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Review

Immunoconjugates: Applications in Targeted Drug Delivery for Cancer Therapy

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Monoclonal antibodies can be produced against virtually any molecule, and unlike polyclonal antisera, they are highly specific. There has been great improvement in the monoclonal antibody production technique since its inception in 1975. The idea behind using monoclonals to direct cancer treatments is based on the fact that surfaces of tumor contain a wide variety of proteins, some of which are specific to the tumor type. Monoclonal antibodies that bind to such tumor-specific antigens could be used, either alone or as conjugates of drugs and toxins (immunoconjugates), to selectively seek out and destroy these tumor cells. Targeted drug delivery therapy of tumor using monoclonals or their conjugates has been reported by many investigators, and the early results are quite promising. However, many obstacles still have to be overcome before immunoconjugates become a valuable agent in the treatment of human diseases including cancer.

KEY WORDS: monoclonal antibodies; immunoconjugates; cancer immunotherapy; targeted drug delivery; liposomes; tumor therapy; drug delivery; cancer treatment; drug delivery systems.

INTRODUCTION

Since the discovery of monoclonal antibodies (MoAbs) in 1975 (5), researchers have a new weapon, "the magic bullet," in their arsenal to devise novel methods of delivering drugs to the target site. Antibodies including monoclonals have found use in sensitive immunodiagnostic tests (1–4). However, the use of MoAbs and their conjugates for therapeutic purposes is still in its infancy. The purpose of this review is to provide up-to-date information on the recent developments in targeted drug delivery and immunotherapy of cancer using antibodies and their conjugates.

In order to appreciate the impact of MoAbs, it is necessary to understand the problems and limitations of conventional polyclonal antisera/antibodies. The production of highly specific antisera is difficult and unreliable; further, it requires highly purified antigen, and different lots of antisera have different specificities and affinities toward the antigen. On the other hand, MoAbs are highly specific. Unlimited quantities of such antibodies can be produced against virtually any molecule, regardless of the purity of immunizing antigens.

MONOCLONAL ANTIBODY PRODUCTION

Each B lymphocyte in an animal expresses an antibody of only one specificity on its membrane. Once driven to differentiate, the B cell becomes a plasma cell with the cytoplasmic machinery to produce and secrete large amounts of its own unique immunoglobulin. For the production of monoclonals, usually a mouse is immunized with the antigen of interest (Fig. 1). Once an immune response ensues, B lymphocytes from the immunized animal's spleen or lymph nodes are harvested in a single cell suspension. These cells are then fused with myeloma cells from the same species. Myeloma cells are immortal and have the cytoplasmic machinery to produce large quantities of immunoglobulin. They also contain an enzyme deletion, hypoxanthine guanine phosphoribosyl transferase (HGPRT), which is required for their survival in the presence of the folic acid antagonist, aminopterine. Fusion of cells is generally triggered by Sendai virus, polyethylene glycol, or electric current. The cell suspension is then distributed into the wells of a microtiter plate in a selection medium such as hypoxanthine–aminopterine–thymidine (HAT), where only hydridomas that have acquired the HGPRT from lymphocytes via cell fusion survive. The hybrids are cloned by limited dilution to one cell per well, which makes it easy to identify an antibody of the desired specificity, titer, and avidity for propogation in mass culture. Alternatively, the antibody-producing cells can be injected into the peritoneal cavity of mice for the production of ascites fluid. Cell supernatant or ascites can later be purified by column or affinity chromatography to obtain the pure antibody.

Since the inception of hybridoma technology in 1975 (5), many improvements in the technique have been made. The use of genetically altered mice for immunization (6), fusing cells in the presence of electric current, hybridoma selection by flow cytometry (7), and *in vitro* immunization (8,9) are some examples.

IMMUNOCONJUGATES

Conjugates of small molecules (drugs, some toxins) and

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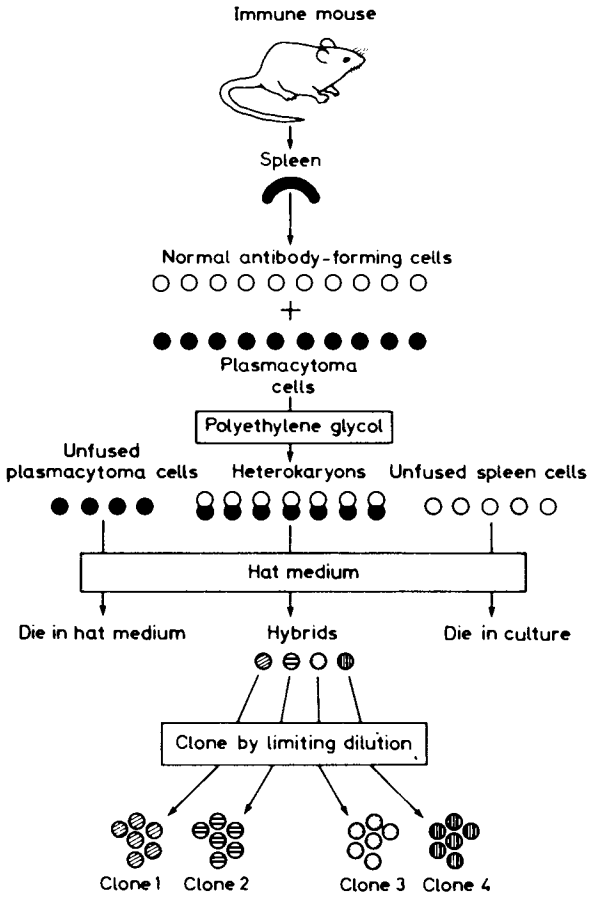

Fig. 1. General scheme for the production of monoclonal antibodies. (Reproduced with permission from Ref. 95.)

large molecules (proteins, enzymes, bacterial and plant toxins) with monoclonal or polyclonal antibodies are used in a myriad of immunodiagnostic assays (10), in immunohistochemistry (11), and as therapeutic agents. Various methods are described in the literature for preparation of protein conjugates (12,13). Antibodies (immunoglobulins) carry several reactive groups, such as the carboxyl groups of the C terminal and of aspartic and glutamic acid residues, the amino groups of the N terminal and of lysine residues, the imidazol of histidine and phenolic function and tyrosine, the sulfhydryl group of cysteine, and the guanidino group of arginine.

Immunoconjugates used in cancer therapy constitute both haptens, with a molecular weight below 1000 daltons (doxorubicin, daunorubicin, vindesine), and large molecules (plant and bacterial toxins such as ricin and diphtheria toxin) (14–16). Ligands with carboxylic groups can be directly coupled to proteins after their activation with a mixed anhydride, a carbodiimide, or *N*-hydroxysuccinimide. Many compounds have reactive groups to which a carboxyl group can be attached, for example, by alkylation of oxygen or nitrogen substituents with halo esters, followed by ester hydrolysis (17,18), and by formation of hemisuccinate esters (12) and carboxymethyloximes (19). Likewise, procedures are available for coupling ligands with the amino group (20,21), hydroxyl group (22), and carbonyl group (23). Multiple methods are available for the production of protein–protein conjugates, for example, using glutaraldehyde as the coupling agent (24,25) or by periodate oxidation (26).

Both haptens and large molecules can be coupled to protein using homo- or heterobifunctional reagents. A general disadvantage of common cross-linking with homobifunctional reagents is a lack of control over the incorporation of protein and hapten molecules into the resulting conjugate. Some of the homobifunctional reagents used for coupling are *N,N′-O*-phenylenedimaleimide (27), 4,4′-difluoro-2,2′-dinitrophenyl sulfone (28), toluene-2,4-diisocyanate (29), benzoquinone (30), and bis-succinic acid *N*-hydroxysuccinimide ester (31). Of these *N,N-O*-phenylenedimaleimide has been extensively used to prepare conjugates of β-galactosidase. The method is suitable for proteins containing sulfhydryl groups, which can also be introduced into the proteins with *S*-acetylmercaptosuccinic anhydride (32), 5,6,4-methylmercaptobutyrimidate (33), or disulfide reduction.

Heterobifunctional reagents (13) are becoming increasingly popular for the preparation of protein–protein and protein–hapten conjugates, as the reactions leading to conjugate formation can be performed in a controlled fashion. The heterobifunctional reagent, *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), has been used (34) to conjugate IgG to toxins (16,35), liposomes (36), tuberculin (37), and enzymes (38,39). The conjugation scheme using SPDP is presented in Fig. 2. Some examples of related heterobifunc-

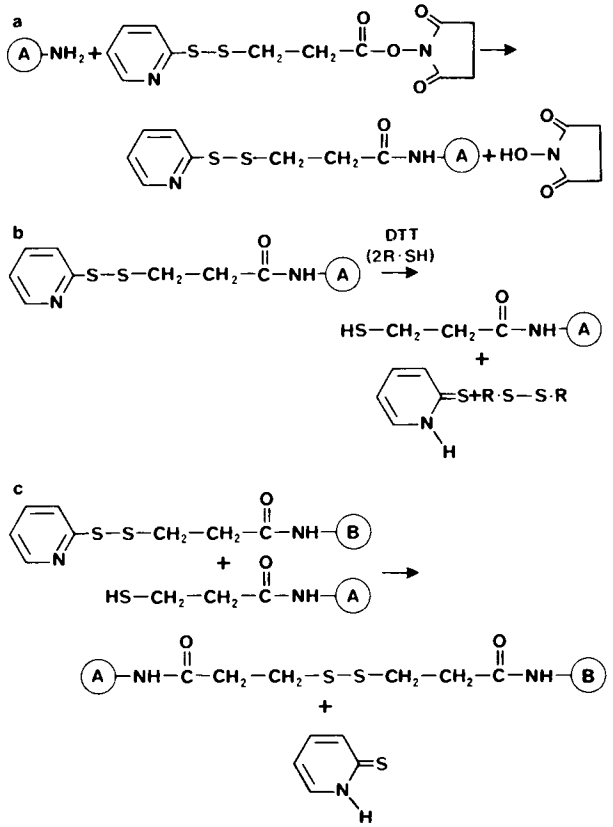

Fig. 2. Scheme for protein:protein conjugation. (a) Introduction of 2-pyridyl disulfide structures into a protein using SPDP. (b) Thiolation of the modified protein. (c) Conjugation of proteins A and B. (Reproduced with permission from Ref. 96.)

tional reagents used in conjugation are *m*-maleimidobenzoic acid *N*-hydroxysuccinimide ester (MBSE) (40) and *N*-(4-carboxycyclohexylmethyl)maleimide *N*-hydroxysuccinimide ester (41).

Assessment of Immunoconjugates for Therapeutic Use

The following criteria must be considered if an immunoconjugate is to be used in a clinical setting: purity, binding properties, and pharmacological activity. For the purification of protein conjugates of drugs of low molecular weight (MW), the free drug can be removed by dialysis or gel filtration on Sephadex G-10 or G-25 (42). Purification of conjugates of two high MW components is achieved by protein fractionation technique using Sephadex G-150 (43) and G-200 (44), Ultrogel AcA-44 (45), ion-exchange chromatography (46), and affinity chromatography (47).

Antibodies may undergo conformational changes in their structure after conjugation, which may affect their binding characteristics and thus their efficacy as a carrier vehicle. However, many antibodies do not lose their specificity and binding characteristics after conjugation (48). For example, of several monoclonal antibodies against carcinoembryonic antigen (CEA) (49), human osteogenic sarcoma (50), p97 melanoma-associated antigen (51), and neuroblastoma cells (52), only the CEA antibody lost some antigen binding affinity when conjugated to the anticancer drug vindesine (VDS).

Loss of antibody activity may also be related to the drug/antibody ratio in the conjugate. Increasing the ratio of drug on the conjugate in general decreases the antibody activity. Three different VDS–antibody ratios gave the following antibody activities relative to the unconjugated antibody: 3:1, 84%; 7:1, 76%; and 9:1, 70% (48).

The pharmacological activity of anticancer drugs in a monoclonal immunoconjugate may decrease or increase in comparison with the free drug. The antimitotic drug VDS, when conjugated to a MoAb raised against the human osteogenic sarcoma cell line, 791T, showed less toxicity on cultured 791T cells than the free drug (53). On the other hand, the conjugate of the drug daunorubicin with melanotropin had three times more toxicity on cultures of melanoma cells than free daunorubicin (54). In addition, the conjugate showed selective toxicity to mouse melanoma cells but not to mouse 3T3 fibroblasts, whereas unconjugated daunorubicin was equally toxic to both cell lines (55). The enhanced selective toxicity of the conjugate may be due to the recognition of conjugate by melanotropic receptors present on melanoma cells (56) but absent from 3T3 cells as hypothesized by the authors (54).

DRUG DELIVERY AND TARGETED THERAPY OF CANCER

Monoclonal antibodies may, by themselves, kill tumor target cells and may therefore serve as anticancer agents. For example, Centocor (Malvern, Pa.) has introduced monoclonals, 17-1A and CA-125, in clinical trials to treat gastrointestinal and ovarian cancers, respectively. Biotherapy System (BTS, Mountain View, Calif.) and Biotherapeutics, Inc. (Franklin, Tenn.), are developing monoclonals tailored to each individual's tumor. A number of complete and partial remissions have been achieved in clinical trials using this antibody approach (57).

Most researchers believe that this approach to treat cancer will not prove feasible in most cases due to several severe limitations of the antibody: (a) cancer cells are heterogeneous, so those cells that are not recognized by the monoclonal antibody can escape and proliferate; (b) many cancer cells can interact with the antibody, rendering it harmless; (c) some tumors contain semidead cores which cannot be reached by antibodies; and (d) the need for readministration will increase the likelihood of immunogenic reactions by patients.

The Immunoconjugate Approach

The use of MoAbs for targeting therapeutic agents to tumors is currently a major area of interest in tumor immunology (58). The aim is to produce conjugates of antibody and a toxic agent that will localize selectively at the tumor site, causing maximum damage to tumor cells but not to normal cells. Much attention has been focused on the use of plant or bacterial toxins such as ricin, the A chain of abrin, gelonin, and the A chain of diphtheria toxin (16,59–62). These molecules alone are relatively nontoxic, but when combined with an antibody they become bound to target cells, followed by their internalization, which leads to death of the tumor cells. The toxic agents or drugs in the conjugates ride piggyback on monoclonal antibodies which carry them to tumor-specific sites.

Homing of Liposomes

Many unwanted reactions arising from the use of drugs, enzymes, and proteins in cancer treatment or prevention of disease could be minimized by the entrapment of such agents in liposomes (63,64). Injected liposome-entrapped agents do not come in contact with blood, and their clearance from plasma and tissue distribution is controlled by their carriers (65,66). The endocytolic mode (67) of liposome uptake by cells warrants the entrance of agents in otherwise inaccessible cells and it also provides a convenient mechanism for the release of entrapped agents by the disruption of liposomes in the lysosomal milieu (64). However, the localization of injected liposomes mainly in the fixed macrophages of the liver and spleen restricts their use (64). It has been suggested that direction of liposomes to alternative targets could be attained by appropriate manipulation of the liposomal surface. Liposomes have been used to deliver drug to the diseased area by using antibodies as homing probes. More relevant to this discussion are drug delivery systems in which the antibody is the actual drug to be delivered rather than its use as a "homing device" (68). The cytotoxic drug bleomycin used in cancer chemotherapy was entrapped in liposomes with antibody (69). It appeared that following its attachment to the cell surface, liposomal IgG effected the uptake of the associated liposomal moiety itself and the drug bleomycin. It also appeared that only in a limited population of such liposomes could anti-cell IgG, available on their surface, effect the association of the liposomal carrier with cells.

Investigation of the subcellular fate of liposomal bleomycin taken up by cells (HeLa) via cell-specific IgG has

shown that only 20.5% of the cellular ^{111}In radioactivity is bound to the plasma membranes, the remainder, presumably interiorized through endocytosis, being recovered in the lysosome-rich particulate fraction (70).

Studies by Gregoriadis have shown that the liposome-associated antibody is able to get to its binding sites on the cell surface resulting from the internalization of complex in the cell's lysosomes, where disruption of liposomes and liberation of entrapped drug capable of reaching their cellular target occur (69,71). Recently, liposomes have been coupled with monoclonal antibody through covalent linkage using the heterobifunctional reagent SPDP discussed earlier in this review (36). The coupling method resulted in efficient binding of protein to the liposomes without aggregation and denaturation of the coupled ligand. Further, liposomes did not leak encapsulated carboxyfluorescein as a consequence of the reaction (36). In another application, a secondary antibody, raised against a primary antibody against a tumor antigen, was encapsulated into liposomes. Injection of the radiolabeled primary antibody into a patient followed by the second antibody in liposomes led to clearance of the double antibody–liposome complex and aided in tumor detection and localization (72).

One of the biggest advantage of using liposomes as a drug carrier is that a large amount of drug can be packed in the liposomes without losing the immunoreactivity of the linked antibody. It is anticipated that the use of antibody as a means of conferring specificity to liposomes would markedly increase their usefulness in the future.

Toxin Immunoconjugates

Koprowski *et al.* (73) have isolated several hybridoma clones, that secrete antibody to epitopes on colorectal carcinoma cells; these antigens have not been detected on normal tissues or other tumor cell lines tested. Conjugates of MoAb 1083-17-1A (designated 17-1A) with A chain of diphtheria-toxin (DTA)-SS-(17-1A) or ricin toxin (RTA)-SS-(17-1A) were evaluated for their binding to human cell lines.

Specificity was demonstrated by measuring conjugate binding to cell lines that lack antigen. As shown in Fig. 3, (DTA)-SS-(17-1A) bound to both SW948 and SW1116 but did not bind to four other cell lines that do not express antigen. These results correlate with the known binding specificity of 17-1A antibody (74). The higher level of binding of the conjugate to SW 948 cells relative to SW 1116 cells reflects a difference in the number of cells in the assay, not in the amount of antigen expressed by SW 948 cells.

The same conjugates were further studied in a separate experiment to see the inhibition of protein synthesis (an indicator of cell growth inhibition) of human cell lines, which provided evidence of 100% protein synthesis inhibition in colorectal carcinoma cell lines but not in other human cell lines.

Allogeneic bone transplantation is an effective therapy for acute nonlymphocytic leukemia (75) and has a role in the therapy of acute lymphocytic leukemia and non-Hodgkin's lymphoma (76). However, the number of patients treated with allogeneic transplants is small and limited by the availability of compatible donors and patient age. Recently, autologous transplantation has been proposed as an alternative

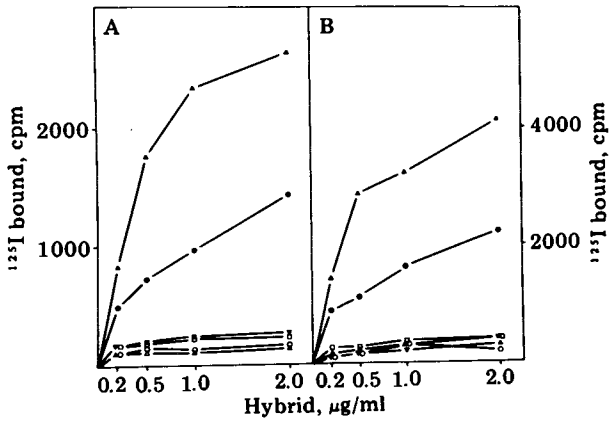

Fig. 3. Specificity of conjugate binding to human cell lines: ▲, SW 948, colorectal carcinoma; ●, SW 1116, colorectal carcinoma; ▽, MRC-5, normal fibroblast; □, WM 56, melanomas; △, MBA-9812, lung carcinoma; ○, HS-0853, lung fibroblasts. (A) (DTA)-SS-(17-1A); (B) (RTA)-SS-(17-1A). (Reproduced with permission from Ref. 16.)

therapy for leukemia and lymphoma patients. In the procedure, marrow is removed from patients and incubated *in vitro* with drugs (77) or specific antibodies (78) to remove residual leukemia cells. The feasibility of this experiment has been demonstrated in experimental animal systems and clinical trials (78,79).

MoAbs are appropriate reagents for purging marrow of malignant cells. Preliminary trials using monoclonal antibodies and complement for autologous transplantation have recently been reported (78). However, many monoclonals do not fix complements or require high antibody concentrations for complement-mediated cell killing (80). Conjugates of ricin with murine monoclonal antibodies have been successfully used in animal transplantation models (62,81). Recently Leonard *et al.* (35) synthesized the conjugates of whole ricin with the pan-T-cell monoclonal antibodies T101 and 3A1 and studied their toxicity for cell lines, peripheral blood T lymphocytes, and normal bone marrow progenitors. In the presence of 0.1 *M* lactose, normal cells and cell lines exhibited the following sensitivities to ricin: 8392 (human malignant B-cell line) < E rosette-positive lymphocytes < bone marrow progenitors < 8402 (human T ALL) < CEM (human T ALL). Ricin sensitivities correlated with ricin binding in the presence of lactose; peripheral blood T cells were resistant to 0.1 n*M* ricin but a similar concentration of T101–ricin inhibited normal and malignant T-colony formation by 98%. 3A1–ricin was slightly less effective. The authors concluded that (a) normal blood cells and malignant cell lines exhibit varying degrees of ricin sensitivity in the presence of lactose; (b) T101–ricin is at least 10-fold more toxic to T lymphocytes than to bone marrow progenitor cells and is effective in mixtures of normal and malignant cells; and (c) treatment of unfiltrated marrow with anti-T-cell immunotoxins should safely remove target T cells without excessively damaging normal progenitors or producing excessive free ricin (35). Thus T101 whole-ricin immunotoxins merit clinical trials for removing malignant T cells from human bone marrow.

Drug Immunoconjugates

An alternative to plant or bacterial toxins is to use conventional anticancer drugs which are already used in clinical practice. Thus, doxorubicin coupled to MoAbs has been reported to have therapeutic effects against a rat mammary carcinoma (14).

Many drug–antibody conjugates for suppression of tumor growth have been reported (82–84). It has been suggested (85) that linkage of cytotoxic drugs to antibody through an inert intermediate carrier offers a wide scope for improved cancer chemotherapy. Rowland *et al.* (85) conjugated *p*-phenylenediamine mustard (PDM) to an antibody against mouse lymphoma cells (EL4) through inert intermediates, e.g., polyglutamic acid (PGA). The PDM–PGA–antibody conjugate showed greater effectiveness *in vivo* as an antitumor agent (data not shown). The antimitotic drug VDS has been conjugated to MoAbs against various carcinomas by many investigators (15,48,53) and shown to have selective toxicity against tumor cells.

Embleton *et al.* (53) used a MoAb originally against the human osteogenic sarcoma cell line 791T to study the cytotoxic effect of VDS immunoconjugates *in vitro* on 791T sarcoma cell lines and other antigenically cross-reactive osteogenic sarcoma cell lines and, also, on tumor cell lines that have no detectable reaction with the MoAb. Continuous exposure to cultured 791T cells indicated that the VDS was partially inactivated following conjugation since the conjugate was less toxic than the free drug. However, MoAb binding activity was essentially preserved following conjugation. Despite diminished drug activity in the conjugate, assays designed to mimic antibody binding to tumor in which target cells were treated with conjugate and washed before culture showed selective cytotoxicity for four osteogenic sarcoma lines, with little or no effect on non-cross-reactive control cells (Fig. 4). In the case of 791T, 2 OS, and T278 the immunoconjugate was more toxic than VDS alone, and with 788T it was less toxic, but in all cases cytotoxicity was highly significant at doses of 10 mg/ml VDS or greater ($P < 0.001$ by Student's t test). Complete cytotoxicity was

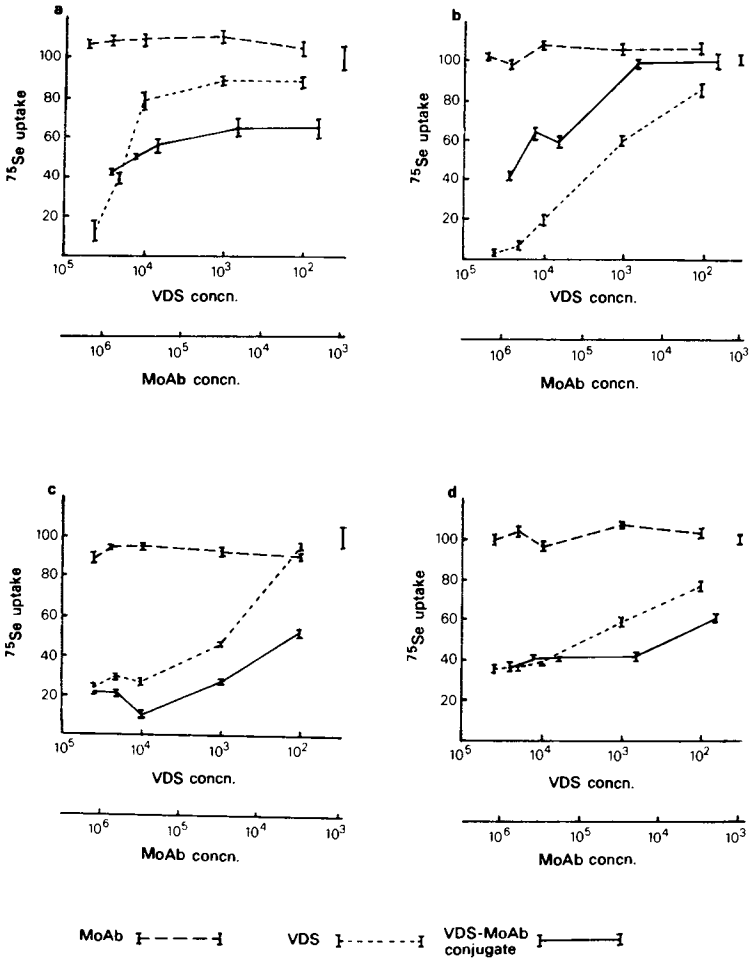

Fig. 4. Relative effects of VDS. VDS–α791T/36 conjugate (VDS–MoAb) and α791T/36 (MoAb) on osteogenic sarcoma cell lines. (a) 791T target cells; (b) 788T cells; (c) 2 OS cells; (d) T278 cells. ^{75}Se uptake is expressed as a percentage relative to that in PBS controls. Vertical bars indicate the SE. The SE at the far right (100%) is that obtained in PBS controls. The concentrations of VDS and MoAb indicated are in ng · ml^{-1}. (Reproduced with permission from Ref. 53.)

not achieved, presumably because not all target cells entered mitosis during the assay. In comparison, free VDS was equally toxic to all cell lines and free antibody was nontoxic.

Conjugates of VDS with MoAbs 96.5, 11.285.14, and 14.95.55 (antimelanoma, IgG2a, anti-CEA, IgGl, and IgG2a, respectively) were evaluated by Rowland *et al.* (15). Conjugate VDS–96.5 was markedly cytotoxic for cells bearing a high concentration of p97 antigen but not for cells expressing low levels of p97. Cells expressing an intermediate p97 level were moderately succeptible to the conjugate.

The effect of conjugate VDS–96.5 was also tested *in vivo* on xenografts of melanoma H2169 in nude mice (Fig. 5). The xenograft initially grew at a lower rate in mice treated with conjugate than in mice treated with free 96.5 antibody or PBS, but from 27 days after implantation (3 days after treatment had ceased) growth rates became similar in all three groups. During the initial phase of retardation (up to 25 days after implantation) the difference between VDS–96.5-treated mice and controls were statistically significant. Antibody alone did not affect tumor growth. Similar results were obtained using VDS conjugated to anti-CEA monoclonal antibodies. However, anti-CEA MoAbs alone were able to suppress the growth of a human colorectal tumor implanted in athymic mice (15,86). Conjugates with drugs methotrexate, chlorambucil, antibiotics, radionuclides, and some alkylating agents have been explored (97). Thus, there seems to exist a potential for application of immunoconjugates in clinical tumor chemotherapy and drug delivery.

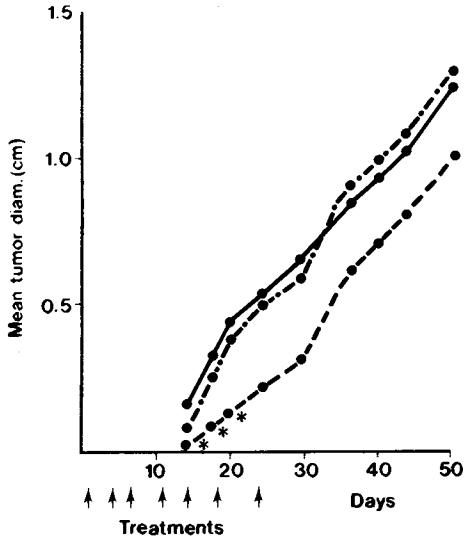

Fig. 5. Effect of VDS-96.5 conjugate or antibody 96.5 alone on growth of human melanoma H2169 xenografts in groups of five athymic nu/nu mice. Mice were given tumor on day 0 and treated on days, 1, 4, 7, 11, 14, 18, and 24 with PBS (arrowheads). 96.5 alone at 124 mg/kg (–●–) or VDS–96.5 at 5.1 mg/kg VDS and 124 mg/kg 96.5 (---). The conjugate-treated group showed a significant difference ($P < 0.02$) from the PBS group at the time points indicated (*) (Student's t test). For clarity, standard error bars are omitted. (Reproduced with permission from Ref. 15.)

MONOCLONAL ANTIBODIES, IMMUNOCONJUGATES, AND CANCER: SOME PROBLEMS AND PROBABLE SOLUTIONS

Although tumor therapy using MoAbs and their conjugates seems to be quite promising, their use as a therapeutic agent has been hampered by many problems and unanswered questions listed below (1).

1. Most of the MoAbs developed against human tumor cells have been made in mice by immunizing the mice with human tumor cells or extracts thereof; hence, these MoAbs are mouse immunoglobulins and represent the way a mouse spleen cell sees a human tumor cell. These MoAbs may cause unwanted side effects in humans by serving as antigens and stimulating an immune response in the patient. This would make the MoAb therapy impossible. This problem may be circumvented by using human MoAbs, which are much less likely to generate an immune response. However, there are two major obstacles to this approach. First, there is no adequate, genetically marked human myeloma cell line to fuse with immune cells producing MoAb. This makes it very difficult to find a MoAb-producing cell after fusion between the immune cells and the parental myeloma cell line has been performed. Second, obtaining immune cells has proved difficult (87–89).
2. MoAbs in direct human therapy may have the potential for generating antigen–antibody complexes. Such complexes have long been known to damage the kidneys, as well as to act as antigens themselves. In a recent study, the induction of human anti-mouse IgG antibody by repeated administration of mouse antibody has been circumvented by the construction of chimeric antibody. Since human antibodies of appropriate specificity are not available, chimeric antibodies with variable regions identical to those of mouse hybridoma and human constant regions may provide antibodies of the appropriate specificity that are less immunogenic than the complete mouse antibodies. Furthermore, the ability to genetically engineer changes in the DNA segments enables one to produce antibody molecules with "tailor-made" effector functions (90–92).

   Following the above approach, Ghrayeb (90) recently described the construction of immunoglobulin genes in which the DNA segments encoding the variable regions from the heavy and light chains of the mouse MoAb 17-1A were joined to the DNA segments encoding human γ^3 and κ constant regions. The transfection of expression vectors containing these chimeric Ig genes into mouse myeloma cells resulted in the production of functional chimeric IgG with the same binding specificity as the original hybridoma antibody. The early results were very promising using the chimeric IgG.
3. For both drugs and toxins, a major problem may be the need for the antibody to attach and translate the agent into each cell. Antigen modulation may play a role as a mechanism by which this translation can occur (87). Where antigenic heterogeneity exists, these conjugates may be limited by the heteroge-

neity. In contrast, radioisotopes will have a certain "field" effect and may circumvent the problem of heterogeneity. Unfortunately, this field effect and toxicity, without intracellular translation, will cause greater toxicity to normal organs where the antibody conjugates might be retained, generally in liver and spleen. An additional feature of isotope-labeled antibody is the potential for imaging in addition to therapy (93,94).

4. Each immunoconjugate has the potential for enhanced therapeutic specificity given the conjugation to the antibody molecule and its inherent specificity for the antigenic site on the tumor cell. However, many problems remain to be defined as to the class of immunoglobulin, the purification of antibody, the route and schedule of administration and the use of immunoconjugates as cancer-specific reagents.
5. Most of the work on the use of immunoconjugates has been done using *in vitro* systems. The results of *in vivo* studies are limited and do not warrant definitive conclusions.
6. Worst of all, an immunoconjugate effective for one type of cancer in one patient may not be active against similar cancers in other patients. This means it may be necessary to raise monoclonal antibody against tumor from each patient to prepare an effective immunoconjugate. Tailoring of MoAb to individual lymphoma patients would present additional practical difficulties if additional work bears out the initial success. In view of this problem, a few companies are producing custom-tailored MoAbs to each patient's lymphoma.
7. The goal is to use an antibody that recognizes a tumor-specific antigen which distinguishes absolutely between tumor cells and normal cells; however, with the notable exception of idiotypes on B-cell tumors, no such tumor-specific antigen has yet been found. Rather, a number of tumor-related antigens have been defined that are more or less restricted to tumor cells and their tissue of origin.
8. Binding of some antibodies to cells causes the target antigen to disappear from the cell surface. This antigenic modulation may decrease the effectiveness of repeat loss of antibody (87,88). However, not all antibodies have this effect.
9. The human pharmacology on antibody-drug conjugate has not been studied at all. Carefully planned clinical trials are needed to learn how to best administer drug-antibody conjugates with the goal to improve drug delivery over the conventional therapy with drug alone (98).

The early reports of tumor therapy with monoclonal immunoconjugates are promising. The improvement in *in vitro* immunization of human lymphocytes, in constructing chimeric antibodies by genetic engineering techniques, and in finding optimal conditions for producing human–human hybrids and, especially, the promise of transfected stable cell lines and of better conjugate synthesis give hope that MoAbs and their immunoconjugates can be used as therapeutic agents in the future.

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