The Appearance and Diversification of Receptors for IgM During Vertebrate Evolution

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Abstract Three different receptors that interact with the constant domains of IgM have been identified: the polymeric immunoglobulin (Ig) receptor (PIGR), the dual receptor for IgA/IgM (FcαμR) and the IgM receptor (FcμR). All of them are related in structure and located in the same chromosomal region in mammals. The functions of the PIGRs are to transport IgM and IgA into the intestinal lumen and to saliva and tears, whereas the FcαμRs enhance uptake of immune complexes and antibody coated bacteria and viruses by B220+ B cells and phagocytes, as well as dampening the Ig response to thymus-independent antigens. The FcμRs have broad-spectrum effects on B-cell development including effects on IgM homeostasis, B-cell survival, humoral immune responses and also in autoantibody formation. The PIGR is the first of these receptors to appear during vertebrate evolution and is found in bony fish and all tetrapods but not in cartilaginous fish. The FcμR is present in all extant mammalian lineages and also in the Chinese and American alligators, suggesting its appearance with early reptiles. Currently the FcαμR has only been found in mammals and is most likely the evolutionary youngest of the three receptors. In bony fish, the PIGR has either 2, 3, 4, 5 or 6 extracellular Ig-like domains, whereas in amphibians, reptiles and birds it has 4 domains, and 5 in all mammals. The increase in domain number from 4 to 5 in mammals has been proposed to enhance the interaction with IgA. Both the FcαμRs and the FcμRs contain only one Ig domain; the domain that confers Ig binding. In both of these receptors this domain shows the highest degree of sequence similarity to domain 1 of the PIGR. All Ig domains of these three receptors are V type domains, indicating they all have the same origin although they have diversified extensively in function during vertebrate evolution by changing expression patterns and cytoplasmic signaling motifs.

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1   The Adaptive Immune System of Vertebrates

In jawed vertebrates the adaptive immune system has appeared in a stepwise manner through several major key events. The first of these was the appearance of the somatically rearranging genes for immunoglobulins (Igs) and T-cell receptors (TCRs). These seem to have occurred at the base of jawed vertebrates, around 450 million years ago. All jawed vertebrates including cartilaginous fish have mostly bona fide Igs or TCRs, whereas lamprey and hagfish, belonging to the jawless fish, the agnathans that separated from other early vertebrates during the early Cambrian Period approximately 550 million years ago, both lack classical Igs and TCRs. However, these jawless fish have other complex antigen receptors named variable lymphocyte receptors (VLRs), which functionally closely resemble IgM and TCRs, but have a completely different evolutionary origin (Hirano et al. 2011; Kasahara and Sutoh 2014). Instead of Ig-like domains they have leucine rich repeats, which are more closely related to Toll-like receptors than to Igs (Hirano et al. 2011; Kasahara and Sutoh 2014). In our minds this is one of the most striking examples of convergent evolution: Starting from a completely different set of genes but ending up with a set of molecules with very similar functions. This convergent nature extends into the three different variants of the VLRs (VLR-A, -B and -C) found in both hagfish and lamprey, which represent functional equivalents of the αβ TCRs, the Igs and the γδ TCRs, respectively (Hirano et al. 2011; Kasahara and Sutoh 2014). These VLR variants are also expressed by three distinct cell types representing equivalents of the B and T lymphocytes in jawed vertebrates (Hirano et al. 2011; Kasahara and Sutoh 2014). Furthermore, the VLR-A, -B and -Cs are encoded from three different loci, all with a high variability generating capacity (Hirano et al. 2011; Kasahara and Sutoh 2014). A recent study on the recognition of influenza virus epitopes in the mouse and lamprey also shows that the response against antigens is almost identical. Both respond against the same regions of the virus, with a relatively similar amount of Igs or VLR-Bs against the different epitopes (Altman et al. 2015). These findings indicate that the two systems are functionally very similar despite being structurally dissimilar.

In jawed vertebrates, the first Ig isotype to appear was most likely IgM as it is found in essentially the same form in all jawed vertebrates, except the coelacanths, which likely secondarily lost the gene for this Ig class (Amemiya et al. 2013;

Boudinot et al. 2014; Kaetzel 2014; Saha et al. 2014). The µ gene of IgM is also located at the 5' end of the locus adjacent to the gene segments for the variable domain of the heavy chain in almost all species studied. An exception to this rule is the zebrafish, where IgZ is located upstream of the IgM gene (Danilova et al. 2005). IgM is also generally the first isotype to be expressed by all B cells. Following the appearance of the first Igs and TCRs, the complexity of the Ig repertoire has increased gradually by gene duplications. The increase in the number of Ig isotypes has made it possible to separate effector functions and thereby increase the regulatory potential of the system. Today, mammals express up to six different Ig classes: IgM, IgD, IgG, IgE, IgA and IgO, and the total number of isotypes can sometimes exceed 15 (Zhao et al. 2009; Magadan-Mompo et al. 2013a, b; Sun et al. 2013; Akula et al. 2014; Kaetzel 2014; Estevez et al. 2016). This facilitates effector functions of the Igs such as complement activation, epithelial transfer and placental transfer, which can be regulated separately. Although we see a large difference in the number of other isotypes and Ig classes in different jawed vertebrates, they all have IgM, which is also, except in coelacanths, present in a very similar form. In fish and mammals, IgM is multimeric; where hexamers are found in fish, and pentamers in mammals (Getahun et al. 1999; Klimovich 2011). In addition, IgM is found as a membrane bound form on all B cells during early B-cell development.

2 The Appearance of Receptors Interacting with the Constant Domain of Igs

Following the appearance of bona fide Ig and TCR genes, a number of additional adaptations have later taken place to increase the roles of these antigen receptors in immunity. One such adaptation has been the appearance of a complex set of proteins interacting with the constant domains of the Igs. These molecules, named Fc receptors (FcRs), due to their interaction with the constant domain of the Igs, have a number of important functions in vertebrates including facilitating phagocytosis by opsonization, constituting key components in antibody-dependent cellular cytotoxicity as well as activating cells to release their granular content. One member of this family, the polymeric Ig receptor (PIGR), also facilitates transfer of Igs across epithelial layers; IgM and IgA in mammals and birds, IgX in amphibians and IgT/IgZ in fish (Fig. 1) (Danilova et al. 2005; Hansen et al. 2005; Zhang et al. 2010; Kaetzel 2014). This receptor therefore makes it possible to target pathogens before they have entered the tissues of the infected individual. In addition to the PIGRs, four major types of classical FcRs for IgG have been identified in mammals as well as one high-affinity receptor for IgE, one for both IgM and IgA, one for IgM and one for IgA (Figs. 1, 2, 3 and 4). All of these receptors are related in structure, where they all contain Ig-like domains. Furthermore they all, with the exception of the IgA receptor, are found on chromosome 1 in humans, indicating that they originate from one or a few common ancestors by successive gene duplications (Fig. 2).
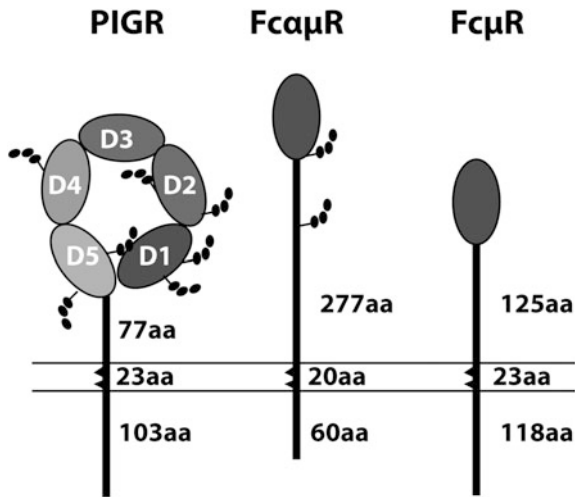
Fig. 1 A schematic presentation of the three receptors for IgM: the PIGR, the FcαμR and the FcμR. The Ig domains are depicted as ovals and potential N-linked glycosylation sites are marked with three small *connected circles*. Approximate sizes in amino acids numbers are also included for each of the three receptors. The Ig domains are generally 100–110 amino acids in size. The figure is modified from Klimovich (2011) and based on the structural information presented in Klimovich (2011), Stadtmueller et al. (2016)

Ig domains are classified as V, C1, C2 or I domains depending on features such as the spacing of cysteine bridges and the number of beta sheets. V domains are generally found in variable regions of Igs and TCRs as well as in cluster of differentiation (CD) markers including CD2, CD4, CD80 and CD86. C1 domains are found in constant regions of Igs, TCRs and in MHC class I and II. C2 domains are found in CD2, CD4, CD80, VCAM and ICAM, and I domains are found in VCAM, ICAM, NCAM, MADCAM and numerous other diverse protein families (EMBL-EBI InterPro). All Ig domains of the FcR-like (FcRL) and classical FcRs are classed as C2 domains and the Ig domains of the PIGRs, IgM receptors (FcμRs) and IgA/IgM receptors (FcαμRs) are V type domains (Nikolaidis et al. 2005; Viertlboeck and Gobel 2011). In a phylogenetic analysis of the individual domains of these receptors, they separate into individual branches clearly separating C2 and V type domains and also individual domains within the PIGRs, FcμRs and FcαμRs (Fig. 5).

As previously described, the first Ig isotype to appear was most likely IgM. One would therefore expect the first FcRs to appear would also be the receptors for IgM. However this is only partly true. The PIGRs are found in all tetrapods and bony fish but not in cartilaginous fish (Akula et al. 2014). One of the first steps in the evolution of the classical FcRs was the appearance of the transfer receptor for transporting IgM and later IgA, IgX or IgT/IgZ over epithelial layers (Figs. 2, 3 and 4). Interestingly, one important signaling molecule for the classical FcRs, the common γ chain, also appeared with bony fish (Akula et al. 2014; Kaetzel 2014). This non Ig-domain-containing signaling subunit is a member of a small family of related molecules

including the TCR zeta chain, DAP10 and DAP12 (Weissman et al. 1988; Blank et al. 1989; Rodewald et al. 1991; Lanier 2009). The latter two proteins serve as signaling components of NK-cell receptors and as well as the related Ig-domain containing receptors (Lanier 2009). A new family of receptors, related in structure to the classical IgG and IgE receptors, was also discovered upon the completion of full genome sequences from a number of mammalian species (Davis 2007; Ehrhardt and Cooper 2011). Eight different such FcRL genes have been identified in the human genome: FcRL1-FcRL6 as well as FcRLA and FcRLB (Figs. 2 and 4). Genes closely related to these mammalian FcRL genes are also found in bony fish but not in cartilaginous fish, indicating a major step in the evolution of FcRs at the base of bony fish with the appearance of the PIGRs, the FcR γ chain and the FcRL molecules (Akula et al. 2014).

In a recent study we show that the classical receptors for IgG and IgE most likely appeared as a separate subfamily of the FcRL molecules during early mammalian evolution (Akula et al. 2014). Related genes are also found in the Western clawed frog (*Xenopus tropicalis*) and the Chinese alligator, indicating that the processes forming the subfamily of receptors that later became the classical IgG and IgE receptors may have started already during early tetrapod evolution. However, this subfamily probably did not appear as a distinct subfamily until the appearance of the mammals (Akula et al. 2014). In the Xenopus these receptors have a structure similar to the human high affinity IgG receptor, FcγRI, with three extracellular Ig domains of the C2 type. In the platypus there are both two and three domain receptors, which are similar to the human three-domain FcγRI as well as the low affinity IgG receptors FcγRII and III, which have two domains. This indicates that the development of high and low affinity receptors also took place during early mammalian evolution. Despite this knowledge, currently none of these amphibian, reptile or non-placental mammalian receptors have been studied for their isotype specificities and affinities.

In contrast to several of the receptors previously described, both the FcαµR and the FcµR seemed to appear relatively late during vertebrate evolution. The FcαµR has only been found in mammals, which indicates that this receptor appeared sometime during early mammalian evolution. A partial clone for FcαµR has been present in the platypus genome assembly but subsequently disappeared from the database, most likely due to incomplete coverage of that specific chromosomal region. However, the Ig domain encoded in this chromosomal fragment did show a high degree of homology to other mammalian FcαµRs, indicating its presence is likely in all three extant mammalian lineages (Fig. 7). The receptor for IgM, FcµR, has until very recently also only been found in mammals. However, in a recent screening of a panel of vertebrate genomes, we also found a gene for the FcµR in both the American and the Chinese alligators (Figs. 2, 4 and 8). This suggests that the receptor appeared during early amniote evolution before reptiles and mammals separated as the diapsid and synapsid lineages, possibly sometime between 320 and 360 million years ago. Interestingly, this receptor may have been secondarily lost in several reptile lineages, as it is not found in the anole lizard genome nor in any of the screened bird genomes (Fig. 2). Birds are known to have gone through massive gene losses followed by re-expansions of gene loci, as many genes found in other species are missing in birds, which may explain the lack of the FcµR gene in birds (International Chicken Genome Sequencing 2004).

◀**Fig. 2** Fc receptor genes from a panel of selected tetrapods focusing on the genes for the three IgM receptors, the PIGR, the FcαμR and the FcμR. The regions of the major FcR gene loci in humans are shown above the figure as a reference. Each *horizontal line* corresponds to a chromosome on which different FcR genes are located. Genes are color coded. The Fc receptor-like (FcRL) genes are shown in *yellow* except the FcRLA and B that are in *light green*, classical IgG receptors in *red* (pseudogenes in *striped red*), IgE receptor gamma chain in *dark green*, the TCR zeta chain in *light brown*, the IgM receptor in *dark blue*, the PIGR and the IgA/IgM receptor in other shades of *blue*. A number of bordering genes have also been included as reference genes for the chromosomal region of interest. The enlarged region containing the three receptors binding IgM are bordered at one side by a number of cytokine genes including IL-10, IL-19, IL-20 and IL-24. In cattle and pigs an inversion has occurred resulting in the movement of the IL-24 gene to the other end of the region encoding the IgM receptors. A rearrangement involving the region containing the cytokine genes has also occurred in the Western clawed frog genome
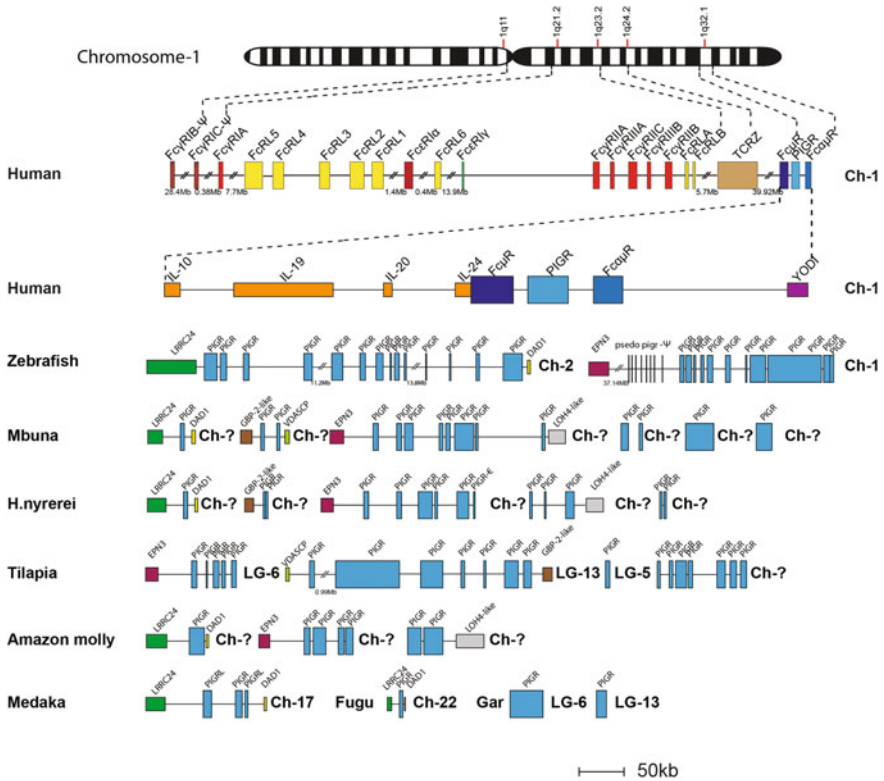

Fig. 3 The PIGR gene loci in a panel of fish genomes. The regions of the major FcR gene loci in humans are shown above the figure as a reference. Each *horizontal line* corresponds to a chromosome on which different FcR genes are located. Genes are color coded as in Fig. 2 with the PIGR genes in *light blue*

◀**Fig. 4** A summary figure of domain structures and signaling motifs of the various vertebrate Fc receptors. The Ig-like domains are depicted as *filled circles* with color-coding according to the similarities in sequences based on phylogenetic analyses (Fayngerts et al. 2007; Guselnikov et al. 2008) and Fig. 5. The domain type D1, D2, D3, D4 and D5 show a relatively conserved pattern in most tetrapods and have therefore been color-coded in *red*, *dark blue*, *yellow*, *light blue* and *green*. A phylogenetic analysis of individual domains of a panel of different FcR is presented in Fig. 5. The color-coding here is based on the result from Fig. 5. The extracellular regions, the transmembrane regions and cytoplasmic tails are not to scale in order to show the positions of potential signaling motifs like immuno-tyrosine activation motifs (ITAMs) (*green boxes*) and immuno-tyrosine inhibitory motifs (ITIMs) (*red boxes*), which regulate the biological function the FcRs. Some of the intracellular proteins contain C-terminal mucin-like regions, which are depicted as *blue triangles*. The PIGR domains are depicted in different shades of *gray*, with domain 1 in *darker gray*, and domain 5 and unassigned domains of fish PIGRs as the lightest shade of *grey*

The relatively late appearance of the specific receptor for IgM is interesting from an evolutionary perspective. IgM is the first Ig isotype to appear but the specific IgM receptor did not enter the scene until relatively late. The only two receptors that show a later appearance are the dual FcαμR and the specific IgA receptor. The latter is only found in placental mammals, where in humans is the only FcR that is located on another chromosome than the other classical FcRs, and is found together with the NK-cell receptors in a gene cluster on chromosome 19 (Bakema and van Egmond 2011; Akula et al. 2014).

The three receptors interacting with IgM are all related in structure; they contain at least one Ig domain of the V type (Fig. 1). However, other parts of the proteins are very different. By analyzing the relatedness between the Ig-like domains of the PIGR, the FcαμR and the FcμR, it is evident they form a separate subfamily in a phylogenetic tree (Fig. 5). They clearly separate from the domains of the IgG, IgE and IgA specific receptors that all contain C2 type domains, which also confirms that the domains of the three different receptors for IgM, with their V type domains, are closely related in structure (Fig. 5) (Nikolaidis et al. 2005). In the following separate sections, we will discuss the structures, functions and possible evolutionary origins of the three different receptors for IgM in more detail.

3 The Polymeric Ig Receptor—PIGR

In mammals, the PIGR is responsible for the transfer of IgM and IgA antibodies from body fluids into secretions including tears, saliva, breast milk as well as into the intestinal lumen by the mucus epithelium and ducts of excretory glands. The antibodies bind at the baso-lateral side of the epithelial cells and the antibody receptor complexes are then transported, by transcytosis, to the apical side of the cells in vesicles. At the apical side proteases cleave the PIGR, leaving the extra-cellular part in complex with IgM or IgA. This part that stays firmly attached to IgM or IgA is termed the secretory component (SC), which has a protective role by limiting proteolytic degradation of IgM or IgA by bacterial and intestinal proteases
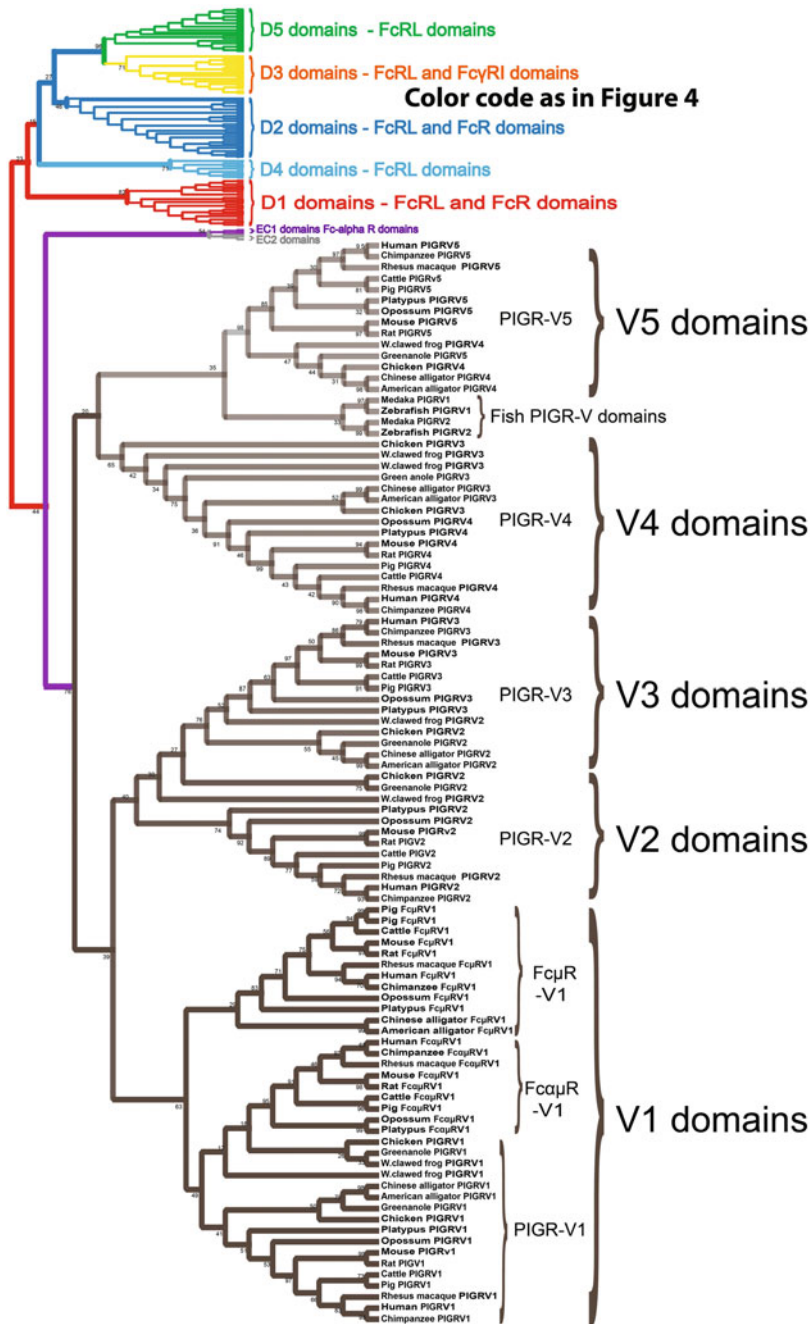
Fig. 5 A phylogenetic tree of individual domains of a panel of different Fc receptor sequences from a number of different vertebrates analyzed for their sequence relatedness. Protein domains were identified with the SMART software (Letunic et al. 2012). The rooted maximum likelihood tree was constructed by using MEGA5.2 software (Tamura et al. 2011). Bootstrap analysis was performed with 500 replicates. Bootstrap values are indicated in the phylogenetic tree. The individual domain types are color-coded as in Fig. 4

(Kaetzel 2005). The PIGR is highly glycosylated, containing 22% carbohydrate by weight although these carbohydrates do not seem to directly influence ligand binding (Sletten et al. 1975; Bakos et al. 1991). This receptor is expressed at its highest levels in the small and large intestines but is also present in the kidneys, pancreas, lungs and endometrium (Krajci et al. 1989; Klimovich 2011).

In all mammals studied, covering all three extant mammalian lineages; monotremes, marsupials and placental mammals, the PIGR has five Ig-like domains each approximately 100–110 amino acids in length. Structural studies show that they most closely resemble Ig variable domains, so called V type domains, where short hinge-like regions are found between domains 1 and 2, and domains 3 and 4 (Pumphrey 1986; Hamburger et al. 2004). Amphibians, reptiles and birds have four domain PIGRs and teleost fish have even more variable numbers of Ig domains in PIGRs. Interestingly, most tetrapods have one or at the most two copies of the PIGR gene, whereas the numbers in bony fish sometimes exceed thirty (Figs. 2 and 3). The fact that all mammals have five domain PIGRs and all reptiles and amphibians have only four domains, indicates that the duplication of the second domain resulting in five domain PIGRs occurred during early mammalian evolution, possibly 200–250 million years ago (Fig. 4). One step in this process has been an internal duplication involving domains 2 and 3, leading to an exon containing two domains, which is in contrast to all other Ig domains within various PIGR genes, which are encoded by a separate exon. This duplication did not appear or was later reverted in the chicken genome (Wieland et al. 2004). The second and third domains appear to be of importance for efficient transport of dimeric IgA (Norderhaug et al. 1999). The reasons for the very large number of PIGR genes in fish as well as the difference in the number of domains within fish PIGR genes are not yet known. In any case, it is strikingly different from the tetrapod scenario and may indicate that PIGRs have more than one function in fish. Previously, fish have only been considered to express two and three domain PIGRs, however, a re-screening of their updated genomes showed that they also have members with four, five and even six Ig domains, although the majority of the consist of two or three domains (Figs. 3 and 4). In the zebrafish databases there are now 34 PIGR sequences of which 27 have two domains, 3 have three domains, 1 has four domains, 2 have five domains and 1 has six domains. Interestingly, many of the fish PIGRs also seem to lack transmembrane regions, which may mean they are found as soluble forms with potentially other functions than epithelial transport (Fig. 4). This is somewhat precautionary, as the possibility of other forms of membrane anchoring has not been studied.

The PIGRs of placental mammals are remarkably well conserved in all regions except the linker region between domain 5 and the membrane anchoring hydrophobic region (Fig. 6). Furthermore, parts of the cytoplasmic region are conserved in all tetrapods from amphibians to mammals (Fig. 6). Conserved tyrosines of the cytoplasmic tail are important for the internalization process; the first step in the transport of the receptor from the baso-lateral to the apical side of the cell (Okamoto et al. 1992). The phosphorylation of serine 664 (marked by green star, Fig. 6) is also important for the translocation into the endosomes and their

Fig. 6 A sequence alignment of a panel of PIGRs. Conserved residues are shown within *black boxes*. The Ig domain, the hydrophobic transmembrane region and the conserved potential signaling motifs in the cytoplasmic region are marked by a *thick black line*, a *red line* and *green lines*, respectively. Cysteine residues involved in intra-domain cysteine bridges are marked by *red stars*. The conserved phosphorylated cytoplasmic serine 664 is marked by a *green star*

subsequent transfer to the apical side of the cell, which is dependent on microtubule dynamics (Casanova et al. 1990; Hunziker et al. 1991). Mutation of this residue into an alanine markedly slows down the translocation of the complex within the cell, and conversely mutation into a negatively charged aspartic acid, resembling a negatively charged phosphorylated serine, enhances the rate of translocation (Casanova et al. 1990).

Studies on the binding properties of PIGRs in different species show that the first domain, domain 1, is essential and sufficient for the binding of IgA and IgM (Norderhaug et al. 1999; Kaetzel 2005; Klimovich 2011). However, this binding affinity increases 20-fold by adding additional domains, suggesting that although domain 1 is essential, other domains are of importance at least in some species (Zikan and Bennett 1973; Klimovich 2011). A recent study of the structure of the human PIGR shows an almost closed ring or triangular structure of the five domain PIGR when not bound to IgA or IgM. In this orientation, domain 1 directly interacts with domains 2, 4 and 5 to form this triangular structure and upon ligand binding this opens up into a more extended structure (Stadtmueller et al. 2016). Analysis of a two domain fish PIGR by the same lab shows that the fish PIGR forms an extended structure even in the absence of direct contact with Igs (Stadtmueller et al. 2016). Interestingly, the addition of domain 2 in mammalian PIGRs seems to facilitate the triangular shape and also to stabilize the complex between the SC and IgA (Stadtmueller et al. 2016). There is also a marked difference in ligand specificity between PIGRs from different species. In primates, PIGRs bind both IgA and IgM, whereas rodent (mouse, rat and rabbit) PIGRs only bind IgA and not IgM (Brandtzaeg and Johansen 2001). The PIGR Ig domains are of the V type and three complementarity determining regions (CDRs) can also, similar to Ig V regions, be identified in the PIGR domain 1, which are important for the interactions between the PIGR and IgA and IgM (Coyne et al. 1994). During a domain swapping experiment between human and rabbit PIGRs, the exchange of CDR2 from the rabbit into the human PIGR results in a loss of affinity for IgM, indicating this region is of major importance for the interaction with IgM (Roe et al. 1999). The increase in domain numbers improves IgA binding and the duplication of the exon for domain 2 has been implicated in this increased IgA affinity (Norderhaug et al. 1999). The binding of IgA and IgM is dependent on the presence of the J chain of both of these isotypes (Ferkol et al. 1995). However, the CH4 domain of IgM also appears to be essential for binding of IgM (Ferkol et al. 1995). It has been proposed that the driving force in the evolution of the PIGR from two domains to four and five domain PIGRs has been interactions with the commensal microbiota (Kaetzel 2014).

The number of PIGR genes in the different teloeost fish is remarkable (Fig. 3). Currently, 34 PIGR genes have been identified in the zebrafish genome and several other fish have more than 5 or even 10 such genes. One exception is the gar, where only one PIGR gene has been observed. The gar represents an early branch of bony fish, which may indicate that the massive expansion in many fish species has occurred after the diversification of the bony fish. One major question that remains
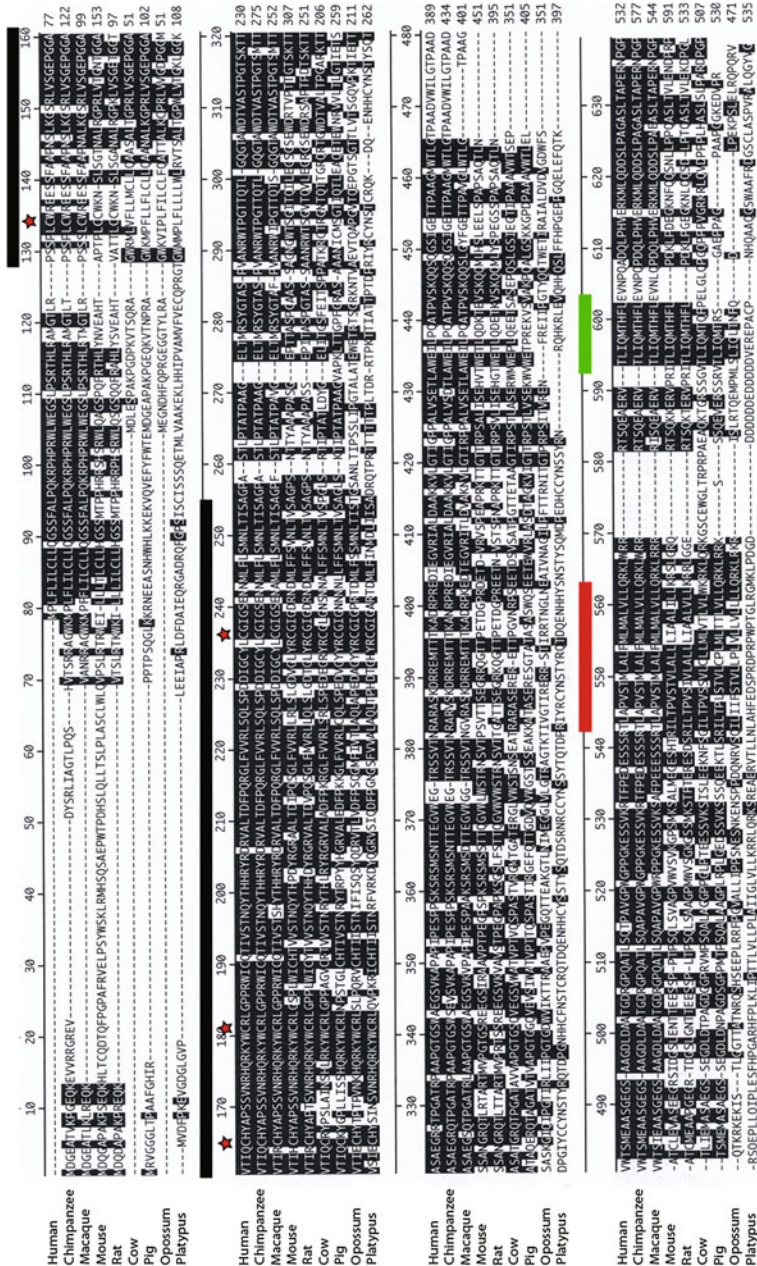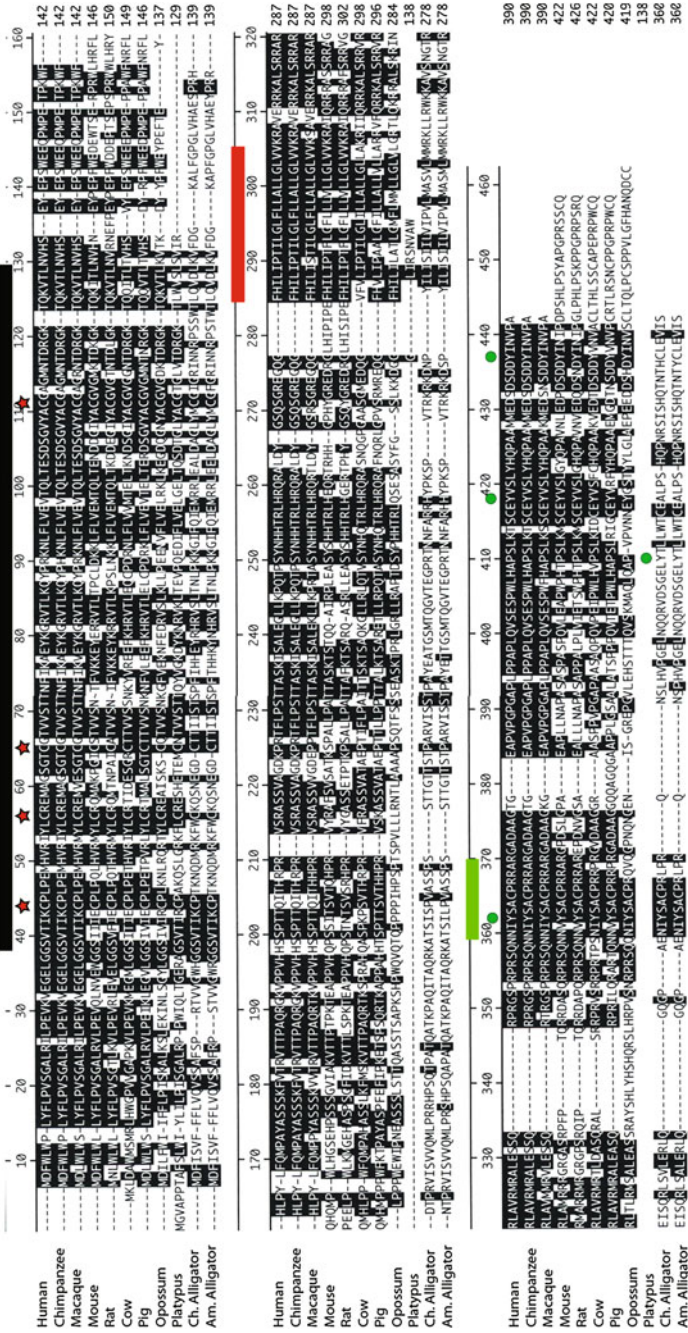
Fig. 7 A sequence alignment of a panel of FcαμRs. Conserved residues are shown within *black boxes*. The Ig domain, the hydrophobic transmembrane region and the conserved potential signaling motifs in the cytoplasmic region are marked by a *thick black line*, a *red line* and *green lines*, respectively. Cysteine residues involved in intra-domain cysteine bridges are marked by *red stars*. The platypus sequence is probably only partly correct. Only the Ig domain shows significant homology to the other FcαμR sequences, and the remaining parts of the protein is likely to be wrongly annotated in the database

to be addressed here are the functions of all of these PIGRs in bony fish. In our minds it is unlikely that they are all involved in epithelial transport.

4 The FcαμR

The dual receptor for IgA and IgM is a 55 kD protein and is involved in endocytosis of IgM coated microparticles and bacteria (Shibuya et al. 2000). The mature FcαμR is a remarkably stable homodimeric glycoprotein with an M_r of 115–135 kD (Kikuno et al. 2007). Among the hematopoietic cells it is expressed primarily on follicular dendritic cells (FDC) in both mice and humans (Kikuno et al. 2007). It is also expressed by certain cell populations in the liver, kidneys, small and large intestines, testes and placenta (Shibuya et al. 2000; Sakamoto et al. 2001; Kikuno et al. 2007). Interestingly the expression level is highest in the kidney, indicating a potent physiological role not only in hematopoietic cells (Sakamoto et al. 2001). In contrast to the PIGR, this receptor has only one Ig-like domain, which is most closely related to domain 1 of the PIGR in tetrapods (Shibuya et al. 2000). The binding affinity of this domain is approximately ten times stronger for IgM than for IgA, 2.9×10^9 M^{-1} and 3×10^8 M^{-1}, respectively, but it clearly binds both isotypes indicating that it is a bona fide dual receptor (Shibuya et al. 2000; Klimovich 2011). In contrast to PIGR, the binding does not appear to be dependent on the J chain as monomeric IgA can also bind, at least to the mouse receptor (Shibuya et al. 2000). Furthermore, as IgA and IgM seem to compete for binding to the mouse receptor, it indicates a common site for their interaction (Yoo et al. 2011). In addition to the N terminal Ig domain this receptor has a relatively long, approximately 277 amino acids (in humans), extracellular mucin-like domain of unknown function (Shimizu et al. 2001). The transmembrane portion of the human receptor is 20 amino acids long with no charged residues (Figs. 1 and 7) and the 61 amino acid cytoplasmic region has no archetypal ITIMs or ITAMs, which are found in the classical mammalian IgG and IgE receptors (Shimizu et al. 2001). A di-leucine motif in the cytoplasmic region of the mouse receptor appears to be involved in the internalization process (Shibuya et al. 2000). However, this motif is not conserved in any of the other species listed (Fig. 7) including the human receptor, indicating that other motifs are of importance for this receptor in other species (Shibuya et al. 2000). Instead, there is another motif that is relatively well conserved between all placental mammals studied and also in the opossum, a marsupial (Fig. 7). This conserved sequence is located approximately 15 amino acids in from the membrane and has the consensus sequence V/I-T/S-L-I-Q-M-T-H-F-L-E/D. This motif may be of major importance for intracellular signaling processes (Fig. 7), although this needs to be studied and verified experimentally.

Knock out experiments in mice show that the absence of the FcαμR does not affect the titers of IgG and IgM in sera nor the T-cell dependent antibody responses (Honda et al. 2009). However, a marked increase in IgG3 levels to thymus independent antigens is observed as well as an IgG3 germinal center dependent memory

◄**Fig. 8** A sequence alignment of a panel of FcμRs. Conserved residues are shown within *black boxes*. The Ig domain, the hydrophobic transmembrane region and the conserved potential signaling motifs in the cytoplasmic region are marked by a *thick black line*, a *red line* and *green lines*, respectively. Cysteine residues involved in intra-domain cysteine bridges are marked by *red stars*. The platypus FcμR sequence is only partial, only containing a signal sequence and the Ig domain. The three Tyr residues conserved in all mammalian sequences are marked by *green dots* (Tyr 315, 366 and 385). A Tyr, possible corresponding to Tyr 366 and/or 385, in the alligator sequences is also marked by a *green dot*

response (Honda et al. 2009). The number of germinal center B cells increases significantly after injection with thymus independent antigens but not with T-dependent antigens. These effects of FcαμR are dependent on complement and complement receptors, as blocking monoclonal antibodies to complement receptors 1 and 2 results in an almost complete loss of these responses (Honda et al. 2009). Concluding, this receptor seems to be involved in the internalization of antigen in B cells, which is most likely for presentation on MHC molecules, but also as a negative regulator of IgG3 responses to thymus independent antigens.

5 The FcμR

The receptor specific for IgM was the last of these receptors to be identified. Indications for the presence of such a receptor has been apparent for many years but it was not until very recently that it was finally identified through expression cloning into mammalian cells by the lab of Hiromi Kubagawa (Kubagawa et al. 2009, 2014). FcμR is a 60 kD transmembrane protein expressed by human B, T and NK cells and binds pentameric IgM with a very high avidity. In mice it is primarily expressed by B cells and not at significant levels on T and NK cells (Shima et al. 2010; Honjo et al. 2012; Ouchida et al. 2012; Honjo et al. 2014). However, there are some controversies regarding the expression pattern of this receptor as independent groups have provided varying results when using different monoclonals or when analysing mRNA levels by qPCR or Northern blotting, which explains why there is still slight doubt over the exact tissue distribution of this receptor (see Chapter of Kubagawa et al.) (Wang et al. 2016). The FcμR is also internalized upon ligand binding, which complicates the analysis by when using monoclonals analyzing its surface expression (Wang et al. 2016). After translation the primary polypeptide is only 41 kD, hence a large fraction of the molecular weight is attributed to carbohydrate residues. Despite this, no N-linked carbohydrate addition sites (N-X-T/S) are found in the human or the mouse sequences (Figs. 1 and 8). Recently several O–linked glycosylation sites have been identified in the linker region by point mutational analyses, showing at least the majority of these carbohydrates are O-linked (Kubagawa et al. 2009; Vire et al. 2011; Kubagawa et al. 2014). The isoelectric point also is markedly different when comparing the 41 kD

polypeptide chain to the 60 kD glycosylated form. The change is from a predicted pI 9.9 to 5, indicating a high percentage of negatively charged sialic acid residues in the carbohydrate chains (Kubagawa et al. 2014). FcμR is also the only receptor that exclusively binds IgM and interestingly, the CDR1 loop of the receptor is considerably shorter than the corresponding region in both the PIGR and the FcαμR; five amino acids compared to nine. Furthermore, the receptor lacks an Arg residue in this region, which is predicted to interact with IgA, suggesting that this difference is responsible for the monospecificity of the FcμR (Kubagawa et al. 2009, 2014).

This receptor is present in all three extant mammalian lineages and it was also recently identified in both the American and the Chinese alligator genomes, indicating that it appeared relatively early in tetrapod evolution. However, as this receptor has not been found in any of the amphibian genomes, it implies that it appeared in an early reptile, which became the ancestor to both reptiles and mammals. The FcμR is involved in a number of important steps in B-cell development including IgM homeostasis, B-cell survival, humoral immune responses and in autoantibody formation (Honjo et al. 2012; Ouchida et al. 2012; Choi et al. 2013; Honjo et al. 2014). Despite these observations, quite contradicting results have come from three independent knockout mice that have been generated (Nguyen et al. 2011; Honjo et al. 2012; Ouchida et al. 2012; Choi et al. 2013; Kubagawa et al. 2014; Wang et al. 2016). Two recent reviews have discussed the effects by functionally inactivating the FcμR and the discrepancies between the three different knockout mouse strains, therefore only a short list of some of the key findings described from studies will be given below. (Kubagawa et al. 2014; Wang et al. 2016). All three knockouts show alterations in B-cell populations, although with varying effects between the different mice. They also all show dysregulated humoral immune responses, impaired B-cell proliferation after ligation of surface Ig, and an increase in autoantibody production. Interestingly, mice lacking secretory IgM, through a deletion of the genomic region encoding the secretory terminal region of the CH4 exon, results in a mouse that only expresses cell surface bound IgM and not secretory IgM, showing a very similar phenotype to the FcμR knockout mice (Kubagawa et al. 2014; Wang et al. 2016). Although there are clear differences between the knockout models, the consensus is that the FcμR has major effects on B-cell differentiation and Ig homeostasis (Kubagawa et al. 2014; Wang et al. 2016). The FcμR also appears to be the only receptor among the FcRs directly involved in IgM homeostasis (Honjo et al. 2012, 2014).

The signaling from the FcμR does not involve classical ITAMs or ITIMs. However, there are several conserved tyrosines and serines in its cytoplasmic tail (Fig. 8) (Kubagawa et al. 2014; Wang et al. 2016). Three tyrosines are conserved within the cytoplasmic tail of all eutherian mammalian FcμRs but this number drops to one tyrosine when alligators are also included (Fig. 8). Four serines are conserved between all mammals and two additional serines are almost fully conserved. However, similarly to the tyrosines, a drop is seen when including alligators, resulting in only two fully conserved residues (Fig. 8). A recently identified Ig tail tyrosine (ITT) phosphorylation motif Glu/Asp X_{6-7}-Asp-Tyr-X-Asn, which is present in membrane bound IgG and IgE, is also found in the C-terminal end of

mammalian FcμRs but not in the corresponding region in the alligator sequences (Fig. 8) (Wang et al. 2016). In Igs this ITT motif is involved in triggering and activating switched memory B cells (Wang et al. 2016). Only one of the motifs is conserved between all species analyzed and has the sequence N-I/V-Y-S-A-C-P-R (Fig. 8, marked with a *green box*). A quite extensive mutational analysis has been performed on the trans-membrane and cytoplasmic tail of the human FcμR. The membrane proximal Tyr (Tyr-315) that is found in the motif marked in green in Fig. 8 has been shown to be of importance for the anti-apoptotic effect of the FcμR. This mutation has almost the same effect on the anti-apoptosis as deleting almost the entire intracellular domain (Honjo et al. 2015). Mutations of the His residue in the transmembrane region and the two membrane distal Tyr residues 366 (Tyr to Phe) and 385 (Tyr to Phe) also affects the anti-apoptosis however to a lesser extent. The two latter mutations also showed a pronounced effect on internalization of the receptor indicating a prominent role of these two Tyr residues in receptor-mediated endocytosis (Honjo et al. 2015). These three Tyr residues are marked by green dots in Fig. 8. In this figure a Tyr possible corresponding to the Tyr 366 and/or 385 in the alligator sequences is also marked by a green dot.

6 Concluding Remarks

Following the appearance of Igs in early jawed vertebrates there has been a parallel increase in the complexity of molecules interacting with these antigen specific molecules during vertebrate evolution. The first molecules to interact with Igs have probably been components of the complement system. All of the essential components of the classical complement system have been identified in a number of cartilaginous fish (Goshima et al. 2016). These components seem to absent in jawless fish, indicating the appearance of this branch of the complement system with the jawed vertebrates. The second additions were most likely the PIGRs, the FcR common γ chain and the FcRL molecules. This was later followed by an increase in complexity of the FcRL molecules in tetrapods, resulting in the appearance of the classical receptors for IgG and IgE in mammals (Akula et al. 2014). Although all of the evidence indicates that IgM is the evolutionary oldest of the different Ig isotypes, two of the three receptors for IgM, FcμR and FcαμR, appeared relatively late during vertebrate evolution. The FcμR may have appeared sometime during early reptile evolution whereas the FcαμR probably appeared during early mammalian evolution. Due to the high sequence similarity in the first Ig-like domain of all three IgM receptors, the most likely scenario is that they have appeared by successive gene duplication events involving domain 1 of the PIGR. The origins of the other parts of both the FcμR and the FcαμRs are still a mystery, as no closely related sequences can be found in either the mouse or human genomes. Following these duplications, these three receptors have diversified quite extensively in function by changing expression patterns and cytoplasmic signaling motifs. In tetrapods, the PIGR primarily functions as a transport receptor for IgM and IgA (IgX in amphibians). The situation in fish is still not fully

known. However, the large complexity of PIGRs in some bony fish, such as the zebrafish, is exciting and needs further detailed analyses to obtain a more complete picture of their roles in fish immunity. What is also somewhat surprising is the complete absence of known FcRs in cartilaginous fish. The obvious question is how have they solved the problems of Ig mediated antigen uptake by phagocytic and antigen presenting cells as well as transport of Ig over epithelial layers and potential triggering of granule release by hematopoietic cells, which is attributed to FcRs in mammals? To our knowledge no good candidates for such molecules have been identified yet. Additionally, the complex role of the FcμR and FcαμR in regulating B-cell responses is a fascinating research area. Originating from a PIGR, with primarily a transport function, they have gained important roles in thymus-independent B-cell responses, B-cell homeostasis and autoantibody formation. In the near future, new insights from the studies of the different knockout animals will most likely shed even more light on these intricate regulatory mechanisms by these three receptors.

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