Regular Review

Monoclonal antibodies in clinical medicine

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The production of monoclonal antibodies by in vitro techniques[1] has opened up many new avenues for the use of antibodies, both for diagnostic procedures and in treatment.[2] Indeed, the potential applications for these new reagents might seem limitless if the enthusiastic predictions of some of those responsible for their production were to be realised. Recent publications have dealt with the production of monoclonal antibodies and with methods for their application in every branch of biological science, from biochemistry to tumour imaging.[3-7] Their value in the elucidation of the finer points of antigen expression, in the study of the antigens of the major histocompatibility complex in health and disease, and in the characterisation of important markers of cell differentiation is evident from the many important papers on these topics in the leading scientific journals.

What will be the place of monoclonal antibodies in clinical medicine and, more particularly, in patient care? Initially, the haematological malignancies have been the object of considerable attention.[8] Probably because monoclonal antibodies were developed to satisfy the demands of immunologists, the lymphocyte was the first human cell type to be studied extensively with murine monoclonals which were prepared using selected normal or leukaemic cells as the stimulating antigen.[9] Their first use was in fluorescence microscopy techniques. Next, automated laser based cell sorters were used to exploit these antibodies, the application of automated machines giving greater scope for large scale studies.[10] These techniques have made heavy demands for antibody on the plentiful supplies available from immunoglobulin secreting hybridomas (the etymologically inelegant but descriptive term applied to the immortal cells derived from the fusion of myeloma cells with B lymphocytes).

The recognition of specific markers for haemopoietic cells of various lineages has clarified the classification of leukaemias far beyond that based on crude morphological criteria, providing a new means of deciding on optimum treatment regimens and even predicting outcome.[11] Further insight into the nature of putative tumour specific antigens in leukaemia and lymphoma has been gained by studying the serological cross reactivities of monoclonal antibodies directed against haemopoietic cells. Many of the antigens presumed to be tumour associated have now been shown to be closely related in structure to blood group substances. Differences are usually detectable, however, between the degree of glycosylation of the normal blood group carbohydrate determinants and the products of the immature or proliferating malignant cells, suggesting that this difference might be used in producing monoclonals selective for tumour cells for treatment.

Other monoclonals which react with tumour associated antigens include murine monoclonal antibodies against some of the antigens found in melanoma cell membranes,[12] colorectal carcinoma,[13] breast cancer,[14 15] brain tumours,[16] and ovarian carcinoma.[14] Some of these antibodies have been used in clinical trials with varying reports of limited efficacy.[17 18] In most reported clinical trials of passive treatment with monoclonal antibodies, however, the patients were mainly those with haematological malignancies, including the lymphoid solid tumours, and were generally suffering from advanced disease which had failed to respond to conventional treatment. Even so, remarkable if short lived responses have been achieved, particularly in cutaneous T cell malignancies[19] and in a few B cell lymphomas with anti-idiotype antibody—that is, a monoclonal antibody directed against the immunoglobin unique to the malignant clone of B cells forming the tumour.[20]

Although the production of tailor made antibody for each individual patient may seem impracticable because of the time required and the expense, potentially monoclonal antibodies could be used to modify or regulate the aberrant behaviour of some of the cells of the immune system in other disorders where dysfunction rather than malignant proliferation is the problem, such as the autoimmune diseases.[21]

The treatment of renal transplant rejection with murine antilymphocyte monoclonals was initially hailed as a breakthrough, but the problems of serum sickness (from hypersensitivity to the mouse protein) limited its value.[22] The methods used for malignant diseases have depended both on the type of disease and on the site of the antigenic determinant against which the antibody is directed. In leukaemia efforts have been concentrated on extracorporeal treatment of leukaemic cells in autologous bone marrow, removed from the patient in remission and then treated in the presence of complement (with monoclonal antibodies selected as cytotoxic for the tumour cells). After the patient's bone marrow has been made aplastic by "supralethal" chemotherapy the treated "clean" marrow is reinfused.[23] This treatment has particular merit in that it does not require the existence of an HLA identical sibling donor. Subsequent relapse with the reappearance of leukaemic cells has been common—probably because of residual tumour in some inaccessible sites such as the testes or cerebrospinal fluid, but perhaps also because the exact amount of antibody required to clear the remission marrow of all leukaemic cells is difficult to calculate. The use of antibody coupled to magnetic beads allows the removal of the antibody coated cells by magnets, and this may be preferable to cytotoxic killing, which may be less than complete. The method is also applicable where the tumour target is resistant to complement dependent killing —for example, neuroblastoma cells.[24]

Imaging

The foregoing are the more dramatic applications of monoclonal antibodies derived from murine hybridomas. Other applications of potential value include imaging of lesions in deep tissues or the localisation of tumour in small foci inaccessible to other diagnostic tests.[25] Imaging with radiolabelled antibody has been successful in studies on animals for both malignant and non-malignant lesions.

Much interest has focused on the delineation of myocardial infarcts using antibodies against cardiac myosin, actin, or enzymes released from damaged muscle.[26] Specificity has been difficult to achieve, and since short lived isotopes have to be used there have been problems in producing a clear picture of the extent of the lesion. More problems may develop if repeat tests are required to monitor the extent and progress of an infarct: radiolabelled material may persist, bound to damaged cells, and there is a risk of hypersensitivity developing after repeated exposure to mouse immunoglobulins.

Such factors are perhaps less critical when monoclonal antibodies are being used to detect tumour cells.[27-29] The use of polyclonal antibodies was always bedevilled by variation among batches of antisera and by cross reactivity between epitopes on tumour cells and on normal tissues, particularly when oncofetal antigens were the target. Monoclonals might have been expected to overcome these problems, but the results have not been clear cut. The problem of cross reactivity still exists and is most troublesome with tumours of the gut, where common antigenic determinants such as carcinoembryonic antigen are no more suited to specific tumour imaging than they were to earlier serological detection.[30] Useful monoclonal antibodies have been produced for in vitro studies directed against melanoma associated antigens—in particular, an iron binding surface glycoprotein which is expressed at a higher surface density on melanoma cells than on normal fibroblasts, lymphocytes, or some other tumour cells, though its presence on the cells of benign naevi reduces its specific application in vivo, limiting it largely to diagnostic immunohistology.[31] Carcinoma of the breast is another obvious candidate for imaging procedures, and some success has been achieved. Both primary and secondary lesions have been detected with murine monoclonals labelled with either indium-111[15] or iodine-123,[14] but the clarity of definition and localisation is below that produced with advanced radiological methods such as computed tomography. An additional problem lies in the modulation of antigen expression in the presence of antibody.[32] This ability of cells to "escape" from the effects of antibody had been known to occur with murine viral leukaemia cells when treated with potentially cytotoxic polyclonal antibodies.[33] In that case the antibody causes an alteration in surface antigen expression leading to the "stripping" of the membrane antigen; this makes the cells insusceptible to subsequent treatment with the antibody. In theory "escape" might be less likely to occur if a cocktail of monoclonal antibodies was employed or if consecutive courses of treatment used antibodies of slightly different specificities, but these solutions would increase both the difficulty and the cost of successful treatment.

Treatment

The greatest potential benefit is likely to be derived from the use of monoclonal antibodies as carriers of drugs or toxins for the efficient localisation of cytotoxic treatment. Polyclonal antibodies were tried for this purpose in man, mainly in leukaemia with chlorambucil, doxorubicin, and methotrexate, but only marginal improvements in antileukaemic effects were seen when compared with the results of drug treatment alone.[34] The non-specific nature of the polyclonal antisera may have contributed to the lack of success as well as the problems inherent in targeting drugs which are largely intracellular in their effect and which might be hindered from reaching the interior of the cell when coupled to an antibody.

Considerable interest has been generated by the possibility of using monoclonal antibodies coupled to bacterial or plant toxins, including diphtheria toxin A chain, exotoxin A of *Pseudomonas aeruginosa*, ricin (from *Ricinus communis*, the castor oil plant), or abrin (from *Abrus precatorius*, the wild liquorice plant), all of which penetrate the cell membrane and inhibit protein synthesis.[35] The advantage of these molecules is that their potency maximises the chance of killing tumour cells even when the antigenic determinant which is specific for the monoclonal carrier is not expressed in high density at the cell surface, as is the case with many tumour specific antigens. The selectivity conveyed by the monoclonal antibody also allows such toxin conjugates to be used to kill selected cells in mixed populations. Murine monoclonal antibodies linked to ricin have been used effectively in the treatment of mouse B lymphoid neoplasms. Experience with human tumours is limited and is mainly based on laboratory studies with cultured cells as targets.[36]

Microbiology

Microbiologists have also been quick to exploit the potential of monoclonal antibodies, though therapeutic applications are likely to await the production of human rather than mouse antibodies, to avoid the problems of immunisation against mouse protein with repeated administration. Numerous murine monoclonals have been produced against a wide variety of microbial antigens, including many of the medically important viruses, bacteria, and parasites.[37] Antibodies have been produced against membrane components, toxins, and other selected target molecules. Herpes viruses, hepatitis B, rabies, and influenza are candidates for the use of monoclonals for the purification of specific antigens with a view to the subsequent production of effective and safe vaccines. Interferon was the first molecule to be purified using murine monoclonals with purification of over 5000-fold with little loss of activity—a protein chemist's dream come true, and a success which has become the first money spinner of the British biotechnology firm Celltech, created out of a marriage of venture capital and Medical Research Council brain power.[38] It is only a matter of time before the potential for the preparation of vaccines is exploited in the same way, with *Bordetella pertussis* and the J5 antigen of the Enterobacteriaceae looking likely candidates.

Diagnostic kits using monoclonals are already replacing older polyclonal antibodies for meningococci, gonococci, legionella, chlamydia, and others. The advantage to the manufacturer lies mainly in the reliability of supply, although initially there may be some market advantage in the novelty. For some organisms there is the possibility of increased specificity compared with that achievable with existing polyclonal xenoantisera. Cost may well preclude a wholesale conversion of diagnostic kits, and most firms will probably depend for development and even for supplies on

researchers anxious to earn cash to supplement their dwindling budgets. Grant giving charities may have to recognise the commercial potential of their award holders and should not be afraid to tackle the realities of exploiting these new discoveries.

Biochemistry, both clinically applied and industrial, will also benefit from the application of monoclonal antibodies to many assays and purification procedures. Small molecular weight peptides, such as parathyroid hormone, have been notoriously difficult to purify but the success achieved with lymphokines such as interferon by the use of columns of monoclonal antibody bound to inert materials should be repeatable. Diagnostic tests using monoclonals are already in use for several biologically important molecules, including α fetoprotein, fibrinogen, alkaline phosphatase, pregnancy associated proteins, and complement components.

Human monoclonals

What of the possibilities for human as opposed to murine monoclonal antibodies? One might suppose that human antibodies would answer many diagnostic (imaging) and therapeutic problems, including antibiotic resistant infections, exotoxin induced disease, endotoxic shock, and deep seated or early lesions in the case of tumours. The difficulty has been in achieving the same runaway success in producing human antibodies in the test tube as for the mouse product. Progress has been slow, but there are optimistic signs that the difficulties of production may be resolved either by using a different pairing of cells to achieve the hybridoma—for example, rodent human hybrids—or by bulk culture of B lymphocytes, transformed by Epstein-Barr virus and capable of long term growth without hybridisation. A preliminary report of the successful production of antirhesus D immunoglobulin by the Epstein-Barr transformation method has stimulated hopes that human monoclonals may not be unattainable.[39]

Those directed against microbial agents which are insusceptible to existing methods of treatment, or for which no appropriate chemotherapeutic agent exists, would find an application, and the commercial biotechnology firms are preparing to enter this research, with possible preliminary trials of human monoclonal antibodies directed against the endotoxins of selected Gram negative bacteria. Some of the rare but potentially fatal viral haemorrhagic fevers might also be candidates for this approach, using lymphocytes from those fortunate enough to survive an attack.

The potential uses of monoclonals in diagnosis are also likely to be greatly extended by the use of cell fusion techniques to produce antibodies which are bispecific—that is, have binding sites directed against two separate antigenic determinants. The antibodies produced by hybrids are the random outcome of the genes present in the original fusion partner cells, and it is possible, by the use of appropriate selection and purification procedures, to arrive at hybrid products which have binding sites for two separate antigens —for example, antiperoxidase and antisomatostatin.[40] Such antibodies are potentially powerful tools in immunocytochemistry for the localisation of tumours.[41] Genetic engineering should also make it possible to produce modified murine monoclonal antibodies, tailor made to avoid some of the problems of hypersensitivity to xenogeneic material, by removing part of the murine immunoglobulin molecule and splicing in appropriate parts of human immunoglobulin, thus making use of the advantages of the ease of production of murine antibodies on a large scale in vitro.[42] We are only at the beginning of a long and exciting series of new developments in antibody technology with great potential in human medicine.

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