Brief Definitive Reports

SYNERGISTIC OR ANTAGONISTIC EFFECT OF DIFFERENT ANTIBODY CONCENTRATIONS ON IN VITRO LYMPHOCYTE CYTOTOXICITY IN THE MOLONEY SARCOMA VIRUS SYSTEM*

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The in vitro study of the combined effects of humoral antibody and lymphocytes has been essential in attempting to understand the paradoxical effect of humoral antibodies leading to the phenomenon of enhancement (1). Part of the mechanism has been considered to be the shielding of cellular antigenic determinants by humoral antibody from the lymphocytes. This could mask antigenic sites preventing recognition and result in an afferent block and also interfere with the cytotoxic effect of specifically sensitized lymphocytes producing an efferent block. Support for both these hypotheses has been demonstrated (2–7). The blocking effect of humoral antibodies has been proposed to be of importance in the phenomena of tolerance, chimerism, and the protection of the fetus from maternal lymphocytes (8). However, it has been well documented in certain systems that humoral antibody and lymphocytes may act in concert to effect target cells' destruction (9, 10). Under different experimental conditions, pretreatment of target cells with humoral antibody may protect them from the cytotoxic effect of immune lymphocytes or mediate their destruction by a normal lymphocyte population. In vivo probably a balance between these two phenomena occurs.

In experiments concerning the combined effects of sera and lymphocytes from Moloney sarcoma virus (MSV)[1]-infected rats vs. MSV-transformed target

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[1] *Abbreviation used in this paper:* MSV, Moloney sarcoma virus.

cells, we found that lymphocyte-mediated cytotoxicity was influenced in two opposite directions depending on the serum concentration used to pretreat the target cells. With some sera relative increased killing of target cells was found at higher serum concentrations which subsided upon dilution and was replaced by better cell survival at lower concentrations.

Materials and Methods

Source of Lymphocytes and Sera.—Nine 5-day old Fischer rats were inoculated intramuscularly with 0.1 ml of MSV prepared by the Moloney method (11, 12). Only two of these animals developed tumors, one of which regressed after 30 days. The other had a progressively growing tumor at the 49th day when it was sacrificed. Lymphocytes were harvested from axillary and inguinal nodes from two rats without tumor 77 and 120 days after injection. Sera were collected from each rat 49, 77, and 106 days after injection with MSV.

Cytotoxic Assay.—The cytotoxic effects were assayed on A78 target cells (Wistar rat MSV-transformed fibroblast line) using the microcytotoxicity method of Takasugi and Klein (13) adapted to the study of serum as well as cell-mediated cytotoxicity. 50–100 target cells seeded in the wells of microplates (No. 3034, Falcon Plastics, Div. B-D Laboratories, Inc., Los Angeles, Calif.) incubated 24 hr at 37°C with 5% CO_2 were exposed to lymphocytes, sera, or both using six replicate wells for each serum dilution or lymphocyte concentration. Guinea pig complement (1:10) was added for assessment of serum cytotoxicity. When lymphocytes and sera were tested together, the target cells were incubated with serial serum dilutions for 45 min, then washed, and the lymphocytes were added. Medium for cultivation, washing, and dilutions was Eagle's minimal essential medium with 10% heat-inactivated fetal bovine serum, 50 units/ml of penicillin, and 50 μg/ml of streptomycin. After 48 hr incubation, the plates were washed with balanced salt solution, fixed with methanol, and stained by Giemsa and eosin methylene blue. The number of cells remaining in each well were counted.

Sera.—All immune sera were cytotoxic but their strength differed considerably varying between 30 and 90% target cell reduction at a dilution of 1:10 with titers between 1:40 and 1:320. The serum collected from the tumor-bearing rat was weak (30% target cell reduction and 1:40 titer). By indirect immunofluorescence the presence of antibodies was demonstrated. In a 1:20 dilution the different immune sera stained 40–70% of the A78 cells while the control sera were negative.

Lymphocytes.—Cytotoxicity varied according to the ratio of lymphocytes to target cells. Since a 50:1 ratio was noncytotoxic with normal lymphocytes, this ratio was used in the experiments concerning the effect of antibodies. The specific cytotoxicity was low which can be explained by the long period elapsing after MSV infection (14).

RESULTS

Fig. 1 shows the combined effects of sera and the immune lymphocyte population (harvested 77 days after MSV infection). The results are expressed as the percentage of target cells in control wells to which the same ratio of normal lymphocytes alone were added. The mean number of target cells without lymphocytes was 177.2 ± 71.2/well.

Immune lymphocytes alone caused a 33.5% reduction in target cell number compared to normal lymphocytes. Pretreatment with high serum concentrations resulted in an increased destruction of target cells by three of the sera. At lower concentrations, one serum was without effect, but the other three re-

duced target cell destruction, and as the sera were further diluted this effect disappeared or diminished. The control serum did not influence the cytotoxicity. Pretreatment with the immune sera alone, without addition of complement was not cytotoxic. In the wells treated with serum from an animal with a progressively growing tumor, the relative target cell survival was higher though the shape of the titration curve was similar to the others. This might be the result

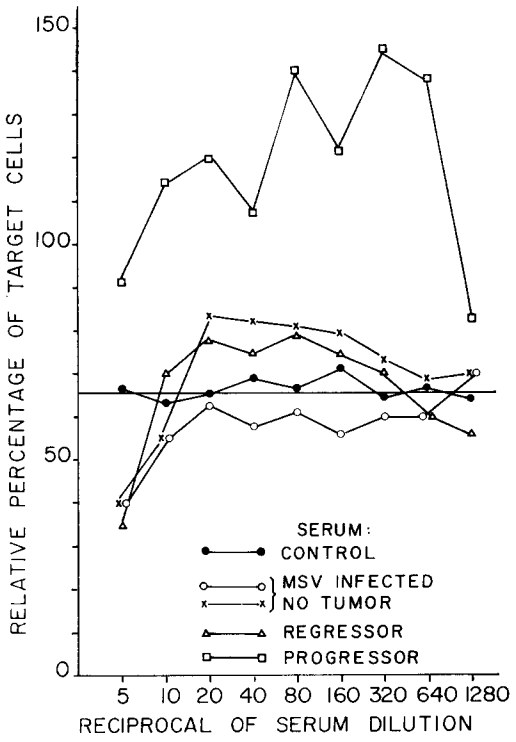

FIG. 1. Influence of individual sera collected 49 and 77 days after MSV infection on the cytotoxic effect of lymphocytes. The lymphocytes were tested 77 days after infection and caused 33.5% target cell reduction as indicated by the horizontal line.

of several interacting factors. A growth-stimulating effect and a concentration-dependent assistance in cytotoxicity or blocking would explain the curve.

Fig. 2 shows a similar experiment in which the sensitized lymphocytes were only slightly cytotoxic (12.9% target cell reduction). The lymph nodes were harvested 106 days after MSV infection. Two immune sera were highly effective in assisting lymphocyte-mediated cytotoxicity. None of these animals had progressively growing tumors. With one serum, the above discussed high relative target cell survival suggesting stimulation was obtained.

DISCUSSION

These data suggest that a synergistic cytotoxic effect of antiserum and lymphocyte population from a sensitized host can take place when high concentrations of antisera are used to coat the target cells. The same serum used in lower concentrations may block lymphocyte-mediated cytotoxicity. The extent of

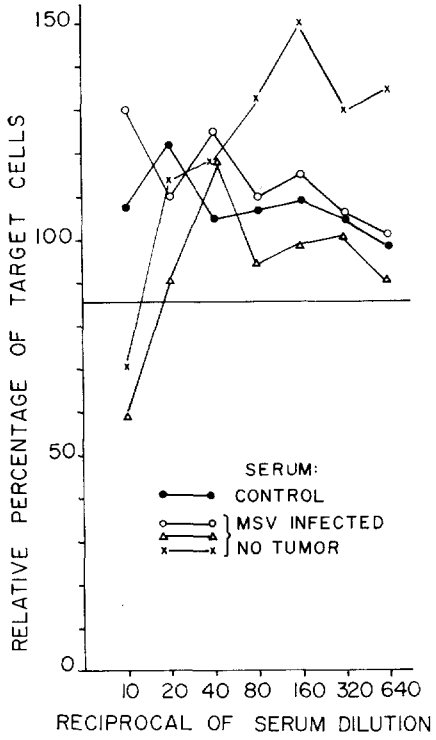

FIG. 2. Influence of individual sera collected 106 days after MSV infection on the cytotoxic effect of lymphocytes. The lymphocytes were tested 120 days after infection and caused a 12.9% target cell reduction as indicated by the horizontal line.

these effects obtained with individual sera varied. The phenomenon of antiserum-treated target cell destruction by lymphocytes can thus be extended to virally determined cell surface antigen(s).

Another fact which is suggested by these data is that the sera of animals without progressive neoplasms may also inhibit the cytotoxic effect of immune lymphocytes at some dilutions as effectively as the serum from a "progressor" animal. A similar blocking effect was obtained by Ankerst (15) with sera from tumor-free mice immunized by repeated injections of small cell numbers in the adenovirus 12 system. The blocking effect was found to be in the 7S fraction

The 19S fraction was cytotoxic in the presence of complement but did not block lymphocyte cytotoxicity.

It seems that the classification of different individual antisera in view of the effect on lymphocyte-mediated cytotoxicity needs to be correlated to the concentrations used.

It may be recalled in this context that the enhancement phenomenon was found to depend considerably on several factors, including the timing of immunization and the antigen dose as well as the cell type used for graft (16, 17). In experiments producing enhancement by passive antibody, the effect was highly dependent on the antibody dose administered (1). Moreover, in experiments with in vivo passive transfer, the synergistic effect of immune serum and a lymphocyte population from sensitized mice was demonstrated (16, 18).

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