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The Potential of the Endocrine System for Tuberculosis Therapy

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Immunity to tuberculosis requires a Th1 cytokine profile accompanied by several types of cytotoxic T cell. However, the disease state in both man and mouse is accompanied by a progressively increasing Th2 component. Since glucocorticoid steroids can drive the immune response towards Th2, we have investigated adrenal function in human tuberculosis, and tested the role of glucocorticoid and antiglucocorticoid hormones in a murine model.

In tuberculous mice the adrenals increase in size, and then atrophy progresssively as the Th2 component increases. In the human disease, there is a relative loss of the antiglucocorticoid steroid dehydroepiandrosterone (DHEA, which is also metabolized abnormally to 16α-hydroxylated derivatives), loss of the evening trough in cortisol levels, and diminished net conversion of cortisol to inactive cortisone. These changes all tend to increase glucocortcoid effects in the tissues, and may account for progressive deregulation of the immune response. When tuberculous mice with late progressive disease are treated with either glucocorticoid or DHEA alone they die quickly, but combined treatment with a critical balance of both steroids is therapeutic. Such therapies may be of use in human cases of multi-drug resistant tuberculosis.

Immunity to Tuberculosis

Immunity to tuberculosis requires a Th1 pattern of response accompanied by a range of cytotoxic cell types that can lyse phagocytes if they fail to destroy ingested organisms (Orme et al 1993; Bloom et al 1994; Tanaka et al 1995). This may allow the organisms to be taken up by fresh activated phagocytes. However, in murine tuberculosis there is a very clear shift towards a Th2-mediated response as the disease progresses, and although less obvious, the same is true in man. For instance, the IL-4 gene is expressed in peripheral blood mononuclear cells (Schauf et al 1993), while there is a deficit in IL-2 expression (Schauf et al 1993). The same two points have emerged from studies using 3-colour flow cytometry to look at actual cytokine production (Thapa et al, unpublished observations). This suggests the presence of a Th2 component in disease, and is supported by the fact that tuberculosis patients often have high circulating levels of IL-10 (Cooper et al 1995) and IgE antibody (IL-4-dependent) (Yong et al 1989). When tuberculin test sites were studied using a laser doppler velicometer it was found that the extent to which blood flow was reduced in the centre of the site at both 6 and 48 h, was related to the level of specific IgE antibody to *M. tuberculosis* (Gibbs et al 1991). This appears to represent incipient necrosis (Koch phenomenon) which is characteristic of the disordered immune response accompanying the disease. But what is the cause of the shift towards Th2 cytokine profile?

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The Sensitivity to Glucocorticoids of the Immune Response to *M. tuberculosis*

A possible cause is a change in the function of the hypothalamo-pituitary-adrenal axis. Reactivation of tuberculosis following administration of glucocorticoids is a well-documented clinical event, and reactivation or progression of the disease is also induced by chronic activation of the hypothalamo-pituitary adrenal axis. Exposure to the stress of war or poverty in man (Spence et al 1993), or to the stress of transportation in cattle, are enough to cause reactivation of disease. The disease-promoting effect of stress has been demonstrated under more controlled conditions in mice (Tobach & Bloch 1956; Brown et al 1995). These effects are thought to be mediated via glucocorticoid release, because cortisol (corticosterone in mice) reduces macrophage activation and antimycobacterial effects (Rook et al 1987), and reduces Th1 T cell activity (Daynes et al 1991), while synergizing with some Th2 functions (Fischer & Konig 1991). Similarly, although in short-term cultures, glucocorticoids will reduce production of Th2 cytokines, it is clear that when lymphocytes are allowed to undergo several cycles of replication in the presence of glucocorticoids, they evolve into Th2 T cell lines (Brinkmann & Kristofic 1995; Ramirez et al 1996). Thus, the mechanisms that control tuberculosis are sensitive to glucocorticoids, probably because these steroids provoke a Th1-to-Th2 shift. Moreover, several other features of tuberculosis are compatible with glucocorticoid-mediated effects. These include a reduced CD4 count and CD4/CD8 ratio (Swanson-Beck et al 1985; Singhal et al 1989; Rook et al 1993, 1994), and a mildly impaired glucose tolerance (Zack et al 1973).

Regulation of Cortisol Levels in Peripheral Tissues

Before discussing the evidence that there is excessive glucocorticoid activity in tuberculosis, it is important to appreciate how the effects of glucocorticoids in any given tissue are actually regulated (Baker et al 1996). Both the concentration and efficacy of cortisol are regulated within target organs, and therefore do not depend only on plasma concentrations. This regulation is achieved partly by conversion of cortisol to inactive cortisone within the tissue, and partly by generation within the tissue of metabolites of DHEA that oppose many effects of cortisol. Such mechanisms, are essential because each peripheral organ needs to adjust the concentration and activity of cortisol to its own requirements.

Interconversion of Cortisol and Cortisone

The regulatory role of conversion of cortisol to inactive cortisone has been known for the kidney for some time. In normal kidneys 11β-hydroxysteroid dehydrogenase type 2 (11β-OHSD-2, an NAD-dependent enzyme) converts cortisol to cortisone and so prevents cortisol from reaching the aldosterone receptor, which would otherwise be swamped by it, leading to salt retention and hypertension (Walker 1993). The same mechanism is now known to occur in lymphoid tissue, where 11β-OHSD is found in stromal cells (Dougherty et al 1960; Berliner & Dougherty 1961), and exerts a potent effect on the Th1/Th2 decision within the lymph nodes (Daynes et al 1995), because glucocorticoids drive the response towards Th2 as discussed above. In healthy individuals the cortisone generated by these enzymes tends to be converted back to cortisol by a reductase present in the liver. This enzyme is usually known as 11β hydroxysteroid dehydrogenase type 1, (11β-OHSD-1) but it is in fact an NADPH-dependent reversible oxido-reductase. Although this enzyme functions in liver as a reductase, it has become apparent that normal lung parenchyma and bronchial epithelium also contain 11β-OHSD-1, but paradoxically in normal lung this enzyme appears to function as a dehydrogenase, inactivating cortisol by converting it to cortisone (Schleimer 1991; Hubbard et al 1994).

DHEA Metabolites

Another major regulator of cortisol function within individual organs is the local production of metabolites of dehydroepiandrosterone sulphate (DHEAS) that have anti-glucocorticoid effects acting via receptors that have not yet been identified. Inhibition of DHEA sulphatase (Foulkes, personal communication) has a profound glucocorticoid-like effect. In contrast, administration of DHEA or of 3,17-androstenediol causes an antiglucocortioid effect, and a Th1 bias (Blauer et al 1991; Daynes et al 1995; Porter & Svec 1995). As can be anticipated, DHEA kills adrenalectomized rodents (Svec, personal communication).

Other Factors Controlling Glucocorticoids Activity in the Lung

Two other regulatory effects also play a role. Inflammation caused by the disease is likely to accelerate equilibration between plasma and tissue cortisol levels. This effect will also be modulated by the presence of steroid-binding proteins in plasma (Rosner 1991). Raised cortisol levels can lead to reduced corticosteroid-binding globulin, further increasing free cortisol, which will equilibrate more rapidly with the lung parenchyma (Rosner 1991; Armario et al 1994). Moreover, neutrophil elastase released in inflammatory sites can cleave corticosteroid-binding globulin, resulting in release of the cortisol (Rosner 1991).

The Endocrine Changes in Human Tuberculosis

Previous studies of adrenal function in tuberculosis (Post et al 1994) were mostly confined to the investigation of adrenal reserve using vastly supraphysiological quantities of adrenocorticotrophic hormone (ACTH), and found no relevant abnormality. However such studies, quite apart from the use of the traditional—but absurd—250 μg dose of ACTH, failed to address the question in relation to the new insights into the way in which the peripheral effects of cortisol on the T-cell system are regulated (Fig. 1). We have assessed tuberculosis patients using gas chromatography and mass spectrometry of 24-h urine collections (Rook et al 1996), and our findings, together with the existing literature, suggest the following four relevant changes.

Firstly, there may be decreased production of DHEA, and metabolism of DHEA to 16α-hydroxylated, rather than to

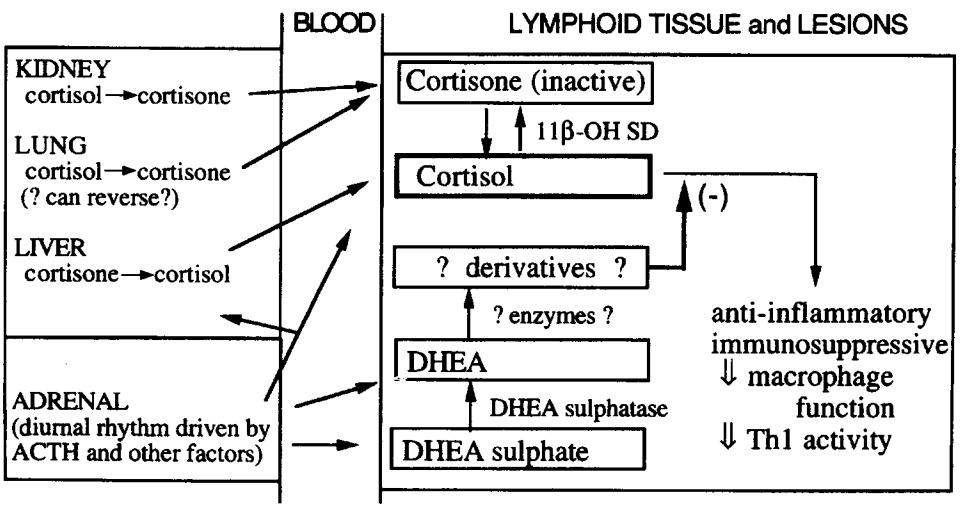

FIG. 1. Representation of the regulation of peripheral effects of cortisol on T cell system. Glucocorticoids released by the adrenal are inactivated in the kidney (cortisol → cortisone), and reactivated in the liver. The lung contains the enzyme found in the liver, but apparently running in reverse, so the lung may turn out to make an important net contribution to cortisone levels and cortisol inactivation. The eventual glucocorticoid effect in any tissue depends partly on the plasma cortisol, partly on the diurnal rhythm of production by the adrenal, and partly on balance of cortisol–cortisone interconversion within that tissue. This is further modulated by conversion of DHEA into unidentified metabolites that reverse many effects of glucocorticoids. Abnormalities of all these factors are evident in tuberculosis.

reduced, derivatives. We do not know which metabolites of DHEA are important, so the significance of the 16α-hydroxylation is unclear. However, it is interesting that the enzyme responsible (CYP 3A7, Kitada et al 1987) is also active in the foetus, which is another situation where there is a striking Th1-to-Th2 shift (Wegmann et al 1993). Whether or not this is significant, it is clear that in tuberculosis, a low DHEA metabolite–cortisol ratio will increase cortisol-mediated effects.

Secondly, a loss of the normal evening trough in plasma cortisol level is suggested (Sarma et al 1990). Therefore, although plasma cortisol is normal or only modestly raised in most tuberculosis patients (Post et al 1994), the tissues are exposed to this concentration for 24 h a day. Therefore, the mean cortisol concentration in the lungs may be high in tuberculosis. In normal individuals the type II cortisol receptors in T cells are unoccupied during the evening trough in cortisol levels, and the diurnal changes in plasma cortisol level correlate with a diurnal rhythm in Th1 and Th2 cytokine production (Petrovsky & Harrison 1995). In tuberculosis patients the cortisol receptors will remain occupied in the evening, and we need to know whether this has dire consequences for Th1 function.

Thirdly, adrenals may be hyperresponsive to very low doses (60 ng) of ACTH. This is a new observation, currently based on few donors (Baker et al, unpublished observation), but if confirmed it may explain the previous item, since production of cortisol may continue during the evening when ACTH levels are low, so eliminating the evening trough in cortisol.

Finally, a striking increase in metabolites of cortisol relative to metabolites of cortisone is suggested. This would indicate a net increase in the activity of 11β-hydroxysteroid reductase (inactive cortisone to active cortisol) relative to dehydrogenase (cortisol to cortisone). We do not know whether the lung is the site of the change. It cannot be due to decreased activity of the kidney enzyme, because this would result in salt retention and hypertension, which are not seen in tuberculosis. It could be due to acceleration of the liver reductase, but some endocrinologists consider that this enzyme is already running close to its maximum rate.

Meanwhile, it can be calculated that the normal lung may be an important net contributor to the systemic cortisone levels, because of its content of 11β-OHSD-1 working as a dehydrogenase. However, as outlined above, 11β-OHSD-1 is reversible. It behaves unidirectionally or bidirectionally in different cell types into which it has been cloned, but efforts to understand its regulation have failed (Jamieson et al 1995). It is known to be subject to regulation by T lymphocyte-derived cytokines, at least in granulosa cell preparations (Erangelatou et al 1996). Therefore it may be that the critical change in the balance of cortisol–cortisone takes place in the tuberculous lung, as a consequence of local cytokine release. If the lung is the site of the change in the ratio of dehydrogenase to reductase activity, then this will greatly exaggerate the consequences in that organ of the other endocrine changes.

The Adrenals in Murine Pulmonary Tuberculosis

The importance of these observations can be tested in a murine model of pulmonary tuberculosis. In these animals there is first an increase in adrenal weight peaking at 2–3 weeks, that accompanies an early Th1-dominated phase of immunity, and then adrenal atrophy to about 50% normal size by day 60 (Hernandez-Pando et al 1995). This phase of the disease shows a mixed Th1 and Th2 pattern of cytokine release. Interestingly, the adrenals start to atrophy immediately after intratracheal infection in mice pre-immunized so that a Th2 component is already established before challenge (Hernandez-Pando et al 1995; Rook & Hernandez-Pando 1996). None of these effects is due to direct infection of the adrenals themselves.

The findings in the human disease led us to try therapeutic studies with glucocorticoids and DHEA derivatives in this mouse model. In the early phase that is accompanied by a predominantly Th1 cytokine profile and adrenal hypertrophy, administration of DHEA is protective. However, in the late phase of adrenal atrophy, administration of either a glucocorticoid alone or of DHEA alone is rapidly fatal. In contrast, administration of both types of steroid together is significantly protective, suggesting that the beneficial effects of each steroid are conserved while the pro-inflammatory effect of DHEA and the Th2-promoting effect of glucocorticoid are opposed.

Future Exploitation in Man

The need for the right balance of the two steroid types is in agreement with epidemiological collaborations with Peter Donald's group in South Africa, where we are investigating hypothesis that the changes in susceptibility to tuberculosis, and in the nature of the resulting disease in small children, may well depend upon the age-related changes in DHEA levels associated with adrenarche and puberty (Donald et al 1995). Meanwhile, similar mixed steroid regimens should be tested in human cases of multidrug resistant tuberculosis.

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