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Impairment of alternate pathway (CD2) of T cell activation in leprosy

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Abstract. Recent studies in basic immunology have been directed towards the understanding of the mechanism of T cell activation. T cells can be activated to proliferate *via* the classical pathway through the antigen receptor (CD3-Ti) or *via* the alternate pathway through the CD2 receptor. Since immunologic unresponsiveness in lepromatous leprosy is considered to be due to the inability of T cells to proliferate upon stimulation, we have been interested in the nature of these receptors and the activation pathways in lymphocytes of leprosy patients. In the present investigation we demonstrate: (i) CD2 receptor (E-receptor) is downregulated in bacterial index positive lepromatous leprosy patients. (ii) The alternate pathway of T cell activation is impaired in lepromatous patients as revealed by the inability of their lymphocytes to proliferate in response to a pair of mitogenic anti-CD2 monoclonals. (iii) The addition of recombinant interleukin 2 *in vitro* restores the ability of lymphocytes from lepromatous patients to proliferate in response to anti-CD2 antibodies. (iv) Interestingly, CD2 modulation and the associated functional impairment could be brought about in peripheral blood lymphocytes from normal subjects by prior treatment with *Mycobacterium leprae in vitro.* This approach would be useful in understanding the molecular events leading to the defective T cell functions in leprosy.

Keywords. Leprosy; CD2; CD3; IL2; *Mycobacterium leprae.*

Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae.* One population of leprosy patients, referred to as lepromatous type (LL), is known to have reduced T cell functions as evidenced by *in vitro* and *in vivo* tests (Nath *et al.,* 1977; Godal, 1978). Though several approaches (Bloom and Mehra, 1984; Kaplan and Cohn, 1985; Nath *et al.,* 1984) have been used in attempts to understand the mechanism of immunologic unresponsiveness in lepromatous leprosy (LL) patients, none has yet gained wide acceptance.

Earlier studies indicated that the number of T cells, as enumerated by sheep erythrocyte-rosette forming cell (E-RFC) assay (Dwyer *et al.,* 1973; Nath *et al.,* 1977; Jaswaney *et al.,* 1980), was reduced in LL patients· Subsequently, when monoclonal antibody to a pan-T-cell marker (CD3) was used with the immunofluorescence technique, the proportion of T cells was found to be normal (Bach *et al.,* 1981; Mshana *et al.,* 1982). We have recently repeated these experiments and found that, while the proportion of CD3-positive cells was normal, E-receptor (CD2) positive cells were significantly reduced in LL patients (Muthukkaruppan *et al.,* 1987). On the basis of our findings in conjunction with the role of CD2 receptor in T cell

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Abbreviations used: LL, Lepromatous leprosy; E-RFC, erythrocyte-rosette forming cell; LLBI + , bacillary index positive LL; IL2, interleukin 2; LLBI –, bacillary index negative LL; PBL, peripheral blood lymphocytes; rIL2, recombinant IL2; LFA3, lymphocyte function associated antigen 3.

biology (Springer *et al.,* 1987), we have proposed a hypothesis to explain the mechanism of immunologic unresponsiveness in leprosy (Muthukkaruppan, 1986).

Our earlier studies (Muthukkaruppan *et al.,* 1987) have clearly demonstrated that (i) the expression of CD2 receptor, but not that of CD3, was downregulated in bacillary index positive LL (LLBI +) patients, (ii) this downregulation was strongly associated with reduced proliferative response to mitogens and antigens, and (iii) CD2 modulation and the associated immunosuppression could be brought about even in lymphocytes from normal subjects by prior incubation with *M. leprae* (Dharmendra lepromin) *in vitro.*

CD2 receptor is expressed on all T cells (Kamoun *et al.,* 1981). Certain monoclonal antibodies to $T11_1$ epitope of'CD2 receptor were shown to downregulate T cell proliferation by blocking the production of interleukin 2(IL2) (Palacios and Martinez-Maza, 1982; Tadmori *et al.,* 1985; Gromo *et al.,* 1987). On the other hand, pairwise combinations of anti-CD2 monoclonal antibodies to certain other epitopes ($T11_2$ and $T11_3$, or $T11_1$ and D66) can induce T cells to proliferate (Meuer *et al.,* 1984; Brottier *et al.,* 1985). CD2 receptor also helps in thymocyte-thymic epithelial interaction (Singer and Haynes, 1987). These findings suggest that CD2 can function in activation signalling (alternate pathway of T cell activation) as well as in cell-to-cell adhesion.

The present study was directed at understanding the nature of the alternate pathway of T cell activation in leprosy. Our data indicate that the alternate pathway of T cell activation is impaired in lymphocytes of LL patients as a result of CD2 modulation by *M. leprae* and this impairment in proliferation is due to a block in IL2 secretion.

Materials and methods

Patients

A total of 19 lepromatous and 8 tuberculoid leprosy patients who were attending Arogya Agam, Leprosy Hospital, Andipatty, were studied. These patients were graded by Ridley-Jopling classification (Ridley and Jopling, 1966) on the basis of bacillary smears and clinical symptoms. The LL patients were subdivided into LLBI + *i.e.* those who demonstrated acid-fast bacilli in slit-skin smears, and bacillary negative (LLBI–) patients. Most of the patients were undergoing multi-drug therapy. Control subjects were healthy volunteers from the university campus.

Mycobacterial preparations

Dharmendra lepromin was kindly provided by Dr U. Sengupta, Central JALMA Institute for Leprosy, Agra. Bacille Calmette-Guerin (BCG) was obtained from BCG Vaccine Laboratories, Madras, and subjected to treatment similar to the preparation of Dharmendra lepromin (Sengupta *et al.,* 1978).

Indirect immunofluorescence assay

The percentages of CD2+ and CD3+ cells were enumerated as already described

(Muthukkaruppan *et al.*, 1987). To 1×10^6 peripheral blood lymphocytes (PBL) in 100 μl of medium monoclonal antibodies OKT3 (for CD3) and OKT11 (for CD2) (Orthodiagnostic Inc., USA) were added and the cell suspensions incubated at 4°C for 30 min with frequent mixing. After washing the cells in RPMI 1640 (Sigma), 50 μl of goat anti-mouse IgG FITC conjugate (Coulter Electronics) were added and the tubes were incubated at 4°C for 30 min. The percentage of fluorescence-positive cells was determined by phase contrast/fluorescence microscopy, counting 200 cells per tube. Ascites fluid (BRL) was used as control for monoclonal antibodies.

To study the effect of *M. leprae* on the modulation of CD2, 1×10^6 PBL from healthy non-contacts were incubated at 37°C for 24 h with Dharmendra lepromin containing 3×10^5 bacilli. As a control, PBL from the same individuals were treated with the same number of BCG separately. After this treatment cells were washed and stained for the detection of CD3 and CD2 receptors.

Lymphocyte transformation test

Triplicate cultures of PBL from leprosy patients were prepared in flat-bottom 96-well culture plates (Greiner, FRG) and cells were stimulated with optimal concentration of phytohaemoagglutinin-M (Bacto, USA) or monoclonal antibodies $T11_1$ and D66 (a kind gift from Dr Alain Bernard, France) either alone or together as previously described (Brottier *et al.*, 1985). In another set of cultures, PBL from healthy non-contacts were pretreated with *M. leprae* or BCG (1×10^5 bacilli per well at 37°C for 24 h) before adding mitogen or antibodies.

Addition of recombinant IL2

Recombinant IL2 (rIL2), a kind gift of Dr Sinigaglia, Hoffman La Roche, Basel, Switzerland, was added at 50 units per ml to cultures, wherever mentioned.

Statistics

Statistical comparisons were made by Student's *t* test.

Results

Alternate pathway of T cell activation in leprosy

We have used a pair of mitogenic anti-CD2 monoclonal antibodies ($T11_1$ and D66) to elucidate the proliferative response of lymphocytes *via* the alternate pathway of T cell activation (figure 1). PBL from healthy non-contacts exhibited a good proliferative response. However, only a minimal response (SI 7·7±2·8) was observed in LLBI + patients, in contrast to the response in borderline tuberculoid and LLBI– patients. Comparable results were obtained when PHA-M was used as a stimulant. Indirect immunofluorescence assay with PBL from the above subjects revealed a significant reduction in the proportion of CD2-positive cells only in LLBI+ patients (figure 1). These results indicate that the alternate pathway of T cell activation is impaired in LLBI+ patients and that this is correlated with the modulation of CD2 receptor.

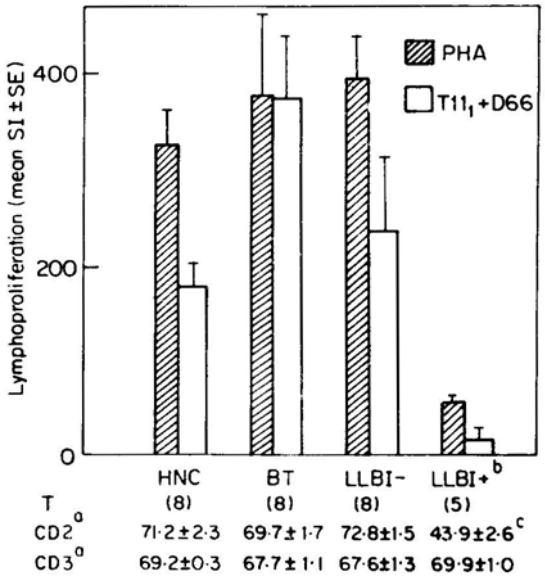

Figure 1. Impairment of alternate pathway of T cell proliferation in leprosy in correlation with CD2 modulation. Figures in parentheses below the histograms are numbers of individuals studied. HNC, Healthy non-contacts; BT, borderline tuberculoid. [a]Percentages of immunofluorescence-positive cells ± SE; [b]P <0·001 between stimulation indices (SI) of LLBI + (BI ranging from 0·4–6) and LLBI– patients; [c]P<0·001 between CD2-positive and CD3-positive cells.

Effect of M. leprae on alternate pathway of T cell activation in PBL of healthy non-contacts

The reduction in the proportion of CD2-positive cells was found in LLBI+ patients, *i.e.* in persons harbouring *M. leprae in vivo.* We therefore wanted to determine the effect of *M. leprae* on PBL of healthy non-contacts. For this purpose we have developed an *in vitro* test in which PBL from healthy non-contacts were treated with *M. leprae* or BCG and the expression of CD2 and CD3 receptors was analysed. As shown in table 1, *M. leprae,* but not BCG, significantly modulated the

Table 1. Percentage of CD2-positive and CD3-positive cells in normal PBL treated with *M. leprae* or BCG·

	Immunofluorescence-positive cells[b] (%)	
Treatment[a]	CD2	CD3
No treatment	71·44 ± 1·80	69·34 ± 1·50
M. leprae	38·18 ± 4·53[c]	67·28 ± 1·94
BCG	70·16 ± 3·28	70·70 ± 3·05

[a]Number of subjects 5 in each group.
[b]Cells were stained with monoclonal antibodies OKT11 for CD2 and OKT3 for CD3. The data are mean ± SE.
[c]P< 0·001 between untreated and *M. leprae* treated groups in CD2-positive cells.

expression of the CD2 receptor, while the percentage of CD3-positive cells remained unaltered. In parallel experiments, PBL from the healthy subjects were incubated with *M. leprae* or BCG for 24h and then stimulated with a pair of anti-CD2 antibodies (figure 2). The data clearly indicate impairment of the alternate pathway

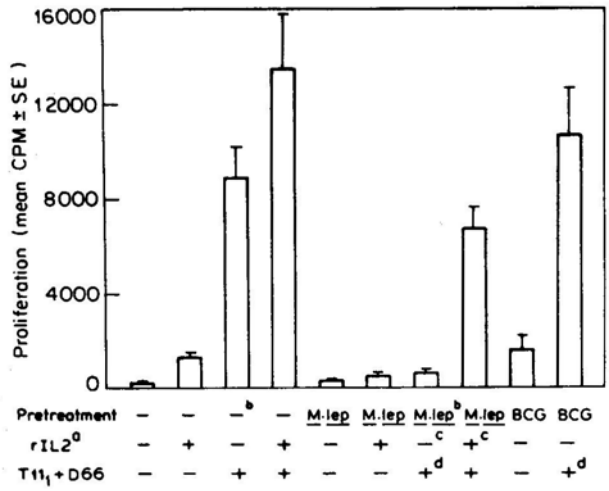

Figure 2. Impairment of alternate pathway of T cell proliferation in PBL of healthy non-contacts by *M. leprae in vitro.* [a]IL2 and/or $T11_1$+D66 were added to untreated PBL cultures or cultures pretreated for 24 h with *M. leprae/BCG;* [b]$P<0{\cdot}001$ between cpm of *M. leprae* treated and untreated cultures stimulated with $T11_1$+D66; [c]$P<0{\cdot}001$ between cpm of *M. leprae* treated cultures stimulated with $T11_1$+D66 with and without the addition of rIL2; [d]$P<0{\cdot}001$ between cpm of *M. leprae* and BCG treated cultures stimulated with $T11_1$ + D66.

of T cell activation as a result of CD2 modulation. The response to PHA-M was also suppressed by *M. leprae,* as shown in figure 3.

Recovery of proliferative response by the addition of rIL2

We next examined the mechanism of unresponsiveness produced by *M. leprae* through CD2 modulation. Table 2 shows that the addition of rIL2 (50 U/ml) could restore the proliferative response to the pair of anti-CD2 antibodies in PBL of LLBI + patients. Further, *M. leprae* pretreated PBL from healthy non-contacts also showed recovery of the proliferative response to anti-CD2 antibodies upon the addition of rIL2 (figure 2). Interestingly, very little proliferative response to *M. leprae* antigens was observed in cultures of normal PBL even with the addition of rIL2 (figure 2).

Discussion

Several studies have clearly demonstrated that the cell-mediated immune response is suppressed in LL patients (Godal, 1978; Nath, 1983; Bloom and Mehra, 1984). Peripheral blood T cells from these patients were unable to proliferate in response

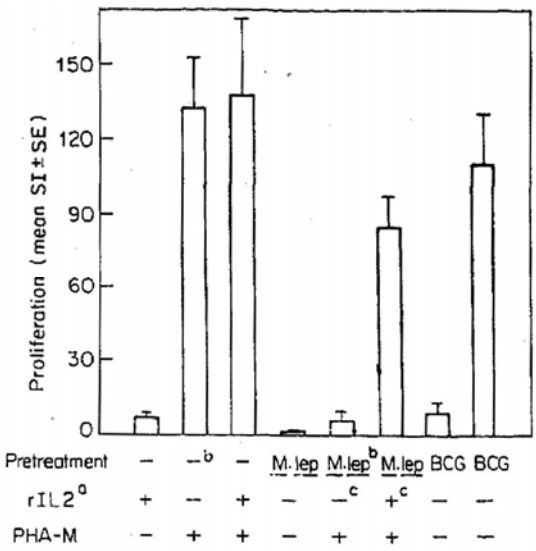

Figure 3. Impairment of proliferative response to PHA-M in PBL of healthy non-contacts by *M. leprae in vitro.* [a]IL2 and/or PHA-M were added to PBL untreated cultures or cultures pretreated for 24 h with *M. leprae/BCG;* [b]$P<0{\cdot}001$ between SI of *M. leprae* treated and untreated cultures stimulated with PHA-M; [c]$P<0{\cdot}001$ between SI of *M. leprae* treated cultures stimulated with PHA-M with and without the addition of rIL2.

Table 2. Recovery of proliferative response *via* alternate pathway by the addition of rIL2 to LLBI + PBL cultures.

Subject[a] (LLBI+)	Mean incorporation of radio-labelled thymidine (cpm)			
	IL2	Medium	D66+T11$_1$	PHA
1.	−	164	1988	7044
	+	3202	28206	115168
2.	−	303	2865	12853
	+	937	33650	75508
3.	−	479	863	3549
	+	421	22683	14987
4.	−	457	6182	10559
	+	580	37159	24829

[a]At the time of study, the bacillary index of the patients ranged between 0·4 and 5. The percentage of CD2-positive and CD3-positive cells were 46·0±1·8 and 62·2±2·6 respectively ($P< 0{\cdot}001$).

to *M. leprae.* This was suggested to be due to reduced IL2 production and IL2 receptor expression (Nath *et al.,* 1984; Kaplan and Cohn, 1985; Mohagheghpour *et al.,* 1985). We have initiated a new approach to explain the mechanism of *M. leprae* specific unresponsiveness in leprosy (Muthukkaruppan, 1986).

The CD2 receptor was found to be significantly downregulated in LLBI+ patients and this condition was strongly associated with impaired proliferative response to PHA-M and PPD (Muthukkaruppan *et al.,* 1987). The modulation of CD2 and the associated impairment of T cell proliferation can be brought about even in lymphocytes from normal subjects by prior incubation with *M. leprae* (Dharmendra lepromin) *in vitro* (Muthukkaruppan *et al.,* 1987). It is of interest to note that tuberculoid leprosy, though caused by the same bacteria, did not show reduction in the proportion of CD2-positive cells in the peripheral blood circulation (Nath *et al.,* 1977; Muthukkaruppan *et al.,* 1987). This means that the CD2 modulation would require the presence of a certain detectable level of bacilli, as in LLBI+ patients.

Our more recent study (Muthukkaruppan *et al.,* 1988) indicates that the CD3 receptor, though apparently expressed in normal proportion in the peripheral blood of LLBI+ patients, is functionally impaired, since CD2 modulated T cells did not proliferate in response to mitogenic anti-CD3 monoclonal antibody. The data presented here demonstrate the functional impairment of the alternate pathway (CD2) of T cell proliferation, since PBL from LLBI+ patients were unable to proliferate in response to a pairwise combination of anti-CD2 monoclonal antibodies (figure 1). Thus, both classical (CD3) and alternate (CD2) pathways of T cell activation are impaired as a result of CD2 modulation. Further, proliferative response to *M. leprae* could not be recovered even by the addition of IL2. However, when such T cells were activated by a potent stimulus like PHA-M or a pair of anti-CD2 monoclonals vigorous proliferation ensued only when exogenous IL2 was present in the cultures (figures 2 and 3).

Accumulating evidence in recent years suggests the importance of a functional interrelationship between CD2 and CD3 receptor complexes in T cell activation (Breitmeyer, 1987). T cells after treatment with certain anti-CD2 monoclonals were unable to proliferate in response to mitogenic anti-CD3 antibodies. Furthermore, the importance of interaction between CD2 receptor and its natural ligand, lymphocyte function associated antigen 3 (LFA3), in T cell functions has been emphasized (Krensky *et al.,* 1983; Springer *et al.,* 1987). In the light of these findings, the present study may suggest the importance of CD2 modulation in unresponsiveness in leprosy. However, further investigations are required to understand the mechanism of CD2 modulation by *M. leprae* as well as the nature of CD2 and IL2 receptor expression in LLBI + patients.

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