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Author: Andrew T. Vaughana Mark S. Cragga Stephen A. Beersa

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Title: Antibody modulation: limiting the efficacy of therapeutic antibodies

Author(s): Andrew T. Vaughan[1,2], Mark S. Cragg[1], Stephen A. Beers[1]

Institution: [1]Antibody and Vaccine Group, Cancer Sciences Unit and [2] Department of Molecular Microbiology, Clinical and Experimental Sciences Unit; Faculty of Medicine, University of Southampton, UK

Address correspondence to: Mark S. Cragg or Stephen A. Beers, Antibody and Vaccine Group, Faculty of Medicine, University of Southampton, MP88, Tremona Road, SO16 6YD

E mail: msc@soton.ac.uk or sab:soton.ac.uk; Fax: (+44) 02380 704061

Graphical abstract

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Abstract

Monoclonal antibodies (mAb) have revolutionised the way in which we treat disease. From cancer to autoimmunity, antibody therapy has been responsible for some of the most impressive clinical responses observed in the last 2 decades. A key component of this success has been their generally low levels of toxicity, and unique mechanisms of action. These two facets have allowed them to a) be integrated rapidly into clinical practice in combination with conventional radio- and chemo-therapies and b) to avoid the resistance mechanisms typically observed with classical small molecule drugs, such as upregulation of drug efflux transporters, dysregulation of apoptosis and mutations in key target enzymes/pathways.

Although success with mAb therapies has been impressive, they are also subject to their own resistance mechanisms. In this perspective we discuss the various ways in which mAb therapeutics can be inhibited, concentrating mainly on the ways in which they can be removed from the target cell surface - a process called modulation. This can be achieved either in a cis-fashion on a single cell or in trans, precipitated by engagement with a second phagocytic cell. The evidence for each of these processes will be discussed, in addition to possible therapeutic strategies that might be employed to inhibit or reverse them.

Abbreviations: Fc gamma receptor, FcγR; monoclonal antibody, mAb

Key Words: Antibodies, Fc gamma receptor, FcγRIIB, Modulation, Shaving, Immunotherapy, CD20, Tumour Resistance

Introduction

The large scale use of antibodies as potential therapeutics first became a reality in 1975 when Kohler and Milstein described how to generate monoclonal antibodies (mAb) [1]. This technical advancement, for which the inventors were awarded the Nobel prize for medicine in 1984, allowed an infinite supply of a single mAb specificity to be produced for the first time, thereby facilitating the careful development and controlled production required for translation into human therapeutics. The intervening decades have not been without challenges: the issue of immunogenicity of the original murine antibodies, and the unexpected toxicity of some antibody specificities have represented significant setbacks in the field but these have now largely been overcome through antibody engineering (chimerisation, humanisation, phage display), the development of mice expressing human antibody genes and more rigorous pre-clinical testing and careful trial management (reviewed in [2] and [3]).

In particular, several mAb have clearly demonstrated the potential benefits of mAb therapeutics. The anti-CD20 mAb rituximab was the first to be approved for use in oncology and heralded in a new era in the treatment of B cell malignancies [4], improving response rates and overall survival in combination with chemotherapy and significantly raising the bar for new therapies. It has more recently also made significant inroads into autoimmune disorders, revealing perhaps surprising efficacy in diseases not previously associated with B cell dysfunction [5]. Its success has been to the extent that frequently clinical data is now assessed as being from either the pre- or post-rituximab era. In autoimmunity, the anti-tumour necrosis factor (TNF)

α mAb infliximab has had a similar impact. Approved first for Crohn's disease in 1998, it has since been approved for ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis [6], and ulcerative colitis. Like rituximab, it has gone on to be administered to millions of patients. However, as well as illustrating the success of mAb therapeutics these two reagents also illustrate a new phenomenon – that of antibody resistance.

As detailed above, mAb function very differently to conventional small molecule therapeutics. They work through a variety of potential effector mechanisms (reviewed previously [7]). In essence, they bind to their specific target molecule and in doing so may block the interaction with the natural ligand (as in the case of infliximab – it binds TNFα - preventing it from binding to its receptor), modulate target molecule signalling (as in the case of Herceptin by preventing Her-2neu dimerization), and/or engage the effector systems of the immune system. These latter may be serum proteins such as complement or cellular effectors such as NK cells and macrophages which are engaged through key receptors on the cell surface known as Fc receptors which bind the Fc region of the antibody.

As discussed elsewhere and in keeping with the fact that most therapeutic mAb are of the IgG class, Fc receptors and particularly Fc gamma receptors (FcγR), are pivotal for the activity of the majority of therapeutic mAb. FcγR represent a family of evolutionary related receptors which in mammals may be broadly subdivided with regards to their affinity for IgG and downstream signalling effects (reviewed in [8]). Humans and mice have a single, high affinity FcγR, capable of binding monomeric

IgG, with the remainder all low-medium affinity, only binding multimeric IgG in the form of soluble or cell-bound immune complexes. The majority of FcγR are activatory receptors and have a positive signalling function, engendered through their association with the common FcR gamma chain, which contains an immunoreceptor tyrosine-based activation motif (ITAM). However, in both mouse and man there is a single inhibitory FcγR, FcγRIIB (CD32B) which has an immunoreceptor tyrosine-based inhibitory motif (ITIM) and serves to reduce intracellular signalling arising from activatory FcγR and other stimulatory receptors via the recruitment of SHIP [9].

Using anti-CD20 mAb as a model, we and others have tried to identify the key roles that FcγR play in mAb effector function [7] and how resistance might be elicited [10,11] (Figure 1). Based on in vitro functional differences CD20 mAb can be classified as type I (rituximab-like) or type II (tositumomab-like) [7]. Type I display a potent ability to activate complement through enhanced recruitment of C1q [12] due to the efficient clustering of antibody Fc regions [13]; an activity directly linked to their ability to redistribute CD20 to lipid raft microdomains of the plasma membrane. In contrast, type II anti-CD20 mAb do not display either of these properties but instead evoke strong homotypic adhesion [14] and a non-apoptotic form of lysosomal cell death [14-17]. In addition, we observed that type I anti-CD20 mAb undergo more rapid internalization from the cell surface, in contrast to type II mAb [18-20]. Below we discuss the various ways in which the study of these two different types of mAb have elucidated a number of mechanisms of mAb resistance, before discussing how they may be overcome.

Antigenic modulation: Antibody internalisation

Historically, CD20 was considered to be an ideal target for mAb therapy due to its high expression on malignant cells, its B cell lineage restriction, absence from antibody-producing plasma cells and stem cells, and apparent lack of antigenic modulation [21,22]. Whilst the first three properties hold true, it is now appreciated that antigenic modulation of CD20 occurs in some circumstances.

Using transgenic mice expressing human CD20 on the surface of the B cell population, we demonstrated that type II anti-CD20 mAb consistently outperformed type I mAb in mediating B cell depletion in vivo [23]. Both the extent and duration of depletion was greater in animals treated with the type II mAb and was independent of differential complement activation [23] and programmed cell death [18] mediated by type I and II mAb. Instead, type I anti-CD20 mAb were internalised and degraded in transgenic mouse B cells in vivo as well as primary and malignant human B cells treated in vitro, in contrast to the type II mAb [18]. Internalisation was associated with a reduction in both antibody half-life [23] and phagocytosis of opsonised cells [24] by effectors (Figure 1), suggesting that internalisation of type I anti-CD20 mAb-ligated CD20 leads to reduced therapeutic efficacy and increased consumption of mAb from the serum.

Mechanisms of internalisation

The mechanism of internalisation of type I anti-CD20 mAb was investigated by Lim et al. who demonstrated that the rate was slower in response to ligation by a $F(ab')_2$ fragment of rituximab, suggesting a potential role for Fc receptor engagement [19].

Internalisation occurred in purified B cells which express only the inhibitory FcγRIIB and engaging FcγRIIB with a specific blocking mAb inhibited the process. Furthermore, there was a negative correlation between the cell surface expression of FcγRIIB and the proportion of rituximab remaining on the cell surface after in vitro culture [19]. We also demonstrated that the ITIM of FcγRIIB was phosphorylated in response to rituximab, indicating that a direct interaction between the Fc domain of the mAb and the Fc-binding domain of the FcγR augmented internalisation.

Type I anti-CD20 mAb and FcγRIIB may interact in either of two ways; engaging adjacent cells in trans, or on the surface of a single cell in cis. By repeating our experiments under conditions in which direct cell-cell interaction was unlikely, Lim et al. demonstrated that a cis interaction between type I anti-CD20 mAb and FcγRIIB was required to augment internalisation [19], a process termed antibody bipolar bridging. Finally, we demonstrated reduced survival after rituximab treatment in patients with mantle cell lymphoma whose tumours expressed high levels of FcγRIIB, after treatment with rituximab-containing immunochemotherapy [19], compared to those expressing low levels. This same observation was also made later in patients with follicular lymphoma treated with rituximab monotherapy [25], supporting the assertion that internalisation may reduce the therapeutic efficacy of type I anti-CD20 mAb therapy when used clinically.

Interaction between type I anti-CD20 mAb:CD20 and FcγRIIB expressed in cis is analogous to the interaction between FcγRIIB and immune complexes formed by antibody-coated antigen. Upon antibody bound-antigen binding to its cognate B cell

receptor (BCR), the immune complex binds to FcγRIIB in cis via the Fc domain of the bound antibody, bringing the inhibitory FcγR and the BCR into close proximity in the plasma membrane; an interaction that inhibits BCR activation and is believed to act as a negative feedback loop for antigen-specific B cell responses [26]. In addition to inhibiting BCR activation, binding of immune complex in the form of heat aggregated IgG has been shown to induce rapid internalisation of the B2 isoform of FcγRIIB that is dependent on the presence of a complete ITIM sequence in the cytoplasmic domain [27,28]. These data suggested that the interaction between type I anti-CD20 mAb and FcγRIIB may bring FcγRIIB and CD20 into close proximity in the plasma membrane, augmenting internalisation of the trimeric complex via phosphorylation of the FcγRIIB ITIM, analogous to the response with immune complex. However, investigations by Vaughan et al. demonstrated that a truncated mutant form of FcγRIIB lacking the entire cytoplasmic domain was able to augment internalisation of type I anti-CD20 mAb-ligated CD20 as effectively as the wild type receptor [29]. This suggested that unlike the interaction between FcγRIIB and immune complex, internalisation of antibody-ligated CD20 was not mediated via FcγR-dependent signal transduction, implying that the role of FcγRIIB was restricted to physical/structural interactions.

Role of lipid rafts

Interestingly, type I anti-CD20 mAb are not unique in their ability to interact with FcγRIIB. In fact, many mAb targeting antigens on B cells interact with FcγRIIB in cis in direct proportion to the amount of mAb bound to the cell surface, including mAb to MHC II, CD40 and CD38 [20]. However, in the majority of cases these interactions

fail to alter the rate of internalisation of the mAb-ligated receptor, with only anti-CD38 and anti-CD19 mAb significantly affected [20].

In an attempt to further elucidate the mechanism of antigenic modulation, we have investigated the role that lipid rafts may play in FcγRIIB-augmented internalisation of mAb-ligated CD20. Type I anti-CD20 mAb mediate redistribution of CD20 to lipid rafts [30,31] in contrast to type II anti-CD20 mAb [12] and many other mAb directed to B cell surface receptors. Furthermore, FcγRIIB also redistributes to lipid rafts upon crosslinking with the BCR [32-34]. Redistribution to lipid rafts and subsequent endocytosis is a well-recognised pathway of internalisation for many receptor:ligand complexes and viruses [35]. We speculated that the interaction between FcγRIIB and rituximab in lipid rafts may be required for augmenting internalisation [29], explaining why the rate of internalisation of type II anti-CD20 mAb and mAb directed to other receptors remain largely unchanged, despite phosphorylation of FcγRIIB.

To investigate the role of lipid rafts, we transfected human myeloma cells with mutant versions of CD20 unable to redistribute to lipid rafts [14,31]. In the absence of FcγRIIB, cells expressing these mutant forms of CD20 demonstrated slower internalisation of type I anti-CD20 mAb than cells expressing wild type CD20 suggesting that redistribution of CD20 to rafts is important for internalisation (unpublished observations). However, internalisation was augmented when cells were co-transfected with FcγRIIB, suggesting that FcγRIIB was able to compensate for the mutation by acting to chaperone mutant CD20 into lipid rafts. To investigate this possibility we prepared a transmembrane mutant form of FcγRIIB based on a similar mutation made in the transmembrane domain of FcγRIIA [36] that is unable to redistribute to rafts. This mutant form of FcγRIIB also augmented internalisation of

CD20, suggesting that its role in the process may be independent of its ability to enter lipid rafts (unpublished observations).

It is still unclear how the interaction between FcγRIIB and type I anti-CD20 mAb augments internalisation of CD20. A prerequisite for endocytosis is the formation of membrane curvature that allows the budding of endocytic vesicles. Recently, Stachowiak et al. demonstrated that steric confinement of highly crowded protein within regions of artificial lipid membranes is enough to drive membrane puckering and lipid tubule formation in the membrane [37], observing that puckering increases with protein concentration. We have observed punctate staining of CD20 that co-localises with FcγRIIB upon ligation with type I anti-CD20 mAb, in contrast to diffuse staining observed with the non-redistributing type II mAb [18-20,29]. The high density redistribution of CD20 and FcγRIIB induced by type I anti-CD20 mAb-ligation resembles the high density staining observed in the artificial membranes generated by Stachowiak et al. [37] and may therefore be sufficient to trigger membrane puckering and subsequent endocytosis. The function of FcγRIIB in this process may be to form high affinity interactions with the mAb-ligated receptor, promoting high density clustering within the membrane, necessary for membrane distortion. This does not fully explain why type II anti-CD20 mAb do not augment internalisation of CD20, which also interact with FcγRIIB expressed in cis. However, the crystal structure of the type II mAb GA101 (obinituzumab) indicates that type II antibodies bind CD20 in a different orientation to type I anti-CD20 mAb [38]. This difference may alter the affinity or density with which type I and II mAb interact with FcγR in cis. Although type II mAb interact with and phosphorylate FcγRIIB, the level of activation is much less [20]. The altered elbow angle of type II anti-CD20 mAb may not be sufficient to drive the clustering required to elicit membrane puckering and

subsequent endocytosis. This, coupled with the inability of type II mAb to induce redistribution of CD20 to lipid raft domains may result in type II mAb and mAb directed to other protein targets, remaining on the cell surface.

Antigenic modulation: Antibody shaving

An alternative explanation for the phenomenon of antibody resistance is antibody shaving or trogocytosis. The shaving reaction was first implicated in resistance to mAb treatment in studies with rituximab in CLL patients by the group of Ron Taylor [39]. They proposed this mechanism to help explain the clinical observation that after initial infusions of rituximab, circulating CLL cells were reduced in number and then replaced by CD20 low/negative CLL cells which persisted in the face of ongoing mAb administrations [40].

In the shaving reaction antibody and antigen complexes are nibbled or plucked (shaved) from the target cell surface in an FcγR-dependent reaction by monocytes or macrophages [39]. Although originally postulated to be mediated by FcγRI, shaving has since been demonstrated to be possible with any, and all, FcγR and seems simply to require productive contact between antigen-antibody complexes and FcγR expressing effector cells. Indeed in one mouse model system the inhibitory FcγRIIB was also demonstrated to mediate shaving [41]. It is noteworthy that these data regarding FcγR usage were obtained using an intraperitoneal tumour mouse model where previously complement had been demonstrated to play a role [42]. It is therefore possible that complement receptors which are expressed on monocytes

and macrophages, and themselves able to mediate phagocytosis [43,44] may have been active in this system making delineation of the role of FcγR difficult.

Taylor and colleagues proposed that the shaving process occurred when effector cell populations become saturated and exhausted (reviewed in [45]). Shaving is proposed to leave the cells viable but refractory to clearance by subsequent effector functions as they are no longer coated with mAb. Although the depletion of complement components [40] and activation of NK cells [46] has been demonstrated after rituximab administration no formal demonstration of reduction in capacity or exhaustion for monocytes or macrophages has been evidenced to date. It is interesting to question whether even in the presence of heavy leukaemic or other tumour burdens saturation of the reticulo-endothelial system is possible given its huge capacity for cellular uptake and clearance. Indeed under normal homeostatic conditions phagocytic cells of the liver and spleen have been calculated to clear 2 million red blood cells per second [47].

Shaving, albeit not so-called at the time, was first demonstrated by Griffin and colleagues who observed that capped antigen-antibody complexes could be internalised by monocytes/macrophages without engulfing or destroying the opsonised cells [48]. They had previously shown that antigen-antibody capping prevented phagocytic uptake of opsonised cells. They showed in a series of elegant studies that the capping process effectively sequestered antibody to one half of the target cell which was brought into intimate contact with the effector cell plasma membrane. This process left the membrane-distal portion of the target cell denuded of opsonising antibody thereby preventing the zipper mechanism of phagocytic

uptake they had previously outlined [49]. Although these data provide good evidence for the ability of antibodies to mediate such a process it should be noted that these early studies were carried out with polyclonal antibodies rather than monoclonals. It is likely that the use of polyclonal Ab raised against highly expressed receptors such as the B cell receptor and their consequent ability to induce extensive hyper cross-linking produce an exaggerated effect when compared to mAb such as rituximab which recognise more discreet antigens and have been demonstrated to produce smaller caps [19] rather than the hemisphere sized caps produced in these early studies. Despite this caveat, several laboratories have shown similar findings with a variety of other mAb (trastuzumab, cetuximab and T101, [50]; epratuzumab, [51]; daclizumab, [52]; CD22/CD20 bispecific, [53] and CD3/Trop-2 bispecific, [54]), demonstrating that shaving does indeed occur on target cells.

The questions that remain regarding shaving are as follows: firstly, whether and to what extent this phenomenon impacts mAb efficacy in patients and secondly which mechanism of resistance, internalisation or shaving, is dominant in limiting responses to rituximab. The first question is important in order to help establish the rules by which one can predict and therefore rationally design mAb specific to antigens on different cell types whilst avoid shaving and potential escape. Secondly, for CD20 therapy it is important to understand which of the two resistance mechanisms dominate as each has been proposed to require very different clinical strategies to reduce or overcome their impact.

In the case of internalisation we have proposed the use of non-internalising type II CD20 mAb such as obinutuzumab or the concurrent administration of mAb which reduce or negate the internalisation process of type I mAb like rituximab (discussed below). Alternatively, in order to overcome the detrimental impact of antigen loss

through shaving Taylor and colleagues have proposed that repeated low dose antibody administration, sufficient to clear cells but not, they suggest to saturate the effector populations, will enhance responses in CLL [55] and potentially to other direct targeting mAb [45].

Taylor and colleagues in [45] themselves concede that in contrast to their own pilot and phase I/II trials [56,57], “the results of a dose escalation trial for CLL indicated a higher level of efficacy for single agent rituximab at (higher) weekly doses of 2250 mg/m^2” [58]. Further, the recent randomised Phase II NCRI Attenuated dose Rituximab with ChemoTherapy In CLL (ARCTIC) trial of low dose rituximab in previously untreated CLL closed the low rituximab arm early as it was inferior to standard FCR [59]. It should be noted that although this would suggest that shaving is not limiting efficacy in this setting and low dose rituximab regimes are not likely to augment responses in CLL these data are confounded by the addition of mitoxantrone.

These results do however highlight the larger question regarding the CD20 low/negative cells observed; are these deletion-resistant circulating cells as Taylor and colleagues propose or are they cells in circulation/transit which have shaved and escaped initial encounters with effector cells but which will eventually be cleared? Taylor and colleagues proposed that shaving was a secondary and separate mechanism only evoked when effectors were saturated or exhausted leading to resistant circulating cells. However, evidence in support of the latter contention, that these are cells in the process of being cleared, comes from a study by Boross et al. [41] where they showed that, “RTX-induced trogocytosis of CD20 is dependent on

RTX concentration and correlates with the therapeutic effect of CD20, confirming that the two processes are intimately related." Our own unpublished data also support the latter statement as we only observe shaving in the presence of phagocytic activity in vivo and in vitro. Indeed, if we artificially saturate murine or human macrophages in vitro using latex beads we see a saturation-dependent loss in shaving which corresponds with a reduction in phagocytosis. Interestingly, and as demonstrated by Pedersen et al., both type I and II CD20 mAb mediate shaving equally [60] and we have confirmed this in unpublished mouse and human studies. Despite this similar propensity to shave, type II mAb outperform type I in mediating cellular clearance in vitro and in mouse models in a manner that can instead be explained by their lack of propensity to internalise [18,24]. Further to this the type II mAb obinituzumab has recently been trialled head to head with rituximab in CLL patients in combination with chlorambucil (CLL11, [61]) and found to nearly double progression free survival (albeit with a higher dosing schedule for obinituzumab). Taken together, these data support that internalisation rather than shaving limits the efficacy of type I anti-CD20 mAb.

Other interactions between therapeutic mAb and inhibitory FcγRIIB that limit therapeutic efficacy

In addition to reduced therapeutic efficacy mediated by the loss of opsonised antigen from the surface of a target cell via internalisation or shaving, there are additional interactions between therapeutic mAb and FcγRIIB that may increase resistance to depletion. These effects are mediated via interaction between FcγRIIB and therapeutic mAb in cis or trans and are discussed below (Figure 1).

Cis effects

It is clear that mAb targeting specific cell-surface receptors on FcγRIIB–expressing cells can, and do, interact with FcγRIIB in cis [20,62]. The consequences of this interaction are both target and mAb specific. Firstly, cis interaction between mAb and FcγRIIB can compete with other FcγR expressed in trans leading to a potential reduction in downstream FcγR-dependent immune effector mechanisms. This was demonstrated by Cassard et al. using an in vivo model in which mice were challenged with a metastatic melanoma cell line transfected with FcγRIIB. Survival of mice challenged with these cells was reduced after treatment with a direct tumour targeting therapeutic antibody, compared to mice challenged with the untransfected FcγRIIB-ve cells. This effect was independent of the FcγRIIB cytoplasmic domain and resulted in lower antibody-dependent cellular cytotoxicity in vitro [62]. We would predict that cis interactions between FcγRIIB and therapeutic mAb would also likely compete with activatory FcγR expressed in trans on professional phagocytic cells, reducing antibody-dependent phagocytosis of cellular targets and thereby clinical efficacy. Further, if the opsonised target acts as an adjuvant to boost the immune response via engagement of activatory FcγR, reduced uptake of opsonised cells by professional antigen presenting cells could also result in reduced antigen-specific responses directed against tumour specific antigens.

Interactions between therapeutic mAb and FcγRIIB expressed in cis may also compete with complement proteins for binding to the Fc domain of antibodies. Type I anti-CD20 mAb efficiently fix complement, activating the classical complement cascade [12]. Although this activity is largely considered dispensable for the therapeutic effects of the antibodies in vivo [23], Wang et al. have demonstrated that

complement components C1q and C3 inhibit the ability of type I anti-CD20 mAb to activate NK cells via FcγRIII expressed in trans, resulting in reduced ADCC [63]. This effect is presumably due to competition between complement and FcγR for binding to the Fc domain of the therapeutic mAb. Although not shown experimentally, a similar effect might occur between complement and FcγRIIB expressed in cis, in which the two proteins may compete for binding to the Fc of the therapeutic mAb. Therefore, with mAb for which complement activation is therapeutically important, cis interactions with FcγRIIB may be detrimental.

Trans effects

We have demonstrated that mAb targeting B cell surface receptor proteins are capable of binding to FcγRIIB expressed in cis and trans [20]. Trans interaction with FcγRIIB expressed on professional phagocytic cells lowers the therapeutic efficacy of direct targeting mAb [64,65], presumably due to competition for Fc binding sites with activatory FcγR on the phagocytes and inhibition of cellular activation via the downstream inhibitory effects of FcγRIIB engagement. The ratio of activatory FcγR to inhibitory FcγRIIB engaged by a therapeutic mAb is termed the activatory:inhibitory ratio [65,66], and is determined by cellular FcγR expression and the mAb IgG subtype. In mice, mouse IgG1 has a higher affinity for the inhibitory FcγR than mouse IgG2a [66], and thus has a lower activatory:inhibitory ratio and reduced therapeutic efficacy [24]. The deleterious effect of trans engagement between therapeutic mAb and FcγRIIB and the importance of activatory:inhibitory ratio was demonstrated by Nimmerjahn et al. using mice challenged with a metastatic melanoma cell line. Treatment of mice with the IgG2a subtype of a direct

tumour targeting mAb, which has an activatory:inhibitory ratio of 70 dramatically reduced the number of lung metastases to almost zero, compared to untreated mice. Conversely, treatment of mice with the IgG1 subtype resulted in no reduction in lung metastases due to the lower activatory:inhibitory ratio (0.1). However, in mice in which the gene encoding FcγRIIB had been deleted so that mAb could only engage activatory FcγR, the therapeutic efficacy of the IgG1 subtype was substantially augmented [65]. Thus, the degree of trans engagement between direct targeting mAb and FcγRIIB may have a direct effect on the outcome of therapy. This may be relevant in the treatment of various human malignancies, including malignant melanoma in which FcγRIIB expression is expressed on up to 40% of metastatic tumours [67]. Such ectopic expression of FcγRIIB on the tumour cells themselves (or on non-haematopoietic cells associated with it) could compete for mAb binding with activatory FcγR expressed on effector cells at the tumour site, lowering the activatory:inhibitory ratio of therapeutic mAb and reducing clinical efficacy.

Future strategies to reverse mAb resistance mechanisms

Co-administration of FcγRIIB blocking mAb and anti-CD20 mAb for the treatment of B cell malignancies

Concentrating on anti-CD20 mAb, we have highlighted the diverse range of mechanisms through which therapeutic mAb can interact with FcγRIIB to result in reduced efficacy. Remarkably, the majority of these inhibitory mechanisms can potentially be abrogated using a single strategy; blocking FcγRIIB. Roghanian et al. recently co-treated mice expressing human CD20 and FcγRIIB with both rituximab and an FcγRIIB-specific blocking mAb, 6G11[68]. As expected, treatment of cells

with the FcγRIIB-blocking mAb reduced the rate of internalisation of mAb-ligated CD20 from the surface of B cells in vitro and this corresponded with an enhanced depletion of B cells in vivo. Furthermore, we observed a similar augmentation of B cell depletion when rituximab was combined with an N297Q mutant form of 6G11 unable to bind FcγR by its Fc domain [68]. This suggested that the enhanced therapy seen was, at least in part, due to the inhibition of rituximab-mediated internalisation by preventing bipolar antibody bridging with FcγRIIB.

In addition to inhibiting internalisation, blocking bipolar antibody bridging should promote interaction between direct targeting mAb and FcγR expressed in trans. Use of an FcγRIIB-blocking mAb such as 6G11 would also be expected to prevent trans interactions between direct targeting mAb and FcγRIIB on phagocytic cells and tumour cells, thereby enhancing the activatory:inhibitory ratio of the antibody, further enhancing therapeutic efficacy.

With such a variety of effector mechanisms potentially augmented by co-administration of 6G11, it will be exciting to see how this mAb performs in up-coming clinical trials [69]. If successful, co-treatment with blocking anti-FcγRIIB mAb may be applicable to many other direct targeting antibody therapeutics, and possibly some agonistic mAb. For example, for mAb which have been chosen for their ability to activate cell-surface receptors, blocking the interaction with FcγRIIB may augment activation due to the tendency of the inhibitory receptor to otherwise terminate downstream signalling pathways [70]. Paradoxically however, cross-linking by FcγRIIB expressed in trans has been demonstrated to be essential for the agonistic

activity of certain mAb such as anti-CD40 [71,72]. Therefore, blocking FcγRIIB in this context may actually be detrimental and so the use of this approach would need to be evaluated for each agonistic antibody depending on its mechanism of action.

Conclusions

In summary, it is clear that mAb have begun to revolutionise medical intervention, particularly in oncology, and this trend is set to continue [73]. Since the approval of rituximab in 1997, a plethora of other mAb have followed and become embedded into clinical practice. A completely different therapeutic modality to conventional chemotherapy, it is unsurprising that its resistance mechanisms also vary. Here we have outlined the way in which internalisation, shaving and other FcγRIIB-mediated mechanisms can reduce mAb efficacy, particularly for anti-CD20 mAb. If in the coming years effective strategies to overcome each of these issues are realised, even more impressive clinical responses with mAb therapeutics will be provided.

Conflict of interest statement

Prof Cragg serves as a consultant for Bioinvent International, has previously served as an ad hoc consultant for Roche and receives grant funding from both parties as well as from GSK. The other authors declare no conflict of interest.

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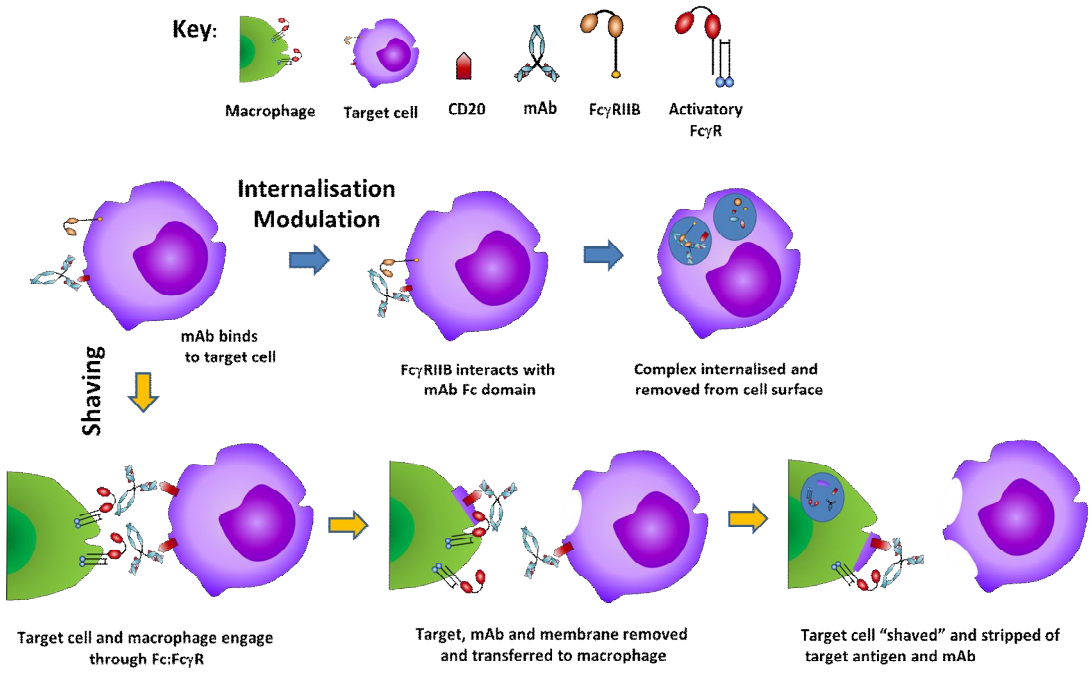
Figure legends

Figure 1. Potential means of mAb resistance. Two key modes of mAb resistance have been proposed; internalisation/modulation or "shaving". In the former, a cell intrinsic process occurs whereby mAb binds to the target antigen (e.g. CD20) and its Fc is engaged by the inhibitory FcγRIIB, precipitating internalisation of the tripartite complex. In contrast, shaving is performed by a secondary phagocytic cell. Under certain conditions, such as target cell saturation, the activatory FcγR rather than mediating phagocytosis of the target cell, rip a portion of the cell membrane containing the target antigen and antibody from the target cell, stripping it of mAb and antigen.



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