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Immunologic Researches in Leprosy

Diagnostic Applications

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I. Manifestation by immunofluorescence of fixation on Hansen bacilli of serum antibodies in human leprosy.

Immunofluorescence and fluorescent microscopy permit the demonstration of fixation of antibody, previously made fluorescent, on a specific antigen. A *direct technic* reveals the antigen to which globulins representing the antibodies of immune serum, previously conjugated with a fluorescent substance, are fixed. An *indirect technic* makes use of antihuman globulins developed in rabbit sera and made fluorescent. The globulin antibodies, fixed on the homologous antigen, are made visible by this technic. The second technic is more widely used, as it demonstrates antibody fixation on any specific antigen without requiring previous conjugation of globulins with fluorescent material for each specific serum. It is of course significant to take essential precautions to avoid nonspecific fixation of excess fluorescent material on the proteins of the specific antigen.

In 1963 we (4) were able to detect the presence of antileprosy antibodies in human leprosy sera, using leprosy bacilli obtained from nasal mucosa smears in lepromatous cases. The smears were fixed for ten minutes in cold acetone and then left for 30 minutes in contact with the serum studied. After an antibody present in leprosy serum has been conjugated with fluorescein isothiocyanate (direct technic) it can be fixed to the bacilli and becomes visible by fluorescent microscopy. After contact for 30 minutes with the serum studied and rinsing in physiologic saline, the smears are covered for 30 minutes with a drop of rabbit antiserum containing antihuman globulin previously made fluorescent, and the antibodies are thus made evident. In contrast, in the case of normal human sera, the bacilli remain nonfluorescent and therefore not visible by fluorescent microscopy by either the indirect or the direct technic. These procedures were followed in all forms of leprosy, whether untreated or still active, i.e., whatever the form of leprosy, including tuberculoid as well as lepromatous types. Morris, Aulisio, Bozeman and Guinto (8), using bacillus smears of splenic origin, had detected circulating antibodies by immunofluorescence, but in only 19 out of 22 lepromatous cases and five out of 17 tuberculoid cases. The same authors demonstrated an antigenic family kinship between Hansen and Koch bacilli. The serum of leprosy patients was able to make tubercle bacilli visible by immunofluorescence, and vice versa. This cross fixation was suppressed by previous absorption of tuberculous sera on tubercle bacilli or leprosy sera on tubercle bacilli.

II. Demonstration by immunofluorescence of close antigenic kinship between the Hansen bacillus of human leprosy and the Stefansky bacillus of murine leprosy.

The leprosy bacillus is practically nonculturable, and cannot be inoculated in other species than man. Moreover it cannot be recognized in a number of forms of human leprosy. It is not possible to detect it in smears except in lepromatous forms, and then only before treatment has reduced their number and changed their morphology. In leprosy research laboratories the bacillus of rat leprosy (*M. lepraemurium*, or Stefansky bacillus) is commonly used because of its numerous analogies with *M. leprae* (morphology, staining properties, impossibility of artificial cultivation, and exclusive species inoculability), in spite of the somewhat different character of the lesions induced. The murine bacillus has

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proved capable of fixing antibodies present in human leprosy sera, and thus of becoming fluorescent, either directly or indirectly, with marked leprosy antibodies or with additional human antiglobulin antibody previously conjugated with the isothiocyanate of fluorescein (while contact with a normal human serum does not lead to fluorescence).

Fluorescence of the Hansen bacillus is inhibited by previous absorption of the leprosy serum by the Stefansky bacillus. This cross fixation of circulating human antibodies shows immune antigenic kinship between human and murine bacilli (4).

At the same time a study was made of the antigenic kinship of the Hansen bacillus, the Koch bacillus, and the bacillus of Calmette and Guerin. Only in the sera of progressive tuberculosis, confirming what is known of the richness of tuberculosis serum in antibodies, was it possible to detect an antibacillary antibody the fixation of which on the Hansen bacillus or Stefansky bacillus was disclosed by immunofluorescence. Previous absorption of tuberculosis serum on a suspension of Calmette-Guerin bacilli prevents induction of immunofluorescence and leads to disappearance, by previous fixation on these biliated bacilli, of antibodies able to attach to the leprosy bacilli. It seems preferable in a leprology laboratory to use Stefansky bacilli, rather than Calmette-Guerin bacilli in the detection of leprosy serum antibodies.

Thus it has been possible to substitute Stefansky bacilli for leprosy bacilli in the detection of leprosy antibodies by immunofluorescence. The Stefansky bacillus is easy to obtain and handle; it is nonpathogenic for man, and is present in the nodules it provokes in the rat. It can be passed repeatedly from rat to rat for many years and collected at will from the tumor-like lesions it causes. It is more likely to have antigenic constancy than the human leprosy bacillus, which is more or less difficult to harvest from old human lesions, the activity and richness of which in bacilli are highly variable.

III. Use of segment of the guinea pig terminal ileum, passively sensitized by murine antigen, to confirm the existence of antibodies in leprosy serum.

This confirmation was made by a technique of localized passive anaphylaxis *in vitro*. A segment of terminal ileum, to which antibodies from a leprosy serum had previously been fixed, was maintained in Tyrode solution adequately oxygenated. Prior to the experiment it had been shown in five rats with Stefansky lepromas that contraction occurred after addition of extract of ground leproma. In control animals (12 rats and 3 guinea pigs) passive sensitization of the terminal ileum, established by contact with serum obtained from lepromatous rats, led to the capacity of the segment of the ileum to contract on addition of extract of murine lepromas. This method of passive anaphylaxis *in vitro* was later extended to study of sera from leprosy patients, utilizing guinea pig ileum, which appears to provide a better test material than rat ileum.

The technic used requires an isolated organ bath and recording equipment with carbon black on a cylinder. The contractions of the isolated ileum are recorded by isotonic myography. The richness of the serum in antibodies is appreciated by comparison with a histaminic contraction obtained on the same ileum.

The terminal ileum, removed after sacrifice of the animal and freeing it from blood, is placed in an isolated organ bath and immersed for ten minutes in Tyrode solution adjusted to pH 7.8, and then for ten minutes in a 5 per cent aqueous glucose solution. Passive sensitization is attained by contact of the ileum *in vitro* for 30 minutes with the serum to be tested, one ml. of which has been diluted in 19 ml. of 5 per cent glucose solution. This contact is followed by three washings in Tyrode solution. Then the ileum is submitted to antigenic challenge while maintained in Tyrode solution at 37° C., suitably oxygenated.

Initially the provocative antigen was a suspension obtained by extraction of a

ground leproma fragment developed in a rat after inoculation of Stefansky bacilli. The suspension, prepared by grinding the tissue in Tyrode solution, using a motor-driven Potter crusher, is centrifuged for 30 seconds at 800 rpm. The supernatant opalescent antigenic suspension should be prepared freshly for each experiment. In a series of later experiments the provoking antigen was made up of Stefansky bacilli themselves, extracted freshly from the ground mass of murine leproma and suspended in 10 ml. of physiologic serum and subjected to successive centrifugations. An initial low speed of centrifugation, 3,000 rpm, for three minutes eliminates gross tissue particles. The supernatant is centrifuged at high speed, 12,000 rpm, for 20 minutes. The centrifugate, suspended in 20 ml. of physiologic saline, is subjected to a new centrifugation at high speed for 20 minutes more. This cycle of operations is repeated four times and a final centrifugation at low speed, 1,500 to 1,800 rpm, removes agglutinated bacilli. The final supernatant is a suspension of living bacilli whose vitality is shown by their capacity to provoke, within four months in the rat, a rapid development of lepromas with extensive bacillary multiplication in the spleen and liver. Whatever the source of the antigenic suspension, whether murine leproma or purified Stefansky bacilli, the antigenic suspension, when ready for use, is opalescent and should display an optical density of about 130 as measured by a Jobin and Yvon labospac with a No. 64 red filter. It should have a minimum content of 300 bacilli per oil immersion field at a magnification of 600.

The presence of antibodies in the serum of leprosy patients has been confirmed by the contraction of a segment of terminal ileum of the guinea-pig, to which the antibodies of the serum had previously been fixed passively. The contraction is provoked by the addition of an antigenic suspension made either from a ground mass of murine leproma or a purified emulsion of Stefansky bacilli.

After checking that the ileum of the guinea-pig when incubated with normal human serum does not contract when antigenic suspension is added, we studied 34 sera of leprosy patients, viz., 26 from lepromatous and 8 from tuberculoid cases. Twenty-three of these were tested with ground murine leproma and 11 with the purified suspension of Stefansky bacilli. In 33 cases a contraction of the guinea-pig ileum occurred on the introduction of antigen with a histamine equivalent ranging from 10^{-4} to 50×10^{-5} μ per ml. (Fig. 1). In one case no response took place, in a woman with lepromatous disease who had been treated for a number of years, and whose serum was practically negative on diagnostic immunofluorescence. It should be noted that in certain cases the responses obtained successively with increasing doses of antigen lead to exhaustion of possibilities of immunologic liberation of histamine by the ileum, or diminution of the response on repetition of the same dose of antigenic suspension (Fig. 2).

It has not been possible, with this technic, to establish clearly that serum antibody values are more elevated in lepromatous than in tuberculoid disease, although the antibody values expressed in histamine equivalents appeared somewhat higher in the sera of lepromatous than the sera of tuberculoid cases([5, 9]).

IV. Lymphoblastic transformation of lymphocytes of leprosy patients, provoked by murine antigen.

The small lymphocyte has long been considered as a fully matured cell at the end of its cycle, definitely differentiated, without possibility of further evolution, and lacking the cytoplasmic components required for the synthesis of proteins. The discovery by Nowell ([7]) in 1960 showed that this theory is wrong. When phytohemagglutinin, extracted from the red bean *Phaseolus vulgaris*, is added to a culture of lymphocytes, multiplication and transformation of the latter are observed. Lymphocytes are transformed into lymphoblastic-type cells with a large nucleus enclosing

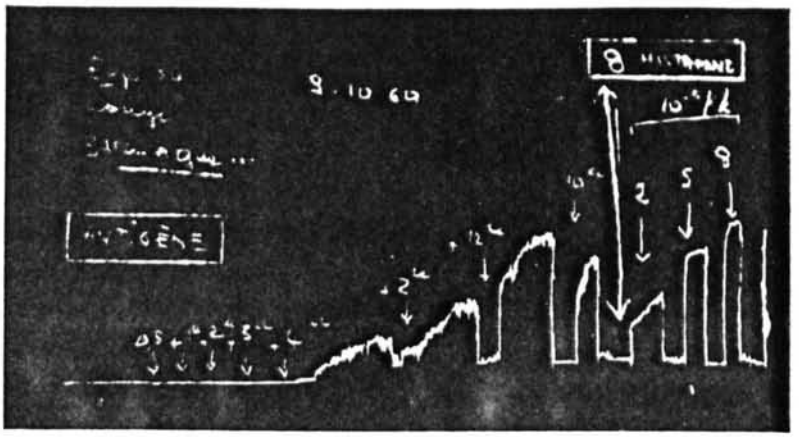

FIG. 1. Experiment 34. Isolated ileum from a guinea pig sensitized by leprosy serum. Contractions provoked by addition of suspension of ground murine leproma. At first, absence of response; then increasingly intense response on addition of increasing doses of antigenic suspension: histamine equivalents.

a nucleolus, surrounded by voluminous basophilic pyroninophilic cytoplasm rich in ribosomes and vacuoles.

An immunologic stimulus also may induce such modifications. The lymphoblastic transformation induced by an antigen to which the donor patient is sensitized, has found many applications in physiopathology (2). It seemed of interest to study the possible blastic transformation of lymphocytes from lepromatous and tuberculoid cases of leprosy by an antigen prepared from a murine leproma.

The lymphocytes were obtained from a fasting leprosy patient and cultured in the usual way. Thirty ml. of blood were taken, 0.5 ml. of calciparin being added to prevent coagulation (corresponding to 12,500 units of heparin as calcium heparinate). The blood was then distributed as rapidly as possible in test tubes placed in an incubator at 37° C, at an angle of 45°, in order

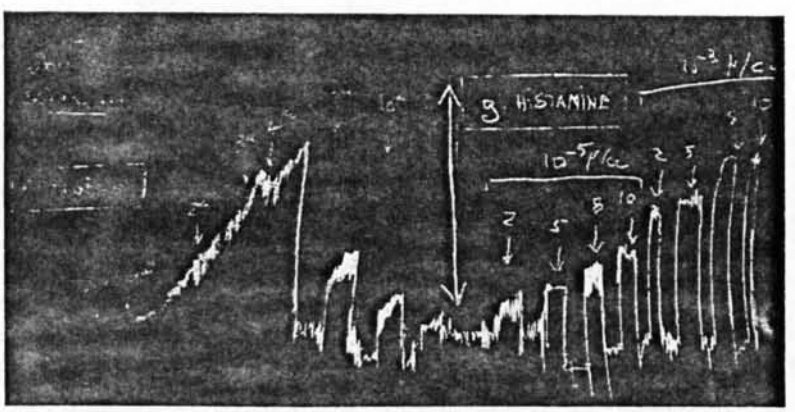

FIG. 2. Experiment 36. Ileum isolated from guinea pig sensitized by leprosy serum. Contractions provoked by addition of suspension of ground murine leproma. Increasing intensity of response with increasing doses of antigenic suspension. Secondary exhaustion of the possibility of liberation of histamine: histamine equivalents.

to obtain satisfactory sedimentation in 30 to 90 minutes. The supernatant was then collected smoothly through a pipette and freed from polynuclear cells by filtration on two or three gm. of carefully washed and dried wool compressed in an Allin tube of grade 2 porosity. The filtrate then contained a suspension of 70 to 80 per cent mononuclear cells, principally lymphocytes. Red cells and polynuclears disappear rapidly from cultures incubated at 37° C, without requiring addition of antibiotics when the suspension is diluted with 70 per cent of Parker 199 medium. The resulting concentration of mononuclear cells is approximately 10^6 per ml.

The provoking antigen was prepared as noted above, for stimulation of the contraction of passively sensitized ileum. In the first series of experiments the antigen was constituted by antigenic suspension freshly prepared from an extract of ground leproma, and in the second series by Stefansky bacilli that had been repeatedly extracted and purified by centrifugation. Three drops of antigenic suspension were added to a test tube containing 3 ml. of lymphocyte culture. The suspension was then incubated for 96 hours at 37° C. Higher doses could have caused toxic lysis. At the same time a control culture without antigen, and a culture exposed to nonspecific provocation by phytohemagglutinin, were incubated (three drops of a solution obtained by addition of Parker 199 medium to a flask of phytohemagglutinin Egic, i.e., lyophilized phytohemagglutinin). Blastic transformation was counted in 500 cells in smears stained by the May-Grünwald-Giemsa method. The proportion of lymphoblasts (20-30 μ in diameter) to lymphocytes (average diameter 8-10μ) was calculated for these two cell types only, excluding intermediate cells too difficult to classify with certainty. The test was considered as positive when, after exposure to the antigen, the cultures yielded over 6 per cent blastic cells against less than 2 per cent in the control cultures.

The provocation of lymphoblastic transformation has been studied with lymphocytes from 43 leprosy patients, made up of 13 tuberculoid and 30 lepromatous cases (23 active and 7 stabilized). Tables 1 and 2 show the results in cases challenged by antigens from extracts of ground murine leproma and emulsions of Stefansky bacilli.

No blastic transformation was observed, with either the murine antigen, or, in a practical way, with phytohemagglutinin (which normally causes transformation in about 60 per cent of cases) in 10 out of 13 cases of tuberculoid leprosy. The two cases only, in which blastic transformation was induced, were of major tuberculoid character. In these the transformation rates were 25 and 12 per cent when phytohemagglutinin was used.

Lymphocytes from seven stabilized lepromatous cases could, on the whole, not be stimulated by either murine antigen or phytohemagglutinin. When murine antigen was used, a significant transformation was noted in only one case (6%), whereas the transformation was not significant at 3 per cent in the one case in which phytohemagglutinin induced a normal transformation rate of 58 per cent.

In contrast lymphocytes from lepromatous cases underwent significant lymphoblastic transformation either with extracts from lepromas (8 out of 19 cases) or with

TABLE 1. *Lymphoblastic transformation in patients with tuberculoid leprosy.*

Case No.	Clinical Type	Murine antigen	Phytohemagglutinin
A. Provocation by extract of ground murine leproma			
1	T	50	56
2	TM	25	8.5
3	T	3	6
4	T	3	3
5	T	2	4
6	T	1	4.5
7	T	1	2
8	T	0.5	1
9	T	0.2	1
10	T	0.2	0.2
B. Provocation by Stefansky bacilli			
1	T	3	1
2	T	0	0
3	TM	12	6

TABLE 2. *Lymphoblastic transformation in patients with lepromatous leprosy.*

Case No.	Type of leprosy[a]	Murine antigen	Phytohemagglutinin
A. Provocation by extract of murine leproma			
1	LA	22	30
2	LA	12	25
3	LA	10	72
4	LA	8	4
5	LA	7	70
6	LA	6.5	6
7	LA	6	45
8	LA	5	50
9	LA	3	12
10	LA	3	6
11	LA	3	2.8
12	LA	2	1.5
13	LA	1	2
14	LA	0.2	0.2
15	LS	6	28
16	LS	3	58
17	LS	3	4
18	LS	1.5	2
19	LS	1	2
B. Provocation by Stefansky bacilli			
1	LA	85	80
2	LA	78	57
3	LA	75	70
4	LA	70	49
5	LA	70	40
6	LA	69	65
7	LA	54	60
8	LA	26	12
9	LA	20	20
10	LS	4	1
11	LS	2	6

[a] LA = patients with active leprosy
LS = patients with leprosy stabilized or in the course of stabilization

Stefansky bacilli (all 9 cases studied). In the latter group the transformation rates were very high, all of them surpassing 20 per cent and even 50 per cent in 6 out of 9 cases. It is to be noted that although lymphocytes from lepromatous cases in general reacted to the nonspecific stimulation of phytohemagglutinin, in 8 out of 9 cases the response was particularly satisfactory ([6, 10]). It is obviously most important to be sure, before studying possible blastic transformation of lymphocytes in Hansen's disease, that no chloroquine (or Nivaquine), or any other drug, has been administered that might inhibit any possibility of blastic transformation.

It was observed by Dierks and Shepard ([1]) also that sensitivity to stimulation by phytohemagglutinin was depressed in cultured lymphocytes from leprosy patients. In contrast with our results, their observations seemed to indicate that this depression was more pronounced in active lepromatous cases than in either long-treated inactive lepromatous cases or tuberculoid cases. In contrast Sheagren, Block, Trautman and Wolf ([11]) observed normal rates of lymphoblastic transformation using phytohemagglutinin, but a marked depression of this transformation with streptolysin O.

It should, however, be recalled that in tuberculoid leprosy intradermal injection of lepromin, i.e., an extract of lepromas rich in leprosy bacilli, induces not only the late Mitsuda reaction, corresponding to the lesion observed in this type of leprosy, but also the delayed allergic Fernández reaction, which seems to correspond to a delayed hyperreactivity in which normally the lymphoblastic transformation test should be positive. In contrast, the absence of lepromin reactivity in lepromatous leprosy suggests a decreased resistance and anergy, but in precisely these cases test for lymphoblastic transformation has given positive results. It is true that from the humoral, but not from the cellular, point of view, immunofluorescence permits the detection of a higher content of serum antibodies in lepromatous than in tuberculoid leprosy patients.

What remains to be explained in our cases is the general lack of lymphoblastic transformation, on antigenic provocation by Stefansky bacilli or nonspecific stimulation by phytohemagglutinin, in lymphocytes from cases of human tuberculoid or arrested lepromatous leprosy.

V. Diagnostic application. Serodiagnosis by immunofluorescence by use of the Stefansky bacillus.

In the present state of our investigations only the detection of circulating antibodies

can be applied practically in the diagnosis of Hansen's disease.

Technique: The *antigenic material* is prepared from extracts of murine lepromas rich in Stefansky bacilli, which have been passed from rat to rat every four to six months for several years. The lepromas themselves can be stored at —20° C. When the central part of the material is crushed, with care to avoid the cortical part, which contains much fatty material and substances interfering with autofluorescence, a smear rich in bacilli is made. This is fixed in anhydrous acetone for 10 minutes.

The *human antiglobulin antiserum* is prepared by injecting a rabbit, at suitable intervals of time, with increasing doses of human antiglobulins. When the antibody titer is adequate, this serum, diluted 1/32, gives a clear-cut precipitation line on an Ouchterlony plate against human gamma globulin, in concentration of 1 mgm./ml., in a well 8 mm. distant. The gamma globulin fraction is obtained by precipitating a pool of such sera by one-third saturation with ammonium sulfate and dialyzing it. This fraction is adjusted in physiologic saline to give a protein concentration of 12 mgm./ml. The product is labelled with fluorochrome by conjugation with fluoresceine isothiocyanate (0.3 mgm./5 mgm. gamma globulins) dissolved in pure anhydrous acetone. The globulin solution is diluted by two volumes of acetate buffer solution at pH 9. After fixation by shaking in an ice-bath for 24 hours in a cold room, the fluorochrome that has not been conjugated is absorbed on vegetable charcoal (2.5 mgm./mgm. of protein) by shaking for one hour at 0° C. and removing particles by centrifugation. Since this procedure deals with immunofluorescence of almost pure bacterial smears, it is not necessary to purify the fluorochrome-labelled product on powder of animal tissues because there is no interference from nonspecific tissue fluorescence.

The slides with smears of Stefansky bacilli are covered for 30 minutes with respective two-fold dilutions of the serum under study, viz., 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512 and 1/1024. The human gamma globulin left unfixed is removed by four successive prolonged washings with saline solution buffered at pH 7.1 with phosphate. The smears are then covered for 30 minutes with the fluorochrome-labelled antihuman gammaglobulin immunoglobulin (one drop of this solution with an equal volume of buffered saline). Next the slide is subjected to prolonged rinsing with buffered saline, delivered by pipette, to remove the fluorochrome left unfixed. The observation is made on a dry slide, best in a dark room, with equipment for fluorescence microscopy (e.g., Ortholux Leitz, with mercury vapor burner Osram HB200 and excitation filters VG 1 and VG 5). A dark field condenser eliminates the use of barrier filters and at the same time avoids use of the apochromatic objective which is sometimes autofluorescent, the light being reflected by an aluminized metallic mirror. The bacilli labelled by successive fixation of leprosy antibodies, and then fluorescent antihuman antiglobulin, appear in bright yellow on a black background. A whitish-blue auto-fluorescence given by debris from collagen fibers, and darkish shadows due to debris of cells and fatty tissue do not lead to appreciable disturbance in the examination. Control slides are used in order to detect possible deconjugation of the labelled immunoglobulin, which is evidence of nonspecific fixation of a poorly conjugated fluorochrome.

The slides are examined in series, starting with the highest dilution of the serum studied. The slide on which fluorescent babilli can be detected corresponds to the titer of antibodies in the serum. Rigorous observance of the technique ensures a satisfactory standardization of results, in spite of some difficulties in the examination, if an adequate ultraviolet lamp is employed.

RESULTS

The risk of false negatives through zone phenomena in sera rich in antibodies, and of false positives due to nonspecific factors in sera, as well as weak cross-reactions with tuberculous sera (apparently serum antibodies are developed only in progressive

cavitary forms) have led us to investigate leprosy antibodies only in sera diluted 1/8 or higher.

Very strong positive results have been observed constantly in cases of untreated leprosy. Titers can reach 1/1024 or more in lepromatous cases, 1/512 in tuberculoid cases, 1/1024 and 1/512 in borderline cases, and 1/128 or more in intermediate forms where the disease is relatively advanced.

This study has been carried out on more than 700 sera, from confirmed leprosy cases, recent cases, and persons wrongly suspected to have leprosy. Serodiagnosis based on the immunofluorescence of Stefansky bacilli appears to have a definite value at least in cases of untreated leprosy. The titer of serum antibodies decreases gradually with efficient antileprosy treatment, corresponding to resultant clinical and biologic improvement. It is rather usual to observe titers of 1/256 and 1/128 in lepromatous patients negative since six months to a year, and in tuberculoid patients recently considered inactive ([3]) (see Table 3).

TABLE 3. *Serum diagnosis of leprosy.*

Sera studied:
25 normal controls
651 pathologic sera from:
244 cases of leprosy (100 lepromatous, 144 tuberculoid)
196 cases of suspected leprosy
Tuberculoid cases:
43 before treatment
1/512 30 cases
1/256 13 cases
45 cases undergoing arrest
1/256 45 cases
31 cases arrested for more than one year
1/128 21 cases
1/64 10 cases
25 cases arrested for more than two years
1/32 25 cases
Lepromatous cases:
53 new patients, not treated
1/1024 53 cases
31 patients in the course of becoming negative
1/512 20 cases
1/526 11 cases
8 patients negative and arrested for more than one year
1/128 8 cases
8 patients completely arrested for more than two years
1/16 8 cases

A threshold of 1/128 for positive reactions can be considered as of definite diagnostic value. But although the possibility of initial diagnosis seems to be excluded with titers of 1/8 or even 1/16, such titers may represent old cases of leprosy that have been actively treated. Possibly a threshold of 1/32 or even 1/64 might be considered. Such titers might correspond to the early course of leprosy that could not be confirmed as cutaneous or neural or characterized by other manifestations. Such titers could also represent a latent infection in a leprosy environment. In this case it should be associated with conversion of the lepromin reaction to positivity (as in the case of tuberculin reactions in latent Koch bacillus infection in a tuberculized environment). In such circumstances it is very difficult, however, to rule out the possibility of latent infection caused by some other mycobacterium of related antigenicity.

During the treatment of leprosy it is interesting to follow the titers of circulating antibodies, using the immunofluorescence of Stefansky bacilli for the test. A sudden change in titer may represent the beginning of a reactional phenomenon or correspond with a confirmed lepra reaction. Increase in antibodies after a period of decrease, may represent reactivation, whatever its cause (e.g., failure of the patient to take prescribed treatment, or loss of drug activity, as in the case of thiourea treatment and therapy with antileprosy sulfonamides the initial activity of which seems indisputable).

In spite of its difficulties, and the eye fatigue inherent to the examination of slides, serodiagnosis through immunofluorecence of Stefansky bacilli would seem to merit extended use in diagnosis and follow-up examinations in leprosy.

SUMMARY

Serum antibodies in human leprosy have been demonstrated by indirect immunofluorescence staining after fixation on the Hansen bacillus as well as on *M lepraemurium* (Stefansky bacillus).

This antigenic kinship of the two bacilli and estimation of the ultimate positive dilution of the sera studied have allowed us to evolve a sound enough serodiagnostic method using Stefansky bacilli. Serum antibodies decrease with treatment.

The presence of antibodies in leprosy sera has been confirmed by the action of minced rat lepromas or by ultracentrifugation, i.e., by the effect of bacilli extracted from the lepromas on guinea pig ileum sensitized by such sera.

Moreover, blastic transformation of lymphocytes from cases of leprosy can be induced by minced rat lepromas or still better by ultracentrifugation yielding bacilli from these nodules, in the case of lymphocytes from active lepromatous cases. In contrast, lymphocytes from tuberculoid cases remain unchanged under such stimulus, as well as under the nonspecific stimulus of phytohemagglutinin.

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