THE EFFECT OF ISONIAZID ON THE IMMUNITY PRODUCED BY VACCINATION OF GUINEA PIGS WITH BCG

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Since vaccination against tuberculosis with BCG (Bacillus Calmette-Guerin) is practiced throughout the world and because of the proposal that isoniazid be given prophylactically to susceptible groups of people (Ferebee and Palmer, 1956:1) it is essential that we determine whether or not the immunity established by BCG vaccination is influenced favorably or unfavorably by the administration of this antimicrobial agent.

Adequate evidence has been presented that vaccination of guinea pigs with the attenuated BCG bovine strain of tubercle bacilli confers a partial immunity against virulent tubercle bacilli (Sher and Czaja, 1956:95). Similarly a comparable degree of protection is obtained when guinea pigs are infected with comparatively small amounts of pathogenic tubercle bacilli and are subsequently treated for ten weeks with sulfone derivatives (Sher and Kloeck, 1953:66) and with isoniazid (Sher and Czaja, 1956:95). This immunity exists even though, as is the case with isoniazid therapy, the disease is suppressed so that there is morphologically or histologically no evidence of tuberculosis 32 weeks after the infection (Sher, *et al.*, 1957:298). It was postulated that the drugs retarded the rate of bacterial growth and thus enabled the animal body to develop a moderate degree of resistance against a subsequent endogenous spread or externally administered infection (Sher and Czaja, 1956:95-96). However, it has been reported recently that when mice were infected with minute, yet lethal, amounts of tubercle bacilli and were subsequently treated with isoniazid, no protection was observed (Dubos, *et al.*, 1956:546). The question, therefore, arises whether isoniazid will enhance or deter the immunity imparted by BCG vaccination. The response could be similar to the action obtained against minute amounts of virulent organisms, because of the attenuated character of BCG, or might be as it is with the larger inoculum of virulent tubercle bacilli.

These studies were intended to compare statistically the average survival times of guinea pigs vaccinated with BCG in combination with isoniazid with those of non-vaccinated tuberculous control animals. For additional "controls", comparisons were also made with BCG vaccinated animals receiving no isoniazid and with guinea pigs with latent or arrested tuberculosis produced by treatment of a virulent infection with isoniazid.

Materials and Methods

Fifty-one guinea pigs weighing between 400 and 600 gms. were adjusted to the environment of the animal quarters and were divided into 5 groups. Two of these groups, A and B, were vaccinated with BCG obtained from the Tice Laboratories. On the same day group C was inoculated with H37Rv, a virulent human strain of tubercle bacilli. Groups D and E received no inoculation at this time.

Group A consisted of 5 animals injected subcutaneously in the right inguinal region with 0.1 mg. BCG; 134 days later each animal was injected in the left inguinal region with 0.1 mg. H37Rv. This group served as the "immunized, tuberculous group" and was observed until death.

Group B consisted of 13 animals vaccinated as in group A. Starting 7 days after the vaccination they received daily treatment for 60 days of 20 mgs. isoniazid per animal in one ml. of 10% sucrose solution mixed with lettuce. One hundred and thirty-four days after vaccination the animals were inoculated with H37Rv as in group A and observed until death. This group served as the "immunized, treated, tuberculous group".

Group C consisted of 10 guinea pigs injected subcutaneously in the right inguinal region with 0.1 mg. H37Rv, a virulent human strain of tubercle bacilli. Starting 7 days after the inoculation they received daily treatment of isoniazid as in group B; 134 days after the primary infection they were reinfected with 0.1 mg. H37Rv subcutaneously in the left inguinal region. This group served as the "treated, reinfected group" and was included in these experiments to compare the effectiveness of immunity obtained by means of a virulent strain of tubercle bacilli suppressed by isoniazid treatment with that obtained by BCG vaccination.

Group D consisted of 12 guinea pigs that received no injection until 134 days after groups A and B were vaccinated with BCG and group C received the primary H37Rv infection. These animals were inoculated simultaneously with the virulent H37Rv infection of groups A and B and the reinfection of group C; thus they served as primarily infected controls for the evaluation of resistance against infection. They served as the "tuberculous controls".

Group E consisted of 11 normal guinea pigs which were neither infected nor vaccinated and received no drug. They served as the "healthy controls".

Groups A, D, and E, received sucrose solution mixed with lettuce during the period that groups B and C received isoniazid treatment.

Immunity was expressed in terms of the average survival time of each group, calculated from the day of infection with H37Rv or 134 days after the vaccination with BCG. The observed difference, "OD" (Table 1), obtained by comparing the average survival time of the non-vaccinated "tuberculous controls" of group D with each of the other groups, gave a measure of immunity, or resistance to tuberculous infection. This observed difference, divided by the standard error of this difference, "SE-diff", gives the re-

liability quotient OD/SE diff (Waugh, 1938:157) for each group thus compared. A quotient of 2.0 or higher was acceptable because of the small numbers of animals used, and indicated that the likelihood was 21 to 1 that these results were not due to chance occurrence. Appropriate precautions based on the small numbers used were taken in each instance in estimating the statistical reliability.

The animals were dissected carefully to establish gross evidence of tuberculosis. The post mortem rating was estimated according to the method of Sher and Kloeck (1946: 251).

Results

All of the animals included in Table 1 gained weight constantly during the 134 days between vaccination and the challenging infection, at which time they weighed between 600 and 800 gms. On this basis the animals reacted similarly to the normal groups up to the date of the challenging infection with H37Rv, regardless of whether or not they were previously vaccinated as in groups A and B or infected and treated as in group C. These results are similar, in effect, to those obtained when BCG vaccination did not increase the mortality rate of guinea pigs (Sher and Czaja, 1956: 94), and isoniazid provided complete protection against a virulent infection during a similar period (Sher, *et al.*, 1957:297).

The non-vaccinated "tuberculous controls" of group D (Fig. 1) showed a rapid and almost constant mortality rate during the seven months following the challenging infection with H37Rv, while the mortality rate was considerably lower for the "immunized treated tuberculous" animals of group B. During the first three months the death rate of the former was three times that of the latter, and at seven months when none of the tuberculous controls were alive one-third of the immunized guinea pigs were living.

There was little difference in the mortality rate between group B and each of the other immunized groups, A and C. Each group showed some degree of protection, for at any time during the survival period the percent of survivors was greater than that of the "tuberculous control" group.

Ninety percent of the "healthy control" animals of group E were alive at 12.5 months when all of the animals of the other groups were dead. The mortality rate of group E was very much lower than that of the other groups.

The average survival time calculated from the day of injection with H37Rv was considerably longer for group B than for the "tuberculous control" animals of group D. The observed difference of 66 days portrays a partial resistance against a virulent infection with a reliability quotient OD/ SE diff of 2.1 (Table 1). Although the observed difference of 88 days between groups A and D was somewhat greater, the reliability or statistical significance was somewhat less than that obtained for group B. However, for a similar group (Sher and Czaja, 1956:93) of 18 guinea pigs the average survival time was 206 days as compared to 209 days for group A and the reliability quotient of 2.8

TABLE 1.—Average Survival Time from Time of Infection with H437Rv, 134 Days after BCG Vaccination.

Group		Number of animals	Vaccination or primary infection	Isoniazid therapy	Infection or reinfection 134 days after vaccination	Average survival time	SE	OD	OD/SE diff.
A.	Immunized, tuberculous	5	BCG	none	H37Rv	209	62	88	1.6
B.	Immunized, treated, tuberculous	13	BCG	60 days	H37Rv	187	26	66	2.1
C.	Treated, reinfected	10	H37Rv	60 days	H37Rv	192	32	71	2.1
D.	Tuberculous control	12	none	none	H37Rv	121[1]	19	.....	.....
E.	Healthy control	11	none	none	none	>502	24	>381	>12.0

[1] Calculated from date of infection.

was highly significant statistically. This comparison permitted greater confidence in the results calculated for group A.

Calculation of the observed difference between the average survival times of the two BCG vaccinated groups, one without and the other with isoniazid treatment (groups A and B), gave a relatively small value of 22 days, which proved to be statistically not significant.

The observed difference of 71 days for the "treated, reinfected group" showed approximately the same degree of immunity as that obtained with BCG vaccination and isoniazid. The quotient of 2.1 was acceptable. This observed difference approximated the value of 75 days obtained from a similar series of 52 animals, and these latter results were highly significant statistically (Sher, *et al.*, 1957:297).

An average survival time of 292 days was calculated for BCG vaccinated animals which received neither isoniazid nor a virulent challenging infection (not tabulated). This result was sufficiently high to require no detailed statistical analysis to establish that a subsequent virulent infection shortened the lives of these vaccinated animals. On the other hand, these BCG vaccinated animals did not live as long as the "healthy controls".

Upon anatomical examination each "tuberculous control" animal showed severe tuberculous changes, except for the one guinea pig that died two weeks after the infection. In this instance the gross, post-mortem rating (Sher and Kloeck, 1946:251) was 20%. In another animal the rating was 60%, and for the remainder it was 100%. The post mortem ratings for the immunized groups ranged from 35 to 100%. These latter showed severe tuberculous involvement, but to a lesser degree than that of group D.

Discussion

When guinea pigs are vaccinated with BCG in combination with isoniazid, measurable benefits in the form of immunity or resistance against tuberculosis are obtained. From Figure 1 it is apparent that during the three months immediately following the inoculation with H37Rv, the mortality rate of the vaccinated animals was markedly lower than that of similarly infected, non-vaccinated, control animals. This may be attributed to the slow initial rate of bacillary growth in the immunized animals which produced only minimal or sub-lethal amounts of tuberculosis up to this point.

Some evidence of immunity was present also during the remainder of the survival period even though the mortality rate was increased. It is shown in Figure 1 that the slope of the curve for the immunized group (B) is shallower than for the control group (D) for this period.

The accelerated mortality rate was associated with extensive tuberculous involvement found on post-mortem examination of the animals dying during this latter period. The immunity described here in the presence of this extensive involvement corresponded to that found when tuberculous guinea pigs were treated with sulfone derivatives (Sher and Kloeck, 1953:66). In this experi-

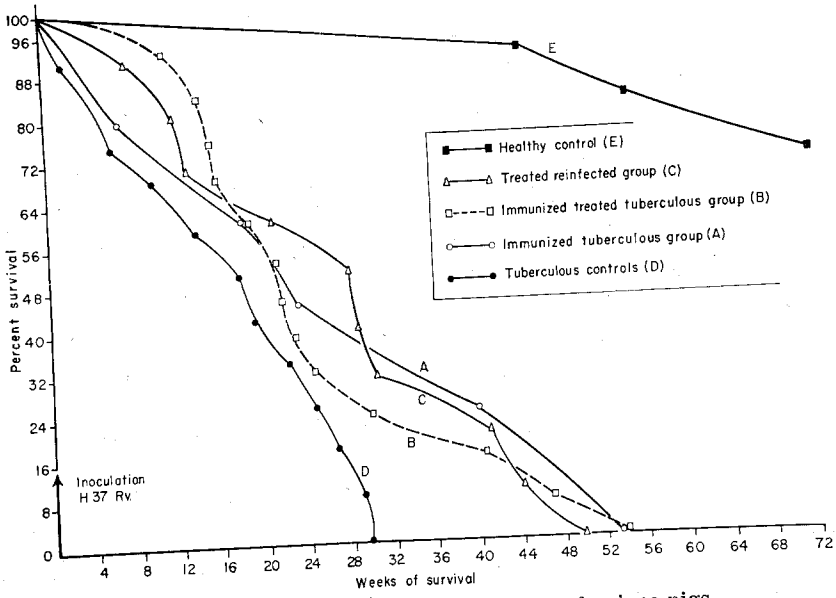

FIG. 1.—Survival of the different groups of guinea pigs. Symbols indicate times of death.

mental study we found that the treated animals lived more than twice as long after treatment ended than the entire life span of the untreated controls, despite the presence of large amounts of tuberculosis.

Examination of the mortality rates and nature of the curves for the vaccinated animals not receiving isoniazid, group A, and for the "treated, reinfected" animals of group C led to practically the same conclusions as were obtained with group B when each of these groups was compared with the tuberculous controls. Also, there was little difference between the immunized groups A, B and C when they were compared with each other.

There is no basis for a detailed discussion of the slight deviations observed in each curve as they appear in Figure 1. However, a summation of these and other factors is inherent in the statistical data of each group tabulated in Table 1.

These data showed that vaccination with BCG produced a moderate degree of immunity, and that this immunity was not significantly influenced by the administration of isoniazid. Although the average survival time was somewhat less with the use of isoniazid, the observed difference between these two groups was too small to be statistically significant.

SUMMARY

Preliminary experiments have shown that the immunity obtained when guinea pigs were vaccinated with BCG was not significantly influenced by the administration of isoniazid.

The immunity obtained when a virulent tuberculous infection was suppressed with isoniazid was approximately equal to that obtained with BCG vaccination.

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