The method of obtaining hematopoietic colonies in chick bone marrow by the use of transplantation of quail cells enables the number of the host's stem cells still remaining in the blood stream and capable of repopulating the bone marrow to be counted and the dynamics of the pool of the donor's stem cells to be studied.

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EFFECT OF THYMECTOMY ON FORMATION OF IMMUNOLOGIC TOLERANCE IN DELAYED-TYPE HYPERSENSITIVITY EFFECTORS

A. D. Chernousov, L. N. Fontalin, N. G. Akopyan, and I. A. Kondrat'eva

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The effect of thymectomy on the formation of tolerance of delayed-type hypersensitivity (DTH) to sheep's red blood cells was investigated. If tolerance was induced by combined injection of a massive dose of antigen and cyclophosphamide, thymectomy did not prevent this process and prolonged the state of tolerance. If area activity was induced by a massive dose of antigen alone, thymectomy restored the ability to form DTH and prevented the formation of suppressor cells. Thymectomy weakened DTH formation somewhat in intact animals, but not in animals receiving cyclophosphamide. The results confirm views regarding the diversity of the mechanism of tolerance (clonal-deficiency and suppressor). It is also suggested that among DTH effectors and their precursors there are two subpopulations which differ in their sensitivity to cyclophosphamide and thymectomy.

KEY WORDS: immunologic tolerance; delayed-type hypersensitivity; cyclophosphamide; thymectomy; suppressor cells.

The use of thymectomy in adult animals enabled the life span of individual subpopulations of thymus-dependent lymphocytes to be determined, and if combined with other procedures, it can provide information on the precise mechanism of individual immunologic phenomena [9], including the mechanism of different forms of immunologic tolerance [6, 7, 10, 13, 14]. The object of the present investigation was to study the effect of thymectomy on the formation of tolerance to sheep's red blood cells (SRBC) in effectors of delayed-type hypersensitivity (DTH) in mice.

EXPERIMENTAL METHOD

(CBA × C57BL/6)F_1 hybrid mice weighing 20-22 g were used. Areactivity of the DTH effectors was obtained either by injection of 6×10^9 SRBC along or by successive intraperitoneal injections of 6×10^9 SRBC, followed after 42-45 h by cyclophosphamide (CP) in a dose of 200 mg/kg [3].

On the 14th day after this treatment the mice were sensitized by intravenous injection of 10^5 SRBC in physiological saline [11]. The DTH level was determined by skin tests [11]. For this purpose, on the 4th day after sensitization, 10^8 SRBC in 40 μl physiological saline was injected into a footpad of the mice. The reaction was read 24 h after the reacting injection of antigen. The difference between the thickness of the footpad

Laboratory of Immunologic Tolerance, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow.

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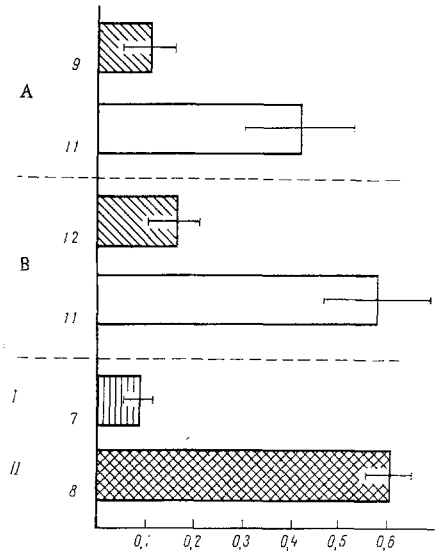

Fig. 1. Effect of thymectomy on formation of areactivity of DTH effectors in animals receiving a massive dose of antigen (A) and on suppressor activity of the spleen cells of such animals (B). Here and in Figs. 2 and 3, intensity of skin reaction plotted along horizontal axis. Obliquely shaded columns denote level of response of nonthymectomized animals receiving a massive dose of antigen (A) and of recipient into which spleen cells of corresponding donors were injected (B); unshaded columns denote level of response of thymectomized animals (A) and of recipients receiving their cells (B). Here and in Figs. 2 and 3, numbers near vertical line denote number of animals. I) negative control; II) positive control.

reflected the level of DTH. The control for these experiments consisted of animals receiving an injection of the same dose of CP as the experimental animals (CP control), sensitized animals (positive control), and animals receiving only the reacting injection of antigens (negative control).

Thymectomy was performed on some of the experimental and control animals two weeks before induction of tolerance [12]. After the skin reaction of all the animals had been read, the completeness of removal of the thymus was verified; mice with remnants of thymus were rejected.

In adaptive transfer experiments, spleen cells of thymectomized and nonthymectomized donors were washed once with medium No. 199, resuspended in the same medium, and injected in a dose of 10^8 cells intravenously into intact recipients. The recipients were sensitized 1 h later by intravenous injection of 10^5 SRBC.

EXPERIMENTAL RESULTS

Data showing the effect of thymectomy on the formation of areactivity of the DTH receptors following injection of a massive dose of antigen are given in Fig. 1. As Fig. 1 shows, injection of 6×10^9 SRBC into nonthymectomized animals significantly reduced their ability to form DTH and also induced the formation of suppressor cells, detectable on transplantation into intact recipients. Thymectomy prevented the formation of areactivity of DTH effector cells and prevented activation of the suppressor cells.

Combined injection of antigen and CP also led to the formation of areactivity of DTH effectors (Fig. 2). However, by contrast with the results given above, if thymectomy was performed before induction of tolerance it did not disturb its formation. Further experiments to study the effect of cells of tolerant nonthymectomized animals revealed absence of a suppressive effect on the immune response of the sensitized recipients. The study of the dynamics of restoration of the function of DTH effectors in the tolerant and thymectomized tolerant animals showed that 6 weeks after combined administration of antigen and CP the thymectomized tolerant animals were unable, as before, to respond with DTH reactions (the mean level of reaction was 0.03 ± 0.01), whereas the ability of the tolerant animals to develop DTH was restored (mean level of reaction 0.33 ± 0.09).

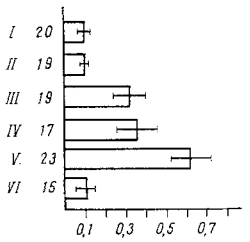

Fig. 2

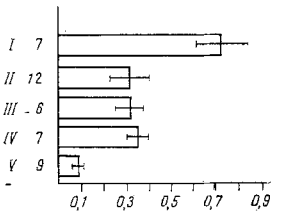

Fig. 3

Fig. 2. Effect of thymectomy on formation of tolerance of DTH effectors in animals treated with a massive dose of antigen and CP. I) tolerant animals; II) thymectomized tolerant animals; III) CP control; IV) thymectomized CP control; V) positive control; VI) negative control.

Fig. 3. Level of DTH in animals subjected to thymectomy, CP, or a combination of both. I) positive control; II) thymectomy; III) CP; IV) thymectomy +CP; V) negative control.

Depending on the method of induction of areactivity, the effect of thymectomy, if performed before induction of tolerance, differed: Thymectomy preceding injection of a massive does of antigen led to the termination of tolerance, whereas thymectomy preceding combined injection of antigen and CP led to prolongation of tolerance.

Restoration of the function of the effector cells as a result of thymectomy performed before induction of tolerance has been demonstrated both as a result of cell transfer and also in situ and on other models: Tolerance to chemical [6] and to disaggregated proteins [13, 14]. Under these circumstances the absence of a suppressor effect both in these and in similar cases was attributed to elimination of short-living suppressor cells, activated by specific tolerogenic treatment, as a result of thymectomy [7, 13, 14]. The experimental results illustrated in Fig. 1 suggest that disturbance of DTH formation to SRBC as a result of injection of a massive dose of antigen is also active in character and is due to the action of suppressor cells sensitive to thymectomy.

The results given in Fig. 2 confirm earlier observations [3] showing that tolerance due to combined injection of antigen and CP is based on a true deficiency of DTH effectors. The deepening of tolerance due to a deficiency of the corresponding clone, as a result of preceding thymectomy, was obtained on this same model during a study of antibody formation [1, 5].

It follows from Fig. 2 that thymectomy does not affect the level of sensitization of animals which subsequently received CP. However, both CP (Fig. 2) and thymectomy, performed at various times before sensitization [9], lead to a fall in the DTH level. The reason for the equal level of sensitization as a result of treatment with CP and CP combined with thymectomy is evidently that both CP and thymectomy act on the same precursors of DTH effectors. To test this hypothesis experiments were carried out to study ability of animals to form DTH if subjected to thymectomy, injection of CP, or a combination of both two weeks before sensitization. The results are given in Fig. 3. They show that all three procedures led to an equal fall in the level of sensitization, which differed significantly from the level of sensitization of the positive control. The results of these experiments suggest that among precursors of DTH effectors there are at least two subpopulations, one of which is insensitive to the action of thymectomy and CP. Since CP acts on actively proliferating immature cells [4, 8], these subpopulations may perhaps differ in their degree of differentiation.

The results of the present investigation confirm views on the diversity of the mechanism of immunologic tolerance [2], due either to elimination of the corresponding clone of immunocompetent cells (after combined administration of antigen and CP) or to activation of suppressor cells of unknown nature (after injection of a massive dose of SRBC).

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NATURE AND MECHANISM OF SYNTHESIS OF NONSPECIFIC IMMUNOGLOBULINS

E. V. Sidorova

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Mice were immunized with Vi-antigen. Suspensions of spleen cells, removed at various times after immunization, were incubated in Eagle's medium in the presence of ^{14}C-glycine. Synthesis of antibodies against Vi-antigen, autoantibodies against mouse IgG, and antigen-dependent nonspecific immunoglobulins (NIg) were determined by the use of specific immunosorbents. Immunization with Vi-antigen sharply intensified in the synthesis of antigen-dependent NIg. The formation of the proteins is thus observed not only during immunization with thymus-dependent antigens, but also in response to thymus-independent antigen. The synthesized antigen-dependent NIg were not autoantibodies against endogenous IgG.

KEY WORDS: Vi-antigen; antibodies; nonspecific immunoglobulins; autoantibodies; immunoglobulin biosynthesis.

It was shown previously [1, 6, 7] that immunization of animals leads not only to antibody synthesis, but also to the formation of antigen-dependent nonspecific immunoglobulin (NIg). It has been suggested that synthesis of the latter takes place in the same cells as antibody synthesis [4]. However, the use of thymus-dependent antigens in these experiments permitted an alternative explanation of the formation of antigen-dependent NIg by the action of nonspecific stimulating T-factors [9, 10]. Meanwhile the report on the discovery of a considerable number of cells synthesizing antibodies against endogenous IGg in the spleen of immunized mice [8] suggested that a substantial part of these antigen-dependent NIg are autoantibodies.

To test both these hypotheses, the synthesis of antibodies, autoantibodies, and NIg by spleen cells in vitro after immunization with T-independent Vi-antigen [3] was investigated.

EXPERIMENTAL METHOD

Female BALB/c and C57BL/6 mice weighing 14-16 g and Salmonella typhi Vi-antigen* were used. The Vi-antigen was injected intraperitoneally or intravenously into the animals in a dose of 1 μg per mouse. The spleen was removed on the 4th, 6th-8th, and 14th days after immunization. Spleens of normal nonimmunized animals served as the control. Cell suspensions were prepared from the spleens and batches of 40-50 million cells were incubated in Eagle's medium with the addition of ^{14}C-glycine at 37°C for 20 h [6]. At the end of incubation the cells were separated by centrifugation for 5 min at 600g and the supernatant was clarified for 30 min at 12,000g with cooling and used for determination of ^{14}C-immunoglobulins with the aid of specific immunosorbents [2]. Antibodies against Vi-antigen were determined with the aid of Vi-sorbent [5], autoantibodies

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