AMYLOIDOSIS IN MICE AFTER IMMUNIZATION WITH TWO ANTIGENS

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During intensive immunization of mice, the replacement of one antigen by another had no substantial effect on the development of amyloidosis in the spleen. It is assumed that amyloid deposition is facilitated by the nonspecific action of large doses of antigens.

In any type of immunologic interpretation of the pathogenesis of amyloidosis, antigenic stimulation of the organism is implied. The role of antigen is revealed most demonstratively in the model of experimental amyloidosis developing after prolonged immunization with casein and other proteins [7]. However, it has not yet proved possible to associate the existence of many different forms of human (hereditary, senile) amyloidosis and spontaneous amyloidosis in animals, or cases of development of amyloidosis after injection of nonantigenic compounds [4], with the action of antigen. The question naturally arises: to what extent is specific immunization necessary for this process to take place?

The object of the present investigation was to determine whether amyloidosis can develop during consecutive administration of two different antigens.*

EXPERIMENTAL METHOD

To exclude both determinant groups, proteins of widely different origin were chosen: egg and human albumin, casein, acid phosphatase (from wheat).

Experiments were carried out on male BALB mice weighing 10-20 g; 5% casein was made up in 0.25% NaOH solution, and the remaining proteins were diluted with physiological saline to the same concentration. The mice received injections of 0.5 ml of each antigen subcutaneously 6 times a week. The results of the experiments were assessed by histological study of sections of the spleen in which amyloid is deposited sooner than in any other organs. Native sections obtained on a freezing microtome were fixed with 96° ethanol and stained with Congo red [9], methyl violet [1], or thioflavin T [2].

Antigens were injected successively: first one, then the other, but on fewer times than would be demanded for amyloid formation if only one antigen was used for immunization. For this reason, the optimal number of injections of each protein before the appearance of the first signs of amyloidosis was first established. For casein this was 13-14, for acid phosphatase 15-16, and for human albumin 17-18. Stopping immunization at this stage led only to regression of the hyperplastic spleen.

EXPERIMENTAL RESULTS

Amyloidosis developed in most cases (under 100%) with different combinations of proteins (Table 1). The intensity of the depositions was sometimes rather less than after adequate injection of one antigen, and

*Preliminary data were presented at the Third Conference on Immunopathology by V.S. Rukosuev and L. V. Litvinova. See Abstracts of Proceedings of the Conference [in Russian], Leningrad (1969), p. 58.

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TABLE 1. Development of Amyloidosis in Mice after Consecutive Injection of Two Different Antigens

First antigen	No. of injections	Second antigen	No. of injections	Number with amyloidosis/ number in experiment
Casein	13	Physiological saline	13	0/5
Egg albumin	13	Physiological saline	13	0/5
Acid phosphatase	15	Physiological saline	15	0/5
Human albumin	17	Physiological saline	17	0/5
Casein	12	Egg albumin	12	6/6
Egg albumin	12	Casein	12	5/5
" "	12	Human albumin	12	4/5
Human albumin	15	Egg albumin	13	6/6
Egg albumin	12	Acid phosphatase	12	6/6
" "	12	Casein	4	4/5
" "	14	Human albumin	2	4/5
Casein	9	Egg albumin	8	2/5
"	9	" "	10	3/5

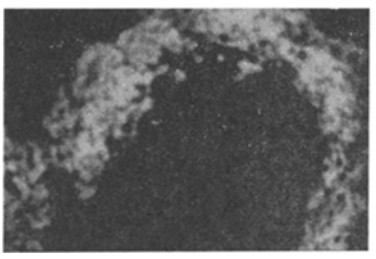

Fig. 1

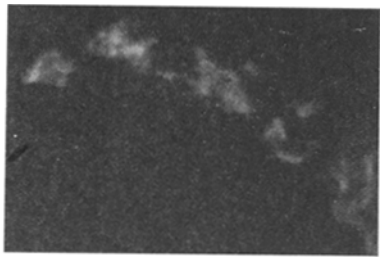

Fig. 2

Fig. 1. Masses of amyloid in the perifollicular zone of a mouse spleen (12 injections of egg albumin followed by 12 injections of acid phosphatase). Thioflavine T, 30×.

Fig. 2. Slight depositions of amyloid at the border of a follicle (12 injections of egg albumin following 4 injections of casein). Thioflavin T, 120×.

it varied within each separate batch of mice (Fig. 1). A definite relationship was found between the positive results and the number of injections of the first antigen.

The total number of injections of two antigens gave the same results as that number of injections of one antigen only in the case of optimal (i.e., on the borderline of formation of amyloidosis) doses of the first first antigen (Fig. 2), and the number was increased if the number of injections of the first antigen was reduced. However, the very small differences in the times of detection of amyloid were not significant compared with the general tendency for the condition to progress with an increase in the duration of immunization, regardless of the nature of the antigenic stimulation.

The results described above show that replacement of one antigen by another has little effect on the induction of amyloidosis in mice. On the basis of modern views regarding narrow specialization of most immunocompetent cells it is difficult to account for this phenomenon otherwise than by some nonspecific action of large doses of protein. This possibly relates to the phenomenon of immunologic tolerance which, like amyloidosis, arises not only to the inducing antigen, but to other antigens injected subsequently, during intensive immunization of adult animals [3, 8, 10, 11].

The role of the second antigen can also be compared with the effect of cytostatic compounds, in that they simply aggravate the severity of amyloidosis produced by preliminary immunization [12]. The possi-

bility is not ruled out that the action of antigens is effected through a nonspecific tissue or humoral factor; the latter has recently been found by American authors [5, 6, 13] during the development of experimental amyloidosis following transplantation, and also by myself, in investigations which have not yet been published.*

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