SCOPE OF MONOCLONAL ANTIBODY TECHNOLOGY IN ONCOLOGY

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ABSTRACT

Monoclonal antibody technology has revolutionised our ability to obtain unlimited quantity of precise antibodies, complementary to recombinant DNA technology for obtaining antigens. Their production from mouse, rat and human myelomas constitutes a quantum jump in oncological studies. Monoclonal antibodies are of immense importance in the field of oncology especially in the typing and classification of malignancies and identification of malignant cells, tumour imaging, chemotherapy and identification and characterization of significant tumour antigens for serodiagnosis and perhaps tumour therapy. Choice of target antigens for monoclonal antibodies and of labels to the monoclonal antibodies for the various applications has been discussed.

INTRODUCTION

Following Kohler and Milstein's epoch-making report[1] on the creation of unlimited quantities of monoclonal antibodies *in vitro* from hybridoma cells (generated by the fusion of mouse myeloma cells and splenic lymphocytes from immunized animals generating an antibody of interest) there has been a knowledge explosion in the applications of such monoclonal antibodies to medicine and biology. The production and testing of monoclonal antibodies have also undergone considerable evolution. Monoclonal antibodies are now available not only from the fusion of lymphocytes with mouse myelomas secreting their own immunoglobulins but also non-secreting mouse myelomas, rat myelomas and now in particular human myelomas. Human type immuno-globulins have obvious advantages over heterologous species for *in vivo* work in human subjects.

In addition there have been reports on the direct transformation of lymphocytes[2] without hybridization with myeloma lines. Another interesting development is the harvesting of lymphocytes already producing antibodies from patients instead of immunizing animals to obtain desired lymphocytes[3].

Monoclonal antibodies engineered an immunological revolution because the polyclonal heterogenous mixtures of antibodies earlier available in limited quantities from precious "lucky strike" animals with varying subclass, avidity, titre and sometimes specificity in various bleeds even from the same animals are now replaced by homogenous immunoglobulins in unlimited quantities with a narrowly defined antigen specificity. In brief the monoclonal technology transformed immunoglobulins from nebulous mixtures to precise chemical reagents. Monoclonal technology which has refined the preparation of antibodies is directly complementary to the recombinant DNA technology which has similarly revolutionized our ability to obtain unlimited quantities of precisely defined antigens[4]. Monoclonal antibodies both of the IgG and IgM classes are available and despite certain limitations (*e.g.* poor immunoprecipitation and sometimes lesser avidity) have found extensive applications in various fields. The limitations in immunoprecipitations: this process involves lattice formation and therefore requires a mix of antibodies. Combinations of monoclonal antibodies can overcome this problem.

POTENTIAL AREAS FOR THE APPLICATION OF MONOCLONAL TECHNOLOGY TO ONCOLOGY

There are numerous exciting possibilities for applying monoclonal antibodies to cancer. Listed below is a brief classification of these applications, some of which are already being realized and others may prove significant in the future.

(i) Typing and classification of tumours and tumour cells such as the classification of leukaemias[5-7].
(ii) Unravelling the antigenic relationship of malignant cells to mature or undifferentiated primitive/precursor normal cells as in haemopoietic malignancies[8] (Greaves, M. F.—Personal communication 1981).

(iii) The identification[9] of malignant cells in aspirates or washings as an extended Papanicolau technique.
(iv) The diagnostic identification of malignant cells or cell aggregates in tissue sections at biopsy or autopsy[10a&b].
(v) The imaging of tumour and tumour metastases in human subjects permitting rational management and accurate localization of external beam radiation therapy or selective arterial regional chemotherapy[11].
(vi) The selective removal of malignant cells from bone marrow specimens intended for autologous transplant rescue after sublethal body irradiation in cancer treatment[12-14].
(vii) The removal of circulating malignant cells through extracorporeal bypass or in modified plasmapharesis[15].
(viii) Therapy of tumours *in vivo* either by antibodies as such[16] or by attaching appropriate warheads-radionuclides[15] cytotoxic drugs or toxins[17-26].
(ix) Identification and characterization of significant tumour antigens by affinity chromatography permitting further duplication or modification by recombinant DNA technology or creation of anticancer vaccines for high risk population.
(x) Creation of sensitive, specific, and robust assay systems for detecting antigens in circulating blood using either radioimmunoassay (RIA) type limited reagent systems or immunoradiometric type (IRMA) excess reagent systems[27-29] for the early diagnosis of cancer[30].
(xi) In addition (as in other areas) monoclonal antibodies can be used for estimating the blood level of drugs and hormones in cancer patients. In addition the following three aspects are also important.

(a) We suggest that monoclonal antibodies could be used as vehicles for efficient delivery of agents such as the retinoids which are stated not only to prevent malignant transformation but also induce reversion[31]. Reports have already appeared on the use of monoclonal antibodies to deliver alpha interferon and other agents of biological response modification to the tumour area to enhance the body's ability to destroy the tumour[32,33]. It is believed that alpha interferon when localized in the tumour may help induce local NK cell activation.
(b) Monoclonal antibodies may also be enshrined in liposomes to induce the uptake by macrophages as well as selectively kill the host T lymphocytes after a better understanding of their role in the creation of blocking or enhancing antibodies in relation to tumour.
(c) The monoclonal antibodies may also be used to delineate and interfere with idiotype and antiidiotypic antibodies existing *in vivo* an area to which attention is now being directed[34].

CHOICE OF TUMOUR ANTIGENS

The early experimental work suggested that tumour antigens exist in experimental carcinogen-induced or virus-induced tumours but whether truly tumour specific antigens existed in naturally occurring human tumours remained doubtful. Tumour antigen may either be an epitope restricted to individual tumour, or histological type of tumour or associated with a range of tumours[35] (Pancarcinoma reactivity) or even shared between tumour and normal cells, or cells affected by benign disease or undifferentiated cells.

Even if a truly tumour specific antigen exists not all the tumour cells may be expressing the epitope and this may vary from time to time—the problem of "antigenic modulation".

It is possible that even if an antigen is shared between normal and malignant cells, it may be utilized if it is present in much greater amount in the tumour cells.

Failing the identification of pancarcinoma tumour antigens, for clinical use of monoclonal antibody therapy in a particular patient one must have a very short turn around time so that one can generate a 'custom-built' antibody against a biopsy specimen from the patient tumour or harvest the patients own lymphocytes and immortalize them, all of which pose technological problems which will hopefully be solved eventually.

On the other extreme perhaps the best example of a truly tumour specific antigen is the idiotype of cell surface IgG of a cell tumour which obviously represents a clonally expressed tumour specific marker. Nadler *et al*[36] have recently reported a lymphoma associated antigen on B-cell lymphomas and even attempted serotherapy, with monoclonal reagents. In another approach B-cell lymphomas have been hybridised to generated hybridomas which can be subsequently utilized to monitor the clinical progress of the lymphoma by making antiidiotype antibodies specific for the IgM secreted by the hybridoma[37]. The earlier studies chiefly used antibodies direct against shed-circulating antigens such as carcinoembryonic antigen[38] alpha fetoprotein[39] HCG[40] and prostatic human phosphatase[41]. Mach *et al*[42] have described their experience of scanning patients with histologically proved carcinoma when injected with ^{131}I label-

led polyclonal goat antibodies (whole, FAb or $F(Ab)_2$ fragments) against carcinoembryonic antigen and observed a 2–4 fold greater concentration in tumour as compared to normal tissues, despite a large fraction of the anti-CEA antibody being picked up by the circulating CEA. They sound a note of caution in their studies of tumour localization with radiolabelled antibodies against CEA in that the total radioactivity localized in the tumour is only about 0.001 of the injected dose.

More recently interest has been aroused in cell-fixed antigens. Unlike circulating antigens they would not mop up the administered antibody and would therefore be more desirable for imaging or therapy *in vivo*.

Epenetos, Britton and co-workers[43] using monoclonal antibodies directed against cell-fixed antigens such as the milk fat globule in mammary tissue observed far greater target to nontarget to ratios. This also exemplifies the ingenious use of an antibody against a normal tissue antigen. The milk fat antigen used exists in normal breast tissues on the interiors of the ducts. In malignant disorganised breast tissue these ducts are exposed in far greater concentration to the antibody (Britton K. E., Personal communication).

There has been recent interest in several tumour markers with alleged pancarcinoma reactivity *e.g.* the Oxford marker and malignin but whether these are tumour-specific is now in doubt.

Most studies have concentrated on surface tumour antigens and early work naturally concentrated on these but it may not be out of place to mention other possible target antigens.

Malignant cells no doubt differ from normal cells in surface properties[44] but the basic alteration in the malignant cells occurs in the DNA and changes in the cell surface or in the other cytoplasmic structures are secondary. So far monoclonal antibodies directed against nuclear antigens have not been employed for radio-nuclidic or immunotoxin targeting but have been employed *in vitro* in histological studies[45]. It is usually thought that interiorisation of antibodies by pinocytosis is too slow a process to permit significant tumour targeting[46], but we have earlier suggested that given a sufficiently great affinity, a dynamic equilibrium favouring antibody localization in living tumour cell nuclei is not impossible and merits exploration[11]. The outer surface of the tumour cell may be in a state of flux permitting antibody entry. Further in the human disease systemic lupus erythematous we have a model of an antinuclear antibody existing *in vivo* and perhaps contributing to the disease process[47]. The recent recognition of specific-altered DNA sequences in malignant cells and of oncogenes related to normal growth factors suggests possible antigenic targets[48]. to the antibody (Britton K. E., Personal com-what will really need targeting is the set of genes which control their activity.

Changes in mitochondrial or other cell organelle, antigens in malignant cells are still unexplored. However, the preferential glucose metabolic pathway that malignant cells follow (which had led to the use of deoxyglucose to inhibit the malignant cell's ability to repair DNA damages) (Jain, M. P. Personal Communication 1981) suggest that malignant cell organelles may possess distinctive antigens. Liposome incorporated radionuclidic labelled antibodies[50] represent a possible method of approaching organelles inside tumour cells.

At the other extreme, instead of homing on the tumour cell surface, antibodies can target on to the gross tumour/non-tumour interface, using antibodies directed against fibrin, fibrinogen or the tumour chemicals, that allegedly promote fibrin formation, repel immune cells and encourage new blood vessel formation[51–53].

CHOICE OF LABEL FOR MONOCLONAL ANTIBODIES

It is possible to follow the antibodies with a variety of labels—radionuclidic enzymes, fluorochromes, chemiluminiscent probes, magnetic ferric oxide and gold sols apart from conventional serological methods such as complement fixation coupled with red cell lysis for demonstrating antigen antibody reactions. The optimal class of labels will be different for the various uses of antibodies described earlier, and even in the radionuclide class the optimal isotope may differ[15].

Although the direct immortalization of lymphocytes without hybridization[2] is of great potential interest, most attempts to make monoclonal antibodies have focused on the earlier discussed methods dependent on fusion with myeloma cells. However as mouse immunoglobulins are usually considered unsuitable for large scale clinical use, it is imperative that the use of secreting human myeloma lines suitable for hybridization be expanded until it becomes possible to immortalize the patients own lymphocytes. However Koprowski[54], has recently indicated that mouse hybridoma antibodies given to human beings in doses as large as 365 to 625 mg did not evoke antimouse antibodies, and thus can be used clinically.

The methods of screening various monoclonal

antibodies for antitumour reactivity have been reviewed[11].

Most commonly, the supernates from hybridoma clones are incubated with tumour cells in microtitre plates followed by detection with an enzyme or ^{125}I labelled anti IgG reagent directed against the species of myeloma.

We have earlier suggested that radiorespirometry which has been advocated for measuring the growth of slow growing mycobacteria could also be adapted to tumour cells[11]. This may be a possible alternative to the chrome release or ^{125}Iododeoxyuridine (IUDR) techniques currently employed with tumour stem cell assays.

CONCLUSION

The use of monoclonal antibodies in research as well as diagnosis in oncology is thus fairly well established. Their use in therepy is at present limited by many problems. However although the road is arduous, the goals are in sight. This optimism is thus triggering extensive research efforts around the globe. In India this work has been taken up at several centres including All India Institute of Medical Sciences, Delhi, S. N. Medical College, Agra and Cancer Research Institute, Bombay. The possible spin-off of this fundamental research in the infective diseases is further justification for our developing expertise in this field.

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