

Glucocorticosteroid inhibition of cytokine production: Relevance to antiallergic actions

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Glucocorticoids are the most clinically effective treatment for asthma available today.¹ These drugs alleviate the major symptoms of asthma by reducing airway inflammation, mucous hypersecretion, and airway reactivity while restoring the integrity of the airways. However, the mechanisms of action that glucocorticoids use to achieve these effects are not fully understood. We believe that the clinical efficacy of glucocorticoid treatment is, to a great extent, the result of their inhibitory effect on leukocyte recruitment into the airways. As such, this review will provide an updated outline of the mechanisms by which glucocorticoids inhibit the infiltration of eosinophils and other inflammatory cells into the airways. Specifically, this review will focus on recent studies that examine the effect of glucocorticoids on chemotactic cytokine gene expression.

GLUCOCORTICOIDS: NATURE'S REMEDY FOR INFLAMMATION

Glucocorticoids are, in a way, nature's remedy for inflammation. As Fig. 1 illustrates, varying levels of endogenous glucocorticoids exist in the circulation at all times.¹ Numerous studies have shown that if the adrenal gland is removed, inflammatory reactions are greatly exacerbated, which suggests that endogenous steroids dampen inflammation; this is true for allergic inflammation as well as for the inflammatory response in general.

During a severe inflammatory response, inflammatory cells release a number of mediators, including interleukin 1 (IL-1), tumor necrosis factor (TNF- α), amines, and so forth. At elevated levels, these mediators will stimulate centers in the brain, which in turn activate the hypothalamic-pituitary-

Abbreviations used

CK- β 10:	Chemokine β 10
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
GRs:	Glucocorticoid receptors
GREs:	Glucocorticoid-response elements
IL:	Interleukin
MCP-3:	Monocyte chemotactic protein-3
mRNA:	Messenger RNA
NF- κ B:	Nuclear factor- κ B
RANTES:	Regulated on activation normal T cell expressed and (presumably) secreted
TNF- α :	Tumor necrosis factor- α

adrenal axis; this results in an increase of cortisol in the circulation, thereby attenuating the inflammatory response.² It is now believed that even at normal levels, endogenous hormones will regulate inflammation. The increased use of glucocorticoids in the treatment of inflammation during the past decade is partly because these drugs are analogs of the endogenous glucocorticoids and share their antiinflammatory activities.³

The development of potent inhaled glucocorticoids has enhanced the popularity of glucocorticoids in the treatment of asthma and other allergic diseases. These agents bind the glucocorticoid receptor with very high affinities and are rapidly metabolized and absorbed; such characteristics facilitate their selectivity for the lung and/or the upper airways when inhaled.^{4,5} The beneficial effects of glucocorticoids in the treatment of asthma are listed in Table I.

MECHANISM OF ACTION OF GLUCOCORTICOIDS: THE PREVAILING VIEW

The prevailing view of the mechanism of action of glucocorticoids has changed over the years. For decades investigators believed that glucocorticoids controlled inflammation by stabilizing the lysosomes within neutrophils, thereby preventing these cells from degranulating and exacerbating the in-

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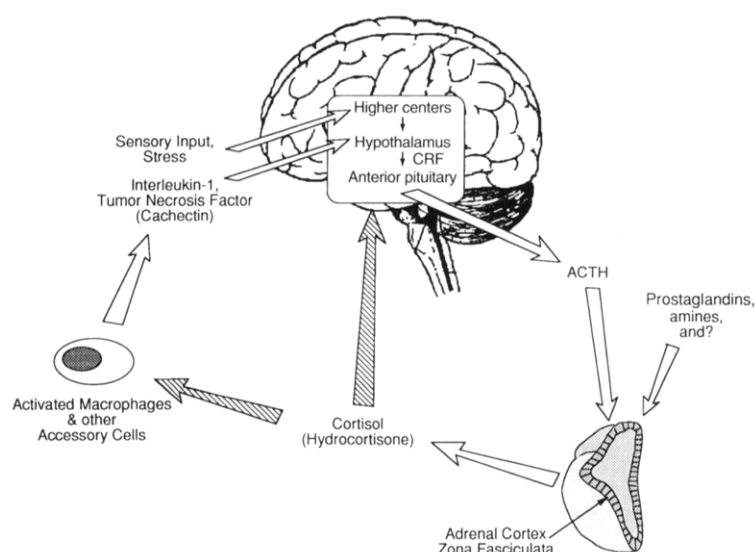


FIG. 1. Schematic representation of endogenous glucocorticoid production. *Filled arrows* indicate inhibitory influences; *open arrows* indicate stimulation.

TABLE I. Beneficial effects of glucocorticosteroids in asthma

Reduce airways inflammation
Improve airways function
Diminish airways secretions
Decrease airways reactivity
Restore airways integrity

flammatory response.⁶ In the mid- to late 1960s, however, this view changed dramatically with the discovery of the steroid receptor and the realization that steroids induce gene expression. A few years later, Flower et al.⁷ and Hirata et al.⁸ demonstrated that glucocorticoids induce the antiinflammatory protein lipocortin; lipocortin inhibits the enzyme phospholipase A₂, thereby preventing generation of prostaglandins and lipoxygenase products. As a result, there was widespread acceptance in the 1980s that the antiinflammatory actions of glucocorticoids were the result of phospholipase A₂ inhibition. Whereas steroids and lipocortin inhibit phospholipase A₂ and thus block generation of arachidonic acid metabolites in many cell types, it is now clear that this is only one of many different mechanisms (discussed below) that glucocorticoids use to attenuate the inflammatory response.

Glucocorticoids exert their antiinflammatory effects through glucocorticoid receptors (GRs). In the cytoplasm of cells, the GR exists as a heterocomplex that contains a single molecule of GR in association with various heat shock proteins, including hsp 90. On binding hormone, the GR

heterocomplex dissociates, and the ligand-activated GR translocates from the cytoplasm to the nucleus. The activated GR may then upregulate the transcription of antiinflammatory genes, such as lipocortin, neutral endopeptidase, and inhibitors of plasminogen activator, presumably through binding to specific DNA sequences termed "glucocorticoid response elements" (GREs). However, it is now appreciated that the ability of glucocorticoids to suppress the transcription of genes involved in inflammation may be their primary activity. Toward this end, the activated GR binds either to negative GREs or interacts with transcription factors, including apolipoprotein 1,¹⁰ or nuclear factor kappa B (NF- κ B)¹¹ to down-regulate gene expression (Fig. 2). Recently Karin et al.¹² have demonstrated that glucocorticoids can also upregulate the expression of inhibitor of nuclear factor- κ B, the specific NF- κ B inhibitor, to decrease NF- κ B's transcriptional ability. As shown in Fig. 3, signals that initiate an inflammatory response trigger the activation of such transcription factors, which in turn stimulate the gene expression of enzymes and cytokines associated with inflammation. In addition, glucocorticoids may decrease the stability of specific messenger RNA (mRNA) molecules to modulate gene transcription.¹³ Notable among glucocorticoid-suppressed genes are collagenase, elastase, plasminogen activator, nitric oxide synthase, cyclooxygenase type II, and most cytokine and chemokine genes. The remainder of this brief review will focus on cytokines and chemokines as steroid targets.

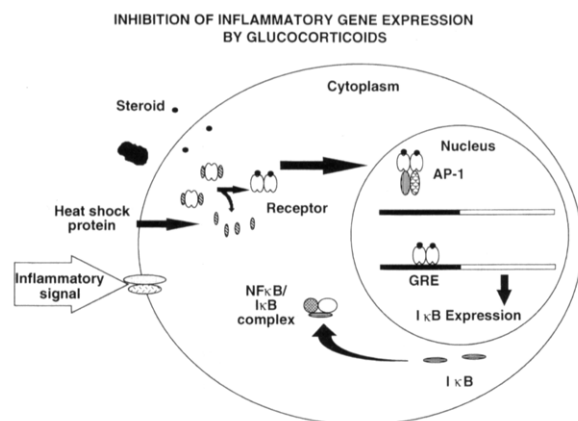


FIG. 2. Inhibition of inflammatory gene expression by glucocorticosteroids. AP-1, Apolipoprotein 1; IκB, inhibitor of NF-κB.

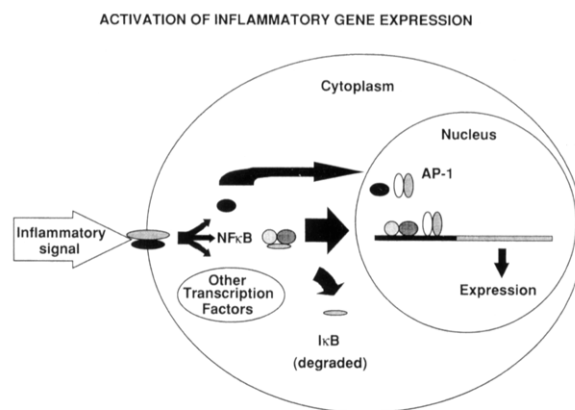


FIG. 3. Activation of inflammatory gene expression. AP-1, Apolipoprotein 1; IκB, inhibitor of NF-κB.

IMPACT OF GLUCOCORTICOIDS ON INFLAMMATION IN ALLERGIC MODEL SYSTEMS

Asthma is characterized by infiltration of eosinophils and lymphocytes into the airways.¹⁴ Until recently, the ability of glucocorticoids to suppress the infiltration of these cells has not been appreciated, because bronchoscopy and bronchoalveolar lavage studies were not performed regularly. Our awareness of this glucocorticoid-mediated effect arose primarily from studies that used antigen challenge models.

Experimental allergen challenge often produces a biphasic response. Recent evidence suggests that in the acute response, activation and degranulation of mast cells occur, resulting in release of inflammatory mediators.¹⁵⁻¹⁷ Characteristics of this acute phase include narrowing of the airways, wheal and flare in the skin, and nasal congestion, depending on the challenge site. During the late response, resident mast cells, as well as eosinophils, lymphocytes, and other mononuclear cells that have infiltrated the airways, release their own panoply of inflammatory mediators. Many investigators now believe that the experimental late phase is a better reflection of the chronic inflammation observed in asthma.

Although use of steroids in the short term does not inhibit human mast cell degranulation in vitro or the acute response in vivo, prolonged administration of inhaled glucocorticoids can dampen the acute response, most likely by decreasing the number of mucosal mast cells.¹⁸⁻²⁰ Studies have demonstrated that oral and inhaled steroids can virtually eliminate the late response.²¹⁻²⁹ We believe this is due in large part to inhibition of the influx of inflammatory cells into the airways.

THE ROLE OF CYTOKINES IN ALLERGIC CELL RECRUITMENT

As stated previously, asthma is characterized by the recruitment and migration of inflammatory cells, specifically eosinophils, into the airways. Cytokine generation in the airways may trigger such infiltration. We believe that four classes of cytokines are important in allergic cell recruitment: (1) nonspecific endothelial activators; (2) specific endothelial activators; (3) those that activate eosinophil functions and prolong their survival, and (4) those that directly stimulate cell migration. More important, one of the most vital antiinflammatory effects of glucocorticoids in the treatment of asthma may be the inhibition of cytokine production.³⁰⁻³⁴

Endothelium-activating cytokines

Cytokines that nonspecifically activate the endothelium, namely, TNF-α and IL-1, are released as the result of experimental antigen challenge³⁵⁻³⁸ and presumably during natural antigen exposure. There is evidence in humans and rodents that IgE stimulation causes the activation of mast cells and release of TNF-α.³⁷⁻⁴⁰ Macrophages in highly allergic individuals, which express both low- and high-affinity IgE receptors, may also produce TNF-α in an IgE-dependent manner.⁴¹⁻⁴⁴ Infiltrating monocytes may also produce these cytokines, as may other cell types. TNF-α and IL-1 induce the expression of the adhesion molecules E-selectin, P-selectin, intracellular adhesion molecule-1, and vascular cell adhesion molecule-1 on the surface of endothelial cells.⁴⁵ When this occurs, the endothelium can actively, but nonselectively, recruit leukocytes, including neutrophils, eosinophils, mononu-

TABLE II. Human chemokines

α (C-X-C) family	β (C-C) family
IL-8	RANTES
PF-4	MCP-1, 2, 3
β -TG	MIP-1 α , β
IP-10	I-309
GRO α , β , γ	ACT-2
ENA-78	MAD 5a, b
NAP-2	FIC
GCP-2	CK β -1, 3, 4, 6, 7, 8, 9, 10, 11
MAD-2	
CK α -1	

PF-4, Platelet factor 4; *β -TG*, β -thromboglobulin; *IP-10*, interferon-inducible protein 10; *GRO α , β , γ* , growth related protein α , β , γ /melanoma growth stimulatory activity; *ENA-78*, epithelial neutrophil activating protein 78; *NAP-2*, neutrophil activating peptide 2; *GCP-2*, granulocyte chemotactic protein 2; *MAD-2*, monocyte adhesion dependent protein 2; *CK α -1*, chemokine α -1; *MIP*, macrophage inhibitory protein; *I-309*, I-309 protein; *ACT-2*, immune activation cytokine; *MAD 5a, b*, monocyte adhesion dependent protein 5a, b; *FIC*, fibroblast-induced cytokine.

clear cells, and basophils from the circulation; all circulating leukocytes have counterligands for at least one of the adhesion molecules expressed on endothelium under these conditions. The actions of these cytokines may in part explain the early nonspecific cell recruitment that occurs after experimental antigen challenge.

Glucocorticoids are very effective and potent inhibitors of TNF- α and IL-1 release from macrophages, monocytes, and other infiltrating cells. Studies in rodents and humans suggest that steroids inhibit TNF- α release in vivo.⁴⁶⁻⁴⁸ Inhibition of TNF- α release may block the antigen-induced secretion of nonspecific endothelial activators, which indirectly result in an inhibition of endothelial activation. There is some evidence that this is the case,⁴⁹ but further study in humans is required.

Cytokines that reduce adhesion marker expression

The second class of cytokines encompasses those that selectively activate the endothelium. This class of cytokines includes IL-4 and IL-13, two cytokines that are associated with allergic diseases.^{43, 50-54} Among resident airway cells, there is some evidence that mast cells may release IL-4,⁵⁵ whereas among the infiltrating cells, lymphocytes, basophils, and now eosinophils have been shown to contain IL-4.^{48, 56} There is some evidence that the lymphocyte is probably the most important source of IL-4 (and probably IL-13) in allergic reac-

tions.^{54, 57} When IL-4 and IL-13 are released, the endothelium is activated to express vascular cell adhesion molecule-1 only; consequently, only circulating cells that have the leukocyte counterligand very late activation antigen-4 can bind. These cells include basophils, eosinophils, monocytes, and lymphocytes. Notably absent from among these cell types are neutrophils. These cytokines are selective in that cells associated with allergic reactions can attach to the endothelial surface, whereas neutrophils, which are generally not associated with asthma and allergic reactions, cannot.^{52, 53, 58-60}

As with the nonspecific endothelial activators, glucocorticoids inhibit the release of IL-4 and IL-13. Steroid inhibition of cytokine release from lymphocytes is well documented,^{24, 61-63} and data now show that glucocorticoids also inhibit IL-4 release from basophils (Schroeder J et al., personal communication); however, it is not yet known whether steroids also inhibit mast cell IL-4 production. At present our hypothesis is that glucocorticoids inhibit the release of activating stimuli, thereby lessening specific endothelial activation in vivo. In our hands, glucocorticoids do not inhibit endothelial activation in vitro induced by either specific or nonspecific activators.^{53, 64}

Cytokines that promote eosinophilia

The next class of cytokines believed to be important in allergic reactions includes those that cause eosinophil priming, namely, IL-3, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon gamma.⁶⁵ These cytokines are probably responsible for differences in the behavior of eosinophils in patients with asthma and allergies compared with eosinophils found in normal subjects. The presence of these cytokines promotes the following: (1) prolonged eosinophil survival; (2) increased adhesion molecule expression; and (3) potentiated eosinophil degranulation and movement across an endothelial barrier.⁶⁶⁻⁷² The source of these cytokines in vivo is difficult to assess, because they may be generated by many different cell types. Resident cells of the epithelium and endothelium, including fibroblasts and macrophages, as well as infiltrating cells such as lymphocytes, monocytes, and eosinophils, may produce GM-CSF.^{73, 74} IL-5 is probably primarily derived from lymphocytes, although it has been detected in mast cells and eosinophils.^{75, 76}

Steroids interrupt the eosinophil priming process in several ways. In addition to inhibiting GM-CSF, IL-3, and IL-5 production,^{24, 61-63, 77-81} steroids directly inhibit survival of eosinophils.⁸²⁻⁸⁷ This probably explains why steroids are so effective

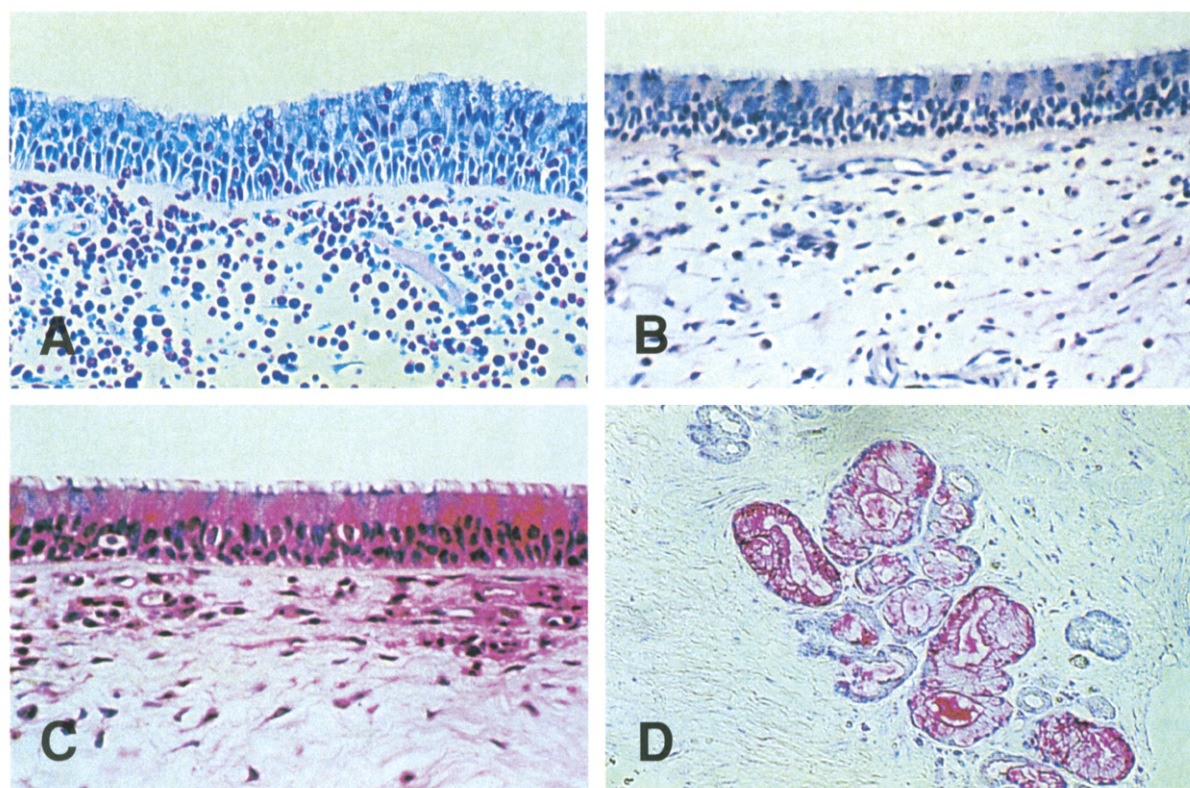


FIG. 4. Sections of nasal polyp tissue. **A**, Photomicrograph of Giemsa-stained tissues demonstrating marked eosinophilia within tissue (original magnification $\times 400$). **B**, Control antibody (IgG_{2a}) staining (original magnification $\times 400$). **C**, Anti-RANTES staining (original magnification $\times 400$). **D**, Glandular epithelium staining with anti-RANTES (original magnification $\times 250$).

in removing eosinophils from patients with allergic disease.

Chemokines

The last class of cytokines that will be discussed are the chemokines, which have been studied extensively during the past few years. These cytokines have many chemotactic properties, including the ability to induce cell migration and to activate selected cell types.^{88, 89} Chemokines may act in part by binding to tissue matrix rather than forming a soluble gradient.⁹⁰ The chemokine superfamily consists of approximately 30 members, all of which are small cytokines with a molecular weight between 8 and 10 kd (Table II). This superfamily is divided into two subfamilies, the α (C-X-C) family and the β (C-C) family. They have 25% to 75% homology at the amino acid level and contain four conserved cysteine residues; the C-X-C subfamily contains an intervening amino acid in the first cysteine pair.

Recent research has focused on the C-C family, which includes several chemoattractants implicated in allergic disease. Of particular interest are those chemokines that induce eosinophil or lym-

phocyte migration such as RANTES (regular on activation normal T cell expressed and [presumably] secreted), monocyte chemotactic protein-3 (MCP-3), eotaxin, and macrophage inflammatory protein-1 α .⁹¹⁻⁹⁵ Recently another chemokine, chemokine $\beta 10$ (CK- $\beta 10$), discovered by Human Genome Sciences, has been shown to induce eosinophil chemotaxis.⁹⁶ Cellular sources of these chemokines are numerous. We speculate, however, that epithelial cells, endothelial cells, and T cells may be the key producers of eosinophil-attracting chemokines such as RANTES, MCP-3, eotaxin, and CK- $\beta 10$.

Among the known chemokines, RANTES is probably one of the most potent eosinophil chemoattractants⁹¹⁻⁹⁴ and induces eosinophil accumulation when injected intradermally in dogs.⁹⁷ Based on this, a study was designed to determine whether RANTES could induce eosinophil migration in humans in vivo.⁹⁸ RANTES was injected into allergic and normal individuals in this study. Large numbers of eosinophils were found to line up along deep vessels within 30 minutes of injection into allergic individuals. At 6 hours after injection, eosinophil degranulation was evident, which indi-

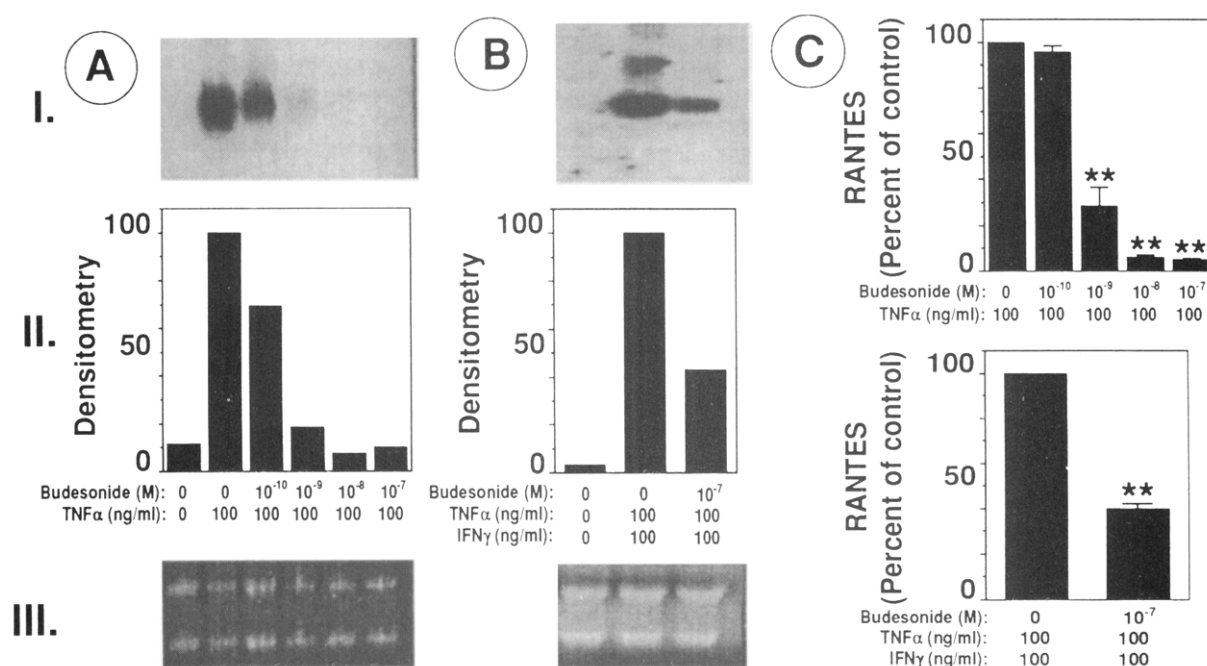


FIG. 5. Inhibition of TNF- α -induced RANTES expression in airway epithelial cells. **A**, Beas-2B epithelial cells were treated with indicated concentrations of budesonide 24 hours before stimulation with TNF- α . **B**, Cells were treated as indicated. **C**, Concentration of RANTES in supernatants detected by enzyme-linked immunosorbent assay ($n = 3$ to 4 , $**p \leq 0.05$ vs control, which was 5.1 ± 2.5 ng/ml (top) and 18.2 ± 5.1 (bottom). **I**, Autoradiography of RANTES mRNA expression; **II**, densitometric analysis of radiography, expressed as percent of maximum; **III**, ethidium bromide staining of agarose gel showing equal loading of lanes and integrity of mRNA.

cates that RANTES induces not only adherence and migration but some activation of eosinophils. RANTES also induced eosinophil migration in non-allergic individuals; a marked infiltration of eosinophils was observed at 24 hours after injection. Interestingly, eosinophil infiltration was not apparent at 6 hours after injection in nonallergic individuals, which is in contrast to allergic subjects. After injection of 4 μ g of RANTES, eosinophils made up most of the cells that migrated into the skin at 24 hours, in association with a number of mononuclear cells, particularly T cells. These findings are not surprising, because RANTES has also been shown to cause migration of memory T cells.^{89, 92, 99, 100} Neutrophils, however, were not induced to migrate into the skin in these studies. Although RANTES challenge resulted in eosinophil degranulation with copious amounts of free major basic protein detected, no detectable signs or symptoms, including wheal, flare or induration, had occurred.⁹⁸

To determine whether RANTES was expressed in allergic reactions, a second study was conducted in which nasal polyps were examined.¹⁰¹ Clinical findings included numerous eosinophils, as well as marked epithelial staining with an antibody for RANTES (Fig. 4). To confirm this finding in vitro,

airway epithelial cells were cultured and then probed for RANTES messenger RNA (mRNA) with Northern blot analysis (Fig. 5). A large increase in RANTES expression was noted; conversely, it was also observed that glucocorticoid treatment virtually completely inhibited the expression of RANTES in human airway epithelial cells.¹⁰²

In addition to RANTES, epithelial cells produce several other cytokines that are steroid sensitive. CK- β 10 mRNA is expressed in activated epithelial cells but not in resting cells.⁹⁶ Moreover, preliminary studies indicate that steroids inhibit the production of this novel chemokine.⁹⁶ Purified recombinant protein CK- β 10 induces eosinophil migration at very low concentrations. Thus the chemokines, like other cytokines involved in allergic inflammation, are susceptible to the inhibitory actions of glucocorticoids.

Evidence is now emerging that chemokines are involved in the pathophysiology of respiratory illnesses. Because most asthma exacerbations, especially those in children, are associated with viral infections of the upper respiratory tract we recently collaborated with Proud et al.¹⁰³ to study chemokine and cytokine expression in airway epithelial cells infected with rhinovirus. These studies revealed that

rhinovirus infection induced IL-8 and RANTES expression within a few hours after infection. Based on these results, it appears that activation of virus-infected epithelium can subsequently activate some of the cytokine mechanisms that cause leukocyte infiltration. The importance of this to viral-induced exacerbations of asthma and whether steroids can inhibit this response are under investigation.

FINDING NOVEL GLUCOCORTICOID TARGETS IN THE AIRWAYS

To uncover new information on the mechanisms of airway inflammation and glucocorticoid action in humans, we have used DNA differential display technology to identify novel genes in human airway epithelial cells that are relevant to inflammatory disease and glucocorticoid action. In particular, we have focused on novel genes that are expressed in airway epithelial cells as a result of cytokine stimulation and for which such induced expression is inhibited by glucocorticoids. DNA differential display involves the reverse transcription of mRNAs with oligo-dT primers anchored to the 5' end of the poly(A) tail, followed by amplification in the presence of random primers, thereby generating double-stranded complementary DNA products of different lengths.^{104, 105} The resulting complementary DNA products (expressed sequence tags) are separated by size on a DNA sequencing gel (Fig. 6). Presently 19 different expressed sequence tags that are TNF- α inducible and dexamethasone sensitive have been identified, subcloned, and sequenced, some exhibiting homologies to cell-surface receptors, cytokines, and signaling molecules (Schwiebert et al., unpublished observations). It is hoped that applications for some of these interesting results will soon be found.

SUMMARY

We believe that there are the following four classes of glucocorticoid-sensitive cytokines that are involved in cell recruitment: (1) those that activate endothelium nonspecifically; (2) those that activate endothelium specifically; (3) those that activate, prime, and prolong the survival of eosinophils; and (4) those that stimulate movement of cells up into the epithelium. Glucocorticoids inhibit the generation of these cytokines and thereby prevent several different aspects of inflammation, including the activation and recruitment of inflammatory cells (eosinophils, basophils, and lymphocytes) and the release of inflammatory mediators. We believe such pleiotropic actions account for the efficacy and widespread use of glucocorticoids in the treatment of asthma.

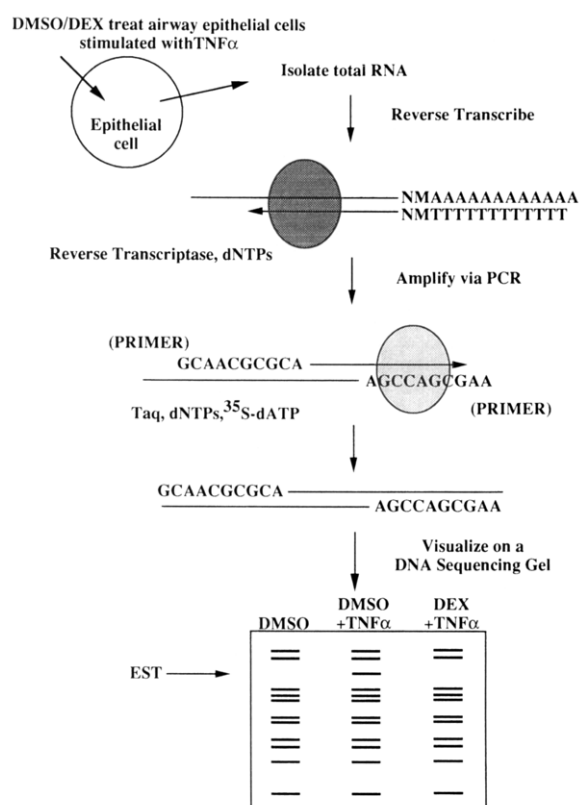


FIG. 6. Schematic representation of DNA differential display technology. Airway epithelial cells are stimulated, total RNA is isolated, and then reverse-transcribed with an oligo-dT primer. Resulting cDNA products are amplified in presence of two random 10-mers and then visualized on a DNA sequencing gel. Bands of interest are eluted from the gel and analyzed.

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