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T-CELL RESPONSE TO MYCOBACTERIAL ANTIGENS IN LEPROMATOUS AND TUBERCULOID LEPROSY

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We showed that a large fraction of lepromatous patients do harbor helper-type circulating T-cells that can be activated in vitro *by* Mycobacterium leprae. M. leprae *and PPD triggered T-cell lines could be then obtained from both tuberculoid and lepromatous patients. The proliferative response of these helper T-cells is predominantly directed against epitopes shared by several species of mycobacteria, in lepromatous patients as well as in tuberculoid patients, but species specific T-cells are also present. When presented in the context of* M. leprae, *these cross reactive epitopes usually fail to stimulate the T-cell lines of lepromatous patients, because of the contamination of the lines by suppressor T-cells activable by* M. leprae. *In one lepromatous patient, PPD and* M. leprae *reactive T-cell lines and clones (of the CD4 phenotype), exhibited a strong cytotoxic activity to autologous target cells coated with antigen: the relevance of this phenomenon to the pathophysiology of lepromatous leprosy remains however unknown.*

The clinical expression of leprosy varies according to the resistance of infected individuals to *Mycobacterium leprae*, along what is called the spectrum of leprosy (Godal, 1978). At the lepromatous pole, the patients, as a consequence of their failure to develop cell mediated immunity to the bacillus, suffer extensive bacillary dissemination and thus produce large amounts of non protective antibodies. Some degree of non specific impairment of T-cell reactivity may be seen but regresses when the treatment reduces the bacillary load. Conversely the specific T-cell anergy to *M. leprae*, whether measured *in vivo* or *in vitro*, remains a permanent and characteristic feature of most lepromatous patients.

At the other end of this spectrum, tuberculoid patients mount strong cellular immune responses which limit the bacilli growth and the extension of the lesions. In between, borderline lepromatous and borderline tuberculoid forms represent intermediate stages of immunological resistance to *M. leprae*.

The mechanism of the T-cell tolerance to *M. leprae* observed in lepromatous leprosy represents a key problem of the pathophysiology of the disease and still remains largely unknown (Bach et al., 1983; Bloom et al., 1984). *Mycobacterium leprae* is an intracellular parasite which can grow in macrophages, unless activated CD4 helper T-cells secrete interferon γ that will promote macrophage activation and bactericidal activity, as it occurs in tuberculoid leprosy. A minority of CD8 T-lymphocytes are also present in tuberculoid granulomas and might contribute to the self limitation of the immune process (Wallach et al., 1984).

Lepromatous granulomas, on the other hand, contain very few T-cells, (a majority of which expressing the CD8 phenotype) and a number of unactivated macrophages heavily loaded by growing *M. leprae*. Thus one may envisage as a primary cause of T-cell tolerance a dysfunction of either macrophages, or T-helper cells or T-suppressor cells. To approach this problem we have analyzed over the past few years the *in vitro* T-cell response to mycobacterial antigens in lepromatous and tuberculoid patients.

Effects of CD8 T-cell elimination on the blood lymphocyte proliferative response to M. leprae – Blood lymphocytes of tuberculoid patients display a good proliferative response to *M. leprae*, whereas lepromatous patients do not. Since we observed both in blood and skin lesions an excess of CD8, suppressor type T-lymphocytes, as compared to CD4 helper-type lymphocytes (Bach et al., 1981; Wallach et al., 1982, 1984), we analyze the proliferative response to *M. leprae* of both subsets separately, in order to detect a possible suppressor effect of the CD8 subset in lepromatous patients (Bach et al., 1983). This was achieved by eliminating either T-cell subset with the corresponding monoclonal antibody in the presence of complement.

Lepromatous patients segregated into two categories, representative examples of which are shown in Table I. For about two third of the

TABLE I

Effect of CD8 and CD4 T-cell elimination on lymphocyte proliferative response of lepromatous patients*

	Antigen	Untreated PBMC	C-treated PBMC **	CD8 + PBMC **	CD4+ PBMC **
Patient 1	*M. leprae*	649	523	1586	589
	PPD	46423	70503	5075	75465
Patient 2	*M. leprae*	1170	76236	43107	115211
	PPD	13841	6727	732	5254

*10^5 PBMC were cultured for six days with the indicated antigen. ^{3}H-thymidine incorporation was measured after an 18 hour pulse.

**PBMC were separated into CD8+ and CD4+ cells by a negative selection procedure using anti CD8 or CD4 monoclonal antibody plus rabbit serum as a source of complement. Control PBMC were either left untreated or incubated at 37°C with rabbit serum alone.

patients, no restoration of the proliferative response to *M. leprae* could be seen after elimination of the CD8 population as seen for patient 1. In one third of lepromatous patients, an unexpected result was noted since the control treatment it-self (that is the incubation of blood lymphocytes at 37°C) induced a dramatic increase of the response to *M. leprae* (see patient 2). These results were the first demonstration that *M. leprae*-reactive T-cells do persist in some lepromatous patients and suggest their inactivation by a suppressor phenomenon, the mechanism of which remains undefined. A recent work by Mohagegpour et al. (1987), suggests that incubation at 37°C inactivates the suppressor activity of a suppressor CD4 T-cell subset.

Partial restoration of T-cell response to M. leprae *by the addition of exogeneous interleukin 2* – A further indication of the existence of *M. leprae*-reactive T-cells in lepromatous patients was given by the ability to restore *in vitro* T-cell response to the bacillus by the addition of exogeneous interleukin 2 (Shankar et al., 1985). About half of the lepromatous patients tested displayed a significant proliferative response to *M. leprae* in the presence of IL2. The T-cell response to PPD was also increased by IL2 in some of those patients who were also unresponsive to PPD.

These data suggest that the suppressor mechanism that inhibits the activation of *M. leprae* specific T-cells might operate at the level of the production of interleukin 2 (Haregewoin et al., 1983).

The restoration of T-cell responsiveness to *M. leprae* only concerns a fraction of the population of the lepromatous patients. Those patients whose response to *M. leprae* was restored by IL2, also showed a significant T-cell response to PPD suggesting that the *M. leprae* reactive T-cells in these lepromatous patients predominantly respond to epitopes shared by *M. leprae* and *M. tuberculosis* or *M. bovis*.

Study of M. leprae *and PPD-triggered T-cell lines* – To further investigate the T-cell response of lepromatous patients to mycobacterial antigens, in terms of function and of antigenic specificity, we developed long term T-cell lines from blood lymphocytes activated by *M. leprae* or PPD (Bach et al., 1985; Shankar et al., 1986). Primary cultures were set up either in the presence of IL2 (for lepromatous patients) or in the absense of IL2 (for tuberculoid patients). Cultures were then restimulated with the same antigen in the presence of irradiated autologous blood mononuclear cells as antigen presenting cells. T-cell lines were then maintained in cultures by periodic restimulation with antigen plus antigen presenting cells, and IL2 supplementation. Their proliferative responses to various mycobacterial antigens was assayed after 2 to 8 weeks of culture, by tritiated thymidine incorporation in a 48 hour assay.

Both *M. leprae* and PPD triggered T-cell lines from tuberculoid patients displayed proliferative responses broadly cross-reacting with other mycobacteria (Table II).

In lepromatous patients (Table III), *M. leprae* lines initially displayed cross reacting responses to PPD and other mycobacteria, as did *M. leprae* lines from tuberculoid patients.

TABLE II

Proliferative responses of T-cell lines from a tuberculoïd patient to mycobacterial antigens*

	Antigens						
	None	*M. leprae*	PPD	BCG	*M. fortuitum*	*M. kansasii*	*M. intracellulare*
M. leprae line	1247	*5568*	*5183*	*4197*	*3842*	*4619*	*4206*
PPD line	1353	*3091*	*4207*	*2587*	*2280*	*3768*	1711

* 10^4 T-cells were cultured for 48 hours with 5 x 10^4 irradiated PBMC plus the indicated antigen. ^{3}H-thymidine incorporation was measured after an 18 hour pulse. Values that significantly differ from control without antigen are in italics.

TABLE III

Proliferative response of T-cell lines from a lepromatous patient to mycobacterial antigens*

	Antigens						
	None	*M. leprae*	PPD	BCG	*M. fortuitum*	*M. kansasii*	*M. intracellulare*
M. leprae	2152	*5126*	*20218*	*8435*	*9457*	*13525*	*7799*
lines	940	*2038*	1155	921	1004	1128	1023
PPD	238	282	*5285*	*1535*	*1913*	*2608*	555
lines	854	631	*4507*	468	779	999	531

* 10^4 T-cells were cultured for 48 hours with 5 x 10^4 irradiated autologous PBMC plus the indicated antigen. ^{3}H-thymidine incorporation was measured after an 18 hour pulse. Values that significantly differ from control value without antigen are in italics.

However, they did not maintain this pattern of specificity and tended to become with time *M. leprae*-specific.

Most PPD lines were also broadly cross reactive, some of them were found PPD-specific, but in contrast to what was observed for PPD lines of tuberculoid patients, they never responded to *M. leprae*.

These data indicate that the T-cell response of lepromatous patients to mycobacterial antigens is for a large part directed at epitopes common to several species of mycobacteria. These epitopes however fail to stimulate T-cells when presented in the context of *M. leprae* in the absence of IL2, suggesting again the coactivation of suppressor cells by the bacillus.

M. leprae *and PPD-reactive T-cell clones* – T-cell lines of some patients could be cloned by limiting dilution. Table IV summarizes the specificity pattern of clones obtained from *M. leprae* and PPD triggered T-cell lines. The majority of both tuberculoid and lepromatous clones derived from *M. leprae* triggered T-cell lines were cross reactive to other mycobacteria. It must be noted that lepromatous clones, at variance with the lines from which they were derived, maintained their specificity pattern over time.

Among T-cell clones obtained from PPD lines, a number of them are also cross reactive, the proportion of species-specific clones varying according to the patient. One important point must be noted: several lepromatous clones do recognize *M. leprae*, although the PPD line from which they originated did not respond to this antigen, which suggests that the line did contain suppressor cells.

CD phenotype of M. leprae *and PPD reactive T-cell lines and clones* – The CD phenotype of both PPD and *M. leprae* T-cell lines was similar in lepromatous and tuberculoid patients: most T-cells initially expressed the CD4 phenotype. Then, all lines got progressively enriched in CD8 cells, losing at that time their ability to grow in culture and to proliferate to the antigen. Clones, on the other hand, were in their majority CD4+, CD8–, a minority of them transiently expressing CD8.

Suppressor and cytolytic activities of M. leprae *and PPD-reactive T-cell lines and clones* –

TABLE IV
Proliferative response of *M. leprae* and PPD reactive T-cell clones: specificity pattern

Patients	Origine of clones	Number of clones	*M. leprae*	PPD	Other mycobacteria
LL1	*M. leprae*	4	++	++	ND
	line	3	++	–	ND
		3	++	++	++
TT1	*M. leprae*	1	++	–	++
	line	2	++	–	–
LL1	PPD	5	++/+	++/+	+
	line	5	+	+++	+
LL2	PPD	6	–	+++	+
	line	4	–	+++	–
TT1	PPD	2	++	++	+
	line	1	–	++	+

Attempts were made to evidence directly suppressor T-cells within these T-cell lines. Indeed all lines tested exerted an inhibitory activity upon the proliferative responses of autologous blood lymphocytes. However this suppressive activity was not specific of mycobacterial antigens and was similarly found in tuberculoid and lepromatous lines, whatever the triggering antigen. It is possible however, that these non specific suppressor phenomena, which may simply result from the absorption of IL2 by activated T-cells, mask a specific suppressor mechanism that could be more relevant to the pathophysiology of lepromatous leprosy. Recent works indeed indicate the presence of *M. leprae*-specific suppressor T-cells in skin lesions and blood of lepromatous patients (Ottenhoff et al., 1986; Modlin et al., 1986).

It was observed on several occasions with the PPD and *M. leprae* lines and clones of one of the lepromatous patient, that antigenic restimulation resulted in a rapid and massive cell lysis (instead of proliferation). It was thus envisaged that T-cells themselves served as targets for an antigen-specific cytotoxic process. Such a cytotoxic activity of these clones could be easily demonstrated in a standard chromium release assay, where autologous EBV-transformed B cells incubated with antigen were used as target cells (Table V). The cytotoxicity activity was strictly antigen dependent and manifested only with autologous targets. Whether this cytotoxicity has some pathophysiological relevance remains unknown.

TABLE V
Cytotoxic activity of T-cell clones from a lepromatous patient towards PPD-coated target cells

Clone no.	Specificity	PPD	target cells*	
			autologous	allogenic
RP3	PPD, *M. leprae*	+	43%**	0%
		–	0%	0%
RP5	PPD, *M. leprae*	+	45%	0%
		–	12%	0%
RM3	PPD, *M. leprae*	+	41%	7%
		–	7%	7%
RM8-8	*M. leprae* alone	+	11%	8%
		–	6%	8%

*51 Cr labelled-EBV-transformed B cells were incubated for 18 hours with T-cells with or without PPD.
**percentage of specific lysis (mean of triplicates).

REFERENCES

BACH, M.A.; CHATENOUD, L.; WALLACH, D.; PHAN DINH TUY, F. & COTTENOT, F., 1981. Studies on T-cell subsets and functions in leprosy. *Clin. Exp. Immunol.*, *44* :491-500.

BACH, M.A.; HOFFENBACH, A.; LAGRANGE, P.H.; WALLACH, D. & COTTENOT, F., 1985. Mechanisms of T-cell unresponsiveness in leprosy. *Ann. Immunol.* (Inst. Pasteur), *134D* :75-84.

BACH, M.A.; WALLACH, D.; FLAGEUL, B. & COTTENOT, F., 1983. *In vitro* proliferative response to *M. leprae* and PPD of isolated T-cell subsets from leprosy patients. *Clin. Exp. Immunol.* *52* :107-114.

BLOOM, B.R. & MEHRA, V., 1984. Immunological unresponsiveness in leprosy. *Immunol. Rev., 80* :5-28.

GODAL, T., 1978. Immunological aspects of leprosy-present status. *Prog. Allergy, 25* :211-242.

HAREGEWOIN, A.; GODAL, T.; MUSTAFA, S.; BELEHU, A. & YEMANEBERHAN, T., 1983. T-cell conditioned media reverse T-cell unresponsiveness in lepromatous leprosy. *Nature, 303* :342-344.

MODLIN, R.L.; KATO, H.; MEHRA, V.; NELSON, E.E.; XUE-DONG, F.; REA, T.H.; PATTENGALE, P.K. & BLOOM, B.R., 1986. Genetically restricted suppressor T-cell clones derived from lepromatous leprosy lesions. *Nature, 322* :459-461.

MOHAGHEGHPOUR, N.; GELBER, R.P. & ENGLEMAN, E.G., 1987. T-cell defect in lepromatous leprosy is reversible in vitro in the absence of exogeneous growth factors. *J. Immunol., 138* :570-574.

OTTENHOFF, T.H.M.; ELFERINK, D.; KLATSER, P.R. & de VRIES, R.R.P., 1986. Cloned suppressor T-cell from a lepromatous leprosy patient suppress mycobacterium leprae reactive helper T-cells. *Nature, 322* :462-464.

SHANKAR, P.; AGIS, F.; WALLACH, D.; FLAGEUL, B.; COTTENOT, F., AUGIER, J. & BACH, M.A., 1986. *M. leprae* and PPD-triggered T-cell lines in tuberculoid and lepromatous leprosy. *J. Immunol., 136* :4255-4263.

SHANKAR, P.; WALLACH, D. & BACH, M.A., 1985. Interleukin 2 induced T-cell response to *M. leprae* in lepromatous leprosy: reversion of a suppressor mechanism of expansion of a small *M. leprae* reactive T-cell pool ? *Int. J. Leprosy, 53* :649-652.

WALLACH, D.; COTTENOT, F. & BACH, M.A., 1982. Imbalances in T-cell subpopulations in lepromatous leprosy. *Int J. Leprosy, 50* :282-290.

WALLACH, D.; FLAGEUL, B.; BACH, M.A. & COTTENOT, F., 1984. Immunohistological analysis of dermal leprous granulomas. *Int J. Leprosy, 52* :318-326.