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RESISTANCE OF ANTIHISTAMINE-TREATED GUINEA-PIGS AGAINST INFECTION WITH *MYCOBACTERIUM LEPRAE* AND *M. LEPRAEMURIUM*[1]

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INTRODUCTION

The guinea-pig is generally regarded as a species natively resistant to infection with *Mycobacterium lepraemurium*. In 1957 Kato (4) reported that he had succeeded in depressing resistance of guinea-pigs to infection with this microorganism by repeated and prolonged treatment with the antihistamine pyrilamine maleate (Neoantergan). Success in his experiments depended upon introduction of the bacteria directly into living macrophages that had accumulated in the peritoneal cavity of the animals in response to a previous injection of glycogen. Kato stated that, in the presence of antihistamines, guinea-pig macrophages failed in their ordinary function of rapidly disintegrating the phagocytized bacteria. According to his observations, guinea-pig macrophages were capable of reducing the rat-leprosy bacillus to acid-fast debris within a few hours or a day. Because of the significance of Kato's observations for elucidating patterns underlying native resistance against murine and human leprosy, the experiments with *M. lepraemurium* were repeated, and extended to include *M. leprae* as an infecting agent.

MATERIALS AND METHODS

1. *Bacterial suspensions.*—(a) M. lepraemurium: Subcutaneous lepromas of C_{57} black mice infected three months previously with the Hawaiian strain of the bacillus were minced with scissors and ground in a mortar with sterile sand and Hanks' balanced salt solution (BSS). The resulting suspension was centrifuged at 5°C for 10 minutes at 1,000 r.p.m. The supernatant was used as the infecting suspension.

(b) M. leprae: The preparation of this suspension was the same, except that the bacilli were obtained from three-months-old subcutaneous embryomas of C_3H mice induced according to the method described by Robertsen (11).

2. *Macrophage peritoneal exudates.*—Multicolored adult guinea-pigs of both sexes

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were given, 5 days before challenge with *M. lepraemurium* or *M. leprae*, intraperitoneal injections of 0.1 mgm. of glycogen in 10 cc. of 0.85 per cent sodium chloride solution.

3. *Antihistamines.*—Pyrilamine maleate (Neoantergan) and diphenhydramine hydrochloride (Benadryl) were used. The drugs were dissolved in saline so that 8 mgm. were contained in a volume of 0.5 cc. The solutions were sterilized by filtration through sintered glass filters.

4. *Experiments with* M. lepraemurium.—Thirty-three adult, multicolored guinea-pigs of either sex were divided into 3 groups, of 10, 13, and 10 animals, respectively, and macrophage peritoneal exudates were provoked in all animals. Five days later they were infected by intraperitoneal injection of 1 cc. of a suspension containing 6×10^8 organisms. Thirty minutes before the challenge injection the 10 animals in one of these groups received a subcutaneous injection of 8 mgm. of Benadryl. Thirteen animals received an equal amount of Neoantergan. The remaining animals received injections of normal saline. These injections were repeated 5 times a week for the duration of the experiment.

5. *Determination of the fate of the ingested bacilli.*—One animal from each of the 3 groups was sacrificed 3, 24, 48, and 72 hours after challenge with the murine bacillus. The peritoneal cavities were washed with 20 cc. of BSS, and the cells were separated by centrifugation at 1,000 r.p.m. for 5 minutes. The supernatants were discarded and the cells resuspended in 2 cc. of fresh BSS. Small aliquots of these suspensions were withdrawn with capillary pipettes and spread on glass slides. These preparations were used for acid-fast staining at room temperature, following fixation in 10 per cent formalin, and for Wright staining and supravital staining with neutral red and Janus green (9). The cell suspensions were then stored at 37°C, and samples were withdrawn after 3, 6, and 12 hours of incubation. From these samples, slides were made for acid-fast and supravital staining. The remaining animals were sacrificed at the end of the eighth week following infection.

All animals were autopsied. Impression slides for acid-fast staining were made in each instance from the peritoneum and the cut surfaces of the spleen and liver. Smears for acid-fast stains were also made from macroscopically visible lesions. Sections were made from the livers, spleens, adrenal glands, and testicles. All tissue sections were processed in an autotechnicon, after 10 per cent formalin fixation, embedded in paraffin blocks, and stained with carbol-fuchsin, and counterstained with eosin-methylene blue.

6. *Experiments with* M. leprae.—Twenty-six adult, multicolored guinea-pigs of either sex were divided into 3 groups of 10, 8, and 8 animals, respectively. Macrophage exudates were provoked in 10 animals in one of these groups in the same way as before. Five days later these guinea-pigs, and those in one of the two other groups, received a subcutaneous injection of 8 mgm. Benadryl. The remaining animals received normal saline. Thirty minutes later all animals were injected intraperitoneally with 1 cc. of a suspension containing approximately 2×10^7 of leprosy bacilli. Injections of Benadryl and saline were repeated 5 times a week for the duration of the experiment. The determination of the fate of ingested bacteria was carried out as before. All additional procedures corresponded with those outlined previously, except that the animals were allowed to survive for 5 months following infection.

RESULTS

1. *Experiments with* M. lepraemurium.—Phagocytosis of the injected bacteria had occurred in the animals sacrificed 3 hours after inoculation. Most of the bacteria were intracellular within macrophages. They stained solidly, regardless of whether or not the animals had been treated with antihistamines. These findings remained unchanged in the animals sacrificed 24, 48, and 82 hours after injection.

Likewise, there was no difference in the appearance of the phagocytized bacteria in any of the cell incubates. Signs of bacterial degradation in the macrophages of animals not receiving antihistamines were lacking entirely, despite the fact that most of the macrophages remained viable for at least 12 hours of incubation as indicated by the results of supravital staining. Figures 1 and 2, respectively, show *M. lepraemurium* within macrophages of an antihistamine-treated and of an untreated guinea-pig 72 hours after injection.

On autopsy, no significant differences were found in the animals that had received antihistamines and the untreated ones. Invariably, round, pus-filled granulomatous lesions were present in the omentum, containing numerous acid-fast rods. Frequently, pea-sized, encapsulated abscesses containing numerous acid-fast rods were found in the peritoneum. Impression slides from the peritoneum and the cut surfaces of liver and spleen ordinarily showed a few acid-fast bacilli. The spleens of most guinea-pigs seemed enlarged. The mean spleen weights were 0.7 per cent of mean body weight in the pyrilamine maleate-treated group, 0.5 per cent in the Benadryl-treated animals, and 0.9 per cent in the guinea-pigs not treated with an antihistamine. Figure 3 shows the enlarged spleen, omental lesions, and peritoneal abscess of one of the untreated guinea-pigs. This particular animal weighed 635 gm. and had a spleen weighing 12.3 gm. (1.9 per cent of body

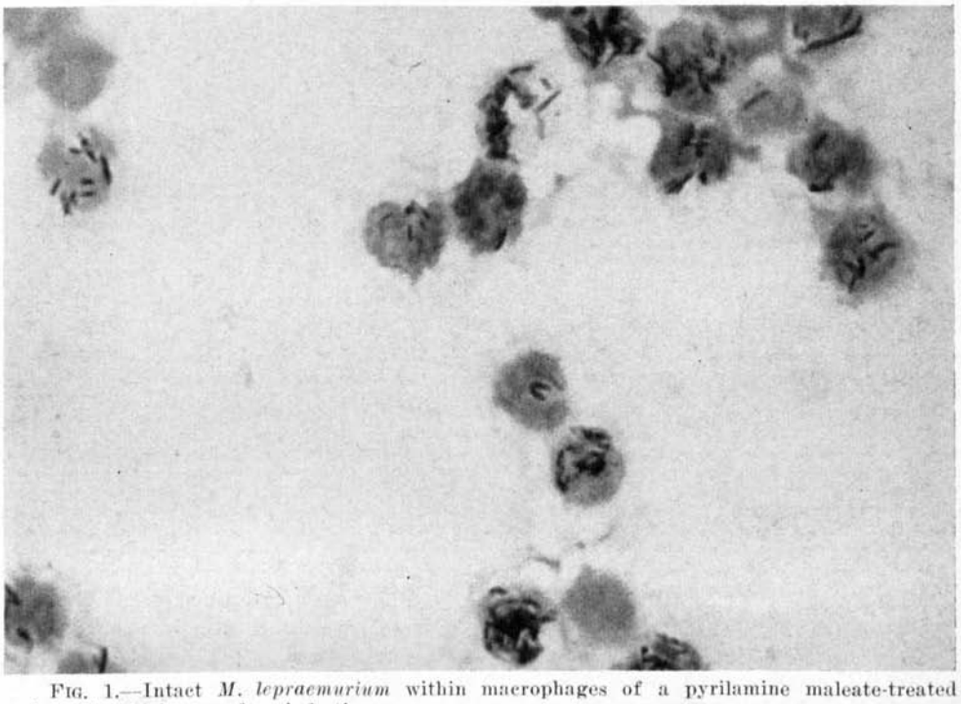

FIG. 1.—Intact *M. lepraemurium* within macrophages of a pyrilamine maleate-treated guinea-pig, 72 hours after infection.

weight). This was the largest spleen found in any of these animals. Histologic examination of the spleens, livers, adrenals, and testicles gave identical results in treated and untreated animals. The testicles and adrenals appeared entirely normal. A few isolated acid-fast bacteria were usually found in the spleens and livers, regardless whether or not the animals had received antihistamines. These bacilli did not seem, however, to have produced tissue changes.

2. *Experiments with* M. leprae.—There were no discernible differences, either *in vitro* or *in vivo*, in the extent of phagocytosis and in the fate of the phagocytized bacilli, whether the animals had been treated with an antihistamine or not. No gross lesions were found in any of these animals. There were no histologic changes that could have been the result of infection with *M. leprae*. Acid-fast organisms were not found in the livers, spleens, adrenals, or testicles of these animals.

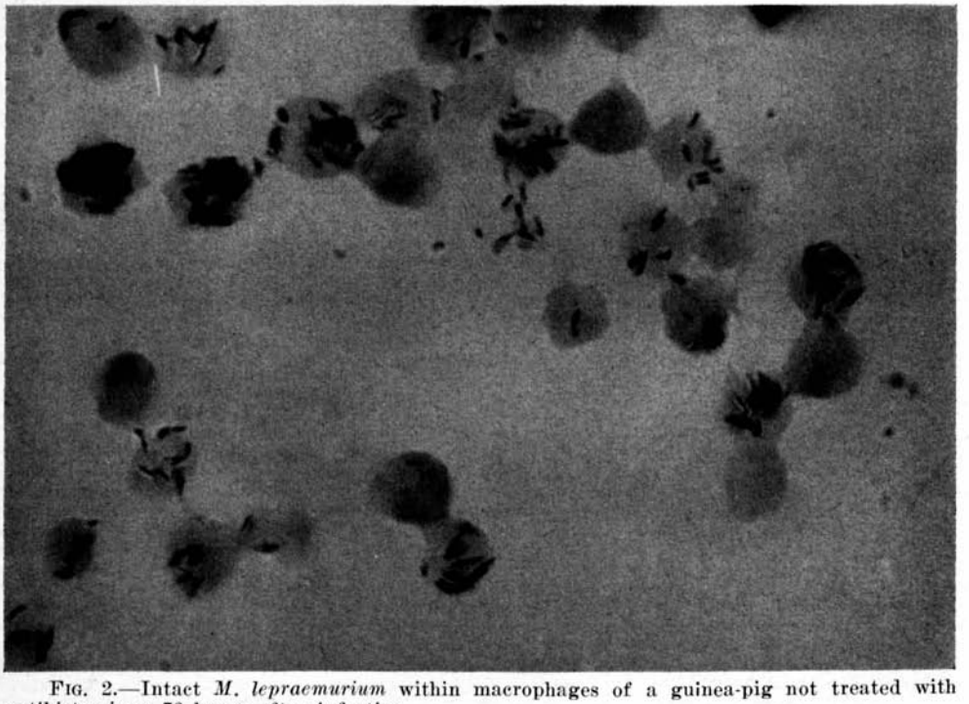

FIG. 2.—Intact *M. lepraemurium* within macrophages of a guinea-pig not treated with antihistamines, 72 hours after infection.

DISCUSSION

Phagocytosis, followed by intracellular destruction, is probably a significant factor in native resistance against bacteria, such as *Diplococcus pneumoniae*, that cause acute infections ([13]). That speedy destruction of certain members of the order *Eubacteriales* takes place within polymorphonuclear and mononuclear phagocytes of various species of animals, including man, has been shown recently by Cohn ([2]).

Using P^{32}- and C^{14}-labeled bacteria, he found that rabbit macrophages caused extensive degradation of *Bacillus subtilis* and *Escherichia coli* within three hours of intracellular residence. These findings were restricted neither to rabbit macrophages nor to the named two bacterial species.

None of the microorganisms included in Cohn's experiments was an intracellular parasite. Intracellular parasitism is of particular interest to the student of leprosy, because of the essentially obligatory intracellular habitat of *M. leprae*. Other mycobacterial infections such as murine leprosy and tuberculosis are characterized by a similar association. Available evidence does not favor the assumption that natural resistance against particular species or strains of the tubercle bacillus is based on its rapid degradation within the host's macrophages. Host-resistance against the $H_{37}Ra$ strain of *M. tuberculosis* seems to be positively correlated with incapability of the microorganism to multiply within host cells, whether *in vitro* ([1, 7, 8, 12]) or *in vivo* ([10]).

Death and degradation are the eventual fate of these mycobacteria. Yet these are consequences rather than causes of avirulence and host resistance. A particular strain of the tubercle bacillus may retain an attenuated capability for intracellular multiplication from the very

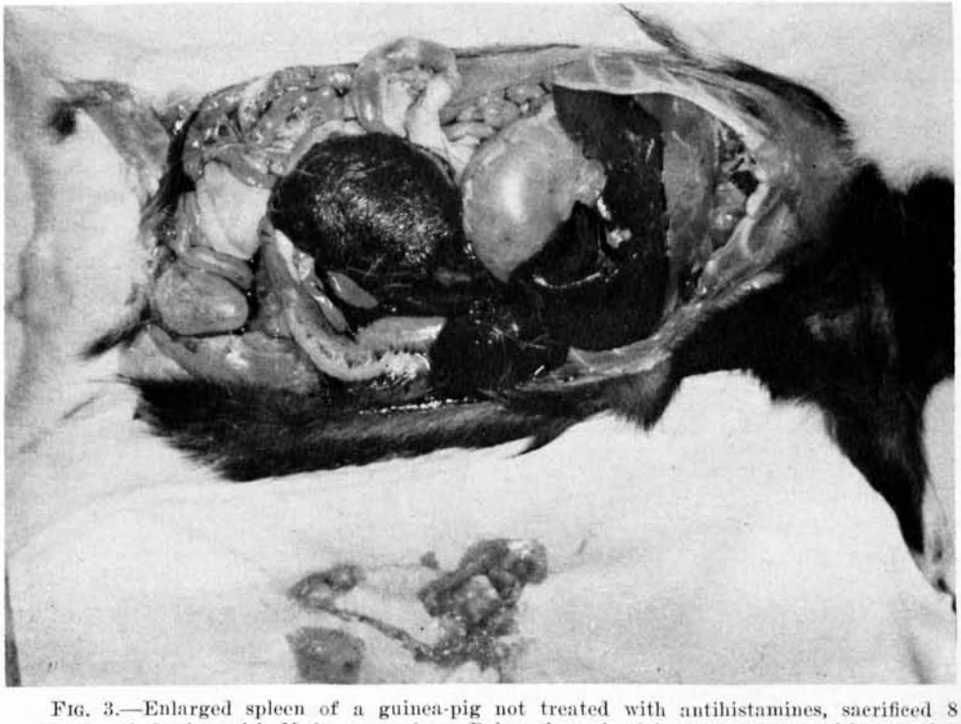

FIG. 3.—Enlarged spleen of a guinea-pig not treated with antihistamines, sacrificed 8 weeks after infection with *M. lepraemurium*. Below the animal is the omentum with granular lesions, and an excised peritoneal abscess.

inception of the infection. Here increased resistance is demonstrated by the hosts' failure to develop progressive disease. Attenuated capacity to multiply within the host is exemplified by the various BCG strains of *M. bovis*.

An additional and different kind of host resistance seems to be operative in the host-parasite relationship between the rabbit and strains of *M. tuberculosis* that are fully virulent for human beings and guinea-pigs but not for most rabbits. Lurie (5) has shown that, at the onset of the infection, multiplication of these strains proceeds at an even faster rate than that of *M. bovis*, which causes progressive and fatal disease in rabbits. Subsequently, however, rabbits ordinarily show increased resistance against the human variety of the tubercle bacillus by inhibiting its further multiplication. It has been speculated that this might result from acquired resistance which now comes into play (5). If this resistance is of the immunologic variety, it would be interesting and important to know why rabbits fail to develop or to invoke the same kind of defenses against the bovine tubercle bacillus.

Possibly these newly-emerged signs of resistance are mediated by mechanisms other than those depending on antibodies. Conceivably, interaction of host tissues and microbes might lead to changes in the physicochemical characteristics of the environment, rendering it unsuitable for proper functioning of the invading bacteria. This would provide the host with attributes of resistance, and force the mycobacterium to behave like an avirulent strain.

It should be mentioned that on the basis of results of later experiments, Lurie and Zappasodi (6) stressed the occurrence of a greater initial destruction of human tubercle bacilli in the lungs of resistant than of susceptible strains of rabbits. This conclusion, however, was based on the results of cultural experiments and therefore does not exclude the possibility of death of bacilli without any actual physical destruction.

Much less is known concerning the fate of *M. leprae* within the cells of natively-resistant animals or individuals. Hanks (3) reported intracellular destruction of *M. leprae* within explanted "fibroblasts" of patients with tuberculoid leprosy. It does not seem, however, that a significant amount of destruction became apparent before the lapse of several weeks. In addition, the possibility cannot be excluded that these fibroblasts stemmed from an individual endowed with acquired resistance on account of the preexisting association of cell donor and parasite.

The results of the present experiments show clearly that *M. lepraemurium* and *M. leprae* do not disintegrate promptly within the macrophages of guinea-pigs. This does not, of course, exclude the possibility that these bacterial species might fail to survive within these cells for any length of time. Nevertheless, prompt intracellular disintegration

does not account for the species resistance of guinea-pigs against these microorganisms.

Numerous unsuccessful attempts have been made in the past to infect laboratory animals with *M. leprae* by depressing their natural defenses. In the present experiments, repeated treatment with Benadryl failed completely to alter the insusceptibility of guinea-pigs to infection with *M. leprae,* even when the bacteria were introduced into macrophages of the animals. Complete refractoriness was also observed in guinea-pigs under identical experimental conditions following repeated and prolonged treatment with cortisone acetate.

The murine leprosy bacillus caused localized lesions when introduced into the peritoneal cavity of guinea-pigs. These lesions contained numerous acid-fast organisms eight weeks after infection. It is not possible to state whether or not bacterial multiplication had occurred in these animals. There was no indication that treatment with Benadryl or pyrilamine maleate had any enhancing effect on the infectivity of the bacillus under the conditions of the experiments.

SUMMARY

Experiments were carried out to observe the effect of guinea-pig macrophages on the integrity of *M. leprae* and *M. lepraemurium* in the presence and absence of antihistamine treatment.

In addition, it was intended to determine the effect of prolonged antihistamine treatment on the infectivity of these mycobacteria for guinea-pigs.

The results of the experiments show that guinea-pig macrophages fail to disintegrate the bacilli within 72 hours *in vivo,* and fail to do so within 12 additional hours *in vitro.*

Treatment wth Benadryl does not modify the solid native resistance of guinea-pigs to infection with *M. leprae,* even when this organism is introduced into macrophages of these animals. Treatment with Benadryl and pyrilamine maleate did not change the infectivity of *M. lepraemurium* introduced in guinea-pig macrophages.

RESUMEN

Se efectuaron experimentos para observar los efectos de los macrófagos de cobayos sobre la integridad de los *M. leprae* y *M. lepraemurium* en la presencia y ausencia de tratamiento con anthistamínicos.

En adición, se intentó determinar el efecto del tratamiento prolongado antihistamínico sobre la infectividad de estas micobacterias para cobayos.

Los resultados de los experimentos mostraron que los macrófagos de cobayos fallaron en la desintegración del bacilo dentro de las 12 horas *in vivo* y fracasaron en los mismos dentro de las 12 horas adicionales *in vitro.*

El tratamiento con Benadril no modifica la sólida resistencia natural de cobayos a la infección con *M. leprae,* aun cuando este organismo es introducido en los macrófagos de estos animales. El tratamiento con Benadril y maleato de pirilamina no cambia las infectividad del *M. lepraemurium* introducido en los macrófagos de los cobayos.

RESUMÉ

Des expériences ont été menées en vue d'observer l'effet des macrophages de cobayes sur l'integrité de *M. leprae* et *M. lepraemurium* en la présence ou en l'absence de traitement anti-histaminique.

De plus, l'intention a été de déterminer l'effet d'un traitement anti-histaminique prolongé sur l'infectivité de ces mycobactéries pour les cobayes.

Les résultats de ces expériences montrent que les macrophages de cobayes ne réussissent pas à désintégrer les bacilles endéans les 72 heurs in vivo, et endéans 12 heurs additionnelles in vitro.

Le traitement par le Benadryl ne modifie pas l'importante résistance naturelle des cobayes à l'infection par *M. leprae,* même lorsque cet organisme est introduit dans les macrophages de ces animaux. Le traitement par le Benadryl et par le maléate de pyrilamine ne modifie pas l'infectivité de *M. lepraemurium* lorsqu'il est introduit dans les macrophages de cobayes.

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