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Studies on the Antigenic Specificity of *Mycobacterium leprae* II. Purification and Immunological Characterization of the Soluble Antigen in Leprosy Nodules[1, 2]

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Previous investigations (1) demonstrated by immunodiffusion, at least two antigens in the water-soluble extract of lepromatous lepromas. One of these antigens appeared to be a heat-stable polysaccharide of *M. leprae*, because it cross-reacted with antisera against BCG and *M. microti*. Another antigen was a heat-labile protein and seemed to be peculiar to leprosy nodules, although its origin was not made clear. This antigen may be similar to abnormal proteins such as C-reactive protein, because many investigators (3, 4, 9, 10, 12) have reported the appearance of C-reactive protein in some sera from leprosy patients. It is also possible that the protein antigen may be derived from *M. leprae*, since Ito (8) has recently found a precipitation reaction between a crude extract of this bacillus and a rabbit antiserum against an extracellular protein of *M. tuberculosis* which has been isolated by Fukui and Yoneda (7). Lepromin reactions in leprosy patients have been used by some investigators (6, 11) for the purpose of identification of *M. leprae*, therefore identification of the antigen in question was attempted by utilization of the Fernández reaction which is known to be a delayed type skin hypersensitivity caused by protein antigens of *M. leprae*.

MATERIALS AND METHODS

Leprosy nodule extract. Leproma-extracts (NE) were prepared from unheated lepromatous nodules by the method previously described (1) and were kept at −20°C until used. Human skin-extract (SE) was as used in previous investigations (1).

Purification procedure. The technic of starch-block zone-electrophoresis has been described elsewhere (2). Gel-filtration chromatography was performed in a cold room by the following method: 3–5 ml containing antigen solution and 200 mg/ml of sucrose were layered at the top of a Sephadex G200 gel column (25 × 450 mm).[4] The column had been equilibrated with 0.1 M Tris-HCl buffer (pH 8.0) containing 0.14 M NaCl. The specimen was poured into the column with the same

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4 A column K25/45 made by Pharmacia Co., Sweden, was used throughout.

buffer at the natural flow rate and fractionated in 3–5 ml by a fraction collector. The optical density at 280 mμ was measured on the effluent fractions and they were collected to three pooled fractions on the basis of the peak of plotted optical density. The major component of protein was contained in the first peak and it was designated as the "nodule-extract protein" (NEPR). The second fraction corresponding to the middle peak was designated as NEG2.

Ultraviolet absorption spectrum. This was obtained with NEPR solution (0.1 mg/ml) in Tris-HCl buffered saline using an automatic recording spectrophotometer (Shimazu UV-50M type, Kyoto). The same apparatus was also used for the measurement of optical density as described above.

Antisera. In addition to anti-NE rabbit serum described previously [1], two kinds of antisera were obtained from rabbits immunized with the materials derived from leprosy nodules. Two rabbits were each given four weekly intravenous one milliliter injections of a crude suspension of leprosy bacilli in saline (0.4 mg/ml). Two months later, intramuscular and subcutaneous injections were given of an emulsion of leprosy nodules in saline (1:20) mixed with Freund's incomplete adjuvant, in a dose of 4 ml per animal divided among a total of eight sites. Six weeks after the last injection the rabbits were sacrificed for exsanguination. The pooled serum was designated as "anti-leproma" serum (anti-Lm). Another antiserum, "anti-NEPR," was prepared in two rabbits immunized with an emulsion consisting of NEPR solution (1 mg/ml) and Freund's incomplete adjuvant containing *B. pertussis* (8×10^{10}/ml).[5] An initial injection of 0.8 ml per animal was divided among the four foot pads. Six weeks later 0.4 ml of the same inoculum per animal was divided among the same sites. Exsanguination was done two weeks after the last injection. Extracellular proteins of *M. tuberculosis*, α and β, and corresponding antisera, anti-α and anti-β,[6] as well as a horse antiserum against human serum[7] were also used for immunodiffusion tests. Anti-CRP serum was a commercial product available for C-reactive protein determination.

Immunodiffusion. The methods of double diffusion and immuno-electrophoresis in agar gel have been described previously [1, 2]. Instead of purified agar (Difco), 1% Agarose (Behringwerke, Germany) in barbital buffer (pH 8.6) was used.

Passive hemagglutination. Formalinized, tanned cell agglutination tests were carried out by the method described by Daniel *et al* [5].

Cutaneous reactions in guinea pigs. Male and female Hartley strain guinea pigs were fed a routine diet of rabbit chow and tap water at constant room temperature. Each group of guinea pigs (4 or 5 animals) were sensitized by one of the four following methods: a) repeated injection of lepromin into the abdominal skin; b) intramuscular injection of 0.5 ml of liquid paraffin containing lyophilized BCG (2 mg/ml); c) four foot pad injections of the emulsion (0.1 ml per site) consisting of one volume of BCG in saline (1 mg/ml) and one volume of Freund's incomplete adjuvant; and d) four foot pad injections of an emulsion composed of fresh leprosy bacilli (2×10^8/ml) and the above adjuvant. As the skin test reagents, NE and its fractions were diluted with saline to the concentration of 0.1 mg/ml and heated at 120/C for 20 minutes. One tenth milliliter of the respective antigens was injected into the dorsal skin from which the hair had been removed. For comparison, standard lepromin containing 160 million bacilli/ml and a solution of protein fraction (10 μg/ml) of BCG culture-filtrate precipitated by 80% saturated ammonium sulfate were also injected in doses of 0.1 ml respectively. The site of injection of each antigen was interchanged on each animal in order to remove variations due to injection-sites. The size of erythema and induration was measured at 5, 24, and 48 hours after administration of the antigens. Maximum reaction was usually observed at the 24

[5] *B. pertussis* was supplied by the Department of Bacteriology I, National Institute of Health, Tokyo.

[6] The author is very grateful to Dr. M. Yoneda of the Research Institute for Microbial Diseases, Osaka University, for supplying these materials.

[7] Kindly supplied from the Department of Serology, Faculty of Medicine, University of Tokyo.

hour reading. Therefore the other readings are omitted from this report.

Cutaneous reactions in leprosy patients. One tenth milliliter each of heated NEPR solution, lepromin and Dharmendra's antigen were injected intracutaneously in 16 leprosy patients of which 13 were lepromatous and three tuberculoid. Three sites of injection (flexor side of both forearms and that of left upper arm) were interchanged on each patient. The readings of skin reactions were made at 48 hours and at one and two weeks after the injection of antigens. Eleven additional patients received intracutaneous injections of NEPR, NEG2, leprolin and tuberculin. NEG2 was the autoclaved saline solution of protein (0.1 mg/ml) obtained by gel-filtration chromatography. Leprolin was prepared from sonicated lepromin by ultracentrifugation at 105,000 g for 90 minutes, and Old Tuberculin was used at 1:2,000 dilution. Four sites of injection were also interchanged among both sides of the upper and lower arms of each patient.

RESULTS

Purification and physio-chemical characterization of the protein antigen in leprosy nodules. As indicated previously (1), the antigen showing precipitation reaction with anti-NE serum was mainly contained in fraction No. 3 obtained by starch-block zone-electrophoresis. This fraction was further purified by Sephadex G200 gel-filtration. An example of elution pattern through this column is shown in Figure 1. Four experiments have been repeated with similar results. Ultraviolet absorption spectrum of fraction one, corresponding to the first peak in Figure 1, showed maximum absorption near 280 mμ, as indicated in Figure 2, which is characteristic of ordinary proteins. This fraction gave a positive pro-

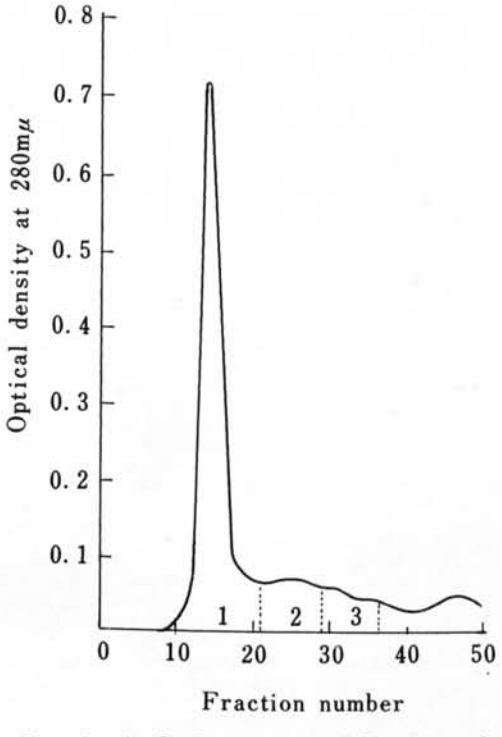

FIG. 1. Sephadex G200 gel-filtration of a preparation of nodule-extract (NE) which had been separated by zone-electrophoresis on starch-block. The eluted fractions were divided into three pooled fractions (1, 2 and 3) as indicated in the figure.

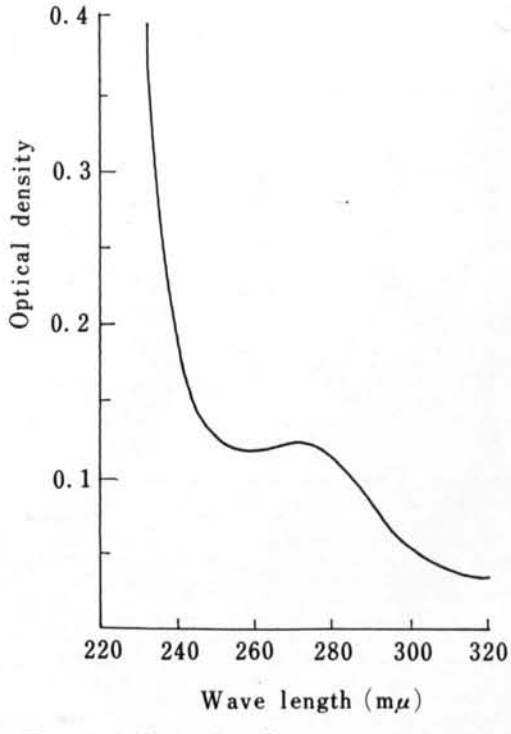

FIG. 2. Ultraviolet absorption spectrum of the first fraction obtained by gel-filtration.

tein reaction with Folin-Ciocalteau's reagent, but negative sugar reaction with tryptophan-sulfuric acid reagent. The yield of NEPR was only about 4 mg from 5.9 gm of lepromas in the single sample so far examined.

Properties of immunodiffusion. Figure 3 indicates the immunoelectrophoretic patterns of the three fractions obtained by Sephadex G200 gel-filtration shown in Figure 1, as compared with that of the original specimen of NE. This specimen produced a single precipitation line with anti-NE serum absorbed with human serum and three precipitation lines with horse antiserum against human serum, the latter being fused with any of parallel lines formed between this antiserum and human serum used for absorption of anti-NE serum. The first fraction, i.e. NEPR, produced a single precipitation line with anti-NE absorbed with human serum, but did not react at all with horse antiserum against human serum. In contrast to this, the second and third fractions caused no precipitation with the former antiserum while yielding one precipitation line with the latter antiserum, the line being fused with the parallel line between the two troughs.

Figure 4 indicates the immunoelectrophoretic patterns of NEPR and NEG2 obtained from another specimen of NE. Because of the use of Agarose, the electrophoretic mobility of these fractions was faster than that shown in Figure 3. Thus NEPR, too, produced a single precipitation line with anti-NE serum, but not with goat antiserum against human serum. Similar precipitation lines were also formed by anti-NE and anti-Lm sera absorbed with human serum. In contrast, NEG2 produced no precipitation line with these antisera, but gave an indistinct line on the anode side with goat antiserum to human serum.

Immunoelectrophoresis was also applied for the purpose of determining whether or not anti-Lm and anti-NEPR sera contained

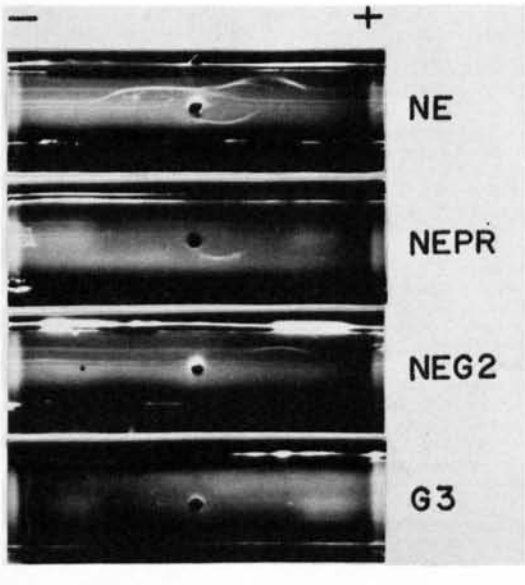

FIG. 3. Immunoelectrophoretic patterns of leprosy nodule-extract (NE) and its fractions (NEPR, NEG2 and G3) obtained by gel-filtration as indicated in Figure 1. These antigens were electrophoresed in a medium of 1.5% purified agar for one hour at 200 volts. After electrophoresis the upper trough of each plate was filled with horse antiserum to human serum (HS) and the lower with rabbit anti-NE serum absorbed with HS.

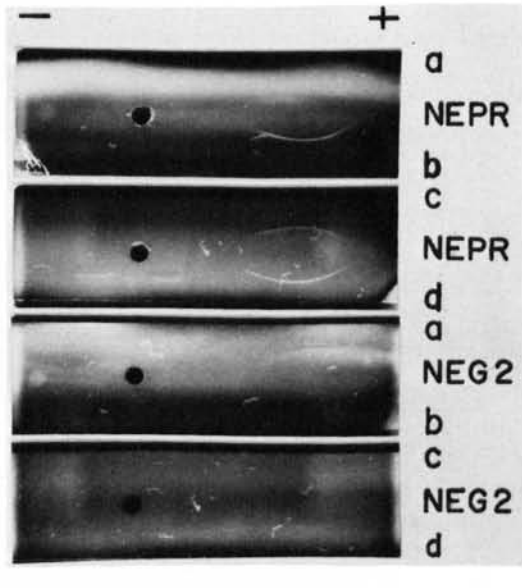

FIG. 4. Immunoelectrophoretic patterns of another preparation of NEPR and NEG2 against anti-HS serum (trough a), anti-NE serum (trough b), anti-NE serum absorbed with HS (trough c) and anti-leproma serum (Lm) absorbed with HS (trough d). Electrophoresis was performed in a medium of 1% Agarose for 45 minutes at 200 volts.

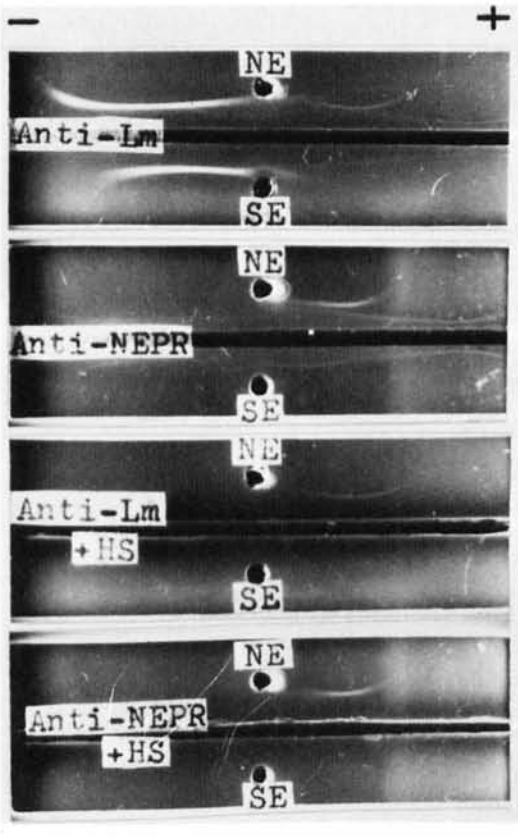

FIG. 5. Immunoelectrophoretic patterns of leprosy nodule-extract (NE) and skin extract (SE) against anti-Lm, anti-NEPR sera and those absorbed with human serum (HS). Conditions of electrophoresis were the same as described in the legend of Figure 3.

antibodies against antigens other than NEPR. The results are shown in Figure 5. Anti-Lm serum produced several precipitation lines against both NE and SE, and one of these lines on the anode side was characteristic for NE, as demonstrated by anti-NE serum. Anti-NEPR serum gave a similar precipitation line with NE and also yielded another line with both NE and SE. This fact seemed to indicate that NEPR still contained some impurities which could produce antibodies in rabbits. These antibodies were easily absorbed by the addition of 1/20 volume of human serum. On the other hand, anti-Lm serum absorbed with human serum produced still another precipitation line with NE near the point of origin, besides the line corresponding to NEPR. The nature of the antigen having slow electrophoretic mobility is not yet clear.

Figure 6 indicates the immunoelectrophoretic differentiation between NEPR and C-reactive protein (CRP). Sera of leprosy patients with *erythema nodosum leprosum* (ENL) produced a precipitation line near the well against anti-CRP serum, but not against anti-NE absorbed with human serum. On the other hand, NEPR did not react at all with anti-CRP serum. Differentiation of NEPR from the α and β antigens of *M. tuberculosis* isolated by Fukui and Yoneda ([7]) was accomplished by means of micro-immunodiffusion tests. The results are shown in Figure 7. Neither anti-α nor anti-β serum reacted with NEPR and neither α nor β reacted with any of anti-NE, anti-Lm and anti-NEPR sera.

Antigenicity in passive hemagglutination tests. NEPR was absorbed to the surface of formalinized, tanned sheep erythrocytes and gave rise to high agglutination titer with anti-NE serum as indicated in Table 1. Minimum concentration of the antigen giving maximum agglutination titer was

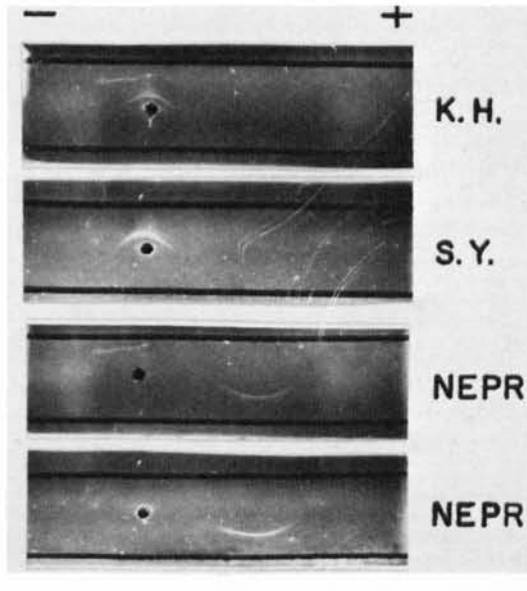

FIG. 6. Immunoelectrophoretic patterns of sera of leprosy patients with ENL (K.H. and S.Y.) and two preparations of NEPR. The upper trough of each plate filled with anti-CRP serum and the lower with anti-NE serum absorbed with human serum. Conditions of electrophoresis were the same as described in the legend of Figure 4.

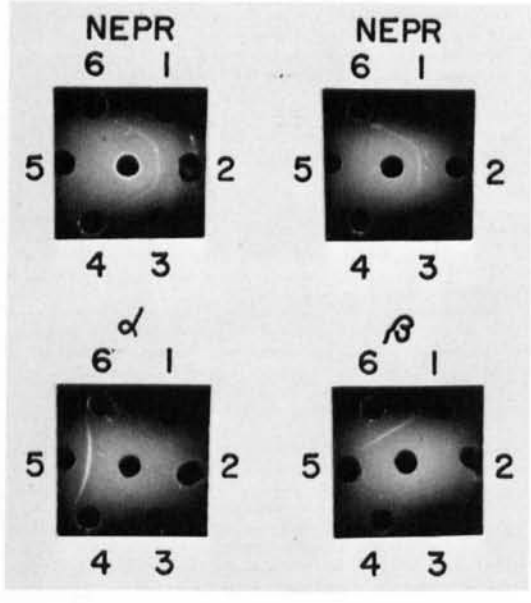

FIG. 7. Micro-immunodiffusion tests using two preparations of NEPR, α and β antigens of *M. tuberculosis* in the respective central wells, against various antisera in the surrounded wells. Well 1 of each plate is filled with anti-NE, well 2 with anti-NEPR, well 3 with anti-Lm, absorbed with human serum, respectively, well 4 with rabbit and anti-*M. tuberculosis* serum, well 5 with anti-α serum and well 6 with anti-β serum.

considered to be 0.125 mg/ml. Erythrocytes treated with this same concentration of antigen were also agglutinated by anti-NE serum absorbed with human serum in the same titer as unabsorbed anti-NE serum. However, passive hemagglutination tests using this suspension of erythrocytes were negative with sera from the 50 leprosy patients thus far tested.

Activity causing delayed skin-hypersensitivity in guinea pigs. Using guinea pigs sensitized with lepromin or BCG in liquid paraffin, the activity of NE and its zone-electrophoretic fractions (F1, F2, F3 and F4) causing cutaneous reactions were compared with that of lepromin and culture-filtrate proteins of BCG (BCGFP). Table 2 indicates the size of reactions at 24 hours after the injection of the antigens. In the animals sensitized with lepromin, F3 produced the largest mean value among four fractions of NE, and F2 as well as F4 showed positive reactions of more than ten millimeters diameter. In contrast, guinea pigs sensitized with BCG caused positive reaction with F3 but not with F2 or F4.

TABLE 1. *Hemagglutination of formalinized tanned sheep erythrocytes sensitized with "nodule extract protein" (NEPR).*

Concentration of NEPR for sensitization (mg/ml)	Agglutination titer of anti-NE serum
1.0	409,600
0.5	102,400
0.25	102,400
0.125	102,400
0.063	25,600
0.032	25,600
0.016	25,600
0.008	1,600
0	0
0.125	102,400[a]

[a] The titer of anti-NE serum absorbed with human serum.

The antigenicity of NEPR and NEG2 was tested in another group of guinea pigs sensitized with BCG or *M. leprae* mixed with incomplete adjuvant. The results are shown in Table 3. The mean size of reactions to NEPR was significantly smaller than that to lepromin in guinea pigs sensitized with *M. leprae*. However, no significant difference was found between NEPR and NEG2. In the guinea pigs sensitized with BCG, a difference between the average sizes of reactions to NEPR and NEG2 was statistically significant at $P < 0.05$.

Comparative skin tests with leprosy patients. NEPR activity in eliciting the Fernández reaction in leprosy patients was compared with that of lepromin (Mitsuda type) and Dharmendra's antigen. The size of reactions are shown in Figure 8. The six patients showing reactions less than 10 mm in diameter against both of the antigens were lepromatous cases. In contrast, NEPR caused reactions of 10 mm or more in two cases of tuberculoid leprosy and in some lepromatous cases who gave positive Fernández reactions against lepromin or Dharmendra's antigen. In another group of patients, comparative skin tests were performed using NEPR, NEG2, leprolin and

TABLE 2. *Cutaneous reactions of guinea pigs to zone-electrophoretic fractions of leprosy nodule extract (NE).*

Guinea pigs sensitized with:	Animal number	Size of reaction (in mm) at 24 hours after injection of: Lepromin	NE	NE-F1	NE-F2	NE-F3	NE-F4	BCG-FP
Lepromin	1	23	26	10	13	17	14	
	2	23	22	7	11	16	13	
	3	20	29	8	17	20	15	
	4	23	26	9	15	22	16	
	Mean	22.3	25.8	8.5	14.0	18.8	14.5	
BCG	5	14	16		4	12	3	24
	6	16	9		4	8	0	19
	7	16	12		6	8	0	17
	8	14	12		5	10	3	21
	9	15	15		6	13	2	23
	Mean	15.0	12.8		5.0	10.2	1.6	20.8

TABLE 3. *Cutaneous reactions of guinea pigs to lepromin and soluble antigens isolated by gel-filtration.*

Guinea pigs sensitized with *M. leprae*	Size of reaction (mm) at 24 hours after the injection of: Lepromin	NEPR	NEG2	Guinea pigs sensitized with BCG	Size of reaction (mm) at 24 hours after the injection of: Lepromin	NEPR	NEG2
No. 1	10	9	12	14	8	4	11
2	12	10	12	15	19	10	19
3	14	9	15	16	7	9	15
4	12	12	15	17	18	7	13
5	16	9	15	18	15	10	14
6	18	13	10	19	14	7	8
7	12	15	16	20	12	5	15
8	15	13	13	21	12	4	11
9	8	5	3	22	11	9	9
10	11	7	5	23	15	14	12
11	15	6	4	24	7	9	5
12	13	4	4	25	11	12	10
13	8	5	4	26	14	11	10
Mean	12.6	9.0	10.2	Mean	12.5	8.5	11.7
Difference	3.6	1.2		Difference	4.0	3.2	
Level of significance	<0.01	>0.05		Level of significance	<0.01	<0.05	

tuberculin. The results are given in Table 4. The size of reaction to NEG2 was correlative with that to tuberculin, while the reaction to NEPR rather resembled that of leprolin. These reactions became negative in most instances at two weeks after the injection of antigens. Table 5 indicates the values of correlation coefficient (r) and correlation ratio (η) calculated on the data shown in Figure 8 and Table 4 and the results of statistical evaluations on these values. All of r were significant at the level less than 0.05, while η was significant only in the correlation between NEG2 and tuberculin.

DISCUSSION

It was presumed that the water-soluble

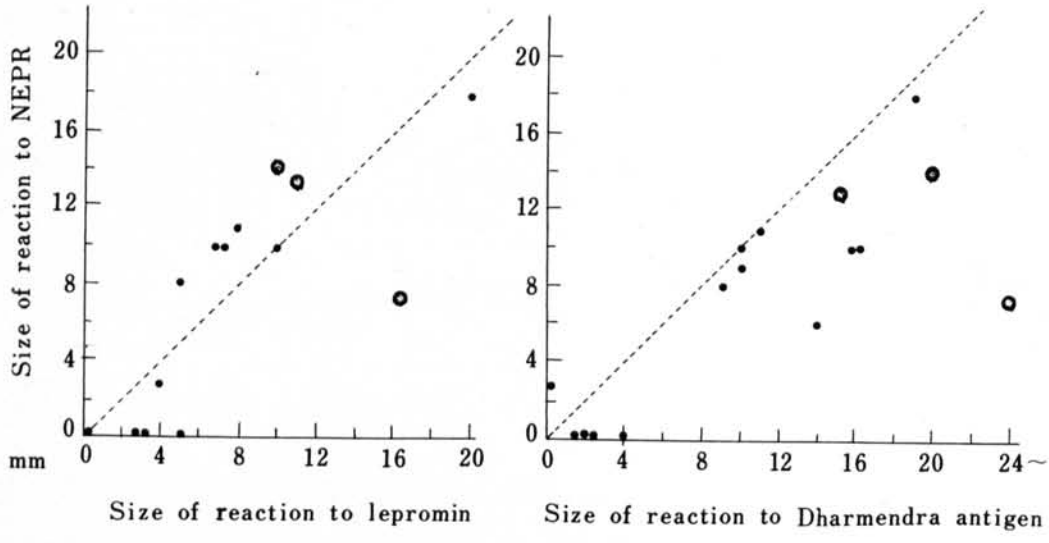

FIG. 8. Correlation between Fernandez' reactions of leprosy patients to NEPR and those to lepromin or Dharmendra's antigen. Solid points indicate lepromatous cases and circles indicate tuberculoid.

TABLE 4. *Comparative skin tests in leprosy patients.*

Patients	Disease type[a]	48 hours reading (mm) of the reaction to: Lepromin	NEPR	NEG2	Tuberculin
K.K.	L	0	0	0	0
M.Y.	L	0	0	0	0
T.M.	L	0	0	0	9
D.I.	L	9	3	1	4
T.M.	L	6	3	0	13
J.Y.	L	0	0	11	16
M.B.	L	4	3	16	17
T.H.	L	4	5	31	30
H.Y.	T	17	6	12	11
Y.I.	T	11	10	13	12
R.O.	B	18	8	27	32

[a] L-lepromatous; T-tuberculoid; B-borderline.

extract of leprosy nodules contained several antigens which might originate in both human tissue and in *M. leprae.* Therefore, it was necessary at first to purify the significant antigen in order to clarify its origin. By using starchblock zone-electrophoresis and Sephadex G200 gel-filtration, a specific protein antigen from leprosy nodules (NEPR) was purified to the extent that it gave a single precipitation line on immunoelectrophoresis. Its molecular weight seemed to be in the range of 200,000—1,000,000 as judged from the pattern of gel-filtration. Rabbits readily produced antibodies against this protein after immunization with the purified antigen and even with a crude suspension of leprosy nodules. This fact seemed to indicate a strong antigenicity in rabbits and stability during pur-

TABLE 5. *Statistical evaluation of the correlation between two kinds of skin reactions in leprosy patients.*

Antigens compared	Number of patients	Correlation coefficient r	ts[a]	Correlation ratio η	Fs[b]
Lepromin—NEPR	14	0.697	3.36**	0.837	2.27
Dharmendra—NEPR	16	0.665	3.57**	0.904	3.76
Leprolin—NEPR	11	0.610	2.31*	0.744	1.96
Tuberculin—NEG2	11	0.895	6.00**	0.890	5.38*

[a] Fisher's t-distribution. ** and * indicate levels of significance less than 0.01 and 0.05, respectively.
[b] Fisher's F-distribution.

ification. This antigen was rarely found in material where the bacillary count was very low. This fact does not exclude the possibility that NEPR may be a substance originating in human tissue, because abnormal proteins may appear in lepromatous tissues with the multiplication of bacilli. C-reactive protein (CRP) may be one of such abnormal proteins. However, it was found that CRP differed from NEPR in immunoelectrophoretic behavior. NEPR also seemed to be distinguishable from the α-antigen of *M. leprae* described by Ito ([8]). This fact may not be discrepant with the specificity of NEPR reported previously ([1]), even if it is assumed that this protein may be derived from *M. leprae*. Evidence supporting this hypothesis was obtained by examining cutaneous reactions in guinea pigs sensitized with BCG or *M. leprae* and in leprosy patients.

A delayed type skin hypersensitivity to an antigen originating in human tissue might occur in guinea pigs sensitized with lepromin or a suspension of bacilli in which a considerable amount of tissue component is present. However, NEPR caused similar reactions in guinea pigs sensitized with BCG which contained no antigen from human tissue. Cutaneous reactions to NEPR in leprosy patients correlated with the Fernández reaction to lepromin and Dharmendra's antigen in the same patients. These facts seem to indicate that NEPR is a protein derived from *M. leprae* and that its activity as a skin test antigen is common to *M. leprae* and BCG. The latter property disagrees with the specificity of NEPR in precipitation reactions. This may be explained by artificial change of specificity after autoclaving because of heat-lability of NEPR.

The common reactivity of lepromin and Dharmendra's antigen in *M. leprae* and BCG infections may also be due to the presence of antigen(s) other than NEPR. In fact, another fraction NEG2 was separated from NEPR by Sephadex G200 gel-filtration. This fraction showed stronger reactions than NEPR in guinea pigs sensitized with BCG, while there was no significant difference between the two in guinea pigs sensitized with *M. leprae*. In leprosy patients, too, cutaneous reactions to NEG2 were more correlative to tuberculin than lepromin. Accordingly, it is thought that NEG2 may be an antigen common to *M. leprae* and *M. tuberculosis*. As the yield of this antigen by gel-filtration was very low, it was not possible to determine by precipitation reactions with known antisera, whether or not this antigen is the same as the α-antigen found by Ito ([8]) or whether it may be the heat-stable polysaccharide reported previously ([1]). In any case, the presence of such a common antigen in leprosy bacilli may be regarded as the basis of cross-reactivity between tuberculin and lepromin.

An alternative hypothesis concerning the origin of NEPR is that this antigen may be produced by interaction between both substances of *M. leprae* and host cells. For instance, there may be a possibility that a waxy substance such as lipopolysaccharide of *M. leprae* forms a complex with certain proteins of host cells and acquires a new serologic specificity which is different from that of the respective antigenic components. Such an antigen-complex might readily produce antibodies in rabbits, for it is heterologous to these animals; while its antigenicity in the human body may be not so strong, because a part of the complex is autologous to the antibody-producing cells. The fact that passive hemagglutination tests using NEPR are negative to the sera of leprosy patients seems to support the above hypothesis.

SUMMARY

A protein substance in the water-soluble extract of leprosy nodules was purified by starch-block zone-electrophoresis and by Sephadex G200 gel-filtration. Its immunologic activities were investigated. Antigenic specificity of this substance in immunodiffusion tests was quite different from that of C-reactive proteins in the serum of lepromatous patients and from that of extracellular proteins of *M. tuberculosis*. However, the heated solution of proteins purified from leprosy nodule-extracts caused skin reactions of the delayed type in guinea pigs sensitized with *M. leprae* or BCG. It also elicited Fernández reactions in leprosy pa-

tients, the reactions being well correlative with that caused by lepromin, leprolin and Dharmendra's antigen. The leprosy nodule-extract contained another substance which gave positive skin reactions in guinea pigs sensitized with BCG and good correlation with tuberculin reactions in leprosy patients. These facts seemed to indicate that both antigens in leprosy nodule-extracts may be derived from *M. leprae.*

RESUMEN

Utilizando los métodos de electroforesis de zona en bloques de almidón y filtración en gel de Sephadex G200, se purificó una sustancia proteica del extracto soluble en agua de nódulos de lepra. Se estudiaron sus actividades inmunológicas. La especificidad antigénica de esta sustancia en pruebas de inmunodifusión fué bastante diferente de la de las proteinas C-reactivas en el suero de pacientes lepromatosos y de las de las proteinas extracelulares del *M. tuberculosis.* Sin embargo, la solución de proteinas purificada a partir de extractos de nódulos de lepra, después de ser calentada, produjo reacciones intradérmicas de tipo tardío en cobayos sensibilizados con *M. leprae* o BCG. También produjo reacciones de Fernandez en pacientes con lepra; estas reacciones tenían una buena correlación con las producidas por lepromina, leprolina y el antígeno de Dharmendra. El extracto de nódulos de lepra contenía otra sustancia que daba reacciones intradérmicas positivas en cobayos sensibilizados con BCG y una buena correlación con la reacción de tuberculina en pacientes con lepra. Estos hechos parecen indicar que ambos antígenos en los extractos de nódulos de lepra pudieran derivarse del *M. leprae.*

RÉSUMÉ

Au moyen d'une technique d'électrophorèse par zone sur bloc d'amidon, et de la filtration sur gel Sephadex G200, on a purifié une substance protéinique dans un extrait aqueux de nodules de la lèpre. Les activités immunologiques de cette substance ont été étudiées. La spécificité antigénique de cette substance, étudiée par des épreuves d'immuno-diffusion, était totalement différente de celle relevée pour les protéines C-réactives du sérum de malades lépromateux et de celle observée avec les protéines extra-cellulaires de *M. tuberculosis.* Néanmoins, la solution chauffée de protéines purifiées à partis d'extraits de nodules de la lèpre, a entraîné des réactions cutanées du type retardé chez des cobayes sensibilisés par *M. leprae* ou par le BCG. Cette solution a également induit une réaction de Fernandez chez les malades de la lèpre cette dernière montrant une bonne corrélation avec les réaction produites par la lépromine, par la léproline, ou par l'antigène de Dharmendra. L'extrait de nodules de la lèpre contenait une autre substance qui donnait des réactions cutanées positives chez les cobayes sensibilisés par le BCG, et qui témoignait d'une bonne corrélation avec les réactions tuberculiniques chez des malades de la lèpre. Ces observations paraissent indiquer que l'un et l'autre de ces antigènes obtenus dans les extraits de nodules de la lèpre pourraient provenir de *M. leprae.*

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