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IMMUNOGLOBULIN-BEARING CELLS IN LEPROSY[1]

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Abstract. Peripheral blood lymphocytes of 28 untreated and 17 treated patients with different types of leprosy were investigated for the occurrence of immunoglobulin (Ig) bearing cells by means of a smear method. Seven healthy Africans served as controls. In a later stage a complementary study was performed on 6 tuberculoid and 6 lepromatous leprosy patients by means of a suspension method. The immunofluorescence technique was used for the detection of Ig-bearing cells. In tuberculoid leprosy an increase of Ig-bearing cells seems to occur during treatment, predominantly expressed by an increase in IgD-bearing cells. In lepromatous leprosy no increased percentages of Ig-bearing cells were observed.

Key words: Leprosy; Immunoglobulin-bearing cells; Immunofluorescence technique

B cells in the peripheral blood can be demonstrated by the fact that they carry immunoglobulin on their membranes (9, 21, 27) or that they have complement receptors (7).

First results from the investigation of the role of B cells in leprosy were published by Gajl-Peczalska et al. (10). They used an immunofluorescence technique for the detection of B cells in the peripheral blood and found increased numbers in patients with lepromatous leprosy. However, Kreisler et al. (13) observed decreased numbers in Mitsuda-positive and Mitsuda-negative patients, though using the same method. Moreover, Rea et al. (22) found normal percentages in patients with lepromatous leprosy.

By means of an autoradiographic technique, Dwyer et al. (8) found an increase in B cells in lepromatous leprosy patients.

Mendes et al. (17) used the rosette formation test with human erythrocytes sensitized with antibody and complement. They observed decreased numbers in patients with lepromatous leprosy.

Nath et al. (19) observed increased numbers of B cells in lepromatous leprosy and normal numbers in tuberculoid leprosy. They used an erythrocyte-antibody-complement rosette formation test and anti-human immunoglobulins conjugated with peroxidase for the detection of B cells.

These conflicting results may be due to differences among the patients examined as well as different methods used for the detection of B cells.

In this study an immunofluorescence technique was used. Our aim was to investigate the percentages of immunoglobulin (Ig) bearing cells in the peripheral blood of patients with different types of leprosy.

Moreover, a distinction was made between untreated or short-term treated (<6 months) and treated (>6 months) patients in each group in order to ascertain if there was any influence due to treatment.

EXPERIMENTAL DESIGN

This study was undertaken with the aim of investigating the percentages of immunoglobulin-bearing cells in the peripheral blood of leprosy patients and in a control group of healthy Africans.

As part of the study was performed under circumstances where the requisites for the immediate examination of the material were not available, a "smear method" was chosen for the IF study of Ig-bearing cells. By this method it is possible that intracellular as well as membrane-bound immunoglobulins are demonstrated.

[1] Based on a paper presented in the workshop "Lymphocytes, antibodies and hapten carriers" at the fourth annual meeting of the European Society for Dermatological Research, April 1974, Amsterdam, The Netherlands.

Table I. *Untreated and treated patients with various types of leprosy and healthy controls*

	Untreated or treated <6 months	Treated >6 months		
			Years approximately	
	No.	No.	Mean duration	Range
Tuberculoid	16	5	5	½–11
Borderline tuberculoid	3	–		
Borderline lepromatous	7	6	10	1–21
Lepromatous	2	6	5	½–11
Controls	7			

Consequently, at a later stage, a complementary study was performed on 6 tuberculoid and 6 lepromatous patients by means of a suspension technique. These two groups were composed of untreated and treated patients.

With respect to the investigation of B cells in leprosy, most attention has hitherto been paid to lepromatous leprosy patients. In this study an attempt was made to ascertain if there are any differences between the various types of leprosy and if there is any influence due to treatment. Therefore an (arbitrary) distinction was drawn between untreated or short-term treated (<6 months) and treated (>6 months) patients.

MATERIAL AND METHODS

Patients and controls

A total of 45 patients and 7 control persons were included in this study. The division of the patients according to the type of the disease and the duration of treatment is shown in Table I.

The drug regimen varied but none of the patients was undergoing or had recently had been on steroid and/or thalidomide treatment.

Patients suffering from ENL or reversal reaction were excluded from the study.

The classification was based on clinical and histopathological criteria (24) with minor modifications (14).

Seven Africans who denied contact with leprosy patients were included as controls. Moreover, all persons investigated were questioned concerning atopic disposition and contact eczema as in these conditions increased numbers of IgE-, respectively IgD-bearing lymphocytes have been observed (3, 4, 5, 6). Although all persons gave negative answers to the above-mentioned questions, some doubt remains regarding the reliability of the answers.

The study was performed on 20 patients attending the Dermatological Department, Binnengasthuis, Amsterdam, nearly all of whom were immigrants from Surinam, and on 25 African patients in Kenya. Nineteen of the 28 untreated and 6 of the 17 treated patients as well as the controls were all Africans in Kenya. In the complementary study, all were patients who attended the Dermatological Department, Binnengasthuis, Amsterdam.

Preparation of lymphocyte suspensions

Ten ml of blood obtained by venepuncture was collected in a 100 ml Erlenmeyer flask with glass beads and was defibrinated. Lymphocytes were isolated by the Ficoll-

Table II. *Characteristics of FTC-protein conjugates*

FTC content of conjugates was determined by the method of McKinney, Pearce & Spillane (16) using fluorescein diacetate (FDA) as reference standard. Protein content was measured by the method of Lowry et al. (15). Antibody unitage of conjugates was determined in a standard gel diffusion test using 1 mg/ml respective antigens against doubling dilutions of conjugates as described by Beutner, Sepulveda & Barnett (1). A 1 mg/ml solution of human IgG, IgM, IgA and IgD contained 6.6, 129, 30.5 and 100 antigen units respectively. Staining properties and specificity of the class-specific conjugates were tested in indirect IF in normal and monoclonal bone marrow plasmacytes

Conjugate	FTC content (μg/ml)	Protein content (mg/ml)	F/P ratio (μg/mg)	Molar ratio (F/P× 0.411)	Antibody unitage	Working dilution
Rabbit antihuman IgG/FTC[a] (RAHuIgG/FTC, batch no 4572)	26	9.6	2.7	1.11	2	1/32
Goat antihuman IgA/FTC[a] (GAHuIgA/FTC, batch no 6–171)	31.04	32.8	0.94	0.38	4	1/32
Rabbit antihuman IgM/FTC[b] (RAHuIgM/FTC, batch no KH 15-10-A2)	81	14.2	5	2.0	2	1/32
Rabbit antihuman IgD/FTC[b] (RAHuIgD/FTC, batch no KH 20-05-A2)	48.75	10.4	4.6	1.9	2	1/32
Rabbit antihuman IgE/FTC[b] (RAHuIgE/FTC, batch no KH 25-3-A2)	34.8	11.2	3.1	1.27	–	1/32

[a] Nordic Immunological Laboratoria, Tilburg, The Netherlands.
[b] The unconjugated sera were obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands, and were labelled in our laboratory by Dr S. S. Asghar, Ph.D.

Table III. *Values (%) of IgG-bearing cells in untreated and treated patients with various types of leprosy and healthy controls*

T=tuberculoid; BT=borderline tuberculoid; BL=borderline lepromatous; L=lepromatous; C=controls. Slightly higher mean values in the groups of patients in comparison with the control group. These differences are not significant at 5% significance level

	T		BT	BL		L		
Cases	Untreated	Treated	Untreated	Untreated	Treated	Untreated	Treated	C
1	2.25	4.50	3	7	0.50	2.50	0	3
2	3	8	7	4.50	17	2.75	4.50	3
3	1	5.25	6.50	4	3.50		2	3
4	4.25	2.25		3.75	0.50		2.25	2
5	1.25	4.75		4.25	4.25		3.75	2.25
6	6.25			6.75	3.75		7.75	2
7	8.25			4				1.25
8	5							
9	6							
10	3.75							
11	2.25							
12	1							
13	2							
14	3.75							
15	5							
16	3.50							
Mean	3.66	4.95	5.50	4.89	4.92	2.63	3.34	2.36
Range	1–8.25	2.25–8	3–7	3.75–7	0.50–17	2.50–2.75	0–7.75	1.25–3

Isopaque method according to Böyum (2). The lymphocytes were washed in isotonic saline solution (pH 7.2) and centrifuged for 10 min at 200 *g* four times. After the second washing, contaminating red cells were hemolysed in Tris-NH_4Cl buffer (pH 7.2) for 30 min. The lymphocytes were suspended in 1 ml isotonic saline solution and were counted in a Bürker counting chamber. The volume of the suspension was adjusted to a dilution of 2.5×10^6 lymphocytes per ml.

Preparation for immunofluorescence staining

One drop of the suspension was put in a Socorex®-microscopic slide and a thick smear was made. The slides were dried in open air, rinsed for 30 min in phosphate-buffered saline (PBS) (0.01 M; pH 7.2), incubated with the conjugate in a moist chamber for 30 min, rinsed again with twice renewed PBS for 30 min, mounted with BactoFa® mounting fluid and sealed with a coverglass and nail polish. The slides were stored a temperatures of −20°C or lower until examination.

In the case of a preparation for examination in suspension, the lymphocytes were resuspended in 15 drops of isotonic saline solution (pH 7.2) after the second washing. Two drops of this suspension were mixed with two drops of 1 : 20 diluted conjugate and incubated for 30 min. The lymphocytes were then washed twice and resuspended in two drops of isotonic saline solution (pH 7.2). The cells were examined in a Bürker counting chamber.

Conjugates

The conjugates with their characteristics and working dilutions are shown in Table II.

Microscopy

The slides were examined with incident illumination. The microscopic equipment consisted of a Leitz Ortholux fluorescence microscope with a vertical illuminator after Ploem (20). The light source was a Xenon XBO 75W high pressure lamp. As excitation filter, a combination of BG 38 (5 mm), KP 490 (2 mm) and GG 475 (2 mm) was used and as barrier filter, OG 515 (3 mm). The objective was a Leitz water immersion objective (×50). Each field was also examined with transmitted conventional light.

Only cells that showed a conspicuous fine or coarse staining pattern were counted. Those cells that showed only sparse fine or coarse granular fluorescence or ring-like fluorescence were excluded. On each slide a total of 400 cells were counted.

Specificity tests

Fluorescein-conjugated swine anti-rabbit antiserum (SwAR/FTC) failed to stain the smear cells. The addition of normal rabbit serum to the cell suspensions did not reduce the number of cells staining. Unconjugated antiserum specifically inhibited staining due to its own conjugated counterpart but did not inhibit staining due to a conjugated antiserum directed towards a different class of immunoglobulin.

RESULTS

The percentages of IgG-bearing cells are shown in Table III. Generally speaking, the mean values in the groups of patients, varying from 2.63 to 5.50,

Table IV. *Values (%) of IgM-bearing cells in untreated patients with various types of leprosy and healthy controls*

T=tuberculoid; BT=borderline tuberculoid; BL=borderline lepromatous; L=lepromatous; C=controls. Slightly lower mean values in the groups of patients in comparison with the control group with a possible exception of untreated borderline tuberculoid patients. These differences are not significant at 5% significance level

	T		BT	BL		L		
Cases	Untreated	Treated	Untreated	Untreated	Treated	Untreated	Treated	C
1	3	4	15.75	1	2.25	1.25	1	4.25
2	5	8	6.50	4.75	0.50	3.75	1.50	6
3	1	3	6.50	3.50	1		3.50	8.50
4	0.25	1.75		1.25	0		2	4.25
5	0.50	4.25		7.25	1.50		2.75	9.75
6	4			6.25	4		2.50	10.25
7	5.25			2				4
8	5							
9	4.75							
10	2.50							
11	2.50							
12	1.50							
13	2							
14	4.75							
15	5.50							
16	1							
Mean	3.03	4.20	9.58	3.71	1.54	2.50	2.21	6.71
Range	0–5.50	1.75–8	6.50–15.75	1–7.25	0–4	1.25–3.71	1–3.50	4–10.25

tend to be slightly higher than in the control group, with a mean value of 2.36. No clear differences are observed between the untreated and the treated patients, nor between the tuberculoid and the lepromatous patients.

As regards the percentages of IgM-bearing cells, the mean values tend to be lower in the patient groups, varying from 1.54 to 4.20, than in the control group, with a mean value of 6.71 and appear not to be influenced by treatment (Table IV).

The percentages of IgD-bearing cells are shown in Table V. In the groups of untreated patients the mean values, varying from 4.20 to 7.93, are almost identical with those found in healthy controls, with a mean value of 7.43. In the treated patients, however, the mean values varying from 7.63 to 15.50 tend to be higher than those of the untreated patients; the most conspicuous difference is noted in the group of the tuberculoid patients, in which the mean value (15.50) of the treated patients is about three times as high as the mean value (4.20) of the untreated patients. A wide range of values, as indicated in Table V, is observed in most of the groups. The impression is gained that during treatment the percentages of IgD-bearing cells tend to increase. Statistical analysis reveals:

(*a*) A significantly ($p<0.05$) higher mean value of IgD-bearing cells in treated tuberculoid patients than applies to these cells in controls.

(*b*) A significantly ($p<0.05$) lower mean value of IgD-bearing cells in untreated tuberculoid patients, even compared with the control mean value, and

(*c*) In addition a significant ($p<0.01$) difference is found between the mean values in treated and untreated tuberculoid patients.

The mean values of the percentages of IgE-bearing cells in the treated patients with various types of leprosy, varying from 5.42 to 13.00, are comparable to the mean values of the patients who were not treated, varying from 6.06 to 12.57. A wide range of values is observed in most of the groups (Table VI).

Low percentages of IgA-bearing cells are observed in all persons examined. The mean values of the groups of patients vary from 1.13 to 3.13; the mean value of the control group is 0.68.

The results of our complementary study are shown in Table VII. The mean values of the percentages of IgG- and, to a lesser extent of IgM-bearing cells, are higher. With the exception of IgA-bearing cells there generally exists a wide range of values for the different classes of Ig-bearing cells in both groups.

Statistical analysis, using Student's *t*-test and a

Table V. *Values (%) of IgD-bearing cells in untreated and treated patients with various types of leprosy and healthy controls*

T=tuberculoid; BT=borderline tuberculoid; BL=borderline lepromatous; L=lepromatous; C=controls. A significant ($p<0.05$) lower mean value in untreated tuberculoid patients and a significant ($p<0.05$) higher mean value in treated tuberculoid patients compared with the mean value of the controls. A significant ($p<0.01$) difference between the mean values in untreated and treated tuberculoid patients

	T		BT	BL		L		
Cases	Untreated	Treated	Untreated	Untreated	Treated	Untreated	Treated	C
1	1.50	14.25	5	5.50	7	2.25	22	4.25
2	3.50	17	7.25	12	5.50	9.50	4	4.75
3	1	8.25	5	6.75	17.50		4.50	4.75
4	0.25	27.25		10.50	14.00		3	6.50
5	6.25	10.75		10.25	3.25		2.75	11.50
6	3.75			5.75	5.25		9.50	14.75
7	4			4.75				5.50
8	4.25							
9	8.25							
10	2.25							
11	3.25							
12	3							
13	4.75							
14	7.25							
15	5.25							
16	8.75							
Mean	4.20	15.50	5.75	7.93	8.75	5.88	7.63	7.43
Range	0.25–8.75	8.25–27.25	5–7.25	4.75–12	3.25–17.50	2.25–9.50	2.75–22	4.25–14.75

significance level of 0.05, was performed to compare the results obtained in the two groups of patients. The average percentages of IgM-, IgD- and IgE-bearing cells were significantly greater in the group of patients with tuberculoid leprosy than in the group with lepromatous leprosy. Between the two groups of patients, there was no significant difference at the level defined in the average percentages of IgG- and IgA-bearing cells.

DISCUSSION

Several studies on the occurrence of B cells in leprosy have been published. Various techniques have been used for the detection of B cells. These studies were mostly confined to the investigation of lepromatous leprosy patients and the results have been conflicting.

Gajl-Peczalska et al. (10) used FTC-labelled antisera against IgG, IgM, IgA and IgE for the detection of B lymphocytes and found an increase in 7 out of 9 lepromatous leprosy patients, 2 of whom were also on corticosteroid treatment. Two patients who were inactive on the basis of clinical and histopathological criteria still showed elevated levels of B lymphocytes. Kreisler et al. (13), using the same method, noted a decreased number of Ig-bearing cells in 13 Mitsuda-positive and in 17 Mitsuda-negative patients. Rea et al. (22) found normal percentages of Ig-bearing cells in their study of 16 lepromatous leprosy patients.

Dwyer et al. (8) found by means of an autoradiographic method an increase of labelled cells in most of 17 untreated and treated patients (ranging from LL to BL).

For the detection of B lymphocytes, Mendes et al. (17) used the rosette formation test with human erythrocytes sensitized with a subagglutinating dilution of normal rabbit serum containing natural heterophil antibodies to human erythrocytes and complement. Thirty-six patients with lepromatous leprosy (LL), treated for at least one year, were investigated and a decreased number of B lymphocytes was found.

Nath et al. (19) used two methods for the detection of B cells: an erythrocyte—antibody—complement rosette formation test and a method using anti-human immunoglobulins conjugated with peroxidase. They observed increased numbers in most of the lepromatous leprosy patients, irrespective of treatment. In untreated tuberculoid patients, normal numbers were found.

Table VI. *Values (%) of IgE-bearing cells in untreated and treated patients with various types of leprosy and healthy controls*

T=tuberculoid; BT=borderline tuberculoid; BL=borderline lepromatous; L=lepromatous; C=controls. No significant differences between the mean values of the various groups at the 5% significance level

	T		BT	BL		L		
Cases	Untreated	Treated	Untreated	Untreated	Treated	Untreated	Treated	C
1	3.25	6	7.25	29	12	4.75	10	3.75
2	5.50	22	16	15	22	8	8.50	9
3	1	6.25	4.75	9	15.50		1.25	6
4	0.50	25		12.25	14		4.50	9
5	4.50	5.75		8.25	2		5.50	6.75
6	7.50			8	8.25		2.75	6
7	3.25			6.50				8
8	10							
9	15							
10	7							
11	3.75							
12	5.25							
13	8.50							
14	5.25							
15	9.25							
16	7.50							
Mean	6.06	13	9.33	12.57	12.29	6.38	5.42	6.93
Range	0.50–15	5.75–25	4.75–16	6.50–29	2–22	4.75–8	1.25–10	3.75–9

In the present study an immunofluorescence technique was used with monospecific antisera directed against the heavy chains of IgG, IgM, IgA, IgD and IgE. After isolation of the lymphocytes we used a "smear" method, by which similar results were obtained as with incubation and counting in suspension. These are in agreement with the findings of Rowe et al. (25). In our complementary study we used a "suspension" method.

We have not been able to confirm the findings of Gajl-Peczalska et al. (10), Dwyer et al. (8) and Nath et al. (19), as we did not find increased percentages in lepromatous leprosy with both methods.

Humoral immunity is not impaired in lepromatous leprosy. These patients show a normal antibody response after immunization with Vi-antigen (26) and TAB-antigen (12). Moreover, high titres of antibodies against mycobacterial polysaccharides (23) are found, as also in a high percentage antimycobacterial antibodies (18).

Thus the possibility may exist that in lepromatous leprosy, chronic antigenic stimulation takes place without an apparent increase in Ig-bearing

Table VII. *Values (%) of Ig-bearing cells in tuberculoid and lepromatous patients*

T=tuberculoid, L=lepromatous. A significant higher mean value (at 5% significance level) of IgM-, IgD- and IgE-bearing cells in tuberculoid patients compared with lepromatous patients

	IgG		IgM		IgA		IgD		IgE	
Cases	T	L	T	L	T	L	T	L	T	L
1	20	5	6	11	1	1	4	2	2	2
2	2	33	8	5	2	1	4	8	4	6
3	12	9	8	1	1	1	3	1	8	1
4	3	2	10	1	2	2	9	2	7	3
5	5	6	7	7	1	5	8	2	4	3
6	8	3	9	2	1	1	11	2	10	3
Mean	8.5	10	8	3.5	1	2	6.5	3	6	3
Range	2–20	2–33	6–10	1–11	1–2	1–5	3–11	1–8	2–10	1–6

cells in the peripheral blood. Moreover, Mendes et al. (17) observed that in lepromatous leprosy, lymph node follicles were slightly reduced in size, as judged by HEAC-adherence. Verma et al. (28) pointed out that the increase in Ig-bearing cells in lymph nodes of lepromatous leprosy patients could also be explained as a relative increase due to depletion of T-cells.

On the basis of our findings in tuberculoid leprosy it is suggested that the percentage of Ig-bearing cells increases during treatment and that this is predominantly due to an increase in IgD-bearing cells. One explanation may be that during treatment a further release of antigen(s) stimulates the IgD cell line in analogy to observations in contact allergic dermatitis after re-exposure to the foreign antigen. Alternatively, it may be suggested that this may be due to an altered T- and B-cell cooperation.

What is apparent from previous studies and also from ours, is the wide range of values found in a given type of leprosy. An explanation for the differing results obtained by various investigators may be the differences among the patients examined. These may derive from the fact that in many patients leprosy is not an immunologically "stable" disease. Therefore more valuable information may be obtained from longitudinal studies, of which the first results have been published by Godal et al. (11) regarding reactions in borderline tuberculoid leprosy. This type of investigation and the assessment of circulating T-cells in various types of leprosy are in progress.

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