Role of Various Subpopulations of B Lymphocytes in Production of Antigen-Induced Nonspecific Immunoglobulins

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Congeneric CBA and CBA/N mice (with and without Lyb5+ B cells, respectively) respond to T cell-dependent antigens by the appearance of antibody-producing cells and accumulation of cells synthesizing nonspecific immunoglobulins. Type 2 cell-independent antigens induce similar changes only in CBA mice having mature B lymphocytes. The data suggest that B lymphocytes are responsible for the appearance of antibody-producing cells and polyclonal activation.

Key Words: *antibodies; nonspecific immunoglobulins; polyclonal activation; B cell subpopulation*

Contact with antigens stimulates the synthesis of specific antibodies and sharply enhances production of nonspecific immunoglobulins [3]. Induction of the immune response usually requires the presence of T helper cells. Some antigens induce the immune response without T helpers, so-called T cell-independent (TI) antigens. TI can be divided into 2 types: TI-1 and TI-2 antigens. TI-1 antigens are polyclonal activators stimulating production of antibodies by mature (Lyb5+) and immature (Lyb5-) B lymphocytes, while TI-2 antigens possess no mitogenic properties and stimulate the production of antibodies only by mature Lyb5+ B lymphocytes [4,6]. The mechanisms of the effect of TI-2 remain unclear. It is unknown whether nonspecific immunoglobulins are synthesized only by mature B lymphocytes or they are also produced by immature B cells. The factors contributing to polyclonal activation are still unstudied.

Here we studied the role of Lyb5+ and Lyb5- B lymphocytes in polyclonal activation induced by TI-2 antigens.

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MATERIALS AND METHODS

Experiments were performed on congeneric CBA and CBA/N mice (with and without Lyb5+ B cells, respectively) obtained from Stolbovaya nursery and gifted by B. V. Nikonenko (Institute of Tuberculosis).

Influenza virus strain A/PR/8 and water-soluble antigen of sheep erythrocytes obtained as described elsewhere [5] were used as T cell-dependent antigens. Polyvinylpyrrolidone with a molecular weight of 350 kD and dinitrophenyl-Ficoll served as TI-2 antigens.

In *in vivo* experiments, the mice were intravenously injected with 1-2 μg polyvinylpyrrolidone, 5 μg influenza virus, or 500-700 μg sheep erythrocytes antigen. Antibody-producing (APC) and immunoglobulin-synthesizing (IGSC) cells of splenocyte suspensions were counted on day 4 using ELISPOT [1].

During *in vitro* induction of the immune response, splenocytes from nonimmunized animals were incubated in 96-well plates with 5 μg/ml influenza virus and 10 ng/ml polyvinylpyrrolidone or 30 ng/ml dinitrophenyl-Ficoll in RPMI-1640 medium containing 10% fetal serum and other additives for 4 days in a CO_2 incubator at 37°C. The number of APC and IGSC

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was estimated in 8-9 parallel cultures by the method described elsewhere [1]. Splenocytes obtained from nonimmune mice or incubated without antigens were used as the control in *in vivo* or *in vitro* experiments, respectively.

The number of cells synthesizing antigen-dependent nonspecific immunoglobulins (NIGSC) was evaluated as the difference between the rise in APC and IGSC contents.

RESULTS

The administration of T cell-dependent antigens (Table 1) and influenza virus (data not shown) to CBA mice led the appearance of APC and increased the number of NIGSC.

The absolute contents of APC and IGSC varied and, therefore, we compared the relative contents (percentage) of APC and NIGSC in the fraction of antigen-induced IGSC. The mean content of APC in this fraction was 18.5±3.9% of the total number of antigen-dependent IGSC (5 observations).

TI-2 antigens also induced the appearance of APC and increased the number of IGSC and NIGSC in CBA mice (5 observations, Table 1). The absolute number of these cells was lower than after administration of T cell-dependent antigens. However, the relative content of APC and NIGSC in the fraction of dinitrophenyl-Ficoll-induced IGSC and the NIGSC/APC ratio [4,5] were similar after immunization with TI-2 and T cell-dependent antigens. Polyvinylpyrrolidone less markedly increased the absolute number of APC, IGSC, and NIGSC (7 observations, Table 1). However, the content of APC was 9.3±2.6% of the total number of induced IGSC, and the content of NIGSC 10-fold surpassed that of specific APC.

In vitro experiments on induced splenocytes from CBA mice gave similar results (Table 1). Thus, *in vivo* and *in vitro* immunization of CBA mice with T cell-dependent and TI-2 antigens caused not only the appearance of APC, but also polyclonal activation of B cells.

Immunization of CBA/N mice with T cell-dependent antigens (influenza virus or antigen of sheep ery-

TABLE 1. Immune Response of CBA Mice to T Cell-Dependent and TI Antigens (*M±m*)

Antigen	Rise per 1 million cells		Number of NIGSC	APC, %	NIGSC, %	NIGSC/APC
	APC	IGSC				
In vivo						
WSSEA	2043±775	10633±941	8590±963	18.5±3.9	81.5±2.3	4.2
DNP-F	772±234	4244±1231	3473±1132	20.0±8.0	80.0±8.1	4.5
PVP	205±85	3380±540	3179±504	9.3±2.6	90.7±2.8	10.0
In vitro						
A/PR/8	712±312	5628±1315	5106±1366	10.2±4.9	89.7±4.9	7.2
DNP-F	410±80	5050±1550	4640±1630	10.0±4.2	91.2±3.1	9.0
PVP	267±78	3767±2100	3547±2030	7.1±2.0	91.0±2.0	13.3

Note. Here and in Table 2: WSASE, water-soluble sheep erythrocyte antigen; DNP-F, dinitrophenyl-Ficoll; and PVP, polyvinylpyrrolidone.

TABLE 2. Immune Response of CBA/N Mice to T Cell-Dependent and TI Antigens (*M±m*)

Antigen	Rise per 1 million cells		Number of NIGSC	APC, %	NIGSC, %	NIGSC/APC
	APC	IGSC				
In vivo						
A/PR/8	1326±150	5567±756	4240±905	24.6±6.1	75.4±6.1	3.2
WSSEA	5775±860	18258±2942	12483±2082	31.7±0.4	68.3±0.3	2.2
DNP-F	10±8	1071±660	1064±656	0.9±0.7	98.9±0.7	—
PVP	75±67	1455±263	1378±75.2±5.1	94.1±5.1	—	
In vitro						
A/PR/8	712±312	7178±2372	6466±2020	8.8±1.1	91.2±2.1	9.1
DNP-F	35±25	—	—	—	—	—
PVP	6±6	567±567	567±567	1.0±1.0	99.0±1.0	-

throcytes) also increased the number of APC and NIGSC (Table 2). The relative content of APC in the fraction of antigen-induced IGSC (27%) in CBA/N mice surpassed that in CBA mice. *In vitro* culturing of splenocytes from nonimmune CBA/N mice with influenza virus caused the appearance of APC and NIGSC, and the number of NIGSC 9-fold exceeded the number of APC (Table 2).

However, the production of APC, IGSC, and NIGSC sharply decreased after immunization of CBA/N mice with TI-2 antigens (Table 2). The increase in the number of NIGSC induced by TI-2 antigens in CBA/N mice was less pronounced than that induced by T cell-dependent antigens. Moreover, this increase in CBA/N mice was 3 times lower than in CBA mice. It should be emphasized that variation of individual parameters in CBA/N mice was greater than in CBA mice.

In vitro experiments demonstrated even more clear differences in the response of CBA and CBA/N mice to TI-2 antigens (Table 2). APC production and increased number of TI-2 antigen-induced NIGSC were not found in CBA/N mice.

Thus, TI-2 antigens lead to the appearance of APC and accumulation of NIGSC, i.e. polyclonal activation of B-lymphocytes. TI-2 and T cell-dependent antigens stimulate APC and NIGSC production, which is consistent with previously reported data [2,3]. TI-2 antigens stimulate production of antibodies by Lyb5+ B lymphocytes [4,6]. Therefore, Lyb5+ B lymphocytes are probably involved in polyclonal activation induced by TI-2 antigens. In the absence of Lyb5+ B lymphocytes, mice do not synthesize antibodies against TI-2 antigens, and the formation of antigen-induced nonspecific immunoglobulins is sharply suppressed (because only Lyb5+ B lymphocytes are involved in these processes or some other factors produced by Lyb5+ B lymphocytes and activating only Lyb5+ B cells play the major role in polyclonal activation). Determination of the Lyb5+ phenotype of TI-2 antigen-induced NIGSC in CBA/N mice will help to answer these questions.

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