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REVIEW ARTICLE

Glucocorticoid Action and Resistance in Asthma

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ABSTRACT

In the past decade it has become clear that asthmatic patients who are clinically unresponsive, or "resistant" to glucocorticoid therapy may have T cells which manifest resistance to glucocorticoid inhibition. This is shown by failure of glucocorticoid not only to repress the production of pro-inflammatory, asthma-relevant cytokines such as IL-5, but also to induce the production of anti-inflammatory cytokines, such as IL-10. Molecular mechanisms underlying this resistance are beginning to be defined, and include inappropriate production of transcriptional regulatory proteins (which may bind to and inactivate the glucocorticoid receptor), cytokine-induced modifications of the glucocorticoid receptor (such as phosphorylation), which alter its capacity to bind to ligand, and complex interactions which regulate histone acetylation status and the quaternary structure of DNA itself. Continued study of these phenomena will lead to a better understanding of the "normal" physiological mechanisms of glucocorticoid action, and may point to new, alternative avenues of therapy for glucocorticoid resistant asthmatics who suffer from severe disease, and for whom as yet no adequate therapy is available.

KEY WORDS

asthma, glucocorticoid, resistance, therapy

INTRODUCTION

Glucocorticoids are very effective therapy for asthma and numerous studies have shown that they reduce asthma symptoms, exacerbations and bronchial hyperresponsiveness. Unfortunately, however, a proportion of patients develop severe disease which is relatively or totally refractory to glucocorticoid therapy. While the numbers of these patients are small, they consume a significant proportion of medical resources in terms of both time and money.[1] Regardless of cost considerations, there is an urgent need to provide alternative therapies for these patients, who often have severely impaired quality of life not only from the severity of their symptoms but from the effects of excessive glucocorticoid exposure.

THE CONCEPT OF GLUCOCORTICOID RESISTANT ASTHMA

The diagnosis of glucocorticoid resistant asthma is essentially one of exclusion. Before it can be diagnosed, it must be ensured that the diagnosis of asthma is correct and that factors contributing to poor asthma control have been eliminated as far as is possible. These stages have been formalised[2] as follows :

·Establish/confirm the diagnosis of asthma

·Ensure that adequate dosages of glucocorticoids are reaching the airways (compliance, inhaler technique, metabolic factors which may increase glucocorticoid clearance)

·Exclude ongoing exposure to provoking agents (smoke, irritants, allergens, etc.)

·Rule out and eliminate as far as possible other potential aggravating conditions (chronic sinusitis, oesophageal reflux, drugs which may exacerbate asthma, etc.)

·Rationalise inhaled β2-agonist therapy

·Introduce a strict management plan to assess response to therapy which will typically last for weeks or sometimes months.

Even when all factors which may abrogate the effects of glucocorticoid therapy are eliminated or minimised as far as possible, there remains a group of pa-

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tients who show little or no response of airways obstruction to glucocorticoid therapy. This concept was first proposed nearly 30 years ago.[3,4]

The first attempt to define glucocorticoid resistant asthma in objective terms[5] was based on changes in FEV_1 following a 14 day course of oral prednisolone (40 mg/day). Patients showing improvements of <15% of baseline were classified as resistant, whereas those showing improvements of 30% or more were considered sensitive. All patients, in contrast, showed marked improvement in FEV_1 in response to inhaled β2-agonists, indicating that, in both patient groups, airways obstruction is potentially at least partly reversible. Clearly, these FEV_1 responses represent opposite ends of a continuum of clinical response. Most subsequent studies have employed definitions of glucocorticoid sensitive and resistant asthma similar or identical to the above, in both adults and children.[6,7] The minimum necessary period of glucocorticoid therapy required to detect a response has never been formally defined. It is possible that 14 days may not always be sufficient, although it was shown[8] that 90% of severe asthmatic children showing an improvement in FEV_1 >15% of baseline on high-dosage oral glucocorticoid therapy did so within 10 days.

On the basis of these studies, a workshop of experts on glucocorticoid resistant asthma proposed that this should be defined by the failure to improve baseline morning pre-bronchodilator FEV_1 by more than 15% of the baseline value following at least 14 days of therapy with prednisolone 40 mg daily or its equivalent.[9] Patients with complete glucocorticoid resistance show not only a failure of response in terms of FEV_1, but also, in general, an ability to tolerate reduction of glucocorticoid dosages without significant change in disease activity. In addition, they typically show little increase in FEV_1 even with more protracted, much higher dosages of systemic glucocorticoids. This is in distinction to glucocorticoid "dependent" asthmatics, who may not show a response in FEV_1 of 15% or more during a 14 day trial of systemic glucocorticoid therapy, but rapidly deteriorate when this therapy is withdrawn. Although glucocorticoid resistant patients may show a degree of fixed airways obstruction, many show marked diurnal variability in PEF and a brisk bronchodilator response.[6,7] Most clinical descriptions of glucocorticoid resistant asthma emphasise the following common clinical features[10] :

·Persistent symptoms despite optimal therapy

·Chronic airflow limitation with FEV_1 < 60% predicted in adults and <80% predicted in children

·Failure to achieve an increase in morning pre-bronchodilator FEV_1 of >60% predicted despite systemic glucocorticoid therapy (at least 40 mg/day of prednisolone or its equivalent given for at least 14 days)

·Frequent nocturnal symptoms with significant "morning dipping" of PEF

·Poor clinical and spirometric response to oral glucocorticoid therapy, with <15% improvement in pre-bronchodilator FEV_1 following a trial of oral glucocorticoid therapy as specified.

MECHANISMS OF GLUCOCORTICOID RESISTANT ASTHMA

Despite several decades of usage of glucocorticoids as anti-inflammatory agents, relatively little is known about the precise mechanisms by which they ameliorate inflammatory diseases. This echoes the fact that little is known about precisely how inflammatory changes in the bronchial mucosa of asthmatics actually cause clinical symptoms. Glucocorticoids exert a number of generalised anti-inflammatory activities, such as capillary vasoconstriction and reduction of vascular permeability, which may be relevant to suppression of inflammation however caused. There is now abundant evidence (reviewed in[11]) that glucocorticoid therapy which results in amelioration of asthma is associated with reduced activation of, and synthesis of asthma-relevant cytokines by activated T cells. For example, elevated percentages of peripheral blood CD4, but not CD8 T cells from patients with exacerbation of asthma expressed mRNA encoding IL-3, IL-5 and GM-CSF but not IL-2 and IFN-γ as compared with controls.[12] Spontaneous secretion of the corresponding cytokines was also demonstrable in these patients using an eosinophil survival-prolonging assay. The percentages of CD4 T cells expressing mRNA encoding asthma-relevant cytokines, as well as spontaneous secretion of these cytokines was reduced in association with glucocorticoid therapy and clinical improvement. In a double-blind, parallel group study,[13] therapy of mild atopic asthmatics with oral prednisolone, but not placebo, resulted in clinical improvement associated with a reduction in the percentages of BAL fluid cells expressing IL-5 and IL-4 and an increase in those expressing IFN-γ. These and many other studies support the general hypothesis that glucocorticoids exert their anti-asthma effect at least partly by reducing the synthesis of cytokines by T cells.

FUNCTIONAL T CELL ABNORMALITIES IN GLUCOCORTICOID RESISTANT ASTHMA

A corollary of the above is that glucocorticoid resistant asthma may at least partly reflect refractoriness of T cells to glucocorticoid inhibition. A pioneering study in this field[14] showed that lectin-induced colony formation in soft agar by peripheral blood T cells *in vitro* (a measure of proliferation) was less susceptible to glucocorticoid (methylprednisolone 10^{-8} M) inhibition in clinically glucocorticoid resistant, as compared with sensitive asthmatics. This observation was followed up with two reports from the author's laboratory[15,16] characterising peripheral blood T cells of glu-

cocorticoid sensitive and resistant asthmatics. In summary, it was demonstrated in these reports that lectin-induced proliferation of peripheral blood T cells was inhibited by dexamethasone at therapeutic concentrations in glucocorticoid sensitive, but not resistant asthmatics. This resistance was not absolute but relative, reflecting a shift in the concentration-response curve for inhibition. In other words, T cells from glucocorticoid resistant asthmatics can be inhibited by glucocorticoids, but only at concentrations requiring glucocorticoid dosages that most physicians would not contemplate using for protracted periods in clinical practice. Consistent with this, it was demonstrated[16] that elevated percentages of peripheral blood T cells expressed the activation markers in CD25 and HLA-DR in glucocorticoid resistant, as compared with sensitive asthmatics, suggesting persistent T cell activation in the resistant patients despite glucocorticoid therapy.

In glucocorticoid resistant asthmatics, clinical resistance to therapy could not be accounted for by differences in absorption and clearance of plasma prednisolone derived from orally administered prednisone. We have subsequently shown[17] that the inhibition of lectin-induced proliferation of peripheral blood T cells from asthmatics by glucocorticoids *in vitro* is reproducible both in the short term and when patients are re-tested after intervals of several months. This suggests that the degree of glucocorticoid sensitivity of peripheral blood T cells from asthmatics, and by inference their clinical sensitivity to glucocorticoid therapy, remains relatively constant, although there is evidence (see below) that sensitivity in individual patients may vary to some degree according to the ongoing severity of their disease.

Glucocorticoid resistant asthmatics are not Addisonian and do not have elevated plasma cortisol concentrations,[15] suggesting that the impaired glucocorticoid responsiveness observed in their peripheral blood T cells is not a generalised, systemic phenomenon. One possibility is that impaired T cell glucocorticoid responsiveness in asthma may be induced by the action of pro-inflammatory cytokines within the local environment of the inflammatory process. In this regard, there exists evidence that the glucocorticoid receptor ligand binding affinity of peripheral blood T cells in asthmatics may be altered in the short term according to disease severity *in vivo* and by exposure to cytokines *in vitro*. The glucocorticoid receptor ligand binding affinities of peripheral blood T cells from a group of poorly controlled asthmatics were reduced as compared with normal controls (median K_d 29.0 *vs* 8.0 nM). Glucocorticoid therapy of these asthmatics, which was accompanied by clinical improvement, was associated with a significant increase in affinity of the T cell glucocorticoid receptors.[18] In a detailed study of glucocorticoid receptor binding in peripheral blood mononuclear cells from glucocorticoid sensitive and resistant asthmatics, two distinct abnormalities were observed.[19] The majority of the asthmatics (15 out of 17) demonstrated a significantly reduced receptor binding affinity (mean K_d 42.1 nM) as compared with sensitive patients (mean K_d 21.6 nM) and normal controls (mean K_d 7.9 nM). This abnormality was confined to T cells, reverted to normal after culture of the T cells *in vitro* for 48 hours, but could be sustained by culture in the simultaneous presence of high concentrations of IL-2 and IL-4. The remaining two glucocorticoid resistant asthmatics had abnormally low numbers of nuclear glucocorticoid receptors with normal binding affinity. This abnormality was not confined to T cells and was not influenced by the presence of exogenous cytokines. It was further shown[20] that this abnormality could be induced by culture of peripheral blood T cells from normal donors with IL-2 and IL-4 *in vitro*, and that this induction was associated with a reduced inhibitory effect of methylprednisolone on the proliferation of the T cells induced by phorbol ester and ionomycin. A similar effect of exogenous IL-13 alone was subsequently demonstrated in monocytes.[21] The physiological significance of these relatively small changes in ligand binding affinity of the glucocorticoid receptor is difficult to assess, but clearly they may contribute to glucocorticoid refractoriness. Alternatively, they may represent an epiphenomenon reflecting more fundamental changes in the glucocorticoid receptor of T cells on exposure to cytokines (see below).

There is some evidence for a differential effect of glucocorticoids on T cells from sensitive and resistant asthmatics *in vivo*.[22] In this study, bronchoalveolar lavage was performed in glucocorticoid sensitive and resistant asthmatics before and after a course of oral prednisone, and expression by BAL cells of mRNA encoding cytokines was measured by *in situ* hybridisation. Whereas prednisone therapy of the glucocorticoid sensitive asthmatics was associated with reductions in the percentages of BAL cells expressing mRNA encoding IL-4 and IL-5 and elevation of the percentages of cells expressing mRNA encoding IFN-γ, only a decrease in the percentages of cells expressing mRNA encoding IFN-γ was observed in association with prednisone therapy of the glucocorticoid resistant asthmatics. These data are compatible with the hypothesis that glucocorticoids exert differential effects on cytokine mRNA expression in T cells from glucocorticoid sensitive and resistant asthmatics *in vivo*.

Although glucocorticoids generally reduce inflammation by inhibiting the production of pro-inflammatory cytokines, one interesting facet of glucocorticoid action, which is receiving increasing attention, is their ability to increase the production of the anti-inflammatory cytokine interleukin-10 (IL-10). Studies in patients, initially in transplantation,[23] and

more recently in other conditions including asthma,[24] have demonstrated that administration of glucocorticoids, intravenously or by inhalation, results in a significant increase in systemic or local IL-10 synthesis. Parallel studies from our own laboratories have shown a concentration-dependent induction by glucocorticoids of T cell IL-10 expression *in vitro*.[25] Interest in the possible therapeutic benefit of IL-10 in asthma already exists, based on its proposed role in regulating immune homeostasis in the lung.[26] IL-10 inhibits pro-inflammatory cytokine production, antigen presentation, T cell activation and mast cell and eosinophil function (reviewed in[27]). Furthermore, synthesis of IL-10 is deficient in the airways of asthmatics as compared with controls,[28] and polymorphisms of the IL-10 gene leading to impaired expression of IL-10 are associated with a more severe disease phenotype.[29] We have recently described a marked deficiency in the capacity of CD4+ T cells from glucocorticoid resistant asthmatics to synthesise IL-10 following *in vitro* stimulation in the presence of dexamethasone, as compared with sensitive patients of equivalent disease severity.[30] Defining new modalities of therapy, involving new drugs alone or in combination with glucocorticoids,[31] which overcome this defect may restore essential anti-inflammatory mechanisms that help limit asthmatic inflammation.

MOLECULAR BASIS OF GLUCOCORTICOID ACTION AND RESISTANCE

Recent observations have increased our knowledge of how the GR regulates transcription, and how this process may be modified both *in vitro* and *in vivo*. The GR comprises of three domains, the N-terminal or immunogenicity domain, the central DNA-binding domain and the C-terminal ligand-binding domain.[32] The GR in its ligand-unbound state is located primarily in the cytoplasm as part of hetero-oligomeric complexes containing the heat shock proteins 90, 70 and 50. Upon binding to ligand, the GR undergoes conformational changes, dissociates from the heat shock proteins, homodimerises and translocates to the nucleus. There, the ligand-activated GR may interact with DNA sequences (glucocorticoid response elements) or with other transcriptional regulators through protein/protein interactions, directly influencing the activity of the latter on their target genes.

Proteins which may bind directly to the GR and alter its activity in this way include AP-1, NFκB, signal transduction and activators of transcription (STATs) and certain of the CCAAT-enhancer binding proteins (C/EBP). These interactions appear to be particularly important in glucocorticoid-mediated suppression of inflammation, and may allow the activated GR to transrepress expression of cytokine genes without binding to DNA at all.[33]

Interaction of GR with AP-1: The pro-inflammatory transcriptional element activator protein-1 (AP-1) is an important contributor to the expression of the asthma-relevant Th2 cytokines IL-4, IL-5 and IL-13. AP-1 comprises of variable heterodimers of Jun (c-Jun, JunB and JunD) and Fos (c-Fos, FosB, Fra1 and Fra2) family members. AP-1 is inducible by a variety of cytokines and growth factors.[34] It is activated through the phosphorylation of c-Jun and the transcriptional regulation of c-Fos. Phosphorylation of c-Jun is the end result of the action of a trilayer of kinases.[35] c-Jun itself is phosphorylated by Jun-N-terminal kinase (JNK), a member of the extracellular signal-related kinases/mitogen-activated protein kinases (ERK/MAPK) family of serine/threonine kinases. JNK is in turn activated by phosphorylation by JNK kinase, a member of the MAPK/ERK kinase (MEK) family of kinases that phosphorylate on both a tyrosine and a threonine or serine residue. Of the seven members of the MEK family, MEK4 or Jun-N-terminal kinase kinase is principally responsible for the phosphorylation of JNK. At the top end of the trilayer, the most upstream kinases in the cascade are the MEK kinases (MEKK), serine/threonine kinases that are diverse in sequence and structure.

We have implicated abnormal regulation of AP-1 in the mechanism of glucocorticoid resistance. Glucocorticoid-exposed peripheral blood mononuclear cells from glucocorticoid-resistant, as compared with glucocorticoid-sensitive asthmatics had fewer activated GR available for DNA binding,[36] but elevated DNA binding of AP-1 following phorbol ester stimulation.[37] These cells also demonstrated elevated basal, as well as phorbol ester-stimulated, transcription and translation of c-Fos. Furthermore, phorbol ester stimulation of cells from glucocorticoid-sensitive patients induced a glucocorticoid-resistant phenotype, which was associated with direct interaction between the activated GR and c-Fos, detected by co-immunoprecipitation.[38] Binding of GR to other pro-inflammatory transcriptional activators (such as CREB and NFκB) was unaffected. We interpret these data to suggest that mononuclear cells from glucocorticoid-resistant asthmatics inappropriately over-express AP-1, which sequesters and neutralises activated GR, thus causing refractoriness to glucocorticoid-induced inhibitory responses.

The role of p38 MAP kinase: p38 MAP kinase is another member of the family of MAPK/ERK molecules. Its activity is regulated by phosphorylation, principally by the p38 MAP kinases MEK3 and MEK6. A recent study[39] has raised the intriguing possibility that changes in the binding affinity of nuclear GR induced by exposure to IL-2/IL-4 may be caused by direct phosphorylation of the GR at serine 226 secondary to the resulting activation of p38 MAP kinase. Although this study did not directly demonstrate that

p38 MAP kinase phosphorylation of the GR is responsible for its reduced binding affinity for glucocorticoids or its defective induction of an inhibitory signal, the hypothesis is certainly plausible. Similarly TNF-α has been shown to induce glucocorticoid resistance in normal human monocytes, possibly through activation of p38 MAP kinase in addition to NF-κB.[40] Several other protein kinases, such as JNK, may also modify activity of the GR in this way, either directly or through phosphorylation of co-factor molecules.[41,42]

The findings with p38 MAP kinase raise the possibility that the small alteration in ligand binding affinity of the nuclear GR induced by IL-2/IL-4 exposure is an epiphenomenon reflecting more fundamental alterations in the function of the GR brought about by phosphorylation. This is in line with the fact that the observed changes in ligand-binding affinity of the GR are relatively small and of doubtful physiological significance *per se*. Such a fundamental alteration in GR function is also suggested by the fact that serine 226 is located in the N-terminal domain of the GR, remote from the ligand-binding pocket which resides in the C-terminal portion of the molecule. There is some precedent for the possibility that remote regions of the GR may mutually interact. For example, there are two trans-activation domains, activation functions (AF) 1 and 2, situated respectively in the immunogenic and DNA-binding domains of the GR, which co-contribute to the full activity of the GR molecule on its responsive promoters. Conceivably, phosphorylation at a remote site might effect such interactions. Furthermore, both AF1 and AF2 interact with several other nuclear proteins and protein complexes, such as members of the p160 family and the co-activators p300/cyclic AMP-responsive element-binding protein (CREB)-binding protein (CRP) [43] which can affect the glucocorticoid-titration response of GR transactivation of its responsive promoters.[44]

The role of the GRβ-isoform : The β-isoform of the GR is a splice variant of the "normal" GR, or GRα, situated in exon 9. The cDNA sequence up until this point encodes a common region of 727 amino acids. Thereafter, the GRα splice adds 50 amino acids whereas the β isoform has only a further 15 residues. The consequence of this alternative splice was shown to be an inability of the β isoform to bind ligand.[45] The structural basis for this has been clarified by the recent crystallisation of the GR ligand-binding domain complexed with dexamethasone and a peptide that was homologous to the GR interaction domain of the co-activator TIF2.[46]

Artificial transfection of cells with GRβ can inhibit GRα-mediated stimulation of gene expression.[47,48] The popular theory to explain this is that GRβ acts as a dominant negative inhibitor of GRα activity (but see reservations below). Physiological expression of GRβ in neutrophils has been suggested as a possible cause of their relative refractoriness to glucocorticoid inhibition.[49] Several studies suggest that GRβ may be induced in cell lines to an extent sufficient to induce glucocorticoid resistance by pro-inflammatory cytokines such as TNF-α,[48] possibly reflecting the location of a consensus NF-κB binding sequence in the 5-flanking sequences of the GR gene. Furthermore, increased GRβ immunoreactivity has been reported in peripheral blood mononuclear cells and bronchoalveolar lavage cells from patients with glucocorticoid-resistant asthma,[50] although possible modulation of expression in association with glucocorticoid therapy was not explored. In studies from our own laboratory using a model of tuberculin-induced cutaneous inflammation, we reported elevated expression of GRβ immunoreactivity in inflammatory cells infiltrating the skin lesions in glucocorticoid resistant, as compared with sensitive asthmatics.[51] Treatment of the patients with systemic glucocorticoids was associated with reduced GRα expression in the glucocorticoid sensitive, but not the resistant patients.

Notwithstanding these observations, it seems likely that there is much yet to be learned about the possible functional role of GRβ. For example :

Effects of GRβ on transcriptional activation : In terms of transcriptional activation, the classical role of GRα is to bind ligand, to dimerise and then to bind to GRE. This binding permits the recruitment of co-activator complexes, because one of the consequences of ligand binding for nuclear hormone receptors is a reduction in their affinity for co-repressor complexes, and replacement of these complexes with co-activators. The crystallisation data referred to above[46] indicate that GRβ lacks residues forming the charge clamp required for docking of the amino terminus TIF2 LXXLL motif, suggesting that GRβ may be unable to recruit co-factors required for transcriptional activation. Thus GRβ, by displacing GRα, could conceivably act as a dominant negative inhibitor of transcriptional activation. There are, however, uncertainties regarding this conclusion. In the first place, GRβ seems to be expressed in much lower quantities : (typically 10- to 100-fold less, at least at the level of mRNA expression) : than GRα. In the second place, whereas in the case of GRα ligand binding appears to be a prerequisite for nuclear translocation, it is not known what governs the cytoplasmic/nuclear partitioning of GRβ or if it is present in sufficient quantities in the cellular nucleus. Finally, it is unlikely that GRβ could dimerise efficiently, so that its ability to compete for binding with high affinity to GREs is questionable.

Effects of GRβ on transcriptional repression : Repression mediated by ligand bound GR is thought to occur through binding to atypical sites on DNA, for example non-consensus NFAT/AP-1 sites, rather than binding to GREs. At these sites, it is thought that the conformation of the DNA binding site, and

the influence of locally bound factors might cause the GR to adopt a structure that is not permissive for recruitment of co-activators, but rather permissive for recruitment of co-repressors. There is at present no information as to whether or not GRβ can bind to such sites and, more importantly, recruit co-repressor complexes.

Inhibition of transcriptional activation mediated by sequestration of transcription factors : So far, there has been no clear demonstration for a role for the extreme carboxy-terminus of the GR in the sequestration (and inactivation) of transcriptional activating proteins such as AP-1. It is conceivable, therefore, that both GRα and GRβ could exert repressive effects on gene expression by sequestration of such transcriptional activators.

The possible role of histone proteins : DNA is packaged into chromatin, a highly organised and dynamic protein-DNA complex. The N-terminal tails of the core histone proteins contain highly conserved lysine residues that are sites for post-translational acetylation. Acetylation of histone residues results in unwinding of the DNA coiled around the histone core. This process opens up the chromatin structure, allowing transcriptional factors and RNA polymerase II to bind more readily to DNA, thereby increasing gene transcription.[52] The large co-activator molecule CREB binding protein (CBP) that binds to the basal transcriptional apparatus has intrinsic histone acetyl transferase (HAT) activity. Additionally, associated co-activator proteins, including steroid receptor co-activator-1 (SRC-1), transcription factor intermediary factor-2 (TIF2), p300/CBP co-integrator-associated protein (p/CIP) and glucocorticosteroid receptor enhancing protein-1 (GRIP-1) may enhance local HAT activity. In genes which are induced by glucocorticoids, high concentrations of glucocorticoids cause binding of the activated GR to CBP and/or associated co-activators, resulting in histone acetylation on lysines 5 and 16 of histone H4 and increased gene transcription. HAT activity may be further enhanced by binding of transcriptional regulatory proteins such as AP-1 and NF-κB.[52,53] Repression of genes is conversely associated with a reversal of this process by histone deacetylation, mediated by histone deacetylases (HDACs).[54] HDACs comprise of a growing family of enzymes of which at least 10 mammalian members have been described.[55] Some, such as HDAC4 and HDAC8 are able to shuttle between the cellular nucleus and cytoplasm. Histone acetylation is a dynamic process in which small changes in acetylase and deacetylase activity can considerably alter the net HAT activity at any particular gene promoter site. Suppression of HAT activity, as well as recruitment of HDAC activity to active transcriptional complexes may play a role in glucocorticoid regulation of gene transcription.[56] This may occur in a variety of ways. Glucocorticoid repression may reflect competition between the activated GR and the binding sites on CBP for other transcriptional activating proteins, such as AP-1,[57] NF-κB, Sp1, Ets, NF-AT and STATs, which may alter local HAT activity. Alternatively, and not exclusively, the GR may bind to one of several co-repressor molecules such as RIP140, NCoR1 and GRIP1 which in turn associate with proteins having differing HAT activity.[58] These complex interactions probably play a major role in the genesis of the infinitely variable and subtle effects of glucocorticoids on individual target cells.

Clearly, then, intrinsic abnormalities of, or external influences on the regulation of HAT activity in individual cells may influence their glucocorticoid responsiveness. Already there are suggestions that external influences such as exposure to cigarette smoke, an oxidative stress, may inhibit the anti-inflammatory actions of glucocorticoids on cells in the lungs of smokers by reducing HDAC expression and activity.[59] Similar phenomena may contribute to glucocorticoid resistance in asthma.

GENETIC BASIS OF GLUCOCORTICOID RESISTANCE

The glucocorticoid receptor gene itself is a plausible candidate for a genetic basis to glucocorticoid resistance. A mis-sense mutation in this gene had previously been found to be responsible for the phenomenon of familial glucocorticoid resistance.[60] These patients have a markedly reduced affinity of the glucocorticoid receptor for ligand and clinically have features of Addison's disease, which is not the case with glucocorticoid resistant asthmatics. Analysis of the sequence of the glucocorticoid receptor protein in six resistant and sensitive patients showed no mutations in any of the subjects.[61] Despite these observations, a growing number of single nucleotide polymorphisms (SNPs) are being described in the glucocorticoid receptor gene and its flanking sequences, which may have functional consequences. Although this work is ongoing, there is no obvious mutation that might account for glucocorticoid resistance. Obviously, relevant mutations do not necessarily have to be within the coding region of the receptor gene itself, but could be in genes encoding products in downstream signalling pathways.

In addition to this, glucocorticoid responsiveness may be governed at least partly by genetic factors. For example, one study suggested that inherited abnormalities might render approximately 7% of the normal population relatively hypersensitive to glucocorticoids.[62] Such influences may reflect genetic variability in the responses of a potentially vast number of genes to glucocorticoids, including genes concerned with airways remodelling, genes encoding chemokines and cytokines and their receptors and genes encoding signalling pathways downstream for receptors for glucocorticoids or other anti-asthma drugs.[63]

MANAGEMENT OF GLUCOCORTICOID RESISTANT ASTHMA

Generally all patients with severe asthma, particularly those admitted as an emergency will be treated with systemic glucocorticoids at high dosage for at least two weeks. It is only in the fullness of time that a pattern of glucocorticoid responsiveness is established. In patients thought to be truly glucocorticoid resistant (that is, patients showing the proscribed failure in FEV_1 response when the diagnosis is confirmed and aggravating conditions excluded), further long-term therapy with systemic glucocorticoids is probably inadvisable since there is little evidence that these drugs will influence asthmatic symptoms and disease activity, but may on the other hand cause considerable unwanted effects. In such cases it may be sensible to reduce or withdraw glucocorticoids and treat instead with adequate dosages of alternative anti-asthma drugs such as bronchodilators, leukotriene receptor antagonists and immunosuppressive drugs (see below). In clinical practice, however, because there is little hard evidence to justify withdrawal of systemic glucocorticoids from any severe asthmatic patient, this process is often ignored or delayed. In view of the possibility that glucocorticoid sensitivity may change with time, it would seem prudent to re-assess glucocorticoid sensitivity periodically[64] although, as discussed above, within individuals T cell glucocorticoid sensitivity appears to be relatively stable at least over a period of months.

The observation that glucocorticoid responsiveness of asthmatics correlates with glucocorticoid inhibition of their T cells suggests that other drugs that inhibit T cells might be useful for asthma therapy, in particular drugs that inhibit T cells by mechanisms distinct from those of glucocorticoids. We have shown[16,17] that the immunosuppressive drugs cyclosporin A, rapamycin and mycophenolate mofetil inhibit proliferation of T cells from glucocorticoid sensitive and resistant asthmatics to an equivalent extent. It has been shown[65,66] that cyclosporin A, when administered to patients with poorly controlled asthma despite continuous systemic glucocorticoid therapy, improved lung function while allowing reduction of oral glucocorticoid dosages in a proportion of the patients. Similarly concomitant therapy of glucocorticoid dependent asthmatics with methotrexate[67] or gold salts[68] has been shown in some trials to spare glucocorticoid therapy, although no trials have suggested that these agents improve lung function. In general, none of these agents is particularly satisfactory in the sense that many patients fail to respond and it is impossible to predict responsiveness *a priori*. Furthermore, chronic immunosuppression raises the risk of development of serious infection or malignancy, and there is in addition a list of not insignificant unwanted effects associated, in some patients, with the use of each particular drug. An urgent appraisal of other immunosuppressive drugs is needed in glucocorticoid dependent and glucocorticoid resistant asthma. It is a priority to produce a global definition of which patients are suitable for treatment, and what constitutes an adequate trial of therapy.

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