Lymphocytes Bearing Immunoglobulin Determinants in Normal Human Lymph Nodes and in Patients with Lepromatous Leprosy[1]

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Immunoglobulin receptors have been demonstrated on the outer surface of lymphocytes by various methods ([1, 3, 5, 6, 16, 17, 20, 25]). Considerable evidence now exists to show that these immunoglobulin determinants have much in common with antibody molecules and it is postulated ([6, 12, 17]) that these may be IgM or IgG type of receptors or that these may represent free light chains.

The indirect or "sandwich" fluorescent antibody test on living cells was first described by Moller ([14]) and later adapted by Osunkoya *et al.* ([16]) to study the fate of "surface-receptor serum globulin" complex on Burkitt's lymphoma cells. More recently Raff *et al.* ([17]) used this approach to determine the pattern of distribution of immunoglobulin determinants on lymphocytes in various lymphoid organs of normal mice. Similar information is lacking so far in human cases. One of the purposes of this communication is to report the proportion of lymphocytes bearing immunoglobulin determinants in human lymph nodes. The function and role of cells carrying immunoglobulin determinants is not precisely defined. However, antisera against total globulins are observed not to react with thymus derived lymphocytes ([17]) and the determinants may thus put in evidence cells committed to humoral immune response. The level of immunoglobulins, in particular IgG, has been observed to be appreciably raised in lepromatous leprosy ([9, 11, 18]). It was, therefore, considered of interest to assess the proportion of lymphocytes bearing immunoglobulin determinants in lymph nodes of lepromatous leprosy patients.

MATERIALS AND METHODS

Fluorescein labelled rabbit antihuman immunoglobulins. Total immunoglobulins were fractionated from pooled normal human sera by half saturation with ammonium sulfate ([19]). Rabbits were immunized with this immunoglobulin fraction emulsified with Freund's complete adjuvant. Rabbit antihuman Ig globulins were separated from rabbit sera by half saturation with ammonium sulfate and conjugated with fluorescein isothiocyanate ([8]). The stock solution of fluorescein labelled rabbit antihuman Ig globulins (Fl-anti-H Ig) had 10 gm. protein per ml.

Lymph nodes. Normal lymph nodes were obtained from subjects undergoing surgical intervention for diseases not affecting the immunologic system. These nodes were, in all instances, examined histologically to exclude any gross abnormality. Only those tissues showing an essentially normal histologic pictures were included in the series. Lepromatous leprosy patients were clinically proven cases with a high bacterial index and were lepromin negative. Patients from Tahirpur Leprosy Sanatorium, Shahadara, Delhi, and a few from our hospital outpatient clinics were chosen as subjects. Inguinal or supratrochlear lymph nodes were removed for examination. The lymph nodes were collected in cold Eagle's Minimum Essential Medium (MEM).

Lymphocyte suspension and Fl-anti-H Ig labelling. The lymph nodes were cut into small pieces in fresh cold MEM within

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two hours after removal. Cells were teased out and passed through a wire mesh. The sieved cells were recovered and washed in veronal-buffered saline of pH 7.2 (VBS) at 4°C. Fluorescent labelling was carried out by incubating equal volumes (O. 15 ml.) of cells (20 x 10^6/ml.) and Fl-anti-H Ig at room temperature for 30 minutes with intermittent shaking. The cells were washed twice and resuspended in VBS. Viability of the cells was tested by the trypan blue exclusion test. The number of injured cells was about 5 per cent in all cases.

Fluorescence microscopy. A wet coverslip preparation was examined for fluorescence under a 45x objective of a Zeiss fluorescence microscope fitted with a high pressure mercury lamp, a blue excitation filter and a 500-530 mμ barrier filter. In each preparation, 100 to 300 cells were counted. In each field the cells were first counted under ordinary light to get the total number of cells. The fluorescent cells were then counted in the same field by switching to ultraviolet light. Fluorescing cells, in most instances, showed a particulate or a complete ring or a sectoral pattern ([14, 16]). The presence of fluorescent "caps" or "foot appendages" was seen in very few cells. Cells showing intracellular fluorescence in the form of a dense fluorescein deposit, a manifestation of nonviability, were ignored in the assessment.

RESULTS

The number of lymphocytes bearing immunoglobulin determinants in six normal lymph nodes is given in Table I. The proportion varied from about 18 to 31 per cent with a mean of 23.4 per cent. Mesenteric nodes showed consistently higher values than the tracheobronchial node. Table 2 gives the values in lymph nodes from leprosy patients. Lymphocytes bearing immunoglobulin determinants constituted a higher percentage of cells in lymph nodes of these patients (mean 42.3; range 31-61).

DISCUSSION

Most of the mononuclear cells in the lymph node cell suspensions were lymphocytes as gauged by microscopic examination with Giemsa's stain. The histiocytes were excluded by adherence to the glassware used in processing; therefore the percentage of these cells in the final suspension was negligible. Very few fluorescing cells were detected in normal human peripheral circulating lymphocytes whereas in the normal lymph nodes 18-31 per cent of the cells showed fluorescence. Raff *et al.* ([17]) using fluorescence antibody technic

TABLE 1. *Lymphocytes bearing immunoglobulin determinants in normal lymph nodes.*

Subject	Age (yrs.)	Sex	Clinical diagnosis	Lymph node examined	Number of total cells counted	Number of cells fluorescing	Percentage of fluorescent cells
1. K.P.	46	M	Chronic duodenal ulcer with pyloric stenosis	Mesenteric	152	31	20.3
2. B.L.	54	M	Deudenal ulcer	Mesenteric	188	59	31.4
3. S.K	12	M	Patent ductus arteriosus	Tracheobronchial	167	30	17.9
4. S.L.	42	M	Gastric ulcer	Mesenteric	145	31	21.4
5. R.D.	38	F	Chronic duodenal ulcer	Mesenteric	214	49	22.9
6. V.K.	2	M	Congenital megacolon	Mesenteric	248	66	26.6
						Mean	23.4

TABLE 2. *Lymphocytes bearing immunoglobulin determinants in lymph nodes from patients with lepromatous leprosy.*

Subject	Age (yrs.)	Sex	Duration of disease (yrs.)	Duration of treatment[a] (yrs.)	Bacterial index	Lymph node examined	Number of total cells counted	Number of cells fluorescing	Percentage of fluorescent cells
1. V.D.	45	M	30	20	2	Supratrochlear	118	62	52.5
2. S.P.	40	M	3	Nil	2	Inguinal	180	55	31.0
3. S.D.	64	M	15	15	1	Supratrochlear	161	50	41.6
4. R.B	47	M	25	20	2	Supratrochlear	251	84	33.4
5. B.L.	35	M	2	Nil	1	Inguinal	244	149	61.0
6. Chand	40	M	30	25	2	Supratrochlear	201	69	34.5
								Mean	42.3

[a] Treated with diaminodiphenyl sulfone, but treatment had not been taken regularly by all patients.

and autoradiographic labelling with ^{125}I-anti-mouse-immunoglobulins showed that the percentage of cells bearing immunoglobulin determinants varied in different lymphoid tissues of normal mice. There was no reaction obtained with the thymus lymphocytes and the proportion of lymphocytes reacting with Fl-anti-mouse-Ig in normal mice ranged from 13 to 23 per cent. The distribution pattern of the cells with immunoglobulin determinants, as demonstrated by Raff *et al.* [17] in different tissues, suggests that thymus derived lymphocytes do not give this reaction.

Impaired cell-mediated immune responses in many cases of lepromatous leprosy have been evaluated by poor response to intradermal injections of bacterial or fungal antigens [2], by difficulty in achieving contact sensitization with haptens [2, 23, 24], by poor blast transformation of lymphocytes from lepromatous patients to various stimulants in cell cultures [2, 4, 21], and by delay in skin homograft rejection in these patients [7, 10]. However, our own observations as well as those of others on the response of lepromatous lymphocytes to phytohemagglutinin *in vitro* [4, 21] and observations on the induction of primary contact sensitisation with haptens [2, 23, 24] show that the depression of cell-mediated immune responses is not a universal manifestation in patients with lepromatous leprosy. Increase in the number of lymphocytes bearing immunoglobulin determinants in lymphoid organs may, therefore, be due, at least in part, to a stimulation of humoral immune responses by mycobacterial antigens. This would be consistent with the observed increase in immunoglobulins [9, 11, 18] in these patients.

SUMMARY

Immunoglobulin determinants on lymphocytes of normal human lymph nodes and in nodes from patients with lepromatous leprosy were demonstrated using an indirect fluorescent antibody technic. The mean percentage of lymph node lymphocytes bearing immunoglobulin determinants in lepromatous leprosy was 42.3 per cent (range 31-61) whereas their proportion in normal nodes was 23.4 per cent (range 18-31). The rise in immunoglobulin

bearing lymphocytes could be a result of both a depletion in the number of cells of thymic origin as well as a nonspecific activation of the humoral immune responses.

RESUMEN

Utilizando la técnica de inmunofluorescencia indirecta, se demostraron determinantes inmunoglobulinicos en los linfocitos de ganglios linfáticos normales y en ganglios de pacientes con lepra lepromatosa. El porcentaje promedio de linfocitos de ganglios linfáticos que llevaban determinantes inmunoglobulínicos en lepra lepromatosa fué de 42.3% (rango 31–61), mientras que la proporcióm en ganglios normales fué de 23.4% (rango 18–31). El aumento de linfocitos que llevan determinantes inmunoglobulínicos pudiera ser el resultado de tanto una depleción en el número de células de origen timico, como de una activación no específica de las respuestas inmunológicas humorales.

RÉSUMÉ

En utilisant une technique indirecte faisant appel aux anticorps fluorescents, on a démontré la présence de constituants provenant des immuno-globulines sur les lymphocytes de ganglions lymphatiques humains normaux, et dans les ganglions prélevés chezdes matades souffrant de lèpre lépromateuse. Le pourcentage moyen de lymphocytes obtenus dans des ganglions lymphatiques et porteurs de ces constituants immunoglobuliniques s'élevait à 42.3% (les mesures individuelles variant de 31 à 61%) dans la lèpre lépromateuse, alors que leur proportion dans des ganglions normaux ne s'élevait qu'à 23.4% (de 18 à 31%). L'augmentation ainsi notée dans la proportion de lymphocytes porteurs d'immuno-globulines pourrait résulter à la fois d'une diminution dans le nombre des cellules d'origine thymique, et d'une activation non spécifique des résponses immunologiques humorales.

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