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Immunological Relationship between Leprosy and Murine Leprosy
I. Antigenicity of Leprosy Bacillus and Murine Leprosy Bacillus in the Skin Reaction inGuinea Pigs

SHINJI NISHIMURA, KENJI KOSAKA AND TADAO YASUKAWA

Department of Leprosy, Research Institute for Microbial Diseases, Osaka University, and Department of Dermatology, Osaka University Medical School, Osaka

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SUMMARY

Studies were conducted on the immunological relationship between leprosy and murine leprosy. This problem is important in appraising results of experiments using the murine leprosy bacillus.

Guinea pigs were sensitized with leprosy bacillus, murine leprosy bacillus and BCG, and the Lepromin, Murine Lepromin and Tuberculin reactions examined. Cross immunization was found to occur showing the presence of an immunological interrelationship between these three types. The murine leprosy sensitized and BCG sensitized animals gave the strongest reaction towards the homologous antigen, and lepromin gave values closer to the murine lepromin than to tuberculin.

INTRODUCTION

The problem of whether immunity towards leprosy is gained by inoculation of BCG has become important in relation to the prevention of leprosy. Most previous reports deal with clinical and immunological problems and there are few basic studies in animals.

Murine leprosy has been used for the most part in model experiments on immunity in animals and Muir and Henderson (1927) reported that BCG was ineffective in preventing murine leprosy. Azulay (1954) however, reported the successful prevention of the onset of murine leprosy by inoculation of BCG subcutaneously or intraperitoneally into rats. Hadler and Ziti (1954), on the other hand, reported that oral or intramuscular administration of BCG failed to prevent infection, and suggested that there was no alteration in the antibacterial activity of the macrophages against the murine leprosy bacillus in the rat.

Nishimura (one of the present authors), Kono, Kodama, Yanagisawa and Nojima (1956) have shown that intraperitoneal injection of BCG into the rat has a greater effect than the subcutaneous injection in suppressing the onset of leprosy. Nishimura and Kodama (1957a, 1957b) obtained similar results in the mouse, and it was shown that blockage of the reticuloendothelial system with indian ink reduced the immunological action. Nishimura, Oshima and Yanagisawa (1958) have shown that the immunity against murine leprosy by BCG lasts for a con-

siderable length of time. This production of immunity by BCG strongly suggests an immunological relationship between murine leprosy and tuberculosis. Kawaguchi (1955) has conducted cross resistence studies in the mouse on the tubercle bacillus and the murine leprosy bacillus and found that animals sensitized with a virulent tubercle bacillus show a strong resistence against the challenge with a virulent murine leprosy bacillus. Animals sensitized with the murine leprosy bacillus showed a weak resistence against tuberculosis infection.

There are as yet no reports on the immunological relationship between leprosy and murine leprosy. If a relation could be found between the two, the significance of experiments on murine leprosy would be greater. In the present paper, the antigenicity of the leprosy bacillus and murine leprosy bacillus was studied in animals by the skin reaction.

Rodriguez (1938) studied the lepromin reaction in animals and reported that there was no reaction in the cat, monkey, fowl, pig or rat but a considerable reaction was obtained in the rabbit, kid and full grown dog. Tanimura (1952) has also reported no reaction in the rat with murine leprosy and Convit *et al.* (1955) stated that the hamster was unsuitable for lepromin reaction experiments. Recently, Yanagisawa and Asami (1958) have sensitized the guinea pig, the animal which is most suitable for allergy experiments, with human leprosy bacillus collected by the Trypsin-digestion method and found that a lepromin reaction was induced. Asami *et al.* (1959a, 1959b) have studied the skin reaction in the guinea pig further using various antigen fractions from acid-fast bacilli. The authors, therefore, selected the guinea pig for use in the present experiments.

MATERIALS AND METHODS

Experimental animals

Female albino guinea pigs, weighing 350–400g, bred in the Shizuoka prefecture, were tested with an intracutaneous injection of 0.1 ml of a 1:100 solution of old tuberculin and only those giving a negative response were used.

Sensitizing antigen

a) *Heat-killed-liquid paraffin suspensions of leprosy bacillus*

Leproma, excised from patients, was heated for 10 minutes at 100°C, homogenized in physiological saline, and collected in pure form by the Trypsin-digestion-ultracentrifugation method. 10mg of the wet bacilli were suspended in 0.5 ml of liquid paraffin.

b) *Heat-killed-dried-liquid paraffin suspensions of leprosy bacillus*

Bacilli collected as above were dried in a dessicator, ground thoroughly in an agate mortar and 10mg of the powder suspended in 0.5 ml of liquid paraffin.

c) *Live bacterial suspensions of murine leprosy bacillus*

Fresh leproma, produced by subcutaneous injection of the Kumamoto strain into rats, was excised, finely ground in a mortar, and homogenized in physiological saline. The bacteria were collected by centrifugation and 10mg suspended in 0.5 ml of saline.

d) *Heat-killed-dried-liquid paraffin suspensions of murine leprosy bacillus*

The above bacteria was heated for 10 minutes at 100°C, dried in a dessicator, and ground thoroughly in an agate mortar. They were suspended in liquid paraffin at a concentration of 10mg per 0.5 ml.

e) *BCG*

BCG, serially maintained in Calmett medium (convaining glycerin ox bile-potato

medium) from the Department of Tuberculosis Research was cultivated in 2 per cent KH_2PO_4--Ogawa medium for 3 weeks. A homogenous suspension in sterile distilled water was obtained by carefully grinding the cells in an agate mortar and a suspension containing 1 mg/0.5 ml was prepared.

Method of sensitization

The method used is shown in Table 1.

Table 1. Method of Sensitization

Exp.	Group	No. of Guinea pigs in group	Antigen	Quantity	Site
I	A	10	Live bacteria suspension of murine leprosy bacillus	10mg/0.5ml	Inguinal subcutan.
	B	10	Heat-killed-dried-liquid paraffin suspension of murine leprosy bacillus	10mg/0.5ml	
	C	10	Suspension of BCG	1.0mg/0.5ml	
	D	10	Untreated control		
II	E	6	Heat-killed-liquid paraffin suspension of leprosy bacillus	10mg/0.5ml	Femoral intramuscul.
	F	6	Heat-killed-dried-liquid paraffin suspension of murine leprosy bacillus	10mg/0.5ml	
	G	6	Suspension of BCG	1.0mg/0.5ml	Abdominal subcutan.
	H	6	Untreated control		
II	I	6	Heat-killed-dried-liquid paraffin suspension of leprosy bacillus	10mg/0.5ml	Femoral intramuscul.
	J	6	Heat-killed-dried-liquid paraffin suspension of murine leprosy bacillus	10mg/0.5ml	
	K	6	Suspension of BCG	1.0mg/0.5ml	Abdominal subcutan.
	L	6	Untreated control		

Skin reaction antigen

a) *Lepromin*

200 γ/ml of lepromin prepared at the National Institute for Leprosy and tested at the National Institute of Health was diluted with 0.5 per cent phenol in physiological saline. Suspension containing 25γ/0.125 ml, 10γ/0.1 ml and 4γ/0.1 ml were prepared.

b) *Murine lepromin*

The dried, killed bacteria collected by the method described above were suspended in 0.5 per cent phenol in physiological saline and 3 concentrations (25γ/0.1 ml, 10γ/0.1 ml and 4γ/0.1 ml) Prepared by the 2.5-fold dilution method.

c) *Tuberculin*

Old tuberculin prepared at the National Institute of Health was diluted 100-fold, 250-fold and 625-fold with 0.5 per cent phenol in physiological saline and 0.1 ml aliquots used.

Intracutaneous injection method

Two days before the injection the hair of the back and side of the animal was shaved,

depilated with depilating cream and the area covered with vaseline to protect the skin. 0.1 ml of each of the 3 types of antigen (0.125 ml in the case of the 25γ lepromin), serially diluted 2.5-fold, that is, 9 antigen solutions were injected intracutaneously into the hairless area. They were injected at distances of 1.5 cm from each other. The reaction might be affected by the site of the injection so care was taken that the same antigen was not always injected into the same site.

Evaluation of the reaction

Antigen was injected 4 weeks and 6 weeks after sensitization and the diameter of the induration and red area measured with the micrometer 24 and 48 hours after the injection. The average for each group was then calculated.

RESULTS

The strongest reaction was observed in Exps. I, II and III 6 weeks after sensitization and the 24 hours reaction was stronger than the 48 hours one.

a) *Murine leprosy bacillus sensitized group*

The reactions toward the various antigens of animals sensitized with the heat treated murine leprosy bacillus liquid paraffin emulsions are shown in Table 2. In Exps. I, II and III, the greatest reaction was observed 24 hours after the injection, both 4 weeks and 6 weeks after sensitization, with the murine lepromin. There was a significant difference from the lepromin and tuberculin reactions. Lepromin gave a stronger reaction than tuberculin. The more concentrated the antigen the strongest was the reaction and with all three antigens the reaction was stronger than in the untreated controls (Table 6). Table 3 shows the reaction against the various antigens in animals sensitized with live murine leprosy bacillus in Exp. I. The red area was about the same size as in the group treated with killed bacillus liquid paraffin suspension. The reaction was stronger with all three antigens than in the control group.

b) *Group sensitized with suspension of killed leprosy bacillus in liquid paraffin*

The reaction against the various antigens are shown in Table 4. A reaction in proportion to the concentration of the antigen was observed 24 hours after the injection, both 4 and 6 weeks after sensitization. The average reaction decreased as the injection decreased (25γ, 10γ and 4γ injections).

In Exp. II, the murine lepromin gave the strongest reaction, lepromin the next strongest and tuberculin the weakest reaction. In Exp. III, the reaction was strongest against lepromin, moderately strong against murine lepromin and weak against tuberculin. The strength of the reaction was in proportion to the concentration of antigen. The weak reaction in the case of tuberculin was significantly greater than that of the control. It is understandable that the reaction in the leprosy bacillus sensitized animal is strongest against lepromin but in Exp. II, the reaction against the murine lepromin is stronger than that against lepromin. The cause for this is not clear but in the leprosy bacillus sensitized animal, the reactions against lepromin and murine lepromin are quite similar while the reaction against tuberculin is weak. The reaction is weak 48 hours after the injection in all cases.

Table 2. Skin Reaction in the Killed-Liquid Paraffin Suspension of Murine Leprosy Bacillus Sensitized Guinea Pigs (mm)

Time after sensitization	Time after injection	4 weeks						6 weeks								
Experiment		II Group F (5 animals)			III Group J (3 animals)			I* Group B (10 animals)			II Group F (5 animals)			III Group J (3 animals)		
Concentration of antigen		25γ	10γ	4γ	25γ	10γ	4γ	25γ	10γ	4γ	25γ	10γ	4γ	25γ	10γ	4γ
Dilution of Tuberculin / Antigen		×100	×250	×625	×100	×250	×625	×100	×250	×625	×100	×250	×625	×100	×250	×625
Lepromin	24 hrs.	† 8.3	6.3	5.1	8.5	7.5	6.8	5.1	4.5	3.7	10.7	6.5	5.9	10.8	7.5	6.0
		††(0.83)	(0.37)	(0.40)	(0.50)	(0.50)	(0.43)	(0.70)	(0.59)	(0.60)	(0.81)	(0.83)	(0.80)	(0.44)	(0.50)	(0.70)
	48 hrs.	5.7	4.9	3.6	5.3	5.0	4.2	4.2	3.4	2.8	7.6	5.4	4.4	8.3	5.8	5.0
		(0.79)	(0.59)	(0.55)	(0.98)	(0.70)	(0.78)	(0.60)	(0.53)	(0.55)	(0.37)	(0.20)	(0.20)	(0.44)	(0.44)	(0.70)
Murine lepromin	24 hrs.	14.3	10.4	9.2	15.0	14.0	11.8	10.2	9.0	7.9	13.8	12.2	9.8	15.0	13.8	10.8
		(0.10)	(0.45)	(0.98)	(0.70)	(0.60)	(0.50)	(0.90)	(0.67)	(0.70)	(0.50)	(0.50)	(0.87)	(0.70)	(0.45)	(1.28)
	48 hrs.	10.2	8.4	5.4	11.0	10.8	10.0	6.5	5.3	4.6	10.5	8.5	7.2	10.3	9.3	7.8
		(1.11)	(0.80)	(0.61)	(0.70)	(0.83)	(0.70)	(0.70)	(0.40)	(0.53)	(0.94)	(0.63)	(0.40)	(0.44)	(0.37)	(0.43)
Tuberculin	24 hrs.	4.9	4.2	3.6	5.8	5.5	4.2	4.7	4.3	3.7	7.0	5.1	4.6	8.5	8.0	7.3
		(0.77)	(0.67)	(0.61)	(0.44)	(0.50)	(0.78)	(0.71)	(0.40)	(0.55)	(0.89)	(0.58)	(0.91)	(0.50)	(0)	(1.12)
	48 hrs.	3.1	2.5	2.4	5.7	4.0	3.5	3.1	3.0	2.7	5.2	2.8	2.2	6.5	5.8	4.5
		(0.87)	(0.70)	(0.47)	(0.87)	(0.70)	(0.50)	(0.30)	(0.22)	(0.33)	(0.74)	(0.40)	(0.81)	(0.50)	(0.95)	(0.50)

* In the case of 1 Group B, skin tests were done at 6 weeks but not at 4 weeks

Data: † Mean

†† (σ =): Standard Deviation of Sample

Table 3. Skin Reaction of Murine Leprosy Bacillus Sensitized Guinea Pigs to Live Bacteria Suspension (mm) (After 6 Weeks)

Experiment / Antigen	Time after injection	I Group A (10 animals)		
Concentration of antigen		25γ	10γ	4γ
Dilution of Tuberculin		×100	×250	×625
Lepromin	24 hrs.	† 5.3 ††(0.91)	4.8 (0.74)	4.2 (0.76)
	48 hrs.	3.7 (1.01)	3.4 (0.78)	2.9 (0.94)
Murine lepromin	24 hrs.	10.7 (0.59)	9.2 (0.84)	7.9 (0.80)
	48 hrs.	6.8 (0.71)	6.1 (0.37)	5.0 (0.44)
Tuberculin	24 hrs.	5.4 (0.81)	4.9 (0.30)	2.7 (0.51)
	48 hrs.	3.6 (0.43)	2.9 (1.04)	2.8 (0.40)

Data: † Mean
††(σ=): Standard Deviation of Sample

c) *BCG sensitized group*

As can be seen in Table 5, the tuberculin reaction was the strongest 24 hours after the injection in Exps. I, II and III, and there was a marked difference compared to the other antigens. The value however, is smaller than that of the reaction in the murine leprosy sensitized animal. The reaction against lepromin and murine lepromin is much weaker than the tuberculin reaction but the reaction against murine lepromin is greater than against lepromin. The reaction was again in proportion to the concentration of antigen.

d) *Untreated controls*

As can be seen in Table 6, the reaction against the various antigens is weaker in Exp. I than in Exps. II and III. The fact that there was little difference in reaction between the various antigens or with various concentrations of antigen is noteworthy. It was also found that the reddening had almost disappeared 48 hours after the injection.

DISCUSSION

From the results in Exps. I, II and III, it can be seen that the murine leprosy bacillus sensitized animals give the strongest reaction to murine lepromin and BCG sensitized animals react most strongly to tuberculin. The reaction in the leprosy bacillus sensitized animals was weak compared to the other two groups but greater than the reaction in the controls, and the reaction was stronger

Table 4. Skin Reaction of Leprosy Bacillus Sensitized Guinea Pigs to Killed-Liquid Paraffin Suspension (mm)

Time after sensitization	Time after injection	4 weeks						6 weeks					
Experiment		II Group E (5 animals)			III Group I (6 animals)			II Group E (5 animals)			III Group I (6 animals)		
Concentration of antigen / Dilution of Tuberculin / Antigen		25γ	10γ	4γ	25γ	10γ	4γ	25γ	10γ	4γ	25γ	10γ	4γ
		×100	×250	×625	×100	×250	×625	×100	×250	×625	×100	×250	×625
Lepromin	24 hrs.	† 5.1	3.8	3.3	8.7	8.0	6.8	6.8	5.1	4.8	10.7	7.8	6.3
		††(0.34)	(0.92)	(0.60)	(0.55)	(0.54)	(0.92)	(0.40)	(0.73)	(0.60)	(0.71)	(0.97)	(0.24)
	48 hrs.	2.7	2.2	2.0	4.7	4.3	3.8	5.1	3.0	2.7	6.8	5.3	4.0
		(0.20)	(0.52)	(0.28)	(0.64)	(0.54)	(0.60)	(0.86)	(0.32)	(0.40)	(0.74)	(0.71)	(0.54)
Murine lepromin	24 hrs.	5.0	4.4	3.6	8.3	7.3	7.1	8.0	7.0	6.4	8.8	7.0	6.3
		(0.76)	(0.76)	(0.32)	(0.84)	(0.84)	(0.30)	(0.89)	(0.54)	(0.58)	(0.80)	(0.77)	(0.47)
	48 hrs.	2.4	2.3	1.7	4.2	3.7	2.5	5.6	4.5	3.6	5.1	4.7	4.3
		(0.52)	(0.84)	(0.14)	(0.74)	(0.50)	(0.55)	(0.80)	(0.83)	(0.48)	(0.88)	(0.75)	(0.71)
Tuberculin	24 hrs.	3.0	2.6	2.3	5.2	5.0	4.5	5.1	4.7	4.2	6.3	4.5	4.3
		(0.28)	(0.67)	(0.52)	(0.81)	(0.54)	(0.50)	(0.80)	(0.97)	(0.40)	(0.98)	(0.50)	(0.71)
	48 hrs.	1.7	1.6	1.0	3.8	3.5	3.3	2.8	2.6	2.4	3.5	3.0	2.5
		(0.47)	(0.51)	(0.83)	(0.81)	(0.50)	(0.64)	(0.50)	(0.37)	(0.73)	(0.50)	(0)	(0.50)

Data : † Mean

†† ($\sigma =$) : Standard Deviation of Sample

Table 5. Skin Reaction in the BCG Sensitized Guinea Pigs (mm)

Time after sensitization		4 weeks						6 weeks								
Experiment	Time after injection	II Group G (5 animals)			III Group K (3 animals)			I* Group C (10 animals)			II Grpup G (5 animals)			III Group K (3 animals)		
Concentration of antigen		25γ	10γ	4γ	25γ	10γ	4γ	25γ	10γ	4γ	25γ	10γ	4γ	25γ	10γ	4γ
Dilution of Tuberculin / Antigen		×100	×250	×625	×100	×250	×625	×100	×250	×625	×100	×250	×625	×100	×250	×625
Lepromin	24 hrs.	† 7.9	6.3	5.7	6.0	5.7	3.7	5.0	3.9	3.6	9.0	6.7	5.1	6.7	5.0	4.3
		††(0.88)	(0.74)	(0.97)	(0.77)	(0.61)	(0.50)	(0.50)	(0.37)	(0.48)	(1.10)	(1.28)	(1.16)	(1.01)	(0.77)	(0.71)
	48 hrs.	6.8	5.3	4.5	4.7	4.0	2.0	3.2	2.9	2.6	6.3	4.4	3.4	5.7	3.7	2.7
		(1.08)	(0.70)	(0.40)	(0.44)	(0.40)	(0.40)	(0.40)	(0.37)	(0.48)	(0.94)	(0.82)	(0.62)	(0.96)	(0.80)	(0.71)
Murine lepromin	24 hrs.	6.4	6.3	5.3	3.7	3.3	2.7	5.1	4.9	3.9	7.6	6.2	5.1	8.7	6.7	6.3
		(0.70)	(0.80)	(0.64)	(0.50)	(0.64)	(0.10)	(0.62)	(0.48)	(0.62)	(1.11)	(0.81)	(1.10)	(1.15)	(1.01)	(0.80)
	48 hrs.	6.0	5.2	4.4	3.0	2.7	2.7	3.7	3.5	3.1	5.4	4.2	3.0	6.3	4.7	4.3
		(1.07)	(0.95)	(0.76)	(0)	(0.63)	(0.24)	(0.40)	(0.44)	(0.37)	(0.97)	(0.69)	(0.50)	(0.47)	(0.53)	(0.64)
Tuberculin	24 hrs.	14.9	13.0	10.3	8.3	7.3	7.0	8.6	8.0	6.0	13.4	11.6	8.3	11.7	10.7	8.3
		(0.96)	(0.40)	(0.60)	(0.95)	(0.84)	(0.77)	(0.80)	(0.97)	(0.74)	(1.17)	(1.33)	(1.20)	(0.89)	(0.33)	(0.84)
	48 hrs.	10.8	9.8	7.2	7.0	5.0	5.0	5.6	5.3	4.2	9.8	9.1	6.5	8.0	7.3	5.3
		(1.05)	(1.01)	(1.05)	(0.77)	(0)	(0)	(0.66)	(0.17)	(0.33)	(0.62)	(0.50)	(0.76)	(0.25)	(0.84)	(0.10)

* In the case of 1 Group C, skin tests were done at 6 weeks but not at 4 weeks

Data : † Mean

†† (σ =) : Standard Deviation of Sample

Table 6. Skin Reaction in the Untreated Control Guinea Pigs (mm)
(6 Weeks after Sensitization, 24 Hours after Injection)

Experiment	I Group D (10 animals)			II Group H (5 animals)			III Group L (3 animals)		
Concentration of antigen	25γ	10γ	4γ	25γ	10γ	4γ	25γ	10γ	4γ
Dilution of Tuberculin / Antigen	×100	×250	×625	×100	×250	×625	×100	×250	×625
Lepromin	† 2.8	2.4	2.3	4.3	3.4	3.0	4.3	3.7	3.3
	††(0.18)	(0.86)	(0.33)	(0.24)	(0.37)	(0.32)	(0.77)	(1.02)	(0.64)
Murine lepromin	3.5	3.3	2.7	4.3	3.9	3.6	5.7	4.7	4.0
	(0.56)	(0.90)	(0.96)	(0.50)	(0.58)	(0.48)	(0.96)	(1.14)	(0)
Tuberculin	2.7	2.3	2.3	3.5	3.0	2.5	5.3	5.3	4.0
	(0.61)	(0.61)	(0.52)	(0.77)	(0.32)	(0.32)	(0.71)	(0.71)	(0.77)

Data: † Mean
†† (σ=): Standard Deviation of Sample
48 after injection: There was no reaction

against lepromin and murine lepromin than against tuberculin. Examination of the interrelationship between the antigens shows that the leprosy bacillus sensitized animals give a stronger reaction against murine lepromin than against tuberculin, the murine leprosy bacillus sensitized animals give a somewhat stronger reaction against lepromin than against tuberculin and the BCG sensitized animals give a similar reaction against both lepromin and murine lepromin. These findings suggest that there is a common antigenic factor in the leprosy bacillus, murine leprosy bacillus and BCG and that the relation between the leprosy bacillus and murine leprosy bacillus is closer than their relation with BCG. Statistically the $t_{0.05}$ values for 4, 8 and 18 degrees of freedom are 3.2–8.97, 4.8–9.7 and 6.8–15 respectively.

According to Asami *et al.* (1959a, 1959b), who analyzed the antigens of various acid-fast bacilli, the murine leprosy bacillus reacts well with Johne's bacillus and avian and bovine types of tubercle bacillus. Hadler and Ziti (1953) inoculated leprosy and murine leprosy bacilli, collected with chloroform (Dharmendra's method), intraperitoneally into the guinea pig and examined the tuberculin reaction. The results were compared to those obtained in a BCG inoculated group. It was found that a hypersensitivity to tuberculin was induced in the murine leprosy bacillus sensitized and leprosy bacillus sensitized animal. The reaction in the murine leprosy bacillus sensitized animal was greater and close to the results seen in the BCG inoculated animal. It is interesting that Hadler *et al.* obtained results similar to ours even though the animals were sensitized with bacilli isolated by Dharmendra's method, a method by which a large part of the lipid of the bacterial cell is lost.

Finally, it should be noted that the relatively low hypersensitivity seen in these experiments could be increased by using Freund's incomplete adjuvant instead of a single dose of liquid paraffin.

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