DIAGNOSTIC AND THERAPEUTIC USE OF MONOCLONAL ANTIBODIES TO HUMAN TUMOR ANTIGENS

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Monoclonal antibodies have been obtained to various differentiation antigens that are more strongly expressed on tumor cells than on normal cells from the adult host, some of these antigens are relatively specific for tumors of a given type, e.g. melanoma. Monoclonal anti-tumor antibodies offer strong promise for clinical use, initially for tumor diagnosis and, ultimately, also for therapy.

Key words: Monoclonal antibodies, Tumor antigens, Oncofetal antigens, Tumor diagnostics, Tumor therapy.

BACKGROUND

Some of the first evidence that human neoplasms of the same type, e.g. melanomas, have tumor-associated cell surface antigens was published in the late 1960s. This evidence included the demonstration that lymphocytes from cancer patients are often reactive to antigens shared by tumors of the same histological type, and that sera from such patients sometimes contain antibodies of a similar specificity. Extensive serological studies have since been performed, in which antibody binding to a patient's own tumor was measured and adsorption techniques used to show specificity. These studies demonstrated antibodies to antigens unique to the patient's own neoplastic cells, as well as antibodies to shared tumor-type associated antigens. However, these antibodies were present in a low frequency of patients and there only in low titers. It was not until the advent of the monoclonal antibody technique that the presence of tumor-type associated antigens could be proven and the nature of some of the antigens elucidated.

We shall summarize some of the evidence for human tumor-type associated antigens, as defined by monoclonal antibodies, and discuss the major implications of this evidence for tumor diagnosis and therapy.

THE ANTIGENS

Most hybridomas making anti-tumor antibodies have been obtained by immunizing mice with cultured human tumors and fusing their spleen cells with mouse myeloma cells. However, hybridomas making human monoclonal antibodies with some tumor specificity have been also established by fusing patient lymphocytes with mouse or human myeloma cells.

Monoclonal antibodies have been obtained that have a relative specificity for melanoma, carcinoma of the colon, breast, and lung and for certain other types of cancer.

None of the monoclonal antibodies described is directed to an antigen expressed only on tumor cells and not on any normal cells. The degree of specificity for tumor is, instead, relative, and it varies for different tumor antigens. We can illustrate the concept of relative specificity with our own work on melanoma: by using a highly sensitive immunoradiometric assay, we found that a melanoma-associated antigen, p97, that initially appeared to be entirely tumor-specific, was expressed in small amounts in various normal adult tissues and, in relatively large amounts, in embryonic colon and intestine. Most melanomas express 50,000–500,000 p97 molecules per cell, while fewer than 10,000 p97 molecules per cell have been detected in normal tissues.

The difference between normal and neoplastic cells in sensitivity to chemotherapeutic agents or to the cytotoxic effect of natural killer (NK) cells is hardly that great.

The nature of some of the tumor antigens defined by monoclonal antibodies has been investigated in depth. Antigen p97 has probably been studied the most: it is a 97,000 mol. wt glycoprotein, forms an integral part of the cell membrane, and is homologous to transferrin.
in its amino-acid sequence.\textsuperscript{25} Like transferrin, p97 binds iron.\textsuperscript{25} The gene for p97 has been assigned to chromosome 3,\textsuperscript{26} and cDNA sequences coding for p97 have recently been cloned.\textsuperscript{27}

**DIAGNOSTIC USE OF ANTIBODIES**

Anti-tumor antibodies can be used diagnostically in at least 3 different ways: for immunohistological assays on sections of tumor, to measure circulating tumor antigens and/or antibodies, and to localize tumor metastases in patients. We shall discuss each use separately.

Immunohistology has two major applications. First, it may be helpful in securing diagnosis, particularly when there is a metastatic tumor of unknown primary origin. This can be illustrated by our own studies on malignant melanoma,\textsuperscript{28–30} which have demonstrated antibody binding to frozen sections from essentially all melanomas by using combinations of antibodies to 3 different melanoma-associated antigens, including p97, a proteoglycan, and a GD3 glycolipid; in less than 10% of cases do sections from other tumors bind antibodies to more than one of the 3 antigens.\textsuperscript{30} Second, immunohistology is probably the best method for selecting antibodies for in vivo diagnosis and therapy.

Some monoclonal antibodies are likely to be useful for the detection of tumor-associated antigens in serum. In the past, polyclonal antibodies to carcinoembryonic antigen have proven to be valuable for monitoring some patients with known neoplastic disease,\textsuperscript{31} and monoclonal antibodies may provide a higher degree of tumor specificity. There is suggestive evidence that a monoclonal antibody to a cell surface antigen of human colon carcinoma can be successfully employed this way.\textsuperscript{21}

Cell surface antigens that are shed from cells, and cytoplasmic antigens released from damaged cells may be the ones that are most easily detected in serum in amounts above background levels. Serum antibodies may be detected as well, for example, by measuring the ability of serum immunoglobulin to inhibit the binding of labelled monoclonal antibody to a given antigen.

A third diagnostic use of monoclonal antibody is for the localization of tumor metastases. The most extensive studies on this have been carried out in melanoma patients, using \textsuperscript{131}I-labelled Fab fragments prepared from anti-p97 antibody.\textsuperscript{32–34} Immunological specific localization of approximately 88% of tumor metastases could be detected using \textsuperscript{131}I-labelled anti-p97 Fab fragments. Metastases that were not detected were generally less than 15 mm in diameter. Antibody localization has been verified in two ways. First, studies were performed on biopsies from patients receiving mixtures of differently labelled specific and control Fab fragments. They showed that the specific, but not the control, fragments preferentially localized in tumors, while both types of fragments localized to about the same extent in normal tissues. Second, experiments were performed, in which the same patient was first given \textsuperscript{131}I-labelled anti-p97 Fab fragments which localized in tumor, and, 1 month later, an \textsuperscript{131}I-labelled control Fab, which did not localize in tumor. When \textsuperscript{131}I-labelled anti-p97 Fab were given a month after the control fragments, they still showed tumor localization. More recently, Fab fragments of antibody 48.7, which is specific for a proteoglycan antigen,\textsuperscript{29} has been also used for tumor localization. The results have been comparable to those obtained for p97. Future work should lead to improved procedures for tumor localization by utilizing isotopes with better imaging properties than \textsuperscript{131}I, for example, \textsuperscript{111}In or \textsuperscript{99m}Tc.

**THERAPEUTIC USE OF MONOCLONAL ANTIBODIES**

Anti-tumor antibodies may be therapeutically beneficial in several ways. They may be inhibitory to tumor cells, for example, by binding to a receptor needed for tumor growth,\textsuperscript{35} cytoxic in the presence of complement,\textsuperscript{36} or they act in consort with K cells and macrophages to mediate antibody-dependent cellular cytotoxicity.\textsuperscript{39} The same antibody can have several of these functions, all three of which have been demonstrated in vitro. Therapeutic effects of antibody in vivo have been described.\textsuperscript{38,39} The most impressive of these were obtained in a patient with a B cell lymphoma,\textsuperscript{38} who was treated with a monoclonal mouse antibody to an idiotypic determinant expressed at the surface of his leukemic cells (and presumably also on the clone of normal B cells from which the leukemia was derived).

Second, antibodies may be used as carriers of a tumoricidal dose of radioisotope, toxin or chemotherapeutic drug. Larson et al.\textsuperscript{33} calculated that antibody fragments specific for melanoma antigen p97 may be used to deliver a therapeutic dose of radiation to the tumor site with acceptable levels of damage to normal tissues. Conjugates between anti-tumor antibody and a toxin, ricin A chain, have been prepared (see, e.g. refs 40–43) and shown to destroy cultured tumor cells as long as these express at least intermediary levels of the target antigens, such conjugates can, under certain circumstances, have an anti-tumor effect also in vivo.\textsuperscript{41} Likewise, conjugates have been prepared between monoclonal antibodies and chemotherapeutic agents. One such conjugate between anti-p97 antibody and vindesine\textsuperscript{44} was found to kill cultured melanoma cells expressing at least 20,000 molecules of p97 per cell without affecting cells such as normal skin fibroblasts which express small amounts of p97. This conjugate was found to have a therapeutic effect against human melanoma cells transplanted into nude mice (Rowland et al., manuscript in preparation). A third approach is to use an