Monoclonal antibodies in cancer treatment: Where do we stand after 10 years?

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Summary

Monoclonal antibodies (MoAbs) with specificity to tumour-associated antigens have become increasingly available during the past years. Presently, they are being applied in various in vitro diagnostic assays. They have contributed to the knowledge of cancer biology to a large extent. The understanding of cell surface characteristics and antigenic phenotype of tumours has in particular influenced the approach in the treatment of leukemias and lymphomas. From successful tumour localization in patients by gamma-emitting radio-labelled MoAbs it became clear, that these proteins offer a unique possibility to target therapeutic agents to tumour sites. The mere administration of MoAbs did not result in sufficient clinical benefit, but with proper precautions high doses of murine antibody were well tolerated. In order to use MoAbs as a carrier system, various toxins, cytostatic drugs, or radionuclides have been conjugated to these proteins. Thus far, specific problems were encountered not only associated with the immunoconjugate itself, but also to its fate in the patient. With regard to the substantial knowledge on the use of MoAbs in vivo obtained from animal tumour models, immunoscintigraphy in patients, and phase I serotherapy trials, we will undoubtedly determine the optimal conditions required for a conjugated anti-tumour agent to achieve enhanced cytotoxicity without increased side-effects. Preliminary results with high doses of $^{131}$I-labelled MoAbs in patients having tumour lesions expressing relevant antigens encourage further studies with immunoconjugates in cancer treatment. While much work needs to be done to further define the role of MoAbs as a new treatment modality in malignancies, this area of immunotherapy deserves great emphasis for the development of effective conjugates for future patients.

Introduction

The production of antibodies of predefined specificity after fusion of an immortal myeloma cell line with antibody-producing B cells from immunized mice by Köhler and Milstein in 1975 [37], has brought about enthusiastic efforts in their use for diagnosis and treatment of cancer. Presently, a
TABLE I
Problems associated with the conjugation of monoclonal antibodies for therapy.

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large number of monoclonal antibodies (MoAbs) of predominantly murine origin are available, which recognize tumour-associated antigens. They are extremely valuable for in vitro diagnostic procedures such as immunohistochemistry, measurement of circulating antigen and/or antibody (radioimmunoassay, enzyme-linked immunosorbent assay), immunofluorescent detection of cell surface markers, and other techniques. Some of these antibodies have entered clinical trials in tumour localization after conjugation with gamma-emitting radionuclides, such as $^{131}$I, $^{125}$I, or $^{111}$In. In this respect, detection of malignant lesions has been successful in various tumour types, such as gastrointestinal cancer [4,13,21,22], ovarian cancer [28,73], breast cancer [68], melanoma [12,41,52] and cutaneous T cell lymphoma [10].

Considerable efforts have been generated to employ MoAbs as a carrier system, whereby therapeutic agents can be targeted specifically or preferentially to malignant tissue. Ideal MoAbs for this purpose recognize antigens which are not or hardly shed, in order to prevent side-effects from circulating antigen-immunoconjugate complexes. Much has been learned already about the potential role of MoAbs in cancer treatment from animal tumour models, immunoscintigraphy in patients and from phase I serotherapy trials as described in a few key papers on these subjects [3,24,30,50,51,53,57,59]. Apart from most important results reached thus far in patients with MoAbs alone or conjugated to toxins, cytostatic drugs, or radionuclides, we will discuss the major problems to be encountered in this type of immunotherapy (Tables I and II).

**Serotherapy**

The administration of unbound MoAbs with a therapeutic purpose mainly involved patients with leukemia and lymphoma, while a few trials included solid tumour types. A study with L17F12, which recognizes the T65 antigen present on mature human T cells, thymocytes and chronic lymphocytic leukemia (CLL) cells, performed in a patient with advanced cutaneous T cell lymphoma (CTCL), showed a transient decrease in circulating malignant cells and a striking partial response of skin and lymph node lesions [47]. A similar drop in circulating tumour cells without reduction of soft tissue lesions was observed in a patient with diffuse, poorly differentiated lymphocytic lymphoma upon infusion with antibody 89 against a lymphoma-associated antigen [49]. In three patients with acute lymphoblastic leukemia, a remarkable reduction of circulating blasts was achieved with the anti-CALLA MoAb, but responses were of short duration [58]. Extensive studies with T101, another MoAb reacting with T65, showed a temporary removal of antibody-bound cells from the circulation in patients with CLL or CTCL [18,25]. T101 doses over 50 mg could be repeatedly given by prolonged intravenous infusions, but rapid administration induced bronchospasms, fever and/or hypotension. Saturation of antigen-T101 binding could be achieved on circulating lymphocytes. In most studies the involvement of the reticuloendothelial system has been assumed of importance to achieve an-

TABLE II
Problems associated with conjugated monoclonal antibodies in vivo.

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tibody-dependent cellular cytotoxicity. While serotherapy in haematologic malignancies resulted in limited clinical benefit, preliminary achievements in patients with melanoma or gastrointestinal cancer have not been superior [38,52,63].

Immunotoxins

Ricins and the diphtheria toxin have attracted most attention while attached to antibodies. Toxins consist of two polypeptide subunits. The A-chain is responsible for inhibition of ribosomal protein synthesis in the cell cytoplasm. The B-chain binds to galactose residues on the cell membrane, thus facilitating transpassage of the A-chain [7]. As whole toxin may be toxic to the host if the immunoconjugate became unlinked in vivo, most studies included the A-chain only. After immunotoxins had shown specific toxicity towards target cells in vitro, further studies with these conjugates were done in animal tumour models. An increased life-span without cures in L1210 leukemia was observed with the anti-L1210 antibody conjugated to ricin A-chain [36]. Similar studies with MoAb 9.2.27, which recognizes a melanoma-associated antigen, conjugated to diphtheria toxin A-chain resulted in growth inhibition of M21 melanoma xenografts, but this effect was non-specific and could also be achieved with the MoAb alone [9]. If immunotoxins can be administered safely to patients is questionable. However, immunoconjugates of either the A-chain alone or whole toxin can be of value in purging bone marrow of malignant cells for autologous bone marrow transplantation [48,66,69] or in preventing graft-versus-host disease by elimination of immunocompetent T cells from donor bone marrow [67,72].

Cytostatic drugs conjugated to monoclonal antibodies

Specific problems have been encountered in the conjugation of cytostatic drugs to MoAbs and in particular in the acquisition of a high specific activity without loss of the anti-tumour potential. Covalent binding is necessary to prevent untimely drug release for which each drug requires an individual approach based on its chemical properties. Only a small number of functional groups per antibody molecule are available for direct binding without significant loss of antibody immunoreactivity and with retention of sufficient anti-tumour activity. Vindesine and probably chlorambucil fulfill these criteria [5,20,26]. In case of daunorubicin and doxorubicin, covalent binding usually results in a ratio of 5 molecules of the drug per molecule protein, but without higher anti-tumour efficacy. An extensive increase of this ratio has been achieved through the incorporation of a dextran bridge [34,35,56,71]. In vivo studies with these conjugates at high specific activity caused enhanced growth inhibition of rodent tumours expressing the relevant antigen as compared to treatment with the free drug. Undesirable physical properties of the polymeric carbohydrates may significantly reduce the anti-tumour properties of the attached drug as was shown for bleomycin [46]. New attempts are in progress to acquire better bifunctional coupling reagents such as human serum albumin or synthetic polypeptides [27,70]. Besides the chemical problems, little is yet known about the specific requirements for a drug attached to a membrane-bound antibody to exert cytotoxicity in target cells. Extensive studies are necessary to solve these questions to eventually develop antibody-drug immunoconjugates with a higher therapeutic index.

Radioimmunotherapy

Ideally, a radionuclide applied for immunotherapy should have a high linear energy transfer within short range, a relatively short physical half-life and reasonable chemical properties for conjugation. Ionizing radiations from alpha or beta particles seem most appropriate for short range damage [6,33]. The alpha emitter $^{211}$At may be of interest, but its short half-life of 7.2 h and the difficulties in the chemical binding to antibodies represent major obstacles in this treatment strategy [74]. The beta-
emitting radionuclide $^{131}$I with a half-life of 8 days has been widely used in the treatment of thyroid disease, and is known for its easy conjugation to a variety of antibodies. High doses of $^{131}$I-labelled anti-ferritin induced remarkable remissions in patients with non-resectable hepatoma [42,54]. Radioimmunotherapy with the Fab fragment specific for p97, a melanoma-associated antigen, bound to $^{131}$I at high specific activity showed a cytotoxic effect in two of three patients with advanced melanoma [12]. These results encourage further clinical studies with $^{131}$I-labelled relevant MoAbs in radio- and chemotherapy resistant malignancies. $^{131}$I has concomitant gamma-ray emission with, not surprisingly, myelosuppression as the dose-limiting factor. Although $^{125}$I has a half-life of 60 days, this isotope may also prove to have clinical value because of its unique subcellular radiation properties. The decay of $^{125}$I is by electron capture followed by a substantial number of Auger electrons with a highly efficient radiobiological effect [6]. In vitro studies with $^{125}$I-9.2.27 in a human melanoma cell line and with $^{125}$I-T101 in human malignant T cell lines have shown specific cytotoxicity of a remarkable degree [8,44]. In these experiments immunocojugates were used at high specific radioactivity with sufficient immunoreactivity and cytotoxicity was achieved without substantial internalization of the complex. Radioimmunotherapy with MoAbs will undoubtedly put forward extensive efforts in determining its clinical usefulness in the near future.

Problems with the conjugation of monoclonal antibodies

The choice of a MoAb for targeted therapy does not only depend on its specificity for a particular non-circulating tumour-associated antigen, but also on the antigen density of the target cell, the extent of immunomodulation, the immunoglobulin subclass and the antigen-antibody binding characteristics.

The antigenic expression is known to vary with the cell-cycle phase and is decreased in density in Go/G1 cells [2,11,40,45]. The expression also depends on the heterogeneous cell populations usually found in tumour tissue. Between patients with the same histological tumour type, the antigenic expression may vary remarkably. That the number of binding sites per cell is important for effective therapy was shown for the varying T65 antigen expression of human T cell lines [8].

Immunomodulation of the antibody after binding to the antigen can be detected by a gradual decrease of fluorescent intensity with flow cytometry. This phenomenon should be investigated for each antibody. For instance, for MoAb 9.2.27 was shown that modulation is absent [52], while shedding of immune complexes from CEA-secreting target cells may be expected with anti-CEA antibodies. Other antibodies as anti-CALLA and T101 were demonstrated to induce a rapid modulation of the immune complex upon binding [55,58,62,64]. Re-expression of CALLA and T65 antigens on malignant cells occurred within a few days after withdrawal of the antibody. Absence of the relevant antigen renders serotherapy futile. However, if modulation is due to internalization of the immune complex into the cell, as was shown for anti-CALLA and T101, this process may be advantageous to the effect of antibodies conjugated to therapeutic agents [55,62].

If a specific immunoglobulin subclass may achieve superior cytotoxicity has yet to be clarified. From studies in colorectal cancer xenografts in nude mice, only MoAb 17-1A, an IgG2a, antibody, was demonstrated to induce growth inhibition in contrast with MoAbs of other isotypes [1,31,65]. Whether an antibody subclass is responsible for activating macrophages to mediate the cytotoxicity in this process, is part of the question to be elucidated.

The antigen-antibody binding characteristics partially determine the time of exposure of the therapeutic agent to the target cell. These characteristics may be important in the choice of MoAbs, where increase of exposure time (especially of interest for radiolabelled MoAbs) can be achieved with antibodies having prolonged binding to the cell [15], or higher affinity to a particular antigen [29].

The choice of a particular therapeutic agent for conjugation to a MoAb depends on its potential
toxicity to a target cell, the type and duration of its side-effects, the amount of drug or radionuclide necessary for cytotoxicity and the residual immunoreactivity and drug activity after the conjugation.

The potential cytotoxicity of conjugated antibodies is not only related to the inherent sensitivity of the target cell, but also to the amount of drug or radionuclide delivered at the tumour site. Tumours, rather insensitive to conventional treatment may be more affected by targeted therapy as was shown for human melanoma cells with high doses of $^{125}$I-9.2.27 [44], whereas melanoma lesions are not very radiosensitive in patients. In addition, internalization of immune complexes may increase the efficacy of conjugated drugs.

Most therapeutic agents mentioned thus far are widely used in the clinic and much is already known about their side-effects. Unexpected new side-effects of immunoconjugates may be caused by cross-reactivity of the antibody with irrelevant antigens, by non-specific uptake, by toxicity in adjacent normal cells at the tumour site or by uptake of immune complexes in the reticuloendothelial system of the spleen and the liver. For instance, cross-reactivity of certain anti-CEA antibodies with an irrelevant antigen on granulocytes induced a severe reduction of circulating granulocytes following MoAb infusion [17]. In the choice of a radionuclide, its half-life and the degree of gamma-emission are also criteria, which may limit its clinical utility.

The amount of toxin, cytostatic drug, or radionuclide delivered at the tumour site is largely related to the specific activity of the immunoconjugate. Covalent binding of antibodies with cytostatic drugs is either direct or requires a bifunctional coupling reagent as described earlier. Also toxins should be bound covalently, but the optimal toxin:protein ratio for effective treatment has yet to be identified. While radiodiodination of proteins can be done by various methods [19], other radionuclides require bifunctional metal chelates for binding as utilized for $^{111}$In in immunoscintigraphy [32,60]. The group of Larson and coworkers has developed a method to conjugate the Fab fragment for pIg to $^{131}$I at high specific activity without excessive chemical damage of the immunoreactivity, which can be done under sterile conditions [23].

The residual immunoreactive fraction can be determined in the presence of antigen excess in vitro in case of radiolabelled antibodies [43]. Incorporation of $^{14}$C or $^3$H may be necessary for drugs before their conjugation to MoAbs, to be able to determine the residual immunoreactivity. Radioactive conjugates can easily be investigated for their chemical stability. While the residual activity of the conjugated therapeutic drug is a result of chemical or physical damage, its efficacy will also depend on the conditions required for cytotoxicity.

**Therapeutic immunoconjugates in patients**

While in vivo imaging provided insight in biodistribution and metabolism, the presence of circulating antigen, and in the uptake in normal tissues and the reticuloendothelial system, serotherapy gave information on the rate and the degree of immunomodulation, cross-reactivity with irrelevant antigens on normal cells, and on the host-immune response to the heterologous antibody.

Biodistribution and metabolism vary for each immunoconjugate, which can be expected from differences in antibody properties, the chemical stability and the degradation of immune complexes. These aspects have to be taken into account in order to determine the optimal treatment schedule with minimal side-effects. An example of such differences is given for $^{131}$I- or $^{111}$In-labelled antibodies in tumour localization, where images with $^{131}$I are inferior because of early deiodination of the antibody with non-specific uptake in the liver and the kidneys [12,21]. The optimal treatment schedule will also depend on the tumour burden of the patient. Doses larger than the saturation concentration of the antibody will only add to toxicity.

Systemic side-effects encountered following rapid infusion of high doses of antibody could be eliminated by their administration at a low infusion rate. The production of human anti-mouse antibodies forms a major problem in MoAb treatment. Important consequences of high titers are alterations in the biodistribution with a rapid clearance in the
liver and a marked reduction of tumour uptake, which limit the further therapeutic value of the antibody [12,18]. Because of their size and prolonged circulation, anti-mouse antibodies are more likely to develop with whole immunoglobulins. By use of immune fragments, i.e. Fab, Fab', or F(ab')₂, the patient's antibody response should be lower [12]. However, the rapid uptake and clearance of immune fragments in tumour sites may decrease the exposure time to bound therapeutic agents [41]. Anti-mouse antibodies reacting with the binding site of the antibody, designated as human anti-idiotype antibodies, have been mentioned to induce tumour regression, but elucidation of its mechanism needs further studies [38]. The production of human MoAbs by human myeloma cells, lymphoblastoid cell lines or by Epstein-Barr virus transformed human B lymphocytes is in progress. These antibodies are expected to be far less immunogenic than murine immunoglobulins [16,39].

The antigenic expression varies with cell-cycle phase, and will also be different in heterogeneous cell populations in tumour tissue [2,61]. The simultaneous application of various MoAbs, either of different immunoglobulin subclasses recognizing separate epitopes on the same antigen or specific as described for anti-CEA antibodies [14,29], or recognizing different antigens may considerably increase the efficacy of conjugated therapeutic agents. Immunohistochemistry of biopsy material or immunofluorescent studies of single-cells may identify those antibodies which are most reactive with an individual patient's malignancy. Furthermore, concomitant administration of agents known for their capability to enhance the expression of tumour-associated antigens as was shown for interferons in vitro may increase the antigen-antibody binding in patients [61]. Finally, the use of a "cocktail" of antibodies will not only be advantageous to the deposited amount of a bound therapeutic agent, but also offers the possibility to deliver various agents at the same time.

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