The Behavior of Leprous Lymphocytes and Macrophages in the Macrophage Migration-Inhibition Test[1, 2]

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When immune lymphocytes are stimulated by specific antigen *in vitro* they secrete biologically active substances which probably represent important contributions to their immunologic functions *in vivo* ([3]).

Diseases in which delayed sensitivity[5] and/or cellular immunity are impaired commonly show alterations in the functional behavior of lymphocytes, such as their *in vitro* response to phytohemagglutinin (PHA) and specific antigens ([14]). However there is no convincing evidence that alterations in the functional behavior of macrophages occur in such diseases.

Lepromatous leprosy is a disease in which delayed sensitivity, cellular immunity and *in vitro* lymphocyte reactivity to various stimuli are profoundly depressed ([15, 18]). Recently we have confirmed the observation of others that the lymphocytes of leprosy patients show subnormal transformation responses to PHA ([7]). In addition we have demonstrated that the ability of patients with lepromatous leprosy to reject allografts of normal skin is depressed substantially ([5]) and that their lymphocytes are impaired with respect to their capacity to form lymphotoxin when stimulated *in vitro* with PHA or with a protein-containing extract of *M. leprae* (leprolin) ([8]).

The present communication is to report our finding that lepromatous lymphocytes lack the capacity to participate in the macrophage migration-inhibition test conducted with leprolin but that the behavior of lepromatous macrophages is essentially normal in this reaction.

MATERIALS AND METHODS

Unless stated otherwise the following materials and methods were used throughout the present investigation. Because it was found in a previous study that human blood monocytes do not respond well to MIF produced by guinea pig lymphocytes, this system was not used in the present investigation ([10]).

Human subjects. The volunteers studied included 16 tuberculoid patients, 22 lepromatous patients and 18 healthy subjects. They were used as donors of lymphocytes and/or macrophages on several occasions. All were well-nourished males ranging from 30 to 50 years of age. The patients were under treatment in the Lo Sheng Leprosarium, Shin-Tsong, Taiwan and Leprosy Unit, 813 Army Hospital, Tau-Yan, Taiwan. The status of their disease was judged on the basis of physical findings, skin biopsy and the lepromin test. All of the patients were (or had been) under treatment with DDS or B663.

Guinea pigs. Albino guinea pigs purchased from a local market were used. They weighed 400 gm to 600 gm.

Blood lymphocytes from patients. Thirty ml of fasting venous blood were withdrawn from each volunteer and transferred to a 50 ml bottle containing a mixture of 3 ml of heparin solution, 100 units/ml, and 3 ml of 6% dextran. The bottles were allowed to stand in a vertical position for one hour at

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[5]The term "delayed sensitivity" is used in preference to the more commonly used term "delayed hypersensitivity."

37°C. The supernatant was carefully collected with a Pasteur pipette and centrifuged for five minutes at 550 × g; the resulting sediment was resuspended and the red cells contaminating the leucocyte preparation were destroyed by osmotic shock ([12]). The cells were suspended in Medium 199 (Difco) supplemented with 20% normal human group AB serum and were allowed to pass a glass bead column to remove the contaminating polymorphonuclear leucocytes and macrophages ([16]). The lymphocyte-rich suspension was centrifuged and the cells were resuspended in the same medium to yield 10×10^6 cells/ml. The final preparation contained approximately 95% viable lymphocytes.

Monocytes from patients.[6] One hundred ml of fasting venous blood were withdrawn from each patient and dextran sedimentation was carried out as described above. The monocyte-rich cell suspension was prepared from a leucocyte sediment obtained from the supernatant by the method of Bennett and Cohn ([2]). The leucocyte sediment was suspended in 27% bovine serum albumin and centrifuged at 2,400 × g for 36 minutes. The sedimented cells were washed twice with Hanks balanced salt solution (HBSS) and resuspended in Medium 199 supplemented with 20% human group AB serum to yield 20×10^6 cells/ml. The final monocyte-rich preparation contained approximately 80% monocytes, 15% polymorphonuclear neutrophils and 5% lymphocytes.

Normal guinea pig macrophages. Macrophage-rich cell suspensions were prepared from peritoneal exudates of guinea pigs as follows: Two days prior to cell collection, 20 ml of liquid paraffin were injected into the peritoneal cavity. The animal was sacrificed under ether anesthesia; the abdomen was opened aseptically and the peritoneal cavity was washed with 80 ml to 100 ml of HBSS containing five units of heparin per ml. The washings were pooled and centrifuged at 55 × g for five minutes to sediment macrophages preferentially. The sedimented cells were washed twice with HBSS and were resuspended in Medium 199 supplemented with 20% normal guinea pig serum to yield 20×10^6 cells/ml. The macrophage-rich cell suspensions contained approximately 60% macrophages, 25% neutrophils and 15% lymphocytes.

Antigen. A protein-containing extract of *Mycobacterium leprae* (leprolin) was used. The leprolin was prepared from live *Mycobacteria leprae* as follows: cutaneous nodules from several active lepromatous leprosy patients were pooled. The pooled tissues were minced with a pair of scissors and each 3 gm (wet weight) of the minced tissues were suspended in 10 ml of PBS and transferred to two special specimen vessels containing one third volume of glass beads. They were vibrated with a Mickle disintegrator (Brinkmann Instruments) with ¼" amplitude for 20 minutes. After disintegration, the suspension was collected and centrifuged lightly to carry down tissue debris. The bacilli were disrupted with a sonifier (High Energy System) at DC 10 amp for 20 minutes. The fluid was clarified by centrifugation at 4,500 × g for 30 minutes and the supernatant, "leprolin" was harvested. It was sterilized by Millipore filtration (0.45 μ), distributed into 5-ml vials and stored at −45°C. The protein content was determined by the method of Kalckar ([13]), and the material diluted with culture medium to a concentration of 100 μg/ml before use.

Migration-inhibition tests. Migration-inhibition tests were conducted in the manner previously reported ([10]) in which a modification of the technic of Thor and associates was employed ([17]). For the testing of lymphocytes, the patient's lymphocyte preparation was mixed with an equal volume of the preparation of normal guinea pig macrophages. For the testing of macrophages, a mixture consisting of equal volumes of the patient's blood monocytes and lymphocytes from tuberculoid leprosy patients was used. The cells were cultivated in Medium 199 supplemented with 20% heat-inactivated normal serum. Human group AB serum was used for human cell mixtures and a mixture of equal volumes of human group AB serum and guinea pig serum was used for mixtures containing human cells and guinea pig cells.

Capillary tubes were filled with the cell mixture, sealed at one end with Vaspar (60% vaseline petroleum jelly, 40% solid paraffin) and centrifuged at 100 × g for five minutes. The capillaries were cut at the cell-medium interface. Duplicate capillaries containing

[6]The term macrophage is used occasionally in this paper to designate a monocyte.

the packed cells were placed in a Mackaness-type chamber which was then closed with a coverslip and sealed with Vaspar. The chamber was filled through a side opening with the medium with or without the antigen and was incubated at 37°C for 24 hours. Two or three chambers were used for each preparation. The areas of migration were estimated by means of a dissecting microscope equipped with a viewing screen. The migration patterns were traced on paper having a constant weight/unit area. The silhouettes were cut out and weighed. The weight of the paper representing the areas of migration was reconverted to actual square millimeters by comparison with a weighed calibrated standard. The results of at least four replicate samples were averaged and the migration index was calculated:

$$\text{Migration index} = \frac{\text{Area of migration in the presence of antigen}}{\text{Area of migration in the absence of antigen}} \times 100$$

RESULTS

The capacity of leprous lymphocytes to produce macrophage migration-inhibition factor (MIF) and the capacity of leprous macrophages to respond to MIF were studied in the modified macrophage migration-inhibition test.

The results of the first experiment presented in Table 1 show that, in the presence of leprolin, the migration of normal guinea pig macrophages was inhibited by tuberculoid lymphocytes but was not inhibited by lepromatous or normal lymphocytes.[7]

In systems confined to human cells it was found that monocytes from either tuberculoid, lepromatous or normal subjects behaved similarly in the macrophage migration-inhibition test. For example, in the presence of leprolin and tuberculoid lymphocytes, the migration of monocytes from tuberculoid, lepromatous and normal subjects was inhibited to essentially the same degree (Table 2).

By contrast, the migration of monocytes from lepromatous and normal subjects was unaffected when in the presence of leprolin and either lepromatous or normal lymphocytes (Tables 3 and 4). The observation that the migration of tuberculoid macrophages was significantly, although not markedly, depressed in the presence of lepromatous lymphocytes and leprolin was unexpected. The possibility that it may have been due to antibodies cytophilic for macrophages is doubtful in view of the uniform migration behavior of normal, lepromatous and tuberculoid macrophages in the presence of leprolin and normal lymphocytes (Table 4).

DISCUSSION

The macrophage migration-inhibition test with leprolin was found to be useful for as-

[7]Results of other trials with higher and lower doses of leprolin did not differ significantly from the results obtained with 100 μg/ml.

TABLE 1. *Migration indices of normal guinea pig macrophages in the presence of leprous lymphocytes and leprolin (100 μg/ml).*

Tuberculoid lymphocytes	Lepromatous lymphocytes	Normal lymphocytes
74.02	95.44	96.68
70.66	67.69	97.91
88.92	87.78	102.70
75.49	114.06	99.07
80.27	94.29	98.99
70.91	95.45	114.16
89.41	98.66	92.05
88.19	111.88	96.96
Average:		
79.73	95.66	99.82

TABLE 2. *Migration indices of leprous monocytes in the presence of tuberculoid lymphocytes and leprolin (100 μg/ml).*

Tuberculoid monocytes	Lepromatous monocytes	Normal monocytes
73.82	86.66	96.24
77.80	68.00	68.08
82.53	78.50	68.64
80.93	73.40	80.40
82.63	89.66	84.62
99.13	87.99	78.64
78.64	87.03	88.42
80.26	78.64	76.82
Averages:		
81.97	81.24	80.23

TABLE 3. *Migration indices of leprous monocytes in the presence of lepromatous lymphocytes and leprolin (100 μg/ml).*

Tuberculoid monocytes	Lepromatous monocytes	Normal monocytes
104.20	98.24	102.24
101.64	108.48	88.48
76.48	104.56	118.64
78.62	82.64	118.22
110.46	104.40	124.16
96.36	102.08	102.00
		104.26
		98.40
Averages:		
94.63[a]	100.07	107.05

[a] The value for tuberculoid monocytes is significantly although not markedly different from the value for normal monocytes.

TABLE 4. *Migration indices of leprous monocytes in the presence of normal lymphocytes and leprolin (100 μg/ml).*

Tuberculoid monocytes	Lepromatous monocytes	Normal monocytes
103.74	109.82	98.06
83.35	85.28	85.12
97.85	114.22	120.46
98.86	106.08	112.22
92.46	121.28	120.40
110.81	101.91	100.68
104.45	98.64	96.64
118.20	100.22	102.42
Averages:		
101.22	104.68	104.50

sessing alterations in the capacity of leprous lymphocytes and macrophages to participate in the cell-mediated immune response. The findings clearly indicate that the ability of lymphocytes to produce MIF under the stimulus of specific antigen is markedly if not totally impaired in patients with lepromatous leprosy but that the capacity of their macrophages to respond to MIF is not altered.

Failure of lepromatous lymphocytes to produce MIF under the stimulus of specific antigen is not surprising since substantial impairment of other lymphocyte activities in lepromatous leprosy has been well documented; for example, the lymphocytes from most lepromatous patients have a reduced capacity to induce the lymphocyte transfer reaction ([9]), to reject skin allografts within a normal period ([5]), and to transform and produce lymphotoxin in response to PHA and antigens ([7, 8]).

The most significant aspect of impairment of MIF production by lepromatous lymphocytes under the stimulus of specific antigen is that it is so complete; this could mean that specifically competent cells are either lacking or that a block of their specific competence exists. Either of these alternatives could result from a genetic defect or a block of lymphocyte function imposed by the disease such as immunologic tolerance. An alternative possibility is that the immunologic defect in lepromatous leprosy might reside in an inherent property of the macrophage ([15]). However, attempts to demonstrate defects in the macrophages of patients with lepromatous leprosy have not yielded conclusive results. Barbieri and Corres ([1]) have reported that the macrophages derived from lepromatous and tuberculoid patients differ in their ability to destroy killed *M. leprae*. A study by Godal *et al* ([4]) using the cells of tuberculoid patients showed that proliferation and activation of macrophages *in vitro* by killed *M. leprae* only occurred when lymphocytes were also present. In the presence of autologous lymphocytes and *M. leprae* lepromatous macrophages did not undergo proliferation and activation. Since the activity of lepromatous macrophages in the presence of tuberculoid lymphocytes was not determined, the report did not provide evidence relative to the question of whether lepromatous macrophages are intrinsically defective in their responses to stimuli provided by sensitive lymphocytes in the presence of antigen.

Our observation that monocytes derived from patients with both polar types of leprosy were inhibited normally when in the presence of tuberculoid lymphocytes and leprolin is of singular interest. It indicates that leprous monocytes are fully capable of responding to MIF. Later findings have indicated that the ability of leprous monocytes to accept cytophilic antibodies is also normal ([6]).

Besides acting as effector cells in cellular immunity, macrophages also play a role in antigen release and processing, which in the case of certain antigens appears to be a necessary step in the afferent limb of the immune response. Our results do not rule out the possibility that this early step in the immune response involving macrophages is unimpaired in lepromatous leprosy. Experiments designed to explore this possibility are in progress.

Our observation (Tables 1 and 2) that tuberculoid lymphocytes in the presence of leprolin yielded only moderate, albeit highly significant depression of macrophage migration of both guinea pig and human macrophages could have resulted because the patients were drug treated. It is possible that the lymphocytes of untreated patients would be more sensitive to leprolin.

SUMMARY

The behavior of leprous lymphocytes and macrophages in the cell-mediated immune response to the specific antigens of leprolin was studied *in vitro* by the macrophage migration-inhibition test using a pure human cell system and a mixed cell system comprised of human lymphocytes and guinea pig macrophages. In the presence of leprolin, the migration of normal guinea pig macrophages was inhibited in the presence of tuberculoid lymphocytes but not in the presence of lepromatous or normal lymphocytes. In the presence of leprolin and tuberculoid lymphocytes, macrophages from tuberculoid, lepromatous and normal subjects showed similar degrees of migration inhibition. Whereas the migration of lepromatous macrophages was not inhibited in the presence of leprolin and either normal or lepromatous lymphocytes, the migration of tu-

berculoid macrophages in the presence of leprolin and lepromatous lymphocytes was inhibited to a slight but significant degree.

The results indicate that the capacity of lepromatous lymphocytes to respond to leprolin with the production of MIF is severely if not totally impaired but that the capacity of lepromatous macrophages to respond to MIF is normal. They also indicated that tuberculoid lymphocytes are sensitive to leprolin and can produce MIF in its presence.

RESUMEN

Se estudió el comportamiento *in vitro* de los linfocitos y macrófagos en lepra, con respecto a la respuesta de inmunidad por células ante los antígenos específicos de la leprolina, por medio de la prueba de inhibición de migración de macrófagos, utilizando un sistema celular humano puro y un sistema celular mezclado, compuesto de linfocitos humanos y macrófagos de cobayos. En presencia de leprolina, la migración de los macrófagos de cobayos normales fué inhibida en presencia de linfocitos tuberculoides, pero no en presencia de linfocitos normales o lepromatosos. En presencia de leprolina y linfocitos tuberculoides, los macrófagos de sujetos tuberculoides, lepromatosos y normales mostraron grados similares de inhibición de migración. Mientras que la migración de los macrófagos lepromatosos no fué inhibida por la presencia de leprolina y linfocitos normales o lepromatosos, la migración de los macrófagos tuberculoides fué inhibida en pequeño grado, pero significativamente, en presencia de leprolina y linfocitos lepromatosos.

RÉSUMÉ

On a étudié *in vitro* le comportement des lymphocytes lépreux et des macrophages lépreux dans la réponse immunitaire transmise par des cellules, à l'égard des antigenes spécifiques de la léproline. Cette étude a été effectuée en ayant recours à l'épreuve d'inhibition de la migration des macrophages, en utilisant un système de cellules humaines à l'état pur, ainsi qu'un système cellulaire mixte comprenant à la fois des lymphocytes humains et des macrophages de cobayes. En présence de léproline, la migration des macrophages normaux de cobayes était inhibée par la présence de lymphocytes tuberculoïdes, mais non par la présence de lymphocytes lépromateux ou de lymphocytes normaux. En présence de léproline et de lymphocytes tuberculoïdes, les macrophages provenant de sujets tuberculoïdes, de sujets lépromateux, ou d'individus normaux, ont montré des degrés semblables d'inhibition de la migration. Alors que la migration des macrophages lépromateux n'était pas inhibée par la présence de léproline, ni par la présence de lymphocytes normaux ou lépromateux, la migration de macrophages tuberculoïdes en présence de léproline et de lymphocytes lépromateux était inhibée à un degré modéré mais significatif.

Ces résultats indiquent que la capacité des lymphocytes lépromateux à répondre à la léproline par la production de MIF est gravement sinon totalement empêchée, mais que la capacité des macrophages lépromateux de répondre aux MIF est par contre normale. Ces résultats indiquent également que les lymphocytes tuberculoïdes sont sensibles à la léproline et peuvent produire MIF en sa présence.

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