Expansion of γδ T Cells in Patients Infected With Cutaneous Leishmaniasis With and Without Glucantime Therapy

Haideh Darabi, Mohsen Abolhassani, Amina Kariminia, and Mohammad H. Alimohammadian

Deptartment of Immunology, Pasteur Institute of Iran, Tehran 13164, IRAN

The expansion of γδ T cells in patients with active cutaneous leishmaniasis, with or without glucantime therapy, was investigated. Twenty patients with local cutaneous leishmaniasis including glucantime-treated (n=10) and untreated (n=10) patients were selected. The controls were healthy individuals (n=10) living in endemic areas. Whole blood was obtained and the T cell subpopulations were analyzed by flow cytometry. Significantly more γδ CD_3^+ T cells were observed in untreated patients (15.9% ± 5.9), when compared with glucantime-treated patients (4.6% ± 1.4) and controls (5.3% ± 2.3). On the other hand, when the percentages of αβ CD_3^+ T-cells were analyzed different results were obtained. A significant increase in αβ T cells was seen in glucantime-treated patients (62.4% ± 7.6), when compared to the untreated patients (55.7% ± 5.5) and controls (55.1% ± 9.6). The percentage of total CD_3^+ T cells was statistically greater in both glucantime-treated (68.8% ± 7.4) and untreated patients (73.4% ± 5.9) when compared to the controls (61% ± 10.3). These results are consistent with previous results on the expansion of γδ T cells during the course of cutaneous leishmaniasis. They also indicate that glucantime therapy can reverse the expansion of γδ T cells and as a result increase the percentages of αβ CD_3^+ T cells.

Key Words: Cutaneous leishmaniasis, gamma-delta T cells, glucatime therapy.

Human leishmaniasis is caused by protozoan parasites of the genus Leishmania, which infects host macrophages. The clinical diseases may vary in form and severity from self-limiting granulomatous lesions of the skin to destructive mucosal involvement, and mild to fatal visceral infections. Both human and murine studies suggest that the progression to disease caused by leishmania infection depends on the types of T cells that are stimulated. The current hypothesis is that the activation of CD_4^+ Th1 cells leading to the production of IFN-γ is critical for recovery from disease. Conversely, the stimulation of CD_4^+ Th2 cells, resulting in IL-4 and IL-10 production, likely contributes to disease progression [1, 2].

Received on 10 June 2002; revised 18 September 2002.

Address for correspondence: Dr. Haideh Darabi, Dept. of Immunology, Pasteur Institute of Iran, Tehran 13164, IRAN. Phone/Fax: (98-21) 649-2596. E-mail: hdarabi@institute.pasteur.ac.ir

The Brazilian Journal of Infectious Diseases 2002;6(5):258-262

1413-8670

CD_4^+ T lymphocytes recognize antigens in the context of self major histocompatibility complex using a T cell receptor (TCR) composed of α and β chains in association with the CD_3 protein complex. Another population of T cells uses a different TCR, composed of γ and δ chains. Most γδ T cells do not express CD_4 or CD_8 markers and are mainly present in the lymph nodes, spleen and blood of mammals [3]. These cells also accumulate in the gut mucosa, pulmonary mucosa, reproductive organs and epidermis [4, 5]. γδ T cells make up <5% of peripheral blood lymphocytes [6]. Although, little is known about the immunological function of the γδ T cells, they have been shown to secrete cytokines such as IL-2, IL-3, IL-4, IL-5, IL-10, TNF-α, IFN-γ and GM-CSF [7]. Furthermore, the presence of γδ T cells in certain lesions has suggested that they play a role in bacterial [7, 8] as well as parasitic infection [9]. Expansion of γδ T cells has been observed in genetically resistant mice following *L. major* infection, indicating that γδ T cells may be involved in host defense against this parasite [10].

Pentavalent antimonials such as meglumine antimoniate (glucantime) are a common drug for treatment of leishmania in human. We studied the expansion of γδ T cells in patients infected with cutaneous leishmaniasis, with and without glucantime treatment.

Material and Methods

Patients. Because of the limited number of cases, 20 patients with local cutaneous leishmaniasis (LCL) (age 36 ± 19.2 years) were selected randomly from the endemic area of Kashan (a city located 230 kilometers south of Tehran, Iran). Diagnosis of cutaneous leishmaniasis was confirmed to be *L. major* by parasite isolation, culture, positive skin test [11] and by clinical identification. Patients were divided into two groups (10 in each group) based on therapy with or without glucantime. Healthy uninfected individuals matched with the same sex and age living in the endemic area (n = 10) were used as controls.

Flow cytometry. Whole blood was obtained from patients and healthy individuals. Samples were collected in sterile tubes containing sodium heparin anticoagulant and processed within 6h. The following reagents were used: mouse anti-human CD_3-FITC, TCR αβ-PE, TCR-γδPE (Becton Dickinson, U.S.). Also, the appropriate immunoglobulins were used as isotype controls. The lymphocyte population was gated by $CD_{45/14}$ (leucogate). Phenotypic analysis was done with two-color staining. Analysis of cells was done with an FACScan and LYSIS II software (Becton Dickinson Immunocytometry systems, USA). At least 10,000 cells were analyzed per sample.

Statistical analysis. Student T-test analysis was used for determining difference between the groups.

Results

Expansion of γδ CD_3^+ and αβ CD_3^+ T-cells in glucantime-treated and untreated patients. Flow cytometry analysis of the blood samples of patients with active cutaneous leishmaniasis without glucantime therapy showed a significant increase of γδ CD_3 T cells ($P<0.05$) when compared with glucantime-treated patients and controls (Figure 1A). On the other hand, a significant increase of αβ CD_3 T cells ($P<0.025$) was shown in glucantime-treated patients (Figure 1B). No significant changes of αβ CD_3 T cells was obtained in the untreated patients, when compared with controls (Figure 1B). When the percentage of CD_3 T cells was analyzed in all groups, a significant increase was obtained in patients, especially untreated individuals. The percentages of CD_3 T cells correlated well with total γδ and αβ T cells (Figure 1C).

Discussion

We report the expansion of peripheral blood γδT cells in patients infected with local cutaneous leishmaniasis without glucantime therapy (15.9% ± 5.9 vs. control 5.3% ± 2.3). No expansion of γδ T cells was found in glucantime-treated patients (4.6 % ± 1.4). The same results were obtained not only in patients suffering from visceral leishmaniasis [12] and in patients infected with *L. amazonensis,* but also in the skin lesion of the patients infected with cutaneous leishmaniasis [13] and American leishmaniasis [4]. The expansion of γδ T cells were also observed when normal human T-cell blasts were cultured in the presence of *L. donovani* amastigotes [14].

Similar results were reported by many investigators in murine system. A significant increase in activated γδ T cells was observed in lymphoid organs and the skin lesions of mice infected with *L. major*. Also, an expansion was observed in mice treated with anti-IgD antibodies, or when infected with *Nippostrongliss brasiliensis* [5]. These agents seem to induce a strong Th2 response. These data are consistent with our results and suggest an implication of γδ T cells in the immune response to leishmania parasites. Recent studies have shown that γδ T cells are involved in the first line of defense against *Leishmania major* infection [4]. Anti-γδ TCR mAb, following *L. major* infection, significantly delayed the resolution of cutaneous lesions in genetically resistant CBA/J mice and resulted in the development of larger lesions containing an increased number of

Table 1. Number of treated and untreated patients, the age and number of lesions

No. of Patients	Untreated patients		Treated patients		
	No. of lesions	Age	No. of lesions	Age	Antimonate*(gr)
1	2	55	2	70	22.5
2	2	28	2	35	22.5
3	4	29	3	20	6 (1.5 local)
4	7	60	3	28	22.5
5	6	69	2	14	15
6	2	55	1	13	22.5
7	1	42	2	13	22.5
8	1	20	5	32	20
9	2	45	6	21	22.5
10	13	59	1	18	22.5

* Antimonid was injected I.M. except patient number 3, that had 6gr I.M. and 1.5gr locally around the lesions. The treatment was maximum for two weeks.

parasites in both BALB/c and CBA/J mice [15]. Also, it was shown that in most patients a subset of circulatory γδ T cells co-expressed a CD_8 marker [12]. This finding raises the possibility that some populations of γδ T cells can interact with antigen in the context of class-I MHC proteins. Both γδ CD_8^+ T cells and the null γδ T cells were expanded in the Leishmania antigen response of human T cells lines [12].

Since the pentamonial therapy such as glucantime is widely used against leishmaniosis, we tried to study the expansion of γδ T cells in patients with cutaneous leishmaniasis undergoing glucantime therapy. We observed that in glucantime-treated patients, the expansion of γδ T cells was reversed (15.9% ± 5.9 for untreated vs. 4.6% ± 1.4 in treated patients). The same results were observed in mice treated with glucantime. As compared to untreated mice, the percentage of γδT cells was reduced by 50% in the spleens of glucantime-treated BALB/c mice [15]. These data may indicate that in the presence of glucantime the load of parasites will decrease and therefore there is no need for the expansion of γδ T cells.

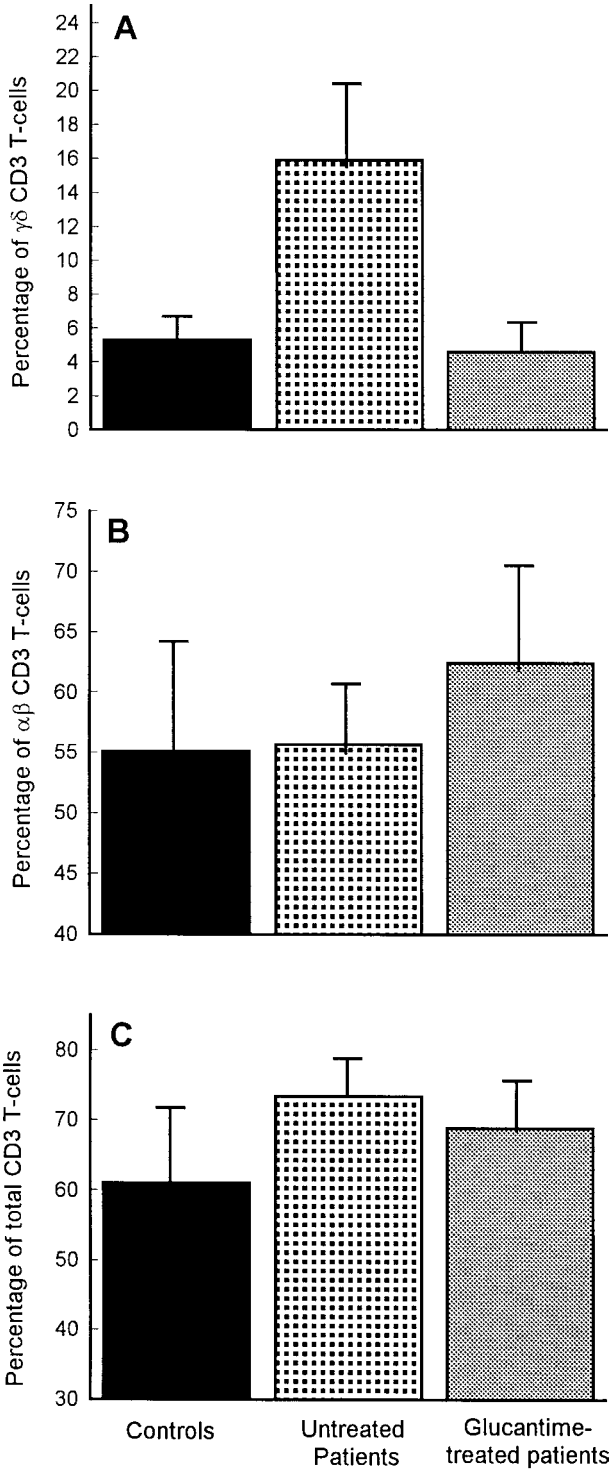
It seems the expansion of γδ T cells *in vivo* depends on the activity of CD_4^+ αβ T cells that secrete Th2 cytokines [5]. Our data indicate that untreated patients have the same percentages of ab T cells (55.7% ± 5.5) as the endemic controls (55.1% ± 9.6), however, the glucantime-treated patients had increased percentages of αβ T cells (62.4% ± 7.6).

γδ T cells play a protective role in infection with various pathogens and expand significantly on days 3 and 6 after infection by *L. monocytogenes* [16]. Mice depleted of αβ-T cells by mAb treatment, showed resistance to infection by *L. monocytogenes* within the first few days after infection [17]. The precise role of γδ T cells during infection with *L. major* and the mechanism by which these cells could influence the outcome of the disease are important issues that remain to be elucidated.

References

1. Russo D.M., Chakrabarti P., and Burns GM., Jr. Native human T cells develope into Th1 or Th0 effectors and exhibit cytotoxicity early after stimulation with Leishmania-infected macrophages. J Infec Dis **1998**;177:1345-51.
2. Kemp K., Kemp M., Kharazmi A., et al. Leishmania-specific T cells expressing interferon-gamma (IFN-γand IL-10 upon activation are expanded in individuals cured of visceral leishmaniasis. Clin Exp Immunol **1999**;116:500-4.
3. Raulet D.H. The structure, function, and molecular genetics of the γδ T cell receptor. Annu Rev Immunol **1989**;26:175-207.

Figure 1. Flow cytometry analysis of T cell subpopulation in patients infected with cutaneous leishmaniasis with or without glucantime therapy. Peripheral blood lymphocytes from patients with or without glucantime therapy and from healthy individuals were isolated and treated with mouse anti-human CD_3-FITC, TCR αβ-PE and TCR γδ-PE. An appropriate Ig was used as an isotype control. The P values of the γδ CD_3, αβCD_3 and total CD_3 T-cells were less than 0.05.



4. Satoskar A., Okano M., David J.R. γδ T cells are not essential for control of cutaneous *Leishmania major* infection in genetically resistant C57BL/6 mice. J Infect Dis **1997**;176:1649-52.
5. Rosat J.P., Conceicao-silva F., Waanders G.A., et al. Expansion of γδ T cells in BALB/c mice infected with *Leishmania major* is dependent upon Th2-type CD_4 T cells. Infec Immun **1995**;63:3000-4.
6. Falcao R.P., Voltarelli J.C., Simoes B.P., et al. Malignant T γδ lymphoproliferative disease with natural killer lytic activity. Am J Hematol **1992**;41:128-31.
7. Has W., Pereira P., Tonegawa S. Gamma/delta cells. Ann Rev Immunol **1993**;11:637-85.
8. Fu Y.X., Roak C.E., Kelly K., et al. Immune protection and control of inflammatory tissue necrosis by γδ T cells. J Immunol **1994**;244: 713-6.
9. Roberts S.J., Smith A.L., West A.B., et al. T cell αβ and γδ deficient mice display abnormal but distinct phenotypes towards a natural, widespread infection of the intestinal epithelium. Proc Natl Acad Sci USA **1996**;93:11774-9.
10. Rosat J.P., MacDonald H.R., Louis J.A. A role for γδ T cells during experimental infection of mice with Leishmania major. J Immunol **1993**;150:550-5.
11. Alimohammadian M.H., Almasi H., Khabiri A., Hatam G., et al. Identification of species and characteristics of an outbreak of cutaneous Leishmaniasis in a New Focus of Iran. Iran Biomed J **1999**;3:31-9.
12. Russo D.M., Armitage R.J., Barral-Netto M., et al. Antigen-reactive γδ T cells in human leishmaniasis. J Immunol **1993**;151:3712-8.
13. Uyemura K., Klotz J., Pirmez C., et al. Microanatomic clonality of γδ T cells in human leishmaniasis lesions. J Immunol **1992**;148:1205-11.
14. Saha A., Chakrabarti G., Sen S., Bandyopadhyay S. *Leishmania donovani* parasites interact with γδ human peripheral blood T cells and induce susceptibility to NK cell-mediated lysis. Scand J Immunol **1999**;50: 588-95.
15. Rosat J.P., MacDonald H.R., Louis J.A. A role for γδ T cells during experimental infection of mice with Leishmania major. J Immunol **1993**;150:550-5.
16. Hiromatsu K., Yoshikai Y., Matsuzaki G., et al. Protective role of γδ T cells in primary infection with *Listeria monocytogenes* in mice. J Exp Med **1992**;175:49-56.
17. Naiki Y., Nishimura H., Itohara S., Yoshikai Y. γδ T cells may dichotomously modulate infection with avirulent *Salmonella cholerasuis* via IFN-γ and IL-13 in mice. Cell Immunol **2000**;202:61-9.