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AGE CHANGES IN IMMUNE RESPONSE OF RATS TO REPEATED INJECTIONS OF DIFFERENT DOSES OF SHEEP'S RED CELLS

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The number of antibody-forming cells and the serum antibody titers were determined in adult and old rats after tenfold immunization with "small" ($4 \cdot 10^8$ cells) and "large" ($4 \cdot 10^{10}$ cells) doses of sheep's red cells. The antibody (hemolysin and hemagglutinin) titers in the animals of both age groups were found to be either similar in magnitude or higher in the younger adult rats (for hemolysins, in the case of injection of the "large" dose of antigen). The number of direct plaque-forming cells in the spleen of the old animals was greater than in the young adults at all times of immunization, but the number of indirect plaque-forming cells was greater only at the end of immunization. The results are evidence of differences in maturation of the immune response in animals of different ages.

KEY WORDS: immune response; age differences.

For a long time the view has been firmly held that with age the antibody-forming ability of the organism diminishes, in connection with a decrease in the number of potential antibody-forming cells [3, 11, 13, 18]. Recently, however, a definite decrease during aging has been found only with respect to the primary immune response [4, 6, 14, 16], whereas the secondary response shows a much smaller decrease or, in general, shows no change [7, 9, 15, 17]. There is evidence that the change in antibody-forming ability in old age may be due not so much to a deficiency of antibody-forming cells as to a disturbance of regulatory mechanisms [4, 6, 8]. This hypothesis seems perfectly probable, for histological investigation of lymphoid tissue in hyperimmune rats showed a well-marked cellular response in old animals [2]. The writer has also shown that old animals can produce antibodies either more or less actively than younger adults, depending on the dose and duration of injection of the antigen [1].

The object of the present investigation was to study the possible mechanism of differences in the state of antibody formation in old animals under different experimental conditions.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats of two age groups: adult (8-10 months) and old (24-26 months).

Antigen – sheep's red cells – was injected intraperitoneally ten times (at intervals of seven days) in doses of $4 \cdot 10^8$ cells (the "small" dose) and $4 \cdot 10^{10}$ cells (the "large" dose). This particular scheme of im-

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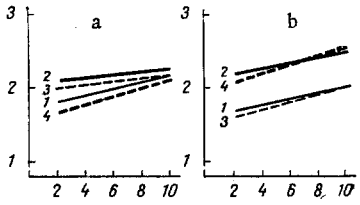

Fig. 1

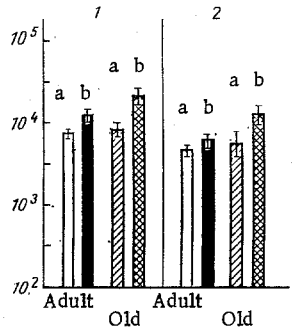

Fig. 2

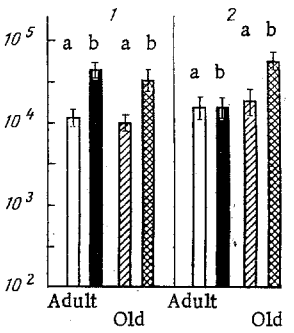

Fig. 3

Fig. 1. Titers of hemolysins (a) and hemagglutinins (b) in adult (1, 2) and old (3, 4) rats during ten immunizations with "small" (1, 3) and "large" (2, 4) doses of sheep's red cells. Abscissa, number of injections of antigen; ordinate, titer of antibodies (log).

Fig. 2. Number of DPFC in spleen of adult and old rats during ten immunizations with "small" (a) and "large" (b) doses of sheep's red cells. Here and in Fig. 3: ordinate, number of antibody-forming cells in spleen; 1) after two injections, 2) after ten injections of antigen.

Fig. 3. Number of IPFC in spleen of adult and old rats during ten immunizations with "small" (a) and "large" (b) doses of sheep's red cells.

munization was used having regard to personal observations [1] and data in the literature [10] indicating that age relations in antibody formation are modified by repeated injection of the antigen.

On the seventh day after the second and tenth injections of antigen the animals were killed and their titers of antibodies (hemolysins and hemagglutinins) in the blood serum and the number of antibody-forming cells in the spleen were determined.

Antibody-forming cells were determined by local hemolysis in gel: Cells forming 19S antibodies (direct plaque-forming cells – DPFC) were detected by the method of Jerne and Nordin [12], and cells forming 7S antibodies (indirect plaque-forming cells – IPFC) by the method of Dresser and Wortis [5].

The data given in this paper are the geometric mean values of results obtained on 10-19 animals.

EXPERIMENTAL RESULTS

After immunization with a "small" dose of antigen the titers of serum hemolysins in the adult and old rats did not differ significantly throughout the period of investigation; after immunization with the "large" dose the hemolysin titers of the young adult animals were higher than those of the old rats (after two injections of antigen $P < 0.05$) (Fig. 1a). So far as the effect of dose of antigen on antibody titer in animals of the same age is concerned, no significant differences were found in either the adult or the old rats.

Titers of the other type of antibodies (hemagglutinins) at these times of the immunization period were equal or similar in value in the adult and old animals after injection of both small and large doses of antigen (Fig. 1b). Dependence on the dose of antigen was found within the same age group: After immunization with the "large" dose of antigen the hemagglutinin titers were much higher than after immunization with the "small" dose (for the adults $P < 0.005$ at both times of testing, for the old rats $P < 0.05$ and < 0.005).

The study of the number of DPFC (Fig. 2) gave the following results. In the course of immunization the number of DPFC in the spleen of the young adult animals fell: $P = 0.05$ and $P < 0.05$ respectively for the "small" and "large" doses of antigen. In the old animals, changes in the number of cells showed the same tendency but they were not statistically significant. The number of DPFC in the spleen increased with an increase in the dose of antigen. This increase was more marked after two injections of the antigen ($P < 0.05$ for the adult and $P < 0.01$ for the old rats) than after ten injections.

The number of DPFC in the spleen of the old animals was greater than in the adults after immunization with the "large" dose ($P < 0.05$ and $P = 0.05$) and was the same as their number in the adult rats after immunization with the "small" dose of antigen.

A rather different dynamics was characteristic of the indirect plaque-forming cells (Fig. 3). During immunization the number of IPFC in the spleen of the adult animals was almost unchanged after injection of the "small" dose of antigen, but after injection of the "large" dose it fell considerably ($P < 0.02$); in the old animals, however, in both cases there was a shift toward an increase. An increase in the dose of the antigen led to an increase in the number of IPFC in the animals of both age groups. In the adult rats this was clearly visible only after the second injection of antigen ($P < 0.01$), but in the old rats it could be seen at both times of testing ($P < 0.01$ and < 0.05).

Age differences in the number of IPFC in the spleen were observed only after ten injections of the "large" dose of antigen: Under these conditions the number of cells in the spleen of the old animals was more than three times greater than their number in the adult rats ($P < 0.005$).

Comparison of the number of plaque-forming cells with the serum antibody titers shows that their dynamics differed during the period of immunization: The antibody titers rose more or less substantially (depending on the dose of antigen and the age of the animals), whereas the number of antibody-forming cells either fell in the animals of both age groups, although less so in the old animals (DPFC), or fell in the adult and rose in the old rats (IPFC). This fact indicates differences in the maturation of the immune response in animals of different ages. It can be postulated that in adult animals antibody production by a single antibody-forming cell increases in adult animals in the course of immunization. In old animals, under the same conditions, the antibody-forming ability of each cell is possibly lower than in younger adults. However, this hypothesis requires direct evidence for its support.

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