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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.

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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 mucin expressed by 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(γ2a), which mediates ADCC with human LAK cells, recognizes a human colon-specific antigen that is widely expressed among colonic 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 (IgG1) and UPC-10 (IgG2a) murine myeloma proteins were purchased from Organon (Durham, NC) while purified human polyclonal IgG was obtained from Jackson Immuno Research (West Grove, Pa). The LS-174T human colonic tumor and the K562 human erythropoietic cell lines were obtained from the American Type Culture Collection (Rockville, Md) while the NIH: OVCAR-3 human ovarian ascites carcinoma was obtained originally from Dr. Thomas Hamilton [14]. Detailed descriptions for the generation of chimeric antibodies have been reported for cB72.3(γ1) [24] and cB72.3(γ4) [52].

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 immunoassays [24].

Effector cells. Human peripheral blood mononuclear cells (hPBMC) were used as effector cells in cytotoxicity assays. These were isolated from buffy coat or leukophoresis 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 leukophoresis or elutriated preparations by adherence to serum-coated flasks according to a modification of the method of Fischer et al. [10]. Briefly, 150-cm^3 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, Moutain View, Calif) plus baby 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 [^{51}Cr]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 ^{111}In-oxyquinoline (Amersham, 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 1×10^4 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% CO_2. 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:

$$\text{Lysis (\%)} = \frac{\text{observed radioactivity (cpm)} - \text{background cpm}}{\text{total (cpm)} - \text{background (cpm)}} \times 100$$

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/10^6 effector cells, in which a lytic unit is defined as the number of effector cells producing 30% lysis of 10^4 labeled target cells.

Table 1. Antibody-dependent cellular cytotoxicity (ADCC) mediated by cB72.3(γ1) and human lymphokine-activated killer (LAK) cells against OVCAR-3 ascites tumor cells[a]

Donor	IL-2	Lysis (%) with antibody			
		Hum IgG	nB72.3	cB72.3(γ1)	17-1A
1	–	0	0.3	4.3	3.7
	+	5.9	15.3	28.6	34.2
2	–	0.8	0.6	4.9	NT
	+	1.1	5.7	22.8	NT
3	–	0	0.2	13.0	NT
	+	4.2	11.6	35.3	NT
4	–	2.5	7.7	22.1	25.6
	+	20.3	44.1	72.2	83.2
5[c]	–	2.0	2.7	3.2	NT
	+	3.3	7.1	8.1	NT

[a] Normal human peripheral blood mononuclear cells (hPBMC) were exposed to 500 U/ml interleukin-2 (IL-2) for 24 h prior to 4-h chromium release assay using 25 μg/ml mAb. The percentage lysis shown was obtained at a 100 : 1 effector/target ratio

[b] Not tested

[c] The effector cells used in this assay mediated ADCC with mAb D612 against LS-174T

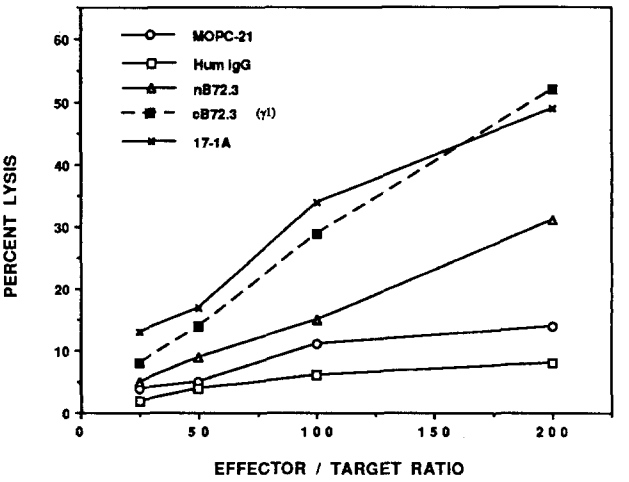

Fig. 1. ADCC mediated by cB72.3(γ1) and lymphokine-activated killer (LAK) cells against OVCAR-3 ascites tumor in 4-h chromium-release assay. Human peripheral blood mononuclear cells (hPBMC) were incubated for 24 h in 500 U/ml IL-2 and then tested against ^{51}Cr-labeled target cells using 25 μg/ml mAb. The results from donor 1 listed in Table 1 are depicted here. Antibodies: MOPC-21 (○); human IgG (□); nB72.3 (▲); cB72.3(γ1) (■); 17-1A (×)

Results

ADCC of nB72.3 and cB72.3 mAb against OVCAR-3

Initial experiments studied the ADCC-mediating properties of nB72.3 and the cB72.3 (γ1) mAb utilizing a 4-h chromium-release assay in which LAK cells (500 U/ml IL-2, 24 h) or untreated hPBMC were used as effectors. The TAG-72 antigen is absent or present in minute amounts in tissue-cultured tumor cell lines [22], necessitating the use of the xenografted OVCAR-3 ascites tumor as a cytotoxicity target. The ascites tumor cells primarily grow as clusters ranging in size from 3 to 40 cells, 60%–95% of which, depending upon harvest, express TAG-72 at the cell surface as determined by immunoperoxidase staining (data not shown). The OVCAR-3 tumor usually had a low sensitivity to non-specific cytotoxicity (antibody-independent) mediated by untreated hPBMC, which was 2% or less at E/T ratios of 100 : 1 among the five donors shown in Table 1. This non-specific cytotoxicity did increase when LAK cells from the same donors were used, but in most cases, maximum lysis was 6% or less. Upon the addition of nB72.3 at 25 μg/ml, there was a modest increase in lytic activity with both untreated hPBMC or LAK cells (Table 1). However, the cB72.3(γ1) mAb was found to elicit much greater ADCC as compared to the nB72.3 mAb. This was particularly evident with LAK cells from four of the five donors listed in Table 1 where the lytic activity in cultures containing cB72.3(γ1) was 2–20 times greater than that of cultures with human polyclonal IgG. Background non-specific cytotoxicity was similar between cultures with or without human IgG. The nB72.3 mAb was 2–4 times less active than the cB72.3(γ1) in mediating ADCC with LAK cells. LAK cells from donor 5 did not show appreciable ADCC with either the nB72.3 or cB72.3(γ1) mAb. Donor 5 LAK cells, however, were active in mediating ADCC with the D612 mAb, previously shown to mediate strong ADCC [36], when tested against the LS-174T colon tumor cell line (data not shown), demonstrating that the absence of ADCC against OVCAR-3 targets with B72.3 mAb was not due to a general defect in the ability of these effector cells to mediate this functional activity. An example of a dose response to the E/T ratio with LAK cells from donor 1 is shown in Fig. 1, which also demonstrates that the cB72.3(γ1) and 17-1A mAb had very similar potency in mediating ADCC with this donor's effector cells against OVCAR-3. The murine 17-1A mAb was included for comparison in these studies because of its well-established ability to mediate potent ADCC with human lymphoid effector cells [20]. The similarity in lytic activity between cultures with and without cB72.3(γ1) mAb at the lower E/T ratios indicates that the antibody-dependent lysis observed at higher E/T ratios was not derived from the action of the mAb by itself, but also required the participation of effector cells.

ADCC against LS-174T tumor cells

The specificity of the ADCC mediated by the cB72.3(γ1) mAb was tested using the LS-174T colon tumor cell line, which has been previously shown to express only trace amounts of cell-surface TAG-72 on only a small percentage (<5%) of cells. When the LAK cells from donors 1–4 (Table 1) were used in assays with the LS-174T cell line as targets, ADCC was not observed with either the nB72.3 or cB72.3(γ1) mAb (Fig. 2). This was not due to the resistance of the LS-174T to antibody-dependent lysis because the murine D612 mAb was able to mediate ADCC against this tumor target.

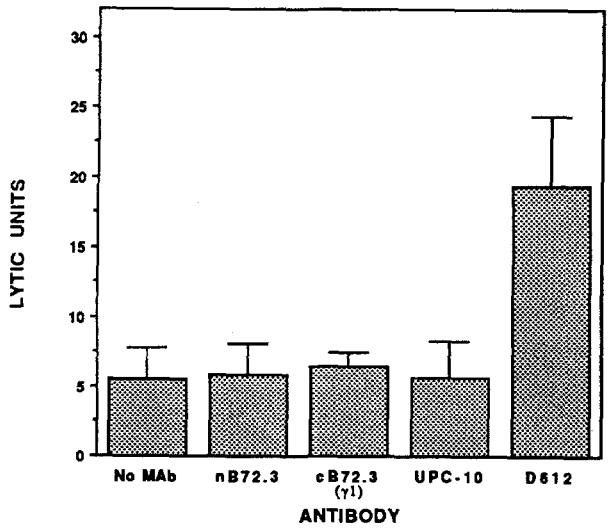

Fig. 2. ADCC mediated by cB72.3(γ1) and LAK cells against LS-174T colon tumor cell line in 4-h chromium-release assay. hPBMC were incubated for 24 h in 500 U/ml IL-2 and then tested against ^{51}Cr-labeled target cells using 25 μg/ml mAb. The mean lytic activity (lytic units ± SD) is shown for four experiments utilizing hPBMC from donors 1–4 listed in Table 1

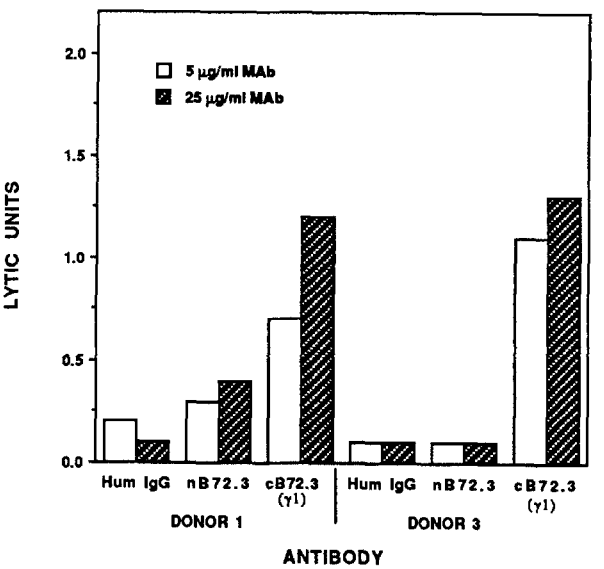

Fig. 3. Effect of mAb protein dose on ADCC mediated by cB72.3(γ1) and LAK cells against OVCAR-3 ascites tumor cells. hPBMC were incubated for 24 h in 500 U/ml IL-2 and then tested against ^{51}Cr-labeled target cells using 5 μg/ml (*open box*) or 25 μg/ml (*hatched box*) mAb in 4-h chromium-release assay. Donors 1 and 3 are the same as those listed in Table 1

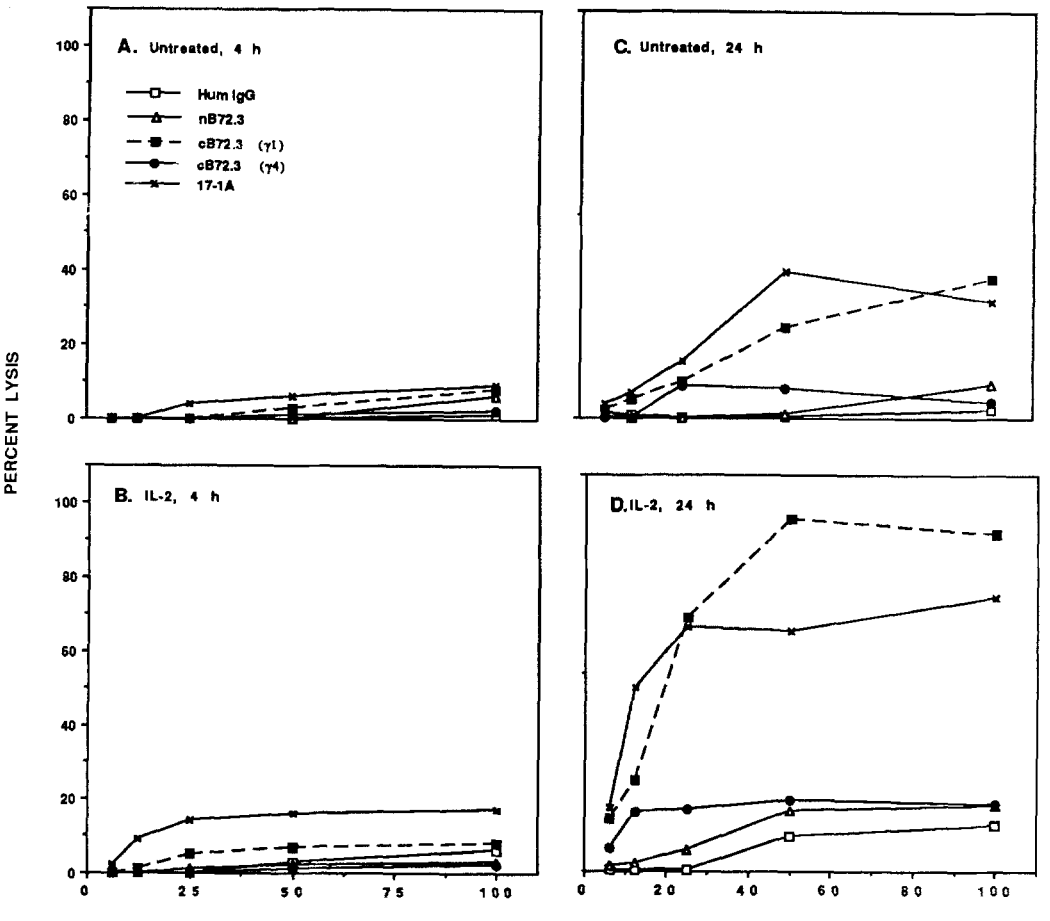

Fig. 4 A–D. Effect of cytotoxicity assay incubation time on ADCC mediated by nB72.3 and cB72.3 mAb against OVCAR-3 ascites tumor. hPBMC were incubated for 24 h in the absence or presence of 500 U/ml IL-2 and then tested against ^{111}In-labeled target cells using 25 μg/ml mAb. Untreated (**A** and **C**) and IL-2-activated (**B** and **D**) hPBMC were incubated with labeled target cells for 4 h (**A** and **B**) or 24 h (**C** and **D**). Antibodies: human IgG (□); nB72.3 (△); cB72.3(γ1) (■); cB72.3(γ4) (●); 17-1A (×)

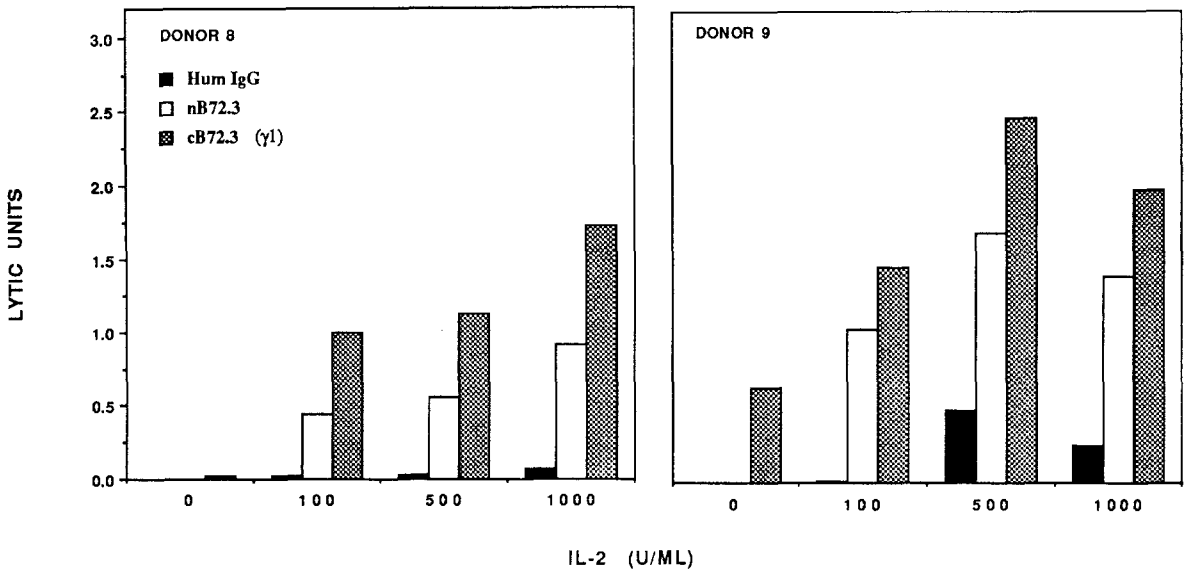

Fig. 5. Effect of IL-2 concentration on ADCC mediated by LAK cells and cB72.3(γ1) mAb. hPBMC were exposed to the designated concentration of IL-2 for 24 h prior to 24 h indium-release assay against OVCAR-3 ascites tumor using 25 μg/ml mAb. Antibodies: human IgG (*closed box*); nB72.3 (*open box*); cB72.3(γ1) (*stippled box*)

Effect of cB72.3(γ1) mAb concentration

The LAK cells from two donors (Table 1, donors 1 and 3) were tested in ADCC with a lower concentration of cB72.3(γ1) mAb (Fig. 3). In donor 1, ADCC with 5 μg/ml cB72.3(γ1) was approximately one-half of that observed at the higher concentration of mAb while in the second donor, ADCC was very similar at the two mAb concentrations. Although ADCC with donor 1 LAK cells decreased somewhat when the concentration of cB72.3(γ1) was lowered five-fold, it remained substantially greater than the lysis obtained with nB72.3 or with the negative control, polyclonal human IgG.

Effect of assay incubation time on ADCC

Although definite ADCC mediated by the cB72.3(γ1) against OVCAR-3 was obtained in 4-h cytotoxicity assays, this was usually best observed at the higher E/T ratios. To determine if a prolonged assay incubation time would enhance measurable ADCC, untreated hPBMC and LAK cells from donors 6 and 7 were incubated for 4 h or 24 h with ^{111}In-labeled target cells. Since effector cells from both donors gave a similar pattern of reactivity with the cB72.3(γ1) mAb, the results from one donor are illustrated in Fig. 4. At 4 h, the 17-1A mAb and at a lower level, the cB72.3(γ1) mAb, showed a small amount of ADCC activity with IL-2-activated effectors but not with untreated hPBMC. By contrast, there was a marked increase in ADCC at 24 h with both the 17-1A and cB72.3(γ1) mAb. ADCC also became evident with untreated effectors, although their activity with the cB72.3(γ1) mAb was 7 times lower than with that of IL-2-activated cells (1.3 vs 10.3 lytic units). The increase in ADCC measured at 24 h was accompanied by a much lower rise in non-specific cytotoxicity. Similar to the results obtained with the short-term ^{51}Cr-release assay, cytotoxicity in the presence of the nB72.3 mAb (0.2 lytic units with LAK cells) was much lower than when the cB72.3(γ1) mAb was added. Thus, the cB72.3(γ1) mAb can be up to 50 times greater than the nB72.3 mAb in its ability to mediate ADCC.

Effect of IL-2 concentration on ADCC

In separate experiments, hPBMC from two additional donors (donors 8 and 9) were incubated for 24 h in different concentrations of IL-2 prior to ADCC assay. At 100 and 500 U/ml IL-2, effector cells from both donors had similar antibody-dependent lytic activity, which tended to increase with cells activated in 500 U/ml IL-2 (Fig. 5). hPBMC exposed to 1000 U/ml showed an increase in ADCC in one donor while there was a decrease with effector cells from the second donor. Non-specific cytotoxicity against OVCAR-3 tumor cells remained low at the different concentrations of IL-2 whereas the dose response of this activity against K562 target cells had a very similar pattern to that observed for ADCC against OVCAR-3 (data not shown). Culturing lymphoid cells for 3 days in the presence of different concentrations of IL-2 did not appreciably alter the pattern of ADCC augmentation obtained after shorter incubation times (data not shown). Results from these experiments as well as those described above show that, depending upon donor, IL-2 increases ADCC mediated by the cB72.3(γ1) mAb 2- to 22-fold compared to the combined cytotoxic activities of LAK cells alone and unstimulated effector cells in the presence of antibody. As depicted in Fig. 5, IL-2 augmented ADCC mediated by both the nB72.3 and cB72.3(γ1) mAb, in which effector cells from these particular donors had 1.5–2 times greater killing activity with the cB72.3(γ1) mAb than with the native antibody.

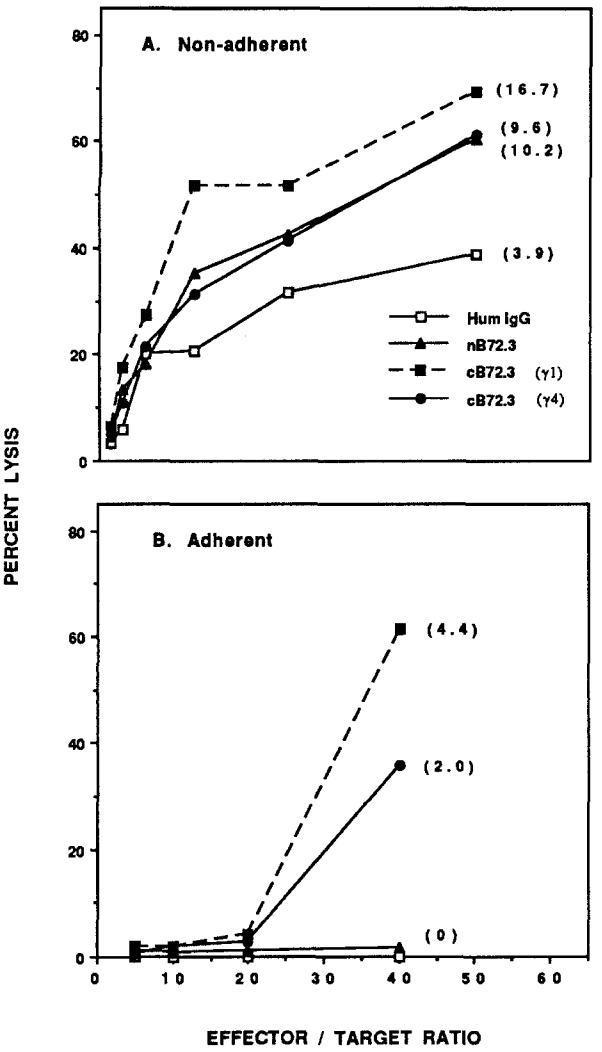

Fig. 6 A, B. ADCC with non-adherent and adherent effector cells. hPBMC were adhered to serum-coated flasks, exposed to 500 U/ml interferon γ for 60 h, and then treated with Leu11b plus complement prior to 24 h cytotoxicity assay against OVCAR-3 ascites tumor using 25 μg/ml mAb. Non-adherent cells were incubated in the presence of 500 U/ml IL-2. Values in parentheses: lytic activity (lytic units). Antibodies: human IgG (□); nB72.3 (▲); cB72.3(γ1) (■); cB72.3(γ4) (●)

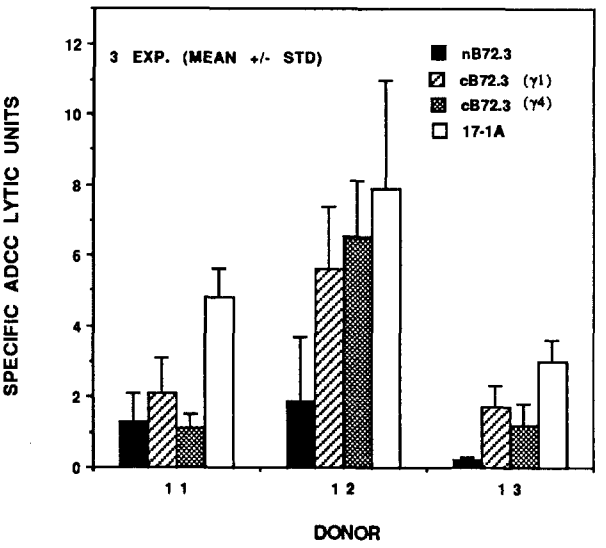

Fig. 7. Comparison between the cB72.3(γ1) and cB72.3(γ4) mAb in ADCC mediated by LAK cells. hPBMC from three donors were tested in three experiments against OVCAR-3 ascites tumor cells using 25 μg/ml mAb. Prior to each assay, frozen effector cells were incubated for 24 h in 500 U/ml IL-2. Results are depicted as specific ADCC lytic activity (lytic units), which is derived by subtracting non-specific cytotoxic activity measured in wells without mAb from the values obtained in wells containing mAb. Antibodies: nB72.3 (*closed box*); cB72.3(γ1) (*hatched box*); cB72.3(γ4) (*stippled box*); 17-1A (*open box*)

ADCC with adherent effector cells

The ability of the native and chimeric B72.3 mAb to mediate ADCC was compared between non-adherent and adherent effector cells obtained from an elutriated preparation (donor 10). Adherent cells that were activated with γ-interferon-mediated ADCC with both of the cB72.3 mAbs but not with the native antibody (Fig. 6B). This activity was only detectable at the highest ratios of interferon-treated adherent cells, and it was totally absent with untreated cells (data not shown). Although maximal lysis of target cells was very similar for non-adherent and adherent effector cells, the overall antibody-dependent lytic potency of IL-2-activated non-adherent cells was approximately three times greater than that of adherent cells. This is shown in Fig. 6A, where significant lysis with non-adherent cells occurred with E/T ratios as low as 5:1, a relationship also reflected in the higher lytic activity generated with these cells in the presence of antibody. Non-adherent effector cells also mediated ADCC with the native antibody (Fig. 6A) while exposure of adherent cells to IL-2 was without effect (data not shown).

Comparison of ADCC mediated by the cB72.3(γ1) and (γ4) mAb

The ADCC-mediating properties of the cB72.3(γ4) were compared to those of the cB72.3(γ1) mAb. Like the nB72.3 mAb, it had definite but usually lower ADCC activity as compared to that of the cB72.3(γ1) mAb with LAK cells (Figs. 4, 6, 7). LAK cells from one donor (donor 12, Fig. 7), however, were equally reactive in ADCC with both of the chimeric antibodies. The stronger ADCC properties of the cB72.3(γ1) were not due to its greater immunoreactivity as compared to that of the cB72.3(γ4) since they both were equally reactive as measured by immunoperoxidase staining of OVCAR-3 (data not shown) and by competition radioimmunoassay [24].

Discussion

Our studies have demonstrated that conversion of the native B72.3 mAb to a recombinant human/mouse chimeric mAb with a human IgG1 (γ1) constant domain leads to a marked improvement in ADCC. With the exception of lymphoid effector cells from one donor that did not mediate ADCC with either the native or chimeric IgG1 mAb, there was an enhanced level of ADCC with the cB72.3(γ1) in all other donors, ranging between a 1.5- and 50-fold increase in lytic activity over that obtained with the nB72.3. With effector cells from some donors, the cB72.3(γ1) also generated as much ADCC activity as the native 17-1A mAb, which has been shown to be very effective at directing the activities of killer cells [20, 47]. Other studies have also shown that chimerization of murine mAb with the human γ1 constant domain can enhance the ability of the native mAb to mediate ADCC with human lymphocytes. The chimeric L6(γ1) mAb, which reacts with tumors of carcinoma and non-carcinoma origin, was found to be 100 times more active than the native IgG2a L6 antibody [29]. However, the chimeric 17-1A(γ1) mAb was shown by two groups of investigators to be comparable to the parent IgG2a 17-1A mAb in its ability to mediate ADCC [44, 47], while in a third study, the chimeric mAb was more effective [33]. In work with lymphoid tumors, chimeric antibody against the common acute lymphocytic leukemia antigen was twice as potent as the parent IgG2a mAb [39] in mediating ADCC. Likewise, Liu et al. [30] found that their chimeric mAb against the B-lymphocyte antigen, CD20, was highly effective in mediating ADCC while the IgG2b native mAb totally lacked this functional activity. Murine mAb that have an IgG2a or IgG3 isotype are much more commonly associated with having ADCC properties than those of the IgG1 isotype, such as the nB72.3 studied here, or IgG2b isotypes [40]. Besides the likely advantage of using chimeric mAb for immunotherapy of cancer because of their decreased immunogenicity [32], our studies as well as those mentioned above show that isotype switching through chimerization of murine mAb to a human IgG1 subclass produces antibodies that are much more potent mediators of ADCC. Native B72.3 has been administered to over 500 patients to date and has been shown to localize approximately 70% of primary and metastatic lesions of colorectal, ovarian, and breast carcinomas [9, 28, 38]. Because chimeric antibodies retain the same antigen-binding characteristics as that of the parent mAb, the cB72.3(γ1) mAb is a promising candidate for use in serotherapy and radioimmunodiagnosis of carcinomas.

Antibody-dependent lysis with both the native and chimeric B72.3(γ1) antibodies was demonstrable with unseparated peripheral blood mononuclear cells. Human natural killer cells have been shown to be a major effector cell type that participates in ADCC reactions mediated by murine monoclonal antibodies against tumor-associated antigens [40]. ADCC of lymphoid cells with murine mAb is also augmented approximately 3-fold when they are cultured in IL-2, an effect that occurs at relatively low concentrations of IL-2 and after a brief exposure time [35, 50]. Previous studies have shown that lymphocytes have an important role in cell killing mediated by human/mouse chimeric antibodies [29, 44, 47], an observation confirmed by our findings as reported here with the cB72.3(γ1) mAb. In addition, our results show that ADCC mediated by cB72.3(γ1) mAb was augmented 2- to 22-fold when IL-2-activated cells were utilized. The enhancing effect of IL-2 was also observed at low concentrations (100 U/ml) and after a short exposure time (24 h). It is expected that enhancement of ADCC in patients receiving IL-2 therapy should occur under typical treatment regimens [42, 46]. Eisenthal et al. [7, 8] reported that therapy with a combination of LAK cells, IL-2, and mAb prevented the development of B16 melanoma liver or lung metastases under conditions where treatment with the individual agents was not nearly as effective. Other studies carried out with syngeneic [1, 26] and xenogeneic [21] animal models have reported similar findings, suggesting that monoclonal antibodies, particularly those that are chimerized and are capable of mediating ADCC such as the cB72.3(γ1), may improve the therapeutic properties of LAK cells and IL-2.

The cB72.3(γ4) was not as active as the chimeric cB72.3(γ1) in mediating ADCC with human lymphocytes from most donors, being variably present and similar in this property to the native antibody. Previous studies have demonstrated, however, that both the cB72.3(γ1) and cB72.3(γ4) mAb react equally in competition radioimmunoassays [24]. This latter work also provided an indication that the cB72.3(γ1) may be active in ADCC. Earlier studies with polyclonal antibodies have shown that among the human IgG subclasses, IgG4 has the poorest capacity to mediate ADCC [4]. This relationship has been confirmed in recent work with chimeric antibodies having the same epitope specificity but with different heavy-chain constant-region domains [3, 44, 47]. However, our work has also suggested that the cB72.3(γ4) can be as active as the cB72.3(γ1) with LAK cells from some donors. The reason for this variation in donor reactivity with the cB72.3(γ4) is unclear at present, but it may represent differences in affinity of Fcγ III receptors for IgG4 among different individuals.

One potential use for the cB72.3(γ1) mAb, is its ability to act as a therapeutic in carcinoma patients via ADCC mechanisms. A possible drawback in the use of antibody-mediated immunotherapy, however, would be the heterogeneity of expression of the TAG-72 antigen. However, potential limitations imposed by antigen heterogeneity may be circumvented in several ways. Previous studies have shown that the heterogeneity of expression of some tumor-associated antigens, including TAG-72, is actually a modulation; i.e., tumor cell populations will express TAG-72 at some times but not others. For example, it has been shown that factors such as spatial configuration, passage in tissue culture, and hormonal milieu will effect TAG-72 expression [22]. Thus, if therapeutic doses of cB72.3(γ1) are given over a range of time, most if not all tumor cells may eventually be targets. Recent studies have also demonstrated that recombinant interferons will greatly upregulate the expression of tumor antigens such as TAG-72 on the tumor cell surface but not on normal cells. This has been demonstrated in cell lines [11], fresh effusions from carcinoma patients [13], and recently by the administration

of recombinant interferon γ to ovarian carcinoma patients with direct analysis of tumor cells before and after interferon treatment [12].

Since the TAG-72 antigen recognized by the B72.3 antibody is secreted by many tumors [27, 45], it was an unexpected finding that the cB72.3(γ1) mAb was quite effective in ADCC. Virtually all antigens that are functional targets for ADCC appear to be membrane-bound, non-secreted molecules [15, 20, 34, 51]. The TAG-72 antigen can be detected by radioimmunoassay in the ascites fluid obtained from animals bearing the OVCAR-3 ascites tumor, but the majority of antigen produced by these cells appears to be cell-associated and in a membrane location (unpublished data). Hence, it will be important to determine in future studies whether other TAG-72-antigen-positive tumor cells that liberate higher quantities of soluble antigen, such as perhaps those obtained from peritoneal effusions of ovarian carcinoma patients, can be killed as effectively through an ADCC mechanism by the cB72.3(γ1) mAb.

Our studies also demonstrated that the cB72.3(γ1), and to a lesser extent, the cB72.3(γ4), mediate ADCC with human monocytes. The lytic potency of monocytes was considerably less than that of LAK cells, but was similar to that reported for the chimeric 17-1A(γ1) mAb [44, 47]. However, unlike the latter studies, antibody-dependent lysis was only observed with interferon-activated monocytes in our experiments. It appears that this difference may be due to the properties of the antigen system and not to differences in monocyte preparations since we found that untreated monocytes mediated ADCC with the 17-1A mAb. The cB72.3(γ1) was more active than the cB72.3(γ4) in monocyte ADCC assays while the native B72.3 was inactive, most likely because of its murine IgG1 isotype. This difference between the chimeric IgG subclasses in mediating monocyte ADCC was also found with 17-1A [47]. The association of chimeric B72.3(γ1) ADCC with interferon-activated monocytic effector cells provides an additional mechanism by which combined monoclonal antibody and lymphokine/cytokine treatment could lead to a potential therapeutic modality.

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