp fragment did not compete with the ERE. Additionally, upon electrophoretic mobility shift assay (EMSA) the ERE could not compete shifted complexes formed from incubation of HeLa or MBA 13.2 nuclear extracts with the labeled 225 bp fragment and this fragment could not bind to ER (116, 117). In summary, these results suggested that ER does not bind to the 225 bp IL-6 promoter

fragment even though it inhibits its activity. These results were not entirely surprising based on the observation that there was no ERE within the 225 bp IL-6 promoter fragment. However, they led to the hypothesis that ER was working through inhibition of positive acting transcription factors by protein:protein interactions.

At nearly the same time, Ray *et al.* reported that wildtype ER, but not ER with mutated or deleted DNA-binding domain (DBD), could mediate repression of IL-1-induced IL-6 promoter activity, yet if the ER DBD was replaced with a GR DBD, the resulting chimeric receptor was capable of mediating repression (116). These results were also observed if the IL-6 promoter activity was induced by co-transfection of HeLa cells with NF-IL6 and NFκB p65 subunit. However, the chimeric receptor, which could mediate repression, could not stimulate a ERE-reporter construct, thus suggesting that repression was not dependent on direct binding to the IL-6 promoter. Furthermore, overexpression of NFκB p65 by transient transfection inhibited ER's ability to transactivate an ERE-reporter construct (116). This result provided evidence for interaction between NFκB p65 and ER.

These studies were extended into the U2-OS human osteoblast and MCF-7 breast carcinoma cell lines by Stein and Yang (141). Similar to the observations in HeLa cells described above, the IL-6 promoter, even when deleted to 109 bp, was stimulated by IL-1β and this activation was repressed by 17β-estradiol in the presence of either co-transfected ER (U2-OS cells) or native ER (MCF-7 cells). Further deletion of the promoter to 49 bp, in which both NF-IL6 and NFκB response elements are deleted, resulted in loss of promoter induction by IL-1b. Based on these data, Stein and Yang concluded that the ER target is between -109 and -49 (141). However, since the 49 bp promoter region was not stimulated, they could not observe ER-mediated repression if it was present, hence this conclusion may be premature.

To deduce which regions of the ER were necessary for repression, Stein and Yang performed a series of transient co-transfection experiments using mutated ER constructs and the IL-6 promoter (141). Deletion of the amino-terminus including the transcription accessory factor (TAF)-1 (Δ1-179) domain still allowed for ER-mediated repression. Extending this deletion to include the DBD (Δ1-281) resulted in loss of repression as did isolated deletion of the DBD (Δ185-251). Additionally, deletion of the carboxy-terminus (Δ271-595) including TAF-2 domain and the LBD resulted in loss of repression. Based on these data, the author's concluded that the DBD contributed to transrepression.

Based on the previous data that ER does not appear to bind to the IL-6 promoter (116, 117), yet can mediate transrepression of the IL-6 promoter, Stein and Yang explored for direct interaction between ER and NFκB p65, NFκB p50, or NF-IL6 (141). They found that all these *in vitro* translated proteins bound with bacterially expressed ER. Intriguingly, this interaction was not dependent on estrogen and deletion of the DBD did not effect the interaction. Furthermore, they demonstrated that ER and NFκB p65 or NF-IL-6 mutually represses each others transactivation abilities through a mechanism which does not induce IκBα. Based on these data, Stein and Yang concluded that binding of NFκB p65 or NF-IL-6 is the driving force which mediates transrepression. However, when considered with the observation described above that isolated deletion of the ER DBD results in loss of transrepression, these data suggest that ER's binding capability for these transcription factors and its ability to mediate transrepression are in fact on different domains of the ER and are mediated by some mechanism which involves more than just binding of these transcription factors. Further studies are needed to resolve these issues.

4.3. Androgen and interleukin-6 expression

Androgens can repress expression of a variety of gene products (142-157). The first demonstration of androgen's ability to repress IL-6 expression was made in +/+LDA11 murine bone marrow stromal cell line which had been stimulated with IL-1 and TNF (18). In this study, 10 nM T was able to repress bioactive IL-6 expression by approximately 20% (as opposed to approximately 60% for 10 nM $17\beta E_{2)}$. Curiously, when HeLa cells were co-transfected with ER and a CAT-reporter plasmid driven by a 225 bp fragment of the IL-6 promoter, 10 nM DHT inhibited PMA-induced activation by approximately 50% (as opposed to approximately 90% for 10 nM 17b-estradiol ($17\beta E_2$) (117). The authors accounted for this effect as due to T's affinity for the ER (117). However, this is unlikely as it has been previously demonstrated that T and DHT inhibits PMA-induced IL-6 promoter activation in HeLa cells transfected with AR, but not ER (158). This supports an earlier study in which it was reported that DHT antagonizes estrogen's effect in the uterus by decreasing estrogen-induced RNA transcription at a point subsequent to estrogen receptor binding (159). Several adrenal androgens which are not known to bind the AR (i.e., androstenedione, androstenediol, and dehydroepiandrosterone sulfate) mediate repression of the IL-6 promoter in HeLa cells transfected with AR. These experiments suggest that androgens are capable of mediating transrepression of IL-6 promoter activation.

We have further demonstrated that DHT requires the AR to mediated DHT's repressive effect in a transient transfection assay system (118). In our system, DHT inhibited PMA-induced activation of the IL-6 promoter by inhibiting translocation of NFkB. This was achieved through maintenance of IkBa levels even in the presence of PMA. Currently, it is unknown how DHT maintains IkBa levels, but decreased phosphorylation or increased protein production are two possibilities.

The *in vivo* relevance of the above observations was demonstrated by Bellido et al. in an orchiectomized mouse model (158). Though they did not report serum or bone marrow IL-6 levels, they found that orchiectomy resulted in increased replication of bone marrow osteoclast progenitors and found that this could be prevented by administration of IL-6 neutralizing antibody or implantation of a slow release form of T. However, because T is converted to $17bE_2$, these results are not conclusive evidence of androgen's action. In fact, the observation of decreased bone density observed in a male patient whom had normal androgen levels, but a mutation resulting in a non-functional ER, suggests that estrogen's effects on bone in men are as important as androgen's (160). This hypothesis is supported by the observation that $17\beta E_2$ can inhibit bone loss observed in men treated by orchiectomy for prostate cancer (161). However, estrogen's ability to inhibit bone loss may be mediated through its transrepression of the IL-6 promoter, and thus these observations are still consistent with androgen loss resulting in increased IL-6 activity. Finally, that orchiectomy of mice without the IL-6 gene (generated by knockout technology) do not demonstrate the increased osteoclast proliferative effects, strongly supports that loss of androgen results in increased IL-6 levels (158).

5. THE INTERLEUKIN-6 RECEPTOR

5.1. The interleukin-6 receptor: Structure and function

The human IL-6R (also known as gp80 and the IL-6Ra subunit), was first cloned by Yamasaki *et al.* from a human natural killer-like cell line, YT, (162) followed by Schooltink et al. from a human hepatoma cell line, HepG2 (163). IL-6R is a 80 Kd protein consisting of 467 aa. Located on chromosome 1 band q21 (164), the IL-6R gene encodes for a 5 Kb mRNA containing a coding region of 1401 bp (162). The 3'-untranslated region (3'-UTR) consists of approximately 1.5 Kb, suggesting that the 5'-UTR compromises approximately 2.1 Kb. The human IL-6R shares 53% identity with the rat hepatic IL-6R aa sequence (165). However, the mRNA structures are strikingly different. Although both human and rat IL-6 mRNAs are approximately 5 Kb, the rat 3'-UTR consists of approximately 3.1 Kb, which combined with the observation that the coding region, similar to the human IL-6R coding region, is 1.4 Kb, suggests that the 5'-UTR is much shorter in the rat than the human IL-6R mRNA (approximately 0.6 vs 2.1 Kb, respectively). A definitive conclusion on the length of the 5'-UTRs of these mRNAs awaits for localization of the transcription initiation site(s).

The structure of IL-6R has been deduced by comparative sequence analysis. A hydropathy plot revealed two major hydrophobic regions; one which encodes for the signal peptide between residues 1 and 20, and the other which encodes for the transmembrane domain in the region of residues 359 to 386 (162). The latter region is followed by a putative transmembrane anchoring stop codon consisting of several positively charged residues. These findings suggest the IL-6R has a 339 aa extracellular region, 28 aa transmembrane region, and 82 aa intracellular region. Intriguingly, the intracellular region does not contain any kinase domains suggesting that this molecule is not capable of signaling activity (162).

Upon homology search, it was identified that the extracellular component of the IL-6R contains a domain which shares extensive homology with the Ig superfamily (162) and two tandem fibronectin type III motifs (166) present in a 200 aa region. This region defines the cytokine receptor family domain, a domain which 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 of the transmembrane region (166, 167). Fibronectin type III domains are observed in cell-adhesion molecules, which implies that cytokine receptors evolved from an ancestral adhesive molecule (168).

As the protein structure suggests, the IL-6R is not capable of inducing signal transduction directly. It is now understood that in order for IL-6 to mediate signal, it first binds to gp-80 forming a low affinity receptor complex. This complex then associates with the non-ligand-binding transmembrane glycoprotein, gp-130 (169). Homodimerization of gp-130 is required for IL-6 signal transduction (170). Although it was originally considered that one unit of IL-6 and the IL-6R bound to a gp-130 homodimer (170), the stochiometry and number of this reaction appears to involve a hexameric complex consisting of two molecules each of IL-6, IL-6R gp-80, and gp-130 (171). This complex forms a high affinity binding site for IL-6, as opposed to the low affinity binding observed with IL-6 and IL-6R gp-80 in the absence of gp-130.

Table 3. Localization and effect of stimulus on interleukin-6 receptor expression.

Cell or Tissue	Method of detection	Treatment	Effect	Reference
Rat hepatocyte	S, R	IL-6, IL-1, Freund's Dex	D I	(179, 180)
Human monocyte	N, F	Endotoxin, *in vitro* maturation, IL-1, IL-6, Dex	D	(181)
Human T-lymphocyte	N, F, S	None		(6, 181)
Human B-lymphocytes	S	SAC	I	(6)
Human hepatocytes, HepG2, Hep3B-2	S, N	IL-1, IL-6, Dex	I	(181-184)
UAC (amnion)	S, N	Dex	I	(183)
YT (NK)	S, N	None		(6)
U937 (myelomonocytic histiocytoma)	S	None		(6)
HL 60 (promyelocytic leukemia)	S	None		(6)
U266 (myeloma)	P	IL-6R antisense. IL-6	D	(185, 186)
Tonsillar lymphocytes	S	None		(6)
U373 (astrocytoma)	S	None		(6)
SK-MG4 (glioblastoma)	S	None		(6)
EBV-immortalized B cells	S	None		(6)
PC12 (pheochromocytoma)	S	None		(35)
Rat brain	P	Development	V	(187, 188)
Lymphoma				(189)
Bladder cancer	P			(190)
Prostate carcinoma and benign hyperplasia	D	None		(191)
Melanoma	S	IL-6	D	(192)
Osteoblasts	D	TNF, PTH, IL-6, IL-1, Dex	None	(193)
Murine serum	E	Age	I	(194)

*Abbreviations: D, Dot blot; S,Scatchard analysis, I,Immunhistochemistry; H, *In situ* hybridization; E, Elisa; EBV, Epstein Barr Virus; F, FACS; W, Western; N,Northern blot; R,RNase protection; SAC, Staphyloccocus aureus Cowan I; P,RT-PCR, Dex, dexamethasone; NK, natural killer; i.p., intraperitoneal; TNF, tumor necrosis factor; PTH, parathyroid hormone, V, varied with area of brain examined.

Mutational analyses of the IL-6R has identified that the region of amino acids 106-322, which comprise the cytokine receptor family domain of IL-6R, is responsible both for IL-6-binding and for binding to gp-130 (172). In fact, the Ig-like domain, whose action in the context of IL-6R is not currently identified, is not required for either of these functions. The IL-6R has an isoform which was first identified in human urine, the soluble IL-6R (sIL-6R) (173). In contrast to other soluble cytokine receptors (e.g., sIL-2R) which inhibit cytokine induced signaling, the sIL-6R forms a fully active hexameric IL-6:sIL-6R:gp-130 complex which induces cell signaling. How sIL-6R is generated is not currently known , but both alternative splicing, resulting in loss of the transmembrane domain (174), or proteolyis of the mature cell surface IL-6R (175, 176) have been proposed.

As mentioned in our opening comments, IL-6 is one of a family of cytokines collectively termed "the interleukin-6-type cytokines". The cytokines which make up this family are IL-6, leukemia inhibitory factor (LIF), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), and interleukin-11 (177). The common characteristic which define this group is they activate gp-130 to induce cell signaling. That these cytokines converge on gp-130 underscores the redundancy present in the cytokine system. However, specificity of cytokine action is mediated, in part, through cell specific repertoire of cytokine specific a chain receptors, such as gp80 for IL-6

5.2. Expression of the Interleukin-6 Receptor

The IL-6R is expressed in a variety of cells (Table 3). In general it is expressed in the range of 100 to 2000 sites/cell (178). However, in myeloma lines and Epstein-Barr virus transformed lines up to 29,000 sites/cell have been identified (178). Except for the effects of dexamethasone, modulation of IL-6 expression by various factors has not given consistent results. Snyer *et al.* demonstrated 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 either CESS (Epstein-Barr Virus B cell immortalized line), HL-60, U937, Hep-G, UAC cell lines. In contrast, several other groups have demonstrated that IL-1 does modulate IL-6R expression in several cell lines and tissues (summarized in Table 3). Perhaps cell specific differences in response account for the discordant results. On the other hand, dexamethasone has been consistently demonstrated to increase IL-6R in a variety of tissues including liver primary cells and

cell lines, monocyte primary cultures, myeloma cell lines, and an amniotic cell line.

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

6. SUMMARY

IL-6 is active in a great number of physiologic and pathophysiologic processes. A wide variety of factors have been demonstrated to modulate IL-6 expression. While may of these stimulate IL-6 expression, only a few factors have been demonstrated to inhibit IL-6 expression. Among the inhibitors of IL-6 gene expression are steroids, including corticosteroids, estrogen, and androgens. Steroids appear to inhibit IL-6 expression thorugh repressing transcriptional activation of the IL-6 gene. In addition to regulation of IL-6 levels, modulation of IL-6R levels is another mechanism by which IL-6 activity is controlled. Both the soluble and membrane bound form of IL-6R mediate IL-6 activity by stimulating cell signalling through activation of gp130. Even though the importance of the IL-6R for manifestation of IL-6 activity is recognized, the molecular mechanisms by which transcriptional control of the IL-6R gene is achieved have not been reported to date. Future studies aimed at elucidating these mechanisms may contribute to understanding how IL-6 is active in a variety of disorders.

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