Antitumor Immune Effector Mechanisms Recruited by Phage Display-derived Fully Human IgG1 and IgA1 Monoclonal Antibodies

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ABSTRACT

We have constructed a recombinant, fully human IgA1 monoclonal antibody, UBS-54/IgA1, against the tumor-associated Ep-CAM molecule and compared its tumor-killing capacity with its IgG1 counterpart in in vitro assays. The data show that phage display-derived fully human IgA1 antibodies efficiently recruit immune effector cells that express the Fc receptor for IgA, FcαRI (CD89). UBS-54/IgA1-mediated killing of tumor cells by isolated polymorphonuclear cells (PMNs) and in whole blood was found to proceed without the necessity to preactivate effector cells with cytokines. In addition, the IgA1 anti-Ep-CAM human monoclonal antibody (huMab) triggered phagocytosis of tumor cells by monocyte-derived macrophages. Strikingly, simultaneous addition of IgA1 and IgG1 anti-Ep-CAM antibodies did not result in enhancement of tumor cell killing unless the effector cells were stimulated with granulocyte colony-stimulating factor. The lack of an additive effect could be attributed to an inhibitory effect of IgG on IgA-mediated tumor cell killing through binding of IgG1 to the inhibitory FcγRIIb receptor expressed by PMNs. These results show that IgA1 antitumor huMabs are capable of recruiting the large population of peripheral blood PMNs for tumor cell killing. This population is not effectively recruited by IgG type antibodies, currently the antibodies most frequently used for clinical application. In addition, the data suggest that a combination of IgG1 and IgA1 antitumor huMabs may collaborate in tumor cell killing in patients treated with granulocyte colony-stimulating factor.

INTRODUCTION

The recent clinical success of engineered chimeric and humanized monoclonal antibodies in tumor immunotherapy (1, 2) has rekindled interest in immune effector functions recruited by antibodies of different isotypes and subclasses. The cytotoxic effect of antibodies is mediated by interaction of their constant (Fc) regions with complement proteins and with immunoglobulin (FcR) receptors expressed on various types of immune effector cells. Fc receptors constitute a family of cell-surface molecules capable of eliciting intracellular signals upon binding of antigen-antibody complexes. Three classes of leukocyte FcR for IgG, FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16), have been identified, encompassing 12 receptor isoforms (3, 4). In peripheral blood, FcγRI is expressed on monocytes and dendritic cells and can be induced in PMNs5 by interferon-γ (5) or G-CSF (6). FcγRI shows the broadest cell distribution, being constitutively present on monocytes, B cells, platelets, dendritic cells, eosinophils, basophils, and neutrophils. FcγRIII is found on monocytes/macrophages and natural killer cells as a transmembrane molecule (FcγRIIa), and on PMNs as a glycosylphosphatidylinositol-linked protein (FcγRIIb). The ability of immunoglobulin molecules to activate immune effector functions is further influenced by the binding to the inhibitory Fc receptor FcγRIIb expressed on macrophages, B cells, and basophils (7). The FcγRIIb molecules harbor a unique ITIM signaling motif in their cytoplasmic tails (8), in which phosphorylation results in recruitment of down-regulating type phosphatases to activating receptor clusters SH2 domain-containing protein tyrosine phosphatase and SH2 domain-containing inositol phosphatase (9–11).

Like FcγRI, cross-linking of FcαRI results in ADCC, phagocytosis, endocytosis, induction of respiratory burst, and release of inflammatory mediators and cytokines (12, 13). Comparison of the distribution of FcγRI and FcαRI on cytotoxic and noncytotoxic cell types suggests a more favorable distribution for therapeutic effect of the latter. FcαRI expression primarily is limited to immune effector cells that demonstrate cytotoxic activities, whereas FcγR receptors are also expressed on noncytotoxic cells (B lymphocytes, platelets) or on effector cells that do not efficiently trigger cytotoxic function (for example, FcγRIIb on PMNs). Recent experiments with bispecific antibodies recognizing FcαRI on effector cells and antigens on tumor cells have demonstrated that in vitro, FcαRI effectively mediates ADCC by PMNs and isolated monocytes (14, 15). Several in vivo studies have suggested an active role for PMNs in the immunosurveillance against malignant tumors (16, 17). To achieve efficient PMN-mediated killing through FcγRI, the PMNs must be preactivated with cytokines (18, 19), whereas a functional FcαRI is constitutively expressed by PMNs.

The tumor-associated antigen Ep-CAM (also known as 17-1A, GA-733, KSA, and EGP-2) has been shown to be an attractive target for antibody-based therapy. Treatment of patients with colon carcinoma with a murine monoclonal antibody against Ep-CAM (17-1A) reduced 5-year mortality by 32% (20). Application of murine antibodies for therapy does not optimally recruit human effector functions and generally leads to the induction of a human antimmunoglobulin response. To circumvent this problem, we recently used a semisynthetic phage antibody display library and subtractive phage selection on colon carcinoma cells to obtain a human scFv antibody fragment specific for human Ep-CAM (21). The V regions encoding this scFv antibody were recloned in eukaryotic expression vectors containing human IgG1 and K constant regions and expressed in eukaryotic cells to generate a fully human monoclonal, IgG1/K monoclonal antibody UBS-54/IgG1 (21). UBS-54/IgG1 has an affinity of 5 nM and has been shown to perform well in in vitro and in vivo models of tumor cell killing (21).

Here we used the V regions of scFv UBS-54 to engineer and produce a fully human anti-Ep-CAM antibody of the IgA1 isotype (UBS-54/IgA1), to our knowledge the first IgA anti-tumor huMab. The biochemical and functional characterization of huMab UBS-54/IgA1 is described in this report. Furthermore, we compared the tumor killing potential of the IgA1 and IgG1 huMabs in in vitro assays and analyzed additive/antagonistic effects induced by simultaneous application of both isotypes.

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3 The abbreviations used are: PMN, polymorphonuclear cell; G-CSF, granulocyte colony-stimulating factor; ITIM, immunoreceptor tyrosine-based inhibition motif; ADCC, antibody-dependent cell-mediated cytotoxicity; sc, single chain; huMab, human monoclonal antibody; BHK, baby hamster kidney cell line; PBMC, peripheral blood mononuclear cell; CDCC, complement-dependent cellular cytotoxicity; MDM4, monocyte-derived macrophage.

MATERIALS AND METHODS

Cells and Antibodies. Cell lines used in this study were maintained in RPMI 1640, DMEM, or Iscove’s modified Dulbecco’s medium (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS (Life Technologies), 100 units/ml penicillin, and 100 μg/ml streptomycin.

The following cell lines were used: the colon carcinoma cell line LS174T (ATCC CL188) and BHK stably transfected with the furin gene (fur-BHK; 22). The L929 fibroblast cell line and the L929 cells transfected with human Ep-CAM cDNA (LME-6) were a kind gift of Dr. S. Litvinov (University of Leiden, The Netherlands; 23). 3T6 cells transfected with human FcγRIIB cDNA have been described elsewhere (24).

The following antibodies were used: FITC-conjugated goat antihuman κ light chain and antihuman IgA and IgG heavy chain (Southern Biotechnology, Birmingham, AL); FITC-conjugated 3G8 (FcγRIII, CD16), AT10 (FcγRII, CD32), and 197 (FcγRI, CD64); Medarex, Annandale, NJ); and phycocerythrin-conjugated anti-CD89 (FcεRI; Immunotech, Hialeah, FL). The F(ab’)_2 fragments directed against Ep-CAM were derived from the murine anti Ep-CAM monoclonal 323/A3 and were a kind gift of Dr. S. Litvinov. For blocking studies, the F(ab’)_2 fragments of 3G8 and AT10, and whole IgG2a of 197 were used.

Construction and Production of Human IgG1 and IgA1 Anti-Ep-CAM Monoclonal Antibodies. We recently described the engineering and production of the human IgG1 anti-Ep-CAM huMab UBS-54 (21). To construct huMab UBS-54 of the IgA1 isotype, the V_H and V_L regions encoding scFv UBS-54 were excised and recloned into vectors for expression of complete human IgA1/κ light chain and antihuman κ light chain and antihuman IgA and IgG heavy chain (Southern Biotechnology, Birmingham, AL); FITC-conjugated 3G8 (FcγRIII, CD16), AT10 (FcγRII, CD32), and 197 (FcγRI, CD64); Medarex, Annandale, NJ); and phycocerythrin-conjugated anti-CD89 (FcεRI; Immunotech, Hialeah, FL). The F(ab’)_2 fragments of 3G8 and AT10, and whole IgG2a of 197 were used.

Complement and Complement-mediated Lysis. The cytolytic activity of huMab in tumor cell killing. The rationale for this approach was based on the functional capacity of an IgA1 isotype variant of UBS-54 and to determine huMab in tumor cell killing. The rationale for this approach was based on the functional capacity of an IgA1 isotype variant of UBS-54 and to determine huMab in tumor cell killing. The rationale for this approach was based on the functional capacity of an IgA1 isotype variant of UBS-54 and to determine huMab in tumor cell killing. The rationale for this approach was based on the functional capacity of an IgA1 isotype variant of UBS-54 and to determine huMab in tumor cell killing. The rationale for this approach was based on the functional capacity of an IgA1 isotype variant of UBS-54 and to determine huMab in tumor cell killing.
a peptide leader sequence from a T-cell receptor α-chain at the 5' end and a splice donor site at the 3' end. Subsequently, the V_h and V_l genes with appended leader and splice donor sequences were inserted into constructs containing the human immunoglobulin Ca1 and Ck constant region genes, respectively. The V_h/Ca1 and V_l/Ck constructs were cotransfected in BHK cells, and methotrexate-resistant clones were expanded and used for large-scale antibody production in a hollow fiber culture system. huMab UBS-54/IgA1 was purified by passing culture supernatant over an anti-IgA1 affinity column, yielding 5 mg of purified protein per liter of culture supernatant. For comparison, UBS-54/IgG1 gave a 10-fold higher yield after production in the same system and purification over a protein A column (21).

The integrity and purity of purified huMab UBS-54/IgA1 was confirmed by denaturing and non-denaturing SDS-PAGE and subsequent staining with Coomassie brilliant blue. Note that the UBS-54/ IgA1 heavy chain runs higher in the gel than the corresponding IgG1 heavy chain, correlating with the 10-kDa difference in molecular weight between these two isotypes (Fig. 1). As determined by immunofluorescence analysis, purified huMab USB-54/IgA1 binds specifically to the Ep-Cam-transfected cell line LME-6 but not to the nontransfected L929 fibroblast parental cell line (Fig. 2).

**Tumor Cell-killing Potential of UBS-54/IgA1.** We compared the efficiency of UBS-54/IgG1 and UBS-54/IgA1 in recruiting immune effector functions in vitro. ADCC assays with PBMCs demonstrated that killing of LS174T colon carcinoma cells by UBS-54/IgG1 was significantly \( P < 0.05 \) more efficiently compared with killing by UBS-54/IgA1 (Fig. 3). In contrast, when purified PMNs were used as effector cells, UBS54/IgA1 mediated tumor cell killing significantly \( P < 0.05 \) more efficiently than UBS-54/IgG1 (Fig. 3). We noted that the combination of IgG1 and PBMCs resulted in a higher percentage of specific lysis compared with the combination of IgA1 and purified PMNs. In addition, maximum lysis was achieved at lower antibody concentrations in the former combination (Fig. 3). In a cytotoxicity assay with whole blood as source of immune effector cells, in which both ADCC and CDCC are measured, the UBS-54/IgG1 antibody more efficiently mediated tumor cell killing than UBS-54/IgA1 (Fig. 3).

To assess the contribution of complement in tumor cell killing, huMabs were added to target tumor cells in the presence of freshly isolated serum as a source of complement (Fig. 3). Only the UBS-54/IgG1 antibody mediated CDCC in the presence of 25% serum \( P < 0.05 \). This was completely abrogated when complement was inactivated by heating the serum at 56°C (not shown). Thus, UBS-54/IgG1 but not UBS-54/IgA1 is capable of activating complement.

**Tumor Cell-killing Potential of Combinations of UBS-54/IgG1 and UBS-54/IgA1.** We evaluated whether UBS-54/IgG1 and UBS-54/IgA1 huMabs were additive in tumor cell killing. To that end, equal quantities of IgA1 and IgG1 huMabs in a (combined) dose range between 0 and 20 \( \mu \)g/ml were incubated with various sources of immune effector cells. With PBMCs, the dose-response curve of the IgG1/IgA1 combination was virtually identical to the dose-response curve of IgG1 alone (Fig. 3). Because IgA1 is not functional in this assay, we conclude that in the dose range used, the simultaneous binding of UBS-54/IgA1 and UBS-54/IgG1 to the Ep-CAM target antigen does not affect IgG1-mediated killing in this assay. Strikingly, with purified PMNs, the combination of UBS-54/IgA1 and UBS-54/ IgG1 consistently induced a significantly \( P < 0.05 \) lower specific lysis of tumor cells than that obtained with UBS-54/IgA1 alone (Fig. 3). In fact, we expected an additive effect on tumor cell killing by combining IgG1 and IgA1 antibodies in this assay. However, apparently the addition of the IgG1 antibody caused a decrease in specific lysis mediated by the IgA1 antibody. A similar effect was noted when whole blood was used as a source of effector cells: here the combination of IgG1 and IgA1 antibodies resulted in a lower specific tumor cell lysis compared with the lysis obtained with IgG1 alone (Fig. 3). It should be noted that in whole blood assays, the eventual tumor cell killing capacity is the net result of complement and FcR-mediated effects. The latter comprise the effects of IgA1 and IgG1 isotypes on the same PMN effector cell as well as the effect of triggering both PMN and PBMC effector cell populations. The negative effect of IgA1 on IgG1-mediated killing in whole blood assays could be explained by interference with the complement-binding capacity of
UBS-54/IgG1. Therefore CDCC was tested with IgG1 and IgA1 huMabs. Indeed, CDCC was completely abrogated when UBS-54/IgG1 and UBS-54/IgA1 were added simultaneously (Fig. 3).

We hypothesized that the antagonistic effect of UBS-54/IgG1 on UBS-54/IgA1-triggered PMNs may be caused by binding of UBS-54/IgG1 to the ITIM-containing inhibitory Fc receptor FcγRIIb. To address this issue, we used a panel of Fab', (Fab')2, and whole murine antibodies specific for the different classes of leukocyte FcγR receptors to block FcγR-mediated effects in ADCC with PMN effector cells. Co-incubation of PMNs with F(ab')2 fragments of antibody 3G8 and whole IgG1 of Mab 197, which block binding of IgG to FcγRIII and FcγRI, respectively, did not affect the antagonistic effect of UBS-54/IgG1 in UBS-54/IgA1-mediated ADCC (Fig. 4). In contrast, F(ab')2 fragments of AT10, which block binding of IgG to FcγRII, abolished the antagonistic effect of UBS-54/IgG1 in ADCC (Fig. 4). In fact AT10 F(ab')2 fragments enhanced IgA1-mediated ADCC (Fig.

**Fig. 3.** ADCC assays mediated via UBS-54/IgG1(●), UBS-54/IgA1(□), and combined addition of UBS-54/IgG1 and UBS-54/IgA1(×). Isolated PBMCs, PMNs, whole blood, and serum were used as sources of effector cells and molecules. In cases of combined addition of huMabs, each antibody was added at the concentration depicted on the X axis. Results from at least five experiments with effector cells from different donors are presented as the mean of the percentage of specific lysis. *, P < 0.05; bars, SE.

**Fig. 4.** Inhibition of UBS-54/IgA1-mediated tumor cell killing by UBS-54/IgG1 can be blocked with AT10 anti-FcγRIIb F(ab')2 fragments. Top panels, analysis of FcγRIII (3G8), FcγRII (AT10), and FcγRI (197) expression on isolated PMNs by flow cytometry. The specific lysis of LS174T tumor cells by PMNs from a single donor was analyzed without blocking antibody and in the presence of 10 μg/ml of the various blocking reagents. Bottom panels, ADCC assays were performed with UBS-54/IgG1(●), UBS-54/IgA1(□), and combined addition of UBS-54/IgG1 and UBS-54/IgA1(×). The experiments are representative for three independent assays, performed with PMNs from different donors.

For more detailed analysis, please refer to the original research article.
ADCC experiments with isolated PBMCs, PMNs, and whole blood from G-CSF-treated healthy individuals as a source of effector cells. ADCC is triggered via UBS-54/IgG1( ), UBS-54/IgA1( ), and combined addition of UBS-54/IgG1 and UBS-54/IgA1( ). Results from three independent experiments are presented as the mean of percentage of specific lysis. *, P < 0.05; bars, SE. Bottom right panel, flow cytometric analysis of FcεRI expression on isolated PMNs after incubation with whole immunoglobulin 197 (dashed line) or F(ab)2 fragments of anti-CD32 mAb AT10 (solid thick line). The thin solid line represents FcεRI expression on isolated PMNs without incubation with blocking reagents. The thin solid line denotes an arrow showing the negative control of the staining.

4; see below). These results suggested that the decrease in UBS-54/IgA1-induced specific lysis of target cells reflects inhibition of FcεRI-mediated activation via FcγRIIb. Similar inhibitory activity of IgG has been described for FcγRII- and FcεRI-mediated signaling (11).

We investigated the mechanism by which F(ab')2 fragments of anti-FcγRII monoclonal antibody AT10 enhanced tumor cell lysis by IgA1 antibodies. First, we determined whether human IgA1, like IgG1, binds to FcγRIIb, thereby down-regulating the effects mediated through binding of IgA1 to FcεR. By flow cytometric analysis, binding of monomeric IgA1 or preformed dimeric IgA1 to FcγRIIb-transfected cells could not be detected (data not shown). Control experiments demonstrated binding of both types of IgA1 to FcεRI transfectants (data not shown). We next determined whether binding of F(ab')2 AT10 and complete antibody 197 affected FcεR expression on PMNs. Isolated PMNs that were incubated for 4 h with various concentrations of the blocking reagents were analyzed for FcεRI expression by flow cytometry. The results demonstrated that incubation of isolated PMN with anti-FcγRII blocking reagent, but not with anti-FcγRI blocking reagent, resulted in up-regulated FcεRI expression (Fig. 5).

PMNs from G-CSF-treated individuals express increased levels of the high affinity FcγRI (18). PMNs from these individuals previously have been shown to exhibit increased tumor cell-killing capacity mediated by a [HER-2/Neu × FcγRI] bispecific antibody (19). Because of the preference of IgG1 for the high affinity FcγRI over the low affinity FcγRI, we expected an additive effect of the combined addition of UBS-54/IgG1 and UBS-54/IgA1 huMabs with effector cells of G-CSF-treated individuals. In ADCC experiments with isolated PMNs from G-CSF-treated donors, a significant (P < 0.05) additive effect of the combination of IgG1 and IgA1 on anti-Ep-CAM huMab-induced tumor cell lysis was observed (Fig. 5). Furthermore, the diminished tumor cell lysis that could be observed after the addition of IgA1 and IgG1 huMabs to untreated whole blood was not observed with whole blood from G-CSF-treated healthy individuals (Fig. 5). Indeed, no significant differences in tumor cell killing could be observed among IgG1, IgA1, and the combination of IgG1/IgA1 when whole blood from G-CSF-treated healthy donors was used (Fig. 5).

UBS-54/IgA1 Mediates Phagocytosis of Tumor Cells by Monocyte-derived Macrophages. Monocytes can be induced to differentiate into macrophages (MDMs) that avidly phagocytize tumor cells (27). Recently, it has been shown that bispecific molecules directed to FcεRI and tumor antigens induce MDM-β-mediated phagocytosis of tumor cells (27). A flow cytometric method was used to compare the phagocytic potential of UBS-54/IgG1 and UBS-54/IgA1 by MDMs (Fig. 6). In the absence of antibody, the CD14-FITC-labeled MDM (X axis) and PKH-26-labeled Ep-CAM-positive LS174T target cells (Y axis) can be distinguished as separate populations in the FACS profile of a mixture of these cells (Fig. 6). Addition of 1 μg/ml UBS-54/IgG1 to the cell mixture and incubation for 1 h resulted in a nearly complete disappearance of the tumor cell population and the appearance of double-positive cell populations in FACS profiles. In the presence of F(ab')2 fragments of murine monoclonal antibody 323/A3, which competes with UBS-54 for binding to Ep-CAM,4 formation of double-positive cells was blocked (Fig. 6). Addition of UBS-54/IgA1 to the cell mixture also caused the formation of double-positive cells that could be blocked with 323/A3. Comparison of the percentage of ingested tumor cells mediated by UBS-54/IgG1 and UBS-54/IgA1 showed that UBS-54/IgG1 is ~2-fold more efficient in mediating phagocytosis than UBS-54/IgA1. Confocal laser-scanning microscopy confirmed that the formation of the double-positive population visualized by fluorescence analysis represented actual inges-

4 G. Huls, unpublished results.
tion of LS174T cells by MDMs (Fig. 6). The optical sections encompassing the central portion of the double-positive cells show the green CD14 fluorescence (MDMf) appearing as a ring surrounding the red PKH-26-stained LS174T cell fragments.

DISCUSSION

The recent observation that bispecific antibodies potently trigger PMN function via FcγRI (14, 15) prompted us to construct a recombinant, fully human IgA1 antibody specific for the tumor-associated Ep-CAM molecule and to assess its tumor cell-killing capacity. Furthermore, we compared the tumor cell-killing potency of this IgA1 huMab with its IgG1 counterpart in in vitro assays. The results of the functional experiments reported here confirm that FcγRI can be exploited to recruit immune effector functions by ADCC, using whole blood or isolated PMNs. We further substantiate that isolated PBMCs, efficient effector cells in IgG1-mediated ADCC, do not kill tumor cells in the presence of IgA1. In addition, the recombinant UBS-54/IgA1 huMab was found to mediate in vitro phagocytosis of tumor cells by monocyte-derived macrophages.

The availability of IgG1 and IgA1 anti-Ep-CAM huMabs allowed us to study activation of immune effector cells upon triggering different FcγR and FcαRI receptors. Because FcαRI is constitutively expressed on PMNs, we anticipated an additive effect of UBS-54/IgG1 and UBS-54/IgA1 in tumor cell-killing assays with whole blood and isolated PMNs. In fact, we observed that in assays with isolated PMNs, the IgG1/IgA1 combination yielded a significantly lower lysis than the maximum lysis obtained with IgA1 antibodies alone. Because the IgG1/IgA1 combination did not affect the dose-response curve of IgG1-mediated tumor cell killing by PBMCs, we reasoned that the reduced specific tumor cell lysis mediated by PMNs could not be attributed simply to competition between IgG1 and IgA1 huMabs for binding to Ep-CAM.

Naive PMNs constitutively express FcγRII and FcγRIII and low levels of FcγRI (Fig. 4; Refs. 3 and 5). The presence of FcγRIIb on PMNs is evidenced by FcγRIIb message levels (28, 29) and functional data with bispecific antibodies (19). The cytoplasmic domain of FcγRIIb contains an ITIM, and the cytoplasmic domain of FcγRIIa contains an ITAM. All other FcγR molecules mediate signaling via immunoreceptor tyrosine-based activation motif signaling motifs provided by association with homo- or heterodimers of γ- or β-chains (3). FcαRI also requires the FcRγ-chain for signal transduction, mediated via electrostatic interaction of amino acids in the transmembrane regions of FcαRI and the γ-chain (30). Blocking studies with monoclonal antibodies against FcγRIII, FcγRII, and FcγRI showed that the inhibitory effect of UBS-54/IgG1 on UBS-54/IgA1-mediated ADCC by PMNs could be alleviated by blocking FcγRII. These results suggest that IgG1 may inhibit FcαRI-mediated effector functions, although we cannot formally exclude that the slight increase in FcαRI expression observed after treatment of PMNs with the blocking F(ab′)2 anti-FcγRII antibody positively affected the IgA-mediated killing capacity. The inhibitory effect of IgG1 on FcαRI-mediated effector functions is indirectly substantiated by the observation that IgG1 and IgA1 act synergistically in tumor cell killing by PMNs from G-CSF-treated individuals. Apparently, the balance of IgG1-mediated
stimulation of PMNs via the up-regulated high affinity FcγRI and the down-regulation of PMN activity by the binding of IgG1 to the low affinity FcγRIIB receptor is in favor of the former. Of note, G-CSF treatment does not result in increased expression of FcγRI (12).

UBS-54/IgG1 was reproducibly more efficient in mediating ADCC in whole blood assays than UBS-54/IgA1. In whole blood ADCC assays, the combined cytotoxic effect of complement-dependent and cell-dependent cytotoxicity is determined. This difference in effectiveness may be explained by the fact that IgG1, but not IgA1, is capable of binding C1q to activate the classical complement pathway (31, 32). Indeed, in the presence of freshly isolated serum, huMab UBS-54/IgG1 but not UBS-54/IgA1 was able to directly lyse tumor target cells. The classical complement pathway is activated by binding of C1q to the Fc portion of antibody molecules complexed to antigen (33, 34). Efficient activation of complement requires a single C1q molecule to simultaneously bind at least two adjacent IgG molecules (33, 34). Our data suggest that binding of IgA1 to the tumor target cells prevents simultaneously bound IgG1 to efficiently form aggregates with C1q and consequently inhibits activation of the complement cascade. No significant differences in tumor cell killing mediated by UBS-54/IgA1, UBS-54/IgG1, or their combination could be observed with whole blood derived from G-CSF-treated individuals as source of effector cells. Here, the additive effect of UBS-54/IgA1 and UBS-54/IgG1, as observed with isolated PMNs, is lost because of the abolishment of UBS-54/IgG1-mediated CDCC in the presence of UBS-54/IgA1. The IgA1 antibody performs well because of the increased numbers of FcγRII-expressing PMNs after G-CSF treatment (19).

The results reported here show that scFv antibodies from phage display libraries may be used to engineer high affinity, functional huMabs of different isotypes. The UBS-54/IgA1 huMab effectively recruits PMNs for ADCC. An additive effect between UBS-54/IgA1 and UBS-54/IgG1 was achieved with PMNs from G-CSF-treated individuals. Combined addition of IgG1 and IgA1 huMabs to non-stimulated PMNs revealed that IgG1 antibodies down-regulate IgA1-mediated recruitment of PMNs in tumor cell killing, presumably via triggering of FcγRIIB (CD32). From these in vitro data, it may be hypothesized that in vivo, the combined application of IgG1 and IgA1 huMabs will result in optimal recruitment of immune effector cells for tumor cell killing in individuals receiving G-CSF treatment.

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