Exposure to considerable stress also leads to a change in the level of cytostatic and cytotoxic activity of spleen cells, and definite correlation is observed in the time course of these two functions after stress [2].

Data on the sensitivity of the cytostatic activity of spleen cells to the effect of interferon and exposure to stress are evidence of definite similarity between the properties of effectors of cytostatic and cytotoxic activity. Meanwhile there are marked differences in the level of their activities in different lymphoid organs and in relation to different target cells.

The possibility cannot be ruled out that effectors of cytostatic activity constitute a heterogeneous population, of which cells with cytotoxic activity constitute one subpopulation. Further investigations are necessary in order to explain this problem.

LITERATURE CITED

1. L. V. Van'ko and G. T. Sukhikh, Byull. Éksp. Biol. Med., No. 12, 84 (1983).
2. B. B. Fuks, Interaction between Normal Killer Cells and Tumor Cells [in Russian], Moscow (1983), pp. 5-22.
3. R. Ehrlich et al., J. Immunol. Meth., 40, 193 (1981).
4. M. Hansson, R. Kiessling, and R. Welsh, Natural Cell-Mediated Immunity against Tumors, New York (1980), pp. 855-872.
5. S. Korec, Natural Cell-Mediated Immunity against Tumors, New York (1980), pp. 1301-1307.
6. M. E. Nunn, R. B. Herberman, and H. T. Holden, Int. J. Cancer, 20, 381 (1977).
7. P. Sertini, B. E. Bozolma, et al., Cell Immunol., 73, 264 (1982).

IN VIVO INDUCTION OF SPECIFIC SUPPRESSOR T CELLS IN H-2K^{bm} MUTANT MICE

E. S. Kan, A. V. Chervonskii, V. R. Isakova, B. D. Brondz, and Z. K. Blandova

UDC 612.112.94-017.1-063:612.6.052

KEY WORDS: H-2 complex; specific suppressor T cells; monoclonal antibodies; H-2K^{bm} mutants.

It has been shown [2] that specific supressor T cells (SSTC) in the H-2 system, appearing as a result of induction *in vivo*, are narrowly specific for a certain epitope of the H-2 molecule. SSTC can recognize determinants similar to serologic determinants [6].

In attempts to induce SSTC in mice mutant for the H-2 complex, with wild-type cells it was found that SSTC are easily formed *in vivo* by a complex mutation of the H-2D^{dm1} molecule if the corresponding mutant (M504), immunized by wild-type B10.D2 (H-2^d) cells, can also form antibodies [6]. Conversely the "point" mutation of the H-2K^{bm1} molecule does not lead to the formation either of antibodies [10], or of SSTC [6], on immunization of the mutant with wild-type C57BL/6 (H-2^b) cells, despite the effective formation of cytotoxic T lymphocytes (CTL) in the same system [10].

In the present investigation, in order to induce SSTC we used a set of mutants with clearly characterized replacement of amino acids in the H-2K^b molecule (Table 1).

EXPERIMENTAL METHOD

Mice of lines BALB/c (H-2^d), DBA/2 (H-2^d), CBA (H-2^k), AKR (H-2^k), and C56BL/6 (abbreviation B6, H-2^b) were obtained from the Stolbovaya Nursery, Academy of Medical Sciences

---

Laboratory of Immunochemistry, All-Union Oncologic Science Center, Academy of Medical Sciences of the USSR, Moscow. Branch of the M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Pushchino, Moscow Region. Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Svetlye Gory, Moscow Region.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Blokhin.) Translated from Byulleten' Éksperimental'nok Biologii i Meditsiny, Vol. 102, No. 8, pp. 203-205, August, 1986. Original article submitted December 13, 1985.

0007-4888/86/0008- 1102$12.50

TABLE 1. Structural Changes in H-2K^b Molecules in Mutant Mice

Line of mics	Abbreviated name	Amino-acid substitution in H-2K^b molecule [12, 13]	Position
B6.C—H-2^{bm1}	bm1	Gly→ Ala	152
		Agr→ Tyr	155
		Leu→ Tyr	156
B6—H-2^{bm3}	bm3	Asp→ X	77
		Lys→ Ala	89
B6.C—H-2^{bm4}	bm4	?	158—173

Note. bm4 has at least two amino-acid substitutions between positions 158 and 173 [9].

TABLE 2. Distribution of Lyt and Thy Antigens on Thymocytes of Some Lines of Mice and G4 MCAB Activity

Line	Lyt1	Lyt2	Lyt3	Thy1	G4
C57Bl/6	2	2	2	2	+
DBA/2	1	1	2	2	+
AKR	2	1	1	1	−
CBA	1	1	2	2	+
BALB/c	2	2	2	2	+
C58	2	1	1	2	+
A	2	2	2	2	+

of the USSR; recombinant line B10.A(4R) ($K^{k}1^{k}/b_D^b$) and mutant lines (bm) B6.C-H-2^{bm1} (bm1), B6-H2^{bm3} (bm3), and B6.C-H-2^{bm4} (bm4), and also lines C58 (H-2^K) were obtained from the Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR.

H-2K^{bm} anti-H-2K^b SSTC were obtained from the spleen of a mouse 4 days after intravenous immunization with $9 \cdot 10^7$ B6 mouse spleen cells, irradiated in a dose of 2000 rads. Activity of SSTC treated with mitomycin C was estimated by inhibition of DNA synthesis in a one-way mixed lymphocyte culture (MLC) with a ratio of SSTC, syngeneic reacting lymph node (LN) cells, and allogeneic stimulator cells, irradiated in a dose of 2000 rads, of 1:3:9.

The inhibition index (II) of DNA synthesis was estimated relative to a control MLC in which, instead of immune lymphocytes, normal lymphocytes treated with mitomycin C were added [2].

To inactivate the SSRC they were treated successively with G4 monoclonal antibodies (MCAB) to antigen Thy 1.2 for 40 min at 20°C, using ascites fluid in a dilution of 1:200, and then with nontoxic rabbit complement absorbed with agarose (final dilution 1:9) for 60 min at 37°C [5].

To obtain MCAB to Thy 1.2 antigen, spleen cells of AKR mice, immunized with CBA thymocytes, were hybridized with the aid of 50% polyethylene-glycol (PEG-1500, Serva, West Germany) with P3-X63-Ag8.653 myeloma cells from a BALB/c mouse. Primary cultures and clones were grown as described previously [3].

The presence of antibodies in the culture fluid was determined by the two-stage complement-dependent cytotoxicity test [8], using rabbit complement and purified T cells.

The T cells were separated from B cells by the "panning" method [11], using purified rabbit antibodies to the L-chain of mouse immunoglobulin (Ig). Under these circumstances the T cells were found in the fraction of cells not adhering to anti-mouse Ig, and the B cells were eluted by pipeting in the cold [11]. The purity of the separated populations were verified in the indirect immunofluorescence test with rabbit antiserum against mouse Ig and with fluorochromed rabbit anti-Ig.

A positive G4 culture was cloned twice by the limiting dilution method, at the rate of one cell per well. The clones of the G4 hybridoma were grown *in vivo* in (BALB/c × AKR)F_1 mice. The ascites fluid thus obtained was kept at −40°C.

EXPERIMENTAL RESULTS

Suppressor cells reacting to H-2K^b antigen were found in only one of the three mutant lines studied (Fig. 1). Only mutant bm3 and the hybrid (BALB/c × bm3)F_1 generated suppressors, whereas no suppressors were found in mutants bm1 and bm4 and in the hybrid (BALB/c × bm1)F_1. The effect of bm3 anti-B6 suppressors was just as specific as that of BALB/c anti-B6, for in both cases the suppressors did not block the proliferative response to "foreign" stimulators B10.A(4R) or CBA, differing from the H-2K^b immunizing agent by the allele H-2K^k.

To determine the nature of the bm3 anti-B6 suppressor cells, MCAB against Thy 1.2 antigen were obtained. For this purpose we selected those samples of hybridomas whose culture fluids had lysed 98-99% of thymocytes of all strains of mice except AKR (Table 2). CBA differs from AKR not only in the allele of the Thy 1 molecule. The possibility of a reaction

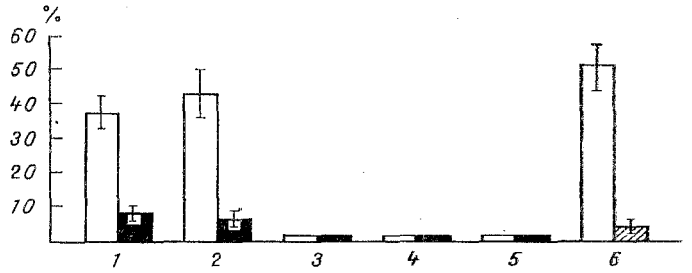

Fig. 1

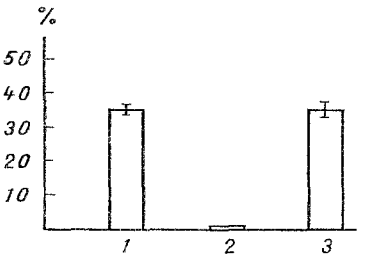

Fig. 2

Fig. 1. Differences in activity of T suppressor cells induced *in vivo* in bm mutants and F_1 hybrids against the wild type H-2K^b. Ordinate, II (in %) of DNA synthesis in mixed lymphocyte culture. 1) bm3; 2) BALB/c × bm3)F_1; 3) bm1; 4) (BALB/c × bm1)F_1; 5) bm4; 6) BALB/c. Stimulators: unshaded columns, B6; black columns, B10.A(4R); obliquely shaded column, CBA. Each column represents average of 2-8 experiments (M ± m).

Fig. 2. T-cell origin of bm3 anti-B6 suppressor cells. Ordinate, II (in %) of DNA synthesis in mixed lymphocyte culture. Cells: 1) intact; 2) treated with G4 MCAB and complement; 3) treated with culture medium and complement. Each column represents mean results of two experiments (M ± m).

with Lyt 1.1 and Lyt 3.2 can be rejected, for G4 MCAB lyse thymocytes of C58 mice which, like AKR, do not possess these alleles of Lyt 1 and Lyt 3 antigens (see Table 2). It was further found that G4 MCAB lyse 30% of mouse spleen cells and 65-70% of LN cells and 100% purified T cells, but do not lyse purified B cells of LN.

Thus G4 MCAB reveal a marker which is identical with Thy 1.2 in its universality and specificity for T cells, and also for its distribution in lymphoid organs of mice of different lines.

G4 MCAB belong to the IgM, κ class, as shown by data on inhibition of radioimmunoadsorption [14].

The effect of bm3 anti-B6 suppressors was completely abolished after their treatment with G4 MCAB and complement (Fig. 2). Since control treatment with culture medium and complement did not affect the degree of suppression, it is evident that T cells were responsible for it.

The selective ability of the bm3 mutant, unlike bm1 and bm4 mutants, to generate SSTC in response to wild-type H-2K^b antigen correlates with the fact that anti-H-2K^b antisera contain antibodies to determinants which are absent only on the K^{bm3} molecule [7], which differs the most from H-2K^b in the number of epitopes compared with other mutant H-2K^{bm} molecules detected by MCAB [4].

Correlation between the ability of the mutants to produce antibodies and SSTC and the total disparity of their ability to produce SSTC and CTL to the same wild-type H-2 molecule *in vivo* [1] confirm data obtained previously on fundamental differences between determinants recognizing SSTC and CTL specific for the H-2 molecule [5].

The authors are grateful to O. V. Rokhlin for providing antibodies against the L-chain of immunoglobulin and to A. G. Tonevitskii for determining the class of MCAB. The work was partly subsidized by WHO.

LITERATURE CITED

1. B. D. Brondz, E. S. Kan, A. V. Chervonskii, et al., Genetika (1986).
2. B. D. Brondz, A. V. Karaulov, I. F. Abronina, and Z. K. Blandova, Genetika, 12, 2151 (1980).
3. A. V. Filatov, A. V. Chervonskii, and B. D. Brondz, Byull. Éksp. Biol. Med., No. 8, 223 (1984).
4. J. A. Bluestone, J. F. C. McKenzie, R. W. Melvold, et al., J. Immunogenet., 11, 197 (1984).
5. B. D. Brondz, I. F. Abronina, M. B. Zaiceva et al., Immunol. Rev., 80, 29 (1984).
6. B. D. Brondz, A. V. Karaulov, I. F. Abronina, and Z. K. Blandova, Mol. Immun., 17, 883 (1980).

7. C. S. David. B. C. Neely, and S. E. Cullen, Immunogenetics, 5 143 (1979).
8. D. K. Dennison, S. S. Rich, and R. B. Rich. J. Immunol., 127, 2176 (1981).
9. G. J. Hämmerling, E. E. Rusch, and N. Tada, Proc. Natl., Acad. Sci. USA, 79, 4737 (1982).
10. J. Klein, Adv. Immun., 26, 56 (1978).
11. M. G. Mage, L. L. McHugh, and T. L. Rothstein, J. Immunol. Methods, 15, 47 (1977).
12. R. Nairn. K. Yamaga, and S. G. Nathenson, Annu. Rev. Genet., 14, 241 (1980).
13. L. R. Pease, D. H. Schulze, G. M. Pfaffenbach, et al., Proc. Natl. Acad. Sci. USA, 80, 242 (1983).
14. O. V. Rokhlin (O. V. Rochlin), T. I. Vengerova, and R. S. Nezlin, Immunochemistry, 8, 525 (1971).

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

M. G. Agadzhanyan, I. N. Smirnova, and E. V. Sidorova

UDC 612.124.017.1.014.46:547.96]-08

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

Laboratory of Isolation, Purification, and Concentration of Biologically Active Substaces, Moscow Scientific-Research Institute of Virus Preparations, Ministry of Health of the USSR.
(Presented by Academician of the Academy of Medical Sciences of the USSR, O. G. Andzhaparidze.) Translated from Byulleten' Éksperimental'noi Biologii Meditsiny, Vol. 102, No. 8, pp. 206-208, August, 1986. Original article submitted November 5, 1985.