

Selection for resistance and susceptibility of deer to tuberculosis using experimental challenge

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Introduction

There is good evidence for a genetic component to the innate resistance to tuberculosis caused by *Mycobacterium tuberculosis* in humans and *Mycobacterium bovis* in cattle, rabbits and mice (Francis, 1958; Stead, 1992). Natural resistance to infection with intracellular organisms such as *M. bovis* is controlled by a dominant gene in mice, designated formerly as Bcg and latterly as Nramp (Vidal *et al.* 1993). This relates directly to the innate ability of the macrophage to kill the organisms residing in phagolysosomes. Recent studies indicated that this gene occurs in cattle and codes for mechanisms responsible for the killing of intracellular organisms such as Brucella and Mycobacteria (Qureshi *et al.* 1995). There is evidence that the Nramp gene is present in deer (Crawford, pers. comm.).

A preliminary study was carried out to select stags based on their resistance or susceptibility to an experimental *M. bovis* challenge. The second phase of the study was to breed offspring from the three most resistant and the three most susceptible stags using artificial insemination of random commercial red hinds with semen collected prior to the challenge. The third phase will be to challenge the offspring with *M. bovis* and to identify genetic and immunological markers for resistance.

Methods

Semen was collected by electroejaculation under sedation from 39 three year old red deer stags of wide genetic origin, and frozen. The stags were moved to the Deer Tuberculosis Research Farm at Milton and they were challenged by instilling 0.2 ml of culture containing 5×10^2 colony forming units (cfu) virulent *M. bovis* organisms into the left tonsillar crypt, while sedated with Fentazin.

The infected deer were monitored for six months by regular blood tests for Tb (BTB), (Griffin *et al.* 1994) cytokine and macrophage assays and a final skin test just prior to slaughter. Necropsies were performed, lesions examined histopathologically and cultures made of lesions and pooled lymph nodes from the head, thorax and abdomen from animals with no visible lesions (NVL).

Results

Of the 30 lesioned animals, 22 had head lymph node lesions, 5 had head and abdominal or thoracic lymph nodes lesions and 3 had head, abdomen and thoracic lesions. The head lesions were predominantly in the left medial

retropharyngeal lymph node. Histologically the lesions were typically caseous, with variable degrees of calcification, varying numbers of neutrophils, a rim of epithelioid cells and Langhans giant cells, very variable numbers of acid fast organisms and some fibrosis. There was a good correlation between BTB, skin test, culture and necropsy results. Macrophage killing assays on a limited number of animals showed good correlation between the ability of an individual's macrophages to kill *M. bovis* BCG organisms and its resistance to Tb challenge.

| Gross lesions | Scale of Lesions Severity | Lesion and NVL Pool Culture Results | Number |
|---|---------------------------|-------------------------------------|--------|
| No visible lesions (NVL) | 0 | Negative | 5 |
| Single small lesion in LMRP* | 1 | <i>M. bovis</i> | 4 |
| Single moderate lesion in LMRP | 2 | <i>M. bovis</i> | 3 |
| Very large single or multiple small lesions in LMRP | 3 | <i>M. bovis</i> | 6 |
| Multiple lesions in head lymph nodes and tonsil | 4 | <i>M. bovis</i> | 7 |
| Large multiple lesions in head, abdomen or thorax | 5 | <i>M. bovis</i> | 5 |
| Large multiple lesions in head, thorax and abdomen | 6 | <i>M. bovis</i> | 3 |

*LMRP - Left medial retropharyngeal lymph node.

Discussion

There was a normal distribution of lesion severity in the Tb challenged stags. Two of the five NVL culture negative animals were skin test negative and BTB negative. The other three NVL stags had low BTB and skin test results, although two had a slight rise in antibody 14 days post skin test. Based on these results, three NVL animals were designated resistant. The three stags with the most extensive lesions were designated susceptible. The semen from these animals was used for subsequent breeding experiments.

The semen from the three resistant and the three susceptible stags were used in an AI programme to inseminate 220 randomly selected commercial hinds. Ultrasound scanning at 40 days post AI showed 109 were pregnant to AI with approximately even numbers of calves per sire. The hinds are due to calve in early December 1995.

To test the hypothesis that resistance to Tb is inherited, it is planned to wean the female offspring of the resistant and susceptible sires at 3 months of age and take them to the Deer Experimental Tb Farm at Milton and challenge them with Tb in the same way as their sires. Concurrently the Nramp genotype, macrophage killing ability and MHC Class II DRB genotype will be studied in order to identify genetic and immunological markers for resistance.

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