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Review Article

Coevolution of Mucosal Immunoglobulins and the Polymeric Immunoglobulin Receptor: Evidence That the Commensal Microbiota Provided the Driving Force

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Immunoglobulins (Igs) in mucosal secretions contribute to immune homeostasis by limiting access of microbial and environmental antigens to the body proper, maintaining the integrity of the epithelial barrier and shaping the composition of the commensal microbiota. The emergence of IgM in cartilaginous fish represented the primordial mucosal Ig, which is expressed in all higher vertebrates. Expansion and diversification of the mucosal Ig repertoire led to the emergence of IgT in bony fishes, IgX in amphibians, and IgA in reptiles, birds, and mammals. Parallel evolution of cellular receptors for the constant (Fc) regions of Igs provided mechanisms for their transport and immune effector functions. The most ancient of these Fc receptors is the polymeric Ig receptor (pIgR), which first appeared in an ancestor of bony fishes. The pIgR transports polymeric IgM, IgT, IgX, and IgA across epithelial cells into external secretions. Diversification and refinement of the structure of mucosal Igs during tetrapod evolution were paralleled by structural changes in pIgR, culminating in the multifunctional secretory IgA complex in mammals. In this paper, evidence is presented that the mutualistic relationship between the commensal microbiota and the vertebrate host provided the driving force for coevolution of mucosal Igs and pIgR.

1. Introduction

The mucosal surfaces of the body form the primary interface with the outside world, providing a conduit for intake of nutrients and air and a home for beneficial microbes that act as "extended self" [1]. Host cells that populate mucosal surfaces must carry out the challenging task of maintaining a mutualistic relationship with the resident microbiota, while protecting the body proper against potential pathogens, toxic environmental substances, and soluble dietary antigens that could act as systemic allergens. Adaptive immune systems, characterized by clonally expressed, somatically diversified antigen receptors in lymphocytes, first emerged in a common ancestor of modern vertebrates [2, 3] (Figure 1). Jawless fish of the superclass Agnatha (hagfishes and lampreys) generate variable-like receptors (VLRs) for antigen (Ag) by a mechanism involving gene conversion. The evolution of the *RAG1/2* dependent mechanism of V(D)J somatic recombination in an ancestor of modern jawed vertebrates led to the first appearance of Ag-specific immunoglobulins (Igs) and T-cell antigen receptors [4, 5]. The basic structural unit of Igs comprises 2 identical light chains, encoded by *IGL* genes, and 2 identical heavy chains, encoded by *IGH* genes (Figure 2). The polypeptide chains of Ig light and heavy chains consist of repeating units of approximately 110 amino acids, so-called "Ig-like" domains with a characteristic 3-dimensional structure [6, 7]. Each domain is encoded by a single exon, suggesting that the *IGL* and *IGH* genes evolved by duplication and diversification of a primordial Ig-like gene. Ig light chains comprise an N-terminal variable (V_L) domain followed by a constant (C_L) domain of either the κ or λ type. Ig heavy chains comprise an N-terminal variable domain (V_H) followed by 3-4 constant domains (C_H1–C_H3 or 4). The sequence of Ig heavy chain constant regions (designated by Greek letters such as α, γ, or μ) defines the Ig class or isotype. Pairing of V_H and V_L domains on adjacent Ig heavy and light chains

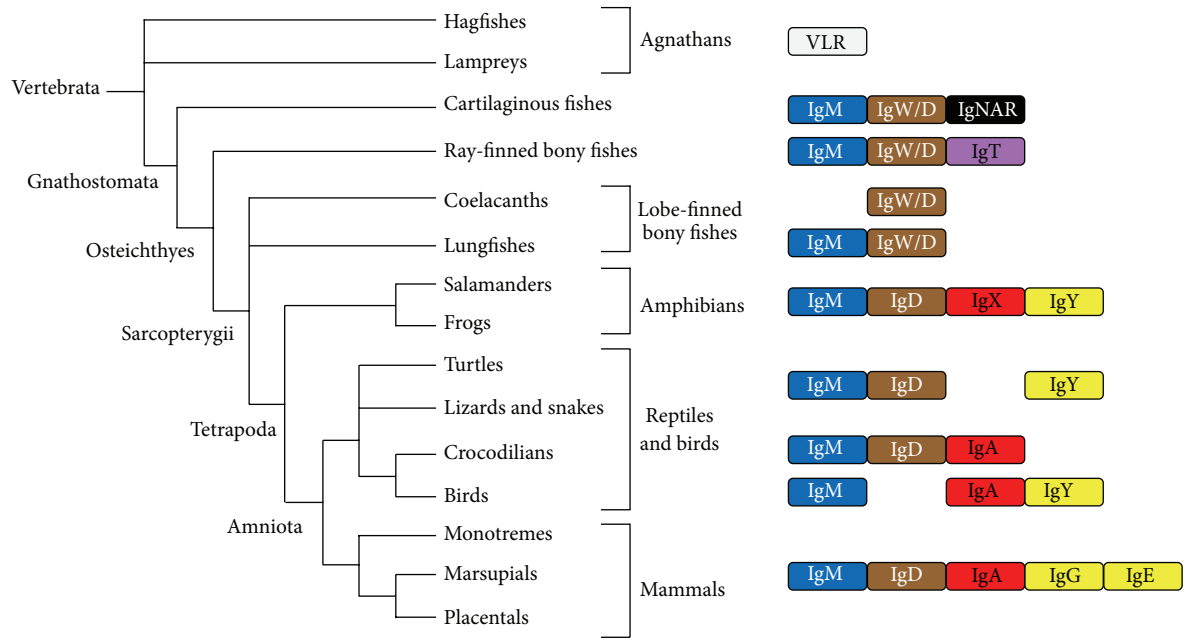


Figure 1: A model for evolution of immunoglobulin isotypes in vertebrates. The antigen-binding variable lymphocyte receptors (VLRs) expressed in extant Agnathans are thought to represent the precursors of modern immunoglobulins (Igs). Genes encoding Ig heavy and light chains apparently emerged prior to the split between cartilaginous and bony fishes, resulting in expression of Ig molecules that are characteristic of higher vertebrates. Cartilaginous fishes express an IgD-like molecule (IgW/D) and IgM, which is found evenly distributed between blood and mucosal secretions. The first evidence of a dedicated mucosal Ig isotype came with the emergence of IgT in an ancestor of modern bony fishes. Subsequent duplication of Ig constant region genes and evolution of Ig class switching mechanisms in a common ancestor of tetrapods allowed the production of multiple Ig isotypes during immune responses. Color coding indicates the presumed evolution of avian and mammalian IgA from a precursor of amphibian IgX and evolution of mammalian IgG and IgE from a precursor of amphibian IgY.

forms the antigen-binding site, while the Fc regions of the Ig heavy chains (comprising the two most C-terminal constant domains) confer isotype-specific immune effector functions. Some Ig isotypes can form higher-order polymers of the basic 4-chain unit, thus increasing the number of Ag-binding sites and enhancing Fc-mediated functions. The emergence of new Ig isotypes during vertebrate evolution allowed for diversification of Ig-mediated immune functions, including those specialized for mucosal surfaces (reviewed in [8]). Many Ig-mediated immune functions involve interactions with isotype-specific cellular receptors for the Fc region of Ig heavy chains (Figure 3). The most evolutionarily ancient Fc receptor is the polymeric Ig receptor (pIgR), a protein expressed selectively by mucosal and glandular epithelial cells that transports polymeric Igs into external secretions. It has been hypothesized that the complex relationship between the vertebrate host and its resident microbiota provided the driving force for evolution of the adaptive immune system [1, 5]. In this review, the concept will be explored that an important consequence of this selective pressure was the coevolution of mucosal Igs and pIgR.

2. Evolution of Mucosal Immunoglobulins

2.1. Cartilaginous Fishes. Primordial Ig-like genes are believed to have arisen in a common ancestor of the Gnathostomata (jawed vertebrates) [2, 3] (Figure 1). The most primitive extant jawed vertebrates comprise the class Chondrichthyes (cartilaginous fishes), including sharks, rays, and skates. Multiple Ig isotypes have been identified in the horned shark (*Heterodontus francisci*) [18], nurse shark (*Ginglymostoma cirratum*) [19, 20], and several species of skates [21]. The primordial IgM of cartilaginous fishes is orthologous to the IgM of higher vertebrates, while a second Ig isotype, initially called IgW, is orthologous to IgD [22]. A third Ig isotype identified in the nurse shark, called the Ig "new antigen receptor" (IgNAR), is homologous to IgM and IgW/D but does not have an ortholog in higher vertebrates [19]. The *IGH* and *IGL* genes are dispersed throughout the genome of cartilaginous fish in hundreds of clusters, each representing an individual isotype [18, 21].

2.2. Bony Fishes. Major changes in the organization of Ig genes occurred during the emergence of the Teleostomi, the clade of jawed vertebrates that includes the superclasses Osteichthyes (bony fishes) and Tetrapoda (four-limbed vertebrates) (Figure 1). The dispersed *IGH* loci characteristic of cartilaginous fishes were replaced with a translocon organization, comprising a 5′ region of V_H, D_H, and J_H gene segments encoding the Ig heavy chain variable region, followed by tandem C_H gene segments encoding Ig heavy chain constant regions for different isotypes (reviewed in [23]). This translocation organization allows a B lymphocyte to "switch" production from one Ig heavy chain isotype to another

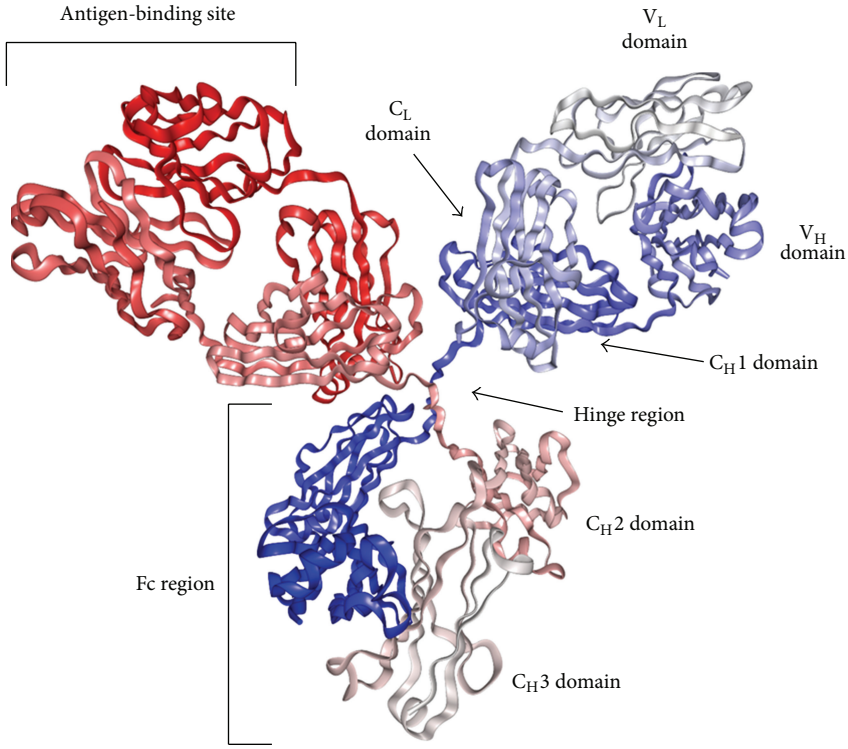


Figure 2: The basic structure of immunoglobulins. The basic structural unit of Igs comprises 2 identical light chains (shown in red and blue) and 2 identical heavy chains (shown in red and blue). The polypeptide chains of Ig light and heavy chains consist of repeating units of approximately 110 amino acids, so-called "Ig-like" domains with a characteristic 3-dimensional structure. Ig light chains comprise an N-terminal variable (V_L) domain followed by a constant (C_L) domain of either the κ or λ type. Ig heavy chains comprise an N-terminal variable domain (V_H) followed by 3-4 constant domains (C_H1–C_H3 or 4). The sequence of Ig heavy chain constant regions (designated by Greek letters such as α, γ, or μ) defines the Ig class or isotype. The structure shown here is characteristic of mammalian IgA, IgD, and IgG, in which the C_H1 and C_H2 domains of the heavy chains are connected by a short cysteine- and proline-rich hinge region. The structure of all Igs in lower vertebrates (as well as IgM and IgE in mammals) differs in that the C_H2 domain replaces the hinge region and the Fc region comprises the C_H3 and C_H4 domains. Pairing of V_H and V_L domains on adjacent Ig heavy and light chains forms the antigen-binding site, while the Fc regions of the Ig heavy chains (comprising the two most C-terminal constant domains) confer isotype-specific immune effector functions.

during immune responses, by a process of DNA rearrangement called class switch recombination (CSR) [24–26]. Most of the studies of Ig structure and function in bony fishes have focused on the Actinopterygii (ray-finned bony fishes), which comprise 99% of the over 30,000 living species of fish, mainly within the infraclass Teleostei. Early studies in several teleost species, including plaice (*Pleuronectes platessa*) [27], common carp (*Cyprinus carpio*) [28], rainbow trout (*Oncorhynchus mykiss*) [29], and channel catfish (*Ictalurus punctatus*) [30], demonstrated that immunization by mucosal routes (oral, anal, and skin) elicited antibody responses in the skin, intestinal mucus, and bile. In 2005, a novel Ig isotype was described in rainbow trout [31], zebrafish (*Danio rerio*) [32], and fugu (*Fugu rubripes*) [33] designated IgT (for teleost) or IgZ (for zebrafish). In the same year, an unusual Ig isotype described in the common carp, which appeared to be a chimera of IgM and IgZ [34], was identified (*Cyprinus carpio*). Subsequent studies in a number of teleost species revealed a common translocon structure in the *IGH* locus, with tandem gene segments encoding the constant regions of IgM, IgD, and IgT(Z) [35–37]. Functional studies revealed that the molar ratio of IgT to IgM was 60-fold higher in gut mucus than in serum of rainbow trout and that specific IgT antibodies were induced in the gut following oral infection with the parasite *Ceratomyxa shasta* [38]. In this study, IgT-expressing lymphocytes were found to represent 54.3% of all B cells in the intestinal *lamina propria*. Interestingly, IgT was found as a monomer in serum but as a tetramer in gut mucus, suggestive of a selective mechanism for polymerization of IgT in mucosal B cells similar to that observed for IgA in higher vertebrates. Thus, IgT appears to be the most primitive Ig isotype with specialized functions at mucosal surfaces.

Although IgT has been identified in most Actinopterygii thus far studied, some exceptions have been noted. The absence of a gene encoding IgT was recently reported in the Siberian sturgeon (*Acipenser baerii*), a species of the order Acipenseriformes, which occupies a unique phylogenetic niche between the cartilaginous fishes and the Teleostei. The absence of IgT was also reported in the medaka (*Oryzias latipes*), a teleost of the order Beloniformes [39]. Surprisingly, IgT is absent in channel catfish, a teleost of the order Siluriformes, which is closely related to the order Cypriniformes

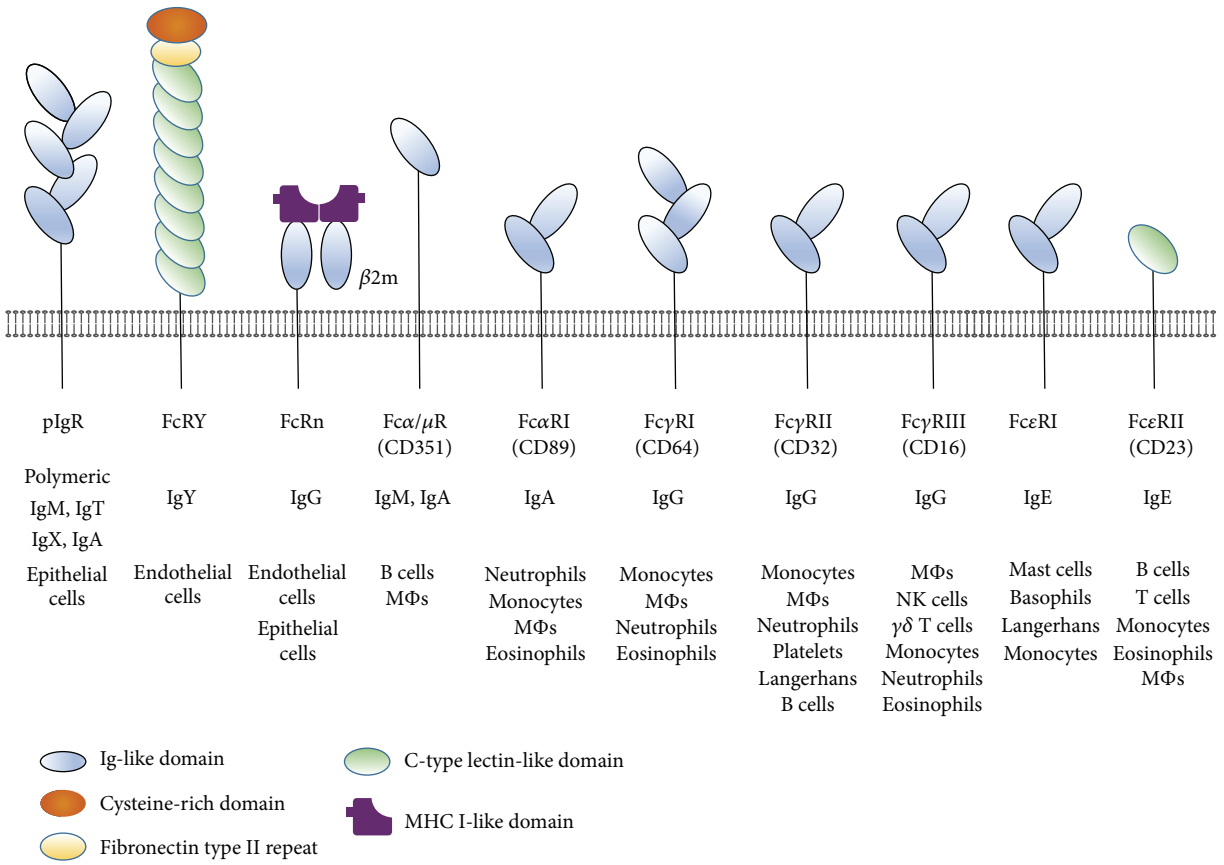


Figure 3: Fc receptors are Ig isotype- and cell type-specific. These schematic diagrams illustrate basic structural features of selected Fc receptors, all of which are Type I transmembrane proteins. The structures are not to scale and do not imply specific 3-dimensional conformations. All of the structures represent the human homolog of the indicated protein except for FcRY, which is from chicken. Binding of the constant (Fc) region of Igs to cellular receptors initiates transport across epithelial cells or intracellular signaling in cells of hematopoietic origin. The membrane-proximal Ig-like domain of FcRn pairs with the Ig-like domain of soluble β2-microglobulin (β2m). The Ig-binding subunits of FcαRI, FcγRI, FcγRII, FcγRIII, and FcεRI (shown here) pair with other protein subunits (not shown) to initiate signaling pathways. This figure was compiled using information from [9–15].

that includes the IgT-expressing carp and zebrafish. The *IGH* loci of the few extant species of the class Sarcopterygii (lobe-finned bony fishes, including coelacanths and lungfishes) differ from those of most of the Actinopterygii (Figure 1). The African coelacanth (*Latimeria chalumnae*), one of only two species of an ancient class of lobe-finned fishes, lacks IgM and has a single Ig heavy chain gene that resembles the IgW/D gene of cartilaginous fishes [40]. By contrast, the African lungfish (*Protopterus aethiopicus*) expresses both IgM and an isotype resembling IgW/D [41]. No ortholog of IgT has been identified in lobe-finned bony fishes. Given the diversity of Ig genes in bony fishes, the evolutionary origin of IgT remains obscure. Furthermore, while IgT shares many functional similarities with IgA in higher vertebrates, the structural genes for IgT and IgA are not orthologous [23]. Thus, the gene encoding IgT appears to be an evolutionary dead-end that emerged in some lineages of bony fishes in response to pressures for immunological protection of mucosal surfaces, likely driven by the resident microbiota as well as mucosal pathogens.

2.3. Amphibians. Duplication and diversification of gene segments within the *IGH* locus in a common ancestor of the Tetrapoda (four-limbed vertebrates) led to the appearance of additional heavy chain Ig isotypes (reviewed in [42]) (Figure 1). Most extant species of tetrapods have a single *IGH* locus, the location of which is syntenic with the human *IGH* locus. Ectothermic vertebrates of the class Amphibia are the most primitive tetrapods, having diverged about 370 million years ago from a common ancestor with reptiles, birds, and mammals. Early studies of the structural and immunological properties of Igs from several species of clawed frogs *(Xenopus)* suggested the emergence of two novel Ig isotypes, IgX and IgY [43–47] (Figure 1). B cells expressing IgX, but not IgY, were found abundantly in the intestinal epithelium, whereas IgX-positive cells were rarely detected in the spleen and IgX levels were very low in serum [47]. Interestingly, thymectomy abolished expression of IgY, but not IgM or IgX, consistent with a T-independent role for IgX in the mucosal immune system of amphibians. Genes encoding IgX and IgY were subsequently identified in 2 species of salamanders [48, 49],

suggesting that these Ig isotypes may have emerged in a common ancestor of amphibians. To date, no studies have been reported on *IGH* loci in caecilians. A recent study in *Xenopus laevis* established the position of amphibian IgX as the evolutionary precursor of IgA in birds and mammals [50]. These investigators found that larval thymectomy did not affect levels of IgX and did not alter the composition of the gut microbiota, confirming the T-independence of the IgX response in *Xenopus*. Phylogenetic comparisons of IgT in bony fishes and IgX in amphibians suggest that these Ig isotypes evolved independently [23], likely responding to similar pressures from the resident microbiota and mucosal pathogens.

2.4. Reptiles and Birds. The clade Amniota within the superclass Tetrapoda can be subdivided into the sauropsids (reptiles and birds) and synapsids (mammals and their close relatives) (Figure 1). The distinction between reptiles and birds is based largely on physical characteristics rather than phylogeny, distinguishing the cold-blooded reptiles with scales from the warm-blooded birds with feathers. Early studies of Igs in turtles, lizards, and snakes were based largely on their physicochemical and antigenic properties, revealing a predominance of IgM and lesser amounts of IgY in bile and gut secretions [51–53]. Subsequent analyses using antibodies to chicken IgA failed to detect a cross-reactive Ig isotype in reptiles [54, 55]. The recent availability of whole genome sequences for 2 species of turtles (*Chrysemys picta* and *Pelodiscus sinensis*) [36, 56] and the green anole lizard (*Anolis carolinensis*) [57] demonstrated the presence of gene segments encoding IgM, IgD, and multiple subclasses of IgY, but no ortholog of IgX in amphibians or IgA in birds and mammals. The leopard gecko (*Eublepharis macularius*) expresses IgM, IgD, and a third Ig isotype that is intermediate in homology between IgA and IgY [50, 58] (designated Ig"A" for the purposes of this review). The recent availability of whole genome sequences for 4 crocodilians (*Alligator sinensis*, *Alligator mississippiensis*, *Crocodylus siamensis*, and *Crocodylus porosus*) revealed significant differences in the *IGH* loci compared to other reptiles, including multiple subclasses of IgM, IgD, and IgA (orthologous to the IgA genes of birds and mammals), but no IgY [59, 60]. Early studies of the immune systems of birds (class Aves) revealed the presence of an ortholog of mammalian IgA in mucosal secretions and an ortholog of IgG (designated IgY) in serum [61–69]. Mapping of the *IGH* locus in chickens and ducks revealed an inverted gene segment encoding IgA, downstream of the IgM gene segment and upstream of the IgY gene segment, and loss of the gene segment encoding IgD [70, 71]. Availability of whole genome sequences for a variety of bird species in the National Center for Biotechnology Information (NCBI) database demonstrates the consistent presence of IgM, IgA, and IgY and the absence of IgD. A recent analysis of Ig gene transcripts in the ostrich (*Struthio camelus*) revealed similarities with chickens, ducks, and other birds, including the expression of IgM, IgA, and IgY and the absence of IgD [72]. Because the ostrich is one of the most primitive extant species of birds, it appears that the distinctive organization of the *IGH* locus evolved very early during the divergence of the avian lineage. Furthermore, a phylogenetic comparison of Ig heavy chain sequences from amphibians with those of higher vertebrates demonstrated with high statistical support that IgX from amphibians and IgA from birds share a recent common ancestor [50]. Taken together, the evidence is consistent with an *IGH* locus in a common ancestor of reptiles and birds that included gene segments encoding IgM, IgD, IgA, and IgY. Instability in the *IGH* locus in individual lineages apparently resulted in the selective loss of IgA in most turtles, lizards, and snakes, IgY in crocodilians, and IgD in birds.

2.5. Mammals. The structure of Ig genes and their encoded proteins has been studied extensively in extant species of the class Mammalia, including the egg-laying monotremes, the pouch-bearing marsupials, and the placentals (eutherians) (reviewed in [8]). The *IGH* locus in all known mammals is organized as a translocon with gene segments encoding IgM, IgD, 2 or more subclasses of IgG, 1 or more subclasses of IgA, and IgE, an isotype unique to mammals (Figure 1). A recent phylogenetic analysis of Ig heavy chain classes in tetrapods indicated with high statistical probability that the IgA gene(s) in mammals evolved from an IgX gene in a common ancestor of all tetrapods, while the IgG and IgE genes evolved more recently from IgY in a common ancestor of amniotes [50]. Continuing diversification in the *IGH* locus in mammals is evidenced by differences in the number of IgG and IgA subclasses and in the structure of Ig heavy chains. Unlike all Ig heavy chains in lower vertebrates, comprising 1 variable Ig-like domain (V_H) and 4 constant Ig-like domains (C_H1–C_H4), the C_H2 domain in mammalian IgA, IgD, and IgG was replaced with a short, flexible hinge region rich in cysteine and proline residues [73]. The heavy chain of mammalian IgE has retained the prototypic 4 constant Ig-like domains (as has IgM), suggesting that the evolution of the hinge region in mammalian IgG occurred after the evolution of IgG and IgE from an ancestral IgY gene (Figure 1).

Determination of the structure of the *IGH* locus in the monotreme duck-billed platypus (*Ornithorhynchus anatinus*) revealed the presence of 8 C_H genes (including 2 subclasses of IgG and 2 subclasses of IgG) arranged in the order C_μ-C_δ-C_o-$C_{\gamma 2}$-$C_{\gamma 1}$-$C_{\alpha 1}$-C_ε-$C_{\alpha 2}$ [74, 75]. Interestingly, the gene encoding platypus IgD lacks a hinge region and is more similar in structure to IgD in lower vertebrates than the IgD of eutherian mammals. Downstream of the C_δ gene in the platypus is a novel C_o gene (omicron for *Ornithorhynchus*), which appears to have evolved from an ancestral IgY gene. In contrast to the platypus, only one IgA gene was identified in another monotreme, the echidna (*Tachyglossus aculeatus*), and in several species of marsupials [76–80]. Similarly, a single structural gene for IgA has been identified in 3 species of the eutherian order Rodentia, mouse (*Mus musculus*) [81], rat (*Rattus norvegicus*) (unpublished; Genbank accession number AJ510151), and gerbil (*Meriones unguiculatus*) [82]. By contrast, the *IGH* locus in the European rabbit (*Oryctolagus cuniculus*, order Lagomorpha) is unique among mammals, apparently having evolved from a common ancestor

prior to the divergence of marsupials and monotremes from eutherians. Distinctive features include a single IgG subclass, 13 functional IgA subclasses, and absence of IgD [83–85]. A more typical organization of the *IGH* locus, including single subclasses of IgM, IgD, IgA, and IgE, and 2 subclasses of IgG has been reported in mammals of the orders Carnivora [86, 87], Cetartiodactyla (even-toed ungulates, whales, and dolphins) [88–91], and Perissodactyla (odd-toed ungulates) [92].

Mammals of the order Primates, including humans, have the greatest number and diversity of Ig classes and subclasses among all of the tetrapods. Mapping of *IGH* loci has now been completed for a number of primate species, including human (*Homo sapiens*), gorilla (*Gorilla gorilla*), chimpanzee (*Pan troglodytes*), orangutan (*Pongo pygmaeus*), gibbon (*Hylobates lar*), baboon (*Papio anubis*), mangabey (*Cercocebus torquatus atys*), and three species of macaques, the crab-eating monkey (*Macaca fascicularis*), pig-tailed monkey (*Macaca nemestrina*), and rhesus monkey (*Macaca mulatta*) [93–97]. A distinguishing feature of primates in the family *Hominidae* (great apes, including humans, gorillas, chimpanzees, and orangutans) and the family *Hylobatidae* (gibbons) is the presence of two IgA subclasses. Although the orangutan is a member of the family *Hominidae*, its *IGH* locus contains only one IgA subclass gene, homologous to the IgA1 gene of other hominoids [95]. By contrast, primates in the family *Cercopithecidae* (Old World monkeys, including baboons, mangabeys, and macaques) have a single IgA gene. Genetic analysis of the *IGH* locus of primates suggested that duplication and diversification of the primordial $C_{\gamma 1}$-$C_{\gamma 2}$-C_{ε}-C_{α} region occurred in a common ancestor of the great apes after the divergence from Old World monkeys [98, 99]. These events resulted in the current organization of the *IGH* locus in extant species of great apes, with four IgG subclasses and two IgA subclasses (the duplicated C_{ε} gene became a nonfunctional pseudogene; hence, only one class of IgE is expressed). The close phylogenetic relationship among the C_{α} genes in the hominoids and *Hylobatidae* suggests that the $C_{\alpha 1}$ and $C_{\alpha 2}$ genes evolved from a single C_{α} gene in a common ancestor of the great apes and that the $C_{\alpha 2}$ gene was subsequently lost in the orangutan. Evidence of continuing evolution in the *IGH* locus of humans was demonstrated by the finding of triplication of the $C_{\gamma 1}$-$C_{\gamma 2}$-C_{ε}-C_{α} region in several families [100]. Subsequent genetic analyses in multiple ethnic groups revealed that gene duplications and deletions in the human *IGH* locus are surprisingly common, with frequencies up to 22% in Japanese and Chinese populations [101].

3. Emergence of the *IGJ* Gene and Evolution of Polymeric Igs

The ability to produce Igs as both monomers (i.e., one unit comprising 2 identical heavy chains and 2 identical light chains) and polymers (covalently linked aggregates of 2 or more Ig units) dates to the emergence of primitive Igs in cartilaginous fish [23, 42, 50]. Whereas pentameric IgM is typically found in serum as well as secretions, the polymeric forms of IgT, IgX, and IgA are generally restricted to mucosal secretions. The discovery of a low molecular weight "J" or "joining" chain as a component of polymeric Igs suggested a potential mechanism for polymerization of individual Ig molecules into higher molecular weight complexes [102–104]. The most primitive extant vertebrates in which the *IGJ* gene has been identified are the cartilaginous fish, concurrent with the presence of polymeric IgM. Although the *IGJ* gene has been identified in almost all lineages of jawed vertebrates, it is surprisingly absent in some lineages of Osteichthyes (bony fishes). No ortholog of the *IGJ* gene has been identified in any species of the class Actinopterygii (ray-finned bony fishes) for which the genome has been sequenced and annotated, including Atlantic cod (*Gadus morhua*), common carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*), zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), and three-spined stickleback (*Gasterosteus aculeatus*). In contrast to the ray-finned bony fishes, *IGJ* genes and functional J chain proteins were recently identified in two species of the class Sarcopterygii (lobe-finned bony fishes, the West Indian Ocean coelacanth (*Latimeria chalumnae*), and the African lungfish (*Protopterus dolloi*) [105]). These findings suggest that the *IGJ* gene was lost during the evolution of the Actinopterygii after their divergence from a common ancestor with the Sarcopterygii. Despite the absence of J chain, ray-finned bony fishes are capable of forming polymeric IgM and IgT that can be transported into mucosal secretions [38].

4. Evolution of Cellular Receptors for Igs

The unique structure of vertebrate Igs allows for simultaneous binding of antigen via the N-terminal variable regions of the Ig heavy and light chains and execution of effector functions via the C-terminal constant (Fc) region of the Ig heavy chains. Evolution of multiple Ig heavy chain isotypes in higher vertebrates expanded the repertoire of Ig-mediated effector functions, including specialized functions at mucosal surfaces. The simplest effector functions involve neutralization of microbes and their toxic byproducts, whereas killing of microbes can be affected by interaction of Igs with soluble factors found in serum and secretions. The concurrent emergence of Igs and proteins of the classical complement pathway in cartilaginous fishes is the earliest example of a specific immune effector function mediated by the Fc region of Ig heavy chains [106]. As the immune system diversified during vertebrate evolution, cellular receptors for Ig heavy chains (Fc receptors) allowed host cells to bind Igs for the purpose of internalization, transport, and/or activation of intracellular signaling pathways. The most evolutionarily ancient of the vertebrate Fc receptors is the polymeric Ig receptor (pIgR), which first emerged in bony fishes (reviewed in [8]) (Figure 3). The primary function of pIgR is to transport polymeric Igs (IgM, IgT, IgX, and IgA) across glandular and mucosal epithelial cells into external secretions (see below). The fact that the extracellular Ig-binding region of pIgR is composed of multiple Ig-like domains suggests that the *PIGR*

gene evolved by duplication of a primordial Ig gene is driven by the need for an efficient mechanism for transport of mucosal Igs into external secretions. Similar forces may have driven the evolution of FcRY, a receptor first described for its function of transporting IgY from maternal blood to the embryo yolk sac in chicken [9, 107–109]. FcRY is not structurally homologous to pIgR or mammalian Fc receptors but is instead homologous to mammalian receptors for mannose and phospholipase A2, with lectin-like extracellular domains (Figure 3). A BLAST search of the National Center for Biotechnology Information (NCBI) nucleotide database for homologs of the gene encoding chicken FcRY (designated *PLA2R1* based on its homology to phospholipase A2 receptor 1) revealed homologous genes in many bird species, as well as turtles, lizards, alligators, and mammals. However, the functional significance of FcRY/*PLA2R1* in the transport of IgY in reptiles (and possibly IgG in mammals) has not been explored. A functional equivalent of FcRY in mammals is FcRn, which mediates transport of IgG across endothelial and epithelial cells in such tissues as placenta and mucosal epithelia (reviewed in [110, 111]). FcRn is homologous in structure to major histocompatibility class (MHC) I molecules, comprising a ligand-binding alpha chain associated with soluble β2-microglobulin (Figure 3).

A unique feature of the mammalian immune system is the expression of Fc receptors for various Ig isotypes by cells of hematopoietic origin (Figure 3) (reviewed in [10]). Engagement of these Fc receptors by Ig (in the presence or absence of bound antigen, depending on the particular Fc receptor) initiates intracellular signaling pathways that can either augment or inhibit immune responses. With the exception of FcεRII, which has a single C-type lectin-like domain, the extracellular Ig-binding regions of these hematopoietic Fc receptors comprise 1, 2, or 3 Ig-like domains [10]. Genes encoding Fcα/μR, FcγRI, FcγRIII, FcεRI, and FcεRII are widely distributed among mammalian species, whereas genes encoding FcαRI and FcγRII are restricted to the primate lineage, suggesting recent evolutionary origins. The genes encoding the α, β. and/or γ chains of FcεRI, FcγRII, and FcγRIII are linked in a cluster on the long arm of human chromosome 1 (1q23), not far removed from the gene for the α chain of FcγRI at 1q21. This tight linkage is consistent with duplication and diversification of a primordial FcR gene in a common ancestor of mammals. The primate-specific genes encoding the α and β chains of FcγRII appear to have evolved more recently. Another cluster of immune-related genes on the long arm of chromosome 1 includes the genes for pIgR (1q31) and Fcα/μR (1q32). Although the overall homology between pIgR and Fcα/μR is relatively low, they share a conserved binding site for IgA and IgM in the N-terminal Ig-like domain. Interestingly, the genes encoding FcRn and FcαRI are closely linked on the long arm of chromosome 19 (19q13.3 and 19q13.42, resp.), although they bear little homology beyond the presence of a single Ig-like domain in the extracellular region. Finally, the gene for the lectin-like receptor FcεRII is located in an unrelated locus on the short arm of chromosome 19 (19p13.3).

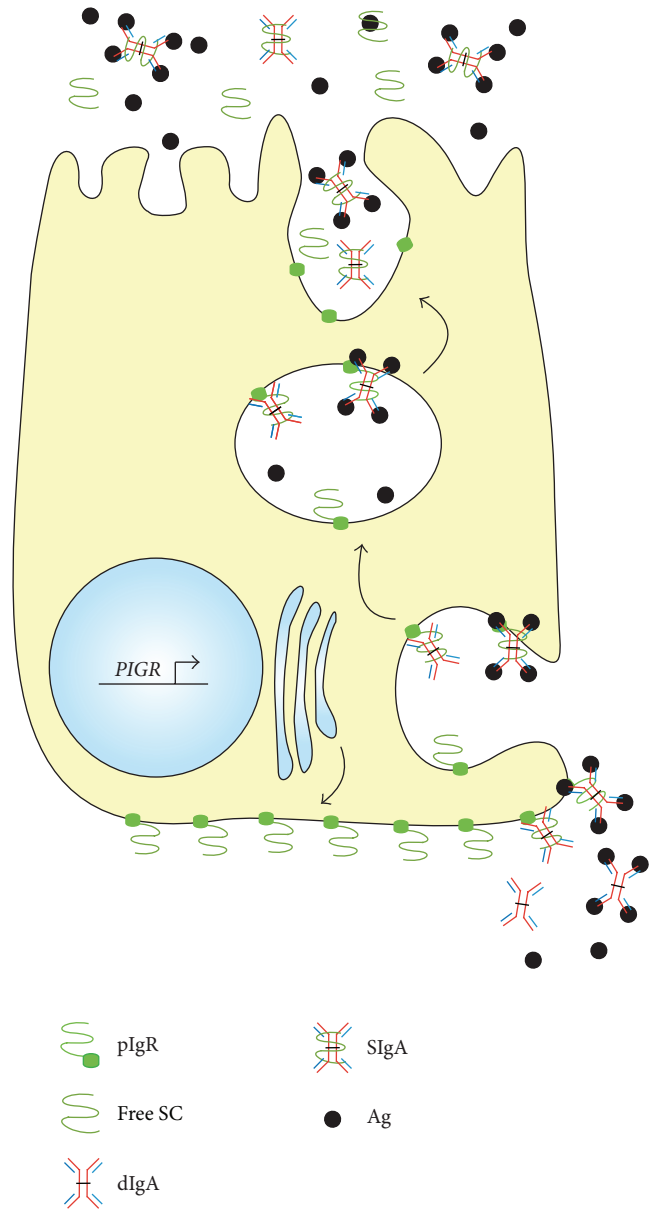
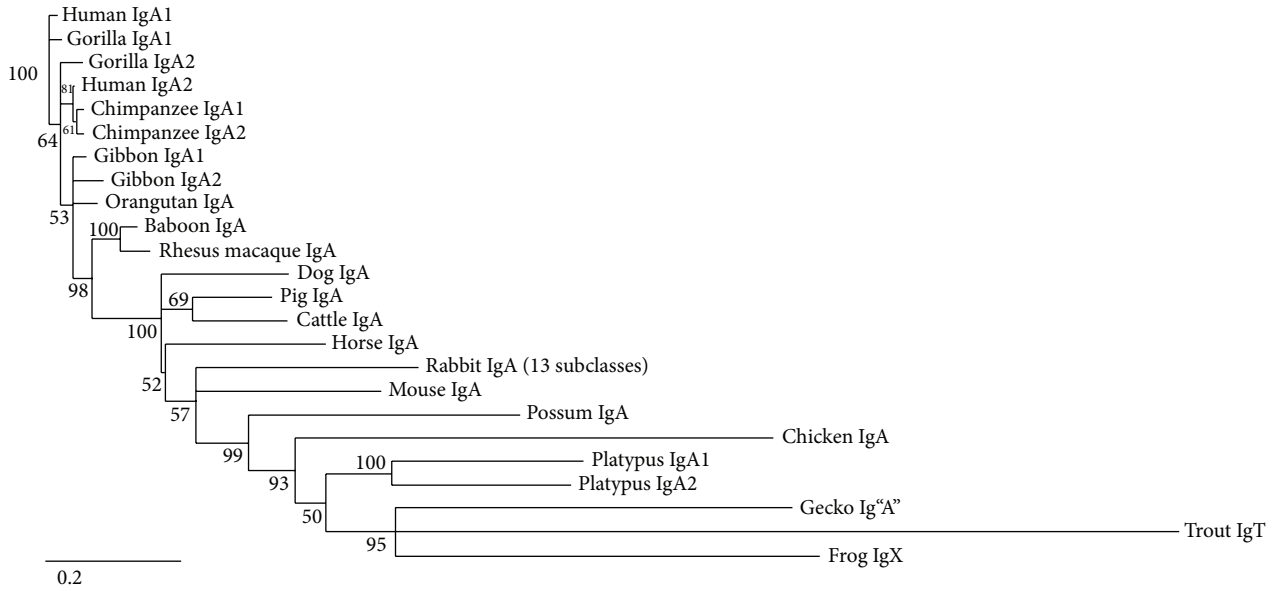


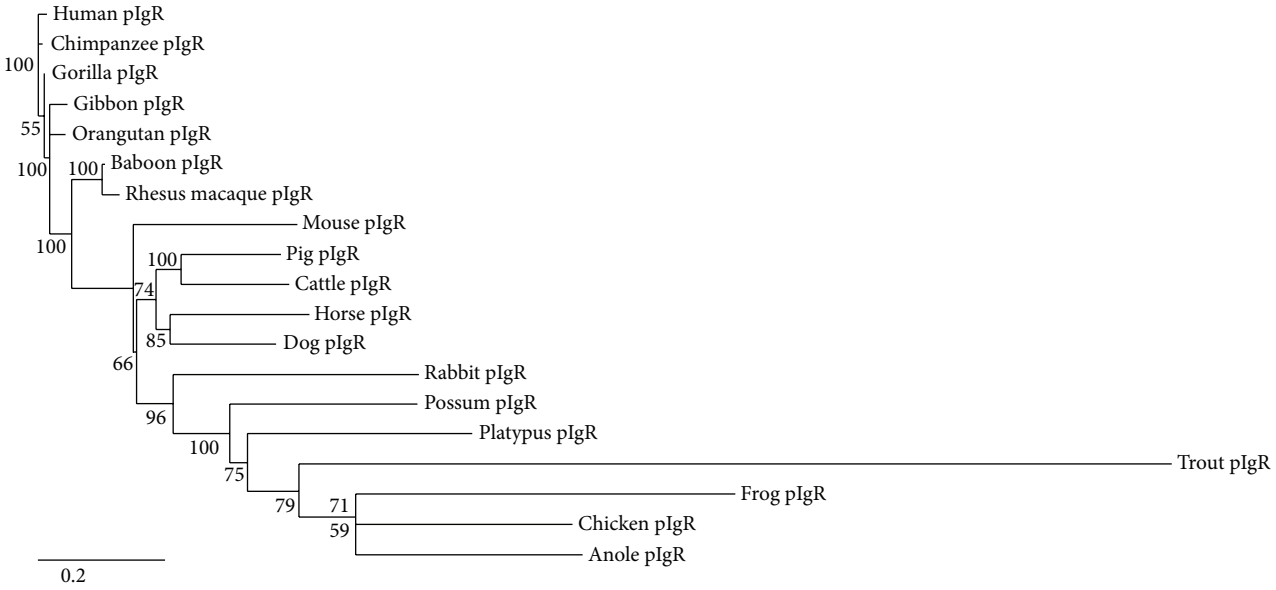
Figure 4: Transcytosis of pIgR through a polarized epithelial cell and formation of secretory Igs. A polarized columnar epithelial cell is illustrated, with the apical surface at the top and the basolateral surface at the bottom. Newly synthesized pIgR is targeted to the basolateral surface, where it binds polymeric Ig (pIg; illustrated here as dimeric (d)IgA) with or without bound antigen (Ag). Following receptor-mediated endocytosis, pIg-bound and unoccupied pIgR molecules are transported through a series of intracellular vesicles to the apical surface. Proteolytic cleavage of pIgR at the extracellular face of the plasma membrane releases free secretory component (SC) and secretory SIg (illustrated here as SIgA). Modified from [13] with permission from John Wiley and sons.

5. Unique Biological Functions of pIgR

The pIgR is expressed on the surface of glandular and mucosal epithelial binds, where it binds selectively to polymeric Ig (pIg) and mediates its transport across epithelial cells into external secretions (reviewed in [17, 111, 112]). Most of



(a)



(b)

Figure 5: Coevolution of mucosal Igs and pIgR. Simplified neighbor-joining phylogenetic trees of vertebrate (a) Ig and (b) pIgR protein sequences, with human IgA1 and pIgR denoted as outgroups. Horizontal distances are proportional to the degree of divergence following a multiple alignment of amino acid sequences. Numbers at nodes denote bootstrap support for each bifurcation after 100 replications. Accession numbers for mucosal Ig sequences: human (*Homo sapiens*) IgA1, J00220; IgA2 (allotype m1), J00221; gorilla (*Gorilla gorilla*) IgA1, X53703; IgA2, X53707; chimpanzee (*Pan troglodytes*) IgA1, X53702; IgA2, X53706; gibbon (*Hylobates lar*) IgA1, X53708; IgA2, X53709; Bornean orangutan (*Pongo pygmaeus*) IgA, X53704; baboon (*Papio anubis*) IgA, DQ868435; rhesus macaque (*Macaca mulatta*) AY039245; pig (*Sus scrofa*) IgA, U12594; cattle (*Bos taurus*) IgA, AF109167; dog (*Canis lupus familiaris*) IgA, L36871; horse (*Equus caballus*) IgA, AY247966; mouse (*Mus musculus*) IgA, D11468; rabbit (*Oryctolagus cuniculus*) IgA1, X51647; brushtail possum (*Trichosurus vulpecula*) IgA, AF027382; duck-billed platypus (*Ornithorhynchus anatinus*) IgA1, AY055778; IgA2, AY055779; chicken (*Gallus gallus*) IgA, AAB22614.2; leopard gecko (*Eublepharis macularius*) Ig"A" (so designated because of its uncertain phylogeny with homology to both IgA and IgY), ABG72684.1; African clawed frog (*Xenopus laevis*) IgX, S03186; rainbow trout (*Oncorhynchus mykiss*) IgT, AAW66981.1. Accession numbers for pIgR sequences, with species that vary from those used for the mucosal Ig alignment in panel A noted in parentheses: human, NM_002644; chimpanzee, XM_003308710; gorilla, XM_004028295; gibbon, XM_003272974; Sumatran orangutan (*Pongo abelii*), NM_001131626; baboon, XM_003893189; rhesus macaque, XM_001083307; pig, NM_214159; cattle, NM_174143; dog, XM_537133; horse, XM_001492298; mouse, NM_011082; rabbit, NM_001171045; brushtail possum, AF091137; duck-billed platypus, XM_001508602; chicken, NM_001044644; green anole (*Anolis carolinensis*), XM_003224013; African clawed frog, EF079076; rainbow trout, FJ940682.

our knowledge of pIgR-mediated transcytosis of mucosal Igs is derived from studies of mammalian secretory IgA (SIgA), as illustrated in Figure 4. Transcription of the *PIGR* gene and translation of pIgR mRNA is accompanied by insertion of the nascent protein into the endoplasmic reticulum. The pIgR undergoes substantial glycosylation during transport through the ER and Golgi apparatus and is then targeted to the basolateral surface of polarized epithelial cells. Membrane-bound pIgR binds pIg and pIg-containing immune complexes, which are internalized along with unoccupied pIgR via receptor-mediated endocytosis and then transcytosed through a series of intracellular vesicles to the apical surface. During transcytosis, intracellular pIg antibodies can neutralize bacteria, viruses, and other antigens that have gained access to the interior of the epithelial cell. At the apical surface, proteolytic cleavage of the membrane-spanning region of pIgR leads to the release of its extracellular domain, known as secretory component (SC), either in free form or covalently bound to pIg. Secretory Igs can neutralize pathogens and antigens within the mucosal lumen and facilitate excretion of antigens that were transported through the epithelial cell as IgA immune complexes. The SC moiety of SIgA has been shown to protect IgA from degradation by host and bacterial proteases and, along with free SC, confers additional innate immune functions that protect the epithelial surface from microbial invasion and limit potentially damaging host inflammatory responses (reviewed in [113]). The finding of an SC-like polypeptide associated with polymeric IgT in rainbow trout [38], polymeric IgX in *Xenopus* [50], and polymeric IgA in birds [114] suggests that all vertebrate pIgRs share the function of transporting polymeric Igs into external secretions. In many mammalian species, pIgR mediates epithelial transcytosis of both polymeric IgM and IgA (reviewed in [111]). Although direct evidence is not yet available, it is reasonable to assume that pIgR transports IgM in lower vertebrates. It will be interesting to investigate whether the SC moiety of secretory Igs in lower vertebrates mediates additional innate immune functions, as has been demonstrated for mammalian SC.

6. Coevolution of pIgR and Mucosal Igs

Molecular cloning of the *PIGR* gene was first reported for mammalian species, including eutherians, marsupials, and monotremes [115–127]. Subsequent elucidation of *PIGR* sequences from diverse species of jawed vertebrates has allowed a comprehensive analysis of the evolutionary origins of the *PIGR* gene [38, 128, 128–132] and additional sequences available in the Genbank database. Whereas no ortholog of the *PIGR* gene has been found in any species of cartilaginous fish, the *PIGR* gene has been annotated in all species of teleost fish for which the genome has been sequenced. No ortholog of the *PIGR* gene has been reported in lobe-finned bony fishes, but further analyses of whole genome sequences will be required to determine whether the *PIGR* gene was lost following the split of the Sarcopterygii. It thus appears that the *PIGR* gene emerged in a common ancestor of higher vertebrates, after the split of cartilaginous fish and prior to the evolution of modern teleosts, likely driven by the selective pressure to provide a mechanism for transport of polymeric Igs such as IgM and IgT into mucosal secretions. This hypothesis is supported by a comparison of phylogenetic trees based on amino acid sequences for IgT, IgX, and IgA heavy chains and pIgR (Figure 5). The predicted evolutionary distance between trout IgT and frog IgX is similar to the distance between trout and frog pIgR, suggesting that the transition from IgT to IgX as the dominant mucosal Ig isotype was accompanied by adaptations in pIgR structure. Similar parallels can be seen in the transition from IgX to IgA in reptiles, birds, and mammals and the concurrent evolution of pIgR.

Alignments of the amino acid sequences of mucosal Ig heavy chains and pIgR from 10 representative vertebrate species provide further support for the theory of coevolution of mucosal *IGH* and *PIGR* genes (Figure 6). A common feature of Ig and pIgR proteins is the presence of repeating Ig-like domains (see Figure 2 for the basic structure of these domains). The structure of IgT, IgX, and IgA heavy chains from fish, amphibians, reptiles, and birds is similar to that of IgM, with 4 constant Ig-like domains. In mammalian IgA (and IgG), the exon for the 2nd constant region was truncated to encode a short "hinge" region, which confers greater mobility to the antigen-binding region at the N-terminus. Thus, the C_H3 domain of IgT, IgX, and IgA in lower vertebrates is structurally and functionally homologous to the C_H2 domain of mammalian IgA. The heavy chains of IgT, IgX, and IgA have retained a characteristic feature of the IgM heavy chain, that is, the presence of a conserved cysteine residue in the tailpiece that allows disulfide bonding between heavy chains of individual Ig proteins to form higher molecular weight polymers. These large Ig structures containing multiple antigen-binding sites are particularly well suited for immune clearance of large antigens found at mucosal surfaces, such as intact microbes and food particles. In mucosal Igs from most vertebrate orders, the J chain protein initiates this polymerization process by disulfide bonding with 2 Ig heavy chains, remaining associated with the polymeric Ig at a stoichiometric ratio of 1 J chain subunit per 2, 3, 4, or 5 Ig units. Despite the absence of J chain, the predominant form of IgM and IgT in mucus secretions in rainbow trout was found to be polymeric, in contrast to monomeric forms of IgM and IgT in the blood [38]. In mammals, IgM is mainly found as a pentamer, whereas the shorter heavy chain of mammalian IgA restricts its polymeric forms to dimers, trimers, and tetramers. Further studies will be required to characterize the polymeric structures of IgM, IgT, IgX, and IgA in lower vertebrates.

Alignment of pIgR amino acid sequences from 10 representative vertebrate species reveals structural changes that were evolved in parallel with structural changes in mucosal Igs (Figure 6(b)). All pIgR proteins have an N-terminal hydrophobic leader peptide, required for targeting of the nascent protein into the ER. The extracellular region of pIgR protein is characterized by repeating "Ig-like" domains, similar to Ig heavy chains [115, 133]. Interestingly, the number of extracellular Ig-like domains has increased during vertebrate evolution, culminating with a total of 5 domains

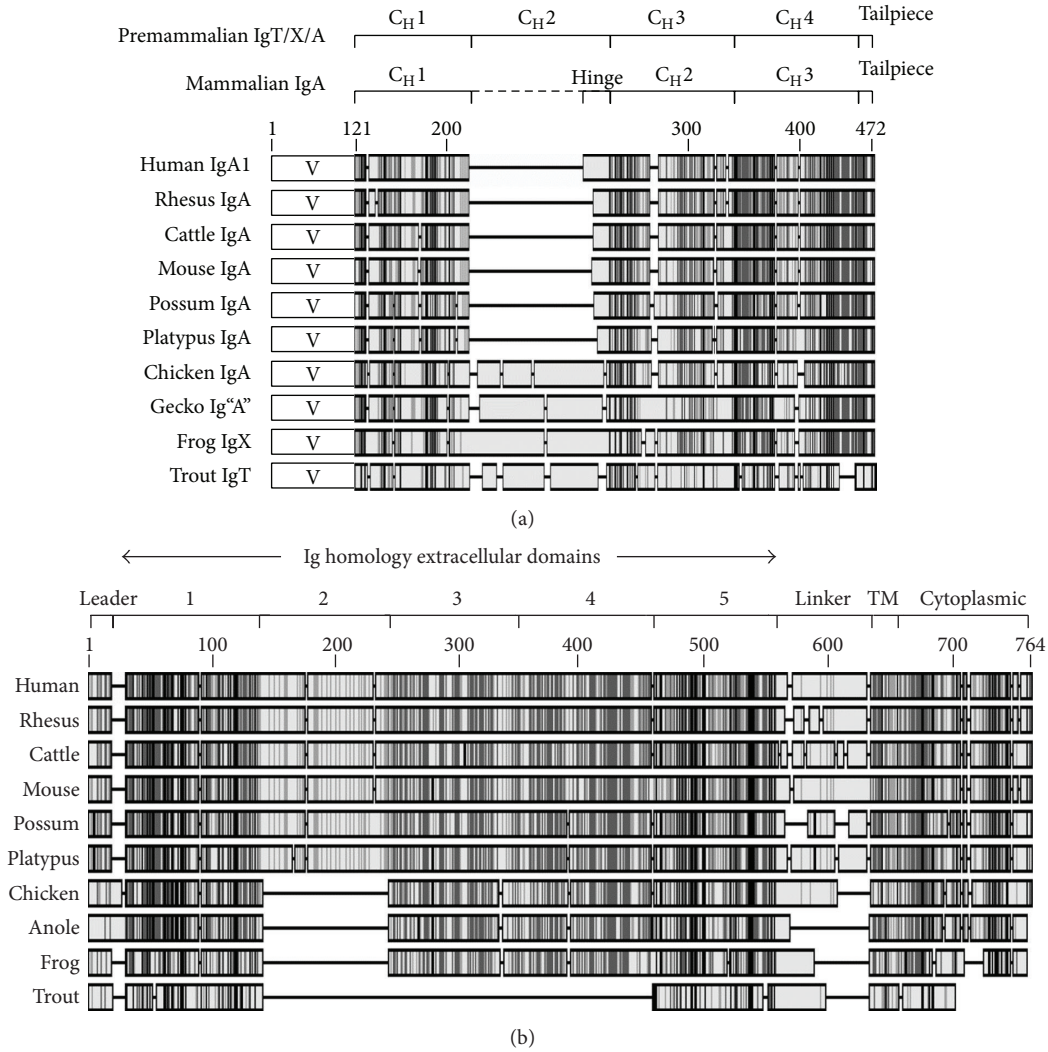


Figure 6: Interspecies similarity in mucosal Ig and pIgR protein sequences from 10 representative vertebrate species. Alignments in amino acid sequences correspond to the phylogenetic trees shown in Figure 5. (a) Numbering of mucosal Ig amino acids corresponds to the sequence of human IgA1, beginning with the first residue of the variable domain. C_H: heavy chain constant region domain. (b) Numbering of pIgR amino acids corresponds to the sequence of human pIgR, beginning with the first residue of the leader peptide. TM: transmembrane. For both alignments, the intensity of shading at each position signifies the degree of similarity among mucosal Ig or pIgR sequences from different species. Gaps in sequence alignments signify regions of limited interspecies homology. Approximate boundaries are noted of key structural elements in mucosal Ig and pIgR proteins.

in mammalian pIgR. By contrast, pIgR from amphibians, reptiles, and birds contains 4 extracellular domains, lacking a homolog of domain 2 of mammalian pIgR. The most primitive form of pIgR found in teleost fish contains only 2 extracellular domains, homologous to domains 1 and 5 of mammalian pIgR. Interspecies homology is greatest in domain 1, which has been shown to be critical for binding of mammalian pIgR to polymeric Igs (reviewed in [111]). Highly conserved internal disulfide bonds are found in all the Ig-like domains of pIgR, characteristic of the immunoglobulin fold. An additional disulfide bond is found in domain 5 of pIgR from mammals, birds, reptiles, and amphibians, but not fish. In human SIgA, this "extra" disulfide bond in domain 5 has been shown to rearrange to form a disulfide bond with cysteine residues in one of the heavy chains of polymeric IgA [134]. Although the structure of SIgA has not been fully characterized for lower vertebrates, it is reasonable to assume that similar disulfide bonds form between domain 5 of pIgR and polymeric IgA or IgX from birds, reptiles, and amphibians. The linker region connecting domain 5 of pIgR to the transmembrane domain has a random structure that is poorly conserved across species. Proteolytic cleavage of pIgR within this domain leads to the release of SC from the apical surface of epithelial cells, either free or bound to polymeric Ig. The cytoplasmic domain of mammalian pIgR contains a number of intracellular sorting motifs that interact with cytoplasmic proteins to direct pIgR through the transcytotic pathway (reviewed in [111]). These motifs are reasonably

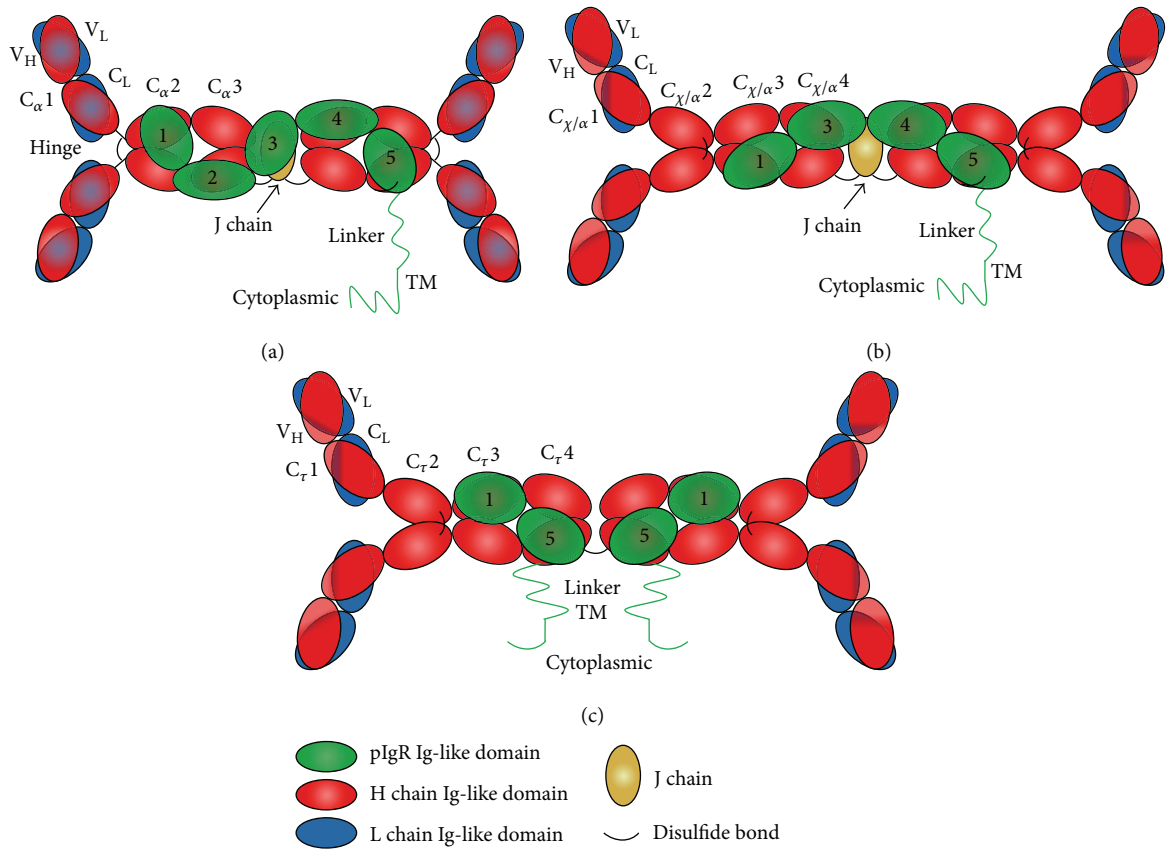


Figure 7: Theoretical model for changes in the structure of secretory Igs during evolution. (a) The structure for mammalian SIgA is adapted from the model of Woof and Russell [16] for human SIgA1, with permission from Nature Publishing Group. (b) and (c): Theoretical models for the structure of IgX/A from amphibians and birds and SIgT from teleost fish, respectively, are predicted from alignment of Ig heavy chain and pIgR sequences with mammalian homologs.

well conserved in pIgR from birds and reptiles, but not in pIgR from amphibians and fish, suggesting that complex mechanisms of intracellular trafficking of pIgR coevolved with the increasing complexity of the mucosal immune system.

7. Hypothetical Models for Changes in Secretory Ig Structure during Coevolution of Mucosal Igs and pIgR

A large number of structural and functional analyses over the past 30 years allowed the development of detailed models for the structure of mammalian SIgA (reviewed in [111, 135]) (Figure 7(a)). Domains 1 and 5 of pIgR, the most highly conserved Ig-like domains, associate with the Cα2 subunits of two adjacent IgA heavy chains in a near-planar structure [136, 137]. During transcytosis, a disulfide bond forms between highly conserved cysteine residues in domain 5 of pIgR and the Cα2 domain of one of the IgA heavy chains [134]. Domains 2, 3, and 4 allow mammalian pIgR to assume a flexible extended structure, which is thought to wrap around the IgA heavy chains and protect against degradation from host and microbial proteases. Binding of mammalian pIgR to polymeric IgA or IgM requires the presence of J chain connecting the Ig subunits (reviewed in [138]), and J chain-deficient mice fail to transport IgA and IgM into external secretions [139–141]. Structure-function studies using mutant forms of human J chain suggested that J chain binds directly to pIgR and also maintains polymeric Ig in an optimal conformation for pIgR binding and transcytosis [142]. The unstructured linker region connecting domain 5 of pIgR to the transmembrane (TM) region gives membrane-bound pIgR the flexibility to bind large polymeric Igs (with or without associated antigens) and provides an exposed site for proteolytic cleavage of pIgR to SC. The C-terminal region of pIgR extends past the cytoplasmic face of the plasma membrane, allowing association with cytoplasmic factors that regulate trafficking of pIgR with or without bound polymeric Ig.

Although detailed structural studies have not yet been performed on secretory Igs from lower vertebrates, hypothetical models can be constructed based on alignments with their mammalian homologs. A model for secretory IgX/A in birds, reptiles, and amphibians is illustrated in Figure 7(b), showing the $C_{\chi/\alpha}2$ domain in place of the hinge region of mammalian IgA. The numbering of the extracellular domains of pIgR corresponds to the homologous domains in mammalian pIgR, noting the absence of domain 2. In

this model, the $C_{\chi/\alpha}3$ domains of two adjacent IgX/A heavy chains (homologous to the $C_\alpha 2$ domain of mammalian IgA) bind to the highly conserved domains 1 and 5 of pIgR. Given the conservation of the analogous cysteine residues in domain 5 of pIgR and the $C_{\chi/\alpha}3$ domain of IgX/A, it is assumed that a disulfide bond forms between pIgR and polymeric Ig during epithelial transcytosis in birds, reptiles, and amphibians. The absence of domain 2 in pIgR from these vertebrate orders necessitates a more rigid structure than that of mammalian pIgR, with a corresponding decrease in its ability to wrap around the IgX/A heavy chains and provide protection against proteoltyic degradation. Despite the shorter length of pIgR in birds, reptiles, and amphibians, the association with the J chain subunit of polymeric IgX/A still appears to be required. Recombinant pIgR from the African frog (*Xenopus laevis*) was shown to bind with moderately high affinity to human polymeric IgA containing J chain, but not to monomeric IgA [125]. The affinity of *Xenopus* pIgR for human pIgA was improved when *Xenopus* J chain was substituted for human J chain in a chimeric polymeric IgA molecule. The lack of sequence conservation across species in the linker region connecting domain 5 of pIgR to the TM region suggests that it acts as a nonspecific "spacer." As stated above, the cytoplasmic region of pIgR appears to have evolved over time to provide enhanced regulation of pIgR trafficking.

The hypothetical structure of secretory IgT from teleost fish in Figure 7(c) illustrates how the most primitive form of pIgR comprising only extracellular domains 1 and 5 could associate with polymeric Ig and mediate epithelial transcytosis. Association of pIgR domain 1 with the $C_\tau 3$ domain of IgT would preserve the functional homology with secretory Igs in higher vertebrates. The short length of teleost pIgR would necessitate the association of domain 5 with a $C_\tau 3$ domain on one of the heavy chains of the same IgT, rather than spanning across to the adjaent IgT subunit. While this model preserves the highly conserved association of pIgR domain 1 with the orthologous domain of the IgT heavy chain, it does not explain the selectivity of teleost pIgR for polymeric rather than monomeric IgT. Although there is no experimental evidence to support this model, it could be hypothesized that dimerization of two pIgR molecules in the plasma membrane is required for high affinity binding of IgT, as illustrated in the model in Figure 7(c). This hypothetical model would also explain the lack of a necessity for J chain in polymeric IgT, since each subunit in the pIgR dimer would only be in contact with one IgT subunit. The unstructured linker region connecting domain 5 of pIgR to the TM region would provide flexibility for association of 2 membrane-bound pIgR molecules with a large polymeric IgT molecule.

Taken together, the structural models for secretory Igs shown in Figure 7 are consistent with the selection of a more functional pIgR molecule over evolutionary time. Duplication and diversification of the extracellular Ig-like domains of pIgR would provide a longer, more flexible surface for binding to polymeric Igs with higher affinity and protecting the Ig heavy chains against proteolytic attack. Expansion of the cytoplasmic domain of pIgR and incorporation of motifs for binding cytoplasmic proteins would allow regulated trafficking of pIgR and polymeric Igs across mucosal and glandular epithelial cells. Importantly, these models illustrate a potential path for coevolution of *IGH* and *PIGR* genes, driven by the common selective pressure to provide antigen-specific humoral immune responses at mucosal surfaces.

8. Evidence that the Commensal Microbiota Provided the Driving Force for Coevolution of Mucosal Igs and pIgR

From the earliest evolution of vertebrate animals, mucosal Igs have been an integral part of the emerging adaptive immune system. Clearly, many environmental factors served as driving forces for the evolution of the adaptive immune system in vertebrates, including a longer lifespan than many (but not all) invertebrates and a more diverse diet that could introduce a broader range of potential gut pathogens. However, it has been proposed that the most significant force may have been the dramatic increase in the numbers and complexity of the resident microbiota in the evolution of vertebrates from their invertebrate ancestors [1, 143, 144]. Antibodies in mucosal secretions contribute to immune homeostasis by limiting access of microbes and their products to the body proper, maintaining the integrity of the epithelial barrier and shaping the composition of the commensal microbiota in favor of metabolically beneficial microorganisms [145]. The predominance of polymeric Igs in these secretions promotes these functions by providing multiple antigen-binding sites per Ig complex, thus increasing the overall avidity of antigen-antibody interactions. Structural and functional refinements in mucosal Igs, culminating with the multifunctional secretory IgA molecule in mammals, have further enhanced their ability to enhance immune homeostasis.

The early emergence of pIgR in vertebrate evolution provided a mechanism for efficient transport of mucosal Igs into external secretions. It is well documented that crosstalk between mucosal epithelial cells and the resident microbiota modulates expression of pIgR (reviewed in [17, 111, 112]), and it is reasonable to assume that these host-microbe interactions provided a driving force for evolution of the PIGR gene in vertebrates. The first demonstration that gut bacteria may modulate pIgR expression in epithelial cells was the finding that butyrate, a bacterial fermentation product and important energy source in the colon, upregulated expression of pIgR in the human colonic epithelial cell line HT-29 [146]. A role for commensal bacteria in pIgR regulation was subsequently demonstrated by the observation that pIgR expression was increased when germ-free mice were colonized with *Bacterioides thetaiotaomicron*, a prominent organism of the normal mouse and human intestinal microbiota [147]. A more recent study, using a model of reversible colonization of germ-free mice with a nondividing mutant of *Escherichia coli*, demonstrated that a long-lived SIgA response could be sustained that specifically recognized the inducing bacteria [148]. However, exposure of *E. coli*-colonized mice to other bacteria limited the duration of the SIgA response against the original colonizer, suggesting a dynamic equilibrium between members of the gut microbial community and host SIgA. A

study from the laboratory of Finn-Eirik Johansen demonstrated that diversity of the bacterial community in the cecal lumen was reduced in pIgR-knockout mice, supporting a role for SIgA in limiting overgrowth of selected species [149]. In contrast, the community of bacteria adherent to the epithelial surface was more diverse in pIgR-knockout mice than in wild-type mice, and these adherent bacteria caused enhanced expression of several epithelial antimicrobial peptides. These findings suggest that bacteria-specific SIgA anchored in the mucus layer restricts adhesion of bacteria to the epithelial surface and limits bacterial stimulation of host epithelial cells. A recent study from the author's laboratory, employing a breeding scheme in which $Pigr^{-/-}$ dams were bred to $Pigr^{+/-}$ males and vice versa, demonstrated that SIgA antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and gene expression in colonic epithelial cells [150].

A direct role for commensal and/or pathogenic bacteria in the regulation of pIgR expression suggests that the innate immune system may "prime" epithelial cells for transport of polymeric Ig produced during the adaptive immune response. Host cells mediate innate immune responses to microbial components through toll-like receptor (TLR) signaling (reviewed in [151, 152]). Intestinal epithelial cells have been shown to express a wide variety of TLRs, the expression of which is upregulated during intestinal inflammation [153–155]. Significantly, expression of pIgR was shown to be upregulated in the HT-29 human intestinal epithelial cell line by bacterial LPS, a ligand for TLR4 [156, 157]. Similar induction of *PIGR* gene transcription was seen following direct binding of bacteria of the family *Enterobacteriaceae*, which express a form of LPS that stimulates TLR4 [158]. A recent study from the author's laboratory demonstrated that shRNA-mediated knockdown of myeloid differentiation primary response protein 88 (MyD88), a cytoplasmic adapter protein that transduces signals from cell surface TLR4, blocked the induction of *PIGR* gene transcription by LPS in HT-29 cells [159]. The *in vivo* significance was confirmed by the finding that mice with a targeted deletion of the *Myd88* gene only in intestinal epithelial cells had reduced expression of pIgR compared to wild-type littermates [159]. Mice with targeted depletion of MyD88 in intestinal epithelial cells also exhibited reduced expression of mucin-2, the major component of the intestinal mucus layer, and several antimicrobial peptides and decreased transmucosal electrical resistance. These alterations in epithelial barrier function were accompanied by significant changes in the composition of the fecal microbiota, increased numbers of bacteria adherent to the intestinal mucus layer, and a dramatic increase in translocation of gut bacteria into the draining mesenteric lymph nodes, including the opportunistic pathogen *Klebsiella pneumoniae*. These findings demonstrate that MyD88-mediated crosstalk between the gut microbiota and intestinal epithelial cells is crucial for optimal expression of pIgR, production of SIgA, development of a healthy gut microbiota, and maintenance of intestinal homeostasis.

A model can be proposed in which continuous stimulation of intestinal epithelial cells by gut bacteria regulates pIgR expression and transport of SIgA and promotes immune exclusion of resident and pathogenic bacteria (Figure 8). A dense layer of mucus, which increases in thickness from the proximal to the distal colon, serves to separate bacteria from direct contact with the epithelial surface. Secretion of microbial-associated molecular patterns (MAMPs) by commensal bacteria stimulates MyD88-dependent TLR signaling, leading to enhanced expression of pIgR and antimicrobial peptides and increased mucus production. Binding of pIgA to pIgR and possibly also signaling via MAMPs

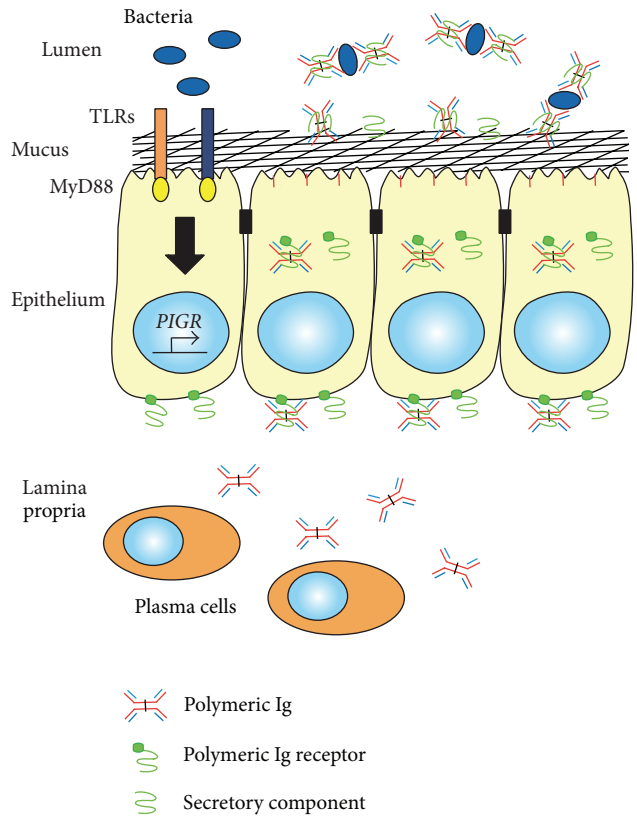


Figure 8: Crosstalk between the polymeric immunoglobulin receptor, secretory immunoglobulins, and the gut microbiota. The single-layered epithelium that lines the gastrointestinal tract is covered with a thick mucus layer, which physically excludes members of the resident microbiota but allows diffusion of shed components of microbial cells. Stimulation of epithelial toll-like receptors (TLRs) with microbial products engages the cytoplasmic adaptor protein MyD88 and initiates NFκB-dependent signaling pathways. Translocation of activated NFκB and other transcription factors to the nucleus enhances transcription of the *PIGR* gene. Activation of TLRs may also stimulate pIgR transcytosis. Polymeric Igs secreted by lamina propria plasma cells bind to pIgR on the basolateral surface of epithelial cells and are transcytosed to the apical surface along with unoccupied pIgR. Proteolytic cleavage of pIgR at the apical surface releases secretory SIg and free secretory component (SC). Binding of SIg and SC to luminal bacteria promotes association with the mucus layer and prevents direct access of bacteria to the epithelial surface. Over time, the continuous crosstalk with SIg shapes the composition of the gut microbiota. Modified from [17] under terms of author's copyright with Nature Publishing Group.

enhances transcytosis of pIgA, pIgA-containing immune complexes, and unoccupied pIgR, which promotes clearance of antigens and pathogens from the *lamina propria*, intracellular neutralization, and release of SIgA and SC at the luminal surface. SIgA and SC serve to trap bacteria in the mucus layer, limiting their access to the epithelial surface. In mice with a targeted deletion of the *Muc2* gene, which have a severely depleted mucus layer in the colon, SIgA, and SC fail to associate with the surface of colonic epithelial cells [160]. The presence of SIgA in the intestinal lumen, whether derived passively from maternal milk or actively via epithelial transcytosis, shapes the composition of the gut microbiota and modulates intestinal epithelial gene expression.

9. Concluding Remarks

The first microbes that emerged on Earth almost 4 billion years ago encountered a hostile world where nutrients were scarce, hazards were everywhere, and life was short. With the evolution of multicellular organisms about 3 billion years later, a select group of microbes chose to sacrifice an independent existence for the benefits of a mutualistic relationship with eukaryotic hosts. Prokaryotic-eukaryotic mutualism reached its apex some 500 million years ago with the emergence of primitive vertebrates, whose gastrointestinal tracts were populated by an abundant and diverse commensal microbiota. In humans, the large intestine is home to some 100 trillion microorganisms, 10 times more than the total number of eukaryotic cells in the entire body. The microbes that inhabit the vertebrate GI tract provide many benefits to the eukaryotic host, including digestion of complex food molecules, synthesis of vitamins, and production of soluble factors that enhance the development of the host immune system. In return, the eukaryotic host provides the microbes with a warm, moist place to live, abundant food, low oxygen tension, and a soft bed of mucus on which to form biofilms. When this mutually beneficial arrangement is challenged by pathogenic microbes, the eukaryotic host and the commensal microbiota join forces to battle the pathogens and protect the body from invasion. The vertebrate immune system is equipped with a well-trained army of immune cells and a sophisticated arsenal of weapons (cell surface molecules and secreted factors) that protect the host from all but the most virulent pathogens. This immune "army" also serves as a constant reminder to the commensal microbes that, while they are free to enjoy the amenities of the mucosal surfaces, they are not welcome in the body proper. The emergence of antigen-specific Igs and T-cell receptors in jawed vertebrates began a process of immunological diversification that led to the development of a specialized mucosal immune system in higher vertebrates, in which secretory Igs play a central role. The relationship between host and microbiota is clearly reciprocal; the mucosal Igs that evolved in response to selective pressure from the commensal microbiota play a central role throughout life in shaping the composition, genetics, and metabolic activity of the microbiota [1, 161, 162].

The epithelial layer lining mucosal surfaces provides a physical and innate immune barrier against penetration of the body proper by microbes, undigested food antigens, and potentially noxious environmental substances. The evolutionary challenge of transporting mucosal Igs into external secretions without compromising the epithelial barrier was met by the emergence of the *PIGR* gene in an ancestor of bony fishes, encoding an epithelial-specific transmembrane protein that actively transports polymeric Igs across mucosal and glandular epithelia (Figure 4). Structural similarities between Igs and the extracellular Ig-binding domains of pIgR (Figure 6) suggest that duplication and diversification of a gene encoding primordial Ig-like domain may have given rise to the *PIGR* gene in a primitive vertebrate ancestor. Parallel refinement of the structures of mucosal Igs and pIgR during tetrapod evolution (Figures 6 and 7) culminated in the evolution of the multifunctional SIgA molecule in mammals, in which the pIgR-derived SC subunit provides innate immune functions and protects the antigen-binding IgA polymer from proteolytic degradation. In addition to acting as a powerful force during vertebrate evolution, the reciprocal relationship between pIgR, mucosal Igs, and the commensal microbiota provides benefits throughout the life of the individual. Microbial colonization of newborn vertebrates stimulates development of mucosal B cells and class switching to mucosal Ig isotypes [1, 163]. Microbial factors upregulate expression of pIgR, enhancing transport of mucosal Igs into external secretions, where they in turn regulate the microbiota. This beautifully orchestrated process allows us to maintain a healthy relationship with our resident microbiota, while providing mutual protection against pathogens.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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