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EFFECT OF DOSE OF T-DEPENDENT AND T-INDEPENDENT ANTIGENS ON FORMATION OF NONSPECIFIC IMMUNOGLOBULIN PRODUCERS*

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KEY WORDS: cells forming antigen-dependent nonspecific immunoglobulins; antibody-forming cells; T-dependent antigens; T-independent antigens.

It was shown previously that, besides a specific immune response, injection of T-dependent or T-independent antigens (TDA and TIA, respectively) into an animal also induces the formation of nonspecific immunoglobulin-producing cells (NIGFC) [1, 2, 4]. The mechanism of this process has not yet been explained. Data have recently been obtained to show that *in vitro*, in the presence of high concentrations of antigen, some B cells bind it nonspecifically [5]. Thus, whereas in low concentrations, antigen is bound by not more than 0.19% of B cells, if high concentrations are present, this cell fraction rises to 3%; immunoglobulin receptors, moreover, have no part to play in this process [5]. It has been suggested that the formation of NIGFC may be due to nonspecific binding of massive doses of antigen.

To test this hypothesis, the effect of low and high doses of TDA and TIA on NIGFC formation was investigated *in vivo*.

EXPERIMENTAL METHOD

Experiments were carried out on BALB/c mice obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR.

Sheep's red blood cells (SRBC) were used as TDA, and polyvinylpyrrolidone, with mol. wt. of 350 kilodaltons (PVP_{350}) and pneumococcal polysaccharide SSSIII (SIII) were used as TIA.

The animals were immunized by a single injection of different doses of antigens. SRBC were injected intraperitoneally in doses of 10^6, 10^7, and $5 \cdot 10^8$ per mouse, PVP_{350} intravenously in doses of 10^{-3}, 10^{-2}, 10^{-1}, and 1 μg per mouse, and SIII intraperitoneally in doses of 10^{-3}, 10^{-2}, and 1 μg per mouse. Nonimmunized animals of the same line were used as the control.

On the 4th day after immunization the number of cells forming IgM-antibodies (AFC) and the number of cells forming immunoglobulins (IGFC) were determined in the spleen of individual animals by methods of direct [9] and reverse [11] hemolysis in gel. The number of NIGFC was calculated as the difference between the number of IGFC and the number of ARC per 10^6 cells. The results are presented in the M ± m form.

SRBC, intact and sensitized with polyvinylpyrrolidone with mol. wt. of 24 kilodaltons (PVP_{24}) [10] or SIII [7] were used as test antigen for AFC assay. To determine IGFC, SRB sensitized with rabbit antibodies to mouse immunoglobulin were used [11]. Rabbit antisera

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TABLE 1. Dependence of Increase in Number of AFC and NIGFC on Dose of TDA and TIA

Antigen injected	Dose of antigen	No. of AFC and NIGFC per 10^6 cells		Increase of AFC and NIGFC per 10^6 cells	
		AFC	NIGFC	AFC	NIGFC
SRBC, millions/mouse	—	1±0,3	1582±218	0	0
	10^6	1,3±0,4	2182±419	0,3	600
	10^7	4,1±1,1	3789±858	3,1	2207
	$5 \cdot 10^8$	1234±255	4677±610	1233	3095
PVP_{350}, μg/mouse	—	1,7±0,4	2559±221	0	0
	10^{-3}	3,3±0,9	3839±429	1,6	1280
	10^{-2}	4,5±1,6	3633±349	2,8	1074
	10^{-1}	30±4	4066±576	28	1507
	1	69±8	4461±332	67	—1902
SIII, μg/mouse	—	0,9±0,3	2743±197	0	0
	10^{-3}	2,6±0,5	2783±336	1,7	40
	10^{-2}	15±4	2521±226	14,1	—222
	1	153±19	2565±418	152,1	—178

Legend. Increase in number of AFC and NIGFC was calculated as the difference between mean numbers of AFC and NIGFC in experiment (immunized animals) and control (intact animals). In each case from 15 to 20 animals were used.

TABLE 2. Effect of Injection of Anti-Thy 1.2-Serum on Increase in Number of AFC and NIGFC in Mice Immunized with DTA or TIA

Treatment of animals	Number of AFC and NIGFC per 10^6 cells			
	AFC			NIGFC
	to PVP_{350}	to SIII	to SRBC	
—	1,7±0,4	0,9±0,3	0,9±0,3	2031±311
Anti-Thy 1.2	2,3±0,5	1,6±0,2	1,3±0,3	1238±178
PVP_{350}	96±17	—	3±0,6	2969±424
PVP_{350} + anti-Thy 1.2	145±19	—	—	3525±235
SIII	—	283±32	0,9±0,2	2026±250
SIII + anti-Thy 1.2 + SRBC	—	430±49	—	2410±303
	—	—	2013±288	6460±948
SRBC + anti-Thy 1.2	—	—	1583±182	6123±882

Legend. From 18 to 25 mice in a group.

specific for mouse immunoglobulin μ-chains or for total mouse immunoglobulins, respectively, were used as enhancing antisera for detection of AFC to SIII and IGFC.

EXPERIMENTAL RESULTS

In the experiments of series I BALB/c mice were immunized with diffetent doses of TDA-SRBC. Injection of SRBC in doses of 10^6 and 10^7 per mouse caused a very low specific immune response on the 4th day, accompanied by insignificant nonspecific stimulation (Table 1).

An immunogenic dose of SRBC ($5 \cdot 10^8$) induced a much more marked increase in the number of both AFC and NIGFC. Thus, whatever doses of TDA were injected into the animal, an increase in antigen-dependent NIGFC was observed (Table 1).

It can be concluded from these results that the formation of antigen-dependent NIGFC *in vivo* was induced not only by high, but also by low doses of TDA. This may indicate that either nonspecific binding of the antigen does not play a role in this process, or that this nonspecific binding also takes place in the presence of low concentrations of antigen.

NIGFC formation in the case of TDA may perhaps be only partially connected with secretion of nonspecific factors, activating all kinds of B blast cells, by activated T cells [3]. To rule out any possible role of T cells and, in addition, to distinguish between the participation of Lyb 5^+ and Lyb 5^- B cells in NIGFC formation, in the experiments of series II NIGFC formation was studied in response to injection of class II TIA (PVP_{350} and SIII) into the animals, stimulating antibody formation only by Lyb 5^+ B cells [12].

Immunization of BALB/c mice with small doses of PVP_{350} (10^{-3} and 10^{-2} μg/mouse) led, just as in the case of SRBC, to a very small increase in the number of specific AFC and to a significant increase in the number of NIGFC (Table 1). If the dose of antigen injected was increased to 10^{-1} μg/mouse, it caused a considerable increase in the intensity of both the specific and the nonspecific immune response (Table 1).

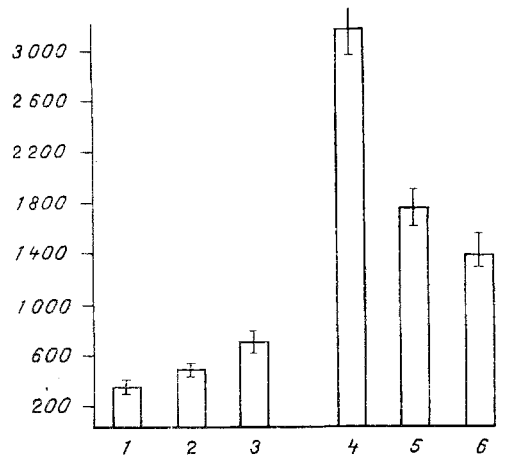

Fig. 1. Dependence of level of specific immune response on quantity of monoclonal anti-Thy 1.2-serum (AS) injected into mice. 1) SIII; 2) SIII + AS (1:100); 3) SIII + AS (1:10); 4) SRBC; 5) SRBC + AS (1:100); 6) SRBC + AS (1:10). Mice received 1 μg of SIII, $5 \cdot 10^8$ SRBC, and 0.2 ml of AS.

Thus on immunization of mice with PVP_{350} a marked increase in the number of NIGFC was observed irrespective of the dose of antigen injected, i.e., results which, in principle, did not differ from those following injection of TDA also were obtained in the model with TIA. These results suggest that the NIGFC appearing under the influence of PVP_{350} are formed from Lyb 5^+ B cells, although the role of Lyb 5^- B cells in this process likewise cannot be completely ruled out on the basis of these results.

When the second TIA was used, namely SIII, the results obtained were different from everything observed previously. Irrespective of dose of antigen injected, no increase in the number of NIGFC could be observed (Table 1), although the specific immune response was well marked and depended on the dose of antigen.

Absence of formation of antigen-dependent NIGFC in animals immunized with various TIA, including with SIII, was reported in one publication [13], but our own results with *Salmonella* Vi-antigen and PVP_{350} [1, 2] contradicted this conclusion. In the present investigation it was found that the two class II TIA differed from each other in their ability to induce a nonspecific immune response. This difference may be due either to differences in the structure of these molecules, affecting their binding with B cells, or to differences in the ability of these TIA to interact, not with B cells, but with T cells. We know that T suppressor cells participate in regulation of the immune response to class II TIA [6, 10]. It can be tentatively suggested that during immunization with SIII more T suppressor cells are induced in the body than during immunization with PVP_{350}. To test this hypothesis, simultaneously with injecting an immunogenic dose of SIII and PVP_{350} into mice, monoclonal anti-Thy 1,2-serum (AS) was injected intravenously in dilutions of 1:10 and 1:100, and the number of ARC and NIGFC in the spleens was determined on the 4th day. Intact and immunized animals and mice receiving AS only were used as the controls.

Preliminary experiments showed that injection of 0.2 ml of AS in a dilution of 1:10 into mice simultaneously with SIII or SRBC led in the first case to an increase of 2.13 times in the intensity of the specific immune response, but a decrease of 2.4 times in the second case (Fig. 1). It was this dilution of AS which was used in the subsequent experiments, the results of which are given in Table 2. Injection of AS simultaneously with SIII and PVP intensified the specific response. At the same time the nonspecific immune response also was enhanced. The greater increase in the number of AFC and NIGFC under the influence of AS was independent greater increase in the number of AFC and NIGFC under the influence of AS was independent of the nature of the antigen used. The number of AFC to SIII and to PVP_{350} was increased by 1.5 times, and the number of antigen-dependent NIGFC by 1.2 times. It can be concluded from these results that suppressor T cells regulate not only the increase in the number of AFC in animals immunized with class II TIA, but also the formation of antigen-dependent NIGFC, and that the contribution of suppressor T cells to the regulation of the nonspecific immune response to SIII is indistinguishable from that observed in the case of PVP_{350}. This means that the absence of formation of antigen-dependent NIGFC in animals immunized with SIII cannot be explained by the action of suppressor T cells.

Data indicating that TIA are not absolutely T-independent, but simply require much smaller numbers of helper T cells than TDA to exhibit their immunogenic action, have been obtained in recent years [8]. If this observation is confirmed, our own results may be evidence that the demand for helper T cells for different TIA to exhibit their immunogenic

action also will differ, and in particular, it will be much smaller for SIII than for PVP_{350} and Vi-antigen.

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EFFECT OF ANTIGEN ON COLONY-FORMING ACTIVITY OF HEMATOPOIETIC STEM CELLS IN TOLERANT MICE

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KEY WORDS: hematopoietic stem cell; immunologic tolerance; regulation.

The polypotent hematopoietic stem cell (PHSC) is known to participate in formation of the immune response [1]. Injection of the most widely differing antigens causes an increase in the number of colony-forming cells and in their proliferative activity [1, 7]. Meanwhile, problems connected with the concrete mechanisms of participation of PHSC in immunogenesis remain largely unsolved.

To determine the character of the cellular mechanism of involvement of PHSC in the immune response, in the investigation described below the effect of an antigen (sheep's red blood cells — SRBC) on the colony-forming activity of PHSC was studied in mice tolerant to that antigen.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL/6)F_1 mice weighing 18-20 g. To induce a state of tolerance SRBC were injected intraperitoneally in a dose of $5 \cdot 10^9$ per mouse 48 h before intraperitoneal test injection of SRBC in a dose of $2 \cdot 10^8$, and the number of antibody-forming cells (AFC) against SRBC was determined in the spleen [5]. The colony-forming activity of PHSC was determined by the method described previously [8], by testing the number of splenic colony-forming units (CFUs) on the 8th day after injection of donors' spleen cells (SC) into the recipients in a dose of $5 \cdot 10^5$ 24 h after, and injection of bone marrow cells (BMC) in a dose of $5 \cdot 10^4$ 72 h after antigenic priming. SC and BMC of intact animals served as the antigenic priming control.

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