Cytokines and Their Receptors: Molecular Mechanism of Interleukin-6 Gene Repression by Glucocorticoids

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ABSTRACT
Recent years have seen the discovery and molecular characterization of a bewildering array of cytokines and hematopoietic growth factors—and an even more complex description of their overlapping functions. The molecular cloning of a wide array of the cell-surface receptors for these cytokines has led to the recognition of classes of structurally related receptor superfamilies. The functional receptors for many of these cytokines (e.g., interleukin (IL)-2R and IL-6R) involve two distinct subunits; strikingly, the same β subunit can interact with distinct α subunits to constitute the receptor for different cytokines (e.g., those for IL-3, IL-5, and granulocyte monocyte colony-stimulating factor). Considerable progress has also been made in defining the molecular mechanisms that underlie clinically relevant cytokine-related phenomena. As an example, the molecular mechanism by which glucocorticoids inhibit IL-6 gene expression has been shown to include the occlusion of the inducible enhancer and the basal promoter elements in the IL-6 promoter. Acute rejection episodes in renal transplant patients are accompanied by increases in serum IL-6 levels; the administration of glucocorticoids during these episodes leads to a rapid and marked decrease in IL-6 levels. It appears that the serum IL-6 level may be a useful diagnostic and prognostic indicator in the transplant patient.

Key Words: Interleukin-6, transplantation, glucocorticoid receptor, promoter occlusion, protein-protein interactions

A plethora of lymphokines and monokines such as the interleukins (IL), colony-stimulating factors, erythropoietin, interferons (IFN), tumor necrosis factor (TNF), lymphotoxin, and leukemia inhibitory factor, as well as the group of polypeptide growth factors and differentiation factors such as epidermal growth factor, nerve growth factor, platelet-derived growth factor, and fibroblast growth factor, are intimately involved in the interactions between cells during the immune response. All of these molecules (including transforming growth factors α and β) are collectively called cytokines (Figure 1).

The application of gene cloning techniques has allowed the structural definition of these cytokines and their respective cell-surface receptors. Studies with both natural and recombinant cytokines indicate that most cytokines have multiple biologic activities on disparate cell types. Cytokines often have overlapping functions and seem to operate within a network. An objective of research in this field is to define precisely which combination of cytokine signals determines particular effects in a given cell type. Any one cytokine may have qualitatively or quantitatively different effects on a particular cell function, depending on what other cytokine signals the cell has received. The detailed temporal kinetics of the induction of particular cytokine genes in the immune system seems to add a further level of complexity to the function of cytokine networks during an inflammatory response. However, it is important to underscore the role of many of these cytokines in "constitutive" hematopoiesis, in the normal bone marrow microenvironment, and in the absence of any immunologic stress. A functional link is maintained between lymphocytes and hematopoietic cells by various cytokines, which culminates in the ultimate biologic response, such as an allergic response, the acute phase response, or graft rejection. Most cytokines function locally as paracrine or autocrine mediators, whereas some that are released into the systemic circulation also serve as "endocrine" mediators. The availability of cytokines and their blocking antibodies for whole-animal experiments and the generation of transgenic animals overexpressing particular cytokine genes should help to provide insight into the role of each cytokine within a functional network. Detailed descriptions of the structural features and biologic activities of the members of the cytokine family can be found in several excellent reviews (1,2).

CYTOKINE RECEPTORS
To understand how a particular cytokine transduces a signal in the responder cell, many investi-
IFN-α, IFN-β, IFN-γ, TNF-α, LT, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IFN-γ (BP), EPO, IL-1α, IL-1β, IL-1 RA, IL-7, CSF-1 (M-CSF), G-CSF, GM-CSF, TNF-α, LT, SCF, IL-2, IFN-y, G-CSF, GM-CSF, IL-3, IL-4, IL-6, IL-12, IL-6, IL-12, TGF-α, LIF, Others

**Figure 1.** Members of the cytokine family. LT, lymphotoxin or TNF-β; TGF, transforming growth factor; EPO, erythropoietin; CSF, colony stimulating factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte monocyte colony-stimulating factor; LIF, leukemia inhibitory factor; SCF, stem cell factor; IL-1 RA, IL-1 receptor antagonist. The terms intercrine-α and intercrine-β have been proposed for the two major subdivisions of the IL-8-related family of cytokines.

**Figure 2.** Cytokines whose receptors have been molecularly characterized. Abbreviations are as defined in Text and legend to Figure 1.

**Figure 3.** (A) Schematic representations of the members of the cytokine receptor superfamily. The curve segments represent Ig-like domains. The horizontal bars represent conserved cysteine residues, and some of the receptors share a second motif at the proximal end of the extracellular domain based on the sequence Val-X-X-Arg-X6-Trp-Ser-X-Trp-Ser, known as the WSXWS motif (3). The horizontal bars in the TNF-R subunits and the IL-2R α-subunit represent repeat units. (B) Schematic diagram of different kinds of multisubunit cytokine receptors. Abbreviations are as defined in Text and legend to Figure 1.

**NEUROENDOCRINE-IMMUNE RESPONSES**

It appears that cytokines and glucocorticoid hormones integrate an immunoregulatory feedback circuit: during stimulation of the immune system, cytokines are released into the peripheral circulation concomitant with an increase in plasma glucocorticoid levels. Both in vivo and in vitro studies indicate that glucocorticoids inhibit the production of multiple cytokines, thereby controlling a harmful prolonged activation of the immune system. These immunosuppressive actions of glucocorticoids form the basis of their use in the treatment of a wide range of diseases.
including autoimmune diseases and the prevention of allograft rejection.

Effects of Cytokines on the Hypothalamic-Pituitary-Adrenal Axis

Cytokines appear to play a key role in the activation of the hypothalamic-pituitary-adrenal axis. In 1985, Woloski et al. (6) reported that hepatocyte-stimulating factor, later identified to be IL-6 (5), stimulated the secretion of adrenocorticotropic hormone (ACTH) from the AtT20 cell line. Later Naitoh et al. (7) showed that IL-6 administered i.v. stimulated the increase of ACTH secretion in conscious, freely moving rats. It was suggested that this action of IL-6 was mediated by the stimulation of corticotropin-releasing factor (CRF), because anti-CRF antibody administered in vivo blocked the increase in ACTH levels produced by IL-6 administration (7). Evidence provided by Vankelecom et al. (8) indicates that the folliculostellate cells in the anterior lobe of the pituitary produce IL-6. Furthermore, IL-6 was found to stimulate hormone secretion (growth hormone, luteinizing hormone, and prolactin) by cultured anterior pituitary cells (9,10). Thus, whereas IL-6 may act on the hypothalamus itself (7), other data suggest that IL-6 might modulate anterior pituitary hormone secretion by the gland itself (9,10). The effect of IL-6 on the anterior pituitary cells was only observed at a high cell density (10), which raises the question of whether the action of IL-6 is determined by the presence of other mediators in the surrounding milieu. The synergism of IL-6 with other cytokines, especially with IL-1, has been reported (11). Because IL-1 immunoreactive cells are also found in the anterior pituitary (12), an interaction between IL-1 and IL-6 seems plausible. In experiments with isolated rat pituitary cells, IL-6 and IL-1 have been found to synergize in the stimulation of ACTH release (13).

There are conflicting reports on the direct effect of IL-1 on ACTH release from the anterior pituitary. Although some investigators have demonstrated the IL-1-activated release of ACTH from cultured anterior pituitary cells in the concentration range of 10⁻¹⁰ to 10⁻⁷ M (16), others were unable to show a direct effect of either recombinant human IL-1α or IL-1β on ACTH release from these cells (17,18). Even at 10⁻¹⁰ to 10⁻¹² M, IL-1β has been reported to stimulate the release of ACTH as well as of growth hormone, luteinizing hormone, and thyroid-stimulating hormone by isolated rat pituitary cells or perfused rat pituitaries (14,15). However, there is increasing consensus that the major site of action of IL-1 is the hypothalamus, causing either a direct release of CRF or via catecholamines (19) or prostaglandins (20).

Data concerning the effects of TNF on ACTH secretion are highly controversial. Although some report a positive effect (21), others indicate either a negative effect (22) or none at all (23).

With respect to glucocorticoid secretion by the adrenal gland, long-term incubation with IL-1 or IL-6 was reported to have a stimulatory effect (24,25). TNF, on the other hand, was shown either to have no effect on the basal level of corticosterone secretion from adrenocortical cells in vitro or to block ACTH-stimulated secretion (26).

Effects of Glucocorticoids on the Production and Action of Immune Cytokines

The use of natural and synthetic analogs of glucocorticoid hormones as anti-inflammatory (antistress) agents is well established. Recent data suggest that glucocorticoids inhibit the production of inflammation-associated cytokines including IL-1, IL-6, TNF, and IFN-γ (6, 27-30). Adrenal insufficiency can therefore be anticipated to result in a massive systemic excess of cytokines during stress, which can have toxic effects. For example, adrenalectomized rats readily succumb to the administration of TNF; this increased susceptibility can be reversed by glucocorticoid administration (31). The toxic effects of excess cytokines are evident in the clinical manifestations of septic shock, which is accompanied by elevated concentrations of IL-1, IL-6, and TNF (32,33). In many instances, steroids have proved effective in controlling the initial manifestations of sepsis (34) and acute respiratory disease syndrome (35). Interestingly, endotoxemia per se is known to induce the endogenous production of glucocorticoids.

The inhibitory effects of glucocorticoids on cytokine gene expression are largely derived from cell culture studies. Recently, the in vivo administration of dexamethasone to mice before endotoxic challenge was shown to drastically inhibit TNF production and moderately affect IL-1 production (36). IL-1β appeared more resistant to inhibition by glucocorticoids than did IL-1α (36).

Although glucocorticoids inhibit the production of inflammation-associated cytokines, they potentiate the action of these cytokines. For example, elevated levels of circulating glucocorticoids synergize with IL-6 in inducing the increased hepatic synthesis and secretion of "acute phase" plasma proteins such as fibrinogen, various antiproteinases, and complement factors (37-39). Another example is that of the dramatic increase induced by IFN-γ together with glucocorticoids in the number of IgG Fc receptors on human mononuclear phagocytes (30).

Mechanisms of Modulation of Cytokine Gene Expression by Glucocorticoids

The consensus sequence for glucocorticoid receptor (GR) binding, i.e., the glucocorticoid responsive ele-
Glucocorticoids strongly inhibit IL-6 gene expression in different tissues (29). The mechanism of repression of the IL-6 gene by dexamethasone was investigated in HeLa cells transiently transfected with plasmid constructs containing IL-6 reporter (bacterial chloramphenicol acetyltransferase) fusion genes, and cDNA vectors constitutively expressing either the wild-type or mutant GR (54). The induction from both MRE I and MRE II was strongly repressed by dexamethasone in a wild-type GR-dependent fashion irrespective of the inducer used (Figure 4: [54]). Dexamethasone also repressed induction by a pseudorabies virus of an IL-6 construct containing the IL-6 TATA box and the RNA start site ("initiator" or Inr element) but not the MRE region (54). The purified DNA-binding domain fragment of GR generated footprints across both the MRE region and the basal promoter elements (TATA box and Inr element in the IL-6 promoter). These observations suggested that ligand-activated GR repressed IL-6 gene function by the occlusion of not only the IL-6 MRE enhancer.

Figure 4. Repression of IL-1, 12-O-tetradecanoylphorbol-13-acetate (TPA), forskolin-, and TNF-induced expression from chimeric IL-6/reporter constructs by dexamethasone (D) in transiently transfected HeLa cells. HeLa cells were transfected with a mixture of pSV2neo, RSVhGRα (expression vector for wild-type GR), and the IL-6/thymidine kinase/chloramphenicol acetyltransferase reporter plasmid pAR10TKC (MRE/TK/CAT; IL-6 sequences from -173 to -151) or pAR11TKC (MREII/TK/CAT; IL-6 sequences from -158 to -145) (54). The cells were left untreated (U) or were treated with IL-1α (5 ng/ml), TPA (100 ng/ml), a combination of forskolin (50 μM) and isobutylmethylxanthine (IBMX; 0.5 mM) (F), or TNF (100 ng/ml) in the presence or absence of D (1 μM). Cells were harvested for CAT activity (54-57).

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Glucocorticoid Modulation of IL-6 Gene Expression

The 5'-flanking region of the IL-6 gene contains a complex network of regulatory elements (54-56). Transient expression studies in HeLa cells show the importance of two overlapping multiple cytokine- and second messenger-responsive enhancer sequences (MRE I and MRE II, respectively) in the induction of the gene (54). MRE I (-173 to -151) contains the typical GACGTCA cAMP-phorbol ester-responsive (CRE/TRE) motif. MRE II (-158 to -145) contains an imperfect 14-base-pair dyad repeat and bears little resemblance to a CRE/TRE motif (54,56). An NF-κB element between -73 and -64 (55) also appears to contribute to the activation of this gene in some cell types (57,58).

Studies on the effects of the synthetic glucocorticoid dexamethasone on both steady-state mRNA levels and the rate of mRNA transcription indicate that glucocorticoids inhibit cytokine gene expression both at the transcriptional and postranscriptional levels.

Dexamethasone inhibits steady-state mRNA levels of IL-1 (49), IL-2 (50), IL-3 (51), IL-6 (29), IL-8 (52), TNF (33), and IFN-γ genes (53). The postranscriptional inhibition of IL-1, IL-8, IFN-γ, and TNF gene expression has also been reported (33, 49, 52, 53).

Among the cytokine genes, the molecular mechanism of the inhibition of the IL-6 gene by glucocorticoids has been elucidated recently in some detail (54).

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region but also that of the basal promoter elements. Figure 5 summarizes the possible mechanisms of transcriptional repression; our data suggest that the repression of the IL-6 promoter by glucocorticoids includes mechanisms A and B.

Further exploration of the relationship between GR structure and IL-6 promoter repression revealed that the deletion of the entire DNA-binding domain or mutations in the second Zn finger abolished repression by GR (59). However, surprisingly, point mutations in the first Zn finger of the DNA-binding domain or deletion of the first Zn finger caused dexamethasone-responsive enhancement of the activity of different IL-6 enhancer and promoter constructs, as well as that of the herpesvirus thymidine kinase promoter, but not that of the murine mammary tumor virus (MTV) long terminal repeat or that of the c-fos promoter (59). In our experiments, it was the fine structure of the transfected GR that determined whether the target IL-6 promoter was repressed or activated (59).

Sequential DNA-binding immunoprecipitation experiments with cell extracts transfected with wild-type or mutant GR showed weak binding of the wild-type receptor to the IL-6 promoter and the absence of any binding by the mutant (first finger) receptors (59). The binding of the wild-type GR to the MTV promoter, which contains a consensus GRE, was strong under the same conditions; the mutant receptors did not bind to the MTV promoter either (59). The weak binding of the wild-type receptor to "negative GRE" sequences in the IL-6 promoter is reminiscent of the weak binding of the full-length receptor to the "negative GRE" sequences in the proliferin promoter (44,45). The involvement of DNA-binding and/or protein-protein interactions of the wild-type receptor and the first Zn finger mutant receptor with positive transcription factor(s) that activate the IL-6 gene (to engender either repression or activation, respectively) is a possibility.

**IL-6 in the Renal Transplant Patient**

Both cytokines and the "soluble" components of cytokine receptors have been detected in the serum and urine of normal and diseased individuals (60). The administration of OKT3 antibody in vivo or in vitro itself induces IL-6 production (61,62). Furthermore, the levels of IL-6 in serum and urine are beginning to emerge as informative indicators of the underlying disease state in patients with renal and bone marrow transplants. Van Oers and colleagues (63) reported that serum and urine IL-6 levels were elevated in renal transplant patients within 2 days after surgery and then declined to pretransplant levels over the next 3 to 5 wk. The IL-6 levels rose markedly 0 to 3 days before clinical acute rejection. In patients with acute rejection, the elevations in serum IL-6 levels preceded elevations in serum creatinine levels. Strikingly, methylprednisolone therapy for the acute rejection episode led to a rapid decline of serum IL-6 levels to the pretransplant levels. These findings have been essentially confirmed by Yoshimura et al. (64), who in addition reported that serum IL-6 levels did not increase in patients with cyclosporine renal toxicity, thus allowing a distinction to be made between cyclosporine toxicity and graft rejection. By in situ nucleic acid hybridization, Vandenbroecke and colleagues (65) consistently observed a uniform high level of expression of IL-6 mRNA, but not of TNF-α or IFN-γ mRNAs, in glomerular cells, tubular epithelia, smooth muscle cells, and vascular endothelia, as well as in the interstitial mononuclear cell infiltrate in renal biopsies of patients undergoing acute renal allograft rejection. Biopsies from kidney transplant
patients with stable renal function did not exhibit significant IL-6, IFN-γ, or TNF-α mRNA expression. These data provide a foundation for the detailed exploration of the diagnostic and prognostic value of measuring serum and urine IL-6 levels in the renal transplant patient. That glucocorticoid therapy used to suppress acute rejection in the renal transplant patient suppresses circulating IL-6 levels has now been clearly established. Although the molecular mechanisms involved in the transcriptional repression of the IL-6 promoter by glucocorticoids are well on their way to being elucidated, the immunological significance of the suppression of gene expression of specific cytokines by glucocorticoids in vivo remains to be defined.

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