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Clinical aspects of glucocorticoid sensitivity

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Recent studies demonstrate that primary (hereditary) abnormalities in the glucocorticoid receptor gene make 6.6% of the normal population relatively "hypersensitive" to glucocorticoids, while 2.3% are relatively "resistant." These abnormalities might explain why some individuals develop severe adverse effects during low dose glucocorticoid therapy, while others do not develop side effects even during long-term therapy with a much higher dose. Awareness of this heterogeneity in glucocorticoid sensitivity in the normal population might eventually allow the prediction of a "safe" dose of glucocorticoid in individual patients.

"Resistance" to the beneficial clinical effects of glucocorticoid therapy in part of the patients with severe rheumatoid arthritis and asthma is probably rarely related to generalized primary (hereditary) glucocorticoid resistance. In the majority of patients this "resistance" seems to be acquired and localized to the sites of inflammation, where it reflects high local cytokine production, which interferes with glucocorticoid action. Recognition of localized, acquired glucocorticoid resistance is of great importance indicating as alternative drug therapy with other immune-modulating drugs like cyclosporin and methotrexate. Chronic high dose glucocorticoid treatment in such patients is ineffective in alleviating symptomatology, while generalized side effects occur, reflecting the patient's normal systemic sensitivity to these drugs. (*Steroids* **61**:157–160, 1996)

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Introduction

Exogenous glucocorticoids play an essential role in the acute and chronic therapy of a number of immune diseases such as asthma and rheumatoid arthritis and contribute considerably to the prevention of allograft rejection after organ transplantation.[1] Two seemingly different aspects of glucocorticoid therapy, however, have reduced our enthusiasm to use these compounds.[2] First is the unpredictable occurrence of severe side effects during chronic therapy,[3,4] with many studies demonstrating that the duration of therapy, the highest steroid dose administered, and the total cumulative dose used are important predictors of adverse events.[5] Unfortunately, it is currently impossible to define for a given patient a "safe" glucocorticoid dose that does not cause side effects.[4,5] Second, the efficacy of glucocorticoid therapy in chronic immune disease is unpredictable. There is clinical and laboratory evidence that patients can be divided into "steroid-sensitive" and "resistant" groups. These differences have been documented in the treatment of asthma[6,7] and rheumatoid arthritis,[8,9] as well as in renal graft recipients.[10,11] In this study we review several recent investigations of the role(s) of hereditary and/or acquired abnormalities in glucocorticoid receptor function in man. The results of these studies are discussed against the background of the two main clinical questions concerning glucocorticoid therapy in immune disease: 1) that of an individual "safe dose," which can be used without the occurrence of side effects, and 2) that of the mechanism of the apparent "resistance" in part of the patient population treated with these drugs.

Hereditary glucocorticoid resistance

Primary hereditary glucocorticoid resistance is a rare disorder, which has only been described in a dozen individuals to date.[12–15] In this syndrome the abnormal receptor alters feedback inhibition of the hypothalamo-pituitary-adrenal axis, which is thus set at a higher level with slightly elevated plasma ACTH levels and increased circulating cortisol concentrations. The diurnal rhythms of ACTH and cortisol secretion remain intact, while the system remains sensitive to external stresses such as that caused by acute hypoglycemia. The elevated circulating cortisol levels do not cause signs or symptoms of Cushing's syndrome, because reduced num-

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bers of impaired glucocorticoid receptors are present in all target tissues. Symptoms of glucocorticoid resistance stem primarily from ACTH-induced adrenocortical overstimulation, resulting in increased serum concentrations of androgens and mineralocorticoids. The wide variation in the nature and severity of the symptoms associated with glucocorticoid resistance can made its diagnosis difficult.[16,17] Hypercortisolism and/or an inadequate response to a 1 mg overnight dexamethasone suppression test can point to this abnormality.[16,17] Hormone profiles (androgens, mineralocorticoids) and analysis of the number and affinity of glucocorticoid receptors in peripheral blood mononuclear leucocytes or in cultured skin fibroblasts are usually carried out. Bioassays to measure glucocorticoid sensitivity can also be performed on lymphocytes or fibroblasts.

The molecular mechanisms of glucocorticoid resistance detected to date reflect at least three different abnormalities. First, missense mutations in the ligand binding domain of the glucocorticoid receptor gene causing decreased ligand binding affinity have been reported in two different families.[18,19] Second, a deletion of four base pairs at the boundary of exon 6 of the glucocorticoid receptor and the following intron has been demonstrated to be responsible for the loss of a splice site and the production of an unstable mRNA. This deletion allows only one allele to be expressed, resulting in a decrease of glucocorticoid receptor protein by 50% in affected members of this glucocorticoid-resistant family.[20] Third, a novel glucocorticoid receptor gene mutation has recently been discovered in the ligand binding domain of the glucocorticoid receptor, in which a point mutation in exon 5 is accompanied by a significant loss of function of the receptor protein, as indicated by transfection studies. Interestingly, in cotransfection studies this mutant receptor exerted a dominant negative effect on the wild type glucocorticoid receptor, causing the lowered glucocorticoid receptor affinity observed in this heterozygous patient's peripheral lymphocytes.[21] In addition to these three distinct molecular mechanisms of glucocorticoid resistance, cases have been reported where increased thermolability of the glucocorticoid receptor or a reduced capacity to bind DNA were the cause of glucocorticoid resistance.[15,22] In these cases, however, abnormalities in the glucocorticoid receptor gene have not yet been reported.

Glucocorticoid receptor abnormalities in the normal population

The recent reports of a significant prevalence of possible abnormalities in the glucocorticoid receptor in patients attending the endocrine clinic for hypokalemia, hypertension, acne, hirsutism, and menstrual disorders prompted us to carry out a cross-sectional study on the prevalence of glucocorticoid receptor abnormalities in a group of 216 healthy elderly individuals (J.W. Koper et al., unpublished observations). In the 1 mg overnight dexamethasone suppression test 17 persons showed "diminished" suppression, with postdexamethasone cortisol levels above 50 nmol/L. In 16 of these "diminished" suppressors and in 10 age- and sex-matched subjects with a normal suppression we analyzed glucocorticoid receptor number and affinity in mononuclear leukocytes and the biological response of these cells to glucocorticoids in a mitogen-stimulated lymphocyte proliferation assay. Of the "diminished" suppressors 10 were found to have abnormal results in the glucocorticoid receptor assays versus none in the control group. Using polymerase chain reaction-based analysis of single strand conformation polymorphism, we screened for mutations in the glucocorticoid receptor gene. Five mutations in the glucocorticoid receptor gene were identified, all occurring in more than one person. Two of these mutations were significantly associated with reduced glucocorticoid receptor function and were calculated to occur in 2.3% of the normal population. Another single point mutation, present in 6.6% of these healthy elderly individuals, was associated with a significantly increased receptor function. These polymorphisms were not associated with clinical abnormalities but may contribute to the variable sensitivity to glucocorticoid therapy observed in the normal population.

Transient and/or acquired glucocorticoid resistance

The best known examples of acquired glucocorticoid resistance are the abnormalities in glucocorticoid receptor activity in the neoplastic cells of most human hematologic malignancies. Glucocorticoid receptor abnormalities, as well as an increasing number of well characterized postreceptor abnormalities,[23–25] play an important role in the ultimate prognosis of patients with acute leukemia and malignant lymphomas.[26] In the differential diagnosis of Cushing's syndrome the degree of glucocorticoid resistance also plays an important role. Most tumors ectopically secreting ACTH are characterized by a high degree of glucocorticoid resistance, which has been demonstrated to be accompanied by changes in ligand binding in several human small cell lung cancer cell lines.[27] In Nelson's syndrome the cells of the expanding, infiltrating, ACTH secreting pituitary tumor have similarly been demonstrated to have a defect in the glucocorticoid receptor gene.[28]

Acquired (localized) glucocorticoid resistance in rheumatoid arthritis and asthma is accompanied by a reduction in the number of glucocorticoid receptors in circulating leukocytes[29] and/or a reversible decrease in the affinity of glucocorticoid receptors in T lymphocytes,[30] respectively. Similarly, in some patients with AIDS, peripheral leukocytes demonstrate a marked decrease in the affinity of glucocorticoid receptors for cortisol.[31] Glucocorticoids are known to be powerful suppressors of the activity of the immune system. Inhibition of chemotaxis and bactericidal activity in neutrophils and monocytes, lymphopenia, decreased macrophage function, and disturbed complement activation are well known effects of glucocorticoid administration.[1] Most of the effects of glucocorticoids on the immune system are thought to be mediated via inhibition of transcription of various cytokine genes, particularly those coding for interleukin-1 (IL-1) and IL-6 in macrocytes/macrophages,[32] and IL-2 in lymphocytes.[33,34] However, higher concentrations of cytokines, especially IL-2, antagonize the suppressive effects of glucocorticoids in a dose-dependent manner, thus counteracting these transcriptional effects.[35] The balance between glucocorticoid action and the production of interleukins in mitogen-stimulated im-

mune cells is in most cases in favor of glucocorticoids, which override the activity of the immune cells. A number of studies suggest that at the site of inflammation in cases of rheumatoid arthritis, asthma, and sepsis, high local concentrations of cytokines in fact induce a localized glucocorticoid resistance, which cannot be overcome by excess exogenous glucocorticoids.[30,35,36,37]

Conclusions

Recent evidence suggests that primary (hereditary) abnormalities in the glucocorticoid receptor gene cause relative "hypersensitivity" to glucocorticoids in 6.6% of the normal population, while 2.3% is relatively "resistant." These differences in ~10% of the normal population may thus contribute to the well known phenomenon of some individuals developing severe adverse effects on low dose glucocorticoid therapy, while others do not develop side effects during long-term therapy with much higher doses. Further studies on the heterogeneity of glucocorticoid sensitivity in the normal population might thus eventually allow the prediction of a "safe" glucocorticoid dose in individual patients.

"Resistance" to the beneficial effects of glucocorticoid therapy in patients with rheumatoid arthritis and asthma is related to generalized primary (hereditary) glucocorticoid resistance in only a small minority of these patients; in this group a higher dose of glucocorticoids might overcome the genetically determined resistance. However, in the majority of patients "resistance" seems to be acquired and localized at the inflammation site(s), where it is a consequence of high cytokine production interfering with glucocorticoid activity. Recognition of localized, acquired glucocorticoid resistance is of great importance, as alternative drug therapy with other immune-modulating compounds such as cyclosporin, methotrexate, and/or gold should be considered early on in the disease. In these circumstances chronic high dose glucocorticoids will insufficiently alleviate the symptomatology of the immune disease and cause general side effects, as the patient has a normal systemic sensitivity to the treatment.

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