Chimeric B72.3 mouse/human (IgG1) antibody directs the lysis of tumor cells by lymphokine-activated killer cells

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Summary. Chimeric mouse/human B72.3 (cB72.3) antibodies having a human IgG1 (γ1) or IgG4 (γ4) constant region were compared to the native murine IgG1 B72.3 (nB72.3) monoclonal antibody (mAb) for their ability to participate with human effector cells in antibody-dependent cellular cytotoxicity (ADCC). Because the TAG-72 antigen recognized by B72.3 is poorly expressed on tissue-cultured tumor cell lines, the xenografted OVCAR-3 human ovarian carcinoma ascites was used as a cytotoxicity target. The lytic activity of the cB72.3(γ1) mAb with peripheral blood lymphocytes was 1.5- to 50-fold greater than that of the nB72.3 mAb and usually the cB72.3(γ4) mAb. However, lymphocytes from some donors had similar ADCC activity with either the cB72.3(γ1) or cB72.3(γ4) mAb. The cB72.3(γ1) and the murine anti-colon carcinoma CO17-1A mAb had comparable activity in mediating ADCC against the OVCAR-3 tumor. Exposure of lymphoid cells to interleukin-2 (IL-2) (100–500 U/ml) for 24 h to generate lymphokine-activated killer (LAK) cells augmented ADCC mediated by the cB72.3(γ1) mAb 2- to 22-fold. By contrast, LAK cells from most donors expressed weak non-specific cytotoxicity against OVCAR-3 ascites tumor cells. The cB72.3(γ1), and to a lesser extent, the cB72.3(γ4) chimera also participated with monocytes in mediating ADCC, but the antibody-dependent lytic potency of monocytic effectors was much weaker than that of IL-2-activated lymphoid cells. These studies show that the cB72.3(γ1) mAb has appreciable ADCC-mediating properties, suggesting a potential role for its incorporation into treatment strategies utilizing adoptive killer cell and/or lymphokine therapy.

Introduction

Studies of antibody-targeted therapy have involved the use of monoclonal antibodies (mAb) as carriers of cytotoxic agents such as drugs, natural toxins, and radionuclides, or as modifiers that act in concert with other elements of the immune system such as cytotoxic lymphocytes and complement [15, 17, 43]. It is well-established that murine mAb against tumor-associated antigens on human carcinomas [20], melanomas [16, 51], and neuroblastomas [34], can participate with lymphocytic or monocytic effector cells of either human or murine origin to cause the destruction of tumor cells in vitro. These antibodies were also effective in vivo as shown by their ability to inhibit the outgrowth of tumor implants in athymic mice [18, 34]. Other findings obtained from work with both xenogeneic [21] and syngeneic [2, 7] animal models have demonstrated that the combination of mAb and interleukin-2 (IL-2) therapies results in a significant improvement in the inhibition of tumor growth over that appearing when either modality is used alone.

Clinical studies based on both the antigen recognition and effector functions of mAb for the treatment of cancer have found definite responses in a few patients [5, 23, 31]. In an effort to enhance the ability of mAb to mediate antibody-dependent cellular cytotoxicity (ADCC) with human effector cells, which may then lead to improved clinical activity, various investigators have constructed mouse/human chimeric antibodies through genetic recombination [3, 29, 39, 41, 44, 47]. As predicted from studies of the functional activities of polyclonal human IgG subclasses [4], mouse/human chimeric mAb having a human IgG1 (γ1) constant domain were most active in mediating ADCC [3, 44, 47]. An important consequence of chimerization is that the immunogenicity of these reagents in humans is expected to be much less than that of the parent murine mAb, as has been found with the mouse/human CO17-1A mAb [32]. The preparation of recombinant mAb also makes it possible to switch the isotype of an antibody that has desirable specificity properties but has an isotype that is ineffective in ADCC.
The murine B72.3 mAb reacts with a variety of human carcinomas including colon, breast, and ovary, and to a lesser extent or not at all by normal adult tissues with the exception of secretory endometrium [45, 48, 49]. The B72.3 mAb has been shown to be useful in tumor diagnostic applications such as in immunoscintigraphy [9], monitoring of disease activity by serum assays [27], and in certain tumor histopathology settings [25]. Since the B72.3 mAb is an IgG1, a murine isotype not commonly associated with ADCC activity, we have recently prepared a chimeric construct that has a human IgG1 (γ1) constant domain [24]. In the latter study we described the immunochemical properties of the cB72.3(γ1), demonstrated its ability to target a human tumor xenograft in athymic mice, and presented preliminary evidence that the cB72.3(γ1) may have the ability to mediate ADCC. In the study reported here, we describe in detail the ability of the cB72.3(γ1) mAb to mediate ADCC with human effector cells in comparison to that of the parent mAb and a chimeric B72.3 IgG4 [cB72.3(γ4)] construct [52]. We also show that IL-2 and interferon-γ can augment the ADCC activity of the chimeric B72.3 IgG1 with lymphocytic and monocytic effector cells, respectively. Furthermore, we describe the variabilities that may exist among different donor populations in mediating ADCC with the chimeric B72.3 mAb.

Materials and methods

**mAb and cell lines.** The generation and properties of the native murine mAb B72.3(γ1), designated nB72.3, have been described previously [6]. nB72.3 IgG was isolated from ascitic fluid by ammonium sulfate precipitation and ion-exchange chromatography. The murine D612 mAb(γ1), which mediates ADCC with human LAK cells, recognizes the human colon-specific antigen that is widely expressed among colon adenocarcinomas [36]. The D612 mAb was isolated from ascitic fluid by protein-A-Sepharose and hydroxyapatite chromatography. Purified 17-1A mAb [19] was generously supplied by Dr. Peter Daddona (Centocor, Malvern, Pa). MOPC-21 (IgG 1) and UPC-10 (IgG2 a) murine myeloma proteins were purchased from Organon (Durham, NC) while purified human polyonal IgG was obtained from Jackson Immuno Research Laboratories (Malvern, Pa). MOPC-21 (IgG 1) and UPC-10 (IgG2 a) murine myeloma proteins were purchased from Organon (Durham, NC) while purified human polyonal IgG was obtained from Jackson Immuno Research Laboratories (Malvern, Pa).

**Chimeric antibody purification.** cB72.3(γ1) was purified from tissue-culture supernatants of transfected SP2/0 cells growing in ABC protein-free medium (Pan-Data Systems Inc., Rockville, Md) using protein-A-Sepharose chromatography as described in detail elsewhere [24]. The nB72.3 and cB72.3 mAb were shown to have very similar reactivity in cross-competition immunosassays [24].

**Effector cells.** Human peripheral blood mononuclear cells (hPBMC) were used as effector cells in cytotoxicity assays. These were isolated from buffy coat or leukopheresis preparations of 13 normal donors by separation on Ficoll/Hypaque gradients (LSM, Organon, Durham, NC).

Cells collected from the gradient interface were depleted of platelets by washing with Ca- and Mg-free Dulbecco’s phosphate-buffered saline (Ca/Mg-free PBS) containing 2% fetal calf serum (Gibco, Grand Island, NY). Cells were then cultured for 24 h at 5 × 10^6 /ml in RPMI-1640 medium supplemented with 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal calf serum (Gibco) (complete medium). Recombinant human IL-2 (Cetus, Emeryville, Calif) was added to cultures at a final concentration of 500 U/ml. hPBMC exposed to IL-2 for 24 h are herein referred to as lymphokine-activated killer (LAK) cells [37]. hPBMC from some donors were stored at -70°C in 90% fetal bovine serum plus 10% dimethylsulfoxide and were activated prior to cytotoxicity assay. Adherent cells (monocytes) were harvested from leukopheresis or elutriated preparations by adherence to serum-coated flasks according to a modification of the method of Fischer et al. [10]. Briefly, 150-cm² plastic tissue-culture flasks (Costar, Cambridge, Mass) were incubated for 15 min at room temperature with 5 ml 1 : 2 dilution of pooled human AB serum (Gibco). The flasks were washed with Ca/Mg-free PBS and then hPBMC were added at 5 × 10^6 cells/ml complete medium. After incubation for 1 h at 37°C, the non-adherent cells were removed and the flasks were washed three times with 10 ml Ca/Mg-free PBS. In some experiments, the non-adherent cells were submitted to a second adherence cycle. The adherent cells were then incubated for 48–60 h in the presence or absence of 500 U/ml interferon γ (Hu-IFN-γ, Hoffman-LaRoche Inc., Nutley, NJ). Before cytotoxicity assay, any cells remaining adherent were released by incubation for 30 min at room temperature with 10 ml 0.1% EDTA in Ca/Mg-free PBS. Adherent cells were treated with Leu11b (Becton-Dickenson, Mountain View, Calif) plus rabbit complement (Pel-Freez, Rogers, Alaska) according to the instructions of the manufacturer of the antibody. The adherent and non-adherent cells contained more than 90% and less than 2% monocytes, respectively, as determined by flow cytometry using fluorescein-labeled LeuM3, Leu 19, and Leu4 mAb (Becton-Dickenson, Mountain View, Calif) plus rabbit complement (Pel-Freez, Rogers, Alaska) according to the instructions of the manufacturer of the antibody. The adherent and non-adherent cells contained more than 90% and less than 2% monocytes, respectively, as determined by flow cytometry using fluorescein-labeled LeuM3, Leu19, and Leu4 mAb (Becton-Dickenson).

**Cytotoxicity assay.** NIH: OVCAR-3 ascites tumor cells, which express the TAG-72 antigen, served as target cells and were obtained from female BALB/c athymic mice (nu/nu) bearing 3–4 week-old ascites tumors. In selected experiments, the LS-174T cell line, which expresses only trace amounts of the TAG-72 antigen [22], also served as a tumor target. For the chromium-release assay, target cells (5 × 10^6 – 10 × 10^6) were labeled with 200 μCi sodium [51Cr]chromate (250–500 mCi/mg Cr, Amersham, Arlington Heights, Ill) in 0.2 ml fetal calf serum for 1 h at 37°C. For assays requiring 24 h incubation, target cells (25 × 10^6) were labeled for 15 min at room temperature with 50 μCi 111In-oxyniquoline (Amerham, code IN.15PA) in a volume of 0.25 ml RPMI-1640 according to a modification of the method of Wiltrout et al. [53]. The cells were washed four times in RPMI-1640 and resuspended in complete medium, and then 1 × 10^6 cells in 50 μl were added to assay plates containing 96 U-bottomed wells (Costar), each of which contained 50 μl complete medium alone or various antibody preparations. Effector cells, which were harvested from flasks by scraping, were washed once and immediately added at different E/T ratios to the assay plates in a volume of 100 μl. The plates were centrifuged for 3 min at 30 g, and then incubated for 4 h or 24 h at 37°C in 7% CO₂. The plates were centrifuged as before, then the supernatant from each well was harvested for gamma counting using Skatron harvesting frames (Sterling, Va). Each measurement was carried out in triplicate; the standard deviation of replicates was usually 10% or less. Specific lysis was calculated using the formula:

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\text{Lysis} \% = \frac{\text{observed radioactivity (cpm) - background cpm}}{\text{total (cpm) - background (cpm)}} \times 100
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Background radioactivity cpm was obtained from radioactivity released by target cells incubated in medium alone, which was between 5% and 10% of the total released after treatment of target cells with 2.5% Triton X-100. Results are also expressed as lytic units/10⁶ effector cells, in which a lytic unit is defined as the number of effector cells producing 30% lysis of 10⁶ labeled target cells.