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Evaluation of chemiluminescence, procoagulant activity and antigen presentation by monocytes from lepromatous leprosy patients with or without reactional episodes

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Summary In this study, we evaluated the activity of peripheral blood mononuclear cells (PBMC), isolated from treated and untreated lepromatous leprosy patients, from lepromatous leprosy patients during and after reactional episodes (erythema nodosum leprosum (ENL) and reversal reaction (RR) , and from normal healthy individuals. We determined reactive oxygen intermediate (ROI) production, procoagulant activity (PCA) and HLA-DR antigen expression of monocytes, besides lymphoproliferation, both in the presence and absence of various stimulatory agents. Phorbol myristate acetate (PMA) stimulated ROI production by monocytes from all the groups studied, with patients during reactional episodes (ENL and RR) showing a significantly higher response ($p < 0{\cdot}009$ and $p < 0{\cdot}00001$). Irradiated *Mycobacterium leprae*, although having little effect when added alone, strongly suppressed PMA-stimulated ROI production. Muramyl dipeptide (MDP) had no influence on either basal or on PMA-induced ROI production. Basal monocyte PCA, as well as *M. leprae* or concanavalin A (ConA)-induced monocyte PCA, was comparable in monocytes from all the groups studied. ConA was able to induce mitogenic activity in mononuclear cells isolated from all the groups studied. *M. leprae*, although stimulatory for normal individuals, did not induce lymphoproliferation in lepromatous leprosy patients, except for cells from patients during RR, which responded equally to *M. leprae* and to ConA. The absence of *M. leprae*-induced lymphoproliferation in lepromatous leprosy patients is not caused by the lack of basal HLA–DR expression, as PBMC from all individuals studied showed the same level of this antigen. Our results suggest an increase of spontaneous or PMA-

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induced monocyte activity, as detected by ROI production, during the reactional episode; addition of *M. leprae* suppressed this response. The increase in monocyte activity could be correlated with the increase of lymphoproliferation response to *M. leprae* during RR, but not during ENL. The importance of a possible immune suppressive action of *M. leprae* is discussed.

Introduction

Leprosy, one of mankind's oldest diseases, is characterized by a very complex immunological response which determines the disease's clinical manifestations. Depending on the capacity of the infected individual to mount an adequate immune response, leprosy appears either in a paucibacillary tuberculoid form (TL), borderline forms such as borderline–tuberculoid (BT), borderline–borderline (BB), and borderline–lepromatous (BL), or in a multibacillary lepromatous stage (LL). However, leprosy is not a stable disease and is characterized by episodes of reactional states such as the reversal reaction (RR), occurring only in borderline patients, and erythema nodosum leprosum (ENL), occurring in both LL and BL patients.[1,2] Cell mediated immunity (CMI) plays a key role in the immunological response to leprosy. In contrast to healthy individuals and TL patients, lepromatous leprosy patients do not mount an efficient CMI response against *Mycobacterium leprae*.[3,4] The absence of a functional T lymphocyte proliferative response[3] and a low level of interferon-gamma (IFN-γ) production[4] in response to infection with *M. leprae* probably results in adequate monocyte activation. Mononuclear phagocytes play a primary role during immune defence; functional deficiency of these cells has been observed in LL patients.

Microbicidal competence of phagocytes in leprosy patients is usually determined by measurement of reactive oxygen intermediate (ROI) production. However, probably because of the complexity of the interaction of macrophage and bacilli, conclusions from studies using this experimental design are highly contradictory.[5–12] One of the initial events in the cascade of steps leading to pathogen killing in the bacterially-induced CMI response is phagocytosis of the bacilli and subsequent expression of bacterial antigens on the surface of the phagocytic cell.[13] Experiments *in vitro* indicate that failure of antigen presentation in an immunogenic form, as observed in monocytes isolated from lepromatous leprosy patients, might lead to the lack of an efficient response.[14] Monocyte activation can also be determined by measuring cellular procoagulant activity (PCA).[15] *M. leprae* has been shown to induce PCA activity in monocytes from normal individuals,[16] but so far no information exists about this activity in lepromatous leprosy patients.

These data prompted us to evaluate various parameters of mononuclear phagocyte activity in patients with distinct clinical manifestations of leprosy. We were able to extend our previous observations on monocyte activity from leprosy patients[10] with new data on monocyte functions such as ROI production, PCA induction, HLA–DR expression, and on lymphoproliferation of mononuclear cells *in vitro*.

Materials and methods

SUBJECTS

All lepromatous leprosy patients were diagnosed at the Souza Araujo Ambulatory

(Leprosy Unit, Oswaldo Cruz Foundation, RJ, Brazil). Clinical diagnosis was confirmed by skin biopsy; disease classification was according to the criteria of Ridley & Jopling.[17] Of the 59 LL and BL patients in this study, 16 had only recently been treated, while 25 had not received any treatment. Another 14 were in a reactional episode (10 patients with ENL and 4 with RR), while 4 patients were in a postreactional state. Multidrug therapy (MDT) was as follows: monthly doses of rifampicin (600 mg) and clofazimine (300 mg) under supervision, as well as daily self-administered doses of dapsone (100 mg) plus clofazimine (100 mg) every other day for 24 months. Patients undergoing a reactional episode did not receive any specific treatment for reactions before blood collection. Patients in a postreactional state were treated by thalidomide and/or prednisone during the reactional episode. Blood collection of these patients was carried out in the first week after the reactional episode, when these patients no longer displayed any associated symptoms. The duration of the reactional episode was variable for each patient. We used 20 laboratory staff as normal controls.

ISOLATION OF MONONUCLEAR CELLS

Mononuclear leukocytes were isolated from heparinized venous blood using Ficoll-Histopaque (Pharmacia Fine Chemicals, Piscataway, NJ, USA) as previously described.[10]

MEASUREMENT OF ROI PRODUCTION

We incubated 1,000,000 mononuclear leukocytes on 13 mm diameter glass coverslips in 24 well microtitre plates (Becton Dickinson, San Jose, CA, USA) in RPMI 1640 medium (Gibco, Grand Island, NY, USA), supplemented with 10% foetal bovine serum (FBS)(Gibco), 2 mM glutamine (Gibco), 100 U/ml penicillin (Gibco) and 100 μg/ml streptomycin (Gibco) at 37°C in 5% CO_2. The cells were incubated with or without 20 μg/ml irradiated, armadillo derived soluble *M. leprae* sonicate (IMMLEP BANK, Mill Hill, UK) and/or 1 μg/ml muramyl dipeptide (MDP; Sigma Chemical Co, St Louis, MO, USA) for 20 h. The medium was removed and the adherent monocytes were washed 3 times with medium containing 2% FBS before use. Measurement of ROI production occurred in the presence of 50 μM luminol (Sigma) as a chemiluminescence CL probe. ROI was induced by the addition of 0·5 μg/ml phorbol-myrystate-acetate (PMA, Sigma) and measured immediately in a Packard liquid scintillation spectrometer (model 1900 CA) as previously described.[10] Results are calculated as follows: Stimulation Index (SI) CL after cell stimulation divided by CL of unstimulated cells. The percentages of basal CL were calculated as follows: (basal chemiluminescence (CL) of monocytes isolated from patients minus CL of monocytes isolated from normal individuals divided by CL of monocytes isolated from normal individuals) $\times$ 100.

DETERMINATION OF PCA

Mononuclear cells (2×10^4/well) were incubated in 96 well plates (Becton Dickinson) in RPMI 1640 medium, supplemented with 10% human serum in the presence or absence of 20 μg/ml *M. leprae* antigen or 10 μg/ml ConA (Sigma). After 20 h the supernatant was removed and the adherent cells lysed by incubation with 100 μl *n*-octyl-B-D-glucopyrano-

side (50 μM; Sigma) for 15 min at 37°C and stored at −70°C until further use. PCA of the monocytes was measured using a modified Edwards & Rickles assay.[15] Briefly, cell lysates were transferred to siliconized tubes, after which 100 μl of plasma was added and coagulation was induced by the addition of $CaCl_2$ to a final concentration of 0·03 M, followed by gentle shaking. Rabbit brain thromboplastin (Baxter Healthcare Corporation, Miama, FL, USA) was used as a standard. For determination of basal monocyte PCA, aliquots of freshly isolated mononuclear cells were sedimented at 200 g for 5 min and lysed; PCA was then determined as described above.

DETERMINATION OF HLA–DR ANTIGEN EXPRESSION

At least 5×10^5 mononuclear cells were resuspended in 20 μl of solution buffer (Earle's balanced salt solution pH 7·2, supplemented with 0·1% sodium azide and 3% FBS) and incubated with OKM1 (Becton Dickinson) or anti-DR monoclonal antibodies (National Institutes of Health, Bethesda, MD, USA), followed by incubation with fluoresceinated goat antimouse Ig polyclonal antibody (Becton Dickinson). The cells were fixed with 1% paraformaldehyde and analysed in a flow cytometer FACScan (Coulter-Miami, USA).

LYMPHOCYTE PROLIFERATION ASSAY

Mononuclear cells were incubated in 96 well plates at a concentration of 2×10^5 cells per well in 200 μl RPMI, supplemented with 10% human autologous plasma, 10 mM HEPES, 2 mM L-glutamine, antibiotics and 50 μM 2-mercaptoethanol. Cells were stimulated with 20 μg/ml *M. leprae* or 15 μg/ml ConA (Sigma). Blast formation was determined by measuring radioactive thymidine incorporation 5 days after the addition of *M. leprae* or 2 days after the addition of ConA. Briefly, cells were labelled by the addition of 1 μCi/well of [methyl-^{3}H] thymidine (5·0 Ci/mmol; Amersham, Bucks, UK) per well for 18 h. Cells were lysed on a glass filter and the amount of incorporated labelled thymidine measured in a liquid scintillation counter. The SI was calculated as follows: incorporated radioactivity in cells stimulated with antigen divided by incorporated radioactivity in unstimulated cells.

STATISTICAL ANALYSES

Significance of the difference between the values of the various groups studied was evaluated by the Student's *t*-test.[18] The *t*-test was modified to correct for the heterogeneity of variances.

Results

ACTIVATION OF MONOCYTES

Figure 1 shows the basal CL of monocytes isolated from normal individuals or from patients with different forms of lepromatous leprosy. Monocytes isolated from patients during ENL or RR showed a significantly higher CL versus normal individuals (20%, $p < 0{\cdot}0001$; and 295%, $p < 0{\cdot}004$, respectively), whereas monocytes isolated from other

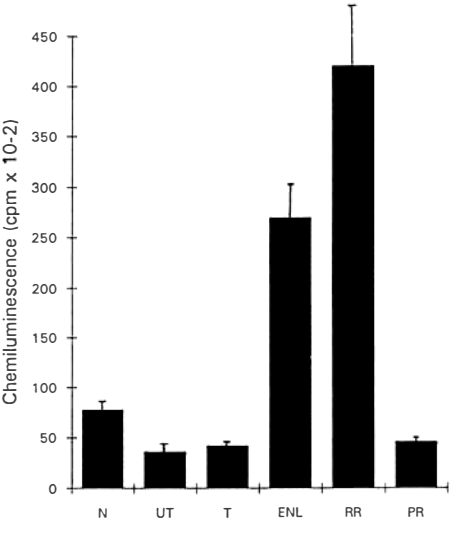

Figure 1. Basal chemiluminescence (CL) of monocytes. Monocytes were cultured for 1 day, washed and further treated for CL determination as described in the Materials and Methods. N, monocytes from normal individuals ($n = 20$); UT, untreated lepromatous leprosy patients ($n = 25$); T, treated lepromatous leprosy patients ($n = 16$); ENL, patients undergoing erythema nodosum leprosum ($n = 10$); RR, patients undergoing reversal reaction ($n = 4$) and PR, patients in post-reactional state ($n = 4$).

lepromatous leprosy patients showed a basal level of CL that was lower than that found in normal individuals ($p < 0{\cdot}04$). Table 1 shows the ROI production of monocytes, isolated from normal individuals or from lepromatous leprosy patients after stimulation with PMA and *M. leprae* or MDP. Monocytes from all individuals studied showed increased ROI production after incubation with PMA. Stimulation with PMA shows a difference in ROI response for LL and BL patients. Regardless of whether BL patients were treated or not, ROI production was comparable to that of normal volunteers; LL patients showed a much lower ROI response. Patients undergoing reaction manifestations (ENL and RR) showed the strongest CL response, differing significantly from normal volunteers. Although no significant change in basal CL was found after incubation of monocytes with *M. leprae*, ROI values were repeatedly slightly lower than in the absence of *M. leprae*. Pretreatment with *M. leprae*, however, strongly inhibited the PMA-induced CL response in all groups studied. Pretreatment of cells with MDP had no influence on either the basal or PMA-induced CL response.

MONOCYTE INDUCED PCA

PCA of monocytes from normal volunteers and lepromatous leprosy patients is shown in Table 2. PCA was only detectable after co-incubation of monocytes with other mononuclear cells (data not shown). Monocytes, isolated from all groups studied, spontaneously induced PCA; values from patients in the PR state, however, were considerably lower ($p < 0{\cdot}02$). Both ConA and *M. leprae* stimulated monocyte PCA without any significant difference between the various groups studied.

Table 1. Activation of monocytes as detected by chemiluminescence

Subjects	Chemiluminescence[a]						
Conditions at the time of the study	PMA	*Ml*[b]	*Ml*+PMA[c]	MDP	MDP+PMA[c]	MDP+*Ml*[d]	MDP+*Ml*+PMA[c]
Normal individuals ($n=20$)	14·0±6·0[e]	0·7±0·4	4·0±3·0	0·6±0·3	10·0±7·0	0·4±0·2	1·7±0·2
Untreated ($n=25$)							
BL[f]	12·0±4·0 (NS[g])	0·8±0·5 (NS)	2·0±1·0 (NS)	0·7±0·1 (NS)	10·0±5·0 (NS)	0·6±0·3 (NS)	1·7±1·1 (NS)
LL	6·0±2·0 ($p<0·01$)						
Treated ($n=16$)							
BL	13·0±3·0 (NS)	0·5±0·3 (NS)	1·8±0·9 (NS)	0·7±0·2 (NS)	20·0±10·0 ($p<0·02$)	0·4±0·1 (NS)	4·2±0·3 ($p<0·02$)
LL	5·0±3·0 ($p<0·01$)						
ENL ($n=10$)	27·0±5·0 ($p<0·0009$)	0·6±0·3 (NS)	3·1±1·5 (NS)	0·7±0·2 (NS)	29·0±11·0 ($p<0·001$)	0·4±0·2 (NS)	2·2±0·7 (NS)
RR ($n=4$)	32·0±5·0 ($p<0·00001$)	0·8±0·4 (NS)	7·2±1·2 (NS)	0·7±0·2 (NS)	25·0±8·0 ($p<0·003$)	0·8±0·3 (NS)	5·4±0·5 ($p<0·002$)
PR ($n=4$)	12·0±6·0 (NS)	0·6±0·3 (NS)	0·9±0·7 (NS)	1·5±0·7 (NS)	11·0±7·0 (NS)	0·3±0·1 (NS)	1·1±0·7 (NS)

[a] Stimulation index = chemiluminescence after stimulation/chemiluminescence without stimulation.
[b] *M. leprae*.
[c] PMA added 24 h addition of *M. leprae* MDP.
[d] MDP and *M. leprae* added simultaneously.
[e] Mean value ± standard deviation.
[f] When not specified, values from BL and LL patients were not significantly different.
[g] Not significantly different from normal volunteers.

Table 2. Procoagulant activity (PCA) of monocytes

Subjects: conditions at the time of the study	PCA (mU thromboplastin)		
		Stimulus-induced	
	Spontaneous	ConA	*M. leprae*
Normal individuals ($n=20$)	653 ± 176[a]	1737 ± 659	1509 ± 310
Untreated ($n=25$)	1058 ± 724	3038 ± 1246	2152 ± 1208
Treated ($n=16$)	510 ± 155	2513 ± 914	2012 ± 627
ENL ($n=10$)	1109 ± 1154	2435 ± 1088	2297 ± 1037
RR ($n=4$)	911 ± 892	2301 ± 568	2073 ± 291
PR ($n=4$)	256 ± 112 ($p<0{\cdot}02$[b])	1361 ± 675	1254 ± 707

[a] Mean value ± standard deviation.
[b] All other values do not significantly differ from normal individuals.

Table 3. Flow cytometric quantification of HLA–DR expression in monocytes as measured by fluorescence

Subjects: conditions at the time of the study	Fluorescence		
	% OKM1 positive cells	% DR-positive cells	% MFI[a]
Normal individuals ($n=20$)	13 ± 7[b]	16 ± 5	80 ± 15
Untreated ($n=20$)	17 ± 8	19 ± 10	77 ± 10
Treated ($n=16$)	12 ± 1	15 ± 6	75 ± 10
ENL ($n=10$)	18 ± 8	16 ± 6	77 ± 12
RR ($n=4$)	17 ± 3	15 ± 4	87 ± 14
PR ($n=4$)	12 ± 4	15 ± 3	81 ± 10

[a] Mean fluorescence intensity of DR-positive cells.
[b] Mean value ± standard deviation.

HLA–DR SURFACE ANTIGEN EXPRESSION

Expression of HLA–DR by PBMC from normal individuals and lepromatous leprosy patients is shown in Table 3. No significant differences emerged between HLA–DR surface antigen expression in any of the groups studied, and the number of monocytes within the mononuclear cell population was the same, independent of the patient's clinical status.

LYMPHOCYTE PROLIFERATION

Table 4 shows that cells from all the groups studied responded to stimulation with ConA. Patients during RR showed a stronger response. Mononuclear cells, isolated from normal individuals, also proliferated strongly after incubation with *M. leprae*. Within the population of lepromatous patients, only lymphocytes isolated from patients with RR were able to respond to the addition of *M. leprae*. Post-RR patients partially retained proliferative capacity.

Table 4. Lymphoproliferative responses of PBMCs from healthy individuals and leprosy patients

Subjects: conditions at the time of the study	ConA		*M. leprae*	
	−	+	−	+
Normal individuals ($n=20$)	1024 ± 213[a]	11733 ± 1667 (11 ± 3)[b]	2057 ± 321	12342 ± 1418 ($6 \pm 0{\cdot}5$)
Untreated ($n=20$)	678 ± 136 (NS[c])	4493 ± 1311 (7 ± 1) (NS[d])	907 ± 158 ($p<0{\cdot}04$[c])	1394 ± 300 (2 ± 1) ($p<0{\cdot}02$[d])
Treated ($n=12$)	708 ± 88 (NS)	2665 ± 199 (4 ± 1) ($p<0{\cdot}001$)	1325 ± 330 ($p<0{\cdot}04$)	1380 ± 302 (1 ± 2) ($p<0{\cdot}001$)
ENL ($n=10$)	341 ± 67 ($p<0{\cdot}04$)	3955 ± 924 ($11 \pm 0{\cdot}5$) (NS)	842 ± 218 ($p<0{\cdot}04$)	757 ± 125 (1 ± 1) ($p<0{\cdot}001$)
RR ($n=4$)	478 ± 41 ($p<0{\cdot}04$)	6597 ± 68 (13 ± 1) (NS)	369 ± 43 ($p<0{\cdot}0001$)	1845 ± 328 (5 ± 1) (NS)
PR ($n=4$)				
ENL	405 ± 51 ($p<0{\cdot}001$)	2638 ± 804 ($7 \pm 0{\cdot}8$) (NS)	412 ± 53 ($p<0{\cdot}001$)	824 ± 126 (2 ± 1) ($p<0{\cdot}001$)
RR	378 ± 137 ($p<0{\cdot}001$)	3529 ± 1204 (9 ± 1) (NS)	525 ± 75 ($p<0{\cdot}001$)	1785 ± 148 (3 ± 2) ($p<0{\cdot}001$)

[a] Mean value in cpm $\pm$ standard deviation.
[b] Stimulation index = mean cpm upon stimulation/mean cpm without stimulation.
[c] p value as compared to normal individuals.
[d] p value as compared to the stimulation index of normal individuals.

Discussion

Previous studies on ROI production by phagocytes from healthy individuals and lepromatous leprosy patients, upon stimulation with *M. leprae* and other agents, are contradictory.[5–9,11,12] We therefore measured various parameters of monocyte activity in the presence or absence of *M. leprae* from patients at given stages along the clinical spectrum of leprosy and its reactional states.

In monocytes isolated from all individuals studied, PMA induced a marked production of ROI, a process that was much more pronounced during reactional episodes. Lepromatous patients without reactional episodes displayed a weak PMA-induced CL response in comparison with normal individuals, as observed previously.[10] The PMA-induced ROI production observed in patients with ENL and RR could be linked to excessive monocyte activity. The high basal ROI seen in patients during reactional episodes strengthens this hypothesis, and the presence of extravascular complex in ENL could play an important role in monocyte activation.[1] No difference in ROI production was found in patients under treatment or not, an observation that could be generalized for all monocyte activities measured in this study. Furthermore, the CL response of LL patients was significantly lower than that of BL patients, who produced ROI to the same extent as did normal volunteers. This suggests some residual monocyte activity in BL patients—a finding that has recently been suggested by us when comparing monocyte activity from TL and LL patients.[10]

Although some groups have proposed a slow and weak ROI stimulatory capacity of *M. leprae*,[7,11,12] several reports claim *M. leprae* to be devoid of any stimulatory activity.[6,19] Such variable results could be partly explained by suppression of monocyte function by some fractions of *M. leprae*. We therefore tested whether *M. leprae* was capable of inhibiting the PMA-induced ROI response and, if so, to what degree this would vary in patients with different clinical forms of leprosy. Indeed, *M. leprae* was found to be strongly inhibitory, to a comparable extent throughout the whole gamut of leprosy patients studied. A suppressor role of various mycobacteria in skin-test responses, and an active role of suppressor factors in the so-called immune defect in LL patients, has been considered.[20] Phenolic glycolipid I (PGL1) is unique to *M. leprae* and was recently shown to decrease superoxide anion production by monocytes from normal donors stimulated with *M. leprae*.[11] It also acts as a scavenger of reactive oxygen species,[21] which may contribute to protecting the bacilli from killing by its host. PGL1 treatment does not inhibit PMA induced ROI production,[11] so it is unlikely that PGL1 alone is responsible for the suppression observed in this study. Synergistic suppression of PGL1 and other components such as lipoarabinomannan (LAM) is not unlikely. LAM has been attributed to have both macrophage stimulatory[22] and inhibitory[23] capacity. In addition, delipified *M. leprae* antigen seems to restore the ability of macrophages from leprosy patients to kill the bacteria.[24] *M. leprae* could also interfere with monocyte activity by inducing the release of prostaglandin E2, a well-known monocyte inhibitor.[25]

MDP is a synthetic analogue of Gram-positive bacterial cell wall peptidoglycan. According to Pabst & Johnston,[26] MDP retains adjuvant properties and primes macrophages to respond with increased ROI production after stimulation with PMA. In agreement with these findings, we observed that MDP exposure did not, in itself, induce ROI production by normal monocytes. However, MDP was unable to influence PMA-induced CL. These contradictory findings could be explained by the fact that the total

mononuclear cell population was incubated with MDP in our experiments. As MDP itself is a synthetic analogue of peptidoglycan, and *M. leprae* suppressed the PMA-induced ROI response when incubated with PBMC under the same conditions as MDP, we can exclude the possibility that peptidoglycan fragments present in *M. leprae* suppress the CL response.

PCA induced by monocyte thromboplastin was also used as a marker for monocyte activity. Monocytes induce an increase in PCA when activated by endotoxin, mitogens, antigens, or cytokines in the absence of lymphocytes.[27] In our study, *M. leprae* was able to induce monocyte PCA in normal individuals, a finding previously reported by Lyberg *et al.*[16] MDP also induced monocyte PCA in all individuals studied (data not shown). Surprisingly, PCA activity of monocytes, isolated from a PR patient, was significantly lower than in the other individuals studied. Perhaps the specific treatment for reaction episodes is suppressing this monocyte activity. Although PCA response to *M. leprae* and PMA was significant and comparable after 20 h of culture, we cannot exclude that PCA is more pronounced or variable between the groups studied at other time points. However, there are conflicting reports as to the time of maximal PCA after stimulation of monocytes; data ranging from 4 to 24 h have been reported.[16,28] As co-incubation of monocytes seems to sustain the PCA response, and our measurements were performed under these conditions, PCA was determined after 20 h.

There are many reports that suggest that patients with lepromatous leprosy are unable to exert a complete CMI response due to failure of their macrophages to present *M. leprae* antigens in an immunogenic form.[14] We could not find any difference in basal HLA–DR antigen expression between PBMC from normal individuals and any of the lepromatous leprosy patients. Our preliminary results indicate that stimulation with *M. leprae* has no influence on HLA–DR expression (data not shown). Comparable levels of HLA–DR expression between normal individuals, tuberculoid and long-term treated lepromatous leprosy patients were reported in another study.[29] If basal HLA–DR expression is a prerequisite for antigen presenting cells (APC) to present immunogenic *M. leprae* antigens, then, at least within this part of the complex CMI response network, APC from lepromatous patients seem to be equally functional. Native antigen presentation does not seem to be sufficient to allow induction of T-cell proliferation in response to *M. leprae*, as we found no increase in T-cell response after incubation of PBMC from lepromatous leprosy patients without reactional manifestations with *M. leprae*. It has been reported that the levels of HLA–DR expression of lepromatous patients reaches that of normal volunteers after stimulation with a combination of *M. leprae* and IFN-γ,[29] without inducing lymphoproliferation.

A T-cell proliferation response towards ConA was seen in all experimental groups studied. It has been demonstrated that the absence of T-cell responses in lepromatous leprosy patients is antigen specific.[3] Indeed, when observed, lymphoproliferation of cells from lepromatous leprosy patients after stimulation with *M. leprae* was smaller than with Con A. *M. leprae* was, however, as potent as Con A in stimulating cells from normal individuals. This is in contrast to the results of Molloy *et al.*[30] who did not observe a mitogenic response of mononuclear cells from normal individuals to *M. leprae*, which they ascribed to a generalized suppressive effect of contaminating LPS in their antigen preparation. In our study, neither treated nor untreated lepromatous leprosy patients showed a lymphoproliferative response towards *M. leprae*. The effect of treatment of lepromatous leprosy patients on CMI is still controversial; data obtained in our

laboratory demonstrate an increase in LTT after long-term treatment.[31] In contrast to this earlier study, the majority of our patients studied here were under drug treatment for only a short time. During RR, significant lymphoproliferation in response to *M. leprae* was observed. We also confirmed our earlier observations[10] that this is not the case in patients during ENL. This could be coupled with the observation of an improvement in clinical status of the patient during and after RR,[32] which probably does not occur during ENL. LTT responses were still elevated in the post-RR.

Recently, it has been proposed that the inhibitory effects of *M. leprae* for various cell functions can be correlated with the content of LPS in the preparations of *M. leprae* and its derivatives.[30] Although the LPS content in our *M. leprae* preparation might be sufficient for some of the effects observed in this study, lipids from *M. leprae* may have LPS-like activity. Sibley *et al.*[23] have demonstrated the ability of LAM to inhibit macrophage activation with a potency comparable to that of LPS. Furthermore, LPS has been shown to stimulate ROI production,[26] an activity that was not shared with our *M. leprae* preparation. Also, *M. leprae* induced a mitogenic response in mononuclear cells isolated from normal individuals and some leprosy patients. If LPS contamination in *M. leprae* preparations is involved in the suppression of mitogenic responses, it would have to be selective for cells from lepromatous leprosy patients.

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