Summary and conclusion
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Historically, tuberculosis (TB) has been a major cause of morbidity and mortality of humankind. The disease has become deadlier in recent times due to emergence of HIV/AIDS as well as drug resistant strains of the pathogen- *Mycobacterium tuberculosis*. In an endemic country like India, the entire population is considered exposed to *M. tuberculosis*. However, it is also a fact that only about 10% of the people infected with the pathogen actually progress to develop TB in their lifetime. The infection remains latent or dormant in the remaining 90% people. This study was an attempt to delineate the factors operating at the level of macrophage-mycobacterium interaction which could determine the final outcome of the ‘latent’ infection in apparently healthy persons living in a TB endemic region.

The exposure of the study subjects to *M. tuberculosis* was confirmed by determining their T and B cell responses against antigens present in cytosol and cell membrane of the bacillus. The donors could be categorized as ‘high’ and ‘low’ responders, based on the ability of their macrophages to either effectively contain an *ex vivo* infection with *M. tuberculosis* or allowing it to progress. An important aspect of this study was to determine if prior opsonization of *M. tuberculosis* with anti-mycobacterial antibodies present in heat-inactivated sera of the donors could influence bacterial clearance by the macrophages.

Using macrophages fed *ex vivo* with heat-killed, live and live-opsonized *M. tuberculosis*, we looked for possible correlations between a protective immune response (or lack of it) and any one or more of the following parameters: Production of cytokines, production of reactive nitrogen- and oxygen- intermediates, and events in intracellular trafficking following phagocytosis. In addition, we also sought a correlation between protection and the presence of anti-mycobacterial antibodies in the sera.

Among the cytokines which were studied TNF-α stood out most prominently as a marker for protective immunity against tuberculosis. Its levels in high responders were significantly raised compared to the low responders. A similar trend was observed with respect to IL-12 and IL-1 though the differences were not statistically significant. The
other two cytokines IL-4 and IL-6 did not show any trend. The serum antibody levels were found to be significantly raised in high responders compared to the low responders.

As for reactive nitrogen intermediates (measured in terms of nitrite levels), live bacilli produced large amounts of nitrite compared to the heat killed bacilli. However, there was no difference in nitrite levels of macrophages of high and low responders. The reactive oxygen intermediates measured in terms of \( \text{H}_2\text{O}_2 \) did not show any discernable trend.

Rab-5, LAMP-2 and Cathepsin-D were used as indicators of phagosome-lysosome fusion. With macrophages infected with live (un-opsonized) \textit{M. tuberculosis}, staining for all the three markers showed that the phago-lysosomal fusion was prevented. The Fc-\( \gamma \) receptor is used in phagocytosis of microbes which are opsonized with IgG type of antibodies. We found that live (un-opsonized) mycobacteria did not colocalize with Fc-\( \gamma \) receptors, whereas the opsonized bacilli did so. Further expression of Fc-\( \gamma \) receptor was found to be increased following phagocytosis of the opsonized bacilli.

Some interesting observations were made with respect to live and live-opsonized bacilli, which were used to infect the macrophages. In low responders, the multiplication of bacilli was reduced by prior opsonization, though such a reduction was not seen in high responders. Further, the microscopy data indicated that phagosome-lysosome fusion was enhanced by prior opsonization of the bacteria.

In conclusion, our results show that: (i) The healthy population of a TB endemic region can be categorized as 'high' and 'low' responders based on their ability to contain the intracellular (\textit{ex vivo}) infection with \textit{M. tuberculosis}. (ii) TNF-\( \alpha \) can be considered as an important indicator of protective immunity against tuberculosis. (iii) Presence of high levels of anti-mycobacterial antibodies in the sera can also be considered as a correlate of protective immunity. (iv) Opsonization of bacilli with antibodies prior to infection of the macrophages has some beneficial consequences, as reflected by faster clearance or containment of the phagocytosed bacilli.