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Cellular Immune Response to *Mycobacterium leprae* Infection in Human Immunodeficiency Virus-Infected Individuals

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The immune responses to *Mycobacterium leprae* and other mycobacterial antigens were studied in 11 leprosy patients with concurrent human immunodeficiency virus type 1 (HIV-1) infection. Three patients manifested borderline lepromatous leprosy, and eight patients had borderline tuberculoid (BT) leprosy. Despite the low $CD4^+$ T-cell count in the peripheral blood, no histologic or phenotypic change in the cellular infiltrate in either the lepromatous or tuberculoid lesions was observed when compared with HIV-1-negative patients. Lepromatous lesions contained heavily parasitized macrophages and few $CD8^+$ T cells. Lesions from the patients with BT leprosy showed extensive $CD4^+$ T-cell infiltration despite a significant reduction in $CD4^+$ T-cell counts in the peripheral blood. No acid-fast bacilli were detected in the tuberculoid lesions. HIV-1 infection did not alter the lack of response in lepromatous leprosy to *M. leprae* antigens either in vitro or in vivo. In contrast, the skin test response to *M. leprae* antigens as well as the in vitro lymphoproliferative responses to mycobacterial antigens that are usually seen in patients with tuberculoid leprosy were abrogated in the BT HIV-1^+ patients. However, production of gamma interferon in response to the same stimuli was preserved in most of the patients. Analysis of cytokine gene expression showed activation of additional cytokine genes in the unstimulated peripheral blood cells of patients with both leprosy and HIV-1 infections as compared with cells from patients with leprosy alone. These results suggest that granuloma formation in leprosy can be independent of the impaired $CD4^+$ T-cell response of the HIV-1 infection. Furthermore, in HIV-1^+ individuals with *M. leprae* infection, activation of cytokine genes is observed even when the circulating $CD4^+$ T-cell count is significantly reduced.

The clinical manifestations of leprosy, a chronic mycobacterial infection, are determined by the cellular immune response of the host (3, 13). There is a wide spectrum in the host response to *Mycobacterium leprae* which is evident in both clinical and histologic findings. When the cellular immune response is not induced, the patient manifests the lepromatous form of leprosy. The lesions of lepromatous leprosy are typically loose infiltrates of macrophages permissive for the intracellular multiplication of *M. leprae*. The uncontrolled growth of the bacilli and spread of infection from cell to cell result in a disseminated cutaneous infection. The low numbers of T cells in the lesions of lepromatous leprosy are almost exclusively $CD8^+$ T cells, with few if any $CD4^+$ T cells present (22). In vitro, cells from these patients fail to respond to *M. leprae* in the lymphocyte proliferation assay (3, 13, 20).

In contrast, in tuberculoid leprosy patients in which there is an adequate host cellular immune response to *M. leprae* infection, the lesions are limited in numbers and consist of organized, lymphocyte-rich granulomas. Few viable *M. leprae* organisms are present in the lesions, with the cellular immune response apparently controlling the growth of the bacteria. The infection can become self-limiting. The predominant T cell observed in the tuberculoid lesions is the $CD4^+$ T cell (22). In vitro, cells from patients with tuberculoid lesions respond strongly to *M. leprae* (3, 13, 20), and in vivo these patients manifest skin test responses to lepromin (Mitsuda test).

The effects of the human immunodeficiency virus type 1 (HIV-1)-induced alterations in the cellular immune response on the progression of mycobacterial infection can be studied in patients suffering simultaneously from both infections. Previous studies have shown that patients with HIV-1 infection are more susceptible to infections with mycobacteria such as *M. tuberculosis* (5) and *M. avium* (2, 6). The course of these mycobacterial infections in the context of HIV-1 infection has been shown to be significantly altered. Since HIV-1 compromises the cell-mediated immune response, the HIV-1-positive individual infected with *M. leprae* might be expected to manifest the lepromatous form of the disease or, alternatively, to show rapid progression from the tuberculoid form to the lepromatous form as HIV-1 infection proceeds.

In order to determine whether HIV-1 infection alters the progression of *M. leprae* infection, 11 patients with both infections were studied. The histology of the lesions, the bacterial load of the lesions, and the cell-mediated immune responses in vivo and in vitro were examined to elucidate the interaction between the two infections.

MATERIALS AND METHODS

Patient population. Patients of the leprosy clinic at the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, and patients from other leprosy clinics in the city of Rio de Janeiro who were referred to this study and who had given informed consent were enrolled. Human experimentation guidelines of the Oswaldo Cruz Foundation Ethical Committee were followed in the conduct of clinical research. The patient population consisted of individuals who had been diagnosed first with leprosy and later shown to be HIV-1 infected and HIV-1-positive individuals who subsequently developed leprosy lesions. Clinical and histologic exami-

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nation of the 11 patients studied revealed that 3 had the multibacillary borderline lepromatous (BL) form of disease and 8 were paucibacillary, borderline tuberculoid (BT) (Table 1). Skin biopsies (6 mm) were obtained from the study patients at the time of diagnosis of leprosy and at the beginning of the study. Biopsies were prepared for histological diagnosis with the Ridley-Jopling classification (17) and for immunohistological evaluation. In addition five BT and three BL HIV-1-negative leprosy patients were evaluated for cytokine mRNA expression in peripheral blood leukocytes (see below) and 48 HIV-1-negative BT leprosy patients were tested for their skin test response to lepromin (see below).

Quantitation of bacterial load. The bacterial index (BI) is an essential component of the diagnosis of leprosy. The BI is a microscopic estimate of bacterial burden and is the number of acid-fast bacilli (AFB) per 100× field (oil immersion). Slit smears for BI determination were taken from six anatomic sites (usually earlobes, elbows, and knees) at the time of diagnosis of leprosy (16).

Immunohistology. Part of each biopsy was snap frozen in liquid nitrogen and kept at −180°C until use. Frozen sections (6 μm) were cut on a cryostat, air dried, and fixed in cold acetone for 10 min. The sections were incubated with the primary antibody and then with biotin-conjugated horse anti-mouse antibody (Vector Laboratory, Inc., Burlington, Calif.) and developed with diaminobenzidene as described elsewhere (18). The presence of a dark brown precipitate indicated a positive reaction. In control sections, the primary antibody was omitted. The sections were evaluated by light microscopy.

MAbs. Mouse anti-human monoclonal antibodies (MAbs) and a polyclonal antiserum were used for the identification of specific cell types, proteins, and surface molecules. Antibodies Leu 4, Leu 2a and Leu 3a (anti-$CD3^+$, -$CD8^+$ and -$CD4^+$ T cells, respectively) and Leu M3 (anti-$CD14^+$; macrophages) (Becton Dickinson and Co., Mountain View, Calif.) were diluted 1:30 in phosphate-buffered saline (PBS) containing 5% fetal calf serum (FCS); anti-HLA-DR (diluted 1:50), anti-LFA-1 (diluted 1:20), and anti-ICAM-1 (diluted 1:50) were obtained from AMAC Inc., Westbrook, Maine. Anti-tumor necrosis factor alpha (TNF-α) serum (diluted 1:100) was donated by H. V. Heuverswyn (Innogenetics, Ghent, Belgium). Concentrations of antibodies used were found to be optimal, providing maximum specific staining with the lowest nonspecific background signal.

Lepromin (Mitsuda) skin test. For the lepromin skin test (Mitsuda reaction), 0.1 ml of Mitsuda antigen containing 4×10^7 armadillo-derived *M. leprae* organisms per ml (National Hansen Disease Center, Carville, La.) was given intradermally in the forearm and the induration was measured 3 weeks after.

Antigens and mitogen for in vitro testing. Armadillo-derived *M. leprae* organisms which had been irradiated and lyophilized were provided by R. J. W. Rees (IMMLEP Bank, The National Institute of Medical Research, Mill Hill, England) and used at 20 μg (dry weight) per ml. Heat-killed *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) (Atauvo de Paiva Foundation, Rio de Janeiro, Brazil) and purified protein derivative (PPD) (Statens Seruminstitut, Copenhagen, Denmark) were used at 10 μg/ml. Phytohemagglutinin (PHA) (GIBCO Laboratories, Grand Island, N.Y.) was used at a 1:100 dilution of the commercial stock.

Stimulation of lymphocytes. Heparinized venous blood was collected, and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.) density centrifugation. PBMC were incubated in triplicate at 10^6 cells per ml at 37°C, in 96-well plates (Costar Corp., Cambridge, Mass.) in a final volume of 0.2 ml of RPMI 1640 medium supplemented with 10% human serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM L-glutamine (all from GIBCO Laboratories). Cells (2×10^5 per well) were cultured in the presence or absence of antigens for 5 days. Culture supernatant was then collected for determination of cytokine production. The remaining cells were pulsed with 1 μCi of [H^3]thymidine overnight and then harvested to determine [H^3]thymidine incorporation. Results are expressed as mean counts per minute ± standard deviation obtained from stimulated cultures and control cultures.

IFN-γ determination. The concentration of gamma interferon (IFN-γ) in culture supernatant was determined using a commercial enzyme-linked immunosorbent assay kit according to the instructions of the manufacturer (Endogen Inc., Boston, Mass.). The detection limit of the assay is 5 pg/ml.

Flow cytometric analysis. PBMC (10^5) in PBS–2% FCS were labelled with the primary mouse anti-human MAb to T-cell subsets (Leu 4, Leu 2a, and Leu 3a) and monocytes (Leu M3) for 30 min on ice, washed in cold PBS–2% FCS, and then stained with a secondary goat anti-mouse immunoglobulin G fluorescein isothiocyanate-labelled polyclonal antibody. The cells were fixed with 1% formaldehyde before analysis on an EPICS analyzer (Coulter Corp., Hialeah, Fla.).

PCR. Fresh PBMC (2×10^6) were pelleted at 4°C and resuspended in 1 ml of RNAzol B (Cinna/Biotecx Lab, Inc., Houston, Tex.). Total RNA was extracted according to the manufacturer's instructions. RNA was stored at −70°C until assay. RNA samples were submitted to a reverse transcription reaction using RNAse H^- reverse transcriptase (GIBCO BRL, Gaithersburg, Md.). Interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-6, IL-8, TNF-α, TNF-β, granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, perforin, and β-actin primers were used to determine expression of mRNA in the cells as described previously (21). PCR amplification was carried out in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) for 30 cycles as described previously (21). The reaction product was visualized by agarose gel electrophoresis.

TABLE 1. Diagnosis and clinical status

Patient no.	Leprosy diagnosis	Yr of diagnosis of leprosy/HIV	Mitsuda response[a] at time of diagnosis	AFB (BI)[b]
1	BL/ENL	1993/1989	−	5+
2	BL	1990/1992	−	6+
3	BL/ENL	1987/1993	−	4+
4	BT	1993/1990	−	−
5	BT	1991/1990	±	−
6	BT	1992/1992	±	−
7	BT	1989/1993	−	−
8	BT	1991/1993	−	−
9	BT	1992/1993	−	−
10	BT	1993/1993	−	−
11	BT/RR	1992/1992	−	−

[a] Skin test response to *M. leprae* administration. ±, 3 to 4 mm of induration; −, no induration.

[b] AFB count in slit smears, not in the lesions, at the time of diagnosis of leprosy. BI, number of acid fast-bacilli per 100× microscopic field: 4+, 10 to 100; 5+, 100 to 1,000; 6+, >1,000.

RESULTS

Lepromatous leprosy (BL) patients. Three patients with multibacillary BL leprosy and HIV infection (BL HIV^+) were studied. Two of these patients were diagnosed with leprosy 2 to 5 years before the diagnosis of HIV-1 infection (Table 1). In contrast, the other patient had been HIV-1 positive for 4 years before the diagnosis of BL leprosy. Two of the lepromatous leprosy patients presented with erythema nodosum leprosum (ENL) during the course of the study. ENL is believed to be an inflammatory response induced by mycobacterial cell wall products released into the circulation either prior to or during antileprosy therapy.

(i) CD4 cell counts. Although some of the BL HIV^+ patients had normal total leukocyte counts (range of 2,000 to 10,800/mm^3), two of three BL patients for whom CD4 T-cell counts were available had counts of 648 and 564 $CD4^+$ T cells per mm^3. $CD8^+$ cells were slightly higher than normal, 584 and 1,226/mm^3. Monocyte numbers were within the normal range.

(ii) Histology of lesions. Slit smears obtained from the BL HIV^+ lepromatous patients showed AFB. When the skin lesions of the patients with concomitant HIV-1 and *M. leprae* infections were examined, the morphology was similar to the histologic characteristics of lesions from leprosy patients who were not HIV infected (17). Lepromatous lesions from BL HIV^+ patients contained multibacillary parasitized macrophages and relatively low numbers of lymphocytes (data not shown).

Immunohistological staining demonstrated relatively high numbers of $CD8^+$ T cells in the lesions of BL HIV-1^+ patients. It has previously been shown that in HIV-negative lepromatous patients, the lesions are enriched in $CD8^+$ T cells (22). Thus, neither the morphology nor the type of immune cells observed in the leprosy lesions was modified by the simultaneous presence of HIV infection or the relatively low number of circulating $CD4^+$ T cells.

(iii) Response to *M. leprae* antigens. Lepromatous HIV-1-infected patients were as nonresponsive both in vivo and in vitro to *M. leprae* antigens as were their HIV-1-negative counterparts. In vivo, all BL HIV^+ lepromatous patients were nonresponsive to Mitsuda skin testing. In vitro, peripheral blood lymphocyte proliferation in response to *M. leprae*, BCG, PPD, and PHA was also analyzed. BL/LL patients are anergic to *M. leprae* antigen stimulation, while 35% of such patients respond to BCG (20). Lymphocytes from lepromatous patients are usu-

TABLE 2. Relationship between CD4 count and lymphoproliferation in vitro in BT HIV-positive leprosy patients

Patient[a]	CD4/CD8 count/mm^3	Lymphoproliferation[b] with stimulus:					
		Control	*M. leprae*	BCG	PPD	Control	PHA
4	185/688	240 ± 21	1,929 ± 67	606 ± 171	1,013 ± 301	272 ± 44	89,072 ± 777
5	74/857	1,060 ± 174	136 ± 28	614 ± 10	550 ± 70	252 ± 22	1,862 ± 196
6	0/453	271 ± 15	529 ± 208	423 ± 197	903 ± 262	696 ± 197	1,536 ± 664
7	64/469	144 ± 7	533 ± 92	610 ± 108	519 ± 18	216 ± 10	4,772 ± 1,405
8	19/ND[c]	115 ± 30	909 ± 236	431 ± 35	401 ± 65	193 ± 12	4,897 ± 814
9	379/536	323 ± 85	3,048 ± 922	16,329 ± 1,365	17,450 ± 658	1,042 ± 573	153,556 ± 11,874

[a] Patients 10 and 11 were not available for study.
[b] Results are expressed as mean counts per minute ± standard deviation. Differences in counts per minute (agonist value-control value) of ≥3,000 for antigens and ≥5,000 for mitogens were considered positive.
[c] ND, not done.

ally responsive to PHA. When the lymphocytes from lepromatous patients who were also HIV-1 infected were stimulated in vitro with *M. leprae*, BCG, PPD, and PHA, a generalized lack of immunoresponsiveness to all antigens and PHA was observed (data not shown).

Tuberculoid (BT) leprosy patients. Eight patients with paucibacillary tuberculoid leprosy and HIV infection were included in this study. Three of these patients were diagnosed with leprosy 1 to 4 years before HIV-1 infection. In three patients both infections were diagnosed at the same time or within 2 to 3 weeks of each other, while the remaining two patients were first shown to be HIV-1 infected and only later (1 month to 3 years) developed tuberculoid leprosy lesions (Table 1). The response of all patients to chemotherapy was excellent, and the lesions of BT patients disappeared in response to therapy (within 6 months).

(i) CD4$^+$ T-cell counts. All patients with concurrent HIV infection and tuberculoid leprosy had from low (2,000) to normal (10,200) numbers of total leukocytes and greatly reduced CD4$^+$ T-cell counts at the time of initiation of this study (Table 2). The number of circulating CD4$^+$ T cells ranged from 0 to 379 CD4$^+$ per mm^3 (normal CD4$^+$ T-cell range, 800 to 1,000 cells per mm^3). In these patients, the numbers of CD8$^+$ T cells were within normal limits or slightly higher than normal, resulting in relatively low CD4/CD8 ratios (normal ratio >1.5). The numbers of circulating monocytes remained within the normal range.

(ii) Histology of lesions. Histologic examination of the skin lesions of patients with both tuberculoid leprosy and HIV infection revealed normal granulomatous differentiation of mononuclear phagocytes and large numbers of infiltrating lymphocytes (Fig. 1A). There were no AFB present in any of the lesions; this is consistent with the diagnosis of BT leprosy. Biopsies taken from these patients during the course of antileprosy chemotherapy demonstrated normal clearance of the tuberculoid lesions.

Immunohistological staining of biopsies obtained at the start of the study demonstrated high numbers of CD4$^+$ (Fig. 1B) and low numbers of CD8$^+$ (data not shown) T cells (CD4/CD8, >1) in the lesions. CD14$^+$ monocytes appeared normal and did not appear to account for the CD4 staining. Dermal cells stained positively for HLA-DR (Fig. 1D), LFA-1, and ICAM-1. In samples from four of five patients, positive staining for TNF-α was observed (Fig. 1C), indicating local cellular activation of TNF-α-producing cells. HLA-DR staining was observed on the surface of keratinocytes overlying the lesions of four of five BT patients studied (Fig. 1D), indicating local production of IFN-γ. Remarkably, the histologic characteristics of the skin lesions of patients with concurrent leprosy and HIV infection did not differ from those of patients with leprosy alone (22). This observation was most surprising in that the BT patients had such low blood CD4$^+$ T-cell counts.

(iii) Response to *M. leprae* antigens. The cellular immune response of BT HIV$^+$ patients was examined in the lepromin skin test. The effect of the intradermal challenge in BT HIV$^+$ patients was strikingly different from that observed in HIV-1-negative BT patients. All BT HIV$^+$ patients were Mitsuda negative (less than 5 mm of induration). Only two of the eight BT patients tested showed induration of between 3 and 4 mm in response to *M. leprae* antigens. This is in marked contrast to results obtained when 48 BT HIV-1-negative patients were challenged in vivo with *M. leprae* antigens. In these BT HIV-negative patients, 91.7% showed a lepromin reaction of ≥3 mm and 39.6% had an induration of ≥5 mm (Fig. 2).

(iv) In vitro response. When lymphocytes from six of the BT HIV-1$^+$ patients were studied in proliferation assays in vitro, all failed to respond to *M. leprae* antigens. This is in marked contrast to the results obtained when lymphocytes from BT HIV-1-negative patients were challenged in vitro with *M. leprae* antigens. Lymphocytes from 57.4% of the BT HIV-negative patients proliferated in response to *M. leprae* antigens (data not shown). Moreover, lymphocytes from five of the BT HIV$^+$ patients also failed to respond to other mycobacterial antigens, e.g., PPD and BCG (Table 2). Lymphocytes from two of the patients responded to PHA stimulation. Thus, patients with concurrent BT leprosy and HIV-1 infection demonstrated significant impaired cellular immune responses to *M. leprae* both in vitro (lymphocyte proliferation) and in vivo (Mitsuda skin test) and manifested significant anergy in vitro in response to other mycobacterial antigens.

IFN-γ release into culture supernatants of PBMC stimulated with the same antigens was less affected by HIV-1 infection. Cells from four of six BT HIV$^+$ patients responded to *M. leprae* with IFN-γ production (Table 4). Lymphocytes from four of the BT HIV$^+$ patients responded to PHA by producing IFN-γ. In addition, PBMC from two of the BT HIV$^+$ patients were capable of IFN-γ release in response to BCG and PPD stimulation in vitro. Interestingly, lymphoproliferation and IFN-γ production in response to antigens and mitogens was observed in PBMC from BT HIV$^+$ patients with higher CD4$^+$ T-cell counts (Tables 2 and 4).

(v) Cytokine mRNA expression by PBMC. The lack of response of PBMC of BT HIV-1$^+$ leprosy patients to soluble antigens in vivo (Mitsuda) and in vitro prompted us to examine the activation of cytokine mRNA in the circulating mononuclear leukocytes of these patients. Cytokine mRNA expression was analyzed in freshly isolated PBMC obtained from BT HIV$^+$ and BT HIV-negative as well as BL HIV$^+$ and BL HIV-negative leprosy patients. The cytokine mRNA profiles observed in cells from BT HIV$^+$, BT HIV-1-negative, BL

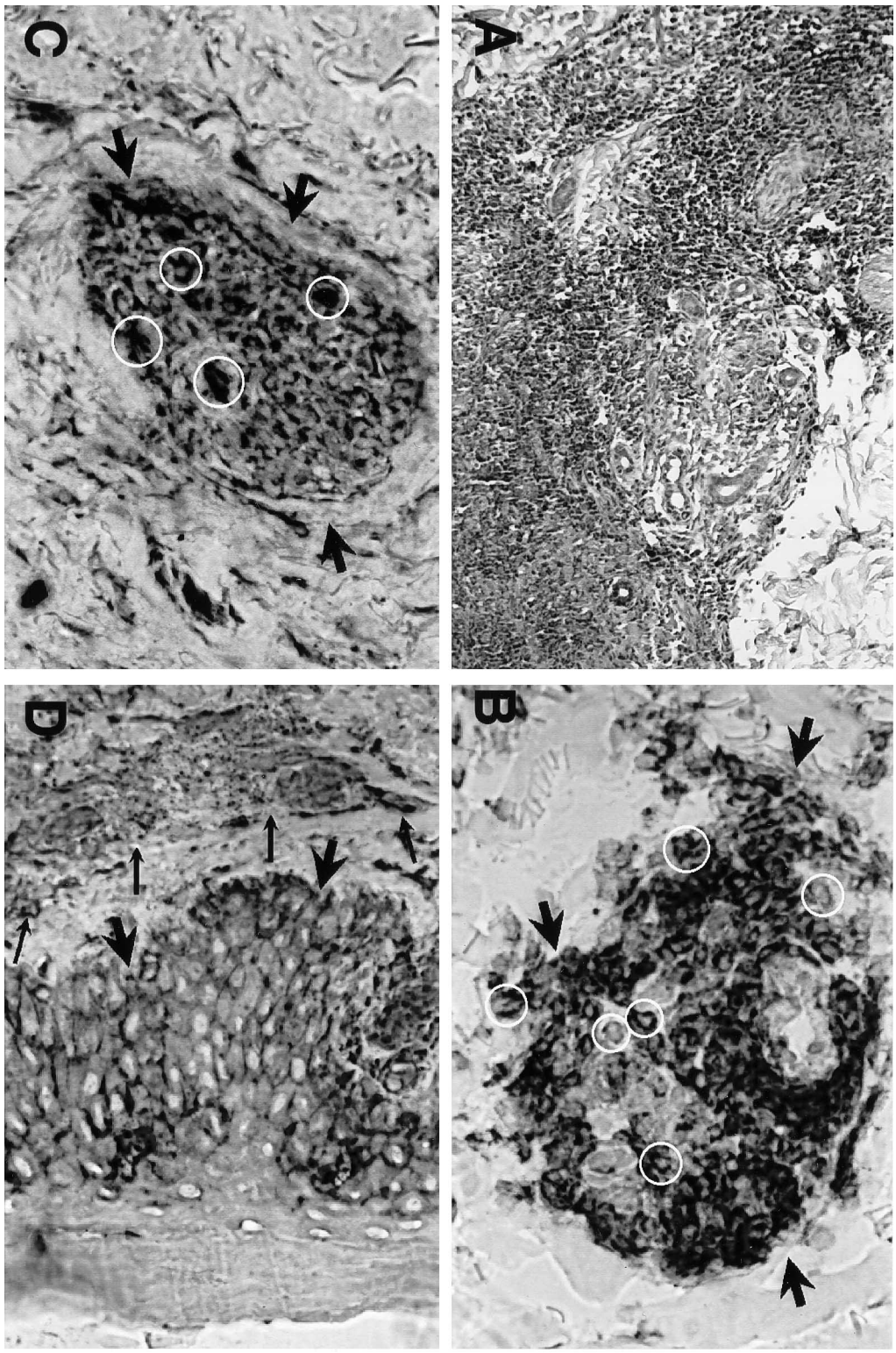

FIG. 1. Morphology of BT lesions. Light microscopy micrographs showing the cellular infiltrate in a representative BT HIV-1$^+$ lesion (patient 4). Histology (A) and immunohistology (B to D) are shown for the same lesion. (A) Hematoxylin and eosin staining. (B) Staining for CD4$^+$ T cells. Arrows show the granuloma; circles mark some of the positively staining cells. (C) Staining for TNF-α. Arrows show the granuloma; circles mark some of the positive staining in the granuloma. (D) Staining for HLA-DR. Large arrows show the epidermis with positive staining in the keratinocytes, and small arrows point to the dermal infiltrate which contains stained cells. Magnifications: panel A, ×25; panels B to D, ×100.

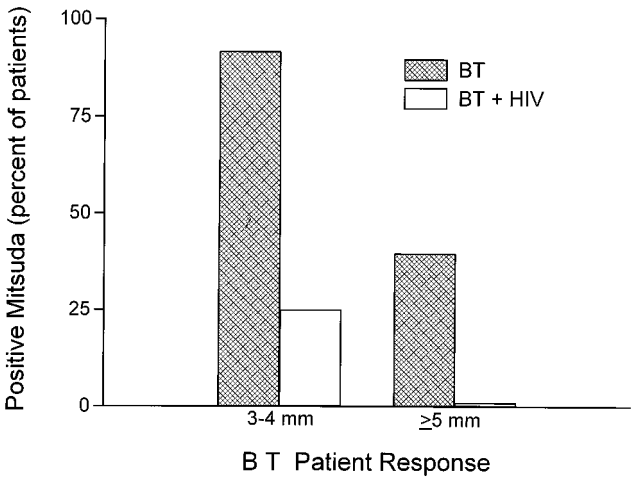

FIG. 2. Skin test response to *M. leprae* antigens (Mitsuda test) in BT patients. Forty-eight BT HIV-1-negative leprosy patients and 8 BT HIV$^+$ leprosy patients were tested. Results are expressed as the percentages of patients that responded with an induration of 3 to 4 mm or 5 mm or more.

TABLE 3. Cytokine gene activation in freshly isolated PBMC of BT leprosy patients

Cytokine mRNA[a]	No. of patients positive for mRNA (n = 5)	
	BT HIV$^-$	BT HIV$^+$
IL-1	0	4
IL-2	0	0
IL-4	1	3
IL-6	5	4
IL-8	5	4
GM-CSF[b]	0	3
TNF-α	5	5
TNF-β	5	5
IFN-γ	0	3
Actin	5	5

[a] Specific band on agarose gel (see Fig. 3).
[b] GM-CSF, granulocyte-macrophage colony-stimulating factor.

HIV$^+$, and BL HIV-negative patients are shown in Fig. 3. Activation of more cytokine genes was observed in the PBMC of BT HIV$^+$ patients when compared with the PBMC of BT HIV-1-negative patients (Table 3). IL-1, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IFN-γ RNAs were expressed in most BT HIV-1$^+$ patients (Fig. 3 and Table 3) but not in HIV-1-negative BT patients (Table 3). A similar enhanced cytokine gene activation was observed in PBMC obtained from HIV-1$^+$ BL patients when compared with PBMC from HIV-1-negative BL patients (Fig. 3). These results show that HIV infection itself in some way leads to activation of cytokine genes.

DISCUSSION

Increased susceptibility to mycobacterial infections is a well-established sequela of HIV infection (2, 5, 7). Moreover, the course of *M. avium* and *M. tuberculosis* infection in the HIV-infected individual tends to be more rapid and fulminant. This is not unexpected, since HIV infection compromises the cell-mediated immune response required for protection against

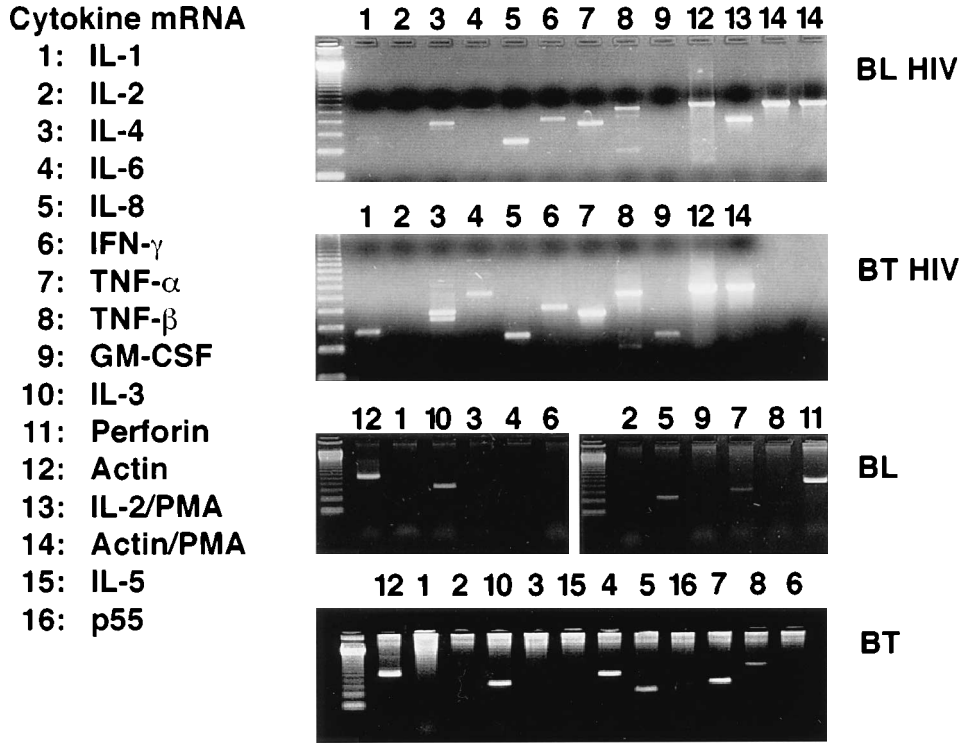

FIG. 3. Cytokine mRNA expression in unstimulated PBMC of leprosy patients. Results shown are for one HIV-1-positive BL patient (no. 2) (top), one HIV-1-positive BT patient (no. 7) (upper center), one BL HIV-1-negative patient (lower center), and one BT HIV-1-negative patient (bottom). p55 is the mRNA for the IL-2 receptor protein p55. The positive control for IL-2 mRNA consisted of RNA extracted from PBMC of a normal donor stimulated with PMA for 5 h (13). mRNA was amplified by PCR and visualized on 2% (wt/vol) agarose gel.

TABLE 4. Relationship between CD4 count and IFN-γ production in vitro in BT HIV-positive leprosy patients

Patient[a]	CD4/CD8 count/mm^3	IFN-γ production (pg/ml)[b] with stimulus					
		Control	*M. leprae*	BCG	PPD	Control	PHA
4	185/688	4	590	320	80	0	20,000
5	74/857	10	206	32	30	40	3,060
6	0/453	5	25	0	0	0	0
7	64/469	10	80	27	12	60	670
8	19/ND[c]	0	0	0	0	0	30
9	379/536	12	80	10,940	3,080	20	19,600

[a] Patients 10 and 11 were not available for study.
[b] Differences in IFN-γ production (agonist value-control value) ≥40 pg/ml were considered positive. The limit of detection of the assay kit is 5 pg/ml.
[c] ND, not done.

these infectious agents. The cell-mediated immune response is of particular importance in the clinical manifestation of infection with another mycobacterium, *M. leprae*. Even in the normal (non-HIV-infected) individual, the cellular immune response to *M. leprae* may be limited, and the patient manifests the lepromatous form of leprosy. However, when the cellular immune response to *M. leprae* is fully functional, the infection is self-limiting or the patient manifests tuberculoid leprosy.

Because the cellular immune response is crucial to the manifestations of *M. leprae* infection, it might be expected that when an HIV-positive individual becomes infected with *M. leprae*, the lepromatous form of the disease would predominate. Alternatively, if a patient manifests the tuberculoid form of leprosy, and then becomes HIV infected, the simultaneous presence of both HIV and *M. leprae* infections might cause rapid progression or a shift towards lepromatous leprosy. This is known as the downgrading reaction often observed in BT leprosy patients (8). When this takes place, the lesions become multibacillary.

Only limited information on the interaction of *M. leprae* and HIV-1 infection exists. Recent reports suggest that in areas of Malawi in which both are endemic, the overlap of the two infections has not altered the prevalence rate of either leprosy or HIV-1 infections (15), although there is a clear association between tuberculosis and HIV-1 in the same study population. In Brazil, the seroprevalence of HIV-1 observed in leprosy patients was the same as that observed for blood donors (1). A case report from the Netherlands suggests that no histological alteration in the lesions of an HIV-1-positive leprosy patient occurred (11). In another case report from Senegal, two patients with both leprosy and HIV-1 were shown to respond satisfactorily to multidrug therapy (MDT) (3), suggesting that HIV infection does not interfere with therapy for leprosy.

To better understand the progression of *M. leprae* infection in the environment of HIV infection, we studied 11 patients with both infections. The results of our study indicate that HIV-1 infection, even as it progresses towards AIDS, does not modify either the expression or the course of *M. leprae* infection. Surprisingly, the phenotypes of the cells present in the cutaneous lesions of BL HIV$^+$ and BT HIV$^+$ patients were the same as those observed in HIV-negative patients. BT lesions are characterized by the scarcity of acid-fast organisms. Even in the lesions of severely immunocompromised BT HIV$^+$ individuals, acid-fast organisms were not observed. Similarly, the local cellular accumulation and differentiation in HIV-1-infected BT patients in response to infection with *M. leprae* were not impaired (Fig. 1) when compared with that in HIV-1-negative BT patients (22).

While the histology and the cellular response in the lesions in response to concurrent HIV and *M. leprae* infections were the same as those observed in HIV-negative individuals, the immune response of PBMC in vitro differed. Cells obtained from BT HIV$^+$ patients failed to proliferate in response to mycobacterial antigens and the mitogen PHA (Table 2). The PBMC did retain the capacity for production of IFN-γ, and mRNA specific for IFN-γ was produced by PBMC (Tables 3 and 4). However, natural killer (NK) cells or γδ T cells as well as CD8$^+$ T cells rather than CD4$^+$ T cells might be involved in the production of IFN-γ. This is currently being investigated.

Another observation indicates that the production of IFN-γ continues in the patients with concurrent HIV and *M. leprae* infections. In BT HIV$^+$ patients, HLA-DR is expressed on the surface of keratinocytes overlying the lesions, suggesting that the leukocytes retained the capacity for local IFN-γ production, which in turn upregulates HLA-DR in keratinocytes. The continued presence of IFN-γ could activate local macrophages, thus inducing granuloma formation. The bacteriocidal and bacteriostatic capacities of the host macrophages appear uncompromised by the presence of HIV or by the course of HIV infection. This is consistent with previous work showing that local injection of IFN-γ into lepromatous leprosy lesions induces the accumulation of lymphocytes and monocytes, with subsequent granuloma formation and local clearance of bacilli (9, 10, 14, 19). Granuloma formation in AIDS patients with other mycobacterial infections (*M. avium* and *M. tuberculosis*) has also been reported (7). The source of IFN-γ in the tissues may be NK cells, CD8$^+$ T cells, or γδ T cells, as well as CD4$^+$ T cells.

Although local macrophage activation does not appear to be impaired by HIV infection, blood mononuclear leukocyte (T cells and monocytes) migration to the site of antigen deposition is compromised. This is shown by the lack of skin test responsiveness to lepromin testing (Mitsuda) in BT HIV$^+$ patients. Thus, HIV infection may not only eliminate memory CD4$^+$ T cells but also alter the cytokine balance that is necessary for cellular traffic control, so that the stimuli needed to bring cells to the site of mycobacterial infection or antigenic stimulation are impaired. Previous studies of HIV-1-infected patients who were anergic to skin test antigens have documented a restoration of skin test responsiveness following intradermal administration of low-dose recombinant human IL-2 (12).

The delay in granuloma response may be a critical factor in the course of mycobacterial infection. However, for an organism that replicates only intracellularly, such as *M. leprae*, this delay may not result in changes in clinical manifestation of the disease. However, for an organism that replicates extracellularly, the delay in recruitment of cells to the site of the infection may leave a critical "window of opportunity" for the infectious organism to proliferate before a cellular response can

occur. The involvement of cytokines in the regulation of the granulomatous response and recruitment of mononuclear cells to the site of mycobacterial infections suggests that modulation of cytokine levels could result in restoration of skin test responsiveness as well as improved host response to mycobacterial infection in the HIV^+ individual. This hypothesis is currently being tested.

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