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Mycobacteria and the host*

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Tuberculosis (TB) and leprosy, two diseases caused by a group of bacterium called Mycobacterium, have been with the Indian population for last several years. Leprosy has been viewed with great respect as a disease caused by Almighty's curse and thus not curable. This has changed significantly during 50 years and during the last 25 years concerted efforts by governmental and non-governmental agencies on survey, education, training and treatment have come to arrest the spread of the disease and contain the foci of the infection. Tuberculosis, on the other hand, has become much more serious and unlike leprosy which is not a killer even if untreated, tuberculosis kills the individual if left untreated. Such circumstances with TB will also lead to spread of the disease. We have increasing number of tuberculosis cases and it is not getting contained, in spite of the availability of effective chemotherapy, to cure the disease within six months. Treated and cured TB patients are in millions, since they were diagnosed and effectively subjected to multidrug chemotherapy. Nevertheless, debility and also mortality in untreated persons are seen in alarming numbers.

MYCOBACTERIA have been recognized as an important group, pathogenic to human, even though they also exist in non-pathogenic forms. Mycobacteria are gram-positive bacteria classified under Actinomycetales and family Mycobacteriaceae. There are several species so far identified under the genus *Mycobacterium*. A major problem faced by microbiologists in understanding mycobacteria, unlike other organisms like *Escherichia coli*, *Bacillus*, or yeast, has been lack of well-understood self-genetic manipulation and very slow growth of the organism *in vitro*. In some cases like *Mycobacterium leprae* there is no demonstrable growth *in vitro*.

The cell wall and membrane of the mycobacteria offer the major difference with other bacteria. The molecules constituting the envelope are responsible for acid-fastness (stainability), aggregation of cells, resistance to several drugs and lytic enzymes. The cell wall has complex lipoidal constitution. Besides the common components like Pthiocerol-dimycocersate, cord factor, sulpholipid, mycolic acid, arabinogalactan, peptidoglycan, there are special components in some species. *Mycobacterium leprae* has a unique phenolic glycolipid which is an immunodiagnostic antigen in the field use now. The significance of the lipoidal envelope becomes important when it is seen that macrophages of the immune system that are able to kill other genera of bacteria are unable to do so with some mycobacteria. This is important in the pathogenesis.

Among the species of mycobacteria that have become very relevant to human are *M. tuberculosis*, *M. leprae*,

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M. avium, *M. intracellulare*, *M. ulcerns*, all pathogenic. These mycobacteria are not fully understood with regard to their metabolism, host–pathogenic interaction, mechanism of infection and damage, resistance by host, etc. The biochemistry and metabolism of cultivable mycobacterial species like *M. smegmatis* do give us some lead information as reported by several groups including the group that was led by T. Ramakrishnan at Bangalore. But these data are inadequate to apply to the mycobacteria growing inside the human cells like *M. tuberculosis* and *M. leprae*. *M. tuberculosis* is stated to be capable of synthesizing its macromolecules, but *M. leprae* depends on the host for purines (nucleic acid precursor) and also other intermediates. However in the intracellular condition many mycobacteria derive their nutrients from the host cell. This is the first indication to the role of the host cell in pathogenesis. The slow growth of the pathogenic mycobacteria even in the host environment may be due to limitation in the permeability of nutrients, rate of metabolism including synthesis of DNA, RNA and proteins. There have been some evidences for this (see ref. 1).

Besides being identified as acid fast stainable organisms, mycobacteria have some interesting features. The guanine plus cytosine content of mycobacterial DNA is high, in the range of 60–67% among the gram positives. However *M. leprae*, the leprosy-causing organism, has a guanine + cytosine content of 56% only. Another interesting feature is that the general size of *M. tuberculosis* is similar to that of *E. coli* (2.5×10^8 Mr) compared to that of *M. leprae* (1.3–2.2×10^8 Mr). Thus within the genus one could see variation[2,3].

The intracellular mycobacteria which are found inside the host cell survive in the cells, like macrophages, Schwann cells, because they are not killed. The tolerance of the pathogenic mycobacteria like *M. leprae*, *M. tuberculosis*, lead to the pathogenesis. We do not have full information how the pathogens get into the cells and after entry how the pathogen paralyses the host cell machinery leading to tolerance. While the answers to these questions are not fully forthcoming now, we have experimental evidence to indicate what probably could happen in the case of *M. leprae*.

As an international effort there have been significant advances in classifying and characterizing mycobacterial species. This includes use of a variety of biochemical features, immunological and antigenic similarity, DNA homology as use of nucleic acid probes. Recently, using species-specific probes DNA homology is being determined between known species and newly isolated organisms. One such success has been the sequence analysis of 16S RNA molecule, which clearly showed that *M. leprae* is a mycobacterium. This has been further confirmed with DNA probe homology.

Chemotherapy

In any bacterial infection the first line of protection is the immune mechanism. But this takes place with a lag period and human efforts have identified chemical molecules that kill the pathogens faster and later host enzymes degrade them into chemical molecules. These are called antibiotics. These are now very efficient and least toxic drugs like sulphones (DDS), rifampicin, clofazamine, ethambutol, isoniazid, ofloxacin (quinolones), protheonamide, etc. These are used for chemotherapy of mycobacterial infections caused by *M. leprae* *M. tuberculosis* and *M. avium*. The patients can be made free of the bacteria by this treatment. The treatment period varies from two months to two years depending on the disease. The present concept is to use these antibiotics in combination as Multi Drug Therapy (MDT) to avoid development of resistance to the drugs by the bacteria. It is expected that this set of drugs with new quinolones recently introduced, should contain the infectious load of the *M. leprae* in the population in the case of leprosy as shown in Figure 1 (ref. 4). For the last several years tuberculosis has been successfully treated with a combination of drugs. However this disease is a serious health problem in this country, more serious than leprosy. More number of cases of tuberculosis patients are coming up. This has led the people concerned, to reorganize the anti-tuberculosis programme in recent years. The years of complacency against the mycobacterial diseases have been over.

The HIV-infected AIDS patients, have been expressing infections from secondary pathogens, which are opportunistic. In the immunity-impaired AIDS patients

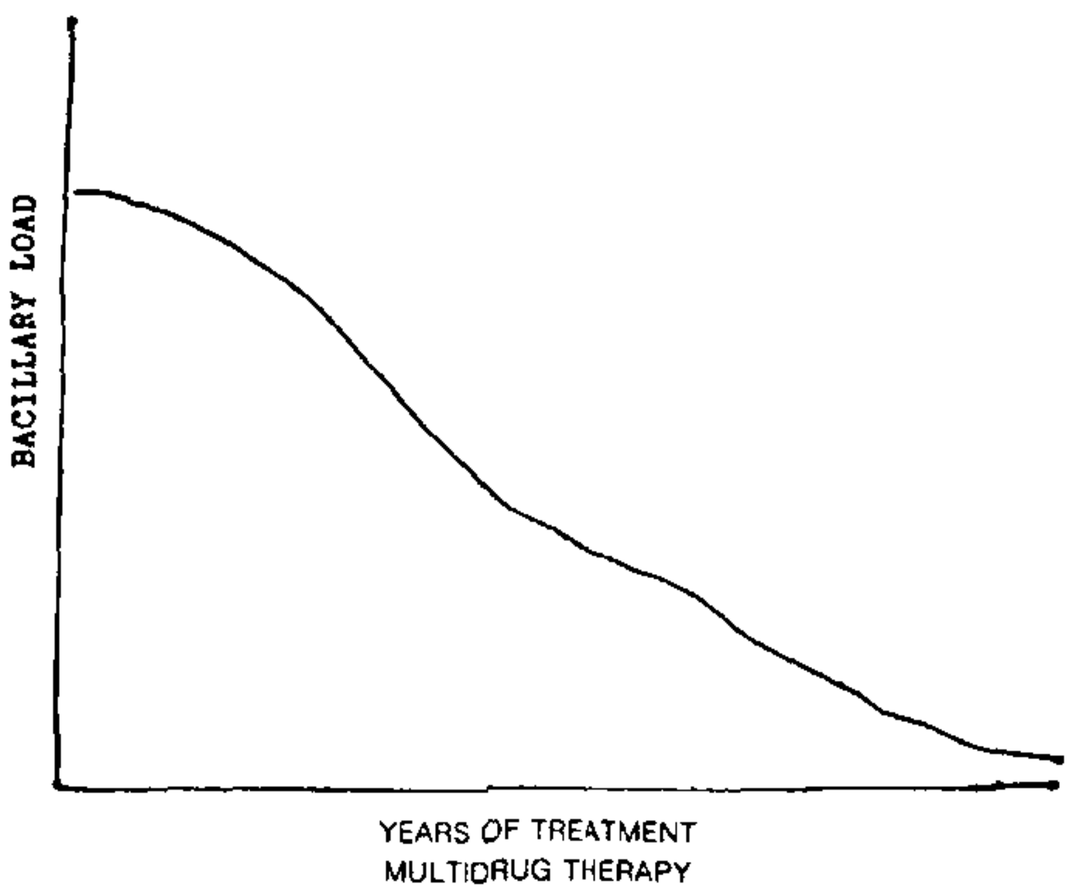

Figure 1. Effect of multidrug therapy on the viability of *M Leprae*

such pathogens find a suitable host. Among such AIDS patients *M. tuberculosis, M. avium, M. intracellulare* have become very important pathogens. This gives added importance in understanding the pathogenesis of mycobacterial infections, and with serious participation of the western world scientists, who otherwise were not concerned too much with leprosy or tuberculosis.

In spite of the poor advance on the microbiology and chemistry of the mycobacteria in general, there has been significant advancement with the molecular biology of the genome of mycobacteria through international efforts.

* We have genomic library of the organisms like *M. leprae, M. tuberculosis, M. avium, M. smegmatis,* etc.
* Genome sequencing of mycobacteria has been started and the entire sequence of the DNA that forms the genome is expected in five years (pers. commun. Dr Steven Cole, Paris).
* Several genes have been identified based on DNA sequence and predicted protein nature.
* Several antigens are being produced as recombinant proteins through various vectors. Some antigens have been predicted to be useful for immunodiagnosis and some as immunoprotective in tuberculosis and leprosy.
* Introduction of outside genes in a host cell like BCG has been perfected[5]. Some antigens from other mycobacteria have been introduced. This exercise could result in a BCG strain with some immunoprotective antigens against leprosy, tuberculosis, etc. Similar transfers of mycobacterial genes have been done into vaccinia virus[6]. In spite of all these advances we do not have the right molecule for immunodiagnostic nor the product for protection as vaccine as of now[7].

Immunity and pathogenesis

In the case of viral pathogens, protective immunity largely operates through the humoral mechanism, by participation of B lymphocytes of the peripheral blood cells to produce specific antibodies to neutralize the relevant antigens of the virus. This also includes a 'recall' memory component to protect against future infection by the same pathogen. This is an ideal system. But there are viral agents that do not behave exactly the same way and the latest and most disturbing are the HIV type of viruses.

On the other hand in the mycobacterial infection, they are not challenged by the humoral antibody participation, but by cell-to-cell co-operation called cell-mediated immunity—by participation of T lymphocytes of the type T_4, T_8 and recently helper cells of TH_1 and TH_2 types, each cell type being responsible in directing a specific pathway in the cell-mediated immune function (Figure 2). In normal healthy individuals

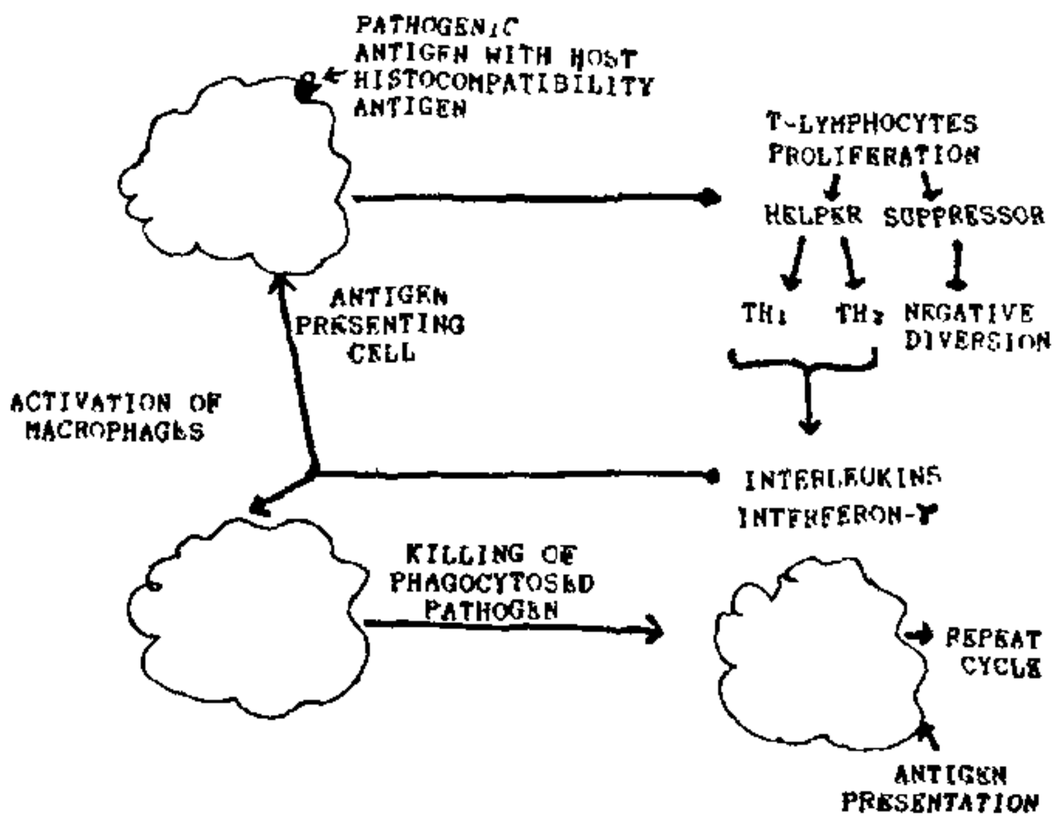

Figure 2. Basic steps in cell-mediated immunity.

invading organisms on contact with phagocytes and neutrophils, which are the first time line of cells in the immune system, are challenged by reactive oxygen species intermediates like hydrogen peroxide (H_2O_2), superoxide (O_2-), hydroxyl radical ($OH\cdot$) and now recently nitric oxide. These are in turn able to inactivate the invading bacteria, during and after phagocytosis. There is some uncertainty whether such an event happens with mycobacteria especially with *M. tuberculosis.* Table 1 shows that macrophages from normal healthy persons are able to inactivate to good extent the leprosy bacteria, but cells from leprosy patients were not able to do so.

This clearly indicated that in the human host, there are those who can kill a mycobacterium like leprosy bacillus; before using the components for effective cell-mediated immunity. Thus the individuals who are unable to kill the bacillus in their macrophages are unable to process them and present the antigens that are to initiate cell-mediated immunity. The data obtained from our laboratory illustrate the probable phenomena and initial steps leading to the pathogenesis in those individuals who are unable to kill the bacteria and initiate good cell-mediated immunity (Figure 3)[8].

Autoimmunity/microbial immunity

Recent identification of several proteins of human pathogens as belonging to the group called heat shock proteins and the remarkable homology regions (conserved regions) in the amino acids of mycobacterial proteins and human host cell proteins, has raised another aspect of host response, namely autoimmunity. The autoimmune phenomenon is basically a response

Table 1. Correlation between reactive oxygen intermediates and loss of viability of *M. leprae* inside the macrophages of various types of individuals

	B(+)LI (Bacteriologically positive)	B(−)II (Bacteriologically negative)	Tuberculoid leprosy	Normal resistant
SOD removable superoxide (nmol/h/10^6 cells)	0.3± 0.3	0.3± 0.2	0.2± 0.3	2.2± 0.5
Hydrogen peroxide (nmol/h/10^6 cells)	25.0± 1.5	89.0± 2.6	62.9±10.1	112.2±10.5
Original smear viability (% viability of *M. leprae* in suspension)	53.0± 9.7	48.0±16.3	55.5± 9.0	56.2±10.6*
Per cent viability of *M. leprae* inside the macrophage	62.0±16.6	50.5± 9.6	59.2± 9.6	21.0± 8.5*

All values are mean of five separate experiments.
*$P < 0.001$.

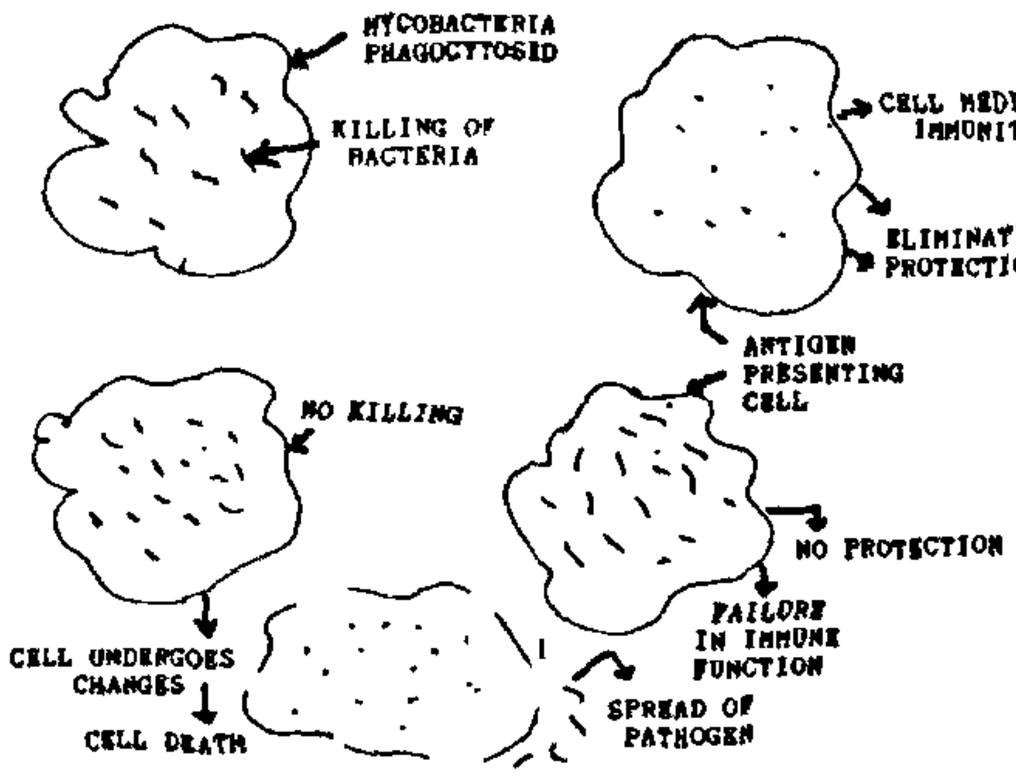

Figure 3. Conclusion based on our results.

of the host immune system to its own antigenic proteins. However when the mycobacterial 65 kDa protein with conserved sequence having homology to some human proteins is released in the host during mycobacterial infection—the host could see the conserved epitopes as one of its own and produce antibodies. This would result in these antibodies reacting to the specific host proteins leading to *autoimmunity*[9,10].

How can microbial immunity be expressed under this condition? This becomes complicated. Cohen and Young[10] had suggested a regulated non-aggressive autoimmune response more appropriate to the self than *M. leprae* might occur in lepromatus leprosy patients. Typical protective immunity in response to microbial epitope may be absent in this type of leprosy.

Is there a common mechanism?

The situation as we see in leprosy does apply to tuberculosis also. The answer is not simple and probably difficult to obtain. The course of pathogenesis, the location of tubercle bacilli in the soft tissue like lungs, and the faster spread of the pathogenesis compared to leprosy, perhaps indicate that susceptibility could be due to a temporary derangement in the immune competence. In that event *M. tuberculosis* gets a foot hold and by the time the body gets ready to eliminate the *infection, the process of damage and effect* on general health lead to very slow recovery or worsening, if not effectively interfered with antitubercular drugs and chemotherapy[11]. Could this be due to the fact that *M. tuberculosis infection in the normal population is also* opportunistic like the situation in an AIDS patient? If so the responsibility of the host to protect oneself from the infection is within the realm of management[12]. In leprosy the situation appears to be different.

However in both the situations the host–pathogen interaction plays a very important role[13]. We have to understand this phenomenon: 1. We should know how the normal resistance manifests in mycobacterial infection. 2. Are these defective in susceptible individuals? 3. How does the host respond *in* molecular terms while allowing expression of *the* disease? 4. What happens to the infecting organisms? 5. How to interfere during infection to stop the progress of the disease? 6. How can we manipulate 'cure' through immunological terms as immunotherapy vaccines (as in leprosy); 7. Is immunoprophylaxis possible? 8. Is susceptibility a hereditary phenomenon?–there appears to be some evidence in mice for BCG infection[14].

We need answers to these and we are still far from getting full information on these in relation to

mycobacterial infections and mycobacteria[15]. This is a challenge for combined well planned scientific efforts of microbiologists, molecular biologists and immunologists of the country.

Having raised the above questions it would be only fair to take the liberty of suggesting some guidelines for future research in India. It is essential to have rapid reliable sero-diagnostic systems available for nonpulmonary tuberculosis and for very early detection of leprosy. Such diagnostic techniques are to be integrated and used by routine pathology laboratories, private and public.

At basic level research efforts should be directed to understand the host–pathogen interaction at molecular level in both diseases, so that tools in the form of antigens/proteins could be screened for immunomodulation leading to protection—in common terms a vaccine— with for immunotherapy and immunoprotection. Such agents should have the potential to activate host cells to initiate effective cell-mediated immunity in spite of metabolic changes introduced by the presence of the pathogen. These agents should also divert the pathway of immune reaction away from self-destructive cytotoxic effects. In achieving these objectives the questions raised in the earlier portion of the text may form working norms.

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