In vitro Tests for Tuberculosis in Farmed Deer

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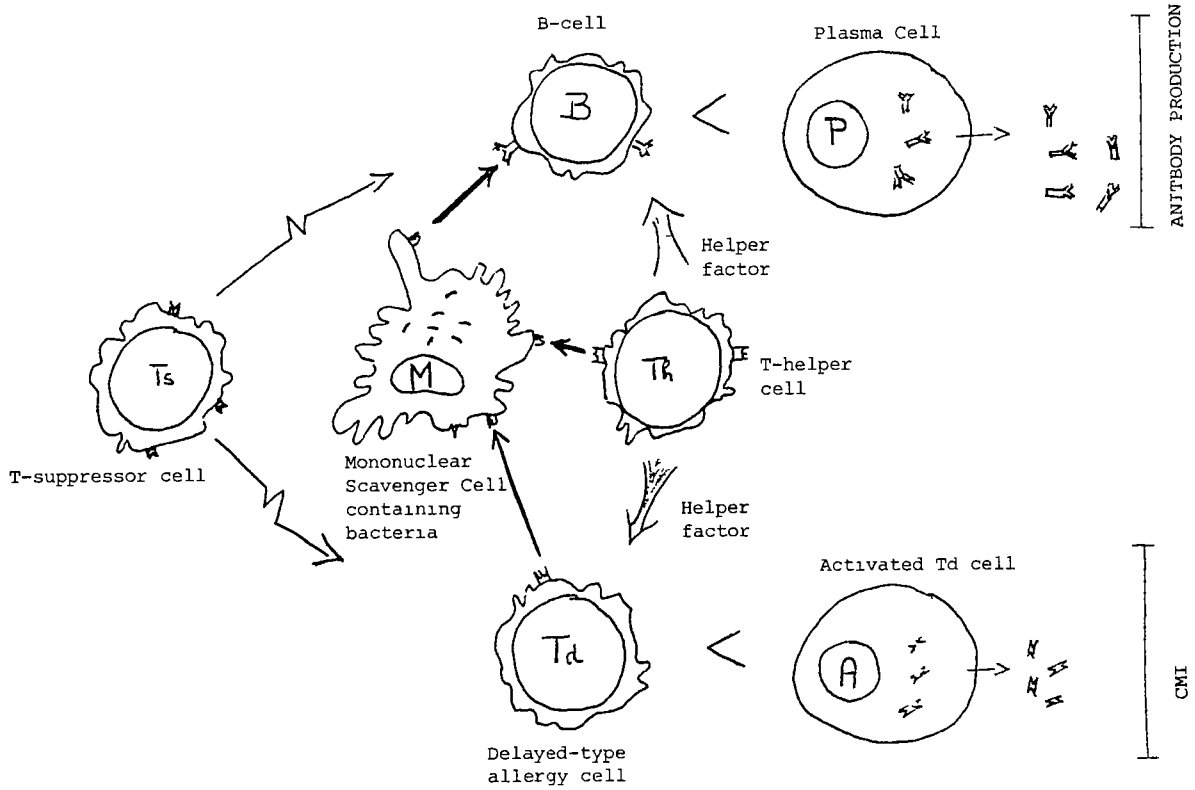
Immunological Perspectives

Metchnikoff (1883) discovered cell mediated immunity (CMI) as the vital immune function in the protective response of guinea pigs to experimental infection with *Myco tuberculosis*. It has subsequently been shown that CMI reactions involve a triad of cells (Fig 1), which require presentation of antigen (macrophage-mononuclear phagocyte - van Furth, 1981), activation by T-helper cells (Mitchison, 1971) and effector activity (Td-cells)(Denis, 1985), to produce the classical delayed type granulomatous response. This reaction is typified by tubercle formation in response to Tb infection. The intradermal skin test has been used to monitor immune reactivity to infection in a number of diseases in which CMI has a central role. Transfer of bovine skin testing (ST) technology for identification of Tb infection in deer has highlighted unique challenges presented by this species in disease control programmes. Central to such considerations are two

FIGURE 1

CELLULAR INTERACTIONS INVOLVED IN IMMUNITY



main issues. Firstly the suppressive effects produced following PPD skin testing requires a test interval of 90 - 120 days. This is due to the activation of suppressor cells (Ts) which are selectively activated by soluble PPD and result in impairment of normal Th and Td function post testing (Thestrup-Pedersen, 1975; Gershon and Kondo, 1970). Secondly False ST reactions appear unacceptable in a deer industry unable to sustain an adequate compensation scheme for slaughter of ST(+) animals. Problems due to False ST reactions can be explained by the special susceptibility of deer to infection by mycobacterial species. Deer appear to be particularly susceptible to tuberculous infections. Infection with *Myco bovis* produces an inordinately high percentage of liquefactive lesions, which do not always produce ST(+) response and may result in FALSE(-) result with ST. Also the significant incidence of immunological reactivity to *Myco avium* found in our studies, suggests that farmed deer are uniquely affected by ubiquitous species of mycobacteria resulting in FALSE(+) ST cross-reactivity, when tested with *Myco bovis* PPD in a CT. (Mid-cervical intradermal tuberculin test)

Laboratory Techniques

The objective of the current programme has been to define laboratory techniques which act as ancillary tests to exclude FALSE(±) reactors in the Tb ST programme. Techniques have been developed which allow us to critically examine individual animal's immune reactivity at the level of Th, Td or Ts cell function. In addition levels of inflammatory cells and proteins are monitored in an attempt to define the levels of Tb infection in a given animal. The initial studies have been concerned mainly with development of techniques which have acceptably high levels of SPECIFICITY and SENSITIVITY, to detect *Myco bovis* infection. Currently work is underway to produce further assays with the greatest degree of FEASIBILITY in terms of breadth of application and cost effectiveness.

The parameters studied during Phase I of the project have assessed the ability of laboratory tests:

1. To identify FALSE(-) ST animals which harbour Tb lesions.

2. To rank CT reactors prior to slaughter in an attempt to predict animals likely lesion status post mortem.

3. To measure the suppressive impact of ST on laboratory assays carried out post ST.

4. To assess the ability of such technology to identify FALSE(+) CT reactors due to *Myco avium*.

Animals Studied

To date 600 deer from 'at-risk' herds with a high level of *Myco bovis* infection (> 50%), and about 2000 control animals, have been studied in the laboratory development programme. Post mortem analysis has been carried out on more than 300 animals and the association between the incidence of lesions at slaughter and laboratory test findings have been evaluated. Samples have been obtained from 28 herds under a diverse range of climatic and management conditions.

Laboratory Findings

From the initial studies of Tb lesion reactor animals a computer model (Fig 2) was designed which incorporates haematological and immunological values considered relevant for the diagnosis of Tb lesions in deer. In Fig 2, N represents the mean values of these parameters in normal animals. L indicates the skewed data found in a typical ST(+) lesion reactor. By extrapolation this model predicted that RB; an animal that gave 11 ST(-) reactions over the past three years, would be a lesion positive, FALSE(-) ST reactor. On autopsy, following a final CCT(-), this apparently healthy animal presented with severe generalised tuberculosis, involving the thorax, lungs and abdominal organs. A further 5 ST(-) animals, all with significant lesions, were identified using the laboratory tests, in this herd. Tests carried out in the herd prior to depopulation gave consistently high predictability as to the lesion status of individual animals (Fig 3). Similar tests carried out on CT(+) animals prior to slaughter have accurately predicted the prospect of *Myco bovis* lesions in each of 6 independent herds without any prior history of tuberculosis.

Studies carried out on animals sampled prior to and following the application of the CT and CCT show that laboratory parameters can be monitored accurately as soon as 72 hours after i.d. inoculation of PPD. It seems likely that the suppressive effects which preclude a short interval between ST' can be overcome with the current laboratory assay systems.

A high degree of antigen specificity is evident using the laboratory assays, which can readily distinguish between reactions due to *Myco bovis* and *Myco avium* (Fig 4). In three separate herds reactivity due to *Myco avium* in CT(+) animals, has been identified unequivocally, before their status was confirmed as 'atypical' reactors in a CCT.

Future Studies

The completion of Phase I of the project will involve the consolidation of our data base by including a further group of animals from *Myco bovis, Myco avium* and non reactor herds.

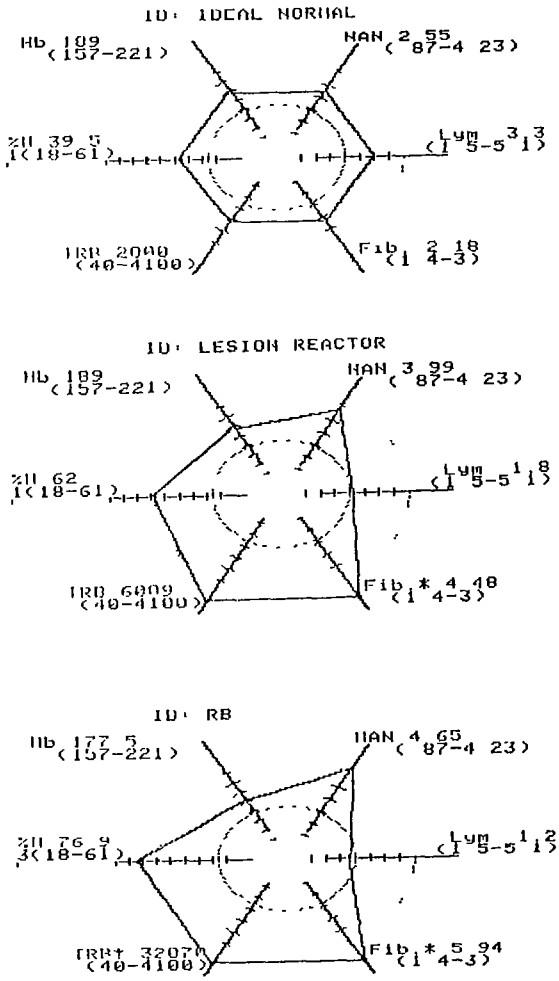
To ensure completion of these controlled experiments, suitable herds have already been selected, so it is inappropriate to consider inclusion of any other herds in the immediate future.

Further critical analysis of the SPECIFICITY and SENSITIVITY of these techniques will be carried out within the next three months, when the histological and microbiological analyses of post mortem samples are completed. Should these composite findings stand the test of critical evaluation then it is probable that this technology would have a role as an ancillary method in conjunction with the ST for control of Tb in deer. This could provide information which would allow the field veterinarian to better implement and manage a comprehensive ST programme. Areas with likely field application would include:

1. The identification and elimination of FALSE(-) ST reactors in *Myco bovis* infected herds.

Fig 2

Composite Analysis of Immunological and Inflammatory Responses in Deer Blood



N

L

R

Hb	Haemoglobin level (g/l)
%	Neutrophils
NAN	Neutrophil count ($x10^9/l$)
Lym	Lymphocyte count ($x10^9/l$)
Fib	Fibrinogen level (g/l)
TRB	Transformation with bovine PPD (Cpm^S)

Fig. 3

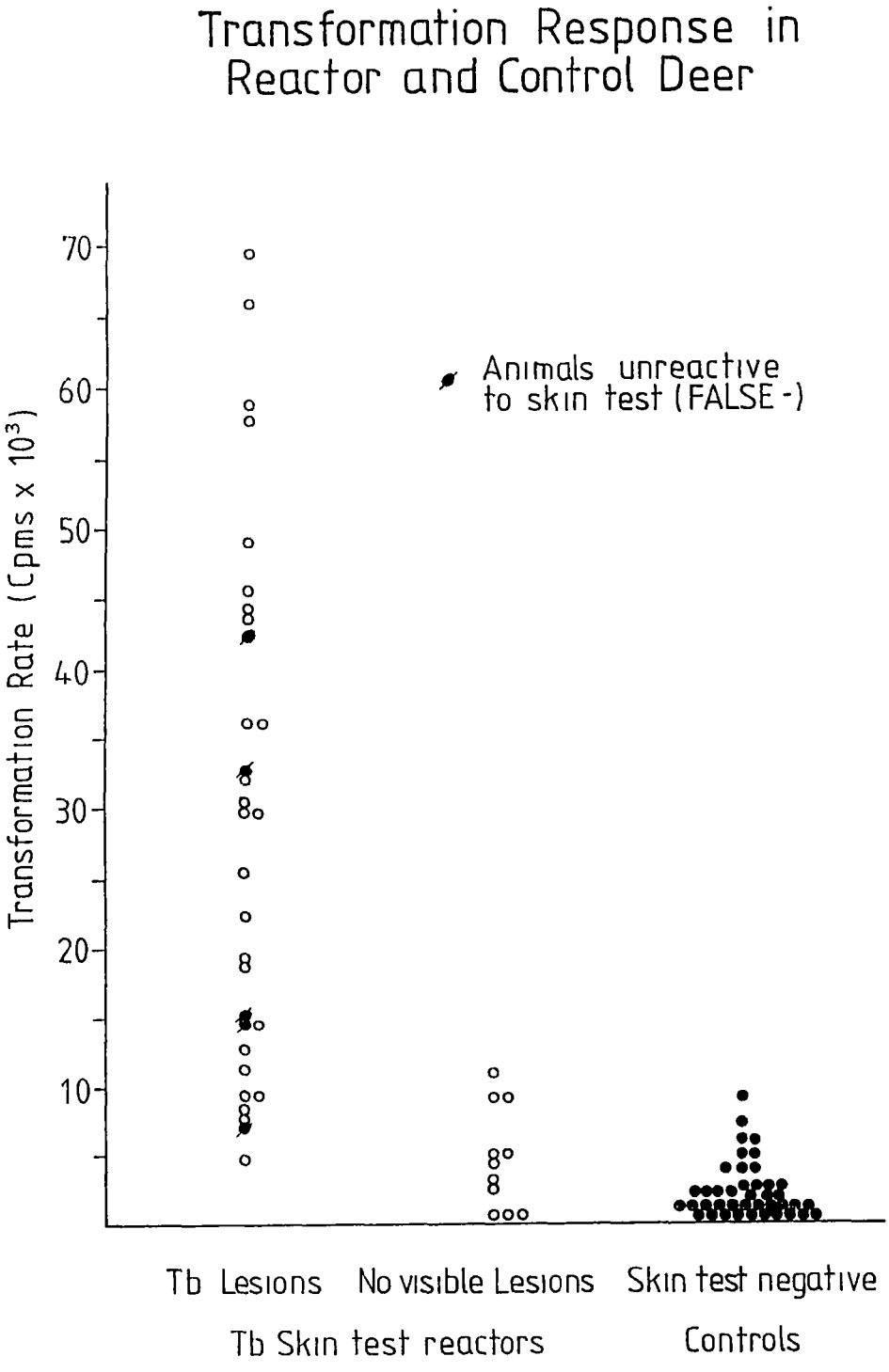
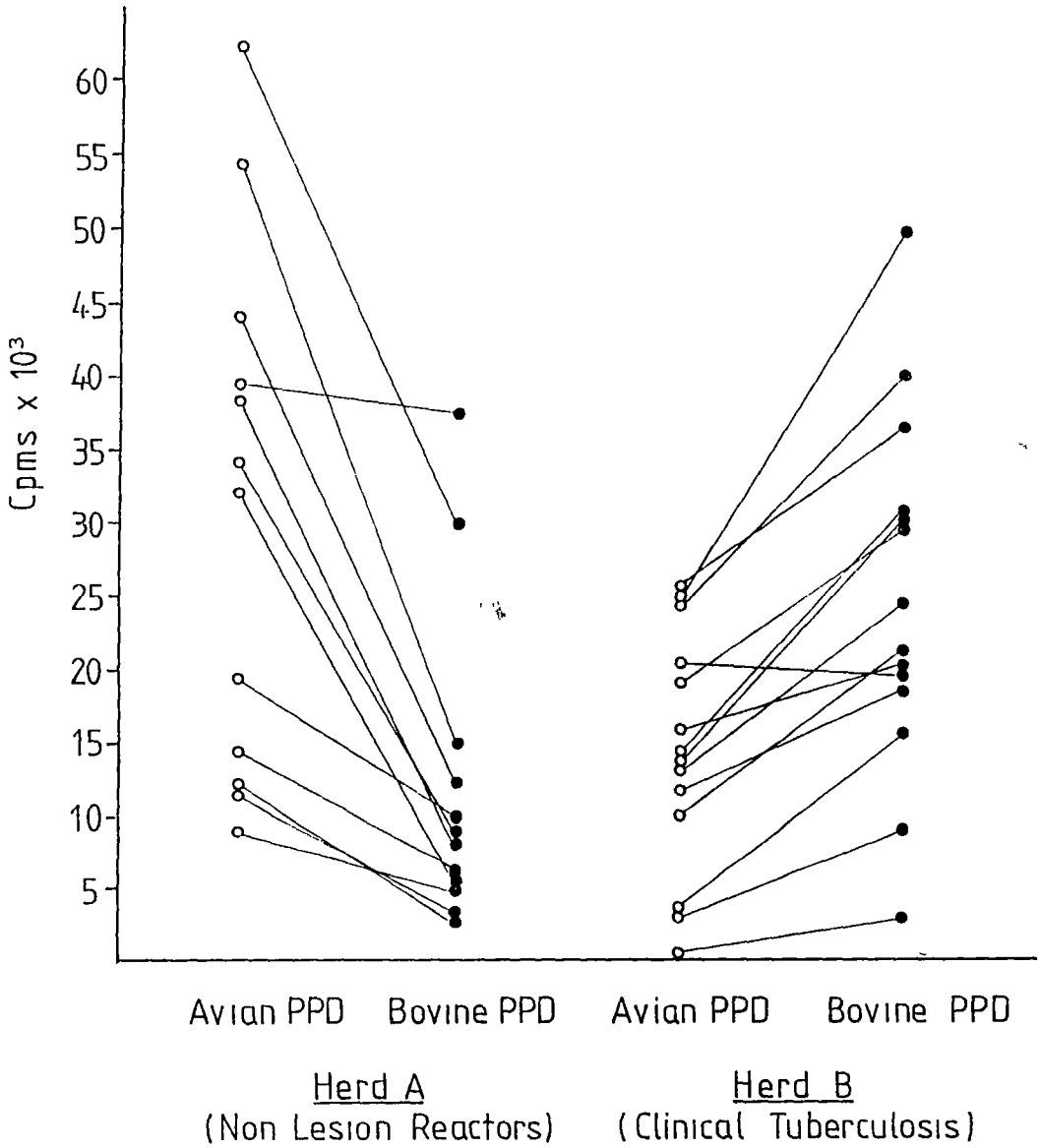

Transformation Response in Reactor and Control Deer
Transformation Rate (Cpms x 10^3)
70
60
50
40
30
20
10
Animals unreactive to skin test (FALSE -)
Tb Lesions
No visible Lesions
Skin test negative
Tb Skin test reactors
Controls

Fig.4

Discrimination between Myco bovis & Myco avium by Lymphocyte Transformation



2. The ranking of CT(+) animals prior to slaughter, to increase the probability of identifying lesion reactors, and optimise the salvage of valuable stock.

3. The discrimination of *Myco avium* reactivity from true *Myco bovis* infection, soon after the application of the ST, in herds of unknown history. This could obviate the concerns of farmers faced with management of significant numbers of CT(+) reactors following skin testing, by eliminating the unduly long interval currently required between successive ST'.

References

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