IDIOTYPE-SPECIFIC INACTIVATION OF T KILLERS, BUT NOT OF T-MIF PRODUCERS IMMUNE TO ANTIGENS OF THE H-2 COMPLEX, BY XENOGENEIC ANTISERA

S. G. Egorova, V. G. Nesterenko, A. P. Suslov, and B. D. Brondz

UDC 612.112.94.017.4.014.46:615.365.018.53

KEY WORDS: T killers; idiotype-specific sera; immunization; T-M.

Idiotypic antigenic determinants of immunoglobulins are expressed on the surface of T lymphocytes, evidently in the region of the antigen-binding receptors [8, 11, 15]. Antisera have been obtained against idiotypes of T cells reacting to transplantation antigens [3-6, 9, 17, 19]. These idiotype-specific sera (ISS) inhibited [3-5, 8] or stimulated [9, 17] functional activity of idiotype-positive lymphocytes. Since different subpopulations of T cells participate in the immune responses to transplantation antigens [2], the study of factors acting selectively on individual subpopulations of T lymphocytes responding to the same antigen is an important task.

In the investigation described below the action of xenogeneic anti-CBA-anti-C57BL/6 (anti-CBA_{B_6}) ISS, induced in rabbits and rats by immunization of CBA-anti-C57BL/6 (CBA-anti-B6) mice on T-killers of different specificity, was studied. It was also intended to compare the effect of ISS on T-killers and T-MIF producers of CBA mice of the same specificity.

EXPERIMENTAL METHOD

Male and female CBA ($H\text{-}2^k$), AKR ($H\text{-}2^k$), C57BL/6 ($H\text{-}2^b$, abbreviated to B6), BALB/c ($H\text{-}2^d$), and DBA/2 ($H\text{-}2^d$) mice weighing 18-20 g were used. Rabbit anti-CBA_{B_6} ISS (ISS_{rab}) were obtained by immunizing rabbits with immune CBA-anti-B6 lymph node cells, and the serum was absorbed with liver, serum, erythrocytes, and thymus, spleen, and lymph node cells from intact mice [4, 5]. THe ISS_{rab} reacted in the complement-dependent cytotoxic test and in the cellular radio-immune test only with CBA T lymphocytes activated by B6 antigens [5, 6], and they also specifically abolished the CBA-anti-B6 graft versus host reaction (GVHR), but had no effect on the CBA-anti-BALB/c and B6-anti-CBA GVHR [4, 5].

T killers from CBA-anti-MCh-11 (k-anti-b) lymph nodes, enriched by absorption-elution on a monolayer of B6 mouse macrophages [1], were injected intraperitoneally five times, with intervals of 2-3 weeks, into Wistar rats (7×10^6 to 10×10^6 cells per injection). The rats were exsanguinated 7 days after the last immunization and the serum absorbed with erythrocytes and spleen, thymus, and lymph node cells of intact mice. Absorption was carried out until complete disappearance of activity of the serum in the complement-dependent cytotoxic test [5] with intact mouse thymus, spleen, and lymph node cells. The ISS_{rat} thus obtained possessed cytotoxicity against CBA-anti-B6 killers eluted from specific target cells. The maximal cytotoxic index and cytotoxic titer for these killers were 0.47 and 1:12, respectively. Antiserum against Thy-1,2 was obtained by repeated immunization of AKR mice with CBA thymocytes [18]. T killers and T-MIF producers were determined in the spleen of CBA or BALB/c mice 10 days after intraperitoneal injection of 25×10^6 EL-4 ($H\text{-}2^b$) leukemia cells or P-815 ($H\text{-}2^d$) mastocytoma cells.

The cytotoxic effect of the lymphocytes was calculated by the formula:

$$\frac{R_{im} - R_{norm}}{R_{max} - R_{norm}},$$

Laboratory of Immunochemistry and Diagnosis of Tumors, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Laboratory of Immunologic Tolerance, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 4, pp. 63-65, April, 1983. Original article submitted May 24, 1982.

 0007-4888/83/9504-0482$07.50

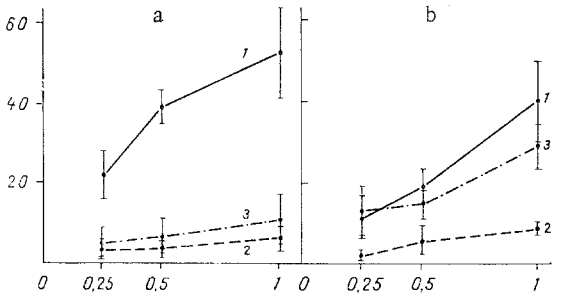

Fig. 1. Effect of ISS_{rab} on T killer activity. Abscissa, number of lymphocytes ($\times 10^6$) per well; ordinate, cytotoxic index. CBA-anti-EL-4 (a) and CBA-anti-P-815 (b) lymphocytes treated (in the presence of complement) with inactivated intact rabbit serum (1), anti-Thy-1,2-serum (2), and ISS_{rab} (3).

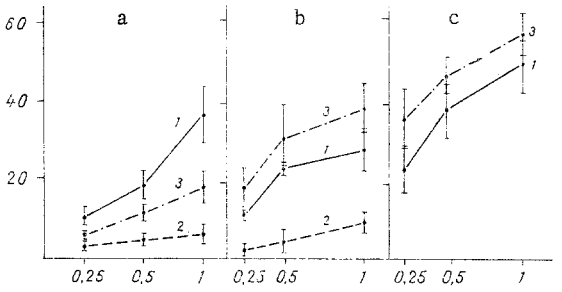

Fig. 2. Effect of ISS_{rat} on T killer activity. CBA-anti-EL-4 (a), CBA-anti-P-815 (b), and BALB/c-anti-EL-4 (c) lymphocytes treated (in the presence of complement) with inactivated intact rat serum (1), anti-Thy-1,2-serum (2), and ISS_{rat} (3). Remainder of legend as in Fig. 1.

where R_{im}, R_{norm}, and R_{max} denote release of ^{51}Cr from labeled mouse macrophages, cultured in microplates after incubation for 16 h with immune and normal lymphocytes and with 2% sodium dodecylsulfate solution, respectively, for 16 h [10]. The macrophage migration inhibition index (II) was determined by the formula $(1 - a/b) \times 100\%$, where a and b are the mean migration zones in the experiment and control, respectively [7]. To treat spleen cells of immune mice with antiserum and complement, 0.6 ml of spleen cells (6×10^7/ml) was incubated with 0.2 ml antiserum at 4°C for 45 min. After removal of the antiserum the cells were incubated in 1 ml rabbit complement (from Cederlane, Canada) in a dilution of 1:20 for 1 h at 37°C, then washed three times, and their ability to give a cytotoxic effect and to produce MIF was then determined. The results were subjected to statistical analysis by Student's t test. Data from three to five experiments are given.

EXPERIMENTAL RESULTS

Results showing the effect of anti-CBA_{B_6} ISS_{rab} on the ability of CBA-anti-B6 (k-anti-b) and CBA-anti-DBA/2 (k-anti-d) killers to induce a cytotoxic effect are given in Fig. 1. Lymphocytes treated with intact rabbit serum (Fig. 1, 1) had a marked cytotoxic action. Their ability to cause lysis of target cells was the same as the cytotoxic effect of immune lymphocytes not treated with the serum (these data are not given). Anti-Thy-1,2-serum (Fig. 1, 2) abolished the cytotoxic effect of k-anti-b and k-anti-d killers. ISS_{rab} inhibited by 73-81% activity of k-anti-b killers but did not affect activity of k-anti-d killers (Fig. 1, 3). ISS_{rat} had a similar effect (Fig. 2). It inhibited the cytotoxic action of k-anti-b killers (by 37-51%) compared with the control but did not act on k-anti-d or d-anti-b killers.

Investigation of the action of antisera on MIF producers showed (Fig. 3) that anti-Thy-1,2-serum inhibited MIF production, whereas ISS_{rab} did not inhibit it.

It can be concluded from these facts that the ISS_{rat} and ISS_{rab}, obtained by different methods [5], acted in the same way. They specifically inhibited activity of k-anti-b T killers. Meanwhile ISS_{rab} did not inhibit k-anti-b T-MIF producers obtained in the same CBA-anti-EL-4

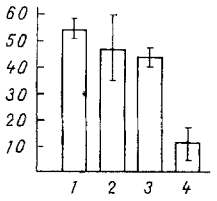

Fig. 3. Effect of ISS_{rab} on T-MIF producers. Ordinate, macrophage migration II. CBA-anti-EL-4 MIF producers, untreated (1) and treated (in the presence of complement) with inactivated intact rabbit serum (2), ISS_{rab} (3), and anti-Thy-1,2-serum (4).

system. The selective action of ISS_{rab} on T killers was not connected with the greater sensitivity of the T killers to treatment with antibodies and complement, for the T-MIF producers were more sensitive than T killers to the action of anti-Thy-1,2-serum [10]. The facts given above directly confirm the indirect data obtained previously on differences between T killers and T-MIF producers with respect to several properties [10, 12, 21], and they show that these cells are different subpopulations of T lymphocytes. Data given previously on the direct action of ISS_{rab} and its absorption [4-6] indicate that these antibodies are directed against idiotypes of antigen-recognizing receptors of CBA-anti-B6 T lymphocytes. This conclusion was confirmed by the present investigation, for idiotypic markers of CBA-anti-B6 T killers were found with the aid of rabbit and rat ISS obtained by different methods.

Various workers also obtained ISS reacting against idiotypes of T killers [8, 13, 16], but there is one report of failure to obtain such ISS [20]. Since ISS_{rab} does not affect CBA-anti-B6 T-MIF producers it can be tentatively suggested that populations of T killers and T-MIF producers which react to the same H-2 haplotype have different sets of idiotypes. This difference may in fact arise because T killers and MIF producers react against unidentical products of the H-2 complex of the k/d and I regions, respectively [14]. However, the hypothesis regarding differences between idiotypes of T killers and T-MIF producers requires further experimental verification. Indirect confirmation of this hypothesis may be provided by data showing that helpers and precursors of T killers which react against antigens of the H-2 complex have different idiotypes [9].

LITERATURE CITED

1. B. D. Brondz and S. G. Egorova, Tsitologiya, No. 11, 1387 (1976).
2. B. D. Brondz and O. V. Rokhlin, Molecular and Cellular Bases of Immunologic Recognition [in Russian], Moscow (1978).
3. S. G. Egorova, A. Yu. Gritsman, and B. D. Brondz, Byull. Éksp. Biol. Med., No. 7, 62 (1980).
4. N. A. Kraskina, Trudy Mosk. Nauch.-Issled. Inst. Épidemiol. Mikrobiol., 17, 171 (1976).
5. V. G. Nesterenko, S. Gruner, E. I. Rubakova, et al., Byull. Éksp. Biol. Med., No. 11, 588 (1980).
6. V. G. Nesterenko, E. I. Rubakova, and S. Gruner, in: Current Problems in Immunology [in Russian], Vol. 1, Moscow (1981), p. 54.
7. A. P. Suslov and A. D. Chernousov, Byull. Éksp. Biol. Med., No. 8, 236 (1977).
8. H. Binz and H. Wigzell, Contemp. Top. Immunobiol., 7, 113 (1977).
9. H. Binz, H. Frischknecht, and H. Wigzell, Ann. Immunol., C-130, 273 (1979).
10. B. D. Brondz, A. P. Suslov, and S. G. Egorova, Scand. J. Immunol., 8, 109 (1978).
11. K. Eichman, Adv. Immunol., 26, 195 (1978).
12. C. S. Hemney, J. Caffney, and B. R. Bloom, J. Exp. Med., 140, 837 (1977).
13. U. R. Kees, J. Exp. Med., 153, 1562 (1981).
14. S. Landdolfo, F. Marcucci, M. Giovarelli, et al., Immunogenetics, 9, 245 (1979).
15. A. Nisonoff, S. T. Ju, and F. L. Owen, Immunol. Rev., 34, 89 (1977).
16. B. Rabinovitz and M. Schlesinger, Immunology, 39, 93 (1980).
17. H. Ramseier, Exp. Cell Biol., 47, 107 (1979).
18. A. E. Reif and J. M. V. Allen, Nature, 203, 886 (1964).
19. B. Rubin and A. Bourgeois, Scand. J. Immunol., 14, 167 (1981).
20. R. Sherman, J. Immunol., 121, 1432 (1978).
21. P. E. Tigelaar and R. M. Gorczynski, J. Exp. Med., 140, 270 (1974).