MICROBIOLOGY AND IMMUNOLOGY

Stimulation of Immune Response: Resistance to Proliferation Inhibitors

E. D. Gavrilova, O. T. Kudaeva, O. P. Kolesnikova, and V. A. Kozlov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 3, pp. 304-307, March, 2010
Original article submitted July 15, 2009

Additional dose of the antigen at the end of the log phase of developing IgM response to T-dependent antigen leads to a drastic increase in the counts of IgM and IgG antibody-producing cells in the spleens of experimental animals. The effect is dose-dependent and more pronounced after the first immunization with the antigen in the suboptimal dose. Elimination of proliferating antibody producers has an ambiguous effect on IgM and IgG antibody production in the spleen: it limits the increase in the count of IgM-producing cells, but does not abolish the stimulation of IgG response. It seems that the increase in the count of IgG producers is not linked with simultaneous active proliferation of IgG producing cell precursors.

Key Words: *proliferative activity of antibody producers; IgM and IgG antibody producing cells; hydroxyurea*

Despite the progress in understanding of the cellular and molecular genetic mechanisms of antibody production, many aspects in the regulation of humoral immune response at the organism level remain unclear [2-4]. We previously showed that additional doses of exogenous antigen during the log phase of developing primary humoral immune response to T-dependent antigen reduce the intensity of the secondary response in (CBA×C57Bl/6)F_1 mice [1]. Here we studied these effects of repeated doses of the antigen on the formation of primary IgM and IgG response.

MATERIALS AND METHODS

The study was carried out on female (C57B1/6×DBA) F_1 mice aged 2-3 months from Experimental Biological Clinics of Laboratory Animals, Siberian Division of the Russian Academy of Medical Sciences. The animals were maintained in accordance with the regulations of the European Convention on the Protection of Animals Used for Experimental and Other Research Purposes (Strasbourg, 1986).

The mice were immunized intravenously with sheep erythrocytes (SE) in the suboptimal (1×10^7) and optimal (2×10^8) doses. The intensity of humoral immune response was evaluated by estimation of IgM and IgG antibody producing cells (APC) by local hemolysis in the mouse spleen at the peak of immune response. The time course of immune response for mice of the studied genotype was determined in preliminary experiments. The maximum count of IgM APC in the spleen is observed 5 days, of IgG APC 9 days after immunization.

Repeated injections of the antigen were carried out also in the suboptimal (1×10^7) and optimal (2×10^8) doses of SE intravenously 24 h before the peak of the response: after 4 days for evaluation of IgM response

Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** edav76@mail.ru. E. D. Gavrilova

0007-4888/10/1493-0328

and after 4 or 8 days for evaluation of IgG response.

In order to eliminate the proliferating cells, the animals pre-immunized with the suboptimal dose were injected with hydroxyurea (HU) in a dose of 1 g/kg (2 injections with a 7-h interval) on day 4 after the primary immunization.

The results were statistically processed by methods of nonparametric statistics; the differences were considered significant at $p<0.05$.

RESULTS

Repeated injection of the antigen in the late log phase of primary IgM response (4 days after immunization) stimulated the development of the primary IgM and IgG response in the spleen (Figs. 1, 2). The increase in APC count was more pronounced after primary immunization with the antigen in the suboptimal dose and repeated injection of the higher dose. After primary immunization with the optimal dose of the antigen, the count of IgM APC increased only after repeated injection of the same dose of the antigen, while the level of IgG APC did not change. The absence of pronounced stimulation of antibody production by repeated doses of the antigen in immunization with high doses was demonstrated previously [3].

Presumably, repeated injection of the antigen at the end of the log phase of primary IgM response prolongs activation of the initial stages of the response, this modulating the normal course of humoral response.

Additional injection of the antigen at the end of the primary IgG response log phase (8 days after immunization) does lead to such an effect. The count of IgG APC does not change. Moreover, their count even decreases significantly, if immunization and reimmunization are carried out with the lower dose of the antigen (Fig. 2).

The difference in response to repeated dose of the antigen after 4 and 8 days can be explained by the regulatory effects of antibodies of different classes. Repeated dose of the antigen was injected after 4 days under conditions of active production of specific IgM, while after 8 days the specific IgG were produced. The IgM antibodies injected together with the corpuscular antigen stimulate the humoral immune response, while IgG antibodies suppress it. The effect was demonstrated for passively injected antibodies and for antibodies produced in the host [5].

After contact with the antigen and Th2 cells, antigen-specific B-cells are stimulated to proliferation and differentiation into APC in primary response. These processes are parallel until the stage of plasma cells [6,8]. The subsequent events are switchover of the immunoglobulin isotypes, formation of short- and long-living plasma cells and memory cells, migration of cells into the bone marrow, and a drop of the count of APC [2-4,7]. Some of these processes are sufficiently well studied, while the details and regulatory factors of other processes remain largely unclear.

The increase in APC count after repeated dose of the antigen at the end of the log phase was very sharp, particularly for IgG APC (by more than one order of magnitude). As for IgM response, the many-fold (3-7-fold after the first immunization with the suboptimal dose) increase in the count of cells producing specific antibodies was rapid (within 24 h). These effects suggest the involvement of potent regulatory factors. One of the mechanisms of realization of their effects can be stimulation of the proliferative processes of B-lymphocytes, differentiating into APC.

The role of proliferative processes in the stimulatory effect of additional dose of the antigen was studied in a series of experiments with injection of HU (DNA synthesis inhibitor). Injection of HU to mice during the log phase of primary IgM response (3-4 days after immunization) resulted in marked inhibition of IgM and IgG antibody production, which completely agrees with the known time course of humoral immune response (HU injection after 3 days inhibited the IgM response by 86% and the IgG response by 77% in the control group; the effects of HU injection 4 days after immunization are presented in Fig. 3). Injection of HU 4 days after immunization simultaneously with repeated dose of the antigen attenuated the stimulatory effect on the IgM response, though the count of IgM-APC in the spleen remained sufficiently high, its level being comparable to that in the control group

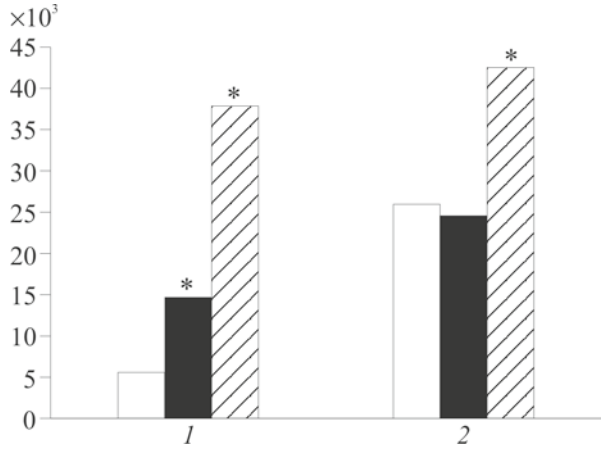

Fig. 1. Count of IgM-APC in the spleens of mice after repeated injection of the antigen 4 days after immunization. Here and in Fig. 2: light bars: control (no repeated injection of the antigen); dark bars: experimental group receiving a repeated dose of the antigen (1×10^7 SE); obliquely hatched bars: experimental group with repeated injection of the antigen in a dose of 2×10^8 SE. First immunization doses: 1×10^7 (*1*) and 2×10^8 (*2*). Here and in Figs. 2, 3: *$p<0.05$ compared to the control.

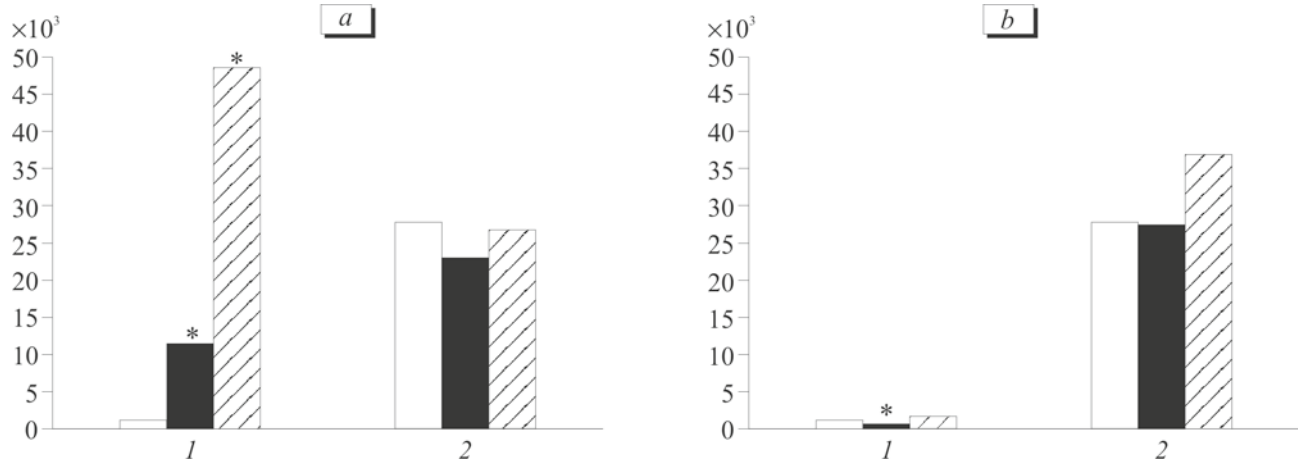

Fig. 2. Count of IgG APC in the spleens of mice after repeated injections of the antigen 4 days (*a*) and 8 days (*b*) after immunization.

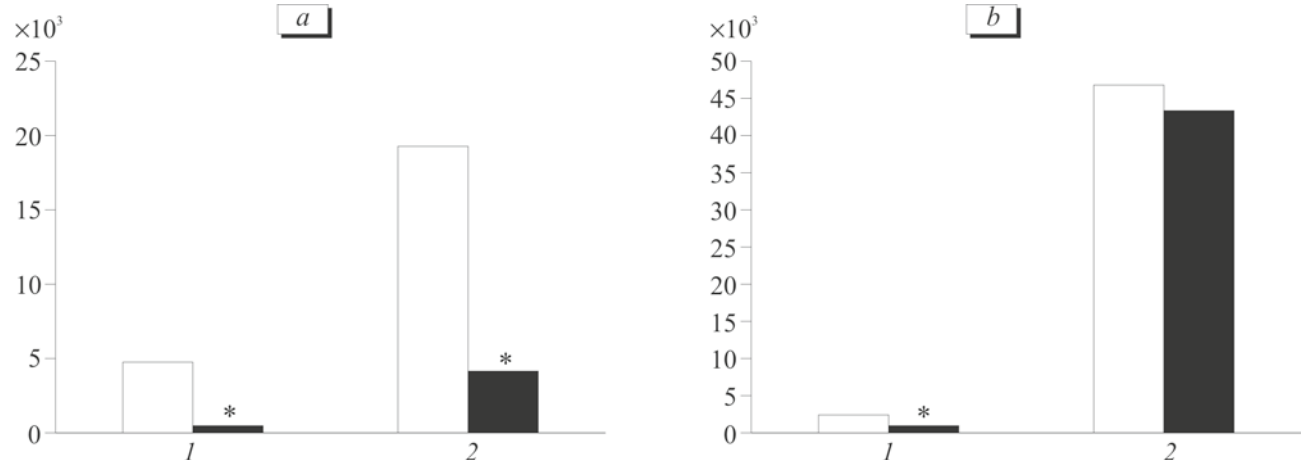

Fig. 3. Counts of IgM-APC (*a*) and IgG APC (*b*) in the spleens of BDF1 mice injected with HU after repeated dose of the antigen 4 days after immunization. *1*) control group (no repeated injection of the antigen); *2*) experimental group receiving additional dose of the antigen (1×10^7 SE). Light bars: no HU injection; dark bars: HU injection.

of mice subjected to a single immunization (Fig. 3). Hence, under conditions of stimulation with repeated dose of the antigen, the greater portion of IgM APC is presented by actively proliferating cells; shortening of cell cycle duration and delayed release of cells from the dividing pool are probable. However, under these conditions a certain portion of IgM APC belongs to the undividing population, this indicating the existence of other mechanisms realizing the stimulatory effect of the antigen (in addition to stimulation of APC proliferation). Presumably, stimulation involves cells less intensely producing antibodies without stimulation in the response, and these cells cannot be detected by standard methods. Presumably, through a possible modification of the cytokine production by antigen-presenting and/or Th-cells, the repeated dose of the antigen stimulates antibody biosynthesis in them. In addition, these can be the cells subjected to apoptosis during single immunization; repeated dose of the antigen can provide signals for their survival [4,7].

Estimation of the count of IgG-APC in our experiment showed no canceling or reduction of the stimulatory effect of repeated antigen dose. The increase in IgG-APC count in the spleen was very high (more than 10-fold; Fig. 3). Presumably, repeated injection of the antigen led to involvement of IgG-APC precursor cells (previously not included in the pool proliferating during that period) in active proliferation; hence, their proliferation started later and was not suppressed by HU.

In addition, repeated injection of the antigen at the end of the log phase of unfolding IgM response can modulate the cell migration processes (stimulate cell migration into the spleen from the bone marrow and periphery and later, *i.e.* during the development of IgG response, inhibit APC migration to the bone marrow) and modify the APC/memory cell proportion in

differentiation processes; these possible mechanisms deserve further studies.

Hence, additional injection of the antigen at the end of the log phase of primary IgM response leads to pronounced stimulation of IgM APC proliferative activity and a significant increase in their count. This is paralleled by a sharp increase in the count of IgG APC. Inhibition of proliferative processes under conditions of primary response stimulation (injection of HU simultaneously with the antigen during the log phase of primary IgM response) inhibits elevation of IgM APC, but does not cancel the stimulation of IgG APC formation, which suggests the involvement in this process of the cells other than the pool of APC actively proliferating during this period.

REFERENCES

1. E. D. Gavrilova, O. T. Kudaeva, and O. P. Kolesnikova, *Vestn. Ural. Med. Akademich. Nauki*, Nos. 3-1, 32-35 (2006).
2. F. D. Batista and N. E. Harwood, *Nat. Rev. Immunol.*, **9**, No. 1, 15-27 (2009).
3. M. D. Cahalan and I. Parker, *Semin. Immunol.*, **17**, No. 6, 442-451 (2005).
4. J. E. Crowley, J. L. Scholz, W. J. Quinn 3rd, *et al.*, *Immunol. Res.*, **42**, Nos. 1-3, 75-83 (2008).
5. F. Hjelm, F. Carlsson, A. Getahun, and B. Heyman, *Scand. J. Immunol.*, **64**, No. 3, 177-184 (2006).
6. S. G. Tangye and P. D. Hodgkin, *Immunology*, **112**, No. 4, 509-520 (2004).
7. D. Tarlinton, A. Radbruch, F. Hiepe, and T. Dörner, *Curr. Opin. Immunol.*, **20**, No. 2, 162-169 (2008).
8. M. C. van Zelm, M. van der Burg, and J. J. van Dongen, *Cell Cycle*, **6**, No. 23, 2890-2895 (2007).