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Reversal of T cell unresponsiveness in lepromatous leprosy

I NATH
Department of Pathology, All India Institute of Medical Sciences, New Dehli, India

Introduction

That antigen-specific unresponsiveness of T cells was the hallmark of borderline and polar lepromatous leprosy was established in the early seventies. During the succeeding years, a large number of mechanisms were investigated to explain this phenomenon. In spite of intense activity around the world, no consensus has been reached as to the central mechanisms that would coherently unify the diverse immunological and clinical phenomena seen in the leprosy spectrum in general and lepromatous leprosy in particular. Recent studies implicate active suppression by T cells (1), monocytes (2, 3) and macrophages (4). With a view to reversing unresponsiveness in LL, our laboratory has been systematically investigating 1) modulation of *M. leprae*-specific *in vitro* cellular interactions (5, 6) and 2) the natural emergence of antigen reactive T cells during the reactional phases of LL both in circulation and in dermal lessions (8).

Results

I. MODULATION OF T CELL RESPONSES IN VITRO

1. Role of IL-2

Since lack of antigen-induced proliferation in LL patients appeared to be associated with concomitant decrease in IL-2 production in our preliminary studies, exogenous IL-2 was added to overcome this defect. Peripheral blood mononuclear cells from 41 freshly diagnosed, bacilliferous lepromatous patients were cultured with *M. leprae* in the presence and absence of IL-2 i) derived from mitogen-induced JR4 human cell line, ii) constitutively released IL-2 from gibbon cell line MLA, and iii) recombinant IL-2. In general, 60 to 65 % of LL individuals showed varying levels of lymphoproliferation in the presence of the T-cell growth factor and antigen. The improvement percentages varied from 190 to 7,200 %, though the majority showed low to moderate increases of 190 to 1700 %. IL-2 had no effect in one third of the individuals studied (Table 1).

Table 1. Effect of IL-2 on M. leprae*-induced lymphoproliferation of peripheral blood mononuclear cells from 41 baccilliferous lepromatous patients.*

No. of patients	Range	
	SI	Mean cmp
3	50 – 75	22,200 – 27,168
4	18 – 28	8,528 – 11,381
19	2.5 –11	4,673 – 5,955
15	2 – <2	141 – 3,711

$$SI = \text{Mean cpm} \frac{\text{IL-2 + } M.\ leprae}{\text{IL-2 alone}}$$

Standard deviation <15 % of mean cpm

2. Modulation by accessory cells

Earlier studies from our laboratory had shown both the presence of inhibitory monocytes in LL patients and the need for accessory cell (AC) presentation of *M. leprae* antigens for an effective T cell proliferation in vitro. Therefore, experiments were made to define whether AC + T cell cocultures could be modulated to reconstitute antigen-induced lymphoproliferation using T cells from LL individuals.

a. HLA-D compatible T + AC cocultures. Two-hour adherent cells from responder healthy/tuberculoid siblings, when cocultured with nylon wool purified (NWC) T cells from lepromatous patients, were able to induce significant ($p < 0.01$) *M. leprae*-induced T cell proliferation in all 4 sibling pairs. ≤ 5 % AC gave maximal proliferation (Table 2).

Table 2. Enhancement of antigen-induced lymphoproliferation with HLA-DR matched T cells from lepromatous and adherent cells (AC) from tuberculoid siblings (representative data).

Patient pairs	HLA –DR haplotypes		△ CPM			
	T	AC	PBMC	T+5% AC	T+10% AC	10+20% AC
I	2/2	2/2	-80	5,010	2,200	ND
II	2/8	2/2	200	2,000	5,025	1,522

△ CPM = Mean cpm of cultures with integral *M. leprae* – Mean cpm of cultures alone.

PBMC = Peripheral blood mononuclear cell cultures from lepromatous patients.

b. Autologous T + AC cocultures. In 9 LL/BL patients similar cocultures with autologous cells also showed improved T cell proliferation to integral and sonicated *M. leprae.* However, in general the cpm in antigen cultures were lower with autologous cocultures and the percentage increase as compared to PBMC ranged from 150-320 %. Interestingly, AC pulsed for 18 h with antigen were superior to nonpulsed AC at reconstituting lymphoproliferation (Table 3).

Table 3. Representative data on improvement in M. leprae*-induced lymphoproliferation with autologous nylon wool purified T cell + adherent cell cocultures from lepromatous patients.*

Patient		△ CPM			
		PBMC	T cells + 5 % AC	T cells + 10 % AC	T cells + 20 % AC
I	a.	73	6,000	3,000	65
	b.	73	4,500	55	– 200
II	a.	– 20	50	3,600	80
	b.	100	– 20	1,300	200
III	a.	100	3,500	3,880	565
	b.	100	2,000	3,000	422

a. Two-hour adherent cells pulsed 16 – 18 h with *M. leprae,* washed 3 times. No further antigen was added in cocultures with T cells.

b. Unpulsed adherent cell + T cells + *M. leprae* antigen. T cells were added to AC at same time and both cultures were harvested on day 6 after 12 – 14 h of prior pulsing with 3H thymidine.

< CMP See note to Table 2.

c. Autologous T + dendritic cell (DC) cocultures. In view of the low level of improvement in T cell proliferation with autologous monocyte rich 2 h adherent cells from LL patients, which would have been contaminated with suppressive monocytes, we next undertook to reconstitute the T cell cultures with dendritic cells as accessory cells. DC were obtained from PBMC of 15 LL patients by the method of Van Voorhis et al. (9), whereby the loosely adherent population removed from overnight culture of 2 h adherent cells contained DC. Monocytes and DC were depleted of contaminating T cells and B cells by complement-mediated cytotoxicity using OKT3 and BA1 antibodies respectively. DC were further depleted of monocytes by 3C 10 monoclonal antibody, kindly provided by Dr. R. Steinman. Routinely 0.8 to 1.5 x 10^6 Ia rich, nonphagocytic, Ig-3C10$^-$, esterase$^-$ DC with distinctive morphology were obtained from 100 ml of heparinised blood. MO and DC were reconstituted with NWC T cells depleted of MO and B cells. A broad concentration of accessory cells, 0.1, 0.3, 1, 3, 5 and 10 % were used in each of the cocultures.

Dendritic cells depleted of monocytes were potent inducers of *M. leprae*-related T cell proliferation in lepromatous leprosy. Of 15 patients studied, improvement was observed to highly significant levels ($p<0.001$) in 9 subjects. △ cpm in T + DC cultures ranged from 2,000 to 7,500 and reached levels observable in tuberculoid leprosy (Table 4). Significantly, increase in *M. leprae* induced gamma interferon production comparable to TT patients was observed in T + DC cocultures of 14 out of 15 LL patients. This was of interest since all of these patients had shown negligible lymphoproliferation.

Table 4. Representative data on improvement in intergral M. leprae*-induced lymphoproliferation with T-cell + dendritic cell cocultures from a lepromatous patient.*

		△ CPM	
PBMC	Accessory cells	T + DC	T + MO
360	10 %	6,525	100
	5 %	6,022	200
	3 %	5,425	- 20
	1 %	4,528	- 40
	0.3 %	4,220	- 42
	0.1 %	4,221	- 54

PMBC, △ CMP – see note to Table 2.

DC = Dendritic cells
MO = Monocytes

Summary
The above studies indicate that modulation of cell interactions and provision of exogenous growth factors can reverse the well documented in vitro antigen specific T cell defects in many lepromatous patients. The improvement with exogenous IL-2 demonstrates that some LL patients possess T cells capable of expressing IL-2 receptors in the presence of *M. leprae* antigens. More significantly, that T cells able to proliferate without exogenous growth factors are also present in the circulation of LL subjects was demonstrated by reconstitution experiments using T + accessory cell combinations. That DC depleted of monocytes is a potent inducer of lymphoproliferation and gamma interferon would suggest that functional T cells capable of microbicidal activity are present in lepromatous leprosy. Their presence is detectable when suppression mediated by monocytes is deleted and another accessory cell is used to present antigen.

II. NATURAL EMERGENCE OF ANTIGEN REACTIVE T CELLS IN VIVO

Some 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. Though immune complexes have been detected in ENL, sporadic evidence of T cell reactivity has also been reported by us as well as others.

T cell functions were studied in 15 LL patients undergoing ENL both during and after the reactional phase. i) Leucocyte migration inhibition to integral *M. leprae* was significantly

increased in all individuals ($p < 0.005$). The mean migration index (0.058 ± 0.01) reached levels commonly observed in tuberculoid leprosy. ii) Similarly, lymphoproliferation to both whole and sonicated bacilli was increased in 9 of 11 ENL patients. Concomitantly, iii) perturbations in T4/T8 ratios were observed with enhanced numbers of cells with T8 phenotype, and iv) increased antigen induced suppressor cell activity was observed in a costimulant assay described earlier.

Thus it would appear that during reactional states there is a natural emergence of antigen-reactive T cells in the circulation of LL patients which are capable of proliferation, lymphokine production and immune regulation. However, after subsidence of reactions following specific therapy, the above T cell reactivity reverted to the basal hyporesponsive levels associated with LL.

That these circulatory events are of biological significance was further shown in studies on dermal lesions. During ENL, the hitherto lymphopenic lesions show entry of moderate to large numbers of T cells with predominant Leu 3a (helper/inducer) phenotype and some $T8^+$ cells. These T cells also show the presence of Ia antigens indicative of an activated state. In addition, the possibility of gamma interferon production at the local site is indicated by the induction of Ia on the surface of keratinocytes. That this may have functional significance and reflect microbicidal activity is also indicated by the presence of granular and broken bacilli in ENL lesions.

Summary
During ENL reactions many LL patients show transient but definitive evidence of circulating functional T cells which produce lymphokines. In addition, activated T cells with helper phenotype enter dermal lesions and may secrete interferon gamma at the local site, thereby inducing Ia on keratinocytes and possible enhanced microbicidal activity in bacilli-laden phagocytes.

Conclusions

Both the natural emergence of T cells during reactional phases and modulation of in vitro cellular interactions indicate that responsiveness to *M. leprae* antigens and thereby reversal of the anergic state is possible in lepromatous leprosy. The initiating causes of the emergence of reactive T cells in vivo and the reasons for the nature of this phenomenon are not yet clear and may need sequential studies. Of the various protocols used to trigger T cell responsiveness in vitro, addition of exogenous IL-2, deletion of monocytes and presentation of antigen by dendritic cells showed impressive results.

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