MAJOR ARTICLE

Selective Expression of the Vβ14 T Cell Receptor on *Leishmania guyanensis*–Specific CD8$^+$ T Cells during Human Infection

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Peripheral blood mononuclear cells from subjects never exposed to *Leishmania* were stimulated with *Leishmania guyanensis*. We demonstrated that *L. guyanensis*–stimulated CD8$^+$ T cells produced interferon (IFN)–γ and preferentially expressed the Vβ14 T cell receptor (TCR) gene family. In addition, these cells expressed cutaneous lymphocyte antigen and CCR4 surface molecules, suggesting that they could migrate to the skin. Results obtained from the lesions of patients with localized cutaneous leishmaniaisis (LCL) showed that Vβ14 TCR expression was increased in most lesions (63.5%) and that expression of only a small number of Vβ gene families (Vβ1, Vβ6, Vβ9, Vβ14, and Vβ24) was increased. The presence of Vβ14 T cells in tissue confirmed the migration of these cells to the lesion site. Thus, we propose the following sequence of events during infection with *L. guyanensis*. After initial exposure to *L. guyanensis*, CD8$^+$ T cells preferentially expressing the Vβ14 TCR and secreting IFN-γ develop and circulate in the periphery. During the infection, these cells migrate to the skin at the site of the parasitic infection. The role of these Vβ14 CD8$^+$ T cells in resistance to infection remains to be determined conclusively.

The clinical outcome of infection with *Leishmania* depends on the infective agents and the specific immune response to *Leishmania* antigens. Cutaneous leishmaniasis is characterized by several clinical, histopathological, and immunological features; however, the immunopathogenesis of different forms of dermal leishmaniasis is poorly understood, and the mechanisms leading to resistance to the various *Leishmania* species are not well defined.

The description of functional CD4$^+$ T helper (Th) cell subsets provided a rational basis for the differentiation of responses elicited by CD4$^+$ T cells during pathogenesis. CD4$^+$ T cells differentiate into 2 functionally distinct subsets, Th1 and Th2, distinguishable by the mutually exclusive patterns of the cytokines that they produce after reactivation. In an experimental murine model of infection with *Leishmania major*, a correlation was found between resistance to infection and the development of a CD4$^+$ Th1 response and between susceptibility to infection and the development of a CD4$^+$ Th2 response [1–3]. However, this murine model does not mimic human dermal leishmaniasis, which presents distinct clinical and immunological profiles. Thus, with regard to human infection, identification of the specific T cell populations that contribute to protective or resistant immune responses is important.

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T cells that recognize foreign antigens track a specific T cell receptor (TCR). The study of the TCR repertoire may contribute to the understanding of disease pathogenesis. In this study, we analyzed the Vβ TCR repertoire by using *Leishmania guyanensis*–stimulated T cells from naive subjects. We showed that interferon (IFN)–γ–producing $CD8^+$ T cells selectively express the Vβ14 TCR gene family. Furthermore, these cells express cutaneous lymphocyte antigen (CLA) and CCR4 molecules, suggesting that they are able to migrate to skin lesions. The preferential expression of the Vβ14 TCR in the lesions of patients with localized cutaneous leishmaniaisis (LCL) supports this hypothesis. The presence of $V\beta14^+$ $CD8^+$ T cells in lesions was not associated with healing but was correlated with a longer duration of lesions.

MATERIALS AND METHODS

Samples. Blood samples were obtained, by venipuncture, from 11 patients with active American cutaneous leishmaniasis caused by *L. guyanensis,* before specific treatment, and were collected into sterile tubes (Veinoject, Terumo). Biopsy specimens were obtained from the border of the healthy skin (5 mm), and all parts of the biopsy were processed. Clinical data for these patients are given in table 1. All patients were seronegative for human immunodeficiency virus. Blood samples from 5 healthy subjects who had never been exposed to *Leishmania* also were included in this study. Absence of prior exposure to *Leishmania* was assessed as described elsewhere [4]. Informed consent was obtained from the subjects, and guidelines issued from the Comité Consultatif de Protection des Personnes dans la Recherche Médicale of Guadeloupe were followed (project no. 99-3).

Antigens. *L. guyanensis* (M4147) promastigotes were cultured in biphasic rabbit blood agar [5]. Extracellular proteins were removed from the parasite pellets by means of 3 washes with PBS. Parasites were used at 10^6 parasites/mL. Whole *M. bovis* bacille Calmette-Guérin (BCG; strain GL2) was used at 5 μg/mL.

Reagents. The reagents for magnetic cell separation using anti-CD8 or anti-CD4 monoclonal antibodies (mAbs) were obtained from Dynal. Mouse anti-human mAbs CD3 (UTCHT1, IgG1), CCR4 (1C1, IgG1), CD62L (Dreg56, IgG1), and CCR7 (2H4, IgM) and rat anti-human CLA (HECA-452, IgM) were obtained from Pharmingen.

Lymphocyte cultures. Peripheral blood mononuclear cells (PBMCs) were isolated, after venipuncture, on a Ficoll-Hypaque gradient ($d = 1.077$) and were resuspended in RPMI supplemented with 2 mmol/L glutamine, 100 U/mL penicillin, and 1 mg/mL streptomycin (all from Sigma) and 5% human heat-inactivated AB serum (EFS Ile de France). Cultures for cytokine production (10^6 cells in 1 mL of culture medium) were plated on flat-bottomed 24-well plates with or without antigens. After 5 days, cells were harvested for analysis of Vβ TCR gene expression.

$CD4^+$ and $CD8^+$ T cells were purified with anti-CD4 and anti-CD8 magnetic beads, in accordance with the manufacturer's instructions (Dynal). This resulted in a population of 92% CD8 and 95% CD4 T cells, as determined by fluorescence-activated cell-sorter (FACS) analysis. For restimulation of Vβ14 and Vβ1 cells, Vβ-positive cells were purified by use of a magnetic activated cell sorter from Dynal. In brief, PBMCs were stimulated for 5 days with *L. guyanensis.* Then, the cells were conjugated with an anti-Vβ14 or anti-Vβ1 TCR, incubated with anti-mouse immunoglobulin-coated magnetic microbeads, and isolated by exposure to a magnetic field. Purity was 92%, as determined by FACS analysis. Positively selected T cells were resuspended in medium, at 10^5 cells/mL, in the presence of mytomicin-treated autologous PBMCs (5×10^6 cells), for use

Table 1. Clinical data for patients with LCL.

Patient	Lesions, no.	Location of lesion(s)	Development of lesion(s), days[a]	Adenopathy	Lymphangitis	Status of lesion(s)
1	2	Wrist	50	No	No	Healing
2	1	Lower limbs	50	No	No	Healing
3	1	Elbow	55	No	No	Healing
4	3	Neck	60	No	No	Healing
5	1	Lower limbs	75	No	No	Not healing
6	2	Foot	105	No	No	Not healing
7	4	Lower limbs	150	No	No	Healing
8	2	Foot	40	No	No	Healing
9	2	Upper limbs	35	No	No	Healing
10	1	Lower limbs	30	No	No	Healing
11	6	Face	15	No	No	Healing

[a] Duration of lesion before patient sought care.

as antigen-presenting cells, and *L. guyanensis* (10^6 parasites/mL) and were further cultured for 72 h. SNs were collected, and IFN-γ production was analyzed by use of a specific IFN-γ ELISA (Pharmingen).

Flow cytofluorometric analysis of IFN-γ–producing CD8⁺ T cells. PBMCs were cultured in the presence of live *L. guyanensis* or whole BCG; after 5 days, $CD8^+$ T cells were purified as described by the manufacturer (Dynal). In brief, cells conjugated with an anti-CD8 mAb were incubated with anti-mouse IgG-coated magnetic microbeads and, subsequently, were isolated after exposure to a magnetic field. Purity was 96%, as determined by FACS analysis. CD8 mAb was released with Detachabeads, as described by the manufacturer (Dynal). Purified $CD8^+$ T cells were labeled first with various anti-human antibodies and incubated for 30 min at 4°C. Anti-mouse IgG or IgM conjugated with fluorescein isothiocyanate (Sigma) was added, and the cells were incubated for 20 min at 4°C. As human anti-CLA was produced, as IgM from rat, an intermediate step with mouse anti-rat was added.

The IFN-γ–secreting cells were isolated from these purified, labeled $CD8^+$ T cells by use of an IFN-γ–secretion assay kit, in accordance with the manufacturer's instructions (Miltenyi Biotec). In brief, a rat anti–IFN-γ mAb (rat IgG1) conjugated to cell surface–specific mAb (mouse IgG2) (i.e., the IFN-γ catch reagent) was attached to the cell surface of $CD8^+$ T cells. Cells then were incubated for 45 min at 37°C, and secreted IFN-γ bound to the IFN-γ catch reagent on the secreting cells. Then, the cytokine was labeled with an IFN-γ–detecting mAb conjugated to phycoerythrin (PE; mouse IgG1). Finally, the IFN-γ–secreting $CD8^+$ T cells were purified by use of anti-PE microbeads. Analysis was done on a FACSscan cell sorter (Becton Dickinson) using Cell Quest software (Becton Dickinson).

RNA extraction from PBMCs and biopsy specimens. Total RNA was isolated from either PBMCs, purified $CD8^+$ and $CD4^+$ T cells, or biopsy specimens, as described elsewhere [6]. First-strand cDNA synthesis was done on total RNA, by use of a first-strand cDNA synthesis kit (Amersham-Pharmacia Biotech).

Polymerase chain reaction (PCR) amplification of Vβ TCR mRNA. Vβ TCR mRNA amplification was performed by use of 28 oligonucleotide primers specific for individual Vβ gene-segment families or for Vβ family members combined with a consensus Cβ primer recognizing sequences conserved between the Cβ1 and Cβ2 genes, as described elsewhere [7]. The PCR conditions were denaturation for 30 s at 94°C and annealing/extension for 1 min at 65°C, for 35 cycles.

ELISA-based quantification of PCR products. Quantification of PCR products was done by use of the PCR-ELISA method described elsewhere [7], using 2 probes specific for Cβ1 (5′-GAAAAACGTGTTCCACCCGAGGTCGCCC-3′) and Cβ2 (5′-GAACAAGGTGTTCCCACCCGAGGTCGCCC-3′) to detect Vβ TCR by means of reverse-transcriptase (RT) PCR.

The 28 Vβ TCR mRNA RT-PCR products were run in triplicate wells in the same ELISA plate, and average optical density (OD) values were obtained for each Vβ gene family. The OD measured for each Vβ gene family was divided by the total OD measured in the repertoire. The percentage of the total Vβ repertoire for each Vβ gene family in tissue was divided by the percentage of the total Vβ repertoire of the same Vβ gene family in stimulated PBMCs and was expressed as the tissue/PBMC ratio. The percentage of the total Vβ repertoire for each Vβ gene family in stimulated PBMCs was divided by the percentage of the total Vβ repertoire of the same Vβ gene family in un-

Table 2. Analysis of expression of Vβ T cell receptor (TCR) genes in peripheral blood mononuclear cells (PBMCs) stimulated with live *Leishmania guyanensis* or whole bacille Calmette-Guérin (BCG; as controls), from subjects never exposed to *Leishmania*.

	L. guyanensis–stimulated PBMCs, by subject					BCG-stimulated PBMCs, by subject			
Vβ TCR gene	1	2	3	4	5	1	2	3	4
1									
2							■		
3		■				■		■	
4									
5.1				■			■		
5.2/5.3									
6.1/6.2/6.3							■		■
6.5/6.8/6.9									
6.6/6.7				■					
7									
8									
9						■			
10						■			
11					■			■	■
12						■			
13.1							■		
13.2									
14	■		■	■					
15									
16	■							■	
17	■								
18									■
19						■			
20		■							
21									■
22									
23						■		■	
24									

NOTE. Blackened boxes indicate a ratio >2 for the percentage of Vβ expression in stimulated PBMCs divided by the percentage of Vβ expression in unstimulated PBMCs.

stimulated PBMCs and was expressed as the PBMCs ratio. A ratio >2 was considered to indicate increased expression of the Vβ gene family.

RESULTS

Expression of the Vβ14 TCR chain in* L. guyanensis*–specific T cells in naive subjects. We had demonstrated previously the existence of *L. guyanensis*–specific $CD8^+$ T cells that produce IFN-γ in PBMCs from healthy subjects never exposed to *Leishmania* [8]. Thus, we investigated whether this $CD8^+$ T cell population showed oligoclonal specificities, by determining the Vβ repertoire in these cells. The RT-PCR–ELISA detection method was used [7] to analyze the relative expression of 28 Vβ gene families in *L. guyanensis*–stimulated PBMCs from subjects who had never been exposed to *Leishmania* infection.

The mean percentage of the total Vβ repertoire for each Vβ gene family was not significantly different within unstimulated T cells from naive subjects (data not shown). The percentage of the total Vβ repertoire for each Vβ gene family in stimulated PBMCs was divided by the percentage of the total Vβ repertoire of the same Vβ gene family in unstimulated PBMCs from 5 subjects never exposed to *Leishmania.* A ratio >2 was considered to indicate increased expression of the Vβ gene family. As shown in table 2, the expression of different TCR gene families was increased in *L. guyanensis*–stimulated PBMCs from naive subjects, when compared with that in unstimulated PBMCs. Although PBMCs stimulated with *L. guyanensis* showed increased expression of at least 1 of the Vβ TCR gene families (such as Vβ3, Vβ5.1, Vβ6.6/6.7, Vβ11, Vβ16, Vβ17, and Vβ20) in 1 naive subject, Vβ14 showed increased expression in PBMCs stimulated with live *L. guyanensis* in 3 of the 5 naive subjects analyzed. As a control, the Vβ repertoire of cells stimulated with whole BCG was analyzed. PBMCs stimulated with BCG showed increased expression of >14 Vβ TCR gene families that did not include Vβ14. Together, these results demonstrate that the increase of Vβ14 TCR expression in PBMCs from naive subjects is specific to *L. guyanensis* stimulation.

To confirm that cells expressing the Vβ14 TCR gene after stimulation with *L. guyanensis* were $CD8^+$ T cells, we isolated $CD4^+$ and $CD8^+$ T cells, stimulated them with *L. guyanensis,* and analyzed the expression of their Vβ TCR repertoire. As shown in table 3, $CD8^+$ T cells stimulated with *L. guyanensis* increased expression of the Vβ14 TCR gene in 3 of 4 naive subjects analyzed. In *L. guyanensis*–stimulated $CD4^+$ T cells, we detected an increase of Vβ3, Vβ5.1, Vβ16, and Vβ20 TCR gene expression.

To confirm that the $CD8^+$ T cells producing IFN-γ in response to stimulation with *L. guyanensis* expressed the Vβ14 TCR gene family, we enriched the IFN-γ–secreting $CD8^+$ T cells obtained at the end of the culture, by using the IFN-γ–secretion assay kit, and analyzed the expression of the Vβ TCR repertoire in these cells. Results from individual amplification of Vβ gene families in cells stimulated and in those not stimulated with *L. guyanensis* are shown in figure 1 for 2 of the 3 naive subjects analyzed. Stimulation of PBMCs from 2 naive subjects (subjects 3 and 4 from table 2) showed restricted Vβ14 expression in subject 3 and restricted Vβ14 and increased Vβ6.6/6.7 expression in subject 4. In contrast, we were unable to detect increased expression of Vβ TCR genes in IFN-γ–secreting cells from subject 2 (data not shown).

In addition, Vβ14 cells were purified from PBMCs stimulated with *L. guyanensis* for 5 days and restimulated with *L. guyanensis* for an additional 3 days, after which IFN-γ production was measured. Purified Vβ14 cells produced higher amounts of

Table 3. Analysis of expression of Vβ T cell receptor (TCR) genes in purified $CD4^+$ and $CD8^+$ T cells stimulated with live *Leishmania guyanensis,* from subjects never exposed to *Leishmania.*

	$CD4^+$ T cells, by subject				$CD8^+$ T cells, by subject			
Vβ TCR gene	1	2	3	4	1	2	3	4
1								
2								
3		■						
4								
5.1				■				
5.2/5.3								
6.1/6.2/6.3								
6.5/6.8/6.9								
6.6/6.7								
7								
8								
9								
10								
11								
12								
13.1								
13.2								
14					■		■	■
15								
16	■							
17								
18								
19								
20		■						
21								
22								
23								
24								

NOTE. Blackened boxes indicate a ratio >2 for the percentage of Vβ expression in stimulated purified T cells divided by the percentage of Vβ expression in unstimulated purified T cells.

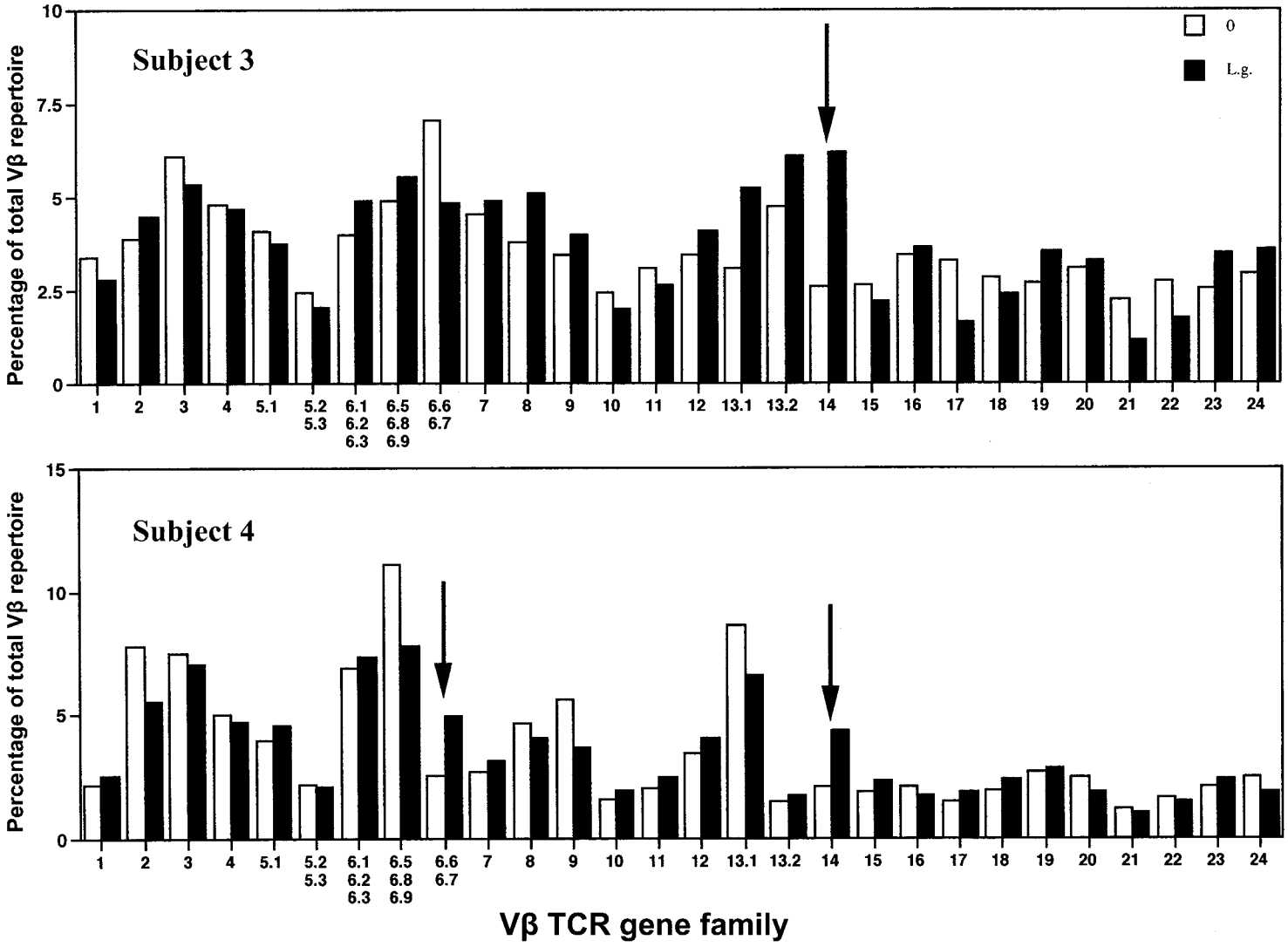

Figure 1. Expression of the Vβ14 T cell receptor (TCR) gene family in interferon (IFN)–γ–producing $CD8^+$ T cells in subjects who have never been exposed to *Leishmania.* For amplification of the Vβ gene families in IFN-γ–producing cells, IFN-γ–secreting cells were enriched from unstimulated or *Leishmania guyanensis* (L.g.)–stimulated peripheral blood mononuclear cells, by use of the IFN-γ–secretion assay kit, and expression of the different Vβ gene families was analyzed as described in Materials and Methods. Percentage of the Vβ repertoire represents the optical density (OD) measured for each Vβ gene family amplification divided by the sum of the total OD for all Vβ genes measured in the repertoire and expressed as the percentage of the total repertoire. Subjects 3 and 4 are the same as in table 1. Arrows indicate significant differences between unstimulated and *L. guyanensis*–stimulated cells.

IFN-γ than did Vβ1 cells in response to secondary stimulation with *L. guyanensis* (1675 ± 240 and 345 ± 265 pg/mL, respectively). As a control, IFN-γ production in all cells (including all TCR-expressing cells) in response to stimulation with *L. guyanensis* was measured (3240 ± 125 pg/mL). Together, these results demonstrate that IFN-γ–producing $CD8^+$ T cells from subjects who have never been exposed to *Leishmania* preferentially express the Vβ14 TCR gene family in response to *L. guyanensis* stimulation.

Migration to the skin of IFN-γ–producing $CD8^+$ T cells that express the Vβ14 TCR chain. The homing capacity of the IFN-γ–producing $CD8^+$ T cells was assessed by measurement of their expression of CLA and the receptors of the CC chemokines (CCR4), which drive the migration of T cells to the skin [9, 10]. In addition, the surface expression of CD62L, which is necessary for migration to lymph nodes [11], was evaluated. By combining the trapping of IFN-γ–producing $CD8^+$ T cells, through use of the IFN-γ–secretion assay kit at the end of the culture, and the analysis of the expression of the above-mentioned markers at the surface of these cells, we showed that IFN-γ–secreting $CD8^+$ T cells expressed the CLA and CCR4 molecules (figure 2*A*). As a control, cells that did not produce IFN-γ (IFN-γ–negative cells) were analyzed and did not express CLA, CCR4, and CD62L (figure 2*A*), demonstrating that the increased expression of these molecules did not result from the culture conditions. Furthermore, after BCG stimulation, IFN-γ–producing cells expressed CD62L at high levels but did not express CLA (figure 2*B*). Together, these results suggest that, in response to live *L. guyanensis*, IFN-γ–producing cells are potentially able to migrate to the skin at the site of *Leishmania* infection.

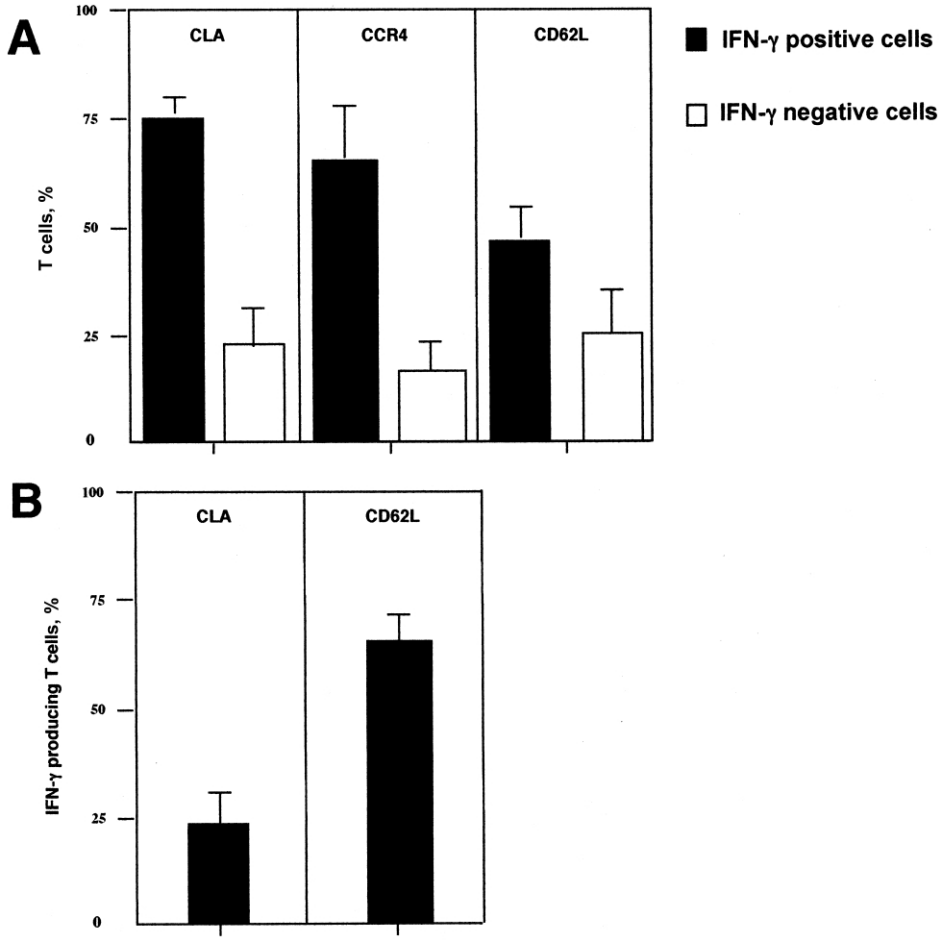

Figure 2. Homing capacity of interferon (IFN)–γ–producing $CD8^+$ T cells from subjects who have never been exposed to *Leishmania*. Peripheral blood mononuclear cells (PBMCs) were cultured as described in Materials and Methods. After 5 days of culture, $CD8^+$ T cells were purified from PBMCs with anti-CD8 magnetic beads (Dynal). Purified $CD8^+$ T cells were first labeled with various purified anti-human monoclonal antibodies (anti–cutaneous lymphocyte antigen [CLA], anti-CCR4, and anti-CD62L), and anti-mouse IgG or IgM conjugated with fluorescein isothiocyanate (Sigma) was added. Because human anti-CLA is a rat IgM, an intermediate step with anti-rat was added. IFN-γ–secreting cells and cells not secreting IFN-γ were isolated from these $CD8^+$ T cells by use of an IFN-γ –secretion assay kit, as described in Materials and Methods. *A,* Stimulation with *Leishmania guyanensis*. *B,* Stimulation with whole bacille Calmette-Guérin.

Table 4. Analysis of expression of Vβ T cell receptor (TCR) genes in leishmaniasis lesions.

	Patient											
Vβ TCR gene	1	2	3	4	5	6	7	8	9	10	11	Patients, %
1	■	■						■	■	■		45.5
2												0
3									■			9.1
4		■										9.1
5.1		■										9.1
5.2/5.3												0
6.1/6.2/6.3	■		■						■			27.3
6.5/6.8/6.9		■						■				18.2
6.6/6.7		■						■				18.2
7	■											9.1
8												0
9		■						■			■	27.3
10								■				9.1
11												0
12												0
13.1		■						■				18.2
13.2						■		■				18.2
14	■	■	■	■	■	■	■					63.5
15												0
16												0
17												0
18												0
19												0
20								■			■	18.2
21												0
22												0
23												0
24	■							■		■		27.3

NOTE. Blackened boxes indicate a ratio >2 for the percentage of Vβ expression in lesions divided by the percentage of Vβ expression in *Leishmania guyanensis*–stimulated peripheral blood mononuclear cells.

Increase of Vβ14 TCR expression in lesions due to* L. guyanensis *infection. To confirm that IFN-γ–producing T cells preferentially expressing the Vβ14 TCR gene migrate to the skin, we determined the Vβ repertoire in lesion samples from patients with LCL. We compared the relative expression of each Vβ gene family in the tissue samples with that in live *L. guyanensis*–stimulated PBMCs from patients with cutaneous leishmaniasis. For the 11 patients analyzed, the expression of several Vβ gene families was increased in tissue. As shown in table 4, expression of Vβ14 was increased in 7 (63.5%) of 11 patients, suggesting that the IFN-γ–producing $CD8^+$ T cells effectively migrated to the skin. Furthermore, expression of the Vβ1 and Vβ6 TCR gene families was increased in lesions from 5 (45.5%) of the 11 patients with LCL, and Vβ9 and Vβ24 expression was increased in 3 (27.3%) of the 11 patients. For all patients, expression of at least 1 Vβ gene family was increased in tissue, when compared with TCR gene expression in PBMCs stimulated with live *L. guyanensis*. Only a small number (1–3) of Vβ gene families were overrepresented in most patients.

Association between increased Vβ14 TCR expression in* L. guyanensis*–infected tissue and a longer duration of lesions. We analyzed the correlation between the increase of Vβ14 TCR expression in tissue and clinical data (tables 1 and 4). We found no association between overexpression of the Vβ14 TCR gene and the number of lesions and/or location. Furthermore, no association was detected between the healing status of the lesions and the expression of Vβ14. Although the 2 patients who presented with unresolving lesions (patients 5 and 6) had increased Vβ14 TCR expression, 4 of 9 patients who presented with healing lesions did not have increased Vβ14 TCR expression. However, we found that increased expression of the Vβ14 TCR gene in tissue was associated with the duration of lesion development: in tissue in which the Vβ14 TCR gene was overexpressed, the duration of lesion development was longer than that in tissue with normal expression of Vβ14 (median of 60 days vs. 32.5 days, respectively) (figure 3). As a control, the increased expression of Vβ6 and Vβ1 TCR genes in tissue

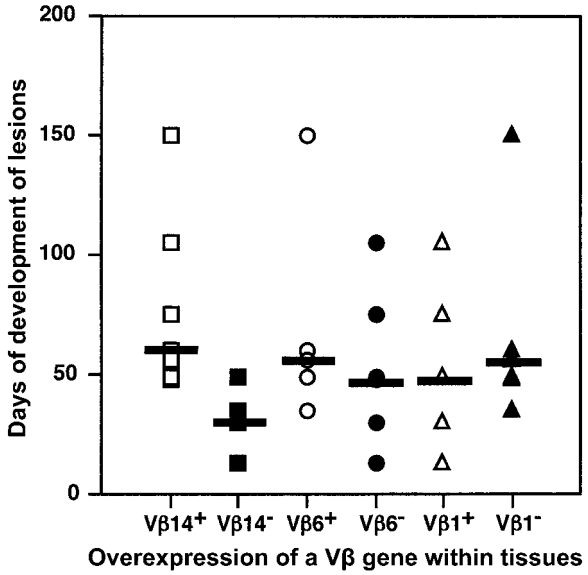

Figure 3. Length of evolution of lesions in biopsy samples, with respect to increased Vβ expression in lesion samples. Results are given as individual plots and median days of the development of lesions with either overexpression of a particular Vβ T cell receptor (TCR) chain (Vβ^+) or normal expression of a particular Vβ TCR chain (Vβ^-). Overexpression of a particular Vβ gene was evaluated as in figure 2.

was analyzed and could not be associated with the duration of lesions.

DISCUSSION

In this study, we demonstrated preferential Vβ14 TCR expression in IFN-γ–producing CD8$^+$ T cells in *L. guyanensis*–stimulated PBMCs from naive subjects. The expansion of cells from naive subjects never exposed to *Leishmania* has been described elsewhere. It has been reported that PBMCs from unexposed subjects may produce IFN-γ in response to *Leishmania aethiopica* stimulation and that NK cells and CD8$^+$ T cells are the source of this IFN-γ [12, 13]. Furthermore, we had demonstrated previously the presence of IFN-γ–producing CD8$^+$ T cells in *L. guyanensis*–stimulated PBMCs from naive subjects [8].

We hypothesized that Vβ14$^+$ CD8$^+$ T cells are able to migrate to the skin to mount their antiparasitic functions. Thus, we investigated the expression of CLA and CCR4 on the IFN-γ–producing CD8$^+$ T cells specific for *L. guyanensis*. By combining the trapping of specific IFN-γ–producing CD8$^+$ T cells and the analysis of the expression of these surface markers, we were able to demonstrate the expression of CCR4 and CLA on these cells, suggesting that these cells can migrate to the skin.

We then measured the diversity of the Vβ chain TCR repertoire in lesions caused by *L. guyanensis*. We found that, although the expression of only a small number of Vβ gene families was increased in tissue samples, Vβ14 was predominant in the lesions of 63.5% of patients with LCL; Vβ1 and Vβ6 were predominant in 45% of patients, and Vβ13 and Vβ24 were predominant in 27% of patients. In accordance with our data, a recent report showed a decrease of Vβ14 expression in CD8$^+$ T cells in the lymph nodes of patients with LCL who were infected with *Leishmania braziliensis*, which may reflect the sequestration of such cells at the site of infection [14].

The fact that the expression of a limited number of Vβ TCR gene families is increased in biopsy specimens from patients with LCL implies the recognition of a limited number of antigens. This result is surprising given the high complexity of the *Leishmania* parasite and the recognition of a high number of antigens from *Leishmania* by the PBMCs of patients with LCL. However, our results are in agreement with those of previous reports showing the dominance of a limited set of Vβ TCR gene families in lesions caused by other pathogens, such as *L. braziliensis* [15], *Wucheria bancrofti* [7], or *Mycobacterium leprae* [16]. We report here that, at the site of infection in patients with LCL, a population of cells preferentially expresses the Vβ14 TCR gene family, a TCR gene that also is expressed in IFN-γ–producing CD8$^+$ T cells specific for *L. guyanensis*, in PBMCs from naive subjects.

Because the expression of a particular Vα chain on the Vβ14$^+$ CD8$^+$ T cells and the diversity of the CDR3 region have not been evaluated, it is not possible to assess whether cells expressing the Vβ14 TCR gene family at the site of infection and PBMCs stimulated with *L. guyanensis* recognize the same antigen. Furthermore, although we have demonstrated that Vβ14-expressing cells in PBMCs from naive subjects are CD8$^+$ T cells, the phenotype of the Vβ14-expressing cells in tissue samples is unknown. However, because CD8$^+$ T cells have been identified in lesions caused by *L. guyanensis* infection [17], we can assume that the Vβ14-expressing cells in tissue could be CD8$^+$ T cells. Purification of CD8$^+$ T cells from tissue samples should confirm this hypothesis. Unfortunately, the number of CD8$^+$ T cells recovered from tissue samples did not allow us to analyze their Vβ repertoire. However, the fact that the Vβ14-expressing cells in naive subjects express markers of migration to the skin suggests that the Vβ14 detected in tissue samples indicates migrating Vβ14 cells.

With regard to the association between the overexpression of a particular Vβ TCR gene family and clinical data, we were unable to detect an association with the number of lesions or location or with the healing status of the lesions. Thus, the Vβ14$^+$ CD8$^+$ T cells did not appear to significantly contribute to the resolution of infection. However, we cannot rule out that the effect of the IFN-γ produced by the Vβ14$^+$ T cells is down-regulated by cytokines produced by cells within the lesion. In a previous study, we described the production of interleukin-10 by *L. guyanensis*–stimulated PBMCs from patients with LCL [18]. Nevertheless, the increase of Vβ14 TCR gene

expression in lesions was associated with the duration of lesions. The duration of lesion development in patients showing increased Vβ14 TCR expression in tissue was longer than that measured in patients with normal Vβ14 expression.

Other skin diseases also have been studied for the nature and function of clonally expanded T cells. In this context, it is interesting to note that Vβ14 overexpression was reported in lesions of human bancroftian filariosis [7]. The Vβ6 TCR gene family was shown to be dominant in the lesions of patients infected with *L. braziliensis* [15] and in tuberculoid leprosy lesions [16]. Recent data have indicated that the Vβ12 TCR gene also may be involved in the response to *L. braziliensis* [14].

Several studies have analyzed the Vβ TCR repertoire in normal human skin. Two of them have reported overexpression of the Vβ6 gene family [19, 20], and a third study has reported overexpression of the Vβ14 gene family [21]. Because Vβ6 and Vβ14 are overexpressed in tissue samples from most patients with LCL, we cannot rule out that *Leishmania* could have stimulated T cells already localized in the skin. Unfortunately, in the present study, for ethical reasons we were unable to obtain normal tissue samples to evaluate expression of the Vβ TCR repertoire in T cells in skin. However, the expression of CCR4 and CLA on *L. guyanensis*–specific T cells producing IFN-γ, as well as the expression of Vβ14 in infected tissue, suggests that *L. guyanensis*–specific T cells migrate actively to the skin during chronic cutaneous infection.

In summary, CD8$^+$ T cells preferentially expressing the Vβ14 TCR gene and secreting IFN-γ develop and circulate in the periphery. These cells migrate to the skin at the site of parasitic infection. However, the exact role of these cells in resistance to infection remains to be firmly established.

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