Immunologic Aspects of Leprosy With Special Reference to the Circulating Antispermatozoal Antibodies[1]

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A striking feature of lepromatous leprosy is its association with some degree of immunologic deficit and formation of a variety of autoantibodies ([19]). Various aspects of thymus-derived lymphocytic functions ([10]), including skin hypersensitivity reactions ([27]), are frequently depressed or even absent. There are often high levels of specific antibodies of IgG and IgM classes against *M. leprae* ([1]). Further, the sera of these patients have high levels of various nonorgan and organ-specific autoantibodies ([5, 20, 26]).

High incidences of antispermatozoal antibodies have been demonstrated in infertile males, in individuals after vasoligation, and even after relief of their vasoobstruction by vasovasostomy ([12]). Spermagglutinins have been detected in some patients with inflammatory diseases and other conditions of the genital tract ([4, 25]). *M. leprae* has been demonstrated in the testis of the patients with lepromatous leprosy ([17]). There is usually microscopic evidence of testicular inflammation resulting in frequent atrophy and gynecomastia ([11]). Recently, it has been demonstrated by Wall and Wright that lepromatous leprosy is associated not only with testicular atrophy, infertility and hypogonadism, but also with autoantibodies which react with testicular germinal cell and spermatozoa ([35]). This study was undertaken primarily to study the possible presence of antispermatozoal antibodies in the sera of patients with leprosy. Three different standard serological technics have been employed to confirm the presence of such antibodies in the test sera. An antihuman globulin consumption test has also been utilized to detect any specifically adsorbed antibody on the surface of normal spermatozoa following incubation with the sera of the leprosy patients.

MATERIALS AND METHODS

Study subjects. The following groups of subjects have been included in the present study.

Thirty-five randomly selected proven cases of leprosy patients, including five females, with ages ranging from 21 to 58 years and living in several leprosy institutions formed the basis of the present study. Clinical examination, lepromin (Dharmendra) tests and skin biopsies were performed in all cases to confirm the diagnosis of leprosy. The patients were grouped on a histopathologic basis following the criteria outlined by Ridley and Jopling ([23]). Borderline (BL and BT) cases were grouped with the respective polar forms; two borderline (BB) patients and one indeterminate case were analyzed separately.

As controls, 50 normal fertile men, with ages ranging from 26 to 45 years, who volunteered for vasectomy in the family planning clinic of Irwin Hospital, Delhi, were used as controls. Samples of blood from these subjects were collected prior to vasectomy.

Collection of sera. The ABO blood groupings of the above individuals were determined using standard technics. Sera were separated from the samples of blood and stored at −20°C.

Collection of semen. Semen samples were collected from normal volunteers for making antigens. Masturbation specimens were collected following five days of sexual abstinence.

Immunologic methods. The following immunologic studies were performed to study antispermatozoal antibodies in the sera of the test and control subjects. Sera from patients with one ABO blood group were tested against spermatozoa collected mostly from donors with the same ABO blood group, or utilizing O blood group when proper matching was not possible.

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Macroscopic spermagglutination method, in gel medium as described by Kibrick *et al* ([18]), and further modified by Rumke ([24]) was employed. Donor semen was diluted with saline to make a sperm density of 40 million/ml which was then mixed with an equal volume of 10% gelatin. The highest dilution of inactivated test serum yielding clear agglutination after incubation and before blending with an equal volume of the gelatin mixture, is registered as the antisperm agglutination titer.

In order to determine the agglutination patterns, one drop of test serum was mixed with one drop of a fresh sperm suspension (40 million spermatozoa/ml), and the mixture was sealed under a cover slip with nail polish, incubated at 37°C for two hours and then examined microscopically.

Sperm immobilization test, as described by Isojima and Tzuzuku ([15]) was employed. Normal donors' semen was diluted to 40 million spermatozoa/ml, mixed with an equal volume of inactivated test serum and a half volume of fresh normal human AB serum as a source of complement. The mixture was then incubated at 37°C for 60 minutes. The proportions of motile spermatozoa were estimated and compared with a similar test using normal human serum.

Tanned red cell hemagglutination test of Boyden, later modified by Stavitsky as previously described ([12]) was used. Antigen was prepared by repeated freezing and thawing of spermatozoa.

Antihuman gamma globulin consumption test, was carried out employing the following technic. Thoroughly washed spermatozoa (40 million/ml) collected from normal fertile donors were mixed with the sera of leprosy patients and fertile individuals. The latter group was taken as control. After incubating the mixtures at 37°C for two hours, they were washed six times with saline. To detect any adherence of gamma globulin on the surface of these sperm samples, they were incubated with broad spectrum antihuman gamma globulin antiserum whose titer was adjusted beforehand to 1:32. After incubation, the supernatant serum was titrated with anti-D-sensitized Rh positive human O group RBC. If the titer of antihuman globulin antiserum was reduced to 1:8 and below, the test was taken as positive.

RESULTS

Table 1 shows the comparison of the incidences of antispermatozoal antibodies tested by four different technics. The antihuman globulin consumption test, presumably a very sensitive method, showed the highest percentage of test sera (82%) having some specific gamma globulin in them which was adsorbed on the normal donor's spermato-

TABLE 1. *Incidence of antispermatozoal antibodies in sera of leprosy patients and in control subjects. All types of leprosy in both sexes are represented.*

Method	Leprosy patients			Control sera		
	Sera tested	Sera positive	% positive	Sera tested	Sera positive	% positive
Macroscopic sperm agglutination test in gelatin[a]	34	14	41	50	1	2
Sperm immobilization test[b]	19	8	37	50	0	0
Tanned red cell hemagglutination test[c]	35	8	23	50	0	0
Antihuman globulin consumption test	23	19	82	6	1	16.6

[a] The titers of sperm agglutination in leprosy patients varied from less than 1:8 to 1:64. Only titer of 1:32 and above has been taken as positive.

[b] Sperm immobilization values (S.I.V.) were calculated by C/T.

C = % of motile spermatozoa in normal serum in the presence of complement, 60 minutes after mixing.

T = % of motile spermatozoa in test serum in the presence of complement, 60 minutes after mixing.

Value of C/T, 2 or more has been taken as significant positive test. In leprosy, C/T varied from 1.0 to 8.5.

[c] The titers of tanned red cell hemagglutination test, 1:4 and above, were taken as positive. Maximum titer was 1:32.

TABLE 2. *Incidence of antispermatozoal antibodies in the sera of male and female leprosy patients of all types.*

Methods	Male			Female		
	Sera tested	Sera positive	% positive	Sera tested	Sera positive	% positive
Macroscopic sperm agglutination test in gelatin	29	13	45	5	1[a]	20
Sperm immobilization test	16	7	44	3	1[a]	33
Tanned red cell hemagglutination test	30	6	20	5	2[a, b]	40
Antihuman globulin consumption test	18	17	94	5	2[a, c]	40

[a] One female tuberculoid leprosy case had all four tests positive.
[b] One patient with BB leprosy.
[c] Extreme polar lepromatous leprosy.

zoa. In the control group, only one of six normal sera consumed antihuman globulin. On the other hand, the incidence of antispermatozoal antibody in the sera of these patients was minimal (23%) when the tanned red cell hemagglutination technic was employed. The occurrence of specific antispermatozoal antibody in these sera by the technic of sperm agglutination in gelatin was 41% and that by the sperm immobilization technic 37%. Thus, the results of these two technics were comparable. In contrast, only 2% of the sera of normal fertile male subjects had only antispermatozoal antibody. Interestingly, the present technics showed that the sera of female leprosy patients had antibody against spermatozoa (Table 2). Sera from five female patients were tested for sperm antibody. Only one showed antispermatozoal antibody. The serum of the same patient also showed positive sperm immobilization, tanned red cell hemagglutination and antihuman globulin consumption. She had tuberculoid leprosy. The serum of another female patient with borderline leprosy (BB) showed positive tanned red cell hemagglutination test. In addition, the sample of serum taken from an extreme polar lepromatous female patient had a positive antihuman globulin consumption test although the other three technics failed to demonstrate antisperm antibody in her serum.

Table 3 depicts the prevalence of antispermatozoal antibodies in sera from lepromatous and tuberculoid forms of leprosy. These antibodies were detected in both forms of leprosy and the type of leprosy did not seem to have any influence on the prevalence of antispermatozoal antibodies in the sera of these patients. However, the frequency of positivity of the antihuman globulin consumption test was more (93%) in the lepromatous form than in the tuberculoid form (66%) of the disease (Table 6).

The results obtained by the various tests were then correlated. Comparison of the data obtained by the sperm agglutination tests with those obtained by the sperm immobilization tests in 18 sera, showed that 22% of the sera proved positive by both tests, while in 33% of the sera both tests were negative. Thus, perfect correlation was obtained in 55% of the sera (Table 3). A similar comparison of the results of the sperm agglutination tests with those of the tanned red cell hemagglutination tests in 19 sera, demonstrated 16% and 52% of the sera proved positive and negative, respectively, by these two tests. Thus, good correlation between these two technics was seen in 68% of the sera. When the results of the sperm immobilization tests and the tanned red cell hemagglutination tests were compared, 2 (14%) of 14 sera showed both tests positive, while 7 (50%) of the same number of sera were negative for sperm antibody by both tests. Thus, a perfect relationship between these two methods was obtained in 64% of the sera. Moreover, when the results of all three tests in 14 sera were compared, perfect correlation between all these three tests was seen in 57% of the sera (Table 4). Thus, after analysis of the present data, it was concluded that correlation between the results of the above three tests for

TABLE 3. *Antispermatozoal antibodies in polar types of leprosy.*

Method	Lepromatous			Tuberculoid		
	Sera tested	Sera positive	% positive	Sera tested	Sera positive	% positive
Sperm agglutination test in gelatin[a]	19	7	37	14	6	42
Sperm immobilization test	13	6	46	6	2	33
Tanned red cell hemagglutination test[b]	22	5	23	12	2	16
Antihuman globulin consumption test[c]	15	14	93	6	4	66

[a] One patient with indeterminate leprosy showed positive sperm agglutination test. Result not included.
[b] One BB leprosy patient showed positive tanned red cell hemagglutination test. Not included in table.
[c] Sera of two BB cases tested, one male, one female. Male showed positive antihuman globulin test. Results not included.

TABLE 4. *Correlation between various tests performed to demonstrate antispermatozoal antibody in the sera of leprosy patients.*

Correlation studied between type of tests done	Sera tested	All tests positive		All tests negative		Perfect correlation between tests		No correlation between any test	
		No.	%	No.	%	No.	%	No.	%
Spermagglutination test in gelatin and sperm immobilization test.	18	4	22	6	33	10	55	8	45
Spermagglutination test in gelatin and tanned red cell hemagglutination test.	19	3	16	10	52	13	68	6	32
Sperm immobilization test and tanned red cell hemagglutination test.	14	2	14	7	50	9	64	5	36
All three tests.	14	2	14	6	43	8	57	6	43

TABLE 5. *Morphology of normal spermatozoa agglutinated by the sera of leprosy patients.*

Type of sperm-agglutination	No. of sera	Percent
Tail-to-tail	4	15
Mixed	9	33
Head-to-head	14	52
Total sera tested	27[a]	100

[a] Of 27 sera, 6 sera were collected from tuberculoid patients. Of these, 4 showed head-to-head and 2 showed mixed type of agglutination.

Titers of spermagglutination in gelatin, 1:32 and above was taken as significant. The spermagglutination in the sera with significant titer showed predominantly head-to-head type of agglutination.

Bunches of spermagglutination contained 15 to 50 spermatozoa.

detecting antisperm antibody in the sera of leprosy patients, was found in about half of the test samples.

Table 5 shows the morphologic types of sperm agglutination. The incidence of head-to-head type of sperm agglutination was maximal (52%), while the tail-to-tail type of agglutination was minimal (15%).

Figure 1 illustrates the kinetics of immobilization of the normal donors' spermatozoa by the sera of leprosy patients. The rate of immobilization of the normal donors' spermatozoa by the sera obtained from the leprosy patients was comparable with that observed in the vasectomized subjects. On the contrary, the rate of immobilization was significantly lower when the test was performed with the sera of normal fertile subjects. These data therefore confirm the presence of

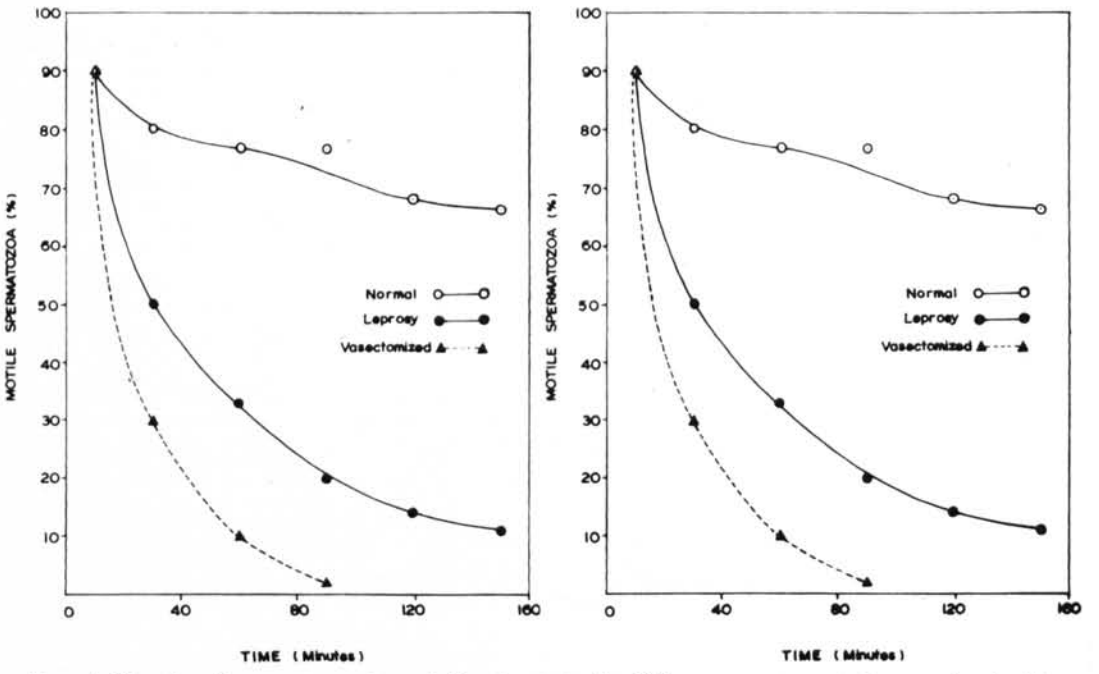

FIG. 1. Kinetics of spermatozoa immobilization test: 40 million spermatozoa/ml were mixed with an equal volume of inactivated test serum and half volume of fresh normal human serum as a source of complement. The mixture was incubated at 37°C for 2.5 hours. The percentages of motile spermatozoa were estimated at different time intervals, and plotted against the time of incubation.

Normal = Average of six tests.

Leprosy = Average of eight tests.

Vasectomy = Average of eight tests.—Data taken from Gupta *et al* ([13]).

TABLE 6. *Antispermatozoal antibody in the sera of leprosy patients by antihuman globulin consumption test.*

Serum group	No. sera tested	Number of sera according to the grade of antihuman globulin consumed[a]					
		4+	3+	2+	1+	±	—
Control	6	0	0	1	1	3	1
Leprosy:	23	10	5	4	1	2	1
Lepromatous	15	10	1	3	1	0	0
Tuberculoid	6	0	3	1	0	2	0
Borderline (BB)	2	0	1	0	0	0	1

[a] Grade of antihuman globulin consumed.

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

1) – : no consumption of antihuman globulin.
2) ± : antihuman globulin consumed up to 1:16 titer.
3) 1+ : antihuman globulin consumed up to 1:8 titer.
4) 2+ : antihuman globulin consumed up to 1:4 titer.
5) 3+ : antihuman globulin consumed up to 1:2 titer.
6) 4+ : antihuman globulin consumed in all tubes.

1)–3) = accepted as negative.
4)–6) = accepted as positive.

complement-dependent sperm immobilizing antibody in the sera of leprosy patients and vasectomized subjects.

Table 6 records that there was specific gamma globulin in the sera of 19 of 23 leprosy patients which was adsorbed to the surface of the normal donors' spermatozoa when the latter was incubated with the test sera. On the other hand, in the control group antihuman gamma globulin was consumed in only one serum when the same test was performed on the sera of six normal fertile subjects. Furthermore, the consumption of antihuman globulin was more intense in the sera of lepromatous patients than in those from tuberculoid patients.

DISCUSSION

Leprosy, especially the lepromatous variety, is associated with a multitude of autoimmune aberrations ([33]). There have been many reports of increased prevalence of autoantibodies in patients with lepromatous leprosy, but the findings have not been uniform. The frequencies of cryoglobulins, antinuclear factors, rheumatoid factors, false positive serological tests for syphilis, antithyroglobulin antibodies and cold autoantibodies are reported as elevated in patients with lepromatous leprosy in some countries but not in others ([5, 22, 26]). Also, cytotoxic antibodies against normal lymphocytes as well as platelet aggregating factors have been found in patients with lepromatous and, to a lesser extent, tuberculoid leprosy, ([21, 26, 34]).

The data in the present communication give strong evidence for the presence of another organ-specific antibody, i.e., antispermatozoal antibodies in the sera of the patients with both lepromatous and tuberculoid leprosy. The results of the three different technics, which have been employed for demonstrating antispermatozoal antibodies, have been correlated in about 57% of test sera (Table 4). Thus, all the three types of antispermatozoal antibodies were not present in every test serum which probably indicates the existence of several spermatozoal antigens, inducing different types of antibodies in different individuals. Parallel observations were made in spermatoxic, spermagglutinating and cytotoxic activities of autoantibodies in guinea pigs ([32]). Similar antispermatozoal antibodies have been recently detected in 141 vasectomized men in this laboratory ([12]). The incidence of positive spermagglutination, sperm immobilization and tanned red cell hemagglutination tests among these vasectomized subjects were 62%, 40% and 9%, respectively, and the occurrence of these various circulating sperm antibodies among these subjects increased with the increase in time of vaso-occlusion. Furthermore, the incidences of these three antibodies in 22 vasorecanalized but infertile male subjects were 86%, 50% and 36%, respectively, which were higher than the respective figures in the vasectomized subjects. The etiology of the formation of the circulating sperm-specific antibodies in leprosy patients seems to be different from that in vasectomized and infertile subjects. Inflammatory condition of testis and the genital tract, e.g., orchitis or epididymitis in these patients ([31]) might have caused obstruction with the formation of such specific autoantibodies. The synthesis of such antibodies might be further accentuated by the adjuvant-like action of *M. leprae* ([6, 30]). Alternatively, it seems probable that in autoimmune organ-specific disorders a subpopulation of T lymphocytes has an important suppressor effect on the tendency of B lymphocytes to form autoantibodies ([2]). In leprosy, especially the lepromatous form, these suppressing or controlling T cells might be lacking, which leads to the emergence of autoimmunity according to the precise nature of the T cell deficiency ([14]). This latter notion supports the recent observation of Saha *et al* ([28]) who noticed the disappearance of rheumatoid factor from the sera of four of five patients with lepromatous leprosy after they had received immunologic reconstitution therapy by intravenous transfusion of normal lymphocytes.

Recently, Wall and Wright ([35]) found testicular germinal cell antibodies, which cross-reacted with mycobacterial antigen (BCG), in the sera of both lepromatous and tuberculoid leprosy patients. This unique characteristic was claimed to be a distinct feature of testicular antibody in leprosy, differing from the testicular antibody occurring occasionally in endocrine patients in the Western countries. In leprosy, testicular autoantibody might be associated with the frequent occurrence of gynecomastia, testicular atrophy and orchitis ([16]). Antibodies

against steroid producing cells have been detected in autoimmune endocrine disorders, although autoimmune testicular failure would appear to be less common in the European countries (14). It is possible that the testicular antibodies in leprosy might cause increased secretion of interstitial-cell-stimulating hormone and estrogen which influence the development of gynecomastia (16).

Although antispermatozoal antibodies were present in both patients of leprosy and vasectomized subjects, the patterns of sperm agglutination were different in the two conditions. In the former situation, head-to-head type of agglutination was predominant (Table 5), while in the latter the tail-to-tail variety of agglutination was mostly seen (12). Recently, antibodies against the main tail piece were found to occur in normal men (13).

Rumke *et al* (25) demonstrated that the head-to-head agglutinin reacts with the acrosomal cap of spermatozoon, and in this respect, this type of spermagglutination evidently resembles the experimental autoantibody produced in guinea pigs by autosensitization with testis (9). Further immunofluorescent tests showed that the sera from affected guinea pigs stained the germinal cells and acrosomes of the spermatozoa contained in the normal guinea pigs testis. Thus, our study showing head-to-head type of sperm agglutination and that of Wall and Wright (35) showing testicular germinal cell antibodies in leprosy, probably suggests that the etiology of these antibodies in leprosy is similar to that in the experimental autoimmune orchitis in guinea pigs. This notion is further supported by the presence of sperm-immobilizing antibodies in our patients because sperm immobilizing antibodies are reported to develop when autologous testis is used for immunizing guinea pigs (25).

Contradictory views have been expressed about the role of the various antisperm antibodies: they could possibly cause infertility (8). In a previous communication, we have reported that circulating sperm agglutinating antibodies with high titer (1:32-1:128) in vasoanastomozied subjects cause antiagglutination of their spermatozoa, of the tail-to-tail variety, and reduced sperm motility. Autoagglutinated spermatozoa are incapable of penetrating the cervical mucus, which may be the likely mechanism of infertility in men (7). Ansbacher and his co-workers (3), on the other hand, suggested that immobilizing antibodies rather than agglutinating antibodies interfere with the fertility of the host. Nevertheless, Southam (29) reported that antispermatozoal antibody detected by the sensitized tanned red cell hemagglutination technic could not interfere with normal fertility. However, the role of the observed circulating sperm antibodies on fertility in leprosy patients is not exactly known and needs further investigation.

The presence of antispermatozoal antibodies in female leprosy patients is interesting and speculative. Our finding is in keeping with that of Wall and Wright (35), who also found autoantibodies reacting with testis in six of nine female patients with lepromatous leprosy. Either the formation of these autoantibodies occurred due to their exposure to spermatozoa through vaginal routes (14), or these antibodies were induced in these females against some other cross-reacting antigens.

Finally, the sera from the patients with tuberculoid leprosy contained antispermatozoal antibodies (Table 3). This observation is in accordance with that of Wall and Wright (35), who found testicular germinal cell antibodies as well as antispermatozoal antibodies in both forms of leprosy by an immunofluorescence technic. It is possible that there may be some cell-mediated tissue damage occurring in the noninfective form of leprosy. Antihuman gamma globulin consumption test (Table 6) further confirmed the presence of antispermatozoal antibodies in the sera of tuberculoid patients, although antispermatozoal antibodies were more prevalent in the sera from patients with lepromatous leprosy than in the alternate form of the illness.

SUMMARY

Macroscopic sperm agglutination in gelatin, sperm immobilization and tanned red cell hemagglutination tests could detect antispermatozoal antibodies respectively in 41%, 37% and 23% sera of 35 leprosy patients, including 5 female cases. Interestingly, all of the above tests were positive in one serum from a female patient with borderline leprosy. Sperm antibodies were detected in both lepromatous and tuberculoid forms of leprosy by the above three technics and no

significant difference was observed in their incidences among the two groups of patients. A three dimensional correlation was observed in 57% of 42 tests performed with 14 sera. Head-to-head type of agglutination was the predominant feature of spermagglutination observed in the sera of these patients. In the control group, only 1 of 50 normal fertile males showed a positive spermagglutination test. Not one in this group showed positive sperm immobilization and tanned red cell hemagglutination tests.

Antihuman globulin consumption test, presumably a very sensitive test, was also employed to demonstrate sperm-specific antibodies in the sera of these leprosy patients. These antibodies were adsorbed on the surface of the normal donors' spermatozoa when the latter were incubated with the patients sera. Antispermatozoal antibody could be demonstrated by this sensitive technic in the sera of two female patients. Moreover, antihuman globulin was consumed more intensely by the antispermatozoal antibodies present in the sera in the lepromatous than in the tuberculoid and borderline leprosy groups.

RESUMEN

Estudiando los sueros de 35 pacientes con lepra, cinco de los cuales eran mujeres, se encontró que las pruebas de aglutinación macroscópica del esperma en gelatina, de inmovilización del esperma y de hemaglutinación con eritrocitos tanizados, permitieron demostrar la presencia de anticuerpos contra espermatozoides en el 41%, 37% y 23% de los casos, respectivamente. Resultó muy interesante que las tres pruebas fueron positivas en el caso de una mujer con lepra del tipo intermedio. Se encontraron anticuerpos anti-esperma en las 2 formas polares de lepra, lepromatosa y tuberculoide, y no se observaron diferencias significativas en su incidencia entre los dos grupos. En 42 pruebas efectuadas con 14 sueros se observó una correlación tridimensional en el 57% de los casos. El tipo predominante de espermaglutinación observado fue el de cabeza con cabeza. La prueba de espermaglutinación resultó positiva en sólo uno de los 50 hombres fértiles del grupo control, pero ninguno de ellos dió positiva la prueba de inmovilización del esperma o la de aglutinación con eritrocitos tanizados. También se usó la prueba del consumo de anti-globulina humana, considerada como muy sensible, para demostrar la presencia de anticuerpos anti-esperma en el suero de los pacientes con lepra. Los anticuerpos anti-globulina humana se adsorben sobre los espermatozoides de donadores normales después de que éstos son incubados en presencia del suero de los pacientes con lepra y sólo cuando tales sueros contienen anticuerpos anti-espermatozoide. Por este método se encontraron anticuerpos antiespermatozoide en el suero de dos mujeres con la enfermedad. Además, la anti-globulina humana se consumió más avidamente por los anticuerpos anti-esperma presentes en el suero de los pacientes lepromatosos que por aquellos presentes en el suero de los grupos tuberculoide o intermedio.

RÉSUMÉ

Dans respectivement 41 pour cent, 37 pour cent, et 23 pour cent des échantillons de serum obtenus chez 35 malades de la lèpre, dont 5 femmes, des épreuves d'agglutination macroscopique du sperme sur gélatine, des épreuves d'immobilisation du sperme et des épreuves d'hémagglutination de globules rouges tannés ont permis de détecter des anticorps antispermatozoaires. Il est intéressant de noter que toutes les épreuves mentionnées ci-dessus étaient positives dans un échantillon de serum provenant d'une femme malade présentant la forme borderline. Des anticorps aux spermes ont été détectés par les trois techniques mentionnées, tant chez les malades lépromateux que chez les tuberculoïdes. Aucune différence significative n'a été observée dans l'incidence relevée parmi les deux groupes de malades. Une corrélation tri-dimensionnelle a été observée dans 57 pour cent des épreuves (42 épreuves) pratiquées sur 14 échantillons de serum. Un type d'agglutination particulier (head-to-head) a constitué la caractéristique majeure de la spermagglutination observée dans le sérum de ces malades. Dans le groupe témoin, seulement 1 homme parmi 50 sujets masculins fertiles a présenté une épreuve positive d'agglutination pour le sperme. Aucun sujet dans ce groupe n'a montré d'épreuves positives d'immobilisation du sperme, ou d'épreuves d'hémagglutination aux globules rouges tannés.

Une épreuve de consommation de globulines antihumaines, qui passe pour être très sensible, a également été utilisée pour démontrer des anticorps spécifiques pour le sperme dans le sérum de ces malades de la lèpre. Ces anticorps ont été absorbés sur la surface de spermatozoaires provenant de donneurs normaux, ceux-ci étant incubés avec du sérum de malades. La présence d'anticorps antispermatozoaires a pu être démontrée par cette technique dans des échantillons de sérum provenant de deux sujets féminins. De plus, la globuline antihumaine était épuisée de façon plus intense par les anticorps antispermes présents dans le sérum de sujets lépromateux que par les anticorps provenant des groupes de malades tuberculoïdes ou souffrant de lèpre borderline.

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