Cellular Immunity in the Aetiology and Diagnosis of Tuberculosis in Farmed Deer

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INTRODUCTION

Immunodiagnostic techniques have been used widely in veterinary medicine for disease control programmes. Immunological reactivity is taken as presumptive evidence of infection, so that animals showing such reactions are culled. The VALIDITY of any given procedure, is influenced by the SENSITIVITY of the technique to identify infected animals, and the SPECIFICITY of the system, which excludes unifected animals.

With Tb, cellular immune mechanisms (Griffin and Cross 1986), are considered central in the host's response to infection by intracellular *M. bovis*. The role of this response in Tb first led to the discovery of cell-mediated immunity (CMI), as the protective immune response in Tb infected guinea pigs, over a century ago (Metchnikoff, 1883). The *in vivo* measure of CMI is the indurated swelling produced intradermally in the skin of reactive individuals, inoculated with soluble extracts ('tuberculin') of the prototype microorganism. The *in vivo* skin-test (ST) reaction is called a delayed-type hypersensitivity (DTH) response and this has been used extensively as the primary method of the control of Tb in cattle throughout the past fifty years.

Factors which influence infection and the efficacy of methods for the control of Tb are:

1. Endemic levels of *M. bovis*
   (a) In the host species.
   (b) In feral animals.

2. Genetic resistance of species and individual groups of animals within a species.

3. Complications due to exposure of animals to other *Mycobacterial species* which cross react with *M. bovis*.

4. Technical difficulties in applying the test.

5. Financial investment in the application of the test, and in providing incentives through compensation of farmers for slaughter of reactor stock.

Rigorous application of the ST in cattle has failed to control Tb in a number of countries, including Australia, New Zealand and Ireland, where one or more of the above factors may have mitigated against its successful conclusion. In all of these countries there is now considerable research efforts into alternative methods for the diagnosis of Tb.

By comparison with bovine schemes, application of skin testing for Tb control in farmed deer in New Zealand, needs to take due consideration of all of the above factors, each of which may influence the test. Firstly, because Tb is endemic in cattle and feral animals in New Zealand, and because farmed deer appear to be highly susceptible to infection, producing lesions which may facilitate spread within and between individual herds. Secondly because animals subjected to undue management stress (e.g. capture, breeding and weaning)may become 'compromised', and be increasingly susceptible to contracting infection. Exposed individuals could have an increased susceptibility to invasive infection, and if infected would be less likely to produce a positive skin test. Farmed deer appear to be highly susceptible to infections caused by other Mycobacteria *(M. paratuberculosis, M. avium)*, which can produce pathological lesions. Saprophytic *Mycobacteria species*, may also cause sensitisation of deer and produce False (+) reactions on testing with tuberculin.

Considering all of the above complexities, we have attempted to produce an alternative test for diagnosis of Tb in farmed deer. The relevance of such a technique is dependent not only on it providing superior SENSITIVITY and SPECIFICITY to the skin test, but also in its inherent FEASIBILITY to be applied effectively under routine veterinary management.

Laboratory Testing for CMI using Lymphocyte Transformation (LT)

Because of the initial success with lymphocyte transformation (LT), this method has been further applied to the blood testing of large numbers of animals, all taken from herds with ST reactors, where Tb was suspected, or atypical reactors, due to *M. avium*, was considered to be a problem. The need to scrutinise the technique using large numbers of samples has limited the effort that could be expended in the study of alternative techniques, such as the production of Interleukin-2 (IL-2), Pro-coagulant (PC) or γ-Interferon (γ-INF), any of which may offer further discrimination in the diagnostic process.

Since the study began, 7248 blood samples have been examined for lymphocyte transformation using tuberculin from *M. bovis* (PPD-B), *M. Avium* (PPD-A) and *M. paratuberculosis* (PPD-J). In essence the method measures the degree of stimulation of blood mononuclear leukocyte (BML) cultures, following the addition of the respective antigens. The level of cell proliferation is estimated by uptake of radiolabelled thymidine (^{3}HTdR), into the DNA of dividing cells.

Positive control cultures have non-specific mitogens added, which cause polyclonal activation and establish the peak reactivity and the normalcy of a given blood sample. Negative control cultures, unstimulated by mitogen or antigen, are used to establish the background proliferation in the test sample. Results, which measure ^3H uptake in individual cultures, are expressed as radioactive counts per minute (cpms).

RESULTS

Classification of herd status following a single blood test

The first question posed in the experimental outline, was whether a single blood test taken from a series of ST reactor animals within a herd, could accurately predict whether the herd under test had clinical tuberculosis, mixed infection; involving *M. bovis* and *M. avium*, or *M. paratuberculosis*, *or Saprophytic Mycobacteria*, or a single infection involving Mycobacteria other than *M. bovis*. Table 1 shows the number of herds where the respective infectious agents were identifiable following a single blood test. An adequate test is defined as a set of results where positive and negative controls are within the normal range.

Table 1. Frequency of herds with different types of Mycobacterial Infection Identified in a single Blood Test

	M. bovis	*M. bovis* + *M. avium*	*M. bovis* + *M. paratb*	*M. avium*	*M. Paratb*	*Saprophytic Myco.*
Number of Herds	4	12	2	17	0	1
Number with Tuberculous Lesions	4	11	2	0	0	0

These results show that the specificity of the blood transformation assay is such that the disease status of a herd is obvious following a single blood test. This has the advantage that it can provide early information which allows a farmer to countenance the prospect of tuberculosis, or to set in place further tests to characterise an 'atypical' reactor problem. Whereas it is accepted that the CCT can effectively identify problems caused by simple *M. avium* reactivity, it is interesting to note that the herd with *Saprophytic Mycobacteria*, showed 15/35 reactors positive for *M. bovis*, when tested by CCT. All of these were excluded as tuberculous animals following the blood test results which showed no dominance of *M. bovis* reactivity.

Influence of the ST on LT in a group of animals with positive reactivity of PPD-B in the LT assay

The use of a laboratory test must ideally be applicable within a short period following the ST, if time is to be gained in the management or exclusion of tuberculosis from reactor animals. The LT response has been monitored in a group of 54 animals with significant reactivity in the LT assay to PPD-B. The animals were first LT tested 90 days prior to ST and resampled just before the ST was used. The animals were rebled 3 days and 14 days after applying the ST. Table 2 shows the results, where each individual animal's reactivity was normalised, and changes measured by comparison with the LT response obtained in the blood sample prior to skin testing.

Table 2. Changes in LT response over a 90 day period, and at 3 and 14 days following ST.

	Time of LT Assay			
Altered* reactivity	D-0	D-90 (ST)	D-93	D-104
None	22(40.8%)	54(100%)	34(62.9%)	33(61.1%)
Increased	25(46.3%)	0	8(14.8%)	14(25.9%)
Decreased	7(12.9%)	0	12(22.2%)	7(12.9%)

*Altered reactivity at any given time, in an individual animal, is taken as a response which alters from the Day 90 response by at least 20%.

The observations of special interest are the changes that occur in the absolute levels of LT activity over a 90 day period in animals which have not been exposed to ST, and the more subtle changes which occur in the LT response, of animals following ST. The changes in LT response which are found in the sequential 90 day blood samples are considered to be of special importance, because they are fundamental in characterisation of CMI reactivity, which may discriminate between IMMUNITY to, and INFECTION by *M. bovis*. To date our observation has been that significant decreases in LT reactivity over time indicates that infection has been resolved and the host is IMMUNE to Tb. By contrast a sustained high level response, or increasing levels of LT suggest that the individual has a persistent INFECTION with *M. bovis*. Clearance of *M. bovis* antigen, through resolution of an infection, results in the dissipation of the LT response. The ability of the LT to discriminate between INFECTION and IMMUNITY will be developed at a later stage in this paper.

The response found 3 days after ST shows no consistent evidence for post ST suppression of LT. Whereas there was more suppressive activity 3 days after ST than at 14 days, only one animal had a ST response after testing which would have caused the LT to be reduced to a level which would have allowed the animal to be classified as LT (-), even if only one blood sample had been analysed. The samples taken at 14 days following ST showed a significant number of animals with increased LT over that found prior to ST. This may result from the activation of memory cells in animals following administration of tuberculin.

SENSITIVITY and SPECIFICITY of LT in herds harbouring *M. bovis*

The results of the LT assay were critically evaluated in six herds where tuberculosis was known to be present. Data is taken only from herds where extensive autopsies were carried out on all animals tested, irrespective of whether they were likely to be tuberculous or uninfected. STs were carried out prior to autopsy and the results from this test is included for comparison. It must be stated that it was recognised that use of the ST was more likely to produce False (+) reactors due to mixed infection of *M. avium* within the affected herds. However, accepting recent recommendations (Wilson, 1986), that the CCT cannot be used where *M. bovis* is known to be present in a herd, it was considered reasonable to use the standard ST in these herds. Autopsies were carried out under controlled conditions on the farms or at DSP premises. Results of macroscopic autopsy findings and laboratory histopathology and microbiology on specimens, were used for the disease classification. The results are given in Table 3.

Table 3. Sensitivity and Specificity of the LT assay carried out on 612 animals prior to autopsy.

	Sensitivity	Specificity
LT	$\frac{158}{166}$ (94.8%)	$\frac{420}{446}$ (91.9%)
ST	$\frac{139}{166}$ (84.1%)	$\frac{365}{446}$ (82.3%)

These comparisons show the superior discrimination obtained by the LT assay over the ST. In the case of assay sensitivity, apart from the incidence of False reactions, another significant difference also emerges. Of the 5% of tuberculous animals which would have passed the LT (False -), no animal had liquefactive lesions present at one or more sites. By contrast among the 16% of animals which passed the ST, at least half had multiple liquefactive lesions, with one quarter of these animals showing generalised Tb. This infers that the LT is superior in the management of Tb, where severely infected animals, likely to cause infections spread within the herd, are not left behind following LT testing.

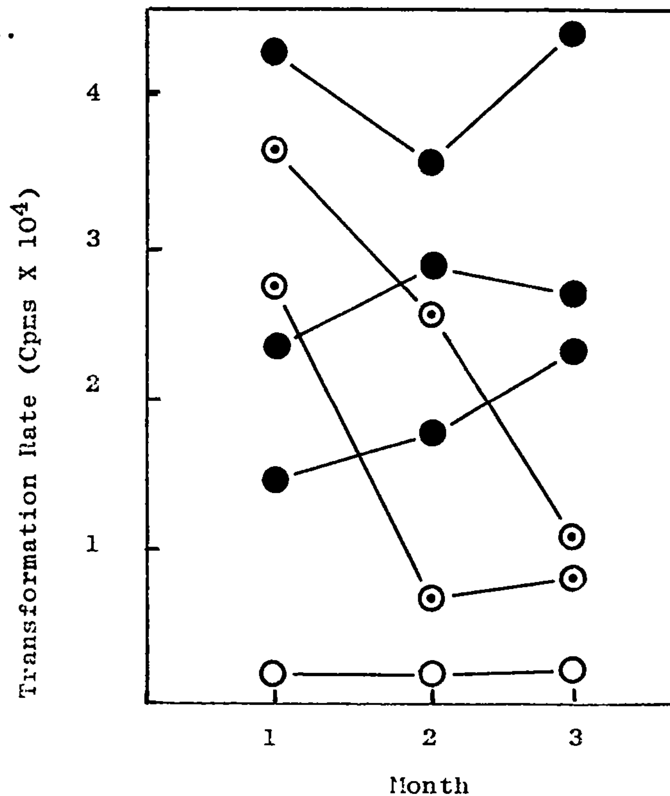
Sequential LT obtained from animals in *M. bovis* infected herds

It was considered likely from our experiences (Table 2) in carrying out sequential LTs on individual animals that the patterns obtained on repeat tests could possibly allow for further discrimination within the LT assay system, to categorise animals as UNEXPOSED, INFECTED, or IMMUNE.

Typical LT responses in individual animals sampled at monthly intervals and tested with PPD-B or PPD-A are shown in Figure 1 and Figure 2.

Sequential LT Response to PPD-B

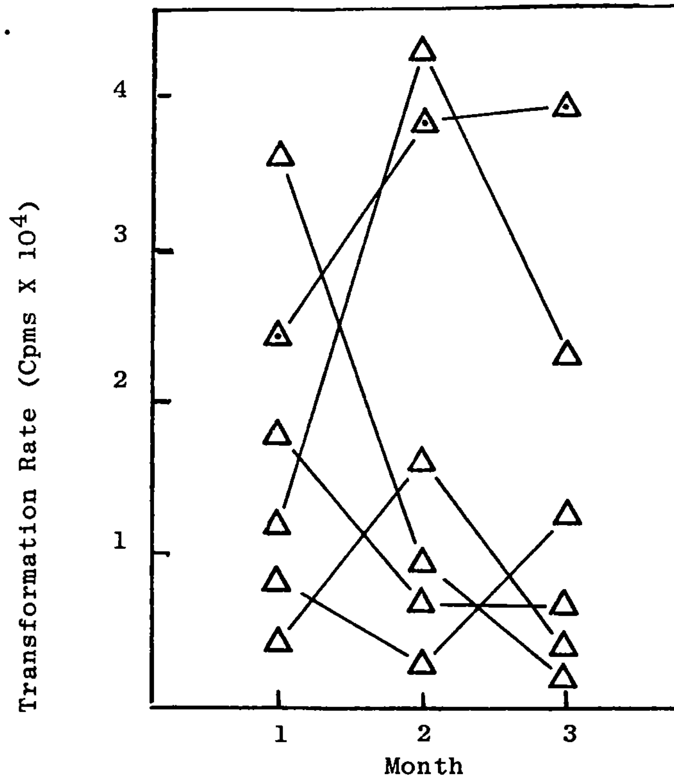
Figure 1.



The results given in Figure 1 show that the patterns of LT reactivity in sequential samples can be used as a predictive indicator as to whether an animal has not been exposed to *M. bovis* (UNEXPOSED - ○), or has been exposed to *M. bovis* with resultant establishment of infection (INFECTED - ●), or has become resistant following exposure to Tb (IMMUNE -⊙). An increasing LT response in sequential samples or persistent high reactivity is indicative of infection, whereas a decreasing LT response is indicative of immunity. Shapiro, Harding & Smith (1974), have shown that DTH reactivity may wane in animals following immunisation with *M. tuberculosis* without a concomitant decrease in immunity resistance. This infers that immune memory may be present in the absence of a detectable CMI response *in vivo*, or as shown above, *in vitro*.

Sequential LT Response to PPD-A

Figure 2.



The individual animal's response to *M. avium* is much more changeable than that found with *M. bovis*. LT reactivity to PPD-A may rise or fall precipitously between samples, with the cell response appearing to be transient, without persistent reexposure to *M. avium*. This response can be influenced by high levels of *M. bovis* within a herd, when LT to PPD-A may be sustained at higher levels than when *M. avium* is present(⚠)

CONCLUSIONS

Alternatives to the ST for the diagnosis and control of Tb must address the following issues, which affect the value of both the ST and the LT, in the control to Tb.

1. Test Interval

The 90 day interval needed because of post ST suppression, may compromise the ability of this assay to eliminate Tb infection, if present at a significant level within a deer herd. The ability of Tb to spread rapidly within some deer herds, may compound this problem. An obvious advantage of the LT is that it can be used on the day the ST is read, without undue interference through ST suppression. Repeat LTs can be carried out without any interference to subsequent testing.

2. Sensitivity

It is widely accepted that anergy to ST may occur in seriously infected tuberculous animals, and that these False (-) reactors may compromise its ability to control Tb spread. The sensitivity of the LT is such that all seriously affected tuberculous deer can be detected by the LT, so that the anergy found in ST is not duplicated in the LT. Independent studies in human tuberculosis have shown that the LT can detect individuals with serious Tb infection who are anergic to the ST (Verma, Gupta and Ghai, 1974 and Steiner & Rao, 1980).

3. Specificity

It is recognised that the CCT offers significantly increased specificity over the ST in the detection of 'atypical' reactors due to *M. avium* Recent recommendations (Wilson, 1986) are that the CCT should not be applied in herds where *M. bovis* is known to occur. In such herds significant stock losses would occur due to atypical reactors,undetectable by the ST. The high degree of specificity of the LT means that it can be applied effectively in herds with mixed infections due to *M. avium* and *M. bovis*. A similarly high degree of discrimination has also been shown using the LT in cattle (Muscoplat *et al.*,1974).

A further complication is that even when the CCT is applied, False (+) reactions may occur,due to exposure to sundry saprophytic Mycobacteria. Results in Table 1 show how such problems can be eliminated by the use of the LT. Independent experimental evidence has shown that slow growing saprophytic Mycobacteria elicit a strong cross reactive ST response with *M. tuberculosis* (Hattikudur & Kamat, 1985).

4, Validity

It is known that the cellular response involved in the DTH reaction, measured by the ST is independent from the cellular response which confers immunity, following exposure to Tb (Orme & Collins, 1984). This means that the ST response cannot be used to accurately identify immune animals. This may explain the low validity in the ST to predict immunity in reactor animals. A validity of 30% (Carter *et al.*, 1985) would mean that valuable uninfected stock must be sacrificed in a ST programme. Repeat LT assays can better distinguish between immune and infected animals. This allows for increased validity which would result in the salvage of stock, especially because uninfected immune animals may be an elite group with superior genetic resistance to Tb. In experimental animals there is evidence that genetic resistance is an important factor in defining the establishment of disease following exposure to *M. bovis* (Stach *et al.*, 1984). Much work is being carried out at present to identify genetic lines of animals with superior resistance to a number of important infectious diseases. Use of the LT may allow for retention of genetically elite animals with increased resistance to Tb.

5. Feasibility

Considerable effort has been expended during the past year to simplify and standardise, the procedures involved in the LT assay. Much work has also been carried out to obviate problems due to sampling, recording and reporting. The laboratory system currently employed, allows for the efficient processing of large numbers of blood samples.

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