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Lepromatous leprosy patients show T helper 1-like cytokine profile with differential expression of interleukin-10 during type 1 and 2 reactions

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SUMMARY

Some leprosy patients suffer from clinical episodes associated with tissue damage which are designated as Type 1 (reversal reaction) when localized to the lesions and Type 2 (erythema nodosum leprosum, ENL) when accompanied by systemic involvement. We had reported earlier that stable, non-reaction lepromatous leprosy subjects show T helper 2 (Th2)- and Th0- but not Th1-like responses in the peripheral blood. To further understand the development of Th-like responses during disease, 32 lepromatous patients undergoing reactions were studied using cytokine-specific reverse transcription–polymerase chain reaction (RT–PCR) and enzyme-linked immunosorbent assay (ELISA) in peripheral blood and some skin biopsies. Of interest was the evidence of a Th1-like response with presence of interferon-γ (IFN-γ) and absence of interleukin-4 (IL-4) mRNA in the peripheral blood mononuclear cells (PBMC) of 85 and 64% of Type 1 and 2 reaction patients, respectively, and in all reaction sites. Whereas a Th0- was seen in some, a Th2-like response was absent. IL-12p40 mRNA was seen in 21/25 ENL and all Type 1 reaction subjects irrespective of the Th phenotype. IL-12p40 and IFN-γ were detectable in unstimulated PBMC suggesting an *in vivo* priming during reactions. IL-10 was mainly associated with adherent cells and showed a differential expression in the two reactions. It was present in the PBMC of ENL but not in reversal reaction patients. Moreover, it was not detectable in the skin lesions of either type of reactions. A Th1-like cytokine profile was associated with immunopathology and persisted up to 6–7 months after the onset of reactions.

INTRODUCTION

Cytokines have emerged as powerful mediators shaping the immune responses of the host to microbial invasion. Both cell-mediated and humoral responses are linked to subtypes of T cells with defined cytokine profiles.[1,2,3] T helper 1 (Th1) cells responsible for the former, are polarised to produce interleukin 2 (IL-2), and interferon-γ (IFN-γ) but not IL-4, whereas Th2 cells which assist in antibody responses have the converse profile. In contrast, Th0 cells, whose role is less clear, produce a wide range of cytokines.[4] Anergy appears to be associated more with a lack of Th1 function. Leprosy is a paradigm for the understanding of human immune responses to intracellular pathogens,[5] presenting not only as a clinicopathological[6] but also an immunological spectrum ranging from the anergic generalized lepromatous form to the more localized tuberculoid form where T-cell responses to the pathogen *Mycobacterium leprae* can be elicited *in vivo* and *in vitro*.

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Leprosy has a relatively benign clinical course where the majority of individuals do not report systemic symptoms. However, a subgroup of patients suffer from clinical episodes designated as Type 1 (reversal) or Type 2 (erythema nodosum leprosum, ENL) reactions. These episodes may present as clinical emergencies requiring immediate attention.

Type 1 is seen mainly in borderline leprosy and is limited to the lesions whereas ENL affects lepromatous leprosy patients with systemic involvement such as fever, joint pains and dermal nodules.[7] Reversal reactions have been mainly linked to delayed-type hypersensitivity, whereas, ENL reflects both enhanced B-cell[5] as well as a transient peripheral T-cell activation[8] with entry of $CD4^+$ cells into the previously lymphopenic lesions.[9,10]

There have been varied reports on the status of Th subsets in leprosy. Both polarized, mixed, as well as varying combinations of Th1- and Th2-like cytokines have been described in the lesions and in the peripheral blood.[11–16] In our studies, half of all leprosy types had a Th0-like cytokine profile and the others polarized Th cell differentiation, where lepromatous leprosy was associated with Th2-like and tuberculoid leprosy with Th1-like cytokine profile.[12] In order to understand the immunological perturbations and the development of polarized

Th subsets in leprosy, we investigated patients undergoing active Type 1 and 2 reactions. Our studies show that during the reactional state lepromatous subjects show a Th1-like cytokine profile, both in circulation and at the reaction site in the skin.

MATERIALS AND METHODS

Subjects

Thirty-two Type 1 and 2 reaction lepromatous patients[6] attending the Hansen's disease clinics of the Leprosy Mission Hospital at Shahadra and Safdarjung Hospital, New Delhi were included in the study (Table 1). All subjects were on antileprosy multidrug therapy consisting of 100 mg dapsone daily, 100 mg clofazamine on alternate days and 600 mg rifampicin monthly. At the time of testing some patients were also on antireaction treatment consisting of steroids or thalidomide.

Antigens

Integral armadillo-derived killed *M. leprae* was kindly provided by Dr R. J. W. Rees through the IMMLEP programme of the World Health Organisation and was used at a concentration of 1×10^7 bacilli/ml.

Isolation of cells for cytokine mRNA

Cytokine mRNA was isolated from peripheral blood as well as skin biopsies as described earlier[12] and cultured in duplicate at 4×10^6 cells/ml/well in 24-well plates in RPMI-1640 (Gibco BRL, Gaithersberg MD) +10% AB serum with or without 10^7 integral *M. leprae* at 37° in 5% CO_2+humidified air for 24 hr.

Adherent cells (AC) were obtained from parallel cultures from the same individuals by plating 4×10^6 PBMC/ml/well for 2 hr under the above conditions. The non-adherent cells (NAC) were removed, their concentration adjusted to 2×10^6 cells/ml and redistributed in fresh wells. The wells containing AC were washed twice and supplemented with fresh medium.

Table 1. Clinical details of patients in leprosy reactions

No	Age (years)	Sex	Leprosy type	Duration (months)	Steroids	No. of episodes	Other
A. Erythema nodosum leprosum (Type 2)							
1	45	M	LL	2	(–)	single	active
2	35	F	BL-LL	6	(–)	multiple	active
3	75	M	LL	2	(–)	multiple	active
4	35	F	BL-LL	<1	(–)	single	active
5	30	F	BL	6	(–)	multiple	subsiding
6	29	M	BL	1	(–)	single	active
7	21	M	LL	2	(–)	single	active
8	75	M	LL	<1	20 mg	multiple	neuritis
9	35	F	LL	<1	20 mg	multiple	active
10	26	F	LL	<1	20 mg	multiple	active
11	38	M	LL	0·5	(–)	single	active
12	40	M	LL	11	20 mg	multiple	neuritis
13	18	M	BL-LL	6	(–)	multiple	active
14	27	M	BL-LL	6	5 mg	multiple	histoid
15	32	M	LL	12	(–)	multiple	active
16	37	M	LL	2	10 mg	multiple	thalidomide
17	40	M	BL-LL	2	20 mg	multiple	active
18	50	M	LL	2	(–)	single	active
19	31	M	LL	1	(–)	single	active
20	18	M	BL	4	(–)	multiple	active
21	35	F	LL	1	(–)	single	active
22	40	M	BL	1	(–)	multiple	thalidomide
23	38	M	LL	2	10 mg	single	active
24	22	F	BL	1	40 mg	single	active
25	30	M	BL-LL	1	(–)	single	active
B. Reversal reaction (Type 1)							
26	26	M	BL	6	(–)	single	subsiding
27	35	F	BL	7	(–)	multiple	active
28	35	M	BL	6	(–)	single	active
29	22	M	BL	1	(–)	single	active
30	25	M	BL	<1	40 mg	single	active
31	42	F	BL	1	(–)	single	active
32	30	M	BL	5	20 mg	single	active

BL; LL: borderline and polar lepromatous leprosy.
Duration refers to the onset of reaction.

Duplicate cultures of equal numbers of peripheral blood mononuclear cells (PBMC), AC and NAC were cultured as above. Culture supernatants were collected and stored at −20° for cytokine enzyme-linked immunosorbent assay (ELISA) and RNA was obtained from lysed cells.

Punch biopsies (6 mm) from the sites of clinical reaction were transported in ice and stored in liquid nitrogen. Fifty 5 μm sections were cut in a cryostat (Leek Technik GmbH, Mainz, Germany) and transferred to 500 μl lysis buffer containing 4 M guanidium isothiocyanate (Gibco BRL) and stored at −70° until RNA isolation.

mRNA isolation

Total cellular RNA isolation was carried out as before using the guanidium isothiocyanate–phenol chloroform method.[12] Briefly, cells were pelleted in 1·5 ml Eppendorf tubes by centrifugation and lysed using 500 μl of lysis buffer containing 4 M guanidium isothiocyanate following which total cellular RNA was isolated by acid–phenol–chloroform extraction and precipitation using isopropyl alcohol.

Reverse transcription

Reverse transcription of the total cellular RNA was undertaken using Oligo d(T) primers as described earlier[12] to yield cDNA from the expressed cellular genes. Briefly, the precipitated RNA sample was washed twice with 80% ethanol-diethyl pyrocarbonate (DEPC)-treated water and the pellet dried. The sample was resuspended in 8 μl DEPC treated water and reverse transcribed using Moloney murine leukaemia virus reverse transcriptase (MMLV)-RT (Gibco BRL) and oligo d(T) (Pharmacia LKB Biotechnology, Uppsala, Sweden) in a total reaction volume of 20 μl. After reverse transcription, the sample was made up to 100 μl and stored at 4° until polymerase chain reaction (PCR) analysis.

Polymerase chain reaction (PCR)

Amplification of the cDNA was carried out using cytokine specific primer pairs. Approximately 1 μg of cDNA was amplified with Taq polymerase (Promega Co., Madison, WI). The nucleotide sequences of cytokine specific primer pairs for (Rama Biotechnologies, Secunderabad, India) IFN-γ, IL-4 and β-actin are as reported earlier.[12] The following 5′ and 3′ pairs were used for: IL-10; ATGCCCCAAGCTGA GAACCAAGACCCA and TCTCAA GGGGCTGGGTCA GCTATCCCA, IL-12p35; CCTCAGTTTGGCCAGAAACC and GGTCTTTCTGGA GGCCAGGC, IL-12p40; CCA-AGAACTTGCAGCAGCTGAAG and TGGGTCTATT CCGTTGTGTC. The following cycling parameters were used in a DNA thermocycler PTC-100 (MJ Research Inc. Watertown, MA): 95°/45 s, 60°/45 s, 72°/90 s for 35 cycles; followed by 5 min of extension cycle at 72°. IFN-γ, IL-12p35 and p40 cDNA were amplified with the cycle time and temperatures of 95°/60 s, 56°/60 s, keeping all other parameters as above.

Ten microlitres of the amplified product was electrophoresed in 2% agarose gel and the bands visualized by ethidium bromide staining and ultraviolet transillumination (LKB Bromma 2011 Macrovue transilluminator; Pharmacia Biotech, Sweden). The relative intensity of the cytokine specific bands were scored against β-actin which was used as a control for both the efficiency of the reaction and for normalising the cytokine signals within and between the samples. A known cytokine positive control from a patient was also included in each run. The intensity of the signals obtained were arbitrarily graded as 1+ to 3+ in increasing order by two independent observers using gel documentation and analysis system (UVP GDS7 600, software Gel-base gel blot pro, Cambridge, UK).

Cytokine secretion

Culture supernatants stored at −20° were assayed for IFN-γ (Quantikine R&D Systems Inc., Minneapolis, MN) and IL-4 and IL-10 (Genzyme Diagnostics, Cambridge, MA) according to the manufacturer's instructions.

RESULTS

Thirty patients had clinical activity at the time of testing of which 15 had a record of multiple episodes of reactions (Table 1). PBMC from 25 lepromatous (BL, LL) patients with ENL and seven borderline lepromatous subjects (BL) with reversal reactions were investigated for cytokine mRNA and product in unstimulated and antigen-stimulated cultures. In addition, in some subjects lesional cells from skin biopsies as well as adherent cells were examined. mRNA expression for IFN-γ, IL-4, IL-10 and p35 and p40 subunits of IL-12 were evaluated by semiquantitative RT–PCR using specific primers and visualized by ethidium-bromide stained gels. Equivalent quantities of cDNAs of each sample were used and the cytokine signals normalized to the intensity of β actin signals from the same sample with each PCR run. Cytokine expression in basal and antigen stimulated cultures were estimated at 2 and 24 hr after plating of cells. Cytokine production was estimated in some antigen stimulated culture supernatants using specific ELISAs.

Presence of IFN-γ and absence of IL-4 was taken as indicative of Th1, the reciprocal pattern indicative of Th2 and expression of both cytokines was used to define the Th0-like response.

Th cytokine expression in PBMC

Stable lepromatous patients had shown a mixed pattern of both Th2- and Th0-like cytokine profile in our earlier studies.[12] Of importance therefore was the presence of Type 1 like pattern in the PBMC of 64% of the lepromatous patients undergoing ENL reactions (Figs 1 and 4). Six other subjects showed Th0-like profile and four were negative for both cytokines. This was further confirmed by specific ELISA (Table 2) where IFN-γ was seen in culture supernatants of nine out of ten ENL patients with concomitant absence of detectable IL-4 (<1 pg/ml). The level of IFN-γ ranged widely from 250–2150 pg/ml even when the cDNA signal was of the same intensity. The apparent discrepancy may be due to the cumulative increase of the cytokine release in the culture supernatants over a 24-hr period whereas the mRNA expression may reflect a one time measure of cell activation. Alternatively, the semiquantitative RT–PCR may be less sensitive to minor variations in amounts of cDNA.

Unlike stable lepromatous subjects who showed cytokine expression and secretion only after antigen stimulation of PBMC[12] the ENL patients showed basal expression of IFN-γ indicating an *in vivo* stimulation during the reactional state

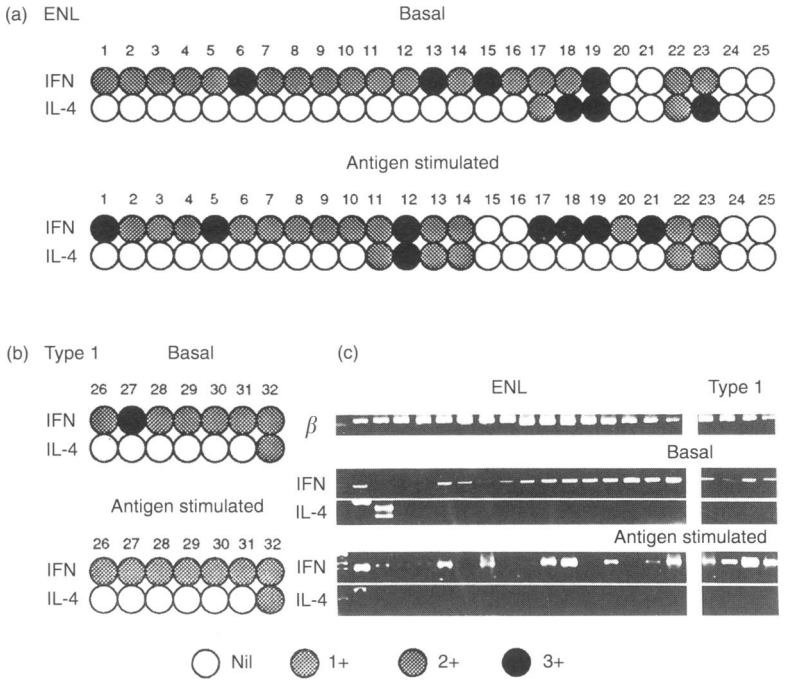

Figure 1. Th1-like cytokine profile in the PBMC of 32 lepromatous leprosy patients undergoing leprosy reactions. Cytokine mRNA was determined by RT-PCR using specific primers. The PCR products were visualised in ethidium bromide stained gels, and *arbitrarily* graded from 1+ to 3+ in increasing order of band intensity. β-actin (β) cDNA was used to normalize the cytokine signals. (a) and (b) depict individual data of 25 and seven lepromatous patients undergoing ENL and Type 1 reactions, respectively. Individual patient numbers are given above the circle. Patient details are as in Table 1. The upper and lower paired rows of circles indicate cytokine status in basal (unstimulated) and antigen stimulated PBMC cultures of the same individuals. (c) Representative samples from 15 ENL and four Type 1 reaction subjects showing signals obtained in ethidium-bromide stained agarose gels. From left to right, lane 1: 1 kb DNA ladder, lane 2: positive cytokine control of proven patient sample, lanes 3–15: ENL patients, lanes 16–20: Type 1 reaction patients. Note presence of interferon-γ (IFN) in all reaction patients and absence of interleukin-4 (IL-4) signals in all except one ENL subject.

Table 2. Interferon-γ and IL-4 cytokine product and mRNA in antigen stimulated PBMC of ENL and Type I (reversal) reaction patients as assessed by specific ELISA (mean pg/ml) and RT–PCR, respectively

	IFN-γ		IL-4	
Patient no.	pg/ml*	cDNA	pg/ml*	cDNA
ENL				
2	450	++	<1	ND
3	980	++	<1	ND
4	350	++	<1	ND
5	2150	+++	<1	ND
6	1870	++	<1	ND
7	250	++	<1	ND
8	1250	++	<1	ND
9	610	++	<1	ND
10	460	++	<1	ND
15	<1	ND	<1	ND
Type 1				
26	1250	++	<1	ND
27	1898	++	<1	ND
28	950	++	<1	ND
29	946	++	<1	ND
30	455	++	<1	ND

*Mean pg/ml of duplicate culture supernatants.

ND: not detectable. Band intensity of PCR products in ethidium-bromide stained gels were scored as given in legend to Fig. 1.

(Fig. 1). Further addition of *M. leprae* in *ex vivo* cultures had no effect except in two patients (pt. nos 15 and 16) where the cytokine mRNA became non-detectable (Fig. 1a).

IL-4 expression was not observed alone in either unstimulated or stimulated PBMC cultures. That the absence of mRNA signal was not related to the experimental conditions was established by a positive signal obtained concurrently from a previously known patient sample. Though we looked routinely for IL-4 mRNA in basal cultures at 2 hr or in antigen-stimulated cultures at 24 hr, additional experiments were undertaken to rule out time kinetic differences. The earlier period of 6 hr or at the later period of 48 hr after antigen stimulation also failed to detect IL-4 (data not shown).

The message for IL-4 appeared to be more influenced by *in vitro* challenge with antigen as PBMC of three subjects (patients 17–19) showing signals for both IFN-γ and IL-4 in the basal state (Th0-like) lost the IL-4 signal on addition of exogenous antigen. Reciprocally, four other patients revealed IL-4 expression on antigenic challenge (patients 11–14) resulting in a shift of Th1- to Th0-like phenotype. In patients with multiple episodes, the Th1 pattern persisted up to 6–7 months after the initial episode. It was also seen in patients receiving antireaction therapy with steroids and thalidomide.

Type 1 reactions

Only lepromatous patients (BL) undergoing Type 1 reactions were consciously selected. The dominant cytokine pattern in

the peripheral blood was that of Th1 (Fig. 1 and Table 2) with the exception of one individual who showed a Th0 profile.

IL-12 p40 and p35 expression

mRNA expression was evaluated for both the constitutive p35 and the inducible p40 subunit of IL-12 in both types of leprosy reactions (Fig. 2). In general, both subunits were expressed in basal and stimulated PBMC. Of importance was the presence of IL-12p40 mRNA in the basal PBMC cultures of 21/25 ENL and all Type 1 reaction patients indicating an ongoing immune response *in vivo*. Antigen stimulation improved the detectability of IL-12p40 in two ENL subjects (pt. nos 13 and 18) who had shown negativity in basal cultures. All Type 1 reaction patients also showed strong signals for IL-12p40 expression in both basal and stimulated PBMC (Fig. 2b). Presence of IL-12 mRNA was seen with both Th1- and Th0-like cytokine profiles.

Differential expression/secretion of IL-10

Patients with the two types of reactions differed in the expression of IL-10 (Fig. 3a,b). Whereas it was expressed in the PBMC of 13/25 of the ENL patients, it was not detectable in the PBMC and adherent cells of Type 1 reaction patients both in the basal and antigen stimulated cultures (Fig. 3b,c and Table 3). IL-10 mRNA and IL-10 protein were primarily derived from adherent cells of the monocyte/macrophage type (Table 3), and were unrelated to the Th type. In two individuals IL-10 mRNA was seen in both the adherent and non-adherent population of cells (data not shown).

Th cytokine expression in dermal lesions

The *in situ* expression of cytokines was also investigated in seven patients (Fig. 4) from skin sites showing histological evidence of ENL and Type 1 reactions. The cytokine pattern in the lesions of both reactions was Th1 like, with concordance between PBMC and skin sites (Fig. 4a,b).

Of interest was the absence of detectable IL-10 expression in skin biopsies of both types of reaction patients (Fig. 4b). Taken together, our data would indicate that whereas IL-12p40 presence is seen in both reactions in both circulatory and lesional cells, adherent-cell derived IL-10 is differentially expressed in the PBMC of the two groups of patients as well as in the two reaction sites.

DISCUSSION

The present study provides evidence of polarized Th1-like responses in the majority of lepromatous patients during clinical reactions. In contrast to the stable form of this disease which was associated with a Th2- or a Th0-like cytokine profile,[12,13] 85% and 64% of lepromatous patients undergoing Type 1 (reversal) and Type 2 (ENL) reactions respectively showed a change to Th1-like cytokine pattern. This was observed even in fresh PBMC indicating an *in vivo* priming during the reactional state. In conformity with the findings of Verghagen *et al.*[17] the change to Th1-like cytokine pattern was also seen in the skin showing the immunopathology associated with reaction sites.[7]

As it is not possible to predict leprosy reactions, the Th type of the individual patients prior to the onset of clinical episodes could not be determined. Nevertheless, it is intriguing that patients with the same clinical type of disease should

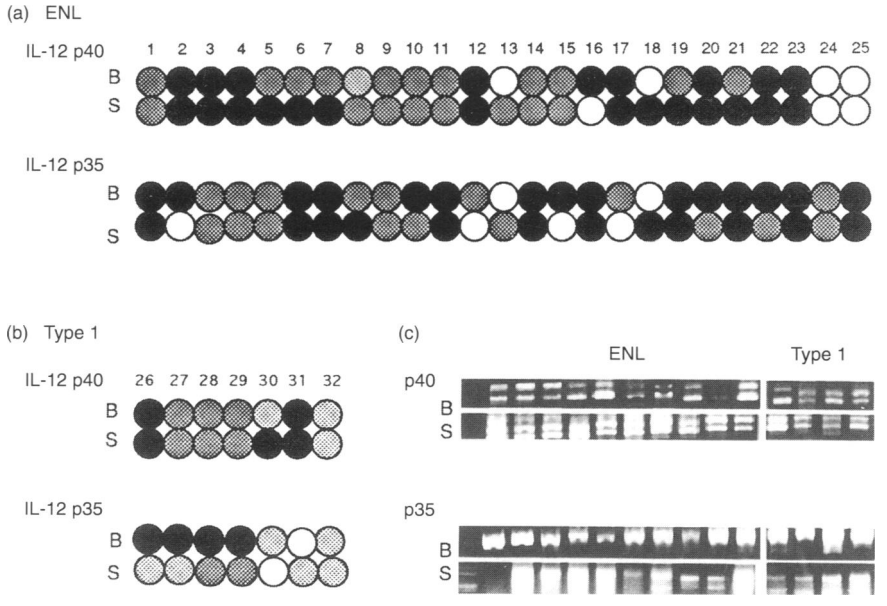

Figure 2. Expression of IL-12, p40 and p35 subunits in basal (b) and antigen stimulated (S) PBMC of (a) ENL and (b) Type I reaction patients evaluated by RT-PCR. Subjects and cytokine grading is given in legend for Fig. 1. (c) Representative PCR products from nine ENL and four Type 1 reaction patients visualized in ethidium-bromide stained agarose gels. From left to right, lane 1: 1 kb DNA ladder, lane 2: positive control from known patient sample, lanes 3–10 ENL patients, lanes 11–14: Type 1 reaction patients. β-actin signal is same as in Fig. 1.

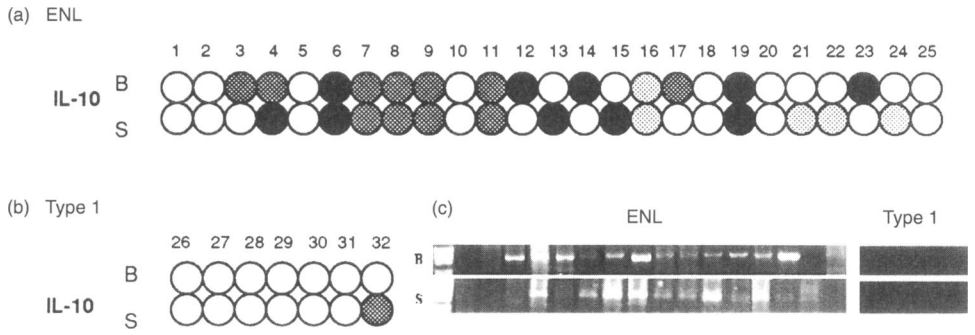

Figure 3. Differential expression of IL-10 in basal (B) and antigen-stimulated (S) PBMC as evaluated by RT–PCR. Presence of IL-10 mRNA was seen in many (a) ENL patients whereas it was non-detectable in (b) Type 1 reaction patients. Subjects and cytokine grading is given in legend for Fig. 1. (c) Representative PCR products from 13 ENL and four Type 1 reaction were visualized in ethidium-bromide stained agarose gels. From left to right, lane 1: 1 kb DNA ladder, lane 2: negative control, lane 3: positive control of known patient sample, lanes 4–15: ENL patients, lanes 16–20: Type 1 reaction patients. β-actin is same as in Fig. 1.

Table 3. IL-10 in leprosy reactions. IL-10 presence in ENL patients is associated with adherent cells. The cytokine released in supernatants and cytokine RNA of cells were assayed, respectively, by ELISA and RT–PCR in antigen stimulated peripheral blood mononuclear cells (PBMC), adherent(AC) and non-adherent cells (NAC)

	PBMC		AC		NAC	
Patient no.	pg/ml*	cDNA	pg/ml*	cDNA	pg/ml*	cDNA
ENL						
1	<1	ND	<1	ND	<1	ND
2	<1	ND	<1	ND	<1	ND
4	120	++	405	++	<1	ND
6	190	++	370	+++	<1	ND
7	117	++	355	+++	3	ND
9	185	++	372	+++	5	ND
Type 1						
27	<1	ND	<1	ND	<1	ND
28	<1	ND	<1	ND	<1	ND
29	<1	ND	<1	ND	<1	ND
30	<1	ND	3	ND	<1	ND

*Mean pg/ml of duplicate culture supernatants.

ND: not detectable. Band intensity of PCR products in ethidium-bromide stained gels were scored as given in legend to Fig. 1.

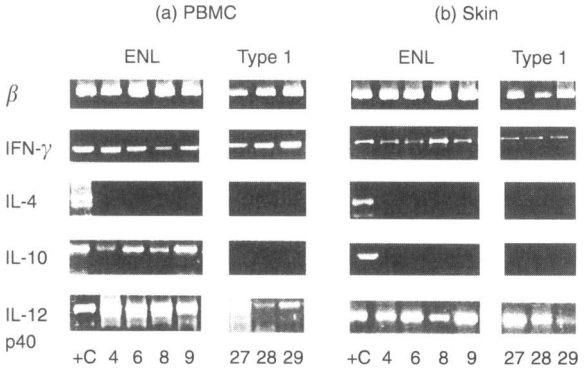

Figure 4. Th1-like cytokine profile seen in both PBMC and skin lesions of the same lepromatous patients undergoing ENL and Type 1 reactions. Equal quantities of cDNA from PBMC and skin of the same patient were amplified with specific primers and visualized in ethidium-bromide stained gels. Presence of IL-12p 40 and IFN-γ is associated with absence of IL-4. Interleukin-10 signals were absent in skin lesions of both reaction types and PBMC of Type 1 reaction patients. Numbers given below the gels refer to patients as given in Fig. 1. (a) stimulated PBMC from four ENL and four Type 1 reaction patients were compared with (b) corresponding skin biopsies of reaction sites. β-actin from PBMC and skin of each subject were normalized and used to compare each set of cytokines from the same individual. M: 1 kb DNA ladder, C+: positive cytokine control from known patient sample, IFN-γ: interferon-γ, IL-4: interleukin-4, IL-10: interleukin-10, IL-12p40, p35: interleukin-12p40 and p35, respectively.

show different Th responses during changes in clinical symptomatology. Current concepts would suggest that Th differentiation occurs early after infection. However, it is possible that in a chronic disease like lepromatous leprosy the host may be subjected to periodic Th perturbations where a Th1 change from a baseline Th2 or Th0 is associated with tissue damage and clinical morbidity. It is not possible from our studies to determine whether this apparent change is caused by the emergence of a new population of Th1 cells or an alteration in population dynamics of a mixture of Th1+Th2 cells presenting as Th0 or Th2 in the stable lepromatous state. A Th1 to Th2 switch has been reported in human immunodeficiency virus-infected subjects and found to be critical for disease progression or seronegativity.[18]

The Th1-like cytokine profile persisted up to 6–7 months from the onset of the clinical reaction and was also observable in patients undergoing antireaction therapy with thalidomide and low dose of steroids. Thalidomide, a powerful inhibitor of tumour necrosis factor – α (TNF-α), a cytokine implicated for the fever and malaise associated with reactions,[19] and steroid therapy known to depress cellular immunity, did not inhibit the development of IFN-γ or IL-12 mRNA in ENL patients indicating a cytokine selectivity induced by these drugs.

IFN-γ expression was prominent even in unstimulated basal cultures of PBMC indicating an ongoing immune response *in vivo*. That this cytokine may have a pathogenetic role in ENL is suggested by the observation that chronic administration of recombinant IFN-γ to lepromatous patients led to development of ENL reactions in 60% of the patients over a 6–7 month period.[19]

Th2-like pattern with IL-4 alone was not seen in lesions or PBMC of the reaction patients studied by us, though its

presence has been reported in lesions of the stable form of the same leprosy type.[16] Antigen stimulation of PBMC increased or decreased the expression of IL-4mRNA over basal levels in seven ENL patients.

It is intriguing that the two clinically dissimilar leprosy reactions should be associated with the same Th1 response. With a view to understanding these differences we looked for regulatory cytokines such as IL-12[1,3,20,21] and IL-10[22–24] which have been implicated, respectively, in the differentiation of Th1 and Th2 cells. In our study inducible IL-12 was strongly associated with leprosy reactions and may have a role to play in the Th1 switch from the previous Th2-like lepromatous form. IL-12 has been shown to prime not only naive $CD4^+$ cells[20] but also established Th2 clones[21] to produce IFN-γ and thereby reduce the inhibitory role of IL-4 on Th1 differentiation. IL-12 has also been shown to be critical for Th1 development in leishmaniasis.[1]

The capacity to induce Th1 and cell-mediated responses to intracellular pathogens/products appears to be linked to their ability to generate IL-12 from macrophages or natural killer cells.[1,3] That certain epitopes of *M. leprae* may be uniquely recognized prior to the onset of the reactional state has been suggested by the presence of sequence specific antibodies that distinguish active and subclinical ENL.[25] Though the type of antigens have not been identified, immune complexes have been reported both in sera and tissues of ENL patients.[5,7] It is tempting to speculate that the emergence of microbial products or unmasking of cryptic epitopes, during the intracellular life of the leprosy bacillus may trigger the production of IL-12 and thereby influence the development of Th1 phenotype during the leprosy reactions.

IL-10, a negative modulator of cell mediated immunity and Th1 development[26] has been shown to be produced by Th1, Th2 cells and macrophages.[22–24] Of importance was the differential expression of IL-10 in ENL and Type 1 reaction patients. Consistent with other studies[27] IL-10 expression was detectable in >55% ENL subjects. It was associated with the adherent cell population as reported earlier by us in the stable disease.[28] Other studies on skin lesions had also shown the presence of IL-10 but differed from ours in the lack of IFN-γ increase.[27] In contrast to ENL, Type 1 reaction patients failed to show detectable IL-10 in both unfractionated PBMC and adherent cells. It would appear therefore that the Th1 development in the two reactions may have different pathways even in subjects with the same leprosy background, which may reflect the varied clinical picture seen in them.

The role of IFN-γ and IL-12 in the down-regulation of IL-10 has been shown in experimental animals and in infections of man.[1,24] In our study, Type 1 reaction patients showed both IFN-γ and IL-12 in the absence of IL-10 which is consistent with the findings of Libraty *et al.*[29] who showed that recombinant IFN-γ had a differential effect with up-regulation of IL-12 and down-regulation of IL-10 in PBMC cultures of leprosy patients. The absence of IL-10 mRNA in reaction sites in the skin in the present and earlier studies[27] is puzzling and requires further investigation, as both reactions occur over a similar background of bacilli-laden macrophage granulomas.

In conclusion, reactional states in lepromatous leprosy show a natural modulation of the immune response which favours a shift towards a Th1-like response. Such a Th switch may serve as a predictive marker of reactional states in leprosy. Strategies to promote Th1-like cytokine pattern while controlling the immunopathology and nerve damage associated with these reactions would be clinically beneficial, as reactions are a major cause of morbidity in leprosy.

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