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Original Article

Composition of T cell immune response in cutaneous leishmaniasis

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Abstract

Background There is wide range of clinical presentations of cutaneous leishmaniasis, which are dependent on different species of the parasite as well as immune response offered by the host. World research is now focused on the host's immune response in this disease. Th 1 type T cell response seems to have a critical role in final outcome of this parasitic infection.

Objective This study was done to analyze quantitatively the cellular immune host response by demonstrating various T cell subsets in the lesions of acute Old World cutaneous leishmaniasis.

Patients and methods The study was jointly conducted in Military Hospital, Rawalpindi and Armed Forces Institute of Pathology, Rawalpindi, Pakistan. 40 biopsies of proven active skin lesions of cutaneous leishmaniasis were evaluated after processing for various immunophenotype cells by using monoclonal antibodies. 15 normal skin biopsies were also studied for comparison and control. Total T cell counts and differential T cell counts were recorded in 10 microscopic fields, using light microscope (x 250) in each tissue specimen. Means and percentages were calculated. Data were analysed by applying ANOVA test using INSTAT programme.

Results Predominant T cell infiltrate was observed in all skin lesions. Total T cell, CD3+ cells, and NK cells counts were seen extremely higher when compared with normal skin biopsies ($p<0.001$). The difference in CD4+, CD8+, CD19+ cells counts was not significant ($p>0.05$).

Conclusion Skin infiltrate is T cell rich and CD3+ and NK cells forms the major portion of the T cell population in cases of acute leishmaniasis.

Key words
Cutaneous leishmaniasis, cellular immune host response, T cell population.

Introduction

Leishmaniasis comprises a spectrum of diseases caused by an obligate intracellular parasite *Leishmania*. There is a wide range of clinical presentations which are dependent on the different species of the parasite as well as immune response offered by the host.[1] Cell mediated immune response is the effecter limb in leishmaniasis. World research is now focused on the host's immune response in all type of leishmaniasis. This is being done for a better understanding of host parasite interaction, evasion mechanisms of the parasites and the effective elimination of the parasite by the host.[2] It may help in

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better understanding of the disease and improvement on treatment modalities. Cutaneous leishmaniasis (CL) is endemic in Pakistan and once restricted to certain geographical regions of the country, has now spread almost all over the country.[1,3,4] This change in the geographic distribution of the disease appears to be due to transportation of the disease from endemic areas to previously non-endemic areas by travelers, tourists, workers and military troops, who are also the frequent victims of the disease.[1,2] Leishmaniasis, being a parasitic infection, presents its host with enormous immunological problems.[5] Parasites are antigenically complex and have complicated life cycles in which the various stages differ antigenically from one another. All parasites though capable of eliciting an immunological response, have evolved numerous ways of evading the consequences of the immune attack. The net outcome is that the infection tends to be long and chronic with the host mounting varied and unsuccessful immunological responses. The immuno-pathological changes caused by these correlate with the magnitude and elevation of infection.[5,6,7] The realisation that both immunity and pathology are controlled by cytokines has provided with means, by which the immune system can be dissected and analysed.[8] In the management of patients, it sometimes becomes necessary to rely on a clinical diagnosis. This can be followed by diagnostic tests. Diagnostic methods can be divided into non-immunological and immunological procedures.[1,9] Non-immunological methods include a parasitic diagnosis. Immunodiagnostic techniques have recently gained importance to aid diagnosis. Sophisticated tests have been devised throughout the world for this purpose.[9] Both limbs of the immune system have been probed, although cell mediated immune response plays an important role in protection of the host as well as the parasite and the expression of the disease itself. In case of old world disease, especially the acute variants, it is the Th 1 type T cell response which plays a critical role in whole immune response.[10,11,12] Granulomatous inflammatory infiltrate forms in response to a number of infectious agents including those causing leishmaniasis. The nature of cells comprising this infiltrate has been an area of active investigations in human and murine models. The particular areas of interest are dissection of the T cell subsets, the antigen recognised by these cells and by the cytokines they produce.[13,14] The characteristics of cellular immunity in cutaneous leishmaniasis have earlier been studied in the peripheral blood[14,15] as well as in lesional tissue.[16,17,18] This study was to analyses the basic components of the cellular immune host response in commonly occurring lesions of cutaneous leishmaniasis in our population.

Patients and methods

69 patients having clinically suggestive lesions of CL were initially included in the study. These patients were mostly the referred cases from various regions of Punjab. Few cases were from the northern areas (NA) and from the North West Frontier Province (NWFP). Most patients were from the military but civilian population was also included in this study. These were the cases of acute cutaneous leishmaniasis (2-8 weeks old) having either a single or multiple (not more than five) lesions. Finally, only 40 cases were

selected on the basis of following strict criteria; (I) All patients belonged to or had lived/travelled through the known endemic area in the past six months before the appearance of the skin lesions. (II) They had skin lesions in the form of either non- healing discrete nodules or ulcers of at least 2-3 weeks duration. (III) All lesions had not healed with one or in some cases two courses of antibiotics. (IV) Systemic examination was non-contributory. (V) Positive skin slit smears for amastigotes. A written consent was taken from all patients after explaining the project in detail. A detailed history of the patients, with reference to duration of illness, appearance of the initial lesion, its spread and state of general health during this period was recorded. The site of lesion, clinical pattern, state of lymphatics, oedema of the area involved was also noted. Elliptical skin biopsies from the edge of the active lesions were performed under aseptic measures. After fixing and processing, slides were made from each specimen, which were stained with routine hematoxylin and eosin stain. The immunophenotyping was done on 40 skin biopsy specimens on formalin- fixed, paraffin-embedded tissue sections. 15 normal skin biopsies were also studied in similar pattern for comparison purpose. Immunophenotyping was done using the strepavidine: biotin method. Primary antibodies used were non-alcohol murine antiCD3, CD4, CD8, CD19, CD57 (ZYMED, San Fransisco, California USA). Second antibody used was Biotinylated Second antibodies (BSA). The kit used for immunohistochemistry was Streptavidine/biotin Histostain SAP kit (ZYMED, San Fransisco California USA). Five slides from each specimen were stained using different monoclonal antibodies. Smears of washed and separated lymphocytes were used as positive controls with each batch. Negative controls without the primary antibody were included in each run and blocking of avidin binding and endogenous peroxidase was done prior to deposition of the first monoclonal antibody. Slides were made by using the strepavidine: biotin method and were read under a light microscope (magnification x 250). Ten randomly chosen microscopic fields in the area of the inflammatory infiltrate were selected. Total cells and number of positive cells were counted in each field. Data was endorsed and analysed by using software SPSS-8 programme. Percentage of cells in granulomas staining with these antibodies was obtained by dividing the number of positive cells by the total number of cells and multiplying by 100.

Results

Our patients belonged to a heterogeneous population of both sexes, but as our study included patients from the military hospitals, the majority of patients were males. There were 38 (95%) males and 2 (5%) female patient. Age of the patients ranged from 19 years to 56 years. The duration of illness was between 2-8 weeks. Almost all the patients had lesions on the exposed parts of their bodies. Majority of the patients (32) had a solitary lesion and few (8) had more than one lesions (four had 2 lesions, three had 3 and one had 4 lesions). The skin sections of 40 acute cases stained routinely with hematoxylin and eosin, showed infiltrates composed of lymphocytes and macrophages arranged diffusely without showing well organised granulomata.

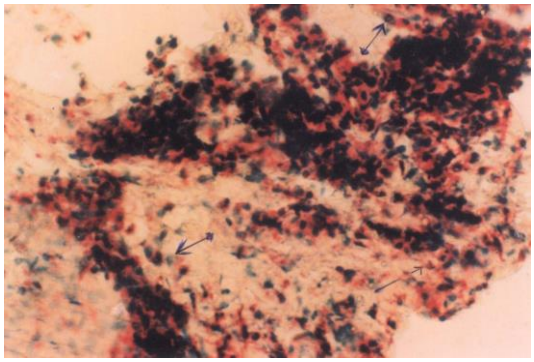

Figure 1 Immunophenotyping of subsets of T cells; CD3+ T cells are demonstrated (indicated with arrows) in this photomicrograph. (X 250)

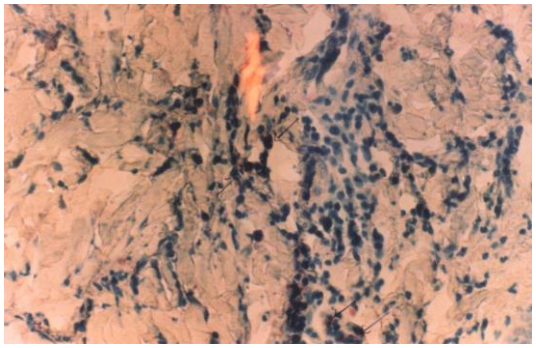

Figure 2 Immunophenotyping of subsets of T cells; CD4+ T cells are demonstrated (indicated with arrows) in this photomicrograph (X 250).

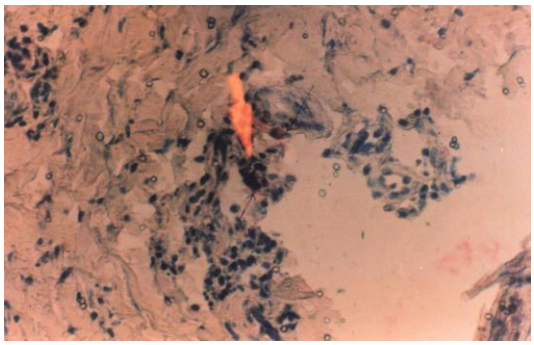

Figure 3 Immunophenotyping of subsets of T cells; CD8+ T cells are demonstrated (indicated with arrows) in this photomicrograph (X250).

Neutrophils, eosinophils, Langhan giant cells and plasma cells were seen. This predominantly mononuclear infiltrate extended from the upper to the lower dermis, sometimes surrounding a zone of necrosis. The overlying epidermis showed moderate ulceration with hyperplasia. Amastigotes were seen lying intra- and extracellularly in 23

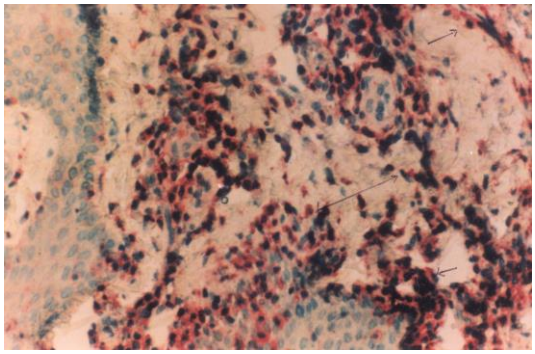

Figure 4 Immunophenotyping of subsets of T cells; CD57+ (NK cells) are demonstrated (indicated with arrows) in this photomicrograph. (X250)

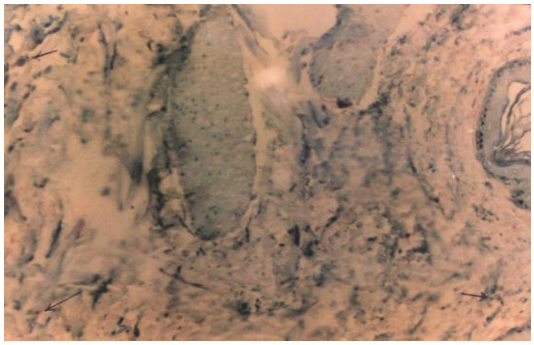

Figure 5 Immunophenotyping of subsets of T cells; CD19+ (Plasma cells) are demonstrated (indicated with arrows) in this photomicrograph (X 250).

histological sections out of 40 cases. All sections showed mild to moderate upper and mid dermal perivascular infiltrate composed of lymphocytes, histiocytes, plasma cells and occasional eosinophils.

Cellular subsets seen in skin sections

Quantitative analysis of cellular subsets (CD3+,CD4 +, CD8+, NK cells and CD19 cells) seen in lesional tissues as well as normal skin tissues (10 fields per sections x 250) is shown in **Table 1** and **2** respectively. The number of CD45RO+ (gamma delta cells) was indirectly calculated by subtracting the sum of CD4 +and CD8+ cells from the total CD3+ cells in each variant. CD3+ cells, CD4 + cells, CD8+ cells, CD57+ (NK) cells and

Table 1 Quantitative analysis of T cell subsets in lesions of cutaneous leishmaniasis.

No.	*Total cells in 10 fields*	*CD3+*	*CD4+*	*CD8+*	*NK cells*	*CD19 cells*
1	211	76	0	0	12	0
2	3340	893	3	2	351	0
3	1395	389	0	1	462	0
4	846	259	3	3	173	0
5	571	221	6	0	30	0
6	970	485	5	0	249	0
7	1697	297	8	5	181	9
8	834	453	8	3	217	4
9	451	200	6	7	141	0
10	1449	659	5	9	182	0
11	1522	692	4	5	378	0
12	651	148	8	5	140	5
13	840	363	0	0	215	8
14	445	130	12	10	56	2
15	491	289	4	8	192	8
16	489	146	8	6	159	6
17	842	393	4	8	183	4
18	993	411	3	5	189	3
19	798	216	6	7	213	4
20	893	378	1	8	182	2
21	921	412	2	4	200	3
22	1011	588	0	3	226	1
23	958	412	3	2	211	2
24	838	381	3	3	213	2
25	798	223	4	2	203	4
26	1239	447	8	8	132	8
27	656	146	8	8	273	5
28	697	299	7	9	226	5
29	593	147	6	7	138	4
30	607	211	8	8	141	3
31	1112	447	8	8	132	8
32	665	146	8	8	273	5
33	679	299	7	9	226	5
34	599	147	6	7	138	4
35	601	211	8	8	141	3
36	831	311	7	2	209	3
37	1679	359	8	6	164	0
38	1196	544	8	10	174	0
39	2158	828	7	5	157	5
40	1831	458	6	8	184	3
Total	39397	13864	189	177	6756	103
%	100	35.19	0.48	0.45	17.15	0.26
Mean	984.92	346.6	4.72	4.42	168.9	2.57

Table 2 Immunophenotyping of normal skin tissue.

No.	*Total cells in 10 fields*	*CD3+*	*CD4+*	*CD8+*	*NK cell*	*CD19+*
1	29	10	0	0	0	3
2	131	0	10	6	20	7
3	50	18	6	4	6	10
4	147	12	4	0	9	1
5	87	9	17	5	0	2
6	46	2	0	2	4	0
7	129	23	4	2	12	2
8	57	15	6	4	6	2
9	22	6	3	0	1	1
10	56	10	4	4	2	3
11	38	11	4	6	3	0
12	29	6	7	4	3	2
13	43	7	3	7	2	2
14	17	6	4	5	0	3
15	28	8	3	4	2	2
Total	907	143	75	55	70	40
%	100%	15.77%	8.27%	5.00%	7.90%	4.41%
Mean	60.47	9.53	5	3.67	4.67	2.67

CD19+ (plasma) cells seen in the sections are shown in photomicrographs (**Figures 1-5**), respectively.

Statistical analysis When cell population in case of lesional tissues was compared with that in normal skin tissues, it showed a marked difference in case of total T cell count, CD3+ cells, and NK cells ($p<0.001$). CD4+, and CD8+ cells were seen more in normal tissues as compared to lesional biopsies but the difference was not statistically significant ($p>0.05$). CD19+ cells counts were almost the same in both cases.

Discussion

There is a wide range of clinical presentations of leishmaniasis which are dependent on the different species of the parasite as well as immune response offered by the host.[1,11] World research is

now focused on the host's immune response in all types of leishmaniasis for better understanding of the disease and possible improvement on treatment modalities. Cutaneous leishmaniasis (CL) is endemic in Pakistan and this study was done to see the composition of T cell immune host response in this disease in our population and to compare it with the immune response seen in other parts of the world. The immunophenotyping of cellular subsets showed some striking features. The total number of cells of the inflammatory infiltrate was more than those stained with different monoclonal antibodies. This indicates that there may be other cells that form part of a chronic granulomatous infiltrate including the fibroblasts, histiocytes, macrophages, dermal dendritic cells and Langhan cells.[17] A rich T cell infiltrate was seen in all the samples as described in earlier studies.[17,19] The inflammatory cellular infiltrate in all active cases contained a large number of CD3 + cells. The presence of 35% CD3+ T cells in our study was lower than that of Modlin[13] (58%), Jaroskava[19] (67%), Barral[20] (48%) and of Ridel[21] (40-75%) but similar to what was reported by Lima[18] and Esterre.[17] The number of CD4+cells and CD8+ cells was markedly lower when compared with other studies.[16,17,19] The relatively low number of CD4+ and CD8+cells may be an indirect indicator for the presence of the significant number of gamma delta cells. Lima *et al*.[18] suggested that these gamma delta subset form about 20% of T cells in early stages of cutaneous leishmaniasis and the number of cells decrease steadily with the age of the lesion and maturation of the granuloma and these cells appear to be critical near or at the commencement of immune response to *Leishmania*.[22,23] Our findings were considered similar to those of Lima *et al*.[18] only the percentage appears to be higher. The reason for the high percentage in our patients thus appears to be the unavailability of the specific monoclonal antibody against gamma delta cells. Plasma cells in our cases were almost negligible similar to previous studies.[21] Natural killer (NK) cells formed a high percentage of the cellular infiltrate in all lesions. It is suggested that presence of NK cells may be a good prognostic marker.[21,24,25] By our study we were able to confirm the previous reports of other researchers that the bulk of the inflammatory infiltrate is made up of T cells.[17,21,26] The predominant cells in the inflammatory infiltrate are CD3+ cells and CD57+ (NK) cells, and a small percentage of CD4+, CD8+ cells. Also a large percentage of cells were seen in the infiltrate, which were CD3+, CD4- and CD8-, these were presumed to be gamma delta cells. This observation further stresses that NK cells and gamma delta cells may be helpful in limiting the disease.

Conclusion

The findings in our study, all point towards the fact that the immune host response developed in cases of acute leishmaniasis in our population is a Th 1 type T cell response and NK cells and gamma delta cells seem to play a critical role in host defence mechanisms against *Leishmania* parasite.

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Manuscript Submission

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