Lymphocyte Transfer Reactions in Leprosy Patients[1, 2]

S. H. Han, R. S. Weiser, J. J. Tseng and S. T. Kau[3, 4]

We have demonstrated that allograft immunity is impaired in leprosy patients, especially patients with lepromatous leprosy ([4]). This finding indicates that the lymphocytes of leprosy patients are immunologically defective with respect to their activities *in vivo*, a concept which is supported by the *in vitro* work of others on lymphocyte transformation ([2, 3, 8]), and our recent work on lymphotoxin production by leprous lymphocytes ([5]). Since the early lymphocyte transfer reaction (LTR) induced by the intradermal injection of allogeneic lymphocytes reflects a graft-versus-host reaction by the donor lymphocytes ([1]), it was used in this investigation to test the *in vivo* immunologic competence of the lymphocytes of leprosy patients.

MATERIALS AND METHODS

Human volunteers. The volunteers studied included 12 healthy individuals, 8 inactive tuberculoid patients, 8 inactive lepromatous patients and 8 active lepromatous patients. The patients were under DDS-treatment in the Lo Sheng Leprosarium, Shin-Tsong, Taiwan. They were well-nourished males ranging from 30 to 50 years of age. Their disease status was classified on the basis of physical findings, tests for acid-fast organisms in skin lesions and the lepromin test (Mitsuda type antigen); only patients with the two polar types of leprosy were used. Individuals who were consistently negative to scraping tests for acid-fast bacilli in skin lesions for one year or longer were classified as "inactive." All donors were carefully screened for a history indicative of viral hepatitis and no subject with a serum glutamic-oxaloacetic-transaminase (SGOT) titer exceeding 40 units was included in the study.

Preparation of lymphocytes. Twenty milliliters of fasting blood were drawn from each donor and transferred to a 30-ml test tube containing 1 ml of heparin (100 units) and 1 ml of 6% polyvinylpyrrolidone. The tube was placed in a vertical position at 37° C for one hour to allow the red cells to settle. The supernatant was collected with a Pasteur pipette and centrifuged at 55 g for five minutes to sediment the larger cells. The resultant supernatant was collected and centrifuged at 550 g for six minutes to sediment lymphocytes. The red cells contaminating the sedimented lymphocytes were lysed by osmotic shock ([6]). The lymphocyte-rich residue was suspended in Medium 199 containing 20% heat inactivated human group AB serum and was allowed to pass a glass bead column to remove the contaminating macrophages and polymorphonuclear leukocytes ([7]). The remaining cells were resuspended in M/100 phosphate buffered saline, pH 7.0 (PBS), to give a concentration of 20 x 10^6 cells per ml. More than 95% of the cells in the final preparation were lymphocytes; approximately 95% were viable.

Lymphocyte transfer reactions in leprosy patients. Suspensions of lymphocytes were prepared from eight healthy subjects, eight inactive tuberculoid patients and eight inactive lepromatous patients. These suspensions were administered intradermally into the volar surface of the left forearm to eight active lepromatous recipients. Each recipient was injected with 2 x 10^6 lymphocytes at each of three test sites. The injected cells were from a healthy donor, an inactive tuberculoid patient and an inactive lepromatous patient, respectively. The injection sites were at least five centimeters

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[3] S. H. Han, M.D., Ph.D. and J. J. Tseng, M.S., Kohlburg Memorial Medical Research Laboratory, National Defense Medical Center, Taipei, Taiwan, Republic of China; R. S. Weiser, Ph.D., Department of Microbiology, University of Washington School of Medicine, Seattle, Washington, 98105, U.S.A.; S. T. Kau, M.D., Leprosy Unit, 812 Army Hospital, Tau-Yan, Taiwan, Republic of China.
[4] Requests for reprints should be directed to Dr. R. S. Weiser.

TABLE 1. *Lymphocyte transfer reactions produced by leprous lymphocytes in lepromatous recipients.*

Lymphocyte Donors	Diameters of erythema in mm at the 24th hr								
	L_9	L_{10}	L_{11}	L_{12}	L_{13}	L_{14}	L_{15}	L_{16}	Average
L_{1-8}[a]	4	0	0	0	0	0	0	2	0.5
T_{1-8}	6	4	0	4	0	0	4	4	2.8
H_{1-8}	4	5	0	5	7	4	5	6	4.5

[a] L = lepromatous patients; L_{1-8} inactive, L_{9-16} active.
T = tuberculoid patients, all inactive.
H = healthy subjects.
Whereas each of the 24 donors donated cells to but one recipients, each recipient received cells from three different donors.

apart and were distant from grossly discernible lesions. The sites were examined after 24 hours and the reactions were expressed as diameters of erythema.

Lymphocyte transfer reactions in healthy subjects. Each of four healthy subjects received three intradermal injections of lymphocytes prepared from the other three respective subjects.

RESULTS

The results of the lymphocyte transfer reaction tests conducted with leprous lymphocytes in lepromatous recipients are summarized in Table 1. The lymphocytes from leprosy patients induced weaker reactions than "normal" lymphocytes from healthy subjects; the lymphocytes of only one of the 16 leprous donors produced a reaction exceeding four millimeters in diameter. The differences in the incidence of reactions among individuals of the groups was even more striking than the size of the reactions; it is especially notable that the lymphocytes of most of the lepromatous donors (6 out of 8) failed to give any observable reaction.

The results of the lymphocyte transfer reaction tests conducted with normal lymphocytes in healthy subjects are presented in Table 2. In general, stronger reactions were produced by normal lymphocytes in healthy recipients than were previously noted to occur in lepromatous recipients.

DISCUSSION

The results of the first experiment using active lepromatous recipients clearly indi-

TABLE 2. *Lymphocyte transfer reactions produced by normal lymphocytes in healthy recipients.*

Lymphocyte Donors	Diameters of erythema in mm at the 24th hr				
	H_9	H_{10}	H_{11}	H_{12}	Average
H_9[a]	—	5	6	7	
H_{10}	10	—	6	6	8.3
H_{11}	8	5	—	5	
H_{12}	25	10	6	—	

[a] H = healthy subjects.
Each subject donated cells to the other three subjects and in turn received cells from them.

cated that, as compared to normal lymphocytes, the lymphocytes of leprosy patients, particularly lepromatous patients, are markedly defective with respect to their capacity to mount a graft-versus-host reaction. Presumably in preparations of lepromatous lymphocytes immunocompetent cells capable of acting against the recipient are either low in numbers or are functionally defective. Since the lepromatous donors were in an inactive stage of their disease it seems remarkable that their lymphocytes were measurably deficient in their capacity to elicit the lymphocyte-transfer reaction. It is probable that the lymphocytes of active lepromatous patients would be even more deficient.

A comparison of the results of the first experiment with those of the second experiment, in which normal donors and recipients were used, emphasizes the contribution of recipient as well as donor leukocytes to the lymphocyte transfer reaction and suggests that lepromatous cells other than lymphocytes may be defective. It is possible that the inflammatory process in the lepromatous recipient is depressed in part because his macrophages and/or other blood leukocytes, as well as lymphocytes, are subnormal in their responsiveness to donor lymphocytes or to effector molecules produced by donor lymphocytes in this graft-versus-host reaction. In the transfer reaction, recipient leukocytes are the principal cells that interact with donor lymphocytes to produce inflammation; consequently in the lepromatous recipient leukocytes may either fail to migrate properly to the test site or once at the site they may fail to make their normal contribution to the inflammatory response. Although the donors of the normal lymphocytes used were not under DDS treatment it is unlikely that such treatment of the leprous donors influenced the results; however, this possibility cannot be ruled out.

SUMMARY

The results of the present studies indicate that the lymphocytes of leprosy patients, particularly lepromatous patients, are defective with respect to their capacity to induce the lymphocyte transfer reaction in lepromatous recipients. They also suggest that other circulating leukocytes in lepromatous patients are functionally subnormal.

RESUMEN

Los resultados de los presentes estudios indican que los linfocitos de los pacientes con lepra, en especial los de los pacientes lepromatosos, son defectuosos con respecto a su capacidad para inducir la reacción de transferencia de linfocitos en recipientes lepromatosos. También sugieren que los otros leucocitos circulantes de los pacientes lepromatosos son funcionalmente subnormales.

RÉSUMÉ

Les résultats des études présentées dans cet article montrent que les lymphocytes de malades atteints de lèpre, et particulièrement ceux provenant de malades lépromateux, sont déficients en ce qui concerne leur aptitude à provoquer une réaction de transfert lymphocytaire chez des récepteurs lépromateux. Ces résultats suggèrent aussi que, chez les malades lépromareux, d'autres leucocytes circulants ne sont pas fonctionnellement tout à fait normaux.

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