9. Ward JF. Biochemistry of DNA lesions. *Radiat Res* 1985, **104**, S103–S111.
10. Ward J. Mechanisms of DNA repair and their potential modification for radiotherapy. *Int J Radiat Oncol Biol Phys* 1986, **12**, 1027–1032.
11. von Sonntag C, Hagen U, Schön-Bopp A, Schulte-Frohlinde D. Radiation-induced strand breaks in DNA: chemical and enzymatic analysis of end groups and mechanistic aspects. *Adv Radiat Biol* 1981, **9**, 109–143.
12. McMillan TJ, Cassoni AM, Edwards S, Holmes A, Peacock JH. The relationship of DNA double-strand break induction to radiosensitivity in human tumour cell lines. *Int J Radiat Biol* 1990, **58**, 427–438.
13. Chiu S-M, Oleinick NL. The sensitivity of active and inactive chromatin to ionizing radiation-induced DNA strand-breakage. *Int J Radiat Biol* 1982, **41**, 71–77.
14. Oleinick NL, Chiu S-M, Friedman LR. Gamma radiation as a probe of chromatin structure: damage to and repair of active chromatin in the metaphase chromosome. *Radiat Res* 1984, **98**, 629–641.
15. Kuo MT. Differential damage of active chromatin by bleomycin. *Cancer Res* 1981, **41**, 2439–2443.
16. Mattes WB, Hartley JA, Kohn KW, Matheson DW. GC-rich regions in genomes as targets for DNA alkylation. *Carcinogen* 1988, **9**, 2065–2072.
17. Wasserman K, Kohn KW, Bohr VA. Heterogeneity of nitrogen mustard-induced DNA damage and repair at the level of the gene in Chinese hamster ovary cells. *J Biol Chem* 1990, **265**, 13906–13913.
18. Riou J-F, Multon E, Vilarem M-J, Larsen C-J, Riou G. *In-vivo* stimulation by antitumor drugs of the topoisomerase II induced cleavage sites in c-*myc* protooncogene. *Biochem Biophys Res Comm* 1986, **137**, 154–160.
19. Darby MK, Herrera RE, Vosberg H-P, Nordheim A. DNA topoisomerase II cleaves at specific sites in the 5' flanking region of c-*fos* proto-oncogenes *in vitro*. *EMBO J* 1986, **5**, 2257–2265.
20. Iliakis G, Okayasu R. The level of induced DNA double-strand breaks does not correlate with cell killing in X-irradiated mitotic and G_1-phase cells. *Int J Radiat Biol* 1988, **53**, 395–404.
21. Cleaver JE, Cortes F, Lutze LH, Morgan WF, Player AN, Mitchell DL. Unique DNA repair properties of a xeroderma pigmentosum revertant. *Moll Cell Biol* 1987, **7**, 3353–3357.
22. Broughton BC, Lehmann AR, Harcourt SA, *et al.* Relationship between pyrimidine dimers, 6–4 photoproducts, repair synthesis and cell survival: studies using cells from patients with trichothiodystrophy. *Mutation Res* 1990, **235**, 33–40.
23. Sinha B, Katkit A, Batist G, *et al.* Differential formation of hydroxyl radicals by adriamycin in sensitive and resistant MCF-7 human breast tumour cells: Implications for the mechanism of action. *Biochemistry* 1987, **26**, 3776–3781.
24. Tewey KM, Rowe TC, Yang L, Halligan BD, Liu LF. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* 1984, **226**, 466–468.
25. Tritton TR, Yee G. The anticancer agent adriamycin can be actively cytotoxic without entering cells. *Science* 1982, **217**, 248–250.
26. Posada J, Vichi P, Tritton TR. Protein kinase C in adriamycin action and resistance in mouse sarcoma 180 cells. *Cancer Res* 1989, **49**, 6634–6639.
27. McMillan TJ, O'Neill P, Peacock JH, Prise K. Workshop report 1st LH Gray workshop. Measurement of radiation-induced damage. *Int J Radiat Biol* 1990, **58**, 391–396.
28. Whitaker SJ, Powell SN, McMillan TJ. Molecular assays of radiation-induced DNA damage. *Eur J Cancer* 1991, **27**, 922–928.
29. Vogelstein B, Pardoll DM, Coffey DS. Supercoiled loops and eukaryotic DNA replication. *Cell* 1980, **22**, 79–85.
30. Deeley JOT, Moore JL. Nuclear lysate sedimentation measurements of peripheral blood lymphocytes from radiotherapy patients. *Int J Radiat Biol* 1989, **56**, 963–973.
31. Roti Roti JL, Wright WD. Visualization of DNA loops in nucleoids from HeLa cells: assays for DNA damage and repair. *Cytometry* 1987, **8**, 461–467.
32. Srinivasan S, Glauert HP. Formation of 5-hydroxymethyl-2'-deoxyuridinein hepatic DNA of rats treated with gamma-irradiation, diethylnitrosamine, 2-acetylaminofluorene or the peroxisome proliferator ciprofibrate. *Carcinogenesis* 1990, **11**, 2021–2024.
33. Tilby MJ, Styles JM, Dean CJ. Immunological detection of DNA damage caused by melphalan using monoclonal antibodies. *Cancer Res* 1987, **47**, 1542–1546.
34. den Engelse L, van Benthem J, Scherer E. Immunocytochemical analysis of in vivo DNA modification. *Mutat Res* 1990, **233**, 265–287.
35. Warters RL, Childers TJ. Radiation-induced base damage in replicating chromatin. *Radiat Res* 1982, **90**, 564–574.
36. Chiu S-M, Friedman LR, Oleinick NL. Formation and repair of DNA-protein crosslinks in newly replicated DNA. *Radiat Res* 1989, **120**, 545–551.
37. Ahn SY, Nevaldine B, Hahn PJ. Direct measurement by pulsed-field gel electrophoresis of induction and rejoining of X-ray-induced double-strand breaks in cultured mouse cells. *Int J Radiat Biol* 1991, **59**, 661–675.

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Antibody Therapy of Malignancy

M.J.S. Dyer

INTRODUCTION

ANTIBODIES HAVE the specificity necessary to deliver targeted therapy. However, despite their widespread clinical application in other situations, and despite intensive research (29% of the US NCI budget is devoted to biological therapy), antibodies have yet to make a significant impact on the treatment of cancer.

The purpose of this article is to outline the nature of the problems associated with antibody therapy of malignancy and to show how they may be overcome by the use of molecular genetic and protein engineering techniques.

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THE ANATOMY, DISSECTION AND REBUILDING OF ANTIBODY MOLECULES

Antibodies have two functions: first to bind to antigen and then to transmit the appropriate stimuli to the rest of the immune system including T cells, macrophages and complement so that the concerted effects of all the 'natural effector mechanisms' can be brought to bear.

These different functions are performed by discrete areas of the antibody molecule. The molecule is Y shaped, the tips of the Y binding to antigen and the stem eliciting the various effector functions (Fig. 1). Furthermore, each of these functions is encoded in distinct protein 'domains'. This greatly facilitates antibody engineering allowing the transfer of antigen binding domains onto the various effector domains to produce human antibodies and the production of isolated antigen binding

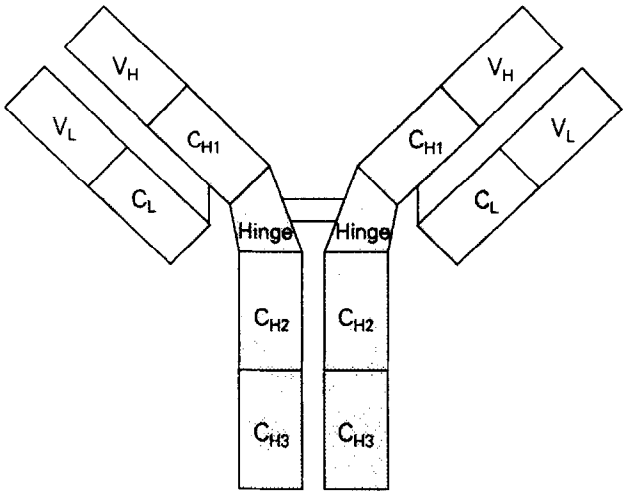

Fig. 1. Antibody molecules consist of the association of two heavy (H) and two light (L) chains. The antigen binding site is formed by the interaction of the variable (V) domains of both H and L chains. Contact with the antigen is made principally by six 'hypervariable' loops; the remainder of the V region domain (the 'framework') essentially serves to maintain the three-dimensional structure of these loops. Effector functions are mediated by constant (C) domains: heavy chains have three such domains (CH1, CH2 and CH3) whilst light chains have a single CL. Different immunoglobulin heavy chains are able to elicit different functions: thus human IgG1 and IgG3 can mediate both antibody-dependent cellular cytotoxicity and complement-mediated lysis. The former property is dependent on binding to Fc receptors expressed on effector cells.

domains for therapy. Reviews on the genetic and protein engineering of antibodies may be found in Refs 1 and 2, respectively.

Manipulation of the antigen binding site

The V regions of monoclonal antibodies (Mabs) can be manipulated to produce the following constructs:

Human Mabs. Difficulties in producing human Mab with antitumour specificity has meant that until recently all therapeutic attempts have been made with rodent Mabs. Human Mabs are preferred not only to reduce immunogenicity but also perhaps to enhance activity: there is evidence that human Mab are transported more efficiently into extravascular spaces. A variety of methods have been described to convert rodent Mab to their human equivalent.

Chimaeric antibodies: genetic manipulation. Cloned VH and VL DNA segments of rodent Mab can be inserted into plasmids containing human C region genes and transfected into myeloma cells for antibody production: secreted Mab therefore has the antigen binding of the rodent Mab but the heavy chain of human immunoglobulin. (See Ref. 3 for a review of these techniques and Ref. 4 for a description of the use of such chimaeric Mab in therapeutic attempts in patients with colorectal carcinoma).

Chimaeric antibodies: protein engineering. Alternatively antibody fragments can be produced by enzymatic digestion and then cross-linked chemically. Fragments of therapeutic rodent Mab containing VH/VL as well as CH1 and CL (the Fab fragment) can be linked chemically to human CH2/CH3 fragments (the Fc fragment) to produce a construct which again retains the specificity of the rodent Mab but now has the effector functions of human antibodies.

Genetically reshaped human Mab. Both the above approaches leave intact rodent V regions and are thus potentially immunogenic. To overcome this problem Riechmann *et al.* [5] developed the techniques of transplanting not the entire V region but just the DNA encoding the six hypervariable loops. This was done by introducing mutations into human hypervariable loop V region DNA sequences so that they resembled the DNA sequence of the original rat Mab. Molecular modelling of the framework residues was then required to ensure that these loops were maintained in the correct conformation. Originally described for the antilymphocyte Mab, CAMPATH-1, this technique has since been successfully applied to several other Mabs.

Small antigen recognition units. An argument against the use of intact Mab molecules for tumour therapy has been that their large size might prevent effective access to many tumour sites. This has been an impetus to produce small fragments of Mab which retain antigen binding. Such fragments include: (a) Fab (VH/CH1-VL/CL domains); (b) Fv (VH/VL); (c) domain antibodies (dAb: single VH domain); (d) minimal recognition units (mru: single hypervariable loop). Whilst these fragments can be readily produced by genetic engineering, a number of features may compromise their use in therapy, such as rapid clearance from the circulation. Chemical trials using Fab fragments with genetically engineered toxin molecules attached are currently in progress in the USA.

Bispecific and monovalent Mabs

Native Mab molecules have the same antigen combining site on both arms of the 'Y'. It may be desirable to have two different specificities in the same Mab molecule (bispecific Mab). As an example it is possible to activate T cells using Mabs against the CD3 molecules associated with the T cell receptor. Bispecific Mabs with CD3 on the one arm and anti-tumour specificity on the other may act not only to activate T cells but also to cause T-cells to lyse the tumour cells, a phenomenon known as 'effector cell retargetting' [6]. *In vitro* this retargetting can lyse tumour cells at very low concentrations of bispecific Mab, in the presence of activated T cells. This approach may therefore be useful clinically if the conditions for T cell activation and expansion *in vivo* without serious side-effects [7] can be established.

Monovalent Mabs in contrast have a single antigenic specificity with no or irrelevant binding on the other 'arm'. This may improve the lytic activity of certain Mabs where bivalent Mabs cause rapid cross-linking and internalisation of the target antigen (see below).

Bispecific and monovalent Mabs can be produced by two methods, either by chemically linking Fab fragments of two different Mab or by producing hybrid-hybridomas. The latter technique utilises the codominant expression of immunoglobulin molecules. The fusion of two cell lines producing two different Mabs results in all four heavy and light chains being produced simultaneously. Because of the random assortment of the different chains, a proportion of the antibody molecules secreted will have the appropriate heavy and light chain combinations to bind to both antigens. These can then be separated from the other species by chromatographic techniques.

Manipulation of the effector function sites

Different classes of immunoglobulin have different functions: human IgM is particularly efficient at complement activation. Through the use of mutations induced within the CH domains the amino acids important for antibody binding both to human

complement and FC receptors have been recognised [8, 9]. It remains to be seen whether perhaps engineered mutations or perhaps duplication of the various CH domains can render a given Mab more effective at activating complement for example.

DESIRABLE FEATURES FOR A TRIAL OF ANTIBODY THERAPY

Given that Mab structure can be manipulated in so many ways which Mab constructs might be most successfully used for cancer therapy? Two divergent approaches have emerged. In the first, the Mab molecule is linked, either chemically or by genetic engineering to plant toxins (such as ricin or abrin), radioisotopes or prodrugs (the ADEPT programme [10]). The Mab therefore serves as a passive vector delivering the agent to the tumour cell, thereby reducing any non-specific side-effects of the drug or toxin and increasing the potency of the Mab. In the second, unconjugated or 'naked' Mabs are used which rely upon the activation of natural effector mechanisms through the CH domains. The aim of this approach must be to refocus the immune system towards tumour destruction, to create in effect an antitumour inflammatory response.

Before comparing these two approaches, a number of features which may be necessary for the development and eventual widespread adoption of antibody therapy need to be considered. Firstly, if Mab therapy is to be successful it is important that the target antigen is expressed not only on the differentiated tumour cell but also on the tumour stem cell. In lymphoma for example these cells have very different phenotypes. The target antigen ideally should also not be expressed on any normal tissues: i.e. it should be a tumour-specific antigen. However, known examples of such antigens such as the surface immunoglobulin of B-cell lymphomas and the tumour-specific deletions of the epidermal growth factor receptor appear to be expressed only in differentiated tumour cells and not the stem cells. It may be therefore that even if Mab therapy can be optimised, that it may not be strictly tumour-specific, but rather lineage-specific. For example, in the lymphoid malignancies, CAMPATH-1 Mabs can induce remission but only at the expense of ablation of any residual normal lymphocytes.

As regards the design of preliminary trials of Mab these are best performed in patients with only slowly progressive but nevertheless incurable disease in whom several therapeutic attempts might be made with different Mab constructs to define the requirements for tumour lysis *in vivo*. Ideally, tumour cells should be assessed for expression of target antigen and susceptibility to lysis *in vitro* both before and after any *in vivo* therapy. There should be a sensitive end-point to the trial, so that minor changes might be detected. However, preliminary studies in the presence of tumour excess need to be performed: once significant effects have been demonstrated, studies on the effects of Mab in the presence of minimal tumour, conditions probably more favourable for effective Mab action can be undertaken. The eventual aim should be to retreat with Mab as soon as the disease becomes detectable once more.

From the practical (commercial) view it is desirable as with any drug, that the Mab be cheap to produce, (biotechnology is expensive) simple to administer, can be administered repeatedly over several courses, be free from serious side-effects and be used to treat as wide a range of malignancy as possible.

BARRIERS TO SUCCESSFUL ANTIBODY THERAPY

Since the advent of Mabs in the early 1980s a large number of clinical trials using both unconjugated and conjugated mouse or rat Mabs have been performed. Most of these trials have failed to demonstrate any significant therapeutic effects but a number of important principles have been established, concerning the natures of the Mab the target antigen and the target cell.

Antibody

Lack of interaction with human effector systems. The use of unconjugated Mabs demands that the Mab activate natural effector mechanisms to elicit lysis of cells by human complement and/or by human cellular effectors such as macrophages, K cells, T cells or neutrophils, or by opsonisation.

An important consideration in this respect is the nature of the Ig H chain, different H chains varying in their functions. This has been shown by the use of hybridoma class-switch variants [11] or by the use of genetically constructed chimaeric Mabs with different H chains [12]. The former depends on hybridoma cells undergoing 'class-switching' spontaneously in culture: thus a hybridoma cell initially secreting rat IgG2a Mab may 'switch' to IgG2b, the same V region gene being expressed on a different H chain gene. Selection of the variants allows comparison of the two different H chains. In the latter, the same end is achieved by genetic manipulation, so that a single V region gene is expressed on all the different H chains [12].

From both *in vitro* and *in vivo* experiments [13] it has been shown that the most effective isotypes at eliciting both complement and cell-mediated lysis are human IgG1 and IgG3 and rat IgG2b. These are therefore the preferred isotypes for therapy with unconjugated Mabs. If on the other hand therapy with Mab conjugated with toxin or radioisotopes is being considered IgG4 would be preferred as this fails to bind to Fc receptors.

In passing it should be noted that conversion of a murine Mab to the preferred human isotype may not necessarily improve potency. No all rat IgG2b Mabs will lyse cells: the nature of the target antigen and also the target cells are also of importance (see below).

Neutralising anti-globulin response. The production of an antibody response against the administered Mab precludes further effective antibody therapy. Early experiments in nonimmunosuppressed patients with immune Mabs resulted in nearly all patients developing neutralising antibody response to the injected Mab within 2 weeks, and in some cases within 5 days. With purer Mab preparations, free from aggregates and impurities it seems that the therapeutic 'window' may be somewhat longer.

The use of human Mabs and particularly completely reshaped Mabs may largely overcome these problems and may allow prolonged and repeated courses to be given. However, individual variations within the heavy chain amongst the population (H chain allotypes) and the unique conformation of the Mab's V region (idiotype) may allow the generation of both anti-allotypic and anti-idiotypic responses. The frequency of these will only be shown by large clinical studies.

Target antigen

Modulation. Some cell surface molecules when cross-linked with bivalent Mab rapidly 'modulate' and internalise within the cell rendering the cell effectively antigen negative. This process can occur within minutes of encountering antibody, but cells may remain devoid of surface antigen for many hours thereafter. Thus, if the antibody-mediated cell lysis is dependent on the persistence of antibody on the cell surface then such antigens

are inappropriate: on the other hand if cell lysis is dependent on antibody getting into the cell such as with Mab conjugated with toxins then this property can be helpful.

Rapid modulation and internalisation of cell surface molecules can be greatly reduced by the use of monovalent Mabs: such monovalent Mabs may have improved lytic efficacy [14].

Efficacy of cell lysis. The target antigen is an important determinant concerning the efficacy of cell lysis. For example, different cell surface molecules vary in their ability to mediate lysis with human complement; although CD45 ('leucocyte common') and CDw52 (CAMPATH-1) are both abundantly expressed nonmodulating glycoproteins only CDw52 Mabs elicit significant cell lysis with human complement [15]. CDw52 exceptional amongst the lymphocyte differentiation antigens in this regard.

Similarly, for Mabs conjugated with toxins, not all modulating surface antigens permit successful cell lysis.

The reasons for these differences are not clear.

Circulating target antigen. Circulating free antigen in the serum will greatly hinder the localisation of Mab to tumour sites.

Target cell

Differences in the nature of the targeted cell populations may also have a significant effect. CDw52 is not only expressed on lymphocytes but also in comparable amounts on monocytes. Lymphocytes and lymphoid tumours can be successfully lysed both *in vitro* and *in vivo* whereas monocytes and monocytic tumours are resistant under the same conditions. Similarly surface expression of CDw52 varies among the lymphoid tumours over a 50-fold range, whereas sensitivity to lysis varies over a 1000-fold. Sensitivity to lysis does not correlate with antigen density [16].

The reasons for these differences are unclear but may be related to the expression of classes of molecules such as CD59 which confer protection against complement-mediated lysis [17].

A final barrier might be the ability of the large Ig molecules to penetrate poorly vascularised tumours. Early experiments in the 1980s and subsequent imaging studies with radiolabelled Mabs have shown that small amounts of Mab will bind to tumour cells in most infiltrated sites. These results were encouraging because the experiments were performed using small doses of Mab in the presence of vast tumour excess. Whether sufficient Mab will localise to deliver tumouricidal effects will depend on the potency of the Mab. In the case of unconjugated Mabs potency means their ability to activate effector mechanisms at that site: this in turn may depend on the number and activation of tumour-infiltrating lymphocytes and macrophages. The administration of prolonged and repeated courses of humanised Mabs may provide a solution to the problem of poor localisation. An alternative might be to use Mab with the patient in remission following conventional therapy as a form of consolidation; small cell lung carcinoma and the non-Hodgkin lymphomas provide excellent models for this type of study.

COMPARISON OF EFFECTS OF VARIOUS ANTIBODY CONSTRUCTS IN LYMPHOMA

Many therapeutic attempts of B cell lymphoma have been made. The ease of tumour biopsy, the accessibility of the tumour to Mab, and the large number of possible target antigens make this an excellent tumour for experimental therapy. Both unconjugated Mab, and Mab conjugated with toxins and radioisotopes have been used which allows some general comparisons between these approaches to be made.

Toxin-conjugated Mabs ('early' experiments reviewed in Ref. 18)

In vitro many of these constructs have been potent, although it is likely that several hundred antibody–toxin conjugate molecules are necessary to kill a tumour cell. Published results have so far on the whole been clinically disappointing, with many patients failing to make more response than might have been expected from unconjugated Mab alone. The reasons for the discrepancy between *in vitro* and *in vivo* potencies remains unclear. Other problems include significant toxicities, including capillary leak syndrome, hepatic and CNS effects, and the immunogenicity of the toxin molecule.

The results of trials with more sophisticated conjugates which should have fewer side-effects due to diminished hepatic uptake using deglycosylated ricin A-chain or 'blocked' ricin are awaited from the Dallas (E. Vitetta/P. Thorpe) and Boston (L. Nadler) groups, respectively.

Radioisotope conjugates

The attraction of this approach is that many lymphomas, even those resistant to chemotherapy may remain radiosensitive: radiolabelled Mab might allow selective delivery of tumouricidal doses of radiation to tumour sites. ^{131}I is commonly used because of ease of coupling but many other radioisotopes have been linked using chelating agents.

Both fractionated, small doses (50 mCi × 12) of radiolabelled Mab [19] and single high dose (600 mCi) [20] approaches have been used. Both showed some spectacular and stable tumour regressions. However, the major problem is the non-specific uptake of radiolabelled Mab in various organs. In the latter study estimated radiation doses to lung were up to 1600 cGy which prevents dose-escalation. Also severe myelosuppression necessitating infusion of stored marrow was required in two of the five cases reported.

The lack of specificity raises the question as to whether external beam radiotherapy might have been as effective. This has not been addressed directly in man, but a study in mice comparing external beam irradiation with radiolabelled specific and nonspecific Mab in a mouse lymphoma model showed that although radiolabelled Mab was more effective than external beam radiotherapy, the specific Mab was only 1.2 times more effective at tumour ablation than the non-specific [21]. The reasons for this are unclear but may include apparently selective uptake of antibody by infiltrated sites [22].

UNCONJUGATED Mabs

Although initial results using unconjugated rodent Mabs for therapy in man were disappointing, in mouse tumour models under certain conditions, similar murine Mabs were able to achieve substantial and long-lasting tumour depletion. This indicated that natural effector mechanisms might be extremely potent once correctly harnessed.

We set out to investigate this hypothesis in man by investigating the effects of a series of rat and human Mab against the CDw52 (CAMPATH-1) antigen. This antigen was chosen because it is exceptional at eliciting complement-mediated lysis. Initially rat CDw52 Mabs of IgM, IgG2a and IgG2b isotype (the IgG2b Mab being a class-switch variant of the IgG2a) were assessed for their ability to activate complement and cell-

mediated lysis of human lymphoid tumour cell *in vitro*. All elicited complement-mediated lysis but only the IgG2b Mab could elicit antibody dependent cellular cytotoxicity (ADCC) with human effector cells through the interaction with human Fc receptors.

In vivo in patients with lymphoproliferative disorders the human IgM and IgG2a induced only transient falls in the peripheral blood white cell count. In contrast the rat IgG2b Mab (CAMPATH-1G) depleted cells from blood marrow and spleen [16]. This was the first Mab to demonstrate consistent anti-tumour effects in man. CAMPATH-1G was less effective against lymph nodes or extranodal disease and was also ineffective when given intrathecally which may reflect a lack of effector cells at these sites.

Subsequently, a genetically reshaped human IgG1 Mab with CAMPATH-1 specificity (CAMPATH-1H) was produced. So far, sufficient Mab has been purified to treat 2 patients with B cell lymphoma in leukaemic phase. Although both patients had floid disease and received only very small doses of Mab (85 and 126 mg) both patients attained complete remission with recovery of normal haemopoiesis during the course of treatment [23]. Neither patient mounted an antiglobulin response despite receiving the Mab for 4 consecutive weeks. A multinational trial of CAMPATH-1H in patients with relapsed/refractory lymphoma will begin in 1992 using material produced by Wellcome.

FUTURE PROSPECTS

The work with CDw52 Mabs indicates that natural effector mechanisms can be extremely potent once correctly harnessed. Whether or not these Mabs find a role in the therapy of lymphomas will depend on their ability to deplete large lymph nodes and extra-nodal masses and whether any serious side-effects emerge from the associated prolonged lymphopaenia. A disadvantage of the CDw52 specificity is that residual normal T cells are depleted along with the lymphoma cells. This may abrogate a potent effector mechanism.

Nevertheless, the tumour regressions seen with only very small, and certainly nonsaturating doses of CAMPATH-1H are very encouraging for antibody therapy of malignancy in general. The question now is whether the same principles can be applied to other Mab specificities and other diseases. At present there are few likely candidate antigens among the solid tumours [24]. Effective antibody therapy for solid tumours may therefore await the characterisation of Mabs with properties similar to those of CAMPATH-1 Mabs: elucidation of the mechanisms by which certain tumours withstand antibody-mediated lysis may also be important.

Other mechanisms for tumour lysis remain largely unexplored. The use of bispecific Mabs are of particular interest. The use of such Mabs along with recombinant cytokines may allow the activation, expansion, arming and retargetting of sufficient numbers of effector cells to mediate large-scale tumour destruction. These studies are currently being undertaken in many centres world-wide. Also of interest are Mabs which may induce programmed cell death or apoptosis *in vivo*.

In conclusion, selection of appropriate target antigen and appropriate immunoglobulin isotypes has allowed the development of Mabs which may have uses in the therapy of many lymphoid malignancies. Similar progress for the more common 'solid' malignancies will depend on the characterisation of suitable targets and the refinement of our ability to manipulate the immune system *in vivo*.

1. Winter G, Milstein C. Man-made antibodies. *Nature* 1991, **349**, 293–299.
2. Stevenson GT. Attack on neoplastic cell membranes by therapeutic antibody. *Mol Cell Biochem* 1989, **91**, 33–38.
3. Shin S-U. Chimeric antibody: potential applications for drug delivery and immunotherapy. *Biotherapy* 1991, **3**, 43–53.
4. Lobuglio AF, Wheeler RH, Trang J, *et al.* Mouse/human chimeric monoclonal antibody in man: kinetics and immune response. *Proc Natl Acad Sci USA* 1989, **86**, 4220–4224.
5. Riechmann L, Clark MR, Waldmann H, Winter G. Reshaping human antibodies for therapy. *Nature* 1988, **322**, 323–327.
6. Clark MR, Gilliland L, Waldmann H. Hybrid antibodies for Therapy. *Prog Allergy* 1988, **45**, 31–49.
7. Richards JM, Vogelzang NJ, Bluestone JA. Neurotoxicity after treatment with muromonoab-CD3. *N Engl J Med* 1990, **323**, 487–488.
8. Duncan AR, Woof JM, Partridge LJ, Burton DR, Winter G. Localisation of the binding site for the human high affinity Fc receptor on IgG. *Nature* 1988, **322**, 563–564.
9. Duncan AR, Winter G. The binding site for Clq on IgG. *Nature* 1988, **332**, 738–740.
10. Bagshawe KD. Towards generating cytotoxic agents at cancer sites. *Br J Cancer* 1989, **60**, 275–281.
11. Hale G, Cobbold SP, Waldmann H, Easter G, Matejtschuk P, Coombs RRA. Isolation of low frequency class-switch variants from rat hybrid myelomas. *J Immunol Methods* 1987, **103**, 59–67.
12. Bruggemann M, Williams GT, Bindon CI, *et al.* Comparison of the effector functions of human immunoglobulins using a matched set of chimaeric antibodies. *J Exp Med* 1987, **166**, 1351–1361.
13. Dyer MJS, Hale G, Hayhoe FGJ, Waldmann H. Effects of CAMPATH-1 antibodies *in vivo* in patients with lymphoid malignancies: influence of antibody isotype. *Blood* 1989, **73**, 1431–1439.
14. Clark MR, Bindon CI, Dyer MJS, Friend P, Hale G, Cobbold S, Calne R, Waldmann H. The improved lytic function and *in vivo* efficacy of monovalent monoclonal CD3 antibodies. *Eur J Immunol* 1989, **19**, 381–388.
15. Bindon CI, Hale G, Waldmann H. Importance of antigen specificity for complement-mediated lysis by monoclonal antibodies. *Eur J Immunol* 1988, **18**, 1507–1515.
16. Dyer MJS, Hale G, Marcus RE, Waldmann H. Remission induction in patients with lymphoid malignancies using unconjugated CAMPATH-1 monoclonal antibodies. *Leukemia Lymphoma* 1990, **2**, 179–193.
17. Lachmann PJ. Protection against complement lysis. Proteins of the Complement System Joint Colloqium. *Biochem Soc Transactions* 1990, **18**, 1159–1160.
18. Hertler AA, Frankel AE. Immunotoxins: a clinical review of their use in the treatment of malignancies. *J Clin Oncol* 1989, **7**, 1932–1942.
19. Denardo SJ, Denardo GL, O'Grady LF, Miller CH, Epstein AJ. Pilot studies of radioimmunotherapy of B-cell lymphoma and leukemia using ^{131}I Lym-1 monoclonal antibody. *Antibody, Immunoconjugates and Radiopharmaceuticals* 1988, **1**, 17–33.
20. Press OW, Eary JF, Badger CC, *et al.* Treatment of refractory non-Hodgkin lymphoma with radiolabelled MB-1 (anti-CD37) antibody. *J Clin Oncol* 1989, **7**, 1027–1038.
21. Knox SJ, Levy R, Miller RA, *et al.* Determinants of the antitumour effect of radiolabelled monoclonal antibodies. *Cancer Res* 1990, **50**, 4935–4940.
22. Rubin RH, Fischman AJ, Callahan RJ, *et al.* In-labeled non-specific immunoglobulin scanning in the detection of focal infection. *N Engl J Med* 1989, **321**, 935–940.
23. Hale G, Dyer MJS, Clark MR, *et al.* Remission induction in non-Hodgkin lymphoma with reshaped monoclonal antibody CAMPATH-1H. *Lancet* 1988, **2**, 1395–1401.
24. Humphrey PA, Wong AJ, Vogelstein B, *et al.* Anti-synthetic peptide antibody reacting at the fusion junction of deletion mutant epidermal growth factor receptors in human glioblastoma. *Proc Natl Acad Sci USA* 1990, **87**, 4207–4211.