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Review

Specific Targeting Immunotherapy of Cancer with Bispecific Antibodies

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Kudo, T., Suzuki, M., Katayose, Y., Shinoda, M., Sakurai, N., Kodama, H., Ichiyama, M., Takemura, S., Yoshida, H., Saeki, H., Saijyo, S., Takahashi, J., Tominaga, T. and Matsuno, S. *Specific Targeting Immunotherapy of Cancer with Bispecific Antibodies*. Tohoku J. Exp. Med., 1999, **188** (4), 275–288 —— In order to enhance cell mediated cytotoxicity, bispecific antibodies (BsAbs), molecules combining two or more antibodies with different antigenic specificities, have been developed as new agents for immunotherapy. Our recent studies revealed that simultaneous administration of two kinds of BsAbs (anti-tumor × anti-CD3 plus anti-tumor × anti-CD28) together with lymphokine activated killer cells with a T cell phenotype (T-LAK cells) inhibited growth of human xenotransplanted tumors in severe combined immunodeficient (SCID) mice, while single BsAb was without effect. Three kinds of BsAbs (anti-tumor × anti-CD3, anti-tumor × anti-CD28, anti-tumor × anti-CD2) showed the highest cytotoxicity against tumor cells when given simultaneously with T-LAK cells or peripheral blood mononuclear cells in vitro and in vivo. BsAbs can be preserved for immediate application, while cytotoxic T lymphocytes (CTLs) must be made-to-order, and are time-consuming to prepare. Tumor associated antigens, such as MAGE antigens, SART antigens, MUC1 antigen, c-erbB 2 antigen or cancer/testis antigens can be served to target antigens for BsAb production. By conjugation with antibodies to effector cells (anti-CD3, anti-CD28, anti-CD16, anti-CD64, anti-CD89 or anti-CD2), many kinds of BsAbs can be produced to cover most types of cancers from different organs. Therefore this strategy might be ubiquitously applicable to most malignancies. ———— bispecific antibody; specific targeting immunotherapy; MUC1; SEA

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Cancer immunotherapy is mainly based on cellular immunity and humoral immunity. Major players in cellular immunity are T cells and those in the latter are monoclonal antibodies. Recent study identified definite tumor associated antigens like cancer/testis antigens, MAGE, SART and MUC1 antigens (Chen et al. 1998; Pardoll 1998). By using dendritic cells pulsed with peptide antigens, we are now able to induce cytotoxic T lymphocytes (CTLs) against cancer cells (Ockert et al. 1999). However, this peptide vaccine therapy has proven only effective in melanoma patients and results have been poor with other epithelial carcinomas up to the present, indicating that there are many problems to be solved in vaccine therapy. The limited effectiveness in clinical trials is attributable to the fact that the induced CTL activity is not enough to control massive tumors, as well as insufficient targeting of CTLs to cancer tissues. For this, the help of humoral immunity is necessary. To develop a suitable strategy, we have constructed bispecific antibodies (BsAbs), defined as molecules combining two or more antibodies with different antigenic specificities (Graziano et al. 1995), to bridge tumor cells and killer cells, and reinforce cell mediated cytotoxicity against cancer.

Construction of BsAbs

BsAbs were constructed according to Nitta's method (Nitta et al. 1989; Saijyo et al. 1996) (Fig. 1). Briefly, anti-killer (T-LAK) cell antibodies were digested with pepsin. Then $F(ab')_2$ fragments were reduced by dithiothreitol (DTT), and reaction was stopped using 5, 5′-dithio-bis (2-nitrobenzoic acid) (DTNB) to give Fab′-S-NB. The MUSE11 antibody (anti-tumor) was digested with preactivated papain to generate $F(ab')_2$. After reduction with DTT, the thus generated Fab-SH fragments of MUSE11 antibody were separated by gel filtration. Fab′-S-NB fragments of anti-killer antibody were then mixed with Fab-SH of MUSE11 antibody at a molar ratio of 1 : 1. After incubation, the resulting preparation was applied to gel filtration chromatography to remove unreactive fragments (Katayose et al. 1996). Thus we obtained three kinds of BsAbs (Table 1) with molecular sizes close to 105 kDa. Since these BsAbs lack Fc portion which is a major stimulus to generation of human anti-mouse antibody (HAMAs), they are considered very convenient for administration to patients. There is a method to produce BsAbs from quadromas (hybridomas × hybridomas) but this have a drawback regarding purification. However, it has become possible to construct BsAbs (bispecific single chain Fv and diabody) by genetic engineering, which entails inserting VHs and VLs from two kinds of hybridomas into an appropriate expression vector (Kipriyanov et al. 1998).

Reactivity of BsAbs

Reactivity of BsAb, namely, bispecificity for tumor cells and killer cells, has to be confirmed by flow cytometry. The reactivity of our BsAbs, anti-tumor ×

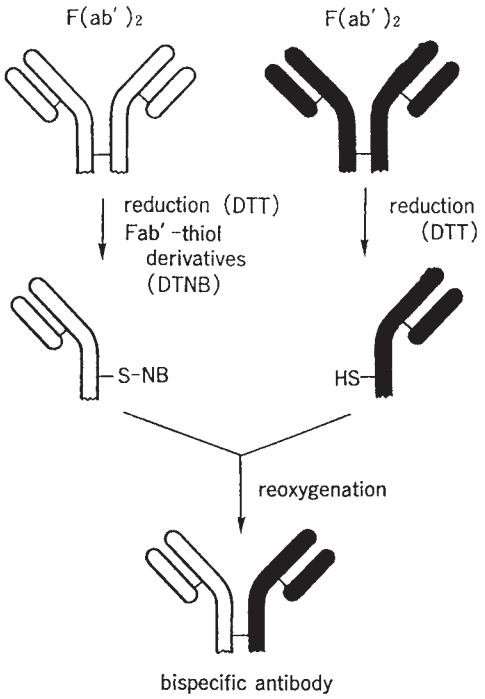

Fig. 1. Reaction scheme for generation of bispecific hetero-F(ab')$_2$ from two different F(ab')$_2$ fragments. F(ab')$_2$ (OKT3 and MUSE11), digested with pepsin or papain, were both reduced to Fab-SH fragments. One Fab-SH fragment was incubated with nitrobenzoic acid to give Fab-S-NB, and then both were reacted together. DTT, (dithiothreitol) $C_4H_{10}O_2S_2$; DTNB, 5, 5'-dithio bis (2-nitrobenzoic acid) $[SC_6H_3(NO_2)COOH]_2$.

Table 1.

BsAbs	Abs served for construction of BsAbs	
MUC1 × CD3	anti-MUC1 (MUSE11) anti-CD3 (OKT3)	anti-tumor T cell, signal transduction
MUC1 × CD28	anti-MUC1 (MUSE11) anti-CD28 (15E8)	anti-tumor activated T, costimulation
MUC1 × CD2	anti-MUC1 (MUSE11) anti-CD2 (TS2/18.1.1)	anti-tumor T, NK cell, signal transduction and adhesion

anti-CD3, anti-tumor × anti-CD28 is illustrated in Fig. 2. Binding of BsAbs to both tumor cells (MUC1-positive bile duct carcinoma TFK-1 cells) and to killer cells (T-LAK cells) was demonstrated (Katayose et al. 1996). In this case, BsAb (anti-MUC1 × anti-CD28) reacted with LAK cells much stronger than the parental anti-CD28 antibody. For this, there is no exact explanation, however, lot-to-lot difference of BsAbs is frequently found at present time. When target tumor cells and killer (T-LAK) cells were co-cultured in the presence of BsAb (anti-tumor × anti-CD3), MUC1-positive tumor cells were observed to attach to surrounding

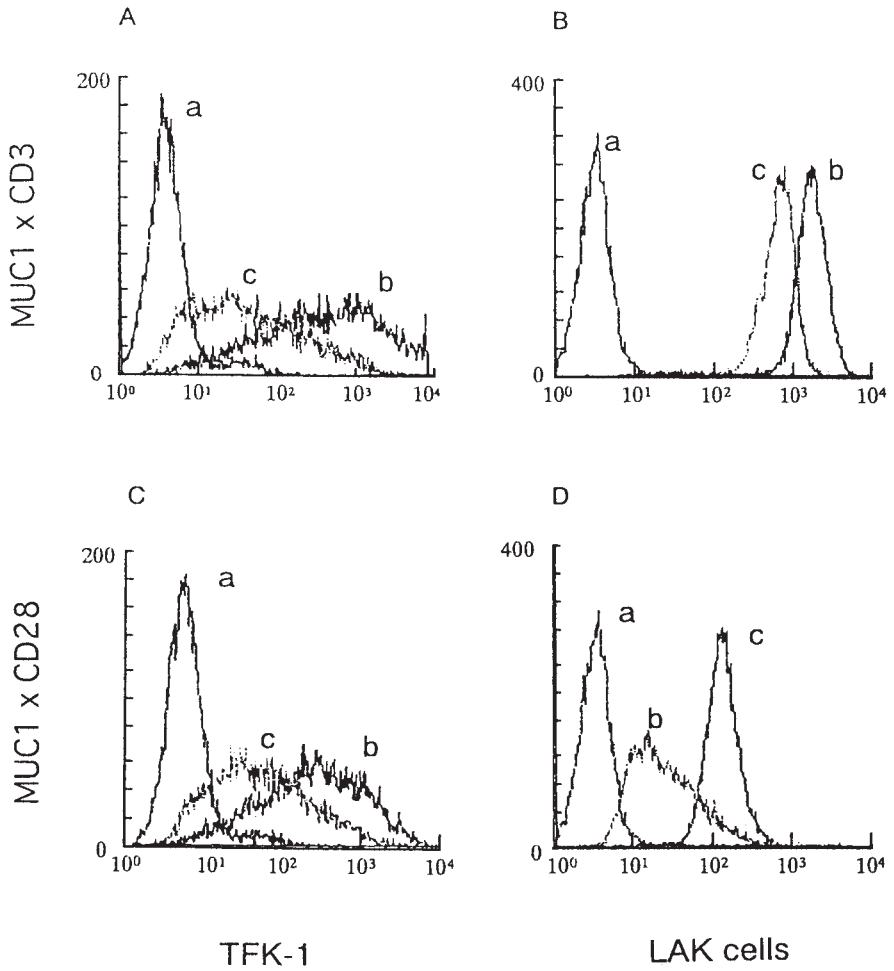

Fig. 2. BsAb reactivities with TFK-1 and LAK cells. A and B, reactivities of $F(ab')_2$ from anti-CD3 mAb (OKT3) and BsAb (anti-MUC1 × anti-CD3); C and D, reactivities of $F(ab')_2$ from anti-CD28 and BsAb (anti-MUC1 × anti-CD28). a, control unstained profile; b and c, profiles of cells reacted with the parental mAb and the BsAb, respectively. Target cells are TFK-1 (A and C) and LAK cells (B and D).

T-LAK cells in the presence of BsAb in a very short time (2–3 hours) from the beginning of cultivation. Thus, retargeting of killer cells rapidly occurs in the presence of BsAb (Katayose et al. 1996).

Enhanced cytotoxicity of killer cells by BsAbs

Cytotoxicity of T-LAK cells to MUC1-positive bile duct carcinoma (BDC) TFK-1 cells was investigated. When a BsAb (anti-MUC1 × anti-CD3) was added to the assay system, remarkable enhancement was apparent. Namely, in vitro cytotoxicity of T-LAK cells alone was about 20%, while it increased up to 60% with 0.5 μg/ml BsAb (anti-MUC1 × anti-CD3). When 2×10^7 T-LAK cells sensitized with both kinds of BsAbs (anti-MUC1 × anti-CD3 and anti-MUC1 × anti-CD28) were administered daily four times i.v. to TFK-1 cell-grafted SCID mice (tumor size 5 mm in diameter), inhibition of tumor growth was clearly observed (Fig. 3). In this in vivo experiment, administration of T-LAK cells alone, or

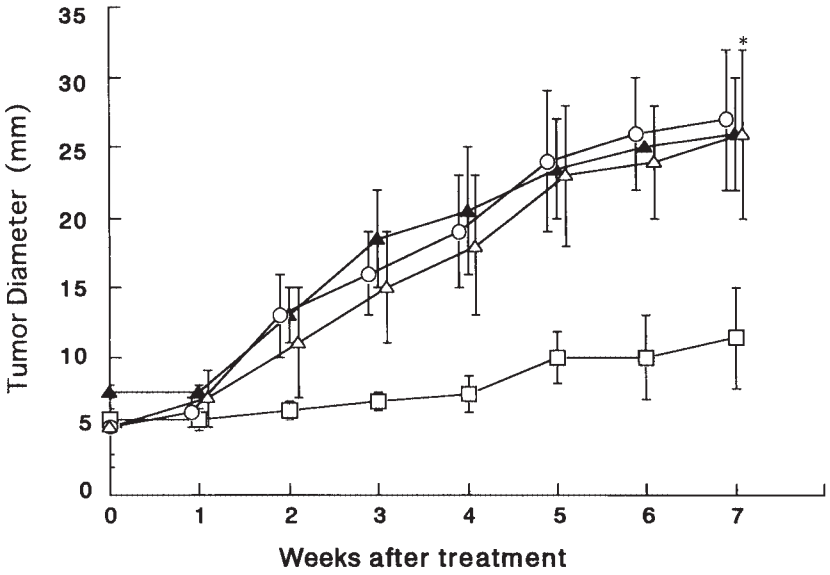

Fig. 3. Experimental adoptive immunotherapy of TFK-1 tumors in SCID mice (5 mice/group) with LAK cells and BsAbs. LAK cells (2×10^7 cells) preincubated with BsAbs were injected i.v. with IL-2 (500 IU/mouse) into xenografted SCID mice on days 10, 11, 12, 13. *Symbols*, mean values; *bars*, SE. Some error bars have been deleted for clarity. BsAbs used for preincubation with LAK cells were (anti-MUC1 × anti-CD3) BsAb (▲) and (anti-MUC1 × anti-CD3) BsAb plus (anti-MUC1 × anti-CD28) BsAb (□). △, results for LAK cells without BsAb; ○, tumor control (no therapy). $^*p < 0.05$ for ▲, △ and ○ groups vs. □ group.

with a single BsAb (anti-MUC1 × anti-CD3) was without effect (Katayose et al. 1996).

The anti-MUC1 × anti-CD3 BsAb has been shown to stimulate T cells, while anti-MUC1 × anti-CD28 BsAb has a costimulation activity when used in combination with the anti-CD3 antibody. Enhanced cytotoxicity with two BsAbs in combination has been reported by Renner et al. (1994). They reported cure of Hodgkin's lymphoma (CD30 positive)-xenotransplanted SCID mice to which had received activated human T cells with two kinds of BsAbs (anti-CD30 × anti-CD3, and anti-CD30 × anti-CD28). The B7 molecule on the tumor cells has been shown to provide costimulatory signal to T cells, and B7 gene-transduced tumor cells have been reported to have vaccine activity in experimental immunotherapy. However, costimulation with two kinds of BsAbs (anti-tumor × anti-CD3 and anti-tumor × anti-CD28) might be superior approach, because BsAbs are ready to use, while establishment of B7 gene-transduced vaccine cells is time-consuming.

Three kinds of BsAbs enhance cytotoxicity of killer cells

We constructed three kinds of BsAbs as listed in Table 1. Anti-CD3/TCR and anti-CD28 give costimulation to T cells (Renner et al. 1994; Li et al. 1999). In addition, remarkable proliferation has been reported when anti-CD28 and anti-CD2 are simultaneously added to T cells (Plet et al. 1997; Van Lier et al.

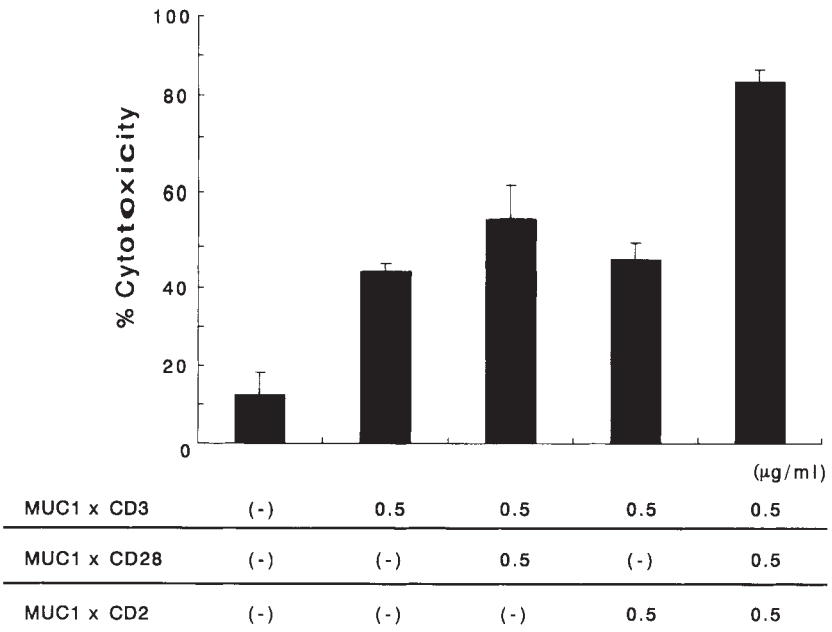

Fig. 4. In vitro targeting of PBMNCs to TFK-1 cells by single, double or triple BsAbs. Cytotoxicity was determined at E/T ratio 5 by 56 hour MTS assays.

1988). Therefore, three kinds of BsAbs, i.e., anti-MUC1 × anti-CD3, anti-MUC1 × anti-CD28 and anti-MUC1 × anti-CD2 were applied to cultures of peripheral blood mononuclear cells (PBMNCs) isolated from a healthy donor, together with MUC1-positive TFK-1 cells, and neither growth factors nor cytokines were added. Remarkably enhanced cytotoxicity was observed when two kinds of BsAbs were added to the culture, but the greatest effects were obtained with all three in combination (Fig. 4). Remarkable interferon γ (IFNγ) production (more than 900 pg/ml) was noted in the 48 hour culture from PBMNCs stimulated with triple BsAbs, while production of IFNγ by T-LAK cells without BsAbs was marginal. In the specific targeting therapy experiment with BDC-xenotransplanted SCID mice, the best result was obtained with combination of three BsAbs (Kodama et al. unpublished data). Since this experimental design is similar to that for in vivo immunotherapy with BsAbs, the results obtained here are very valuable when considering clinical applications.

Fc receptors as triggering molecules

The human IgG Fc receptor type I (FcγRI, CD64) is expressed in a variety of leukocytes, including monocytes, macrophages, dendritic cells and $CD34^+$ myeloid progenitor cells. In addition, polymorphonuclear cells (PMNs) can be induced to express CD64 with G-CSF and IFNγ. CD64 mediates antibody-dependent cell mediated cytotoxicity (ADCC), phagocytosis and release of inflammatory mediators. Several antibodies have been developed that are specific for CD64. Among them, monoclonal antibody (mAb) 22 binds to CD64 at a site distinct from the Fc-ligand binding domain, allowing binding of mAb22 in the presence of physiological concentrations of IgG. Several BsAbs have been constructed with mAb22 and anti-tumor mAbs. Antibody dependent cell mediat-

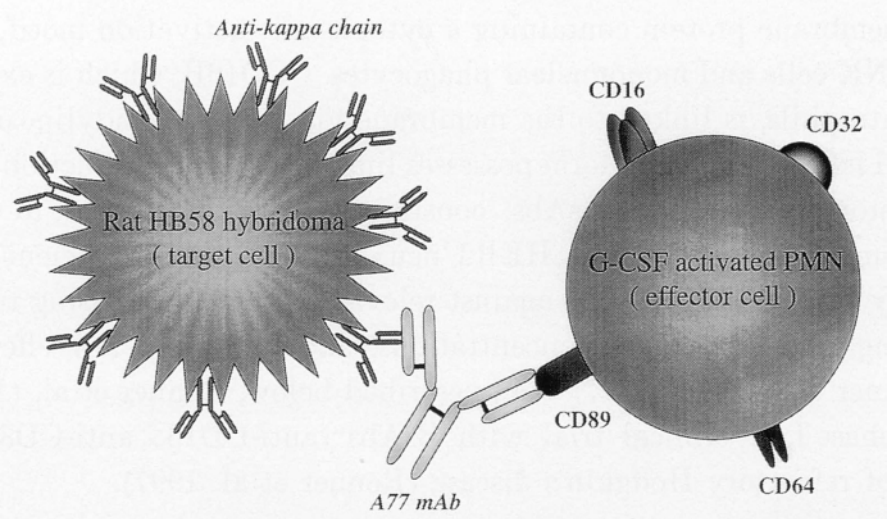

Fig. 5. Reverse ADCC to compare the efficacy of different Fc receptors as trigger molecules for cytotoxicity induction. In this assay, G-CSF primed PMNs are effector cells. ^{51}Cr-labeled rat hybridoma (HB58) cells which secrete anti-mouse kappa and selected for high membrane expression of surface Ig are used as target cells. mAbs directed to different Fc receptors (FcγRI, FcγRII, FcγRIII and FcαRI) are tested for the ability to induce target cell lysis.

ed cytotoxicity (ADCC), phagocytosis and superoxide generation are mediated by these BsAbs through CD64 stimulation (Granziano et al. 1997; Valerius et al. 1997b).

Valerius et al. (1997b) have established a sophisticated method (reverse ADCC assay) to compare directly the efficacy of different Fc receptors as trigger molecules for redirected cytotoxicity, using rat HB58 hybridoma cells as the target cells (Fig. 5). These latter produce an anti-mouse k light chain antibody and are selected for high membrane expression of surface immunoglobulin. In the presence of murine Fc receptor antibodies, they are sensitized for reverse ADCC. In the assay, granulocyte colony stimulating factor (G-CSF) primed PMNs induce the highest level of target cell killing with mAb A77 directed to FcαRI (CD89), and are also effective with antibodies to CD64 or CD32, but not CD16. The role of FcαRI (CD89) as a novel cytotoxic trigger molecule on PMNs was confirmed by blocking IgA mediated ADCC with CD89 antibodies (Valerius et al. 1997a, b), and by using FcαRI (CD89)-directed BsAbs. BsAbs (Anti-FcαRI $\times$ anti-epidermal growth factor receptor EGF-R) were observed to trigger ADCC with whole blood from G-CSF-primed patients against renal carcinoma cells, which were highly resistant to lysis with other EGF-R-directed antibody constructs such as chimeric human IgG1 or FcγRI-directed BsAbs. These results suggest that human IgA antibodies or FcαRI-directed bispecific constructs are interesting for immunotherapy (Valerius et al. 1997b).

FcγRIII (CD16) expressed by neutrophils, a small portion of circulating monocytes, mononuclear phagocytes and natural killer (NK) cells, also serves as an activation molecule for BsAbs. Of the two isoforms of human CD16, CD16A

is a transmembrane protein containing a cytoplasmic activation motif, expressed by human NK cells and mononuclear phagocytes. CD16B, which is expressed by human neutrophils, is linked to the membrane via a phosphatidylinositol glycan moiety, and is shed. This isoform possesses limited signal transduction properties upon receptor engagement. BsAbs, constructed with antibodies to the extracellular domains of CD16 and HER2/neu, showed highly efficient restricted cytotoxicity by human NK cells against relevant antigen-expressing tumor cells, at exceedingly low antibody concentrations, and relatively low effector-target ratios (Weiner et al. 1993, 1997). As described below, Renner et al. (1997) have started a phase I/II clinical trial with BsAbs (anti-CD16×anti-CD30) for the treatment of refractory Hodgkin's disease (Renner et al. 1997).

Clinical trials

From the results of in vitro pioneer study with BsAb and LAK cells, specific targeting therapy against malignant gliomas was initiated (Nitta et al. 1990a, b). LAK cells, which do not affect normal cells, can lyse fresh tumor cells irrespective of their major histocompatibility complex (MHC) status. While the results of local administration of LAK cells for the treatment of malignant gliomas have not been good, pretreatment of peripheral blood T cells with a BsAb (anti-CD3× anti-glioma) greatly enhanced their in vitro killer activity against target glioma cells. Both in vitro studies and animal model experiments have shown that specific targeting with BsAb not only augments the lytic potential of effector T cells, but also causes their cross-linking to target cells. BsAb-treated LAK cells are thus better able to recognize target cells than untreated cells. Specific targeting therapy in 10 glioma patients resulted in regression of the tumor in 4 patients and eradication of glioma cells remaining after surgery in another 4, as demonstrated by computed tomography and histology. No recurrence was detected in the 10 to 18 months of follow-up. Control study group patients who received LAK cells had recurrences within 1 year except in 1 case, and 8 patients died within 4 years (Nitta et al. 1990b).

Based on the excellent outcome of preclinical BsAb studies on CD30 positive Hodgkin's lymphoma cells, a phase I/II clinical trial of HRS-3/A9 BsAb CD16× CD30 for relapsed Hodgkin's disease opened in 1995 (Hartman et al. 1997). Fifteen patients were treated with this BsAb for 1-hour infusions 4 times every 3–4 days, starting with 1 mg/m^2. They reported that toxicity was low at doses below 16 mg/m^2, though HAMAs were detected in 9 patients, and allergic reactions (fever, pain in involved lymph node, and a maculopapulous rash) appeared in 4 patients after attempted retreatment. Clinical evaluation has been encouraging (Morein and Junghans 1998). One complete and 1 partial remission, 3 minor responses, and 1 mixed response were achieved. Activated neutrophils have been recently served as effector cells for BsAbs, their numbers in patients being dramatically increased by application of hematopoietic growth factors such as

granulocyte-macrophage colony-stimulating factor (GM-CSF). Simultaneously, G-CSF and GM-CSF enhance neutrophil function and induce up-regulation of CD64 (FcγRI). Curnow (1997) reported tentative results of phase I/II clinical studies with anti-FcγRI$\times$anti-HER-2/neu (MDX-H210) BsAbs in combination with GM-CSF. This therapy is now on-going, but evidence of antitumor activity has been obtained. Phase II trials of 15 mg/m^2 i.v. administration of MDX-H210 BsAb, given with GM-CSF, has been tried for 11 patients, all of whom had not responded to standard therapy. Among them, four have shown responses, including two with renal cell carcinomas and two with the prostate cancers. One renal cell carcinoma patient has had a 54% reduction in the size of a hepatic metastatic lesion and the other demonstrated a 49% decrease in the size of a lung metastasis with simultaneous clearing of other non-measurable lung lesions. Regarding the two prostate cancer patients, one has had a 90% reduction in serum prostate specific antigen (PSA); the other patient exhibited a 70% reduction of serum PSA after the first treatment. Accordingly, these early-stage findings clearly indicate that MDX-H210 BsAb has exciting potential in the therapy, at least, of otherwise refractory cancers. Some patients treated with MDX-H210 BsAbs have shown evidence of active antitumor immunity following this therapy. Biopsies of metastatic lesions before and after treatment suggest tumor localization of MDX-H210 BsAb and local immunological activity (Curnow 1997).

Superantigen-conjugated antibody therapy

The results obtained with combined use of BsAbs indicate the importance of activation of killer cells, as well as bridging between tumor cells and killer cells. Accordingly, we prepared bacterial superantigen SEA (staphylococcus enterotoxin A) conjugated anti-tumor antibody (anti-MUC1, MUSE11) by a chemical conjugation method. Since T-LAK cells bind to SEA through class II antigens expressed on the cell surfaces, SEA-MUSE11 antibodies could bridge MUC1-positive tumor cells and T-LAK cells in a similar way to anti-MUC1$\times$anti-CD3 BsAbs. In addition, the SEA-conjugated MUSE11 antibody is a potent activator of T cells. In vitro assays, where TFK-1 cells were co-cultured with T-LAK cells in its presence, remarkable enhancement of T-LAK cytotoxicity was found. SEA alone enhances T-LAK cell cytotoxocity, though T-LAK cells were already stimulated with solid phase anti-CD3 antibody and IL-2 (Shinoda et al. 1998). Therefore we tried an in vivo experiment in xenotransplanted SCID mice. First, we transplanted s.c. 5×10^6 TFK-1 cells, then T-LAK cells (2×10^7) sensitized with SEA-anti-MUSE11 antibody (2 μg/ml) together with IL-2 (500 IU) were i.v. injected, on days 10, 11, 12, 13 (when tumor size was 5 mm in diameter). Remarkable tumor growth inhibition was observed with this therapy (Shinoda et al. 1998), while T-LAK cells with either SEA, IL-2, or anti-MUC1 antibody had no effect (Fig. 6). Retargeting of T-LAK cells to tumor tissue was verified by immunostaining of T-LAK cells with anti-CD3 antibodies, CD3-positive T-LAK

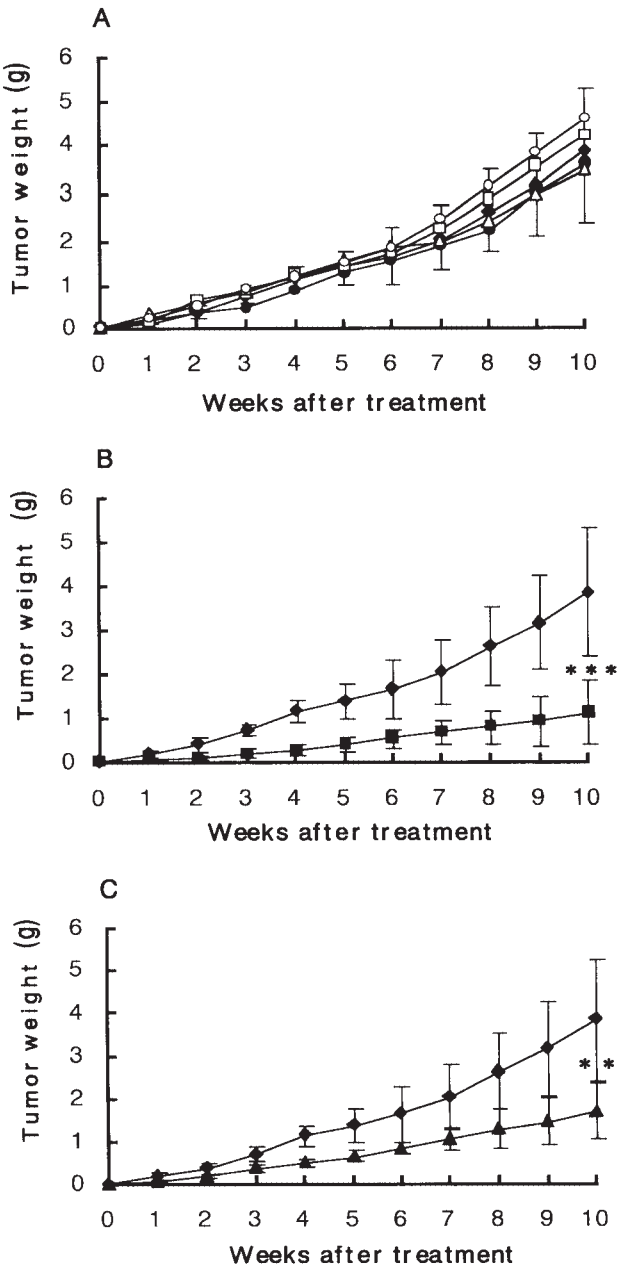

Fig. 6. Results of in vivo adoptive immunotherapy of TFK-1 tumors in SCID mice. T-LAK cells were incubated with 0.15 ml PBS containing 2 μg of SEA-MUSE11 (■), SEA-F(ab')$_2$ (▲), MUSE11 Ab (△), F(ab')$_2$ of MUSE11 Ab (○), or SEA (□) at 4°C for 1 hour. Then, each mouse received i.v. 500 IU IL-2 and 0.15 ml preincubated cell suspension without washing the cells. Data for an experimental group receiving T-LAK cells incubated with PBS (●) and a PBS control group (◆) are included in these figs. *Symbols* show mean values for tumor weights. *Bars*, SDs of the control, SEA-F(ab')$_2$ groups. The SEA-MUSE11 and SEA-F(ab')$_2$ groups each consisted of 25 mice (B and C), and the other groups consisted of 15 mice (A), $^{***}p < 0.001$ for SEA-MUSE11 Ab vs. control group; $^{**}p < 0.01$ for SEA F(ab')$_2$ MUSE11 Ab vs. control group.

cells being more numerous in tumor tissue when given with SEA-antibodies to xenotransplanted SCID mice. Recently, Sakurai et al. (1999) created an SEA-MUSE11 simple chain Fv antibodies (scFv) fusion protein by gene engineering,

which enhanced the cytotoxic activity of T-LAK cells as in the case with SEA-conjugated MUSE11 antibody. Affinity of scFvs is generally much lower than parental antibodies. In order to obtain positive results with flow cytometry, more than 20 μg/ml concentration of scFv is usually required, while about 1 μg/ml is enough for parental antibodies, indicating the need of technical improvement in scFv production. However, mass production of fusion proteins is possible in bacterial systems, and therefore this approach should be suitable for clinical application.

SEA binds class II and activates T cells expressing certain Vβ TCRs, unlike the situation with conventional antigen recognition. However, binding to class II may cause serious side effects when given to in vivo. In order to decrease class II binding, mutated SEA was constructed by changing Asp at 227 to Ala. This mutated form (SEA-D227A) demonstrated very low affinity for class II, but showed strong stimulation of human T cells. When SEA-D227A-conjugated antibodies (anti-MUC1) were given to co-cultures of a MUC1-positive bile duct carcinoma cell line (TFK-1) and PBMNCs, remarkable enhancement of cytotoxicity was observed in a 48-hour MTS assay. Similarly, SEA-D227A enhanced T-LAK cell cytotoxicity to TFK-1 cells. Furthermore, SEA-D227A conjugated-BsAbs (anti-MUC1 $\times$ anti-CD28) demonstrated strong cytotoxicity in TFK-1 cells co-cultured with PBMNCs (Kodama et al. 1999). Taken together, the results indicate that SEA-D227A-conjugated BsAbs (anti-MUC1 $\times$ anti-CD28) might be applicable for cancer immunotherapy.

Future prospects

As an antibody-based immunotherapy, application of BsAbs or SEA-conjugated antibodies has the potential to enhance cell-mediated cytotoxicity of killer cells, not only T-LAK, but also PBMNCs and NK cells. Using the three kinds of BsAbs described here, NKT cells which are very effective against metastatic lesions can be retargeted to cancer tissues.

One major advantage with BsAbs is that they can be produced and stored ready for use when necessary, while CTLs must be made-to-order. Antibodies to MAGE antigens, SART antigens, MUC1 antigen, c-erbB 2 antigen or cancer/testis antigens can be served to BsAb production. By conjugating antibodies (anti-CD3, anti-CD28, anti-CD2, anti-CD16, anti-CD64, anti-CD89) to effector cells with antibodies to anti-tumor antigens, numerous BsAbs can be produced. These various kinds of BsAbs would cover most cancer tissues, and they might enable to start immunotherapy immediately after diagnosis.

Cancer vaccines used to generate tumor-specific CTLs are not effective against tumor cells that have lost, or suppressed expression of their class I MHC proteins. This loss has been observed in primary lesions but more frequently in metastatic lesions (Progador et al. 1997), the difference pointing to a partial success of the patients immune system. T cells specific for tumor-associated peptide epitopes

presented by class I, eradicate class I-positive metastasizing tumor cells, thus causing class I-negative mutant cells in the primary lesions to be selected for metastasis more frequently (Progador et al. 1997). It follows that metastatic lesions are unresponsive to CTL therapy. However, combination therapy with activated killer cells and BsAbs allows this problem to be overcome, provided that metastatic cancer tissues express cancer-associated antigens detectable by antibodies. With regard to the fundamental concern of whether BsAbs to cancer-associated antigens might mask cancer epitopes for CTLs and block their cytotoxic activity, a recent study with CTLs to MUC1 and BsAb (anti-MUC1 $\times$ CD3) was instructive. Cytotoxic activity of CTL cells induced by MUC1 peptide was examined in the presence of BsAb (anti-MUC1 $\times$ anti-CD3) in vitro. The test revealed that BsAb (0.2 μg/ml) did not block cytotoxic activity of CTL, though slight decrease was noted when 1.0 μg/ml BsAb was added (personal communication from Dr. Kontani, Shiga Medical University). These results suggest the applicability of BsAbs for clinical purposes in combination with peptide vaccine therapy, since T-LAK cells and resting T cells can thus be readily retargeted by BsAbs, and BsAbs would not interfere with CTL activity at the usual concentration of BsAbs. BsAb production by gene engineering methods, such as diabody production (Kipriyanov et al. 1998), is necessary for clinical applications which require large amounts compared with in vitro experiments. Humanization of BsAbs will surely decrease side effects caused by employment of mouse antibodies. Collectively, BsAb can augment cellular immunity to tumors, and this approach to therapy now warrants further clinical trials.

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