Antibody Isotype Switching in Vertebrates

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Abstract The humoral or antibody-mediated immune response in vertebrates has evolved to respond to diverse antigenic challenges in various anatomical locations. Diversification of the immunoglobulin heavy chain (IgH) constant region via isotype switching allows for remarkable plasticity in the immune response, including versatile tissue distribution, Fc receptor binding, and complement fixation. This enables antibody molecules to exert various biological functions while maintaining antigen-binding specificity. Different immunoglobulin (Ig) classes include IgM, IgD, IgG, IgE, and IgA, which exist as surface-bound and secreted forms. High-affinity autoantibodies are associated with various autoimmune diseases such as lupus and arthritis, while defects in components of isotype switching are associated with infections. A major route of infection used by a large number of pathogens is invasion of mucosal surfaces within the respiratory, digestive, or urinary tract. Most infections of this nature are initially limited by effector mechanisms such as secretory IgA antibodies. Mucosal surfaces have been proposed as a major site for the genesis of adaptive immune responses, not just in fighting infections but also in tolerating commensals and constant dietary antigens. We will discuss the evolution of isotype switching in various species and provide an overview of the function of various isotypes with a focus on IgA, which is universally important in gut homeostasis as well as pathogen clearance. Finally, we will discuss the utility of antibodies as therapeutic modalities.

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1 Evolution of the IgH Locus

Antibody is composed of four polypeptide chains including two heavy chain (H) and two light (L) chains joined by several disulfide bonds. Each class of Ig defines an individual set of C heavy chain (C_H) domain that corresponds to a single H chain constant region gene. The C_H gene Cμ initially displayed on developing B cells as IgM can be altered by IgH isotype switching to produce IgG, IgE, or IgA. In this review, we focus on IgH genes across evolution.

Immunoglobulin genes are found in all gnathostomes (jawed vertebrates) analyzed to date. Their earliest appearance is in jawed fish (Fig. 1). Among fish, two distinct arrangements of variable (V), diverse (D), joining (J), and heavy chain constant (C_H) regions are found (Stavnezer and Amemiya 2004; Stavnezer and Schrader 2014). Cartilaginous fish such as sharks have many (10s–100s) copies of immunoglobulin genes, each consisting of a V–D–J-like region coupled to an associated constant region (Fig. 1a). This is known as a cluster arrangement of immunoglobulin genes. Interestingly, recent data in shark suggests that V(D)J can be expressed with constant regions from different clusters, although IgH genes are spatially distant, at >120 kb (Zhu et al. 2012). The cluster arrangement gave way to the translocon arrangement seen earliest in bony fish (e.g., zebrafish), in which each constant region is paired with a varying number of V, D, and J segments (Fig. 1b). Rearranged V(D)J segments in bony and lobe-finned fish seem to be limited only to their adjacent C region exons within one cluster (Fig. 1b–d), although in bony fish there is the innovation of using alternative splicing to express IgD instead of IgM (Chen and Cerutti 2011). Extensive splicing events within expressed clusters have also been reported, which could further diversify the antibody genes in lower species (Zhang et al. 2013). Further specialization of the immunoglobulin locus is seen in amphibians such as the frog *Xenopus*, where the translocon arrangement of V–D–J is associated with a set of individual isotypes, each of which can carry out specialized functions (Fig. 1e). In this arrangement, the recombined V(D)J can make use of any of the available isotypes through class switch recombination. This proved to be a very successful arrangement, utilized in nearly all other tetrapods examined from crocodiles to humans (Fig. 1f–m).

Among the constant region sequences, Cμ (IgM) appears to be common across all immunoglobulin-bearing clades (Figs. 1 and 2, red and purple coloring). Cδ/Cω (IgD/IgW) also appears early, being present in elasmobranchs, like sharks, bony fish, and lobe-finned fish, such as coelacanths. However, there is no evidence to date of Cδ/Cω in the holocephalans, like ratfish (Rast et al. 1998) and elephant sharks (Venkatesh et al. 2014). In addition, other isotypes like IgNAR may also be present depending on the species. Cζ, which is the constant chain for IgZ ["Z" for "zebrafish" (Danilova et al. 2005), also known as IgT ("T" for "teleost") (Hansen et al. 2005)], is found in ray-finned fishes and is the earliest Ig known to play a role in mucosal biology. IgZ is highly expressed as a multimer in gut mucus and is transported across epithelial cells in a manner similar to IgA, which will be discussed later.

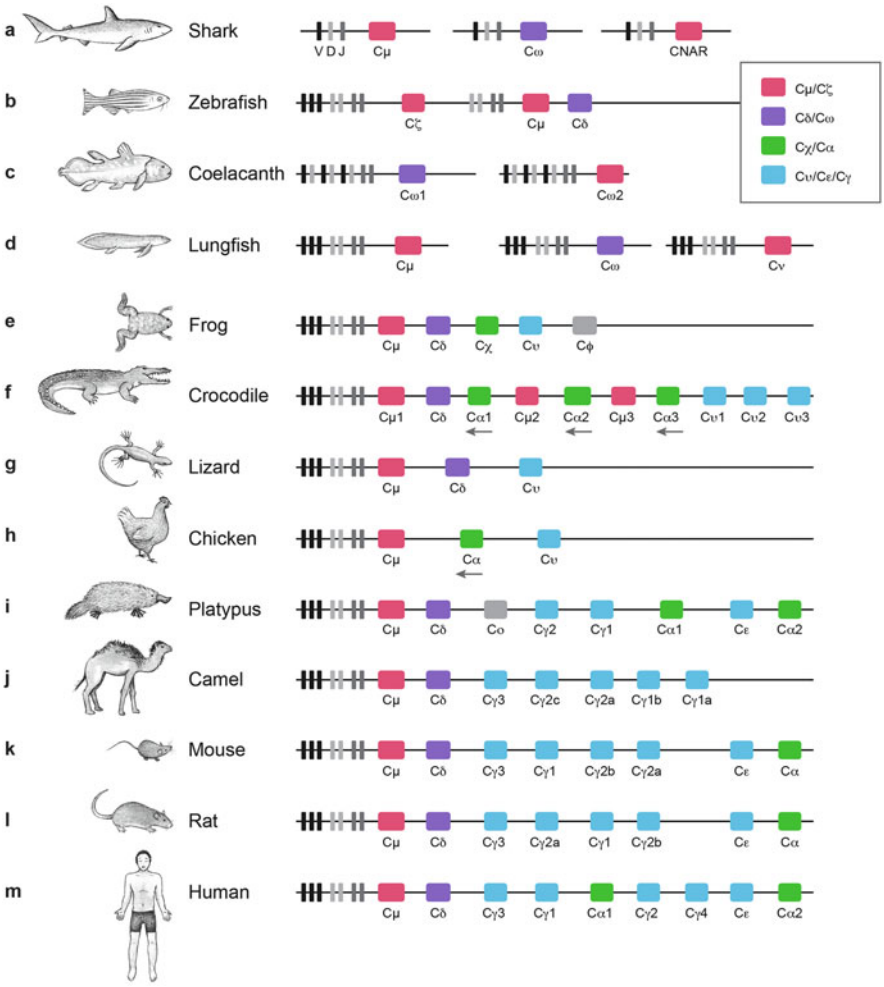

Fig. 1 Genomic arrangement of IgH loci. The arrangement of V–D–J and C regions from selected species is shown. Sharks (**a**) have a cluster arrangement comprised of individual V–D–J segments (*gray*) coupled to a constant region, with this arrangement duplicated anywhere from 10 to 100s of times. Ray-finned fish such as zebrafish (**b**) have a translocon-based arrangement of many V, D, and J segments coupled to constant region exons, but so far do not show class switch recombination. Coelacanth (**c**) and lungfish (**d**) have a similar translocon arrangement, but it is not currently known if their heavy chain loci are on the same chromosome or on different chromosomes. Salamanders, frogs, and all other vertebrates shown have a translocon arrangement of multiple V, D, and J segments coupled with several constant regions (**e–m**). These clades show class switch recombination and have S regions preceding the constant region exons. *Arrows* indicate the orientation of heavy chain coding sequences. Pseudogenes are excluded. Illustration not drawn to scale

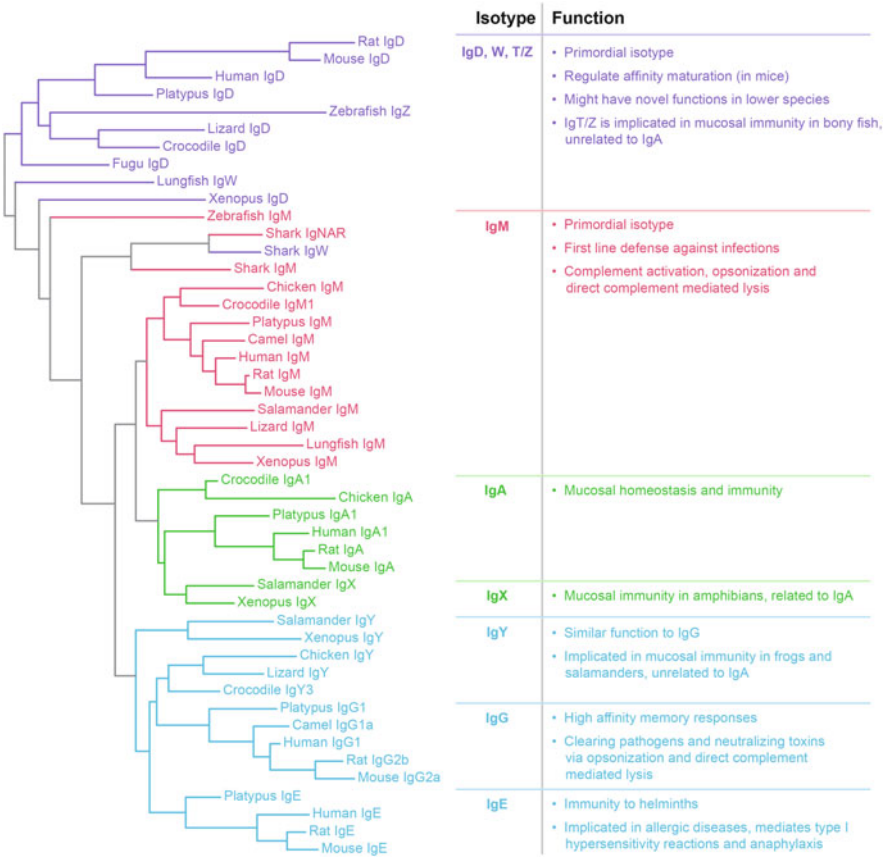

Fig. 2 Phylogenetic tree and properties of IgH constant regions. Heavy chain constant regions, from the first amino acid of the first constant region Ig domain, were retrieved from GenBank. Protein sequences were aligned using MUSCLE (Edgar 2004), and the phylogenetic tree was constructed using PhyML (Guindon et al. 2010). The resulting tree was visualized using TreeDyn (Chevenet et al. 2006). Accession numbers for sequences: shark (*Triakis scyllium*) IgM BAJ20181.1, IgW BAJ20183.1, IgNAR BAJ20188.1; zebrafish (*Danio rerio*) IgM AAK69167.1, IgZ ACH92959.1; fugu (*Takifugu rubripes*) IgD BAD34541.1; lungfish (*Protopterus dolloi*) IgM AGT03500.1, IgW AGT03500.1; salamander (*Ambystoma mexicanum*) IgX CAO82107.1, IgY CAA49247.1; *Xenopus laevis* IgM AAA49878.1, IgX AAH72981.1, IgY CAA33212.1; *Xenopus tropicalis* IgD ABC75541.1; crocodile (*Crocodylus siamensis*) IgM AFZ39206.1, IgD AFZ39209.1, IgA1 AFZ39210.1, IgY3 AFZ39221.1; lizard (*Anolis carolinensis*) IgM ABV66128.1, IgD ABV66130.1, IgY ABV66131.1; chicken (*Gallus gallus*) IgM CAA25762.1, IgA AAB22614.2, IgY CAA30161.1; platypus (*Ornithorhynchus anatinus*) IgM AAO37747.1, IgD ACD31540.1, IgG1 AAL17703.1, IgA1 AAL17700.1, IgE AAL17702.1; camel (*Camelus dromedarius*) IgM BAD00196.1, IgG1a CAD13185.1; mouse (*Mus musculus*) IgM CAC20701.1, IgD 0912262A, IgG2a CAA24179.1, IgE AAZ05128.1, IgA AAD56886.1; rat (*Rattus norvegicus*) IgM AAH92586.1, IgD AAO19643.1, IgG2b ADX94419.1, IgE CAA25439.1, IgA CAD52870.1; and human (*Homo sapiens*) IgM IGHM_HUMAN, IgD IGHD_HUMAN, IgG1 IGHG1_HUMAN, IgA1 CAC20453.1, IgE AAB59395.1

Amphibians retain Cμ and Cδ and add two new heavy chains: Cυ (IgY, Figs. 1 and 2, blue coloring), which is derived from Cμ, and Cχ (IgX, Figs. 1 and 2, green coloring), which arose from a fusion of Cμ and Cυ. Both IgY and IgX are implicated in defending the gut. In the newt *Pleurodeles waltl*, IgY-expressing B cells are mainly found in the mucosa (Schaerlinger et al. 2008), and in young axolotls, IgY localizes to the cells of the stomach and intestinal mucosa by immunofluorescence (Fellah et al. 1992a). IgX has been studied extensively in the African clawed toad (Hsu et al. 1985). It is mainly expressed as a multimer in mucosal tissues, though it is thought to lack the J chain often found linking multimeric Igs such as IgM and IgA (Amemiya et al. 1989; Fellah et al. 1992b, 1993; Haire et al. 1989; Hsu et al. 1985; Mussmann et al. 1996; Schaerlinger and Frippiat 2008; Schwager et al. 1988). The C_H1 and C_H2 domains of Cχ bear clear homology to C_H1 and C_H2 of Cυ, while the C_H3 and C_H4 domains of Cχ are most similar to Cμ.

The Cα heavy chain (IgA), found in reptiles, birds, and mammals, is highly related to Cχ in amphibians, sharing a branch in the phylogenetic tree (Figs. 1 and 2, green coloring). This isotype plays a key role in mucosal defense in all amniotes examined to date with the exception of several reptiles. For example, IgA is present in the leopard gecko *Eublepharis macularius* (Deza et al. 2007), but has not been detected in several snake species (Gambon-Deza et al. 2012), the American anole *Anolis carolinensis* (Gambon-Deza et al. 2009; Wei et al. 2009), or the Chinese soft-shelled turtle *Pelodiscus sinensis* (Xu et al. 2009). Interestingly, in birds such as chicken and ducks, the Cα gene is inverted relative to other heavy chain coding sequences (Mansikka 1992; Magor et al. 1998; Lundqvist et al. 2001). Likewise, the order Crocodilia, which houses the closest relatives to living birds, carries inverted Cα genes. The crocodile and alligator genomes have an unusual high number of Cα genes—3 plus one pseudogene (Cheng et al. 2013), and all are in a reverse orientation (Cheng et al. 2013; Magadan-Mompo et al. 2013). This genomic configuration provides a phylogenetic link between crocodiles and birds and places the inversion of the Cα gene prior to their evolutionary divergence.

The vast majority of mammals examined have a single Cα gene in the same orientation as the other C regions (Woof and Kerr 2004), with intriguing exceptions. Rabbit has 13 Cα genes (not shown), of which 11 have confirmed expression. These IgA subclasses have highly diverse C_H1 domains that are proximal to the hinge region, likely affecting their susceptibility to bacterial proteases. It has been suggested that proteases may have, in fact, been a key factor in shaping IgA diversity in lagomorphs. Another exception to the single Cα gene "rule" is the Hominoid primates (gibbons, gorillas, chimpanzees, humans), which have two IgA subclasses, IgA1 and IgA2. These subclasses arose through gene duplication and are highly similar. The major difference between them lies at the hinge between the two Fab arms and the Fc region. IgA1 has an extended hinge due to a duplicated stretch of amino acids, which may allow for higher avidity bivalent interactions with distantly spaced antigens (Boehm et al. 1999; Furtado et al. 2004). Secreted IgA1 is predominantly found in the gastrointestinal and upper respiratory tracts. Secreted IgA2 is the dominant subclass in secretions and is localized to the

lower ileum and large intestine, possibly because it has decreased susceptibility to bacterial proteases.

Cγ (IgG) and Cε (IgE) are two other heavy chains present in all mammals studied, including the most primitive egg-laying mammals such as platypus (Figs. 1 and 2, blue coloring). Both isotypes appear to have arisen from a duplication of C*υ*. IgG is required for high-affinity memory responses and clears pathogens and toxins via opsonization and complement-mediated lysis. IgE is involved in immunity to helminths and is responsible for allergies and anaphylaxis. In addition to these "classical" isotypes, there are also several species-specific isotypes that arise throughout evolution, such as Cϕ (IgF) in *Xenopus* and Co (IgO) in platypus (Fig. 1e and i, respectively). The functions of these isotypes remain undiscovered. It is important to emphasize common ancestry among isotypes does not necessarily predict functional similarity. There is much to be learned about the function of various isotypes among species.

2 Antibody Isotype Switching

Isotype switching is the process by which C_H domains encoding one isotype are exchanged for another, thereby influencing the effector function of the resulting antibody. In mammals such as mice and humans, this is regulated by T cell-dependent or T cell-independent mechanisms. T cell-dependent isotype switching involves T helper cells (Th) that are activated by antigen (Ag) and B7 co-stimulation, which in turn can activate CD40 ligand (CD40L). CD40 receptor engagement on B cells can activate the isotype switching machinery. Activated T cells also produce a variety of cytokines such as IL-13, IL-4, IL-5, or IFN-γ that can selectively impact the induction of various antibody isotypes. The net effect of Th-dependent isotype switching is production of high-affinity antibodies such as the IgG1 subclasses. Polysaccharide antigens derived from certain bacterial capsules stimulate mainly IgM antibodies, which can bind to bacteria, activate complement, and induce the phagocytosis of opsonized bacteria. During bacterial or viral infections, Th cells are activated to elicit IgG antibodies, which can block pathogen entry to tissues and facilitate their phagocytosis. Helminth infections can induce Th cells to produce Th2 cytokines to induce the IgE response. In addition, B cells localized within the gut produce IgA as a first line of defense, which is mediated by factors like TGF-β (transforming growth factor beta) that is produced by various cell types. Additional cytokines including BAFF (B cell-activating factor) and APRIL (a proliferation-inducing ligand) have been implicated as T cell-independent stimuli for IgA isotype switching via the receptor TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor). The molecular mechanism of isotype switching is known as class switch recombination (CSR).

3 Mechanism of Class Switch Recombination (CSR)

In many vertebrates, the IgH locus contains a battery of C_H encoding exons with common or distinct genomic configurations. In mice, for example, C_H regions are organized in the following order: 5′-Cμ-Cδ-Cγ3-Cγ1-Cγ2b-Cγ2a-Cε-Cα-3′ (Fig. 3). Each C_H gene is a component of a germ-line transcription (GLT) unit consisting of an intronic (I) promoter, a noncoding I exon, switch (S) region, and C_H coding exons. CSR is a genetically programmed gene recombination process in which the exons for constant region Cμ, encoding IgM, can be exchanged for downstream C_H regions, yielding B cells that express IgG, IgE, or IgA. I promoters are normally silent in naïve B cells except for Iμ, which is continuously active. Upon B cell activation, one or more downstream I promoters become activated while activation-induced cytidine deaminase (AID) is upregulated. Germ-line transcription facilitates the access of AID to S regions by creating single-stranded DNA (ssDNA), which is the optimal target for AID. AID recognizes cytosines within hotspot motifs and converts them to uracil. This DNA lesion is then processed by mismatch repair (MMR) and base excision repair (BER) pathways, resulting in point mutations and/or double-stranded DNA breaks (DSBs). Distant DSBs in two switch regions are joined mainly via nonhomologous end-joining or microhomology-dependent alternative end-joining pathways. Typically, recombination of the donor S region (Sμ) with a downstream acceptor S region is accompanied by deletion of the intervening sequences such as between Sμ and Sα, producing the large circular DNA shown in Fig. 3. In the next section, we will discuss observations on how this process evolved.

4 Evolution of CSR in Vertebrates

4.1 Evolution of Switch Regions

Switch (S) regions are 1–10 kb repetitive intronic sequences upstream of C_H regions that are the sites of DSB formation and subsequent end joining during CSR. It appears the main function of S regions is to direct AID to C_H intronic regions, causing the formation of double-stranded breaks (DSBs). Ancient *Xenopus* Sμ in place of mouse Sγ1 can support CSR, suggesting CSR substrates are interchangeable (Zarrin et al. 2004). In addition, the function of S regions and AID can be replaced by DSBs induced by the yeast endonuclease I-SceI, suggesting S regions are not required for the joining of two lesions within the IgH locus (Zarrin et al. 2007), and that any means of forming DSBs may be sufficient for isotype switching.

Mammalian S regions are G-rich on the non-template strand. Certain motifs, such as TGGGG, GGGGT, GGGCT, GAGCT, and AGCT, are common. Among these motifs, AGCT is evolutionarily conserved in amphibians (Mussmann

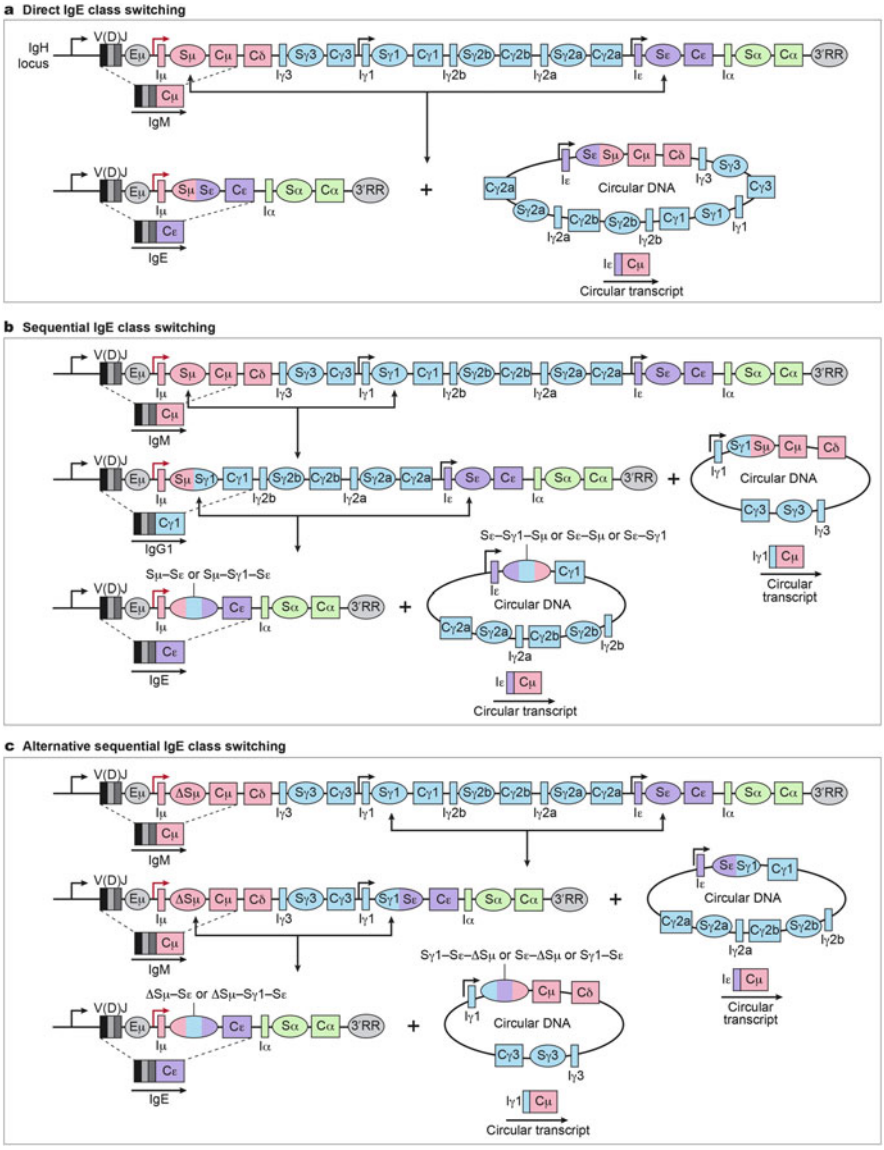

Fig. 3 Mechanism of class switch recombination (CSR). The recombination event that results in the replacement of Cμ with Cα to produce IgA is illustrated. The organization of the mouse IgH locus is diagrammed, with the *red arrow* indicating constitutive germ-line transcription through Sμ. The *black arrow* represents inducible transcription through Sα. AID targets transcribed donor Sμ and acceptor Sα to initiate double-stranded DNA breaks, which is accompanied by deletion of the intervening DNA. The recombined locus is shown *lower left*. The intervening DNA forms a short-lived circle, shown *lower right*, from which transcripts are generated

et al. 1997; Zhao et al. 2006; Ohta and Flajnik 2006) and birds (Lundqvist et al. 2001). Additional palindromic sequences such as CATG, AGCA, and TGCA are also abundant in amphibians and may be involved in CSR (Mussmann et al. 1997). The key enzyme that targets these sequences, AID, preferentially targets RGYW/WRCY motifs within S regions (where R = A/G; Y = C/T; W = A/T; hotspot = G:C) downstream of transcriptionally active start sites (Xue et al. 2006). The overlap and density of AID hotspot sequences on two DNA strands correlates with the quality of the regions and their CSR efficiency (Zarrin et al. 2004, 2005; Xue et al. 2006; Han et al. 2011). Switch regions have not yet been found in fish, although in shark the V(D)J region from one cluster has been found expressed with C_H regions from different clusters, due to recombination within the J–C intron via an unknown mechanism (Zhu et al. 2012).

Structures such as R-loops and G quartets can facilitate AID targeting and CSR. Upon transcription of GC-rich S regions, the RNA remains associated with the template DNA strand, generating an R-loop structure. These R-loops are thought to mediate AID function at the S region (Huang et al. 2007; Roy et al. 2008, 2010; Roy and Lieber 2009; Shinkura et al. 2003; Yu et al. 2003). Experiments using a mouse B cell line have shown that the propensity of a region to form R-loops is an important variable for determining the efficiency of switching to an S region (Zhang et al. 2014). The more G-rich motifs (e.g., TGGGG) seem to have been acquired recently in mammals (Tian and Alt 2000; Shinkura et al. 2003; Yu et al. 2003; Zarrin et al. 2004; Huang et al. 2007; Roy et al. 2008) suggesting that these sequences represent a more efficient target for AID, which itself has evolved over time.

4.2 Evolution of AID

AID was initially proposed to function as an RNA-editing enzyme based on sequence homology to known RNA-editing enzymes, such as the bacterial RNA cytidine deaminase and mammalian APOBEC (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like) family of enzymes, which contains 11 members (Navaratnam et al. 1998; Conticello et al. 2005). It has since been established that AID targets single-stranded DNA (ssDNA) and ssDNA–RNA hybrid structures within transcription bubbles (Petersen-Mahrt et al. 2002; Dickerson et al. 2003; Bransteitter et al. 2003, 2004; Martin and Scharff 2002; Ramiro et al. 2003; Chaudhuri et al. 2003; Yu et al. 2003). Nonimmunoglobulin loci are also susceptible to AID activity, and this has been associated with highly transcribed, topologically complex super-enhancers and regulatory clusters, demonstrating the involvement of these genomic components in AID recruitment (Qian et al. 2014; Meng et al. 2014).

The first appearance of AID is in sharks (Conticello et al. 2005; Conticello 2008), the earliest vertebrate known to have all pivotal components of the modern adaptive immune system, including RAG genes, V(D)J recombination, and major

histocompatibility complex (MHC) proteins. Putative cytidine deaminases have also been identified in the lamprey, a non-jawed vertebrate with a primordial form of adaptive immune receptors, suggesting they predate the appearance of AID (Rogozin et al. 2007; Tasumi et al. 2009; Deng et al. 2010). AID and SHM are found in bony fish, while CSR does not make an appearance until amphibians, suggesting that SHM is more ancient. It follows that the extreme C-terminal portion of AID interacts with CSR-specific factors (Barreto et al. 2003; Ta et al. 2003) and the amino acid residues within this region are divergent in bony fish.

In humans, AID is a small protein of 198 amino acids which folds into a single globular domain containing a central β-sheet that is flanked by α-helices and loops (Fig. 4a). AID contains an N-terminal nuclear localization sequence (NLS) and a C-terminal nuclear export signal (NES), and the heart of the enzyme is formed by conserved HXE and SWSPCXXC motifs which together bind the catalytic Zn^{2+} ion in the active site (Fig. 4a, b). Either directly or indirectly through other proteins, AID interacts with a number of cellular factors to regulate its localization and activity in executing SHM, CSR, and gene conversion (Reynaud et al. 2003), but the molecular basis underpinning the interactions which assemble the immunoglobulin mutasome remains an important area of discovery. Multiple crystal and solution NMR structures have been determined for the APOBEC family of enzymes in recent years, providing a framework to begin to understand the function and regulation of AID.

As seen through structural modeling, AID contains a conserved electropositive surface patch surrounding the active site cleft, as expected for interaction with ssDNA substrates (Fig. 4c). Conservation analysis reveals two additional surface patches on AID of potential biological significance, one engulfing the proximal C-terminal α-helix and surrounding β-sheet (Fig. 4d). The phosphorylation of AID at Ser38 by protein kinase A (PKA) has been implicated in regulating CSR (Basu et al. 2005, 2009) and in some instances SHM. This PKA phosphorylation site is absent in fish AID, but a neighboring acidic residue, unique to the fish, may represent a constitutive phosphomimetic. In a structural model of human AID, Ser38 is found on an exposed loop distal to the active site, where six independent experimental coordinates available for the related APOBEC3G enzyme (~45 % sequence identity) reveal an incredible structural plasticity (Fig. 4b). In APOBEC3G, the conformation of the "Ser38 loop" appears critical for positioning the HXE and SWSPCXXC motifs within the active site. It is therefore possible that phosphorylation of Ser38 by PKA serves as a direct allosteric modulator of AID activity. As with any posttranslational modification that impacts protein conformational dynamics, these may also directly impact other protein–protein interactions across the surface of AID. The field is clearly awaiting an in-depth structural and biophysical characterization of AID, particularly in complex with immunologically relevant targeting and modulatory factors.

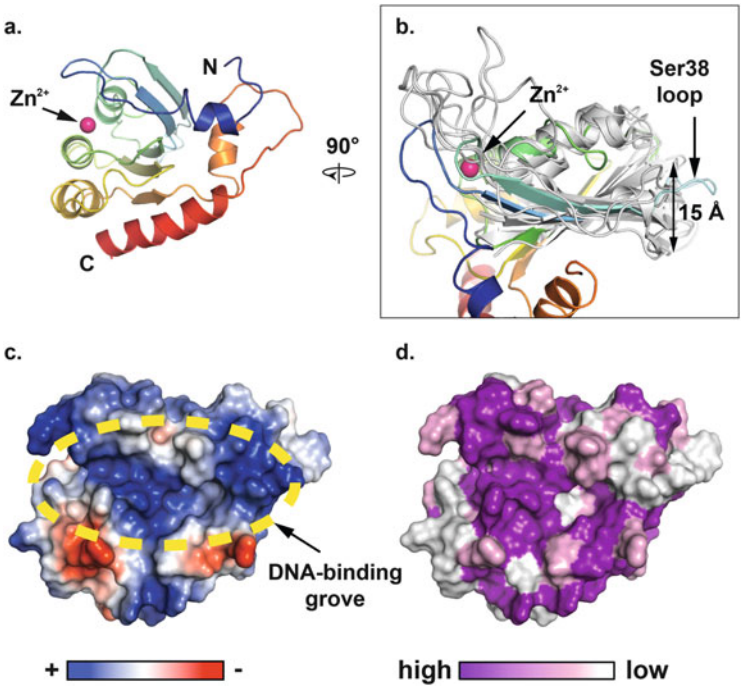

Fig. 4 Structural model of human AID based on its homology to ancient APOBEC3. (**a**) Cartoon rendering highlighting the localization of the essential Zn^{2+} ion in the catalytic site of a homology model of human AID built using the APOBEC2 structure as a template (PDB ID: 2NYT). (**b**) Available X-ray and NMR structures of APOBEC3G superimposed onto the human AID model (PDB IDs: 2JYW, 2KBO, 2KEM, 3E1U, 3IR2). In APOBEC3G, structural transitions observed in the Ser38 "phosphorylation" loop appear to be coupled to structural changes within the active site that could directly regulate enzyme activity. (**c**) Electrostatic surface potential of human AID homology model reveals a large electropositive cleft primed to bind ssDNA substrates. Coloring is as follows: *blue*, basic regions; *red*, acidic regions; *white*, hydrophobic regions. Orientation is the same as in part (**a**). (**d**) Conservation of AID across species shows a region of high sequence conservation mapped onto the active site and presumed substrate binding cleft. The surrounding regions of sequence divergence could represent functionally relevant targeting or modulatory sites on the enzyme. Coloring is as follows: *purple*, high conservation; *pink*, good conservation; *white*, low conservation. Orientation is the same as in parts (**a**) and (**c**)

5 Conserved or Diversified Biological Functions of Antibody Isotypes in Mammals

5.1 IgM

IgM is the primordial Ig from which mammalian immunoglobulins other than IgD are descended. IgM is the first isotype expressed during B cell development, forming a pool of "natural" antibodies that constitute the first line of defense during

infections. Surface-bound IgM cross-linked by its cognate antigen serves as the B cell antigen receptor. Secreted IgM exists as pentamers or, less often, hexamers that are connected via a J chain. The major function of IgM antibodies is to activate the classical pathway of complement and effectively opsonize various bacterial pathogens, as well as cause direct lysis through the assembly of the membrane attack complex.

5.2 IgD

IgD is primarily coexpressed with IgM on the surface of mature B cells before antigenic stimulation and regulated by alternative splicing (Chen and Cerutti 2011). Although typical S regions are not found upstream of Cδ, direct CSR to CδIgD has been reported (Kluin et al. 1995; White et al. 1990). Secreted IgD is detected in blood, in mucosal secretions, and on the surface of innate immune effector cells such as basophils. IgD is produced both as a surface-bound antigen receptor and in secreted form. Unlike IgM, which has been stable during evolution, the structural differences among IgDs across species are quite noticeable, suggesting it might have diverse functions in vertebrates. Ancient IgW and IgD heavy chains are orthologues, and thus both IgD and IgM were present at the inception of the adaptive immune system (Ohta and Flajnik 2006). Unlike other isotypes, the function of IgD remains controversial. In mice, IgD deficiency delays affinity maturation and impacts the localization of B cells during antigen-driven immune responses (Roes and Rajewsky 1993). Recent evidence suggests that secreted IgD enhances immune surveillance by activating antimicrobial, proinflammatory, and B cell-stimulating programs in basophils (Chen et al. 2009).

5.3 IgG

IgG is an abundant isotype in the blood and extravascular compartments. It is highly stable in circulation and has a half-life of about 3 weeks, making it suitable for a variety of bioengineering and therapeutic applications (see below). There are various subclasses of IgG in humans and other species. Common functions of IgG include crossing the placenta and the establishment of the memory humoral immune response. IgG antibodies play an important role in clearing viral and bacterial pathogens and neutralizing toxins. This isotype is also important in direct complement-mediated lysis (e.g., *Neisseria meningitis*) and can also mediate opsonization and destruction within phagosomes (e.g., *Streptococcus pneumoniae*).

There are two general classes of IgG receptors, known as FcγRs: the activating receptors (ITAM-containing) and the inhibitory receptors (ITIM-containing), which usually function in concert. The dual inhibitory and activating function of FcγRs sets the threshold for the magnitude of effector cell responses. The FcγRs

serve as a bridge between adaptive and innate immune responses. FcγRs are expressed on monocytes, macrophages, DCs, neutrophils, NK, NKT, and B cells. Upon engagement, these receptors initiate phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC), and cytokine release. FcγRIIb is an inhibitory receptor, and its co-engagement with other FcγRs results in inhibition of FcγR-mediated signaling. In the absence of FcγRIIb, mice are prone to autoimmunity due to a higher frequency of autoreactive B cells. In addition, polymorphisms associated with a lower expression of FcγRIIb are risk alleles for developing autoimmunity (Nimmerjahn and Ravetch 2008; Tsuchiya and Kyogoku 2005).

5.4 IgE

IgE is the least abundant isotype and is involved in immunity to helminthes and allergic diseases such as asthma, allergic rhinitis, and atopic dermatitis. In addition, IgE mediates type I hypersensitivity reactions, which include both systemic and localized anaphylaxis. IgE has two major receptors, "high-affinity" FcεRI and FcεRII (CD23). FcεRI is predominantly expressed on mast cells and basophils, and its activation mediates cellular degradation and cytokine production. Upon ligation with multivalent antigens, FcεRI transduces signals that lead to the production of proinflammatory mediators such as histamine, which is associated with IgE-mediated pathology as manifested in asthma, allergic rhinitis, and anaphylaxis. FcεRII regulates IgE production and facilitates antigen processing and presentation on B cells. FcεRII on macrophages or epithelial cells facilitates the uptake of IgE-antigen complexes.

5.5 IgA

IgA maintains mucosal homeostasis by controlling host interactions with the microbiota as well as protecting the host from pathogens and their products. The human digestive tract can be considered the largest immune organ in the body and is continuously challenged by microbiota and dietary antigens (Xu and Gordon 2003). IgA is a predominant class in mucosal secretions such as intestine, breast milk, saliva, tears, colostrum, and the mucus of the bronchial, genitourinary, and digestive tracts. Unlike other isotypes of the immune system, IgA is predominantly considered noninflammatory. IgA binds a wide array of pathogenic bacteria, viruses, and toxins and tethers them to mucus, a process known as immune exclusion (Strugnell and Wijburg 2010). IgA can also be transported from the lumen to the basolateral side of intestinal epithelial cells (IECs), bringing antigens along with it for presentation to the headquarters of the mucosal immune system, the gut-associated lymphoid tissue (GALT). IgA is thought to have evolved from

amphibian IgX based on phylogeny, genomic synteny, and function (Mashoof et al. 2013) (Figs. 1 and 2).

In most mammals, IgA has three immunoglobulin domains (Cα1, Cα2, Cα3) connected by a hinge region to the variable domain. In serum, it exists as a monomer, although polymeric (dimer, trimer, tetramer) forms can occur at lower levels. Mucosal IgA, in contrast, is predominantly a dimer, formed when two antibodies are covalently linked end to end by a J-chain polypeptide with variable regions facing outward (Bastian et al. 1992; Krugmann et al. 1997). Dimeric IgA binds to a receptor known as polymeric immunoglobulin receptor (pIgR) present on the basolateral membrane of IECs. This dimeric IgA–pIgR complex is internalized by receptor-mediated endocytosis and transported to the luminal surface. The shuttling pIgR is then enzymatically cleaved, leaving a portion of itself complexed to the now soluble IgA (SIgA) dimeric antibody. This pIgR fragment is termed secretory component (SC). SC protects and extends the half-life of SIgA in the mucosa. SC also confers hydrophilic properties to SIgA, helping to retain it to the mucus environment. In effect, SC keeps SIgA in a location where it interacts with microbiota and pathogens and prevents its diffusion into the lumen (Deplancke and Gaskins 2001; Johansson et al. 2011).

6 Regulation of IgA Production

It has long been appreciated that IgA production is tightly linked to bacterial colonization of the gut. Mice raised in germ-free conditions produce little to no mucosal IgA (Benveniste et al. 1971a, b; Moreau et al. 1978), and inoculation of germ-free mice with certain strains of bacteria is capable of inducing high-titer IgA responses (Hapfelmeier et al. 2010; Geuking et al. 2012). Research into (1) how bacteria communicate with the gut, (2) where, anatomically, does CSR to IgA occur, (3) whether there are T cell-dependent (TD) and T cell-independent (TI) pathways, and (4) what the supporting cell types are has made great strides in recent years.

GALT is organized into distinct structures known as Peyer's patches (PPs), isolated lymphoid follicles (ILFs), and the lamina propria (LP) (Fig. 5) (Suzuki et al. 2010). Peyer's patches are prominent structures that form during embryogenesis. They contain multiple (>5) B cell follicles and comprise the major sources of T cell-dependent IgA synthesis. ILFs, on the other hand, have only one B cell follicle and can generate IgA in a T cell-independent manner (Tsuji et al. 2008). Unlike PPs, ILFs develop postnatally. This was originally believed to be in concert with bacterial colonization (Hamada et al. 2002; Pabst et al. 2006), but new evidence suggests diet plays a role (Kiss et al. 2011; Lee et al. 2012). Together, PPs and ILFs are considered "inductive sites," where most IgA immune responses are initiated through interactions with commensal bacteria. The lamina propria (LP) is a connective tissue underlying the mucosal epithelium. Plasmablasts that

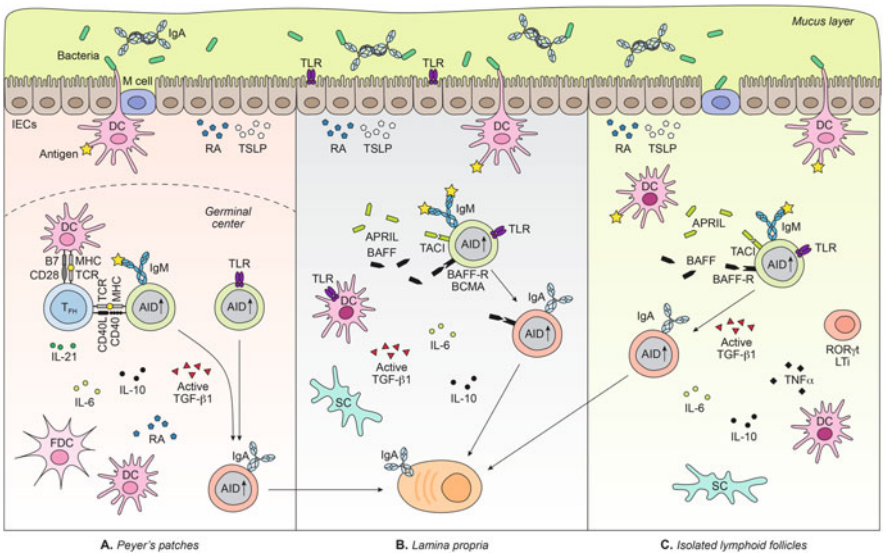

Fig. 5 IgA regulation within gut-associated lymphoid tissues (GALT). (**a**) Peyer's patches: intestinal epithelial cells (IECs) condition underlying dendritic cells (DCs) by releasing thymic stromal lymphopoietin (TSLP) and retinoic acid (RA). M cells and CX3CR1^{+} DCs transport microbial products from the lumen, which are conveyed to germinal center T cells by CD103^{+} DCs. Additional subsets of DCs contribute IL-6, IL-10, TGF-β, and RA, which polarize T cells toward Th2 responses and promote IgA CSR. Inside the germinal center, stromal cells release BAFF, APRIL, and TGF-β1, enhancing IgA CSR and promoting T_{FH} function. Once activated, B cells upregulate AID and switch to Cα. Activated B cells differentiate into plasmablasts within the germinal centers, moving into the lamina propria for terminal differentiation into IgA-expressing plasma cells. (**b**) Lamina propria: DCs respond to bacterial products through TLR signaling and release IL-6, RA, BAFF, and APRIL, which support B cell activation and differentiation. IECs also release BAFF and APRIL in a TLR-dependent manner. An IgA-secreting plasma cell is denoted in *light orange*. (**c**) Isolated lymphoid follicles: Similar to the LP, several DC types and stromal cells (SC) are thought to contribute signals conducive to IgA CSR. Subepithelial CX3CR1^{+} DCs capture and present TI antigens to local follicular B cells. CD11c^{+} DCs produce TNF, inducing matrix metalloproteinases that process precursor TGF-β1 into its mature form. These DCs can also produce BAFF and APRIL. Stromal cells can also produce BAFF in addition to molecules that attract B cells and DCs to the gut

have moved out into the lamina propria and matured into plasma cells are considered the "effector sites" of the GALT (Macpherson et al. 2008).

6.1 *T Cell-Dependent and T Cell-Independent Locations of IgA CSR*

Determining where actual switching to IgA occurs (PP, ILF, or LP) and whether it requires T cell help has been a subject of intense study. Years ago, it was proposed

that PPs were an enriched source of precursors of IgA-producing cells (Craig and Cebra 1971). These structures were hypothesized to "shed" lymphoid cells into circulation, which would "seed" the gut, proliferate, and differentiate into IgA-producing cells. While IgA CSR can take place within PPs, recent evidence suggests these organized lymphoid structures are unnecessary for the gut to be populated with IgA^+ plasma cells.

Peyer's patches are perhaps the best-studied component of the mucosal immune response (Fig. 5a). It is here that T cell-dependent reactions occur to produce high-affinity IgA. Under steady-state conditions, IECs release thymic stromal lymphopoietin (TSLP) and retinoic acid (RA) in response to TLR signals, factors that "condition" underlying DCs to be noninflammatory. The road to IgA synthesis in one of these structures begins when "snorkeling" $CX3CR1^+$ DCs capture bacterial antigens directly from the lumen (Bogunovic et al. 2009; Varol et al. 2009), or M cells convey them via transcytosis (Mabbott et al. 2013). Neither M cells nor $CX3CR1^+$ DCs can migrate to interfollicular areas to present antigens to T cells themselves; instead, a migratory $CD103^+$ DC subtype may act as a courier (Schulz et al. 2009; Bogunovic et al. 2009; Varol et al. 2009; Coombes et al. 2007). Additional DC subsets are found in and around GCs (Rescigno and Di Sabatino 2009; Salazar-Gonzalez et al. 2006; Sun et al. 2007) that together secrete a cocktail of factors (TGF-β, IL-10, IL-6, IL-21, and RA) that promote IgA responses.

Inside the GC, follicular dendritic cells (FDCs) form a scaffold for B and T cell interactions. These stromal cells can release BAFF, APRIL, and large amounts of TGF-β1 that act to enhance IgA CSR and promote T_{FH} function (Suzuki et al. 2010). Tregs in the GC make IL-10, TGF-β1, and CD40L, which drive CSR to IgA and repress inflammatory Th1 responses (Cong et al. 2009). Tregs can also respond to mucosal GCs by downregulating Foxp3 and turning into T_{FH} cells (Tsuji et al. 2009). Once activated, B cells make Iα transcripts, upregulate AID, and switch to Cα, leaving behind transient byproducts of the reaction including Sα–Sμ switch circles and Iα–Cμ circle transcripts. Activated B cells differentiate into plasmablasts within the germinal centers, subsequently moving into the lamina propria for terminal differentiation into IgA-expressing plasma cells (Tarlinton et al. 2008; Yoshida et al. 2010), or, as will be discussed next, bypass GC reactions and switch to IgA within the lamina propria itself.

The first indication that IgA switching could happen outside of follicular structures such as PPs and ILFs came when hallmarks of CSR to Cα, such as AID expression, Sα–Sμ switch circles, and Iα–Cμ circle transcripts, were detected in LP tissue (Fig. 5b) (Fagarasan et al. 2001). These findings were corroborated by others (He et al. 2007; Crouch et al. 2007). However, not every group was able to detect these products in the LP (Barone et al. 2009; Bergqvist et al. 2006, 2010), raising the question of whether the results were simply due to ILF contamination. The LP switching hypothesis was shored up by studies in genetic backgrounds that lack PPs and ILFs, yet still show intestinal IgA production and signs of CSR in the LP (Kang et al. 2002; He et al. 2007; Crouch et al. 2007). Taken together, these findings demonstrate that a visit to a germinal center is not necessary for a B cell in the gut to

switch to IgA. In fact, new evidence suggests the LP may even harbor developing B cells undergoing V(D)J recombination. This would likely be a mechanism to specifically control the gut Ig repertoire and suggests that early B cell development may not be limited to the bone marrow (Wesemann et al. 2013).

Multiple lines of evidence suggest LP switching happens in a T cell-independent manner. The key ingredients for T-independent switching likely involve the activities of several types of dendritic cells with the IECs (He et al. 2007; Tsuji et al. 2008; Tezuka et al. 2007; Shang et al. 2008; Uematsu et al. 2008; Xu et al. 2007, 2008; Uematsu and Akira 2009). DCs respond to bacterial products through TLR signaling and release factors supportive of B cell activation and differentiation, such as RA, IL-6, and B cell factors BAFF and APRIL. IECs are likewise capable of releasing BAFF and APRIL via TLR recognition of bacteria (He et al. 2007; Xu et al. 2007). APRIL is particularly effective at inducing IgA2, the dominant IgA isotype in the distal intestine, when coupled with TLR ligands like flagellin, and can do so in a CD40L-independent manner (He et al. 2007). Also, IgA+ plasma cells secrete iNOS and TNF-α, additional drivers of de novo IgA production (Fritz et al. 2012). Interestingly, evolutionary studies suggest these T cell-independent mechanisms may be ancient ways for regulating mucosal defense. In *Xenopus*, for example, mucosal IgX levels do not change when T cells are ablated via larval thymectomy (Turner and Manning 1974; Mashoof et al. 2013).

ILFs are also believed to induce IgA CSR without T cell help (Fig. 5c). In $Tcrb^{-/-}Tcrd^{-/-}$ mice, which lack T cells, ILFs were found to contain numerous IgA^+ B cells and plasmablasts (Tsuji et al. 2008). Additionally, in a RORγt KO model where lymphoid tissue-inducer (LTi)-like cells were injected, ILFs, but not PPs, developed. In these mice, $CD4^+$ T cells in the LP were greatly reduced, yet B cells were able to populate the ILFs and express AID, and gut IgA levels approached that of WT mice. Similar to the LP, in the absence of T cells, several DC types and stromal cells are thought to contribute signals conducive to IgA CSR. Subepithelial $CX3CR1^+$ DCs have been observed in ILFs (Hamada et al. 2002) that may capture and present T cell-independent antigens to local follicular B cells. $CD11c^+$ DCs present in ILFs are capable of producing TNF, which is a potent inducer of matrix metalloproteinases (MMPs)-9 and -13, which process precursor TGF-β1 into its mature form. These DCs can also produce BAFF and APRIL in a manner that is enhanced by TLR ligands (Tsuji et al. 2008; Suzuki et al. 2010). Coculture of stromal cells (SCs) with LTi cells caused BAFF upregulation as well as key molecules for B cell migration to gut and chemokines associated with DC migration to gut, and expression was enhanced by bacterial products (Tsuji et al. 2008).

7 Utility and Applications

The practical applications gained from our understanding of Ig regulation, structure, and functions are numerous, ranging from research tools to diagnostic and therapeutic applications (Fig. 6). Most prominent is the development of monoclonal

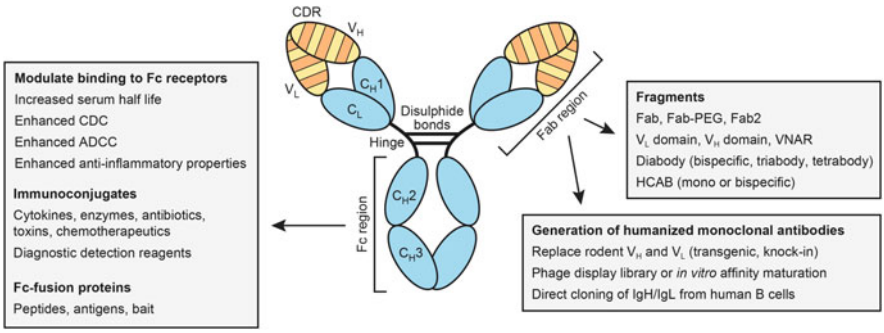

Fig. 6 Utility of antibodies and their domains. Shown is a subset of modifications to the traditional antibody to enhance functionality. *Left panel*: Fc regions can be mutated to enhance or reduce their binding affinities for Fc receptors, conjugated to various proteins or chemical moieties in order to deliver them to target cells, or isolated from the Fab and fused to various proteins. *Right upper panel*: Fab domains can be used in isolation with polyethylene glycol (PEG) added for stability, separated into V_L and V_H domains, or joined together to form bi-, tri-, and tetrabodies with multiple specificities. HCAB refers to heavy chain-only antibody (Janssens et al. 2006). *Right lower panel*: The antigen-binding pocket can be modified to have higher affinity and specificity by replacing the natural sequences with those derived from directly cloned human sequences or phage display libraries

antibodies (mAbs) for the treatment of disease that function to block or activate signaling pathways, deplete pathogenic cell types, or clear infectious agents. Several mAbs are currently used in the clinic for the treatment of various conditions, ranging from cancer to infectious and inflammatory diseases, and hundreds more are at various stages of development. Bispecific antibodies are also emerging as therapeutic modalities offering better therapeutic efficacy. The various properties of Ig molecules have been prodded and tweaked to create therapeutics with increased or decreased half-lives, muted or enhanced effector functions, multivalent antigen interactions, and smaller sizes to penetrate tumors. Other aspects of Ig regulation, such as the mutagenic properties of AID, have been used to enhance commercial antibody affinities, and switch region swapping has been used to create a hyper-IgE mouse model for the study of asthma and allergies. It is important to mention that in the coming years, the analysis of V(D)J and SHM/CSR repertoires, combined with human genome sequencing across various disease indications, will rapidly advance, helping to generate therapeutic and diagnostic hypotheses for a variety of human diseases.

7.1 Generation of Monoclonal Antibodies

Since the discovery of the Nobel prize-winning hybridoma technology for isolating mAbs (Kohler and Milstein 1975), methodologies to generate mAbs have advanced substantially and are still rapidly evolving. Various strategies have been adopted to generate mAbs in vivo by taking advantage of the de novo immune responses using

animal models amenable to genetic engineering such as mouse or rat. After immunization, various strategies such as hybridoma fusion technology or single cell cloning are utilized to obtain a panel of antigen-specific mAbs. Mouse mAbs have been widely used in research, but unfortunately have a low success rate in the clinic (Reichert et al. 2005). This is because they are highly immunogenic, resulting in rapid clearance, reduced efficacy, and an increased risk of adverse reactions ranging from rash to anaphylactic-like symptoms (Lonberg 2005). Furthermore, mouse mAbs react only weakly with human FcγRs, resulting in inefficient effector function, and do not bind the human salvage receptor FcRn (Ober et al. 2001), resulting in a less than 20 h half-life (Carter 2001; Presta 2002). These major hurdles were overcome by transgenic rodent models in which human IgH or IgL V regions are stitched together with mouse or rat constant regions with its known regulatory elements (Fig. 5) (Morrison et al. 1984; Boulianne et al. 1984; Green et al. 1994; Lonberg et al. 1994; Lonberg 2005). Various combinations of V and C regions are utilized to optimize these models. To avoid competition with endogenous loci, these transgenic models are often generated in an IgH/IgL knockout background. One drawback, however, of transgenic humanized rodents seems to be that components of B cell development and/or AID-dependent diversifications processes are suboptimal (Green and Jakobovits 1998; Pruzina et al. 2011). This could be due to reduced B cell numbers, defects in B cell development, a limited V_H repertoire, mis-regulation of the transgene, or a lack of other human compatible components to invoke a robust immune response. Aside from transgenic models, the generation of mouse models with the replacement of endogenous mouse V_H IgH or V_L loci with their human counterparts appears to be more efficient for producing mAbs.

mAbs can also be generated by technology in which human Abs are displayed on the surface of a simple organism such as phage, bacteria, or yeast. This approach has proved successful; however, it is labor intensive and repertoires are limited by the capacity of the library. mAbs have also been directly isolated from humans. This strategy has been effectively utilized to identify mAbs against various pathogens.

7.2 Variables Affecting Heavy Chain Choice

The specific heavy chain used in therapeutics is selected based on desired properties such as half-life, effector function, and valency. IgG1 is the isotype used in the majority of therapeutics (Salfeld 2007). IgG1 has a high affinity for FcγRs, which allows it to activate processes termed antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). ADCC results when an antibody binds to an undesirable target cell and is recognized by FcγIIIRA on the surface of NK cells, monocytes, and macrophages (Congy-Jolivet et al. 2007; Strohl 2009; Biburger et al. 2011), which subsequently destroy the cell. By introducing mutations to the IgG1 Fc domain, its affinity for Fc receptors can be

increased, thereby enhancing target cell clearance. This is particularly useful in tumor therapy. The best evidence showing clinical efficacy mediated by an Fc domain comes from studies of rituximab in patients with non-Hodgkin's lymphoma. It was found that patients carrying an FcγRIII polymorphism responded to the drug, strongly implicating ADCC as its mechanism of action (Cartron et al. 2002; Weng and Levy 2003).

CDC occurs when antibodies directly bound to cell surface antigens are recognized by complement C1q, triggering cell death via the complement cascade (Idusogie et al. 2001; Meyer et al. 2014). In some cases, this can be counteracted by tumors, which secrete complement regulatory proteins (Treon et al. 2001). Outside tumor therapy, activating complement is not considered ideal for many applications, such as neutralizing cytokines. Inappropriate activation of FcR-expressing cells can actually cause cytokine release and associated toxicities. In these cases, mutations can be introduced to IgG1 to lower its propensity to activate complement. IgG2 and IgG4 Fc domains can also be substituted for IgG1 since they have lower affinities for complement, a feature that has made them appealing for drug development recently (Bruhns et al. 2009).

Isotypes other than IgG1/2/4 are less common but have their own selling points. IgM, for example, is a natural adjuvant in vaccines and forms polymers that may mimic the adjuvant "depot effect" (Czajkowsky et al. 2010; Harte et al. 1983; Stager et al. 2003). IgM also binds C1q, which may make it suitable for complement-dependent cytotoxicity. The IgA isotype, with its affinity for mucosal surfaces, may be useful in preventing infections via the nasal cavity and gastrointestinal tract (Corthesy 2003). In these cases, IgA antibody would be applied directly to vulnerable mucosal surfaces, which is known as passive immunization. Many studies in humans and animals using passive immunization have demonstrated that administering antibodies orally, intranasally, via the intrauterine route, or by lung instillation can prevent, reduce, or even cure viral and bacterial infections. Due to the difficulty in generating SIgA with a J chain and SC, many of these studies use monomeric IgG, IgY, or a combination of IgG and IgA. Thus, there is room for improving these reagents, and greater efficacy may result from using complete SIgA.

7.3 Antibody Domains and Fragments

Worth mentioning is the notion that a heavy chain may not be desirable, or even necessary, in drug development. Traditional antibodies are quite bulky (~150–180 kDa) and have difficulty penetrating tumors and tissues, or targeting antigens in recessed spaces such as enzyme active sites. Researchers are now dismembering antibodies into their component parts, forming a toolkit from which to assemble therapeutics with exciting properties (Fig. 6). These "domain antibodies" come in numerous forms: fragment antigen binding (Fab, ~55 kDa), Fab_2 bispecific (~110 kDa), Fab_3 trispecific (~165 kDa), single chain variable fragment (scFv,

~28 kDa), scFv in diabody (~50 kDa), triabody (~75 kDa), and tetrabody (~100 kDa) formats and even the most minimal V_H and V_L domains (~15 kDa each) (Weir et al. 2002; Holliger and Hudson 2005). One issue with isolated V domains, however, is they can have problems with aggregation, poor solubility, and reduced half-life in circulation. The covalent attachment of polymers such as polyethylene glycol (PEG) to Fab regions is one way to alleviate some of these issues. Another problem is that these fragments rarely display affinities close to their parent antibodies. An interesting solution to this problem was provided by comparative biology. Certain antibodies from camelids (camels, llamas) and cartilaginous fish (wobbegong, nurse sharks) do not have light chains, so one would predict their single heavy chain V domain (termed VhH in camelids and V-NAR in sharks) is structured in such a way as to confer high antigen specificity and affinity (De Genst et al. 2004; Dooley and Flajnik 2005; Streltsov et al. 2004). Indeed, the CDR3 region of these V domains, where most of the domain's diversity is concentrated, is elongated and stabilized by disulfide bonds. These small camelid VhH domains have the potential to recognize uncommon epitopes such as clefts on antigen surfaces. Future therapies using minimal V domains will probably be based on these unique species.

Humanized heavy chain-only antibodies, known as Fc-fusion proteins, are also emerging as platforms to generate mAbs with unique properties and come in a variety of formats (bivalent monospecific, tetravalent bispecific). These proteins have the potential for broad clinical and nonclinical applications since their first description as CD4-Fc antagonists (Czajkowsky et al. 2012). Fc-fusion proteins serve as well-behaved (stably and independently folding) modules to which many different proteins (e.g., ligands, antigens, baits) can be attached and remain functional.

7.4 Other Applications

Information gleaned about the mechanistic basis for CSR has been applied in other ways. For example, AID, a potent mutagen, has been used by several groups to enhance the affinity of commercial antibodies (King et al. 2014). In this method, an antibody library is expressed in mammalian cells (B cell or non-B cell origin), and antigen-coupled magnetic beads are used to capture those bearing Ag-specific antibodies. AID is then transfected into the selected cells, recapitulating SHM in vitro, and clones bearing enhanced affinity Abs are screened for and retained. This process has been used to evolve non-Ig proteins as well. Expression of AID with a fluorescent protein in Ramos cells endowed it with increased photostability and a far-red emission.

Even switch regions are finding an application. Recently, these highly repetitive regions of DNA were utilized to create a mouse that expresses high levels of IgE (Misaghi et al. 2013). IgE is a major driver of allergic reactions and asthma because it activates mast cells and basophils via FcεRI. As a result, switching to Cε has been

regulated over the course of evolution, in part by limiting the size of Sε to a relatively small region (~0.8 kb) that contains relatively few AID hotspots. Replacement of Sε with Sμ, which is much larger in size and very rich in deamination motifs, resulted in greatly enhanced switching to the epsilon locus. These Sμ knock-in (Sμ-KI) mice displayed greatly elevated IgE levels upon infection, which could make them useful in the study of allergic responses, asthma, and FcεRI/II function. Humanized IgH constant regions will also allow for efficient generation of selected and humanized isotypes in an efficient manner (Duchez et al. 2010).

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