DEFECTIVE γ INTERFERON PRODUCTION IN LEPROSY

Reversal with Antigen and Interleukin 2

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Leprosy exhibits a wide range of cell-mediated responses resulting in disease states ranging from benign tuberculoid to multibacillary, lepromatous states (1). The skin lesions of tuberculoids show organized granuloma, many Leu 3a/OKT4 helper T cells, and good T cell proliferative responses to the specific organisms. In contrast, lepromatous disease is characterized by a deficient cellular response with the predominance of Leu 2a/OKT8 suppressor/cytotoxic cells in the dermis, the presence of bacilli-laden macrophages, and the absence of T cell proliferative responses (2–3).

Recent evidence has implicated γ interferon (γ-IFN) as the important macrophage-activating factor in lymphokine preparations in both tumoricidal and microbicidal systems (4–7). These results led us to examine the production of this lymphokine in patients with a spectrum of lepromatous, intermediate, and tuberculoid disease. During the course of these studies, a report by Haregewoin et al. (8) suggested that lepromatous lymphocytes failed to make interleukin 2 (IL-2) and that a crude supernatant containing IL-2 restored their proliferative responses to specific antigen.

We now report that the peripheral blood lymphocytes of patients with lepromatous leprosy failed to produce γ-IFN upon exposure to antigen and had reduced responses to mitogen. This deficiency was restored by the addition of a purified human IL-2.

Material and Methods

Patients. After informed consent, skin and heparinized peripheral blood was collected from 20 patients in Brazil and 14 patients in the U. S. Brazilian patients were studied in collaboration with the Departments of Dermatology and General Pathology, Hospital das Clinicas, Universidade do Estado do Rio de Janeiro. In the U. S., patients were obtained through the Staten Island Public Health Hospital in New York. Clinical diagnosis was established by Dr. Jarbas A. Porto in Brazil and Dr. W. Levis in the United States. Histologic diagnosis was established according to the Ridley-Jopling classification (9) by Dr. E. Sarno in Brazil and, in the U. S., Dr. C. K. Job of the National Hansen's Disease Center, Carville, LA.

Production of γ-IFN by Peripheral Blood Mononuclear Cells.   Peripheral blood mononuclear cells (PBMC), obtained as previously described (10), were suspended to 3×10^6/ml in RPMI containing 20% autologous human plasma, penicillin (1,000 U/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM). Cells were incubated in 24-well Costar plates (Costar, Data Packaging, Cambridge, MA) in the presence of *Mycobacterium leprae* (2.5 μg/ml) or concanavalin A (Con A) (15 μg/ml). Controls included cells cultured in medium alone, as well as cells first cultured in medium alone for the appropriate time, with Con A and *M. leprae* added at the end of the incubation period. *M. leprae* was kindly provided by Dr. R. J. W. Rees (IMMLEP Bank), The National Institute of Medical Research, The Ridgeway, Mill Hill, England and Dr. P. Brennan of the Department of Microbiology, Colorado State University, Ft. Collins, Colorado. Culture media were harvested as a source of immune IFN at 2 d (Con A) or 5 d (*M. leprae*) unless otherwise stated, and kept frozen (−20°C) until used.

γ-IFN Assay.   Antiviral activity was determined by the reduction in encephalomyocarditis virus cytophatic effect on human foreskin cells (FS4) grown on microtiter plates (11). Antiviral activity expressed in IFN units was calculated as the reciprocal of the highest dilution of the sample that reduced the viral cytophatic effect by 50%. A laboratory standard for human γ-IFN was included in each assay. All titers were expressed in actual laboratory units without correction. Antiviral activity was determined to be γ-IFN by neutralization assays using a rabbit anti-γ-serum (12).

IL-2 Purification.   PBMC purified as described above (3×10^6/ml) were stimulated for 30 h in the presence of 10 μg/ml Con A and 10 ng/ml phorbol myristate acetate (PMA) in RPMI medium containing 2% human serum albumin. One liter of conditioned medium was concentrated 10× using a YM 10 Amicon membrane (Amicon Corp., Danvers, MA), dialyzed against 20 mM Na citrate, pH 5.5, and loaded on 20-ml column of SP-Sephadex C-25 (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated in 20 mM Na citrate, pH 5.5. The column was washed with the equilibration buffer and IL-2 activity was eluted with increasing salt concentration. Fractions containing IL-2 activity (between 0.15 and 0.30 M NaCl) were pooled and dialyzed against 50 mM Tris HCl, pH 7.8, and loaded on a 20-ml column of DEAE-52 cellulose (Whatman Laboratory Products, Inc., Clifton, NJ) equilibrated with 50 mM Tris HCl, pH 7.8. IL-2 was eluted using a linear gradient of NaCl (0–0.2 M) in 50 mM Tris HCl, pH 7.8. The active fractions (0.05–0.07 M NaCl) were pooled, dialyzed against phosphate-buffered saline (PBS), and loaded in 10-ml column of blue agarose (Bio-Rad Laboratories, Richmond, CA) equilibrated in PBS. IL-2 activity was eluted using a linear gradient of NaCl (0.15–1 M) in PBS. The resulting fractions with IL-2 activity (0.6–0.75 M NaCl) were pooled, concentrated, and dialyzed against 0.05% sodium dodecyl sulfate (SDS). Aliquots were electrophoresed in a 12.5% SDS-polyacrylamide gel electrophoresis (PAGE). The gel was cut into 1-mm slices, eluted in PBS for 18 h at 4°C, and assayed for IL-2 activity. The active fractions were pooled and kept at 4°C. This IL-2 preparation contained 250 U/ml and displayed a single band when run in SDS-PAGE and stained with the silver method, and was completely devoid of IFN activity. One unit corresponded to the minimum amount of IL-2 required to double the [^{3}H]thymidine incorporation observed in untreated control cultures using for the IL-2 bioassay 2×10^4 Con A-stimulated spleen cells (13).

[^{3}H]Thymidine Incorporation.   After the appropriate incubation, cells were harvested and resuspended to 2×10^6 cells/ml in 20% autologous plasma as described above. Triplicate samples containing 2×10^5 cells/well were plated in 96-well round-bottomed trays (Linbro) and 1 μCi [^{3}H]thymidine added per well. The cells were incubated for 18 h at 37°C, harvested, and counted in a scintillation counter.

Immunofluorescence Assays.   Assays were carried out as previously described (4).

Results

γ-IFN Release by PBMC from Leprosy Patients.   PBMC from 34 patients were analyzed for their ability to generate γ-IFN in response to the specific antigen *M. leprae* or to the mitogen Con A. 17 of 18 lepromatous leprosy and borderline

TABLE I

γ-IFN Release by PBMC from Leprosy Patients

Patient	Treatment duration	Clinical diagnosis*	Histological diagnosis	γ-IFN titer	
				+ M. leprae	+ Con A
1 (SRG)	None	L	LL	<4	<4
2 (EAS)	None	L	LL	<4	<4
3 (AA)	1 yr	L	LL	<4	<4
4 (CL)	2 yr	L	BL	<4	<4
5 (APA)	None	L	LL	<4	<4
6 (FL)	<1 yr	L	LL	<4	<4
7 (DMCO)	None	L	I	<4	<4
8 (CCB)	None	L	LL	<4	<4
9 (FBB)	None	L	LL	<4	32
10 (AR)	>5 yr	L	LL	<4	8
11 (SK)	>1 yr	L	LL	8	32
12 (AW)	>5 yr	L	LL	<4	8
13 (CBS)	None	L	BL	<4	<4
14 (RM)	3 yr	L	BL/LL	16	8
15 (FEG)	<1 yr	L	BL	8	15
16 (AW)	>1 yr	B	LL	<4	64
17 (R)	None	L	LL	4	32
18 (JB)	None	B	BL	<4	256
19 (CTR)	None	B	BB	4	16
20 (MDV)	None	L	BB	<4	32
21 (MA)	None	T	TT	<4	256
22 (S)	>1 yr	T	NSI‡	64	16
23 (ML)	10 yr	T	ND§	32	64
24 (GB)	>5 yr	L	BB	32	2,048
25 (JTL)	None	T	TT	16	512
26 (MGSC)	None	B	BT	16	256
27 (RJF)	None	T	BT	16	256
28 (VS)	>5 yr	T	ND	64	64
29 (RCMP)	None	B	BT	128	512
30 (RS)	5 yr	T	BT	128	128
31 (MV)	1 yr	T	BT	256	ND
32 (BFBS)	None	T	TT	256	256
33 (CR)	None	T	TT	128	4,096
34 (IP)	>1 yr	T∥	ND∥	128	ND

* L, lepromatous; B, borderline; T, tuberculoid.
‡ Nonspecific infiltrate; active erythema nodusum leprosum (ENL) reaction.
§ Not done.
∥ BT neural.

lepromatous patients (LL and BL) failed to generate γ-IFN in response to antigen and were similarly unresponsive to Con A (Table I), despite their showing proliferative responses to mitogen (not shown). Patients with the mid-borderline form of leprosy (BB) and a few patients with a histological diagnosis of borderline tuberculoid and tuberculoid leprosy (BT-TT) displayed intermediate responses. Six typical tuberculoid patients (BT-TT) showed vigorous γ-IFN production in response to both antigen and mitogen. Low γ-IFN production in response to antigen was observed not only in untreated lepromatous patients, but also in lepromatous patients who had been under chemotherapy for different lengths of time (Table I).

Omission of the antigen or mitogen during the incubation period (medium alone) or the addition of antigen or mitogen at the end of the incubation period (Con A control and M. leprae control) were without effect on γ-IFN production. In all cases, the antiviral activity could be specifically neutralized by a rabbit

TABLE II

Effect of IL-2 on γ-IFN Release

Patient	Treatment	Clinical diagnosis*	Histological diagnosis	γ-IFN Titer					
				Without M. leprae			With M. leprae		
				−IL-2	+IL-2		−IL-2	+IL-2	
					1 U/ml	5 U/ml		1 U/ml	5 U/ml
Lepromatous									
FEG 1	<1 yr	L	LL	<4	16	ND‡	8	128	ND
RM 2	3 yr	L	BL/LL	<4	8	ND	16	128	ND
CL 3	2 yr	L	BL	<4	32	32	16	128	512
GB 4	>5 yr	L	BL/BB	<4	<4	32	32	64	1,024
AL 5	1 yr	L	LL	<4	<4	8	<4	4	16
EG 6	1 yr	L	LL	<4	<4	32	<4	32	64
SK 7	>1 yr	L	LL	<4	<4	<4	<4	64	64
AR 8	>5 yr	L	LL	<4	<4	<4	<4	4	4
AW 9	>5 yr	L	LL	<4	<4	<4	<4	4	8
Tuberculoid									
IP	>1 yr	T§	ND	<4	8	16	128	128	256
US	>5 yr	T	ND	<4	<4	<4	64	64	64
Control individuals									
AW				<4	<4	4	<4	<4	<4
JH				<4	4	8	4	<4	16
JA				<4	ND	ND	<4	ND	ND
A				<4	<4	4	16	16	64
PK (male)				<4	<4	4	<4	16	64
PK (female)				<4	8	4	<4	4	32

* L, lepromatous; T, tuberculoid.
‡ Not done.
§ BT neural.

antiserum against γ-IFN (not shown). This includes the antiviral activity observed in PBMC from lepromatous patients stimulated by antigen or mitogen in the presence of a purified IL-2.

Restoration of γ-IFN Production in Lepromatous and Tuberculoid Patients. In view of the absent IL-2 response by lepromatous cells (9), we examined the effects of a purified IL-2 preparation on γ-IFN formation. The addition of 1–5 U/ml of IL-2, in the presence of *M. leprae* antigen, led to a significant increase in γ-IFN production in lepromatous patients (Table II). The levels achieved were similar to those produced by antigen-triggered PBMC from tuberculoid patients and seemed related to their initial responsiveness to *M. leprae*. IL-2 also restored responsiveness to Con A and was accompanied by a 10-fold increase in [³H]-thymidine incorporation (not shown). Neither unsensitized control subjects nor patients with tuberculoid leprosy demonstrated enhanced γ-IFN release in the presence of IL-2 and *M. leprae* antigen. Control subjects were unresponsive to the antigen and tuberculoid patients were already making good amounts of γ-IFN in response to antigen and mitogen in the absence of added IL-2.

Correlation of Helper Suppressor Ratio in Skin Biopsies and PBMC γ-IFN Release. Levels of γ-IFN released correlated more closely with the ratio of helper/suppressor (OKT4/OKT8) cells in cutaneous lesions than with histological diagnosis, particularly in borderline cases (Tables I and II); ie., γ-IFN unresponsiveness was associated with a low helper/suppressor ratio (not shown). In some cases, patients classified as TT or BT by histological means (not shown) displayed low γ-IFN levels and a low OKT4/OKT8 ratio.

Discussion

We describe in this report a selective defect in the production of γ-IFN by PBMC of patients with lepromatous leprosy, whereas cells from those with tuberculoid disease release readily detectable γ-IFN. Hyporesponsiveness was most strikingly demonstrated in response to specific *M. leprae* antigen, but was also observed when cells from lepromatous patients were challenged with the polyclonal activator Con A. This is in keeping with the recently reported deficit in IL-2 production (9) and the well-known absence of a proliferative response (2, 3). Indeed, the addition of purified human IL-2 as well as antigen resulted in a marked restoration of γ-IFN production. This fact, in turn, leads to the conclusion that IL-2 is a necessary but not sufficient element in the chain of events that ultimately results in the production of γ-IFN.

The preponderance of Leu 2a/OKT8 (suppressor/cytotoxic) T cells in skin lesions was associated with the lack of IL-2 production by PBMC. This raises the possibility that suppressor T cells, or their products, may influence IL-2 production as they do in other systems (14, 15). The absence of this mediator might then lead either to defective expansion of specifically sensitized T cells able to release γ-IFN or the failure to trigger them for γ-IFN release. Consequently, no activation of macrophages occurs at the site. These hypotheses are being currently investigated. Recent studies (16) have indicated that IL-2 induces B cell helper factor production in stimulated mouse T cells.

These results suggest that the absence of γ-IFN production is correlated with the defect in cell-mediated immunity. In the lepromatous state, the lack of macrophage activation may provide a fertile intracellular milieu for the uncontrolled replication of the bacilli. In addition, the small local T cell infiltrate and the absence of helper T cells complicate dermal responsiveness.

The unresponsiveness of peripheral blood T cells is more closely correlated with the helper/suppressor T cell ratios of the skin than with the histological scoring index. This may reflect both the heterogeneity of the local skin lesions and the general lack of parenteral responsiveness. In this sense, the quantitative assay of γ-IFN production may prove to be a useful prognosticator of cellular immunity and may reflect on the patient's subsequent course.

The presumed in vivo importance of macrophage activation for the control of bacillary replication and the restorative qualities of IL-2 or γ-IFN production suggests future clinical applications of these molecules. Both γ-IFN and IL-2 will soon become available in relatively large quantities through recombinant DNA technology. Their local and/or parenteral administration as replacement immunotherapy in lepromatous disease may serve as an adjunct to chemotherapy.

Summary

Antigen and mitogen-induced γ interferon (γ-IFN) production was studied in peripheral blood mononuclear cells from 34 leprosy patients. 17 of 18 lepromatous leprosy and borderline lepromatous patients (LL and BL) failed to release γ-IFN in response to specific antigen (*Mycobacterium leprae*) and displayed reduced responses to mitogen (concanavalin A) stimulation. In contrast, cells from six tuberculoid and borderline tuberculoid patients (TT and BT) produced considerable levels of γ-IFN under the same experimental conditions. Normal controls

failed to respond to *M. leprae* and most displayed good responses to concanavalin A. Mid-borderline patients (BB) showed intermediate levels of γ-IFN release. γ-IFN release by lepromatous patients could be partially restored with purified interleukin 2 and *M. leprae* antigen but not with interleukin 2 alone.

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