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Letters to the Editor

SEVERITY OF LEPROSY EYE LESIONS IN ARMADILLOS INFECTED WITH *MYCOBACTERIUM LEPRAE*

Sir,

Although leprosy as a cause of bindness is not mentioned in some leading books about blindness,[1,2] the scope of blindness due to leprosy has always been very large. It was estimated that up to 250,000 leprosy patients could be blind.[3] The importance of prevention and treatment of ocular leprosy and its consequent blindness is well recognized by some; and a meeting was held in 1987 to examine various aspects concerning eye leprosy, and the need of some basic biomedical research on ocular leprosy was emphasized, including histopathological and immunopathological examination of human ocular tissues taken from surgical procedures. Due to the paucity of human eye tissues at any stage of the disease, the use of animal models was also emphasized.[3]

The discovery that an armadillo could be experimentally infected *Mycobacterium leprae* made it a model for many in research purposes.[4] One of them is to gain some insight into the pathogenesis and pathology of eye lesions, even though this animal is not a primate and the severity of the lesions caused by the infection are not exactly like those in human beings. But the pathology of the eye of the *M. leprae* infected armadillos could serve as a ground for further investigations with different approaches.

During our study of the pathology of 127 eyes of 66 armadillos infected experimentally with *M. leprae* in 3 research institutes, we found that although these animals were infected with almost the same dose of the bacilli intravenously, the lesions in the eyes of different animals were not of the same severity. We examined the possible factors causing the variance in these lesions.

The experimental infection of armadillos with M. leprae

The armadillos (*Dasypus novemcinctus*) used for the experiments were all caught in Florida, USA. The animals were kept and infected in 3 institutes: Medical Research Institute in Melbourne, Florida, USA, Forschungsinstitut Borstel in Borstel, FRG and the Royal Tropical Institute, Amsterdam, Holland.

After capture in the wild, they were transported to the institutes. Within a period of 6 weeks of quarantine, examinations for acquired leprosy and treatment of parasitic infestations were carried out.

Then a dose of 10^8 *M. leprae* in 1 ml of saline was inoculated into the femoral vein of each animal. After the inoculation, the animals were kept at 25° $\pm$2°C, humidity of 50–60% and examined by experienced personnel till the death of the animal or until they were killed.

At the time of autopsy the liver and spleen of the animals were weighed and the amount of *M. leprae* in these tissues measured.

The eyes of the animals were removed, many with the eyelids and intra-orbital tissues, fixed in buffered formaldehyde or glutaraldehyde and histological preparations were made and examined.

Grading of eye lesions

We categorize the lesions of the eyes of these animals into 4 groups, according to the severity of the reactions. The group with the slightest lesions (the $\pm$group) included the eyes with a very small

amount of lymphocyte, plasma cell infiltrations, usually around the anterior chamber angle, in the ciliary body, and occasionally in the iris and choroid. If there were several macrophages present, the cytoplasm of these cells was not very abundant. The 1 plus (+) group included the eyes with some lymphocyte, plasma cell and large macrophage (with abundant cytoplasm) infiltrations around the anterior chamber angle and in the iris, ciliary body and choroid. The 2 plus (++) group included the cases with significant infiltrations of plump macrophages in the ciliary body, iris and choroid, with slight to moderate thickening of these structures. The 3 plus (+++) group included the cases with the whole uvea tract densely infiltrated with plump macrophages and the uvea was thickened significantly.

Besides each eye being categorized, each animal was also put into one of the four grades, making the higher grade of the two eyes its grade when the grades of their two eyes were not identical. The numbers of animals in the four grade groups from the three institutes are shown in Table 1.

Facts and discussions

The details of the pathology of the lesions of the eyes of these armadillos was reported in a separate article.[5]

No bacillus was found in the liver and spleen of 5 of the inoculated animals. The percentage of the inoculated but not infected (resistant) armadillos, 7·5% (5/66), was similar to other reports.[6] The eyes of these animals showed very slight cell infiltrations around the anterior chamber angle area, which made us put them in the ± group when we viewed the slides without knowing the AFB content in the liver, spleen and eyes of the animals. AFB were found in the eyes of 12 of the 20 ± group animals. In view of this finding in these uninfected and uninoculated normal control animals, the ± lesion could mean either a mild or early reaction to *M. leprae* or to some non-specific irritant.

In the great majority, 56/66 (85%), two eyes of the same animal had the same severity of lesion. In 6/66 (9%) the grades of the lesions in the two eyes were of adjacent grades, only in 4/66 (6%) were they of one or more grades apart. As our specimens were not serial sections and the lesions in the uvea sometimes segmental, the possibility of the chance manifestation in showing the lesions in different cutting planes of the same eyeball was considered, when the discrepancies of the grades of lesions in the two eyes of the same animal and of different animals was reviewed.

Since ± and 1+ groups were those with slight lesions and 2+ and 3+ groups were those with severe lesions, the ± and 1+ groups were combined as one group and the 2+ and 3+ as another in the following analysis.

In the Borstel and Florida experiments, the number of animals on the ±,+ group were of the great majority: 23/28 (82%) and 20/23 (87%), while those in the 2+,3+ group were 5/28 (18%) and 3/23 (13%) respectively. When the duration of infection and the total amount of the AFB in the spleen and liver of these two groups of animals were considered (Table 2), no meaningful relationship between these factors and the lesion severity was found.

Table 1. Number and percentage (in parenthesis) of animals from 3 institutes in different lesion grade groups

Eye lesions	±	+	++	+++	Total
Borstel	18 (64%)	5 (18%)	2 (7%)	3 (11%)	28 (100%)
Amsterdam	2 (13%)	1 (7%)	7 (47%)	5 (33%)	15 (100%)
Florida		20 (87%)	2 (8·7%)	1 (4·3%)	23 (100%)
Total	20 (30%)	26 (39%)	11 (17%)	9 (14%)	66 (100%)

Table 2. The duration of infection and the total amount of AFB in the spleen and liver of the Florida and Borstel armadillos

Number of animals (lesion grade)	Duration of infection (Mo), range (average)	No. of AFB in spleen (10^9) range (average)	No. of AFB in liver (10^9) range (average)
18* (±,+) Florida	13–37 (22·1)	30·42–3600·6 (495·67)	111·3–12676·0 (564·4)
20† (±,+) Borstel	15–60 (26)	0·5–24990·0 (2976·41)	1·2–1844700 (117698)
7† (2+,3+) Florida Borstel	15–49 (23·8)	3·04–810·0 (245·19)	6·8–4116·8 (1477·3)

* AFB in 2 additional animals were not known; † AFB in 3 additional animals were not known; ‡ AFB in 1 additional animal were not known.

But when the animals of the Amsterdam group was considered, the situation was different (Table 3).

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Table 3. The duration of infection and the total amount of AFB in the spleen and liver of the Amsterdam armadillos

Number of animal (lesion grade)	Duration of infection (Mo), range (average)	No. of AFB in spleen (10^9) range (average)	No. of AFB in liver (10^9) range (average)
3 (±)	8–55* (26)	0·0–3·0† (1·77)	0·5–3810‡ (1270·6)
12 (2+, 3+)	12–15	112·5–3760 (1052·3)	128·0–5019 (2276·9)

* 8, 16, 55 in these 3 animals each; † 0·0, 2·1, and 3·0 in these 3 animals each; ‡ 0·5, 1·4, and 3810 in these 3 animals each.

In the Amsterdam group, although the duration of the infection in the ±,+ and 2+,3+ groups were not of significant difference, the amount of the AFB in the spleen of the animals of these groups were significantly different, as shown in Table 3, i.e. $0{\cdot}0–3{\cdot}0 \times 10^9$ in the ±,+ group *vs* $112{\cdot}5–3760 \times 10^9$ of the 2+,3+ group. These figures suggested that there seemed a parallel relationship between the eye lesion severity and the amount of AFB in the spleen of these animals. If the AFB amount of the spleen could be considered as representing something about the degree of bacteremia, the degree of generalization of the infection or the condition of the immunity of the infected animal, then the more severe lesions that occurred in the high spleen AFB concentration animals could be explained accordingly. But the differences of the amount of AFB in the liver of these two groups seemed to be of no meaningful significance.

The amounts of AFB in the spleen and liver of the infected armadillos seemed not always parallel to each other and there seemed to be no distinctive regular relationship between them. They randomly surpassed (or were similar to) each other without known reasons.

In contrast to the Borstel and Florida animals in which the animals with higher grade eye lesions were significantly less (8/51, 16%), the lesions in the animals from Amsterdam were more similar and mostly in the higher grade (12/15, 80%). There was some differences in performing the inoculations in these groups. In the Borstel and Florida animals, the inoculations of *M. leprae* were not done the same day, in the same month and or even the same year. That might mean that the

inoculated bacteria might not be of the same viability or virulence since different manipulations of the same batch, not mentioning different batches, of bacteria might occur. But the Amsterdam animals were inoculated on the same day with *M. leprae* of one Borstel armadillo's spleen (animal number 74, with ± eye lesion grade and $6{\cdot}9 \times 10^9$ AFB/g of spleen). The spleen was taken and held at soft ice temperature for 2 days before the experimental intravenous inoculation. The suspension for inoculation was prepared and dispersed by mild ultrasonic treatment for 5 minutes just before inoculation. This procedure would fragment the particles in the suspension and enhance the distribution of *M. leprae* in infected animals and lead to development of more lesions.[7] This may be one of the causes of the enhancement of the eye complication in the animals infected in Amsterdam. A higher viability of *M. leprae* in the suspension might also be considered, since the *M. leprae* in the suspension used in the Amsterdam group were not subjected to freezing and thawing during the preparation of the inoculum. These might be the reasons why the Amsterdam animals had more similar and severe eye lesions.

Since our present study is a retrospective one, we could not have the desired control over groups of animals included in the study as in a prospective one, for example a control group in the Amsterdam animals using the same batch of *M. leprae* but without sonication before the inoculation. The aforementioned possibilities should be examined when a prospective experiment is possible.

In inducing an eye leprosy animal model for further research, a method guaranteeing a higher rate of forming intra-ocular lesions and a more uniform intra-ocular infection, through systemic inoculation of *M. leprae*, would be very beneficial. We would like to see if the method used in the Amsterdam experiment could give a more constant result.

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References

[1] International Agency for the Prevention of Blindness (under the direction of Sir John Wilson) (ed.). *World Blindness and its Prevention*. Oxford: Oxford University Press, 1980.
[2] Lim ASM, Jones BB (eds). *World's Major Blinding Conditions*. Singapore: FESPIC Enterprises Pte Ltd, 1982.
[3] Courtright P, Johnson GJ (eds.). *Prevention of Blindness in Leprosy*. London: The International Centre for Eye Health, 1988, pp. 25–8.
[4] Kirchheimer WF, Storrs EE. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. *Int J Lepr*, 1971; **39:** 693–702.

[5] Brandt F, Zhou HM, Shi ZR, Kazda J, *et al.* The pathology of the eye in armadillos experimentally infected with *M. leprae*. *Lepr Rev*, 1990; **61,** 112–131.
[6] Job CK, Sanchez RM, Hastings RC. Manifestations of experimental leprosy in the armadillo *Am J Trop Med Hyg*, 1985; **34:** 151–61.
[7] Kazda J. A more malignant course of leprosy infection in armadillos after inoculation with sonicated suspension of *Mycobacterium leprae*. *Int J Lepr*, 1986; **54:** 129–31.