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Effect of Treatment on the Cellular Composition of Cutaneous Lesions in Leprosy Patients[1]

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Leprosy is a chronic infectious disease with a spectrum of clinical manifestations which reflect the cell-mediated immune responses of infected individuals ([1, 2]). It is now believed that lepromatous leprosy patients have a specific unresponsiveness to *Mycobacterium leprae* antigens which is expressed by defective T lymphocyte function ([15]). Mehra, *et al.* ([6]) have postulated that suppressor T cells mediate this specific unresponsiveness although other possibilities exist.

Recent studies have described the cellular composition of cutaneous infiltrates of patients demonstrating the full spectrum of leprosy. By means of immunofluorescent monoclonal antibodies, the lepromatous infiltrate has been shown to contain few lymphocytes and the majority of these are of the Leu 2a/OKT8 (suppressor/cytotoxic) T cell subset. The lesions from tuberculoid patients contained a higher percentage of T cells, and the largest proportion were of the Leu 3a/OKT4 (helper) T cell subset ([8, 9, 15]).

Since the number of tuberculoid leprosy patients studied by Van Voorhis, *et al.* ([16]) was small, we have extended these studies by investigating nine patients with tuberculoid and six with indeterminate disease. We have, in addition, followed the effect of chemotherapy on the cellular composition of cutaneous lesions.

MATERIALS AND METHODS

Patient population. Skin lesions from 38 leprosy patients were studied. After informed consent was obtained, ellipsoid biopsies of 2 × 0.5 cm were taken deep enough to include the lower dermal layers (usually 1 cm in depth). Biopsies were divided into three portions for histologic diagnosis, immunofluorescent studies ([16]), and electron microscopy ([4]). Histologic diagnosis was established according to the Ridley-Jopling classification scheme ([14]). Indeterminate (I) patients were defined according to Liu, *et al.* ([5]). The bacterial index (BI) was established according to Ridley ([13]).

After diagnosis, all of the patients were started on anti-*M. leprae* treatment. Lepromatous patients were given dapsone (100 mg daily) together with rifampin (600 mg daily) for the first three months, and then dapsone alone. Tuberculoid patients were treated with dapsone alone. Newly diagnosed patients in erythema nodosum leprosum (ENL) were not used for the study. Patients who developed ENL during the follow-up period were treated with thalidomide (Patients No. 74 and 80, Table 3). Patient No. 39 was treated with both thalidomide and prednisone.

After 12–18 months of treatment, three groups of patients were identified: 1) disappearance of the lesions (1 TT), 2) regression of the lesions (7 TT, 2 BT, 6 BL, 5 LL, 2 I), and 3) persistence of the lesions (3 LL). Twelve patients were not followed up. Consenting patients of groups 2 and 3 were biopsied a second time, and the biopsies were processed as above.

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Immunofluorescence assays. A sensitive biotin-avidin system was used to identify and enumerate the inflammatory cells in frozen sections by use of mouse anti-human monoclonal antibodies as described by Van Voorhis, *et al.* ([16]). In short, acetone-fixed frozen sections (cryostat set at 2 μm) were exposed for 1 hr to monoclonal antibodies against surface antigens of leukocytes (T29/33, Dr. I. S. Trowbridge, Salk Institute), lymphocytes and their subsets (Leu 1, Becton-Dickinson, Oxnard, California, U.S.A.; OKT4 and OKT8, Ortho), and mononuclear phagocytes (OKMI, Ortho) as listed by Van Voorhis, *et al.* ([16]). Unbound antibody was washed away and the sections were exposed to affinity-purified, biotinylated anti-mouse IgG (Vector Laboratories, Inc., Burlingame, California, U.S.A.) for 30 min, rinsed again, and exposed to fluorescein-labeled avidin D (Vector) for 30 min. Sections were examined by epifluorescence. The numbers of mononuclear phagocytes, lymphocytes, and their subsets recognized by the specific antibodies in 5, 40× microscopic fields were counted. The results are expressed as (mean of number of each cell type per field/mean of total inflammatory cells per field) × 100.

RESULTS

Histologic evaluation

M. leprae were found in the biopsies of all lepromatous (LL) patients and in half of the borderline patients. No bacteria were found in the lesions of polar tuberculoid (TT) patients. All of the tuberculoid (TT and BT) patients were found to have a positive Mitsuda reaction, while all of the LL patients and all but one of the borderline patients were negative (not shown).

The histological findings in the post-chemotherapy sections were significantly different from those of the first biopsy in all patients studied. In the two persistent lepromatous lesions, macrophages were found alone with a few scattered lymphocytes and fibroblasts. Areas with fragmented, degenerating cells with pyknotic nuclei were common in these patients. In the five regressing lepromatous lesions, necrotic cells were less common and the number and density of total inflammatory cells was reduced along with the percentage of lymphocytes. A striking reduction in bacterial load occurred during treatment in all five of these patients.

In the one tuberculoid leprosy case, typical granuloma could not be seen and macrophages were mixed with lymphocytes in diffuse patterns. On an average, the total percentage of lymphocytes in this lesion had increased slightly during the treatment period.

Immunofluorescent identification of T lymphocytes and their subsets in cutaneous infiltrates. The percentage of T cell subsets identified by immunofluorescence in serial sections from cutaneous infiltrates of 38 patients is presented in Tables 1 and 2. An increase in the percentage of T cells and changes in the relative numbers of the T cell subsets were observed as one progressed from the lepromatous to the tuberculoid pole. In patients with tuberculoid leprosy about 30% of the cells stained with anti-T cell antibodies, 60% of which were identified as OKT4 (helper) positive T cells. The T cells were often found in clusters and mantles around the central area of the granulomas. As one approached the lepromatous pole, a drop in the percentage of OKT4 T cells was observed with the T cells dispersed among the phagocytes. The mean OKT4/OKT8 ratio for LL patients was below 0.25 and for the TT patients was above 1.0. Borderline patients had an intermediate ratio. The results confirm some of the earlier observations made by Van Voorhis, *et al.* ([16]) and extend them to include indeterminate patients and more patients at the tuberculoid pole.

In indeterminate patients, the mean percentage of total T cells was found to be over 40%. Although the mean ratio of OKT4 to OKT8 positive cells was 1.78, these patients varied greatly from each other and covered a wide range of OKT4/OKT8 ratios (Table 2). The three patients with low OKT4/OKT8 ratios had negative or low Mitsuda reactions, and one had *M. leprae* in the lesions. Of the other three patients with high ratios, two were Mitsuda positive and one was a low responder.

About 70% of the skin lesions of lepromatous leprosy (LL and BL) patients was found to contain cell-associated immunoglobulin. Only 1 out of 13 tuberculoid (TT and BT) patients had cell-associated immunoglobulin (not shown). These results

TABLE 1. *Distribution of lymphocyte subsets in cutaneous lesions of untreated leprosy patients.*

Histological diagnosis	No. of patients	Sex		Average age in years (range)	Percentage of total infiltrating cells[a]		Ratio OKT4/OKT8
		M	F		OKT4	OKT8	
TT	9	3	6	31 (9–58)	17.07 ± 4.10	13.56 ± 4.78	1.35 ± 0.44
BT	4	2	2	45 (9–61)	8.32 ± 7.24	20.62 ± 7.87	0.39 ± 0.28
BB	2		2	50 (49–51)	20.00 ± 7.07	20.00 ± 7.07	1
BL	7	3	4		9.6 ± 8.39	16.89 ± 6.37	0.48 ± 0.30
LL	10	5	5	37 (17–61)	2.05 ± 1.43	18.03 ± 3.41	0.19 ± 0.15

[a] Results are expressed as mean ± S.D.

correspond with observations made by electron microscopy ([4]) in which lepromatous lesions (LL and BL) were found to contain higher numbers of plasma cells.

Effect of treatment on cellular composition of cutaneous lesions of leprosy patients. After 12–18 months of treatment, the patients from groups 2 and 3 were biopsied a second time and the inflammatory cells in their lesions were identified and counted. Although the bacterial load was reduced drastically in lepromatous patients, there was little change in the OKT4/OKT8 ratios following treatment. The OKT4/OKT8 ratios remained close to or below 0.25 (the cut-off ratio for untreated lepromatous patients) even when the lesions were in regression and the total number of inflammatory cells was reduced. In most of the patients a slight reduction in the percentage of OKT4 (helper) T cells was associated with therapy (Table 3).

DISCUSSION

In an earlier paper, Van Voorhis, *et al.* ([16]) reported that the relative numbers of T helper cells found in the cutaneous lesions of leprosy patients decreased as one approached the lepromatous pole. We have now confirmed these observations and extended them by examining more tuberculoid patients and six indeterminate patients, a group not studied previously. We suggest that the ratio of OKT4/OKT8 T cells is a useful indicator of the cellular immunological status of leprosy patients. The large range of ratios found in the indeterminate patient group suggests that these individuals do not have uniform immune response. Clinical follow-up of indeterminate patients shows that some develop lepromatous disease and others develop tuberculoid disease or even undergo spontaneous cure ([7]). The OKT4/OKT8 ratio could be indicative of the expected course of the disease in at least those

TABLE 2. *Cellular composition of the cutaneous infiltrate of indeterminate leprosy patients.*

Patient no.	Mitsuda reaction	*M. leprae* in lesion	Percentage of total cells		Ratio OKT4/OKT8
			OKT4	OKT8	
16	10 mm	Neg.	7.47	1.78	4.19
57	10 mm	Neg.	61.60	36.60	1.68
70	3 mm	Neg.	18.36	7.14	2.57
90	4 mm	Neg.	27.91	30.59	0.91
32	Neg.	Yes	13.54	12.43	1.08
6	Neg.	Neg.	8.57	31.13	0.27
Mean ± S.D.			22.81 ± 20.34	19.94 ± 14.60	1.78 ± 1.41

patients who progress to polar disease. Low ratios would predict the development of lepromatous disease, and high ratios could suggest the development of polar tuberculoid disease and/or cure.

Wallace, *et al.* ([17]) reported that the number of suppressor T cells in the peripheral blood of multibacillary leprosy patients was elevated, while treated patients had normal T cell subset distribution. On the other hand, the immunological responsiveness of leprosy patients treated with anti-*M. leprae* drugs has been reported to be similar to that of untreated patients ([11, 12]). Horwitz, *et al.* ([3]) have found no effect of long-term treatment on the levels of thymidine incorporation into the blood leukocytes of patients following *in vitro* stimulation with *M. leprae.* Nogueira, *et al.* ([10]) have reported that treatment of lepromatous leprosy patients did not affect their lack of ability to release gamma-interferon in response to *M. leprae* stimulation.

The lesions of all of our treated patients showed some degree of regression, with a lower number of both bacteria and total infiltrating cells. In spite of these signs of regression, the percentage of T cells remained similar to that observed before treatment was started. In addition, the OKT4/OKT8 ratios in lepromatous patients remained low following 12–18 months of treatment. These observations suggest that although treatment arrests the clinical progression of the disease it does not change the immunological status of the patient. This, in turn, suggests that there may be a useful role for immunotherapy in the total care of the lepromatous patient.

SUMMARY

We report on the cellular composition of the cutaneous lesions of 38 leprosy patients prior to and after 12–18 months of chemotherapy. All of the patients with tuberculoid (TT) disease had OKT4/OKT8 ratios that exceeded 1.0. In the indeterminate patients, the ratio varied depending upon the immune status of the individual. A low ratio of OKT4/OKT8 cells (0.25) was found in the LL patients as previously reported. The low ratio of OKT4/OKT8 in the lesions of lepromatous patients did not undergo significant changes after treatment in spite of

TABLE 3. *Clinical, histological, and immunological information on rebiopsied patients.*

Patient no.	Diagnosis			Treatment time (mo.)	Bacterial index		Percentage of total cells				Ratio of OKT4/OKT8	
	Before		After				OKT4		OKT8			
	Clinical	Histologic			Before	After	Before	After	Before	After	Before	After
89	L	LL	Persistence	12	3+	–	<1	<1	18.67	2.23	0.05	0.45
82	L	BL	Regression	18	4+	–	<1	<1	9	53.91	0.11	0.01
74	L	LL	Regression	18	4+	–	2	<1	13	15.36	0.15	0.06
80[a]	L	LL	Regression	18	2+	–	2	<1	13	4.12	0.15	0.24
39[b]	L	LL	Regression	18	6+	1+	3	5.45	27	19.63	0.11	0.27
98	B	LL	Persistence	12	1+	1+	10.55	1.48	17.30	15.35	0.60	0.09
81	L	BB/BL	Regression	12	–	–	7	<1	13	16.28	0.60	0.06
93	T	TT	Regression	12	–	–	13.64	10.75	7.61	14.93	1.79	0.72

[a] Erythema nodosum.
[b] Erythema multiphorme.

a marked reduction in the number of intracellular bacilli.

RESUMEN

Se describe la composición celular de las lesiones cutáneas de 38 pacientes con lepra antes y después de 12–18 meses de quimioterápia. Todos los pacientes con la enfermedad tuberculoide (TT) tuvieron relaciones OKT4/OKT8 que excedieron de 1.0. En los pacientes indeterminados, la relación varió dependiendo del estado inmune del individuo. Como se reportó previamente, los pacientes LL tuvieron relaciones de células OKT4/OKT8 bajas (0.25). La baja relación de OKT4/OKT8 en las lesiones de los pacientes lepromatosos no sufrió cambios significantes después del tratamiento, no obstante la ocurrencia de una marcada reducción en el número de bacilos intracelulares.

RÉSUMÉ

On présente ici les résultats des études sur la composition cellulaire des lésions cutanées chez 38 malades de la lèpre avant chimiothérapie, et ensuite après 12 à 18 mois. Tous les malades atteints de lèpre tuberculoïde (TT) présentaient des rapports OKT4/OKT8 supérieurs à 1. Chez les malades souffrant de lèpre indéterminée, ce rapport était variable et dépendait de l'état immunologique de chacun. Un faible rapport entre les cellules OKT4 et OKT8 (0,25) a été observé chez les malades LL, ainsi que ceci avait déjà été relaté. Le rapport peu élevé OKT4/OKT8 absent dans les lésions de malades lépromateux ne s'est pas modifié de manière significative après le traitement, et ceci malgré une diminution prononcée du nombre de bacilles intracellulaires.

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