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Corticosteroid Resistant Asthma

Stephen J. Lane, Tak H. Lee

INTRODUCTION

The airflow obstruction of the majority of patients with chronic and severe bronchial asthma will improve following treatment with corticosteroids but despite their clinical efficacy their mechanism of action is unknown. One way in which the mechanisms can be explored is to study a subgroup of patients in whom systemic or inhaled treatment with corticosteroids, even when given in large doses, does not lead to any improvement in airflow obstruciton. The asthma in such patients is usually severe and they are seriously disabled for long periods of time. We have defined corticosteroid resistant(CR) asthma as an improvement in FEV_1 of less than 15% after a 14 day course of 40 mg of prednisolone whereas corticosteroid sensitive(CS) asthma has been defined as an improvement of greater than 30% in FEV_1 after a similar course of prednisolone[1]. CR asthma is associated with disease chronicity, a more frequent family history of asthma and impaired *in vitro* and *in vivo* responsiveness of peripheral blood mononuclear cells(PBMC) to the suppressive effects of glucocorticoids[2~5].

Correspondence and request for reprints should be addressed to
Professor TH Lee; Department of Allergy and Respiratory Medicine 4th Floor Hunt's House Guy's Hospital, London SE1 9RT England
Tel: 071-955-4571, Fax: 071-403-8640

IN VITRO DEFECTS IN CR ASTHMA

In an *ex vivo* study, complement receptor expression on monocytes from CS subjects was reduced after one week's treatment with 20mg oral prednisolone daily as compared with cells from untreated patients[3]. The reduction in complement receptor expression induced by prednisolone was not observed in the monocytes of those patients exhibiting CR asthma. This work was extended by Poznansky who demonstrated that 10^{-8} to 10^{-9} M methylprednisolone, which substantially inhibited growth of colonies from phytohaemagglutinin-stimulated mononuclear cells of CS asthmatics, had little effect on colony growth from the mixed mononuclear cells of CR asthmatic individuals. In subsequent cross-over experiments the origin of this in vitro resistance was found to be monocyte-, rather than lymphocyte-derived[7]. In contrast to non-asthmatic controls, we have shown that monocyte supernatants from asthmatic subjects generated a neutrophil priming activity(NPA) which was selectively suppressed *in vitro* in a dose-dependent and rank order fashion by corticosteroid treatment in the CS, but not in the CR, group[1,8,9]. The degree of *in vitro* suppression by corticosteroids of NPA correlated significantly with *in vivo* airways responsiveness to oral prednisolone in the CS group[9]. Physico-chemical analysis has shown this activity to be a 3kD heat and pronase sensitive molecule which may be related to the small molecular weight chemo-

kine family[1]. In addition we have demonstrated that the enhanced monocyte expression of the activation antigens, complement receptors 1 and 3 and class 2 molecules, seen in bronchial asthma is suppressed by hydrocortisone in CS, but not in CR asthmatic subjects[10].

Glucocorticoid resistance is not cell specific and there is evidence for T lymphocyte dysfunction in CR asthma. Corrigan has shown enhanced interleukin-2 (IL-2) and HLA-DR receptor expression on peripheral T lymphocytes in CR as opposed to CS asthma[5]. In addition he has shown that PHA-induced T cell proliferation and the elaboration of IFN-γ and IL-2 from mitogen-stimulated T lymphocytes was inhibited by 10^{-7}M dexamethasone in CS, but not in CR subjects[5,11]. Interestingly cyclosporin-A was seen to partially reverse this *in vitro* resistance, suggesting a potentially therapeutic role for this treatment in CR asthma.

IN VIVO DEFECTS IN CR ASTHMA

We have provided evidence for an *in vivo* defect in the responsiveness of the macrophage-T-cell interaction to the suppressive effects of corticosteroids in CR asthma and that resistance to glucocorticoids is not organ specific[12]. We have used the classical tuberculin cutaneous delayed hypersensitivity immune response to investigate *in vivo* defects in mononuclear cell function in 9 corticosteroid resistant(CR) and 6 corticosteroid sensitive(CS) asthmatic subjects who demonstrated sensitivity to intradermal purified protein derivative(PPD) of Mycobacterium tuberculosis. In a double-blind, crossed-over placebo-controlled study, patients were given oral prednisolone/placebo starting on day 0, a predetermined intradermal dose of PPD on day 7 and on day 9 the site of the induration was measured and biopsied for immunohistochemical analysis. There was no difference in skin induration between the CS and CR groups during the placebo limb of the study($p=0.38$).

Prednisolone significantly suppressed the cutaneous induration($p<0.003$) in the CS but not in the CR group. As compared to placebo, there was suppression by prednisolone of the number of macrophages ($p=0.018$), eosinophils($p=0.009$) and T memory cells ($p=0.009$) in the CS, but not in CR group. There was no significant suppression by prednisolone in the number of neutrophils or monocytes/immature macrophages in either group. There was no difference in ICAM-1, VCAM-1 and ELAM-1 expression in blood vessels or epidermis between the CS and CR groups with no suppression by prednisolone in either group. These findings suggest a generalized *in vivo* defect in the responsiveness of cellular immune mechanisms to the suppressive effects of corticosteroids in steroid resistant asthma. The differential suppressive effects of corticosteroids on cellular recruitment in the PPD response between the CS and CR individuals are not due to modulation of expression of endothelial adhesion molecules. Further evidence for *in vivo* abnormalities in CR asthma comes from a study by Brown *et al* who demonstrated that the cutaneous vasoconstrictor response to beclomethasone dipropionate was significantly reduced in patients in CR asthma as opposed to patients with either mild or severe steroid-dependent CS asthma suggesting that cells other than mononuclear cells may demonstrate impaired steroid responsiveness in this condition[13].

HYPOTHALAMIC-PITIUTARY-ADRENAL(HPA) AXIS IN CR ASTHMA

Corticosteroids are hormones which are synthesised in the adrenal cortex. They may be classified in relation to their pharmacologic effects as glucocorticoids and mineralocorticoids. The glucocorticoids are synthesised in the zona reticularis and fasciculata and

they play a central role in carbohydrate, protein and lipid homeostasis, as well as exerting anti-iflammatory effects. They are secreted in response to pituitary-derived adrenocorticotrophic hormone (ACTH) and hypothalamic corticotrophin releasing hormone(CRH), respectively. The mineralocorticoids e.g. aldosterone are synthesised in the zona glomerulosa and influence electrolyte and water balance under the control of the renin-angiotensin-aldosterone system independent of pituitary control. Mineralocorticoid receptors have an equal affinity for aldosterone and the physiological glucocorticoids, cortisol and corticosterone, which circulate at concentrations higher than those of aldosterone. In mineralocorticoid target tissues, the glucocorticoids are selectively converted by the 11-β- hydroxysteroid dehydrogenase into their 11-keto analogs, which do not bind to the mineralocorticoid receptor.

We have examined whether the lack of clinical resposnse to corticosteroids seen in CR bronchial asthma is reflected in abnormalities of endogenous cortisol secretion and in the sensitivity of the hypothalamic-pituitary-adrendal(HPA) in CR subjects by using a modification of the standard dexamethasone suppression test in response to 0.25 and 1mg oral dexamethasone[14]. Five CS and 5 CR asthmatic subjects were studied on two occasions one month apart. On the first limb of the study subjects received 0.25mg of oral dexamethasone and on the second limb 1mg was administered. Urinary cortisol was measured by fluorimetry after extraction and plasma cortisol and ACTH levels were estimated by ELISA and immunoradiometric assays, respectively. On day 1, a 24 hour urine was collected for estimation of urinary free cortisol. On day 2, a fasting blood was taken at 09.00 for estimation of plasma cortisol and ACTH. At 23.00, 0.25mg(1mg) of dexamethasone was taken orally by each subject. On day 3, blood was taken at 09.00 and at 15.00 for similar estimations. The levels of urinary free cortisol(nmol/24hrs) and predose plasma ACTH(ng/L) and cortisol (nmol/L) were 199$\pm$42, 27.4$\pm$5.7 and 300$\pm$48(mean $\pm$SEM) in CS group and 210$\pm$74, 23.4$\pm$6.7 and 263 $\pm$32(mean$\pm$SEM) in the CR group, respectively($p>0.05$ for all comparisons). Plasma ACTH and cortisol levels were not significantly suppressed in either group after 0.25 mg dexamethasone but were equally suppressed in both groups to undetectable levels by 1mg dexamethasone. These data indicate that CR asthma is not reflected in an altered secretory rate of endogenous cortisol or in a different sevsitivity of the HPA axis to dexamethasone suppression.

GLUCOCORTICOID BIOAVAILABILITY IN CR ASTHMA

Interest has also focused on whether impaired bioavailability of glucocorticoids can account for the differences in therapeutic responses in steroid dependent and steroid resistant asthma. May *et al* measured the pharmacokinetic profile of a single does 15 mg oral prednisolone in 12 steroid dependent asthmatic subjects by radiommunoassay(RIA) and found no inter-individual differences in these subjects with respect to C_{max}, plasma half life and area under the concentration/time curve and concluded that differences in prednisolone bioavailability is not a factor in determining the dose required to control asthma[15]. Rose and colleagues compared the bioavailability of a single dose of 40 mg prednisolone intravenously in 7 severe steroid dependent asthmatic subjects and 13 healthy non-asthmatic volunteers[16]. Plasma prednisolone was measured by HPLC over an 8 hr test period. They found no differences in plasma half lives, apparent volumes of distribution or concentration-dependent protein binding between the two groups. The apparent plasma clearances were 201$\pm$54 and 198$\pm$38ml/min/1,73m^2 for the asthmatic and the

normal groups, respectively. They concluded that the plasma protein binding, distribution and clearance of prednisolone are not responsible for the large prednisolone requirement of steroid dependent asthmatics. He extend the above studies to steroid dependent and resistant asthmatic children and again found no difference in bioavailability parameters[17]. We have examined the pharmacokinetic profile of an oral dose of 40 mg of prednisolone in CS and CR asthmatic subjects. Peak prednisolone concentrations (C_{max}) in plasma, as measured by HPLC, occurred at 1 hour after the oral prednisolone dose[18]. The area under the concentration time curve (AUC) in the CS group was 2778±374 hrs/ng/ml (mean±SEM) and 2510±206 hrs/ng/ml(mean±SEM) in the CR group (p=0.92). Estimated clearance values were 155±8 mls/min/1.73 m^2(mean SEM) in the CS group and 157±17 mls/min/1.73 m^2(mean±SEM) in the CR group(p=0.9). There was no significant difference in AUC, C_{max} and estimated clearance values between the normal group studied(2089±124hrs/ng/ml, 595± 15ng/ml and 188±7mls/min/ 1.73m^2, respectively) and each of the asthmatic groups. This implies that clinical corticosteroid resistance in asthmatic subjects is not reflected in any gross abnormality of the absorption or elimination of prednisolone. These data are in agreement with pharmacokinetic studies carried out in CR asthma by other groups who observed no differences in estimated clearance values of a single dose of oral prednisolone between groups of well characterised CR and CS asthmatic subjects[11,19].

GLUCOCORTICOID LIGAND BINDING AND NUCLEAR TRANSLOCATION CHARACTERISTICS IN CR ASTHMA

Glucocorticoids mediate their effects through soluble receptor proteins(GR; glucocorticoid receptor) that act by transcriptionally regulating a small number of target genes[21]. Our understanding of the nature of these receptors has increased following purification of the receptor protein by chromatographic and antibody isolation, identification of specific DNA recognition sites for the receptor, cloning of the cDNA for the receptor protein and determination of its genomic structure[21~23]. Glucocorticoids enter the cell by passive diffusion where they bind the GR non-covalently by hydrophobic and hydrogen ion interactions. This results in a conformational change in the GR described as "activation". This process modifies the receptor structure allosterically, whereupon the GR undergoes dephosphorylation, dissociates two 90Kd associated heat shock proteins(HSP), forms dimers and translocates to the nucleus in temperature-dependent fashion. In the nucleus the GR interacts with other transcriptionally active molecules and finally binds to sequences of DNA known as glucocorticoid response elements(GRE) in the promoter region of the glucocorticoid responsive genes. *In vivo* binding to ligand is the only known event which converts the glucocorticoid receptor to a transcriptionally competent factor.

The GR receptor complex is a 300 kD phosphoprotein complex which has been shown by immunocytochemical techniques to be located mainly in the cytoplasm in nearly all human cell types including macrophages, lymphocytes, eosinophils and neutrophils and so can be considered an essential housekeeping protein and has a receptor density(R_0) of 2,000~30,000 binding sites per cell[24,25]. The R_0 for monocytes and lymphocytes have been reported to range from 1 to 9×10^3 receptor sites per cell and the dexamethasone binding affinity(K_d) from 2 to 8 nmol/L[26~28].

Competitive binding studies on nuclear extracts derived from peripheral blood monocytes using[^{3}H]-dexamethasone have demonstrated no difference in the K_d, R_0 or nuclear translocation of the activated

GR complex between CS and CR groups of asthmatics. We have shown that maximal specific saturation of nuclear translocated GRs occurs at 15 minutes and is maintained over a 60 min period in human monocytes[8]. We calculated the dexamethasone K_d to be 2.45±0.58 nM(mean±SEM) in the CS asthmatic group and 1.6±0.35 nM(mean±SEM) in the CR asthmatics(p=0.14). Furthermore, the R_0 was 3605±984(mean±SEM) and 4757±692(mean±SEM) binding sites per nucleus in the CS and CR groups, respectively. These findings indicate that corticosteroid resistance in bronchial asthma cannot be explained by abnormalities in GR nuclear translocation, density or binding affinity and are similar to findings in lymphocytes in this condition[11].

It is interesting that a transient *in vitro* state of steroid resistance can be induced by addition of cytokine combinations or by liposaccaride(LPS). This may reflect the fact that glucocorticoids have different actions in different cell types which depends on the tissue specific expression of steroid responsive genes and on the presence or absence of inflammation in the tissue and on the state of cellular differentiation. Kam *et al* have shown that a reversible decreased ligand binding affinity of the nuclear translocated GR, but not of the cytoplasmic GR, can be induced *in vitro* in CD4 positive lymphocytes derived from normal subjects by incubation with a combination of both IL-1 and IL-4 and can be reversed by serum deprivation for 48 hours[30]. These data suggest that factors present in the nucleus inducible by IL-2 and IL-4 can interfere with the binding affinity of the nuclear translocated GR for its ligand which may aftect the efficiency of subsequent gene transcription. In addition, we have demonstrated that the addition of LPS to PBM derived from CS asthmatic subjects in culture decreased the subsequent responsiveness of NPA to dexamethasone suppression by 30-fold[8,9]. These findings may represent an *in vitro* model of *in vivo* glucocorticoid dependance in asthma which is more likely to be a secondary phenomenon consequent upon a more severe disease phenotype. Alternatively they may represent heterogeneous mechanisms underlying the primary glucocorticoid resistant state. This "inflammation dependent" *in vitro* glucocorticoid resistance may give insights into why CR asthmatics are not clinically "Addisonian" in that the anti-inflammatory responsiveness of an individual to glucocorticoids may be determined locally at the site of inflammation and may depend upon the relative concentrations of transcriptionally active molecules generated locally at the site of the inflammatory insult.

DEFECTIVE DNA BINDING BY THE GR IN CR ASTHMA; STRUCTURE-FUNCTION PROPERTIES OF THE GLUCOCORTICOID RECEPTOR

The GR belongs to a highly conserved superfamily of nuclear hormone receptor proteins characterised by a remarkable overall structural unity with impressive functional diversity[21]. The gene coding for the GR lies on chromosome 11 and contains a total of 10 exons and has a minimum size of 80 kb[22]. Exon 1 contains only untranslated sequence and the amino terminal residues are found in exon 2. The DNA binding domain is encoded by exons 3 and 4 and the ligand binding domain is formed from exons 5~9. The use of GR cDNA expression vectors has revealed that these hormone receptors are structurally organised into five homologous domains, each responsible for different functions and each with different degrees of conservation within the superfamilly[30,31]. These are ligand and HSP90 binding, receptor dimerisation, nuclear localization, DNA binding and *transactivation* of gene expression[32].

DNA binding is encoded by a central domain which is the most highly conserved region of the receptor[33]. This is a cysteine-rich 70 amino acid sequence which folds into two zinc finger motifs, each of them with a zinc atom tetrahedrically co-ordinated to four cysteines[34,35]. It corresponds to base pair cDNA sequence 1333~1542. A Gly-Ser- Val sequence in the root of the N-terminal zinc finger determines hormone response specificity and binds as dimers to the major groove on the GRE[36,37]. The GR sub-family of nuclear hormone receptors, which includes the androgen and progesterone receptors, has this amino acid sequence whereas the estrogen receptor sub-family has not. The C-terminal finger binds to a sugar-phosphate flanking sequence of the GRE and is possibly involved in receptor dimerisation. The steroid binding domain of the GR is located at the C-terminal end and is the next most conserved region within the superfamily. It corresponds to base pair cDNA sequence 1675~2466[36]. This region binds the ligand in a hydrophobic pocket and participates in several other functions including dimerisation, nuclear translocation and is the binding site for the two heat shock proteins. It also contains a 30 amino acid region which is involved in hormone-dependent transcriptional activation. The major *transactivating* domain of the hGR has been identified at the N-terminal domain which is independent of hormone binding[37]. The N-terminal domain is the least conserved among members of the superfamily and possesses a marked cell-type and promoter specificity. It is also the immunogenic site of the receptor. In the GR it corresponds to base pair cDNA sequence 133~1333. The nuclear translocation domain is a short sequence which resembles that of the SV40 tumour antigen[38].

We have examined the binding of the activated GR complex to its GRE using gel retardation assay[39]. PBMC from 6 CS, 6 CR and 6 non-asthmatic control subjects were incubated with 1 μM dexamethasone for different time points up to 60 minutes after which the nuclear protein was extracted by detergent lysis. 10μg of nuclear protein was then incubated with a radiolabelled double stranded GRE construct(with or without a 200 fold excess of unlabelled construct) for 20 minutes at room temperature after which the products were resolved by non-denaturing PAGE and the retarded bands detected by autoradiography and quantitated by laser densitometry. Dexamethasone was seen to induce a significant rapid and sustained 2-fold increase in GRE binding in the mononuclear cells from the CS subjects and non-asthmatic control subjects that was markedly reduced over all time points in the CR subjects. Furthermore, Scatchard analysis of GR-GRE binding demonstrated a reduced number of receptors available for DNA binding with a normal binding affinity in the CR group. These results suggested that the ability of the GR to bind to its GRE is impaired in CR asthma and indicated that there may be a defect in the DNA binding or in the transactivating domains of the hGR. The ligand binding experiments, however, indicate that similar numbers of receptors are translocating to the nucleus in both CS and CR subjects[8]. Therefore, these data also suggest that there may be interference with the GR by other transcriptionally active molecules with subsequent effects on gene transcription.

CHEMICAL MUTATIONAL ANALYSIS OF THE GLUCOCORTICOID RECEPTOR IN CR ASTHMA

In view of the above data demonstrating a defect in DNA binding of the GR in CR asthma we have tested the hypothesis that CR asthma results from a consistent polymorphism in the functionally diverse GR cDNA using the sensitive screening technique of PCR amplification and chemical mutational

analysis[40]. Total RNA was extracted from peripheral blood monocytes derived from 6 CS and 6 CR asthmatic subjects. The RNA was reversed transcribed and overlapping GR cDNA fragments were amplified by nested PCR. Double stranded hGR cDNA fragments were hybridised to corresponding ^{32}P-5′-labelled wild type fragments, chemically modified with osmium and hydroxylamine and cleaved with piperidine. The resultant cleaved strands were detected by autoradiography. As controls, single base pair mutated hGR cDNA fragments sensitive to hydroxylamine and osmium modification were used. Using this technique we did not detect any base pair mismatch between the 6 CS and 6 CR patients and the corresponding wild type hGR, despite a 100% detection of control mutations indicating that the defect in CR asthma does not lie in the structure of the GR. This was further confirmed by dideoxy sequencing using linear PCR elongation and chain termination[41]. CMA has advantages over other techniques which are currently being actively used for screening of mutations in that its sensitivity approaches 100% and it allows screening of long stretches of DNA without the need for specialised apparatus. Its main disadvantage is the number of steps involved. It compares very favourably with single strand conformation polymorphism(SSCP) which, although a relatively uncomplicated procedure, allows screening of only short lengths of DNA and at only 80~90% detection rate. G-C clamped denaturing gradient gel electrophoresis(DGGE) has a similar sensitivity to CMA but allows screening only of short sequences of DNA and requires specialised apparatus. Ribonuclease protection is limited in that its sensitivity is only about 70%. CMA is now currently widely applied in screening for mutations in inherited diseases and in cancer.

GENE REPRESSION BY GLUCOCORTICOIDS

The above findings suggest that corticosteroid resistance may be associated with trancriptional interference with a structurally normal GR and abnormally regulated transcriptionally active molecules with resultant impairment of gene transcription. In order to further study these abnormalities it is critical to understand the mechanisms by which glucocorticoids cause repression of transcription. Enhancement of gene expression occurs by direct binding of the GR to positive cognate DNA binding elements, namely glucocorticoid responsive elements (pGRE), in a relatively straightforward fashion. Based on the sequence of their DNA-binding motifs, two groups of steroid receptors can be distinguished: group I receptors recognizing the motif 5′-TGACCT- 3′ as the 3′ palindrome(estrogen receptor, thyroid hormone, retinoic acid and vitamin D receptor), whereas group II receptors(GR, mineralocorticoid receptor, progesterone receptor and androgen receptor) bind to 5′-TGTCCT-3′[42]. Most GREs consist of two half site hexamers separated by three base pairs, with a sequence resembling the consensus sequence GGTACAnnnTGTYCT(where n is any nucleotide and where Y=T or C)[43]. In fact T is found in 65% of GREs. The 3′ half site of the consensus GRE is more conserved among the various GREs than is the 5′ counterpart, and a palindromic GRE maintaining activated GRE-dependent transcription as well as did a consensus GRE. A systematic study on the effects point mutations in the GRE revealed changes of the T in position 3 are not tolerated, and that position 6 must be occupied by a pyrimidine[44]. GREs have been detected as close as 39 base pairs and far away as 2.6kb pairs downstream of the transcription initiation site.

It has recently become evident that many of the important anti-inflammatory effects of steroids appear to be mediated via direct repression of gene transcription[45]. One major form of negative regulation is based on transcriptional interference between the GR and other transcription factors, such as AP-1 at a protein-protein level[46,47]. In this case, the liganded GR bind to and prevents activating protein-1 (AP-1), a heterodimer of Fos and Jun proteins, or other positively acting transcription factors from effective interaction with their respective transcription initiation complexes, thus modulating an effect that they would otherwise have on gene transcription[48]. This interaction is ligand dependent and is independent of the constituents of AP-1. This may be of particular relevance in chronic inflammatory diseases since cytokine-induced and protein kinase C (PKC)-induced activation of transcription factors involved in mediating chronic inflammatory events at a transcriptional level may be inhibited by corticosteroids[49]. Interations between AP-1 and GR have been demonstrated in cultured cells[47,50], in human lung[51] and PBMC[52], and may be an important aspect of the antiinflammatory effect of steroids[53].

A second mechanism of GR-mediated repression of gene expresion has been demonstrated in the mouse proliferin gene involving co-occupancy of the same "composite" response element by two transcription factor[54]. The expression of the proliferin gene is enhanced by AP-1 and repressed by GR by binding of both GR and AP-1 to the same 25 bp element in the proliferin promoter. Transfection of the proliferin promoter linked to a reporter gene into HeLa cells showed that this "composite" response element had no effect on proliferin expression when the cells were cultured with dexamethasone alone, showed minimal enhancement in the presence of cotransfection with Jun homodimer-generating expression vectors demonstrated a marked enhancement of gene expression whereas marked repression was seen after co-transfection with GR and Fos expression vectors. Therefore the GR mediated both positive and negative regulation by direct action on the proliferin promoter depending on the constituents of AP-1 which bind to the same response element. These effects were only seen with high protein levels and do not apply to other members of the nuclear family of hormone receptors indicating that this mechanism of repression is unlikely to be widespread.

A third form of negative regulation by nuclear receptors is based on the binding of nuclear receptors to specialised negative hormone response elements (nHREs). The search for nHREa has been elusive and most cases of gene repression, originally thought to be due to these elements, have subsequently been shown to be due protein-protein interaction. Binding of the unliganded receptor to such an element results in constitutive activation, which is terminated by the addition of ligand. Only one *bonafide* nHRE has been described in the Rous Sarcoma Virus long terminal repeat which is directly activated by the T_3 receptor in the absence of T_3 ligand and activated in its presence[55].

The collective data accrued in CR asthma suggests that there is impairment of interaction of the GR with other transcription factors resulting in defective regulation of gene transcription. It is apparent that structurally normal liganded GRs translocate to the nucleus in a normal quantities and bind their DNA recognition sites with normal affinity. What is not clear is why there is an apparent reduction in the quantity of GRs available for effective DNA interaction. This may indicate that the intranuclear GRs may be being "sequestered" by other pro-inflammatory transcriptionally active molecules which are themselves abnormally regulated in the presence of local inflammation. It is important to remember that anti-inflammatory steroid resistance, albeit rare,

is not confined to bronchial asthma and has been described in other conditions such as rheumatoid arthritis and organ transplant rejection and so may represent a rare primary phenomenon in its own right which only becomes apparent in the presecne of coincidental inflammatory disease. What is interesting is that we must now look beyond the GR in order to unravel the underlying mechanisms involved.

SUMMARY

CR asthma is associated with disease chronicity, a positive family history of asthma and *in vitro* and *in vivo* defects in mononuclear cell function. The HPA axis in CR asthmatics is suppressed normally by dexamethasone and the pharmacokinetic profile of an oral dose of prednisolone is similar to that found in CS subjects. In addition, competitive binding studies have shown that the ligand binding and nuclear translocation functions of the GR are similar in the two groups. Studies using gel retardation assay have indicated a defect in DNA binding in CR subjects. Chemical mutational analysis of the GR has shown that is not due to a defect in its structure at the cDNA level. Scatchard analysis of the GR/DNA and GR/ligand interactions suggests that there may be transcriptional interference of the GR with other transcriptionally active molecules leading to defective gene transcription.

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