CYTOTOXIC POTENTIAL OF MOUSE SPLEEN CELLS ON H-2 ANTIBODY-TREATED TARGET CELLS*

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The interaction of antibodies and lymphocytes in target cell destruction received attention already in the early phase of the studies concerning the mechanism of graft rejection. The enhancement phenomenon indicated that this interaction can be decisive for the fate of an antigenic graft (1). The demonstration of at least partial abolition of cytotoxic activity of sensitized lymphocyte population in vitro when antibody-coated cells were used as targets was pointing to a possible shielding effect of the antigen sites by antibodies. Lately much attention was focused on the nature and significance of this blocking effect (2). In certain systems, however, antibody treatment of the target cells was shown to bring about cytotoxicity effected by nonimmune lymphocyte population (3, 4). The demonstration of these antibody-dependent effects with opposite direction raises the question of their relative importance in vivo and also in the cytotoxic systems studied in vitro. We have previously demonstrated that when cells carrying the MSV-determined surface antigen were pretreated with specific antibody and exposed to sensitized lymphocytes, thereafter the direction of the antibody effect and consequently the resulting cytotoxicity depended on the concentration (5). High concentration resulted in more efficient destruction while lower concentration protected the cells.

These experiments prompted us to continue these studies with attention focused on the relative efficiency of these two mechanisms bearing in mind that each pattern obtained would certainly be relevant in details only in the particular systems used.

Materials and Methods

Tissue Culture Medium.—Eagle’s medium (Grand Island Biological Co., Grand Island, N.Y.) containing 10% heat-inactivated (at 56°C for 30 min) fetal calf serum (Bio-Cult, Glasgow, Scotland) with penicillin and streptomycin were used throughout the experiments as washing fluid, as diluent, and as culture media.

Target Cells.—Two different types of target cells were used. (a) A Moloney leukemia virus-induced ascitic lymphoma of A/Sn origin (YAC) (6), harvested 4–6 days after inoculation of 10⁶ cells by washing the peritoneal cavity with sterile balanced salt solution (BSS).

(b) A tissue culture line of P815-X2, a methylcholanthrene-induced mastocytoma (7).

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Target Cell Labeling.—Twice-washed YAC cells were incubated for 30 min at 37°C at a concentration of 2 × 10⁷ cells suspended in 0.5 ml of BSS with 200 μCi of ⁵¹Cr (The Radiochemical Centre, Amersham, England). The P815 cells were incubated for 4 h at 37°C at a concentration of 2 × 10⁶ cells/10 ml of tissue culture medium with 200 μCi of ⁵¹Cr. The cells were washed twice, counted, and adjusted to the desired cell density.

Effector Cells.—The spleen or the regional lymph nodes of adult CBA mice were pressed through a fine mesh wire and washed twice. Cell viability was checked with trypan blue. For immune cells CBA mice were injected intraperitoneally one to three times with 3 × 10⁷ YAC cells 3-6 wk apart.

Immune Sera.—Hyperimmune sera were obtained from CBA mice immunized with 3 × 10⁷ YAC cells intraperitoneally with 2-wk intervals during 6-8 wk. This yielded antibodies against the D end of the H-2 complex and also against Moloney virus-determined antigen specificities present on the YAC cell. All sera were inactivated at 56°C for 30 min.

Pretreatment of Target Cells with Sera.—Labeled target cells were incubated with different concentrations of normal or immune serum at room temperature for 30 min. In order to remove excess antiserum the cells were washed once, except in the experiment presented in Fig. 3 where the serum was present during the incubation with lymphocytes.

Assay Procedures.—Cell-mediated cytotoxicity was measured by a modification (8) of Brunner’s method (9). 1-ml aliquots of effector cell suspensions were added to 35 × 10 mm Petri dishes (NUCLON®). To each dish 4 × 10⁴ ⁵¹Cr-labeled target cells were added (0.05 ml). The target cells were also incubated without effector cells to estimate the level of spontaneous ⁵¹Cr release. Each cell sample was separated for spontaneous release. Various effector cell concentrations were used to obtain the desired immune target cell ratio. All combinations were prepared in duplicates. The dishes were incubated in a 37°C CO₂ incubator for 3.5-4 h (in the experiment given in Fig. 3, 17 h). After incubation 1 ml of cold medium was added to each dish and the contents were transferred to test tubes. After centrifugation the cell-free supernatants were counted for the presence of ⁵¹Cr. Maximum release was determined by treating the target cells with distilled water, which released approximately 70% of the total ⁵¹Cr.

Evaluation of the Results.—After subtraction of the background, the ⁵¹Cr release is expressed as percentage of the maximum release.

RESULTS

We have found regularly that spleen cells damaged antibody-pretreated YAC or P815 target cells. This effect was only seen with spleen cells and to a smaller extent with peritoneal cells and not with lymph node cells (Fig. 1).

In three experiments antibody-coated cells were lysed by spleen cells from thymus-less nude mice. In two experiments these cells were more efficient than those of the conventional mice. Fig. 2 gives the results using anti-D serum-pretreated P815 target cells showing the relatively higher lytic potency of nude spleen cells.

To assess the interaction of immune cells and antibodies the cytotoxic effect of immune cells after a single immunization was investigated after different intervals both without and with precoating the P815 target cells with immune serum (Table I). The efficiency of lysis of antibody-coated cells fluctuated but was higher in this experiment when compared with the lysis effected by the immunopotent cells alone. It seems that the former effect tends to be weaker as the immune cell population becomes more effective. As there is an inverse cor-
**Fig. 1.** Cytotoxic effect of CBA spleen (●), lymph node (○), and peritoneal exudate (X) cells on YAC cells pretreated with 1:20 diluted CBA anti-YAC (---) and CBA normal (---) sera. The spontaneous release by the serum-treated samples is indicated (NS, IS).

**Fig. 2.** Cytotoxic effect of spleen cells from nude BALB/c (X) or (DBA/2 × Leaden)F1 (●) mice on P815 target cells pretreated with 1:20 diluted CBA anti-YAC (---) serum with variation in the effector to target cell ratios. Normal serum-pretreated target cells (---). The spontaneous release by the serum-treated samples is indicated (SR).

**TABLE I**

| Time after Immunization | no. of cells per spleen X10^6 | Sera used for pretreatment (1/10) | Normal cpm | % Cr release | Anti-D cpm | % Cr release |
|------------------------|--------------------------------|-----------------------------------|------------|-------------|------------|-------------|
| days                   |                               | Untreated                         |            |             |            |             |
| 4                      | 48                            | 904                               | 94         | 17          | 3,160      | 58          |
| 10                     | 44                            | 1,110                             | 20         |             | 3,565      | 65          |
| 13                     | 66                            | 1,576                             | 29         |             | 2,236      | 41          |
| 22                     | 83                            | 1,027                             | 19         |             | 1,741      | 32          |
| 22, 14th day after     | 70                            | 1,420                             | 26         |             | 2,252      | 41          |
| 2d challenge           | 70                            | 2,043                             | 37         |             | 2,190      | 44          |

Maximum release 5,450 cpm; spontaneous release 498-608 cpm.

* Target cells P815; lymphocyte to target cell ratio, 100:1.

† Each population was derived from two mice. The experiment was performed on the same day, the mice being immunized previously with different intervals.
relation with the total cell number in the spleen, the cells responsible for the increased number after immunization may not be operative in the antibody-mediated lysis. Consequently the relative proportion of efficient cells becomes lower because a constant cell number was used throughout. Only 4 days after immunization higher $^5$Cr release was noted compared with the spleen cells from the untreated controls. The lower efficiency of spleen cells from immunized animals in mediating the lysis of antibody-coated P815 or YAC target cells was observed in five further experiments. Under these conditions no blocking effect of the antiserum was observed as the antibody-treated target cells confronted with the immune cells released in all samples higher amount of $^5$Cr than those treated with normal serum.

The clear opposite effect of antibody in relation to the effector cell population was obtained under slightly different conditions (Fig. 3). Antibody excess was not removed and the interaction of P815 target and effector cells was prolonged (17 h). Dependent on the serum concentration a clear blocking effect of the immune effector lysis is seen. The blocking serum concentrations in combination with normal spleen cell population result however in lysis. The fact that blocking effect is present may indicate that the cells responsible for mediating the antibody complexed cell lysis are scarce in the immune cell population.

**DISCUSSION**

Both the mouse lymphoma cells YAC harvested from animals and the in vitro line of P815 mastocytoma cells are killed either by immune spleen cells alone or
by normal spleen cells if the cells are coated with antibodies directed against the
H-2 (and Moloney virus determined for the YAC) surface antigens. Similarly to
the previously often demonstrated effect of antibodies on the immune cell
killing, here also a blocking was demonstrated, the extent of which dependend
on the conditions. In spite of the fact that in some experiments the lytic effects
were only moderately effective and thus synergism could have been detected,
this was observed only in one case when an immune cell population was used.
On the contrary it seems that the immune spleen cell population is less potent in
bringing the antibody-dependent cytotoxicity in effect. This might be due to a
change in the population after immunization resulting in lowering the propor-
tion of the relevant cells.

It is noteworthy that the results obtained here resemble that of an entirely
in vitro system. In the chicken erythrocyte target-rabbit antisera-human
lymphocyte effector cell system, Perlmann et al. (10) have shown that preceding
interaction with the antigen-antibody complex made the lymphocytes acquire a
low but definite cytotoxic potential on target cells without antibody coat. The
important finding relevant to our results was that these lymphocytes were con-
siderably less active in lysing antibody-coated chicken erythrocytes. McLennan
has also shown that unrelated antigen-antibody complexes are also inhibitory
(11). It is also possible therefore that the weaker potential of the immune spleen
cell population is due to the effect of immune complexes on the cells in vivo.

The fact that only spleen cells were found to mediate lysis of antibody-coated
target cells corresponds well to the analysis of the mechanism regarding the
source of the effector cell. It is proposed that the cell is bone marrow-derived
(B cell) (12) and lymph nodes are relatively poor in B cells (13). The relatively
higher efficiency of cell population derived from the thymus-less nude mice, the
lower efficiency of immune spleen in which probably thymus-derived cells are
enriched provides further evidence that the cytolytic effect is bound to non-
thymus-derived cells.

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