# USE OF MONOCLONAL ANTIBODIES IN TROPHOBLASTIC NEOPLASIA

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## SYNOPSIS

**B lymphocytes respond to antigenic stimulation by producing polyclonal antibodies. These are not as specific as monoclonal antibodies, produced naturally in patients with myelomas, and in-vitro with hybridoma technology. Monoclonal antibodies to human tumour are produced at the fusion stage in 3 systems: mouse, rat and human. While the human-human hybrid has been shown to be stable (without preferential loss of human chromosomes), the immunoglobulin output is about one-tenth the output of the corresponding mouse hybridoma system. Clinically, monoclonal antibodies can be used in diagnosis and monitoring, in histological evaluation, in tumour localisation, and in therapy. Their use in trophoblastic neoplasia has not been clinically proven, but has vast potential mainly in monitoring.**

## INTRODUCTION

Since the discovery of monoclonal antibodies by Kohler and Milstein in 1975 (1) there have been hopes of improved understanding of basic processes in biology and pathology, of more sensitive methods of diagnosis, and of more precise ways of therapy. These hopes have been especially high in the field of oncology; however, their realisation has been slow. The diagnostic approach is the most important, especially in trophoblastic neoplasia.

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What is a monoclonal antibody?

Immunological response to antigenic stimulation is polyclonal, with many different B lymphocytes stimulated and producing antibodies. These antibodies have different molecular structures and in turn recognise different molecular configuration 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 the molecular level. This is particularly so with complex antigens such as the tumour cell surface.

However, there are monoclonal antibodies occurring naturally, in patients with myeloma. Here, neoplastic transformation occurs in a clone of B lymphocytes, and large quantities of identical immunoglobulins are produced. Unfortunately, the antigens to which most of the myeloma immunoglobulins are directed are usually unknown and are likely to be unimportant.

In 1975, Kohler and Milstein produced a hybrid myeloma (hybridoma) which produced a monoclonal antibody directed against a specific antigen. Mice were immunised with sheep red cells as antigens and their spleen lymphocytes were collected. The lymphocytes were fused with an established myeloma line and hybrids were 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 immunized mouse). The hybridomas were cloned by diluting the cells and growing up colonies from single cells. These cloned hybridomas now contained only 1 set of new immunoglobulin genes. 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 defined single antigens in a complex mixture such as the molecules on tumour cell surfaces.

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. 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 system (2, 3). Serological analysis and assays of lymphocyte function have shown that the immune system in man can actually recognise the tumour cell surface (4, 5). Whether immune mechanisms are able effectively to destroy tumour cells in vivo remains in question.

With the development of monoclonal antibody technology, it is now possible to sort out the antigenic complexity of human tumour cell surfaces. However, it may be many years more before the data can be meaningfully interpreted.

Production of monoclonal antibodies to human tumours

1. Fusion system

Currently there are 3 systems in which anti-tumour monoclonal antibodies can be raised: mouse, rat and human (6). 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 (7). 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 ul/ml which is one tenth 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 (8). 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 now 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. Lymphocytes in the lymph nodes draining a tumour are more likely to be involved in anti-tumour activity, especially for breast, lung and colorectal cancer. Another source of lymphocytes come from the tumour itself. Certain tumours, eg. gliomas, are often heavily infiltrated with lymphocytes, which can be collected, separated from the tumour and fused to a human myeloma line (9).

2. Immunisation schedule

For xenogenic immunisations the choice of antigenic material and the schedule in which it is used for immunisation varies considerably. Different sources and schedules result in different spectra of antibodies. However, 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 lymphocyte — peripheral blood, spleen, lymph node or intratumour — results in a higher frequency of the required antibodies.

3. 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, eg. a melanoma. The fusion products are screened on that melanoma in an indirect binding radioimmunoassay and the activity of any positive supernatants determined on other melanomas as well as on cell lines of different types, both normal and malignant. 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 walls and used in a solid phase radioimmunoassay to screen the activity of resulting monoclonal antibodies. A variant is to use sections of normal and tumour material to look at the activity of monoclonal antibodies histologically by immunofluorescence on frozen sections or by modified cyto-chemistry techniques with peroxidase, ferritin or colloidal gold techniques.

In the screening of human monoclonal antibodies, one problem is the ubiquitous presence of variable amounts of human immunoglobulin in human tumours. The detecting anti-human Ig, whether flourescein coupled or radiolabelled, binds to this resulting in high background levels in tumour membrane preparations. 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 3H-lysine and screen in a direct binding assay.

## Clinical uses

Monoclonal antibodies have been or are being raised against a wide variety of human tumour, including colorectal carcinoma, melanoma, breast cancer, lymphoma, leukemia, glioma, neuroblastoma, choriocarcinoma, lung cancer as well as cancer of the bladder, prostate and testis. Their uses can be in diagnosis and monitoring, histological evaluation, tumour localisation and treatment.

### 1. Diagnosis and Monitoring

A major problem in 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 alpha-fetoprotein in hepatoma and teratoma; human chorionic gonadotrophin in choriocarcinoma; and C.E.A. 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 value at several stages in the management of cancer patients.

Firstly, patients presenting with symptoms suggestive of malignancy have no tissue readily accessible for biopsy. Investigations for them are often expensive, time-consuming and cause the patient considerable discomfort. Early carcinoma of the pancreas is good example. Secondly, monoclonal antibodies can be used for regular screening of patients known to be predisposed to neoplastic changes, eg. those with ulcerative colitis, polyposis coli, molar pregnancy and certain forms of hepatic cirrhosis. Thirdly, the serial monitoring of tumour marker concentration in an individual patient's serum could provide a reliable index of the behaviour of the tumour and its response to treatment.

### 2. Histological evaluation

Previously unobtainable information may be obtained with a panel of monoclonal antibodies in immunohistology. Using such techniques more can be learnt about the different types of lymphoma and their response to treatment. Antibodies are also now available which can discriminate between different histological types of common solid tumours. They would also be of considerable use in evaluating histological material from patients presenting with metastatic disease.

### 3. Tumour localisation

Monoclonal antibodies have several advantages over polyclonal anti-tumour antibodies for tumour detection. Firstly, their defined specificity may allow the contrast required for effective imaging. Secondly, their production is reproducible on a large scale. Thirdly, they represent a concentrated form of immunoglobulin with defined activity and thus the total foreign load given to an individual is much lower.

### 4. Treatment

Monoclonal antibodies are currently being used by several groups in attempts to assess their value as therapeutic agents, eg. T cell nepolasm (10) and lymphosarcoma cell leukemia (11). There are several mechanisms by which tumour cell destruction can occur. These include the activation of complement; the triggering of antibody-dependent cell-mediated cytotoxicity; and opsonisation resulting in macrophage killing. It is possible that such mechanisms alone are unlikely to destroy large tumour masses but could deal effectively with well vascularised micrometastases. There is no report for such an approach in choriocarcinoma, but this tumour may be effectively treated in this manner.

## Monoclonal antibody to human chorionic gonadotrophin (hCG)

Monoclonal antibodies have been raised against hCG (12). Thirty-nine monoclonal antibody producing hybridoma cell lines derived from infusions of mouse myeloma cells with spleen cells from mice immunized with hCG have been established. Their products have been tested in RIA using 1251-labelled hCG, LH, FSH, TSH, the alpha- and beta-subunits of hCG and LH, and the C-terminal peptide 109-145 of chorionic gonadotrophin. All monoclonal antibodies were, in addition, tested in indirect immunofluorescence on paraffin sections of human pituitary glands. According to the intramolecular localization of the determinants recognised, 3 main groups of monoclonal antibodies can be distinguished; (1) those directed against epitomes on the alpha chain, (2) those directed against beta-chain determinants, and (3) those reacting with a conformational determinant only present on the native hormone and not on either subunit. The monoclonal antibody against beta-hCG is considered to eliminate completely LH cross-reactivity (13). Its value in tracking trophoblastic tumour behaviour is yet to be tested in clinical studies. The specificity of monoclonal antibodies to precise sequence structures may be a limitation as it has been shown that choriocarcinoma cell types may be heterogenous (14). Moreover, Cole and Hussa (15) have reported that hCG produced by cancer cells may be different structurally from hCG found in pregnancy urine; cancer cell hCG subunits may have an alternative oligosasccharide structure (16), or a reduced carbohydrate content or are sugar-free (17). Even slightly altered forms of hCG may not be detected; for example, removal of amino acids from either the N-terminal or C-terminal portion of the B-subunit eliminates recognition (18).

Beta-hCG is important as a tumour marker in trophoblastic disease. On the basis of in vitro and in vivo

TABLE 1
COMMERCIAL MONOCLONAL ANTIBODY PREPARATIONS TO HUMAN CHORIONIC GONADOTROPHIN

| Name | Company | Sensitivity |
|---|---|---|
| HCG MAIA Clone | Serono Diagnostics | 1 mIU/ml serum |
| Neo-Planotest Duoclon | Organon Teknika | 50 mIU/ml urine |
| Gravindex-B-hCG | Ortho Diagnostics | 80 mIU/ml urine |
| Pregnanstick | Monoclonal Antibodies, Inc | 175 mIU/ml urine |

studies, one tumour cell produces an average approximately $10^{-4}$ to $10^{-5}$ IU hCG per day. Thus at least $10^4$ to $10^5$ cells are necessary to show a serum or urine level of 1 miu hCG/ml (or its equivalence of about 0.2ng/ml) (19). There should, therefore, be allowance for extrapolation down to the theoretical "zero cell" level to obtain an indication of the minimum duration of treatment. The sensitivity of most assays has yet to reach that critical level. Under present conditions, the radioreceptor assay and the solidphase radioimmunoassay lack the sensitivity of the double antibody liquid radioimmunoassay (20, 21).

Mixtures of certain monoclonal antibodies appear to bind hCG in a "cooperative" fashion because they form circular complexes with the hormone. This property has been exploited to develop very sensitive immunoassays for hCG or any other antigen. Since the assays are not based on competitive inhibition between radiolabelled and unlabelled antigen, they are much more sensitive than a traditional radioimmunoassay in which either one of the same antibody is used alone (22).

Their use at the moment is mainly diagnostic and is available commercially to this effect (Table 1). The hCG MAIA clone (Serono Diagnostics, from Inter-Yeda, Israel) is a quantitative 2-site immunoradiometric assay for intact hCG and its B-subunit, with a 15 minute incubation at room temperature. The Neo-Planotest is based on the principle of direct latex agglutination with the complete hCG molecule specifically detected and "sandwiched" by two different monoclonal antibodies. One antibody reacts with a conformational determinant which only occurs on the fused alpha-beta subunits, thus being specific for the intact hCG: the other antibody reacts with an antigenic determinant on the B-subunit. The Gravindex B-hCG claims to have specifically-treated latex particles which are chemically bonded to the hCG molecule; the monoclonal antibody is B-specific. The Pregnan Stick is a dipstick with B-specific monoclonal antibody on it. Techniques utilising monoclonal antibodies to hCG include radioimmunoassay (23) and immuno-histochemistry (24). Stenman et al (25) have also reported its use for gynaecologic emergencies. There is also possibility of its use in immunointerception of fertility (26).

## CONCLUSION

The use of monoclonal antibodies in trophoblast neoplasia has not been clinically proven. However, its potential, especially in tracking the tumour by its marker, is vast. Only time will tell whether it will be of use in enhancement of chemotherapy.

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