Glucocorticoid Therapy for Immune-mediated Diseases: Basic and Clinical Correlates

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■ **Glucocorticoids are pleiotropic hormones that at pharmacologic doses prevent or suppress inflammation and other immunologically mediated processes. At the molecular level, glucocorticoids form complexes with specific receptors that migrate to the nucleus where they interact with selective regulatory sites within DNA; this results in positive and negative modulation of several genes involved in inflammatory and immune responses. At the cellular level, glucocorticoids inhibit the access of leukocytes to inflammatory sites; interfere with the functions of leukocytes, endothelial cells, and fibroblasts; and suppress the production and the effects of humoral factors involved in the inflammatory response. Clinically, several modes of glucocorticoid administration are used, depending on the disease process, the organ involved, and the extent of involvement. High doses of daily glucocorticoids are usually required in patients with severe diseases involving major organs, whereas alternate-day regimens may be used in patients with less aggressive diseases. Intravenous glucocorticoids (pulse therapy) are frequently used to initiate therapy in patients with rapidly progressive, immunologically mediated diseases. The benefits of glucocorticoid therapy can easily be offset by severe side effects; even with the greatest care, side effects may occur. Moreover, for certain complications (for example, infection diathesis, peptic ulcer, osteoporosis, avascular necrosis, and atherosclerosis), other drug toxicities and pathogenic factors overlap with glucocorticoid effects. Minimizing the incidence and severity of glucocorticoid-related side effects requires carefully decreasing the dose; using adjunctive disease-modifying immunosuppressive and anti-inflammatory agents; and taking general preventive measures.**

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Dr. Dimitrios T. Boumpas (Kidney Disease Section, National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK], National Institutes of Health [NIH], Bethesda, Maryland): Since 1949, when Hench and colleagues first introduced cortisone for the treatment of rheumatoid arthritis, glucocorticoids have revolutionized the treatment of immunologically mediated diseases. Although substantial complications associated with glucocorticoids have tempered enthusiasm for their use, they have remained the cornerstone of therapy for virtually all immunologically mediated diseases. In recent years, an explosion of new information has occurred relevant to both basic and clinical aspects of glucocorticoid therapy.

We describe the molecular mechanisms, sites of action, and effects of glucocorticoids on various cells involved in inflammatory and immunologically mediated reactions. Treatment principles are also provided with examples of specific glucocorticoid regimens in prototypical conditions. We also review selective complications of glucocorticoid therapy and discuss recent information about their pathogenesis and management.

Mechanisms of Action

Dr. George P. Chrousos (Chief, Pediatric Endocrinology Section, Developmental Endocrinology Branch, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland): Glucocorticoids exert most of their effects through specific, ubiquitously distributed intracellular receptors (1). The classic model of glucocorticoid action was described more than two decades ago and is briefly updated here (Figure 1, *panel A*). Glucocorticoids circulate in blood, is either in the free form or in association with cortisol-binding globulin. The free form of the steroid can readily diffuse through the plasma membrane and can bind with high affinity to cytoplasmic glucocorticoid receptors (the role of receptors primarily residing in the nucleus is controversial). The formation of the ligand-receptor complex is followed by its "activation" (that is, translocation into the nucleus and binding to what are called "acceptor sites"). The bound complex modulates transcription of specific genes that encode proteins responsible for the action of glucocorticoids.

Glucocorticoid Receptors

In 1985, the complementary DNA of the human glucocorticoid receptor was cloned (2); it contains three main functional domains (Figure 1, *panel B*): first, the DNA-binding domain in the center of the molecule that recognizes specific sequences of the DNA called hormone-responsive elements; second, the ligand-binding domain in the carboxyl terminal region that interacts

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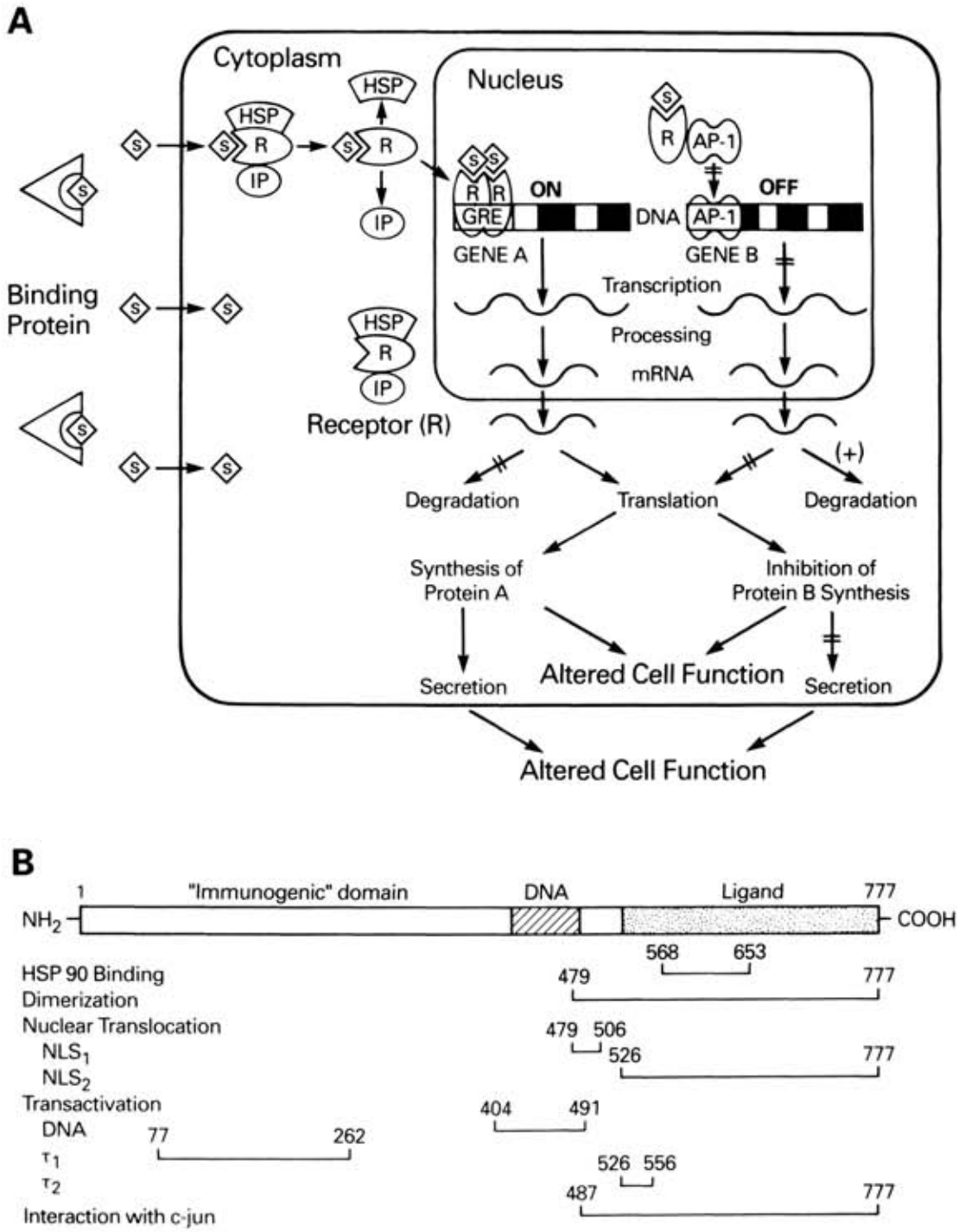

Figure 1. Mechanisms of glucocorticoid action. Panel A. Steroid hormone (*S*) circulates as a free molecule or as a complex with plasma-binding protein. After the steroid enters the cell, it binds to receptors (*R*) that reside in the cytosol complexed to heat-shock protein (*HSP*) and immunophilin (*IP*). Binding of the ligand to the complex causes dissociation of HSP and IP. The receptor-ligand translocates into the nucleus where it binds at or near the 5′-flanking DNA sequences of certain genes (glucocorticoid-responsive elements [*GRE*]). Receptor binding to the regulatory sequences of the responsive genes increases or decreases their expression. In the first instance (*ON*), glucocorticoids increase the transcription or stability or both of messenger RNA, which is translated on ribosomes to the designated protein. In the second instance (*OFF*), glucocorticoids repress (*cross-hatched arrows*) certain genes at the transcriptional level by interacting with and preventing the binding of nuclear factors required for activation of the gene (for example, activator protein (AP)-1 nuclear factor). In other instances, glucocorticoids exert their effects post-transcriptionally by either increasing the degradation of messenger RNA or by inhibiting the synthesis or secretion of the protein. **Panel B.** The three main domains (immunogenic, DNA-binding, and ligand-binding) of the glucocorticoid receptor represented in a linear model. At left are the indicated domains and amino acid sequences of the receptor (*see* text for details). HSP 90 = heat-shock protein 90; NLS_1 and NLS_2 = nuclear localization sequences 1 and 2; τ_1 and τ_2 = transactivation domains 1 and 2.

with the specific steroid; and third, the "immunogenic" domain in the amino terminal region.

The nonactivated glucocorticoid receptor resides in the cytosol in the form of a hetero-oligomer with other highly conserved proteins (3). This molecular complex comprises receptor, heat-shock proteins, and immunophilin (Appendix Table 1) (4). The binding of the receptor to the heat-shock protein 90 facilitates its interaction with the ligand (5). When the ligand binds, the receptor dissociates from the rest of the hetero-oligomer and translocates into the nucleus. Before or after the translocation, the receptor forms homodimers through sequences present in the DNA and ligand-binding domains (6).

Gene Regulation

After specific interaction with pore-associated proteins, the hormone-receptor complexes enter the nucleus through the nuclear pores (7). The interaction is facilitated by two nuclear localization sequences in the receptor, both in the ligand-binding domain. Inside the nucleus, the hormone-receptor complexes bind to specific glucocorticoid responsive elements within DNA (8). The complexes modulate the transcription rates of the corresponding glucocorticoid-responsive genes (9), apparently by stabilizing the initiation complex, composed of RNA polymerase II and its ancillary factors A through F. The hormone-receptor complex may interact directly with factor IIB (10), but it also interacts with other nuclear proteins to produce the conditions necessary for effective transcription (11). These proteins may be able to relax the DNA away from the nucleosome and thus make it easier for the polymerase to exert its effects. In addition, glucocorticoid receptors may interact with DNA-binding proteins that are associated with different regulatory elements of the DNA (12, 13). At least two such proteins have been described: One is the glucocorticoid modulatory element-binding protein and the other is the CACCC–box-binding protein. Both of these transcription factors potentiate the modulatory effects of glucocorticoids after transcription of specific genes.

Transcription appears to be important in the regulation of genes involved in growth and inflammation. Glucocorticoid response elements can act both positively and negatively on transcription, depending on the gene on which the complex acts (14, 15). One major way by which glucocorticoids exert down-modulatory effects on transcription is through noncovalent interaction of the activated hormone-receptor complex with the c-Jun/c-Fos heterodimer (16–18), which binds to the activator protein (AP)-1 site of genes of several growth factors and cytokines. The glucocorticoid-receptor complex prevents the c-Jun/c-Fos heterodimer from stimulating the transcription of these genes. Another mechanism by which glucocorticoids may suppress gene transcription is by an interaction between the hormone-receptor complex and glucocorticoid response elements that are in close proximity to responsive elements for other transcription factors (19). Thus, the promoter region of the glycoprotein hormone-α subunit, which is stimulated by cyclic AMP through the cyclic AMP-responsive element, contains a glucocorticoid response element in close proximity, so that when the receptor dimer binds to its own element, it hinders the cyclic AMP–binding protein from exerting its stimulatory effect on that gene.

Post-Transcriptional Effects

In addition to modulating transcription, glucocorticoids also have effects on later cellular events, including RNA translation, protein synthesis, and secretion. They can alter the stability of specific messenger RNAs of several cytokines and other proteins, thereby altering the intracellular steady-state levels of these molecules (20, 21). This may occur through modulation of transcription of still unknown proteins that bind RNA and alter its translation and degradation rates. Also, glucocorticoids influence the secretion rates of specific proteins through mechanisms that have not yet been defined. Finally, the receptor itself has guanylate cyclase activity, and glucocorticoids can rapidly alter the electrical potential of some cells (22, 23).

Anti-inflammatory and Immunosuppressive Effects

Dr. Dimitrios T. Boumpas: Although the cause and pathogenesis of many immunologically mediated diseases are not completely understood, it is known that the localization of leukocytes at sites of inflammation, their subsequent activation, and the generation of secretory products contribute to tissue damage, as shown in Figures 2 and 3 (24–26). Glucocorticoids inhibit the access of leukocytes to inflammatory sites, interfere with their function and the function of fibroblasts and endothelial cells at those sites, and suppress the production and the effects of humoral factors. In general, leukocyte traffic is more susceptible to alteration by glucocorticoids than is cellular function; in turn, cellular immunity is more susceptible than humoral immunity to these agents.

Even though the effects of glucocorticoids on the different types of inflammatory cells will be discussed separately, each cell type is actually involved in complex interactions with other cells. Glucocorticoids affect many, if not all, the cells and tissues of the body, thus provoking a wide range of changes that involve several cell types concurrently.

Effects on Nonlymphoid Inflammatory Cells

Dr. Ronald L. Wilder (Chief, Inflammatory Joint Diseases Section, Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland): Glucocorticoids are among the most potent anti-inflammatory agents available in clinical medicine. Pharmacologic doses of glucocorticoids dramatically inhibit exudation of plasma and accumulation of leukocytes at sites of inflammation. Several factors influence the magnitude of these effects, including the dose and route of administration of the glucocorticoids used, as well as the type and differentiation state of the target cell population (27). Several host variables also modify the anti-inflammatory response to glucocorticoids. For example, some persons (those with active systemic lupus erythemato-

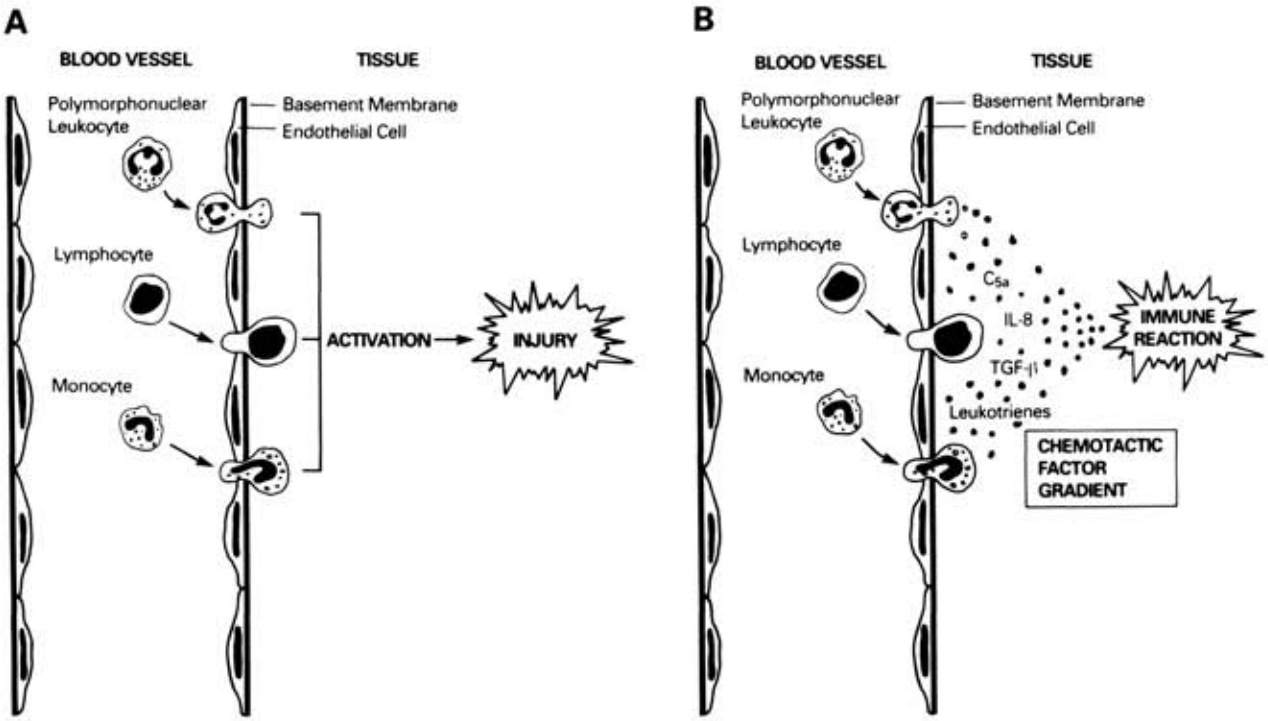

Figure 2. Models of the pathogenesis of inflammation and immune injury. Panel A. The recruitment of leukocytes at sites of inflammation, their subsequent activation, and generation of secretory products contribute to tissue damage. Panel B. Circulating leukocytes exit the vascular bed in response to chemotactic stimuli (for example, complement 5a [*C5a*], leukotrienes, interleukin-8 [*IL-8*], and transforming growth factor [*TGF-β*]) released at sites of inflammation. The interaction of leukocytes with endothelial cells lining blood vessel walls represents the first critical step in leukocyte movement into the tissue. This is mediated by cell surface glycoproteins called adhesion molecules. The persistent migration and tissue accumulation of leukocytes to local inflammatory sites is paramount in initiating or modifying or both many disease processes (24).

sus) appear to have an accelerated rate of glucocorticoid catabolism (28). Various levels of target tissue resistance may exist in some patients with systemic lupus erythematosus and rheumatoid arthritis (29). These factors, alone or in combination, may explain the observation that different patients and diseases have variable therapeutic responses to glucocorticoids (30, 31).

Macrophages

Glucocorticoids antagonize macrophage differentiation and inhibit many of their functions (27). These agents 1) depress myelopoiesis and inhibit expression of class II major histocompatibility complex antigens induced by interferon-γ; 2) block the release of numerous cytokines, such as interleukin-1, interleukin-6, and tumor necrosis factor-α; 3) depress production and release of proinflammatory prostaglandins and leukotrienes; and 4) depress tumoricidal and microbicidal activities of activated macrophages.

Neutrophils

The major effect of glucocorticoids on neutrophils appears to be the inhibition of neutrophil adhesion to endothelial cells. This effect diminishes trapping of neutrophils in the inflamed site and probably is responsible for the characteristic neutrophilia. At pharmacologic doses, glucocorticoids only modestly impair important neutrophil functions, such as lysosomal enzyme release, the respiratory burst, and chemotaxis to the inflamed site. Lower doses do not affect these neutrophil functions (27, 30).

Eosinophils, Basophils, and Mast Cells

Just as they affect macrophages, glucocorticoids decrease circulating eosinophil and basophil counts. They also decrease the accumulation of eosinophils and mast cells at sites of allergic reactions. Functionally, glucocorticoids inhibit IgE-dependent release of histamine and leukotriene C_4 from basophils, and they also inhibit degranulation of mast cells (27).

Endothelial Cells

These cells form the barrier between the blood and the tissues and are critical regulators of the inflammatory cascade. They affect hemostasis, vascular permeability, trapping, and exudation of leukocytes into inflammatory sites. Glucocorticoids have profound effects on the activation and subsequent function of these cells (27) and clearly inhibit vascular permeability. Moreover, they inhibit numerous molecular events associated with activation. For example, they inhibit up-regulation of the expression of class II major histocompatibility complex antigens, as well as endotoxin-induced up-regulated expression of the adhesion molecules (endothelial leukocyte adhesion molecule–1 [ELAM-1] and intercellular cell adhesion molecule–1 [ICAM-1]), which are cell surface molecules critical to leukocyte localization (24–26, 32) (*see* Figure 3). Further, glucocorticoids in-

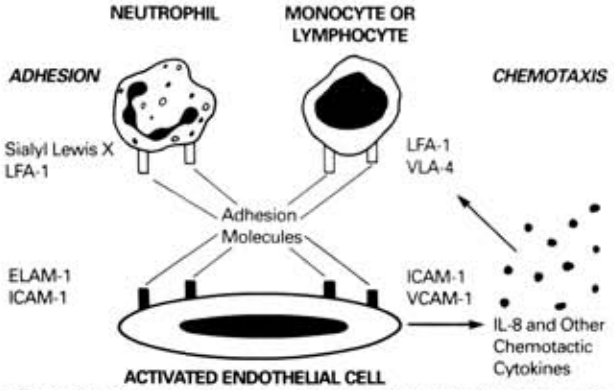

Figure 3. Cellular adhesion molecules. Several adhesion molecules participate in the binding of leukocytes to endothelial cells (25, 26). These molecules belong to at least three groups of glycoproteins: immunoglobulin supergene families, integrins, and selectins. Members of the immunoglobulin supergene family (intercellular cell adhesion molecule–1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1]) are found exclusively on the endothelial cells. Their counter-receptors on leukocytes belong to the integrin group of adhesion molecules (lymphocyte (leukocyte) function–associated antigen-1 [LFA-1], very late antigen–4 [VLA-4]). Members of the selectin group (endothelial leukocyte adhesion molecule–1 [ELAM-1], sialyl Lewis X) group can be found in both cell types. These adhesion molecules mediate the initial tethering and subsequent shear-resistant attachment of leukocytes to the endothelium as well as their penetration into the inflamed tissue. The cytokines interleukin-1, tumor necrosis factor-α, and interferon-γ are also involved in this process through the activation of endothelial cells and the induction of adhesion molecules on their surface. Activated endothelial cells secrete interleukin-8 and other chemotactic cytokines that further stimulate the extravasation of leukocytes bound to endothelial cells.

hibit the secretion of the complement pathway proteins C3 and factor B; they also inhibit the formation of interleukin-1 and arachidonic acid metabolites and are potent inhibitors of the expression of cyclooxygenase type 2.

Fibroblasts

These cells contribute to the inflammatory process and are major targets of glucocorticoids (27). At supraphysiologic concentrations, glucocorticoids suppress proliferation and suppress growth factor-induced DNA synthesis and protein synthesis, including synthesis of collagens. They induce fibronectin messenger RNA transcription, inhibit interleukin-1, inhibit tumor necrosis factor-α–induced metalloproteinase synthesis, and inhibit arachidonic acid metabolite synthesis. They are also potent inhibitors of the up-regulated expression of cyclooxygenase type 2 (Crofford LJ, Wilder RW, Hla T. Unpublished observations). These observations suggest that glucocorticoids might retard bone and cartilage destruction in diseases such as rheumatoid arthritis.

Prostaglandins

The cellular actions of glucocorticoids may be affected by prostaglandins. Prostaglandins are metabolites of arachidonic acid, which is released from phospholipids by the action of phospholipase A_2. Previously, it was assumed that glucocorticoids directly inhibited the enzymatic activity of this enzyme. New evidence, however, indicates that suppression of phospholipase A_2 activity is mediated by the activation of inhibitors of the enzyme itself or by inhibition of enzyme synthesis. The primary mediators of the inhibition of enzyme activity appear to be members of the "annexin" family of proteins, which includes proteins such as lipocortin-1 (33). This topic, however, remains controversial (34–36).

A second step in prostaglandin synthesis is the formation of prostaglandin H_2 from arachidonic acid by enzymes called cyclooxygenases. Recent data have shown the existence of at least two cyclooxygenase (COX) genes, COX-1 and COX-2; the proteins have 61% amino acid identity. The COX-2 gene and protein, but not COX-1, are strongly up-regulated in macrophages, endothelial cells, and fibroblasts by mediators such as endotoxin and interleukin-1, although they are inhibited by glucocorticoids. In contrast, COX-1 is constitutively expressed and relatively unaffected by glucocorticoids.

The role of the differential expression of COX-1 and COX-2 in inflammation and their dramatically different responses to glucocorticoids is unclear, but the available data suggest that glucocorticoids affect the production of proinflammatory arachidonic metabolites (37–40; Crofford LJ, Wilder RW, Hla T. Unpublished observations).

Effects on Lymphocytes

Dr. Dimitrios T. Boumpas: The immunosuppressive effects of glucocorticoids are also directed at the traffic and function of lymphocytes.

T Lymphocytes

In humans, a single dose of glucocorticoids produces a marked but transient lymphocytopenia involving all lymphocyte subpopulations (41). The mechanism of the lymphopenia involves the redistribution of circulating lymphocytes to other lymphoid compartments, particularly the bone marrow (42). Changes in the expression of adhesion molecules (*see* Figure 3) may be responsible for that redistribution of lymphocytes (32). In contrast to other species (such as the mouse, rat, or rabbit), glucocorticoid-induced lymphopenia in humans is not due primarily to cell death. Mature, resting human lymphocytes are not lysed even by suprapharmacologic doses of glucocorticoids. However, immature human T cells (thymocytes and transformed lymphocytes) and, in some instances, activated T cells may be susceptible to lysis (43, 44) by programmed cell death (apoptosis).

In addition to their effects on cell distribution, glucocorticoids also affect the initiation and the progression of the T-cell cycle. During the initiation phase (activation), antigen binds to the T-cell receptor and initiates a cascade of events that leads to production of interleukin-2 (and other cytokines) and induction of high-affinity interleukin-2 receptors. Binding of interleukin-2 to its receptor promotes T-cell proliferation and generation of effector, suppressor, and cytotoxic functions. As shown in Figure 4, glucocorticoids inhibit, through various mechanisms, several events associated with T-cell activation (45–48). In addition to depressing interleukin-2 production, they interfere with the action of interleu-

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