Blocking Activity of Non-cytotoxic $F(ab')_2$ Tumor Cell Antibody Fragments* (32871)

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The study of antibody fragments has helped to correlate antibody structure with multiple antibody activities. Tumor cell antibody can enhance as well as inhibit growth of animal tumors. The reasons for this are complex and involve both cellular and humoral responses of the host (1). One *in vitro* model which could aid in explaining enhancement utilizes two types of 7S guinea pig antibodies, one of which can protect tumor cells against the cytotoxic activity of the other (2). Experimental results presented below suggest another model in which the cytotoxic activity of a 7S guinea pig $gamma_2$-globulin is blocked by non-cytotoxic 5S $F(ab')_2$ fragments derived from the globulin by pepsin degradation.

Materials and Methods. Five million freshly harvested Ehrlich ascites tumor (EAT) cells in 0.5 ml of 0.85% NaCl plus 0.5 ml of Freund complete adjuvant were injected intramuscularly into 500–800 gm male guinea pigs once a week for 6 weeks. Animals were bled by cardiac puncture 7 days after the last injection and sera harvested and pooled. $Gamma_2$-globulin was obtained by a combination of ammonium sulfate precipitation and DEAE-cellulose chromatography (3). Immunoelectrophoresis of the purified $gamma_2$-globulin gave a single arc when developed with rabbit anti-guinea pig serum. The $F(ab')_2$ antibody fragments were prepared by pepsin digestion of immune $gamma_2$-globulin (4) with a pepsin–globulin ratio of 1:50, pH 3.4, 5°C, 72 hours using 0.05 *N* HCl and NaOH for pH adjustment and neutralization. In the same way, normal $gamma_2$-globulin and $F(ab')_2$ fragments were obtained from normal guinea pig sera.

Cytotoxic activity of $gamma_2$-globulins for Ehrlich ascites tumor cells was evaluated by dye-uptake of the cells (4). Doubling dilutions of 0.5–0.016 mg/ml of globulin were prepared in 0.5 ml 0.85% NaCl, and 3×10^6 EAT cells in 0.1 ml of 0.85% NaCl, and 0.4 ml fresh or heat-inactivated (56°C, 0.5 hour) guinea pig serum complement (C′) were added. Mixtures were incubated for 0.5 hour at 37°C. One volume of cell mixture was added to 1 volume of 0.05% Safranin O dye in Alsever solution. This dye-cell suspension was examined within 1–2 min by microscope at 490× magnification, and percentage of cells taking up dye was used as an index of cytotoxicity.

Blocking activity of $F(ab')_2$ fragments for globulin cytotoxicity was determined by adding 6×10^6 EAT cells in 0.1 ml of 0.85% NaCl to 0.5 ml of 0.85% NaCl containing $F(ab')_2$ fragments to give concentrations of 2.5 to 0.16 mg/ml. Mixtures were incubated at 37°C for 0.5 hour, and 0.8 ml of C′ and 0.6 ml of 0.85% NaCl containing $gamma_2$-globulin were then added to give a final $gamma_2$-globulin concentration of 0.06 mg/ml. Mixtures were reincubated for 0.5 hour. At the same time the cytotoxic activity of globulins, $F(ab')_2$ fragments, or C′ alone was determined by substituting 0.85% NaCl for appropriate components of this system. The EAT cell viability was assayed by exclusion of Safranin O dye as described above and also by the ability of 2×10^6 EAT cells in 0.67 ml of cell mixture to produce tumors when injected subcutaneously into mice. One week after injection, tumor diameters were measured along 3 perpendicular axes, and the root mean cube was determined.

Results. Figure 1 shows the relative cytotoxic activity of guinea pig $gamma_2$-globulins. Immune globulin in concentrations of 0.06 mg/ml or more plus C′ is cytotoxic for essentially all EAT cells. However, with inactivated C′, immune globulin is not cytotoxic. Normal globulin plus C′, or C′ alone, has essentially no cytotoxic action.

Figure 2 clearly indicates that the $F(ab')_2$

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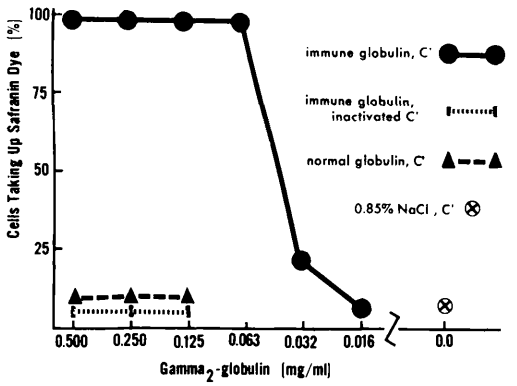

FIG. 1. Relative cytotoxicity of guinea pig gamma$_2$-globulins for Ehrlich ascites tumor cells in the presence of C'. Cytotoxicity is indicated by cell uptake of Safranin O dye.

fragment of immune gamma$_2$-globulin does not have the cytotoxic activity of the globulin from which it is derived. Although 0.06 mg/ml of immune gamma$_2$-globulin plus C' is cytotoxic for essentially all EAT cells, the immune F(ab')$_2$ fragment plus C' is no more cytotoxic than the normal F(ab')$_2$ fragment plus C', or C' alone. Figure 2 also shows blocking by non-cytotoxic immune F(ab')$_2$ fragments of the cytotoxic immune gamma$_2$-globulin. The EAT cells incubated with high concentrations of F(ab')$_2$ before exposure to 0.06 mg/ml of immune gamma$_2$-globulin show lack of cytotoxicity similar to that of cells in other non-cytotoxic preparations. Blocking decreases as the concentration of immune F(ab')$_2$ fragments used for preincubation decreases. Normal F(ab')$_2$ fragments show no blocking activity.

There is good correlation of the *in vitro* and *in vivo* indices used to determine cytotoxicity in Figs. 2a and b, respectively. Cell preparations with a high percentage of stained cells produced tumors of negligible size, and preparations with a low percentage of stained cells produced large tumors.

Discussion. Results presented above show that the cytotoxic activity of immune 7S gamma$_2$-globulin plus C' is absent in the 5S F(ab')$_2$ fragment prepared by pepsin degradation. However, this F(ab')$_2$ fragment is capable of blocking the cytotoxic activity of the globulin. Loss of much of the F_c globulin subunit (Porter fragment III) occurs during pepsin degradation (5). This fragment is the predominant mediator of C'-dependent reactions (6), although it has been shown that some C' is bound by washed antigen-F(ab')$_2$ complexes or by complexes formed in high concentrations of F(ab')$_2$ fragments (7). Lack of *in vitro* C'-fixation by rabbit 5S F(ab')$_2$-EAT cell complexes and a corresponding lack of cytotoxicity has been demonstrated previously (4). Blocking activity of the F(ab')$_2$ fragment is present because antibody combining sites remain on the F(ab')$_2$ fragment during pepsin degradation (8,9).

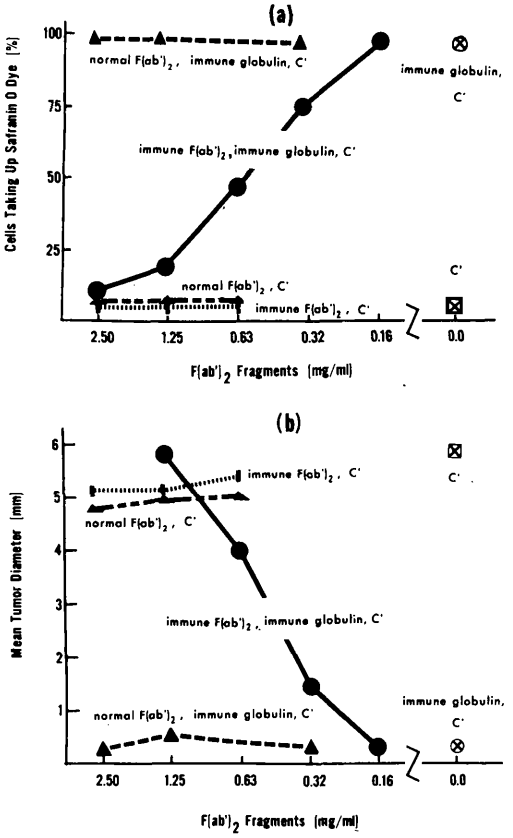

FIG. 2. Relative cytotoxic and blocking properties of F(ab')$_2$ fragments. Cytotoxicity for Ehrlich ascites tumor cells is indicated (a) *in vitro* by cell uptake of Safranin O dye, and (b) *in vivo* by small tumor size following injection of cells into mice. Cytotoxicity—cells were incubated with: F(ab')$_2$ fragments in the concentration indicated plus C', C' alone, or 0.06 mg/ml of immune globulin plus C'. Blocking—cells were incubated with F(ab')$_2$ fragments in the concentration indicated followed by 0.06 mg/ml of immune globulin plus C'.

These fragments combine with EAT cell antigenic sites and appear to prevent intact antibody from combining with the cell thereby preventing approximation of C′ and cell damage.

Tumor-bearing animals produce both cytotoxic lymphocytes and cytotoxic antibodies (1). Also, sarcoma cell antiserum protects sarcoma cells from sensitized lymphoid cells *in vitro* (10). In addition, human sera contain globulins which resemble pepsin-degraded antibody fragments (11). The present paper has shown the blocking properties of noncytotoxic $F(ab')_2$ antibody fragments. Evidence for immunologic enhancement of sarcoma SA-1 in Strain A mice by the 5-S $F(ab')_2$ fragment of rabbit anti-SA-1 antibody has been presented previously (12). These findings suggest the possibility that antibody fragments produced *in vivo* by synthesis, or by degradation of previously formed antibody, at times may be one factor responsible for immunologic enhancement of tumors.

Note: After completion of this manuscript, a report of enhancement of C57Bl leukemia E.L. 4 in mice following injection of Fab fragments of isoantibody has been noted (13). The suggested mechanism of blocking is the same as that proposed above.

Summary. Immune guinea pig $gamma_2$-globulin is cytotoxic *in vitro* for tumor cells in the presence of guinea pig complement. $F(ab')_2$ antibody fragments, prepared by pepsin degradation of the $gamma_2$-globulin, are not cytotoxic. Preincubation of tumor cells with $F(ab')_2$ fragments blocks the cytotoxic activity of $gamma_2$-globulin in high $F(ab')_2$/globulin ratios. The implication of these findings for immunologic enhancement of tumors is discussed.

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