# MECHANISM OF HAPTEN-SPECIFIC T-SUPPRESSOR FACTOR INHIBITION OF HAPTEN-MODIFIED PROLIFERATION OF HUMAN AND MOUSE LEUKEMIA CELLS

A. D. Chernousov and N. V. Medunitsyn

UDC 612.112.94.017.1.064.08

KEY WORDS: proliferation, hapten, leukemia, T-suppressor cells

It has recently been shown that suppression of the immune response by specific T-suppressor factors (TSF) is largely determined by their antiproliferative activity [6]. It was found that antiidiotypic T-suppressor cells (TSC) and their TSF can suppress not only antigen-activated lymphoid cells, but also idiotypic-positive ($Id^+$) B-lymphomas and B-B hybridomas [7]. However, it was not clear whether antigen-binding TSF, like antiidiotypic TSF [7] can suppress proliferation of antigen-carrying cells.

To resolve this problem the ability of supernatants of hybridomas of antigen-binding TSC, specifically suppressing the immune response to a hapten, namely 2,4,6-trinitrophenylsulfonic acid (TNPSA), can suppress proliferation of TNPSA-modified human and mouse leukemia cells [3-5].

## EXPERIMENTAL METHOD

Human leukemia cell lines (Table 1) and mouse B-B hybridoma, secreting antibodies to *Mycobacterium tuberculosis* antigen were used (the hybridoma was generously provided by V. I. Litvinov). The cells were cultured at 37°C in medium RPMI-1640 containing 10% embryonic calf serum, 2 mM L-glutamine, 10 mM HEPES, and gentamicin (80 U/ml) as additives. During cultures of the B-B hybridoma the medium contained $5 \times 10^{-5}$ M 2-mercaptoethanol and 0.2 mM sodium pyruvate besides the above-mentioned additives. To modify the cells [2] we used TNPSA or azobenzenearsonate (ABA) in a final concentration of 0.005 mM. After incubation for 20 min at 20°C with TNPSA or 5 min at 4°C with ABA the cells were washed off 3 times with medium 199 and resuspended in medium RPMI-1640 with additives. To investigate the effect of the hybridoma supernatants on DNA, RNA, and protein synthesis we used cells cultured beforehand for 48 h at 20°C. Next, $(2\text{-}4) \times 10^4$ hapten-modified cells were cultured for 24 h in a 96-well panel at 37°C in the presence of supernatants of hybridomas SE1C7 (C7), SE2B6 (B6), FL2B8 (B8), and FI2G4.K (G4K) [5] in a dilution of 1:10. When modified B-B hybridoma cells were used as targets the supernatants were diluted 30 times. The label was introduced 4 h before the end of culture. When incorporation of $^3$H-thymidine was determined, 0.2 mCi was introduced into each well, when $^3$H-uridine or $^{14}$C-amino acids were used, the dose added was 1 mCi. Cells were transferred to glass filters with the aid of a Titertek cell harvester. Incorporation of the label

TABLE 1. Suppression of Proliferation (in %) of Human Leukemia Cells by Supernatants of T Hybridomas ($M \pm \sigma$)

| Supernatant | «Jurkat» | | CEM | | H-9 | | GL-4 | | CESS | | K562 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 843 (205) | 35 053 (1294) | 8560 (444) | 11 483 (428) | 7581 (1385) | 15 835 (385) | 6575 (117) | 8527 (426) | 22 504 (1354) | 27 053 (756) | 7606 (230) | 13 192 (854) |
| BW 5147 | 9,1 | 0,3 | 3,2 | 0,7 | 7,2 | 1,6 | 3,1 | 0,2 | 8,1 | 1,4 | 4,8 | 0,11 |
| SE.1C7 | 41,7 | 4,1 | 38,3 | 2,1 | 51,0 | 3,9 | — | — | — | — | 39,4 | —0,9 |
| SE.2B6 | 37,1 | —3,1 | 39,4 | 1,5 | 40,4 | 2,5 | 38,0 | 0,17 | 44,2 | —0,24 | 35,6 | —0,8 |
| FL.2B8 | 38,0 | —0,2 | 35,2 | 0,9 | 39,7 | —0,4 | 39,4 | 2,2 | 43,9 | —3,5 | 39,2 | —2,1 |
| FI.2G4 | 50,0 | 0,11 | 40,0 | 2,0 | 46,5 | —0,3 | 46,3 | 0,29 | 51,2 | —4,4 | 43,7 | 0,8 |
| ST.2D4 | 7,6 | 0,17 | 3,1 | 0,1 | 3,5 | —0,6 | — | — | — | — | — | — |

Legend. The first column of each pair represents incorporation of $^3$H-thymidine by TNPSA-modified cells, the second column — incorporation by unmodified cells.

Laboratory of Molecular Immunology, Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 67-69, July, 1989. Original article submitted June 10, 1988.

 0007-4888/89/0007-0982$12.50 

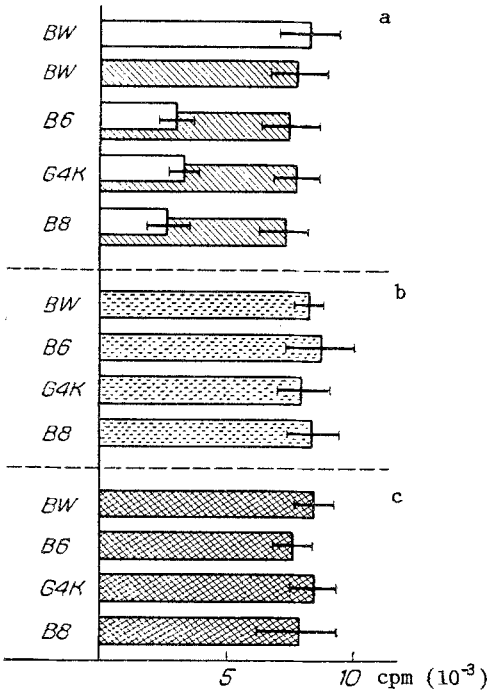


Fig. 1. Effect of supernatants of antigen-binding T hybridomas on incorporation of $^3$H-thymidine by B-B hybridoma cells modified by TNPSA. a) Unshaded columns represent incorporation on treatment of TNPSA-modified hybridomas with unexhausted supernatants, oblique shading — incorporation of $^3$H-thymidine on treatment with supernatants of ABA-modified cells; b) incorporation on treatment of cells with supernatants exhausted on TNP—BSA-sepharose 4B; c) incorporation of adsorption of supernatants on sorbent with anti(TNP) idiotypic antibodies. Level of $^3$H-thymidine incorporation under influence of supernatants exhausted on ABA—BSA-sepharose 4B (control to group b) or on sorbent with rabbit antibodies against mouse immunoglobulins (control to group c) did not offer from level of incorporation of label by modified cells under the influence of unadsorbed supernatants.

was determined on a Mark III scintillation counter. Unmodified cells and cells modified by ABA were used as the control. In some experiments the functional activity of the supernatants was estimated after adsorption on TNP—BSA-sepharose 4B, ABA—BSA-sepharose 4B, or a sorbent with anti(TNP) idiotypic rabbit antibodies or rabbit antibodies against mouse immunoglobulins [3]. Synthesis of cyclic AMP (cAMP) was determined by the standard method using a kit from "Amersham International" in the presence of a 1 mM solution of theophylline.

## EXPERIMENTAL RESULTS

The investigations showed that supernatants of hybridomas of TSC inducers (C7 and G4.K), TSC effectors (B8) and antigen-binding hybridoma SE2B6, suppressing antigen-dependent proliferation [3, 4], can also inhibit proliferation of human leukemia cells (Table 1) and mouse leukemia cells (Figs. 1 and 2), modified by TNPSA. It follows from these data that the suppressor effect is specific, for supernatants of hybridomas do not suppress unmodified or ABA-modified cells. The suppressor effect depends on the antigen-binding $Id^+$ material, for it is abolished by adsorption of the supernatants on the corresponding sorbent (Fig. 1). Adsorption of the supernatants on the ABA—BSA sorbent and on rabbit antibodies against mouse immunoglobulins (control) did not affect antiproliferative activity.

Data showing that the action of the supernatants was similar in type (Fig. 2) and that they suppress incorporation of $^3$H-thymidine into DNA, synthesis of which takes place in the S phase, but they do not affect synthesis of RNA and protein, which takes place in the $G_1^a$- and $G_1^b$-phases of the cycle, i.e., they prevent the passage of the cell from the $G_1^b$ phase into the S phase of the cycle. Phenomenologically this is manifested as accumulation of labeled RNA and

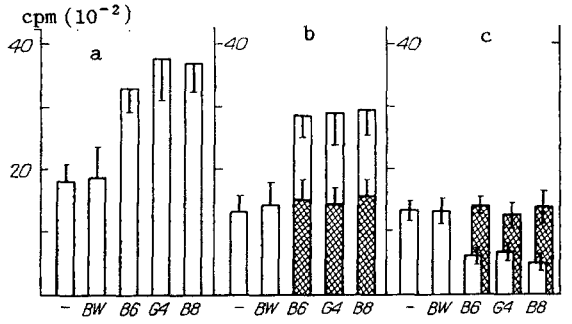


Fig. 2

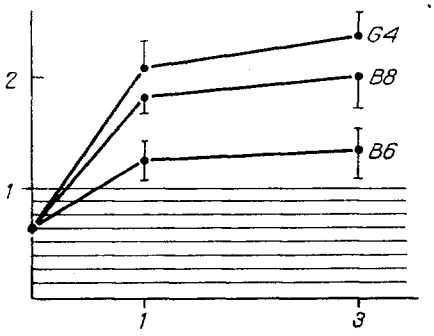


Fig. 3

Fig. 2. Effect of supernatants of hybridomas on incorporation of $^{14}$C-amino acids (a), $^{3}$H-uridine (b), and $^{3}$H-thymidine (c) by B-B mouse hybridoma cells modified by TNPSA. Cross-hatching — level of incorporation of label under the influence of supernatants exhausted on sorbent with rabbit anti(TNP) idiotypic antibodies.

Fig. 3. Effect of supernatants of hybridomas on cAMP synthesis by B-B hybridoma cells modified by TNPSA, in the presence of theophylline. Abscissa, time after treatment with supernatants of cells (in h); ordinate, cAMP concentration (in pmoles/$10^7$ cells). Horizontal shading indicates maximal level of cAMP on treatment of modified cells with BW5147 supernatant or on treatment with supernatants of hybridomas of unmodified cells.

protein. These effects are due to the $Id^+$ material, for they are abolished by adsorption on a sorbent with antiidiotypic antibodies.

On the basis of the data in Fig. 2 it can be postulated that the action of supernatants of the hybridomas is due to activation of mechanisms blocking passage of the cell from the $G_1^b$ phase into the S phase of the cycle and, in particular, activation of adenylate cyclase [8]. It follows, in fact, from Fig. 3 that supernatants of the hybridomas increased the cAMP concentration in cells of a modified B-B hybridoma.

On the whole the data in this paper are evidence that antigen-binding suppressor T cells, like antiidiotypic suppressor T cells, can suppress proliferation of leukemia cells. This result evidently opens up the prospects for obtaining immunotherapeutic preparations based not only on antiidiotypic, but also on antigen-binding suppressor factors, suppressing proliferation of antigen-carrying leukemia cells. On the other hand, the results suggest that the regulatory antiproliferative effects of TSF are connected with cAMP accumulation in lymphoid cells.

LITERATURE CITED

1. A. D. Chernousov, N. V. Molodtsov, S. G. Zadorozhnyi, et al., Immunologiya, No. 6, 21 (1986).
2. A. D. Chernousov, N. V. Molodtsov, and N. V. Medunitsyn, Immunologiya, No. 5, 20 (1982).
3. A. D. Chernousov, O. P. Terekhov, N. E. Surnakova, and N. V. Medunitsyn, Immunologiya, No. 4, 21 (1987).
4. A. D. Chernousov, A. V. Chervonskii, N. V. Molodtsov, et al., Immunodeficiencies and Allergy [in Russian], Moscow (1986), p. 104.
5. A. D. Chernousov, A. V. Chervonskii, P. R. Poznakhirev, et al., Byull. Éksp. Biol. Med., No. 4, 450 (1986).
6. J. Klein, Natural History of the Major Histocompatibility Complex, New York (1986).
7. R. G. Lynch, Leukemia, Berlin (1983), p. 83.
8. R. Watson, Transplant. Rev., 23, 223 (1975).