

IMMUNOLOGICAL STATUS OF ENL (ERYTHEMA NODOSUM LEPROSUM) PATIENTS: ITS RELATIONSHIP TO BACTERIAL LOAD AND LEVELS OF CIRCULATING IL-2R

Leila M.M. VIEIRA(1), Elisabeth P. SAMPAIO, José A.C. NERY, Nadia C. DUPPRE, Edson C.A. ALBUQUERQUE,
Morton A. SCHEINBERG(2) & Euzenir N. SARNO

SUMMARY

Recent data suggest that the clinical course of reactional states in leprosy is closely related to the cytokine profile released locally or systemically by the patients. In the present study, patients with erythema nodosum leprosum (ENL) were grouped according to the intensity of their clinical symptoms. Clinical and immunological aspects of ENL and the impact of these parameters on bacterial load were assessed in conjunction with patients' *in vitro* immune response to mycobacterial antigens. In 10 out of the 17 patients tested, BI (bacterial index) was reduced by at least 1 log from leprosy diagnosis to the onset of their first reactional episode (ENL), as compared to an expected 0.3 log reduction in the unreactional group for the same MDT (multidrug therapy) period. However, no difference in the rate of BI reduction was noted at the end of MDT among ENL and unreactional lepromatous patients. Accordingly, although TNF- α (tumor necrosis factor) levels were enhanced in the sera of 70.6% of the ENL patients tested, no relationship was noted between circulating TNF- α levels and the decrease in BI detected at the onset of the reactional episode. Evaluation of bacterial viability of *M. leprae* isolated from the reactional lesions showed no growth in the mouse footpads. Only 20% of the patients demonstrated specific immune response to *M. leprae* during ENL. Moreover, high levels of soluble IL-2R (interleukin-2 receptor) were present in 78% of the patients. Circulating anti-neural (anti-ceramide and anti-galactocerebroside antibodies) and anti-mycobacterial antibodies were detected in ENL patients' sera as well, which were not related to the clinical course of disease.

Our data suggest that bacterial killing is enhanced during reactions. Emergence of specific immune response to *M. leprae* and the effective role of TNF- α in mediating fragmentation of bacteria still need to be clarified.

KEYWORDS: Leprosy, *M. leprae*; TNF- α , interleukin-2 receptor; ENL; Cytokines; Mycobacterial antigens.

INTRODUCTION

Erythema nodosum leprosum (ENL) is a serious complication of lepromatous leprosy that affects the skin which, as a result of multiple organ involvement, is

often associated with systemic manifestations. The mechanisms underlying ENL have been attributed to immune complex-mediated injury^{29, 30}. However, evi-

Leprosy Laboratory, Oswaldo Cruz Institute/Fiocruz, Avenida Brasil 4365, Manguinhos, 21045-900 Rio de Janeiro, RJ, Brazil.

(1) Pathology Department, State University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

(2) Laboratory of Diagnostic Investigation, São Paulo, SP, Brazil.

Correspondence to: Dr. Euzenir N. Sarno, Leprosy Unit, Oswaldo Cruz Foundation, Avenida Brasil 4365, Manguinhos, 21045-900 Rio de Janeiro, RJ, Brazil.

dence of transient T-cell reactivity has been observed by many authors^{8, 13}. Recent data suggest that the clinical course of leprosy is closely related to the cytokine profile released locally or systemically by the patients^{12, 18, 23}. Elevated levels of inflammatory cytokines (TNF- α , IL-1 β) in the serum^{12, 18} as well as the presence of HLA-DR antigen and adhesion molecules in the ENL lesion^{17, 24} support the hypothesis that cell-mediated immunity (CMI) contributes to the immune reactivation in these reactional patients. Nevertheless, the effect of reactions on bacterial viability is not yet clear.

In the present study, ENL patients were grouped according to the intensity of their clinical symptoms. Clinical and immunological aspects of ENL were assessed in conjunction with patients' *in vitro* immune response to mycobacterial antigens. The impact of these parameters on bacterial load was also analyzed. In addition, levels of circulating antibodies to specific components of mycobacteria and human nerve, known to be present in lepromatous leprosy¹⁰, were assessed.

MATERIAL AND METHODS

Patient population: Leprosy patients from the Leprosy Out-Patient Unit, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, were diagnosed according to the Ridley-Jopling classification¹⁴. A total of 95 multibacillary patients (62 males and 33 females), 16 to 67 years of age (mean \pm SD = 34 \pm 16), were studied. Histopathologically, the patients were classified as lepromatous leprosy (LL, n = 30) and borderline lepromatous (BL, n = 65). All were lepromin negative and had been treated for 24 months with multidrug therapy (MDT, Rifampin 600 mg and clofazimine 300 mg monthly supervised and dapsone 100 mg and clofazimine 50 mg daily unsupervised). Patients have been followed up clinically for the incidence of leprosy reaction. Fifty-one patients presented with crops of erythematous, painful, subcutaneous nodules, and were taken as ENL patients. Patients with ENL were divided into three clinical groups according to the severity of their clinical symptoms: Group I - ENL characterized only by the presence of dermal erythematous nodules, in the absence of any systemic manifestations (n = 12); Group II - ENL associated with systemic symptoms, such as fever, nerve thickening with pain, bone or muscle pain and weakness, lymphadenopathy, epistaxis, conjunctivitis, orchitis, skin rash, malaise, weight loss, leukocytosis, nasal obstruction, and edema (n = 31); Group III - This group comprised those patients with the same characteristics as described in Group II who, in addition, presented aggravating symptomatology characterized by erythema multiforme lesions, with or without bullous or necrotic aspects (n = 8). In all cases, the clinical diagnosis of ENL was confirmed by

histopathological examination. Whenever present, the systemic ENL manifestations (Groups II and III) were subsequently treated with thalidomide (300 mg/day, progressively reduced by 100 mg/week), associated or not with steroids, until total remission of the inflammatory symptoms.

Enumeration of bacilli: The bacterial index (BI) is an essential component of the diagnosis of leprosy. The BI is a microscopic estimate of bacterial burden and represents bacterial numbers of acid-fast bacilli (AFB) per 100X microscopic field (oil immersion). Slit smears for BI determination¹⁵ taken from six anatomic sites (usually earlobes, elbows, and knees) were performed at the time of diagnosis, and for their rate of reduction during the 24 months of MDT and at the onset of the reactional episode. BI is expressed on a base 10 logarithmic scale as follows: neg = absence of bacteria; 1+ = 1-10 bacteria per hundred 100X fields; 2+ = 1-10 bacteria per ten 100X fields; 3+ = 1-10 bacteria per one 100X field; 4+ = 10-100 bacteria per 100X field; 5+ = 100-1000 bacteria per 100X field; 6+ = > 1,000 bacteria per 100X field. The BI of patients tested at diagnosis ranged from 2 to 5+.

Mouse footpad assay: A 4-mm punch biopsy of reactional lesions was obtained from 11 ENL patients and used for *M. leprae* isolation. *M. leprae* recovered from each biopsy was counted, diluted and injected into both hind footpads of BALB/c mice. A total of 5 x 10³ bacilli were injected into each footpad in 0.03 ml saline, and bacterial growth was evaluated 6, 9, 12 and 15 months after footpad inoculation. *M. leprae* growth was evaluated by sacrificing the mice, isolating the bacilli from the hind footpads, and by directly counting the bacteria as described^{21, 22}.

Serum collection: Serum samples from reactional and unreactional leprosy patients were collected aseptically, processed under sterile conditions, and kept frozen (-20°C) until use. The amount of soluble interleukin-2 receptor (sIL-2R), tumor necrosis factor α (TNF- α) and circulating anti-nerve components and anti-mycobacteria antibodies were determined as described below.

Antigens: Nerve antigens named ceramide and galactocerebroside were donated by Dr. Rama Mukherjee (Department of Microbiology, National Institute of Immunology, New Delhi, India). Natural disaccharide conjugated to BSA (ND-BSA) was provided by Dr. Patrick Brennan (Department of Microbiology, Colorado State University, Fort Collins, CO). For the *in vitro* tests, armadillo-derived *M. leprae* antigen was provided by Dr. R. J. W. Rees (IMMLEP Bank, The

National Institute of Medical Research, Mill Hill, England). Optimal antigen stimulating concentration was found to be 20 µg/ml.

Lymphocyte transformation test (LTT): Heparinized venous blood was collected for in vitro tests and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density centrifugation². PBMC were incubated in triplicate at 2×10^5 cells/well at 37°C in 96-well U-bottom plates (Costar Corporation, Cambridge, MA) in 200 µl of RPMI 1640 medium (Gibco Laboratories, Grand Island, NY), supplemented with 10% pooled AB serum, 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine (Gibco Laboratories). Cells were cultured for 6 days in the presence or absence of antigen and the assay performed as described⁶. Results obtained as counts per minute (cpm) are expressed as Δ cpm (cpm obtained in stimulated cultures minus cpm obtained in control cultures).

Interferon-gamma (IFN-γ) production: The amount of IFN-γ on a 5-day culture supernatant was assayed us-

ing a commercial RIA kit (IMRX Corp., Centocor Malvern, PA), specific for the active human IFN-γ as described⁴. Levels of IFN-γ are expressed as units/ml (U/ml) in stimulated cultures minus U/ml obtained in control cultures.

TNF-α ELISA assay: TNF-α concentration in serum samples was determined using a commercial specific ELISA kit, processed according to the manufacturer's recommendation (N. V. Immunogenetics S.A., Antwerp, Belgium). Cytokine levels are expressed as pg/ml of protein. Detection limit of the assay is 4 pg/ml.

Soluble IL-2R assay: Levels of soluble IL-2R (sIL-2R) were determined using an immunosorbent assay according to the manufacturer's recommendation (Immunotech, France). Briefly, plates were coated with 150 µl of the monoclonal anti-IL-2R antibody (1 µg/ml) or in buffer alone. Following an overnight incubation at 4°C, the plates were washed and 100 µl of the samples added to the coated and control wells. After a 2 hour incubation, fluorescein isothiocyanate (FITC) was added to each well. The plates were incubated for another 2

TABLE 1
Bacterial index and serum TNF-α levels from multibacillary reactional patients evaluated at leprosy diagnosis and at the onset of the first reactional episode (ENL).

Patient Number	Diagnosis	Bacterial Index* (BI)		Time of MDT (Months)	Serum TNF-α Levels** (pg/ml)	
		Leprosy Diagnosis	ENL		Leprosy Diagnosis	ENL***
1	LL	3+	2+	4	0	0
2	BL	3+	2+	6	62.5	10,000
3	LL	4+	3+	<1	0	0
4	BL	4+	2+	3	125	260
5	LL	5+	3+	5	60	0
6	LL	4+	2+	8	0	0
7	LL	4+	2+	12	0	0
8	LL	4+	3+	23	1,240	3,300
9	BL	5+	4+	<1	-	2,300
10	BL	5+	4+	15	0	400
11	BL	2+	2+	16	300	300
12	LL	3+	3+	4	1,080	300
13	LL	3+	3+	5	900	1,320
14	LL	3+	3+	8	2,480	2,600
15	LL	4+	4+	11	140	360
16	BL	4+	4+	13	0	70
17	LL	4+	4+	17	160	1,100

* BI, number of acid fast-bacilli per 100X microscopic field: 2+, 1 to 10; 3+, 1 to 10; 4+, 10 to 100; 5+, 100 to 1,000; 6+, > 1,000.

** Cut-off values for positive response = 45 pg/ml

*** Reactional patients with negative TNF-α values comprised ENL patients with no systemic manifestation at the time of serum collection.

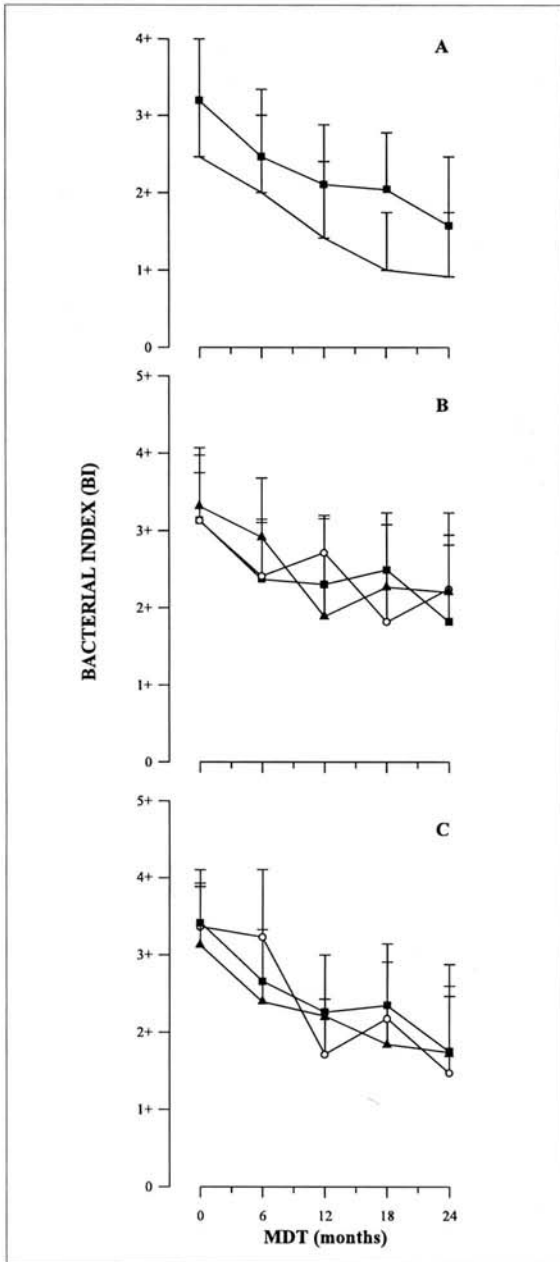


Fig. 1 - (A) Rate of BI reduction in 95 multibacillary leprosy patients along the 24 months of MDT. No difference was noted among the (□) ENL patients (n = 51, 1.65 mean log reduction) as compared to the (Δ) unreactional group (n = 44, 1.67 mean log reduction). (B) Rate of BI reduction in patients who presented with one ENL episode (□), 2 ENL episodes (Δ) and 3 ENL episodes (o). When analyzed in short MDT periods (up to 12 months), a 0.9, 1.5 and 0.5 mean log reduction was noted respectively. (C) Rate of BI reduction in ENL patients who were grouped according to the intensity of their clinical symptoms. Patients in Group I (□), Group II (Δ) and Group III (o) showed 1.75, 1.5 and 2.0 mean log reduction respectively.

hours, and 100 µl of an alkaline phosphatase-conjugated rabbit anti-FITC was added to the wells. Colour was developed using the substrate p-nitrophenyl phosphate (Sigma Chemical Co., St Louis, MO) and absorbance was then determined at 450nm.

Detection of anti-nerve and anti-mycobacterial antibodies: For the ELISA tests, 96-well U-bottom polyvinyl Titertek plates were coated either with 200 ng ceramide, 400 ng galactocerebroside (50 µl per well in 100% ethanol), or 10 mg ND-BSA antigen (in 0.2 M carbonate bicarbonate buffer, pH 9.5). Dilution of serum samples (1:100 - 1:200) were incubated for 1 hour at 37°C. The presence of antibodies was revealed with a rabbit anti-human immunoglobulin conjugated to horse-radish-peroxidase (Dakopatts, Copenhagen, Denmark). Colour was developed using the substrate orthophenylene diamine (OPD, 0.5 mg/ml) in 0.15 M citrate phosphate buffer (pH 5.0) and the absorbance determined at 490 nm in a microplate ELISA reader (EL 309, Biotek Instruments). Antibody titers were expressed as the average optical density (OD) of duplicate experimental wells subtracted from the mean OD of control wells (Δ OD).

RESULTS

BI analysis in ENL patients - Relationship to circulating TNF- α levels: Bacterial load evaluated in 17 lepromatous leprosy patients (BL/LL) at the time of ENL development showed a 0.86 mean log reduction from the leprosy diagnosis (mean \pm SD = 3.76 \pm 0.7) to the first ENL episode (mean \pm SD = 2.9 \pm 0.8), with a mean MDT period of 9 \pm 6 months, ranging from 0 to 23 months (Table 1). The expected rate of BI reduction for this period in patients under chemotherapy was a 0.6 mean log reduction, as determined in a control patient population, comprised of 44 unreactional lepromatous patients who have never developed ENL or reversal reaction. Moreover, BI was reduced by at least 1 log in 10 of the 17 ENL patients analyzed (Table 1). In 8 patients (patients with less than 15 months of chemotherapy, numbers 1-7 and 9), the decay in BI (mean of 1.5 log in 4.7 \pm 3.0 months) seemed to not be related to the duration of multidrug therapy (expected rate of reduction for this period = 0.3 log). These values indicated a possible accelerated reduction in systemic BI in these 8 patients following the development of ENL.

Presence of circulating TNF- α was also assessed in the serum of ENL patients at leprosy diagnosis and at the onset of their first reactional episode. As seen in Table 1, high levels of TNF- α (mean \pm SEM = 409 \pm 172 pg/ml) were detected in 62.5% of the patients (10 out of 16 individuals) at leprosy diagnosis, and were related,

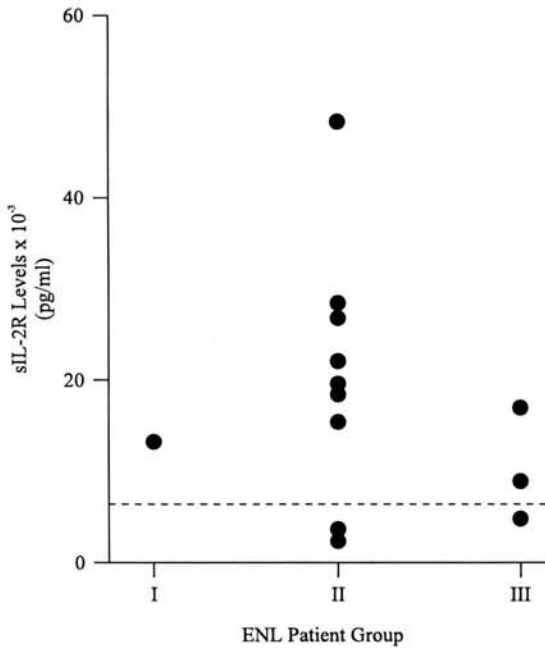


Fig. 2 – Serum levels of soluble IL-2 receptor (sIL-2R) assessed in 14 ENL patients grouped according to the intensity of their clinical symptoms (Group I, n = 1; Group II, n = 9; Group III, n = 4). Results are expressed as pg/ml of protein. Each point represents a single patient. (—) Cut-off value.

TABLE 2

Levels of sIL-2R assayed in six ENL patients with two serum samples collected along the clinical follow-up.

Patient Number	Reactional Episode	Treatment for Reaction*	sIL-2R Levels (pg/ml)
1	Before ENL	-	2,910
	ENL	-	27,295
2	ENL	P	26,422
	ENL*	P	26,073
3	ENL	P	2,045
	ENL**	-	5,325
4	ENL	P	4,145
	ENL#	-	8,862
5	ENL	P/T	11,596
	After ENL	T	7,337
6	ENL	-	14,343
	After ENL	-	6,917

Serum samples collected during the ENL episode at (*) 15 or (**) 60 days after the first sample.

Serum sample collected on a second ENL episode.

* P = Prednisone; T = Thalidomide.

as already described¹⁷, to the presence of systemic inflammatory manifestations, in the absence of full-blown clinical ENL. Accordingly, most ENL patients (70.6%) showed elevated TNF- α levels in the serum at the time of ENL development (mean TNF- α values \pm SEM = $1,312 \pm 597$ pg/ml). Patients with asymptomatic ENL (presence of few ENL nodules in the absence of systemic symptoms) had no detectable TNF- α (Table 1).

No relationship was noted between circulating TNF- α levels and the decrease in BI detected at the onset of the reactional episode. Only 50% of the patients (5/10) with lower BI showed elevated TNF- α levels in the sera as compared to 100% in the group of patients with no changes in BI.

In order to evaluate the decay in BI along the MDT period, slit smear analysis was performed regularly in ENL (51 patients) and a control unreactional lepromatous population (44 patients), at 6, 12, 18 and 24 months of MDT. No difference in the rate of BI reduction was noted at the end of MDT period between the 2 groups (1.65 and 1.67 mean log reduction respectively) (Figure 1A). When analyzed during short MDT periods (up to 12 months), reactional patients who presented with 2 ENL episodes (Figure 1B) and patients belonging to Group III (see description in the Methods section) (Figure 1C) showed a more dramatic reduction in their bacterial load as compared to the other groups.

Evaluation of M. leprae viability in a reactional leprosy lesion: Under the conditions used for inoculation²¹, the presence of 1×10^5 *M. leprae* per mouse (2 hind footpads pooled) was considered to be evidence of bacterial viability and growth. In contrast to the control samples (*M. leprae* isolated from lesions of untreated unreactional lepromatous patients), *M. leprae* isolated from ENL lesions of 11 patients showed no growth in the footpads. Inhibition of bacterial growth was also demonstrated when different numbers of bacilli were injected into the mice (not shown).

Lymphoproliferation and IFN- γ assays during a ENL episode: Immunological studies were performed during active ENL, prior to initiation of anti-reactional therapy and, in some cases, 1 to 2 weeks after thalidomide treatment, when clinical signs and symptoms of ENL had subsided. Twenty ENL patients were tested for their in vitro reactivity to *M. leprae* antigen. Four individuals (20%) showed positive lymphoproliferative response to *M. leprae* during ENL (mean Δ cpm \pm SEM = $14,063 \pm 4,157$). Moreover, the increased proliferative response seen in these patients was not present when the test was performed at leprosy diagnosis and subsided following the remission of the clinical symptoms

(not shown). Only one patient released IFN- γ (> 100 U/ml) in these cultures.

Measurement of circulating IL-2R in ENL patients sera: Presence of soluble IL-2R (sIL-2R) was assayed in the serum of ENL patients by an immunosorbent assay as described. A previous evaluation in normal individuals showed that sIL-2R is commonly detected in the sera of normal controls (mean \pm SD = 2,940 \pm 890 pg/ml, ranging from 1,050 to 4,230 pg/ml). Therefore, levels above 5,610 pg/ml (corresponding to 3 SD above mean values) were considered positive in relation to the control population. Fourteen ENL patients were tested (Figure 2). High levels of sIL-2R were detected in 11 individuals (mean \pm SEM = 15,809 \pm 3,643 pg/ml). Six patients had at least 2 serum samples collected during clinical follow-up (Table 2). In one patient, IL-2R levels detected before the reaction enhanced during the ENL episode (patient number 1, Table 2). Three ENL patients had their serum collected while in treatment with steroids. At that time, two of them showed lower amounts of IL-2R in the circulation which increased following the suspension of treatment or at the time of a second ENL episode (patients number 3 and 4, respectively, Table 2). In the other two patients (number 5 and 6), elevated IL-2R levels decreased following the reactional episode. No relationship between levels of sIL-2R and the decrease in BI during MDT was noted.

Presence of circulating antibodies in reactional patients: Serum levels of anti-ND-BSA antibodies (IgM antibodies) were enhanced in lepromatous leprosy patients (LL/BL, mean OD \pm SD = 0.75 \pm 0.06) as compared to tuberculoid patients (TT/BT, mean OD \pm SD = 0.32 \pm 0.1) or normal individuals (not shown). In addition, reactional patients (ENL, n = 22) grouped according to the intensity of their clinical symptoms were assessed. Although elevated antibody levels were found in ENL patients (mean OD \pm SD = 0.91 \pm 0.54), no difference among reactional and unreactional lepromatous patients was noted (Figure 3A).

Previous reports demonstrated increased amounts of circulating antibodies against neural antigens in leprosy patients' sera, as compared either to normal individuals or to patients who presented neuropathy not related to leprosy¹⁰. Like the lepromatous patients, ENL patients showed high levels of anti-ceramide IgM antibodies in their sera (Figure 3B). However, diminished amounts of anti-galactocerebroside antibodies were detected in reactional as compared to unreactional patients (Figure 3C).

DISCUSSION

Reactional states in leprosy constitute a serious inflammatory complication in which immunological

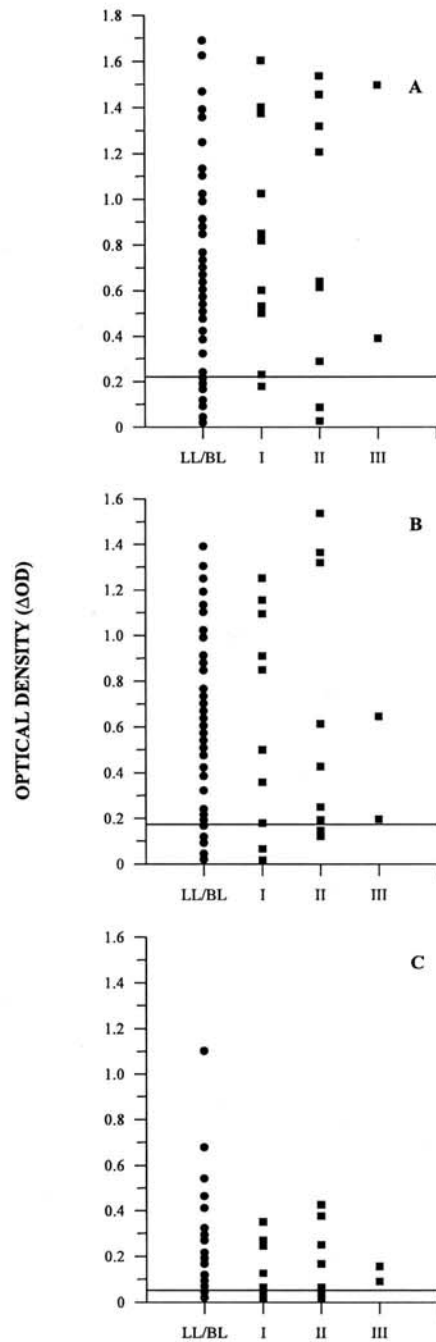


Fig. 3. Serum levels of (A) anti-ND-BSA, (B) anti-ceramide and (C) anti-galactocerebroside antibodies assayed in 44 unreactional lepromatous leprosy patients (LL/BL) and 22 ENL patients grouped according to the intensity of their clinical symptoms (Group I, n = 11; Group II, n = 9; Group III, n = 2). Antibody titers are expressed as the average optical density readings (Δ OD) for each individual. Horizontal bars represent mean OD values for each group tested. Each point represents a single patient. (-) Cut-off values.

mechanisms seem to trigger acute inflammatory episodes aggravating the silent course of the disease. The prompt beneficial effect obtained with corticosteroids and thalidomide therapy strongly suggests that these episodes are not due to the aggravation of the infection itself, but are rather the result of the generation and excessive release of host inflammatory mediators. It is well known that reactional episodes in leprosy can be triggered by several factors such as vaccination, associated virus or bacterial infections, drugs, among others. It has been recently demonstrated that local injection of IFN- γ in lepromatous patients precipitated a ENL episode in 60% of the injected patients¹⁶. This finding suggests that a transient immune reactivation could play a role in the induction of ENL.

In the present study, an attempt was made in order to correlate bacterial load to the occurrence of ENL and *in vitro* immunological response. Some constraints were detected in the course of the study. One concern is the impossibility to have available blood specimens of all patients for performing all immunological tests. This was however overwhelmed trying to perform each test in a representative number of patients for further analysis. The other constraint concerns the fact that slit smears collected during the reaction presented with large amounts of fragmented bacilli making it difficult the counting of bacteria. Thus precise BI was difficult to determine and could be even overestimated. Quantitation of viable *M. leprae* in human lesions can only be carried out *in vivo* by following the bacillus multiplication in the murine footpad²¹. So, in order to evaluate *M. leprae* viability in ENL lesions, the mouse footpad model was used. Bacilli obtained from the reactional lesions were inoculated in the mouse footpads as described by SHEPARD^{21, 22}. No growth was noted in any of the 11 patients tested. These data indicate that bacterial killing is enhanced during reactions. It was previously demonstrated that monocytes obtained from patients who had developed ENL exhibited higher respiratory burst activity as compared to unreactive patients¹⁶. Emergence of T-cell reactivity in ENL could lead to a temporary production of cytokines, induction of monocyte activation, and consequent inhibition of bacterial growth. However, only 4 out of the 20 patients tested (20%) demonstrated specific immune response to *M. leprae* during ENL. It is believed that for most of the patients, transient immunologic events must have occurred previous to the ENL episode. Detection of high levels of soluble IL-2R in 78% of the patients tested seems to confirm this hypothesis. However, the function of sIL-2R remains uncertain. The release of soluble IL-2R seems to be a characteristic marker of T lymphocyte activation and might have an important immunoregulatory function. Elevated sIL-2R serum levels have been detected in patients with

a variety of inflammatory conditions^{3, 25}, in leprosy patients with reversal reaction²⁶, as well as in lymphoid malignancies²⁸, and appear to correlate with the clinical stage disease.

Over the past 4 years, several studies have indicated that TNF- α plays a central role in granuloma formation⁷, antibacterial resistance²⁷, and in the tissue necrosis of mycobacterial disease¹. TNF- α detected in ENL patients sera confirmed previous reports^{11, 16, 18}, thereby implicating TNF- α to the clinical and histopathological findings in this syndrome. It was also demonstrated that TNF- α induces both the superoxide radical and manganous-superoxide dismutase²⁰. Therefore, the effect of TNF- α on macrophage activation in leprosy cannot be ruled out. Its effective role in bacterial killing remains to be clarified.

In this study, it was demonstrated that, similar to lepromatous patients, ENL patients present high levels of anti-PGL1 and anti-nerve antibodies in their sera, which were not directly related to the clinical course of disease. Recently, myelin basic protein (MBP) was detected in circulating immune complexes present in lepromatous sera with no association to bacterial load or to the clinical course of leprosy⁵. It seems that in chronic infections, polyclonal activation might lead to high levels of antibodies, which can also be seen in many lepromatous patients, but are not related to the pathogenesis of the disease.

RESUMO

Perfil imunológico de pacientes com ENL (Erythema Nodosum Leprosum): relação entre carga bacilar e os níveis de IL-2R circulantes

Dados recentes sugerem que o curso clínico dos estados reacionais na lepra está estritamente relacionado à liberação local ou sistêmica de citocinas. Neste estudo, pacientes com ENL (erythema nodosum leprosum) foram agrupados segundo a intensidade de seus sintomas clínicos. Os aspectos clínicos e imunológicos do ENL e os efeitos desses parâmetros na carga bacilar foram estabelecidos em conjunção com a resposta imune *in vitro* desses pacientes, a antígenos microbacterianos. Em 10 de 17 pacientes testados, o índice bacteriano (IB) foi reduzido em pelo menos 1 log desde o diagnóstico da lepra até o aparecimento do primeiro episódio reacional, comparado a uma redução esperada de 0.3 log no grupo não reacional no mesmo período de MDT (multidrogaterapia). Entretanto, nenhuma diferença na taxa de redução do IB foi notada no final da MDT entre os pacientes ENL e os lepromatosos não reacionais. Assim também, embora os níveis de TNF- α (fator de necrose tumoral) estivessem

aumentados no soro de 70,6% dos pacientes com ENL testados, nenhuma relação foi notada entre os níveis circulantes de TNF- α e a diminuição do IB detectada no aparecimento do primeiro episódio reacional. A avaliação da viabilidade bacteriana do *M. leprae* isolado de lesões reacionais não mostrou crescimento na pata de camundongo. Somente 20% dos pacientes demonstraram resposta imune específica ao *M. leprae* durante o ENL. Além disso altos níveis de IL-2R (receptor de interleucina 2) solúvel estiveram presentes em 78% dos pacientes. Anticorpos circulantes anti-nervo (anti-ceramídeo e anti-galactocerebrosídeo) e antimicobacterianos foram também detectados no soro dos pacientes, e não estiveram relacionados ao curso clínico da doença.

Nossos dados sugerem que a morte bacteriana está aumentada durante as reações. A emergência de resposta imune específica ao *M. leprae* e o papel efetivo do TNF- α na mediação de fragmentação bacteriana necessita ainda ser esclarecida.

ACKNOWLEDGEMENTS

We thank to R.B. Oliveira for technical assistance. Thanks are also due to A.R. de Moura and M.M.S.B. Pinheiro for the graphic work and E.R. Leite for efficient secretarial help. Dr. L.M.M. Vieira is a Visiting Professor at Leprosy Laboratory, Oswaldo Cruz Foundation, and a FAPERJ fellow. This study was supported in part by UNDP/World Bank/TDR-WHO.

REFERENCES

1. ATTIIYAH, R.A.; MORENO, C. & ROOK, G.A.W. - TNF- α -mediated tissue damage in mouse footpads primed with mycobacterial preparations. *Res. Immunol.*, 143:601-610, 1992.
2. BOYUM, A. - Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Invest.*, 21 (suppl.):97-105, 1968.
3. CAMPEN, D.H.; HORWITZ, D.A.; QUISMORIO JR., F.P.; EHRESMANN, G.R. & MARTIN, W.J. - Serum levels of interleukin-2 receptor and activity of rheumatic diseases characterized by immune system activation. *Arthr. and Rheum.*, 31:1358-1364, 1988.
4. CHANG, T.W.; MCKINNEY, S.; LIU, V. et al. - Use of monoclonal antibodies as sensitive and specific probes for biologically active human γ -interferon. *Proc. nat. Acad. Sci. (Wash.)*, 81:5219-5222, 1984.
5. CÔRSICO, B.; CROCE, M.V.; MUKHERJEE, R. & SEGAL-EIRAS, A. - Identification of myelin basic proteins in circulating immune complexes associated with lepromatous leprosy. *Clin. Immunol. Immunopath.*, 71:38-43, 1994.
6. KAPLAN, G.; WEINSTEIN, D.E.; STEINMAN, R.M. et al. - An analysis of in vitro T cell responsiveness in lepromatous leprosy. *J. exp. Med.*, 159:917-929, 1985.
7. KINDLER, V.; SAPPINO, A.P.; GRAU, G.E.; PIGUET, P.F. & VASSALI, P. - The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. *Cell*, 56:731-740, 1989.

8. LAAL, S.; BHUTANI, L. K. & NATH, J. - Natural emergence of antigen-reactive T cells in lepromatous leprosy patients during erythema nodosum leprosum. *Infect. Immun.*, 50:887-892, 1985.
9. MEDEIROS, G.N.; CAVALIERI, H.; KNOBEL, M. et al. - Thyroid autoantibodies and soluble interleukin-2 receptor levels in patients with active and inactive acromegaly. *Endocr. J.*, 2:289-295, 1994.
10. MUKHERJEE, R.; THOMAS, B.M.; VEMURI, N. & TALWAR, G.P. - Nerve antigen based serological tests for the diagnosis and prognosis of leprosy. *Trop. Med. Parasit.*, 41:357-358, 1991.
11. PARIDA, S.K.; GRAU, G.E.; ZAHFER, S.A. & MUKHERJEE, R. - Serum tumor necrosis factor and interleukin 1 in leprosy and during lepra reactions. *Clin. Immunol. Immunopath.*, 62:23-27, 1992.
12. PISA, P.; GENNENE, M.; SODER, O. et al. - Serum tumor necrosis factor levels and disease dissemination in leprosy and leishmaniasis. *J. infect. Dis.*, 161:988-991, 1990.
13. RAO, R.D. & RAO, P.R. - Enhanced cell-mediated immune response in erythema nodosum leprosum reactions of leprosy. *Int. J. Leprosy*, 55:36-41, 1987.
14. RIDLEY, D.S. & JOPLING, W.H. - Classification of leprosy according to immunity. A five group system. *Int. J. Leprosy*, 34:255-273, 1966.
15. RIDLEY, D.S. - Bacterial indices. In: COCHRANE, R.G. & DAVEY, T., ed. *Leprosy in theory and practice*. 2. ed. Bristol, John Wright & Sons, 1964. Appendix III, p. 612-622.
16. SAMPAIO, E.P.; MOREIRA, A.L.; SARNO, E.N.; MALTA, A.M. & KAPLAN, G. - Prolonged treatment with recombinant interferon- γ induces erythema nodosum leprosum in lepromatous leprosy patients. *J. exp. Med.*, 175:1729-1737, 1992.
17. SAMPAIO, E.P.; KAPLAN, G.; MIRANDA, A. et al. - The influence of thalidomide on the clinical and immunological manifestation of ENL. *J. infect. Dis.*, 168:408-414, 1993.
18. SARNO, E.N.; GRAU, G.E.; VIEIRA, L.M.M. & NERY, J.A. - Serum levels of tumour necrosis factor-alpha and interleukin-1 β during leprosy reactional states. *Clin. exp. Immunol.*, 84:103-108, 1991.
19. SCHEINBERG, M.A.; IKEJIRI, M.; SILVA, H.M. & MOTA, M.I. - Interleukin-2 receptor membrane and in soluble form in the aqueous humor and peripheral blood of patients with acute untreated uveitis. *J. Reum.*, 29:1362-1363, 1962.
20. SCHULZE-OSTHOFF, K.; BAKKER, A.C.; VANHAESEBROECK, B. et al. - Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. *J. biol. Chem.*, 267:5317-5320, 1993.
21. SHEPARD, C.C. - The experimental disease that follows the injection of human leprosy bacilli into footpads of mice. *J. exp. Med.*, 112:445-454, 1960.
22. SHEPARD, C.C. & McRAE, D.H. - *Mycobacterium leprae* in mice: minimal infectious dose, relationship between staining quality and infectivity and effect of cortisone. *J. Bact.*, 89:365-372, 1965.
23. SILVA, C.L. & FOSS, N.T. - Tumor necrosis factor in leprosy patients. *J. infect. Dis.*, 159:787-790, 1989.
24. SULLIVAN, L.; SANO, S.; PIRMEZ, C. et al. - Expression of adhesion molecules in leprosy lesions. *Infect. Immun.*, 59:4154-4160, 1991.
25. SYMONS, J.A.; WOOD, N.C.; DI GIOVINE, F.S. & DUFF, G.W. - Soluble IL-2 receptor in rheumatoid arthritis. Correlation with disease activity, IL-1 and IL-2 inhibition. *J. Immunol.*, 141:2612-2618, 1988.

26. TUNG, K.S.K.; UMLAND, E.; MATZNER, P. et al. - Soluble serum interleukin 2 receptor levels in leprosy patients. *Clin. exp. Immunol.*, **68**:10-15, 1987.
27. VOGELS, M.T.E.; HERMSEN, C.C.; HUYS, H.L.P.; ELING, W.M.C. & MEER, W.M. - Role of tumor necrosis factor alpha, granulocyte-macrophage colony-stimulating factor, platelet-activating factor, and arachidonic acid metabolites in interleukin-1 - induced resistance to infection in neutropenic mice. *Infect. Immun.*, **62**:2065-2070, 1994.
28. WASIK, M.A.; SIOUTOS, N.; TUTTLE, M. et al. - Constitutive secretion of soluble interleukin-2 receptor by human T cell lymphoma xenografted into SCID mice. Correlation of tumor volume with concentration of tumor-derived soluble interleukin-2 receptor in body fluids of the host mice. *Amer. J. Path.*, **144**:1089-1097, 1994.
29. WATERS, M.F.R.; TURK, J.L. & WEMAMBU, S.N.C. - Mechanisms of reactions in leprosy. *Int. J. Leprosy*, **39**:417-428, 1971.
30. WEMAMBU, S.N.C.; TURK, J.L.; WATERS, M.F.R. & REES, R.J.W. - Erythema nodosum leprosum: a clinical manifestation of the arthus phenomenon. *Lancet*, **2**:933-935, 1969.

Recebido para publicação em 25/09/1995
Aceito para publicação em 14/02/1996