A COMPARISON OF THE CELLULAR REACTION IN EXPERIMENTAL TUBERCULOSIS OF THE CORNEA IN ANIMALS OF VARYING RESISTANCE *

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Previous experiments by Vorwald [1] have shown that a definite correlation does not exist between the intensity of cellular reaction in the lung in different animals after implantation of tubercle bacilli, and the subsequent development of tuberculosis in this organ. His studies at the same time indicated that a large percentage, perhaps the majority, of the cells taking part in the formation of the developing tubercles under the conditions of his experiment came from the blood stream. A study by Long, Vorwald and Donaldson [2] on the early cellular reaction to tubercle bacilli in the testis in normal and tuberculous guinea pigs and guinea pigs immunized with dead bacilli, led to the same conclusions.

Vorwald's observations brought out again with unusual clarity the facts in the course of tubercle development first described by Borrel [3] and his associates, and recently emphasized by Albert-Weil,[4] *viz.*, the immediate influx of polymorphonuclear leukocytes at the point of lodgement of tubercle bacilli and the subsequent phagocytosis and replacement of these cells by large mononuclear cells. The apparent vascular origin of most of the cells concerned suggested the value of a similar study of tubercle formation in different animals in the non-vascular cornea. It seemed possible that differences in the rate of tubercle growth in different animals might be still more conspicuous in the latter organ.

Four series of animals were inoculated with human type tubercle bacilli (H 37 of Saranac Lake), *viz.*, normal and tuberculous guinea pigs, rabbits and cats. Interlamellar injections were made with sharp, short beveled, fine gauge hypodermic needles in or near the center of the cornea in each case. It was impossible to fix the dosage exactly because of the minuteness of the amount of fluid that could be injected into the cornea without detrimental trauma or uncon-

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trolled spreading, but the approximate amounts of tubercle bacilli injected were 0.01 mg. in the rabbits and cats, and 0.005 mg. in the guinea pigs. A sudden, sharply limited, white opacity 2–3 mm. wide in the cornea in the region inoculated, on slight pressure of the loaded syringe after the needle was in place, signalized the success of the injection.

The course of development of the infection in the different animals is summarized in Figure 1. The relative sizes of the lesions are shown by fine shading at the site of infection, and blood vessels are indicated by small circles, at the limbus in all animals and at the site of infection in the sensitized guinea pig killed at 2 weeks and the normal guinea pig and rabbit killed at 1 month. Approximate simple differential counts are given of the cells partaking in the reaction, the region at which the count was made being indicated by an arrow in each case.

At 6 hours no reaction was apparent in any animal except at the limbus. After the passage of 24 hours a cellular reaction was seen in the injected area, in or bordering on the center of the cornea, in every animal. In the figure attention is drawn to the limbus reaction at the early and to the central reaction at the later periods.

Significant facts from observation of sections, including those stained for tubercle bacilli, are as follows, and the following conclusions seem warranted in their explanation.

1. The first cellular response apparent in the infected cornea was around the blood vessels of the limbus in every instance; it was notable here 6 to 24 hours before a reaction could be detected at the actual site of injection. This seemed good evidence that the cells responding immediately to the infection came from the blood vessels at the margin of the cornea, and not from the conjunctival secretion along the needle track, as was claimed by many of the early observers.

2. A considerable variation in the limbus response was seen in the different animals of the series at the 6 hour period. The most intense reaction occurred in the sensitized (tuberculous) guinea pig. The severity of reaction in the normal guinea pig, rabbit and cat decreased in the order named. The prompt outpouring of cells in the already infected guinea pig appeared simply another example of the well known hypersensitivity in this type of animal. It could quite appropriately be considered a tuberculin reaction, and indeed other

experiments by ourselves have shown that the same divergence in intensity of reaction in the cornea occurs in normal and tuberculous guinea pigs following the intracorneal injection of purified tuberculin protein. The difference in intensity of initial reaction in the normal animals was similar to that observed by Vorwald in the lung, and is further evidence that intensity of reaction at the outset does not predicate corresponding speed of suppression of the infection. On the contrary, the least reaction was observed in the cat, the animal of the highest general resistance to infection.

3. The predominant cell in the limbus reaction at 6 hours, the earliest period studied, was the polymorphonuclear leukocyte in all instances. At 24 hours cells of this type had reached the point of inoculation in large numbers, whereas few mononuclears had yet appeared at this point. Large mononuclears were present at 24 hours in considerable number, however, in the zone of reaction at the limbus. As time went on, as shown by sections at later periods, mononuclears gradually increased in number in the central zone of reaction at the site of infection, and rapidly increased when blood vessels reached this point. These facts, taken together, seemed to warrant the conclusions that (*a*) both the polymorphonuclear leukocyte and the large mononuclear in the developing tuberculous lesion in the cornea come from the vascular system, and (*b*) the former reaches the infected region sooner because it is a more rapidly moving cell. The failure of large mononuclears in the early reaction at the site of infection seems good evidence that the local cells do not partake in the reaction, as claimed by Baumgarten [5] in his early work.

In passing it should be said that objection may be made to these conclusions because of the size of the dose of tubercle bacilli used in the experiments here recorded. Krause [6] has stressed the point of dosage in the following words: "If we eliminate all disturbing factors that may arise through the inoculation of too large quantities of bacilli we may arrive at a true appreciation of the character of the process which gives rise to the aggregation of epithelioid cells which form primary tubercle. In response to the localization of a very few bacilli and during the first few days after localization, we find that not a vestige of tissue disturbance occurs except at and around the immediate point of localization of bacilli. Between this site and any other point we cannot trace the slightest evidence (anatomical) that the bacilli have exerted the least effect on any other cells than those

of the immediate neighborhood. Save for the completely isolated aggregation of epithelioid cells, enclosing tubercle bacilli, the microscopic field discloses entirely normal tissue — of lung or cornea for instance. At the same time, within the epithelioid masses cells in active mitosis are frequently to be observed. These two phenomena compel the opinion that the *origin* of nodular tubercle, that is, the first aggregation of epithelioid cells, is accomplished through the *proliferation*, in other words the new growth, of cells at or near (within microscopic distance of) the point of localization of tubercle bacilli, and normally existing there."

The question of the origin of the cells entering into the reaction will be considered in detail, in connection with further experiments, in the next chapter.

In explanation of the size of the dose used in our experiments we should say that we desired to follow the fate of the tubercle bacilli injected, and for this reason injected a quantity making this effort easy. The amount injected is not excessive according to the usual standards for the inoculation of tubercle bacilli. Nevertheless, in order to answer the question of relation of type of reaction to dosage we diluted the suspension of bacilli used in the experiments here reported 100 times for an additional, otherwise similar experiment in the rabbit cornea. It is significant that although the intensity of reaction was very much reduced, qualitatively no difference was observed from that seen and here described with the larger dose. Accordingly we felt that valid conclusions for the general subject of tuberculous infection could be drawn from our more extensive experiments with the doses of 0.005 to 0.01 mg. of bacilli.

4. The tubercle bacilli injected were practically all phagocytozed by polymorphonuclear leukocytes within the first 24 hours. With the arrival of the large mononuclear phagocytic cells, ingestion of the polymorphonuclears by these cells occurred, with a corresponding transfer of the tubercle bacilli first taken up by polymorphonuclears. The situation was like that described and illustrated by Vorwald, except that in the present case the arrival of the large mononuclears was a much delayed process. At 72 hours the predominant cell at the site of infection in all animals was still the polymorphonuclear leukocyte, but at the two later periods studied, 2 weeks and 1 month, the large mononuclears were in considerable excess, and the bacilli were chiefly within their cytoplasm.

5. The final picture showed characteristic differences. The sensitized guinea pig was dropped from the series recorded in the accompanying figure, because at this period in reinfected guinea pigs ulceration occurred and heavy secondary infection complicated the picture. In the other animals the intensity of the reaction, as measured by the proportion of the cornea in a state of inflammatory reaction, varied in the guinea pig, rabbit and cat, with decrease in the order named. At 1 month vascularization of the infected area was well developed in the guinea pig and rabbit, and in these animals the cells of the lesion were now predominantly mononuclear. In two out of three cats, on the other hand, vascularization of the infected area had not yet occurred and the proportion of mononuclear cells of inflammation was much lower. As the cat had the highest general resistance to infection with the strain of tubercle bacillus used, and the guinea pig the least, the intensity of reaction at 1 month, the period of full tubercle development, was seen to be in inverse proportion to the general resistance.

Summary

Central interlamellar corneal injections of 0.01 to 0.005 mg. of moderately virulent tubercle bacilli of human type were made in a series of normal and tuberculous guinea pigs, and normal rabbits and cats. Animals of each kind were killed at 6 hours, 1, 3 and 14 days and 1 month, and the nature and extent of the inflammatory response determined by microscopic examination.

In all cases the first reaction noted was at the limbus, and not at the site of injection. The reaction consisted of an outpouring of polymorphonuclear leukocytes from the marginal vessels. It was most intense in the tuberculous guinea pig (tuberculin reaction), less in the normal guinea pig, still less in the rabbit and least in the cat.

The first cellular reaction at the site of injection in the center of the cornea consisted of leukocytes migrating in from the margin. The reaction was well developed in all animals at 24 hours, and the cells taking part were almost exclusively polymorphonuclear leukocytes.

After 24 hours there was a gradual increase in the proportion of large mononuclears in the reaction. The rate of increase rose with the closer approach of blood vessels, as vascularization of the cornea

developed. The paucity of these cells at the earlier periods and abundance in the vascularized state of the cornea seemed good evidence that the large mononuclear leukocyte in corneal tuberculosis is not locally derived, as formerly claimed. In all animals the large mononuclears, as they reached the site of infection, engulfed the polymorphonuclear leukocytes already there, taking over the tubercle bacilli contained by the latter cells.

From 3 days to 2 weeks the inflammatory reaction progressed most rapidly in the tuberculous guinea pigs, and less rapidly in the normal guinea pigs, rabbits and cats in the order named. Ulceration occurred in the tuberculous pigs in 2 weeks, and these pigs were dropped from the series at this point.

At 1 month, the last period studied, the intensity of inflammatory reaction, as determined by the size of the lesion and proportion of the cornea involved, was greatest in the guinea pig, less in the rabbit and least in the cat. This order is in inverse relation to the general resistance of these animals to the strain of tubercle bacillus used.

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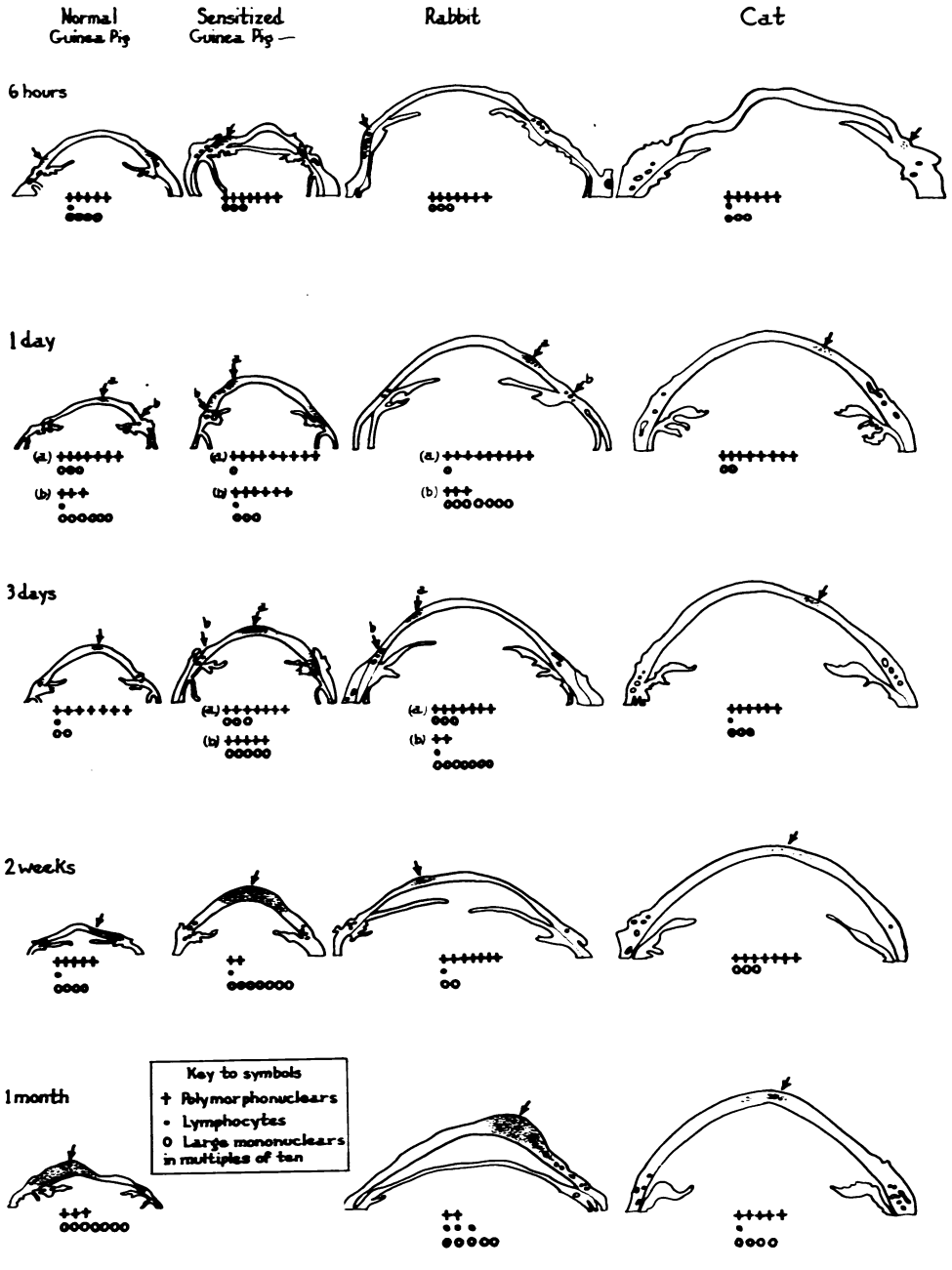
DESCRIPTION OF PLATE

PLATE 53

FIG. 1. Course of development of tuberculosis of the cornea in different animals. The size of the lesion is indicated by fine shading. Blood vessels are indicated by small circles and ovals. Approximate differential counts of the cells are given, the regions at which the counts were made being marked by arrows.


Normal Guinea Pig
Sensitized Guinea Pig
Rabbit
Cat
6 hours
1 day
3 days
2 weeks
1 month
Key to symbols
+ Polymorphonuclears
• Lymphocytes
0 Large mononuclears
in multiples of ten