CORRESPONDENCE

Bispecific Antibody That Binds Carcinoembryonic Antigen and Ricin Toxin A Chain Cytotoxic for Gastrointestinal Tract Tumor Cells

Monoclonal antibodies have many attractions as targeting agents for site-specific delivery of drugs (1). An alternative approach to chemical conjugation is to use a bispecific antibody with dual binding activity to link a toxic moiety to a binding moiety with targeting specificity. This approach is being explored with drugs (2), drug-carrier conjugates (3), and ribosome-inhibiting toxins (4, 5). With such bispecific antibodies, the toxic and targeting moieties could be administered separately, with the smaller size of the separate components giving potentially advantageous tissue penetration. It would also be possible to prelocalize the antibody, giving considerable flexibility in the design of schedules for optimum localization of the toxic moiety. However, it is important to examine in vitro whether a monovalent form of an antibody against a selected tumor-associated antigen can deliver sufficient toxin of choice to an appropriate intracellular compartment to kill cells of the proposed type of target tumor. In this study, a bispecific antibody with specificities for carcinoembryonic antigen (CEA) and the A chain of ricin toxin (RTA) has been produced and examined for its ability, in the presence of RTA, to mediate selective target-cell damage against CEA-expressing gastrointestinal tract tumor cells.

Bispecific antibodies can be produced by chemical conjugation of antibody fragments (5), but fusion of two hybridomas or a hybridoma with immune spleen cells (2, 6-8) can produce hybridomas giving a continual supply of antibody. Hybridoma to hybridoma fusion was used in the present study, and selection of heterohybrids using a fluorescence-activated cell sorter was found to be a rapid and convenient method of isolating hybrid hybridomas. From a fusion of an anti-RTA hybridoma (designated 596/192) and an anti-CEA hybridoma (NCRC-23), a hybrid hybridoma designated 636, which was found to secrete high levels of antibodies binding to CEA and RTA, was selected and recloned twice.

The maximum yield of bispecific antibody from hybrid hybridomas is 50% of the total immunoglobulin synthesized, but lower proportions of bispecific antibody are produced if heterologous heavy and light chain reassociations occur (9). In the present study, we separated antibodies using agarose-encapsulated hydroxyapatite (IBF Biotechnics, Life Science Laboratories, Luton, UK): anti-RTA reactivity eluted at about 0.087 M phosphate, both anti-CEA and anti-RTA reactivities eluted at about 0.087 M, and binding activity for CEA alone eluted at approximately 0.158 M. Antibody fractions were then tested for their ability to bridge between CEA and RTA in a flow cytometric test. Latex beads with CEA covalently coupled to the surface (10) were mixed with putative bispecific antibody and RTA labeled with fluorescein; bound fluorescence was quantitated by flow cytometry. A positive reaction was seen in the intermediate fractions from the column, but not in the early or late fractions or the parent antibodies.

Purified 636 bispecific monoclonal antibody acted synergistically with RTA to achieve highly significant cytotoxicity (table 1) at an RTA concentration as low as 4 × 10⁻⁹ M against CEA-expressing MKN-45 gastric carcinoma cells. These results compare with those of independent experiments performed with chemically prepared conjugates between RTA and a different CEA-specific antibody (228). In those experiments, the most active conjugates killed 50% of MKN-45 cells at a concentration of about 1.5 × 10⁻⁹ M RTA (11). The specificity of the cytotoxic effect delivered by bispecific antibody 636 was demonstrated by the lack of increased damage to 791T cells by RTA in the presence of bispecific antibody (table 2), although this cell line is highly sensitive to RTA delivered as an immunotoxin following conjugation to an appropriate antibody (12).

Thus, monovalently bound, anti-CEA bispecific antibody can deliver RTA to an appropriate intracellular compartment to kill gastrointestinal tract tumor cells. Further experiments, in xenograft systems, are planned to investigate the effect of bispecific antibody on the biodistribution and tumor localization of RTA preparations, particularly deglycosylated forms such as recombinant products, which do not have rapid hepatic

Table 1. Cytotoxicity mediated by RTA chain and bispecific antibody 636

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<tr>
<th>Antibody</th>
<th>% cytotoxicity ± SE at antibody concentration</th>
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<tr>
<td></td>
<td>0 μg/mL</td>
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<tr>
<td>636 (bispecific)</td>
<td>12.6 ± 2.8</td>
</tr>
<tr>
<td>Anti-RTA (monospecific)</td>
<td>12.6 ± 3.8</td>
</tr>
<tr>
<td>Anti-CEA (monospecific)</td>
<td>12.6 ± 3.8</td>
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*Cytotoxicity was assessed by inhibition of incorporation of [³⁵Se]selenomethionine as described in Embleton et al. (12). RTA concentration = 250 ng/mL.
†P < .01 by Student's t-test.
‡P < .05 by Student's t-test.
clearance. These experiments will also indicate whether RTA delivered to an epithelial tumor by bispecific antibody can exert a therapeutic effect.

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