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Review article

Monoclonal antibodies in oncology

KAROL SIKORA

From the Ludwig Institute for Cancer Research, The Medical School, Hills Road, Cambridge CB2 2QH

SUMMARY Molecular biology has made tremendous strides over the last five years. The new biology allows us to prepare monoclonal antibodies to defined antigens; to detect, isolate and clone specific genes; and to insert these genes into defined sites in different cells giving new functions to old organisms. These revolutionary developments have been followed closely by researchers, businessmen, politicians and philosophers, as well as by those involved in the clinical care of patients. Although our understanding of human molecular biology is increasing rapidly, it is the development of monoclonal antibodies that has the most immediate application in the clinic. There have been several reports of their use in the diagnosis, localisation and treatment of human malignant disease. This review describes developments that are likely to have direct relevance to patient care in the near future.

What is a monoclonal antibody?

The immunological response to any foreign antigen is polyclonal: many different clones of B lymphocytes are stimulated to produce antibodies. These antibodies have different molecular structures and in turn recognise different molecular conformation patterns on the stimulating antigen—the antigenic determinants. It is this complexity of antibody response that makes the antigen-antibody interaction difficult to analyse at a molecular level. This is particularly so with complex antigens such as the tumour cell surface. Monoclonal antibodies occur naturally in patients with myeloma. Here neoplastic transformation occurs in a clone of B lymphocytes with the result that large quantities of identical immunoglobulin molecules are produced. It was by using myelomas that the chemical structure of the immunoglobulin molecule was discovered.[1] However, the antigens to which most myeloma immunoglobulins are directed are usually unknown and are unlikely to be important. In 1975 Köhler and Milstein[2] constructed a hybrid myeloma (hybridoma) which produced a monoclonal antibody directed against a specified antigen. Mice were immunised with the antigen (sheep red cells) and their spleen lymphocytes collected. The lymphocytes were fused with an established myeloma line and hybrids selected by growth in selective tissue culture medium. The resultant hybrids were rapidly growing (a property conferred by the myeloma) and yet contained new immunoglobulin genes (from the lymphocytes of the immunised mouse). The hybridomas were cloned by diluting the cells and growing up colonies from single cells. These cloned hybridomas now contained only one set of new immunoglobulin genes (Fig. 1). After growing in tissue culture the supernatant containing the secreted antibody was tested for activity against the immunising antigen. Using this system, antibodies can be isolated which define single antigens in a complex mixture such as the molecules on tumour cell surfaces. These molecules can now be compared to those appearing on non-malignant cells from the same tissue of origin.

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Do tumour antigens exist?

There is considerable evidence that the immune system responds to antigens on tumour cells, both in experimental animal systems and in human neoplasia. These tumour antigens are defined by assays which utilise the various modes of immune response to them. It is important to distinguish the antigens present on the tumour cell surface that are unique to tumours and are not shared with normal cells. There are several documented examples of such antigens within experimental tumour systems.[3,4]

Until the development of the monoclonal antibody technology, it has been impossible to sort out

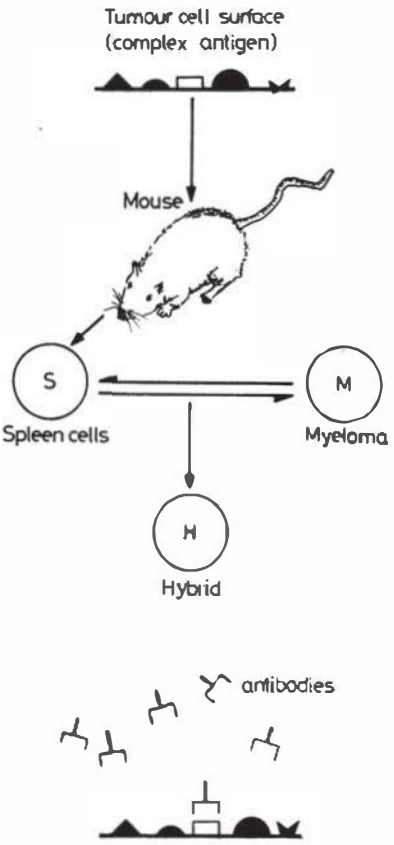

Fig. 1 *Making a monoclonal antibody. A complex antigen, such as a tumour cell surface, is used to immunise mice. The spleen cells (S) are removed and fused with a myeloma line (M). Hybrids are cloned and those antibodies binding to the antigen selected.*

the antigenic complexity of human tumour cell surfaces. The evidence for the existence of unique tumour specific antigens in man analogous to the tumour-specific transplantation antigens in animals is circumstantial. The natural history of certain tumours, the waxing and waning of tumour masses and the occurrence of spontaneous regression suggests that there may be some host control of tumour growth.[5] Similarly, the relation between histological evidence of tumour infiltration by immunocompetent cells and prognosis suggests that these infiltrating cells have some controlling influence of tumour growth.[6] Further circumstantial evidence comes from the increased incidence of malignancy in immunosuppressed patients, although here the spectrum of tumour types found is not similar to that found in the normal population.[7] Serological analysis and assays of lymphocyte function have shown that the immune system in man can actually recognise the tumour cell surface.[8 9] Whether immune mechanisms are able effectively to destroy tumour cells in vivo remains in question.

Production of monoclonal antibodies to human tumours

FUSION SYSTEM

Currently there are three systems in which anti-tumour monoclonal antibodies can be raised; mouse, rat, and human. For human tumours, mice and rats have the obvious advantages of responding to a wide variety of antigens and are thus the choice for an exhaustive analysis of tumour cell surface components. This wide response may be a disadvantage in that xenogeneic immunisations often result in antibodies directed against histocompatibility antigens and blood group substances.

It is now possible to fuse human lymphocytes directly from patients with tumours, either with mouse or rat myelomas, so obtaining mixed species hybrids which produce human monoclonal antibodies. The frequency of hybridisation and the quantity of human immunoglobulin produced by interspecies hybrids is considerably less than in mouse-mouse or rat-rat fusions. A further problem is the preferential loss of human chromosomes in rodent-human hybrids which results in frequent loss of immunoglobulin production. There are now, however, several human myeloma lines available which are suitable for fusion.[10 11] Such lines must be rapidly growing and have an appropriate genetic selection mechanism to enable the parent myeloma to be killed in the hybridoma mixture. Once established, human-human hybrids show no apparent preferential loss of chromosomes and thus the stability of the hybrid is assured. The quantity of immunoglobulin secreted by these human-human hybrids is usually of the order of 1 μg/ml which is one tenth of the output of the corresponding mouse hybridoma system. There are several advantages in using human lymphocytes to produce monoclonal antibodies. The spectrum of the human immune response which serologically defines tumour-specific antigens can be examined. There is abundant evidence that patients with cancer at some time in the natural history of the tumour have in their serum antibodies which recognise their own tumours.[12] The titre of these antibodies is low and so far there have been no good studies on the chemical nature of the determinants recognised by such antibodies. By obtaining the antibodies in monoclonal form and in sufficient quantity such chemical studies are possible. Lymphocytes from cancer patients can be collected from several sites. Peripheral blood lymphocytes may not represent a good starting

population from which to perform fusions. More likely to be involved in antitumour activity are the lymphocytes in the lymph nodes draining a tumour. Such lymphocytes can easily be collected in large quantities from patients with breast, lung and colorectal cancer. Another source of lymphocytes for fusion comes from the tumour itself. Certain tumours, for example gliomas, are often heavily infiltrated by lymphocytes. These lymphocytes can be collected, separated from the tumour and fused to a human myeloma line.[13]

IMMUNISATION SCHEDULE

For xenogeneic immunisations the choice of antigenic material and the schedule in which it is used for immunisation has varied considerably. Very little detailed work has been performed on optimising these schedules. Sources of tumour material for immunisation can come from cell lines grown in vitro, pieces of fresh tumour tissue, membrane preparations from fresh tissue, or fractionated solubilised components from fresh tumour cell membranes. These different immunisation procedures will almost certainly result in different spectra of antibodies.

In the production of human monoclonal antibodies immunisation is not possible and the choice lies in the source of lymphocytes for fusion. There is as yet no evidence to suggest that any particular source of lymphocytes—peripheral blood, spleen, lymph node or intratumour—results in a higher frequency of the required antibodies.

SCREENING METHODS

The production of antibodies against human tumour cell surfaces requires the screening of many fusion products to find suitable immunoglobulins. Several strategies have been developed. The commonest method is to immunise mice with a chosen tumour cell line, for example a melanoma. The fusion products are screened on that melanoma in an indirect binding radioimmunoassay (see Fig. 2) and the activity of any positive supernatants determined on other melanomas as well as on cell lines of different types, both normal and malignant (Fig. 3). In this way the specificity of the monoclonal antibody is characterised and its ability to distinguish tumour cells from their normal counterparts is determined.

Screening can also be performed using primary tumour material. Membrane preparations of tumours can be used to immunise rodents; the same membrane preparation can be bound to plastic wells and used in a solid phase radioimmunoassay to screen the activity of resulting monoclonal antibodies. A variant of this screening procedure is to use

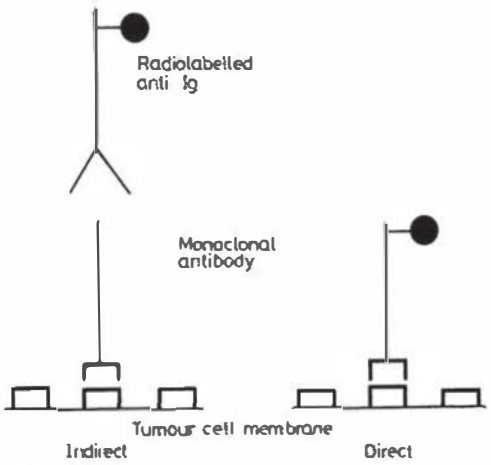

Fig. 2 *Binding assays for monoclonal antibodies. In the indirect assay bound monoclonal antibody is detected by a radiolabelled anti-immunoglobulin. In the direct assay internally labelled—for example, ^3H-lysine, antibody is used.*

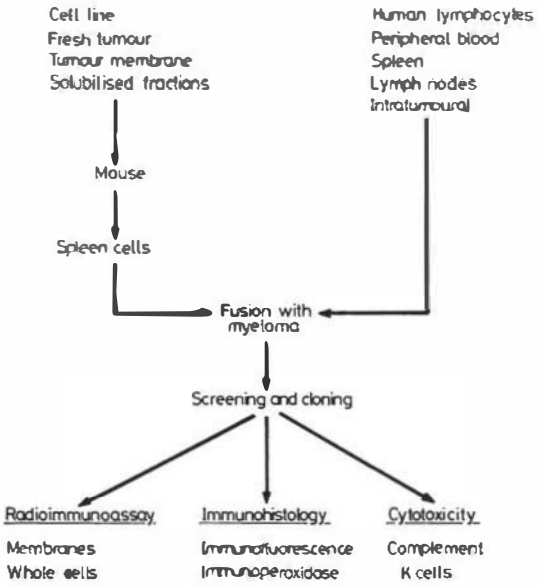

Fig. 3 *Strategies for making and screening monoclonal antibodies to human tumour antigens.*

sections of normal and tumour material to look at the activity of monoclonal antibodies histologically by immunofluorescence on frozen sections or by an immuno-peroxidase technique. This latter technique has the advantage of allowing retrospective surveys of paraffin block material readily available from hospital pathology departments. By comparing tumour samples from different patients with cancer

of the same or different tissues, additional information of diagnostic value can be sought.

In the production of human monoclonal antibodies screening strategies are less well worked out. One problem is the ubiquitous presence of variable amounts of human immunoglobulin in human tumours. The detecting anti-human Ig, whether fluorescein coupled or radiolabelled, binds to this resulting in high background levels in tumour membrane preparations. This can obscure binding by relatively small amounts of high affinity monoclonal antibody. This problem can be overcome by using cell lines for screening although of course this results in selection. A more laborious technique is to radiolabel internally each human immunoglobulin produced by the hybrids by incorporating a radioactive amino acid such as ^{3}H-lysine and screen in a direct binding assay.[14]

Antitumour monoclonal antibodies currently available

COLORECTAL CARCINOMA

Colorectal cancer is a common problem in clinical oncology. Diagnosis is often difficult, requiring extensive endoscopic or radiological investigation. The assessment of recurrent disease following primary surgery is usually impossible until large masses of neoplastic tissue have accumulated. For the last 15 years much effort has been spent investigating carcinoembryonic antigen (CEA), an antigen detected by an antiserum produced in rabbits after immunisation with extracts from colonic cancer. This antigen, a glycoprotein with a molecular weight of 180 000, is found in several gastrointestinal tumours, some lung and breast tumours as well as in normal fetal colon.[15] Considerable interest has been aroused in the possibility that the measurement of CEA in the blood would relate to the tumour load in an individual patient, thus producing both a diagnostic test and a marker for monitoring progress of the disease. A major problem in the use of CEA for these purposes has been the extensive cross-reaction between CEA and a variety of similar glycoproteins such an non-specific cross-reacting antigens (NCA), biliary glycoprotein (BGP) and a glycoprotein found in washings of normal colon (NCW). These glycoproteins share antigenic determinants with CEA and therefore confuse the serological analysis since different immunisation and absorption protocols result in the production of different antibodies in the resulting antiserum. Monoclonal antibodies give more precise information about the interrelations between these cell surface components and thus lead to more selective and sensitive assays for truly tumour-related products.

Several groups have now produced monoclonal antibodies to either CEA or other antigens present on colorectal carcinomas. So far these attempts have been carried out by immunising mice with either purified CEA preparations or colorectal carcinoma cell lines. After fusion, screening on either CEA or the immunising cell line has been used to identify interesting supernatants. Accolla and his colleagues[16] raised 400 hybrids from mice immunised with purified CEA and found two which secreted antibodies reacting specifically with two different antigenic determinants present on CEA molecules. The affinities of these antibodies are relatively high and could be used to characterise solubilised CEA immunochemically. Herlyn *et al*[17] immunised mice with cells grown in vitro. The screening assays included radioimmunoassay, mixed haemabsorption assays and immunofluorescence on the immunising cell line. Two hybridomas were found which secreted antibodies binding specifically to human colorectal carcinomas, either growing in culture or obtained directly from patients. These antibodies do not bind to normal colonic mucosa, to other malignant cells or to CEA.

MELANOMA

Melanoma is a tumour studied frequently by immunologists. Serology, using panels of patient sera and melanoma cells, has been used to construct large serological matrices. The biochemical separation of thedifferent serologically recognised antigens has been hampered by the low titres of the sera. There are several monoclonal antibodies against a variety of human melanoma antigens. Some of these antibodies are directed against the human DR (HLA D locus-related) antigen. In one study,[18] three out of six hybridoma secreted antibodies were found to bind to the majority of melanoma cell lines and to astrocytomas, as well as to all normal and Epstein-Barr virus-transformed lymphocytes tested (the same distribution as the DR antigen). Two of the remaining antibodies, however, were found to detect two different antigens common to melanoma and astrocytoma cells only. The most elegant analysis of the use of monoclonal antibodies in characterising antigenic systems on the surface of human tumours comes from the work of Dippold and his collaborators.[19] Mice were immunised with the melanoma cell line SK-MEL 28 and the 18 antibodies derived were tested on a large panel of human cell lines from a variety of tumour types, as well as on early cultures of normal tissue. Serological studies, in conjunction with immunoprecipitation analysis of radiolabelled cell extracts and antibody inhibition tests with solubilised antigens

indicated that the 18 monoclonal antibodies recognised six antigenic systems. Two of the systems are glycoproteins with molecular sizes of 95 000 and 150 000 daltons, and two systems have characteristics of glycolipid antigens. The biochemical nature of the remaining two antigenic systems has not been determined.

BREAST CANCER

Xenogeneic monoclonal antibodies have been raised against breast tumour lines, although the number of antibodies available is less than in colorectal and melanoma systems. A monoclonal antibody that may have considerable clinical use is that raised against the human oestrogen receptor.[20] It is known that the presence of oestrogen receptors in breast cancer tissue is an indicator of the likelihood of response to hormone treatment. The derivation of monoclonal antibodies which can be used for immunohistological detection of receptors would greatly increase the pathologist's ability to provide information of prognostic value to the clinician.

By using lymphocytes derived from axillary lymph nodes from patients with breast cancer, human immunoglobulins which bind to breast carcinoma cells have been produced.[21] A human IgM monoclonal antibody produced in this way has been shown to discriminate between mammary carcinoma cells and normal mammary epithelial cells. This antibody also reacted significantly with metastatic mammary carcinoma cells in lymph nodes of breast cancer patients with no binding to the normal lymphocytes or to the stroma of the same node.

LYMPHOMA AND LEUKAEMIA:

A wide range of monoclonal antibodies has been raised against myeloid and lymphoid neoplasms. Normal lymphocytes with different biological functions—for example, helper and suppressor effects on antibody synthesis, can be distinguished by their surface markers. Not surprisingly neoplastic transformation in cells of the lymphoid series results in the clonal expansion of a population of cells bearing a distinct surface marker pattern. Using conventional serology such patterns have already been related to prognosis as in the subclassification of lymphatic leukaemia into T, B and common ALL types. With monoclonal antibodies a much finer discrimination can be made and used to plan therapeutic approaches to these diseases.[22] The range of monoclonal antibodies available to different lymphoid subpopulations is outlined in the Table. It should be stressed that these antibodies do not recognise tumour antigens but clonally expanded normal antigens.

Commercially available monoclonal antibodies to lymphocyte differentiation antigens

Antibody	*Reactive populations*
*OKT 3 †L17 F12 ‡NEI-016	peripheral blood T lymphocytes. T cell leukaemia, mycosis fungoides
*OKT 4 † SK 3 †SK 4	helper/inducer T cells, certain leukaemias, mycosis fungoides, Sézary syndrome
*OKT 6	thymic lymphocytes, some thymomas
*OKT 8 †SK 1	suppressor/cytotoxic T lymphocytes, certain T cell neoplasms
*OKT 10	immature T cells, certain leukaemias
anti HLA, DR (Ia) anti Ig	B lymphocytes and B cell neoplasms (nodular lymphoma, most chronic lymphocytic leukaemias, myeloma)

Commercial suppliers:
*Ortho Pharmaceuticals, Denmark St, High Wycombe, Bucks HP11 2ER.
†Becton Dickinson, 490-3, Lakeside Drive, California 94086 USA.
‡New England Nuclear, 2 New Road, Southampton SO2 0AA.

OTHER TUMOURS

Monoclonal antibodies have been or are being raised against a wide variety of human tumours, including gliomas, neuroblastomas, sarcomas, lung cancer as well as bladder, prostrate and testicular tumours.

Clinical uses

DIAGNOSIS AND MONITORING

A major problem in clinical oncology is the measurement of tumour load in an individual patient. Less than 10% of all cancer patients have disease which can be reliably assessed by conventional techniques, such as palpation or diagnostic radiology. This hampers the evaluation of different treatment methods. Certain relatively rare tumours shed products into the circulation; and the concentration of these tumour markers can be related to the total tumour cell burden. Examples include α-fetoprotein in hepatoma and teratoma; human chorionic gonadotropin in choriocarcinoma and CEA in some colorectal carcinomas. Other tumour-related molecules are also shed into the serum but until now there has been no way of detecting them. By using specific monoclonal antibodies in a suitable radioimmunoassay, picogram quantities of these shed products can be measured. A large panel of well characterised monoclonal antibodies will therefore have considerable diagnostic use at several stages in the management of cancer patients.

Firstly, patients presenting with symptoms suggestive of malignancy may have no tissue readily accessible for biopsy. Investigations now necessary, are often expensive, time-consuming and cause the patient considerable discomfort. Early carcinoma of the pancreas is a good example. The second use of

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