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ACKNOWLEDGEMENT. I thank DST, New Delhi for financial assistance for preparing this manuscript.

Received 21 May 1996; accepted 17 July 1996

Immunodiagnostic approaches to detect bovine tuberculosis

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Even though tuberculosis is an ancient disease, its early diagnosis is still a global problem. Conventional diagnostic tests along with some improved techniques and molecular biological approaches have been reviewed. The current status and the pitfalls of the diagnostic procedures are reported. An attempt has also been made to give some insight of the future prospect of the field of research.

A recent report of the World Health Organization shows that multidrug resistant tuberculosis, which often leads to death; has emerged as a new global challenge. The

WHO, which has declared TB as a global emergency, has warned that the TB crisis will continue to grow unless immediate action is taken to stop its growth. The public health risk is more in agrarian countries like India, as bovine tuberculosis is a serious problem even now. The relationship between bovine tuberculosis and human tuberculosis has been reviewed earlier and it has been established that tuberculosis in cattle may be transmitted to man via milk, milk products, meat and directly in the cowbyre¹. The resurgence of the devastating disease has led to renewed interest in the development of improved diagnostic tests for tuberculosis in animals and a review of control measures.

The basis of the TB eradication programme in other countries has been systemic application of the standard tuberculin test and the slaughter of reactors. But in India test and slaughter method cannot be used due to high incidence of reactors and moreover, this procedure would result in the loss of highly productive cows and working animals. Hence, the accurate diagnosis of the infected animals remains a major concern for eradication of the bovine tuberculosis.

Early approaches

Efforts have been made to develop *in vitro* tests for tuberculosis. The conventional methods for detection and diagnosing bovine tuberculosis have been tried. A variety of serological tests, viz. complement fixation test^{2,3}, bentonite flocculation test^{4,5}, kaolin agglutination test⁶ and indirect fluorescent antibody test⁷ have been evaluated for the diagnosis of bovine tuberculosis. But these assays are less sensitive and less specific due to antigenic cross reactivity between mycobacteria often encountered by all mammalian species.

Cellular vs humoral immune response

It is well established that tuberculosis in both human and cattle induces a strong cellular response⁸⁻¹⁰ and hence a standard test for detection of cell-mediated immune response against tuberculosis in cattle should be targeted. The reaction mediated in a tuberculous cattle as a result of an intradermal injection of tuberculin, an antigenic extract derived from the tubercle bacillus, is of delayed hypersensitivity type. The reaction to tuberculin in a sensitized animal is an immunologically specific inflammatory reaction mediated by T cells. Because positive tuberculin reaction occurs only animals that have or have had tuberculosis, skin testing may be used to identify animals infected by this disease. But the tuberculin tests fails to detect some infected humans and cattle in advanced stages of the disease and it has been reported that the antibody responses in such cases of advanced disease were elevated^{10,11}. Therefore, a serological test coupled with a cellular test should give the greatest degree of accurate diagnosis¹². Moreover, the intradermal tuberculin test is known to lack both specificity and sensitivity due, in part, to the complex nature of the antigen purified protein derivative (PPD) used, which includes several cross-reactive proteins common to a range of other mycobacteria.

Improved diagnostic tests

Several workers recommended the use of enzyme linked immunosorbent assay (ELISA) as a complementary test

with tuberculin testing. The use of ELISA for bovine tuberculosis using tuberculin PPD as the source of antigen was first reported¹³ in 1983. Subsequently, it has been reported that ELISA can be used for the detection of *Mycobacterium bovis* infected animals, anergic to the tuberculin test. The specificity of ELISAs using sonicate preparations of *M. bovis* was reported to be low¹⁴. This may be due to the presence of higher concentration of the widely cross-reactive heat-shock proteins in these preparations compared with culture filtrate preparation¹⁵.

DNA synthesis is the most commonly measured parameter used for quantitative lymphocyte blastogenesis. This is usually accomplished by measuring the uptake and incorporation of radioactivity labelled base analogue or other molecules associated with cellular DNA synthesis. ³H-thymidine uptake measures the rate of DNA synthesis. ³H-thymidine uptake by tuberculin-stimulated lymphocytes is highly correlated with the degree of delayed skin reactivity. *In vitro* lymphocyte stimulation test tend itself as a practical and reliable test to aid in the diagnosis of *M. bovis* infection in cattle¹⁶. Lymphocyte transformation test is more sensitive and often positive when the skin test is negative, doubtful or feeble¹⁷. However, the incubation time and number of laboratory manipulations make it unsuitable for field work.

Therefore, a rapid, whole blood incubation system was developed and the release of a cytokine, interferon gamma (IFN- γ), as the indicator of a positive response to *M. bovis* antigen (bovine PPD) was proposed. A bioassay to detect the presence of IFN- γ , which showed good correlation with results obtained with the lymphocyte proliferation assay was developed initially¹⁸. Subsequently, monoclonal antibodies to recombinant bovine IFN- γ were produced¹⁹. These monoclonal antibodies were used to develop a sensitive enzyme immunoassay (EIA) for bovine IFN- γ (ref. 20). The bovine IFN- γ EIA when used in conjunction with the whole blood culture system resulted in a simple, rapid (24 h) and sensitive *in vitro* assay for detecting specific cell-mediated immune responsiveness in *M. bovis*-infected cattle. The IFN- γ assay has been accredited by the Standing Committee on Agriculture as a diagnostic test for bovine tuberculosis in Australia. The IFN- γ EIA is produced commercially and field trials of this assay, for the diagnosis of bovine tuberculosis, are currently in progress in the USA, New Zealand and Ireland²¹.

Molecular biological approaches

In the last couple of years, molecular biology has provided new approaches which have enabled detailed studies to be made of the molecular characteristics of *M. bovis*. These characteristics have been investigated for their potential use in diagnosis and epidemiological

studies. Restriction fragment analysis of genomic DNA from isolates of *M. bovis* has provided a highly discriminating typing system which has been extensively used for epidemiological studies. DNA elements in *M. bovis* have been investigated for their potential use in diagnostic assay based on the polymerase chain reaction (PCR). PCR-based diagnostic tests have the potential to detect DNA from a single organism of a predetermined species in a few days or less. This ability to provide a test that is specific and sensitive has encouraged development of many such tests for *M. tuberculosis* and these tests would also be expected to detect *M. bovis*. But unfortunately, techniques from sputum are not adequate for extracting very small number of *M. bovis* organisms from tissue lesions. Until this problem is overcome, diagnostic tests for *M. bovis* based on PCR will not have the sensitivity of current culture methods²².

Conclusion

Limited specificity and sensitivity is still a major problem in the development of a suitable diagnostic test for tuberculosis. Mycobacterial antigens consisting of a few or no crossreacting components, are needed to differentiate between tuberculous and non-tuberculous infections. In the past, efforts have been made to isolate specific antigen present in *M. bovis* BCG and *M. bovis* AN5 culture filtrates^{15,23-25}. Unfortunately, the cross reactivity of all the antigen purified so far was unacceptably high¹².

Recombinant mycobacterial antigen produced in *Escherichia coli* appears to be of limited use as diagnostic agents, because they are not consistently recognized by infected animals. The potential benefits of PCR assay approaches will remain the subject of intensive investigation. Hence, to identify the critical antigen(s) for the diagnosis of bovine tuberculosis, a lot more work is needed to improve the specificity without undue loss of sensitivity.

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Received 26 June 1996; accepted 11 July 1996