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Review Article

Applications of bispecific antibodies in therapeutics

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Introduction

Since Nisonoff and Rivers first introduced the concept of bispecific antibodies (bsMAbs) into academia 40 years ago, this area had not been really attractive to either the commercial or the scientific community until hybridoma technology established itself as one of the cornerstones of modern biotechnology (1-3). Today, bsMAbs have been used to specifically recruit a variety of different effector mechanisms including cell-mediated cytotoxicity by targeting CTLs, NK cells, neutrophils and monocytes/phagocytes (4-12) as well as other cytotoxic mechanisms by recruiting toxins, drugs, enzymes or radioisotopes (13, 14). Recently, they have also been used in gene therapy for specific targeting of therapeutic vehicles like liposomes or viruses (15-18) (Fig. 1). Some of these exciting explorations have already extended to redirecting cytotoxicity to tumor cells, HIV and other infectious agents, targeting enzyme to achieve site-specific activating anticancer prodrugs, localizing fibrinogen activator to dissolve fibrin clots and delivering antigen specifically to antigen-presenting cells as vaccines. Due to their great potential for the development of new therapeutic applications, enormous research efforts have been devoted to this new area, allowing for its rapid growth. This review paper will focus primarily on the main applications of bsMAbs and highlight recent advances.

Drug delivery

BsMAbs have shown the potential to deliver chemotherapeutic drugs specifically to the tumor. BsMAbs have been constructed to deliver various drugs, *e.g.*, doxorubicin (19-21), epirubicin (22), methotrexate (23) and vinca alkaloids (24-27). Ford *et al.* have successfully demonstrated a bsMAb targeting of doxorubicin to carcinoembryonic antigen (CEA) expressing colon cancer cell lines *in vitro* and *in vivo* (19). In *in vitro* studies, 3 human colon cancer cell lines (COLO320DM, LS174T and SKCO1) have been used with no, medium and high CEA expression, respectively. The IC_{50} values for doxorubicin with COLO320DM, LS174T and SKCO1 were 1163, 324 and 28.5 ng/ml, respectively. The various concentrations of bsMAbs at 1, 0.1 and 0.01 g/ml all resulted in statistically significant reduction in doxorubicin IC_{50} values with the CEA-expressing cell lines SKCO1 and LS174T but not with COLO320DM. *In vivo*, there was also a statistically significant inhibition of the growth of CEA-expressing LS174T cells growing as xenografts in nude mice treated with a bsMAb and doxorubicin compared to control mice. This effect was not seen with COLO320DM xenografts. This study demonstrated that the bsMAb recognizing CEA and doxorubicin could reduce the IC_{50} for doxorubicin *in vitro* and inhibit tumor growth *in vivo* (19).

BsMAbs have been used to target toxins, *e.g.*, ricin A (28), saporin (29-32) and gelonin (33), to the tumor. Some clinical studies have successfully utilized a synergistic effect of two bsMAbs to deliver saporin (ribosome-inactivating protein) for the treatment of low-grade, end-stage B-cell lymphoma. Both bsMAbs have one arm directed at saporin and another arm at CD22 on target B cells. However, the two bsMAbs recognized different, non-overlapping epitopes on saporin. This strategy allowed high-avidity double attachment of saporin to the target. In a small-scale clinical trial, 5 patients were treated with weekly doses of 2-4 mg of saporin for up to 6 weeks. All patients showed a rapid and beneficial response with minimal toxicity (31, 32).

Another pair of bsMAbs (anti-gelonin/anti-CD30) has shown a similar beneficial synergistic effect on Hodgkin's lymphoma cell lines (33). Gelonin is a ribosome-inactivating protein that displays a lower toxicity compared to other ribosome-inactivating proteins. These two bsMAbs were produced using the same anti-CD30 MAb and two different anti-gelonin MAbs, directed to unrelated epi-

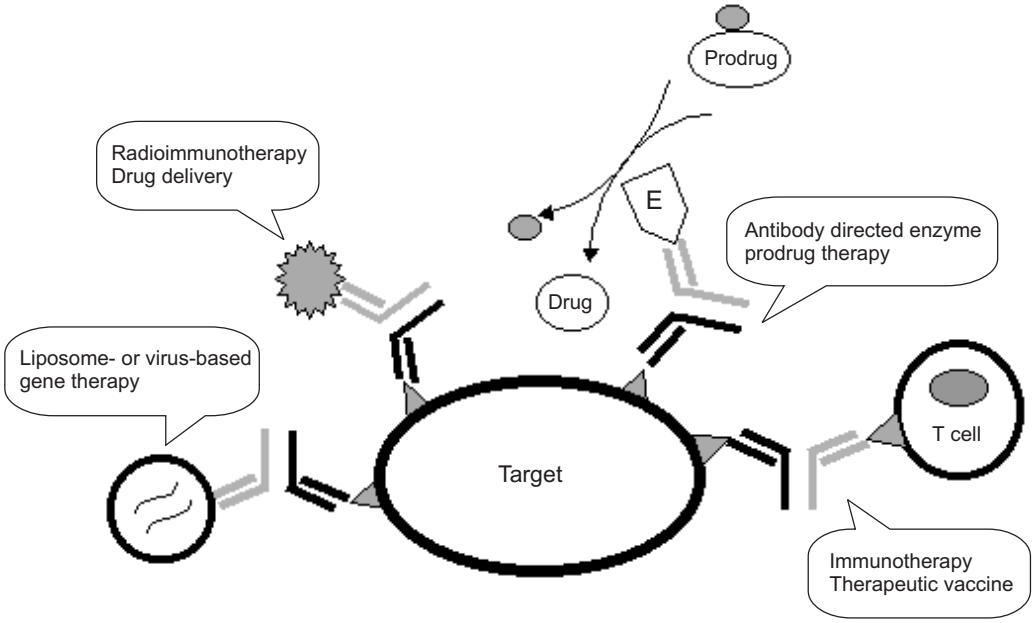

Fig. 1. Schematic representation of different therapeutic applications of bsMAbs.

topes of the gelonin molecule. *In vitro*, both bsMAbs enhanced gelonin toxicity against the CD30 positive L540 Hodgkin's lymphoma cell line. In the presence of either bsMAb, protein synthesis was inhibited with an IC_{50} of 5 x 10^{-10} M or 8 x 10^{-11} M, respectively, compared to IC_{50} 5 x 10^{-8} M in the absence of bsMAb. A combination of two bsMAbs had shown a synergistic effect with an IC_{50} of 6 x 10^{-12} M. Among CD30 positive tumor cell lines, the Hodgkin's lymphoma L428 was also sensitive to gelonin delivered by bsMAb (IC_{50} 6 x 10^{-11} M) (33). This result further indicates the beneficial effect of combining two bsMAbs to deliver toxins.

BsMAbs are also used to deliver cytokines. The use of tumor necrosis factor α (TNFα) in cancer therapy is limited by its short circulatory half-life and its severe systemic side effects. To overcome these limitations, a bsMAb directed against CEA and human TNFα has been constructed to target this cytokine to the tumor (34). A two-step injection protocol was used to target TNFα to the human colorectal carcinoma T380 in nude mice. First, a variable dose of ^{125}I-labeled bsMAb was injected, followed 24 or 48 h later by 1 μg of ^{131}I-labeled TNFα. Mice pretreated with 3 μg of bsMAb and sacrificed 2, 4, 6 or 8 h after the injection of TNFα showed a 1.5- to 2-fold increased concentration of ^{131}I-labeled TNFα in the tumor as compared to control mice, which received TNFα alone. With pretreatment of 25 μg bsMAb, mice showed a better targeting of TNFα with a 3.2-fold increased concentration of ^{131}I-labeled TNFα in the tumor. In a one-step injection protocol using a premixed bsMAb-TNFα preparation, similar results were obtained 6 h postinjection (3.5-fold increased TNFα tumor concentration). Moreover, a longer retention time of TNFα was observed leading to a 8.1-fold increased concentration of TNFα in the tumor 14 h postinjection (34).

The advantage of using bsMAbs over MAbs to deliver high-molecular weight toxins or drugs to tumors is to avoid the complex chemistry involved in direct linking of the antibody carrier to the cytotoxic agent. This provides a more uniform cross-linking between the target and the effector molecule. However, if the toxin or drug is a low-molecular weight effector molecule, the advantage may be outweighed by the dose limitation of this approach. To circumvent this limitation, a strategy combining drug attached to polymeric biodegradable carriers or encapsulated in liposomes with bsMAb may be adopted.

Antibody-directed enzyme prodrug therapy

Antibody-directed enzyme prodrug therapy (ADEPT) targets an enzyme specifically to a tumor where it converts a relatively nontoxic prodrug to a potent cytotoxic drug. Since each enzyme molecule has a capacity to convert a large number of nontoxic prodrugs to cytotoxic drugs, ADEPT has the potential to increase the concentration of cytotoxic drugs at cancer sites by a substantial margin. ADEPT also addresses the issue of slow or uneven tumor penetration by antibody-drug conjugates through generating small, toxic molecules within tumor mass; such molecules having better diffusion characteristics than antibody-drug conjugates. The diffusion characteristics of small molecules also addresses the issue of heterogeneity of antigen expression by clonogenic cells since their access to cells was not limited by antigen expression, giving rise to the so-called bystander effect.

ADEPT also circumvents the problem associated with releasing drugs from the carrier molecules (35).

BsMAbs or bifunctional MAbs provide an alternative approach to antibody-enzyme conjugate in the ADEPT system (36-38). A recombinant bifunctional fusion protein comprised of anti-CEA single chain Fv (scFv) antibody, fused to the amino-terminus of the enzyme carboxypeptidase G_2 (CPG_2) has been constructed to achieve ADEPT in nude mice bearing CEA-positive LS174T human colon adenocarcinoma xenografts (36). Bifunctional antibody cleared rapidly from circulation and catalytic activity in extracted tissues showed tumor to plasma ratios of 1.5:1 (6 h), 10:1 (24 h), 19:1 (48 h) and 12:1 (72 h). ^{125}I-Bifunctional antibody was retained in kidney, liver and spleen but its catalytic activity was not, resulting in excellent tumor to normal tissue ratios 48 h after injection. These were 371:1 (tumor to liver), 450:1 (tumor to lung), 562:1 (tumor to kidney), 1477:1 (tumor to colon) and 1618:1 (tumor to spleen). Favorable tumor to normal tissue ratios occurred at early time points when there was still 21% (24 h) and 9.5% (48 h) of the injected activity present per gram of tumor tissue. The high tumor concentrations and selective tumor retention of active enzyme delivered by bifunctional antibody demonstrate that bifunctional fusion protein has the potential to give improved clinical efficiency for ADEPT (36).

Sahin *et al*. (38) developed a bsMAb, HRS-3/AP-1, with reactivity against the Hodgkin's- and Reed-Sternberg cell-associated CD30 antigen and alkaline phosphatase, respectively. After an active incubation with alkaline phosphatase, purified whole immunoglobulin molecules and $F(ab')_2$ fragments of the bsMAb were equally effective in converting a noncytotoxic prodrug, mitomycin phosphate (MOP), into cytotoxic mitomycin alcohol. The cytotoxicity of MOP was unaffected when the cells were pretreated with either the bsMAb or the enzyme alone. HRS-3/AP-1 did not bind to HPB-ALL cells (CD30 negative) and was not able to activate MOP on these cells. However, in cocultivation experiments with HPB-ALL and L540 cells, the activation of MOP by HRS-3/AP-1 and alkaline phosphatase led to considerable cytotoxicity against the antigen-negative bystander cells. Thus, this immunotherapeutic approach might be effective in tumors in which not all the tumor cells express the respective tumor antigen (38). The advantage of a bsMAb plus enzyme/prodrug approach over bifunctional fusion protein is that the pharmacokinetics and targeting site accumulation of each component could be controlled separately. A limitation of this approach is the requirement of multistep injection; however, it may be argued that multistep injection is still less complex than many widely used chemotherapy protocols.

Besides cancer therapy, bsMAbs can be used to improve fibrinolytic efficacy of tissue plasminogen activator (tPA) in thrombosis therapy. tPA triggers the conversion of plasminogen to the fibrinolytic enzyme plasmin, which causes the dissolution of thrombi. Branscomb *et al.* have demonstrated that a bsMAb against human fibrin and tPA could enhance fibrinolysis 10-fold *in vitro* than with tPA alone (39). Some researchers have also tried to use bsMAbs to target urokinase plasminogen activator (uPA) to fibrin-containing clots (40), which resulted in 13-fold more fibrinolysis *in vitro* and 6-fold more fibrinolysis *in vivo* than with urokinase alone. In another study using baboon bearing ^{125}I-fibrin clots in femoral vein, continuous i.v. infusion of a bsMAb resulted in a 5-fold enhanced thrombolytic potency of uPA than that of uPA alone (41).

Radioimmunotherapy

Conventional radioimmunotherapy with MAb conjugates damages critical organs due to exposure to high radiation doses from long-circulating radiolabeled antibody and nonspecific accumulation of radiolabeled antibody in exposed organs. Pretargeted therapy with bsMAbs is a multistep strategy which allows delivery of the radioisotope (^{90}Y, ^{131}I, ^{188}Re and ^{32}P) to a tumor quickly and specifically with minimal radiation exposure and hence lowered toxicity to normal organs (42-48). Pretargeting involves administration of a bsMAb with high affinity for both a tumor antigen and a small rapidly excreted radioisotope molecule. After the bsMAb has concentrated in the target tumor, a clearing agent is administered to remove the excess bsMAb from the circulation. Following the clearing agent, the radioisotope molecule is given, and the maximum tumor concentration and tumor-to-blood ratio is achieved in 1-3 h. Unbound radiolabel is rapidly excreted via the kidney by glomerular filtration mechanism (48).

Gautherot *et al.* have compared the potential of pretargeting a bsMAb to the directly labeled $F(ab')_2$ fragment for the treatment of LS174T colorectal xenografts (44). A total of 6 groups of tumor-bearing mice were treated using anti-CEA x anti-diethylenetriamine pentaacetic acid (DTPA) $bsF(ab')_2$ and ^{131}I-labeled di-DTPA bivalent hapten. Three groups of mice were injected with various activities of ^{131}I-labeled bivalent hapten (75, 96 and 112 MBq) 20 h after administration of $bsF(ab)'_2$. Three other groups were injected with an almost constant activity of labeled hapten (102 MBq) at 3 time periods (15, 30 and 48 h) after $bsF(ab')_2$ administration. For conventional radioimmunotherapy, mice were treated with 96 MBq ^{131}I-labeled anti-CEA $F(ab')_2$. Conventional radioimmunotherapy induced severe toxicity and resulted in death of several treated animals. Nevertheless, all surviving animals treated with ^{131}I-labeled anti-CEA $F(ab')_2$ relapsed shortly after treatment (tumor growth delay = 48 ± 13 days). For animals treated with a pretargeting bsMAb, toxicity varied with the pretargeting time interval and the administered activity. For 20-h pretargeting time, the maximum tolerated dose was 96 MBq. For all pretargeting groups except one (with 48-h pretargeting time interval and growth delay of 82 ± 26 days), no tumor growth was observed over a period of 8 months. Furthermore, based on clinical and histologic criteria, 33% of the treated mice were considered cured. High cure rates of LS174T colon carcinoma were achieved with the pretargeting strategy.

Although results have been encouraging for pretargeted ^{131}I radioimmunotherapy, the radionuclide used is not optimal because of its long half-life, strong gamma emission, poor specific activity and low β particle energy. ^{188}Re, though unsuitable for direct antibody labeling, could be used with a two-step pretargeting technique. ^{188}Re has a greater range than ^{131}I, which should allow the eradication of solid tumors around 1 cm in diameter. One study has compared the distribution and dosimetry of a bivalent hapten labeled with ^{188}Re or ^{125}I (43). After preliminary injection of a bsMAb (anti-CEA/anti-hapten), AG 8.1 or AG 8.0 hapten radiolabeled with ^{188}Re or ^{125}I, respectively, was injected into a nude mouse model grafted subcutaneously with a CEA positive human colon carcinoma cell line (LS174T). This study indicates that ^{188}Re can be used for radiolabeling of hapten in a two-step radioimmunotherapy. Although the method used for hapten radiolabeling did not provide optimal tumor uptake, the use of a bifunctional chelating agent associated with AG 8.1 should solve this problem.

Pretargeting combines the pharmacokinetics of long-circulating MAbs with rapidly excreted small molecules to give high tumor concentrations and high tumor-to-blood ratios. The total radiation dose to normal tissue is greatly reduced in the pretargeting method by two ways: (i) absence of radiation during the localization phase, since the bsMAb is not radiolabeled at this time; (ii) rapid renal excretion of excess radiolabeled hapten that is not bound to the bsMAb in the tumor. The flexibility of the pretargeting approach allows the control of hematological toxicity, which is the main limitation to dose escalation with conventional radioimmunotherapy. However, from the drug development perspective, this multistep strategy places additional manufacturing burden and complicates the design of clinical trials. Another limitation is that bsMAbs directed to rapidly internalizing antigens would fail to capture and localize the radiolabeled hapten. In this instance, a bsMAb that is poorly internalized would be more appropriate.

Alternative pretargeting methods have incorporated the use of biotin- or avidin-labeled monospecific antibody followed by radiolabeled avidin or biotin, respectively (49). However, the immunogenecity of the hen egg avidin or streptavidin is the major hurdle for human use. Furthermore, a bsMAb has been made against polyhedral boron anion and a tumor-associated antigen so that it can deliver a large amount of stable boron atoms to tumor cells. After irradiation with low energy thermal neutrons, boron atoms will release lethal α particles to destroy tumors locally (50, 51). The interior of the tumor mass usually lacks radiosensitivity due to low level of oxygenation; bsMAb fragments, therefore, may be more attractive than whole immunoglobulin molecules for tumor radioimmunotherapy.

Immunotherapy

BsMAbs have the capability of activating and targeting the cellular immune defense system to kill tumor cells or other pathogens. A number of different effector cell populations have been studied extensively in this application. They include T cells, natural killer cells, macrophages and polymorphonuclear cells (8). Many groups have already demonstrated lysis of target cells by T effector cells using bsMAbs in a non-MHC restricted fashion (52, 53). The primary cytotoxic trigger on T cells is the TcR/CD3 complex, which is normally antigen specific and major histocompatibility complex (MHC) restricted. However, a bsMAb can react with this complex and initiate targeted cytotoxicity by activating the T-cell in a non-MHC restricted manner. Many efforts have been made to target bsMAbs to surface molecules such as TcR heterodimer (54), CD3 (55, 56) and CD2 (57, 58). Cytotoxicity was not due to bystander lysis, since direct contact between effector and a target cell is required (59). The therapeutic success was mainly based on the retargeting of the small existing cytotoxic T cell pools and was further augmented by anti-CD28 costimulation (60). Other types of effector molecules that have been used are FcγRIII receptor (CD16) (61, 62), complement receptor CR3 of macrophage (63), Fc receptor (FcγRI) (64) and anti-γ/δ T cell receptor (65).

Cancer immunotherapy involving bsMAb-mediated killing has been explored by several groups, including anti-epidermal growth factor receptor for breast cancer (55), anti-sialyl Lewis^a for colon cancer (66), anti-ovarian cancer (67, 68), anti-renal cell carcinoma (69), anti-lung small cell carcinomas (70), anti-CD19 for B lymphoma (71, 72), anti-CD13 for acute myeloid leukemia (73) and anti-tenascin for gliomas (55). The exciting preclinical studies have prompted several clinical studies using bsMAbs. The toxicity associated with this immunotherapeutic approach appears to be minimal and early results show promising clinical benefits as well. Guyre and Franger have developed a bsMAb, MDX-210, which recognizes FcγR1 on monocytes and macrophages and the cell surface product of the HER-2/neu oncogene overexpressed on some breast and ovarian cancers. Clinical trials have demonstrated that treatment with MDX-210 is well tolerated. Optimization of the dose and schedule of MDX-210 and development of combination treatments with cytokines that modulate immune effector cells will greatly enhance the efficacy of this novel bsMAb approach for treatment of tumors that overexpress HER-2/neu (74). Another bsMAb, 2B1, with specificity for the c-erbB-2 and FcγRIII extracellular domains has also been tested in humans. This bsMAb promotes the targeted lysis of malignant cells overexpressing the c-erbB-2 gene product of the HER2/neu proto-oncogene by human natural killer cells and mononuclear phagocytes expressing the FcγRIII A isoform. In a phase I clinical trial, 2B1 treatment induced more than 100-fold increase in circulating levels of tumor necrosis factor α, interleukin 6 and interleukin 8 (62).

BsMAbs have also been tested for their immunotherapeutic applications in viral infections (75). Chamow *et al.* have developed a humanized bsMAb, CD4/anti-CD3, to target HIV-infected cells. First, the bsMAb targets to

HIV-infected cells by the natural affinity of CD4 for gp120. Second, it recruits and activates CTL to lyse target cells through its anti-CD3 moiety in a non-MHC restricted manner. The authors demonstrated that the bsMAb could specifically lyse HIV-infected cells using purified CTL and whole peripheral blood lymphocyte (PBL). In contrast, a human anti-gp120 MAb can lyse HIV-infected target cells only with PBL fractions and not with purified CTL. Moreover, the lytic activity of anti-gp120 antibody was completely blocked by human serum (which competes for Fc γ receptor binding with abundant human IgG), whereas bsMAb-mediated lysis of target cells was not affected. This reflects a potential advantage of bsMAbs over MAbs for HIV-directed therapy (76, 77).

Gene therapy

Efficient gene transfer by recombinant adenoviral vectors depends on expression of the coxsackievirus/adenovirus receptor (CAR) and alpha (v)-integrins on target cells. Because normal cells may express these receptors, the use of adenoviruses for gene therapy is limited by their lack of specificity. Targeting of the adenoviral vector may allow the administration of fewer adenovirus particles, thus decreasing vector-related toxicity. In addition, it may enhance the infection efficacy of adenoviral vectors on specific cells that express a low amount of receptors for these viruses. Hence, targeting the adenoviral vector has important applications for *in vivo* use of these vectors. Since most human adenoviruses bind to the CAR via the carboxyl-terminal knob domain of their fiber protein, the targeting of adenovirus to specific cell lines can be achieved by using bsMAbs to redirect the binding to CAR to a new cellular receptor on the target cells (78-84). Nettelbeck *et al.* have developed a bispecific scFv (anti-CD105 x anti-knob) targeting the adenovirus to the endothelial cell surface protein endoglin (CD105), for vascular targeting of adenoviruses. Endoglin (CD105) is a component of the transforming growth factor beta-receptor complex representing a promising target for antivascular cancer therapy. This bispecific molecule enhanced selective adenovirus transduction of target cells and demonstrated the utility of bsMAbs for the retargeting of adenoviruses in cancer gene therapy (78).

Another bsMAb (anti-knob x anti-TAG72) has been investigated clinically for gene transfer to both ovarian cancer cell lines and primary ovarian cancer cells cultured from malignant ascites fluid (80). This bsMAb augmented gene transfer to primary ovarian cancer cells 2- to 28-fold relative to untargeted gene transfer, while also decreasing gene transfer to autologous cultured mesothelial cells 4- to 9-fold. Data *in vivo* demonstrated that bsMAb retargeting improved the selectivity of adenoviral gene transfer for ovarian tumors 8- to 252-fold on i.p. injection. These results suggest that bsMAb retargeting may improve the therapeutic index of cytotoxic gene therapy for ovarian cancer in clinical trials (80).

In addition, Reynolds *et al.* have used a bsMAb to target the angiotensin-converting enzyme (ACE), which is preferentially expressed on pulmonary capillary endothelium, thus enabling gene therapy for pulmonary vascular disease (79). Administration of retargeted vector complex into rats resulted in at least a 20-fold increase in both adenoviral DNA localization and luciferase transgene expression in the lungs, compared to the untargeted vector. Furthermore, targeting led to reduced transgene expression in nontarget organs, especially the liver, where the reduction was over 80%. These studies so far show that bsMAb retargeting can specifically modify the i.v. behavior of an adenoviral vector given systemically and thus encourage the further development of injectable adenoviral vectors. For bsMAb retargeting gene therapy, bsMAb fragments may be the better candidate since they can circumvent the problem of viral clustering, which may occur with bivalent MAbs and trapping in the reticulo-endothelial system through the Fc domain of whole immunoglobulins. Besides the viral vector, other vectors like liposomes can also be retargeted by bsMAbs to tumor or disease site specifically. The further exploration of bsMAb redirected gene therapy will definitely open new horizons for the treatment of cancer and other deadly diseases.

Therapeutic vaccines

A major goal of therapeutic vaccines is the induction of a systemic immune response including cellular and humoral responses against the target antigens. Costimulatory molecules are often needed for augmentation of this response to completely eradicate the target and prevent its relapse. BsMAbs with their unique two-armed structure can enhance the immune response by directly linking the target and T cell or through binding to those costimulatory molecules, like CD28 or B7 (85-93). Haas *et al.* have generated a tumor vaccine modification procedure that involves infection with Newcastle disease virus to mediate the cell surface binding of costimulatory molecules (85). Following the infection of tumor cells with a nonvirulent strain of Newcastle disease virus, the cells were further modified by incubation with a bsMAb, specifically against the viral hemagglutinin-neuraminidase (HN) molecule on the infected tumor cells and against CD28 to augment antitumor T-cell responses. A second bsMAb (anti-HN x anti-CD3) was produced to deliver T-cell receptor-mediated signals either alone or in combination with anti-CD28. In human T-cell stimulation studies *in vitro*, the bsMAb (anti-HN x anti-CD28) vaccine caused an upregulation of early (CD69) and late (CD25) T-cell activation markers on CD4 and CD8 T lymphocytes from either normal healthy donors or cancer patients and induced tumor cytostasis in nonmodified bystander tumor cells. In addition, in combination with the bsMAb (anti-HN x anti-CD3) vaccine, augmented antitumor cytotoxicity and T-cell proliferative responses were observed.

Guo *et al.* reported a nonviral way that relies on simple cell culture techniques to develop a cancer vaccine. In

a two-step process, tumor cells removed from a patient are first modified in the laboratory with a combination of cytokines to enhance the expression of tumor antigens and immune activating molecules needed to generate the body's immune response. Treated tumor cells are then armed with a bsMAb that has two different binding sites: one recognizing an antigen on tumor cells and another that binds to a key immune activating molecule (CD28 antigen) expressed on T cells. By specifically targeting CD28 molecules on T cells, the bsMAb on the cytokine-treated tumor cells creates a "bridge" that facilitates tumor and T-cell interactions. This bond greatly enhances T-cell activity and the immune response. Experiments were performed on laboratory mice susceptible to fast-growing tumors. Disease-free mice were vaccinated with the modified cell vaccine, and 2 weeks later were injected with cancer cells. Ordinarily, tumors would rapidly develop, but in this case no cancer was detected in the mice treated with the vaccine. In another experiment, T cells harvested from vaccinated mice were shown to have the ability to kill cancer cells grown in laboratory culture, providing evidence that an enhanced T-cell immune response had been stimulated (92).

The human MAb repertoire to *Haemophilus influenzae* type b (Hib) polysaccharide (PS) is dominated by antibodies that express an idiotype designated Hibld-1. Reason *et al.* prepared a bispecific vaccine consisting of the $F(ab')_2$ fragment of a MAb specific for Hibld-1, coupled to the $F(ab')_2$ fragment of a MAb specific for CD3. This bispecific idiotypic vaccine stimulated production of human MAbs to Hib PS in severe combined immunodeficient mice engrafted with normal human adult PBLs. The induced MAbs uniformly expressed Hibld-1 and protected neonatal rats from Hib bacteremia (86).

Mocikat *et al.* explored the use of trioma (secreting bsMAb)-based vaccine against B-cell lymphoma that confers long-lasting tumor immunity (91). Lymphoma cells are fused to a xenogeneic hybridoma cell line that secretes an antibody against a surface molecule on APCs. The resulting "trioma" cells produce a bispecific antibody containing the lymphoma Id and the APC-binding arm, which redirects the Id to APCs. Processing and presentation of the Id led to T-cell activation and tumor immunity was specific and long-lasting (90).

Summary

Bispecific/bifunctional antibodies attract much attention from the research community due to their unique structure against two different antigens. The two-armed structure of bsMAbs allows researchers to place an item on one arm and allow the other arm to specifically target the disease site. Such an item can either be a drug, toxin, radioisotope or any therapeutic agent that can be delivered to the affected sites. Futhermore, bsMAbs make it possible to combine disease cells and immune effector cells specifically in a single molecule, thus allowing the effector cells to redirect their cytolytic activity towards the diseased cells in a highly efficient manner. The plethora of applications for bsMAbs as unique therapeutic systems is expanding with major advances in recombinant DNA technology, the increased number of identified disease targets with the completion of the human genomic map project, as well as a better understanding of the human immune system. Although still in their infancy, bsMAbs hold great promise for numerous therapeutic needs such as cancer, heart disease, arthritis, infectious diseases, *etc*.

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