3. A. Afeltra, M. Galeazzi, G. M. Ferri, *et al.*, *Ann. Rheum. Dis.*, 52, 457-460 (1993).
4. A. N. Akbar, M. Salmon, and G. Janossy, *Immunol. Today*, **12**, № 6, 184-188 (1991).
5. E. Beckmann and P. J. Connolly, *Clin. Microbiol. News Lett.*, **9**, № 6, 41-46 (1987).
6. R. Biselly, P. M. Matricardi, R. Damellio, and A. Fattorossi, *Scand. J. Immunol.*, **35**, 439-447 (1992).
7. A. Boyum, *Scand. J. Clin. Lab. Invest.*, **21**, 77-82 (1968).
8. C. J. Corrigan and A. B. Kay, *Int. Arch. Allergy*, **94**, 270-271 (1991).
9. C. J. Corrigan and A. B. Kay, *Amer. Rev. Resp. Dis.*, **141**, № 4, 970-977 (1990).
10. D. Matour, M. Melnicoff, D. Kaye, and D. M. Murasko, *Clin. Immunol. Immunopath.*, **56**, 82-99 (1989).
11. H. Zola, *Immunol. Cell Biol.*, **70**, 337-341 (1992).

MICROBIOLOGY AND IMMUNOLOGY

Variants of Secondary Immune Response in CBA and C57Bl/6 Mice

E. Yu. Gusev, V. L. Ponosov, and N. N. Kevorkov

UDC 612.017.1:57.04

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 11, pp. 486-488, November, 1994
Original article submitted January 25, 1994

The manifestation of a secondary immune response to intraperitoneal and subcutaneous injection of sheep red cells in various doses was studied in CBA and C57Bl/6 mice. The parameters under study were the delayed-type hypersensitivity reaction and antibody production assessed from the levels of antibody-producing cells of classes M and G in the lymph node and spleen. The manifestation and correlations between delayed-type hypersensitivity and antibody production were found to depend on the route of antigen administration and its first immunizing dose, interval between the two immunizations, and genetic control of the immune response.

Key Words: *antibody production; delayed-type hypersensitivity; secondary immune response*

Different types of immune response in CBA and C57Bl/6 mice are determined by the priority of development of antibody production (ABP) or delayed-type hypersensitivity (DTH) in response to suboptimal doses of sheep red cells (SRC) in the local immune response and to suboptimal and optimal doses thereof in the generalized response [1]. These differences increase for immunization 7 days before with SRC doses subthreshold for ABP but optimal for the DTH reaction [4]. At the same time, the data reflect only one variant of secondary response.

The purpose of this research was to evaluate different variants of secondary immune response of CBA and C57Bl/6 mice to SRC, namely, to use various doses of SRC for primary immunization at different intervals between the two immunizations.

MATERIALS AND METHODS

Four hundred CBA and C57Bl/6 mice weighing 18 to 22 g were used. Primary immunization

Biochemistry Department, Medical Institute, Perm. (Presented by K. P. Kashkin, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0011-1183$12.50

was performed after two protocols: 1) intraperitoneally in doses of 10^4, 10^6, and 10^8 SRC at various times before reinjection of the antigen and 2) subcutaneously in the pad in a dose of 10^8 SRC 21 days before reimmunization with SRC in various doses. According to our findings [2,3], injection of 10^4 SRC after scheme 1 induces selective production of antigen-specific cyclophosphamide-sensitive DTH T suppressors (DTH-Ts); the dose of 10^6 SRC (in C57Bl/6 mice) is optimal for the generation of DTH T effectors (Te); 10^8 SRC is the dose hyperimmune for DTH but optimal for ABP; in parallel with immunization after scheme 2, SRC in a dose of 10^9, hyperimmune for DTH and ABP, but optimal for the production of ABP T suppressors (ABP-Ts) were injected after scheme 1. It should be emphasized that immunization with SRC after protocol 1 [1] did not induce an immune response in the area of the popliteal lymph node.

Secondary immunization of animals of both strains with SRC was performed after scheme 2 in the dose of 10^8 optimal for the local immune response [1]; CBA mice, in addition to this, were injected SRC after scheme 1 in various doses, from 10^6 to 10^9, 21 days after immunization with SRC in a dose of 10^8 according to scheme 1. The secondary immune response was assessed 5 days after the last immunization: DTH as described previously [4], ABP from the count of class M antibody-producing cells (APC) in the regional popliteal lymph node or (after reimmunization after scheme 1) in the spleen by direct local hemolysis [5]. In addition, in CBA mice immunized according to schemes 1 and 2 the counts of class G APC in the spleen or lymph node were estimated by indirect local hemolysis 21 days before reimmunization with 10^8 SRC [6]. Control mice instead of primary immunization were injected 0.5 ml normal saline according to scheme 1 or 0.05 ml according to scheme 2. The results were statistically processed using the Student t test.

RESULTS

Figure 1 shows ABP and DTH in response to reinjection of the antigen. Out of all the SRC doses used (from 10^6 to 10^9) for immunization after scheme 1 and simultaneous immunization after scheme 2 (10^8 SRC) only one, the maximal, effectively depressed local ABP in CBA and C57Bl/6 mice (Fig. 1, *a*). The DTH reaction in CBA mice is depressed at SRC doses of 10^8 and 10^9, whereas in C57Bl/6 mice this occurs only at SRC dose of 10^9. On the other hand, immunization of C57Bl/6 mice after scheme 1 performed 5, 10, and 15 days previously with an SRC dose of 10^8 is associated with a more marked reduction of ABP (as soon as on day 5 postimmunization) in comparison with CBA mice (Fig. 1, *b*). Since these cells are capable of limiting the generation of Te as well, these strain-specific immune reactions may be attributed to a different genetically determined sensitivity of ABP and DTH to the regulatory action of ABP-Ts [7]. Injection of SRC in doses of

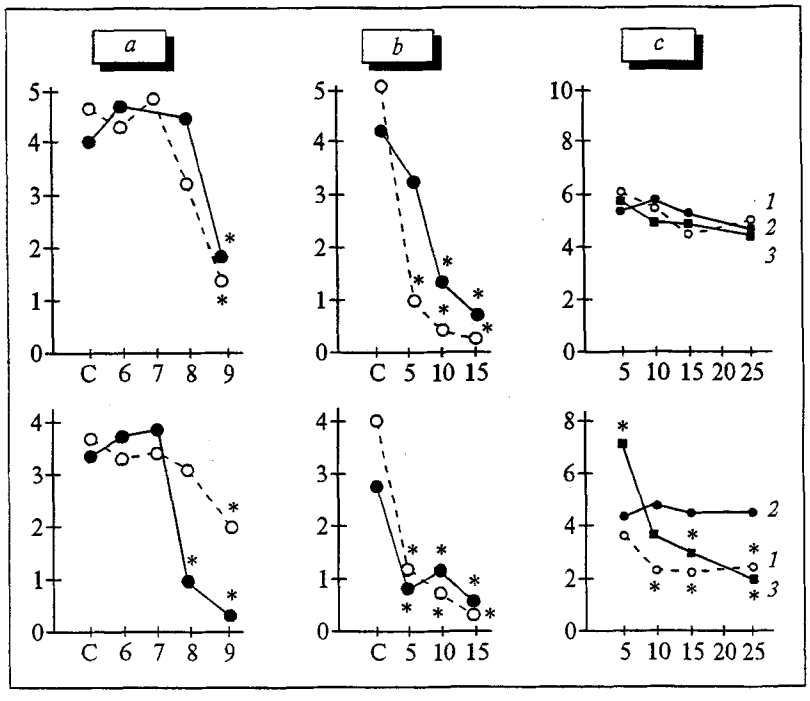

Fig. 1. Effect of immunization after scheme 1 on the manifestation of local ABP and DTH reaction in response to injection of 10^8 SRC after scheme 2. Abscissa: *a*) log SRC dose injected after scheme 1 simultaneously with immunization after scheme 2; *b*) period between immunizations with 10^8 SRC after schemes 1 and 2, days; c) the same, but for immunization of C57Bl/6 mice with 10^4 and 10^6 SRC. Ordinate: count of M–APC ($\times 10^3$) per lymph node (above) and degree of DTH in U (below). *a*, *b*) *C*: controls, continuous line – CBA; broken line – C57Bl/6; *c*) *1*) injection of 10^4 SRC after scheme 1; *2*) control; *3*) injection of 10^6 SRC after scheme 1. Asterisk shows the data reliably ($p<0.05$) differing from the control.

10^4 and 10^6 to C57Bl/6 mice after scheme 1 at various times (5, 10, 15, 25 days) prior to immunization after scheme 2 in a dose of 10^8 SRC did not influence the local ABP (Fig. 1, *c*). On the other hand, SRC in a dose of 10^4 injected after protocol 1 10 to 25 days before immunization according to protocol 2 suppressed the development of DTH. An SRC dose of 10^6 stimulated DTH if injected 5 days prior to scheme 2 immunization and depressed it if injected 15 to 25 days before it. Such an effect may be attributed to the short life of Te (up to 10 days), which are capable of boosting the DTH reaction in response to reinjection of the antigen [4], and the relatively long life of DTH-Ts [2,3].

Figure 2 presents the data reflecting the pattern of immune response to SRC injection in various doses according to schemes 1 and 2 21 days after preimmunization with 10^8 SRC. This pattern of response is characterized by the following features, in comparison with the primary antigen "dose-effect" relationship [1,3,4]: a marked increase of the threshold antigenic sensitivity for the local (by 3 orders of magnitude) and generalized (by 2 orders of magnitude) immune response; predominance of G-APC over M-APC, whose level is negligible; complete absence of DTH manifestations.

The immune response in this experiment seems to be mediated mainly at the expense of memory cells to the detriment of repeated generation of APC and formation of Te.

Hence, the secondary immune response, besides the genetic control, depends on the specific conditions of primary and secondary immunization.

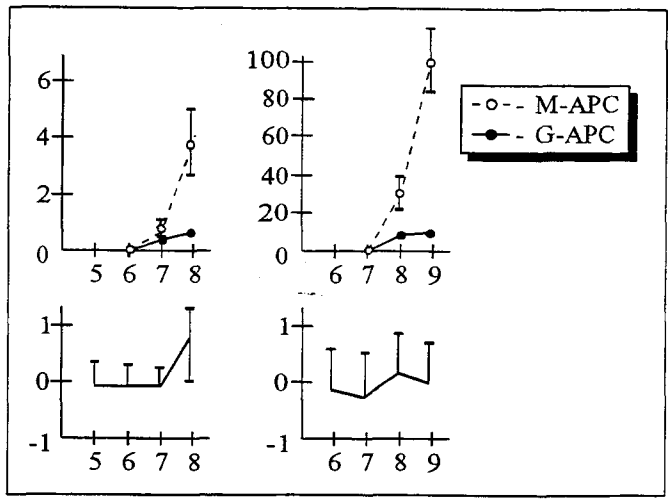

Fig. 2. Local (*a*) and generalized (*b*) secondary immune responses to different doses of SRC after immunization according to schemes 2 (*a*) or 1 (*b*) 21 days before reimmunization with 10^8 SRC in CBA mice. Abscissa: log SRC dose for reimmunization after schemes 2 (*a*) or 1 (*b*); ordinate: above – count of APC ($\times 10^3$) per lymph node (*a*) or spleen (*b*); below – DTH reaction in U.

REFERENCES

1. E. Yu. Gusev, V. L. Ponosov, and N. N. Kevorkov, *Byull. Eksp. Biol. Med.*, **112**, № 9, 271 (1991).
2. E. Yu. Gusev, *Deposited at Vsesoyuz. Inst. Nauch. Tekh. Informatsii*, № 1459-B92.
3. E. Yu. Gusev, V. L. Ponosov, and N. N. Kevorkov, *Pat. Fiziol.*, № 1, 39 (1993).
4. E. Yu. Gusev., N. N. Kevorkov, and V. L. Ponosov, *Byull. Eksp. Biol. Med.*, **118**, № 11, 499-501 (1994).
5. N. K. Jerne and A. A. Nordin, *Science*, **140**, № 3365, 405 (1963).
6. J. Sterzl and I. Riha, *Nature*, **208**, № 5013, 858 (1965).
7. R. L. Wisler and J. D. Stodo, *J. Immunol.*, **121**, № 2, 539 (1978).