Immunologic Aspects of Leprosy as Related to Leucocytic Isoantibodies and Platelet Aggregating Factors¹

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Leprosy is an infectious disease associated with a multitude of immunologic aberrations. Both the cell-mediated and antibody immune systems are involved. There exists an extraordinary dichotomy between the antibody forming and delayed hypersensitivity mechanisms in patients with lepromatous leprosy; the former exhibiting a generalized hyperactive response in sharp contrast to the hyporeactive state of the latter system. Furthermore, many of the antibody-mediated immune aberrations, observed in the sera of variable numbers of lepromatous leprosy patients, resemble autoimmune disorders (²⁹). These features include biologically false-positive tests for syphilis, low as well as high complement levels, high levels of nonspecific and specific antibodies with depressed specific as well as nonspecific cellular immunity, antinuclear factors, L.E. cells, rheumatoid factors, antithyroglobulin autoantibodies, circulating immune complexes and cryoglobulins (4. 17, 21, 22, 23, 24, 28, 30). Recently, Dutta and Saha (11) demonstrated the presence of I-specific cold isoantibody directed against erythrocytes in the sera of leprosy patients. The purpose of the present study is to present evidence of isoantibodies against leucocytes and antiplatelet factors in the sera of leprosy patients.

MATERIALS AND METHODS

The study group consisted of 112 unselected patients with leprosy, hospitalized either in the Leprosy Home, Shahdara, Delhi or the Army Leprosarium, Agra. Their diagnosis was based on characteristic clinical features, testing with Dharmendra lepromin and skin biopsy. Cases with borderline tuberculoid leprosy were included under tuberculoid leprosy while patients with borderline lepromatous leprosy were grouped with lepromatous leprosy. Random blood samples were collected from each patient. Sera were rapidly separated and part of these samples were frozen at -20° C.

Leucoisoagglutinins (LIA). Leucoisoagglutinins were demonstrated according to the principle described by Dausset, Colombani and Okachi (8). In brief, fresh leucocytes from a panel of six ABO blood group compatible donors were pooled after defibrinating the blood. The pooled leucocytes (4,000 per cu mm) were mixed with equal volumes of heat inactivated (56°C for half an hour) test sera. Then the leucocytes were incubated in nonsiliconed test tubes at 37°C for two hours. One drop of 1:100 dilution of glacial acetic acid was then added to dissolve the traces of RBC. The tubes were gently shaken and the agglutination of leucocytes was observed under a microscope.

Negative and positive controls were included with each test. A test was regarded as positive when out of 100 fields definite clumps of leucocytes were observed in 75 or more fields. The positive sera were further titrated for LIA titer using the serial dilution technic.

Leucoisocytotoxins (LIC). Leucoisocytotoxins were detected according to the method described by the Indian Council of Medical Research (13) with slight modifications. The leucocytes were separated from the pooled defibrinated blood of a panel of ABO blood group matched donors after sedimentation with dextran. The lymphocytes separated by passing through a cotton column. The red cells in the elute were lysed with a solution containing nine volumes of 0.83% ammonium chloride and one volume of tris buffer (pH 7.6); the pH of the final solution was adjusted to 7.2. Then one volume of test serum, one volume of lymphocytes containing 2,000 cells per cu mm and two volumes of selected rabbits' pooled serum (as a source of complement) were mixed and the final solution was incubated for two hours at 37°C. With every test the following controls

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were always included: a) a known positive equine antihuman lymphocytic serum and a negative serum obtained from healthy individuals; b) test sera with lymphocytes only but no complement; and c) cells with complement only but no test sera. The percentage of dead cells was determined by the trypan blue exclusion method. The percentage of dead (stained) cells being less than 30 was considered to be negative for the lymphocytotoxic activity of the test sera. The dead cells in the control groups never exceeded 10%.

Sera from 12 multiparous women (number of pregnancies including abortions varying between 2 to 7) and two subjects who received multiple transfusions (number of transfusions varying between 5 to 15) were also regarded as the control group for LIA and LIC tests.

Platelet aggregating factor (PAF). PAF was detected according to the principal described by Dausset *et al* (⁸). The method of Dacie and Lewis (⁵) was followed.

Briefly, the platelets were obtained from the blood taken from a panel of six O group donors using plastic syringes. Nine volumes of blood were mixed with one volume of 2%EDTA (ethylene diamine tetra acetic acid) in saline. The platelets were washed and suspended as described by Dacie and Lewis (⁵). The platelet count was adjusted to 1×10^6 per cu mm.

The test sera were inactivated by heating to 56°C for 30 minutes. As an alternative to absorption of the sera with barium sulphate the agglutination test was carried in 2× 10-2 M EDTA (5). Two sets of platelet agglutination reaction with test sera were done: one in saline, and another in 2×10^{-2} M EDTA. The later medium obviated any risk of agglutination of platelets due to clotting factors (5). Two volumes of the platelet suspensions and one volume of test sera were incubated on a nonsiliconed Kline slide at 37°C for one hour. Intense platelet aggregation was noticed. The sera showing clumping of platelets in 80 of 100 microscopic fields were considered to be positive for PAF. The sera showing positive platelet agglutination by the above screening test were further titrated using the serial dilution technic. A positive and a negative control were always included.

Platelet aggregating factor demonstrated by antihuman globulin consumption test. Due to the large number of platelets required to perform this test, it was done in only 21 selected sera from leprosy patients showing positive PAF. The principle described by Dausset et al (8), and the method described by Dacie and Lewis (5) were followed. In short, nine volumes of group O blood were collected with a plastic syringe and mixed with one volume of 2% EDTA solution kept in a siliconized tube. The platelet rich supernatant (PRS) was collected by centrifugation at 800-1000 rpm for 15 minutes. The final platelet button was then obtained by centrifuging the PRS at 3000 rpm for 15 minutes on five occasions after washing it with 1% EDTA in saline each time.

Two drops of the platelets (4×10^9) were then sensitized with two drops of the test serum by incubating the mixture at 37°C for two hours. After incubation, the sensitized platelets were washed six times with 1% EDTA in saline and were collected as a platelet button. To the washed platelet button sensitized with the test serum, three drops of wide spectrum antihuman globulin serum were added, whose titer was adjusted before to 1:32. The mixture was allowed to stand for 15 minutes and then centrifuged. The supernatant serum was titrated with anti-D-sensitized Rh positive human RBC. The reduction of the titers of the antihuman globulin antisera was then noted. A control with unsensitized RBC was always included during the test. The sera obtained from ten patients suffering from different hematologic diseases (Table 3) formed in the control group.

Cryoglobulins (CG). Fresh sera were sucked into 7 cm long capillary tubes of 0.5 mm diameter. The tubes were kept vertical for 48 hours at 4°C. The precipitates of cryoglobulins deposited at the bottom were measured in millimeters and graded accordingly as follows:

- -height of precipitate below 0.5 mm
- +height of precipitate between 0.5-1.0 mm
- ++ height of precipitate between 1.0-2.0 mm
- +++ height of precipitate between 2.0-3.9 mm and more

Antinucleoprotein factor (ANF). Latexnucleoprotein reagent (Hyland Laboratories, USA) was employed to detect the ANF using the slide latex agglutination technic. In each instance positive and negative controls were included with test sera. The positive sera, detected by this screening test, were then serially diluted and titrated with latexnucleoprotein reagent to determine the ANF titer.

Thyroglobulin autoprecipitin (TA). Commercial thyroglobulin coated latex particles (Hyland Laboratories, USA) were mixed on glass slides with patients' sera, which were previously incubated at 56°C for 30 minutes. Both diluted ($20 \times$ in glycine-saline buffer) and undiluted sera were screened to avoid prozone phenomenon. Positive and negative sera were included as controls with every test. The sera showing positive TA test were further diluted serially and titrated with TA reagent to determine their titer.

Sera from 30 normal adults and 2 proven cases of thyrotoxicosis were included in the control group and tested for CG, ANF and TA.

RESULTS

Leucoisoagglutinins (LIA) and leucoisocytotoxins were tested in the sera of 61 unselected leprosy patients and 14 controls. The latter group consisted of 12 normal multiparous women and 2 patients who had received multiple blood transfusions. It is evident from Table 1 that LIC were positive in a higher number of leprosy patients as well as controls than LIA, and all the sera except one positive for LIA also exhibited positive reaction for LIC. Also, LIC were detected in a larger percentage of sera of lepromatous patients (44%) than in the less virulent form of the disease (28%). The agglutination of normal leucocytes by the sera of leprosy patients was very spectacular and average number of cells in each clump was found to be 25. Table 2 shows the percentage of dead (stained) cells observed during the LIC tests. The titrations of the positive sera for LIC tests showed that the titers of leucocytotoxicity varied from 1:2 to 1:20. The total and differential leucocytic counts were carried out on the blood samples of the leprosy patients who were tested for LIA and LIC. The number of the total leucocytic, neutrophilic and lymphocytic counts were 8.52 (±0.09), 5.11 (±0.69), and 2.75 (±0.23) million per milliliter of blood respectively.

Platelet aggregating factor (PAF) was studied in the sera of 81 unselected patients and 10 controls (Table 3). The latter group included patients having idiopathic thrombocytopenic purpura (ITP), secondary purpura, leucopenia, aplastic anemia and thalassemia. PAF was tested in saline as well as in 2×10^{-2} M EDTA. Its incidences were 51.2% and 45% in the lepromatous and tuberculoid leprosy patients respectively, in saline as well as in EDTA. The titer of PAF tested in saline varied between 1:4 to 1:32. Although the incidences of PAF in both saline and EDTA were the same, there was a twofold fall in the titer of PAF when tested in EDTA, as compared with the test in saline.

Coomb's antihuman globulin consumption test was done in 21 selected sera of leprosy patients which were positive for PAF. Platelet aggregating factor could be demonstrated

	Leu	coisoaggluti	nins	Leucoisocytotoxins				
Type of subjects	No. sera tested	No. positive	% positive	No. sera tested	No. positive	% positive 44 28		
Leprosy: lepromatous tuberculoid	25 36	2 ^a 3 ^a	8 8.3	25 36	11 ^b 10 ^b			
Control: multiparous women multiple blood	12	0	0	12	2°	16.6		
transfused subjects	2	0	0	2	1 ^d	50		

TABLE 1. Incidence of leucoisoagglutinins and leucoisocytotoxins in sera of leprosy patients.

All the sera except one, exhibiting positive leucoisoagglutinins, were also positive for leucoisocytotoxins.

^aThe ABO blood group distribution of the positive sera was: A = 1, B = 1, AB = 1, O = 2.

^bBlood group distribution of the positive sera was: A = 5, B = 5, AB = 1, O = 10.

^cSix and seven pregnancies in one patient each. ^dReceived 15 blood transfusions.

The titer of leucoisoagglutinins varied from 1:2 to 1:4. The titer of leucoisocytotoxin varied from 1:2 to 1:20.

		Percentage of dead cells ^a						
Group ·	tested	0-25	26-50	51-75	76-100			
Leprosy: lepromatous tuberculoid	25 36	14 (56) 23 (64)	5 (20) 7 (19.4)	3 (12) 5 (13.9)	3 (12) 1 (2.7)			
Control: multiparous women multiple blood	12	10 (83)	2 (17)	0	0			
transfused subject	2	1 (50)	0	1 (50)	0			

TABLE 2. Leucoisocytotoxicity test results in sera of leprosy patients and controls.

^aFigures in parentheses show percentages of cases in the respective groups. Sera showing more than 30% dead cells were considered to be positive for leucoisocytotoxins.

One equine antihuman lymphocytic serum was titrated for its leucocytotoxin which was also included in the positive control for leucoisocytotoxicity test: 1:80 dilution of this serum could kill 30% cells. The percentage of dead cells in the negative controls never exceeded 10%.

TABLE 3. Incidence of platelet aggregating factor in sera of leprosy patients and controls.

Groups		Platelet aggregat	Antiplatelet factor by antihuman globulin consumption test		
	No. sera tested	No. positive sera (in saline)	No. positive sera (in 2 × 10 ² M EDTA)	No. sera tested	No. sera positive
Leprosy: lepromatous tuberculoid	41 40	21 (51.2) ^d 18 (45.0)	21 (51.2) 18 (45.0)	9 ^a 12 ^a	6 (66.6) 6 (50.0)
Controls ^b	10	1	not done	10	·Ic

^aDetection of antiplatelet factor using antihuman globulin consumption test was carried out in the sera of 21 selected leprosy patients who had positive platelet aggregating factor in their sera.

^bThe controls consisted of: idiopathic thrombocytopenic purpura, 1 case; secondary purpura, 5 cases; leucopenia, 2 cases; aplastic anemia, 1 case; thalassemia, 1 case.

^cCase of idiopathic thrombocytopenic purpura.

^dFigures in parentheses show percentages of positive cases.

by antihuman globulin consumption test in only 12 of these 21 selected sera. The isoantibodies were positive in 66.6% and 50% of the sera of the lepromatous and tuberculoid patients respectively (Table 3). Table 4 shows the detailed results of the antihuman globulin consumption test. The sera of the lepromatous leprosy patients exhibited higher antihuman globulin consumption in comparison to those of the tuberculoid patients. In the control group, the serum from one case of ITP also showed grade IV consumption of antihuman globulin.

Total platelet count was also undertaken on 10 tuberculoid and 20 lepromatous patients who had exhibited positive PAF in their sera. Their counts were $214,000 \pm 26,000$ and $199,000 \pm 76,000$ per cu mm of blood in the two groups respectively.

Table 5 shows the incidence of cryoglobulins (CG), antinucleoprotein factor (ANF) and thyroglobulin autoprecipitin (TA) in the sera of leprosy patients, as well as the age and sex of matched adult subjects. The CG were present in 12.7% of sera of normal adults but grade III CG was positive only in one case. Of the leprosy patients, the CG was present in 58.9% of lepromatous and 9.5% of the tuberculoid cases. In the latter group only grade I CG were observed in 11 of the patients in contrast to the lepromatous group where grades I, II and III CG were found in 35.5%, 5.2%, and 18.2% of the sera respectively. In the control group, ANF and TA were not detectable in the sera of any of the 30 normal subjects, although TA was demonstrated in serum of two female patients of thyrotoxicosis, aged 42 and 50 years.

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					i i i	No. of sera according to the grading of antihuman globulin consumed ^a							
Group			No. sera tested			++++	++	+++	++	+	-	±	
Leprosy: lepromatous tuberculoid Controls ^b			9 12 10			1 1 1		1 00	2 0 0	2 5 0	1 3 0	2 3 9	
^a Grades: — No consumption of antihum ± Antihuman globulin was cor + " " " " +++ " " " "				man glo onsume "	obuli d up " "	n. to "	1:16 1:8 1:4 1:2 the tubes	<pre> Taken as negative Taken as positive</pre>					

TABLE 4. Antiplatelet factor by antihuman globulin consumption test in the sera of leprosy patients and controls.

The titer of antihuman globulin antiserum was adjusted beforehand to 1:32.

 TABLE 5. Incidence of cryoglobulin, antinucleoprotein factor and thyroglobulin autoprecipitin in the sera of controls and leprosy patients.

Groups	Tests									
	Cryoglobulins			Antinu	cleoprotei	n factor	Thryoglobulin autoprecipitin			
	No. sera	Cases positive	% positive	No. sera	Cases positive	% positive	No. sera	Cases positive	% positive	
Leprosy: lepromatous tuberculoid	39 21	23 ^a 2 ^a	58.9 9.5	41 40	10 ^b 4 ^b	24.4 10	25 33	6° 3°	24 9.1	
Control: normal adults thyrotoxicosis	47	6ª	12.7	30	0	0	30	0	0	
patient	NT^d	NT	NT	NT	NT	NT	2	2	100	

^aOf the 23 lepromatous leprosy patients with positive cryoglobulins, grades 1, 11, and 111 cryoglobulins were present in 35.5%, 5.2%, and 18.2% respectively. In tuberculoid patients only grade 1 cryoglobulin was observed, while in normal controls grade 1 was found in 10.5%, and grade 111 in 2.2%.

^bImmediate agglutination occurred within two minutes. It occurred in three lepromatous and two tuberculoid cases. In the remaining cases agglutination took place within 30 minutes (late agglutination). The titer of ANF varied from 1:2 to 1:20.

^cThyroglobulin autoprecipitins were tested in undiluted as well as in 20× diluted serum. In lepromatous patients the test was positive in undiluted sera alone in three patients, in diluted serum in one patient, but positive in both in two patients. In tuberculoid leprosy the test was positive in diluted, undiluted and in both in one case each. The titer of TA varied from 1:2 to 1:40.

dNT = not tested.

ANF was present in 24.4% and 10% of lepromatous and tuberculoid patients respectively, while TA were detected in 24% and 9.1% of the respective groups.

The overlap of the various abnormal serum factors in one serum sample was rarely observed. Only one patient having tuberculoid leprosy exhibited positive tests for ANF, TA, LIA, LIC and platelet antibodies.

DISCUSSION

During the last decade several reports have appeared suggesting that leprosy, especially the lepromatous type, is associated with a multitude of autoimmune aberrations (²⁸). Analysis of results of the present study not only supports this view but further suggests that the circulating autoantibodies in general do not seem to be directly deleteri-

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ous to the host.

Leucocytic isoagglutinins were detected in 8% of leprosy patients while leucocytic isocytotoxins occurred in 44% of these cases. Their incidence was much higher in lepromatous leprosy sera (Table 1). Antileucocyte isoantibodies are mostly IgG, active especially at 37°C, and are demonstrable by leucoagglutination, cytotoxicity, complement fixation or antiglobulin consumption technics. Of these, the cytotoxicity technics seem to be most sensitive (6) which explains the present finding of the higher incidences of positive leucocytoxicity than leucoagglutination. Thus, these observations suggest that another type of immune aberration is being observed in the sera of leprosy cases especially the lepromatous type. Leucocytic antibodies are also known to occur in multiparous women or patients who have received multiple transfusions (19), individuals suffering from atypical pneumonia, pancytopenia, leukemia, Hodgkin's disease, systemic lupus erythematosis, and paroxysmal nocturnal hemoglobinuria (10).

The titer of leucocytic antibodies in leprosy patients was low varying between 1:2 and 1:20. These were associated neither with leucopenia nor with lymphopenia, thereby suggesting that the leucocytic antibodies whenever present in leprosy patients were probably not pernicious to them. The incidence of leucoisocytotoxins was found to be more in blood group O among leprosy patients (Table 1). However, this apparently increased frequency of positive leucocytotoxicity in the sera of group O leprosy patients might be due to the more than expected incidence of O blood group among leprosy patients (12). More recently lymphocytotoxins were detected in 40 leprosy patients by Parez et al (20). The exact nature of these cytotoxic antibodies is not known. Whether these antibodies are HLA-specific or non-HLA specific and belong to Le system or MN and P system (16) is yet to be determined. It has recently been reported that plasma from some untreated lepromatous leprosy patients can inhibit the transformation of normal lymphocytes by phytohemagglutinin (15). The forementioned data further substantiates the findings of high occurrence of leucocytic cytotoxins in the lepromatous sera which might be acting as an enhancement antibody by specifically being adsorbed on to the lymphoid cells and thus rendering them unresponsive to the mitogenic stimulus.

The platelet aggregating factors were detected in saline as well as EDTA in 50% and 41% of lepromatous and tuberculoid patients respectively. This has been recently described by Wager et al (29). Antiplatelet isoantibodies can be detected by agglutination, complement fixation, and antiglobulin consumption technics. The thromboagglutination technic seems to show only antibodies active against the antigens restricted to platelets, while the results of complement fixation technic seem to parallel those of the antiglobulin consumption test (6). The platelet aggregating factor could be demonstrated by antihuman globulin consumption test in only 12 of 21 sera which were all positive for PAF. The titer of PAF was low and varied between 1:4 and 1:32 while the Coomb's antihuman globulin consumption test was comparatively intense in quantity as well as in frequency. The discrepancy observed between the two tests is either due to the difference in the sensitivity of the two tests, agglutination being positive only when strong agglutinins are present (26), or may be due to the nature of antiplatelet isoantibody resulting in incomplete blocking antibody of the 7S class while aggregating antibody of the 19S type (26). Further, according to Shulman (²⁶), agglutination, antiglobulin consumption, and complement fixation are the only serological tests available for determination of antibodies against platelet antigens, but no single test is reliable for measuring all antibodies. Antiglobulin consumption test can detect only PLA1, PLE1, and DUZO antigens on platelets, while agglutination can detect PLAI, PLA2, and Koa antigens on platelets. Platelet isoantibodies have already been reported in 1% to 2% of women after three or more pregnancies (9), or in sera of patients who have received multiple transfusions (²⁶), or in patients suffering from thrombocytopenic purpura (7). The platelet counts in the present series of leprosy patients have been found to be within normal limits. Similarly normal platelet counts in leprosy patients have also been noted earlier (18). Therefore, it is suggested that the platelet aggregating factor observed in variable numbers of leprosy patients are probably harmless as far as the count of the thrombocytes is concerned. Circulating immune complexes have been

demonstrated to cause the aggregation of thrombocytes in dengue fever (¹). Whether the similar complexes already demonstrated in the sera of the leprosy patients (²⁸) play a similar role, is difficult to comment on because unrelated IgG immune complexes, if adsorbed onto platelets, could as well be responsible for increased adhesiveness as well as a positive antiglobulin test. The high incidence of cryoglobulins (59%) in the sera from lepromatous leprosy patients of the present study further suggests the presence of such immune complexes in these test sera.

Table 5 shows the incidence of cryoglobulins (CG), antinuclear factor (ANF), and thyroglobulin autoprecipitin (TA) in the sera of leprosy patients. It has been well documented that cryoglobulins are immune IgG-IgM complexes (4). Cryoglobulins were detected in 58.9% of lepromatous leprosy and 9.5% of tuberculoid cases. Mathews and Trautman (14) reported positive results in 82% lepromatous leprosy while Ulrich et al (²⁷) found cryoglobulins with an almost equal frequency in the sera of lepromatous and tuberculoid cases. The ANF were positive in 24.4% and 10% of cases of lepromatous and tuberculoid leprosy respectively. Bonomo et al (2) studied 55 unselected lepromatous patients for ANF and reported an incidence of 18.2% while Petachclai et al (21) did not find ANF in even a single serum. Bonomo et al (3) also studied the incidence of thyroglobulin antibody in leprosy cases and found it to be positive in 48% of lepromatous sera and 11% of tuberculoid sera. Mathews and Trautman (14) found TA in 38% of lepromatous patients. They also studied protein bound iodine (PBI) in those cases where TA were demonstrated and found PBI within normal limits in all of them. Petachclai et al ⁽²¹⁾ could find antithyroglobulin antibody only in 6.9% of lepromatous cases. In the present study, TA were detected in 24% and 9.1% of lepromatous and tuberculoid leprosy cases respectively. Sehgal and Basu (25) have studied thyroid functions based upon radioiodine uptake studies in 17 well-advanced lepromatous leprosy patients. They found that, with two exceptions, the values were within normal range. Though the thyroid functions were not done in the present study, the two studies taken together suggest that TA present in the sera of a few leprosy patients appear to be functionally harmless

and support the findings of Mathews and Trautman (14).

Simultaneous occurrence of auto- and isoantibodies in the serum of one patient is a rare finding. Only two lepromatous and one tuberculoid patient had both ANF and TA in their sera. Of these, only in one patient, each of tuberculoid and lepromatous leprosy, could LIA be detected. Interestingly all the factors (TA, ANF, LIA and PAF) could be simultaneously demonstrated in the serum of an isolated tuberculoid patient. Thus the present study shows that the frequencies of these already well-documented autoantibodies and circulating immune complexes (i.e., cryoglobulins) are comparable to those reported earlier by other authors.

Finally, the immune complexes present in the sera of leprosy patients as shown by the high incidence of cryoglobulins in the present study as well as in other studies (4), may increase the adhesiveness of the leucocytes and platelets which may explain the observed leucocyte and platelet aggregation by the sera of leprosy patients. The remarkable antihuman globulin consumption by the platelets might also be due to the adsorption of these immune complexes onto the thrombocytes. But the leucocytotoxicity cannot be mediated by these antigen-antibody complexes. Thus, while the present data decisively demonstrate the presence of antileucocytic antibodies in the sera of these leprosy patients, they cannot conclusively prove that the platelet aggregation factor present in these sera is antibody in nature.

SUMMARY

The incidences of various iso- and autoantibodies in a random population of 112 unselected leprosy patients is presented. Low titers of leucocytic isoantibodies and platelet aggregating factor were detected in the sera of a variable number of such patients. The leucoisoagglutinins were found in 8% of the sera of tuberculoid as well as lepromatous leprosy patients, whereas the leucoisocytotoxins were detected in a larger percentage of the lepromatous (40%) as well as tuberculoid (28%) cases. The platelet aggregating factors (PAF) were positive in 51.2% and 45% of lepromatous and tuberculoid cases respectively. Of the 21 positive sera for PAF, the antiplatelet factor by antihuman globulin consumption test could be demon-

strated only in 66.6% and 50% of lepromatous and tuberculoid sera respectively. To study the frequencies of these newly detected antibodies or antibody-like factor and to compare their occurrences with other welldocumented autoantibodies present in the sera of leprosy patients: cryoglobulins, antinucleoprotein antibody and thyroglobulin autoprecipitin were also studied in the sera of the same population of leprosy patients. It has been observed that the simultaneous occurrence of all these auto- and isoantibodies in the serum of one patient is a rare phenomenon. Leucocytic and platelet counts of these patients having antibodies against leucocytes and platelets were found to be within normal limits. Accordingly, it is suggested that the low levels of antileucocyte antibody and antiplatelet factor are probably harmless to the hosts. On the other hand, it is postulated that these antibodies may act as enhancing factors by being specifically adsorbed on the lymphoid cells, thus rendering them unresponsive to mitogenic stimulus in vitro. From these studies it seems that leprosy, especially the lepromatous type, is associated with some of the serological features suggestive of an autoimmune aberration.

RESUMEN

Se presenta la incidencia de los diversos iso y auto-anticuerpos en una población no seleccionada, elegida al azar, de 112 pacientes con lepra. Se detectaron títulos bajos de iso-anticuerpos leucocitarios y factor agregador de plaquetas en el suero de un número variable de estos pacientes. Se encontraron leucoisoaglutininas en el 8% de los sueros de pacientes, tanto tuberculoides como lepromatosos, mientras que se encontraron leucoisocitoxinas en un porcentaje mauor de los lepromatosos (40%) que de los tuberculoides (28%). Los factores agregadores de plaquetas (FAP) fueron positivos en 51.2% y 45% de los casos lepromatosos y tuberculoides, respectivamente. De los 21 sueros positivos para FAP, se pudo demostrar el factor antiplaquetas, por medio de la prueba de consumo de globulina antihumana, solo en 66.6% y 50% de los sueros lepromatosos y tuberculoides, respectivamente. Para estudiar la frecuencia de estos anticuerpos o factores similares a anticuerpos recientemente detectados y para comparar su frecuencia con la de otros autoanticuerpos bien documentados presentes en el suero de pacientes con lepra, tales como las crioglobulinas, anticuerpos antinucleoproteínas y autoprecipitinas tiroglobulínicas, estos últimos se estudiaron también en el suero de la misma población de pacientes con lepra. Se ha observado que la

presencia sumultánea de todos estos auto o isoanticuerpos en el suero de un solo paciente es un fenómeno poco corriente. Se encontró que los recuentos de leucocitos y plaquetas de estos pacientes que tenían anticuerpos contra leucocitos y plaquetas, estaban dentro de límites normales. Por lo tanto, se sugiere que niveles bajos de anticuerpos antileucocitos y factor antiplaquetas son probablemente inocuos para el huésped. Por otra parte, se postula que estos anticuerpos puden actuar como un factor estimulante al ser absorbidos específicamente por las células linfoides, volviéndolas así no-responsivas a los estímulos mitogénicos in vitro. De estos estudios parece desprenderse que la lepra, especialmente de tipo lepromatoso, está asociada con alguna de las características serológicas que sugieren una aberración auto-inmune.

RÉSUMÉ

On présente ici les chiffres se rapportant à la fréquence d'iso-anticorps et d'auto-anticorps dans un groupe de 112 malades de la lèpre non selectionnés choisis au hasard. Des titres peu élevés d'isoanticorps leucocytaires et du facteur d'agrégation des plaquettes ont été observés dans le sérum d'un nombre variable de ces malades. Des leucoïisoagglutinines ont été relevées chez 8 pour cent des malades tuberculoïdes, et dans une même proportion des malades lépromateux, alors que des leucoïsocytotoxines ont été détectées dans un plus grand pourcentage des malades lépromateux (40%) que tuberculoïdes (28%). Les facteurs d'agrégation des plaquettes (PAF) se sont révélés positifs chez respectivement 51,2 pour cent et 45 pour cent des malades lépromateux et tuberculoïdes. Parmi les 21 sérums positifs pour le facteur d'agglutination des plaquettes, le facteur antiplaquette par épreuve d'épuisement de la globuline anti-humaine n'a pu être démontré que dans 66,6 pour cent des serums lépromateux et dans 50 pour cent des serums tuberculoïdes. Dans le but d'étudier la fréquence de ces anticorps, ou de ce facteur semblable aux anticorps, récemment détectés, et de comparer leur apparition avec d'autres anticorps présents dans le sérum des malades de la lèpre et sur lesquels on possède des données abondantes, on a également procédé à l'étude des cryoglobulines, de l'anticorps antinucleoprotéine et de l'autoprécipitine pour la thyroglobuline, dans le serum de ces mêmes malades. On a observé que l'apparition simultanée de tous ces isoanticorps et autoanticorps dans le sérum du même patient était un phénomène rare. La numération des leucocytes et des plaquettes se situait dans des limites normales chez les malades qui avaient des anticorps contre les leucocytes et les plaquettes. En conséquence, on suggère que des taux faibles pour l'anticorps antileucocytaire et pour le facteur antiplaquettes sont vraisemblablement sans effets nocifs sur le malade. On postule que ces anticorps peuvent agir comme facteurs stimulants par suite d'une adsorption spécifique sur les cellules lymphocytaires, ce qui empêche ainsi la réponse de celles ci à la stimulation mitogénique *in vitro*. Ces études font penser que la lèpre, et particulièrement la lèpre lépromateuse, pourrait être associée avec certaines des caractéristiques sérologiques suggestives d'un trouble autoimmun.

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