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Commentary

Monoclonal antibodies targeting cancer: 'magic bullets' or just the trigger?

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Abstract

The first monoclonal antibodies (mAbs) approved for cancer therapy are now in Phase II and III trials, but the critical mechanism(s) determining efficacy and response in patients are still largely undefined. Both the direct antigen-binding (Fab) and constant (Fc) regions of mAbs can contribute to their biological activity. However, Clynes *et al* (*Nat Med* 2000, **6**:443) recently suggested that the latter (at least in experimental models) might be the dominant component in *vivo*, triggering host responses to destroy cancer cells. Those workers showed that in mice lacking 'activation' Fc receptors (FcγRI and FcγRIII), anti-tumour effects of certain mAbs were significantly reduced. In contrast, mice deficient in the 'inhibitory' receptor FcγRIIB responded with tumour growth inhibition and enhanced antibody-dependent cellular cytotoxicity (ADCC). These observations suggest that mAbs might be engineered for preferential binding to FcγRIII to maximise therapeutic benefit. However, further work is needed to establish a definitive cause–effect relationship in experimental models that are more clinically relevant, to determine whether human FcγR isoforms behave in a similar fashion, and to confirm that therapeutic mAbs and host cells can adequately access solid tumour deposits to mediate effective ADCC *in situ*. Finally, the 'cost–benefit' ratio of such modified macromolecules will need to be measured against mini-mAb constructs, antisense oligonucleotides, peptidomimetics and emerging drugs capable of inhibiting key tumour cell signalling pathways.

Keywords: antibody-dependent cellular cytotoxicity, Fc receptors, Herceptin, monoclonal antibody, Rituxan

Introduction

The Holy Grail of cancer therapy is to develop agents capable of selectively destroying disseminated tumour cells while sparing normal tissues. With this aim, major efforts have been directed at harnessing the exquisite specificity of the immune response. Hybridoma technology has enabled the development of tumour selective monoclonal antibodies (mAbs) [1,2], and the past few years have witnessed the approval by the Food and Drug Administration of the first mAbs for the therapy of cancer: Rituxan (anti-CD20) for non-Hodgkin's lymphoma and Herceptin [anti-(c-*erb*B-2/HER-2)] for metastatic breast cancer. The purpose of this commentary is to summarise known and recently reported properties of these mAbs and consider whether recent findings might lead to more effective therapies for cancer.

Targeted therapy for breast cancer

Although the earlier detection of breast cancer and improvements in surgery and adjuvant therapy have

ADCC = antibody-dependent cellular cytotoxicity; bsAbs = bispecific antibodies; CDC = complement-dependent cytotoxicity; EGFR = epidermal growth factor receptor; FcγR = receptors for the Fc region of IgG antibodies; mAb = monoclonal antibody; NK = natural killer; scFv = single-chain antibody variable region.

improved survival rates, there are still around 15,000 deaths in the UK each year and 43,000 in the USA. This is due primarily to the development of drug-resistant metastatic disease. An increasing number of genetic changes have been identified in breast and other cancers, which are now being actively explored for targeted therapy [3]. One of the most exciting new targets is the c-*erb*B-2/HER-2/neu proto-oncogene, which is expressed in 20–30% of breast and other carcinomas. Clinical observations and laboratory experiments have demonstrated convincingly that, together with the related epidermal growth factor receptor (EGFR), it is causally related to maintenance of the malignant phenotype, functioning as a critical signalling molecule in tumour cell proliferation, motility, angiogenesis and metastasis [4]. The accessibility of c-*erb*B-2 at the cell surface, low expression on normal adult tissues and relatively homogeneous distribution within 'positive' tumours and their metastases makes it an ideal candidate for immunotherapeutic intervention [5].

Development of therapeutic mAbs and determination of their mechanisms of action

Initially, attention focused on specificity and affinity, with the selection of mAbs being based primarily on their ability to inhibit tumour cell growth *in vitro*. Some mAbs are extremely potent, with IC_{50} values (concentrations giving half-maximal inhibition) in the nanomolar range, competing well in this regard with low-molecular-mass tyrosine kinase inhibitors. Once good target selectivity had been achieved, mAbs were chemically or genetically modified to decrease their immunogenicity in patients and to improve their physicochemical properties. Antibodies are structurally complex macromolecules with multiple functions. Some, but by no means all, of their activities depend on the complementarity-determining regions within the specific antigen-binding site. When directed against signalling molecules such as CD20, c-*erb*B-2/HER-2 and EGFR, mAbs can exert either agonistic or antagonistic (potentially therapeutic) effects. Simply stated, antagonistic mAbs can be shown to 'remove' and/or to 'switch off' their target antigen, resulting in anti-proliferative effects. For example, 4D5 (the murine mAb from which Herceptin was derived) partly blocks heregulin-induced receptor phosphorylation and transphosphorylation. However, the major effect of these mAbs seems to be receptor downmodulation, potentially preventing heterodimerisation and activation of other HER family members and downstream signalling [6]. Cell cycle progression is inhibited and cells are arrested in G_0/G_1; they can subsequently undergo terminal differentiation or apoptosis, depending on the cell type.

Some antagonistic mAbs preferentially enhance ubiquitination and degradation of their target [7] and yet others (exemplified by certain anti-EGFR mAbs [8]) do not significantly downregulate receptor expression but effectively compete with the cognate growth factors for receptor binding and activation. With Rituxan, it has been shown that the target antigen CD20 is not downregulated, but the mAb induces apoptosis and sensitises cells to the effects of conventional therapy [9]. Thus, even considering the direct effect of mAbs, it is clear that there are a multiplicity of possible responses determined by the properties of the antigen, the antibody, and the cellular context.

Engineering mAbs for improved clinical utility

The major problems of mAb therapy are related to the immunogenicity of rodent proteins and the relatively poor penetration of intact immunoglobulin molecules into solid tumours. The former has been addressed by making chimeric mAbs (human constant region plus mouse variable region) or 'humanised' mAbs in which the human framework Ig contains only rodent sequences encoding the three complementarity-determining regions, as in Herceptin. Another method of reducing immunogenicity and assisting penetration into solid tumours is to remove the constant (Fc) region and to prepare monomeric or dimeric antibody fragments such as Fab, $F(ab')_2$ and single-chain antibody variable region (scFv). However, it was noted that some mAbs were more active *in vivo* than *in vitro*, and this benefit was lost if the Fc portion was removed. Although this is partly explained by the lower affinity and/or shorter half-life of these molecules, results with chemically and genetically modified Fc regions led to an appreciation of the possible contribution of indirect host effects mediated by interactions between IgG Fc and receptors for the Fc region (FcγR) [10].

The recent paper by Clynes *et al* [11] highlights further subtleties relating to mAb interaction with specific FcR subtypes; the authors now suggest that this is a dominant component of the activity of Herceptin and similar mAbs. The experiments described, although elegant, leave several unanswered questions about the interpretation of the data and their clinical relevance. We need to consider whether the differential effects of mAb therapy observed in the genetically modified mice are linked directly to their FcR status, and if so whether similar effects are likely to occur in humans, and finally whether antibody-dependent cellular cytotoxicity (ADCC) is a feasible goal for effective therapy in cancer patients.

The contribution of host effector mechanisms to mAb activity *in vivo*

FcγRs are the key link between humoral and cellular immune responses. They are important in immune regulation and mediate ADCC, endocytosis, phagocytosis, the release of inflammatory cytokines and antigen presentation. FcγR comprise three classes: FcγRI (CD64) FcγRII (CD32) and FcγRIII (CD16). Each class also contains isoforms that exhibit different binding affinities for IgG subclasses, and further complexities arise from their differential expression on host cell populations and the

presence of variant alleles in the low-affinity II and III receptor subtypes [12]. FcγRI and III are multimeric 'activation' receptors, containing both a ligand-binding subunit and a signalling subunit, the immunoreceptor tyrosine-based activation (ITAM) motif, but if co-ligated to the monomeric FcγRIIb (which contains an inhibitory ITIM motif), responses are downregulated.

Herceptin contains a human γ1 Fc and interacts primarily with FcRγIII on natural killer (NK) cells and monocytes. The binding of free mAb is quite weak, but once bound with high affinity to c-*erb*B-2 on the tumour cell surface, it mediates effective ADCC *in vitro* [6]. Clynes *et al* [11] now show that Herceptin (and Rituxan) also bind FcγRIIB (present on monocytes and macrophages, but not NK cells) and that if this interaction is prevented, ADCC is enhanced. They have also shown, with three different experimental systems (including therapy of breast carcinoma xenografts with Herceptin) that the efficacy of mAbs *in vivo* was, first, reduced if the Fc portion was deleted; second, reduced in mice deficient in FcγRI and RIII, and third, enhanced in mice deficient in FcγRIIB. However, first, it should be noted that the breast carcinoma (and lymphoma) were grown ectopically (subcutaneously) and in all cases therapy was commenced on day 0, maximising the opportunity for the administered antibody (or activated host cells) to exert therapeutic effects. Second, the behaviour of mAbs in congenitally athymic mice is not equivalent to that in immunocompetent hosts [13]. In the former, the effector function of NK cells and monocytes is enhanced in compensation for a lack of T cell function, and circulating Ig levels are abnormally low. Although a third model used melanoma cells injected intravenously into inbred C57/bl mice, these are known to be a 'high responder' strain immunologically. On balance, it would therefore be premature to use the current data to predict responses in heterogeneous, generally aged, often immunodeficient, human cancer patients in which the clinical problem is established, disseminated disease.

There are also several extra studies that could strengthen the conclusion that FcR status is causally related to mAb therapeutic efficacy *in vivo*. Although it was shown that the tumours grew similarly in the *nu*/*nu* hosts and those crossed with different FcγR-deficient strains, it would be important to show the following: (1) that the HER2 expression and kinase activity of the transplanted tumours were equivalent in all hosts, (2) that tumours with different expression levels responded as predicted, and (3) that the tumour response to direct-acting agents was equivalent – these could be HER-2 tyrosine kinase inhibitors, non-ADCC-mediating mAbs, drug or radioisotope conjugates. These studies should exclude any epigenetic modifiers of response that could inadvertently have been introduced by selective breeding. For example, it has been shown that HER-2 expression (and Herceptin) can alter the sensitivity of tumour cells to cytokines such as tumour necrosis factor-α, and it might be that the different hosts vary in their endogenous (or mAb-activatable) cytokine profiles.

With Rituxan, which is a chimeric mAb comprising human γ1 Fc plus mouse anti-CD20 Fab regions, both ADCC and complement-dependent cytotoxicity (CDC) have been demonstrated *in vitro*, although the main determinants of its clinical efficacy have not been defined. Indeed, it has never been formally proved that ADCC operates in patients, and some mAbs that perform well in ADCC assays fail in clinical trials. Clynes's data (confirming reports by Funakoshi [14]) show a significant FcγRIII-dependent host component of anti-CD20 (Rituxan) in the response of xenografts in *athymic* mice. However, in B lymphomas in immunocompetent animals, Tutt *et al* [15] found that in most cases crosslinking and inhibitory signalling by mAbs directed against surface immunoglobulin idiotype, CD19 and CD40 were more important than the recruitment of host effectors.

Thus, although host mechanisms clearly can contribute to mAb-induced therapeutic responses, their importance varies in different situations. Activity *in vitro* (either direct or mediated via ADCC or CDC) does not seem to predict activity *in vivo*, so we must understand that patients' individual responses are the sum of multiple factors including expression of the target antigen (and other signalling molecules that might compensate if the former is inactivated), levels of circulating Ig or immune complexes and the functional status of their effector cells.

How does the manipulation of FcR interactions compare with other strategies?

Host immune responses can also be induced by the use of bispecific antibodies (bsAbs) in which one Fab arm recognises tumour antigen and the other engages epitopes on T cells (CD3) [16], or specific FcγR [17]. These constructs, unlike Herceptin and Rituxan, are monomeric, which might be a disadvantage (because of their lower affinity), but a new class of bsAbs has been designed that recognises tumour cell EpCAM antigen and CD3, and has an Fc composed of a mouse γ2a heavy chain and a rat γ2b heavy chain: like human γ1, two very potent activators of FcR. mAb BiUII has been shown to activate T cells expressing CD3, monocytes and macrophages expressing FcγRIII, NK cells expressing RI, but not B cells expressing RII/CD32 [18]. Although clinical use might be limited by human anti-mouse and anti-rat (HAMA and HARA) responses, these heterologous Fc regions proved more active than either homologous Fc and showed the advantage of recruitment of multiple classes of host effectors. In contrast, bsAbs recognising HER2 and FcγRIII (for example 2B1) have had limited clinical utility owing to toxicity, although bispecific scFv might overcome some of the problems [19]. The relative merits of these different constructs remain to be fully explored clinically [20].

Do the recent observations by Clynes *et al* have implications for the future design of therapeutic mAbs, and how will these measure up to other agents? Theoretically, if the Fc region could be engineered to give selective binding to FcγRIII relative to FcγRIIB, activation of host effector cells could be maximised. This group found that a single amino acid change at residue 265 of the CH2 domain of murine mAb 4D5 was sufficient to reduce its binding to FcR, abrogate ADCC activity and compromise efficacy *in vivo*. However, binding to both the activation RIII and the inhibitory RIIb receptors was decreased, with no evidence of selectivity. The specificity of binding of Ig isotypes to different FcR isoforms and allotypes is complex, with both CH2 regions 234–237 and the CH2/CH3 interface being implicated [21]. In addition, there is a high degree of homology between the ectodomains of many receptors, suggesting very similar binding profiles.

Careful and comprehensive analysis of the binding specificities of mutated mAbs to human effector cells, ideally harvested from cancer patients, would therefore be essential to predict the net effect of changing FcR interactions. FcγRIIB expressed on follicular dendritic cells in germinal centres has also recently been shown to be important for the regulation of B cell recall responses, and it is possible that this could contribute to host anti-tumour responses in patients [22]. A further consideration is the expression of the FcγR variant alleles in control and disease populations, which might also influence response to therapeutic mAbs [23,24].

Many trials with Herceptin are under way, but as previously stated there is as yet no hard evidence that ADCC is contributing to therapeutic response. It would seem logical to explore this further before steps are taken to generate FcγRIII-selective mAbs as proposed by Clynes *et al*, because if patients are unable to mobilise effector cells to sites of metastasis, the strategy will fail. The CAMPATH 1 mAb (which is a rat γ2b isotype directed against CD52) is a powerful inducer of CDC and ADCC against antigen-positive lymphoid malignancies. However, although it depleted target cells effectively from blood, spleen and bone marrow, it was much less effective against solid tumour deposits [25].

Increasingly, emphasis is being placed on pharmacodynamic endpoints and surrogate markers of response in trials of novel therapies, and mAbs should be no exception. It should be possible to check that patients have adequate levels of NK cells and monocytes before therapy and that these are responsive in ADCC assays. Once therapy is under way, trials could be designed (similar to those with bsAbs) to assess immunological function, for example evidence of CD16-positive host cell recruitment into accessible tumour deposits and circulating cytokine levels. In addition, to examine whether other mechanisms are operating, it would be worth checking for the induction of idiotypic antibody cascades and cell-mediated responses to HER-2.

Finally, the efficacy and cost of novel engineered mAbs must be critically compared with new low-molecular-mass agents that inhibit c-*erb*B-2 and EGFR tyrosine kinases. These include a 1.5 kDa exocyclic anti-HER2/neu peptide mimic [26] and also orally active agents with cellular inhibitory activity in the nanomolar range [27]. Whatever the final outcome, the use of increasingly sophisticated genetically engineered antibodies, cells and animal models will lead to a much greater understanding of tumour–host interactions and how they can best be manipulated for therapeutic benefit. Even when tumours are preselected to be antigen positive, only a minority of patients respond to mAb therapy. If Clynes *et al* are correct, this might be due to the ability of their NK cells and monocytes to become 'triggered' by the therapeutic mAb to kill tumour cells. These are exciting times and effective targeted therapy for cancers is close to being a reality. Whether the 'magic bullets' will be mAbs, their derivatives or synthetic drugs remains to be seen.

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