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Human monocyte-mediated cytotoxicity towards erythrocytes induced by hybrid mouse monoclonal antibodies: effect of antibody binding valency on IgG-FcγR interaction

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SUMMARY

In this study, we describe the ability of hybrid mouse monoclonal antibody (mAb) to induce monocyte-mediated cytotoxicity towards human IgA1-coated E (HuIgA1-E), and the effect of mAb binding valency on FcγRI-mediated ADCC. All hybrid monospecific (ms) anti-HuIgA1 and bispecific (bs) anti-HuIgA1/HRP mAb were capable of inducing monocyte-mediated lysis of HuIgA1-E, in spite of differences in mAb densities essential for optimal lysis. The cytotoxicity induced by hybrid mAb which consist of one or more mIgG2a H chains was predominantly mediated via FcγRI, as shown by inhibition studies on monocytes with FcγRI-blocking mAb TB-3 (≈80% inhibition). However, partial inhibition of mIgG1-2a and mIgG2a-2b-induced cytotoxicity (20–50%) was observed by using FcγRII-blocking mAb IV.3 or CIKM5. For hybrid mIgG1-1 mAb the opposite was true; the cytotoxicity was predominantly mediated via FcγRII (70–80%) and less via FcγRI (20–30%). Comparing the hybrid ms anti-HuIgA1 mAb-induced cytotoxicity with the cytotoxicity induced by hybrid bs anti-HuIgA1/HRP mAb of the same isotype, we observed a decrease in cytotoxicity towards HuIgA1-E sensitized with univalently bound bs anti-HuIgA1/HRP mAb. This decrease was only found for FcγRI-mediated ADCC (mIgG2a-2a, mIgG1-2a and mIgG2a-2b). This diminished recognition of univalently bound IgG relative to bivalently bound IgG by FcγRI was also observed with U937 effector cells. In conclusion, this work shows that hybrid mAb are able to induce monocyte-mediated cytotoxicity towards E-HuIgA1 and that there appears to be an effect of Ag-IgG binding valency on FcγRI-mediated cytotoxicity.

INTRODUCTION

In recent years, monoclonal antibodies (mAb) have been used as immunotherapeutic agents against cancer, both in human patients and animal models. The isotype of the mAb used plays an important role in anti-tumour activity both *in vivo* and *in vitro*. Studies using animal models have shown that mIgG2a is the most efficient mouse isotype. In most of these studies, macrophages appeared to be the major source of effector cells by elimination of the tumour cells via antibody-dependent cellular cytotoxicity (ADCC).[1,2] In the human system, large granular lymphocytes, neutrophils, and monocytes are potential mediators of tumour cell destruction by virtue of their ability to mediate ADCC.[3]

New developments in the hybridoma technology made it possible to raise hybrid bispecific (bs) mAb.[4,5] There are several applications of bs mAb, e.g. in immunohistochemistry, in immunoassays[6] and in immunotherapy. Bs mAb are potential tools in targeting tumour cells to effector mechanisms, e.g. cytotoxic T cells[7,8] or cytotoxic agents.[9] Furthermore, bs mAb may display enhanced recognition of cells that co-express two-surface antigens (Ag) relative to cells expressing only one Ag, resulting in an enhanced C-mediated lysis of these cells in the presence of rabbit C.[10] This enhanced recognition of cells co-expressing two surface Ag by bs mAb may also be relevant in ADCC. The ability of (bs) mAb to activate effector cells and

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; anti-HuIgA1/2a, anti-HuIgA1 mAb of the IgG2a isotype; anti-HuIgA1/HRP/1-2b, bispecific anti-HuIgA1/HRP mAb of the IgG1-2b isotype; bs, bispecific; FcαR, receptor(s) for the Fc moiety of IgA; FcγR, receptor(s) for the Fc moiety of IgG; HR, high responder; HRP, horseradish peroxidase; hu, human; HuIgA1, human IgA1; LR, low responder; mIgG2a, mouse immunoglobulin(s) of the IgG2a isotype; ms, monospecific; PE, phycoerythrin; RFL, red fluorescence; TRITC, tetramethylrhodamine isothiocyanate.

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induce ADCC activity depends on the interaction between FcγR on the effector cells and the (bs) mAb.

Human monocytes may express three classes of IgG FcR: FcγRI, FcγRII and FcγRIII. FcγRI binds monomeric IgG. The other two FcγR interact well with only complexed or aggregated IgG.[11,12] Only FcγRI and FcγRII are expressed by freshly isolated or 2-day cultured monocytes,[13] and both types of FcγR are involved in ADCC.[3]

Bs mAb may possess recombinant Fc parts consisting of two different H chains. Recently, we observed the binding of hybrid mIgG1-2a and mIgG2a-2b mAb to FcγRI on the human monocytic cell line U937.[14] Furthermore, we demonstrated activation of these U937 cells after cross-linking of FcγRI-bound hybrid mAb either by anti-Ig antibodies, resulting in Ca^{2+} mobilization, or by target cell Ag, resulting in lysis of HuIgA1-E via ADCC.[15]

In this report we describe the ability of hybrid mIgG to mediate cellular cytotoxicity towards Ag-sensitized E using human monocytes as effector cells. Moreover, we show that there is an effect of antibody binding valency on the interaction between FcγR and mIgG.

MATERIALS AND METHODS

Cell lines

Hybridomas producing mAb recognizing horse-radish peroxidase (anti-HRP, mIgG1) or human IgA1 (anti-HuIgA1, mIgG1), both kindly provided by Dr J. J. Haayman (TNO, Rijswijk, The Netherlands), their isotype switch variants (isolated as described in ref. 14), hybrid hybridomas (see below) and the monocytic cell line U937 were grown in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS) as described in ref. 14. All cell lines were mycoplasma free.

Hybrid hybridoma and mAb isolation

Hybrid hybridomas were isolated as described in ref 5. Parental, monospecific (ms) hybrid, and bispecific (bs) hybrid mAb were isolated from ascites fluid using a combination of protein A chromatography and Ag-affinity chromatography as described in ref 14. Isolated mAb were concentrated and the protein concentration was determined using the method described by Lowry.[16] To remove aggregates, the Ag-affinity- and protein A-purified mAb were ultracentrifuged (1·5 hr at 100,000 ***g***, 4°), snap-frozen in small aliquots in liquid nitrogen and stored at −70° (referred to as monomeric mAb). The purity of ms and bs hybrid mAb preparations was determined to be more than 99·5%, using mouse isotype specific ELISA.[14]

Preparation of monocytes

Monocytes were obtained from blood of normal volunteers. Mononuclear cells were isolated by Ficoll-Paque centrifugation (d = 1·077 g/ml, Pharmacia, Uppsala, Sweden, 1000 ***g*** for 20 min). The interface layer was washed twice and resuspended in complete medium. Monocytes were allowed to adhere to polystyrene tissue culture flasks for 1–2 hr at 37°. Thereafter, non-adherent cells were removed by washing twice with cold medium and adherent cells were stimulated with recombinant interferon-gamma (rIFN-γ) (100 IU/ml). After 2 days, adherent cells were collected, washed twice, resuspended in complete medium at a concentration of $0{\cdot}5 \times 10^6$/ml and kept on ice until used. Between 70 and 85% of these adherent cells (referred to as monocytes) appeared to be $CD14^+$, as determined by immunofluorescence. The remaining cells were lymphocytes.

Preparation of HuIgA1-E

Human erythrocytes, isolated from the same blood samples from which the monocytes were isolated, were washed five times with 0·9% NaCl. Fifty-microlitre packed erythrocytes were resuspended in 0·8 ml NaCl and 100 μl of serial dilutions of HuIgA1 (0·5–50 μg) was added. HuIgA1 was coupled to the erythrocytes by incubation in 1 ml $CrCl_3$ (25 μg/ml in 0·02 M sodium acetate, pH 5·5) for 10 min. The reaction was stopped by washing the HuIgA1-E in phosphate-buffered saline (PBS). HuIgA1-E with the highest amount of bound HuIgA1, as detected by immunofluorescence, were stored at 4° in 0·9% NaCl supplemented with 10% FCS, and used within 2 days.

mAb used for the characterization of FcγR binding sites

Ascites fluid of TB-3 mAb[17] was generously donated by Dr René Van Lier (CLB, Amsterdam, The Netherlands). Culture supernatants of anti-FcγRI mAb 32.2[18] and anti-FcγRII mAb IV.3[19] were provided by Dr Paul Guyre (Dartmouth Medical School, NH) and IgG fraction of anti-FcγRII mAb CIKM5[20] by Dr Glenn Pilkington (The Cancer Institute, Melbourne, Australia).

Antibody-dependent cellular cytotoxicity assay

A total of 10^7 HuIgA1-E were incubated with 150–200 μCi ^{51}Cr ($Na_2{}^{51}CrO_4$, Radiochemical Centre, Amersham, Bucks, U.K.) for 1 hr at 37°. After washing, $2{\cdot}5 \times 10^4$ ^{51}Cr-labelled HuIgA1-E (in 25 μl complete medium) were incubated in round-bottomed microtitre plates (Costar, Cambridge, MA) with serial dilutions of parental anti-HuIgA1, hybrid monospecific anti-HuIgA1 and hybrid bispecific anti-HuIgA1/HRP mAb (25 μl) for 30 min at room temperature in triplicate. Then 50 μl ($0{\cdot}5 \times 10^6$/ml) monocyte suspension was added, the plates were centrifuged for 5 min at 50 ***g*** and incubated at 37° in a humidified CO_2 incubator. After 90 min, ^{51}Cr release was promoted by addition of 100 μl of a hypotonic (17 mM) NaCl solution. Finally, the plates were centrifuged (5 min, 500 ***g***), and 100 μl of supernatant was collected and quantitated using a gamma counter. The ADCC, expressed as percentage of specific cytotoxicity was calculated as $100 \times (E-S)/(M-S)$, where E = experimental release, S = spontaneous release, and M = maximal release. S was determined by incubation of the target cells without effector cells, and M was obtained by addition of saponin (3% final concentration). S was always $< 5\%$ of M. For blocking experiments, the monocytes were incubated with putative blocking antibodies for 30 min at 4°, before being added to the ^{51}Cr-labelled HuIgA1-E, which were sensitized with 0·1 μg/ml parental or hybrid mAb.

Flowcytometric analysis

HuIgA1-E were washed twice with PBS, 0·5% bovine serum albumin (BSA), and 0·02% NaN_3 (CFG buffer), and 5×10^5 HuIgA1-E were incubated with 0·5 ml of serial dilutions of purified parental and hybrid mAb, diluted in CFG buffer, for 30 min at room temperature. After washing with CFG buffer, the cells were incubated with rat anti-mouse kappa-phycoerythrin (κ-PE) (Becton Dickinson, Mountain View, CA) for 30 min at 4°. Finally the cells were washed twice and analysed using an Ortho 50-H flowcytometer.

Table 1. Types of hybrid monospecific and bispecific mAb used in this study

Parental hybridomas		Hybrid		
I	II	hybridoma	Isotype	Specificity*
Anti-HuIgA1/1	Anti-HuIgA1/2a	Anti-HuIgA1/1-2a	IgG1-2a	ms
Anti-HuIgA1/1	Anti-HuIgA1/2b	Anti-HuIgA1/1-2b	IgG1-2b	ms
Anti-HuIgA1/1	Anti-HRP/1	Anti-HuIgA1/HRP/1-1	IgG1	bs
Anti-HuIgA1/2a	Anti-HRP/2a	Anti-HuIgA1/HRP/2a-2a	IgG2a	bs
Anti-HuIgA1/2b	Anti-HRP/2b	Anti-HuIgA1/HRP/2b-2b	IgG2b	bs
Anti-HuIgA1/1	Anti-HRP/2a	Anti-HuIgA1/HRP/1-2a	IgG1-2a	bs
Anti-HuIgA1/2a	Anti-HRP/1	Anti-HuIgA1/HRP/2a-1	IgG1-2a	bs
Anti-HuIgA1/1	Anti-HRP/2b	Anti-HuIgA1/HRP/1-2b	IgG1-2b	bs
Anti-HuIgA1/2b	Anti-HRP/1	Anti-HuIgA1/HRP/2b-1	IgG1-2b	bs
Anti-HuIgA1/2a	Anti-HRP/2b	Anti-HuIgA1/HRP/2a-2b	IgG2a-b	bs
Anti-HuIgA1/2b	Anti-HRP/2a	Anti-HuIgA1/HRP/2b-2a	IgG2a-2b	bs

* ms, monospecific; bs, bispecific.

RESULTS

Monospecific or bispecific hybrid mAb-producing cell lines

To investigate the ability of hybrid mAb to mediate ADCC, we generated a panel of 11 different hybrid hybridomas producing hybrid mAb. Isotype switch variant cells specific for HRP (anti-HRP) or human IgA1 (anti-HuIgA1) were used to obtain two different groups of hybrid mAb-producing hybridoma cell lines. One group was made by the fusion of two isotype switch variant hybridomas recognizing HuIgA1. The hybrid mAb produced by these hybrid hybridomas are monospecific (ms) and differ only in their recombinant Fc part from the parental anti-HuIgA1. The other group of hybrid hybridomas was obtained by the fusion of an anti-HuIgA1 cell line and an anti-HRP hybridoma. The hybrid mAb, produced by this second group of hybrid cell lines are bispecific and recognize both Ag (HuIgA1 and HRP). These so-called hybrid bispecific (bs) mAb may differ in the combination of isotype H chains used to form their Fc parts (see Table 1).

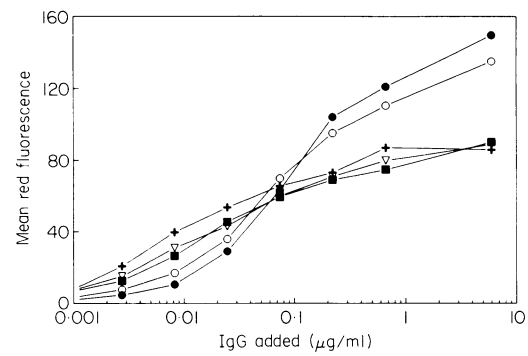

Figure 1. Binding of different parental and hybrid ms anti-HuIgA1 and hybrid bs anti-HuIgA1/HRP mAb to HuIgA1-E. Serial dilutions (in complete medium) of purified anti-HuIgA1/1 (▽), anti-HuIgA1/2a (+), hybrid ms anti-HuIgA1/1-2a (■), hybrid bs anti-HuIgA1/HRP/1-2a (○) or hybrid bs anti-HuIgA1/HRP/2a-2a (●) mAb were added to $0{\cdot}5 \times 10^6$ HuIgA1-E. After washing, bound IgG was detected using PE-conjugated rat anti-mouse κ mAb.

Binding characteristics of monospecific and bispecific hybrid mAb

The Age binding sites of the protein A and/or Ag-affinity purified isotype switch variant mAb and monospecific (ms) hybrid mAb remained unchanged as determined using an inhibition ELISA.[21] However, the binding characteristics of hybrid bs anti-HuIgA1/HRP mAb differed from those of the parental anti-HuIgA1, hybrid ms anti-HuIgA1 or anti-HRP mAb. Figure 1 shows a representative immunofluorescence experiment using HuIgA1-coated erythrocytes (HuIgA1-E). At low antibody concentrations, more (bivalent) parental or hybrid ms anti-HuIgA1 mAb bind to HuIgA1-E relative to bs mAb. At higher antibody concentrations, however, approximately twice as much bs anti-HuIgA1/HRP mAb was bound by the same HuIgA1-E, compared to parental or hybrid ms mAb. This is most likely due to the univalent interaction of the bs anti-HuIgA1/HRP mAb with HuIgA1. The fit of these experimental data is excellent in the theoretical model of mAb binding to cell surface antigens recently proposed by Larsson.[22] The same results were obtained using polyclonal goat anti-mouse Ig-FITC antibodies to detect bound IgG (data not shown). This excluded the possibility that the differences in fluorescence intensities between parental anti-HuIgA1 mAb and hybrid anti-HRP/HuIgA1 mAb were due to a decreased recognition of the hybrid mAb by the monoclonal rat anti-mouse κ-PE antibodies.

Induction of ADCC towards HuIgA1-E by parental and hybrid ms anti-HuIgA1 mAb

The ability of hybrid mIgG molecules to induce monocyte-mediated lysis was investigated using HuIgA1-E as target cells. In order to be able to compare lysis of HuIgA1-E induced by conventional parental anti-HuIgA1 mAb with that induced by hybrid ms anti-HuIgA1 or bs anti-HuIgA1/HRP mAb, we determined the (relative) amount of IgG bound per HuIgA1-E using a rat anti-mouse kappa mAb conjugated to PE and measured the immunofluorescence intensity. These immunofluorescence experiments were performed under exactly the same conditions as the ADCC experiments. The percentage lysis of HuIgA1-E was then plotted versus the relative amount of IgG

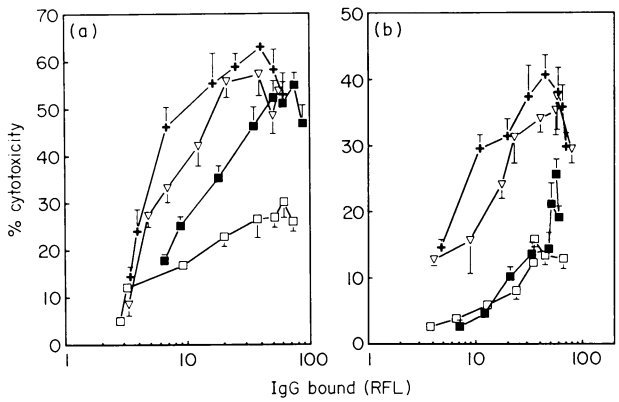

Figure 2. Parental and hybrid monospecific anti-HuIgA1 mAb-mediated lysis of HuIgA1-E by human monocytes. ^{51}Cr-labelled HuIgA1-E were incubated with serial dilutions of anti-HuIgA1/1 (■), anti-HuIgA1/2a (+), anti-HuIgA1/2b (□) or hybrid anti-HuIgA1/1-2a mAb (▽) for 30 min at room temperature before rIFN-γ-stimulated monocytes were added to react for 90 min at 37 . Chromium release was measured after hypotonic lysis of damaged HuIgA1-E and plotted versus the relative amount of IgG bound to HuIgA1-E. Two different reaction patterns were observed (a and b). Results represent mean ± SD of triplicate wells and are representative for nine (a) and two (b) experiments, respectively.

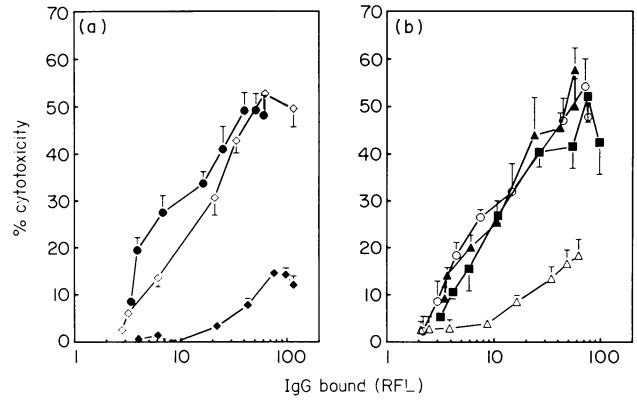

Figure 3. Hybrid bispecific anti-HuIgA1/HRP mAb-mediated lysis of HuIgA1-E by human monocytes. ^{51}Cr-labelled HuIgA1-E were incubated with serial dilutions of (a) anti-HuIgA1/HRP/1-1 (◇), anti-HuIgA1/HRP/2a-2a (●) or anti-HuIgA1/HRP/2b-2b (◆) or (b) anti-HuIgA1/HRP/1-2a (▲), anti-HuIgA1/HRP/2a-1 (○), anti-HuIgA1/HRP/2a-2b (■) or anti-HuIgA1/HRP/1-2b (△) mAb for 30 min at room temperature before rIFN-γ-stimulated monocytes were added to react for 90 min at 37 . Chromium release was measured after hypotonic lysis of damaged HuIgA1-E and plotted versus the relative amount of IgG bound to the HuIgA1-E. Results represent mean ± SD of triplicate wells and are representative for four individual experiments.

(RFL) bound to HuIgA1-E. Monocytes of healthy donors were isolated and cultured for 2 days in the presence of rIFN-γ, and used as effector cells. When we evaluated the ability of the parental anti-HuIgA1 mAb to induce lysis of HuIgA1-E, two types of monocytes could be distinguished: monocytes able to mediate lysis of HuIgA1-E, which were sensitized with relatively low densities of mIgG1 (Fig. 2a, $n=9$) and monocytes killing only HuIgA1-E sensitized with high densities of mIgG1 (Fig. 2b, $n=2$). The mIgG2a-induced cytotoxicity (very efficient at low Ig densities) and mIgG2b-induced cytotoxicity (very little lysis even at high densities) did not differ between the two types of monocytes. No cytotoxicity was observed in the absence of anti-HuIgA1 mAb. The difference in level of mIgG2a-mediated cytotoxicity between the two types of monocytes is most likely due to differences in Ag (and thus mAb) density on the HuIgA1-E targets, since the experiments shown were performed on different days with freshly prepared targets.

Of the hybrid monospecific antibodies, anti-HuIgA1/1-2a mAb were less effective in inducing lysis of HuIgA1-E, when compared with parental anti-HuIgA1/2a mAb. A higher mIgG1-2a mAb density was needed to obtain similar levels of lysis as induced by the parental anti-HuIgA1/2a mAb. Moreover, both types of monocyte lysed HuIgA1-E very efficiently in the presence of hybrid mIgG1-2a antibodies. Hybrid ms anti-HuIgA1/1-2b mAb demonstrated the same cytotoxicity pattern as the parental anti-HuIgA1/2b mAb (data not shown).

Induction of ADCC towards HuIgA1-E by hybrid bs anti-HuIgA1/HRP mAb

We subsequently evaluated the ability of bs anti-HuIgA1/HRP mAb to induce ADCC. The only difference beween the parental or hybrid ms anti-HuIgA1 mAb and hybrid bs anti-HuIgA1/HRP mAb is the binding valency of the mAb to Ag: a bivalent binding of the parental and ms hybrid anti-HuIgA1 mAb and a univalent binding of the hybrid bs anti-HuIgA1/HRP mAb to the HuIgA1-E targets. All hybrid bs anti-HuIgA1/HRP mAb did lyse HuIgA1-E, but again we observed a difference in mAb density to achieve similar percentages of lysis. Hybrid bs mAb with homologous H–H chain combinations (mIgG1-1, mIgG2a-2a and mIgG2b-2b) showed the same reaction pattern as the parental anti-HuIgA1 mAb: mIgG2a-2a > mIgG1-1 > mIgG2b-2b (Fig. 3a). Of the hybrid bs anti-HuIgA1/HRP with heterologous H–H chain combinations, the mIgG1-2a and mIgG2a-2b isotypes induced approximately the same cytotoxicity towards HuIgA1-E irrespective of whether the mIgG2a H chain was or was not involved in Ag binding (Fig. 3b). As already shown for ms hybrid anti-HuIgA1 mAb-mediated cytotoxicity, the mIgG1-2a and mIgG2a-2b-induced cytotoxicity was less than the cytotoxicity induced by the anti-HuIgA1/HRP/2a-2a mAb. The bs anti-HuIgA1/HRP/1-2b mAb induced a cytotoxicity similar to the lysis induced by the mIgG2b-2b isotype (Fig. 3b).

Characterization of FcγR-mediating hybrid mAb-induced cytotoxicity

The characterization of the type of FcγR involved in hybrid mAb-induced cytotoxicity was performed using antibodies that specifically block different classes of FcγR. The cytotoxicity induced by hybrid mAb, consisting of at least one mIgG2a H chain, was efficiently blocked (≈80%; Fig. 4) after preincubation of the monocytes with mIgG2a mAb TB-3, which blocks FcγRI via its Fc part;[14,23] the 'Kurlander phenomenon'.[24] When monocytes were preincubated with either FcγRII-blocking mAb IV.3 or mAb CIKM5, the cytotoxicity induced by hybrid mIgG1-1 and mIgG1-2b (data not shown) mAb was blocked for more than 80%. Furthermore, we found a significant inhibition (18–47%) of the mIgG1-2a and mIgG2a-2b-induced lysis when FcγRII was blocked, whereas the inhibition of mIgG2a-induced cytotoxicity was less than 7·5%. This may indicate that hybrid

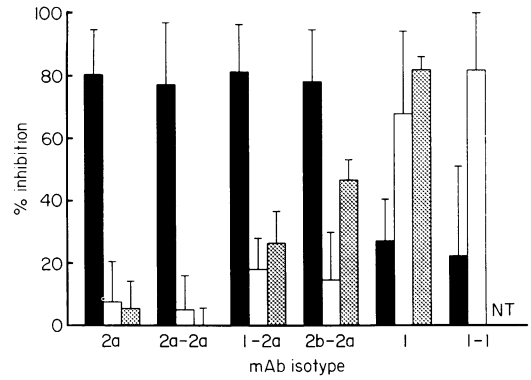

Figure 4. Effect of FcγR-blocking mAb on hybrid mAb-mediated lysis of HuIgA1-E by human monocytes. IFN-γ-stimulated monocytes were preincubated with 50 μl of saturating amounts of either FcγRI-blocking mAb TB-3 (■), anti-FcγRII mAb IV.3 (□) or anti-FcγRII mAb CIKM5 (▩). After 30 min at 4°, HuIgA1-E and 50 μl of parental or hybrid mAb (0·22 μg/ml) were supplemented and ADCC experiments were performed as described. Results are expressed as mean percentage inhibition ± SD of at least six (maximal 16) experiments. NT, not tested.

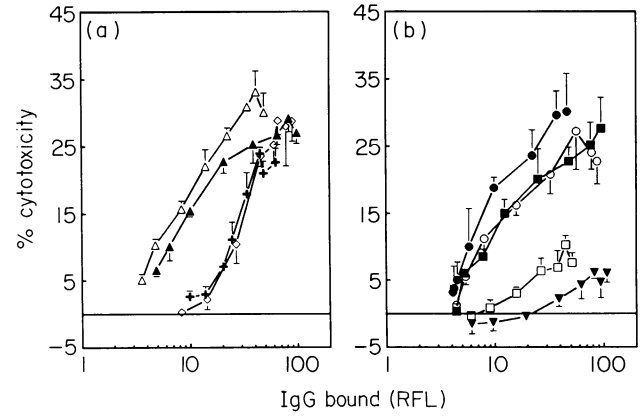

Figure 5. Comparison of bivalently and univalently bound mAb-mediated lysis of HuIgA1-E by monocytes. ^{51}Cr-labelled HuIgA1-E were incubated with serial dilutions of (a) anti-HuIgA1/1 (◇), anti-HuIgA1/2a (△), hybrid bs anti-HuIgA1/HRP/1-1 (+), or anti-HuIgA1/HRP/2a-2a (▲) or (b) anti-HuIgA1/2b (□), hybrid ms anti-HuIgA1/1-2a (●), hybrid bs anti-HuIgA1/HRP/1-2a (■), anti-HuIgA1/HRP/2a-1 (○), or anti-HuIgA1/HRP/2b-2b (▼) mAb for 30 min at room temperature before rIFN-γ-stimulated monocytes were added to react for 90 min at 37°. Chromium release was measured after hypotonic lysis of damaged HuIgA1-E and plotted versus the relative amount of IgG bound to HuIgA1-E. Results represent mean ± SD of triplicate wells and are representative for six experiments.

mIgG1-2a and mIgG2a-2b-induced cytotoxicity is predominantly mediated via FcγRI and to a lesser extent via FcγRII.

Effect of antibody binding valency on ADCC mediated by monocytes

Bs mAb bind univalently to target cells expressing only one type of Ag, recognized by the bs mAb. In contrast, the parental or hybrid ms mAb may interact with both F(ab′) fragments. We assessed the effect of differences in mAb binding valency on ADCC by comparing the lysis of HuIgA1-E induced by univalently bound (bs) anti-HuIgA1/HRP mAb with the cytotoxicity induced by bivalently bound (parental or hybrid ms) anti-HuIgA1 mAb. Figure 5, a representative experiment (out of six), shows that bivalently bound IgG was more effective in inducing monocyte-mediated lysis of HuIgA1-E compared with univalently bound IgG. This phenomenon was only observed when bivalently bound mIgG2a-2a (Fig. 5a), mIgG1-2a, mIgG2b-2b (Fig. 5b) and mIgG2a-2b (data not shown) mAb were compared to their univalently bound counterparts. Cytotoxicity of all these mAb is predominantly mediated via FcγRI (see Fig. 4). No difference was observed when univalently bound anti-HuIgA1/HRP/1-1 mAb was compared with the bivalently bound parental anti-HuIgA1/1 mAb (Fig. 5b). The decreased cytotoxicity mediated via FcγRI for univalently bound IgG was also found when U937 cells were used as effector cells (data not shown).

DISCUSSION

The ability of (bs) mAb to activate effector cells and induce ADCC activity depends on the interaction between FcγR on the effector cells and the (bs) mAb. Since the Fc parts of hybrid bs mAb may consist of two H chains of different isotypes, e.g. mIgG1 and mIgG2a H chain combination, we studied the interaction between FcγR and hybrid (bs) mAb. In order to be able to study the influence of H–H chain combination and the effect of Ag binding valency of the hybrid mAb on ADCC activity, we generated a panel of hybrid mAb recognizing either HuIgA1 alone (monospecific mAb), or HuIgA1 and HRP (bispecific mAb). We tested the ability of these hybrid mAb to induce lysis of HuIgA1-E in the presence of human monocytes and compared this lysis with cytotoxicity induced by the parental (isotype switch variant) anti-HuIgA1 mAb.

Human monocytes, freshly isolated or after 2-day culturing, express only FcγRI and FcγRII,[13] and both FcγR are involved in the triggering of monocytes to mediate ADCC activity.[3] It is important to note that some authors have reported the presence of FcγRIII on 2-day cultured monocytes. This receptor, however, was cytotoxically inactive.[25] FcγRII displays a functional polymorphism in anti-CD3-induced T-cell proliferation, rosette formation and ADCC activity.[11,23] Monocytes of so-called 'high-responder' (HR) individuals do support mIgG1 anti-CD3-induced T-cell proliferation and are able to lyse E, sensitized with relatively low amounts of mIgG1 mAb. Monocytes of low-responder (LR) individuals, however, do not support anti-CD3-induced mitogenesis, and they are only able to kill target cells, sensitized with very high amounts of mIgG1 mAb.[11] The two types of monocytes we observed with regard to their ability to mediate mIgG1-induced ADCC (Fig. 2) most likely reflect this functional polymorphism of FcγRII. The first type of monocytes (HR) did lyse HuIgA1-E with low numbers of mIgG1 molecules. The other type (LR) was only able to lyse HuIgA1-E sensitized with high densities of mIgG1.

All hybrid mAb tested in this study exhibited the ability to induce ADCC activity towards HuIgA1-E. Only the degree of (hybrid) mAb sensitization of HuIgA1-E essential for optimal cytotoxicity differed between the individual hybrid ms and bs mAb. The FcγR specificity for hybrid mAb with homologous

H–H chain combinations (IgG1-1, IgG2a-2a) was identical to that for the parental anti-HuIgA1 mAb as determined by using mAb that specifically block different classes of FcγR. ADCC induced by hybrid mIgG2a-2a mAb was predominantly mediated via the high affinity FcγRI (>80%) and only to a minor extent via FcγRII (Fig. 4). This is in contrast to mIgG1-1-induced cytotoxicity, which was mainly mediated via FcγRII (70–80%), and less via FcγRI (20%). The FcγR specificities for hybrid mAb with homologous H–H chain combinations is in accordance with data obtained with normal mIgG1 and mIgG2a antibodies, as reported by others (see ref. 11). FcγR modulation studies with high densities of complexed mIgG2a mAb[26] and ADCC experiments using monocytes preincubated with blocking anti-FcγR mAb[23] revealed that mIgG2a presented at high density interacts with both types of FcγR. The interaction with FcγRI, however, is always stronger than with FcγRII. The reverse is found for mIgG1 mAb (which interacts well with FcγRII and much less with FcγRI). Lysis of E sensitized with high densities of mIgG1 anti-glycophorin A mAb could be blocked by an anti-FcγRII mAb for more than 80%. The remaining cytotoxicity was sensitive to inhibition by an FcγRI-blocking mAb.[23] These data may indicate that the specificities of FcγRI and FcγRII for mouse hybrid (ms and bs) IgG are only relative and that the results depend critically on the IgG density used in the experiments. We could not detect any ADCC towards HuIgA1-E in the absence of mouse mAb, this excludes the possibility that the observed ADCC towards the HuIgA1-E was mediated via FcαR, also present on human monocytes.[27]

Lysis of HuIgA1-E induced by hybrid mIgG1-2a or mIgG2a-2b mAb was predominantly mediated via FcγRI. Both types of monocytes from HR and LR individuals mediated mIgG1-2a (Fig. 2) and mIgG2a-2b (data not shown) induced cytotoxicity to a similar extent. Furthermore, the FcγRI-blocking mAb TB-3 inhibited this cytotoxicity for more than 80% (Fig. 4). However, the contribution of FcγRII to the lysis induced by these hybrid mAb was higher compared to the FcγRII contribution in mIgG2a (or mIgG2a-2a)-mediated cytotoxicity. These data are in accordance with observations in Ca^{2+} mobilization studies in which we found that anti-FcγRII mAb could inhibit the Ca^{2+} mobilization, induced by cross-linking of FcγRI-bound hybrid mIgG1-2a mAb.[15] All our data support the fact that both classes of FcγR are involved in mIgG1-2a or mIgG2a-2b-induced cytotoxicity towards E-HuIgA1.

By comparing bivalently bound hybrid ms anti-HuIgA1-induced cytotoxicity with the lysis induced by univalently bound bs anti-HuIgA1/HRP mAb we observed a decreased recognition of univalently bound mIgG2a, mIgG1-2a and mIgG2b compared with their bivalently bound counterparts in each individual experiment. Since no difference was observed in mIgG1-induced cytotoxicity, this may indicate that only FcγRI-specific IgG recognition is sensitive to binding valency. Further studies need to be performed in order to determine whether this difference in FcγRI recognition is due to either a conformational change in the Ig molecule, induced by the bivalent binding of the Ig molecule, or to a more favourable orientation of the bivalently bound Ig molecule, facilitating a better recognition of IgG by FcγRI. In conclusion we found that hybrid mAb are able to induce monocyte-mediated cytotoxicity towards HuIgA1-E and that there is an effect of Ag-IgG binding valency on the FcγRI-mediated cytotoxicity.

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