ANALYSIS OF NATURALLY OCCURRING DELAYED-TYPE HYPERSENSITIVITY REACTIONS IN LEPROSY BY IN SITU HYBRIDIZATION

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Leprosy provides a useful model for understanding immunoregulatory mechanisms in man. The disease forms a spectrum in which the immunologic response of the patient correlates with the clinical and histopathologic classification (1–3). At one end of the spectrum, patients with tuberculoid leprosy have one or several skin lesions in which bacilli can rarely be identified. $CD4^+$ T lymphocytes predominate in these lesions (4–7) and respond to *Mycobacterium leprae* in vitro (8). At the other end of the spectrum, patients with lepromatous leprosy have diffuse infiltration of skin and nerves with bacilli-laden macrophages. $CD8^+$ cells predominate in lepromatous lesions and function as antigen-specific Ts cells in vitro (9, 10). The $CD4^+$ lymphocytes derived from these lesions are unresponsive to *M. leprae*. Imposed upon this spectrum are the so-called "reactional states" of reversal reaction and erythema nodosum leprosum (ENL)[1] that are of immunologic interest in understanding mechanisms of immunoregulation in man.

The reversal reaction syndrome is associated with a marked rise in lymphocyte transformation to *M. leprae* antigens in vitro, and is therefore thought to be a delayed-type hypersensitivity (DTH) reaction against *M. leprae* antigens (11–15). Clinically, the condition is often associated with reduction or elimination of bacilli in lesions and sometimes followed by upgrading of the clinical and histological classification toward the tuberculoid pole. The pathogenesis of ENL reactions is somewhat controversial, thought to be due to immune complex deposition in the lesions (16–18) and/or increases in cell-mediated immunity (19, 20).

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[1] *Abbreviations used in this paper:* AEC, aminoethyl carbazole; DTH, delayed-type hypersensitivity; ENL, erythema nodosum leprosum.

Immunohistologic study of skin lesions has thus far not distinguished between the immune processes underlying the pathogenesis of reversal reaction and ENL (21–25). The inflammatory infiltrates in both reactional states are characterized by a predominance of $CD4^+$ lymphocytes. IL-2-containing cells are present in the lesions of both conditions. Furthermore, keratinocyte Ia expression, a putative marker of IFN-γ production, and Langerhans cell hyperplasia are apparent in the epidermis overlying both reactional states.

The present study was undertaken to investigate the immunoregulatory processes that underlie these reactional states of leprosy, utilizing newly developed reagents and technology to analyze the inflammatory infiltrates. We performed in situ hybridization to cellular mRNA using probes for IFN-γ and human serine esterase (huHF), a marker for cytotoxic cells, in order to define functional T cell subpopulations in these reactions. The findings of these studies were then analyzed in the context of immunostaining of lesions with mAbs that define subpopulations of $CD4^+$ and $CD8^+$ lymphocytes. The results suggest that reversal reactions are more characteristic of classical DTH reactions than ENL, and represent a window into the complex immunoregulatory mechanisms underlying the pathogenesis of DTH in man.

Materials and Methods

Patients. Patients with leprosy were classified according to the clinicopathological criteria of Ridley and Jopling (1). Clinical criteria for the diagnosis of reversal reactions included torpid lesions becoming erythematous and tumid, the abrupt onset of new erythematous, tumid lesions, and the sudden onset of neuritis. Histological changes were not uniform but included edema in the granulomas, increased numbers of lymphocytes, giant cells, and desmoplasia of the connective tissue. The diagnosis of ENL was made using clinical and histopathologic criteria. Patients presented with the distinctive picture of crops of painful and tender erythematous nodules in association with fever, malaise and arthralgias. Histopathologic examination of lesions revealed the presence of neutrophils and lymphocytes superimposed over a lepromatous infiltrate. In addition, lepromin skin tests (3-wk Mitsuda reactions) were studied from tuberculoid patients (26). All specimens were obtained with informed consent from tuberculoid patients (26). All specimens were obtained with informed consent from patients at the Outpatient Clinic of Los Angeles County/University of Southern California Medical Center, San Francisco Medical Center (with gratitude to Dr. Robert Gelber), and McKean Rehability Institute, Chiang Mai, Thailand. The patients were distributed among the different diagnostic groups showing no segregation according to sex, race, or age.

Tissues. Skin biopsy specimens, obtained by punch or scalpel technique, were divided for histological diagnosis on conventional paraffin sections and for immunoperoxidase and in situ hybridization study by snap freezing in liquid nitrogen tissue embedded in OCT medium (Ames Co., Elkhart, IN). The tissues were stored at −70°C until sectioning.

In Situ Hybridization

The in situ hybridization technique described below was performed as previously described (27).

Tissue Preparation. Biopsy specimens were collected and frozen as described above. 3-μm cryostat sections were placed on poly-L-lysine hydrobromide-coated slides (Sigma Chemical Co., St. Louis, MO) and air dried for 2 h. Dried sections were fixed for 20 min in 4% paraformaldehyde-PBS (pH 7.4), rinsed through several changes of PBS (Flow Laboratories, Inc., McLean, VA), and dehydrated through graded alcohols. Sections were stored dessicated at 4°C until use.

Probe Preparation. Two probes were utilized in the present study: IFN-γ cDNA, which was provided by Hoffmann-La Roche, Inc. (Nutley, NJ), and huHF gene coding for human serine esterase, as described previously (28). The cDNA was recloned into an expression vector system

that would allow for the generation of both sense as well as antisense ^{35}S-labeled RNA probes. IFN-γ cDNA was inserted into pGem-1 (Promega Biotec, Madison, WI) and the huHF gene into a pGem-1 like vector, pSPT671 (27). Template DNA was prepared in each case by linearizing plasmid DNA with restriction enzymes that cut once at the distal end of the cloned insert; this restricted in vitro RNA synthesis to probe-specific sequences. Both inserts were cloned in the 5′ to 3′ direction so that RNA transcripts generated in vitro by transcription from the 5′ SP6 promotor-generated sense-strand RNA, while the 3′ T7-directed synthesis produced antisense RNA. ^{35}S-labeled transcripts were reduced to an average 200-bp length by alkaline hydrolysis (29), then ethanol precipitated after adding 7.5 μg yeast tRNA/10^6 cpm. The probe was subsequently resuspended at 1–2 × 10^6 cpm in 10 mM Tris with 1 mM EDTA, pH 8, and stored at −70°C until hybridization to tissue sections.

Hybridization. Sections were digested in 1 μg/ml proteinase K in 100 mM Tris/50 mM EDTA, pH 8, at 37°C for 30 min, then refixed for 20 min in 4% paraformaldehyde/PBS to stabilize cellular mRNA within the proteolyzed matrix. Free amino groups on tissue sections were acetylated by treatment with 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8) for 10 min; the tissues were rinsed briefly in water then air dried while awaiting hybridization. For hybridization, probe (denatured at 80°C for 3 min) was mixed with formamide (50% final concentration), NaCl (300 mM), Tris-HCl, pH 7.5 (20 mM), EDTA (5 mM), Denhardt's solution (1 ×), dextran sulfate (10%), and dithiotreitol (100 mM) at a final concentration of 10^5 cpm/μl hybridization mix. 10 μl of this hybridization solution containing 10^6 cpm probe was added to each slide, the tissue covered with a siliconized coverslip, and sealed with rubber cement. Slides were hybridized for 17–20 h at 47°C. Unhybridized probe was washed from the slides in a solution of 50% formamide, 2 × SSC, and 5 mM EDTA in four changes for a total of 2 h at 56°C. After the second wash, the slides were digested with RNase A (20 μg/ml) and RNase T1 (1 U/ml) for 30 min at 37°C. After the last wash, slides were dehydrated through graded alcohols containing 400 mM ammonium acetate and air dried. For autoradiography, slides were dipped into Kodak NTB-2 emulsion, diluted 1:2 with 400 mM ammonium acetate, and exposed at 4°C for 10–28 d. The slides were then developed using Dektol developer (1:2 in water) for 2 min and Fixer for 5 min (photography supplies from Eastman Kodak; Rochester, NY) and counterstained with either hematoxlyin and eosin (IFN-γ) or 4% Giemsa (huHF). Each tissue was hybridized with sense-strand (homologous to cellular mRNA) as well as antisense strand (complementary to cellular mRNA) probes; known positive and negative control tissues were included in each run.

Evaluation of Slides. Cells were considered to be positive for the expression of IFN-γ when they contained greater than twice the number of grains of the most positive cell on the control (sense) slide for the same tissue. Positive cells were enumerated over the entire tissue section and the percentage of positive cells was then calculated (8). Cells positive for huHF were numerous and were estimated by three independent observers whose percentages were within five percentage points. Gene expression levels were evaluated by determining the median grain number over background of positive cells in each section.

Immunostaining Studies

mAbs. Primary mouse anti-human lymphocyte mAbs were used at optimal concentrations (CD3, pan T cell marker, anti-Leu-4 at 1:200; CD8, T suppressor/cytotoxic marker, anti-Leu-2a at 1:50; CD4, T helper/inducer marker anti-Leu-3a at 1:50 [all from Becton Dickinson & Co., Mountain View, CA]) as determined by checkerboard titration on cells from reactive tonsils.

Recently, these CD4 and CD8 subsets have been further divided into subpopulations by new mAbs (30). $CD8^+$ cells that bear the 9.3 marker (CD28) have cytotoxic function, and cells that are $CD8^+$ 9.3^- have suppressor function in vitro (31). Similarly, evidence suggests that $CD4^+$ cells bearing the 4B4 (CDw29) or UCHL1 (CD45) markers belong to a T helper/memory subpopulation (32, 33) whereas those expressing 2H4 (CD45R) or Leu-8 antigens comprise a population of naive T cells (34–36). Anti-2H4 was used at 1:50, anti-4B4 at 1:400 (both from Coulter Electronics Inc., Hialeah, FL), UCHL1 (Dr. P. Beverley, London) at 1:20, anti-Leu-8 at 1:100 (Becton Dickinson & Co.), and anti-9.3 (Dr. J. Hansen, Seattle, WA) was used at 1:50. Controls in each experiment included omission of the primary anti-

body and the use of irrelevant antibodies of the same IgG subclass on sections for the same period of incubation. Blocking was used for some mAbs that gave significant levels of background staining, (e.g., anti-4B4, -9.3, and -Leu-3a). Where appropriate, slides were preincubated in normal serum (1:20 in PBS) derived from the species that generated the secondary antibody; i.e., normal goat serum was used when the linking antibody was biotinylated goat antimouse.

Single Immunoperoxidase Staining. All sections were acetone fixed and incubated sequentially as described (21): first with the mAbs, then peroxidase-conjugated goat anti-mouse IgG 1:20 (Tago Inc., Burlingame, CA) for 30 min with 10-min PBS washes between each incubation. Slides were then incubated with the chromogen aminoethyl carbazole (AEC) in the presence of hydrogen peroxide for 15 min, counterstained with hematoxylin, and then mounted in glycerine-gelatin. The IFN-γ-induced peptide, IP-10, was visualized in tissue sections using a rabbit polyclonal antibody as previously reported (37).

Double Immunoperoxidase Staining. This procedure allows for the visualization of two antigens on the same section. For the monoclonals that gave significant levels of background staining, the sections were first blocked with normal goat or horse serum (diluted 1:20 in PBS), as specified above. Each section was stained first with primary mAbs followed by goat anti-mouse IgG-peroxidase conjugate. Throughout both single and double immunostaining procedures, each antiserum (or Avidin-Biotin complex) was applied to the tissue section for 30 min, followed by a 10-min PBS wash. The first reaction was developed by a short (8-min) incubation in AEC. The development period was shorter than that used in single staining so that the strong AEC precipitate would not obscure the weaker blue precipitate deposited in the second stage of the procedure. The sections were washed with PBS for 10 min. Before the second stain, all sections were blocked with normal horse serum at 1:20 (Vector Laboratories, Inc., Burlingame, CA). Subsequently, the slides were incubated with the second primary antibody, washed and then incubated with the secondary biotinylated horse anti-mouse IgG 1:50 (Vector Laboratories, Inc.), followed by an incubation with the ABC-GO kit (Vector Laboratories, Inc.). The blue color was developed with a glucose oxidase substrate kit (containing nitroblue tetrazolium as chromogen) or alternatively using β-galactosidase kit (Vector Laboratories, Inc.) using X-gal as the chromogenic substrate. Slides were then rinsed in tap water for 10 min and mounted with glycerine-gelatin. Control slides, on which either of the primary antisera were omitted, demonstrated that the second labeling system did not cross-react with the antibodies already added in the first reaction.

Quantitation of Stained Cells. Single staining cells were numerous, such that the percentage of positive cells was determined by averaging the estimates of two independent observers; their reading invariably agreed within five percentage points. For double immunostaining, cells were counted to be double positive when both red and blue colors could be seen on the same cells or a purple color distinct from either red- or blue-colored cells in the same section was identified. Percentages of double-stained cells were determined as described previously (38).

Results

IFN-γ Gene Expression in Lesions. To assess functional activity of T lymphocytes in lesions, we initially performed in situ hybridization to detect IFN-γ mRNA. 16 patients with reversal reaction were studied, all of whom were classified as having borderline lepromatous leprosy. 10 patients with ENL were classified as having lepromatous disease. Findings were compared with specimens from patients with borderline lepromatous leprosy not in reversal reaction (n = 7), tuberculoid patients (n = 15), and lepromatous patients (n = 10). Since the "Mitsuda" reaction to *M. leprae* is an established measure of DTH, skin test reactions in three responsive individuals were studied. Cells showing positive hybridization were most numerous in reversal reactions, followed by lepromin skin tests and tuberculoid lesions (Figs. 1 and 2). In comparison, lesions from nonreactional lepromatous patients and patients with ENL

contained few positive cells, generally an order of magnitude lower than in reversal reactions. The positive cells were centrally located within the granuloma in reversal reactions and tuberculoid lesions, suggesting that they are of the Th phenotype (Fig. 1). The relative level of mRNA was estimated by determination of the number of grains per average positive cell. IFN-γ mRNA expression was five times higher in reversal reactions than tuberculoid lesions or lepromin skin tests (Fig. 2). Interestingly, the few positive cells in ENL lesions contained levels of IFN-γ mRNA that were five times greater than found in lepromatous cases.

To obtain evidence that IFN-γ mRNA was translated into functional protein, immunostaining for IP-10 was undertaken. This peptide has been reported to be specifically induced by IFN-γ in cells, including keratinocytes (37). The majority of kerotinocytes overlying reversal reactions and tuberculoid leprosy stained positive for IP-10 (Fig. 1), indicating the presence of IFN-γ in these lesions. On the other hand, few keratinocytes in lepromatous epidermis were positive for IP-10. ENL lesions gave an intermediate staining pattern.

huHF Gene Expression in Lesions. We investigated lesions for the presence of T cells of cytotoxic function using a riboprobe for the enzymatic marker associated with cytotoxic cells, serine esterase (huHF). Cells positive for huHF were more numerous in tuberculoid, lepromin skin tests, and reversal reaction lesions, as compared with ENL and nonreactional lepromatous lesions (Figs. 3 and 4). The microanatomic location of huHF$^+$ cells within granulomas of tuberculoid leprosy and reversal reaction was similar to that of the CD4$^+$UCHL1$^+$ subpopulation (see Fig. 3). The relative gene expression was higher in reversal reaction than in tuberculoid lesions (Fig. 4).

T Cell Subsets in Lesions. The nature of the T lymphocyte infiltrates in lesions of leprosy reactional states was established by using mAbs in conjunction with immunohistochemical techniques. These studies were undertaken to provide experimental support for the in situ hybridization studies.

The distribution and location of the CD4 and CD8 T lymphocytes subsets were found to be similar to that described in earlier studies (21, 22). CD4$^+$ cells predominated in reversal reaction and ENL lesions, lepromin skin tests, and tuberculoid lesions, but not in nonreactional borderline lepromatous or lepromatous lesions. The pattern of cell distribution in reversal reaction specimens was similar to that found in tuberculoid granulomas: CD4$^+$ cells were distributed throughout the granulomas with CD8$^+$ cells confined to the surrounding mantle zone. In contrast, in ENL and nonreactional lepromatous or borderline lepromatous lesions, both CD4$^+$ and CD8$^+$ cells were admixed throughout the biopsy sections.

CD4$^+$ Subpopulations. To analyze in more detail the nature of the immunologic processes underlying the reactional states, lesions were analyzed with mAbs used to define subpopulations of CD4 and CD8 cells. In reversal reactions, as in tuberculoid granulomas and Mitsuda reactions, 2H4$^+$ and Leu-8$^+$ cells were invariably restricted to the mantle area surrounding the granuloma, whereas the cells associated with macrophages in the granuloma were of the UCHL1 and 4B4 phenotype (Fig. 3). These markers have been associated with differentiated or memory T cells and Th cells. In contrast, in ENL and nonreactional lepromatous and borderline lepromatous, all phenotypes were admixed with macrophages.

Double immunostaining was undertaken to quantify the various CD4 subpopulations since some markers, such as UCHL1, are present on different cell types (Fig. 5).

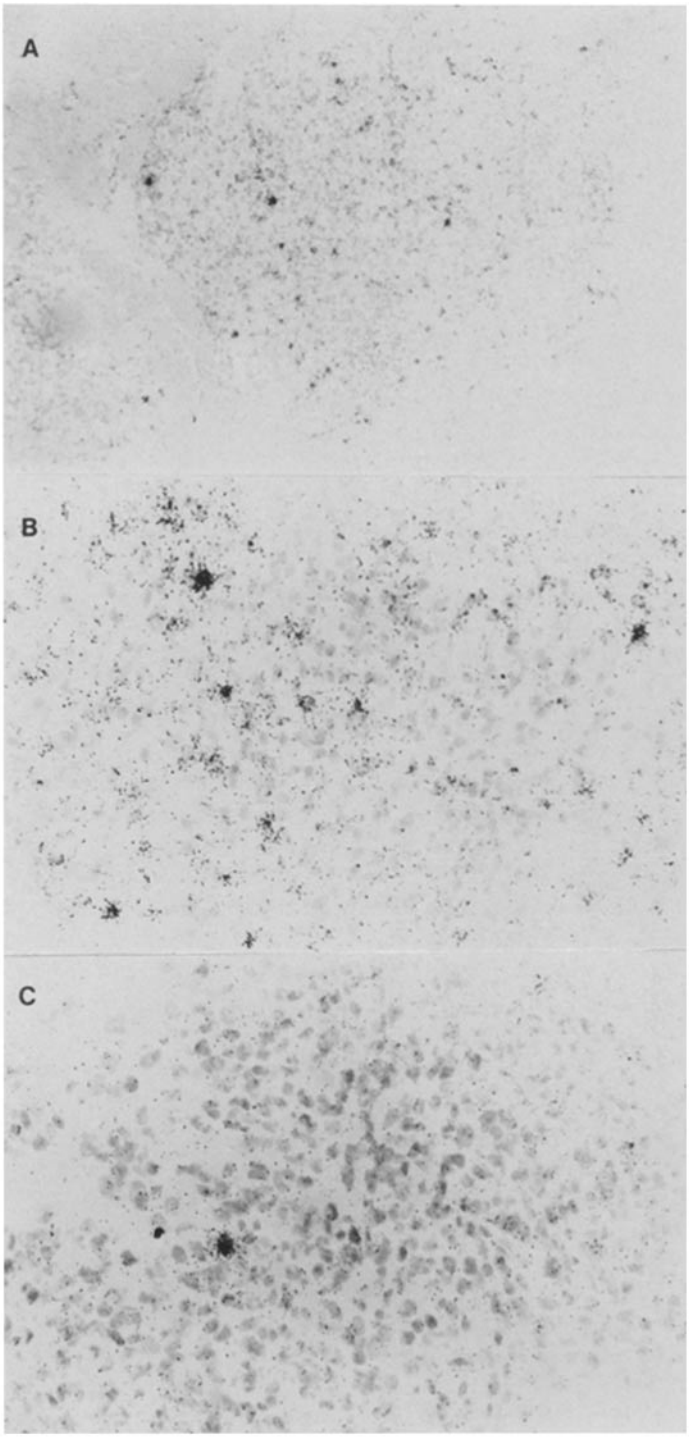


FIGURE 1. Continued on following page.

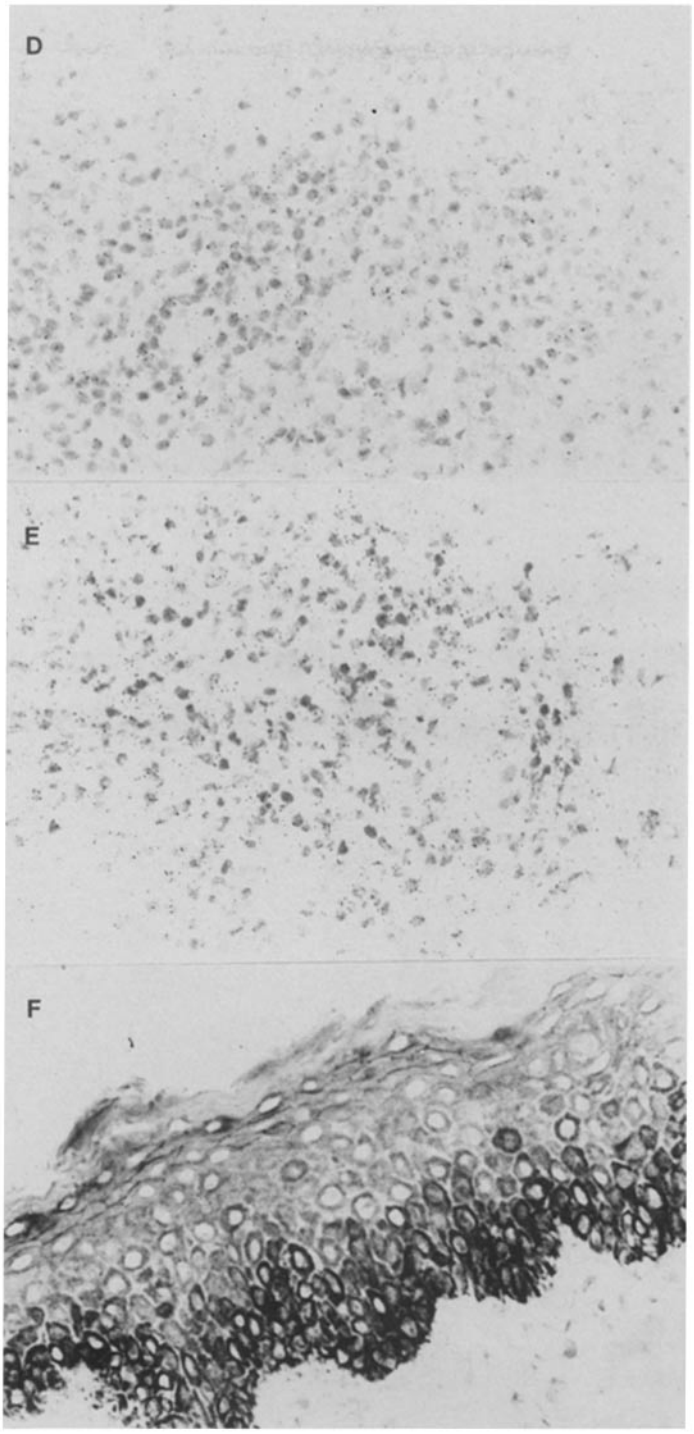


FIGURE 1. In situ hybridization for IFN-γ cellular mRNA in leprosy lesions. (*A*) IFN-γ mRNA-positive cells can be identified by clustering of silver grains above cells in this reversal reaction lesion (× 10 objective). (*B*) Reversal reaction, higher power of (*A*) demonstrating positive cells (× 25). (*C*) ENL lesions with a single positive cell (× 25). Control sections for reversal reaction (*D*) and ENL (*E*) hybridized with sense probe show no hybridization (× 25). (*F*) Expression of the IFN-γ inducible peptide, IP-10, by keratinocytes overlying reversal reaction lesions is evidence that the IFN-γ mRNA is translated into functional protein (immunoperoxidase × 40).

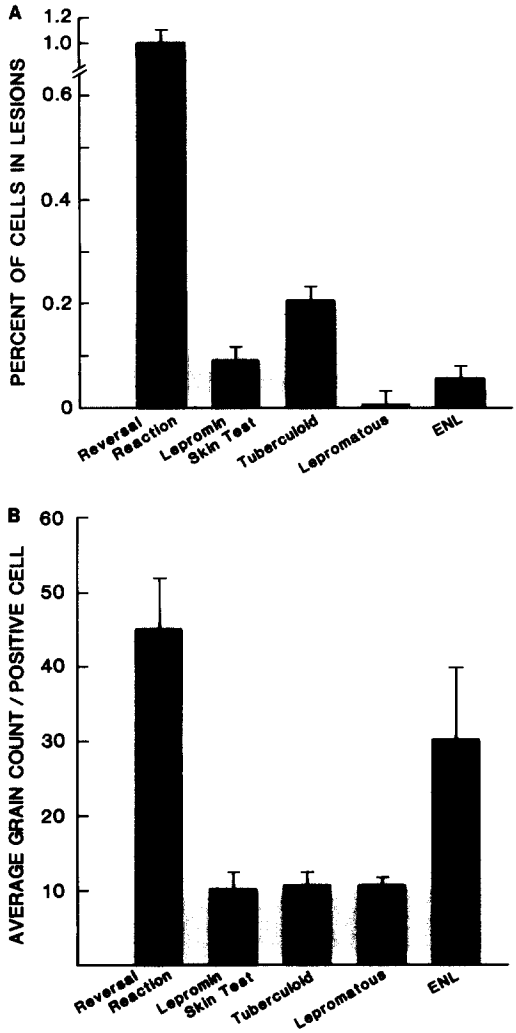


FIGURE 2. IFN-γ mRNA in leprosy lesions by in situ hybridization. (*A*) Percent of positive cells in lesions. (*B*) Gene expression as measured by median grain count over background in positive cells.

In the more immunologically active forms of leprosy, the ratio of T helper/memory ($CD4^+2H4^-UCHL1^+$) to T naive ($CD4^+2H4^+$) cells were high; however, the converse was true for immunologically unresponsive forms of leprosy. The memory to naive ratio was 9:1 in reversal reaction, 6:1 for ENL, 13:1 for lepromin skin tests, 14:1 for tuberculoid lesions, and 1:1 for nonreactional patients with borderline lepromatous or lepromatous disease. Double immunostaining with UCHL1 and Leu-8 gave results that were consistent with 2H4 results. $CD4^+UCHL1^+$ (T helper or memory) cells were numerous in reversal reactions, ENL, and tuberculoid leprosy; whereas, $CD4^+Leu\text{-}8^+$ (putative Ts-inducer) cells were a significant component of the CD4 response in lepromatous individuals.

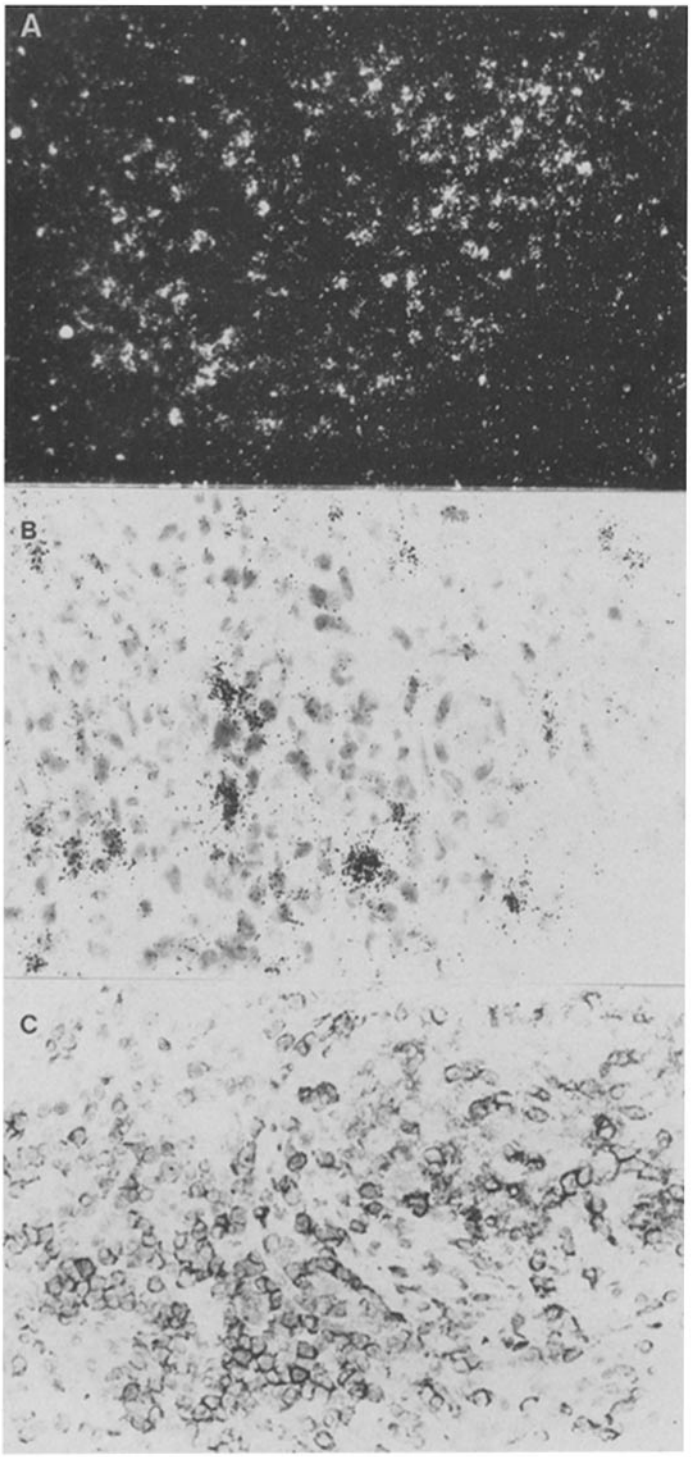
$CD8^+$ Subpopulations. Antibody 9.3 was used in conjunction with Leu-2a (anti-CD8) to divide $CD8^+$ cells into two subphenotypes, Ts (9.3^-) and T cytotoxic (9.3^+) cells (31). Double immunostaining revealed that cells of the T cytotoxic phenotype were the predominant $CD8^+$ subset in reversal reactions, lepromin skin tests, and in tuberculoid lesions, while cells of the Ts phenotype were the predominant $CD8^+$ subset in nonreactional lepromatous and ENL lesions (Fig. 5). However, since the numbers of $CD8^+$ cells in ENL lesions are few, the actual percentage of Ts phenotypes in ENL lesions are far fewer than in nonreactional lepromatous lesions, on the order of that in tuberculoid lesions.

Discussion

Analysis of tissue lesions of the major reactional states of leprosy was undertaken to study the immune mechanisms underlying regulation of cell-mediated immunity and DTH in man. Study of the reactional states of leprosy provides new insights into these immune processes. Reversal reactions in leprosy are characterized by a reduction, or occasionally the elimination, of organisms from lesions with upgrading of the clinical classification, associated with a sudden gain in cell-mediated immunity against *M. leprae* in vitro. Cells infiltrating the reversal reaction lesions contained mRNA coding for IFN-γ, as well as human serine esterase, and expressed cell surface antigenic determinants of the Th and T cytotoxic subpopulations. These results provide in vivo evidence that reversal reactions represent a naturally occurring DTH reaction in leprosy. In contrast, the study of ENL lesions provides evidence suggesting that this reactional state represents a transient cell-mediated immune process clearly differentiated from the classical DTH response occurring in reversal reaction.

In situ analysis of tissue lesions revealed greater numbers of cells expressing IFN-γ mRNA, as well as cells of the T helper/memory phenotype ($CD4^+UCHL1^+$), in reversal reactions, tuberculoid leprosy, and lepromin skin tests in comparison with lepromatous leprosy. Such Th cells derived from leprosy lesions may contribute to DTH by proliferating and producing IFN-γ in response to *M. leprae* in vitro (38). IFN-γ is likely to be involved in the reduced numbers of bacilli observed in reversal reactions since this lymphokine has been shown to facilitate the intracellular killing of mycobacteria in vitro and in vivo (39, 40).

Cells expressing serine esterase mRNA (huHF), a marker for cytotoxic cells, were more abundant in reversal reactions, lepromin skin tests, and tuberculoid lesions than in nonreactional lepromatous leprosy, and correlated in number with the $CD8^+9.3^+$ T cytotoxic population. However, the microanatomic location of these $huHF^+$ cells throughout the granuloma indicate that some are likely to be either macrophages or $CD4^+$ cells. Since macrophages do not appear to express this gene (41) and CD4 killers can release this protease (42), it appears that $CD4^+$ cells in reversal reaction lesions contain this cytotoxic marker. Although NK cells have been shown to produce serine esterase, immunohistologic analysis of leprosy lesions has failed to demonstrate the presence of cells bearing NK phenotypes. We have not yet obtained functional evidence that cells in lesions have cytotoxic activity, and in fact, Th cells have been shown to express the huHF marker (43); however, EM has shown that cytolysis of parasitized macrophages occurs within leprosy lesions (44).



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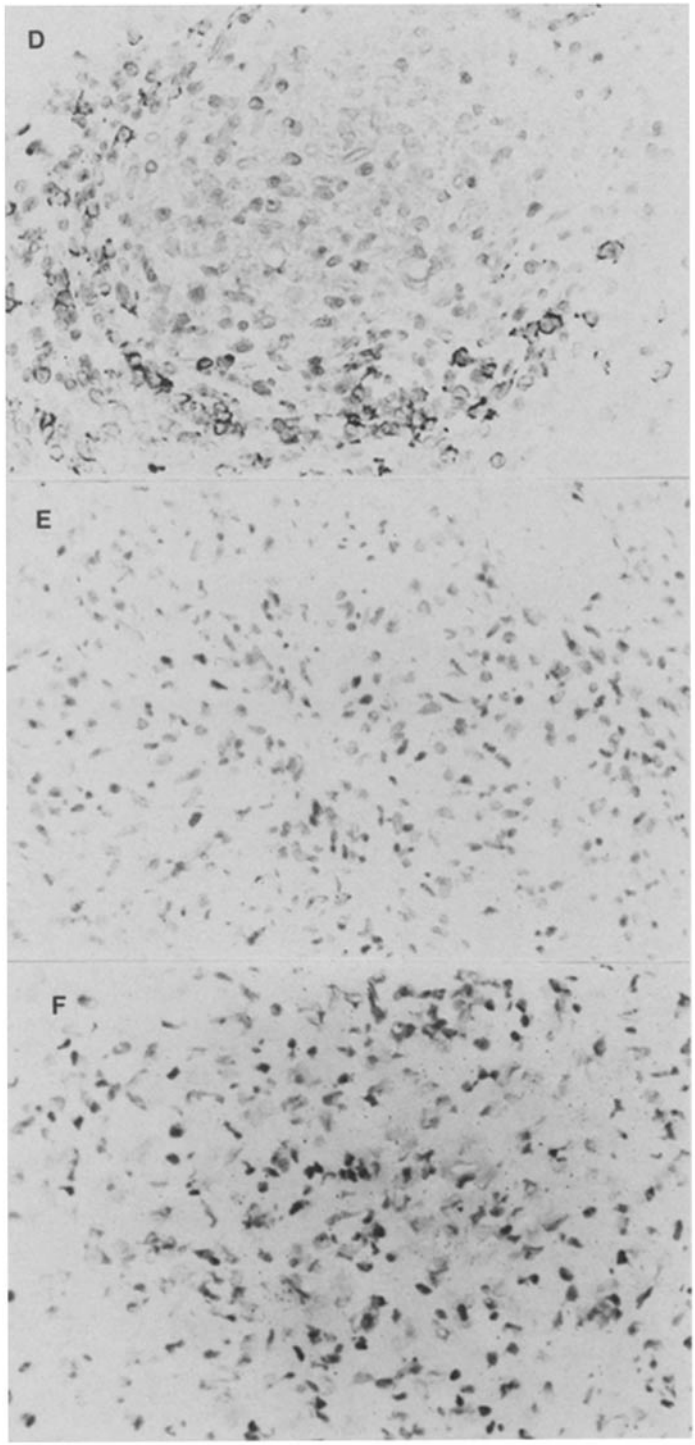

FIGURE 3. In situ hybridization to detect serine esterase (huHF), a marker for T cytotoxic cells. (*A*) Positive cells in this darkfield micrograph of a reversal reaction granuloma are identified by the clustering of white dots into spots and are distributed throughout the granuloma in the same distribution of $CD4^+$ cells (× 25). (*B*) A clustering of positive cells is observed in the ENL granuloma (× 40). The sense control hybridizations are negative in both reversal reaction (*E*) and ENL (*F*) (× 25). Immunoperoxidase staining of reversal reaction granulomas revealed that $UCHL1^+$ cells (*C*) are distributed throughout the granuloma; whereas, $2H4^+$ cells (*D*) are in the lymphocytic mantle surrounding the granuloma (× 40).

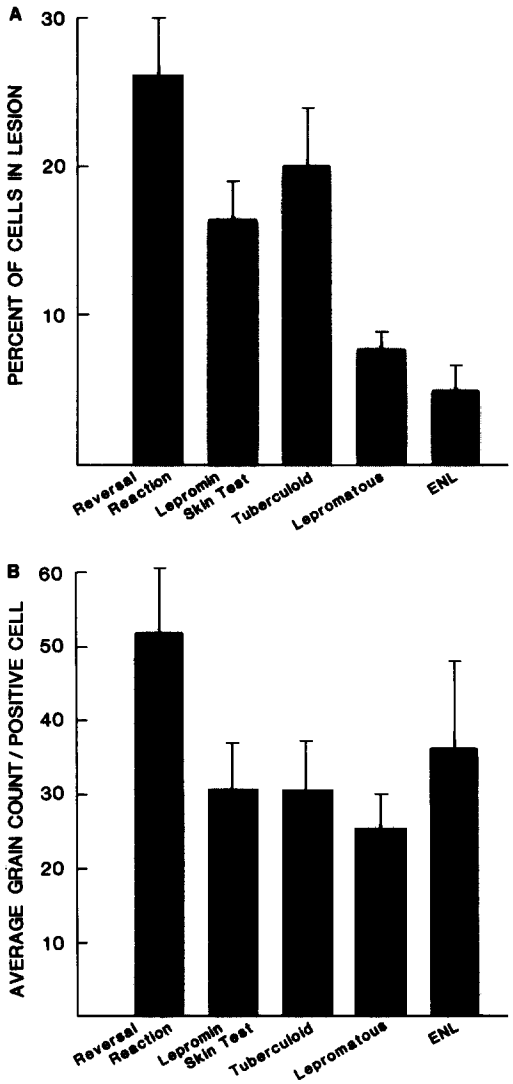

FIGURE 4. Serine esterase (huHF) mRNA in leprosy lesions by in situ hybridization. (*A*) Percent of positive cells in lesions. (*B*) Gene expression as measured by median grain count over background in positive cells.

In addition, $CD4^+$ antigen-specific killers have been isolated from the peripheral blood of leprosy patients (45). These T cytotoxic cells are thought to contribute to host defenses against mycobacterial infection by lysing infected targets in vitro (46). The lysis of these infected targets in tuberculoid lesions may then eventuate in the elimination of bacilli, allowing the dilution of organisms from heavily parasitized macrophages into fresh IFN-γ activated phagocytes, where destruction can then take place.

The data presented demonstrate that reversal reactions are a naturally occurring DTH reaction within leprosy. Although elimination of reduction of bacilli is the desired result, concomitant nerve damage is often a serious consequence. Of interest, the level of IFN-γ and serine esterase gene expression in reversal reaction lesions was markedly elevated compared with lepromin skin tests and tuberculoid leprosy le-

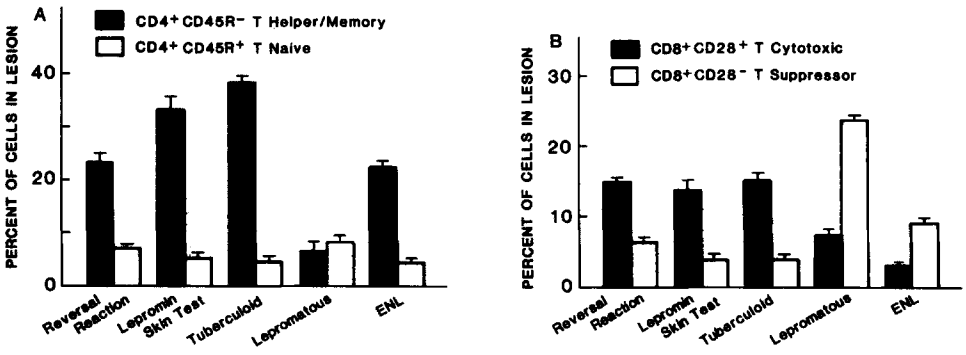

FIGURE 5. (*A*) Percentages of $CD4^+$ subpopulations in leprosy lesions. The T helper/memory subpopulations was identified as $CD4^+UCHL1^+$ or $CD4^+2H4^-$; whereas, the T naive subpopulation was identified as $CD4^+2H4^+$. (*B*) Percentages of $CD8^+$ subpopulations in leprosy lesions. The T cyotoxic subpopulations was identified as $CD8^+9.3^+$; whereas, the Ts subpopulation was identified as $CD8^+9.3^-$.

sions. Since Kaufmann (47) has shown in the mouse that induction of class II MHC on *M. leprae* antigen-containing Schwann cells by IFN-γ permits them to be killed in vitro, it is intriguing to speculate that reversal reactions represent a hyperimmune DTH response in man in which T cell cytotoxicity mediates severe nerve damage.

Analysis of ENL lesions revealed clear differences from that of reversal reaction (Fig. 6). Although phenotypic Th cells predominated in ENL lesions, the high levels of IFN-γ gene expression, characteristic of reversal reactions, were not observed. ENL granulomas lacked the characteristic microanatomic separation of T cell subpopulations found in tuberculoid and reversal reaction granulomas. In addition, T cytotoxic cells as measured by 9.3 antigen expression and huHF serine esterase gene expression were relatively low in these ENL specimens. The relative absence of a DTH-like response in ENL correlates with the inability of the immune cells in these lesions to clear bacilli.

The differences observed between reversal reactions and ENL reactions in leprosy can be conceptualized as representing fundamentally different dynamic events occurring in the lesions of the patients. Reversal reactions would appear to reflect a

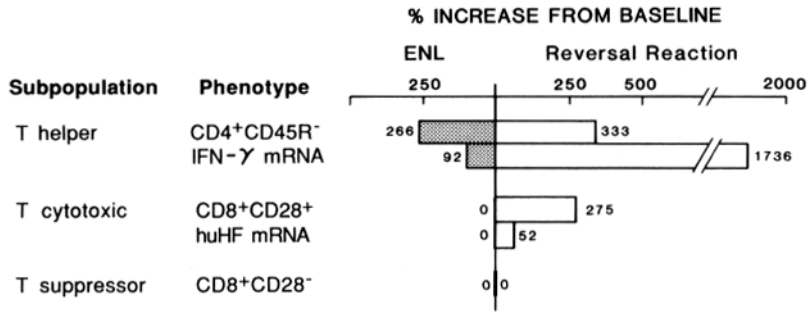

FIGURE 6. Differential increases over background of various T cell subpopulations in reversal reaction compared with ENL lesions.

selective increase of $CD4^+$ IFN-γ producing and $CD4^+$ and/or $CD8^+$ CTL activity in the lesions, with the number of $CD8^+$ Ts cells remaining almost constant. In contrast, ENL reactions may be visualized as a transient diminution of Ts activity, leading to partial augmentation of Th activity. We have reported a transient diminution of Ts activity in vitro during ENL episodes (24). The diminution of Ts regulation could account for the augmentation of both cell-mediated immunity and antibody formation characteristic of ENL reactions. However, ENL reactions would appear to lack significant numbers of lymphokine-producing cells and fail to exhibit a significant increase in CTL cells. This picture could lead to the immune complex disease symptoms associated with reversal reactions. The changes in T cell subsets and function in these reactional states of leprosy continue to offer new insights into regulation of cell-mediated immunity in man.

Summary

Analysis of tissue lesions of the major reactional states of leprosy was undertaken to study the immune mechanisms underlying regulation of cell-mediated immunity and delayed-type hypersensitivity (DTH) in man. In situ hybridization hybridization of reversal reaction biopsy specimens for INF-γ mRNA expression revealed a 10-fold increase in specific mRNA-containing cells over that observed in unresponsive lepromatous patients. Expression of huHF serine esterase, a marker for T cytotoxic cells, were fourfold increased in reversal reaction and tuberculoid lesions above that detected in unresponsive lepromatous individuals. Immunohistology of reversal reactions confirmed a selective increase of Th and T cytotoxic cells in the cellular immune response. Of interest, the microanatomic location of these serine esterase mRNA-containing cells was identical to the distribution of $CD4^+$ cells.

Analysis of erythema nodosum leprosum (ENL) lesions revealed differences in the underlying immune processes in comparison with reversal reaction lesions. Although phenotypic Th cells predominated in ENL lesions, IFN-γ and serine esterase gene expression were markedly reduced. We suggest that reversal reactions represent a hyperimmune DTH response characterized by a selective increase of $CD4^+$ IFN-γ producing cells and T cytotoxic cells, which result in the clearing of bacilli and concomitant tissue damage. In contrast, ENL reactions may be viewed as a transient diminution of Ts cells and activity leading to a partial and transient augmentation in cell-mediated immunity, perhaps sufficient to result in antibody and immune complex formation, but insufficient to clear bacilli from lesions.

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