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AMYLOIDOSIS DEVELOPING IN EXPERIMENTAL NOCARDIA INFECTIONS

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Summary.—Swiss white and C57/BL/6J mice inoculated repeatedly with either *Nocardia asteroides* or *Nocardia brasiliensis* organisms developed amyloidosis over a 7-month period. Amyloidosis also developed in these mice within 6 weeks following a single large inoculum of either organism, but not in other in-bred mouse strains, suggesting a genetic influence in the pathogenesis of this form of secondary amyloidosis.

THERE are clinical and pathological similarities between nocardia infections and tuberculosis, leprosy and actinomycosis (Drake and Henrici, 1943; Brown and von Lichtenberg, 1970). Moreover, nocardiae share certain antigens with *Mycobacterium tuberculosis* and with *Mycobacterium leprae* (Humphreys, Drowder and White, 1975). Whereas human tuberculosis, leprosy and actinomycosis may be complicated by the development of amyloidosis of the secondary type (Williams *et al.*, 1965; Powell and Swan, 1955, Symmers, 1973), amyloid deposition has not been described as a complication of nocardiosis. We report here a series of experiments in which it was demonstrated that the development of amyloidosis was associated with nocardia infection. Our investigations have been based on a mouse model of nocardiosis in which a localized and persistent infection is produced, without the addition to the inoculum of adjuvant or mucin (Folb, Jaffe and Altmann, 1976), as these two agents may in their own rights alter the pathological and immunological responses of the host (Uesaka *et al.*, 1971).

MATERIALS AND METHODS

Nocardia organisms.—The identification and confirmation of the Nocardia organisms which were used and the method of inoculation have been described by us previously (Folb *et al.*, 1976).

Animals:—Swiss white, C57/BL/6J, New Zealand black (NZB), BALB/C, CBA/LAC, C_3H/eB and congenitally athymic ("nude") (ICR strain) mice weighing 25–30 g were obtained from the Veterinary Department, Weizmann Institute, Rehovot.

Inoculation and histological examination:—Twelve female Swiss white mice were injected i.p. with 5 mg (wet weight) of *Nocardia asteroides* suspended in 0·5 ml sterile saline on 3 separate occasions spaced at 2-month intervals. They were killed 7 months after the start of the experiment and 3 months after the last injection. A further 8 Swiss white mice with the same characteristics were similarly inoculated with 5 mg (wet weight) *Nocardia brasiliensis* suspended in 0·5 ml sterile saline 5 times at monthly intervals, and they were killed 7 months after the start of the experiment and 3 months after the last injection. In each case a full post-mortem examination was performed, and it was verified pathologically and bacteriologically that a chronic, active nocardia infection was present, and that there was no other apparent disease. The presence of amyloid was determined by histological examination. Preparations were stained with haematoxylin and eosin, and with

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Congo red, and the latter were examined under a polarized light microscope.

In each case equal numbers of Swiss white mice injected i.p. with 0·5 ml sterile saline served as controls.

In a separate experiment 2 groups of 10 C57/BL/6J mice were injected i.p. with a single dose of *N. asteroides* and *N. brasiliensis* respectively in the same manner as previously, but with an inoculum twice as large (10 mg wet weight of organism). In the same way groups of 10 Swiss white, New Zealand black (NZB), BALB/C, CBA/LAC, C_3H/eB and congenitally athymic ("nude") mice (ICR strain) were inoculated with *N. asteroides* and *N. brasiliensis* respectively. Ten mice of each strain injected i.p. with 0·5 ml sterile saline served as controls. At 2 and 4 weeks after inoculation 2 mice were killed from each group, and at 6 weeks the experiment was terminated and the remaining animals were examined. A postmortem examination was performed in each case and the search for amyloid was made in the manner described above.

RESULTS

Amyloid was found in 8 of the 12 Swiss white mice inoculated with *N. asteroides*. The heaviest infiltration was in the spleen. Here, the amyloid was noted in the walls of the sinuses and the adjacent connective tissue framework constituting the red pulp. The deposits tended to coalesce to form large masses and sheet-like areas. The follicles were relatively unaffected. The distribution of amyloid in the walls of the splenic blood vessels was of a perireticulin pattern (Heller *et al.*, 1964). The splenic trabeculae were replaced by amyloid. The liver was invariably involved in those animals in which the spleen was affected. Here the distribution was limited to blood vessels and to the sinusoids. In the kidneys amyloid deposition was minimal, and variable, and limited to blood vessel walls. Of the 8 mice inoculated with *N. brasiliensis* amyloidosis was found in 6 and deposited in the same manner as that of the *N. asteroides*-infected animals.

The results of the second experiment in which inoculations of *N. asteroides* and *N. brasiliensis* were administered to Swiss white mice and to different inbred genetic mouse strains are shown in the Table. It was found that in the case of both C57/BL/6J and Swiss white mice amyloid had appeared by 4 to 6 weeks in about two-thirds of the animals injected with either organism. No differences in the incidence, quantity or distribution of the amyloid deposited in these two mouse strains were noted. No amyloidosis developed in the other genetic mice strains over the 6-week period. The statistical significance of these findings is indicated in the Table.

None of the control animals developed amyloidosis.

DISCUSSION

This finding of amyloidosis developing in the course of nocardia infections is a new one. Despite marked differences in

TABLE.—*The Incidence of Amyloidosis Following Inoculation of Different Mouse Strains with* N. asteroides *and* N. brasiliensis

	N. asteroides			*N. brasiliensis*		
Mouse-strain	2 weeks	4 weeks	6 weeks	2 weeks	4 weeks	6 weeks
Swiss white	0/2	1/2	4/6	0/2	1/2	3/6
C57/BL/6J	0/2	1/2	4/6	0/2	1/2	4/6
NZB	0/2	0/2	0/6	0/2	0/2	0/6
BALB/C	0/2	0/2	0/6	0/2	0/2	0/6
CBA/LAC	0/2	0/2	0/6	0/2	0/2	0/6
C_3H/eB	0/2	0/2	0/6	0/2	0/2	0/6
Congenitally athymic (ICR)	0/2	0/2	0/6	0/2	0/2	0/6

Statistical testing (binomial test) showed that in the case of both *N. asteroides* and *N. brasiliensis* infections the Swiss white mice differed from the individual groups of NZB, BALB/C, CBA/LAC, C_3H/eB and congenitally athymic (ICR strain) mice respectively in terms of their development of amyloidosis at 6 weeks at a significance level of $P < 0{\cdot}001$.

There was no statistical difference between Swiss white and C57/BL/6J mice.

the histopathological responses of Swiss white mice to *N. asteroides* and *N. brasiliensis* respectively (Folb *et al.*, 1976), and despite apparent differences in the immune responses of this mouse to the two organisms (personal observations, unpublished), these factors do not appear to have any bearing on the incidence, rate of onset or distribution of the amyloidosis. This amyloid model is reasonably consistent and, in that deposits may appear within 4–6 weeks of inoculation in certain mouse strains, the model may be of use in the study of secondary amyloidosis.

The findings add further weight to the contention that nocardia produces a disease similar to that of mycobacterial infections, in particular tuberculosis and leprosy. The development of amyloidosis in certain strains of mice but not in others suggests that, in addition to the antigenic stimulus, genetic factor(s) may play a role in determining whether or not secondary amyloidosis develops.

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