Authentic IgM Fc Receptor (FcμR)

Hiromi Kubagawa, Christopher M. Skopnik, Jakob Zimmermann, Pawel Durek, Hyun-Dong Chang, Esther Yoo, Luigi F. Bertoli, Kazuhito Honjo and Andreas Radbruch

Abstract Since the *bona fide* Fc receptor for IgM antibody (FcμR) was identified eight years ago, much progress has been made in defining its biochemical nature, cellular distribution, and effector function. However, there are clearly conflicting results, especially about the cellular distribution and function of murine FcμR. In this short article, we will discuss recent findings from us and other investigators along with our interpretations and comments that may help to resolve the existing puzzles and should open new avenues of investigation.

Contents

H. Kubagawa (✉) · C.M. Skopnik · J. Zimmermann · P. Durek · H.-D. Chang · A. Radbruch
Deutsches Rheuma-Forschungszentrum in Berlin, 10117 Berlin, Germany
e-mail: Hiromi.Kubagawa@drfz.de

E. Yoo
Department of Microbiology, Immunology and Molecular Genetics,
University of California, Los Angeles, CA 90095, USA

L.F. Bertoli
Brookwood Medical Center, Birmingham, USA

K. Honjo
Department of Medicine, University of Alabama at Birmingham,
Birmingham, AL 35209, USA

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1 Introduction

It has been established from studies of mutant mice deficient in IgM secretion that both preimmune "natural" IgM and antigen-induced "immune" IgM are important in responses to pathogens and self-antigens (Ehrenstein and Notley 2010). Effector proteins interacting with the Fc portion of IgM, such as complement and its receptors, have so far mainly been elucidated in the context of IgM-mediated immune protection and regulation (Heyman 2000) (see also Chap. 4 by Anna Sörman and Birgitta Heyman). The role of IgM Fc receptor (FcμR) in such effector functions has just begun to be explored, since the *FCMR* was identified in 2009 (Kubagawa et al. 2009). In this chapter, recent findings about the FcμR in both humans and mice are reviewed, along with emphasis on its significance and the discrepancies among different reports especially in murine studies. Several review articles on FcμR and its ligand IgM have already been published elsewhere, and the authors recommend them for further information on both of these topics (Baumgarth 2016; Ehrenstein and Notley 2010; Klimovich 2011; Kubagawa et al. 2014a, c; Panda and Ding 2015; Wang et al. 2016).

2 Lymphocyte-Restricted Expression of FcμR

The cellular distribution of FcμR in mice is still not completely resolved. In our studies, FcμR expression at *the level of transcription* is only detectable in B-lineage cells and not in other cell types. This was investigated by reverse transcriptase polymerase chain reaction (RT-PCR) using RNAs from the following tissue/cell samples. (i) Splenocytes or liver tissues of mice deficient for recombination-activating gene 1 (*Rag1*), which are devoid of B and T cells but contain abundant granulocytes and macrophages and (ii) CD19-positive B or CD19-negative non-B cells and Gr1- or Ly6G-positive myeloid cells enriched twice to high purity by fluorescence-activated cell sorting (FACS) from wild-type (WT) mouse bone marrow (BM) and spleen (Honjo et al. 2012a, 2013). By contrast, Choi et al. (2013) using a similar RT-PCR analysis of FACS sorted cells found clear expression of FcμR by the splenic Gr1-positive cell population in addition to B cells, but curiously, total BM cells, which should contain more abundant Gr1-positive myeloid cells than B cells, did not express significant levels of FcμR. The B cell-restricted expression pattern of FcμR is also documented in two large-scale expression databases of immune cells: Immunological Genome Project (https://www.immgen.org) and Reference Database of Immune Cells (http://refdic.rcai.riken.jp/welcome.cgi). Intriguingly, however, another study has recently reported that by single-cell RNA sequencing along with complex algorithmic assessments and its functional annotation, FcμR is suggested as one of the critical regulators of Th17 pathogenicity in myelin oligodendrocyte glycoprotein (MOG)-induced autoimmune encephalomyelitis (EAE) (Gaublomme et al. 2015). In our studies using the T cell

transfer colitis model in *Rag1*$^{-/-}$ mice, however, none of the sorted T cell subpopulations with the phenotype of IL-17^{+}, IFNγ^{+}, or IL-17^{+}/IFNγ^{+} express FcμR transcripts as determined by gene array analysis (Zimmermann et al. unpublished). In this regard, transcriptome analysis of even homogenously isolated cell populations (e.g., Th17 cells) is a mixed snapshot of asynchronously propagated, metabolically heterogeneous cell populations. On the other hand, single-cell RNA sequence analysis along with multiple algorithmic assessments may distinguish distinct states of cells within such populations. Even based on single-cell RNA sequencing data, however, it is difficult to distinguish whether such a small subpopulation of Th17 cells may indeed express full-length FcμR transcripts or may passively acquire them through exosomes or membrane vesicles from FcμR-bearing B cells via trogocytosis (Gyorgy et al. 2011). It will also be difficult to prove formally that such a minor population of Th17 cells expresses functional FcμR at low levels on their cell surface and plays a major regulatory role in the pathogenesis of MOG-induced EAE. The experimental data leading to this idea were based on the ex vivo data of Th17-polarizing cells from *Fcmr*-deficient (KO) and WT mice. This issue will be further discussed in the section of *Fcmr* KO mice (Sect. 4).

With respect to *the cell surface expression* of FcμR using receptor-specific monoclonal antibodies (mAbs) and IgM-ligand binding, we and others have found that the expression of FcμR is restricted to adaptive immune lymphocytes: both B and T cells and, to a lesser extent, natural killer (NK) cells in humans (Kubagawa et al. 2009; Murakami et al. 2012) and only B cells in mice (Honjo et al. 2012a, 2013; Lapke et al. 2015; Ouchida et al. 2012; Shima et al. 2010). [NK cells are the only known non-adaptive immune cell in humans to express FcμR, but this cell is now thought to have both adaptive and innate immune cell features (Vivier et al. 2011)]. This lymphocyte-restricted expression suggests a distinct function of FcμR compared to FcRs for switched Ig isotypes (e.g., FcγRs, FcεRI, and FcαR), which are also expressed by various innate immune cells. The species difference in cellular distribution of FcμR indicates that the results from murine FcμR studies may not necessarily reflect the human situation. Given the fact that IgM is the first Ig isotype appearing during phylogeny, ontogeny, and immune responses and that IgM is considered as a first line of defense against infection, the lymphocyte-restricted expression pattern is somewhat unexpected and may have some functional significance. In this regard, it is also noteworthy that unlike the phylogenetically broad distribution of IgM from jawed vertebrates (i.e., cartilaginous fish) onward, analysis of currently existing genomic sequence databases indicates that the IgM FcR first appears in early reptiles and is found in all three major living (extant) groups of mammals (i.e., egg laying, marsupial and placental mammals) (Akula et al. 2014) (also see Chap. 1 by Srinivas Akula and Lars Hellman).

Contrary to the B cell-restricted expression pattern in mice described in the above-mentioned studies, other groups using a rat mAb (B68 clone) against mouse Toso, an original name of FcμR (Hitoshi et al. 1998; Kubagawa et al. 2015), reported the weak but "functional relevant expression" of FcμR by myeloid cells, dendritic cells (DCs), and T cells (Brenner et al. 2014; Lang et al. 2013a, b; Nguyen

et al. 2011). Strangely, in their analyses, Ly6G-positive BM myeloid cells were weakly positive for B68 mAb staining, whereas Ly6G-negative cells, which should contain abundant B-lineage cells, were completely negative, suggesting a non-optimal assessment with this mAb (Lang et al. 2013a, b). In fact, Lapke et al. (2015) have recently demonstrated that the expression of Toso/FcμR in mice is restricted to B cells using the same B68 and additional A96 mAbs, consistent with the results from our analysis using a panel of five different mAbs (MM1, MM2, MM3, MM4, and MM6 clones) (Honjo et al. 2012a, 2013). Notably, most studies dealing with the predicted function of FcμR in granulocytes, monocyte/macrophages, and DCs were based on comparative analysis in adoptive transfer experiments of WT *versus Fcmr* KO BM cells (Brenner et al. 2014; Lang et al. 2013a, b, 2015) and not on any actual convincing data of the cell surface FcμR expression by non-B cells, hence the use of the phrase "functional relevant expression." Apart from this, there is another concern regarding the specificity or cross-reactivity of several commercially available polyclonal or monoclonal reagents raised against synthetic peptides of both human and mouse FcμRs, which might account for the reported expression of Toso/FcμR by non-hematopoietic cell types such as pancreatic ß cells (Dharmadhikari et al. 2012).

The lymphocyte-restricted expression pattern of FcμR is thus distinct from the expression of other IgM-binding receptors. Polymeric Ig receptor (pIgR) is predominantly expressed by mucosal epithelial cells (Kaetzel 2005), and FcR for IgA and IgM (Fcα/μR) is expressed by follicular dendritic cells and other cell types including Paneth cells in small intestinal crypts, the proximal tubular epithelial cells in kidneys, and the serous acini and small epithelial cells of salivary glands (Kikuno et al. 2007).

Several types of stimulations or conditions have been shown to modulate cell surface expression of FcμR. Upon antigen receptor ligation with antibodies or phorbol myristate acetate stimulation, FcμR expression in humans is up-regulated on B cells but is down-modulated on T cells, suggesting differential regulation of FcμR expression during B cell and T cell activation (Kubagawa et al. 2009; Nakamura et al. 1993; Sanders et al. 1987). Stimulation of T and NK cells with IL-2 in vitro also down-regulates FcμR expression in a STAT5-dependent manner (Murakami et al. 2012). In diseases, the enhanced expression of FcμR is a hallmark of chronic lymphocytic leukemia (CLL) B cells, as first demonstrated many years ago by rosette formation with IgM-coated erythrocytes (Ferrarini et al. 1977; Pichler and Knapp 1977), followed by IgM-ligand binding (Ohno et al. 1990; Sanders et al. 1987), gene expression (Catera et al. 2008; Pallasch et al. 2008; Proto-Siqueira et al. 2008; Rosenwald et al. 2001; Wang et al. 2004), and receptor-specific mAbs (Li et al. 2011; Vire et al. 2011). Intriguingly, surface FcμR levels are also significantly elevated in the non-CLL B cells and T cells in CLL patients (Li et al. 2011). Immunotherapies targeting for the FcμR have been designed for CLL cells. One is an immunotoxin-coupled IgM Fc (Cμ2-Cμ4) (Vire et al. 2014), and another is chimeric antigen receptor-modified T cells using a single-chain fragment (scFv)-containing the variable regions of an anti-FcμR mAb (6B10) (Faitschuk et al. 2016). In both cases, patient CLL B cells appear to be

selectively eliminated in vitro without affecting the non-leukemic B and T cells. In patients with selective IgM immunodeficiency, cell surface FcμR levels on a particular blood B cell subset with a marginal zone (MZ) phenotype ($IgM^+/IgD^+/CD27^+$) are significantly diminished as compared to age-matched controls, but the molecular basis for this reduction remains unclear (Gupta et al. 2016).

3 Unique Ligand-Binding Activity

3.1 Fcμ-Specificity, Ligand-Binding Avidity, and Glycosylation

After identifying the FcμR cDNA from human B-lineage cell-derived cDNA libraries by a functional cloning strategy (i.e., IgM-ligand binding), cell lines stably expressing FcμR have mainly been used to investigate their ligand-binding specificity. The FcμR-bearing cells clearly bind IgM in a dose-dependent manner, but not other Ig isotypes (i.e., IgG1-4, IgA1-2, IgD, or IgE) (Kubagawa et al. 2009). The inability of FcμR to bind polymeric IgA clearly indicates that FcμR is distinct from pIgR and Fcα/μR, both of which bind IgM and polymeric IgA and are clustered within the *FCMR* locus on chromosome 1q32.2. [Recent domain swapping analysis has revealed that unlike pIgR, Fcα/μR can bind J chain-deficient IgM hexamers (Yoo et al. 2011); hence, all three IgM-binding receptors are different in terms of ligand-binding specificity]. Binding of IgM by FcμR is mediated by its Fc5μ fragments, consisting mostly of Cμ3/Cμ4 domains, but not by Fabμ fragments, thereby confirming its IgM Fc-binding specificity (Kubagawa et al. 2009; Murakami et al. 2012). Recent domain swapping analysis reveals the Cμ4 as the target of FcμR (Lloyd et al. 2017). By Scatchard plot analysis assuming a 1:1 stoichiometry of FcμR to IgM ligand, FcμR binds IgM pentamers with a strikingly high avidity of $\sim$10 nM. This in turn suggests that serum IgM, the concentration of which is $\sim$1 μM, constitutively binds FcμR on the surface of B, T, and NK cells and explains why detection of cell surface FcμR is enhanced by preculture of cells, especially T cells, in IgM-free media (Kubagawa et al. 2009; Nakamura et al. 1993). In this regard, Vire et al. (2011) found that FcμR on CLL B cells was rapidly internalized upon IgM binding and shuttled to the lysosomes for degradation. The configuration of IgM is also important for FcμR binding, as higher concentrations (>100-fold) are required for binding of monomeric IgM to the $FcμR^+$ cells than pentameric IgM. In addition to IgM pentamer, J chain-deficient IgM hexamers are also present in normal sera, albeit at unknown concentrations, but the complement activation activity of the IgM hexamers is 50- to 100-fold higher than IgM pentamers (Randall et al. 1992; Wiersma et al. 1998). Intriguingly, our preliminary findings show that the dissociation constant (K_D) of a recombinant IgM hexamer for FcμR is only twofold to threefold higher than that of IgM pentamers (Fig. 1).

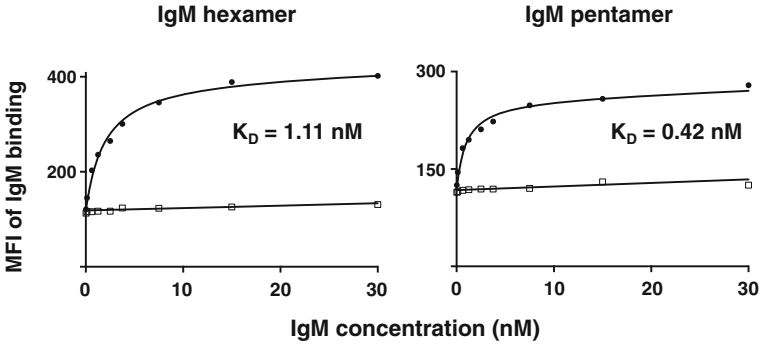

Fig. 1 Binding of hexameric and pentameric IgM to FcμR. An equal mixture of murine thymoma line BW5147 stably expressing both FcμR and green fluorescent protein (GFP) (•), and WT ($Fc\mu R^-/GFP^-$) control cell line (□) was incubated with various concentrations (25 pM–30 nM) of recombinant hexameric (*left* IgM-αTp) or pentameric (*right* rIgM) human IgM ligands, the preparation and purity of which were described elsewhere (Yoo et al. 2011) and were confirmed prior to use in the present studies. The bound IgM was assessed by addition of phycoerythrin-labeled goat antibodies specific for human μ heavy chain, followed by flow cytometry. The mean fluorescent intensity (MFI) of IgM binding at each concentration was plotted, and the K_D was calculated by nonlinear regression analysis using GraphPad Prism software. Two independent experiments yielded similar K_D values (nM), and one of them is shown

FcμR does not have N-linked glycosylation motifs (NxS/T; x indicates any amino acid) in the extracellular region, consistent with our previous biochemical characterization of the IgM-binding protein (Ohno et al. 1990). Since the core peptide of FcμR is predicted to have a M_r of ∼41 kDa and the FcμR expressed on B and T cells has a M_r of ∼60 kDa, one-third of the M_r of the mature FcμR is thus made up of O-linked glycans. Potential glycosylation sites were determined by mutagenesis experiments: Thr residues at positions of 161, 164, 165, 181, 182, and 185 and Ser at 178 and 179 (Vire et al. 2011). Removal of sialic acids from $Fc\mu R^+$ cells by neuraminidase treatment slightly enhanced IgM-ligand binding, suggesting that desialylated FcμR has better ligand-binding activity (Kubagawa et al. 2009). Notably, Colucci et al. have recently shown that natural IgM, which is rich in terminal sialic acid residues, is internalized by T cells in humans and inhibits T cell responses such as anti-CD3/anti-CD28 mAbs- or PHA-mediated proliferation ex vivo and expression of pro-inflammatory cytokine genes. In contrast, desialylated natural IgM is not internalized and has poor inhibitory activity (Colucci et al. 2015). It remains to be elucidated how carbohydrate moieties of FcμR and its IgM ligands affect their interaction, as has been well documented in the case of FcγRs and IgG ligands (Pincetic et al. 2014; Schwab and Nimmerjahn 2013). In this regard, it has recently been reported that IgM binds FcμR and is internalized irrespective of its glycosylation (Lloyd et al. 2017).

3.2 *Cis Engagement*

In addition to the above findings of ligand binding in solution, a unique ligand-binding property of FcμR was found in the assay system using cell surface-attached IgM such as an agonistic IgM anti-Fas mAb (CH11 clone). Apoptosis-prone human Jurkat cells stably expressing FcμR were shown to be protected from Fas-/CD95-mediated apoptosis when ligated with the IgM anti-Fas mAb (Hitoshi et al. 1998), but not when ligated with an agonistic IgG anti-Fas mAb or Fas ligand (Honjo et al. 2012b; Kubagawa et al. 2009; Murakami et al. 2012). Notably, co-ligation of FcμR and Fas with the corresponding IgG mAbs plus a common secondary reagent [e.g., $F(ab')_2$ fragments of anti-mouse γ Ab] had no inhibitory effects on the IgG anti-Fas mAb-induced apoptosis (Kubagawa et al. 2009). This suggests that the anti-apoptotic activity of FcμR depends on usage of the IgM anti-Fas mAb, and not on physical proximity of two receptors by artificial co-ligation as observed in immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing receptors such as FcγRIIb (Ravetch and Lanier 2000) and paired Ig-like receptor of inhibitory isoform (PIR-B) (Bléry et al. 1998).

To determine how FcμR protects from apoptosis induced by IgM anti-Fas mAb, we added various concentrations of soluble IgM or its immune complexes as inhibitors into the apoptosis assays. The results are summarized in a cartoon fashion in Fig. 2. Addition of IgM anti-Fas mAb at 10 ng/ml induced robust apoptosis of FcμR-negative WT control, but not of FcμR-positive Jurkat cells.

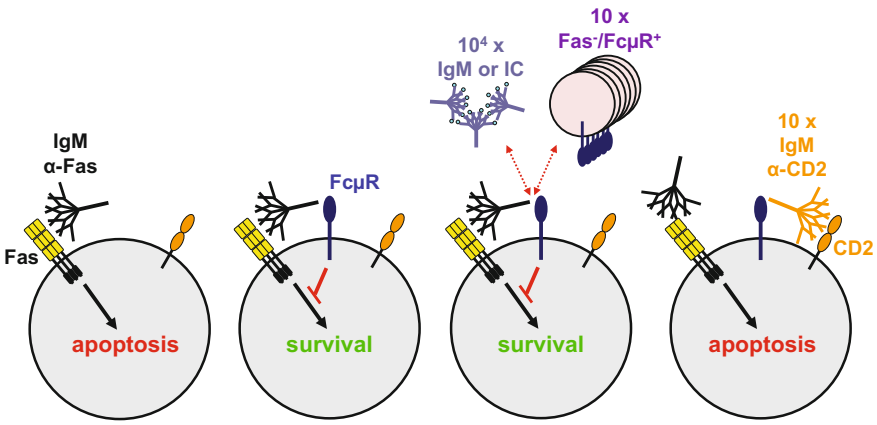

Fig. 2 Cis engagement of FcμR. Ligation of Fas/CD95 death receptor trimer (*yellow*) with agonistic IgM anti-Fas mAb (*black broom shape*) induces apoptosis in WT Jurkat cells (1st *gray circle*), but not in FcμR (*blue tennis racket shape*)-positive Jurkat cells (2nd). This FcμR-mediated protection is not blocked by addition of 10^4 molar excess of IgM or its immune complexes (*pale blue broom*) or tenfold excess of FcμR-positive, Fas-negative cells (pale purple small circles), suggesting an efficient *cis* interaction of IgM Fas mAb and FcμR on the same cell surface, but not a *trans* interaction between neighboring cells (3rd). Addition of tenfold excess of IgM mAb (*orange broom*) reactive with CD2 (*orange peanut-shell shape*) on Jurkat cells can efficiently block the interaction of IgM Fas mAb and FcμR, resulting in apoptosis (4th)

This FcμR-mediated protection was found to occur in *cis,* but not in *trans,* interactions of the Fc portion of IgM anti-Fas mAb with FcμR, because addition of excessive FcμR-positive/Fas-negative cells did not diminish the protection (Honjo et al. 2015). Addition of more than 10,000 molar excess of IgM Ab or its immune complexes (e.g., 1-21 IgM anti-α1-3 dextran mAb/α1-3 dextran) was required for partial, but significant, blockade of the *cis* interaction of the Fc portion of IgM anti-Fas mAb with FcμR. This suggests that the soluble IgM immune complexes are not potent competitors in the FcμR-mediated protection from apoptosis. When the IgM mAb reactive with CD2 (C373 clone) (Weiss and Stobo 1984) on the surface of Jurkat cells was employed as a potential competitor for the interaction of IgM Fas mAb with FcμR, a tenfold excess of IgM anti-CD2 mAb was sufficient to efficiently block the above *cis* interaction, thereby permitting the $FcμR^+$ cells to undergo apoptosis. [In particular, same results were also obtained with an IgM anti-TCR mAb (C305 clone)]. Collectively, these findings show that although FcμR binds soluble IgM pentamers at a high avidity of $\sim$10 nM, FcμR binds more efficiently to the Fc portion of IgM antibody when it is attached to a membrane component via its Fab region on the same cell surface. The preferential *cis* engagement of FcμR is thus distinct from the *trans* engagement of FcγRIIb, an inhibitory FcγR, in death receptor-mediated apoptosis. The interaction of agonistic IgG mAbs against death receptors, including Fas/CD95, with FcγRIIb is essential for the death receptor-mediated apoptosis and occurs in *trans,* but not in *cis* (Bando et al. 2002; Li and Ravetch 2011; Xu et al. 2003). The *cis* engagement of FcμR in turn implies that FcμR can modulate the functional activity of lymphocyte surface receptors or proteins recognized by either natural or immune IgM antibody.

3.3 Modulatory Effect of FcμR by Cis Engagement

The physiological relevance of the *cis* engagement of FcμR may be related to the unique features of IgM antibody, especially natural IgM antibody. Serum levels of IgM in mice raised in germfree conditions are similar to those in mice maintained under conventional or specific pathogen-free housing conditions (Hashimoto et al. 1978; Haury et al. 1997; Thurnheer et al. 2003). Two-thirds of the newly generated B cells in BM react with self-antigens such as double- or single-stranded DNA, insulin, and lipopolysaccharide (Wardemann et al. 2003). In our assessments, one-fourth of the IgM secreted from Epstein–Barr virus-transformed B cell lines derived from neonatal B cells reacts with lymphocyte surface components (unpublished observation). IgM anti-lymphocyte antibodies are often present in individuals with autoimmune diseases or chronic viral infections and recognize many different surface antigens (e.g., CD45, CD175/Tn, CD3ε, CD4, chemokine receptors, sphingo-sine-1-phosphate receptor 1), and some of those antibodies regulate T cell-mediated inflammatory responses in vitro (Cappione et al. 2004; Daniel et al. 1989; Koren et al. 1992; Liao et al. 2009; Lobo et al. 2008; Muller et al. 1994;

Silvestris et al. 1989; Warren et al. 1988; Winfield et al. 1997) (see also Chap. 5 by Peter Lobo). It is thus quite possible that these IgM antibodies reactive with lymphocyte surface components engage FcμR in a *cis* interaction on the shared membrane surface, thereby modulating the functional activity of lymphocyte surface antigens or receptors by FcμR (see Fig. 4).

To explore this possibility, we compared Ca^{2+} mobilization upon ligation of lymphocyte surface antigen alone with co-ligation of lymphocyte surface antigen plus FcμR (Honjo et al. 2015). In the *first* experiment, an equal mixture of WT (i.e., FcμR-negative/GFP-negative) and FcμR-positive/GFP-positive Jurkat cells was preloaded with Ca^{2+} dye and then simultaneously stimulated by IgM anti-CD2 mAb. As shown in Fig. 3a, the rise in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) occurred significantly faster in $Fc\mu R^+$ cells than in $Fc\mu R^-$ WT cells when ligated with the anti-CD2 mAb. In contrast, the ionomycin-induced $[Ca^{2+}]_i$ increase occurred at the same time in both cell types. These findings suggest that the co-ligation of CD2 and FcμR induces a more rapid increase in $[Ca^{2+}]_i$, presumably release from the intracellular store, than does the ligation of CD2 alone. In the *second* experiment, Ca^{2+} mobilization by freshly prepared blood B cells was assessed following stimulation with a mitogenic IgM anti-κ mAb (END-5C1 clone) in the presence of IgG2bκ anti-FcμR mAb with blocking (HM7 clone) or non-blocking (HM3 clone) activity for IgM-ligand binding (Kubagawa et al. 2014b). IgM anti-κ mAb-induced Ca^{2+} mobilization was the same in the absence or presence of FcμR non-blocking mAb. In contrast, the FcμR-blocking mAb diminished the IgM anti-κ mAb-induced Ca^{2+} mobilization of blood B cells (Fig. 3b), suggesting that FcμR provides a stimulatory signals upon B cell receptor (BCR) cross-linkage with IgM mAbs. Collectively, FcμR expressed on B, T, and NK cells may thus have a potential to modulate the function of target antigens or receptors when they are recognized by natural or immune IgM antibodies, on the same cell surface (see the model shown in Fig. 4).

3.4 *Key Residues in the Transmembrane and Cytoplasmic Tail for FcμR Function*

In the case of pairs of activating and inhibitory receptors with highly homologous ectodomains, such as FcRs, killer cell Ig-Like receptors (KIRs), and PIRs, a general receptor structural rule has become appreciated. Namely, when the ligand-binding α chain has a short cytoplasmic tail with no signal-transmitting potential, then it contains a charged residue in the transmembrane segment that facilitates non-covalent association with another transmembrane protein containing immunoreceptor tyrosine-based activation motifs (ITAMs). This association allows for the transmission of activating signals to cells, as seen with FcγRI, FcγRIII, FcαR, FcεRI, KIR2DS or KIR3DS, and PIR-A (Blank et al. 1989; Clevers et al. 1988; Ernst et al. 1993; Kubagawa et al. 1999; Morton et al. 1995; Ravetch 1994).

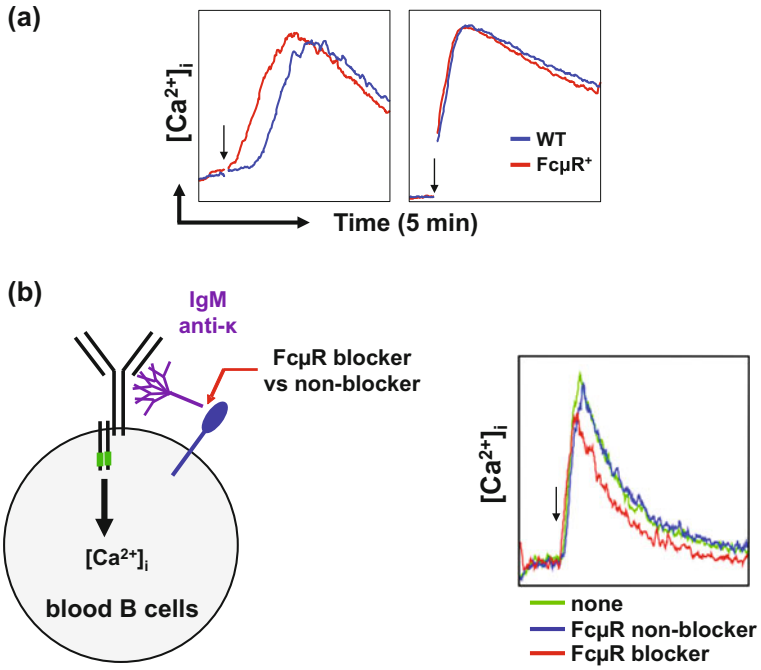

Fig. 3 Ca^{2+} mobilization by IgM mAbs against CD2 or Igκ. **a** An equal mixture of WT ($Fc\mu R^{-}$/GFP^{-}; *blue lines*) and $Fc\mu R^{+}$/GFP^{+} (*red lines*) Jurkat cells preloaded with the Ca^{2+} dye Indo-1/AM was stimulated with IgM anti-CD2 (373 clone) mAb at 10 μg/ml (*left*) or by 1 μM ionomycin (*right*) at the time points indicated by *arrows*. The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was assessed by the 405/485 nm fluorescence ratio in each viable cell population using an LSR II flow cytometer. Note that the $[Ca^{2+}]_i$ rise occurs faster in co-ligation of CD2 and FcμR than ligation of CD2 alone and that the ionomycin-induced $[Ca^{2+}]_i$ rise occurs at the same time in both cell types. **b** The experimental design is depicted in a cartoon fashion (*left*). Fluo-4-loaded blood B cells were treated with IgM anti-human Igκ mAb (END-5C1; 10 μg/ml) in the absence (*green line*) or presence of IgG2b, FcμR-blocking (HM7 clone; *red line*) mAb, or FcμR-non-blocking (HM3 clone; *blue line*) mAb (100 μg/ml) at the time point indicated by an *arrow*. The $[Ca^{2+}]_i$ levels were assessed by fluorescence intensity during a 5-min period. Note that FcμR provides a stimulatory signal upon BCR cross-linkage with IgM mAbs

By contrast, when the ligand-binding α chain has a long cytoplasmic tail, then it contains a conventional hydrophobic transmembrane segment and ITIMs in the cytoplasmic tail (Vély and Vivier 1997). Upon phosphorylation of ITIMs after receptor ligation, the ITIM recruits either the polyphosphate inositol 5-phosphatase or the tyrosine phosphatases 1 and 2 to attenuate signaling, as seen with FcγRIIb, KIR2DL or KIR3DL, and PIR-B.

Unlike this general consensus, FcμR is unusual in having both features (Fig. 5): a charged His residue (H^{253}) in the transmembrane segment and a long cytoplasmic tail containing three conserved Tyr (Y^{315}, Y^{366}, Y^{385}) and five conserved Ser (S^{283}, S^{299}, S^{359}, S^{368}, S^{382}) residues, when comparing seven different species (Kubagawa et al.

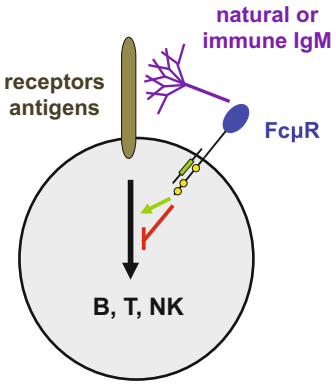

Fig. 4 Working hypothesis for FcμR function in humans. FcμR expressed on the plasma membrane of lymphocytes (B, T, and NK cells) consists of two subunits. The α chain (*blue badminton racket shape*) has ligand-binding activity via its Ig-like domain (head part) and signal-transducing ability via its conserved Tyr (three small *yellow circles*) and Ser (not shown) residues in the cytoplasmic tail. The other subunit termed the adaptor is non-covalently associated with the α chain and contains an ITAM (*green square*) signaling motif. (The molecular identity of this adaptor is presently unknown.) FcμR can either negatively or positively modulate the functional activity of lymphocyte surface protein/receptors (*brown sausage shape*) recognized by natural or immune IgM (*purple broom shape*) through the *cis* engagement

2009). The C-terminal Tyr matches the recently described Ig-tail tyrosine (ITT) motif (DYxN) (Engels et al. 2009; Engels and Wienands 2011), but the other two Tyr residues do not correspond to an ITAM ($D/Ex_2Yx_2L/Ix_{6\text{-}8}Yx_2V/I$), ITIM ($I/VxYx_2L/V$), or switch motif ($TxYx_2V/I$). Collectively, these characteristics suggest a dual signaling ability of FcμR: one from a potential as yet unidentified adaptor protein non-covalently associating with the FcμR via the H^{253} residue and the other from its own Tyr and/or Ser residues in the cytoplasmic tail (see Figs. 4 and 5).

To explore whether the aforementioned amino acid residues in FcμR are responsible for the receptor function, we made human FcμR cDNA constructs with point mutations (H253F, Y315F, Y366F, or Y385F) or a deletion of most of the cytoplasmic tail (A281–A390; ΔCy) and expressed them in Jurkat T cells. The results (Honjo et al. 2015) are summarized as follows (see Fig. 5). (i) Although non-mutated and mutated FcμR-bearing cells expressed comparable levels of cell surface FcμR as judged by receptor-specific mAbs, IgM-binding activity was significantly increased in the ΔCy mutant. [The ΔCy mutant lacks most of the cytoplasmic tail but includes an eight post-transmembrane basic amino acid-rich region (K^{273}–K^{280})]. Our subsequent data suggested that this enhancement was likely due to the formation of oligomeric FcμR due to its presumably mobile nature within the plasma membrane, rather than to the inside-out regulation of FcμR ligand binding by its cytoplasmic tail as seen in adhesion molecules (Kinashi 2005). (ii) The His^{253} residue was found to be important in the anchoring of FcμR in the plasma membrane. When examining the fate of IgM bound to FcμR by immunofluorescence microscopy, enhanced cap formation was clearly observed with the H253F mutant

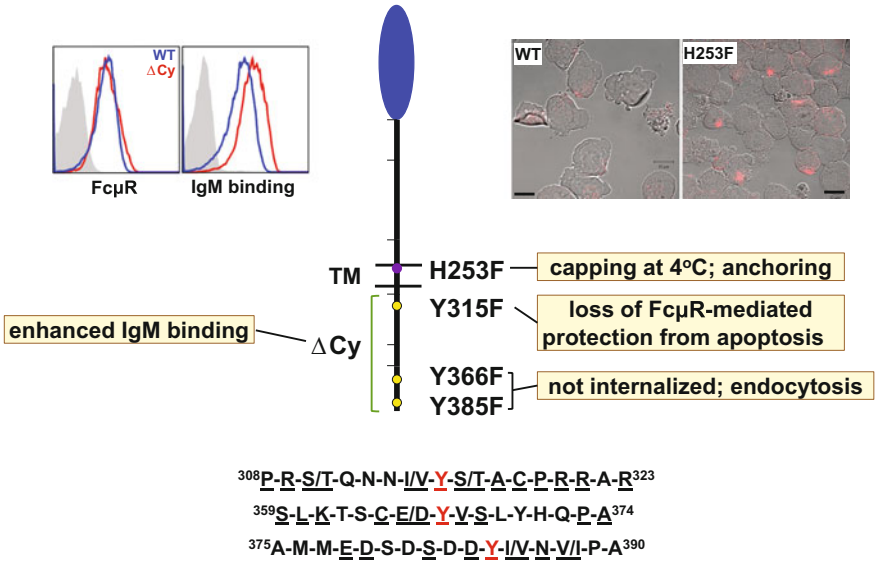

Fig. 5 Summary of FcμR mutational analysis. The human FcμR cDNA encodes a type I transmembrane protein that consists of a single V-set Ig-like domain (*blue oval shape*), an additional extracellular region (stalk) with no known domain structure, a transmembrane (TM) segment (between two *thick lines*) containing a charged His residue (*purple circle*), and a relatively long cytoplasmic tail containing three conserved Tyr residues (*yellow circles*). Point mutations are indicated, and the extent of the deletion of the cytoplasmic tail is shown by the green bracket. *Hatch marks* indicate exon boundaries in the *FCMR* gene. In representative flow cytometric profiles (*upper left*), cells stably expressing FcμR WT (*blue*) and ΔCy (*red*) were stained with biotin-labeled anti-FcμR (*open*) or isotype-matched control (*shaded*) mAb for cell surface expression of FcμR (*left panel*) and with biotin-IgM (*open*) or PBS (*shaded*) for ligand binding (*right panel*). Because profiles with control mAb or PBS were the same between FcμR WT and ΔCy cells, only one shaded profile is shown. Note the enhanced IgM binding by FcμR ΔCy cells as compared to FcμR WT cells, despite their equivalent levels of surface FcμR. In representative epi-fluorescence microscopic images (*upper right*), the FcμR WT (*left*) and H253F cells (*right*) were incubated with Alexa Fluor 555-IgM (without NaN_3) on ice, washed, and cytocentrifuged. Fluorescence images were combined with phase contrast cell images (scale bars = 10 μm). Altered phenotypes observed in mutant FcμR cells or potential function of the indicated residues are shown in the *yellow-filled boxes*. The unique sequences around three conserved Tyr residues are also shown with *underlines* indicating conserved amino acid residues

even at 4 °C as compared to the cells expressing non-mutant or other mutant FcμRs (except the ΔCy mutant), which exhibited a more broadly localized staining pattern. Notably, unlike other multi-chain FcRs, the FcμR H253F mutant was expressed on the surface of Jurkat cells without a potentially associated membrane protein. (iii) Consistent with the findings of Vire et al. (2011), the two C-terminal conserved Tyr residues were involved in receptor-mediated endocytosis. (iv) The FcμR-mediated protection from IgM anti-Fas mAb-induced apoptosis was significantly diminished in the Y315F and ΔCy mutants, as the frequency of apoptotic cells in these mutants was indistinguishable from those in $FcμR^-$ control cells.

This is of interest, given the unique sequence around the Y^{315} residue: ^{308}**P·R·S/T·**Q·N·N·**I/V·Y·S/T·A·C·P·R·R·**A· $\mathbf{R}^{323}$ (bold type indicates conserved amino acids). This does not match any known Tyr-based signaling motif. The mechanism of Toso-/FcμR-mediated protection from apoptosis was suggested to result from potentiation of the cellular FLIP [FADD-like IL-1ß-converting enzyme (FLICE)-like inhibitory protein], a master anti-apoptotic regulator (Hitoshi et al. 1998), or alternatively by prevention of internalization of Fas, an important step for apoptosis signaling (Vignaux et al. 1995; Yamauchi et al. 1996), owing to simultaneous cross-linkage of both Fas and FcμR with IgM Fas mAb (Murakami et al. 2012).

In addition to the mutational analysis, our previous findings indicated that ligation of FcμR with preformed IgM immune complexes induced the phosphorylation of both Tyr and Ser residues of the receptor. Intriguingly, the phosphorylated FcμR migrated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis faster than the unphosphorylated form, unlike most proteins, which usually run slower when phosphorylated (Kubagawa et al. 2009). It remains unclear whether the phosphorylation causes a global structural change of FcμR leading to increased mobility as seen, e.g., in CD45 on activated myeloid cells (Buzzi et al. 1992) or if proteolytic cleavage occurs in the cytoplasmic tail of FcμR after receptor ligation as observed in FcγRIIa on platelets (Gardiner et al. 2008). Ligation of FcμR on NK cells with IgM immune complexes was shown to lead to the phosphorylation of PLCγ2 and ERK1/2 (Murakami et al. 2012).

4 FcμR Deficiency in Mice

Fcmr KO mice have been independently generated by three different laboratories [K. H. Lee (Hannover Medical School, Hannover, Germany); H. Ohno (RIKEN, Yokohama, Japan); and T. W. Mak (Princes Margaret Cancer Center/University Health Network, Toronto, Canada)] and have been characterized by five different groups of investigators (Brenner et al. 2014; Choi et al. 2013; Honjo et al. 2014; Honjo et al. 2012a; Lang et al. 2013a; Nguyen et al. 2011; Ouchida et al. 2012). Clear differences in the reported phenotypes exist among these mice that are well summarized in a recent review article (Wang et al. 2016). While the basis for these differences requires further investigation, they could in part be attributed to: (i) different strategies for gene targeting (i.e., deletion of exon 4-7, exon 2-4 *versus* exon 2-8 and/or the absence *versus* presence of the *Neo* gene in the mouse genome); (ii) embryonic stem (ES) cells of C57BL/6 *versus* 129 origin as well as the extent of the 129 mouse-origin DNA around the *Fcmr* gene remaining after backcrossing onto C57BL/6. [In this regard, the region closely flanking the targeted gene, called the passenger genome, remains of donor origin and typically contains mutations called passenger mutations. Annotating these passenger mutations to the reported genetically modified congenic mice generated using 129-ES cells revealed that nearly all these mice possess multiple passenger mutations potentially influencing

the phenotypic outcome (Vanden Berghe et al. 2015)]; (iii) investigators' ideas regarding the cellular distribution of FcμR/Toso in B cells *versus* myeloid and T cells and its function as an IgM Fc-binding protein *versus* an anti-apoptotic protein; and/or (iv) other factors (e.g., age of the mice, environments including intestinal microbiota or reagents used). Nevertheless, the abnormal phenotypes commonly observed in *Fcmr* KO mice are as follows: (i) alterations in B-lineage cell subpopulations; (ii) dysregulation of humoral immune responses; and (iii) predisposition to autoantibody production (Choi et al. 2013; Honjo et al. 2012a; Ouchida et al. 2012). Notably, many of the abnormalities seen in *Fcmr* KO mice mirror those observed in mice deficient in IgM secretion, suggesting that FcμR is a critical sensor of secreted IgM. In *Fcmr* KO mice on autoimmune backgrounds, FcμR was shown to play important regulatory roles in: (i) the autoantibody production; (ii) the differentiation of MZ B cells into plasma cells; and (iii) the formation of Mott cells, aberrant plasma cells with intracytoplasmic Ig inclusion bodies (Honjo et al. 2014).

Apart from these mutant mice, the laboratory of N. Baumgarth has recently generated a mouse strain in which the second stalk region exon (exon 4) of *Fcmr* is flanked by loxP sites allowing the B cell-specific deletion of *Fcmr* by crossing with *Cre-Cd19* transgenic mice (Nguyen et al. 2017). Several interesting findings were obtained by comparative analysis between mice with such a B cell-specific FcμR deletion and control mice. FcμR directly interacts with membrane-bound IgM BCR in the *trans*-Golgi network of BM immature B cells, thereby regulating the surface expression of BCR and eventually resulting in limiting tonic BCR signaling. B cell-specific FcμR deficiency results in dysregulated spontaneous activation and differentiation of B-1 and B-2 cells and development of a lympho-proliferative disorder. This suggests that FcμR constrains BCR expression to regulate the fundamental homeostasis and biology of B cells. [It is worth noting, however, that deletion of exon 4 could still allow production of soluble form of FcμR by the mutant B cells via a reading frame shift in exon 5 (TM) (see hatch marks in Fig. 5). Such a soluble FcμR could have unexpected consequences on B cell function. In fact, we have identified another splice variant in CLL patients that results from the direct splicing of exon 4 to exon 6 (1st cytoplasmic), skipping exon 5. This splice event results in a reading frame shift in exon 6 and generates a novel 70-amino acid hydrophilic, carboxyl-terminal tail and the resultant soluble FcμR protein is clearly elevated in many patient's sera as determined by enzyme-linked immunosorbent assays (Li et al. 2011)]. Contrary to the above FcμR-mediated suppression of BCR signaling, FcμR has also been shown to enhance survival of mature B cells upon BCR cross-linkage ex vivo with $F(ab')_{2\gamma}$ anti-μ antibodies by activation of the non-canonical NF-κB pathway, but not upon CD40 ligation or LPS stimulation (Ouchida et al. 2015). Thus, FcμR may have a potential to transmit both positive and negative signals to cells.

Another difference in *Fcmr* KO mice between T. W. Mak and H. Ohno is that splenic B cells from our (the latter) *Fcmr* KO mice produce significantly less IL-10, but comparable amounts of IL-6, ex vivo upon stimulation with *Salmonella* bacteria or with ligands for Toll-like receptor 4 (TLR4), TLR7, or TLR9 (Fig. 6). Since B cell-derived IL-10 has been implicated as an important negative regulator of

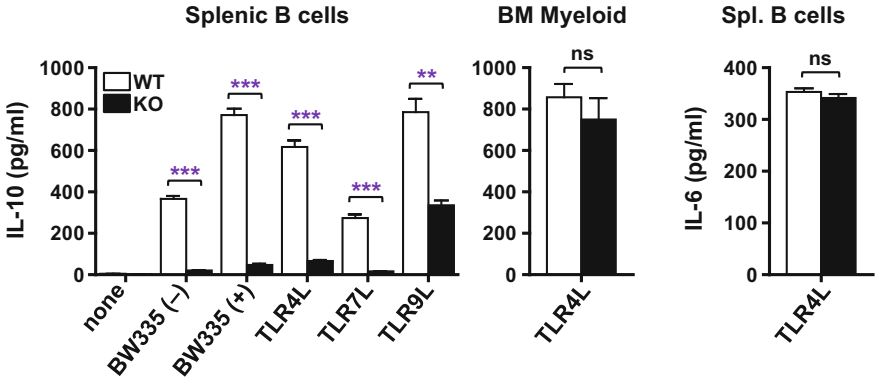

Fig. 6 Diminished IL-10 production by FcμR-deficient B cells. Splenic B (*left* and *right*) and bone marrow myeloid (*middle*) cells (4×10^5 cells) from sex and age-matched *Fcmr*-deficient (*black* columns) or littermate control (*white* columns) mice were cultured for 2 days in the absence (none) or presence of heat-killed, non-opsonized (−) or serum-opsonized (+) BW335, an LT2 strain of *Salmonella enterica* serovar Typhimurium (4×10^5 cfu), or the indicated TLR ligands (TLRL): LPS (10 μg/ml) for TLR4, Gardiquimod (1 μg/ml) for TLR7, and ODN1826 (2 μM) for TLR9. The concentration (pg/ml) of IL-10 (*left* and *middle*) and IL-6 (*right*) in the culture supernatants was assessed by ELISA in triplicate. The symbols ** and *** and "ns" indicate $p < 0.01$, $p < 0.001$, and "not significant," respectively, as assessed by Student's *t* test. *Note* (i) diminished production of IL-10, but not IL-6, by FcμR-deficient B cells and (ii) comparable production of IL-10 by marrow myeloid cells from both groups of mice. Identical results were obtained seven times for IL-10 and twice for IL-6, and representative experiments are shown

MOG-induced EAE (Fillatreau 2015), we thought that our *Fcmr* KO mice would be more *susceptible* than WT to MOG-induced EAE. However, the results reported by Brenner et al. (2014) suggest that this is not the case. Their *Fcmr/Toso* KO mice are *resistant* to MOG-induced EAE, because their *Fcmr/Toso* KO DCs are immature and tolerogenic and weak stimulators of inflammatory T cell responses. *Fcmr/Toso* KO Th17-polarizing cells secrete significantly less IL-17 and IL-10 than WT control mice (Gaublomme et al. 2015). Intriguingly, passive administration of a recombinant, soluble human FcμR–IgG fusion protein ameliorates MOG-induced EAE in WT mice (Brenner et al. 2014). The basis for this discrepancy (susceptible *versus* resistant) remains unclear at the moment, because we have never examined the susceptibility of our *Fcmr* KO mice to MOG-induced EAE and Brenner et al. have never assessed IL-10 production by B cells in their *Fcmr/Toso* KO mice. A side-by-side analysis of these two different strains of *Fcmr/Toso* KO mice would facilitate the resolution of these conflicting results, and it is highly likely that this discrepancy results from different strategies for gene targeting (deletion of exon 2-4 without *Neo* for ours *versus* deletion of exon 2-8 with remaining of *Neo* in the mouse genome for Brenner et al.).

5 Epilogue

FcRs for switched Igs are expressed by many different cell types, including myeloid cells, and are considered to be central mediators coupling innate and adaptive immune responses. Rewardingly, much of the knowledge gained from studies of these FcRs has been translated into clinical fields. On the other hand, the long elusive IgM FcR was finally identified eight years ago by functional cloning. However, since the cloned FcμR cDNA was identical to the cDNA-encoding TOSO or Fas apoptosis inhibitory molecule 3 (FAIM3), which was also previously identified by functional cloning as a potent inhibitor for Fas-mediated apoptosis, there have been spirited debates regarding the real function of this receptor, Fcμ-binding *versus* anti-apoptotic activity. Notably, there is now a general consensus that TOSO/FAIM3 is an authentic Fcμ-binding protein and not a Fas inhibitory protein per se (Kubagawa et al. 2015). Several interesting findings about the FcμR have recently been reported: FcμR binds more efficiently to the Fc portion of IgM antibody when it is attached to a membrane component via its Fab region on the same cell surface (i.e., *cis* interaction) than to the Fc portion of IgM in solution (*trans* interaction). FcμR directly interacts with membrane-bound IgM BCR in the *trans*-Golgi network of BM immature B cells, thereby regulating the surface expression of IgM BCR and eventually resulting in limiting tonic BCR signaling. FcμR can also regulate the differentiation of MZ B and B1 cells. By contrast, FcμR may enhance survival of mature B cells upon BCR cross-linkage via activation of the non-canonical NF-κB pathway. Immunotherapy targeting the FcμR is now designed for CLL cells. Many conflicting results still exist, but we hope that this short article may help to resolve these existing puzzles and will open new avenues of investigation.

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References

Akula S, Mohammadamin S, Hellman L (2014) Fc receptors for immunoglobulins and their appearance during vertebrate evolution. PLoS ONE 9:e96903

Bando M, Miyake Y, Shiina M, Wachi M, Nagai K, Kataoka T (2002) Actin cytoskeleton is required for early apoptosis signaling induced by anti-Fas antibody but not Fas ligand in murine B lymphoma A20 cells. Biochem Biophys Res Commun 290:268–274

Baumgarth N (2016) B-1 cell heterogeneity and the regulation of natural and antigen-induced IgM production. Front Immunol 7:324

Blank U, Ra C, Miller L, White K, Metzger H, Kinet JP (1989) Complete structure and expression in transfected cells of high affinity IgE receptor. Nature 337:187–189

Bléry M, Kubagawa H, Chen CC, Vely F, Cooper MD, Vivier E (1998) The paired Ig-like receptor PIR-B is an inhibitory receptor that recruits the protein-tyrosine phosphatase SHP-1. Proc Natl Acad Sci USA 95:2446–2451

Brenner D, Brustle A, Lin GH, Lang PA, Duncan GS, Knobbe-Thomsen CB, St PM, Reardon C, Tusche MW, Snow B, Hamilton SR, Pfefferle A, Gilani SO, Ohashi PS, Lang KS, Mak TW (2014) Toso controls encephalitogenic immune responses by dendritic cells and regulatory T cells. Proc Natl Acad Sci USA 111:1060–1065

Buzzi M, Lu L, Lombardi AJ Jr, Posner MR, Brautigan DL, Fast LD, Frackelton AR Jr (1992) Differentiation-induced changes in protein-tyrosine phosphatase activity and commensurate expression of CD45 in human leukemia cell lines. Cancer Res 52:4027–4035

Cappione AJ, Pugh-Bernard AE, Anolik JH, Sanz I (2004) Lupus IgG VH4.34 antibodies bind to a 220-kDa glycoform of CD45/B220 on the surface of human B lymphocytes. J Immunol 172:4298–4307

Catera R, Silverman GJ, Hatzi K, Seiler T, Didier S, Zhang L, Herve M, Meffre E, Oscier DG, Vlassara H, Scofield RH, Chen Y, Allen SL, Kolitz J, Rai KR, Chu CC, Chiorazzi N (2008) Chronic lymphocytic leukemia cells recognize conserved epitopes associated with apoptosis and oxidation. Mol Med 14:665–674

Choi SC, Wang H, Tian L, Murakami Y, Shin DM, Borrego F, Morse HC III, Coligan JE (2013) Mouse IgM Fc receptor, FCMR, promotes B cell development and modulates antigen-driven immune responses. J Immunol 190:987–996

Clevers H, Alarcon B, Wileman T, Terhorst C (1988) The T cell receptor/CD3 complex: a dynamic protein ensemble. Annu Rev Immunol 6:629–662

Colucci M, Stockmann H, Butera A, Masotti A, Baldassarre A, Giorda E, Petrini S, Rudd PM, Sitia R, Emma F, Vivarelli M (2015) Sialylation of N-linked glycans influences the immunomodulatory effects of IgM on T cells. J Immunol 194:151–157

Daniel V, Schimpf K, Opelz G (1989) Lymphocyte autoantibodies and alloantibodies in HIV-positive haemophilia patients. Clin Exp Immunol 75:178–183

Dharmadhikari G, Muhle M, Schulthess FT, Laue S, Oberholzer J, Pattou F, Kerr-Conte J, Maedler K (2012) TOSO promotes ß-cell proliferation and protects from apoptosis. Mol Metab 1:70–78

Ehrenstein MR, Notley CA (2010) The importance of natural IgM: scavenger, protector and regulator. Nat Rev Immunol 10:778–786

Engels N, Konig LM, Heemann C, Lutz J, Tsubata T, Griep S, Schrader V, Wienands J (2009) Recruitment of the cytoplasmic adaptor Grb2 to surface IgG and IgE provides antigen receptor-intrinsic costimulation to class-switched B cells. Nat Immunol 10:1018–1025

Engels N, Wienands J (2011) The signaling tool box for tyrosine-based costimulation of lymphocytes. Curr Opin Immunol 23:324–329

Ernst LK, Duchemin AM, Anderson CL (1993) Association of the high-affinity receptor for IgG (FcγRI) with the γ subunit of the IgE receptor. Proc Natl Acad Sci USA 90:6023–6027

Faitschuk E, Hombach AA, Frenzel LP, Wendtner CM, Abken H (2016) Chimeric antigen receptor T cells targeting Fcμ receptor selectively eliminate CLL cells while sparing healthy B cells. Blood 128:1711–1722

Ferrarini M, Hoffman T, Fu SM, Winchester R, Kunkel HG (1977) Receptors for IgM on certain human B lymphocytes. J Immunol 119:1525–1529

Fillatreau S (2015) Regulatory plasma cells. Curr Opin Pharmacol 23:1–5

Gardiner EE, Karunakaran D, Arthur JF, Mu FT, Powell MS, Baker RI, Hogarth PM, Kahn ML, Andrews RK, Berndt MC (2008) Dual ITAM-mediated proteolytic pathways for irreversible inactivation of platelet receptors: de-ITAM-izing FcγRIIa. Blood 111:165–174

Gaublomme JT, Yosef N, Lee Y, Gertner RS, Yang LV, Wu C, Pandolfi PP, Mak T, Satija R, Shalek AK, Kuchroo VK, Park H, Regev A (2015) Single-cell genomics unveils critical regulators of Th17 cell pathogenicity. Cell 163:1400–1412

Gupta S, Agrawal S, Gollapudi S, Kubagawa H (2016) FcμR in human B cell subsets in primary selective IgM deficiency, and regulation of FcμR and production of natural IgM antibodies by IGIV. Hum Immunol 77:1194–1201

Gyorgy B, Szabo TG, Pasztoi M, Pal Z, Misjak P, Aradi B, Laszlo V, Pallinger E, Pap E, Kittel A, Nagy G, Falus A, Buzas EI (2011) Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci 68:2667–2688

Hashimoto K, Handa H, Umehara K, Sasaki S (1978) Germfree mice reared on an "antigen-free" diet. Lab Anim Sci 28:38–45

Haury M, Sundblad A, Grandien A, Barreau C, Coutinho A, Nobrega A (1997) The repertoire of serum IgM in normal mice is largely independent of external antigenic contact. Eur J Immunol 27:1557–1563

Heyman B (2000) Regulation of antibody responses via antibodies, complement, and Fc receptors. Annu Rev Immunol 18:709–737

Hitoshi Y, Lorens J, Kitada SI, Fisher J, LaBarge M, Ring HZ, Francke U, Reed JC, Kinoshita S, Nolan GP (1998) Toso, a cell surface, specific regulator of Fas-induced apoptosis in T cells. Immunity 8:461–471

Honjo H, Kubagawa Y, Suzuki Y, Takagi M, Ohno H, Bucy RP, Izui S, Kubagawa H (2014) Enhanced autoantibody and Mott cell formation in FcμR-decient autoimmune mice. Int Immunol 26:659–672

Honjo K, Kubagawa Y, Jones DM, Dizon B, Zhu Z, Ohno H, Izui S, Kearney JF, Kubagawa H (2012a) Altered Ig levels and antibody responses in mice deficient for the Fc receptor for IgM (FcμR). Proc Natl Acad Sci USA 109:15882–15887

Honjo K, Kubagawa Y, Kubagawa H (2012b) Is Toso an antiapoptotic protein or an Fc receptor for IgM? Blood 119:1789–1790

Honjo K, Kubagawa Y, Kubagawa H (2013) Is Toso/IgM Fc receptor (FcμR) expressed by innate immune cells? Proc Natl Acad Sci USA 110:E2540–E2541

Honjo K, Kubagawa Y, Kearney JF, Kubagawa H (2015) Unique ligand-binding property of the human IgM Fc receptor. J Immunol 194:1975–1982

Kaetzel CS (2005) The polymeric immunoglobulin receptor: bridging innate and adaptive immune responses at mucosal surfaces. Immunol Rev 206:83–99

Kikuno K, Kang DW, Tahara K, Torii I, Kubagawa HM, Ho KJ, Baudino L, Nishizaki N, Shibuya A, Kubagawa H (2007) Unusual biochemical features and follicular dendritic cell expression of human Fcα/μ receptor. Eur J Immunol 37:3540–3550

Kinashi T (2005) Intracellular signalling controlling integrin activation in lymphocytes. Nat Rev Immunol 5:546–559

Klimovich VB (2011) IgM and its receptors: structural and functional aspects. Biochemistry (Mosc) 76:534–549

Koren E, Reichlin MW, Koscec M, Fugate RD, Reichlin M (1992) Autoantibodies to the ribosomal P proteins react with a plasma membrane-related target on human cells. J Clin Invest 89:1236–1241

Kubagawa H, Chen CC, Ho LH, Shimada TS, Gartland L, Mashburn C, Uehara T, Ravetch JV, Cooper MD (1999) Biochemical nature and cellular distribution of the paired immunoglobulin-like receptors, PIR-A and PIR-B. J Exp Med 189:309–318

Kubagawa H, Oka S, Kubagawa Y, Torii I, Takayama E, Kang DW, Gartland GL, Bertoli LF, Mori H, Takatsu H, Kitamura T, Ohno H, Wang JY (2009) Identity of the elusive IgM Fc receptor (FcμR) in humans. J Exp Med 206:2779–2793

Kubagawa H, Oka S, Kubagawa Y, Torii I, Takayama E, Kang D-W, Jones D, Nishida N, Miyawaki T, Bertoli L, Sanders S, Honjo K (2014a) The long elusive IgM Fc receptor, FcμR. J Clin Immunol 34:35–45

Kubagawa Y, Honjo K, Kang DW, Kubagawa H (2014b) Monoclonal antibodies specific for human IgM Fc receptor inhibit ligand-binding activity. Monoclon Antib Immunodiagn Immunother 33:393–400

Kubagawa H, Kubagawa Y, Jones D, Nasti TH, Walter MR, Honjo K (2014c) The old but new IgM Fc receptor (FcμR). Curr Top Microbiol Immunol 382:3–28

Kubagawa H, Carroll MC, Jacob CO, Lang KS, Lee KH, Mak T, McAndrews M, Morse HC 3rd, Nolan GP, Ohno H, Richter GH, Seal R, Wang JY, Wiestner A, Coligan JE (2015)

Nomenclature of Toso, Fas apoptosis inhibitory molecule 3, and IgM FcR. J Immunol 194:4055–4057

Lang KS, Lang PA, Meryk A, Pandyra AA, Boucher LM, Pozdeev VI, Tusche MW, Gothert JR, Haight J, Wakeham A, You-Ten AJ, McIlwain DR, Merches K, Khairnar V, Recher M, Nolan GP, Hitoshi Y, Funkner P, Navarini AA, Verschoor A, Shaabani N, Honke N, Penn LZ, Ohashi PS, Haussinger D, Lee KH, Mak TW (2013a) Involvement of Toso in activation of monocytes, macrophages, and granulocytes. Proc Natl Acad Sci USA 110:2593–2598

Lang KS, Lang PA, Meryk A, Pandyra AA, Merches K, Lee KH, Mak TW (2013b) Reply to Honjo et al.: Functional relevant expression of Toso on granulocytes. Proc Natl Acad Sci USA 110:E2542–E2543

Lang PA, Meryk A, Pandyra AA, Brenner D, Brustle A, Xu HC, Merches K, Lang F, Khairnar V, Sharma P, Funkner P, Recher M, Shaabani N, Duncan GS, Duhan V, Homey B, Ohashi PS, Haussinger D, Knolle PA, Honke N, Mak TW, Lang KS (2015) Toso regulates differentiation and activation of inflammatory dendritic cells during persistence-prone virus infection. Cell Death Differ 22:164–173

Lapke N, Tartz S, Lee KH, Jacobs T (2015) The application of anti-Toso antibody enhances CD8 (+) T cell responses in experimental malaria vaccination and disease. Vaccine 33:6763–6770

Li F, Ravetch JV (2011) Inhibitory Fcγ receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies. Science 333:1030–1034

Li FJ, Kubagawa Y, McCollum MK, Wilson L, Motohashi T, Bertoli LF, Barton JC, Barnes S, Davis RS, Kubagawa H (2011) Enhanced levels of both membrane-bound and soluble forms of IgM Fc receptor (FcμR) in patients with chronic lymphocytic leukemia. Blood 118:4902–4909

Liao JJ, Huang MC, Fast K, Gundling K, Yadav M, Van Brocklyn JR, Wabl MR, Goetzl EJ (2009) Immunosuppressive human anti-lymphocyte autoantibodies specific for the type 1 sphingosine 1-phosphate receptor. FASEB J. 23:1786–1796

Lloyd KA, Wang J, Urban BC, Czajkowsky DM, Pleass RJ (2017) Glycan-independent binding and internalization of human IgM to FCMR, its cognate cellular receptor. Sci Rep 7:42989

Lobo PI, Schlegel KH, Spencer CE, Okusa MD, Chisholm C, McHedlishvili N, Park A, Christ C, Burtner C (2008) Naturally occurring IgM anti-leukocyte autoantibodies (IgM-ALA) inhibit T cell activation and chemotaxis. J Immunol 180:1780–1791

Morton HC, van den Herik-Oudijk IE, Vossebeld P, Snijders A, Verhoeven AJ, Capel PJ, Van De Winkel JG (1995) Functional association between the human myeloid immunoglobulin A Fc receptor (CD89) and FcR γ chain. Molecular basis for CD89/FcR γ chain association. J Biol Chem 270:29781–29787

Muller C, Kukel S, Schneweis KE, Bauer R (1994) Anti-lymphocyte antibodies in plasma of HIV-1-infected patients preferentially react with MHC class II-negative T cells and are linked to antibodies against gp41. Clin Exp Immunol 97:367–372

Murakami Y, Narayanan S, Su S, Childs R, Krzewski K, Borrego F, Weck J, Coligan JE (2012) Toso, a functional IgM receptor, is regulated by IL-2 in T and NK cells. J Immunol 189:587–597

Nakamura T, Kubagawa H, Ohno T, Cooper MD (1993) Characterization of an IgM Fc-binding receptor on human T cells. J. Immunol. 151:6933–6941

Nguyen XH, Lang PA, Lang KS, Adam D, Fattakhova G, Foger N, Kamal MA, Prilla P, Mathieu S, Wagner C, Mak T, Chan AC, Lee KH (2011) Toso regulates the balance between apoptotic and nonapoptotic death receptor signaling by facilitating RIP1 ubiquitination. Blood 118:598–608

Nguyen TT, Klasener K, Zurn C, Castillo PA, Brust-Mascher I, Imai DM, Bevins CL, Reardon C, Reth M, Baumgarth N (2017) The IgM receptor FcμR limits tonic BCR signaling by regulating expression of the IgM BCR. Nat Immunol 18:321–333

Ohno T, Kubagawa H, Sanders SK, Cooper MD (1990) Biochemical nature of an Fcμ receptor on human B-lineage cells. J Exp Med 172:1165–1175

Ouchida R, Mori H, Hase K, Takatsu H, Kurosaki T, Tokuhisa T, Ohno H, Wang JY (2012) Critical role of the IgM Fc receptor in IgM homeostasis, B-cell survival, and humoral immune responses. Proc Natl Acad Sci USA: E2699-E2706

Ouchida R, Lu Q, Liu J, Li Y, Chu Y, Tsubata T, Wang JY (2015) FcμR interacts and cooperates with the B cell receptor To promote B cell survival. J Immunol 194:3096–3101

Pallasch CP, Schulz A, Kutsch N, Schwamb J, Hagist S, Kashkar H, Ultsch A, Wickenhauser C, Hallek M, Wendtner CM (2008) Overexpression of TOSO in CLL is triggered by B-cell receptor signaling and associated with progressive disease. Blood 112:4213–4219

Panda S, Ding JL (2015) Natural antibodies bridge innate and adaptive immunity. J Immunol 194:13–20

Pichler WJ, Knapp W (1977) Receptors for IgM-coated erythrocytes on chronic lymphatic leukemia cells. J Immunol 118:1010–1015

Pincetic A, Bournazos S, DiLillo DJ, Maamary J, Wang TT, Dahan R, Fiebiger BM, Ravetch JV (2014) Type I and type II Fc receptors regulate innate and adaptive immunity. Nat Immunol 15:707–716

Proto-Siqueira R, Panepucci RA, Careta FP, Lee A, Clear A, Morris K, Owen C, Rizzatti EG, Silva WA Jr, Falcao RP, Zago MA, Gribben JG (2008) SAGE analysis demonstrates increased expression of TOSO contributing to Fas-mediated resistance in CLL. Blood 112:394–397

Randall TD, Brewer JW, Corley RB (1992) Direct evidence that J chain regulates the polymeric structure of IgM in antibody-secreting B cells. J Biol Chem 267:18002–18007

Ravetch JV (1994) Fc receptors: rubor redux. Cell 78:553–560

Ravetch JV, Lanier LL (2000) Immune inhibitory receptors. Science 290:84–89

Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, Yang L, Pickeral OK, Rassenti LZ, Powell J, Botstein D, Byrd JC, Grever MR, Cheson BD, Chiorazzi N, Wilson WH, Kipps TJ, Brown PO, Staudt LM (2001) Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 194:1639–1647

Sanders SK, Kubagawa H, Suzuki T, Butler JL, Cooper MD (1987) IgM binding protein expressed by activated B cells. J Immunol 139:188–193

Schwab I, Nimmerjahn F (2013) Intravenous immunoglobulin therapy: how does IgG modulate the immune system? Nat Rev Immunol 13:176–189

Shima H, Takatsu H, Fukuda S, Ohmae M, Hase K, Kubagawa H, Wang JY, Ohno H (2010) Identification of TOSO/FAIM3 as an Fc receptor for IgM. Int Immunol 22:149–156

Silvestris F, Edwards BS, Sadeghi OM, Frassanito MA, Williams RC Jr, Dammacco F (1989) Isotype, distribution and target analysis of lymphocyte reactive antibodies in patients with human immunodeficiency virus infection. Clin Immunol Immunopathol 53:329–340

Thurnheer MC, Zuercher AW, Cebra JJ, Bos NA (2003) B1 cells contribute to serum IgM, but not to intestinal IgA, production in gnotobiotic Ig allotype chimeric mice. J Immunol 170:4564–4571

Vanden Berghe T, Hulpiau P, Martens L, Vandenbroucke RE, Van Wonterghem E, Perry SW, Bruggeman I, Divert T, Choi SM, Vuylsteke M, Shestopalov VI, Libert C, Vandenabeele P (2015) Passenger mutations confound interpretation of all genetically modified congenic mice. Immunity 43:200–209

Vély F, Vivier E (1997) Conservation of structural features reveals the existence of a large family of inhibitory cell surface receptors and noninhibitory/activatory counterparts. J Immunol 159:2075–2077

Vignaux F, Vivier E, Malissen B, Depraetere V, Nagata S, Golstein P (1995) TCR/CD3 coupling to Fas-based cytotoxicity. J Exp Med 181:781–786

Vire B, David A, Wiestner A (2011) TOSO, the Fcμ receptor, is highly expressed on chronic lymphocytic leukemia B cells, internalizes upon IgM binding, shuttles to the lysosome, and is downregulated in response to TLR activation. J Immunol 187:4040–4050

Vire B, Skarzynski M, Thomas JD, Nelson CG, David A, Aue G, Burke TR Jr, Rader C, Wiestner A (2014) Harnessing the Fcμ receptor for potent and selective cytotoxic therapy of chronic lymphocytic leukemia. Cancer Res 74:7510–7520

Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, Yokoyama WM, Ugolini S (2011) Innate or adaptive immunity? The example of natural killer cells. Science 331:44–49

Wang J, Coombes KR, Highsmith WE, Keating MJ, Abruzzo LV (2004) Differences in gene expression between B-cell chronic lymphocytic leukemia and normal B cells: a meta-analysis of three microarray studies. Bioinformatics 20:3166–3178

Wang H, Coligan JE, Morse HC 3rd (2016) Emerging functions of natural IgM and its Fc receptor FCMR in immune homeostasis. Front Immunol 7:99

Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC (2003) Predominant autoantibody production by early human B cell precursors. Science 301:1374–1377

Warren RQ, Johnson EA, Donnelly RP, Lavia MF, Tsang KY (1988) Specificity of anti-lymphocyte antibodies in sera from patients with AIDS-related complex (ARC) and healthy homosexuals. Clin Exp Immunol 73:168–173

Weiss A, Stobo JD (1984) Requirement for the coexpression of T3 and the T cell antigen receptor on a malignant human T cell line. J Exp Med 160:1284–1299

Wiersma EJ, Collins C, Fazel S, Shulman MJ (1998) Structural and functional analysis of J chain-deficient IgM. J Immunol 160:5979–5989

Winfield JB, Fernsten PD, Czyzyk JK (1997) Anti-lymphocyte autoantibodies in systemic lupus erythematosus. Trans Am Clin Climatol Assoc 108:127–135

Xu Y, Szalai AJ, Zhou T, Zinn KR, Chaudhuri TR, Li X, Koopman WJ, Kimberly RP (2003) FcγRs modulate cytotoxicity of anti-Fas antibodies: implications for agonistic antibody-based therapeutics. J Immunol 171:562–568

Yamauchi A, Taga K, Mostowski HS, Bloom ET (1996) Target cell-induced apoptosis of interleukin-2-activated human natural killer cells: roles of cell surface molecules and intracellular events. Blood 87:5127–5135

Yoo EM, Trinh KR, Lim H, Wims LA, Morrison SL (2011) Characterization of IgA and IgM binding and internalization by surface-expressed human Fcα/μ receptor. Mol Immunol 48:1818–1826