The peak of proliferation of thymus and splenic lymphocytes is thus observed on the third to fourth day of immunization. The cytotoxicity of thymus lymphocytes is still present on the fourth to fifth day but falls by the sixth day of incubation, whereas SPL-CTL are active on the fourth to sixth day.

Both DNA synthesis and the proportion of blast cells among stimulated splenic lymphocytes were considerably higher than those of stimulated thymus lymphocytes, whereas the cytotoxicity of these cells was similar. The Thym-CTL are thus a cell population with a high content of cytolytic T lymphocytes which can be used with advantage for the study of the intimate mechanisms of transplantation and antitumor immunity.

LITERATURE CITED

1. G. I. Drizlikh, A. V. Andreev, I. F. Kotomina, et al., Byull. Eksp. Biol. Med., No. 3, 340 (1976).
2. L. C. Andersson, Scand. J. Immunol., 2, 75 (1973).
3. W. Andersson and H. Blomgren, Cell Immunol., 1, 362 (1970).
4. B. Bennett, J. Immunol., 95, 656 (1965).
5. J. C. Cerottini, H. D. Engers, H. R. McDonald, et al., J. Exp. Med., 130, 703 (1974).
6. M. Nabholz, J. Vives, H. M. Young, et al., Eur. J. Immunol., 4, 378 (1974).
7. P. Perlmann and G. Holm, Adv. Immunol., 11, 117 (1969).
8. I. A. Ramshaw and C. R. Parish, Cell. Immunol., 21, 226 (1976).
9. R. M. Torer and C. S. Henney, Nature (London), 262, 75 (1976).
10. H. Wagner, Immunology, 109, 630 (1972).
11. H. Wagner, J. Exp. Med., 138, 1679 (1973).
12. H. Wagner, A. W. Harris, and M. Feldman, Cell. Immunol., 4, 39 (1972).

EFFECT OF SOLUBLE H-2 ANTIGENS ON THE CYTOTOXIC EFFECT AND ADSORPTION OF IMMUNE LYMPHOCYTES

I. F. Kotomina and T. P. Pletneva

UDC 612.112.94.017.4

Serologically active preparations of soluble H-2 antigens were obtained by extraction with 3 M KCl from ascites cells of leukemia L1210 ($H\text{-}2^d$) and sarcoma MCh-11 ($H\text{-}2^b$). These preparations had no specific effect on the cytotoxic action of immune lymphocytes on target cells in vitro and did not inhibit adsorption of lymphocytes on a monolayer of the corresponding target cells.

KEY WORDS: *soluble H-2 antigens; cytotoxic effect; T cells.*

Conjecturally the receptors of T cells are molecules of special (T cell) immunoglobulins [10, 13], products of the Ir gene [8], or a complex of the V region of immunoglobulin with the H-2 molecule [5, 12]. To study the nature of receptors of cytotoxic lymphocytes, direct destruction of their membrane [18] or discovery of the conditions or interaction between living T cells and preparations of soluble H-2 antigens [19] have been used.

In the investigation described below the effect of soluble H-2 antigens, extracted with 3 M KCl, on the cytotoxic effect and adsorption of immune lymphocytes was studied.

Laboratory of Immunochemistry and Diagnosis of Tumors, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Central Research Laboratory, Saratov Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 9, pp. 333-336, September, 1977. Original article submitted February 22, 1977.

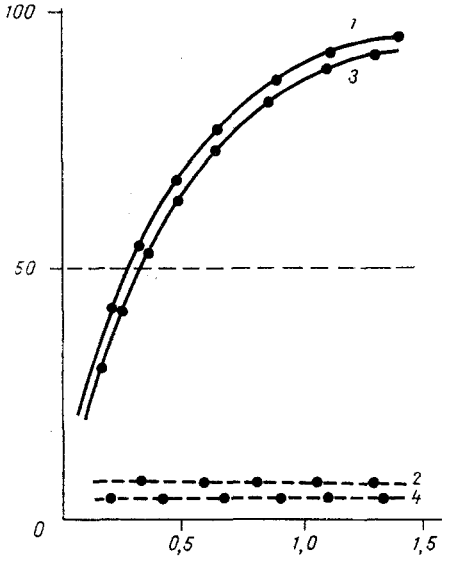

Fig. 1. Inhibition of cytotoxic reactions by soluble H-2 antigens from L1210 and MCh-11. Inhibition of cytotoxic action of anti-H-2^d antigen from L1210 (1) and MCh-11 (2). TC – C57BL/6 lymphocytes. Inhibition of cytotoxic action of anti-H-2^b antigen from MCh-11 (3) and from L1210 (4). TC – DBA/2 lymphocytes. Antisera used in dilution causing 95% death of TC. Abscissa, dilutions of serum (in $\log_2$); ordinate, inhibition of cytotoxic reaction (in %).

EXPERIMENTAL METHOD

Mice of hybrid strains C57BL/6 (H-2^b) and DBA/2 (H-2^d) were obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, and mice of strains B10.D2 (H-2^d) and C57BL/10 (H-2^b) (abbreviated to B10) were obtained from the nursery of the N. F. Gamaleya Institute of Epidemiology and Microbiology. Leukemia L1210 was maintained by passage through DBA/2 mice and sarcoma MCh-11 by passage through BL/6 mice. Ascites cells of both tumors were used for one-stage immunization of allogeneic mice at 6 points [2].

The cytotoxic effect (CE) of regional lymph node cells was tested in vitro in microplates (Microtest II Plates No. 3040, Falcon Plastics), using either 2-day cultures of peritoneal macrophages [6] or tumor cells [9] as the target cells (TC). In both cases the TC were first labeled with ^{51}Cr. Various doses of lymphocytes in 0.2 ml medium No. 199, containing 0.01 M Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) and antibiotics (10 units/ml each of penicillin and streptomycin) were added to $5 \cdot 10^4$ TC. After incubation for 3 h at 37°C in an atmosphere of 5% CO_2 the supernatant was transferred to tubes for counting the label in a Nuclear Chicago gamma spectrometer. The cytotoxic index (CI) was calculated by the formula:

$$\frac{IL - NL}{ML - NL} \times 100,$$

where IL, NL, and ML represent the immune, normal, and maximal liberations respectively of ^{51}Cr, in counts/min, after incubation of the TC with immune and normal lymphocytes, Triton X-100 (to destroy the tumor cells), and a 2% solution of sodium dodecyl sulfate (to destroy macrophages). In some experiments, before carrying out the tests for CE, the lymphocytes were adsorbed on a monolayer of TC [3]. For this purpose, $15 \cdot 10^6$–$20 \cdot 10^6$ lymphocytes were applied to a monolayer of allogeneic or syngeneic macrophages cultures in 3.5 × 1-cm plastic petri dishes and, after incubation for 3 h at 30°C, the unattached lymphocytes were centrifuged, counted, and their CE on TC determined.

Soluble H-2 antigens from L1210 and MCh-11 ascites cells were obtained by extraction with 3 M KCl [16]. The resulting samples were concentrated on an Amicon cell with XM-50 filter to 10 mg/ml (as protein). The protein content was determined by Lowry's method, using bovine serum albumin as the standard. The concentrated antigen was kept at –20°C.

Anti-H-2^d and anti-H-2^b sera were obtained by sixfold subcutaneous immunization of (C57BL × A)F_1 mice with B10.D2 spleen cells and B10.D2 mice with C57BL spleen cells respectively. Antibodies were determined in the mouse sera by the modified [1] lymphocytotoxic test [14]. Antigenic activity of the preparation of soluble antigens was determined by neutralization of cytotoxic antibodies in sera against H-2 antigen [15]. The quantity of soluble antigen, in micrograms, causing 50% neutralization of antibodies was taken as the antigen unit (ID_{50}).

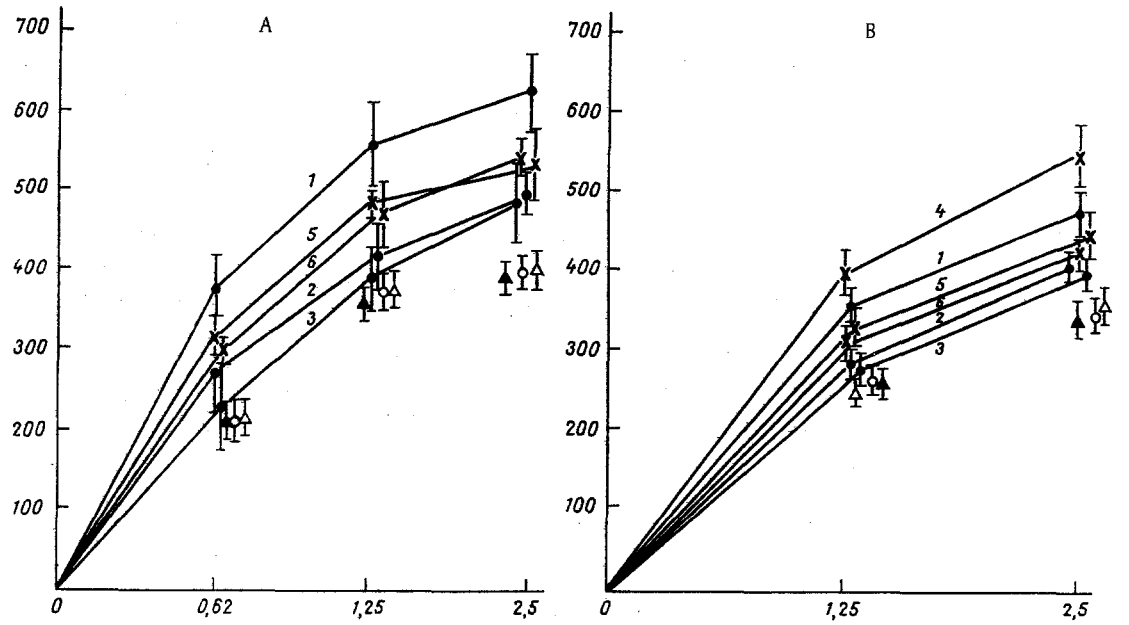

Fig. 2. Effect of soluble H-2 antigens on CE of immune lymphocytes. A: b anti-d lymphocytes, TC — B10.D2 macrophages; B: b anti-d lymphocytes, TC — L1210 (1, 2, 3) or d anti-b lymphocytes, TC — MCh-11 (4, 5, 6). Lymphocytes untreated (1, 4), preliminarily treated with antigen from L1210 (2) or MCh-11 (3) in concentration of 500 μg/ml, treated with antigen (5) or MCh-11 (6) constantly present in culture medium, NL without antigen (empty circles), in presence of L1210 antigen (empty triangles), or of MCh-11 antigen (filled triangles). Abscissa, number of lymphocytes per well ($\cdot 10^6$); ordinate, liberation of ^{51}Cr (in counts/min).

To treat the immune lymphocytes concentrated antigen was diluted with culture medium and sterilized by filtration through a Millipore filter (0.45 μ). In experiments to determine CE the lymphocytes were either pretreated with antigen (500 μg/ml for 30 min at 37°C) and then washed twice, or added to the TC together with antigen in a concentration of 100 μg/ml. In the experiments to study adsorption of lymphocytes antigen was added in the same concentration to the culture medium for the whole period of adsorption. In all cases after treatment with antigen the lymphocytes were washed off twice, counted, and adjusted to the same number of living cells. Besides the active antigen preparation, it was also used in the denatured form (80°C, 10 min) or after partial (by 60-70%) neutralization with alloantibodies.

EXPERIMENTAL RESULTS

Soluble H-2 antigens were specifically neutralized by antibodies in the corresponding antisera (Fig. 1). Antigen from L1210 ascites cells (ID_{50} = 0.28 μg) blocked the cytotoxic action of anti-H-2^d serum but did not inhibit the action of anti-H-2^b serum, while conversely,

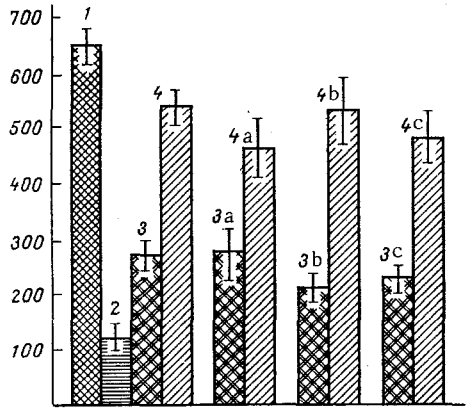

Fig. 3. Effect of soluble antigen from L1210 on adsorption of b anti-d lymphocytes on B10.D2 target cells. Original immune (1) and normal lymphocytes (2), immune lymphocytes not adherent to DBA/2 (3a, b, c) or C57BL/6 (4a, b, c) target cells. Adsorption in presence of native antigen (3a, 4a), antigen–antibody complex (3b, 4b), or denatured antigen (3c, 4c). Ordinate, liberation of ^{51}Cr (in counts/min) after incubation of lymphocytes with B10.D2 target cells.

antigen from MCh-11 ascites cells (ID_{50} = 0.26 μg) blocked the action of anti-H-2^b serum but did not inhibit the action of anti-H-2^d serum. In the experiments to study CE either C57BL anti-L1210 (b anti-d) lymphocytes were used together with B10.D2 macrophages and L1210 cells as TC (Fig. 2A, B) or B10.D2 anti-MCh-11 (d anti-b) cells were used with MCh-11 cells as TC (Fig. 2B). Since similar results were obtained in four experiments, the results of only one of them are shown in Fig. 2. In all cases, after preliminary treatment of the immune lymphocytes with soluble H-2 antigen, CI was reduced from 12-22 to 7-12%, i.e., by 40-50%. Blocking of CE by 60% also was observed if H-2 antigen was constantly present in the culture medium throughout the period of the test (Fig. 2A). This blocking was nonspecific, for each antigen — H-2^d (from L1210) and H-2^b (from MCh-11) — blocked equally the CE of lymphocytes immune to either of the haplotypes (d anti-b and b anti-d). The preparations of soluble H-2 antigens did not inhibit specific adsorption of immune lymphocytes on the corresponding TC (Fig. 3). It will be clear from Fig. 3 that soluble H-2 antigens modified by thermal denaturation or by partial neutralization with antibodies likewise had no effect on adsorption of the immune lymphocytes.

Soluble H-2 antigens thus do not specifically block the CE of immune lymphocytes and do not inhibit their specific adsorption on the corresponding TC. Nonspecific inhibition of CE may be due to the presence of impurities nontoxic to lymphocytes, but blocking their activity, in the preparations of soluble H-2 antigens. The results agree with those of other investigations ([19], etc.) in which neither specific inhibition of CE of lymphocytes by soluble H-2 antigens nor specific binding of cytotoxic lymphocytes with those antigens was obtained. Blocking of the CE of immune lymphocytes by soluble H-2 antigen, demonstrated in some investigations [20] was on a small scale and its specificity was established in only one investigation [11].

Cytotoxic T lymphocytes (CTL) bind with structures of the cell membrane (CTL determinants) which are products of the *H-2*^{k} and *H-2*^{d} genes of mice [4], linked with serologically determinable special H-2 specificities; these latter are evidently components of the CTL determinants [17]. The results are evidence that CTL receptors do not react with serologically determinable H-2 specificities separated from the cells. This could mean that the structure of the CTL determinants either is destroyed during extraction of the soluble H-2 antigen or that, for contact with the CTL receptors, it must be built into the target cell membrane. This last hypothesis is supported by observations showing that receptors of immune T cells react with soluble antigens only if these antigens are represented on the surface of immune B cells [7].

LITERATURE CITED

1. B. D. Brondz, Byull. Éksp. Biol. Med., No. 5, 64 (1964).
2. B. D. Brondz, Folia Biol. (Prague), 14, 115 (1968).
3. B. D. Brondz and A. E. Snegireva, Immunology, 20, 457 (1971).
4. B. D. Brondz, I. E. Egorov, and G. I. Drizlikh, J. Exp. Med., 141, 11 (1975).
5. G. I. Drizlikh and B. D. Brondz, Molekul. Biol., No. 2, 253 (1977).
6. G. I. Drizlikh, A. V. Andreev, I. F. Kotomina, et al., Byull. Éksp. Biol. Med., No. 3, 340 (1976).
7. A. Basten, J. F. A. P. Miller, and R. Abraham, J. Exp. Med., 141, 547 (1975).
8. B. Benacerraf and H. O. McDevitt, Science, 175, 273 (1972).
9. G. Berke, K. Sullivan, and B. Amos, J. Exp. Med., 135, 1334 (1972).
10. U. Binz and H. Wigzell, Scand. J. Immunol., 5, 359 (1976).
11. B. Bonavida, J. Immunol., 112, 926 (1974).
12. M. Braun, Cell. Immunol., 25, 1 (1976).
13. R. E. Cone, J. Immunol., 116, 847 (1976).
14. P. A. Gorer and P. O'Gorman, Transplant. Bull., 3, 142 (1956).
15. D. Götze and R. Reisfeld, J. Immunol., 112, 1643 (1974).
16. R. A. Reisfeld, M. A. Pellegrino, and B. D. Kahan, Science, 172, 1194 (1971).
17. R. D. Stulting, R. F. Todd, and L. R. Gooding, Transplantation, 21, 71 (1976).
18. R. F. Todd and G. Berke, Immunochemistry, 11, 313 (1974).
19. R. F. Todd, R. D. Stulting, and D. B. Amos , Cell. Immunol., 18, 304 (1975).
20. H. Wagner and W. Boyle, Nature (London) New Biol., 240, 92 (1972).