The effect of dexamethasone on the protective efficacy of *Mycobacterium bovis* BCG in deer

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Introduction

At first glance it may appear as though the immune system functions autonomously, completely devoid of any meaningful communication or interaction with any other system. There now exists overwhelming evidence that this is not the case and that the immune system is connected via innumerable structural and functional bridges with both the endocrine system and the nervous system, so as to constitute an extraordinarily complex multisystem, often referred to as the Neuroendocrineimmune system (Jankovic, 1994). It is remarkable that in light of this evidence, the importance of these interactions continues to be underestimated or completely overlooked by most immunologists who study both the pathogenesis and development of vaccines against infectious diseases (Rook *et al.* 1994) and tuberculosis research is no exception.

Stress and immunity

Stress can be considered as the detrimental physiologic outcome of an individual's inability to adapt adequately to a changing environment or to cope with an unpredictable and/or uncontrollable situation. Both physical and psychological stressors elicit responses developed to reduce the impact of stressors and restore balance or homeostasis (Gold *et al.* 1988). In general the stress response functions beneficially at an acute level with few adverse effects, although if it is activated chronically, without appropriate adaptation, suppression of vital immune function may result (Chrousos and Gold, 1992). The major consequence of a suppressed immune response is the increased susceptibility to infectious disease and there exists a large body of evidence showing that stress can predispose, exacerbate and reactivate the infectious process in a large number of animal models and in humans. Tuberculosis provides a good example of this (Thomson *et al.* 1994).

The stress response and corticosteroids

A variety of endocrine changes including activation of the sympathetic-adrenomedullary axis (SPA) and/or the hypothalamic-pituitary-adrenal (HPA) axis occur during the individual's response to stress (Solomon, 1987). Hyperactivity of the HPA axis results in an increased production of corticosteroid hormones (Chrousos and Gold, 1992). The role of corticosteroids in regulating virtually every component of the immune system suggests that it is hugely important in the mammalian stress response.

Corticosteroids and the immune system

Corticosteroid hormones are known to increase the susceptibility of animals to infectious disease, by exerting profoundly suppressive effects on the immune system (Roth and Kaeberle 1982). Corticosteroids inhibit many functions of granulocytes, monocytes/macrophages and lymphocytes. They influence their trafficking patterns, decrease cytokine production and the expression of cytokine receptors, they also decrease the production of many mediators of inflammation and reduce the effect of such mediators on certain target tissues (Cupps and Fauci, 1982). Corticosteroids impair monocyte/macrophage function by altering both phagocytosis and intracellular killing (Schaffner, 1985). Recently corticosteroids have also been shown to regulate T-helper cell subset development, suppressing production of T_H1 cytokines, interleukin-2 and gamma-interferon, whilst having negligible effects on the production of T_H2 cytokines, such as IL-4 (Wilder, 1995). The combination of all these effects when considering infectious diseases like tuberculosis results in a significant alteration in the ability of an individual to resist infection or contain disease.

The vaccine paradox: who are we trying to protect?

Corticosteroid alteration of immune function is most evident during the establishment of a novel immune response, the memory lymphocytes inherent in long lasting immunity being relatively resistant to its suppressive effects. This means that if individuals are vaccinated against an infectious agent like tuberculosis, then the effects of stress on the pathogenesis can be minimalised. However, if an individual is exposed to stressful stimuli during primary vaccination, development of protective immunity may be compromised.

Herein lies the problem We need vaccines to protect the immuno-compromised host, but vaccines can't establish immunity in these individuals. The solution exists at the level of vaccine design. There is a need to produce vaccines that confer protection even in the immunocompromised or stressed host. To achieve this we need to incorporate models of stress into vaccine development and test new candidate vaccines in such models.

An appropriate model to test vaccine efficacy

The corticosteroid model of stress-induced immunosuppression has several advantages over more traditional methods where animals are exposed to physical stress paradigms. These include: alleviation of individual variation to stress; reproducible and dose dependent suppression; sustainable suppression by continued administration without problems of adaptation and; the fact that corticosteroids cause very little distress to the animal.

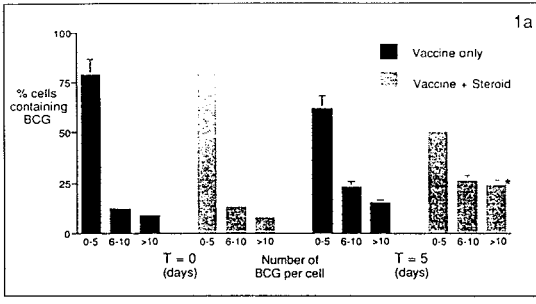
Methods and results

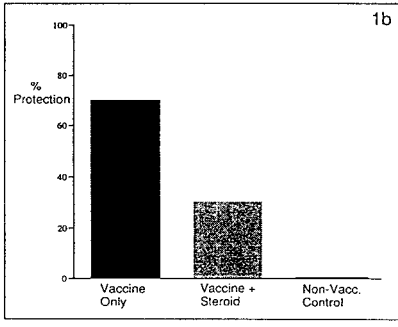
In the present study we examined the effect of dexamethasone, a potent corticosteroid, on the ability of red deer to mount an effective immune response following vaccination with BCG. Animals were treated with dexamethasone for

Figure 1: The effect of dexamethasone on cervineimmunity.

(1a) The effect of in vivo *dexamethasone on the anti-bacterial activity of blood derived monocytes. The percentage of cells infected after phagocytosis (T = 0) and following 5 days incubation (T =5) is shown. (* sig. different, p>0.05) Columns represent the mean ±SE of 200 cells per animal (10 per group).*

(1b) The effect of in vivo *dexamethasone on the efficacy of BCG in protecting against experimental infection with virulent M. bovis. Data from 30 deer (10 per group).*





three weeks, during which time they were vaccinated with live BCG; they were subsequently challenged with a low dose (2.5×10^2 cfu's) of live virulent *Mycobacterium bovis*. Measurements of haematological and immunological parameters were made, along with an evaluation of both vaccine response and protective efficacy. Comparisons were made with untreated controls to confirm that any observed effects were due to the steroid treatment.

Total numbers of white blood cells increased with steroid treatment, accounted for by a significant increase in the number of circulating neutrophils ($p>0.05$). Numbers of mononuclear leucocytes were significantly reduced in the peripheral blood of steroid treated animals ($p>0.05$), as were the levels of eosinophils ($p>0.05$). The functional capacity of peripheral blood lymphocytes was evaluated using a nonspecific, mitogen driven, lymphocyte transformation assay and was shown to be significantly suppressed during the period of steroid treatment ($p>0.05$). The ability of macrophages, derived from the peripheral blood of treated animals, to both phagocytose and inhibit the replication of live BCG *in vitro* was examined. Although there was no difference in the initial uptake of bacilli in the steroid treated group, the ability monocytes from animals treated with dexamethasone to restrict the intracellular growth of BCG was significantly reduced (Figure 1a). The efficacy of the vaccine was assessed by ranking the disease prevalence post-challenge, using necropsy and histopathological data. The efficacy of BCG in protecting against tuberculosis was significantly reduced in animals treated with dexamethasone (Figure 1b).

Conclusion

We have shown that along with suppression of immune function, dexamethasone influences vaccine efficacy. These results indicate that the administration of corticosteroids to red deer represents an appropriate model for stress induced immunosuppression in which to assess the efficacy of new vaccines against tuberculosis. Stress is a major effector in the pathophysiology of disease, but by incorporating stress into the study of immunity, we can overcome some of its deleterious effects and design better vaccines with improved efficacy.

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