MACROPHAGE MIGRATION INHIBITION REACTION IN EXPERIMENTAL AMYLOIDOSIS IN MICE

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Evidence has now been obtained to show that cellular immunity is depressed in amyloidosis [3, 5, 7-11]. The presence of a factor acting on responses of cellular immunity and, in particular, on macrophage migration, in the serum of patients with amyloidosis seems very probable.

The object of this investigation was to seek a macrophage migration inhibiting factor (MIF) in the blood serum of patients with amyloidosis and of animals with experimental amyloidosis and to study spontaneous cell migration during amyloid formation.

EXPERIMENTAL METHOD

Male CBA and C57BL mice aged 1.5-2 months and weighing 18-20 g, and guinea pigs were used. During the experiment the animals were kept on a standard diet. Amyloidosis was induced in the mice by subcutaneous injections of 1 ml of 5% casein solution in 0.25% NaOH solution five times a week. Altogether, 40 injections were given. For the morphological control, sections of the spleens were stained with hematoxylin-eosin and Congo red. The macrophage migration inhibition test [6] was used. Sensitized lymphocytes from patients with amyloidosis and from animals with experimental amyloidosis were used as producers of MIF. Its presence in the sera was determined.

Glass capillary tubes 0.6 mm in diameter with the cell suspension were placed in microchambers, in which 20% calf serum and 10% of the test serum were added to medium No. 199. In the experiments of series I the sera of mice with casein-induced amyloidosis (40 injections) were studied, with sera of intact animals as the control. The migrating cells were spleen cells from intact CBA mice. The cell suspension was prepared individually for each mouse in the usual way [1]. There were four capillary tubes per chamber (eight zones of migration). In each of the two groups of this series there were 40 mice.

In the experiments of series II sera from patients with secondary amyloidosis (15 patients) and clinically healthy subjects (10) were used. The migrating cells were spleen cells from intact C57BL mice.

The action of sera from 104 patients with various diseases, including secondary amyloidosis (25 patients), systemic lupus erythematosus (19), chronic active hepatitis and cirrhosis of the liver (20), and chronic nephritis (25) and from 15 clinically healthy persons was next investigated. The migrating cells were peritoneal exudate cells from intact guinea pigs. To obtain peritoneal macrophages, 15 ml of sterile mineral oil was injected intraperitoneally into the guinea pigs 2 days before the test. The suspension of macrophages was prepared by the usual method [1]. Cells of guinea pig peritoneal exudate and from spleens of intact mice were used as indicators of the presence or absence of macrophage MIF in the test sera.

The migration chambers were hermetically covered with coverslips and incubated at 37°C for 20-22 h. The zones of migration of cells from the capillary tubes were then projected on x-ray film, cut out, and weighed. The migration index was calculated by the equation:

$$\frac{\text{Zone of migration in experiment}}{\text{Zone of migration in control}} \times 100.$$

In the experiments of series III spontaneous migration of spleen cells from CBA mice during induction

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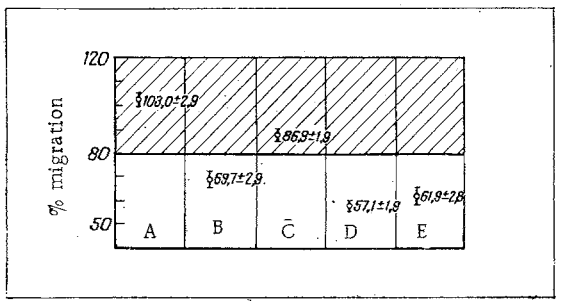

Fig. 1. Effect of patients' sera on migration of guinea pig peritoneal exudate macrophages. A) Donors; B) secondary amyloidosis; C) chronic nephritis; D) hepatitis; E) systemic lupus erythematosus. Shaded zone represents limits of variations of macrophage migration under normal conditions.

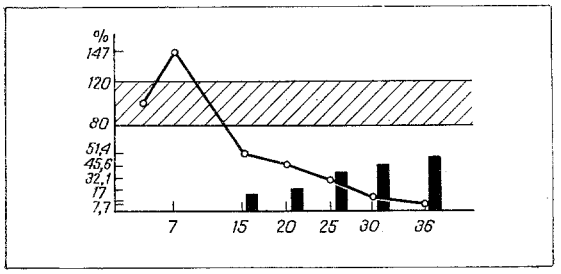

Fig. 2. Migration of spleen cells of CBA mice at different stages of induction of amyloidosis. Abscissa, intensity of amyloid deposition in the spleen (in conventional units); ordinate, migration index (%).

of casein amyloidosis was studied. For this purpose 70 mice were divided into seven groups (with 10 animals in each group): group 1 consisted of intact mice (control); group 2 of mice receiving eight injections of casein; group 3 of mice receiving 15 injections of casein; group 4, 20 injections; group 5, 25 injections; group 6, 30 injections; and group 7, 36 injections of casein. The cell suspension also was prepared individually for each mouse. In this case, only 20% calf serum was added to the medium No. 199 in the microchambers to create optimal conditions for cell culture. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

Comparison of the ability of splenic macrophages of intact mice to migrate under the influence of "intact" and "amyloid" sera revealed a decrease in the ability of the cells to migrate in the presence of sera from animals with amyloidosis (migration index 69.4 ± 2.9 compared with 114 ± 3.9 with intact sera). Similar results also were obtained when migration of splenic macrophages from intact C57BL mice in the presence of sera from patients with secondary amyloidosis and from clinically healthy subjects was compared (the migration indices were 73.3 ± 2.6 and 119.5 ± 5.3, respectively).

Having obtained these results, the next step was to study whether this test can be used for the diagnosis of amyloidosis. Sera from patients with various diseases were tested (Fig. 1).

The migration index was considerably reduced after the addition of sera from patients with chronic active hepatitis and cirrhosis of the liver, systemic lupus erythematosus, and secondary amyloidosis to the incubation medium. The migration index of patients with chronic nephritis and of clinically healthy subjects was within normal limits. The results of these investigations suggest that the MIF found in the serum of patients with amyloidosis and of animals with experimental amyloidosis is also present in patients with chronic active hepatitis and cirrhosis of the liver, with systemic lupus erythematosus and, to a lesser degree, in patients with chronic nephritis, and is evidently connected with particular features of the immunologic disturbances in

these diseases. The practical value of the results of this investigation is that they rule this test out as a diagnostic test for amyloidosis. The theoretical aspect, however, is more interesting, for it suggests once again that mechanisms of cellular immunity are concerned in amyloidosis, for the factor inhibiting macrophage migration is a mediator of cellular immunity.

In the next series of experiments an attempt was made to discover changes in spontaneous migration of splenic macrophages from CBA mice during induction of casein amyloidosis in them. Changes in spontaneous migration were found during the development of the immune response (Fig. 2).

After eight injections of casein, sharp stimulation of cell migration was found. Morphologically, this period coincides with the first stage of experimental amyloidosis (pyroninophilia [12]), which is characterized by proliferation of pyroninophilic, reticulo-endothelial, and plasma cells, and clinically it is expressed as hypergammaglobulinemia, i.e., it is a normal response (process of immunoglobulin synthesis) to injection of an antigen. This is in agreement with the observations of Koval'chuk et al. [2], who found an increase in spontaneous migration of peritoneal exudate cells from CBA mice on the 6th day after immunization with Freund's complete adjuvant when studying interlinear differences in reactions of cellular immunity. Later in the course of amyloid formation the index of the cells corresponds to the morphological picture of increasing amyloid deposition in the spleen.

It can be tentatively suggested that during amyloid formation, as a result of prolonged antigenic stimulation, sensitized lymphocytes produce a factor which inhibits migration of macrophages, under the influence of which the macrophages not only lose their ability to migrate, but they also modify their macrophagal activity, with the result that the process of resorption of amyloid is inhibited and it accumulates in the organs.

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