Defucosylated Chimeric Anti-CC Chemokine Receptor 4 IgG1 with Enhanced Antibody-Dependent Cellular Cytotoxicity Shows Potent Therapeutic Activity to T-Cell Leukemia and Lymphoma

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ABSTRACT

Human IgG1 antibodies with low fucose contents in their asparagine-linked oligosaccharides have been shown recently to exhibit potent antibody-dependent cellular cytotoxicity (ADCC) in vitro. To additionally investigate the efficacy of the human IgG1 with enhanced ADCC, we generated the defucosylated chimeric anti-CC chemokine receptor 4 (CCR4) IgG1 antibody KM2760. KM2760 exhibited much higher ADCC using human peripheral blood mononuclear cells (PBMCs) as effector cells compared with the highly fucosylated, but otherwise identical IgG1, KM3060. In addition, KM2760 also exhibited potent ADCC in the presence of lower concentrations of human PBMCs than KM3060. Because CCR4 is a selective marker of T-cell leukemia/lymphoma, the effectiveness of KM2760 for T-cell malignancy was evaluated in several mouse models. First, to compare the antitumor activity of KM2760 and KM3060, we constructed a human PBMC-engrafted mouse model to determine ADCC efficacy with human effector cells. In this model, KM2760 showed significantly higher antitumor efficacy than KM3060, indicating that KM2760 retains its high potency in vivo. Second, KM2760 suppressed tumor growth in both syngeneic and xenograft mouse models in which human PBMCs were not engrafted. Although murine effector cells exhibited marginal ADCC mediated by KM2760 and KM3060, KM2760 unexpectedly showed higher efficacy than KM3060 in a syngeneic mouse model, suggesting that KM2760 functions in murine effector system in vivo via an unknown mechanism that differs from that in human. These results indicate that defucosylated antibodies with enhanced ADCC as well as potent antitumor activity in vivo are promising candidates for the novel antibody-based therapy.

INTRODUCTION

Antibody-dependent cellular cytotoxicity (ADCC), a lytic attack on antibody-targeted cells, is triggered on binding of lymphocyte receptors (FcγRs) to the antibody constant region. ADCC is considered to be a major therapeutic function of antibodies, although there are others (e.g., antigen binding, induction of apoptosis, and complement-dependent cellular cytotoxicity; Refs. 1, 2). FcγRIIIα, the FcγR mainly expressed on natural killer cells and responsible for ADCC activation, has two isoforms, 158Val and 158Phe. The FcγRIIIα-158V allele shows higher binding capacity for IgG1 antibody compared with the FcγRIIIα-158F isoform (3, 4). Importantly, Cartron et al. (5) have reported recently that the anti-CD20 chimeric IgG1 antibody Rituxan was more effective for follicular non-Hodgkin lymphoma patients with FcγRIIIα-158Val compared with patients with FcγRIIIα-158Phe. Similar results have been reported by Anolik et al. (6) of Phase I/II trials of Rituxan in the treatment of systemic lupus erythematosus. These reports underscore the importance of ADCC in the clinic.

One IgG molecule contains two asparagine N-linked oligosaccharides sites in its Fc region (7). The general structure of IgG N-linked oligosaccharides is complex-type, characterized by a mannosyl-chitobiose core with or without bisecting N-acetylgalactosamine (GlcNAc)/L-fucose and other chain variants including the presence or absence of galactose and sialic acid. Several groups have reported that ADCC enhancement can be achieved by manipulating human IgG1 subclass antibody oligosaccharides. ADCC requires the presence of oligosaccharides in the Fc region and is sensitive to change in the oligosaccharide structure (8–10). Among all the sugar components in the oligosaccharide, galactose (11, 12), bisecting-GlcNAc (13, 14), and fucose (15, 16) have been reported to affect ADCC. We clarified recently the greater importance of fucose among these sugar components; defucosylation of humanized anti-interleukin 5 receptor antibody or chimeric anti-CD20 antibody enhanced their ADCC >50-fold (16). Compared with fucose, the involvement of bisecting-GlcNAc in ADCC was minimal, and galactose did not contribute to ADCC (16). The influence of defucosylated oligosaccharide on ADCC has been also reported by Shields et al. (15) using humanized anti-HER2 IgG1 and humanized anti-IgE IgG1. They showed that the improved ADCC of defucosylated IgG1 resulted from its improved binding to FcγRIIIα. Defucosylation of human IgG1-type antibody is, thus far, one of the most powerful ways to improve antibody effector function. However, the superiority of defucosylated antibodies in vivo has yet to be proven.

Chinese hamster ovary (CHO) cell lines are one of the most widely used host cell lines for the production of recombinant pharmaceutical proteins. Many approved or developing therapeutic antibodies are produced by CHO cells, including Rituxan and the anti-HER2 IgG1, Herceptin, both of which are increasingly used in the treatment of non-Hodgkin’s lymphoma (17) and breast cancer (18), respectively. Several in vivo and clinical studies indicate that ADCC is one of the essential therapeutic mechanisms of Rituxan and Herceptin (2, 5, 6). However, the content of fucose in oligosaccharides of CHO-produced antibodies is relatively high, and their ADCC is much lower than that of defucosylated antibodies (15, 16).

Chemokine receptors mediate leukocyte migration through binding of soluble ligands. CC chemokine receptor 4 (CCR4) is a chemokine receptor that binds specifically to its ligands thymus and activation-regulated chemokine and macrophage-derived chemokine. CCR4 is expressed mainly on Th2-type CD4+ helper T cells in normal conditions (19–21). Th2 cells regulate humoral immunity and are also thought to play a key role in immune disorders, such as allergies and autoimmune diseases (22).

Several groups reported recently the selective expression of CCR4 on certain subsets of T-cell leukemia/lymphoma. Yoshie et al. (23) reported the frequent expression of CCR4 on adult T-cell leukemia (ATL) cells (22 of 24 cases were CCR4-positive by reverse transcription-PCR). ATL is the most aggressive and fatal leukemia, and there is currently no curative therapy. The very high rate of CCR4 positivity in ATL was also reported most recently by Ishida et al. (24). Inter-
Estingly, their data demonstrated for the first time that the CCR4 expression correlates with poor prognosis. With respect to other T-cell malignancies, anaplastic large-cell lymphoma (25, 26), mycosis fungoides in transformation (25, 27), and cutaneous T-cell lymphoma (27) have been observed for the frequent expression of CCR4.

To verify the potential of defucosylated antibody with enhanced ADCC for anticancer drugs, we established the defucosylated chimeric anti-CCR4 IgG1 antibody KM2760. KM2760 has lower fucose content than an otherwise identical CHO-produced anti-CCR4 IgG1, KM3060, and exerts much higher ADCC when human peripheral blood mononuclear cells (PBMCs) are used as effector cells. We additionally established a T-cell leukemia mouse model entailed with the generation of CB-17/Icr- scid nu mice, and used CB-17/Icr- scid nu as the secondary reagent. The stained cells were analyzed using an EPICS XL-MCL flow cytometer (Beckman Coulter, Tokyo, Japan).

Preparation of Murine Effector Cells. Splenocytes were taken from C57BL/6 mice and stimulated in 50 ng/ml (>500 units/ml) recombinant human interleukin 2 (Peprotech EC, London, England) for 7 days. Thioglycollate-induced macrophages were taken from i.p. cavities of mice 4 days after the i.p. injection (2 ml/mouse) of 3% Brewer thioglycollate medium containing 0.3 mM thioglycollate (Difco, Detroit, MI).

ADCC Assay. ADCC was determined by 4 h 51Cr release assay using PBMCs from healthy volunteers or interleukin 2-activated murine effector cells as effector cells as described previously (16). For macrophage-mediated ADCC, the incubation time was extended to 18 h.

Construction of Human PBMC-Engrafted Mice. Human PBMCs were isolated from the peripheral blood of a healthy donor using Lymphoprep (Axis Shield, Dundee, United Kingdom). PBMCs (5 × 10^6) were injected i.p. in a volume of 0.2 ml suspended in PBS into SCID mice under sterile conditions. The mice were pretreated with a combination of γ irradiation using a 137Ceium irradiator (2.5 Gy, 3 days before PBMC injection) and 50 μl anti-asialo GM1 antiserum (Wako, Richmond, VA) given i.p. 1 day before PBMC injection.

Assessment of Antitumor Activity in Hu-PBMC-SCID Mice. CCR4/ EL4 cells (5 × 10^6) were injected i.p. into C57BL/6 mice on day 0. On days 1, 3, and 5, anti-CCR4 IgG1s were injected i.v. Twelve days after tumor injection, mice were anesthetized and sacrificed by exsanguination. Livers and kidneys were taken and their weight were measured as an indication of metastases. The organ weights were normalized by dividing by body weights. Treated versus control group value was also used as an indication of metastasis, which was calculated as follows: treated versus control group (%) = [(Organ weight of treated group) – (Organ weight of naive group)] / (Organ weight of naive group) × 100. For in vivo depletion of natural killer (NK) cells, 50 μl anti-asialo GM1 antisera were injected i.p. on days 3, 5, and 7, and metastases were evaluated on day 11. Almost complete elimination of NK cells in spleens of the treated mice on day 6 was confirmed using flow cytometry.

Experiments in a Syngeneic Disseminated Tumor Model. CCR4/EL4 cells (5 × 10^6) were injected i.v. into C57BL/6 mice on day 0. On days 1, 3, and 5, anti-CCR4 IgG1s were injected i.v. Twelve days after tumor injection, mice were anesthetized and sacrificed by exsanguination. Livers and kidneys were taken and their weight were measured as an indication of metastases. The organ weights were normalized by dividing by body weights. Treated versus control group value was also used as an indication of metastasis, which was calculated as follows: treated versus control group (%) = [(Organ weight of treated group) – (Organ weight of naive group)] / (Organ weight of naive group) × 100. For in vivo depletion of natural killer (NK) cells, 50 μl anti-asialo GM1 antisera were injected i.p. on days 3, 5, and 7, and metastases were evaluated on day 11. Almost complete elimination of NK cells in spleens of the treated mice on day 6 was confirmed using flow cytometry.

Experiments in a Human T-Cell Leukemia Xenograft Model. BALB/c- nu nu mice were injected s.c. with CRRF-CEM cells (2 × 10^7). Three h, 3 and 6 days later, antibodies were injected i.v. Tumor volume was calculated by the following equation:

\[
\text{Tumor volume (mm}^3\text{)} = 0.5 \times \left(\text{major diameter}\right) \times \left(\text{minor diameter}\right)^2.
\]

Statistical Analysis. Statistical significance of differential findings between experimental groups of animals was determined by two-tailed unpaired t test.

RESULTS

Generation and Characterization of Chimeric Anti-CCR4 IgG1. We have demonstrated previously that human IgG1 antibodies produced by rat myeloma YB2/0 cells have lower fucose contents in their N-linked oligosaccharides compared with those produced by CHO cells (16). In this study, we generated chimeric anti-CCR4 IgG1 produced by YB2/0 cells, designated KM2760, and that produced by CHO/DG44 cells, designated KM3060, to compare the antitumor activity of defucosylated IgG1 to highly fucosylated IgG1. These two IgG1s have the same amino acid sequence; only their N-linked oligosaccharide structures vary. Oligosaccharide profile analyses showed that the content of nonfucosylated oligosaccharides in KM2760 was 93%, whereas that in KM3060 was only 9% (Fig. 1;
A significant increase in ADCC was observed when KM2760 was compared to KM3060. The ADCC of KM2760 was significantly higher, particularly in the presence of human peripheral blood mononuclear cells (PBMCs) from different donors. The difference in potency was prominent for donor A (Fig. 2A), where the maximum cytotoxicity of KM2760 was 51% and 8% for KM3060, respectively. A similar result was observed for donor B (Fig. 2B), with maximum cytotoxicities of 100% and 4%, respectively. KM2760 also exhibited higher potency for donor C, where the maximum cytotoxicity of KM3060 was 4%, while KM2760 achieved 86% (Fig. 2C). These results suggest that defucosylated IgG1 antibodies exhibit considerably higher ADCC than highly fucosylated CHO-produced IgG1s. This is consistent with previous reports on the enhanced antitumor activity of defucosylated IgG1s among individuals.

ADCC was also measured in the presence of varying numbers of PBMCs from donor A at a constant antibody concentration (3 μg/ml). Notably, KM2760 needed much fewer effector cells to achieve the same cytotoxicity as shown by KM3060 (Fig. 2D). A similar advantage of defucosylated IgG1 in E/T ratio-based observation has also been reported for humanized anti-HER2 IgG1 (15).

To further elucidate the mechanism behind the enhanced ADCC of KM2760, we analyzed the oligosaccharide profiles. The oligosaccharide compositions of KM2760 and KM3060 were determined by high-performance liquid chromatography (HPLC) and are presented in Table 1. Although the difference in the contents of fucose was most prominent, there were also some changes in other sugar components, such as galactose or GlcNAc, their differences were not considered sufficient to contribute to the difference in ADCC (16). The CCR4-binding activities of KM2760 and KM3060 were measured by ELISA, and the two antibodies showed identical binding to CCR4 (data not shown).

KM2760 also exhibited higher ADCC than KM3060 in all three of the experiments. The difference in potency was prominent especially for donor A (Fig. 2A), where the maximum cytotoxicity of KM2760 and KM3060 was 51% and 8%, respectively) and donor B (Fig. 2B, 100% and 4%, respectively). KM2760 also showed higher potency for donor C, however, the maximum cytotoxicity of KM3060 was relatively high in this case (Fig. 2C, 100% and 71%, respectively). These results suggest that defucosylated IgG1 antibodies exhibit much higher ADCC than highly fucosylated CHO-produced IgG1s. This is true despite the considerable variation in ADCC of CHO-produced IgG1s among individuals.

Table 1: Oligosaccharide composition of anti-CCR4 IgG1s

<table>
<thead>
<tr>
<th>IgG1</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
<th>i</th>
<th>Total</th>
<th>Fuc(−)</th>
<th>Bis(+)</th>
<th>G0</th>
<th>G1</th>
<th>G2</th>
</tr>
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<tr>
<td>KM2760</td>
<td>76</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
<td>2</td>
<td>100</td>
<td>93</td>
<td>6</td>
<td>86</td>
<td>14</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>KM3060</td>
<td>9</td>
<td>ND</td>
<td>ND</td>
<td>37</td>
<td>34</td>
<td>11</td>
<td>9</td>
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<td>100</td>
<td>9</td>
<td>46</td>
<td>45</td>
<td>9</td>
<td>ND</td>
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* CCR4, CC chemokine receptor 4; HPLC, high-performance liquid chromatography; ND, not detected.

Pyridylaminated oligosaccharides were analyzed for their compositions by reverse-phase HPLC as described under “Materials and Methods.” The percentages of the total oligosaccharide are given on a molar basis. The structures of each oligosaccharide are represented in Fig. 1. Fuc(−) is total percentage of non-fucosylated oligosaccharides. Bis(+) indicates total percentage of bisection GlcNAc-binding oligosaccharides. G0, G1, and G2 indicate total percentage of non-galactosylated, mono-galactosylated, and di-galactosylated oligosaccharides.
We also confirmed by flow cytometry that KM2760 or KM3060 did not bind to parental EL4 cells not expressing human CCR4. Likewise, KM2760 and KM3060 did not exert any ADCC on the parental EL4 cells (data not shown), indicating that the potent cytotoxicity of KM2760 depends on the presence of CCR4.

**T-Cell Lymphoma and Leukemia Cell Lines Frequently Expressing CCR4 Are Lysed by KM2760-Mediated ADCC.** To confirm the reactivity of KM2760 to neoplastic T cells, we used biotinylated KM2760 to stain eight randomly selected human T-cell leukemia/lymphoma cell lines. CCR4/EL4 cells were stained as the positive control. As shown in Fig. 3, five of eight cell lines were significantly KM2760-positive (Fig. 3, A–E), indicating that T-cell leukemia/lymphoma frequently express CCR4. This finding coincides well with recent reports (23, 25–27).

Using fresh human PBMCs (donor D) as effector cells, we measured ADCC of KM2760 against five of the eight cell lines selected above. As shown in Fig. 4, all of the target cell lines were lysed in the presence of KM2760, although the extent of antibody-dependent and independent cytotoxicities were varied among cell lines.

**KM2760 Shows Potent in Vivo Antitumor Activity in a T-Cell Leukemia Mouse Model Engrafted with Human PBMCs.** To evaluate the in vivo antitumor activity of chimeric anti-CCR4 antibodies in a mouse model in which ADCC was effected by human effector cells, we constructed a human PBMC-engrafted SCID mouse model. According to the method reported by Shpitz et al. (34), SCID mice were pretreated with irradiation and anti-asialo GM1 antisera, followed by i.p. injection of human PBMCs. The engrafted PBMCs were derived from donor A, whose PBMC-mediated ADCC was shown in Fig. 2, A and D. Our preliminary study showed that the engrafted PBMCs survive for at least 2 weeks in the peritoneal cavity, and human PBMCs obtained from peritoneal cavities of engrafted mice ex vivo induced potent ADCC by KM2760 and weak ADCC by KM3060 (data not shown), in the same manner as when fresh PBMCs were used as effector cells.

The antitumor activity of antibodies in human PBMC-engrafted mice is shown in Fig. 5A. When CCR4/EL4 cells were injected i.p. 4 days after the engraftment of human PBMCs, without additional treatment the mice survived only for an additional 15.2 days on average because of the ascites tumor development. Significant survival prolongation was observed in mice that received either KM2760 or KM3060. However, both 1 μg and 10 μg of KM2760 were significantly more potent (the mean survival time, 22.7 days and 23.2 days, respectively) than the same and larger doses (10 μg and 100 μg) KM3060 (17.7 days and 18.0 days, respectively). In contrast, 10 μg KM2760 showed no significant survival prolongation in mice without human PBMCs (Fig. 5B), indicating that the antitumor efficacy shown in Fig. 5A entirely depended on human PBMCs.

**In Vivo KM2760 Antitumor Activity in a Syngeneic Disseminated T-Cell Leukemia Model.** Human IgG1 exerts antitumor activity in vivo via murine Fc receptor signaling (2). To additionally verify whether advantage of defucosylated IgG1 is evaluable in the conventional mouse model without introducing human effector cells, we measured KM2760- and KM3060-mediated ADCC with effector cells from C57BL/6 mice (interleukin 2-stimulated splenocytes and thioglycollate-induced peritoneal macrophages; Fig. 6A). When splenocytes were used as effector cells, they showed increased natural cytotoxicity in the absence of IgG1 primarily induced by lymphokine-activated killer cells. However, regarding IgG1-dependent cytotoxicity, the activated splenocytes or macrophages could only show marginal activities, and no significant differences were found between the two IgG1s. Taken together, these data indicate that murine effector cells do not reproduce the enhanced ADCC of KM2760; consequently, murine effector system might differ from that of human regarding the mechanism of action of defucosylated IgG1.

Next we investigated the in vivo antitumor efficacy of KM2760 and KM3060 in a syngeneic tumor model that does not bear human effector cells. When CCR4/EL4 cells were injected i.v. in syngeneic mice, KM2760 showed no significant survival prolongation in mice without human PBMCs (Fig. 5B), indicating that the antitumor efficacy shown in Fig. 5A entirely depended on human PBMCs.
In this study, we observed that KM2760 ADCC with PBMCs from donor C (Fig. 2E, which reproducibly formed a s.c. solid tumor in nude mice (data not shown). As shown in Fig. 7, KM2760 showed a significant tumor formation delay (3–4 weeks later than the control group).

**DISCUSSION**

In the present study, we generated the defucosylated chimeric anti-CCR4 IgG1 antibody KM2760, and demonstrated its extremely potent ADCC and antitumor activity to T-cell leukemia/lymphoma in various mouse models.

The high frequency of CCR4 expression on neoplastic T cells has been reported (29, 31–33) and is especially high in ATL (80–90%). Our results also showed that CCR4 is frequently expressed on neoplastic T-cell lines (5 of 8) and that all of these CCR4-expressing cell lines were lysed by KM2760-mediated ADCC. KM2760 is also expected to recognize and lyse ATL cells in patients, because Ishida et al. (24) have shown that neoplastic cells from ~90% of ATL patients can be lysed by KM2160, the original murine antibody of chimeric KM2760 sharing the same variable region with KM2760. Because CCR4 expression is mostly localized to Th2 cells (19–21), we expect low toxicity of CCR4-targeted therapy. There is currently no curative therapy for ATL, and the 4-year survival rate is only 5–6% for acute or lymphoma type patients (35). CCR4-targeted antibody therapy with potent effector function is, therefore, a promising candidate to overcome this difficulty.

KM2760 with low fucose-containing oligosaccharides (93% defucosylated) exerted potent ADCC with human PBMCs as effector cells. In this study, we observed that KM2760 ADC with PBMCs from three donors was much higher than that of KM3060 (9% defucosylated oligosaccharides). Interestingly, KM3060 ADC was relatively high in the experiment with donor C (Fig. 2C). We did not investigate the FcγRIIIA genotypes of PBMC donors in this study; however, it may be that one of three donors used in this study carries the FcγRIIIA-158Val genotype, which corresponds to an isoform capable of stronger binding to IgG1. In addition, KM2760 can exert potent ADCC in the presence of fewer effector cells (Fig. 2D). This feature could be therapeutically beneficial, because the number of effector cells able to penetrate into a large tumor mass is much less than available for circulating tumors.

In this report, the therapeutic efficacies of KM2760 and KM3060 were compared in a CCR4-positive T-cell leukemia model with human PBMC-engrafted mice. Human PBMCs can be engrafted into the peritoneal cavity of SCID mice for a limited duration (34). We adapted this mouse model to reflect therapeutic efficacy of ADCC by human effector cells. In this mouse model, KM2760 was shown to be significantly more effective in survival prolongation than larger amounts of KM3060. This result suggested that the high potency of KM2760 was retained in vivo. In support of the clinical relevance of the model, the antitumor activity of KM2760 entirely depended on the presence of engrafted PBMCs. This is the first report to demonstrate the superiority of defucosylated IgG1 as a therapeutic agent in vivo. The potent in vivo efficacy of defucosylated IgG1 achieved with a very low dose could ameliorate the problem of cost, which is one of the major defects of antibody drugs.

Additional evidence of the effectiveness against T-cell malignancy was shown in a syngeneic or a xenograft mouse model that does not
To deplete natural killer cells, all mice were additionally given anti-asialo GM1 antisera. Cytotoxicity (%) is indicated on the Y axis as mean (n = 3); bars, ± SD. Dashed lines indicate the natural cytotoxicity in the absence of antibody (29% for splenocyte cytotoxicity and 2.6% for macrophage cytotoxicity). E:T ratios are shown above each panel. In splenocyte cytotoxicity assay, similar data were also obtained with E:T = 30, with 15% natural cytotoxicity and very weak antibody-dependent cytotoxicities for the two IgG1s, which were not significantly different from each other (data not shown). B, effect of KM2760 and KM3060 on syngeneic disseminated tumor. Experimental metastases were induced by i.v. inoculation of CCR4/EL4 cells into C57BL/6 mice. Of the two IgG1s or control saline, 0.1 or 1 μg was injected on 1, 3, and 5 days after inoculation i.v. Each group consisted of five or six mice. On day 12, livers (upper) and kidneys (lower) were taken from all mice including healthy ones that did not receive tumor inoculation (naïve) and measured their relative weight as a percentage to the total body weight to evaluate the metastasis. Horizontal lines represent mean value in each group. Treated versus control (%) of each animal group were shown above each panel. Significant differences between the two IgG1s are indicated by * (P < 0.05) or ** (P < 0.01). Similar results were found in two repeat experiments. C, effect of natural killer cell-depletion on the antitumor activities of KM2760 and KM3060.

To deplete natural killer cells, all mice were additionally given anti-assialo GM1 antiseras (50 μg, i.p.) on day −3, −1, and 1 to the same experimental protocol as described in B.

Fig. 6. Antitumor activity of KM2760 and KM3060 in murine effector system. A, antibody-dependent cellular cytotoxicity of KM2760 and KM3060 with murine effector cells. In vitro cytotoxicities of the two IgG1s were determined in 15Cr release assay using interleukin 2-activated splenocytes (upper) or thioglycollate-induced macrophages (lower) as effector cells. Cytotoxicity (%) is indicated on the Y axis as mean (n = 3); bars, ± SD. Dashed lines indicate the natural cytotoxicity in the absence of antibody (29% for splenocyte cytotoxicity and 2.6% for macrophage cytotoxicity). E:T ratios are shown above each panel. In splenocyte cytotoxicity assay, similar data were also obtained with E:T = 30, with 15% natural cytotoxicity and very weak antibody-dependent cytotoxicities for the two IgG1s, which were not significantly different from each other (data not shown). B, effect of KM2760 and KM3060 on syngeneic disseminated tumor. Experimental metastases were induced by i.v. inoculation of CCR4/EL4 cells into C57BL/6 mice. Of the two IgG1s or control saline, 0.1 or 1 μg was injected on 1, 3, and 5 days after inoculation i.v. Each group consisted of five or six mice. On day 12, livers (upper) and kidneys (lower) were taken from all mice including healthy ones that did not receive tumor inoculation (naïve) and measured their relative weight as a percentage to the total body weight to evaluate the metastasis. Horizontal lines represent mean value in each group. Treated versus control (%) of each animal group were shown above each panel. Significant differences between the two IgG1s are indicated by * (P < 0.05) or ** (P < 0.01). Similar results were found in two repeat experiments. C, effect of natural killer cell-depletion on the antitumor activities of KM2760 and KM3060.

To deplete natural killer cells, all mice were additionally given anti-assialo GM1 antiseras (50 μg, i.p.) on day −3, −1, and 1 to the same experimental protocol as described in B.

Fig. 7. Effect of KM2760 on tumor growth of human T-cell leukemia cells in nude mice. CCRF-CEM (2 × 10⁶) cells were injected s.c. into nude mice. Three h, and 2 and 5 days later, mice were treated i.v. with indicated dose of KM2760 or control human IgG. Each group consisted of five mice. Significant differences versus control group are indicated by * (P < 0.05) or ** (P < 0.01). Similar results were found in two repeat experiments.

Fig. 7. Effect of KM2760 on tumor growth of human T-cell leukemia cells in nude mice. CCRF-CEM (2 × 10⁶) cells were injected s.c. into nude mice. Three h, and 2 and 5 days later, mice were treated i.v. with indicated dose of KM2760 or control human IgG. Each group consisted of five mice. Significant differences versus control group are indicated by * (P < 0.05) or ** (P < 0.01). Similar results were found in two repeat experiments.
tance of macrophages in FcγR-mediated IgG1 therapy in the mouse model has also been pointed out by Clynes et al. (2), as they found that the therapeutic efficacies of Rituxan and Herceptin were potentiated in mice that lack inhibitory receptor FcγRIIB, which are expressed on macrophages but not on NK cells. In contrast to NK cells, which selectively express FcγRIIa/Ib, macrophages express all of the three subtypes of FcγRs both in human and mouse systems. One possible explanation is that the in vivo superiority of KM2760 in mouse system may be due to the differential binding of the two fucose variant IgG1s to at least one of the FcγRs on macrophages. At this point, we have no additional clues to how KM2760 functions in mice that lack inhibitory receptor FcγRIIB. At this point, we have no additional clues to how KM2760 functions in mice that lack inhibitory receptor FcγRIIB. Many therapeutic antibodies currently approved or under clinical development are produced by CHO cells. Compared with recombinant human monoclonal IgG anti-D is reduced by β-galactosidase treatment. Hum Antib Hybrid 1995;6:82–8.


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