The Therapeutic Potential of a Novel PSMA Antibody and its IL-2 Conjugate in Prostate Cancer

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Abstract. *Prostate-specific membrane antigen (PSMA) is an attractive target for treatment of prostate cancer. Using the PSMA-recognizing mouse monoclonal antibody 2C9 obtained in our previous study, the biological activities of PSMA antibody were evaluated. Mouse-human chimeric IgG1 of 2C9 (KM2777) showed antibody-dependent cellular cytotoxicity activity against PSMA-expressing prostate cancer cells in the presence of human peripheral blood mononuclear cells (PBMCs). To increase lymphocyte-mediated cytotoxicity of KM2777, C-terminus interleukin-2 (IL-2)-fused KM2777 (KM2812) was constructed. KM2812 retained binding activity to PSMA and exhibited growth-stimulating activity equivalent to IL-2 on the IL-2-dependent T-cell line CTLL-2. Moreover, KM2812 exhibited enhanced cytotoxic activity against PSMA-expressing prostate cancer cells in the presence of PBMCs compared with KM2777. In a xenograft tumor model using PSMA-expressing prostate cancer cells, KM2812 exhibited marked antitumor activity, accompanied by complete regression of tumor in some of the KM2812-treated mice. These results suggest that KM2812 has a therapeutic potential for prostate cancer by stimulating lymphocyte-mediated antitumor cytotoxicity.*

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Prostate cancer is a common neoplastic disease in males. According to statistics, in the United States in 2012 the morbidity and the number of deaths are high for prostate cancer compared to other cancer types (1). As for treatment of prostate cancer, surgery or radiation therapy are applied to low-risk patients, whose tumors are not advanced or not metastasized (2). Hormone therapies such as androgen suppression are applied to patients with locally advanced or metastatic prostate cancer (2). Although these treatments are effective, a large number of patients experienced relapse (3). Acquisition of hormone-refractory property by prostate cancer leads to poor prognosis. Although docetaxel was approved for treatment of hormone-refractory prostate cancer (4, 5), chemotherapy is not effective in large proportion of these patients (6). Therefore, novel therapeutic strategies are required.

Since several monoclonal antibodies have exhibited clinical efficacy for various types of tumors (7), monoclonal antibodies targeting prostate cancer might be effective in treating hormone-refractory prostate cancer. One such candidate target protein is prostate-specific membrane antigen (PSMA), which is a type-II transmembrane protein expressed in almost all patients with prostate cancer (8). The expression of PSMA is high in patients with poorly-differentiated, metastatic, and hormone-refractory prostate cancer (8-11). Although prostate-specific antigen (PSA) and prostate acid phosphatase (PAP) are also considered markers for prostate cancer, these molecules are secreted proteins and their functional roles in prostate cancer cells are unclear. PSMA is expressed on prostate cancer cells and has enzymatic activity of folate hydrolase and N-acetyl-linked acidic dipeptidase I; expression of PSMA is reported to

correlate with the progression of prostate cancer (8-11). Structurally, PSMA has a large extracellular domain (717 amino acids) (8), which allows for effective antibody access to PSMA-expressing cancer cell surface. Thus, PSMA is considered an attractive target for antibody-based therapy of patients with prostate cancer.

In our previous study, a monoclonal antibody, 2C9, was obtained from immunization of mice with LNCaP cells (12). PSMA was determined as the target antigen of the antibody (12). 2C9 antibody stained poorly-differentiated prostate tumors that were not positive with antibodies targeting PSA or PAP (12). The staining pattern with 2C9 antibody was quite similar to that with other anti-PSMA antibodies but differed from those with anti-PSA or anti-PAP antibodies (12). Moreover, 2C9 was shown to recognize an extracellular portion of PSMA (12). Therefore, 2C9 might be effective in treating poorly-differentiated and hormone-refractory prostate cancer.

In the present study, biological activities of 2C9 and its mouse-human chimeric IgG1, such as antibody-dependent cellular cytotoxicity (ADCC), were evaluated. ADCC activity is considered one of the mechanisms of tumor-targeting by monoclonal antibodies (7). Moreover, immunocytokine, which is a cytokine-fused monoclonal antibody, is considered an effective approach (13-21) and some immunocytokines have been evaluated in clinical trials (13, 22-27). Since mouse-human chimeric IgG1 of 2C9 was shown to have ADCC activity, IL-2 fused mouse-human chimeric IgG1 of 2C9 was also constructed to increase cytotoxic activities of the 2C9-derived chimeric antibody against cancer cells by stimulating natural killer (NK) cells and other immune effector cells.

Materials and Methods

Mice and cell lines. Fox CHASE C.B-17/Icr-scid Jcl (SCID) mice were purchased from CLEA Japan (Tokyo, Japan). These mice were maintained under pathogen-free conditions. All experiments were performed in conformity with Institutional guidelines and in compliance with national laws and policies. Hybridoma 2C9 was established in the Aichi Medical University as described previously (12). YB2/0, LNCaP and CTLL-2 were purchased from ATCC (Manassas, VA, USA). PC-3 was purchased from DS Pharma Biomedical (Osaka, Japan). HEK-293F was purchased from Invitrogen (Carlsbad, CA, USA).

Establishment of a PSMA-expressing prostate cancer cell line. cDNA encoding human PSMA was subcloned into pKANTEX93 (28). The established PSMA expression vector was named pKANTEXPSMA12. pKANTEXPSMA12 linearized with *Aat* II was electroporated into PC-3 cells. The electroporated cells were selected with 0.2 mg/ml G418 in 96-well plates and screened based on expression of PSMA determined by flow cytometry. G418-resistant clones with high PSMA expression were subjected to cloning by limited dilution method. The 9-7-11 clone which has high expression of PSMA was obtained and named as PSMA/PC-3.

Preparation of mouse-human chimeric IgG1 antibody, KM2777. mRNA was isolated from mouse hybridoma 2C9 using Fast Track mRNA Isolation Kit (Invitrogen, Carlsbad, CA, USA). cDNA which had *Eco* RI-*Not* I adaptor sequence in both ends was synthesized from 2C9 mRNA using TimeSaver cDNA Synthesis Kit (GE Healthcare Biosciences, Pittsburgh, PA, USA). cDNA of heavy chain and κ light chain were isolated from agarose gel electrophoresis. Each cDNA was digested with *Eco* RI, followed by subcloning of these fragments into λZAPII vector and packaged into λ phage using Gigapack III Gold Packaging Extracts (Agilent Technologies, Santa Clara, CA, USA). cDNA phage libraries of 2C9 heavy chain and light chain were isolated and immobilized onto HybondN+ filter. Constant region sequence on the filter was probed using ECL Direct Nucleic Acid Labelling and Detection Systems (GE Healthcare Biosciences). Highly positive phage clones were converted into plasmids with *in vivo* excision method. Full-length cDNA of heavy chain and light chain was obtained. cDNAs encoding 2C9 heavy chain and light chain were subcloned into pKANTEX93 which can be used for the expression of antibodies whose constant region is human IgG1 (hIgG1). The established mouse-human chimeric IgG1 of 2C9 expression vector was named as pKANTEX2C9.

pKANTEX2C9 linearized with *Aat* II was electroporated into YB2/0 cells. The electroporated cells were selected with 1.0 mg/ml G418 in 96-well plates and screened based on production of antibody. G418-resistant clones with high antibody production were subjected to gene amplification of dehydrofolate reductase (*DHFR*) using methotrexate. The clone KM2777, which has a high production of mouse-human chimeric IgG1 antibody, was obtained.

KM2777 was seeded at a density of $1-2 \times 10^5$ cells/ml and cultured in Hybridoma SFM (Invitrogen) medium containing 5% Daigo's GF21 (Nippon Pharmaceutical, Tokyo, Japan) [H-SFM(GF5)] for 5-7 days. The culture supernatant was collected and subjected to purification of chimeric antibody, KM2777, with Prosep-A (Millipore, Billerica, MA, USA).

Preparation of immunocytokine, KM2812. Gene encoding human Cγ1 (hCγ1) pKANTEX2C9 was substituted with the cDNA encoding human Cγ1 fused with human interleukin 2 (IL-2) (hIL-2) to its *C*-terminus. The established hIL-2-fused mouse–human chimeric IgG1 of 2C9 expression vector was named as pKANTEX2C9-hIL-2. pKANTEX2C9-hIL-2 linearized with *Aat* II was electroporated into YB2/0 cells. The electroporated cells were selected with 1.0 mg/ml G418 in 96-well plates and screened based on production of antibody. G418-resistant clones with high antibody production were subjected to gene amplification of *DHFR* using methotrexate. Clone KM2812 was obtained, which has a high production of hIL-2 fused mouse–human chimeric IgG1 antibody.

KM2812 was seeded at density of $1-2 \times 10^5$ cells/ml and cultured in H-SFM(GF5) medium for six days. The culture supernatant was collected and subjected to purification of hIL-2-fused chimeric antibody, KM2812, with Prosep-A (Millipore).

Biotinylation of antibody. Antibody solution (1 mg/ml in PBS) was mixed with 1/4 volume of sodium carbonate buffer (pH 9.2) and 1/4 volume of 1 mg/ml NHS-LC-biotin (Thermo Scientific, Rockford, IL, USA) solution (in *N,N*-dimethylformamide). The reaction was performed under room temperature for 3 h. The biotinylated antibody solution was then subjected to dialysis with a buffer containing 150 mM NaCl and 10 mM citric acid (pH 6.0).

Flow cytometry. Cells (2-5×10⁵) were incubated with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) (BSA-PBS) in the presence of 10 μg/ml primary antibody at 4°C for 30 min. After washing the cells with PBS three times, the cells were incubated with BSA-PBS in the presence of fluorescein isothiocyanate (FITC)-labeled secondary antibody or FITC-labeled streptavidin (when biotinylated antibodies were used as primary antibody) at 4°C for 30 min. After washing the cells with PBS three times, the cells were resuspended with 1 ml PBS. The cells were analyzed using EPICS Elite flow cytometer (Beckman Coulter, Brea, CA, USA).

Kinetic analysis of the binding of anti-PSMA to PSMA. To evaluate the kinetics of the binding of anti-PSMA to PSMA, *N*-terminal FLAG epitope (DYKDDDDK)-tagged human PSMA extracellular domain (FLAG-PSMA) was transiently expressed in HEK-293F cells and purified by anti-FLAG affinity chromatography (Sigma, St. Louis, MO, USA). Kinetics were examined by Biacore T100 (GE Healthcare Biosciences). Antibody to PSMA was immobilized on a Series S CM5 sensor chip (GE Healthcare Biosciences). Different concentrations of FLAG-PSMA in HBS-EP buffer (0.01 M HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20) were passed over the sensor chip and the interactions monitored for 3 min. The sensor surface was washed with HBS-EP buffer to detect dissociation for 30 min and then regenerated with 3 mM MgCl$_2$ at the end of each experiment. The association rate constant (k_a), dissociation rate constant (k_d), and dissociation constant (K_D) were calculated by Biacore T100 evaluation software (GE Healthcare Biosciences).

Antibody-dependent cellular cytotoxicity of chimeric antibody, KM2777. Peripheral blood mononuclear cells (PBMCs) were separated from peripheral blood of healthy donors using Lymphoprep (Axis-Shield, Oslo, Norway) and used as effector cells. Human prostate cancer cell line PC-3, PSMA/PC-3, or LNCaP (1×10⁶) were incubated with Na$_2$⁵¹CrO$_4$ (Perkin Elmer, Waltham, MA, USA) at 37°C for 1 h. After washing the cells three times, the ⁵¹Cr-labelled cells were used as target cells. Aliquots of the target cells (1×10⁴ cells/well) and effector cells (2×10⁵ cells/well, effector/target ratio of 20/1) were put into 96-well plates and incubated with different concentrations of KM2777 at 37°C for 4 h. After centrifugation, the released ⁵¹Cr in the supernatant was detected with a γ-counter. Percentage-specific lysis was calculated from sample counts according to the formula: % cytotoxicity=100×($E–S$)/($M–S$), where E is experimental release (count in the supernatant from target cells incubated with antibody and effector cells), S is spontaneous release (count in the supernatant from target cells incubated with medium alone), and M is the maximum release (count released from target cells lysed with 1 M HCl). Blood donors were randomly selected from healthy volunteers registered at Kyowa Hakko Kirin. All donors gave their written informed consent prior to participation.

hIL-2 activity of immunocytokine, KM2812. CTLL-2 cells (5×10⁴) were incubated with KM2812 or hIL-2 (Pepro Tech, Rocky Hill, NJ, USA) for 72 h. WST-1 was then added to the cells, followed by incubation with WST-1 for 3 h. The OD$_{450}$ of each mixture was measured as an index of cell proliferation.

Effector cell activation and cytotoxic activity of immunocytokine, KM2812. Effector cells and target cells were prepared as described in the previous section. Effector cells (5×10⁴) were put into 96-well plates and incubated with KM2550, KM2777, or KM2812 at 37°C for 72 h. Then, target cells (1×10⁴) were added and incubated at 37°C for 4 h in the presence of each antibody. After centrifugation, the released ⁵¹Cr in the supernatant was detected with the γ-counter. Percentage-specific lysis was calculated from sample counts as described in the previous section.

Antitumor activity of anti-PSMA antibody in vivo. Anti-asialo GM1 anti-serum (Wako Pure Chemical Industries, Osaka, Japan) was administered to 5-week-old male SCID mice intraperitoneally. After two days, LNCaP cells (1×10⁷) suspended in Matrigel (BD Biosciences, San Jose, CA, USA) were injected into the SCID mice subcutaneously. The mice were grouped based on tumor volume (average volume: 177-178 mm³) 19 days after implantation of cancer cells. KM2777 (70 μg: 467 nmol), KM2812 (84 μg: 467 nmol) or saline was injected intravenously once a day for five days from the day after grouping. The rate of tumor-free mice (on day 56) was used as the criterion for antitumor activity.

Results

Binding activity of the chimeric antibody, KM2777, to prostate cancer cell surface. Binding activity of mouse-human chimeric IgG1 antibody of 2C9, which ia an anti-PSMA antibody established in the previous study, to prostate cancer cell surface was evaluated. The chimeric antibody, KM2777, stained PSMA/PC-3 cells which were produced by overexpression of PSMA on PC-3 cells (Figure 1B), while KM2777 did not stain PC-3 cells (Figure 1A). This clearly shows the specific reactivity of KM2777 to PSMA after chimerization of 2C9. KM2777 also bound to the surface of LNCaP cells which express PSMA endogenously (Figure 1C).

Antibody-dependent cellular cytotoxicity of the chimeric antibody, KM2777. Since ADCC activity has been reported as one of the important mechanisms of antitumor activity of monoclonal antibodies, ADCC activity of anti-PSMA was evaluated using human PBMCs. KM2777 exhibited ADCC activity towards PSMA/PC-3 cells but not PC-3 cells (Figure 2A and B). Consistent with the result of the flow cytometric study, KM2777 also exhibited ADCC activity against LNCaP cells which express PSMA (Figure 2C).

On the other hand, KM2777 did not influence the viability of LNCaP cells in the absence of PBMCs (data not shown).

Binding activity of immunocytokine, KM2812, to prostate cancer cell surface. To increase the antitumor activity *in vivo*, hIL-2 was fused to the *C*-terminus of KM2777. To examine the binding activity of hIL-2-fused antibody through its variable region, flow cytometric studies were performed. As a result, KM2812 bound to LNCaP cell surface to the
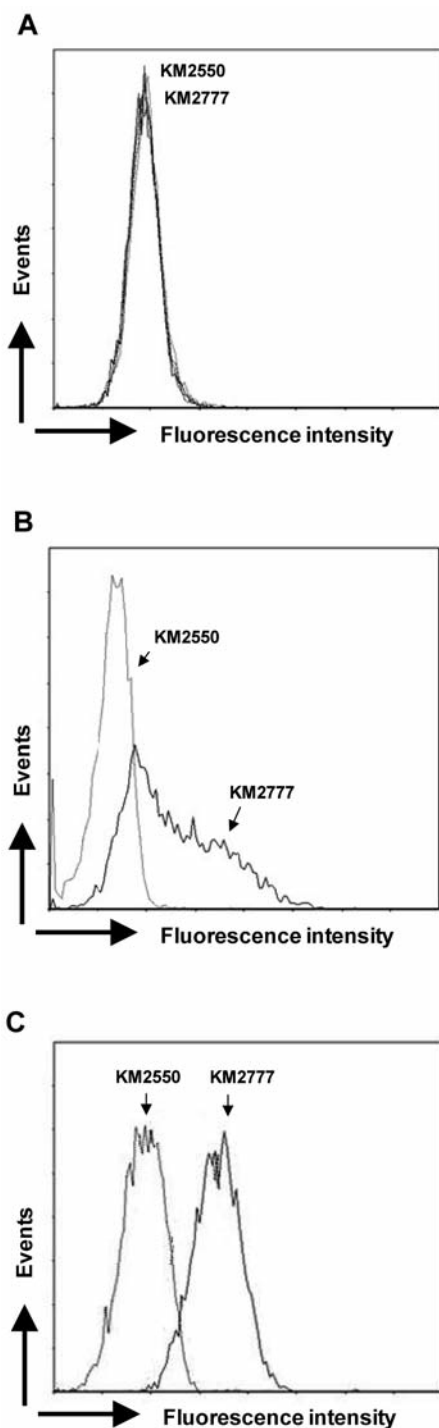
Figure 1. *Reactivity of antibodies to prostate-specific membrane antigen (PSMA) towards human prostate cancer cell lines. PC-3 (A), PSMA/PC-3 (B), or LNCaP (C) cells were incubated with 10 μg/ml of KM2777. Anti-fms-like tyrosine kinase-1 (Flt1) mouse-human chimeric IgG1, KM2550, was used as a negative control for KM2777. In (A) and (C), primary antibodies were biotinylated. After washing, cells were stained with fluorescein isothiocyanate (FITC)-labeled secondary antibody (B) or FITC-labeled streptavidin (A, C). After washing cells were analyzed using a flow cytometer.*

Table I. *Kinetic rate and binding constants of KM2777 and KM2812 to human prostate-specific membrane antigen (PSMA). KM2777 or KM2812 was captured on anti-human IgG antibody immobilized CM5 sensor chip. Kinetics of binding of human PSMA protein (FLAG-PSMA) to KM2777 or KM2812 were examined by Biacore.*

	k_a (1/Ms)[a]	k_d (1/s)[b]	K_D (nM)[c]
KM2777	5.29×10^3	5.93×10^{-5}	11.2
KM2812	5.51×10^3	5.17×10^{-5}	9.39

[a]association rate constant; [b]dissociation rate constant; [c]dissociation constant.

same extent as KM2777, while it did not react with PC-3 cells (Figure 3A and B).

Kinetics of the binding of anti-PSMA to PSMA. Binding of anti-PSMA to PSMA protein was evaluated using Biacore. KM2777 and KM2812 bound to human PSMA protein immobilized on a sensor chip and its dissociation from PSMA was slow (Figure 4). From the results of kinetics analysis, KM2777 bound to PSMA with K_D of 11.2 nM, and its dissociation rate (K_D) was determined to be 5.93×10^{-5} (1/s) (Table I). KM2812 also bound to human PSMA protein with similar binding kinetics to KM2777 (Table I). K_D and k_d of KM2812 were calculated to be 9.39 nM and 5.17×10^{-5} (1/s), respectively (Table I)

Biological activity of immunocytokine, KM2812. To examine the biological activity of the hIL-2 portion of KM2812, an IL-2-dependent T-cell line (CTLL-2) was incubated with KM2812 or hIL-2, followed by measurement of cell growth. As a result, KM2812 was as potent and efficacious as hIL-2 on the proliferative effects of CTLL-2 cells (Figure 5A).

KM2812 exhibited ADCC activity comparable to that of KM2777 (data not shown). Next, in order to evaluate effector cell activation and cytotoxic activity of KM2812, PBMCs pre-incubated with each antibody were reacted with target cancer cells, LNCaP, in the presence of the same antibody, followed by the determination of injury of the cells. KM2812 exhibited enhanced cytotoxic effect towards LNCaP cells compared with KM2777 (Figure 5B).

Evaluation of antitumor activity of anti-PSMA antibody using human prostate cancer cell-xenografted mice. Antitumor activities of KM2777 and KM2812 were evaluated *in vivo* using human prostate cancer cell LNCaP-xenografted mice. Administration of anti-PSMA resulted in complete remission in several mice. On day 56, when the evaluation ended, the rate of tumor-free mice *via* complete regression was 0%, 20% and 60%, in the saline-, KM2777-, and KM2812-treated mice, respectively.
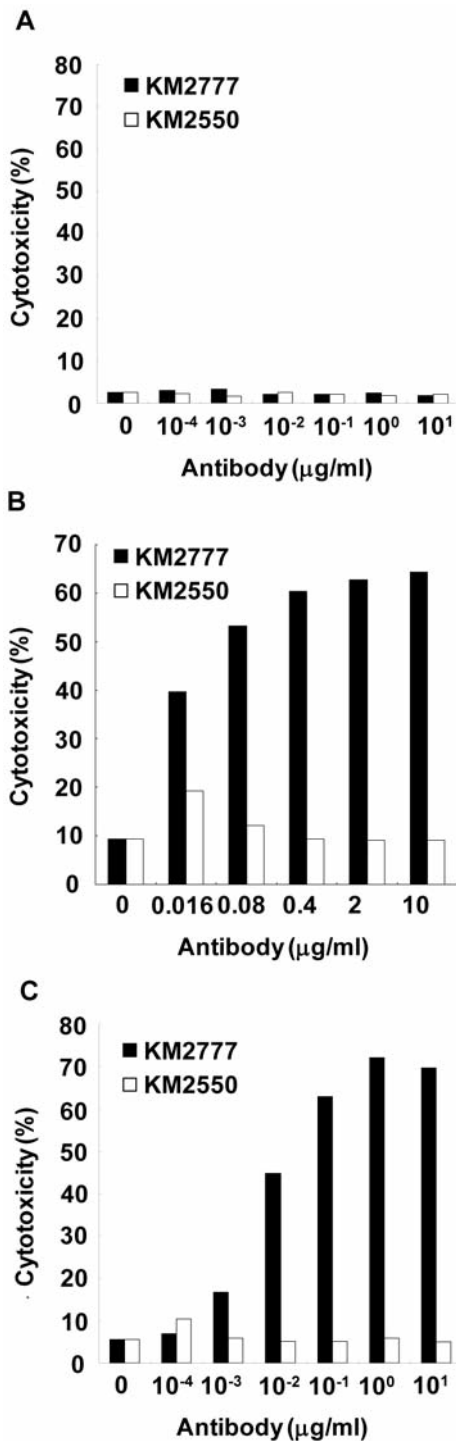
Figure 2. *Antibody-dependent cellular cytotoxicity (ADCC) activities of antibodies to prostate-specific membrane antigen (PSMA) against human prostate cancer cells. PC-3 (A), PSMA/PC-3 (B), or LNCaP (C) cells were labeled with $Na_2{}^{51}CrO_4$. The ^{51}Cr-labeled cells were incubated with human peripheral blood mononuclear cells (PBMCs) (effector/target ratio 20/1) and different concentrations of anti-PSMA at 37°C for 4 h. The released ^{51}Cr in the supernatant was detected with the γ-counter and percentage-specific lysis was calculated as described in the text.*

Discussion

2C9 is a novel PSMA-specific antibody obtained by immunization of mice with human prostate LNCaP cancer cells (12). In the present study, biological activities of anti-PSMA were evaluated. Since 2C9 was shown to bind to PMSA-expressing cell surface in the previous study (12), 2C9 might be effective in treating prostate cancer. To evaluate therapeutic applications of the PSMA antibody 2C9, constant region of 2C9 was converted to that of hIgG1 to prepare a chimeric antibody, KM2777. From the experiment using not only PSMA-transfected cells (PSMA/PC-3) but also endogenously PSMA-expressing LNCaP cells, KM2777 was shown to bind to cell surface of PSMA as with 2C9. Having the biological features of therapeutic antibody, KM2777 elicited ADCC activity in the presence of human PBMCs. In addition, KM2777 bound to PSMA with a slow dissociation rate constant. Since it was reported that slower off-rate CD20 antibody veltuzumab derived from rituximab had improved therapeutic activity compared to rituximab in xenograft models (29), KM2777 with a slow dissociation constant might have a superior antitumor activity.

Since *in vivo* antitumor activities of reported PSMA antibodies seemed to be limited (30), it was considered necessary to modulate antibodies in order to enhance their antitumor activities (30, 31). For such an approach, conjugation with radioactive materials (31, 32) or cytotoxic drugs (30, 33-36) has been tested in clinical trials. Immunocytokine is also considered an effective approach. Immunocytokines were reported to increase antitumor activities *in vivo* through immunomodulatory activities (13). IL-2 has been used for immunotherapy of melanoma and renal cancer (37). According to the clinical trial that examined the effect of PSMA antibody HuJ591 with low-dose IL-2 on prostate cancer, it was reported that PSA stabilization was observed in some patients (38). Since IL-2 is known to have potent immunostimulatory effects, such as activation of cytotoxic T-cells and NK cells, IL-2-fused antibodies were expected to have a therapeutic potential. There is another benefit in using immunocytokine, other than enhancement of effector cell function. When IL-2 is administered intravenously, a high dose is needed to maximize its effect because of its short half-life and its non-specific tissue localization (13, 39, 40). It is important to avoid non-specific tissue localization because systemic administration of high doses of IL-2 leads to toxic side-effects on cardiovascular and respiratory systems (13). Conjugation of PSMA antibody with IL-2 is considered to improve such problems (13). Therefore, IL-2-fused PSMA antibody is suggested to be effectively delivered to prostate tumor and to activate lymphocytes *in situ* for a longer period. Several IL-2 immunocytokines
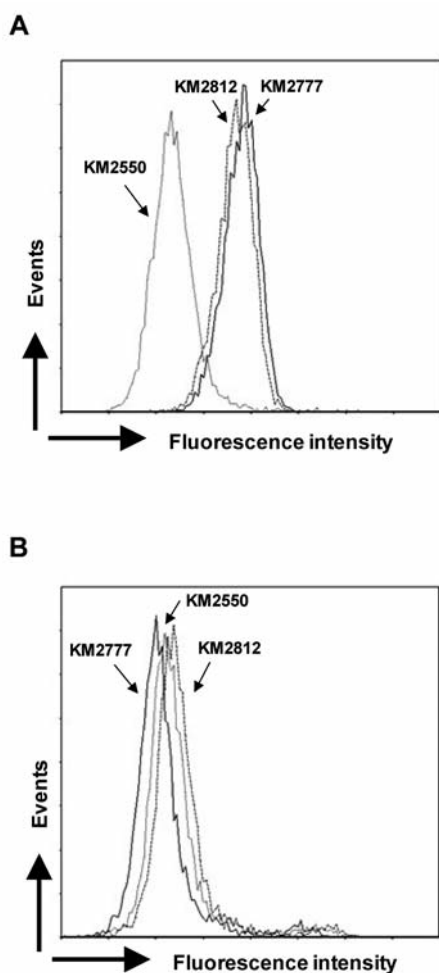
Figure 3. *Binding of interleukin-2 (IL-2)-fused antibody KM2812 to cell surface of prostate cancer cells. Flow cytometric analysis of KM2812 using LNCaP (A) and PC-3 (B) cells. Concentration of the antibody was 10 µg/ml.*

Figure 4. *Binding activity of KM2777 and KM2812 to prostate-specific membrane antigen (PSMA) protein. KM2777 (A) or KM2812 (B) was captured on an anti-human IgG antibody immobilized CM5 sensor chip. Kinetics of binding of human PSMA protein (FLAG-PSMA) to KM2777 or KM2812 were examined by Biacore.*

such as epithelial cell adhesion molecule (EpCAM)-targeting huKS-IL-2 (EMD273066) (25, 26) and disialoganglioside (GD2)-targeting hu14.18-IL-2 (EMD273063) (22-24) have been evaluated clinically. In both trials, immunostimulatory activities of these immunocytokines were confirmed (25, 41). Since IL-2-fused PSMA antibodies have not been reported, the IL-2-fused antibody, KM2812, was constructed by adding the sequence of hIL-2 to the C-terminus of KM2777. As expected, KM2812 retained its binding activity, specificity and ADCC activity of KM2777, and exhibited a proliferative activity towards the T-cell line CTLL-2 equivalent to that of IL-2, confirming that the IL-2 portion of KM2812 was functional. To examine the ADCC-enhancing effect of IL-2-fused PSMA antibody, ADCC was m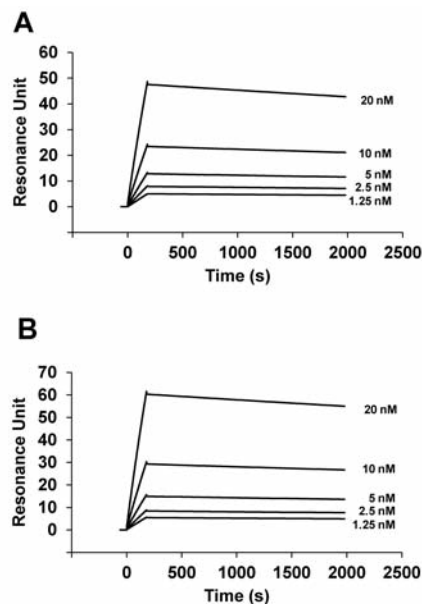easured under the conditions in which PBMCs were pre-activated for 72 h in the presence of KM2812. Since LNCaP-specific cytotoxic T-cells were considered to be very rare in this *in vitro* assay condition, NK cells were considered to be the major effector cell in the cytotoxic effect in this assay. As a result, cytotoxic effects of KM2812 in the presence of PBMCs were greater than those of KM2777 after the pre-activation of PBMCs, suggesting activation of NK cells. Based on these results, we herein focused on antitumor activity *via* NK cells in subcutaneous xenograft tumor models in SCID mice. While antibody to asialo GM1 was administered to the mice for facilitation of tumor engraftment, it was reported that NK cells were recovered several days after transient depletion (42). Therefore, we consider that NK cells worked on day 19 when the treatment was started in this experiment. Administration of KM2812 resulted in complete remission in several mice, while KM2777 showed limited activity. In mice, it was reported that monocytes were major effector cells *in vivo* (43-45). While there might be differences in effector cells between humans and mice, the enhancement of antitumor activity by KM2812 (through the IL-2 portion) is suggested to be attributed to the activity of NK cells in our mice model. Our results suggested that IL-2-fused PSMA antibody, KM2812, has a therapeutic potential for prostate cancer by stimulating lymphocyte-mediated antitumor cytotoxicity.
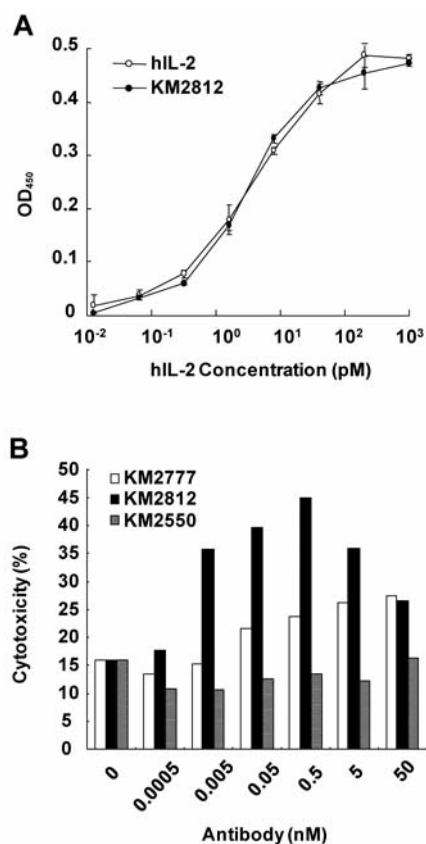
Figure 5. *Biological activities of interleukin-2 (IL-2)-fused antibody KM2812. A: Growth-stimulating activity of KM2812 against CTLL-2 cells. CTLL-2 cells were incubated with different concentrations of KM2812 or hIL-2 for 72 h. Cell viability was evaluated using WST-1 assay. OD_{450} was measured as an index of cell proliferation. B: Cytotoxicic activities of KM2812. Human peripheral blood mononuclear cells (PBMCs) were incubated with KM2550, KM2777, or KM2812 at 37°C for 72 h. Then ^{51}Cr-labeled LNCaP cells were incubated at 37°C for 4 h in the presence of each antibody. The released ^{51}Cr in the supernatant was detected with a γ-counter. Percentage-specific lysis was calculated as described in the text.*

According to the activities of KM2812 found in the present study, it is concluded that IL-2-fused PSMA antibody has potential as a candidate next-generation drug for prostate cancer treatment.

Acknowledgements

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