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DR NITYA ANAND ENDOWMENT LECTURE, 1987

Immunological Unresponsiveness and its Reversal in Lepromatous Leprosy

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I am honoured to have been awarded the first Dr Nitya Anand Endowment Lecture by INSA. I first met Dr Nitya Anand when he chaired the discussions on the future strategies for research in Leprosy as part of the Eradication of Leprosy Programme initiated by Dr S Swaminathan Committee. My experiences during the meetings gave insight into the leadership quality of Dr Nitya Anand and his ability to encourage young persons like me to express our views.

The studies I plan to describe synthesise some of our observations on the immunological features of leprosy as a natural response in man to exposure to the pathogen. They do not totally cover our studies of a decade and a half, but focus on these aspects which help us to draw some conclusions in continuum.

Leprosy is a unique infectious disease caused by the incultivable organism *Mycobacterium leprae*. Quarter of the world's population of leprosy patients live in ·ia. The leprosy bacillus has a prediliction for skin ıerves, resides within macrophages and Schwann and leads to a clinicopathological spectrum in ı. Over the last decade and a half, it has been well ıblished that the leprosy spectrum is due to the ·erse nature of the host immune responses to *leprae*. At one pole of the spectrum lies tuberculoid)sy (TT), which consists of a well circumscribed ı, distinct from the surrounding healthy skin but ·ng the neighbouring nerve. Such lesions show of bacilli indicating thereby the ability of host nt a healthy immune response capable of g the pathogen by granulomatous ation. In contrast, the other end of the pole is ıcted by lepromatous leprosy (LL) wherein the ent shows disseminated multibacillary lesions tered throughout the body and recognised microscopically by abundant collections of macrophages filled with leprae bacilli and lacking the accompanying lymphocytes seen in TT leprosy. Such individuals are difficult to cure and are a source of infection to the community. Between these two poles lie three clinical types of borderline leprosy which represent an unstable form of the disease. Depending on their clinical proximity to the polar forms described above, they are variously designated as Borderline Tuberculoid (BT), Borderline Borderline (BB) and Borderline Lepromatous (BL) leprosy.

Immunological Features of Leprosy

It is well established that humoral immunity mediated by antibodies and cellular immunity affected by thymus-processed T-lymphocytes are the major limbs by which immunological reactions lead to protection or pathological reactions to foreign antigens. Since antibodies do not penetrate living cells, pathogens such as *M. leprae* evade humoral immunity by their location within phagocytic cells. Tables 1 & 2 show the T and B cell functions in leprosy reported by us and others. TT patients have paucity of antibodies but mount good cellular immunity and are able to contain the spread of bacilli. LL patients have abundant general mycobacterial and specific anti *M. leprae* antibodies and are yet unable to destroy the bacilli.

They lack cellular responses to *M. leprae* as indicated by unresponsiveness to the specific antigens both by *in vivo* skin tests and by *in vitro* lymphocyte function tests. Interestingly, these patients respond to other cross-reacting mycobacterial antigens. Indian LL patients during active disease show low general T cell functions in terms of T cell numbers and their responses to T cell mitogens. On treatment and

Table 1 *Immunological features of Leprosy*

	Tuber-culoid	Lepro-matous
T Cells:		
Numbers		
E rosettes	—›	
CD 3+	—›	
Functions		
Skin Tests:		
KLH, DNCB	—›	
PPD	—›	
M. leprae	—›	
Lymphoproliferation:		
Mitogens	—›	
Cross reacting ags	—›	
M. leprae	—›	
Leucocyte migration Inhibition:		
H 37 RA	—›	
M. leprae	—›	
IL 2 Production:		
Mitogens	—›	
PPD	—›	
M. leprae.	—›	

Antigen specific T cell anergy is longlasting. Depression in general T cell function is secondary and reversible

Table 2 *Immunological features of Leprosy*

	Tuber-culoid	Lepro-matous
HUMORAL		
B cell nos.	—›	↑
Serum Ig	—›	↑
Mycobacterial abs:		
Other mycobacteria	—	↑
65Kd	—	↑
Arabinomannan	—	↑
M. leprae specific abs:		
FLAbs	—	↑
Phenolic glycolipid	—	↑
MLO 4, 6	—	↑

Decrease with treatment and drop in bacillary load.
Secondary to disease.
Not protective in nature.
May have a diagnostic role

decrease in bacillary load these defects are corrected (Nath et al. 1974, 1975).

That T cell mediated cellular immunity is of protective value has been clearly demonstrated in Leprosy. With a view to understanding the natural immune response to *M. Leprae* our recent studies have centered around the characterisation of T cell lines and T cell clones in individuals who respond effectively to the leprosy bacillus. We have been able to enrich for leprae reactive T cells by developing long term T cell lines and cloning to homogeneity some of these T cells. All the clones we have developed to date bear the $CD4_+$ (helper/inducer) phenotype. Some of them produce the T cell growth factor (IL–2) and the lymphokine interferon gamma (INF–γ) thought to be an inducer of class II MHC antigens Ia and to have the ability to activate macrophages (Nathan et al. 1984). In terms of antigen specificity the T cell clones and lines fall into three groups: (i) crossreactive proliferative responses to 10–15 mycobacterial species and unrelated tetanus toxoid; (ii) with restricted responses to 3–4 mycobacteria; and (iii) with responses to *M. leprae* and not to other mycobacteria. All the lines/clones required exogenous IL-2 for maintenance. Moreover, the time kinetics of IL-2 and INF-γ varied not only within the same clone but also in response to various mycobacteria. We have not as yet produced T cell lines/clones from lepromatous patients.

Mechanisms underlying Uresponsiveness in Lepromatous Leprosy

The predominant antigen specific anergy observed in LL patients is the most intriguing feature of leprosy. Whereas specific antibodies to the leprosy bacillus are abundant, the T cells are unable to respond to the same pathogen. The mechanisms underlying this defect have been difficult to detect since, ethical considerations limit the scope of investigation in man and there is no suitable animal model. Our studies have therefore been limited to the peripheral blood or skin lesions.

Anergy or lack of responsiveness to an antigen may be due to various factors, such as (i) genetic predisposition, (ii) active suppression due to cells or soluble factors, (iii) lack of antigen reactive cells due to clonal deletion, (iv) defects in antigen presentation, and (v) reduction in growth factors. We explored in-depth the role of active suppression and the growth factors in patients with leprosy.

Immunological Suppression in Leprosy

Suppressor T-Cells

Subsets of T cells with phenotypic characteristics play an immunoregulatory role by exerting positive help promote an immune response or by supressing ongoing response. In disease states both these functio may play a pathological role. The role if any of

suppressor T cells in inhibiting the responses in lepromatous leprosy was investigated by us using various methodologies. Peripheral blood T cells from leprosy patients, were induced to generate suppression by both a general mitogen Con A and the specific antigen *M. leprae* (Nath et al. 1979, 1980). It was consistently shown that suppressor T cell activity though detectable in tuberculoid leprosy was absent or low in lepromatous leprosy. It appeared that during the natural course of the disease, exposure to *M. leprae* induced suppressor T cells in individuals with good T cell responses, as part of a general T cell reactivity. The lack of these cells as seen in LL patients may explain the lack of control leading thereby to overproduction of antibodies and auto-antibodies. We further confirmed these results using HLA-D compatible siblings from the Wardha area which had been typified for HLA haplotypes by Drs N K Mehra, M C Vaidya from AIIMS and Drs JJ van Rood and R R DeVries from Leiden. T cells from LL patients were mixed with PBMC from his genetically matched tuberculoid or normal siblings. Such cocultures were stimulated with antigen to study the effect on lymphoproliferation. These studies indicated unequivocally that LL patients did not have suppressive T cells in circulation (Nath et al. 1980). Recent studies from our laboratory showed that phenolic glycolipid I considered to be a unique antigen of *M. leprae* (Hunter et al. 1982) when incorporated in the form of liposomes generated general suppression of lymphoproliferation *in vitro* (Prasad et al. 1987). This suppression was observed in both tuberculoid and lepromatous patients. We concluded that suppression by T cells was not the major mechanism underlying anergy in Lepromatous Leprosy. Furthermore, the proposed role of PGL-1 as a unique suppresor epitope (Mehra et al. 1984) appeared unlikely to be the central mechanism responsible for the unresponsiveness as it showed a general suppressor role and did not explain the differential features of the two poles of the leprosy spectrum.

Supressor adherent cells

Using HLA compatible siblings, we observed that monocyte-rich adherent cells of LL patients inhibited antigen-induced T cell proliferation of tuberculoid patients and healthy responder individuals (Nath et al. 1980). Such inhibition was mediated by soluble factors released de novo into the culture medium by monocytes of untreated LL and not by cells from tuberculoid patients. The de novo factors were observed to be decreased in treated patients, but could be induced on exposure of monocytes to antigen (Sathish et al. 1983). Interestingly, *M. leprae* induced maximal suppression in comparison to 5 other mycobacteria. Morever, addition of such factors to lymphocytes stimulated with various mycobacteria also showed maximal inhibition of *M. leprae* related lymphoproliferation. Further characterisation on HPLC and by radioimmunoassay indicated that arachadonic acid metabolites, PGE_2, leukotriences and thromboxane were present in greater amounts in suppressor factors from LL individuals and in low or nondetectable amounts in healthy and tuberculoid individuals. Interestingly, these factors inhibited the production of T cell growth factor — IL2. (Nath et al. 1984). It had no effect on its utilisation. Though these factors are currently being further characterised, we are inclined to believe that monocytes from lepromatous patients during contact with *M. leprae*, produce multiple factors which may play a role in suppressing T cell responses by inhibiting the production of T cell growth factors. We are currently investigating whether T cells play a role in influencing the monocytes in this function in order to explain the antigen induced suppresion observed in leprosy.

The Status of T Cell Growth factors in Leprosy

Concurrently with our studies on suppression, efforts were made to evaluate the defect if any in the production of T cell growth factors. It is now well established that monocytes/macrophages on contact with antigen release interleukin 1 (IL-1) which in turn helps the T cells to produce interleukin 2 (IL-2) which is required for the clonal proliferation of T cells. In our hands, both tuberculoid and lepromatous patients showed normal ability to produce IL-1 on stimulation with the general mitogen PMA and the specific antigen *M. leprae*. However, IL-2 production by T cells of LL patients was low to absent (Nath 1986). Though these individuals produced IL-2 to other antigens such as PPD or to T cell mitogens, they were unable to do so when stimulated with the specific antigen. It is possible from our earlier data on monocyte suppression that the peripheral blood cells being a mixture of monocytes, T cells and B cells would show defect in IL-2 production due to concurrent inhibition by monocyte factors.

Reversal of T Cell Anergy in Lepromatous Leprosy

Since the above studies implied that LL patients may have T cells which were suppressed by monocyte factors and which failed to clonally expand due to IL-2 defect, studies were undertaken to reverse the T cell anergy *in vitro* and *in vivo*.

In vitro modulation of T Cell Responses

Role of monocytes

Adherent cells from tuberculoid or healthy responder individuals were cocultured with HLA-compatible nylon wool purified T cells from LL siblings in the presence of leprae antigens. Five such sibling pairs showed efficient lymphoproliferation indicating the presence of antigen-reactive T cells in these lepromatous patients (Nath et al).

Role of autologous dendritic cells

Since lepromatous individuals possess suppressive monocytes and transfer of monocytes from tuberculoid patients would not be operationally feasible as a therapeutic measure, we explored the possibility of replacing monocytes with other accessory cells capable of presenting antigen to T cells (earlier studies had shown and it is well established that T cells only see antigen processed by accessory cells and in the context of MHC Class II antigens). Peripheral blood of man contain 1% of Dendritic Cells (DC) which are rich in MHC class II antigens and which can reconstitute lymphoproliferative responses in the absence of monocytes (Mittal & Nath 1987). DC were obtained from 15 LL patients and reconstituted in concentrations varying from 0.1 to 10% with purified autologous T cells and *M. leprae*. Interestingly, significant lymphoproliferation was observed in 9 patients. More importantly, 14/15 patients showed production of INF-γ, a macrophage activating lymphokine (Mittal et al. 1988)

Role of IL-2

Since our above studies had shown a defect in IL-2 production, we sought to reverse this by addition of exogenous IL-2 to *M. leprae* stimulated peripheral blood lymphocytes of 41 LL patients. IL-2 was obtained from 3 sources (i) mitogen induced JR-4 cells (ii) constitutively released IL2 from the gibbon cell line MLA; and (iii) recombinant IL-2 obtained commercially. In general 60–65% of LL individuals showed varying levels of improvement in antigen induced lymphoproliferation. (Nath et al. 1984).

These investigations provide proof that modulation of cell interactions and provision of exogenous T cell growth factors can reverse the well documented antigen specific T cell defect *in vitro*. It would appear that many LL patients possess antigen reactive T cells with IL-2 receptors which can be triggerred to clonal expansion by IL-2. That DC constituted T cells could release INF-γ would suggest that T cells capable of microbicidal activity are present in many LL patients.

In vivo Emergence of Antigen Reactive T Cells

Leprosy reactions

Some BL and LL patients suffer from acute episodic reactional states called erythema nodosum leprosum (ENL) which are characterised by dermal nodules and systemic manifestations of fever, neuritis and arthralgia. We undertook investigation of such patients both for (i) T cell functions in peripheral blood, and (ii) phenotypic characteristics of cells in skin lesions.

Surprisingly, the hitherto anergic LL patients during ENL reactions showed enhanced T cell functions in terms of lymphoproliferation and lymphokine production (Laal et al 1985). Moreover, the usual lymphopenic lesions showed entrance of $CD4_+$ helper inducer T cells in significant numbers (Narayanan et al. 1984). Such cells were seen in the dermis and epidermis. That these cells were in a functional state was indicated by the appearance of Ia or MHC Class II antigens on the previously negative keratinocytes. Since antigen presentation to T cells is linked to the presence of MHC Class II antigens and they can be induced by INF-γ, we conclude that $CD4_+$ T cells entering ENL lesions is indicative of release of INF-γ in the local site (Thangaraj et al. 1988).

These studies confirm the natural emergence of antigen reactive T cells into the peripheral blood and lesions of lepromatous patients. Their presence becomes discernible during ENL reactions which we feel represent a state of T cell perturbation. The factors that trigger their emergence and traffic into lesions are not clear. That it is of importance to identify these events is evident from the fact that ENL lesions show fragmentation of bacilli and indicate that LL patients are capable of killing *M. leprae* given the right stimulus.

In vivo Injection of PPD

With a view to further understanding whether the lack of $CD4_+$ helper/inducer T cells in LL lesions were due to a general defect in emigration and accummulation or represented specific unresponsiveness, we generated tuberculin reactions by injecting PPD into lesions. In a collaborative study with Drs G Kaplan and Z A Cohn of the Rockefeller University, the epidermis and dermis were studied for phenotypic markers of the various cells using monoclonal antibodies. Similar to the ENL lesions described above we noted that $CD4_+$ cells entered LL lesions, keratinocytes expressed MHC Class II antigens and showed increased layers. These results indicated once more that LL lesions were permissive to the entry of helper/inducer T cells, and that these cells released lymphokines such as INF-γ and epidermal growth factors (Kaplan et al. 1986, 1987).

Thus, both the *in vitro* and *in vivo* studies indicate that many of the hitherto anergic lepromatous patients would be amenable to immunological modulation whereby the antigen specific unresponsiveness may be reversed. One consistent feature that emerged was that approximately 1/3 of the individuals showed little responsiveness under similar conditions. Strategies to promote the total LL population to show improvement in immunological reactions leading to the killing of intracellular *M. leprae* are being currently explored by us.

Showing foresight and determination, India has introduced multidrug regimen on a large scale to

control leprosy. Current reports indicate that though these drugs have a significant impact in clearing bacilli from the majority of multibacillary patients, yet a small foci of individuals continue to harbour *M. leprae* for long periods. Apart from individual morbidity, such foci pose a threat of infection to the community at large. Immunological modulation may be required as an adjunct to chemotherapy in these patients. Moreover, immunotherapy may reduce the length of treatment required with drugs. We hope that studies such as above would contribute strategies for the better control of not only leprosy but other infectious diseases where the intracellular location of pathogens makes eradication difficult.

Thus a decade and a half of careful dissection of the leprosy engima has given us some answers but has not provided total consensus. This enticing disease bekons us to explore further the mystery that is Leprosy.

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