SUMMARY AND CONCLUSIONS
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1. Peripheral T cell responses were studied in vitro in multibacillary leprosy patients vaccinated with *Mycobacterium w*, unimmunized lepromatous leprosy patients, tuberculoid patients and healthy contacts. The analysis was carried out with fractionated antigens of *M. w* using the technique of T cell blotting. The cells were also stimulated with PPD, intact *M. leprae* and *M. w*.

2. Except unimmunised LLs, all the subjects responded to a number of antigens of *M. w*. The responses were predominantly directed against low molecular weight components of 14-42KDa. There was no correlation between the clinical status and response to the antigens.

3. The *M. w*-vaccinated subjects were classified into three categories depending upon their lepromin conversion and disease status. These were lepromatous patients who remained lepromin negative following vaccination, lepromatous patients who converted to lepromin positivity following vaccination and borderline lepromatous patients. In the lepromin positive group the responses were predominantly against 26-42KDa, 49KDa and 57KDa fractions. The lepromin negative vaccinated patients mainly recognised antigens of 15-19KDa, 26-31KDa and 57KDa molecular weight while the BL patients showed responses predominantly against 28-31KDa and 39KDa.

4. In the tuberculoid group of patients, the in vitro proliferative responses were frequently directed against
16-42KDa and 49KDa.

5. PBMCs from healthy contacts recognised predominantly 14-16KDa, 19KDa and 24-36KDa antigens of M.w.

6. In the unimmunised group, except three LL patients one of whom recognised a 42KDa antigen, the second 28 & 33KDa fractions of M.w and the third a 22KDa fraction the rest 7 did not respond to any of the fractions.

There was no correlation between responsiveness to PPD, intact M.leprae and M.w, and M.w fractions.

7. A borderline tuberculoid patient who did not recognise M.leprae, M.w or PPD or any of the fractions, responded to several fractions of M.w after a single dose of the vaccine. The immunisation also restored responsiveness to PPD, M.leprae and M.w.

8. Although, the responses to the individual fractions varied considerably from one subject to another in all the groups, the antigenic fractions of 28K and 31K triggered T cell responses in 80% of the subjects. These two antigenic fractions ran as a single major band on SDS-PAGE.

9. B cell responses to this antigen(s) were studied in sera obtained from vaccinated individuals, unimmunised LLs, TT and apparently healthy subjects. Sera from all the categories studied responded to this antigen(s). However, only 5/10 sera from non-endemic areas obtained through WHO showed a weak B cell response to this antigen(s). The other 5 did not show any reactivity.
The T cell unresponsiveness to this antigen(s) in 9/10 LLs and B cell responsiveness in all the LL subjects further exemplifies the anergy seen at the level of T cell recognition in these patients. The strong antibody response in apparently healthy subjects could be attributed to prior exposure of these subjects to mycobacterial antigens since mycobacterial infections are endemic in India.

10. Interestingly even sera from pulmonary tuberculosis patients recognised this antigen(s) thus showing that this antigen shares epitopes with *M. tuberculosis* as well.

11. This antigen(s) was isolated from SDS-polyacrylamide gel by extraction with SDS for further characterisation. It reacted with Con A suggesting that it is a glycoprotein.

12. Polyclonal antibodies raised against this antigen(s) recognised sonicate antigens of *M.w, M.lepra* as well as *M.tuberculosis*.

13. Localisation studies using immunofluorescence and ELISA demonstrated that this antigen(s) was associated with the cell surface of *M.w*.

14. The polyclonal antibodies recognised 28-31KDa and 24KDa antigens in *M.w, 24KDa in M.lepra* sonicate and 24KDa and 26KDa antigens in *M.tuberculosis* in the western blot.

15. Guinea pigs immunised with this antigenic fraction
gave a DTH response to *M.w, M.leprae* as well as *M.tuberculosis*. The kinetics of this response suggests Jones-Mote type of response seen in mice.

These results demonstrate conclusively that 28-31KDa antigen(s) shares T and B cell determinants with *M.leprae* as well as *M.tuberculosis*.

This study shows that *M.w* contains a large number of T cell-stimulating antigens which trigger T cell responses in vitro in immunologically privileged TT and healthy contacts and following vaccination do so in otherwise unresponsive LL patients. There were a number of low molecular weight antigens which induced T cell responses in TT, healthy contacts and vaccinated patients. While it is possible that antigenic determinants recognised by the subjects from various groups were different, the study suggests an important role for these antigens in cell mediated immunity against leprosy.

The study also demonstrates the presence of an immunodominant 28-31KDa antigen(s) carrying T as well as B cell determinants which it shares with *M.leprae* as well as *M.tuberculosis*. This antigen(s) is associated with cell surface of *M.w* which is in conformity with its immunodominant character. This antigen(s) was recognised by T as well as B cells from immunised LLs, TT and healthy subjects while lepromatous leprosy patients only produced antibodies against this fraction. This suggests an important role for this antigen(s) in cell mediated immunity against leprosy.
immunity against *M. leprae*. The Jones-Mote type of reaction seen in guinea pigs immunised with this antigen(s), has been shown in mice to be partially mediated by IFN-γ. Therefore, it is possible that this antigen(s) might be activating/recruiting T cells which secrete IFN-γ. The latter has been shown to play a major role in mycobacterial immunity.

The study reports for the first time a detailed analysis on identification of T cell-stimulating antigens of candidate anti-leprosy vaccine, *Mycobacterium* and the results shown should help in characterising the antigens that are relevant to cell mediated immunity against leprosy.