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Evaluation of a New Blood Test for its Potential to Indicate Leprosy Infection Prior to the Appearance of Clinical Symptoms[1]

Claude V. Reich, Ricardo S. Guinto, Roland V. Cellona, Tranquilino T. Fajardo, and Monina G. Madarang[2]

A previous study ([4,5]) in this laboratory had indicated that a specific reaction could be demonstrated in lepromatous sera by using a laser nephalometer to detect the interaction of highly diluted sera from leprosy patients with dilute standardized leprosy tissue suspensions. With this procedure, the reactions of 70% of 132 lepromatous sera from Cebu, Philippines, were greater than the reactions of 99% of 100 normal controls from the same area. Abe, *et al.* ([1]), using an indirect fluorescent antibody procedure, reported that the reaction titers of adsorbed lepromatous sera were directly related to the severity of the clinical leprosy of the patients from whom they were taken. Godal ([2]) reported that Harboe, *et al.*, using radioimmunoassay, had confirmed the presence of leprosy specific antibodies in patients' sera.

While all of these studies reported the presence of specific antibodies in lepromatous sera, it is most likely that the antibodies are not directly involved in effective immunity to the disease. Reich ([7]) later again confirmed that 70% of lepromatous patients were positive to this light scatter test in another group of 50 sera collected from persons within the colony on Culion, Philippines. This same study reported that for Culion residents, this value of 70% for active lepromatous leprosy increased to 76% in a group of 50 former lepromatous patients who at bleeding had been bacteriologically negative for at least a year, and this value increased still further to 84% for a group of 35 patients who were relapsed at the time of bleeding but who, prior to relapse, had been bacteriologically negative for at least a year. However, when sera were taken from a group of 100 bacteriologically negative ex-patients whose residences at the time of bleeding were in Cebu City, removed from routine contact with active cases of leprosy, the frequency of high reactors dropped to less than 50%. These results can be explained by the assumption that, in the absence of active infection, routine contact with leprosy is necessary for the maintenance of serum reactivity to this test. This possibility had been demonstrated earlier, in Table 1 and Fig. 1 of the previously mentioned paper by Reich ([5]), where a difference between contact and non-contact normal sera was indicated by the data presented.

In 1954, Guinto, *et al.* ([3]) reported that the average annual attack rate for household associates of cases of lepromatous leprosy was more than four times that for the total population and about six times that for non-exposed persons. In the study reported here, the blood test reactions of persons with no clinical leprosy who are living in routine daily contact with clinical cases of leprosy are compared with test results of sera taken from a comparable group of non-infected persons living in the same community but not residing in a leprosy household. The experimental hypothesis assumes that the contact that is responsible for the differences in attack rates between house-

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[2] C. V. Reich, Ph.D., Associate Research Professor, The George Washington University Medical Center, Department of Microbiology, 2300 I Street, Washington, D.C. 20037, U.S.A., and Chief, Philippine Division, Leonard Wood Memorial; R. S. Guinto, M.D., Chief, Epidemiology; R. V. Cellona, M.D., Epidemiology; T. T. Fajardo, M.D., Chief, Clinical Branch; M. G. Madarang, M.T. (ASCP), Chief, Technical Services, Laboratory, Leonard Wood Memorial, Cebu City, Philippines.

hold contacts and others will result in comparable differences in values when tested by an effective blood test.

MATERIALS AND METHODS

The blood test was accomplished by mixing the serum specimen at a fixed dilution into a standardized suspension of leprosy biopsy tissue and measuring any possible haze formation or particle enlargement that resulted from the interaction of serum and tissue. A Differential I laser light scattering photometer (Science Spectrum, Santa Barbara, California, U.S.A.) was used for this purpose (the PDQ Laser Nephalometer of Hyland Laboratories was subsequently tested and also appeared to be applicable. A new set of dilutions would need to be devised to fit the characteristics of the Hyland instrument). The photometer was operated in the log mode at 600 V on the photocell. All values were recorded in terms of Relative Intensity (RI) of light scatter at a constant angle of 90° from the incident angle of a helium-neon laser (0.5 W, 632.8 nm).

The antigen suspension was prepared from human leprosy biopsy tissue which had been autoclaved for one hr. After autoclaving, fat and epidermis was removed, and the remaining bacteria rich tissue was finely minced with a scissors and then ground with (0.5%) phenolized physiologic saline in a Ten Broek grinder. The resulting smooth suspension was poured from the grinder with the pestle in position to retain the gross aggregates of tissue. The preparation was allowed to stand overnight at room temperature, and the supernatant was carefully withdrawn with a Pasteur pipet after which the turbidity was adjusted with phenolized saline to an OD of 0.4 at 525 nm with a Coleman Jr. Spectrophotometer. A microscopic count according to the technique of Shepard and McRae ([8]) was made, and the final dilution was made with phenolized saline to yield a count of 30 to 40 $\times$ 10^6 acid-fast organisms per ml. This material was placed in 10 ml vials and held at 4°C until needed.

Prior to use, these 10 ml tissue preparations were shaken vigorously and vortexed to achieve as homogenous an antigen suspension as possible. The well-shaken antigen mixture was then taken into a 10 ml syringe, which was subsequently fitted with a "Swinney type" Nucleopore filter holder with a 5.0 μ Nucleopore membrane (the Nucleopore unit supports both sides of the cylindrically pored membranes and makes this kind of back flushing filtration possible). The antigen suspension was then slowly passed back and forth through the filter about 1 ml at a time, slowly increasing the total amount passed through until the entire 10 ml had been screened of particles larger than 5.0 μ. This working preparation could be held at 4°C for at least one month provided it was well shaken each time before use.

A total of 310 sera was taken for this investigation from residents of Mactan, Philippines. Half of the specimens were taken from persons who were residing in households with an active case of leprosy (HC). Twenty-four leprosy households were studied. Twelve of the leprosy index cases were classed as borderline and twelve as lepromatous. No preliminary estimation was made of the relative activity of the disease state of any of the index cases.

An equal number of sera were taken from non-household contact (NLH) residents of the community surrounding these leprosy households. While no overt effort was made to match the household and non-household serum donors, there appeared to be no significant discrepancy in the characteristics of the two test populations with regard to economic circumstances, age, sex, and medical history.

All sera were preserved at −20°C until needed. All tests were conducted using working solutions of sera diluted 1:10 with distilled water and filtered through 0.2 μ membranes. These working solutions were prepared fresh daily and held in an ice bath until used.

The test was conducted by placing 100 μl of the working antigen suspension into 10 ml of 0.2 μ filtered distilled water in the standard plastic throw away cuvettes used in the Differential I instrument. The cuvette was then centered in the laser beam and the Relative Intensity (RI) of light scatter was recorded for a period of 1 min to establish the RI baseline for the antigen alone. The pen of the recorder was then returned to the zero time origin of the curve and restarted at the moment that 50 μl of the

TABLE 1. *Distribution of light scatter serum reactions among leprosy household contacts vs other residents of Philippine communities where leprosy is endemic.*

RI[a]	NLH[b]	HC[c]	RI	NLH	HC	RI	NLH	HC	RI	NLH	HC
0			10	10	2	20	3	5	30		6
1	1[d]		11	10	8	21	1	11	31		4
2			12	12	5	22	3	8	32		1
3			13	7	5	23	2	5	33		3
4	6		14	9	8	24	3	6	34		1
5	2		15	15	13	25		4	35		3
6	5		16	9	5	26	1	3	36	2	1
7	5	1	17	9	11	27	1	4	37		
8	8	1	18	2	7	28		3	38	1	1
9	16	2	19	7	12	29	3	3	≥39	2	3
Total	43	4		90	76		17	52		5	23
Ratio	1:0.1			1:1			1:3			1:5	

[a] RI = Relative intensity of serum reaction.
[b] NLH = Sera from persons not living in a household with an active case of leprosy.
[c] HC = Sera from household contacts of leprosy.
[d] Numbers of sera reacting at each RI level.

working dilution of the serum specimen was mixed into the cuvette of the antigen mixture whose RI baseline had just been recorded. The RI of the antigen-serum mixture was then recorded for exactly 5 min at room temperature.

The test RI value for each serum was the measure, at exactly 5 min, of the light scatter increase over that of the antigen baseline. Each RI unit indicated a 0.05 inch (20 RI units per inch) increase between the baseline curve and the serum added curve at 5 min. These units can be converted directly to a value equivalent to the output voltage of the photocell and can be equated to comparable readings with standardized suspensions of latex particles of known size.

Most of the RI values recorded in this experiment were derived from a single test for each specimen. However, every day's run of serum testing included at least one and usually two specimens chosen from previously run samples. These repeat specimens were usually selected from the most reactive sera in order to confirm the validity of high test values, but occasional repeat control specimens were selected from low value sera, and some were selected at random. If the test result for a repeat specimen differed by more than 15% from its original reading, that serum was usually repeated again in another day's run of tests. When more than one test was run for a single serum, the reported value is the mean for all tests for that serum.

RESULTS

The mean RI for all 155 sera from persons not of a leprosy household (NLH) was 13.74, and for the same number of household contact sera (HC), the mean RI was 20.79. Of equal significance is the fact that there were only five NLH sera with an RI of 30 or greater while the HC serum group yielded 23 of these high level reactors ($p = 0.0004$). Opposed to this, there were 53 NLH sera with RI values of 10 or less as compared to six HC sera in this low level reaction range.

Table 1 summarizes the number of both NLH and HC sera that reacted at each RI value between 0 and 39 or greater. The RI values in the table are divided into four groups of 10 RI units each. This division is intended to illustrate the tendency for the NLH serum reactions to cluster in the lower RI values while the HC sera were clustered in the higher ranges. The ratio for numbers of NLH sera vs numbers of HC sera in each successive unit of 10 RI values was 1:0.1, 1:1, 1:3, and 1:5, going from low RI values to the higher RI values. The highest reactions recorded were 42, 43, and 44 for three HC sera.

It appeared that there was a significant relationship between the degree of serum response in this test and residency in a

TABLE 2. *Distribution of blood test reactions.*

Group	Subdivision	No. of specimens	Mean RI	Number ≥ 30
Sex	Male	68	21.2	11
	Female	87	20.5	12
Age	6–12	23	21.0	3
	13–17	26	22.5	6
	18–22	26	20.0	3
	23–31	26	20.8	4
	32–45	25	20.4	4
	>45	29	20.0	3
Family relation to index case	Son	15	22.6	3
	Daughter	18	20.1	3
	Sister	11	19.4	1
	Brother	19	19.5	2
	Aunt	5	20.4	1
	Uncle	4	30.0	2
	Cousin	22	22.4	5
	Niece/nephew	16	22.5	2
	In-law	15	23.9	4
	Husband/wife	8	15.8	0
	Mother/father	15	16.9	0
	Grandrelative	5	15.8	0
	None	2	22.5	0
Clinical class of index case	BL	66	20.1	9
	LL	89	21.3	14

household that included an active case of leprosy. Table 2 was designed to explore the possibility that factors other than mere residency in a leprosy household may have been responsible for this relationship. It can be seen that no significant differences exist between the test results for HC sera when evaluated on the basis of sex, age, family relationship to the index case, or clinical status of the index case.

Male HC sera had a mean RI value of 21.2, and female sera had a mean of 20.5.

The six age groups of HC sera within the range of from 6 to over 45 years had in the order of 25 specimens in each group. The means for each of the six groups were remarkably close to the mean for all 155 HC sera.

There was a similar, very close relationship between the mean serum reactions of the various family relationship groups described in Table 2. There is some question concerning the portent of the reduced serum reactivity for the groups: wife-husband, mother-father, or grandrelatives. The serum reactions of these particular groups appear to merit further investigation; however, this uniqueness is on the low side, and this in turn adds further significance to the overall increased RI resulting from residency in a leprosy household.

It also did not matter whether the index case was borderline (BL) or lepromatous (LL). The respective RI values were 20.1 and 21.3.

Table 2 shows that the same conditions that were true for the mean of all RI values were also true for the distribution of those sera that had RI values of 30 or more. These high level reactors appear to be evenly distributed among all groups regardless of sex, age, family relationship, or clinical status of the index case.

DISCUSSION

The blood test described here is demonstrating an excellent capacity to identify individuals on the basis of their apparent degree of contact with leprosy index cases. All of the serum donors had close physical examinations at the time of bleeding, and many of them had a second examination after the data were tabulated and the key was broken. No clinical leprosy was detected in any person who contributed a specimen to this study. Other tests have demonstrated differences between known infected persons and known non-infected

persons. This trial indicates that there are detectable differences in the sera of persons with no clinical disease. The serum factor that is responsible for the increasing RI of positive reactors in this test was previously shown to be immunoglobulin G ([6]) and so it must be assumed that the test is a function of an immune challenge. Whether this challenge is the result of purely constant extraneous antigen stimulation or is a function of a subclinical infection is not known. It is difficult to see how sufficient antigen could be constantly transmitted from an external source to the household contacts to induce and maintain their immune responses at the 1:2000 serum dilution levels that were used in this trial. A far more defensible conclusion would be that the contact state induces and maintains a subclinical infection that in turn provides the immune challenge that was being measured in this study.

It is impossible to define any degree of serum test response as being positive in a study of this kind since the degree of correlated subclinical infection is not possible to grade. The RI value of 30 or over was selected as an arbitrary value for the demonstration of the distribution of test values purely on the basis of convenience of tabulation. Serendipitously, the value of 30+ seems to have divided leprosy household sera and nonleprosy household sera into almost the identical ratio that could be anticipated for the incidence of new leprosy within these two populations, 5 to 1. However, it is just as obvious that, even if all 30+ serum reactors do have subclinical leprosy, not all 30+ reactors will develop clinical leprosy. On the basis of an estimated leprosy incidence rate of one per 1000 for the area under study, the best theory would be that about one case of clinical leprosy would occur among every seventy 30+ reactors. While this study indicated that leprosy may be easier to transmit than we care to admit, it also demonstrated that once transmitted, its progression to clinical leprosy is rare.

There is another interesting aspect of leprosy transmission that is not evident in the tabulated data but becomes apparent only after a closer analysis.

Table 2 indicates that there was no difference in serum reactivities between the BL and LL groups of index cases. A total of 66 contacts of 12 BL index cases had a mean RI of 20.1, and a total of 89 contacts of 12 LL index cases had a mean RI of 21.3. There were nine 30+ reactors in the BL contact group and fourteen 30+ reactors in the LL group. However, the nine 30+ reactors in the BL group came from among the contacts of only six of the 12 BL index cases, and the fourteen 30+ reactors in the LL group came from among the contacts of six of the 12 LL cases. If these groups are evaluated on the basis of the presence or absence of 30+ reactors, then the mean RI for the 29 BL contacts without 30+ reactors was 15.0, and the mean RI for 37 BL contacts with 30+ reactors was 24.0. The corresponding values for LL contacts without and with 30+ reactors was 16.0 and 23.0, respectively. It would appear that not all leprosy cases can be associated with the development of high serum test reactivity among the contact members of their households. The reason for this is not apparent from the information gathered for this study. The phenomenon is under further investigation.

Since the 30+ reactors were grouped among only a portion of the index cases, there was a significant variation between the mean RI values for the index cases when their contact groups were considered individually. The subdivision into index case groups resulted in instances of sample sizes being too small for evaluation. However, it can be said that, generally, the RI values of groups without 30+ reactors had mean RI values that were not significantly different from the mean for all 155 NLH sera. In those index case groups that had a 30+ reactor, the presence of the 30+ reactor did not appear to be totally responsible for the high RI of the group itself. Even if the 30+ reactor reading was not considered, the mean RI value for the remaining contact sera in the group was still notably higher than the mean for all 155 NLH sera.

The test reported here does seem to be indicating the existence of some discrete relationships between man and the disease of leprosy. While this appears to be an immune function, the evidence is not clear in favor of its being directly associated with immunity and, in fact, the reverse appears

to be true. However, the test is relatively simple, rapid, and can lend itself to the testing of a large population. It could provide another practical tool for investigating leprosy.

SUMMARY

A test is described that utilized a laser nephelometric measure of the interaction between human serum and a leprosy biopsy suspension to demonstrate household contact with a leprosy index case. None of the test sera was from persons with clinical evidence of the disease. There was a 5:1 ratio of higher level reactors in sera from household contacts vs high level reactors in sera from persons in the surrounding community. This did not appear to be a result of age, sex, family relationship to the index case, or clinical character of the index case. Some index cases had high level serum reactors among their contacts; others did not. This phenomenon was not a function of the clinical classification of the index case. The reason is unknown. This test should provide another practical means to study leprosy.

RESUMEN

Se describe una prueba que utiliza la nefelometría de rayos laser para medir la interacción entre el suero humano y una suspensión preparada a partir de biopsias de lesiones leprosas con el fin de demostrar el contacto de los convivientes con un enfermo de lepra. Ninguno de los sueros probados correspondió a personas con evidencias clínicas de la enfermedad. La relación de reactores a títulos altos entre los contactos convivientes con los pacientes y las personas no convivientes pero de la misma comunidad, fue de 5:1. Esto no pareció ser el resultado de la edad, sexo, relación familiar con el paciente, o tipo clínico de lepra. Algunos pacientes tuvieron convivientes reactores a títulos elevados, otros no. Este fenómeno no estuvo en función de la clasificación clínica del paciente. La razón de esto se desconoce. Esta prueba constituye otra forma práctica para el estudio de la lepra.

RÉSUMÉ

On décrit ici une épreuve basée sur l'utilisation d'une mesure néphélométrique au laser, de l'interaction entre le sérum humain et une suspension de biopsie de lèpre. Cette épreuve est destinée à mettre en évidence des antécédents de contact au domicile avec un cas index de lèpre. Aucun des échantillons de sérum étudiés ne provenaient d'individus présentant des signes cliniques de la maladie. On a observé cinq fois plus d'échantillons avec des niveaux élevés de réaction dans les spécimens de sérum provenant de contacts domiciliaires, que parmi ceux provenant de personnes choisies dans la communauté environnante. Ces différences ne semblent pas être la conséquence de l'âge, du sexe, de la relation familiale au cas index, ou du caractère clinique de ce dernier. On a trouvé des personnes avec des niveaux élevés de réaction dans le sérum, parmi les contacts de certains cas index. Dans d'autres cas, il n'a été observé aucune personne avec des niveaux élevés de réaction parmi les contacts du cas index. Ce phénomène ne dépendait pas de la classification clinique du cas index. La raison en reste inexpliquée. Cette épreuve devrait se révéler un complément pratique utile pour l'étude de la lèpre.

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