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Limited T-Cell Receptor β-Chain Diversity of a T-Helper Cell Type 1-Like Response to *Mycobacterium leprae*

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Delayed-type hypersensitivity (DTH) is the standard measure of T-cell responsiveness to infectious organisms. For leprosy, the Mitsuda reaction, a local immune response to cutaneous challenge with *Mycobacterium leprae*, is considered to represent a measure of DTH against the pathogen. We analyzed the diversity of the T-cell receptor β-chain repertoire in Mitsuda reactions to determine the breadth of the mycobacterial antigens involved. The polymerase chain reaction was used to compare Vβ usage in the Mitsuda reaction T-cell lines established and unstimulated peripheral blood. These molecular analyses revealed a skewed T-cell receptor Vβ gene usage in the Mitsuda reaction and in T-cell lines from lesions. To examine the reactivity of T cells from these lesions, T-cell lines were tested against the available native and recombinant antigens of *M. leprae*. T-cell lines derived from Mitsuda reactions responded more strongly to the 10-kDa *M. leprae* antigen, a homolog of GroES in *Escherichia coli*, than to other *M. leprae* proteins. T-cell lines were also shown to proliferate strongly in response to the 17- and 3-kDa proteins. The pattern of the lymphokine mRNA of these cells was reminiscent of the pattern of murine T_H1 cells, positive for interleukin-2 and gamma interferon and weakly positive for interleukin-4. These data indicate that a limited array of T cells, perhaps recognizing stress proteins, secrete a type 1 lymphokine profile in the DTH response to mycobacteria.

Delayed-type hypersensitivity (DTH) to infectious pathogens has been traditionally assessed by performing skin tests with a crude antigen preparation of the causative organism. For leprosy, the skin test used for clinical classification of patients is called the Mitsuda reaction (23) and is thought by many to be a measure of DTH against *Mycobacterium leprae* (9, 15, 16, 26). Skin testing is performed with preparations containing intact killed *M. leprae* organisms, called lepromins, which are derived from infected human or armadillo tissues. The Mitsuda reaction occurs 3 to 4 weeks after intradermal injection of lepromin and is characterized histologically by the presence of granulomas, organized collections of T lymphocytes and macrophages. The test is positive in patients with resistance to progressive *M. leprae* infection (tuberculoid patients and patient contacts) and negative in those individuals with widespread disease (lepromatous patients). The Mitsuda reaction, which is readily accessible for biopsy, provides an opportunity to study the T cells which mediate DTH against pathogens.

The majority of T cells infiltrating these DTH reactions are $CD4^+$ T cells bearing αβ T-cell receptors (TCRs), although there is a significant presence of γδ T cells (5, 9, 19). The T-cell and cytokine patterns as directly measured in Mitsuda reaction lesions are strikingly similar to those of reversal reactions in leprosy, which are considered to be naturally occurring DTH responses (5, 19, 31). In the present study, we measured the diversity of the TCR β-chain repertoire of T cells in Mitsuda reactions, in order to gain insight into the set of antigens recognized in the local immune response. This was accomplished by analyzing variable (V) gene usage in a Mitsuda reaction and T-cell lines derived from biopsy specimens. The T-cell lines were evaluated for reactivity against native and recombinant *M. leprae* antigens and characterized according to lymphokine mRNA patterns.

MATERIALS AND METHODS

Patients. Patients with leprosy were evaluated at the Los Angeles County Hansen's Disease Clinic and classified according to the criteria of Ridley and Jopling (24). The eight patients selected for skin testing in the present study were diagnosed as having tuberculoid leprosy. Lepromin A (armadillo derived), 0.1 ml of 2×10^6 autoclaved bacilli per ml, was injected intradermally into the lateral upper arm. At 21 days, a punch biopsy was performed for all reactions with a 5-mm or larger diameter. A portion of the specimen was processed for routine histology, a portion was stored at −180°C for molecular studies, and the remainder was placed in RPMI 1640 for cell culture.

Extraction of lymphocytes from biopsy specimens. Lymphocytes were extracted from punch biopsy specimens by adopting the strategy described earlier (17, 18). Specimens were cut into 1-mm pieces with a surgical scalpel and extruded through a tissue sieve fitted with a 64-μm-mesh filter (Bellco Glass, Inc., Vineland, N.J.). Mononuclear cells were then isolated by Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) centrifugation. Yields ranged from 10,000 to 500,000 cells. These mononuclear cells were directly cultured for further study.

Generation of T-cell lines. T-cell lines of in situ-activated cells were established by culturing cells in media supplemented with interleukin-2 (IL-2) and/or sonicated *M. leprae* (10 μg/ml, provided by V. Mehra, Bronx, N.Y.). To generate such lines, T cells were seeded in 96-well round-bottom microtiter plates at approximately 1,000 cells per well. Each

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well contained 10^5 lethally irradiated (3,000 rads) autologous peripheral blood mononuclear cells (PBMC) in RPMI 1640 with 1% penicillin-streptomycin, sodium pyruvate, glutamine, 10% heat-inactivated human AB serum, and 10% IL-2 (Pharmacia Fine Chemicals). Cultures were incubated at 37°C in 7% CO_2, examined with an inverted-phase microscope every 3 or 4 days, pooled, and expanded to 2-ml flat-bottom wells as necessary. To all wells, 50% fresh IL-2 containing media was added every 3 or 4 days. These lines were restimulated every 2 weeks with the addition of antigen and fresh feeder cells. T-cell lines were enriched for αβ T cells by immunomagnetic depletion of γδ T cells (1).

Measurement of TCR β-chain repertoire in T-cell lines. Total RNA was isolated from 50 5-μm cryostat sections from T-cell lines and PBMC by acid guanidinium lysis (4). cDNA was synthesized by reverse transcription as described previously (30). Polymerase chain reaction (PCR) analysis of the TCR Vβ repertoire was accomplished by using paired Vβ and Cβ oligonucleotide primers. Each Vβ primer is specific for a particular Vβ family or subfamily and the Cβ primer recognized sequences invariant between the Cβ1 and Cβ2 gene segments. PCR was performed by using 40 s at 94°C for denaturation and 1 min at 65°C for annealing and extension. In addition, the time at 65°C was increased by 2 s per cycle to ensure complete chain extension of amplified product. PCR products were resolved by electrophoresis through a 1.5% agarose gel before transfer to nylon membranes. The membranes were then hybridized to an internal Cβ probe labeled at the 5′ position with [γ-^{32}P]ATP and polynucleotide kinase. Filters were washed in SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7]) and sodium dodecyl sulfate solutions, visualized with autoradiography, and quantitated with an Ambis blot analyzer (Automated Microbiological Systems Inc., San Diego, Calif.). The intensity of each Vβ band was expressed as individual Vβ cpm/total of all Vβ cpm. Primer and probe sequences have been previously reported (8).

Validation of PCR. Choi et al. have demonstrated the oligonucleotides employed for the PCR analysis provide meaningful estimates of the Vβ repertoire (3). Furthermore, DerSimonian et al., whose method we have slightly modified, performed a number of controlled experiments to validate this approach (8). PCR primers had been designed to be of both similar length and GC content in order to be of similar primary efficiency. To validate that similar primer efficiency was achieved, a cDNA sample was serially diluted and subjected to PCR amplification with Vβ primers. The PCR products were then quantified by β emission, and the counts per minute versus amount of cDNA was plotted. For each primer set, linear responses were obtained, with similar slopes, indicating that primer efficiencies were equivalent, as has previously been shown by others (27). It should be noted that in comparing Vβ gene usage in different specimens (i.e., lesion versus PBMC) from the same individual, we account for small biases in primer efficiency.

Comparison of the Vβ repertoire requires that similarly sized populations of T cells be sampled so that one sample is not on the nonlinear part of the PCR titration. Prior to repertoire analysis, individual samples were standardized by PCR analysis of the CD3δ mRNA. CD3δ was used as a marker for T-cell mRNA, as previously reported (30), although other markers could be employed. Serially diluted cDNAs were PCR amplified with CD3δ oligonucleotide primers. Titrations indicated that amplification is linear throughout the range of substrate. Our rationale was to limit the amount of substrate cDNA in the PCR mixture to avoid overamplification, which might cause one or more reactants to become rate limiting. Typically, we found that the cDNA concentration meeting these criteria for optimal Vβ-Cβ PCR amplification yielded CD3δ PCR product in the lower end of the visible linear response range.

We have validated the PCR in our laboratory to ensure accuracy and reproducibility. To verify the results of Vβ analysis by PCR, we compared results to those obtained by fluorescence-activated cell sorter (FACS) analysis. This was accomplished in a study of pleural fluid from patients with tuberculosis (20a). For one patient, we determined that Vβ8 comprised 14% of the repertoire by PCR and 13% by FACS with an anti-Vβ8 monoclonal antibody. Similar results were obtained for four other patients for whom Vβ8 could be measured by FACS.

Repeat experiments with the same cDNAs yielded reproducible results with Vβs, usually within one percentage point. Serial sectioning of tissue samples also yielded a similar Vβ repertoire. We have previously determined that 35 cycles of PCR amplifications are necessary to measure small differences in mRNA from the limited number of T cells obtainable from skin biopsy specimens (31). Furthermore, we have established that 35 cycles of PCR yield an amount of product similar to that yielded by 30 cycles but with a significant improvement in sensitivity.

Precautions taken to avoid PCR contamination included the use of aerosol-resistant pipette tips (Continental Lab Products, San Diego, Calif.), assembling PCR mixtures in a laminar flow hood, and inclusion of negative controls. PCR amplification of RNA from samples alone yielded no detectable Vβ-Cβ product under these conditions, indicating that the PCR product did not contaminate samples.

Measurement of antigen-induced proliferation of T-cell lines. T-cell lines, washed free of IL-2, were cultured in the presence of autologous irradiated PBMC as antigen-presenting cells. Antigens employed include sonicated *M. leprae*, the purified native proteins of *M. leprae* including the 3-, 10-, 17-, 22-, and 35-kDa proteins, which were kindly provided by P. Brennan (Fort Collins, Colo.) (11). Purified recombinant 65- and 70-kDa proteins were provided by J. van Embden (National Institute of Public Health, Bilthoven, The Netherlands), and the 18-kDa protein was supplied by J. Watson (University of Auckland, Auckland, New Zealand). Triplicate 200-μl flat-bottom wells contained cells at a density of 10^4 cells from the T-cell line per 10^5 feeders in the presence of *M. leprae*. Control cultures received 10% IL-2 alone to demonstrate viability of clones or lines, no antigen to detect background proliferation, or an irrelevant antigen such as tetanus toxoid. After 3 days, cultures were pulsed with 1 μCi of [^{3}H]thymidine and harvested 4 h later. [^{3}H]thymidine incorporation was determined by standard scintillation measurement.

Measurement of lymphokine mRNAs in T-cell lines. To determine the cytokine pattern of T-cell lines, lines were stimulated with anti-CD3 antibodies (19). RNA was isolated and cDNA was synthesized as described above. cDNAs were examined for lymphokine message by PCR with oligonucleotide primers specific for IL-2, IL-4, and gamma interferon (30). Cytokine PCR product was electrophoresed on 2% agarose gels. The sensitivity of the PCR was determined to be in a semiquantitative range and equivalent for each cytokine on the basis of titration of cytokine plasmid cDNAs. PCR amplification, as assessed by ethidium bromide staining, was sensitive to the order of 10^2 to 10^3 copies for each cytokine and proportional to the number of copies of plasmid cDNA up to at least 10^8 copies.

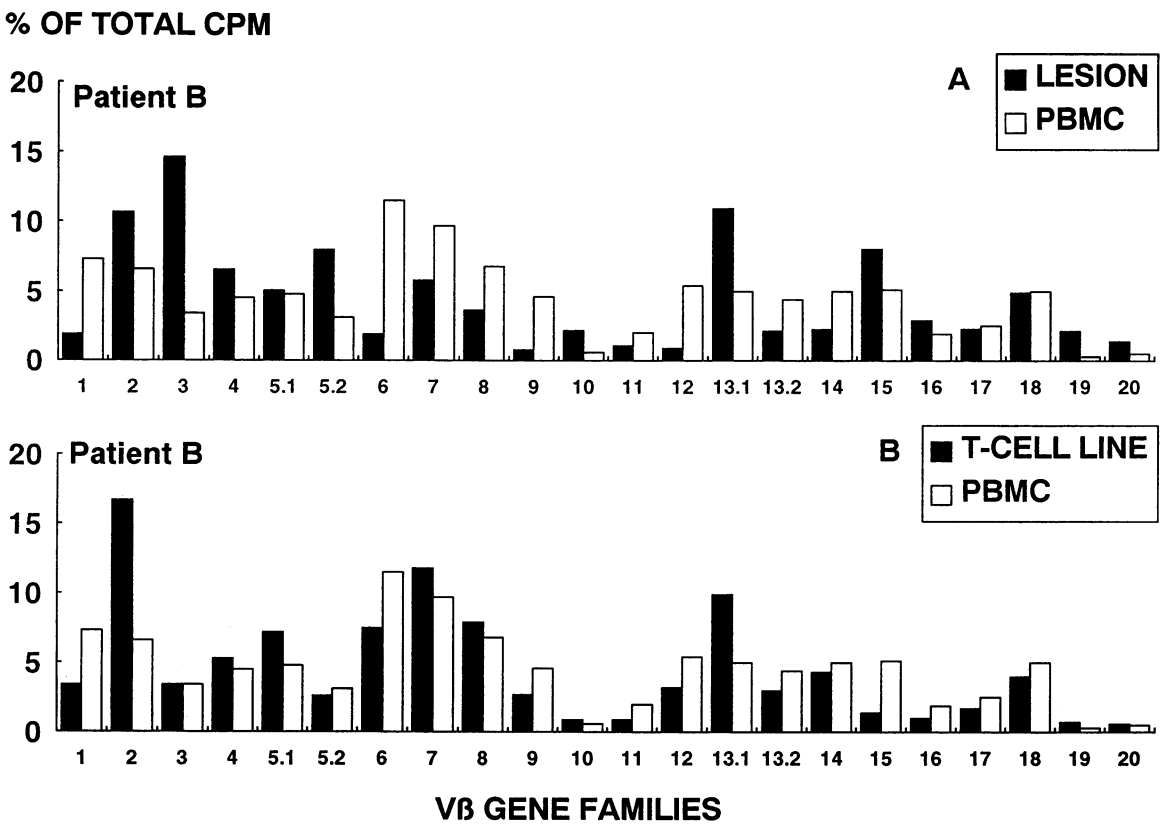

FIG. 1. Vβ repertoire in a Mitsuda reaction in leprosy. (A) Vβ repertoire in a lesion compared with that in PBMC. mRNA from patient B was isolated from frozen sections of the biopsy specimen and PBMC and used to synthesize cDNA. Vβ repertoire analysis was accomplished by PCR with paired Vβ and Cβ primers. Amplified products were resolved in agarose and subjected to Southern blot analysis. Results of individual Vβ usage were expressed as the percentage of the total Vβ repertoire. (B) Vβ analysis of a T-cell line derived from a Mitsuda reaction and PBMC. Repertoire analysis was performed on a T-cell line derived from the lesion shown in panel A and compared with that of PBMC.

RESULTS

TCR β repertoire in the Mitsuda reaction. To determine whether specific T-cell populations were overrepresented in DTH lesions, we compared the TCR Vβ repertoire in a Mitsuda reaction versus unstimulated PBMC from the same individual. RNA was extracted from a frozen section of the biopsy specimen, and the TCR β-chain repertoire was determined by PCR. A small number of Vβ families were strikingly overrepresented in the lesion studied. Vβ2, Vβ3, and Vβ13.1 gene families each accounted for approximately 10% or more of the total Vβ repertoire, according to the percentage of the total counts per minute (Fig. 1A). These Vβ families were also two- to threefold overrepresented in the Mitsuda reaction compared with unstimulated PBMC. We reasoned that *M. leprae*-specific T-cell lines established from Mitsuda reactions would be further skewed in terms of Vβ gene usage if the antigen-specific T cells recognized a limited number of antigens. We therefore explored the extent of the TCR αβ repertoire in the T-cell line derived from this patient's Mitsuda reactions. The Vβ repertoire in this individual's T-cell line generated from the Mitsuda reaction was similar to direct analysis of the lesion Vβ repertoire (Fig. 1B). Again, the Vβ2 and Vβ13.1 gene families were overrepresented in the T-cell line by two- to threefold compared with the PBMC. The Vβ3 gene family, which was overrepresented in the lesion, was not overrepresented in the line. The data indicate that the T-cell line is generally representative of the skewed TCR repertoire of the Mitsuda reaction.

TCR β repertoire of T-cell lines from Mitsuda reactions. To more fully evaluate the TCR β-repertoire in Mitsuda reaction, three additional T-cell lines were established from other Mitsuda reactions. The repertoires in these lines were compared to those of unstimulated PBMC from the same individuals. In the three additional lines expanded in the presence of *M. leprae* antigen, skewed TCR Vβ gene usage was observed. For patient D, Vβ3, Vβ4, and Vβ9 were overrepresented in the T-cell line from the reaction, at least twofold in comparison to this individual's PBMC (Fig. 2A). In the T-cell line derived from the Mitsuda reaction of patient M, Vβ2 and Vβ11 were prominent in the T-cell line from the lesion compared with those of blood (Fig. 2B). Vβ12 was prominent in this lesion but not significantly overrepresented compared with that from peripheral blood. Finally, in the T-cell line from patient D, Vβ12 and Vβ13.1 were relatively overrepresented (Fig. 2C). The results indicate a limited TCR Vβ gene usage in T-cell lines derived from Mitsuda reactions and further suggest that a limited set of antigens are recognized by such T-cell lines. Overall, it was found that the Vβ2 and Vβ13.1 gene families were prominent in two of four T-cell lines studied, accounting for more than 10% of the repertoire and/or being at least twofold overrepresented in lesions compared with those in blood.

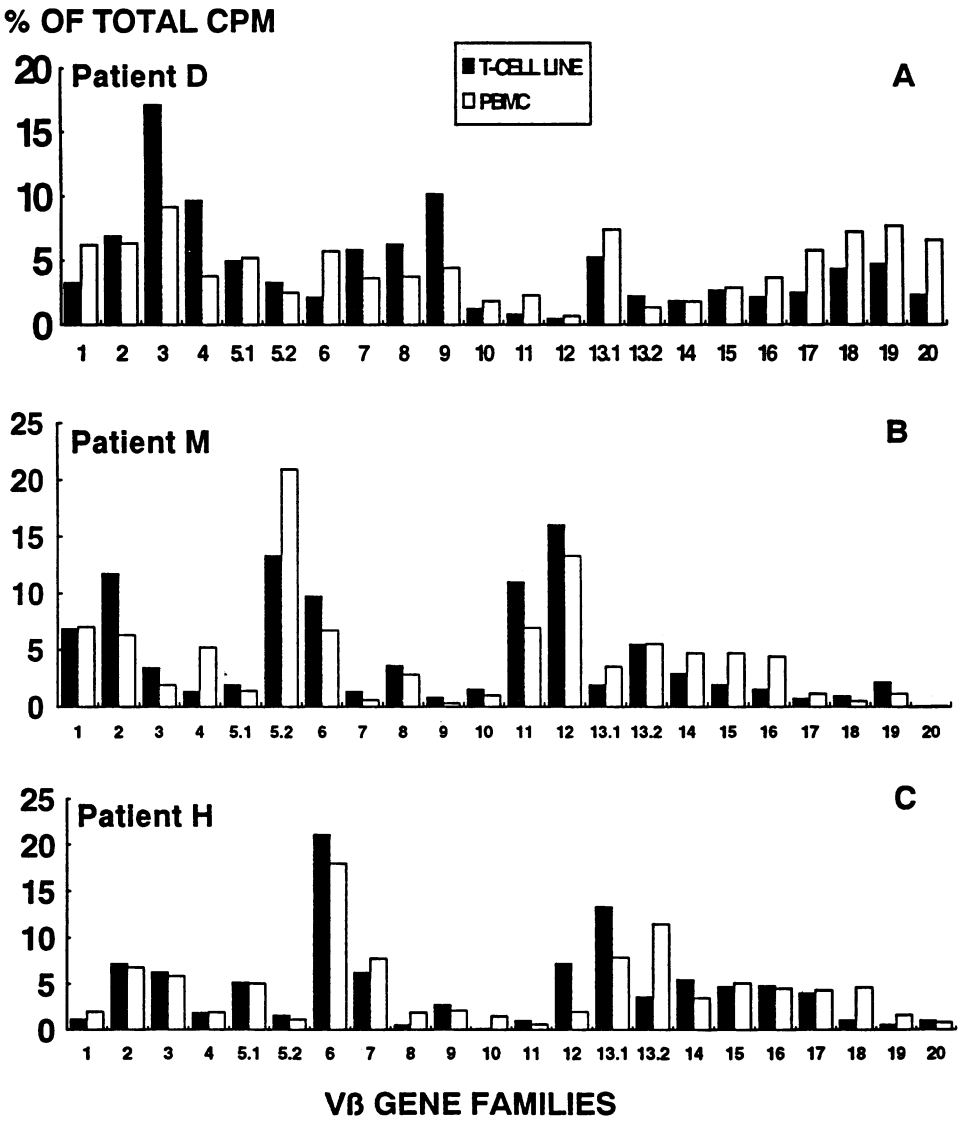

FIG. 2. PCR analysis of TCR β-chain gene segment usage in T-cell lines from Mitsuda reaction lesions and unstimulated PBMC. Vβ gene (families 1 to 20)-Cβ PCR amplifications were performed on T-cell lines from lesions and peripheral blood of three patients. Southern blot analysis of Vβ-Cβ products was performed by using an internal Cβ probe, and the amounts of the products were quantified. Each Vβ gene usage was expressed as a percentage of the total repertoire. Results for patients D, M, and H (panels A, B, and C, respectively) are shown.

To verify that the twofold differences in Vβ repertoire observed were significant, reproducibility of PCR amplifications was examined for two T-cell lines derived from two Mitsuda reactions (Table 1). The table shows repeat determinations for Vβs, which were initially found to account for approximately 10% or more of the total repertoire in lesions and were approximately twofold overrepresented compared with those from the blood. In the T-cell line from patient B, Vβ2 and Vβ13.1 were reproducibly overrepresented relative to the patient's PBMC Vβ repertoire in two separate experiments. Similarly, triplicate PCR amplifications of patient M's T-cell line revealed skewed expression of Vβ2 and Vβ11 relative to that from PBMC. The remaining Vβ families were consistent from each PCR experiment (data not shown).

Antigen reactivity of T-cell lines derived from Mitsuda reactions. To determine the antigens to which these T-cell lines were reactive, proliferation of seven T-cell lines derived from Mitsuda reactions from a total of seven patients against the available purified and recombinant *M. leprae* antigens was assessed. The T cells were most strongly reactive against the native antigens with molecular masses of 10 kDa (Fig. 3). Marked proliferative responses were also observed for the native 17- and 3-kDa antigens. Similar responses were observed for the PBMC of tuberculoid patients (13). In these patients, the responses to the 70-, 65-, 35-, 22-, and 18-kDa proteins were weak but present.

Lymphokine patterns of T-cell lines derived from Mitsuda reactions. To determine the lymphokine profile of these TCR αβ T-cell lines, three T-cell lines derived from Mitsuda reactions were stimulated with anti-CD3 antibodies for 18 h. Following RNA extraction and cDNA synthesis, PCR amplification was performed by using lymphokine specific primers. Strong ethidium bromide bands were obtained by using primers for IL-2 and gamma interferon, but no visible

TABLE 1. Reproducibility of TCR Vβ mRNA levels by PCR[a]

Patient and gene family	% of total cpm[a]					
	T-cell line			PBMC		
	1	2	3	1	2	3
B						
Vβ2	17.6	16.7	ND	5.6	6.6	ND
Vβ13.1	9.6	9.9	ND	4.0	5.0	ND
M						
Vβ2	10.8	11.4	12.4	8.9	6.3	7.0
Vβ11	10.9	16.0	15.8	5.7	6.9	6.1

[a] To assess the reproducibility of PCR, duplicate or triplicate PCR reaction experiments for TCR Vβ gene usage were performed. Repeat PCRs were performed on cDNA prepared from T-cell lines derived from Mitsuda reactions and PBMC from two patients (designated B and M). Amplified products were identified by Southern analysis. Results of the overrepresented (>10% of total Vβ repertoire) and predominant (nearly two- or threefold difference between the T-cell line and PBMC) Vβ gene transcripts are shown; values are given for three separate experiments (1, 2, and 3). Vβ gene families equally represented in lines and PBMC are not shown. ND, not done.

band was detected for IL-4 (Fig. 4). The inclusion of positive controls and the simultaneous titration of plasmid containing cDNAs indicate the efficiency of our PCR method (data not shown).

DISCUSSION

The outcome of the host response to infection by intracellular pathogens is dependent on the presence of antigen-specific effector T cells. DTH is the classic measure used to assess T-cell responsiveness to an infectious agent. Mitsuda reactions, considered to be a measure of DTH in leprosy, provide an excellent opportunity to study the T cells which mediate immunologic resistance against a pathogen. We chose to study the TCR repertoire in Mitsuda lesions and T-cell lines derived from such reactions to gain information about the set of antigens recognized. We found that the TCR β-chain repertoire in Mitsuda reactions was skewed compared with that from peripheral blood, suggesting that a limited set of antigens is recognized by T cells mediating DTH.

In the comparison of T-cell lines derived from Mitsuda reactions from PBMC from the same donor, several Vβ families were overrepresented and enriched two- to threefold. Additionally, the Vβ families overrepresented in one T-cell line derived from a Mitsuda reaction were similarly overrepresented in the tissue when directly measured. This indicates that the T-cell line is representative of the in vivo response. Overall, Vβ2 and Vβ13.1 gene expression was prominent in two of four lesion-derived T-cell lines studied. The finding that several Vβ gene families were overrepresented in Mitsuda reactions suggests that the number of specific T-cell populations that accumulate at the site of disease activity in these responses is limited. However, further analysis of Vα gene usage for each overrepresented TCR Vβ population would more precisely define the repertoire.

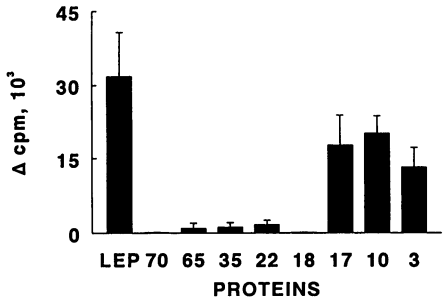

FIG. 3. Antigen reactivity of seven T-cell lines derived from Mitsuda reactions. T-cell lines were stimulated with whole *M. leprae* cells and the native and recombinant proteins (with molecular masses in kilodaltons as indicated). of *M. leprae*. The T cells were most reactive against 17-, 10-, and 3-kDa antigens. Data are presented as the means ± standard errors of the means for the seven lines. LEP, sonicated *M. leprae*.

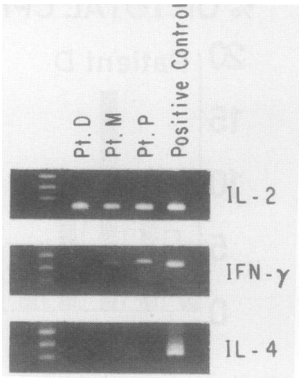

FIG. 4. Lymphokine mRNA patterns of Mitsuda reaction T-cell lines. T-cell lines from three patients, D, M, and P, were stimulated with anti-CD3 monoclonal antibody for 18 h and collected. cDNA was prepared from total RNA and quantitatively normalized for CD3 by PCR. PCR amplification for lymphokine message revealed products for IL-2 and gamma interferon but not IL-4.

Limited TCR V gene usage has also been described for a number of autoimmune conditions (2, 6, 12, 21, 22, 29). The finding of limited TCR diversity in these autoimmune diseases is thought to reflect the recognition of a limited set of antigens, perhaps autologous self-peptides. We find it surprising that the T-cell response mounted against a complex foreign pathogen such as *M. leprae* is mediated by a selective increase of a small number of T-cell families.

In examining the TCR repertoire in naturally occurring DTH responses in leprosy called reversal reactions, we found that infiltrating T cells also expressed a limited TCR Vβ repertoire (28). Limited TCR Vβ gene usage was detected in reversal reaction lesions compared with that in both the peripheral blood of the individual and the prereaction leprosy lesions. In the reversal reaction lesions, Vβ6, Vβ12, Vβ14, and Vβ19 predominated and are a different set than the predominant Vβs reported here for Mitsuda reactions. In general however, the predominant Vβ gene used varied from individual to individual. A factor which may determine Vβ gene usage is the major histocompatibility complex class II haplotype (7). In the study of reversal reaction lesions, we found that HLA-DR15 individuals expressed Vβ6 in lesions, suggesting that DR15 may present a small set of *M. leprae* peptides, with subsequent selection of Vβ6 TCRs. Similarly, preliminary analysis indicates that variations in predominant Vβ gene usage at the site of Mitsuda reactions are probably related to differences in individual major histocompatibility complex. A more comprehensive immunogenetic analysis of

the TCR repertoire may reveal association between particular Vβ-chain usage and major histocompatibility complex.

To gain insight about the set of antigens recognized by the local immune response, we tested TCR αβ T-cell lines derived from Mitsuda reactions for their ability to respond to the available recombinant and native *M. leprae* proteins. Our results indicated that several antigens, especially the 10-kDa antigen, induced strong proliferative responses in these T-cell lines. Significant T-cell responses were observed for the 17- and 3-kDa antigens. Small but significant responses were elicited by the 70-, 65-, 35-, 22-, and 18-kDa proteins in some of the patients. This pattern of reactivity is similar to that previously observed in a study of the peripheral blood of tuberculoid leprosy patients and patient contacts (13). We have previously shown that T-cell lines derived from Mitsuda reactions in the presence of IL-2 alone (to expand those cells that had been activated by antigen in situ) responded strongly to the 10-kDa *M. leprae* antigen (13). Moreover, approximately one-third of *M. leprae* reactive cells in two skin test-positive individuals reacted to the 10-kDa antigen. The present data further indicate that the 10-kDa protein, a homolog of *Escherichia coli* GroES, is a major stimulator of the DTH response against *M. leprae*. Clearly, more extensive analysis of antigen reactivity, such as T-cell Western blot (immunoblot) (14), will be required to define the set of antigens recognized by the limited TCR β response.

The immunologic role of these TCR αβ T cells from Mitsuda reactions was examined in terms of their pattern of lymphokine production. TCR αβ T-cell lines derived from Mitsuda reactions were found to contain abundant IL-2 and gamma interferon mRNA but not IL-4, a pattern characteristic of T_H1 or type 1 cells (20). Previously, we have demonstrated that the resistant immune response to *M. leprae* infection, tuberculoid leprosy, was characterized by the presence of type 1 lymphokine mRNAs in lesions, whereas susceptibility to disseminated *M. leprae* infection, lepromatous leprosy, was characterized by the presence of type 2 lymphokine mRNAs in lesions (30). The type 1 response is characteristic of mycobacterium-reactive T-cell clones derived from the blood of patient contacts and the lesions and blood of patients with tuberculoid leprosy (10, 25). In contrast, $CD8^+$ T-suppressor clones from the blood and lesions of lepromatous patients produce type 2 lymphokines. The data presented here indicate that a type 1 cytokine pattern can be detected directly in lesions of Mitsuda reactions, as well as the naturally occurring DTH responses, reversal reactions (31).

In order to develop an understanding of the cellular events involved in immunologic resistance against a pathogen, such as *M. leprae*, we have defined in molecular terms the TCR repertoire of the local response. Our results suggest that the TCR αβ T cells are of limited genetic diversity, respond to the 10-, 17-, and 3-kDa native *M. leprae* proteins, and express a T_H1 pattern of lymphokines. Such information about the cell-mediated immune response against *M. leprae* can be utilized to more competently design an effective vaccine against leprosy. Our results suggest that it should be possible to design an effective vaccine by selecting a small number of relevant antigens which engender resistance against infection. It should be feasible to derive T-cell clones bearing overrepresented TCR Vβs from Mitsuda reactions. These clones will be useful in further defining immunodominant antigens which mediate immunologic resistance against *M. leprae*.

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