STUDIES ON THE IMMUNOLOGY OF MURINE LEPROSY

EFFECTS OF VACCINATIONS AND PREVIOUS INFECTION

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So long as attempts to cultivate the leprosy bacillus and to infect animals, and other fundamental experiments, remain unsuccessful, studies on the immunology of murine leprosy may be considered important. Work in this field has been reported by various authors beginning in 1909—Wherry (6), Markianos (2), Watanabe (5), Sato (3), Urabe (4), and Iketani (1) among others. It has been said that vaccines of heat-killed bacilli or of bacilli extracted of lipids are effective to a certain degree, with respect to both prophylaxis and therapy.

The studies here reported concern (1) the prevention of infection (prophylaxis), and (2) the inhibition (delay) of the onset of leproma formation, by the use of a vaccine of killed murine leprosy bacilli and one to which liquid paraffin had been added to delay absorption. Furthermore (3) superinfection was employed in an attempt to ascertain if there was a possibility of immunization by living bacilli.

MATERIALS AND METHODS

Young white rats of a mixed strain, 70-80 gm. in weight, were used for the experimental animals. The lepromas used were of the Kumamoto strain of murine leprosy, passed in rats. This material was used for making the vaccines, and also for infection of the controls and for the challenge inoculations.

The vaccine used mostly was a saline suspension made of lepromas that had been heated for 30 minutes at 100°C to kill the bacilli, ground up in a mortar, diluted with physiological salt solution at 20 cc. per gram of leproma. This was filtered through four layers of gauze, and phenol was added in 0.5 per cent concentration.

A water-in-oil emulsion of leproma was used in one of the experiments. For this preparation the leproma was heat-killed and then ground up in 2 volumes of saline, after which sterile liquid paraffin was added to make a 5 per cent (1:20) emulsion. This was then filtered through gauze and carbolized.

A liquid-paraffin suspension of a nonpathogenic acid-fast bacillus was also used. This bacillus, designated No. 16, had been isolated from the lymph node of a wild rat. A two-weeks growth on Petragnani's medium was harvested and suspended in liquid paraffin, 30 mgm./cc. This preparation was heated at 100°C for 30 minutes and carbolized.

For the challenge inoculations a saline suspension of fresh leproma, 10^{-3} dilution, was used—a very dilute inoculum in comparison with what has been employed by other investigators. It was chosen on the assumption that if the degrees of immunity obtained in the various experiments should be small, it might in this way be less difficult to differentiate them from the results in the control animals.

The control groups ordinarily consisted of 10 normal rats, inoculated with 0.5 cc. of the 10^{-3} suspension.

All inoculations were subcutaneous. The degrees of the lesions produced were graded as follows:

Infiltrations (barely palpable to 30 mm²)	±
Small-sized tumor (31-150 mm²)	1+
Medium-sized tumor (151-300 mm²)	2+
Large-size tumor (over 300 mm²)	3+

The lesions at the sites of inoculation were followed for five months. The surviving animals were then sacrificed and the changes in the lymph nodes and viscera, and the distribution of bacilli, were determined and compared with the findings in the control group.

1. EFFECTS OF SALINE VACCINE

EXPERIMENT 1. *Prevention of infection (immunization) by vaccination before inoculation.*

In this experiment 3 groups of 5 rats each were injected subcutaneously with 0.5 cc. doses of the saline vaccine at different frequencies during one week. Group 1A got 2 injections, Group 1B got 3, and Group 1C got 4. One month after the last vaccine injections each rat received a challenge inoculation of 0.5 cc. of the 10^{-3} suspension of living bacilli subcutaneously in the lower abdomen.

Results, Experiment 1

The results of monthly examinations of the animals in this experiment are shown in Table 1, in which are shown also the results of inoculations of the same dose of living bacilli in the 10 control rats.

In none of the groups vaccinated was there any sign of infection after one month (although there were signs in 3 of the 8 control rats alive at that time), but at two months a small infiltration was found in a single animal in Group 1B and in Group 1C. After three months, infiltrations were palpable in all of the surviving animals of those two groups, but in Group 1A three of the five showed no infiltration. Even after four months one animal of that group was without infiltration. At five months, however, the differences between experimental groups and the control group were regarded as not significant. No significant differences were observed in the pathologic changes and distribution of bacilli on autopsy of the animals that had lived that long.

In short, it was possible by prior injection of vaccine to inhibit infection—to delay the onset and development of lesions—to a certain degree, but complete protection was not achieved. It is of interest that more immunity effect was noted in Group 1A, in which the vaccine had been given only twice, in one week, than in those which had had it three and four times.

EXPERIMENT 2. *Inhibition (delay) of development of lesions by vaccination after inoculation.*

In this experiment three groups of 5 rats each were inoculated subcutaneously over the lower abdomen with 0.5 cc. of the 10^{-3} suspen-

TABLE 1.—*Results of postvaccination inoculations in the prevention (immunization) experiment with saline vaccine, compared with the control group.*[a]

Time after postvaccination inoculation (months)	Control group (10 rats) (not vaccinated)					Inoculated groups (5 rats each)														
						Group 1A (2 vaccine injections)					Group 1B (3 vaccine injections)					Group 1C (4 vaccine injections)				
	—	±	1+	2+	3+	—	±	1+	2+	3+	—	±	1+	2+	3+	—	±	1+	2+	3+
1	5	2	1			5					5					5				
2		3	5			5					4		1			3	1			
3			4	3		3	1	1				2	2				3	1		
4			1	3	1	1		2	2			1	1		2		1		1	
5			1	1	3		1	1	3				2	1	1			1		1

a Animals which died during the observation period are dropped from the tabulations.

TABLE 2.—*Results of prevaccination inoculation in the inhibition (delay) experiment with saline vaccine, compared with the control group.*[a]

Time after prevaccination inoculation (months)	Control group[b] (10 rats) (not vaccinated)					Inoculated groups (5 rats each)														
						Group 2A (2 vaccine injections)					Group 2B (3 vaccine injections)					Group 2C (4 vaccine injections)				
	—	±	1+	2+	3+	—	±	1+	2+	3	—	±	1+	2+	3+	—	±	1+	2+	3+
1	5	2	1			5					5					5				
2		3	5			5					4	1				4	1			
3			4	3			3	2				2	3				1	2		
4			1	3	1			3	1				3	1			1	1	1	
5			1	1	3				3	1			1	1	2			1		2

a Animals which died during the observation period are dropped from the tabulations.
b Repeated from Table 1, for convenience of comparison.

sion, before administration of vaccine. On the following day 2.5 cc. of the saline vaccine was injected subcutaneously on the back. The animals of Group 2A received one further injection of the same dose of vaccine after a week (2 doses total); those of Groups 2B and 2C received two and three more doses, respectively, at weekly intervals.

Results, Experiment 2

The findings in the monthly examinations in this experiment are shown in Table 2, those in the control animals being repeated (from Table 1) for convenient comparison.

Here again, as in the first experiment, none of the experimental animals showed palpable infiltration after one month, although 3 of the control group did so. At two months a barely palpable infiltration was discovered in a single animal in each of Groups 2B and 2C, whereas there was infiltration in all of the animals in the control group. After three months all of the rats had lepromas, but those in the control group were materially larger than those in the experimental groups. In the subsequent examinations, however, the groups could not be differentiated on that basis.

In the earlier months there were seen no significant difference in the degree of inhibition dependent on the number of injections of vaccine. Examination of the pathologic changes and the distribution of bacilli in the animals autopsied at the end of five months failed to reveal any significant differences.

It is concluded that by prior injection of heat-killed murine leprosy bacillus vaccine, onset of the infection is delayed to a certain degree; that although it is not possible completely to prevent infection—i.e., to achieve immunization—some inhibition is possible.

2. EFFECTS OF OIL-BASED VACCINES

In this experiment were used the oil-based vaccines described, one made of the rat leprosy bacillus and the other of a nonpathogenic acid-fast organism. Two groups of rats, 15 each, were employed. The vaccines were injected once, subcutaneously on the back, with 0.5 cc., and one month afterward the animals were given challenge inoculations in the same way as in Experiment I.

Results With Oil-Based Vaccines

The results of the challenge inoculations in this comparison experiment are shown in Table 3.

In the first place, it is seen that among the animals vaccinated with the murine-bacillus liquid-paraffin emulsion no infections were perceptible for the first two months (Section A of Table 3), but that 5 out of 10 showed signs of infection after three months, and 8 out of 10 after four and

five months. The severity of the infection was only slightly greater at five months than at four months.

In the rats which had received the emulsion of the nonpathogenic bacillus (Section B of Table 3), infections appeared earlier and on the average the local lesions were more severe. After one month 2 out of

TABLE 3.—*Results on subsequent challenge inoculations of administration of oil-based emulsions of* (a) *murine bacillus, and* (b) *nonpathogenic bacillus.*

Time after postvaccination inoculation (months)	A. Results in animals (15) vaccinated with murine-bacillus emulsion					B. Results in animals (15) vaccinated with nonpathogen emulsion				
	—	±	1+	2+	3+	—	±	1+	2+	3+
1	15					12	2			
2	13					7	3	2		
3	5	3	2			2	2	5		
4	2	1	7			2		2	3	
5	2		7	1		1	1		3	2

14 animals had become infected, 5 out of 12 in two months, 7 out of 9 in three months, 5 out of 7 in four months, and 6 out of 7 in five months.

Examination of the pathologic changes in the lymph nodes and organs, and the dissemination of bacilli in the body, of the animals that survived at the termination of the experiment failed to reveal a significant difference with the unvaccinated controls, as was the case in the previous study. However, the changes and dissemination were inhibited most strongly in Group A, which had received the oil-based murine-bacillus vaccine, and the results coincided rather closely with those observed in the study on the development of lepromas locally.

A comparison of these results with the findings in the unvaccinated controls in Tables 1 and 2 shows that, although the murine-bacillus oil vaccine did not prevent infection completely, it did inhibit, or delay, infection quite strongly. The effect of the oil preparation of the nonpathogen was much weaker, but it is believed that a slight degree of immunity was gained.

3. STUDIES ON SUPERINFECTION IN MURINE LEPROSY

The question of whether or not a leproma would be formed at the site of a second inoculation with murine leprosy bacilli, in already-infected rats, was regarded as of interest immunologically. It was also believed that findings of significance from the standpoint of immunology could be obtained by observing the effect of secondary inoculations on the primary lesion, and also the relationship between the size of the primary lesion and the local skin reaction and spread of the leproma of the secondary infection.

MATERIAL AND METHOD

The infected rats used in this experiment were the 40 survivors of a lot of about 100 young rats, 50-70 gm. in weight, which 5 months previously had been inoculated subcutaneously with a 10^{-3} suspension for another purpose. These animals were divided into 3 groups and reinoculated as follows: Group A (12 animals) was reinoculated with the 10^{-2} suspension, Group B (15 animals) with the 10^{-3} suspension, and Group C (13 animals) with the 10^{-4} suspension. The inoculations were all given subcutaneously in the back, the amount 0.5 cc. in each case.

For controls, 29 healthy rats similarly divided into three groups and inoculated with the three grades of bacillus suspensions.

The sites of inoculation were shaved clean of hair and the local skin reactions examined every day. The effects were judged in the same way as in the previous study. The pathologic changes in the lymph nodes and organs and the distribution of the bacilli are usually proportional to the size of the lepromas, so in this study only the size of the lepromas was taken into consideration in the evaluation.

RESULTS

Results of superinfection.—The results of the secondary inoculations in the previously infected rats are shown in the first part of Table 4, and for comparison the results of the control inoculations made at the same time in healthy rats are given in the second part of the table.

Of the rats reinoculated with the 10^{-2} suspension, all were negative at the sites of the secondary inoculations through the third month; after 4 months only 3 had become infected, while at 5 months 6 out of 11 were infected. Among the animals reinoculated with the 10^{-3} suspension, 1 became infected in 3 months and 1 more in another month; at 5 months, 8 out of the 15 animals were infected. Among the rats given the 10^{-4} suspension, none showed evidence of reinfection at the end of the 5-month period of observation.

In the control groups the results were very different. Even with the dose that had caused no new superinfection lesions, 6 of the 9 surviving at the end of 2 months had lesions, and all of the 7 remaining after 5 months had them.

It is seen that even with the largest reinoculation dose there was definite inhibition of production of reinoculation lesions in the animals already infected with murine leprosy as compared with the newly infected controls, and the differences were relatively more marked with the smaller doses. The inhibition was evident not only in the proportions of animals that developed new lesions—approximately one-half with the two larger doses, none (total inhibition) with the smallest one—but also in the size of those lesions.

Skin reaction at the site of secondary inoculation.—The skin over the sites where the bacilli were deposited in the secondary inoculations was observed for any local reaction which might resemble a Koch phenomenon. None was seen in any instance, regardless of the degree of the lesion of the primary inoculation. It is concluded that in murine leprosy sub-

TABLE 4.—*Comparison of the results of inoculations of threedilutions of lepromа suspension in (1) previously infected rats (superinfection groups), and (2) normal rats (control groups).*

Time after test inoculations (months)	Dilution group and degree of lesions														
	A. 10^{-2} dilution					B. 10^{-3} dilution					C. 10^{-4} dilution				
	—	±	1+	2+	3+	—	±	1+	2+	3+	—	±	1+	2+	3+
Superinfected group[a]															
1	12					15					13				
2	12					15					13				
3	12					14	1				13				
4	8	1	2			13	1	1			13				
5	5	3	3			7	3	4	1		13				
Control group[b]															
1	2	3	2			9	2				11				
2		2	4			6	3	1			3	4	2		
3		1	4	1		1	2	5	1		1	2	4	1	
4		1	3	2		0	1	3	5	1	0	1	4	2	
5		0	2	2	2	0	0	3	4	3	0	1	3	2	1

a Actually there were 43 rats in this group when the experiment was set up, but 3 died shortly afterward and were dropped from consideration.

b These control groups totalled 42 rats at the outset, but 13 of them died within one month from other causes than rat leprosy and are therefore not included.

cutaneous reinfection does not induce any acute reactional change in the overlying skin.

Size of the primary lesion and the results of secondary inoculation.—The data have been analyzed comparatively in the manner here indicated, but the results have so little significance that they are not given. There was no definite trend in the direction of fewer reinfection lesions with upward gradation of the sizes of the original lesions. At most it can be said that there was almost no instance in which a secondary lesion was larger than the primary lesion.

Effect of the secondary infection on the primary lesion.—The data were also examined to ascertain whether the progress of the primary lesion was either inhibited or intensified by the secondary infection. It was found that the primary lesion progressed steadily even after secondary infection, and there was no inhibition in that respect or in the pathologic changes.

DISCUSSION

The studies here reported were conducted to determine whether, in rats, injections of a saline vaccine or a liquid-paraffin vaccine of heat-killed murine leprosy bacilli (leproma suspension) would give rise to immunity, and also to ascertain whether secondary infection would be prevented in rats with primary lesions. A point that we wish to emphasize is that in these experiments 0.5 cc. of a 10^{-3} leproma suspension was used for the challenge inoculation in the vaccinated groups, and that for the secondary inoculations of infected rats three concentrations of the suspension (10^{-2}, 10^{-3} and 10^{-4}) were used. The point of importance is that numerous past studies have shown that careful use of small challenge doses is required in studies of immunity in order to measure resistance. Another essential point is that judgments were based on the size of the induced lepromas, because the pathologic changes in the lymph nodes and organs and the distribution of bacilli are proportional to the size of the leproma.

SUMMARY

1. Injection of a saline vaccine of heat-killed murine leprosy bacilli some time prior to inoculation of live bacilli, causes inhibition of onset (prophylaxis) in a relatively high degree, although not totally. When the vaccine is given simultaneously with inoculation of live bacilli, there is a distinct but lesser degree of inhibition of onset.

2. Injection of a vaccine prepared by adding liquid paraffin to killed murine leprosy bacilli did not completely prevent infection by a challenge inoculation of live bacilli, but onset was strongly inhibited. The effect of this vaccine was stronger than that of the saline vaccine.

3. Administration of a liquid-paraffin vaccine of nonpathogenic acid-

fast bacilli in place of murine leprosy bacilli resulted in a slight degree of immunity.

4. When secondary infection was attempted in rats already infected with murine leprosy, resistance greater than that induced by injection of the liquid paraffin vaccine was observed. When the challenge inoculum was a 0.5 cc. dose of a 10^{-4} leproma suspension, complete prevention was observed.

5. An acute reaction of the skin over the site of the reinoculation dose, resembling the Koch phenomenon in tuberculosis, was not observed. The size of the primary lesion did not influence the results of secondary inoculation. The secondary inoculation does not influence the progress of the primary lesion.

It is indicated that a certain degree of immunity can be attained by injection of killed or live murine leprosy bacilli in rats, and that it is also possible to obtain such an effect nonspecifically but in lesser degrees by injection of vaccines of other acid-fast bacilli.

RESUMEN

1. La inyección de una vacuna salina de bacilos de lepra murina, matados al calor, antes de la inoculación de bacilos vivos, ocasiona inhibición de la iniciación (profilaxis) de un tenor relativamente alto, pero no totalmente. Cuando se administra la vacuna simultáneamente con la inoculación de los bacilos vivos, se presenta una inhibición bien definida, pero menor, de la iniciación.

2. La inyección de una vacuna preparada agregando parafina líquida a bacilos muretos de lepra murina no impidió totalmente la infección por una inoculación provocadora de bacilos vivos, pero sí inhibió poderosamente la inciación. El efecto de esta vacuna fué mayor que el de la vacuna salina.

3. La administración de una vacuna en parafina líquida de bacilos ácidorresistentes anapatógenos, en lugar de bacilos de lepra murina, dió por resultado una inmunidad leve.

4. Cuando se ensayó una infección secundaria en ratas ya infectadas con lepra murina, se observó una resistencia mayor que la inducida por la inyección de la vacuna de parafina líquida. Cuando el inóculo provocador consistía en una dosis de 0.5 c. c. de una suspensión de leproma al 10^{4}, se observó prevención absoluta.

5. Sobre el sitio de la dosis de reinoculación, no se observó una reacción agudo de la piel, semejante al fenómeno de Koch en la tuberculosis. El tamañn de la lesión primaria no afectó los resultados de la inoculación secundaria. La inoculación secundaria no afecta el avance de la lesión primaria.

Queda indicado que puede alcanzarse cierto grado de inmunidad por la inyección de bacilos matados o vivos de lepra murina en las ratas y además que es posible obtener ese efecto inespacíficamente, pero en menores grados, con la inyección de vacunas de otros bacilos ácidorresistentes.

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