DEVELOPMENT OF AMYLOIDOSIS IN MICE UNDER THE INFLUENCE OF CERTAIN ANTIGENS

V. S. Rukosuev

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Consecutive or combined injection of between 4 and 10 protein antigens in different combinations caused the deposition of amyloid in the spleen of mice at about the same time as after immunization with casein only. These results are evidence against the leading role of the specific immunological response of the organism in the pathogenesis of experimental amyloidosis.

Not only casein but also many other proteins can induce amyloid formation [4, 11, 17]. It has recently been shown that the replacement of one antigen by another in the course of immunization has no significant effect on the development of amyloidosis in mice [1].

The object of the present investigation was to study the possibility of amyloid formation during consecutive or simultaneous injection of various protein antigens.

EXPERIMENTAL METHOD

Experiments were carried out on male BALB mice weighing 18-20 g. The following 10 proteins were used in 5% solutions: casein, human γ-globulin, rabbit γ-globulin, bovine γ- and β-globulins, human albumin, bovine albumin, egg albumin, alkaline (from chicken intestine) and acid (from wheat) phosphatases. The proteins were diluted in physiological saline except casein, which was dissolved in 0.25 M NaOH. In preparation of the mixture equal proportions of the proteins were estimated on the basis of a 5% solution. Subcutaneous injections of 0.5 ml of the protein solutions were given six times a week.

Altogether six series of experiments were carried out with simultaneous or consecutive injection of the different proteins (Table 1).

The mice were sacrificed in groups after 16, 18, 20, and 24 injections. The experimental results were assessed by histological study of frozen sections of the spleen fixed in 96° ethyl alcohol and stained with methyl violet and thioflavine T.

EXPERIMENTAL RESULTS

In the five experimental series (Table 1) the first signs of deposition of amyloid in the peripheral zone of the spleen follicles in isolated animals were observed after 18-20 injections, regardless of the combinations of proteins given [4, 6, 10] or whether they were injected simultaneously or consecutively. If casein alone was injected (series VI) amyloidosis developed several days earlier. However, the general tendency toward an increase in the number of affected animals and an increase in the mass of amyloid deposited in proportion to the quantity of protein injected remained unchanged in all cases.

The slight slowing of amyloid deposition in the experimental series is of no significance in principle and was evidently due to the physicochemical properties of the proteins used, for at least some proteins

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TABLE 1. Development of Amyloidosis in Mice after Consecutive and Simultaneous Injection of Different Proteins

Series No.	Number of injections of each protein or of mixture of proteins	Order in which proteins injected	Number of cases with amyloidosis after total number of injections of proteins			
			16	18	20	24
I	6	1. Casein 2. Egg albumin 3. Bovine γ-globulin 4. Human albumin	0/5	2/5	2/5	4/5
II	4	1. Egg albumin 2. Bovine γ-globulin 3. Human albumin 4. Casein	0/5	1/5	3/5	3/4
III	2	1. Alkaline phosphatase 2. Bovine albumin 3. Casein 4. Bovine β-globulin 5. Human γ-globulin 6. Acid phosphatase 7. Human albumin 8. Egg albumin 9. Bovine γ-globulin 10. Rabbit γ-globulin	–	1/4	6/10	–
IV	20	Mixture of 4 proteins from series I	–	1/5	4/6	–
V	20	Mixture of 10 proteins from series III	–	1/5	3/7	–
VI	24	Casein	2/5	3/5	5/5	5/5

with a lower molecular weight than casein induce amyloidosis correspondingly later [1, 17]. The important fact is that induction of amyloidosis takes place through the action of different antigens, for which it is difficult to postulate common determinant groups, especially if they are injected consecutively. It must therefore, be questioned whether a specific immune response is necessary in the pathogenesis of amyloidosis and, consequently, whether the various immunological interpretations of amyloid as an antigen–antibody complex [16, 18], a product of activity of the cells of the reticulo-endothelial system [22], or of lymph glands in the final stage of tolerance [6],* are justified.

The evidence for the presence of humoral immunity in experimental amyloidosis is in fact contradictory [8, 10, 13]. Nevertheless, amyloidosis readily arises in the presence of wasting and depression of the lymphoid system caused by bursectomy and thymectomy [9, 12, 13, 19], x-ray irradiation [14, 19], or antilymphocytic serum [9, 20], in artificial tolerance [6, 7], or in "secondary disease" [5]. It is also confirmed by recent work on the stimulation of amyloid formation in mice under the influence of a special factor even if obtained by heterologous transfer [2, 3, 14, 21].

Without completely denying the role of a specific immune response in experimental amyloidosis, attention must evidently be concentrated on some as yet unknown mechanism of nonspecific adaptation of the immunocompetent system [15] as part of a general series of responses of the organism to the disturbance of homeostatis produced by massive doses of exogenous proteins.

*The authors suggest that tolerance passes through two phases: an early, inductive phase and a late phase of actual amyloid deposition.

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