Immunological Aspects of Experimental Amyloidosis

In the last four decades, an immunological nature of experimental and secondary amyloid deposition has often been proposed[1,2] but never fully accepted, because the proofs in favour of such a pathogenetic mechanism have been incomplete.

It seems clear that, if amyloidosis is caused by some antigen-antibody reaction, it should not be possible to bring about the disease by means of repeated injections of casein (the most common amyloidogenic agent) in susceptible animals whose normal immunological responsiveness has been in some way erased.

A new approach to this problem has been offered by the recent advances regarding the possibility of suppressing the normal immunological function of vertebrates in several ways; such as the removal of the thymus in newborn animals, the uniform X-irradiation of the body and treatment with various drugs, the injection of antigens during foetal development or soon after birth.

In order to test, then, whether amyloid deposition rests on a classic immunological basis, domestic rabbits were exposed to casein soon after birth and subjected, after weaning, to a parenteral treatment with the same protein for several months. The induction of a stage of immunological tolerance to the antigen, its duration and the eventual amyloid deposition in the organs were then periodically checked.

Two female rabbits were mated with one male, and when the females were observed to be obviously pregnant, they were placed in separate cages. The cages were checked twice daily and the time of birth of each litter was recorded. One half of each litter was kept as controls; the other half was injected subcutaneously daily for 8 days with 1 ml of a sterile 10% solution of casein in sodium and potassium hydroxide at final concentration of 0.25%. 1 ml of solution was also injected at 15 days of age. We refer to the inoculated half-litters as the initially exposed group.

A month and a half after birth, initially exposed and control animals were subcutaneously injected with 0.2 ml of the above-mentioned casein solution incorporated into 0.2 ml of complete Freund's adjuvant; 10 days later the rabbits received a further 0.2 ml of the antigen solution without adjuvant.

On the fifth day following the last injection, blood was drawn from the initially exposed and control groups to test for casein antibody. We refer to the control group now as sensitized controls. Individual sera were tested for their casein antibody titer by means of the passive haemagglutination procedure performed on Takatsy plates with formalinized sheep erythrocytes[3], tanned and coated with casein[4]. All the animals were then inoculated subcutaneously for a period of four months, five times a week, with 5 ml of the usual 10% casein solution; 40,000 IU sodium penicillin were given twice a week in order to prevent infections.

Initially exposed and sensitized controls were bled every fifteen days in order to test the antibody titer in their sera; some of the animals were killed at intervals of two months from the beginning of treatment and their spleens, livers and kidneys were fixed and stained with haematoxylin-eosin, methyl-violet, Congo red, Alcian blue and with periodic acid-Schiff's reagent (PAS. reaction) for histological studies.

Under our experimental conditions, all the sensitized controls tested five days after subcutaneous challenge with casein plus complete Freund's adjuvant gave rise to serum antibody titers ranging from a minimum of 1:1280 to a maximum of 1:10,240, while none of the initially exposed rabbits, contemporaneously tested, showed the presence of circulating antibody against casein. This failure of sensitization to casein following neonatal exposure can reasonably be ascribed to acquired immunological tolerance as defined by MEDAWAR[5].

Initially exposed and sensitized animals subjected to the amyloidogenic treatment maintained their state either of immunological tolerance or of sensitization throughout the entire experiment, as demonstrated by the periodic 15 day passive haemagglutination test on the individual sera.

The main pathomorphological lesions were localized in the spleen after two months of amyloidogenic treatment. In fact, in all animals tested, the initially exposed as well as the sensitized controls, this organ was enlarged as compared to normal, friable and in section showed large follicles. The marginal zones of the follicles were crowded with cells resembling plasma and reticular cells, plus some multinuclear giant cells, whose cytoplasm was strongly Alcian blue and PAS positive. Some amyloid substance could also be seen in certain areas. No relevant modification could be observed in the kidneys and livers.

After four months of the same treatment, the spleens of animals belonging to both experimental groups were still larger and firmer than before. Besides at the periphery of the follicles, isolated nodular deposits in the red pulp, or small intrafollicular deposits could be observed. Renal involvement was conspicuous; amyloid was deposited within the glomerular tufts and, to a quite marked extent, in the pyramids of the kidneys. Only in a few cases was amyloid found in the livers, deposited perivascularly at the periphery of the lobules.

From the above evidence it may be inferred that amyloidosis is not the product of a classic immunological reaction; these results are also in accord with previous investigations by ourselves on the experimental induction of amyloidosis in mice tolerant to casein[6].

It is possible, however, that the disease may be a consequence of some auto-immune process, as suggested by PAVLIKHINA and SEROV[7], according to whom the administration of large doses of foreign protein (casein) to a rabbit brings about an abnormality of the protein-synthesizing function of the reticuloendothelial system, resulting in the formation of anomalous proteins which act as auto-antigens.

It should be pointed out that such an abnormality may take place also in our initially exposed animals, since immunological tolerance is highly specific, i.e. it does not prejudice the rabbit's responsiveness to other antigens or auto-antigens, but only prevents the production of antibodies to those antigens with which it was confronted early in life.

In order to exclude or accept such an auto-immune mechanism, it would be advisable to take advantage of

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the other methods (although more traumatizing and perhaps with side-effects) used to produce non-specific suppression of the normal immunological function in rabbits, and to repeat the present experiment.

Riassunto. Conigli immunologicamente tolleranti alla caseina trattati parenteralmente con la stessa proteina vanno soggetti ad amiloidosi in misura uguale a quella degli animali di controllo, dimostrando che la patogenesi della malattia non è riconducibile a schemi immunologici classici.

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Thyroid and Pituitary Gland Activity during Compensatory Renal Hypertrophy

Though there have been several investigations as to endocrine factors controlling renal compensatory hyperplasia and hypertrophy, the exact roles of the thyroid and pituitary glands are not fully understood. Despite its consequent low metabolism, thyroidectomy does not prevent compensatory renal enlargement[1,2]. On the other hand, thyroxine administration enhances kidney hypertrophy in uninephrectomized rats[3]. The effect of hypophysectomy is more controversial. Several investigators have reported failure of compensatory renal hypertrophy in the absence of the pituitary[1,4–6]. However, compensatory renal hypertrophy can be demonstrated if allowance is made for the rapid drop in weight or deoxyribonucleic acid (DNA) synthesis after hypophysectomy[7–9].

This investigation explored the cellular activity and morphologic changes of the thyroid and pituitary glands during renal compensatory hypertrophy in rats. Autoradiographic techniques with tritiated thymidine were used to measure deoxyribonucleic acid (DNA) synthesis and cell proliferation rates.

Materials and methods. Female albino rats of the Sprague-Dawley strain (Charles River Laboratories, Wilmington, Mass.)weighing between 135 and 150 g were kept in a room where the temperature was maintained between 65 and 80°F. They were housed two to a cage and fed Purina Laboratory Chow and water *ad libitum*. A left nephrectomy was performed on the experimental group through a lateral abdominal incision. The control animals were sham operated; the left kidney was manipulated and replaced. The animals were sacrificed on various days after the operation. An intraperitoneal injection of tritiated thymidine (1 μc/g body weight) was given 4 h prior to death. The tritiated thymidine had a specific activity of 2.0 c/m*M* and was purchased from New England Nuclear Corp., Boston (Mass.) All animals were sacrificed between 1 p.m. and 3 p.m. to avoid variations in diurnal mitotic activity[10,11]. After death, the thyroid and pituitary glands were removed, weighed, fixed in formalin, embedded in paraffin, cut at 5 μ, and mounted on glass slides for autoradiography according to the method of Messier and Leblond[12]. After 4 weeks' exposure, the autoradiographs were developed, fixed and stained with hematoxylin and eosin. The degree of labelling of the thyroid and anterior pituitary parenchymal cells was obtained by counting the number of labelled nuclei with 8 or more silver grains. At least 10,000 parenchymal cells were counted per gland. Sections were selected 25 μ apart to avoid recounting the same labelled cell. After initial examination, the slides were relabelled and renumbered, and counted as unknowns.

Results and discussion. Kidney hypertrophy began 24 h post nephrectomy and appeared to reach a plateau 20 days after operation (Table). The findings agree with reports by ADDIS and LEW[13], and ROLLASON[14].

The results of tritiated thymidine autoradiographic studies are also seen in the Table. The thyroid and pituitary glands of the uninephrectomized rats exhibited a marked increased incorporation of tritiated thymidine. A

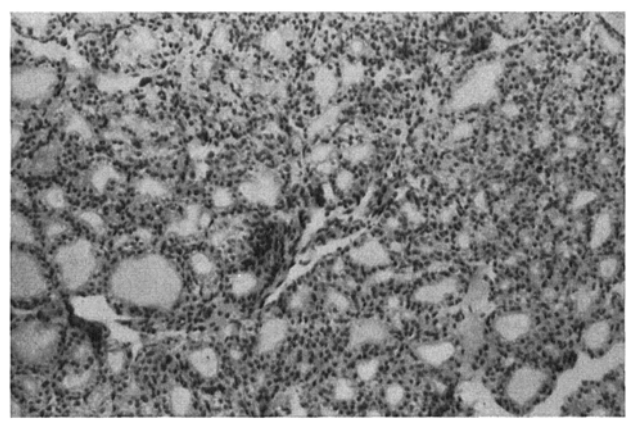

Fig. 1. Section of thyroid gland from 5th day uninephrectomized rat. The follicle lining cells are enlarged and many follicles show colloid depletion (125 × H & E stain).

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