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Lymphocytotoxins in Leprosy [1]

J. M. Kreisler, A. Arnaiz, B. Perez and A. Bootello [2]

Leprosy has been divided into two "polar" forms, with a clear cut pattern which is defined on the basis of several clinical, histopathologic and immunologic parameters. In contrast, a third clinical borderline group lies between the polar forms and is frequently associated with difficulties in its definition ([15]). Lepromatous leprosy is characterized by the finding of several abnormalities in the immunologic response, either *in vivo* responses to tuberculin, DNCB, *M. leprae*, etc. ([1, 16]) or *in vitro* responses to antigens and mitogens ([12]). Furthermore, several reports on the presence of autoantibodies in the sera of lepromatous patients have also been published ([15]).

On the other hand, in certain situations, such as systemic lupus erythematosus and rheumatoid arthritis ([13]), viral infections ([10]), chronic infections ([8]) and after active immunizations ([7]), cytotoxic antibodies have been found. Such antibodies required special temperature conditions and the presence of complement to produce cell damage in lymphocytes from a random population.

Sera from leprosy patients show a variety of autoantibodies and are inhibitory for normal lymphocyte cultures ([2]), similarly to that described in some of the above mentioned conditions ([4, 11]). Therefore, leprosy sera appeared to be an interesting material for the investigation of cold lymphocytotoxins. A correlation between the different clinical forms of leprosy and the presence of such lymphocytotoxins was also intended.

MATERIALS AND METHODS

Patients. Samples of blood from 59 leprosy patients were collected under sterile conditions. Sera were kept frozen at 20°C until tested. Leprosy patients were classified as tuberculoid (TT), lepromatous (LL), borderline tuberculoid (BT), borderline lepromatous (BL) and borderline (BB), according to the recommendation of the Ninth International Leprosy Congress and the WHO standards. All the cases came from the same geographic and ethnic areas of Spain and were under medical control and treatment. Patients with concomitant diseases were excluded. As controls we used sera from healthy voluntary donors.

Cytotoxicity. All sera were tested with lymphocytes from 40 random healthy human donors. Donors were tested for ABO and HL-A groups and their sera were autoanalyzed. The conditions for the microlymphocytotoxicity test were as follows. One microliter of serum was mixed with one microliter of a suspension of lymphocytes (1,000 cells) and incubated for 30 minutes at 15°C prior to the addition of 5 ml of rabbit complement. After additional incubation at 15°C for three

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[2] J. M. Kreisler, M.D., Head, Department of Immunology, Clinica Puerta de Hierro; A. Arnaiz, M.D., Resident, Department of Immunology, Clinica Puerta de Hierro; B. Perez, M.D., Director, Instituto Leprologico de Trillo, Guadalajara, Spain; A. Bootello, M.D., Head, Department of Immunology, Clinica Puerta de Hierro, Madrid, Spain.

hours, the reactions were stained, fixed and read as described by Mittal *et al* (9). Scores of 6 and 8 with one or more different lymphocyte populations were considered as positive.

Autoantibodies. Antinuclear antibodies (ANA), antimitochondrial antibodies (MTA) and antismooth muscle antibodies (SMA) were tested by immunofluorescent technic as described by Holborow (6). The sections were stained with fluorescein isothyocyanate antisera (Hyland Travenol Laboratories, Inc., U.S.A.).

Immunoglobulins and C3. These levels were detected by radial immunodiffusion with commercial plates (Hyland Travenol Laboratories, Inc., U.S.A.).

Statistical calculations. The X^2 test with Yates' correction and the Fischer's Z test were used for the calculations.

RESULTS

Cold lymphocytotoxins were detected in 46% of the sera from leprosy patients whereas in control sera only 13% of the cases gave positive reactions ($p < .01$), Table 1. In control sera, positivity was only present once in 40 different reactions for each serum, whereas in patient sera, positivity was present in an average of 6 of 40 different reactions. There was no clear difference in the strength of the reactions in both groups, the most frequent percentage of cell death being between 15% and 90% of the total number of cells (score 6, according to the Terasaki method).

When positive sera were tested at normal cytotoxic conditions (9) of one hour incubation time at room temperature, none of them gave a positive reaction.

The presence of cold lymphocytotoxins

TABLE 1. *Cold lymphocytotoxins in leprosy.*

Leprosy type	Positive sera	Negative sera	Percentage
Control (30)[a]	4	26	13
LL (34)	18	16	52
BL (10)	6	4	60
BB (4)	2	2	50
BT (8)	0	8	—
TT (3)	0	3	—
Total (59)	26	33	46

[a] Number of sera in parentheses.
Leprosy patients *vs* controls: $p < .01$ (X^2 & Z).

TABLE 2. *Incidence of autoantibodies and alloantibodies in leprosy.*

Leprosy clinical form	ANA	MTA	SMA
Sera with alloantibodies:			
LL (18)[a]	5	1	4
BL (6)	2	1	0
BB (2)	1	0	2
BT (0)	—	—	—
TT (0)	—	—	—
Control (4)	0	0	0
Total (30)	8	2	6
Sera without alloantibodies:			
LL (14)	2	1	5
BL (4)	1	0	0
BB (2)	0	1	0
BT (8)	0	0	0
TT (3)	0	0	0
Control (26)	0	0	0
Total (57)	3	2	5

[a] Number of sera in parentheses.

TABLE 3. *Immunoglobulins and C3 levels in leprosy.*

Leprosy clinical form	Normal values			
	IgG	IgA	IgM	C3
	700-1700	100-350	50-150	125-225
LL (32)[a]	1378±328	517±280	194±169	148±32
BL (10)	1678±241	512±149	187± 88	155±14
BB (4)	1152±276	383± 79	145± 45	137±27
BT (8)	1430±397	480±229	153± 74	147±29
TT (3)	1416±378	490± 88	325±217	161±53

[a]Number of sera in parentheses.
Values in mg% (±) Standard Deviation.

was higher in lepromatous leprosy cases and in borderline cases. Tuberculoid cases did not present such antibodies. Table 2 shows a comparison between the presence or absence of cold lymphocytotoxins. MTA and SMA were found in a similar percentage in both groups. ANA were slightly more frequent among the sera with cold alloantibodies.

Levels of immunoglobulins and C3 in the sera were calculated in each leprosy clinical form. The high standard deviation for each mean of values did not allow us to arrive at any correlation between alloantibodies and immunoglobulin levels. C3 level was normal in all groups (Table 3).

DISCUSSION

The present study shows that in certain clinical forms of leprosy it is possible to find cold lymphocytotoxins in a significantly higher frequency than in normal people.

Such cytotoxins are complement dependent, the complement used being from rabbits. Several reports have been published on the cytotoxic effect of rabbit serum ([3, 5]). It may be argued that positive reactions are due to the xenoantibodies present in the rabbit serum, but we believe that the effect is dependent on the antibodies present in patients since sera from normal persons are not cytotoxic under the same conditions, and rabbit complement tested without human serum did not produce any cytotoxicity. Furthermore, in a previous study we showed that cold lymphocytotoxins were not influenced by the absorption of rabbit complement ([8]).

On the other hand, we do not know what is the specificity of these lymphocytotoxins. We have not been able to find any correlation with the HL-A and ABO groups of the target cells. Similar lack of specificity has been reported in earlier publications ([8, 13]), ignoring which lymphocyte membrane antigens are responsible for this reaction. The possibility that such cytotoxicity is a passive phenomenon due to the reaction of human immunoglobulins with a xenoantigen at the membrane level cannot be ruled out. Nevertheless, if this is the case such antibodies must only appear in certain situations or during the evolution of certain diseases.

The significance of cold lymphocytotoxins remains obscure. They appear to occur whenever immunization takes place ([7]). Their frequent high occurrence in autoimmune disorders and chronic infections ([8, 13]) also supports this generalization. The fact that auto- and alloantibodies are frequently found in lepromatous leprosy correlates with our findings.

Inhibitory factors have been detected when normal lymphocytes are cultured in the presence of leprosy serum ([2]). Whether or not such factors correspond to the presence of cold lymphocytotoxins will be the subject of further investigation. If this turns out to be the case, the mechanism of action of lymphocytotoxins should be by competition with the antigen or mitogen at the membrane cell level rather than by direct cell damage since cold lymphocytotoxins require complement and certain conditions which are not present during a lymphocyte culture. This kind of mechanism has been suggested similarly by Thorsby ([14]) to explain the MLC inhibition by anti-HL-A sera.

SUMMARY

Occurrence of cold lymphocytotoxins has been observed in 59 leprosy sera. In 46% of the patients, cold lymphocytotoxins were present whereas in healthy controls only 13%

showed such antibodies. The highest incidence of alloantibodies was detected in lepromatous leprosy. Levels of autoantibodies, immunoglobulins and C3 were tested in parallel without finding any significant correlations.

RESUMEN

Se estudió la presencia de crío-linfocitoxinas en 59 sueros de pacientes con lepra. Las crío-linfocitoxinas se observaron en el 46% de los sueros de pacientes, mientras que solamente 13% de los controles sanos mostraron estos anticuerpos. La más alta incidencia de aloanticuerpos se detectó en la lepra lepromatosa. Se estudiaron en forma paralela los niveles de autoanticuerpos, inmunoglobulinas y C3, sin encontrar relaciones significativas.

RÉSUMÉ

On a relevé l'apparition de lymphocytotoxines à froid dans le sérum de 59 malades de la lèpre. Chez 46% de ces malades, les lymphocytotoxines à froid étaient présentes, alors que seulement 13% de témoins sains présentaient de tels anticorps. L'incidence la plus élevée de ces alloanticorps a été détectée dans la lèpre lépromateuse. On a déterminé simultanément les niveaux d'autoanticorps, d'immunoglobulines et de C3, sans relever aucune corrélation significative.

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