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Serum levels of tumour necrosis factor-alpha and interleukin-1β during leprosy reactional states

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SUMMARY

The possible role of cytokines in leprosy reactions was investigated by analysing the levels of tumour necrosis factor (TNF) and interleukin-1 (IL-1) in serum samples from 39 leprosy patients, 22 of them presenting either type I (upgrading) or type II (ENL) reactions. Fifty per cent of the patients showed elevated concentrations of TNF and IL-1 in at least one of the serum samples tested. This included all four patients undergoing type I reversal reaction and nine (50%) of the ENL patients studied. Concentrations of TNF above 1000 pg/ml were found in four patients with ENL. Development of erythema multiforme in these ENL patients represented an aggravating factor and all four patients suffering from this type of lesion demonstrated increased serum TNF levels. All BT patients tested presented elevated IL-1 levels, while only half of them presented elevated levels of TNF. No correlation was found between any particular systemic symptoms and the levels of TNF and IL-1. These results suggest that TNF and IL-1 may be implicated in leprosy reactions, either acting directly or in synergism with other cytokines.

Keywords cytokines leprosy reactions nerve damage corticosteroids thalidomide

INTRODUCTION

Leprosy is a chronic inflammatory disease caused by an obligate intracellular pathogen and characterized by a broad spectrum of clinical forms which depend in part on the host's immune response. Lepromatous leprosy patients (LL/BL forms) are characteristically unresponsive to challenge with *M. leprae* antigen(s), in contrast to tuberculoid (TT/BT) patients. The immune responsiveness correlates with the ability of the host's immune system to limit the spread of infection (Godal, 1978; Nogueira *et al.*, 1983). However, leprosy is not a stable disease in any of its clinical forms, and, depending on the studies, 15% (Sehgal, 1987), to 50% (Vásquez-Botet & Sanchez, 1987), of all leprosy patients have reactions that are acute, episodic inflammatory states unrelated to secondary infections. These reactional states are classified as either type I (reversal reactions) or type II (erythema nodosum leprosum, ENL) (Jopling, 1959; Bjune, 1983), in view of the clinical characteristics of the acute episode and its immune background.

The precipitating factors in 'reactions' and the physiopathological mechanisms involved both remain ill defined. Many studies have indicated that alterations occur in the cellular immune response(s) in type I reactions, which often result in improvements within the clinical spectrum (Laal, Mishra & Nath, 1987). Some recent studies have also referred to a contribution made by cellular immunity during ENL (Rao & Rao, 1987), which are in disagreement with earlier reports which attributed a significant role to the presence of pathogenic immune complexes in ENL (Sehgal, 1987).

Cytokines have been demonstrated to play a role in the inflammatory response initiated by infections or trauma.

Interleukin-1β (IL-1β) and tumour necrosis factor-alpha (TNF-α), which mediate both local and systemic effects accompanying many diseases, are produced rapidly by monocytes and macrophages in response to a number of stimuli and are able to induce wide-ranging changes in a variety of cells. Recently it has been indicated that relatively few cytokines are capable of mediating the status of diseases which bear no obvious pathophysiological relationship to each other, such as Gram-negative sepsis (Beutler & Cerami, 1987), cancer cachexia (Oliff, 1988), cerebral malaria (Grau *et al.*, 1989c) and other parasitic diseases (Scuderi *et al.*, 1986). TNF-α and IL-1β are among the most widely studied cytokines.

Both cytokines are monocyte/macrophage-derived mediators, although it is now known that virtually all nucleated cell types are capable of producing IL-1α (Billingham, 1987). First described as tumouricidal and cachexia promotor, TNF overlapping with IL-1 in many of its inflammatory properties (Grau

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et al., 1989a). There is increasing evidence that both cytokines primarily involved in host defence are also involved in the pathogenesis of various manifestations of human inflammatory diseases, as well as mediating certain experimental tissue lesions such as induced granuloma and graft-*versus*-host reaction (Piguet *et al.*, 1987; Kasahara *et al.*, 1989; Kindler *et al.*, 1989). Serum cytokine levels have been evaluated in many clinical situations and range from being highly beneficial (Scuderi *et al.*, 1986; Blanchard *et al.*, 1988) to extremely toxic (Grau *et al.*, 1987; Waage, Halstensen & Espevik, 1987).

Studies concerning TNF levels in sera from leprosy patients have frequently been open to controversy. For example, Silva & Foss (1989), by way of a cytotoxic bioassay, reported that 75% of tuberculoid patients tested showed 280–340 TNF U/ml whereas all lepromatous patients were considered to be within the normal range (<60 U/ml). On the other hand, the ELISA method (which does not reflect biological activity) showed sera of (multibacillary) lepromatous patients to contain higher TNF levels than those found in pacibacillary forms (Pisa *et al.*, 1989).

Serum studies of IL-1 levels in leprosy patients have not been reported to date. However, *in vitro* studies with adherent cells derived from lepromatous patients did not demonstrate production of IL-1 levels above normal ranges, in contrast to cells from certain tuberculoid patients, which were able to produce IL-1 spontaneously (Watson *et al.*, 1984).

Here we have quantified the TNF and IL-1 levels in the serum of leprosy patients during different types of reactional states and have demonstrated that IL-1 is regularly produced during ENL and that the observed TNF concentrations greatly exceeded previously reported levels in other disease states.

PATIENTS AND METHODS

Study population

Thirty-nine patients classified according to the Ridley & Jopling scale (Ridley & Jopling, 1966) were included in the study. All patients surveyed were from the out-patient unit of the Leprosy Department of the Oswaldo Cruz Foundation. Nineteen were borderline lepromatous (BL, BL/BB), 14 were lepromatous (LL) and six borderline tuberculoid (BT) patients. Twenty-two (12 BL or BL/BB, and 10 LL) were diagnosed based on clinical and laboratory tests as undergoing a 'reaction'. Eighteen presented tender crops of erythematous subcutaneous nodules and were considered to be in ENL, whereas four were found to have an upgrading type I reaction. Eleven lepromatous and six borderline tuberculoid (BT) patients with no evidence of typical reversal reaction or ENL were included. All lepromatous patients except eight were under treatment on the following regimens: (i) MDT, monthly doses of 600 mg rifampicin and 300 mg clofazimine under supervision for a 24-month period; and daily self-administered doses of 100 mg dapsone plus self-administered doses of 100 mg clofazimine to be taken every other day during the same 24-month period; (ii) national regimen: rifampicin 600 mg daily self-administered for 3 months and 100 mg of dapsone daily for 5 years; and (iii) for paucibacillary patients, MDT, monthly doses of 300 mg of rifampicin under supervision for 6 consecutive months; daily self-administered doses of dapsone for the same 6-month period. Treatment duration varied from 0 to 25 months with a mean of $12{\cdot}10 \pm 8{\cdot}77$ months.

For study purposes, the total number of patients in ENL were distributed into three clinical groups according to the degree of severity of their clinical symptoms at the moment of serum collection for cytokine assays.

Group I. ENL characterized by the presence of dermal erythematous nodules alone or simultaneous with one of the constitutional symptoms described below in group II ($n=6$).

Group II. ENL accompanied by systemic involvement comprised of the following symptoms: fever, shivering, nerve thickening with pain, bone pain, muscle pain and weakness, lymphadenitis, epitaxis, eochymosis, conjunctivitis, epididymoorchitis, skin rash, mental stress, sleepiness, malaise, weight loss, leucocytosis, nasal obstruction and edema. Nodule ulceration occurred in some patients ($n=8$).

Six patients were under prednisone and/or thalidomide treatment for current reactional episodes or for previous episodes which had occurred within the previous 2 months.

Group III. This group comprised those patients with the same characteristics described in group II which in addition presented aggravating feature characterized by erythema multiforme—purplish iris lesions manifesting simultaneous bullous or necrotic aspects ($n=4$). Two patients experienced these manifestations before initiation of chemotherapy.

Two other patients were under prednisone medication at the time of bleeding. Reactional episodes occurring at the moment of serum collection were labelled 'last episode'.

Serum samples

Serum samples from leprosy patients and normal volunteers (Brazilian blood donors) were collected in the course of routine laboratory testing. Serum samples were immediately frozen ($-20°C$) after being drawn and stored at the same temperature for up to 10 months. Most serum samples were not frozen and thawed more than once. All assays were performed blind with no prior knowledge of the patients' clinical status.

TNF and IL-1 assays

TNF and IL-1 levels were determined by using immunoradiometric assays (IRMA) (Medgenix, Fleurus, Belgium), as previously described (Grau *et al.*, 1989a). Briefly, tubes were coated using a combination of several monoclonal antibodies (oligoclonal system) recognizing distinct epitopes of the relevant cytokine, the samples were added and revealed by a ^{125}I-labelled anti-cytokine antibody. The sensitivity limits were 15 pg/ml for TNF and 50 pg/ml for IL-1, respectively (Fig. 1).

RESULTS

In total, 47 serum samples from Brazilian leprosy patients were assayed for TNF and 39 for IL-1 levels. Elevated TNF levels (i.e. >30 pg/ml) were found in 21 cases and for IL-1 (i.e. >150 pg/ml) in 25 cases. However, there was no statistical correlation between the levels of these two cytokines ($r=0{\cdot}12$).

The TNF levels in the normal volunteers ($n=20$) were in the range from 0 to 15 pg/ml.

Borderline (BT, BB) leprosy patients

Serum TNF and IL-1 levels were measured in non-lepromatous patients including six BT patients and four BB and BB/BL patients in type I reaction (Table 1). High levels of TNF were observed in two BT patients, 6 and 12 months after completion

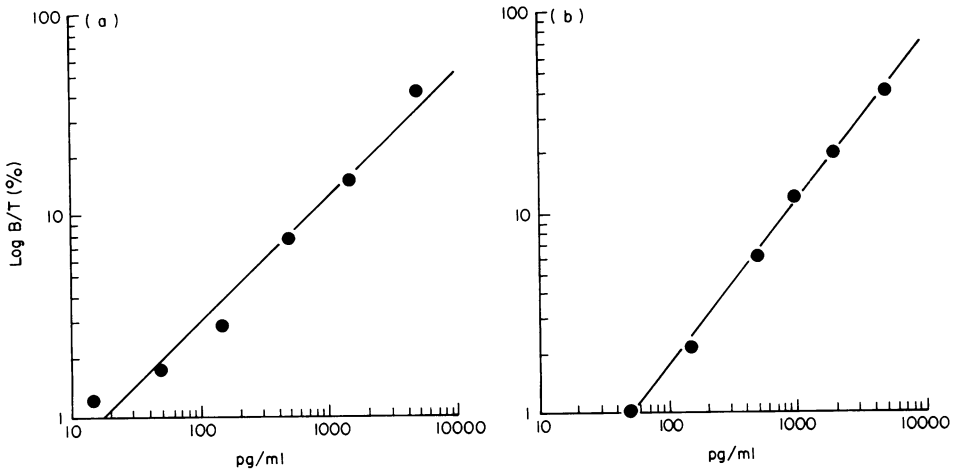

Fig. 1. Standard curves for TNF (a) and Il-1 (b) IRMA. B, bound ct/min; t, total ct/min. Background ct/min in the absence of cytokine.

Table 1. Serum cytokine levels in leprosy patients

Patient no. (BT, BB/BL)	Sex	Age (years)	BI	Treatment (months)	TNF (pg/ml)	IL-1 (pg/ml)
BT patients without reaction						
1	M	15	0	2	0	340
2	F	51	0	6	0	310
3	F	29	0	4*	50	360
4	F	56	1	6*	0	520
5	F	24	0	6*	270	220
6	F	45	0	12*	530	560
BB/BL patients in type I reaction						
7	M	69	3	21	64	5320
8	M	23	2·5	0	60	100
				5	64	160
9	M	38	2	2	96	80
10	M	18	2	21	170	—
BL and LL patients without reaction (type I and II)						
11†	M	65	3·5	18	0	140
12†	M	17	3	1	15	420
13	M	61	2	0	<15	—
14	F	17	3	0	30	—
15	M	56	2	13	<15	—
16	M	34	3	18	50	—
17†	M	30	3	0	20	—
18	M	26	3	0	<15	—
19†	F	53	4	2	20	—
20	M	48	4	0	1100	120
21	F	16	3	10	1250	260

* Number of months under surveillance after completion of 6 months of MDT.

† Lepromatous leprosy patients.

BI, bacteriological index, average of six sites.

of MDT. In contrast, TNF was undetectable in the two BT patients still under treatment. All BT patients tested who were not in reaction showed elevated serum levels of IL-1, irrespective of duration of chemotherapy. One out of the four BB patients in type I reaction had markedly elevated serum levels of IL-1 (5320 pg/ml).

Lepromatous (BL, LL) leprosy patients

The clinical features of BL and LL patients with ENL are presented in Table 2. These patients were subdivided into three main groups, according to the number and the severity of clinical complications, and serum cytokine levels were determined. The levels of TNF observed in 25 serum samples from

Table 2. Characteristics of leprosy patients studied who presented with erythema nodosum leprosum

Patient no.	Sex	Age (years)	Diagnosis	BI	Regimen	Treatment duration (months)	Clinical group	Number of episodes	Duration of last episode (months)
22	M	33	LL	4	MDT	24	I	3	2
23	M	23	LL	4	MDT	18	I	3	<1
24	F	60	BL	3·5	National	13	I	2	2
25	F	64	BL	3	MDT	6	I	1	<1
26	M	22	BL	5	MDT	15	I	1	3
27	F	52	LL	3·5	National	8	I	3	<1
28	M	28	LL	2	National	18	II	1	<1
29	M	45	BL	3	National	1	II	1	<1
30	M	37	BL	3	National	8	II	2	2
31	M	18	LL	3·5	National	24	II	3	<1
32	F	38	LL	3	National	25	II	3	<1
33	M	32	BL	1	National	1	II	1	<1
34	M	24	LL	2·5	National	13	II	2	2
35	F	26	LL	2	MDT	11	II	3	2
36	M	31	BL	3	National	14	III	3	8
37	M	58	LL	4	National	10	III	1	3
38	M	28	LL	2	None	0	III	2	<1
39	M	40	BL	3·4	None	0	III	2	<1

BI, bacteriological index, average of six sites.

those patients undergoing ENL varied widely, ranging from undetectable (six samples) to extremely high levels (four samples from 1000 to 5000 pg/ml). There was no obvious correlation between severity of ENL and cytokine levels (Table 3), although the four patients with TNF levels exceeding 1000 pg/ml did belong to groups II and III, i.e. with the most severe type of complications (see Patients and Methods). Similarly, IL-1 levels were widely scattered, although the two highest values (2300 and 13 540) were seen in patients with most severe clinical status. In addition, neither TNF nor IL-1 levels appeared to correlate with the number or duration of recorded ENL episodes.

However, cytokine levels did appear to be lower in patients under treatment for reaction with either steroids or thalidomide; in the majority of treated patients TNF but not IL-1 levels remained within normal range. Indeed, markedly elevated TNF levels were seen primarily in untreated patients.

Nine of eleven lepromatous patients who were chosen to constitute a non-reactional control group since no ENL or reversal reactions had been diagnosed showed low levels of TNF. Two demonstrated high TNF levels with moderate to low IL-1 levels (Table 1). One patient manifested painful acute oedema of the left hand and arm and the remaining patient had a typical neuritic crisis at the time of bleeding.

DISCUSSION

This study clearly links, for the first time, elevated concentrations of TNF and IL-1 with reactional episodes in leprosy. The most significant finding here is that TNF concentrations exceeding 5000 pg/ml were observed in the absence of a life-threatening pathological condition(s). Recently, Waage *et al.* (1989) have reported that TNF and IL-1 are released into the serum of septic shock patients, in addition to interleukin-6 (IL-6).

The presence of markedly elevated levels of TNF and IL-1 in the absence of a life-threatening condition, as well as the discrepancy observed in some sera between immunoreactive and bioactive cytokines, suggest that leprosy reactions are accompanied by an increased production of TNF and IL-1 inhibitors, which have been recently identified (Engelmann, Novick & Wallach, 1990; Arend *et al.*, 1990; Seckinger *et al.*, 1990). This possibility is under current investigation (manuscript in preparation).

All patients whose sera were tested during the clinical pattern of erythema multiforme (group III) showed high levels of TNF and the highest levels of IL-1 seen in this study (Table 3). The pathophysiology of these lesions is not fully understood, but small dermal vessel involvement is evident. It is well known that TNF alters vascular endothelial cells to provide an effective surface for thrombotic phenomena and ensuing coagulopathies (Nawroth & Stern, 1986; Nawroth *et al.*, 1986). TNF also enhances aggregation and adherence of polymorphonuclear leucocytes, initiating inflammatory events directly or through the stimulation of endothelial cell IL-1 generation (Libby *et al.*, 1986; Kapp, Zech-Kapp & Blohm, 1989). IL-1 exerts similar effects on endothelial cells (Billingham, 1987). Recently, this cytokine has been implicated in the pathogenesis of systemic necrotizing vasculitis (Girardin *et al.*, 1988; Grau *et al.*, 1989b). The role of these cytokines in the localized vascular lesions observed either in ENL or erythema multiforme or even in Lucio's phenomenon (a reaction characterized by generalized vascular damage) remains to be determined. Reactional episodes in leprosy traditionally have been treated with corticosteroids and/or thalidomide. However, steroids have a limited efficacy in many patients with acute symptoms of ENL.

Human monocyte secretion of TNF-α is sensitive to glucocorticoid inhibition, but an optimal level of blockade in the cytokine secretion is achieved only if the drug is present during

Table 3. Serum levels of TNF and IL-1 in lepromatous patients during erythema nodosum leprosum (ENL)

Clinical group	Patient no.	Treatment for reaction	TNF (pg/ml)	IL-1 (pg/ml)
I	22	None	40	270
	23	None	0	410
	24	None	70	120
	25	None	90	190
	26	None	0	240
	27	None	15	120
II	28	None*	25	290
	28	None	20	300
	29	None	> 5000	450
	30	Prednisone	0	70
	31	Thalidomide	0	230
	32	Prednisone	0	370
	33	Both	15	100
	34 (0 time)	Prednisone	20	0
	34 (1 month)	Thalidomide	25	530
	34 (2 month)	Thalidomide	80	110
	35 (0 time)	Prednisone	25	110
	35 (2 month)	None	2600	2300
III	36	Prednisone†	0	140
	36	Prednisone‡	260	410
	37	Prednisone‡	350	13 540
	37 (6 month)	None§	1000	13 430
	38	None‡	880	120
	38 (2 month)	None§	30	150
	39	None‡	> 5000	210

* Before ENL appeared.
† Prednisone given 12 days after initial serum sample taken.
‡ Episode with erythema multiforme lesions.
§ Second episode of ENL.

the first moments following the contact between the monocyte and the stimulus for TNF-α secretion (Debets *et al.*, 1989). This observation is consistent with the observations that glucocorticoids inhibit TNF-α gene transcription as well as post-transcription steps in TNF synthesis (Sariban *et al.*, 1988). This effect of corticosteroids might explain their limited clinical efficacy in some cases of ENL. Steroids exhibit a dual effect on IL-1 production. On the one hand, an anti-inflammatory effect of corticosteroids blocks IL-1 production in tissues, and on the other hand, glucocorticoids induce the expression of IL-1 receptors on many cells (Larrik, 1989). It would be interesting to evaluate non-steroid drugs with similar properties for their ability to control leprosy reactions. Thalidomide has an excellent effect in controlling the acute symptoms of ENL. No information exists to date concerning the potential effect of thalidomide on any inflammatory cytokines, although it has been reported that thalidomide is able to decrease the T helper : T suppressor cell ratio in healthy individuals (Gad *et al.*, 1985). It cannot be ruled out that thalidomide might interfere with TNF or IL-1 production, either by blocking their release or by blocking its effect on at least some of the target cells. This possibility is under study. The possibility of thalidomide and/or corticoids having an effect on TNF production but not on that IL-1 is suggested by the fact that among the patients of groups II and III, low levels of TNF were detected when one of these drugs were taken. In two patients (Table 3, patients no. 35 and 37) withdrawal of the prednisone was associated with increased TNF levels in the sera.

It has not yet been possible to establish a direct correlation between concentrations of TNF and IL-1 with any particular clinical symptomatology. Neuropathological symptoms, however, were frequently manifested during the reactions and in 50% of the patients, the re-evaluation of disabilities after ENL episodes demonstrated a clear deterioration. The complex symptomatology seen in ENL therefore strongly suggests that inflammatory cytokines can be an important mediator and may be involved in the progression of the lepromatous deformities. In this connection Selma & Raine (1988) recently demonstrated that TNF but not interferon-gamma (IFN-γ) or interleukin-2 (IL-2) produced demyelination accompanied by the selective necrosis of oligodendrocytes, using organotypic cultures of mouse spinal cord. The effects of TNF or other cytokines in relation to peripheral nerve damage need to be clarified.

In this study nine lepromatous patients that had never presented any features of reactional episodes showed low levels of TNF. However, two lepromatous patients (although without typical reactional episodes) revealed elevated concentrations of TNF. These two patients demonstrated clinical abnormalities during sample collection, such as neuritis and noticeable painful oedema of the left arm and hand, signs which might well be related to a local effect(s) of this particular cytokine. All BT patients showed high levels of IL-1 which did not correlate with the observed levels of TNF. The interpretation of these data with respect to the cellular immunity directed against *Mycobacterium leprae* in this form of leprosy depends upon further studies. The patients undergoing type I reversal reactions in this study appear to form a fairly homogeneous group. All but one had markedly elevated serum levels of IL-1 and all showed moderately elevated levels of TNF. This may suggest that production of these cytokines occurs concomitantly, in addition to other mediators produced during the cellular immune reactivity observed during reversal reactions.

The cytokine serum levels observed in our leprosy patients were in apparent disagreement with previous observations. Two major aspects could explain this discrepancy. Firstly, the data presented here discriminated accurately the patients in the course of reactional states from those without this clinical condition. Secondly, the groups analysed here showed heterogeneity in the course of the disease even in the reactional state group. The response to anti-inflammatory treatment and the presence of cytokines inhibitors might explain part of this heterogeneity.

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