Evaluating Antibodies for Their Capacity to Induce Cell-mediated Lysis of Malignant B Cells

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ABSTRACT

Promising results from clinical trials have led to renewed interest in effector mechanisms operating in antibody-based therapy of leukemia and lymphoma. We tested a panel of B-cell antibodies from the Sixth Human Leukocyte Differentiation Antigen workshop for their capacity to mediate antibody-dependent cellular cytotoxicity, often considered to be one of the most potent effector mechanisms in vivo. As effector cells, mononuclear cells and polymorphonuclear (PMN) cells from healthy donors were compared with FcγRI (CD64)-expressing PMN cells from patients receiving granulocyte colony-stimulating factor (G-CSF) treatment. Of the 29 IgG workshop antibodies binding most strongly to the targeted malignant human B-cell lines, only 3 consistently induced target cell lysis. These three antibodies were determined to be HLA DR reactive. Experiments with a panel of HLA class II antibodies showed the involvement of individual Fcγ receptors on effector cells to be strongly dependent on the antibody isotype. We then compared killing mediated by chimeric IgG1 antibodies with that from FcγRI-directed bispecific antibodies, targeting classical HLA class II, or the Lym-1 and Lym-2 antigens. The latter two are variant forms of HLA class II, which are highly expressed on the surface of malignant B cells but which are found only at low levels in normal cells. With blood from G-CSF-treated donors, bispecific antibodies showed enhanced killing compared to their chimeric IgG1 derivatives, because they were more effective in recruiting FcγRI-expressing PMN cells. G-CSF- and FcγRI-directed bispecific antibodies to HLA class II, therefore, seem to be an attractive combination for lymphoma therapy.

INTRODUCTION

Malignant lymphomas are the most common neoplasm of young adults, with increasing mortality over the last decades (1). In the Western world, most cases are of B-cell origin, and, although chemotherapy and radiotherapy have proven to be effective treatments, the majority of patients with disseminated low-grade lymphoma or relapses of high-grade lymphoma will ultimately die from their disease. The application of MoAbs1 has the potential to become another therapeutic option (2). Hematological malignancies seem to be particularly promising targets for antibody therapy, because antibodies to well-defined and rather specific surface molecules are available, therapeutic antibodies usually reach their targets, and induction of human antimouse or antichimeric antibody is less pronounced than in patients with solid tumors (3). Clinical trials with customized antibodies to patients' tumor idiotype were the first to show encouraging results in lymphoma patients (4), and IDEC-C2B8 (a chimeric CD20 antibody (5)) was the first MoAb to be approved by the United States Food and Drug Administration for treatment in oncology.

MoAbs mediate their antitumor effects either by directly acting on tumor cells (e.g., by blocking growth factors, inhibiting cell proliferation, or inducing programmed cell death or dormancy) or by recruiting immune effector mechanisms such as cell- or complement-dependent cytotoxicity. Studies with isotype switch variants showed a positive correlation between the capacity to induce ADCC in vitro and therapeutic efficacy in vivo, suggesting that ADCC can be an important mechanism of antibody action in vivo (6). Neutrophils, the most abundant Fc receptor-expressing effector cells, showed cytolytic activity against a broad spectrum of tumor cells in vitro (7) and were critically involved in the rejection of cytokine-transfected tumor cells in vivo (8). In vitro, we found cell-mediated target cell lysis by neutrophils to be a major effector mechanism for HER-2/neu-directed MoAbs (9). The contribution of neutrophils can be further enhanced by clinical application of hematopoietic growth factors, such as G-CSF or granulocyte-macrophage CSF, which dramatically raise neutrophil numbers in vivo and at the same time stimulate important functions, such as phagocytosis, release of oxygen radicals, and ADCC (10).

Cell-mediated effects of MoAbs require interaction between the Fc region of antibodies with activating Fc receptors on immune effector cells (11). Depending on their specificity for the heavy chains of IgA, IgE, or IgG, Fc receptors are grouped as Fca, Fce, or Fcγ receptors, respectively (12, 13). The majority of Fc receptors consist of ligand-specific α chains, which associate with shared molecules for signaling (14, 15). Neutrophils constitutively express the myeloid receptor for IgA (FcaRI, CD89) and two low-affinity IgG receptors, FcγRIIa (CD32) and FcγRIIb (CD16; Ref. 16). IFN-γ (17) or G-CSF (18) induces neutrophils to additionally express the high-affinity IgG receptor (FcγRI, CD64). Tumor-cytolytic activity on neutrophils has been established for FcγRI, FcγRII and, more recently, for FcaRI (19) but not for FcγRIIb, which is glycosylphosphatidylinositol-linked on PMN cells. Monocytes/macrophages mediate tumor cell killing via molecules belonging to all three Fcγ receptor classes, whereas NK cells express only the cytotoxically active FcγRIIa (11).

In a previous study, comparing the capacity of B cell-directed antibodies to induce ADCC of malignant cells, we observed an unexpected antigen restriction whereby PMN cells induced high levels of target cell lysis with antibodies to HLA class II but not with antibodies to classical B-cell antigens, such as CD19, CD20, CD21, CD37, or CD38 (20). Here, we report on our results with an extended panel of B-cell antibodies from the Sixth Human Leukocyte Differentiation Antigen workshop and with engineered antibody derivatives. The results extend our understanding of HLA class II-directed reagents and their ability to recruit effector cells and underscore the potential of bispecific antibodies as therapeutically reagents in the treatment of lymphoma.
PATIENTS AND METHODS

Blood Donors. Experiments reported here were approved by the Ethical Committee of the University of Erlangen-Nürnberg, in accordance with the Declaration of Helsinki. After informed consent was obtained, peripheral blood was drawn from healthy volunteers or from patients receiving G-CSF therapy. Patients were treated with rh-met-G-CSF (Neupogen; 3–5 μg/kg of body weight) from Hoffmann-La Roche (Basel, Switzerland), based on clinical indications. In G-CSF treated patients, FcγRII expression on PMN cells was significantly (P < 0.001) higher than in healthy donors, as reported (18).

Isolation of Mononuclear and Neutrophil Effector Cells. Mononuclear and neutrophil effector cells were isolated as described (20). Briefly, 10–20 ml of citrate anticoagulated blood was layered over a discontinuous Percoll (Seromed, Berlin, Germany) gradient. After centrifugation, neutrophils were collected at the interphase between the two Percoll layers, and MNCs were collected from the Percoll/plasma interface. Remaining erythrocytes were removed by hypotonic lysis. Purity of neutrophils was determined by cytospin preparations and exceeded 95%, with few contaminating eosinophils and <1% MNCs. Viability was tested by trypan blue exclusion and was higher than 95%.

Cell Lines. Malignant human B-cell lines REH (O-acute lymphoblastic leukemia), RAJI (Burkitt’s lymphoma), ARH-77 (mature B cells), HUT-78 (T-acute lymphoblastic leukemia) and L cells (mouse fibroblasts) were obtained from the American Type Culture Collection (Manassas, VA). RM-1 (an EBV-transformed B-cell line) was from Dr. G. Bonnard (Bethesda, MD; Ref. 21), and JK-6 (plasmacytoma) was established at our institution by Dr. R. Burger (22). All cells were kept in RPMI 1640 medium containing RPMI 1640 (Life Technologies, Inc., Paisley, United Kingdom) supplemented with 10% heat-inactivated FCS, 100 units/ml penicillin, 100 units/ml streptomycin, and 4 mmol/liter L-glutamine (all from Life Technologies).

Generation of HLA Class II Transfectants. Vectors containing DRA *0101 (DRα 120; ATCC 57392) and DRB1 *0101 (45.1 DRβ 008; ATCC 57081) were obtained from the American Type Culture Collection. Sense primers CCC-AGA-CTG-GTA-GCT-CTG-TTC (DRβ), and antisense primers CCC-TCT-TCC-TTG (DRα) and CCC-AAG-CTT-CGA-GCT-CTA-CTG-ACT (DRα) and CCC-AAG-CTT-CGA-GCT-CTA-CTG-ACT (DRβ) were used to amplify DRA and DRβ, respectively, cDNA from the plasmids and to generate singular HindIII and Xbal restriction sites at 5′ and 3′ ends. PCR products were cloned into pGEM-T-vector (Promega, Madison, WI) and sequenced by the dye terminator method on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). For eukaryotic expression, inserts were ligated via their unique restriction sites into pRcCMV (Invitrogen, NV Leek, the Netherlands). Transfection of 10^5 L-cells was carried out by simultaneous electroporation of 10 μg of each of the DRA and DRβ expression vectors at 250 V and 960 μF (Bio-Rad, Richmond, CA). After 24 h, G418 (Life Technologies) was added to the culture medium for selection of stable transfected lines. High-expressing cells were obtained by fluorescence-activated cell sorting on an EPICS ELITE ESP (Coulter, Hialeah, FL) after staining with HLA class II antibody F3.3 and FITC-labeled goat antimouse IgGl (all from Life Technologies).

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Table 1 Binding of B-cell panel antibodies to B-cell lines of different maturation levels
One hundred two antibodies from the Sixth Human Leukocyte Differentiation Antigen workshop were tested for binding to four different human B-cell lines. Relative fluorescence intensities, antibody isotypes, and antibody specificity, as determined by the workshop, are listed for the 25 most strongly binding antibodies.

F(ab')2 fragments were produced by limited digestion with pepsin and were then reduced with mercaptoethanol amine to provide F(ab') with free hinge-region sulfur hydroxyl (SH) groups. The SH groups on one of the Fab'-γ (SH) partners were then fully derivatized with excess o-PDM to provide free maleimide groups. Finally, the F(ab')-o-PDM and F(ab')-SH were combined at a ratio of 1:1 to generate heterodimeric F(ab')-o-PDM-F(ab') constructs. After purification by size exclusion chromatography and characterization by high-performance liquid chromatography, samples were sterilized by filtration and stored at 4°C. All bisppecific antibodies showed binding to effector and target cells as expected from their parental antibodies.

Chimeric FabFcγ constructs of HLA class II antibody F3.3, consisting of F(ab') fragments from the mouse antibody chemically conjugated to two human Fc fragments, were prepared as reported (26). Briefly, F(ab')-o-PDMs of F3.3 were produced as described above. To prepare Fcγ, normal human IgG was digested with papain, and the resulting Fcγ fragments were separated and purified. Following reduction of Fcγ fragments, incubations were made with F(ab')-o-PDM to yield FabFcγ constructs with mainly human Fcγ fragments.

Immunofluorescence Analysis. During incubation of effector cells with MoAbs, polyclonal human IgG (4 mg/ml) was added to inhibit nonspecific binding to FcγRIL. FITC-labeled F(ab')2 fragments of goat antimouse or anti-human MoAbs were used for staining. Cells were analyzed on an EPICS PROFILE flow cytometer (Coulter). For each cell population, RFI was calculated as the ratio of mean linear fluorescence intensity of relevant to irrelevant, isotype-matched antibodies.

ADCC Assays. ADCC assays were performed as described (20). Briefly, target cells were labeled with 200 μCi of ^51Cr for 2 h. Effector cells, sensitizing antibodies, and RF10+ were added into round-bottomed microtiter plates. In some experiments, Fcγ receptor-blocking antibodies were used at a final concentration of 10 μg/ml. Assays were started by adding the target cell suspension, giving an E/T cell ratio of 40:1. For whole blood assays, 50 μl of whole blood were added instead of isolated effector cells. After 3 h at 37°C, assays were stopped by centrifugation, and ^51Cr release from triplicates was measured in cpm. The percentage of cellular cytotoxicity was calculated using the formula:

\[ \text{cytotoxicity} = \frac{\text{cpm}_{\text{target cells}} - \text{cpm}_{\text{cell free release}}}{\text{cpm}_{\text{target cells}} - \text{cpm}_{\text{cell free release}}} \times 100 \]
B-CELL ANTIBODIES IN ADCC

\[
\text{% specific lysis} = \frac{\text{Experimental cpm} - \text{basal cpm}}{\text{Maximal cpm} - \text{basal cpm}} \times 100
\]

with maximal \(^{51}\text{Cr}\) release determined by adding perchloric acid (3% final concentration) to target cells and basal release measured in the absence of sensitizing antibodies and effector cells. Only very low levels of antibody-mediated, noncellular cytotoxicity (without effector cells) were observed under these assay conditions (<5% specific lysis). Antibody independent killing was seen in whole blood assays and with mononuclear effector cells, but not with PMN cells. For analysis of effects induced by Fc receptor antibodies, percentage of inhibition was calculated as follows:

\[
\text{% inhibition} = \frac{\text{% lysis without } - \text{ % lysis with FcyR antibody}}{\text{ % lysis without FcyR antibody}} \times 100
\]

**Statistical Analysis.** Data are reported as mean ± SE from an indicated number of experiments with different blood donors. Differences between groups were analyzed by unpaired (or, when appropriate, paired) Student’s t test. Levels of significance are indicated.

**RESULTS**

ADCC Activity of the B-Cell Panel Antibodies from the Sixth Human Leukocyte Differentiation Antigen Workshop. A total of 102 B-cell antibodies from the Sixth Human Leukocyte Differentiation Antigen workshop were tested for binding to four human B-cell lines representing different stages of maturation: REH (O-acute lymphoblastic leukemia), RAJI (Burkitt’s lymphoma), RM-1 (mature B cells), and JK-6 (plasmocytoma). Binding intensities of the 29 most strongly binding IgG antibodies, their isotypes, and their antigen specificities, as determined by the B-cell workshop, are summarized in Table 1. These antibodies were then analyzed for their capacity to mediate ADCC against respective target cell lines. As effector cells, we compared PMN cells (data not shown) and MNCs from healthy donors with PMN cells from patients during G-CSF treatment (Fig. 1). Antibodies B069, B070, and B071 were found to give the highest levels of killing on the broadest spectrum of target cells. These three antibodies, as well as B065 and B068, were determined by the workshop to be HLA class II reactive. B065 and B068, however, bound only weakly to most of the tested target cells (Table 1) and did not mediate ADCC. MNCs, additionally, mediated ADCC against RAJI cells in the presence of antibodies B025 and B099, which were clustered as CD37 and CD21, respectively. Plasmocytoma cell line JK-6 is HLA class II negative and was not lysed with any of the tested panel antibodies. However, JK-6 cells, coated with the hapten nitroiodophenyl, were killed by PMN cells and MNCs in the presence of nitroiodophenyl-directed antibodies, excluding the possibility that they were resistant to the lytic mechanisms of ADCC (data not shown). Interestingly, PMN cells were cytotoxic only in the presence of HLA class II antibodies, as previously reported (20).

Isotype Dependency of MNC- and PMN Cell-mediated ADCC. Antibody isotype has been reported to be a critical factor for effective ADCC (6). Therefore, we analyzed HLA class II antibodies of different isotypes for their capacity to mediate ADCC with PMN cells and mononuclear effector cells. As expected from the isotype specificities of FcyRIIIa on NK cells (16), MNCs were most effective with antibodies of human IgG1 or murine IgG3 isotypes. PMN cells from healthy donors or from G-CSF-treated patients induced significant target cell killing with antibodies of human IgG1, as well as with all

![Fig. 1. ADCC capacity of B-cell panel antibodies against malignant B-cell lines. The 12 IgG antibodies with the highest staining intensities on four human B-cell lines (see Table 1) were tested at 2 μg/ml in ADCC against malignant B cells of different maturation levels. As effector cells, G-CSF-primed PMN cells (□) or MNCs (♦) were used at an E:T ratio of 40:1. Significant ADCC (*) was observed most consistently with antibodies B069, B070, and B071, which were directed against HLA DR and, therefore, stained negative on plasmocytoma cell line JK-6. Results from four experiments with different donors are presented as mean ± SE of the percentage of specific lysis.](image-url)
murine isotypes (Fig. 2). G-CSF-primed PMN cells were significantly more effective than healthy donor PMN cells with human IgG1, murine IgG2a, and murine IgG3 antibodies. From these data, we conclude that negative results with G-CSF-primed PMN cells and B-cell panel antibodies (Fig. 1) were not explained by insufficient interaction of sensitizing murine antibodies with human Fc receptors on PMN cells.

**Isotype-dependent Involvement of FcγR in ADCC by G-CSF-primed PMN Cells.** MNC-mediated ADCC was, as expected, completely blocked by F(ab')2 fragments of FcγRIII antibody 3G8 (data not shown). In contrast to healthy donor PMN cells, which express both the low-affinity FcγRII (CD32) and FcγRIII (CD16), G-CSF-primed PMN cells additionally express the high-affinity FcγRI (CD64). The contribution of each of these three FcγR classes to ADCC was analyzed in assays via different target antibody isotypes by selectively blocking FcγRI, FcγRII, or FcγRIII with antibodies 197, IV.3, or 3G8, respectively (Fig. 3). Under our assay conditions, these Fc receptor antibodies are well documented to block selectively their respective Fc receptors (Refs. 13 and 16). Involvement of FcγRI was highest in assays via mlgG3, mlgG2a, and hlgG1 antibodies. These three isotypes were also more effective with G-CSF-primed PMN cells compared to healthy donor PMN cells (Fig. 2), indicating that expression of FcγRI on G-CSF-primed PMN cells causes enhanced killing via these isotypes. Blocking of FcγRII, on the other hand, had the most prominent influence in assays via mlgG1 and mlgG2b isotypes (in decreasing order). Interestingly, blockade of the most strongly expressed FcγRIII did not induce significant inhibition in assays via any of these target antibody isotypes, a finding that might relate to the glycosylphosphatidylinositol anchorage of FcγRIIIb in PMN cells. However, F(ab')2 fragments of FcγRIII antibody 3G8 stimulated ADCC in FcγRII-dependent assays. In combination with data from Fig. 2, these results suggest that particularly the therapeutic
efficacy of antibody isotypes reacting with FcγRI may benefit from a combination with G-CSF.

Comparing HLA DR, DP, DQ, and Invariant Chain (II, CD74) as Target Antigens. With the B-cell panel antibodies, PMN cells only mediated ADCC against malignant B-cells with HLA DR antibodies but not with antibodies to other B cell-related antigens (Fig. 1). Next, we analyzed whether PMN cells could induce B-cell lysis with antibodies to other HLA class II isosforms, or to the associated invariant chain (ii, CD74). RM-1 mature B cells served as targets for these experiments because they were found to express high levels of all four antigens (Fig 4A). As shown in Fig 4B, both healthy donor and G-CSF-primed PMN cells induced significant ADCC with antibodies to HLA DR, DP, DQ, or via invariant chain antibodies. G-CSF-primed PMN cells were again more effective than healthy donor PMN cells.

To analyze whether neutrophil-mediated cytotoxicity by HLA class II antibodies is B cell specific, we used the HLA class II expressing T-cell line HUT-78 (RFI = 207) as the target for ADCC. In these experiments, healthy donor PMN cells were highly effective (40 ± 15% specific lysis; n = 6). Next, we tested whether HLA DR transfected into nonlymphoid cells can trigger ADCC in the presence of HLA class II antibodies. For this purpose, HLA DRα and DRβ were stably cotransfected into L cells (RFI = 55), which were then used as targets in ADCC. Again, effective ADCC was mediated by PMN cells in the presence of HLA class II antibody F3.3 (17 ± 7% specific lysis; n = 4).

Comparison of FcγRI-directed Bispecific Antibodies and Chimeric Human IgG1 Antibodies against HLA Class II and Its Variants Lym-1 and Lym-2. Results reported thus far were obtained with isolated effector cells at constant effector to target cell ratios. In addition to inducing FcγRI expression on PMN cells, in vivo application of G-CSF also dramatically increases PMN cell numbers. To assess the role of increased effector cell numbers, we established whole blood ADCC assays, in which 50 μl of freshly drawn blood from healthy donors or from patients receiving G-CSF were used as the effector sources. These assays, in addition to cell-mediated effects, measured antibody-mediated complement-dependent lysis. FcγRI-directed bispecific antibodies (all [F(ab')2 × F(ab')2]) to classical HLA class II (22 × F3.3)), Lym-1 (22 × Lym-1), or Lym-2 (22 × Lym-2) antigens were compared with their respective mouse/human chimeric IgG1 constructs. ARH-77 mature B cells were used as targets in these assays because they expressed all three target antigens (Fig 5A). All three chimeric IgG1 constructs mediated significant lysis with blood from healthy donors (data not shown) or from G-CSF-treated patients (Fig 5B), especially at high antibody concentrations. However, there was no significant difference between healthy donor blood and blood from G-CSF-treated donors. Interestingly, whole blood from G-CSF-treated donors mediated ADCC with all three bispecific antibodies, and this was significantly higher than the cytotoxicity achieved with chimeric antibodies, especially with Lym-1- or Lym-2-directed bispecific antibodies (Fig 5B). As expected from the low numbers of FcRI-expressing cells in healthy donors, FcγRI-directed bispecific antibodies were not effective with blood from healthy donors (data not shown).

When whole blood was then fractionated into plasma, MNCs, and PMN cells, the chimeric antibody against classical HLA class II was significantly more effective in inducing lysis with fresh human plasma than the Lym-1 or Lym-2 antibodies. This activity was completely abolished when plasma was heat inactivated, suggesting that complement activation was the underlying mechanism. Chimeric IgG1 antibodies directed against Lym-1 (data not shown) or Lym-2 (Fig 6) mediated lysis mainly with mononuclear effector cells. However, FcγRI-directed bispecific antibodies also effectively recruited G-CSF-primed PMN cells and therefore were significantly more active with G-CSF-primed blood than chimeric IgG1 antibodies.

DISCUSSION

Results reported in this study extend previous observations that HLA class II antibodies are excellent in eliciting effector cell-mediated killing of malignant B cells (20, 27, 28). Antibodies to HLA class II were also reportedly effective in mediating complement-dependent lysis (29), in inhibiting cell proliferation (30), and in inducing apoptosis by Fas-mediated pathways (31), all of which are considered important effector mechanisms for therapeutic antibodies in vivo. HLA class II antigens are highly expressed on a broad spectrum of malignant B cells, are absent on hematopoietic stem cells and plasma cells, do not modulate, and are only found at low levels in soluble form. Furthermore, antibodies to HLA class II showed therapeutic efficacy against malignant B cells in syngeneic (32) and xenografted mouse tumor models (30, 33). Importantly, no side effects were observed in these mice, and extended immunological experiments did not reveal long-lasting immunosuppression after this treatment (32). However, HLA class II is not specific for malignant B cells but is also found on antigen-presenting cells like normal B cells, monocytes/macrophages, and dendritic cells. On these cells, HLA class II serves important functions in presenting antigens to CD4-positive T cells, and HLA class II antibodies were shown to induce tolerance under
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Fig. 5. Comparing chimeric IgG1 and FcγRI-directed bispecific antibodies against “classical” HLA class II and against HLA class II variants Lym-1, or Lym-2. A, whole blood from G-CSF-treated patients was used as effector source against ARH-77 mature B cells, which stained positive with HLA class II antibody F3.3, Lym-1, and Lym-2. B, significant lysis (P < 0.05, indicated by *) was observed with all six constructs. However, bispecific antibodies were significantly more effective (P < 0.05, indicated by #) than chimeric IgG1 antibodies, especially when Lym-1 or Lym-2 was targeted. Data from three experiments with different donors are presented as mean ± SE of the percentage of specific lysis.

certain experimental conditions (34). In addition, HLA class II expression is inducible on virtually every cell type by proinflammatory cytokines, such as IFN-γ. Subsequently, HLA class II antibodies showed severe toxicity in nonhuman primates, probably due to uncontrolled complement activation on cytokine-activated endothelial cells (35). These latter results have delayed the clinical development of HLA class II antibodies as therapeutic reagents because constructs with a lower complement-activating capacity were needed.

Reduced complement activation by MoAbs can be achieved by different approaches. For example, the C1Q-binding site of human IgG could be mutated, or antibody isotypes with less activity in complement activation, such as human IgG4 or human IgA, could be selected. Human IgA does not activate complement-dependent lysis, which seems beneficial when HLA class II is considered as target antigen, but is very effective in inducing cell-mediated lysis of tumor cells (19). Furthermore, antibodies to glycosylation variants of HLA class II, such as Lym-1 or Lym-2, have been shown to activate human complement (29) but were less potent than classical HLA class antibodies. Lym-1 and Lym-2 may have the additional advantage that they bind preferentially to HLA class II in malignant human B cells.

Fig. 6. Analyzing effector mechanisms of a chimeric IgG1 and an FcγRI-directed bispecific antibody against Lym-2. Lysis of ARH-77 mature B cells was measured comparing mouse/human chimeric IgG1 or (FcγRI × Lym-2) bispecific antibody directed to the Lym-2 antigen (both 2 µg/ml). As the effector source, whole blood from G-CSF-treated patients (G-CSF) or from healthy donors (HD) was compared and then fractionated into plasma, isolated MNCs or PMN cells. Interestingly, plasma was completely ineffective, indicating that no complement-mediated lysis occurred. In healthy donor blood, lysis with the chimeric IgG1 antibody resided mainly in the MNC fraction, whereas in G-CSF-treated patients, PMN cells were also recruited more effectively. As expected, the FcγRI-directed bispecific antibody was only effective with G-CSF-primed samples, in which it induced significantly higher killing (P < 0.05) than the chimeric antibody. *, significant lysis; #, differences between healthy donors and G-CSF-treated patients (P < 0.05).
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compared to normal B cells and monocytes (23). A clinical Phase I trial with murine Lym-1 showed minimal toxicity in lymphoma patients (36). However, clinical responses with the unconjugated antibody were unsatisfactory, and the Lym-1 antibody is currently evaluated as a radioimmunoconjugate (37, 38). Meanwhile, antibodies Lym-1, Lym-2, and 1D10 (another antibody with similar binding characteristics) have been expressed as human IgG1 antibodies (30, 39), and clinical trials with these reagents are expected to start soon. However, in whole blood assays, effector cell recruitment by human IgG1 antibodies proved less optimal, as shown in Fig 5B.

Human IgG1 antibodies effectively activate human complement, interact well with FcγRIIIa on NK cells and macrophages (40), and have an extended half-life in vivo because they are protected from degradation by binding to FcγRβ (41). However, human IgG1 was less effective in recruiting PMN cells, the most populous Fc receptor-degradation by binding to FcγRβ (41). However, human IgG1 was less effective in recruiting PMN cells, the most populous Fc receptor-degradation by binding to FcγRβ (41). However, human IgG1 was less
tive, thereby allowing specific engagement of activating Fc receptors on effector cells (e.g., platelets or B cells) or to Fc receptors on effector cells. This issue is especially critical for FcγRI because this high-affinity receptor binds monomeric IgG and therefore is not available as cytotoxic trigger molecule in the presence of serum concentrations of human IgG (42). In addition, therapeutic antibodies may bind to Fc receptors on non-effector cells (e.g., platelets or B cells) or to Fc receptors on effector cells, which do not trigger cytolytic cascades (e.g., FcγRIb or FcγRIIib). Fc receptor-directed bispecific antibodies represent an elegant solution to many of these problems (43). These genetically or chemically constructed molecules combine specificity for a tumor cell with the ability to interact well with FcγRI on NK cells and macrophages as well as eosinophilic and neutrophilic granulocytes, as shown in vivo by chimeric mouse human monoclonal antibody to CD20. Blood, 83: 435–445, 1994.


Evaluating Antibodies for Their Capacity to Induce Cell-mediated Lysis of Malignant B Cells

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