

Dependence of surface monoclonal antibody binding on dynamic changes in Fc γ RIIb expression

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Introduction

Receptors for the Fc portion of immunoglobulin G (IgG), the Fc gamma receptors (Fc γ Rs), are cell surface glycoproteins of the immunoglobulin superfamily. These receptors bind IgG through a conserved extracellular structural motif, comprising two or three immunoglobulin-like domains.¹ Fc γ Rs then transmit signals to the cell via highly divergent transmembrane and cytoplasmic regions, to control antibody production and link the humoral response to effector mechanisms.²

In mice, four classes of Fc γ R have so far been identified, which vary with regard to their cellular expression patterns and effector functions.³ Fc γ RI (CD64), Fc γ RIII (CD16), and the recently identified Fc γ , are activatory receptors and mediate signalling through a common immunoreceptor tyrosine-based activation motif-containing γ chain. Fc γ RIIb (CD32b), on the other hand, is an inhibitory receptor with an immunoreceptor tyrosine-based inhibitory motif in its cytoplasmic tail. In addition to their divergent signalling potential, the family of Fc γ Rs

Summary

Receptors for the Fc region of immunoglobulin G (Fc γ Rs) are expressed on a broad range of haematopoietic cell types and are responsible for regulating antibody production and linking the humoral and effector responses. In response to a number of stimuli, such as cytokine signals or inflammation, Fc γ R expression at the cell surface is dynamically regulated. On B cells, we observed what appeared to be a correlation between CD22 expression and Fc γ RIIb expression when the latter was varied in a number of models. Further investigation revealed that this was specific to a particular anti-CD22 monoclonal antibody, which appeared to require stabilization by interaction with Fc γ RIIb for optimal binding to CD22. Since alterations in the regulation of Fc γ R expression are important in controlling immune responses and have been associated with a number of immune-mediated disease states, we suggest that it might be prudent to confirm the expression of cell surface markers by two independent methods. Furthermore, because the efficacy of therapeutic antibodies may depend upon their interaction with Fc γ Rs, our results are relevant to their design and assessment.

Keywords: B cell; CD22; Fc γ RIIb; interleukin-4; therapeutic antibody

vary with regard to their affinity for IgG and their preference for the different IgG subclasses. Fc γ RI is a high-affinity receptor with a dissociation constant of 10^{-8} – 10^{-9} M for IgG that is capable of binding monomeric IgG.² Fc γ RIIb and Fc γ RIII on the other hand are low-affinity IgG receptors ($K_d \sim 10^{-6}$ – 10^{-7} M) and bind immunoglobulin of the IgG1, IgG2a and IgG2b isotypes.² Fc γ RIV has an intermediate affinity for immunoglobulin ($K_d \sim 10^{-8}$ M), but only binds the IgG2a and IgG2b isotypes.⁴ These lower-affinity receptors interact only with multivalent IgG, presented as immune complexes. The functions of the Fc γ Rs *in vivo* therefore reflect both their signalling properties and their varying affinities for the different IgG subclasses presented in different contexts.

Fc γ Rs are expressed on a broad range of haematopoietic cell types, including macrophages, eosinophils, neutrophils, dendritic cells, natural killer cells and lymphocytes.^{2,4} Expression of Fc γ Rs on these different cell types varies between individuals and is dynamically regulated in response to various stimuli and the effects of inflammation.^{5–9} For example, murine B cells exhibit an approximately

Abbreviations: Anti- μ , anti-immunoglobulin M (μ -chain); Fc γ R, Fc γ receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IgG, immunoglobulin G; IL-4, interleukin-4; LPS, lipopolysaccharide; mAb, monoclonal antibody; α 2,6Sia, α 2,6-linked sialic acid.

threefold increase in Fc γ RIIb expression upon activation, which is abrogated if the cells are cultured in the presence of interleukin-4 (IL-4), or possess an Fc γ RIIb promoter polymorphism.^{5,10,11} Expression of Fc γ RIIb, and indeed other Fc receptors, is therefore subject to multiple levels of control and is dynamically regulated in response to many stimuli, both *in vitro* and *in vivo*.

In this study, we have identified an apparent correlation of CD22 expression with Fc γ RIIb expression on murine B cells using flow cytometry. Further investigation has, however, revealed that this is specific to a particular anti-CD22 monoclonal antibody (mAb), which apparently requires stabilization through its interaction with Fc γ RIIb for optimal binding to CD22. Since Fc γ R levels on cells are frequently altered in disease states,¹² and artificially when studying the mechanisms involved in disease,¹³ this result has important implications for the study of other cell surface molecules in these contexts. In addition, the potential for surface-bound mAbs to interact with Fc γ Rs expressed *in cis* on the cell surface has implications for the design and assessment of therapeutic antibodies.

Materials and methods

Mice

Fc γ RIIb-deficient mice on a C57BL/6 background were provided by J. Ravetch and S. Bolland (Rockefeller University, New York, NY). CD22-deficient mice on a C57BL/6 background were provided by M. Neuberger (Laboratory of Molecular Biology, Cambridge, UK). B-cell-specific Fc γ RIIb transgenic mice and non-transgenic littermate controls were on a mixed CBA/C57BL/6 background. ST6Gal I-sialyltransferase knockout splenocytes were a kind gift from L. Nitschke (University of Erlangen, Germany). All other mice were purchased from Charles River Laboratories (Margate, UK).

Cell lines

The A20 cell line was maintained in Dulbecco's modified Eagle's medium (Sigma-Aldrich, Poole, UK), supplemented with 10% fetal bovine serum (Invitrogen, Paisley, UK), 1 mM glutamine (Sigma-Aldrich), antibiotics and 1×10^{-5} M 2-mercaptoethanol. The Fc γ RIIb-deficient cell line IIA1.6, described in ref. 14 was maintained in a similar way.

Cell purification and stimulation

Splenic B cells were selected by magnetic cell purification using anti-CD19 beads (Miltenyi Biotech, Woking, UK), to > 95% purity, according to the manufacturer's instructions. Cells were cultured (4×10^6 cells/ml) in 12-well flat-bottomed plates (Corning Inc., Artington, UK) in

RPMI-1640 (Sigma-Aldrich), supplemented with 10% fetal bovine serum, 1 mM glutamine, antibiotics and 1×10^{-5} M 2-mercaptoethanol. Stimulation was with either 10 μ g/ml goat anti-mouse IgM μ -chain specific F(ab')₂ (Jackson ImmunoResearch Laboratories, West Grove, PA) or 10 ng/ml lipopolysaccharide (LPS) from *Salmonella minnesota* (Sigma-Aldrich) for 48 hr, with or without 10 ng/ml IL-4 (Peprotech, London, UK).

Flow cytometry

The fluorescein isothiocyanate-conjugated anti-CD22 mAbs 2D6 (NIM-R6) and Cy34.1 were obtained from Southern Biotech and BDPHarmingen (Oxford, UK), respectively, and used at a 1 : 400 dilution for flow cytometry. Other antibodies were purchased from BDPHarmingen. Biotinylated *Sambucus nigra* agglutinin was obtained from Vector Laboratories (Peterborough, UK), and used at 2 μ g/ml. Allophycocyanin-conjugated streptavidin was from Molecular Probes (Invitrogen, Paisley, UK) and biotinylated sialoside probes (used at 10 μ g/ml) were provided by the Consortium for Functional Glycomics (grant number GM62116; compound numbers PA211 and PA209).¹⁵ The probes were: Neu5Gc α 2-6Gal β 1-4GlcNAc β -SpNH-PAA and Neu5Gc α 2-3Gal β 1-4GlcNAc β -SpNH-PAA, abbreviated to α 2,6 NeuGc and α 2,3 NeuGc, respectively. Cells were stained with the appropriate antibody/probe combination in phosphate-buffered saline (PBS) containing 0.5 μ g/ml 2.4G2 (anti-Fc γ RII/III) and 1% normal rat serum. Cells were counterstained with 7-aminoactinomycin D to exclude dead cells and analysed using a FACSCalibur™ flow cytometer (Becton Dickinson, Plymouth, UK) and FCS PRESS software (Ray Hicks, University of Cambridge, UK).

Semi-quantitative real-time polymerase chain reaction

RNA was extracted from cells using RNeasy™ columns and reagents (Qiagen, Crawley, UK) and reverse transcribed using Super reverse transcriptase (HT Biotechnology, Cambridge, UK). Levels of CD22 messenger RNA (mRNA) were assessed relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using real-time semi-quantitative polymerase chain reaction (ABI Prism 7700 Sequence Detection System; Applied Biosystems, Foster City, CA). The GAPDH control primers and probe were Taqman rodent control reagents (Applied Biosystems). The CD22 primers and probe were designed using PRIMER EXPRESS software (Applied Biosystems) and manufactured by Sigma-Genosys (Haverhill, UK).

Western blotting

Cells were lysed in radioimmunoprecipitation assay (RIPA) lysis buffer (Upstate, Lake Placid, NY), supplemented with

1 × Complete™ Mini, ethylenediaminetetraacetic acid-free protease inhibitors (Roche, Burgess Hill, UK), 2 mM phenylmethylsulphonyl fluoride, 2 mM Na₃VO₄ and 2 mM NaF. Lysates were clarified by centrifugation (14 000 g for 15 min at 4°), boiled for 5 min in Laemmli buffer containing 0.1 M dithiothreitol and resolved by 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis. CD22 was detected using the anti-CD22 mAb MB22-1, which was a kind gift from T. Tedder (Duke University, Durham, NC). β-Actin was used as a loading control and was detected with the mouse AC-15 mAb (Abcam, Cambridge, UK). Densitometry was performed using QUANTITY ONE software (Bio-Rad Laboratories, Inc., Hemel Hempstead, UK).

Antibody deglycosylation

Fluorescein isothiocyanate-conjugated 2D6 mAb was deglycosylated by treatment with peptide N-glycosidase F, purified from *Flavobacterium meningosepticum* (Roche Applied Biosciences, Burgess Hill, UK), for 24 hr at 37°. One unit of enzyme was used per microgram of antibody.

Results

Binding of mAb 2D6 to CD22 correlates with FcγRIIb expression

Previous studies in our laboratory, and others, have demonstrated that when murine B cells are activated in the presence of IL-4, surface expression of FcγRIIb, CD22 and CD72 is reduced compared to in cells activated in the absence of cytokine.¹⁰ It appeared that expression of these receptors was co-ordinately regulated, so we sought to establish whether deficiency in one receptor, namely FcγRIIb, could affect the expression of other receptors. B cells were isolated from the spleens of FcγRIIb-deficient mice and C57BL/6 control mice and the expression of CD22 (Fig. 1) and CD72 (data not shown) was examined by flow cytometry. Whether the cells remained untreated or were cultured for 48 hr in the presence of LPS or anti-μ F(ab')₂ (data not shown), staining of cells by the rat anti-CD22 IgG1κ mAb 2D6 was reduced in FcγRIIb-

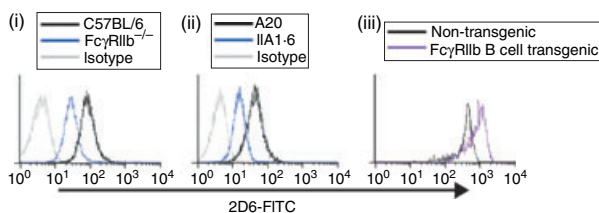


Figure 1. Expression of CD22 as assessed by monoclonal antibody (mAb) 2D6 is affected by expression of FcγRIIb. Binding of the anti-CD22 mAb 2D6 to B220⁺ splenocytes (i and iii), or B-cell lines (ii), was examined by flow cytometry. Histograms are representative of at least four independent experiments in each case.

deficient cells compared to control cells. This effect was also observed in the FcγRIIb-deficient cell line IIA1.6, which demonstrated reduced 2D6 staining compared to the FcγRIIb-sufficient A20 parental cell line (Fig. 1).

Since FcγRIIb deficiency could reduce binding of the mAb 2D6, we wondered whether overexpression of this receptor might serve to enhance antibody binding. This was indeed the case, as demonstrated by the staining of B cells from an FcγRIIb-overexpressing transgenic mouse (RJ Brownlie, KE Lawlor, HA Niederer, AJ Cutler, Z Xiang, MR Clatworthy, RA Floto, DR Greaves, PA Lyons, KGC Smith, unpublished data). Compared to B cells from non-transgenic littermates, FcγRIIb-overexpressing B cells bound considerably more 2D6 mAb (Fig. 1).

Expression of CD22 is unaffected by FcγRIIb deficiency

Given that binding of mAb 2D6 to murine B cells correlated with the expression of FcγRIIb, we investigated whether CD22 expression was reduced in the FcγRIIb-deficient mouse. CD19⁺ B cells were purified from the spleens of FcγRIIb-deficient and control mice by magnetic cell sorting. Cells were either left untreated, or were cultured for 48 hr in the presence of anti-μ F(ab')₂, or LPS. Expression of CD22 mRNA was examined by semi-quantitative real-time polymerase chain reaction and normalized to GAPDH. Compared to control B cells, FcγRIIb-deficient cells expressed similar levels of CD22 mRNA (Fig. 2a). Similarly, the abundance of CD22 protein in FcγRIIb-deficient B cells was comparable to controls, as demonstrated by Western blotting and subsequent densitometry (Fig. 2b). Upon stimulation with anti-μ F(ab')₂ or LPS, no significant difference in CD22 expression was observed at either the mRNA or protein level.

Since expression of CD22 in resting cells is not altered by FcγRIIb deficiency, we examined the CD22 surface expression by flow cytometry using an alternative anti-CD22 antibody; the mouse anti-CD22 IgG1κ mAb, Cy34.1. In agreement with our expression data, this antibody bound equally well to both FcγRIIb-deficient and control B cells. Furthermore, Cy34.1 showed no significant difference in its ability to stain A20 and IIA1.6 cells, nor could it differentiate between the FcγRIIb transgenic and control B cells (Fig. 2c). Therefore, we conclude that surface expression of CD22 is not affected by FcγRIIb deficiency but that binding of mAb 2D6 to CD22 depends upon the abundance of FcγRIIb at the cell surface.

Alterations in 2D6 binding do not reflect changes in CD22 accessibility

On the B-cell surface, CD22 exists predominantly as homomultimeric clusters, located in clathrin-rich membrane microdomains.¹⁶ Maintenance of this distribution

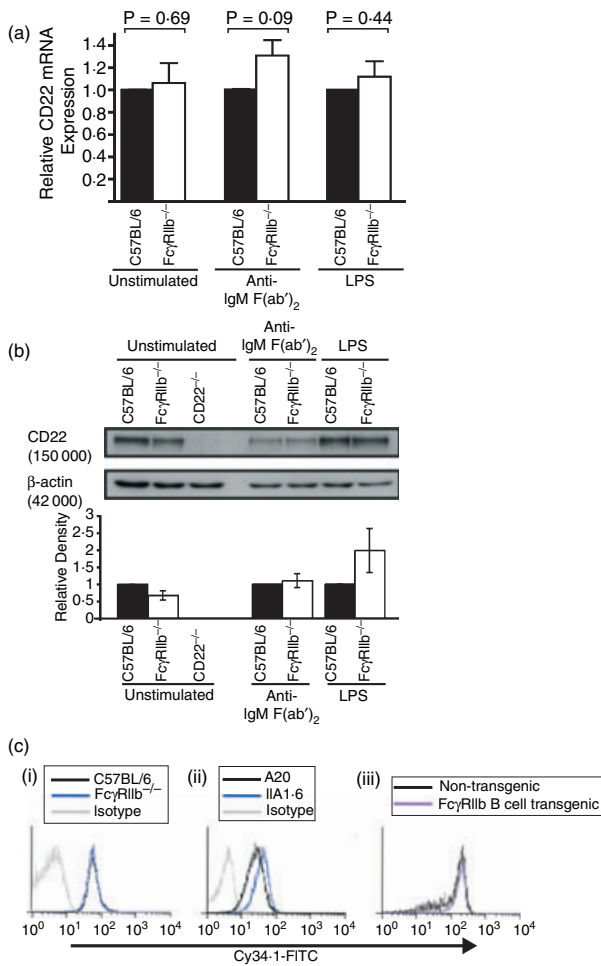


Figure 2. CD22 expression as assessed by semi-quantitative real-time polymerase chain reaction (SQRT-PCR), Western blot and monoclonal antibody (mAb) Cy34.1 is not affected by expression of Fc γ RIIb. To determine CD22 expression at the messenger RNA (mRNA) and protein levels, B cells were purified by magnetic cell sorting and cultured with anti- μ F(ab')₂ or lipopolysaccharide (LPS) for 48 hr. Levels of CD22 mRNA were quantified (relative to GAPDH) using SQRT-PCR, and expressed relative to controls for each stimulation condition (a). CD22 protein was detected by Western blotting and normalized to a β -actin loading control by densitometry (b). Binding of the anti-CD22 mAb Cy34.1, to B220⁺ splenocytes (i and iii), or B-cell lines (ii), was examined by flow cytometry (c). Results for SQRT-PCR and protein analysis are expressed as mean + SD for three independent experiments. *P*-values refer to a Student's *t*-test analysis.

is critically dependent upon *cis* interactions between CD22 and its ligand; α 2,6-linked sialic acid (α 2,6Sia).¹⁷ In the absence of α 2,6Sia, CD22 becomes more dispersed throughout the cell membrane and its binding site becomes 'unmasked' such that it becomes more available to bind exogenous ligands, such as synthetic sialoside probes.¹⁸ The mAb 2D6 binds to a more membrane-proximal epitope than Cy34.1,^{19,20} so one might envisage that accessibility to this epitope would be more sensitive

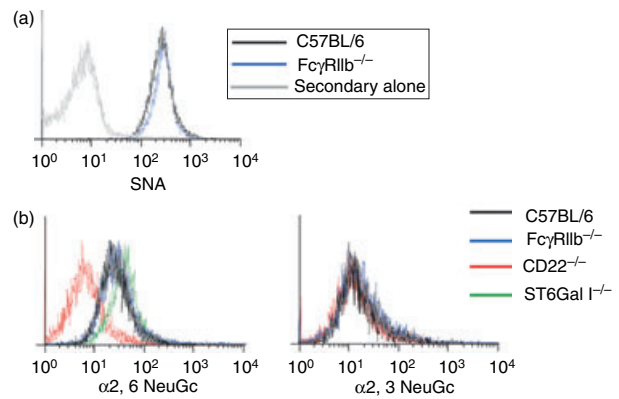


Figure 3. CD22 ligand abundance and masking of the CD22 binding site are unaffected by Fc γ RIIb expression. Splenocytes from Fc γ RIIb^{-/-}, CD22^{-/-} and ST6Gal I-deficient mice were incubated with biotinylated *Sambucus nigra* agglutinin (SNA) lectin (a) or sialoside probes (b), followed by allophycocyanin-conjugated streptavidin. Staining of B220⁺ cells was compared to C57BL/6 controls and traces are representative of cells from three mice in each case.

to CD22 clustering than that of Cy34.1. We therefore sought to establish whether clustering of CD22 was altered in the absence of Fc γ RIIb by examining two parameters: the abundance of CD22 ligand (α 2,6Sia) at the cell surface, and the ability of CD22 to bind exogenous sialoside probes. Using *Sambucus nigra* agglutinin, a lectin that binds specifically to α 2,6Sia, we observed that ligand abundance at the B-cell surface was unaffected by the absence of Fc γ RIIb (Fig. 3a). We next examined the ability of an α 2,6-linked synthetic sialoside (α 2,6 NeuGc) to bind to CD22 on Fc γ RIIb-deficient and control B cells (Fig. 3b). Binding of α 2,6 NeuGc to B cells was demonstrated to be specific for CD22, because only background levels of staining were achieved with CD22-deficient cells, and because binding of a negative control (α 2,3 NeuGc) was equivalent for cells of all genotypes tested. In accordance with published results,²¹ B cells from ST6Gal I knockout mice, which lack the enzyme required to produce the CD22 ligand, stained very strongly with the α 2,6-linked probe, indicating that CD22 on these cells is unmasked and available to interact with ligands delivered in *trans*. Less staining was observed in control C57BL/6 B cells, as has been described previously, and critically this was equivalent to the level of staining achieved in Fc γ RIIb-deficient cells. We can therefore conclude that masking of the CD22 ligand binding site is not affected by Fc γ RIIb and, as a result, it seems unlikely that changes in CD22 membrane localization are responsible for the differential binding of the two anti-CD22 mAbs.

The mAb 2D6 requires stabilization by Fc γ RIIb for optimal binding to CD22

Since optimal binding of 2D6, but not Cy34.1, to B cells is dependent upon the presence of Fc γ RIIb, we wondered

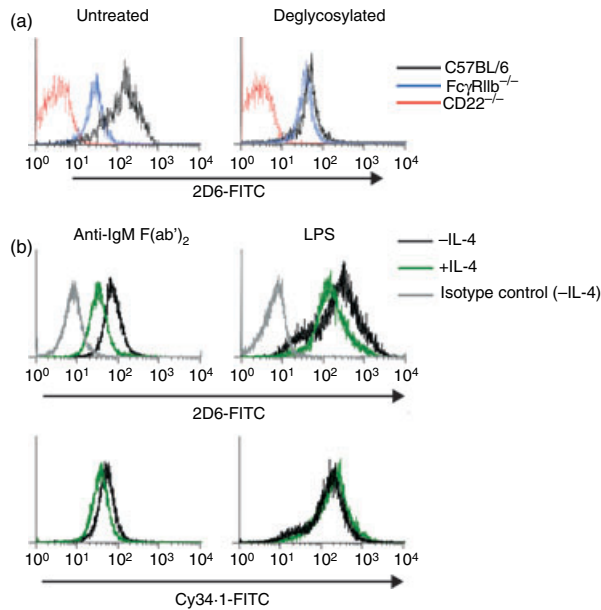


Figure 4. The monoclonal antibody (mAb) 2D6 interacts with FcγRIIb on B cells and gives rise to artefactual changes in CD22 expression upon interleukin-4 (IL-4) treatment. Binding of protein-*N*-glycosidase F-treated, or mock-treated, mAb 2D6 to FcγRIIb-deficient and control B220⁺ splenocytes was examined by flow cytometry (a). (b) Purified splenic B cells were stimulated with anti-μ F(ab')₂ or lipopolysaccharide (LPS) for 48 hr, in the presence or absence of 10 ng/ml IL-4 as indicated. Binding of mAbs 2D6 and Cy34.1 was assessed by flow cytometry. Traces are representative of cells from three mice in each case.

whether the Fc portion of mAb 2D6 might interact with FcγRIIb. This hypothesis appeared unlikely, because all staining was conducted in the presence of the FcγRII/III-blocking antibody 2.4G2, and 1% normal rat serum. Furthermore, staining of B cells with mAb 2D6 was clearly dependent upon the presence of its cognate antigen, because no staining was observed on CD22-deficient B cells (Fig. 4a, 'untreated'). However, the possibility remained that binding of 2D6 to CD22 could be stabilized through concomitant interaction of its Fc portion with FcγRIIb. Since rat IgG1 antibodies are notoriously difficult to fragment to high purity,²² we chose an alternative method to abrogate binding of this mAb to FcγRs. Removal of the glycan attached at Asn-297 of the Fc portion of antibodies completely prevents binding of IgG to Fc receptors.^{23,24} We therefore treated mAb 2D6 with protein-*N*-glycosidase F (PNGase F) to remove all the *N*-linked glycans, and compared its ability to bind FcγRIIb-deficient and control B cells with that of the mock-treated antibody. While binding of native, and PNGase F-treated, antibody was similar in FcγRIIb-deficient B cells, enzymatic treatment reduced staining of control B cells to a level that was approximately equivalent to that in FcγRIIb-deficient cells (Fig. 4a). Therefore,

binding of the Fc portion of mAb 2D6 to FcγRIIb accounts entirely for the apparent correlation of staining with FcγRIIb expression, and this FcγRIIb-dependent binding persisted when higher concentrations of the mAb 2D6 were used (data not shown).

Immunophenotyping with mAb 2D6 is affected by FcγRIIb expression

The effect of FcγRIIb on the binding of this mAb, and potentially others, has important implications for immunophenotyping. Changes in FcγR expression, in response to various physiological and experimental stimuli, may give rise to differential staining by certain antibodies, which could be incorrectly interpreted as changes in expression of the corresponding cell surface molecules. In light of this finding, we reconsidered the effect of IL-4 on CD22 expression on activated B cells. We have previously reported that IL-4 reduces CD22 expression, but that changes in CD22 mRNA were significantly less pronounced than those observed for other inhibitory receptors.¹⁰ We therefore re-examined expression of CD22 on activated B cells in the presence or absence of IL-4 using Cy34.1 mAb, which is unaffected by FcγRIIb expression (Fig 4b). We discovered that while CD22 measured by mAb 2D6 was apparently reduced in IL-4-treated cells, this was not the case when detected by mAb Cy34.1. Although a small 'true' difference in CD22 expression was observed in cells stimulated with anti-μ F(ab')₂, this was of a much smaller magnitude than that previously determined using mAb 2D6 (Fig. 4b and ref. 10). It is interesting to note, therefore, that while IL-4 has little impact on CD22 expression, it completely abolishes its function.¹⁰ This suggests another level at which the inhibitory activity of CD22 might be regulated, and is the focus of further investigation.

Discussion

In this study, we demonstrate that binding of the anti-CD22 mAb 2D6 to murine B cells correlates directly with FcγRIIb expression. This appears to be a consequence of stabilization of the mAb through interaction with FcγRIIb, because the effect is completely abrogated upon deglycosylation of the antibody. Nevertheless, we cannot completely rule out the possibility that stabilization of mAb may also involve a third-party molecule, whose expression or accessibility is affected by FcγRIIb. This result has important implications for the study of other cell surface molecules, because FcγRIIb expression is highly variable and may give rise to changes in mAb binding that could be incorrectly interpreted as changes in expression or conformation of the corresponding cell surface molecules. Unlike common staining artefacts, where antibody binds directly to FcγRs, the effect that we

observe is antigen specific, and absolutely dependent on the presence of CD22. It would therefore appear that co-expression of FcγRIIb on the cell surface stabilizes the interaction of 2D6 with CD22, shifting the binding equilibrium to favour a greater degree of staining. Even at higher concentrations of 2D6, maximal staining cannot be achieved in the absence of FcγRIIb. It is not entirely clear why this should be so, and why FcγRIIb expression does not affect Cy34.1 binding in the same way. One possibility is that mAb 2D6 has a particularly low affinity for CD22, and is therefore highly susceptible to the increase in avidity afforded by binding to the Fc receptor. However, the affinity of this antibody for CD22 has been estimated at $1.2 \times 10^8 \text{ M}^{-1}$,²⁵ well within the normal range for mAbs. Nevertheless, FcγRIIb does appear to be a limiting factor for 2D6 binding and this effect appears to be 'dose-dependent'. Remarkably, over a range of FcγRIIb densities, from zero to the 10-fold excess (relative to controls) expressed by FcγRIIb transgenic B cells, we do not appear to reach a level at which the effects of FcγRIIb become saturating. This would indicate that CD22 is present in substantial excess on the cell surface. Consistent with this prediction, the stoichiometry has been estimated at 10 : 1 CD22 molecules to FcγRIIb, though this is likely to be an underestimate given that it was obtained using mAb 2D6 (which from the data presented in this paper would be expected to bind only a fraction of the total CD22 molecules present on the cell surface).^{25–27}

This example of FcγRIIb-dependent mAb binding is not an isolated one. A similar binding pattern was observed with an anti-CD72 mAb,²⁸ and this was interpreted to be the result of the creation of a 'neo-epitope' that is influenced by FcγRIIb. It seems likely, however, that this was in fact the result of FcγRIIb-dependent binding of the anti-CD72 mAb, because the effect occurred on resting cells (in the absence of CD72 : FcγRIIb colocalization) and because binding of a F(ab')₂ fragment to B cells showed no dependence on FcγRIIb expression. While these examples relate to murine B cells, and the role of FcγRIIb, it is likely that this phenomenon is more widespread, extending to multiple cell types and involving other members of the Fc receptor family. It would seem prudent that immunophenotyping of surface antigens be verified using an alternative reagent, or independent method, where possible.

Our results are also relevant to the design of therapeutic mAbs, because interaction of these agents with Fc receptors is critical for their efficacy.²⁹ Many therapeutic mAbs recruit FcγR-expressing effector cells to facilitate the elimination of target cells through mechanisms such as phagocytosis and antibody-dependent cell-mediated cytotoxicity. Consistent with these functions, the effectiveness of anti-tumour mAb therapy and of B-cell depletion by rituximab (anti-CD20) in the treatment of systemic lupus erythematosus correlates with FcγR genotype.^{30–33}

Numerous attempts have therefore been made to develop mAbs with enhanced FcγR affinity, and these modifications have been accompanied by improvements in the efficacy of these therapies.^{34–37} Our results show that the degree of binding of mAbs to cell surface antigens may vary up to 10-fold depending on the level of expression of FcγRIIb. This would be expected to impact on the therapeutic efficacy of such antibodies, especially when binding to circulating cells, and may also influence the effect of relevant autoantibodies (e.g. anti-lymphocyte antibodies in systemic lupus erythematosus and anti-neutrophil antibodies in immune-mediated neutropenia^{38,39}). This should be borne in mind when developing and assessing therapeutic mAbs.

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Conflict of interest

The authors declare that they have no competing financial interests.

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