Proc. R. Coll. Physicians Edinb. 1997; **27**: 145-152

THE RETURN OF THE MAGIC BULLET: MONOCLONAL ANTIBODIES IN MODERN CANCER MEDICINE*

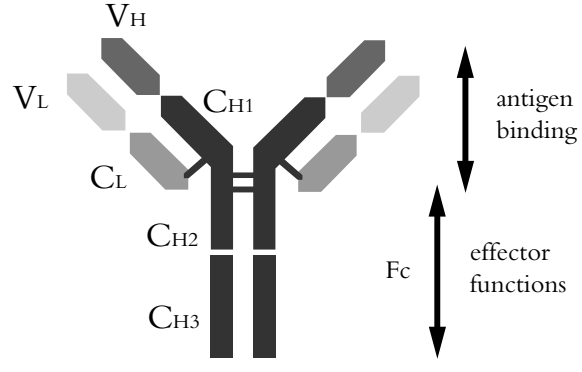
S. Nicholson, ICRF Clinical Research Fellow, Imperial Cancer Research Fund Oncology Unit, Royal Postgraduate Medical School, Hammersmith Hospital, London

Paul Ehrlich's concept of 'Magic Bullets' is currently used most to promote the future promise of gene therapy in all its guises. While gene therapy is in its infancy our experience of monoclonal antibodies has matured, enabling an ever greater role for this technology in various aspects of cancer diagnosis and therapy.

NUTS AND BOLTS

The schematic diagram of immunoglobulin G (Fig 1) will be familiar to many. The antibody is composed of two heavy chains and two light chains, each chain possessing one variable domain and one or more constant regions. The variable domains are collectively modified by 'gene shuffling' to produce antibodies with a binding affinity for any specified antigen; while the constant domains are responsible for the antibodies' effector functions, e.g. activation of the Complement cascade.

Although this diagram is useful, it is insufficient to describe the many antibody fragments that can be produced which, while retaining the ability to bind antigen, lose the particular structure of naturally-occurring immunoglobulins. These may be generated by simple enzymatic digestion of chemical bonds, however it is increasingly fashionable to produce such small molecules through the techniques of molecular biology. The principles behind this approach are beyond the scope of this paper, but such molecular approaches allow these antigen-binding molecules to be produced without recourse to immunizing experimental animals. The paradigm for these molecules is the fusion of the variable region (Fv) from one heavy chain and one light chain, joining them by an inert peptide linker to produce the single-chain antibody, or ScFv.



C = constant region or domain, V = variable region or domain
H = part of Heavy chain, L = part of Light chain

FIGURE 1.
Schematic Diagram of Antibody

* Based upon a lecture delivered at the Symposium on the *The Hazards of Life: Living Dangerously* held in the College on 18 October 1996

HYBRIDOMAS FOR BEGINNERS

The generation of an hybridoma in the manner of Kohler and Milstein[1] remains important both for the majority of commercial antibody production and also for illustrating the limitations of these antibodies. An experimental animal, usually a mouse, is repeatedly immunised with the antigen of interest (Fig 2). Splenocytes from the animal are disaggregated into a suspension of single cells, some of which can then be persuaded by chemical means to fuse with a mouse myeloma cell line. Some of the viable cells from this fusion retain the ability to secrete their single immunoglobulin subtype, and in a proportion of these that antibody will have an affinity for the antigen with which the animal was immunised. Cell cultures generated from individual fusion cells are screened and selected to enable the establishment of a cell line (the hybridoma) all of whose cells produce a single antibody of known subtype and binding specificity - the monoclonal antibody (MAb).

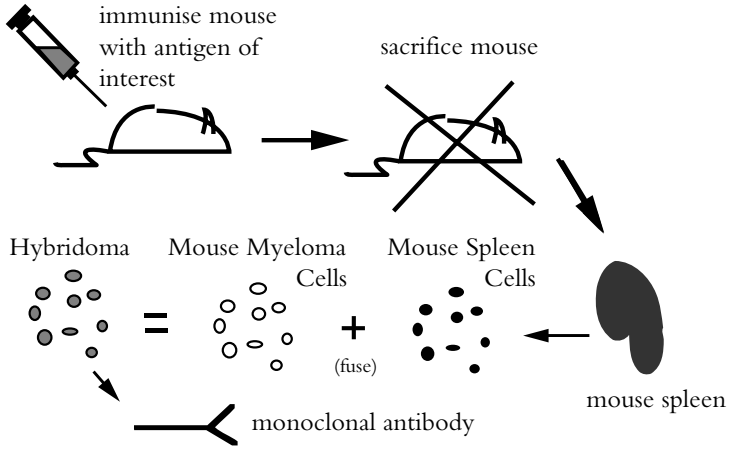


FIGURE 2.
Hybridomas for beginners

THE TARGET

All tissue cells express antigen on their surface. Tumour-specific antigens are few and in their place are a selection of tumour-associated antigens whose expression on malignant tissues differs quantitatively or qualitatively from that on normal tissue. Such antigens constitute targets for antibodies for either therapeutic or diagnostic purposes.

An example of a truly tumour-specific antigen, and one which has been found amenable to MAb therapy (see below), is the surface immunoglobulin found on the malignant clone of B lymphocytes in B cell Non-Hodgkin's Lymphoma. While the overall structure of the antibody is the same as for any other human antibody of its class, the antigen-binding site of this surface antibody is unique to the malignant clone. Other antigens such as CD19 or CD20 are found on all B lymphocytes. Since in the diseased state the malignant B cell clone outnumbers all other B cells, these antigens may be regarded as tumour-associated.

The situation in solid tumours is perhaps best illustrated by the non-secretory cell-surface glycoprotein known as Polymorphic Epithelial Mucin (PEM), encoded by the gene MUC1. Although present on normal glandular epithelium, most notably

in the lactating breast, in epithelial tumours of the breast, ovary and pancreas PEM is overexpressed, distributed more broadly over the cell surface and aberrantly glycosylated. This in turn reveals novel epitopes for antibody binding which are hidden in the non-malignant situation.[2,3]

THE USES OF MONOCLONAL ANTIBODIES

The diagnostic and therapeutic uses of MAb are summarised in Table 1.

Table 1
Uses of monoclonal antibodies

Diagnostic	
1.	Immunohistochemistry
2.	Tumour markers
3.	Radioimmunoscintigraphy
4.	Per-operative radioimmunodetection
Therapeutic	
1.	Antibody alone, e.g. Antibody dependent cellular cytotoxicity, cancer vaccines
2.	Radioimmunotherapy
3.	Anti-neoplastic conjugates
4.	Immunotoxins e.g. Ricin
5.	Enzyme conjugates - ADEPT

DIAGNOSTIC USES OF MONOCLONAL ANTIBODIES

Immunohistochemistry

Tissue diagnosis in cancer practice can never be over-emphasised, and anyone coming into contact with an oncologist is doubtlessly aware of their obsession with the histology of the neoplasus which they treat. Immunohistochemistry goes beyond providing merely confirmatory evidence of tumour type, however. An example of the emerging role of immunohistochemistry as a guide to therapy is the immunochemical detection of the cell surface protein c-erbB2 in breast cancer. The product of a classical oncogene, it has been demonstrated in a number of studies that lymph node-positive breast cancer that stains for c-erbB2 has a poorer prognosis than that which does not, and that c-erbB2 tumours seem to be more dependent on the intensity of chemotherapy than those that are c-erB2 negative.[4] This may have future import for the use of myeloablative chemotherapy followed by bone marrow/peripheral stem cell transplantation, a technique which is currently under study in a number of clinical trials.

Tumour markers

The principle of enzyme immunoassay and radioimmunoassay for circulating tumour markers is illustrated in Fig 3. A classical 'sandwich' immunoassay uses two monoclonal antibodies (MAb), each recognising different antigens on the molecule of interest. The first MAb, the 'catcher', is bound to a solid phase, typically a polystyrene bead or microtitre plate. This is exposed to the patient's serum and left to incubate. The plate/bead is then washed and re-incubated with the second antibody, the 'tracer,' which is conjugated with either a radio-isotope

(radioimmunoassay) or an enzyme that produces a colour signal when exposed to a chromogenic substrate. When excess tracer is washed away, the intensity of the signal, radioactive or colour, is proportional to the concentration of the tumour marker in the patient's serum.

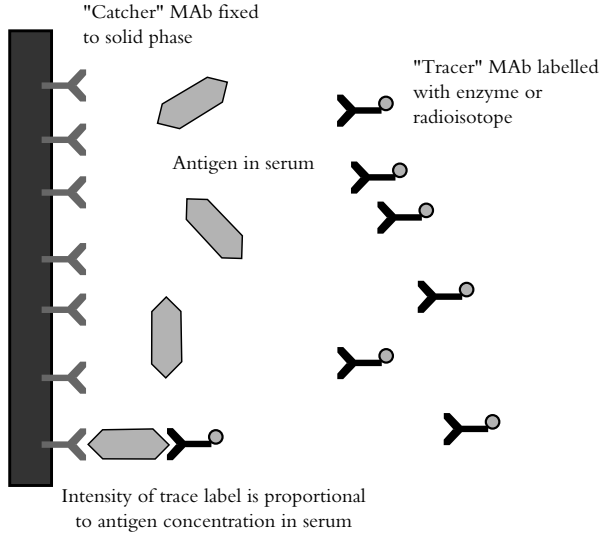


FIGURE 3.
'Sandwich immunoassay'

The tumour markers of germ cell malignancies i.e. alpha fetoprotein (AFP) and β (human chorionic gonadotrophin (HCG) were the first to be appreciated for their value in monitoring the response to chemotherapy and watching for early relapse. The utility of the tumour marker, Prostate Specific Antigen (PSA) for monitoring therapy and for predicting disease stage at presentation has been well-defined; however the current debate about its utility as a marker is becoming increasingly politicised. The question now being addressed is whether all men above a certain age should undergo annual screening; this would include digital rectal examination and a blood test for PSA. The controversies surrounding this issue are beyond the scope of this paper, but suffice it to say that these arguments are likely to be repeated in the near future, when the tumour will be carcinoma of the ovary and the marker CA125.

Radioimmunoscintigraphy (RIS)
Labelling an antibody with an isotope that emits gamma radiation enables the antibody to be imaged where it localises after administration. Although this may allow tumour-specific imaging, cancer patients will still need to undergo routine radiological assessment. Where RIS is superior to CT or U/S scanning, and may have a major role to play, is in its ability to differentiate between benign and malignant tumours. A particularly good example is that published by the Barts group,[5] who imaged 45 patients with suspected ovarian cancer prior to surgery. The antibody used, SM3, recognises that malignant form of PEM and this was labelled with the isotope technetium 99m. When compared with histological examination, this imaging was found to be both highly sensitive and specific, and had a negative

predictive value of 100% i.e. those masses that did not image on RIS, were all found to be benign at laparotomy. Although this was a small study it may prove to be immensely important in the context of setting up a national ovarian cancer screening programme, particularly when another study which considered 234 laparotomies for asymptomatic ovarian masses detected on ultrasound, showed that only nine patients were suffering from malignancy.[6] Clearly any technique which could make such screening more cost-effective should be taken seriously.

Per-operative radioimmunodetection

This is the most recent application of MAbs to the field of diagnostics and involves the administration of a radiolabelled MAb pre-operatively, followed by the use of a hand-held detector probe intra-operatively. Although the most obvious technique would be the scanning of the entire operative field, in fact this is less likely to be valuable than the more subtle approach of scanning tissues *ex vivo* in the operating theatre. This would involve the surgeon comparing the excised tumour with negative and positive controls, perhaps then enabling the rapid confirmation of malignancy of breast lumps, rather than waiting for frozen section histology. The surgeon would be able to take biopsies from suspicious sites, or at random, and compare their activity with that of the tumour and of control tissues: this would enable a better surgical clearance of small volume disease, which is of known prognostic significance, for example, in ovarian cancer.[7]

THERAPEUTIC USES OF MONOCLONAL ANTIBODIES

A basic observation about any new treatment for malignant disease, is that if it is going to have a chance of proving valuable in the treatment of solid tumours, it will first have been demonstrated to be effective in the 'hematological' malignancies.

Radioimmunotherapy

The paradigm for the use of MAbs to target toxic therapy at cancer cells is radioimmunotherapy (RIT) of B lymphocyte Non-Hodgkin's Lymphoma (NHL), a tumour which is radio-sensitive. Here a malignant clone of lymphoid cells has supplanted the normal B cell population offering the opportunity to use radio-labelled MAbs directed against B cell antigens. Kaminski *et al.* have shown good responses to low activity RIT[8], but the outstanding work arguably comes from the Seattle group, who used dose-intensification with iodine-131 labelled MAb, followed by autologous bone marrow rescue[9,10]. At the last report, of 21 patients treated in relapse there had been 16 complete remissions and 2 partial remissions; at a mean follow-up of 2 years, the overall survival was 93%, with progression-free survival of 63%. Considering that this population of patients had all been multiply pre-treated, these results are outstanding.

Advance in the treatment of solid tumours is perhaps best represented by the work of the Hammersmith group in ovarian cancer.[11] Patients in complete remission were treated with a MAb which recognises the tumour-associated antigen PEM, labelled with low activity of the pure β emitter yttrium-90. These patients have a high risk of relapse: the overall 5 year survival from ovarian cancer is in the region of only 30%. In a phase II study the small number of patients who received a single intraperitoneal administration of this labelled MAb seemed to demonstrate a survival advantage when compared to historical controls, with a crude five year survival of 80% (unpublished). This work is now being examined in a randomised phase III

controlled trial. The level of anti-tumour activity that could be ascribed to the low radio-activity delivered locally raises some doubt that this is the mechanism of action: it seems more likely that any beneficial effect is being mediated immunologically (see below).

Antibody-directed enzyme prodrug therapy
This technique, devised by Professor Bagshaw of Charing Cross Hospital involves the use of a MAb to convey an enzyme into the region of the tumour. Once extraneous antibody-enzyme conjugate has been allowed to clear from the circulation, a prodrug is administered which is activated by the enzyme, thus directing cytotoxic drug release to tumour tissue. The pilot-scale clinical trial of this approach produced partial remissions in 4 of the 8 assessable patients suggesting that it has potential as an important therapeutic modality for the future.[12]

Antibody alone
The most publicized success, and the trial which generated new interest in antibody therapy, is the work of Riethmüller and colleagues in colorectal cancer.[13] Using relatively large doses of MAb (500mg iv initially, followed by 100mg iv monthly) as adjuvant therapy in patients with Dukes' stage C cancer of the colon, 189 patients were entered into a phase III trial of antibody versus observation - (this work predated the adjuvant chemotherapy results). The survival at 5 years was 49% in the observation group and 64% in the treatment group. No data has been published on the scientific basis for this activity, but it seems likely that the antibody is acting by directing cytotoxic cells to tumour deposits - Antibody-Dependent Cellular Cytotoxicity (ADCC).

Monoclonal antibodies as cancer vaccines
An alternative approach is to use unconjugated MAb in an attempt to generate an immune response by virtue of the immunogenicity of the MAb.

(i) Idiotypic Networks
Antibodies administered to patients are treated as foreign antigens by the human immune system; this is particularly true of murine antibodies. The principal response is the generation of a broad spectrum of Human Anti-Mouse Antibodies (HAMA), most of which are directed against the Fc portion of mouse immunoglobulin. A small proportion of HAMA - perhaps 5% - is generated against the antigen-binding site of an administered MAb, and is termed 'anti-idiotypic'. If the complementarity of antigen and antibody is considered, it can be appreciated that the antigen-binding site of the anti-idiotypic antibodies may resemble the original human antigen to which the administered MAb binds. This is often referred to as the generation of an 'internal image.' Furthermore, as originally predicted by Jerne,[13] the production of anti-idiotypic antibody by the immune system leads to the generation of a further round of 'anti-anti-idiotypic' antibody, directed against the binding site of the anti-idiotypic antibody, and whose own antigen-binding site therefore resembles that of the original mouse MAb administered. 'Idiotypic Networks' have been demonstrated in a wide variety of patients given MAbs for imaging or therapy[14] and this immunological mechanism is the rationale for using MAbs as cancer vaccines.

(ii) Why Use MAbs as Cancer Vaccines?

The concept of tumour vaccines has re-surfaced in recent years with its proponents tending towards a cellular/gene therapy approach, aiming to modulate tumour cells by transfecting into them genes which may render those cells more immunogenic. MAbs present an alternative approach for several reasons

- there are now over 20 years of experience in their use.
- there are established methods for producing large volumes of pure MAb.
- the safety of MAbs is well-established and issues which need to be addressed with gene therapy are avoided: these mainly concern the tranforming potential of new DNA sequences if incorporated into the genome of attendants or the wider population.
- the immunology of MAb therapy is relatively well-characterised, compared to other cancer vaccine approaches.
- it may well be that the Fc region of murine immunoglobulin, by being recognised as a foreign protein, may confer on MAbs an intrinsic adjuvanticity, whereas other vaccines usually need to be combined with vaccine adjuvants to stimulate a cellular or humoral immune response.

(iii) Clinical Experience

A body of experience has been built up using idiotypic/anti-idiotypic vaccination in the treatment of B cell Non-Hodgkin's Lymphoma and colorectal cancer.

The surface-bound immunoglobulin on the malignant clone in B cell NHL is antibody which, once purified, has been used both as an immunogen itself, and also to develop a patient-specific anti-idiotypic vaccine.[15] Idiotypic vaccination in nine patients led to the development of appropriate immune responses in seven, and Complete Remission (CR) was achieved in the two patients who had assessable disease. Patient-specific anti-idiotypic vaccines were used in 34 patients who had been heavily pre-treated: the overall response rate (CR + Partial Remissions) was 68%.[16]

The situation in solid tumours is not so clear, but a great deal of research has been directed at the idiotypic/anti-idiotypic vaccination of colorectal cancer (CRC). Ten years ago Herlyn *et al* published results of anti-idiotypic vaccination in 30 patients with advanced CRC, all of whom had hepatic metastases.[17] The antibody used had been raised against the MAb 17-1A.[13] Small doses of anti-idiotypic antibody (up to 4mg) were administered intradermally at varying intervals. Although this study was not clinically well designed, nevertheless Partial Remissions were obtained in six patients (20%), a response rate which is comparable with most chemotherapeutic responses in this patient group. More recently the Nottingham group have used 100-200μg of a different anti-idiotypic antibody in a similar patient population: cellular anti-tumour immune responses were generated and the patients had an improved median survival as compared to the usual population of patients at that institution.[18,19] Trials of this MAb are still in progress.

Ovarian cancer is the more recent candidate for this approach. Much interest developed following the observation by a number of investigators that patients who have undergone repeated radioimmunoscintigraphy for recurrent disease seem to have a better than expected prognosis.[20] A number of trials of idiotypic vaccination are now underway and it is postulated that the effectiveness of intraperitoneal radioimmunotherapy in the Hammersmith trial is due to an idiotypic vaccine effect.[6]

THE PRESENT AND THE FUTURE

Far from being discredited, monoclonal antibodies have an expanding role in modern oncological practice; I would like to compete with those who enjoy speculating about molecular diagnosis and gene-directed cure of cancers by expanding on three areas I have mentioned above. It has been shown that a dose of radiolabelled antibody used for RIS would also serve for intra-operative radioimmunodetection in the same patient the following day, and that antibody administration seems to confer a survival advantage in some patients. It seems reasonable to propose that in the future one dose of MAb will enable definitive pre-surgical diagnosis, ensure optimum primary surgery and form part of the therapy for the tumour. Well, it seems reasonable to me, anyway.

REFERENCES

1 Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; **256:** 495-7.

2 Taylor-Papadimitriou J, *et al.* The polymorphic epithelial mucin as a target for immunotherapy. *Ann N Y Acad Sci* 1993; **690:** 69-79

3 Ho SB, *et al.* Heterogeneity of mucin gene expression in normal and neoplastic tissues. *Cancer Res* 1993; **53:** 641-51.

4 Muss HB, *et al.* c-erbB-2 expression and response to adjuvant therapy in women with node-positive early breast cancer [published *erratum* appears in *N Engl J Med* 1994 Jul 21;**331**(3):211.] *N Engl J Med* 1994; **330:** 1260-6.

5 Granowska M, *et al.* Breast cancer 99mTc SM3 radioimmunoscintigraphy. *Acta Oncol* 1996; **35:** 319-21.

6 Campbell S, *et al.* Transabdominal ultrasound screening for early ovarian cancer. *Br Med J* 1989; **299:** 1363-7.

7 Ind TE, *et al.* Peroperative radioimmunodetection of ovarian carcinoma using a hand-held gamma detection probe. *Br J Cancer* 1994; **70:** 1263-6.

8 Kaminski MS, *et al.* Iodine-131-anti-B1 radioimmunotherapy for B-cell lymphoma. *J Clin Oncol* 1996; **4:** 1974-81.

9 Press OW, *et al.* Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. *N Engl J Med* 1993; **329:** 1219-24.

10 Press OW, *et al.* Phase II trial of 131I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. *Lancet* 1995; **346:** 336-40.

11 Hird V, *et al.* Adjuvant therapy of ovarian cancer with radioactive monoclonal antibody. *Br J Cancer* 1993; **68:** 403-6.

12 Bagshawe KD, *et al.* Antibody directed enzyme prodrug therapy: a pilot-scale clinical trial. *Tumor Targeting* 1995; **1:** 17-30.

13 Riethmuller G, *et al.* Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. German Cancer Aid 17-1A Study Group. *Lancet* 1994; **343:** 1177-83.

14 Courtenay-Luck NS, *et al.* Development of anti-idiotypic antibodies against tumour antigens and autoantigens in ovarian cancer patients treated intraperitoneally with mouse monoclonal antibodies. *Lancet* 1988; **2:** 894-7.

15 Kwak LW. *et al.* Induction of immune responses in patients with B-cell lymphoma against the surface-immunoglobulin idiotype expressed by their tumors. *N Engl J Med* 1992; **327:** 1209-15.

16 Vuist WM, Levy R, Maloney DG. Lymphoma regression induced by monoclonal anti-idiotypic antibodies correlates with their ability to induce Ig signal transduction and is not prevented by tumor expression of high levels of bcl-2 protein. *Blood* 1994; **83:** 899-906.

17 Herlyn D, *et al.* Anti-idiotype immunization of cancer patients: Modulation of the immune response. *Proc Nat Acad Sci* 1987; **84:** 8055-9.

18 Denton GW, *et al.* Clinical outcome of colorectal cancer patients treated with human monoclonal anti-idiotypic antibody. *Int J Cancer* 1994; **57:** 10-4.

19 Durrant LG, *et al.* Enhanced cell-mediated tumor killing in patients immunized with human monoclonal antiidiotypic antibody 105AD7. *Cancer Res* 1994; **54:** 4837-40.

20 Baum RP, *et al.* Activating anti-idiotypic human anti-mouse antibodies for immunotherapy of ovarian carcinoma. *Cancer* 1994; **73:** (Suppl 3): 1121-5.