SYNTHESIS OF NONSPECIFIC IMMUNOGLOBULINS IN TOLERANT ANIMALS

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Immunization of mice with sheep's red cells leads not only to antibody synthesis, but also to a sharp increase in the formation of nonspecific immunoglobulins. Injection of antigen into tolerant animals does not stimulate the synthesis of nonspecific immunoglobulins.

KEY WORDS: *antibodies*; *nonspecific immunoglobulins*; *cyclophosphamide*; *tolerance*.

A previous investigation showed that immunization of animals with protein antigens leads not only to antibody formation, but also to a sharp increase in the rate of synthesis of nonspecific immunoglobulins (NIG) [3]. Under the influence of certain inhibitory or stimulating factors, the two processes are modified similarly [2, 5]. It has accordingly been postulated that the same cells participate in the synthesis of antibodies and also of the NIG formed under the influence of antigen (antigen-dependent NIG) and also that the injection of specific antigen into animals tolerant to that antigen ought not to lead to an increase in the NIG titer [4].

In this investigation NIG synthesis was studied in tolerant animals.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice and on (CBA × C57Bl/6)F hybrids. Sheep's red cells were used as the antigen. Tolerance to the red cells was produced by injecting $6 \cdot 10^9$ red cells into the animals and by injecting cyclophosphamide (CP) in a dose of 200 mg/kg body weight 2 days later. Control mice received CP only.

To induce an immune response and as the test injection to verify the state of tolerance, $5 \cdot 10^8$ red cells were injected into the animals.

The formation of antibodies against sheep's red cells was assessed from the number of antibody-forming cells (AFC) in the spleen, determined by Jerne's method, and from the hemagglutinin and hemolysin levels in the serum of the mice.

To determine the synthesis of antigen-dependent NIG, spleen cells from control and experimental animals were incubated in Eagle's medium containing ^{14}C-glycine at 37°C for 8-20 h. At the end of incubation the cells were separated by centrifugation and the concentration of ^{14}C-NIG in the culture medium after exhaustion with sheep's red cells was determined with the aid of a specific immunosorbent [1].

A BFL-25 end-window counter or an SL-40 scintillation counter (Intertechnique, France) was used to count the radioactivity.

EXPERIMENTAL RESULTS

It has first to be made sure that correlation was present between antibody synthesis and the synthesis of antigen-dependent NIG after immunization of the animals with corpuscular antigens. With the scheme used to immunize the mice with sheep's red cells, the formation of cells producing 19S antibodies is known to be observed on the 4th day after injection of

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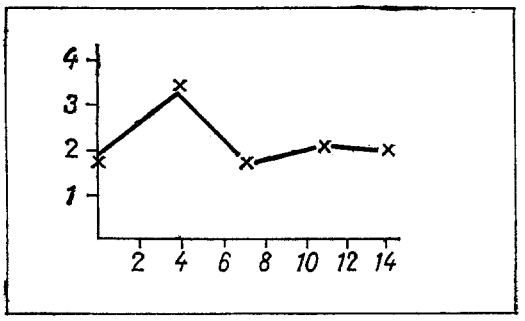

Fig. 1. Synthesis of nonspecific immunoglobulins by mouse spleen cells at various times after immunization with sheep's red cells. Abscissa, time after immunization (in days); ordinate, ^{14}C activity (counts/min/10^8 cells $\times 10^{-2}$).

TABLE 1. Effect of Tolerance on Synthesis of Antigen-Dependent NIG in Mice 28-30 Days after Induction of Tolerance

Group of animals	NIG synthesis (counts/min/10^8 cells)	
	before immunization	after immunization
Control (receiving CP only)	173±17 (19)	258±27 (17)
Tolerance	289±37 (15)	173±24 (19)

Note. Number of animals in group given in parentheses.

the antigen, after which the intensity of this process falls off rapidly. It was accordingly interesting to discover how the synthesis of antigen-dependent NIG changed at various times after immunization. To do this, their formation was determined 4, 7, 11, and 14 days after injection of the red cells into the mice. The results are given in Fig. 1. Clearly synthesis of antigen-dependent NIG reached a maximum on the fourth day after immunization also, after which the rate of their formation rapidly declined to reach the initial level by the seventh day. After immunization of mice with the corpuscular antigen, the same parallel was accordingly observed between synthesis of antibodies and NIG as during immunization of animals with protein antigens [4].

It was shown previously that antibody synthesis in the spleen cells of mice immunized with sheep's erythrocytes 1-2 weeks after induction of tolerance in the animals is virtually absent on the 4th day after the test injection [7]. The number of AFC in these animals does not exceed 1.1 per 10^6 spleen cells (compared with 400-700 in the control) and the titers of hemagglutinins and hemolysins are between 1:10 and 1:80 (compared with 1:320 and 1:1280 in the control). Determination of NIG synthesis at these times, however, gave very high results. Their synthesis 2-3 weeks after induction of tolerance amounted to 400% of its level in the intact animals. Injection of antigen into tolerant mice did not increase, but decrease NIG formation by about 20% compared with their level in the control, unimmunized animals. In normal mice immunization under these conditions led to a marked increase in the titer of antigen-dependent NIG, the synthesis of which was approximately at twice the rate of the control.

Previous results [6] indicated that these phenomena can be attributed to the action of CP. Since it has been shown that after injection of CP it takes about 1 month for normal NIG synthesis to be restored in the spleen, it was decided to study the effect of tolerance on the synthesis of antigen-dependent NIG at that time (it must be noted that tolerance to sheep's red cells still persists after 1 month in most animals).

The results of some of the experiments to determine the synthesis of antigen-dependent NIG in animals in which tolerance was induced 28-30 days before immunization are given in Table 1.

They show that immunization of mice receiving CP 1 month previously leads to an increase in the level of antigen-depent NIG. This is in agreement with data showing an increase in the number of AFC in the spleen of these animals and an increase in the titer of hemolysins and hemagglutinins in their serum [6]. On the other hand, in mice with induced tolerance the synthesis of antigen-dependent NIG not only did not increase but was actually reduced to lower levels than before the test injection.

Regardless of the time elapsing after induction of tolerance, it was thus impossible to speed up the synthesis of antigen-dependent NIG in mice tolerant to that antigen. Moreover, injection of antigen into tolerant animals always caused the NIG titer to fall below its initial level.

In the modern view, at least three types of cells participate in antibody synthesis: T, B, and A. The role of the T cells consists essentially of the isolation of a special factor (factors) stimulating antibody formation by B cells under the influence of the antigen.

With the scheme of induction of tolerance used in these experiments the function of the T cells was the first to be disturbed [8], although damage to some B cells likewise cannot be completely ruled out. The absence of an immune response in the tolerant mice in the present experiments may have been due, perhaps, chiefly to a deficiency of the factor produced by the T cells. The parallel cessation of formation of antigen-dependent NIG suggests that this same factor is also essential for their synthesis.

These results are in good agreement with those obtained in a different form of tolerance [9].

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