REPRODUCTION OF AMYLOIDOSIS IN MICE DURING STIMULATION INHIBITION OF IMMUNOGENESIS

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Using the passive hemagglutination, agar diffusion, and indirect Coon's tests amyloidogenesis was studied in the spleen of BALB/c mice during active immunogenesis (injection of egg albumin in adjuvant) and during the development of partial tolerance (injection of a massive dose of egg albumin). The different states of the immunocompetent system were not reflected in the formation of amyloid masses. The results make it unlikely that the specific immunological response of the organism plays an essential role in the pathogenesis of experimental amyloidosis.

Changes in the state of immunity in amyloidogenesis, especially of the experimental type, had been firmly established [9, 12, 16]. However, the concrete role of the immunological system in this process is uncertain. The old concept, laying stress on immunogenesis and regarding amyloid as a product of antigen–antibody precipitation [14, 15], has increasingly met with objections [8, 12, 19]. It has recently been postulated that amyloid, on the contrary, is the natural outcome of induced tolerance [6, 7].

The object of this investigation was to study the pattern of development of experimental amyloidosis during stimulation and inhibition of immunogenesis.

EXPERIMENTAL METHOD

Two series of experiments were carried out on male BALB/c mice weighing 18-20 g, with 30 animals in each series.

In series I (stimulation of immunogenesis) the animals received a single intraperitoneal injection of 0.4 ml (25 mg) egg albumin in Freund's adjuvant (Difco). A model of the reproduction of amyloidosis with the aid of adjuvant has been suggested recently [17], for this substance is known to be a nonspecific stimulator of immunogenesis [1].

In series II (inhibition of immunogenesis) mice received subcutaneous injections of 0.5 ml 10% egg albumin in physiological saline six times a week for 6 weeks, in accordance with the classical model of casein amyloidosis [10]. The injection of larger doses of soluble proteins induced tolerance in the animals [11].

The mice were sacrificed in groups of three or four at a time 3 days and 1, 2, 3, 4, 5, and 6 weeks later, and also 1 week after a further subcutaneous injection of 5 mg egg albumin in physiological saline. The sera of each group of mice were collected in the same tube. The passive hemagglutination test (PHT) was carried out with formalinized erythrocytes, and Ouchterlony's agar diffusion test was carried out in the micromodification [2]. The distribution of cells containing antibodies against egg albumin and nonspecific γ-globulin was studied by the indirect Coons' method in paraffin sections of the spleen. Rabbit antibodies against egg albumin, labeled and unlabeled with fluorescein isothiocyanate, ass antibodies against

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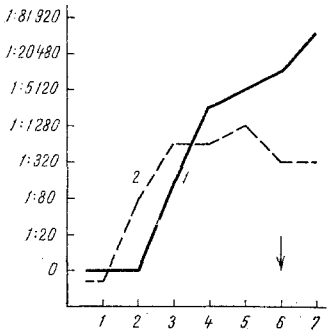

Fig. 1

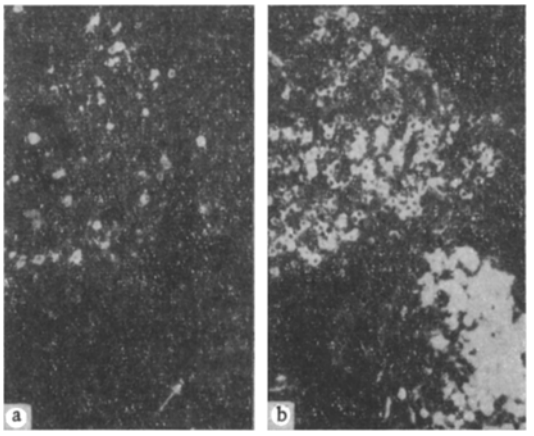

Fig. 2

Fig. 1. Dynamics of antibody formation (passive hemagglutination test against egg albumin in mice during production of experimental amyloidosis in various ways: 1) single intraperitoneal injection of 25 mg egg albumin in Freund's adjuvant; 2) injection of 50 mg egg albumin solution subcutaneously six times a week for 6 weeks. At the beginning of the 7th week all surviving mice received a further subcutaneous injection of 5 mg egg albumin solution (arrow). Abscissa, weeks after beginning of immunization; ordinate, dilution of serum.

Fig. 2. Spleen of mouse 6 weeks after injection of 25 mg egg albumin in Freund's adjuvant: a) group of cells producing specific antibodies lies some distance from the slightly luminescent amyloid (arrow); b) nonspecific γ-globulin in cells and amyloid. Indirect Coons' method. Serial sections, 60×.

rabbit γ-globulin, labeled with fluorescein isothiocyanate, and monospecific rabbit serum against mouse γ-globulin* were used. Serial sections were stained with hematoxylin and eosin, methyl green and pyronine, Congo red, methyl violet, and thioflavine T.

EXPERIMENTAL RESULTS

Judging from the PHT (Fig. 1), the increase in antibody titer in the mice in series I took place a little later than in the mice in series II. Later the titer continued to rise only in the mice of series I, while in the mice of series II it remained at a low level and even decreased, so that by the end of the experiment the difference between the antibody titers in the mice of the two series was significant. After the 5th week the serum of the mice in series I formed three precipitation bands in agar with egg albumin in dilutions of between 2 and 0.005 mg/ml, but no such bands appeared if the serum of the mice of series II was tested. Single cells containing antibodies against egg albumin were identified directly in the spleen sections of most mice of both series 1 week after immunization. Later their number increased considerably only in the animals of series I, but they always remained fewer in number than the cells containing nonspecific γ-globulin. They were arranged in groups, usually along the course of the trabeculae and vessels in the red pulp, less frequently in the follicles, and as a rule, they were not in contact with amyloid (Fig. 2a, b). Meanwhile, in the mice of series II, in which antibody-containing cells were almost completely absent (Fig. 3a) extensive groups of cells synthesizing nonspecific γ-globulin were found more frequently. On histological investigation increasing hyperplasia of the cells was found in the pulp and follicles, and was more marked in the mice of series II. Amyloidosis was first found after 3 weeks in two of the four mice in series I, and in all animals of both series subsequently. Irrespective of the method of immunization, amyloid always began to be deposited at the periphery of the follicles, gradually spreading to adjacent regions of the spleen tissue. The masses of amyloid possessed the typical staining properties (Fig. 3b) and they contained much γ-globulin (Fig. 2b), with slight and inconstant incorporation of specific antibodies (Fig. 2a).

*The last two preparations were generously provided by N. V. Éngel'gardt (N. F. Gamaleya Institute of Epidemiology and Microbiology), to whom the author is grateful.

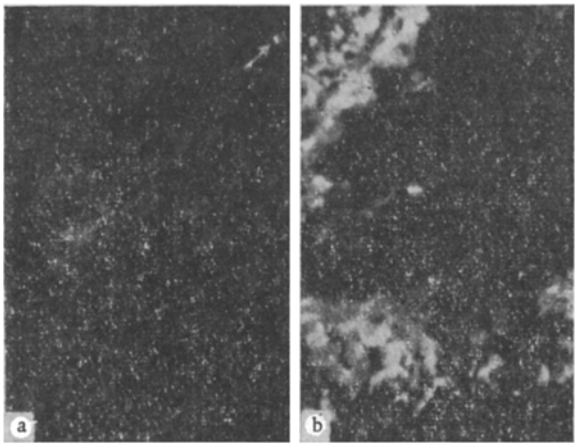

Fig. 3. Spleen of mouse after receiving 36 injections each of 50 mg egg albumin: a) single cell synthesizing specific antibodies (arrow); amyloid remains dark; indirect Coons' method; b) fluorescence of masses of amyloid stained with thioflavin T. Serial sections, 60×.

Consequently, whereas injection of a small dose of egg albumin in adjuvant promoted active immunogenesis, injection of massive doses of antigen led to a sharp decrease in the synthesis of specific antibodies.

The development of only partial tolerance in the latter case is most probably explained by the heterogeneous character of the egg albumin preparation used [21], for slight contamination with conalbumin is known to appreciably increase the total titer of antibodies, especially in the PHT [13]. However, despite difference in the state of immunity, amyloid formation in both cases took place identically. In agreement with these observations, the isolated statements to the effect that humoral immunity is preserved in experimental amyloidosis [3, 11], together with data on the formation of amyloid when the immunocompetent system is inhibited or exhausted [6, 7, 8, 18], do not appear quite so contradictory. Since amyloid formation is not directly dependent on the character of the immunological response to the injected antigen, for amyloidosis can arise by the action of nonantigenic compounds [9], and in view of the existence of forms of hereditary [5] and senile amyloidosis [20], it can be postulated that amyloidogenesis is an extreme form of disturbance of the predominantly nonimmunogenic function of the reticulo-endothelial system provided that each system of the body is only relatively isolated from the rest.

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