The development of a new, multipurpose, animal model of tuberculosis

Euan Lockhart, Lynn Slobbe, Sarah Hook, Frank Griffin, Colin Mackintosh and Glenn Buchan
Tb Research Group, University of Otago

The re-emergence of tuberculosis as a major human and animal health problem has led to renewed interest in the immunological response to *Mycobacterium tuberculosis* complex. Although the mouse immune system is the best characterised of any species, many of the pathological and immunological responses to infection with tubercle bacilli are unlike those of naturally susceptible species. Its high level of natural resistance to tuberculosis makes it an unsuitable model in which to test vaccines. The most productive approach to better understand protective vaccine responses against tuberculosis may be to use the immunological power of the murine system to direct research in naturally susceptible host species.

We have proposed that domesticated deer are highly suited as a model for naturally susceptible host species (Buchan and Griffin, 1990). The deer model has already contributed to our understanding of TB diagnosis. Experiments to identify *M. bovis* specific antigens that could be used to improve diagnostic assays have shown that single purified antigens lack the sensitivity for use in the field. The model has also confirmed that a combination of assays that measure both T cell and B cell responses to *M.bovis* are necessary to effectively diagnose all diseased animals (Griffin *et al.* 1994).

We have developed the immunological tools with which to characterise the cervine immune response to tuberculosis. We have cloned and sequenced cervine IL-1β, IL-2, IL-4, IL-10, IL-12 (p40), GM-CSF, TNFα and IFN-γ. Expression systems have been developed to produce large quantities of purified recombinant cytokines. Using eukaryotic (baculovirus) and prokaryotic (*E. coli*) expression systems recombinant IL-1β, IL-2, IL-4 and IFN-γ have been produced. These recombinant cervine cytokines are being used to generate monoclonal and polyclonal antibodies which can be used to measure *in vitro* cytokine production and neutralise cytokine activity. The characteristics of deer cytokines studied so far are outlined in Table 1.

A variety of monoclonal antibodies against cervine lymphocyte subpopulations have been identified. Phenotyping antibodies to $CD4^+$, $CD8^+$, Tγδ cells and B cells are available. Antibodies against activation markers such as Proliferating Cell Nuclear Antigen, MHC II and IL-2 receptor (IL-2) are also now

Table 1: Immunological studies on deer cytokines.

Cytokine	Size (amino acids)	% Homology to Human	Recombinant Cytokine	Antibody
IL-β	266	55	Yes	Yes (ovine)
IL-2	162	58	Yes	Yes
IL-4	135	62	Yes	Yes
IL-10	179	78	No	No
IL-12	nd	nd	No	No
GM-CSF	147	79	Yes	No
TNFα	266	80	No	+/–(ovine)
IFNγ	166	67	Yes	Yes
IL-2 receptor α	276	52	Yes	No

available. Increasingly it is becoming possible to undertake detailed experimental investigations of the cervine immune response.

Experiments are underway to identify differences between the disease related immune response and the protective immune response. We have previously reported that immunisation and boosting with live BCG produces a cell mediated immune (CMI) response without antibody (Griffin and Buchan, 1993). This response has also been found in some naturally infected animals. Immunisation with killed BCG in oil adjuvant produces a CMI response but with a concurrent antibody response. Naturally infected animals with this type of immune response are invariably found to be diseased at autopsy (95%+). These immunisation schedules are being used to develop *in vitro* assays for discriminating immune protection from disease related pathogenesis in tuberculosis.

Experimental findings generated from the deer model include:

- T cells from severely diseased animals have a dysfunction in their ability to produce IL-2 and/or IL-2 receptors.
- Low doses ($5x10^4$ cfu) live BCG can induce a protective immune response against virulent challenge with *M. bovis*. High doses ($1x10^8$ cfu) of live BCG give inferior protection.
- High dose killed BCG in oil induces an immune response which is similar to that observed in diseased animals.
- There is a high degree of homology between the cytokines of deer, cattle and sheep. The homology between deer and human is greater than that between human and mouse.

- There is clear evidence to suggest that deer have *type 1* and *type 2* regulatory subpopulations of T cells. These are not, however, mutually exclusive as has been reported in mice. Early disease is characterised by a mixed response, with IL-4, IL-2 and IFN-γ all being produced. It is not until later in the disease process that IL-2 and possibly IFN-γ are turned off. This helps explain why IFN-γ can be used as a diagnostic marker for disease as well as protection. Protection appears to be characterised by the presence of IFN-γ but the absence of IL-4. IL-4 is a good diagnostic marker of disease even in the presence of IFN-γ.
- Effective vaccination appears to require conversion of an immune response from a *type 0* (IL-2, IL-4 and IFN-γ) to one that expresses IFN-γ in the absence of IL-4; *type 1* response. Prior exposure to cross-reactive antigen (imprinting) may be important in skewing the immune response in either a positive (*type 1*) direction or negative (*type 2*) direction.
- Peripheral blood is a convenient source of recirculating, antigen specific, memory lymphocytes which can be used to study immune responses to vaccination and disease. Antigen specific response can be detected in peripheral blood lymphocytes for considerably longer than they can be detected in the draining lymph node.

The characterisation of the cervine immune system combined with the development of experimental immunisation and infection procedures have established the deer as an important, naturally susceptible animal model in which to study the immunological response of naturally susceptible mammals to infection with pathogenic mycobacteria.

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Bibliography

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