Structure and function of immunoglobulins

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Immunoglobulins are heterodimeric proteins composed of 2 heavy and 2 light chains. They can be separated functionally into variable domains that bind antigens and constant domains that specify effector functions, such as activation of complement or binding to Fc receptors. The variable domains are created by means of a complex series of gene rearrangement events and can then be subjected to somatic hypermutation after exposure to antigen to allow affinity maturation. Each variable domain can be split into 3 regions of sequence variability termed the complementarity-determining regions (CDRs) and 4 regions of relatively constant sequence termed the framework regions. The 3 CDRs of the heavy chain are paired with the 3 CDRs of the light chain to form the antigen-binding site, as classically defined. The constant domains of the heavy chain can be switched to allow altered effector function while maintaining antigen specificity. There are 5 main classes of heavy chain constant domains. Each class defines the IgM, IgG, IgA, IgD, and IgE isotypes. IgG can be split into 4 subclasses, IgG1, IgG2, IgG3, and IgG4, each with its own biologic properties, and IgA can similarly be split into IgA1 and IgA2. (J Allergy Clin Immunol 2010;125:S41-52.)

Key words: *Antibody structure, antibody function, immunoglobulin structure, immunoglobulin function, immunoglobulin gene rearrangement, class switching, somatic hypermutation*

Abbreviations used

ADCC:	Antibody-dependent cellular cytotoxicity
AID:	Activation-induced cytosine deaminase
C:	Constant
CDR:	Complementarity-determining region
CSR:	Class-switch recombination
FcR:	Fc receptor
FcRn:	Neonatal Fc receptor
FR:	Framework region
Fv:	Fab variable fragment
H:	Heavy
IgSF:	Immunoglobulin superfamily
J:	Joining
L:	Light
NHEJ:	Nonhomologous end-joining
pIgA:	Polymeric IgA
pIgR:	Polymeric immunoglobulin receptor
ΨLC:	Surrogate or pseudo-light chain
RAG:	Recombination-activating gene
RSS:	Recombination signal sequence
SC:	Secretory component
SHM:	Somatic hypermutation
sIgA:	Secretory IgA
V:	Variable

In 1890, von Behring and Kitasato reported the existence of an agent in the blood that could neutralize diphtheria toxin. The following year, reference was made to "Antikörper," or antibodies, in studies describing the ability of the agent to discriminate between 2 immune substances. Subsequently, the substance that induces the production of an antibody was referred to as the "Antisomatogen + Immunkörperbildner," or the agent that induces the antibody. The term "antigen" is a contraction of this term. Thus an antibody and its antigen represent a classic tautology.

In 1939, Tiselius and Kabat used electrophoresis to separate immunized serum into albumin, α-globulin, β-globulin, and γ-globulin fractions. Absorption of the serum against the antigen depleted the γ-globulin fraction, yielding the terms γ-globulin, immunoglobulin, and IgG. "Sizing" columns were then used to separate immunoglobulins into those that were "heavy" (IgM), "regular" (IgA, IgE, IgD, and IgG), and "light" (light chain dimers).

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More than 100 years of investigation into the structure and function of immunoglobulin has only served to emphasize the complex nature of this protein. Typically, receptors bind to a limited and defined set of ligands. However, although individual immunoglobulin also bind a limited and defined set of ligands, immunoglobulins as a population can bind to a virtually unlimited array of antigens sharing little or no similarity. This property of adjustable binding depends on a complex array of mechanisms that alter the DNA of individual B cells. Immunoglobulins also serve 2 purposes: that of cell-surface receptors for antigen, which permit cell signaling and cell activation, and that of soluble effector molecules, which can individually bind and neutralize antigens at a distance. The molecular mechanisms that permit these many and varied functions are the focus of this chapter.

STRUCTURAL ELEMENTS

The immunoglobulin domain: The basic immunoglobulin superfamily building block

Immunoglobulins belong to the eponymous immunoglobulin superfamily (IgSF).[1-3] They consist of 2 heavy (H) and 2 light (L) chains (Fig 1), where the L chain can consist of either a κ or a λ chain. Each component chain contains one NH2-terminal variable (V) IgSF domain and 1 or more COOH-terminal constant (C) IgSF domains, each of which consists of 2 sandwiched β-pleated sheets pinned together by a disulfide bridge between 2 conserved cysteine residues.[1] Each V or C domain consists of approximately 110 to 130 amino acids, averaging 12,000 to 13,000 kd. Both immunoglobulin L chains contain only 1 C domain, whereas immunoglobulin H chains contain either 3 or 4 such domains. H chains

with 3 C domains tend to include a spacer hinge region between the first (C_H1) and second (C_H2) domains. A typical L chain will thus mass approximately 25 kd, and a 3 C domain Cγ H chain with its hinge will mass approximately 55 kd. Considerable variability is allowed to the amino acids that populate the external surface of the IgSF domain and to the loops that link the β strands. These solvent-exposed surfaces offer multiple targets for docking with other molecules.

Antigen recognition and the Fab

Early studies of immunoglobulin structure were facilitated by the use of enzymes to fragment IgG molecules. Papain digests IgG into 2 Fab fragments, each of which can bind antigen, and a single Fc fragment. Pepsin splits IgG into an Fc fragment and a single dimeric $F(ab)_2$ that can cross-link, as well as bind, antigens. The Fab contains 1 complete L chain in its entirety and the V and C_H1 portion of 1 H chain (Fig 1). The Fab can be further divided into a variable fragment (Fv) composed of the V_H and V_L domains, and a constant fragment composed of the C_L and C_H1 domains. Single Fv fragments can be genetically engineered to recapitulate the monovalent antigen-binding characteristics of the original parent antibody.[4]

Intriguingly, a subset of antibodies in a minority of species (camelids[5] and nurse shark[6]) lack light chains entirely and use only the heavy chain for antigen binding. Although these unusual variants are not found in human subjects, there are a number of ongoing attempts to humanize these types of antibodies for therapeutic and diagnostic purposes.[7]

Paratopes, epitopes, idiotypes, and isotypes

Immunoglobulin-antigen interactions typically take place between the paratope, the site on the immunoglobulin at which the antigen binds, and the epitope, which is the site on the antigen that is bound. *In vivo* immunoglobulins tend to be produced against intact antigens in soluble form and thus preferentially identify surface epitopes that can represent conformational structures that are noncontiguous in the antigen's primary sequence. This ability to identify component parts of the antigen independently of the rest makes it possible for the B cell to discriminate between 2 closely related antigens, each of which can be viewed as a collection of epitopes. It also permits the same antibody to bind divergent antigens that share equivalent or similar epitopes, a phenomenon referred to as cross-reactivity.

Immunization of heterologous species with mAbs (or a restricted set of immunoglobulins) allowed the identification of both common and individual immunoglobulin antigenic determinants. Individual determinants, termed idiotypes, are contained within V domains. Common determinants, termed isotypes, are specific for the constant portion of the antibody and allow grouping of immunoglobulins into recognized classes, with each class defining an individual type of C domain. Determinants common to subsets of individuals within a species yet differing between other members of that species are termed allotypes and define inherited polymorphisms that result from gene alleles.[8]

IMMUNOGLOBULIN GENE ORGANIZATION AND REARRANGEMENT

Immunoglobulin heavy and light chains are each encoded by a separate multigene family,[9,10] and the individual V and C domains are each encoded by independent elements: *V(D)J* gene segments for the V domain and individual exons for the C domains. The primary sequence of the V domain is functionally divided into 3 hypervariable intervals termed complementarity-determining regions (CDRs) that are situated between 4 regions of stable sequence termed framework regions (FRs; Fig 1).

Immunoglobulin rearrangement

Each *V* gene segment typically contains its own promoter, a leader exon, an intervening intron, an exon that encodes the first 3 framework regions (FRs 1, 2, and 3), CDRs 1 and 2 in their entirety, the amino-terminal portion of CDR3, and a recombination signal sequence (RSS). Each joining (J) gene segment begins with its own recombination signal, the carboxy terminal portion of CDR3, and the complete FR4 (Figs 1 and 2).

The creation of a V domain is directed by the RSSs that flank the rearranging gene segments. Each RSS contains a strongly conserved 7-bp (or heptamer) sequence (eg, *CACAGTG*) that is separated from a less well-conserved 9-bp (or nonamer) sequence (eg, *ACAAAACCC*) by either a 12- or 23-bp spacer. These spacers place the heptamer and nonamer sequences on the same side of the DNA molecule separated by either 1 or 2 turns of the DNA helix. A 1-turn *RSS* (12-bp spacer) will preferentially recognize a 2-turn signal sequence (23-bp spacer), thereby avoiding wasteful *V-V* or *J-J* rearrangements.

Initiation of the *V(D)J* recombination reaction requires recombination-activating genes (RAGs) 1 and 2, which are almost exclusively expressed in developing lymphocytes.[11] RAG1 and RAG2 introduce a DNA double-strand break between the terminus of the rearranging gene segment and its adjacent RSS. These breaks are then repaired by ubiquitously expressed components of a DNA repair process, which is known as nonhomologous end-joining (NHEJ), that are common to all cells of the body. Thus although mutations of RAG affect only lymphocytes, loss or alteration-of-function mutations in NHEJ proteins yield susceptibility to DNA damage in all cells of the body. The NHEJ process creates precise joins between the *RSS* ends and imprecise joins of the coding ends. Terminal deoxynucleotidyl transferase (TdT), which is expressed only in lymphocytes, can variably add non–germline-encoded nucleotides (N nucleotides) to the coding ends of the recombination product.

Typically, the initial event in recombination will be recognition of 12-bp spacer *RSS* by RAG1. RAG2 then associates with RAG1 and the heptamer to form a synaptic complex. Binding of a second RAG1 and RAG2 complex to the 23-bp, 2-turn *RSS* permits the interaction of the 2 synaptic complexes to form what is known as a paired complex, a process that is facilitated by the actions of the DNA-bending proteins HMG1 and HMG2.

After paired complex assembly, the RAG proteins single-strand cut the DNA at the heptamer sequence. The 3′ OH of the coding sequence ligates to 5′ phosphate and creates a hairpin loop. The clean-cut ends of the signal sequences enable formation of precise signal joints. However, the hairpin junction created at the coding ends must be resolved by renicking the DNA, usually within 4 to 5 nucleotides from the end of the hairpin. This forms a 3′ overhang that is amenable to further modification. It can be filled in through DNA polymerases, be nibbled, or serve as a substrate for TdT-catalyzed N addition. DNA polymerase μ, which shares homology with TdT, appears to play a role in maintaining the integrity of the terminus of the coding sequence.

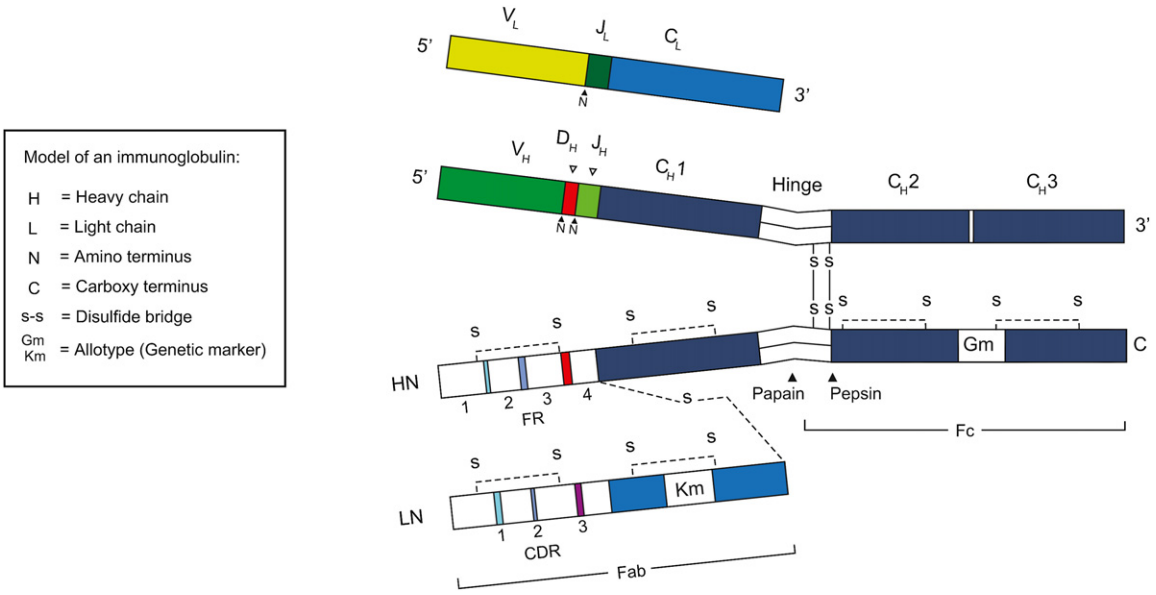

FIG 1. Two-dimensional model of an IgG molecule. The H and L chains at the top deconstruct the antibody at a nucleotide level. The chains at the bottom deconstruct the protein sequence. See the text for further details.

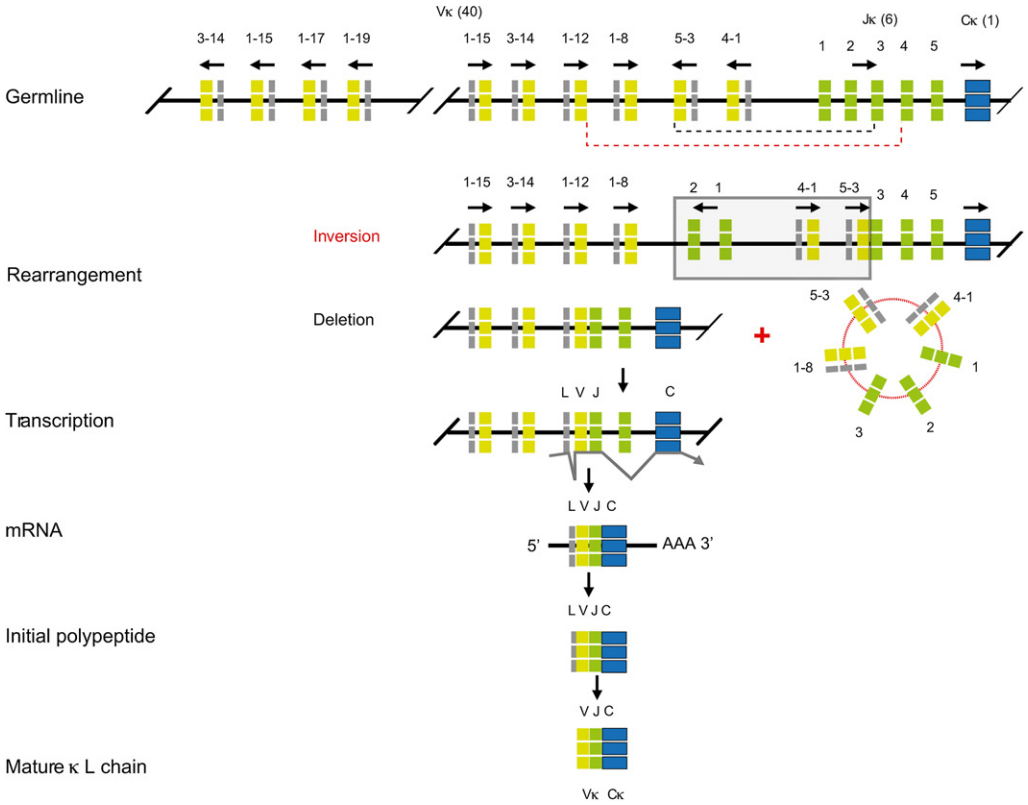

FIG 2. Rearrangement events in the human κ locus. See the text for further details.

The cut ends of the coding sequence are then repaired by the NHEJ proteins. NHEJ proteins involved in *V(D)J* recombination include Ku70, Ku80, DNA-PKcs, Artemis, XRCC4, and ligase.[4] Ku70 and Ku80 form a heterodimer (Ku) that directly associates with DNA double-strand breaks to protect the DNA ends from degradation, permit juxtaposition of the ends to facilitate coding end ligation, and help recruit other members of the repair complex. DNA-PKcs phosphorylates Artemis, inducing an endonuclease activity that plays a role in the opening of the coding joint hairpin. Finally, XRCC4 and ligase 4 help rejoin the ends of the broken DNA. Deficiency of any of these proteins creates sensitivity to DNA breakage and can lead to a severe combined immunodeficiency phenotype.

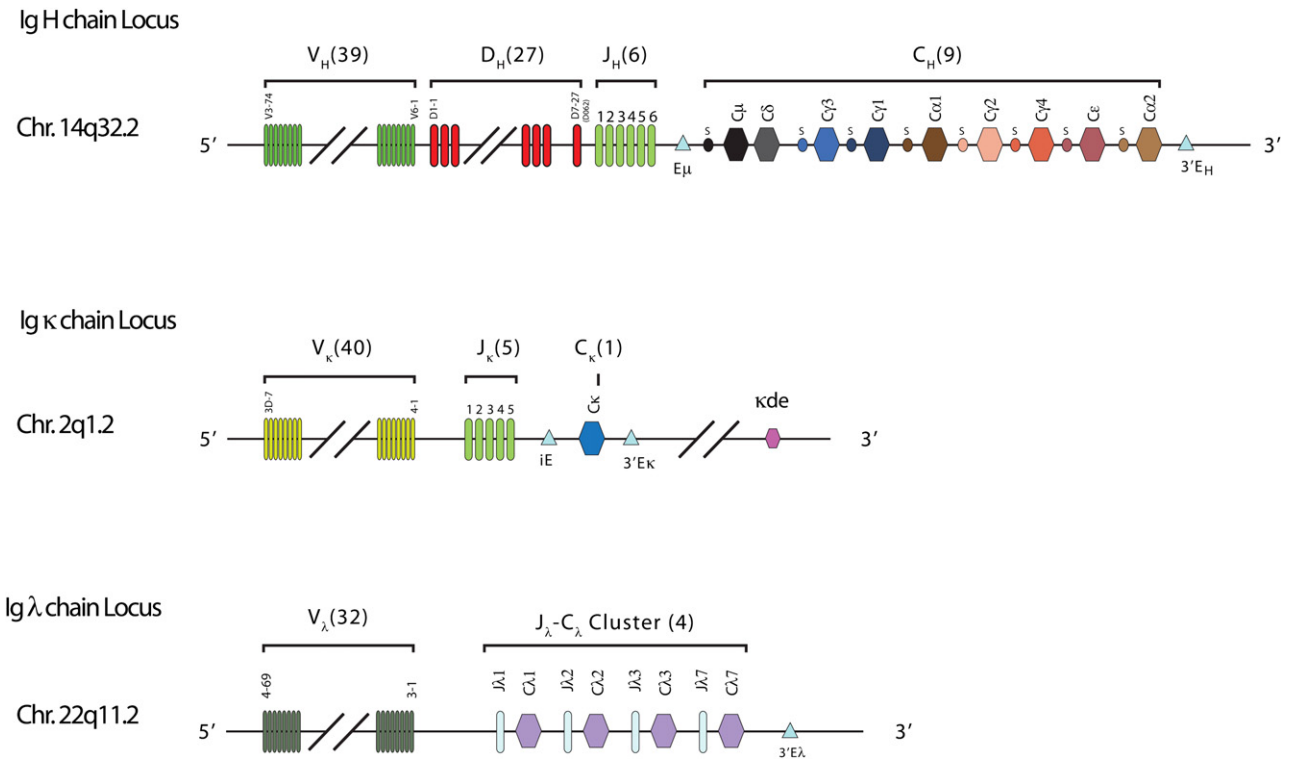

FIG 3. Representation of the chromosomal organization of the immunoglobulin H, κ, and λ gene clusters. The typical numbers of functional gene segments are shown. The κ gene cluster includes a κ-deleting element that can rearrange to sequences upstream of Cκ in cells that express λ chains, reducing the likelihood of dual κ and λ light chain expression.

The κ locus

The κ locus is located on chromosome 2p11.2.[12] κ V domains represent the joined product of *V*κ and *J*κ gene segments (Fig 2), whereas the κ C domains are encoded by a single *C*κ exon. The locus contains 5 *J*κ and 75 *V*κ gene segments upstream of *C*κ (Fig 3). One third of the *V*κ gene segments contain frameshift mutations or stop codons that preclude them from forming functional protein, and of the remaining sequences, less than 30 of the *V*κ gene segments have actually been found in functional immunoglobulins. *V* gene segments can be grouped into families on the basis of sequence and structural similarity.[13,14] There are 6 such families for *V*κ.

Each active *V*κ gene segment has the potential to rearrange to any of the 5 *J*κ elements, generating a potential "combinatorial" repertoire of more than 140 distinct *VJ* combinations. The *V*κ gene segment contains FR1, FR2, and FR3; CDR1 and CDR2; and the amino-terminal portion of CDR3. The *J*κ element contains the carboxy terminus of CDR3 and FR4 in its entirety. The terminus of each rearranging gene segment can undergo a loss of 1 to 5 nucleotides during the recombination process, yielding additional junctional diversity. In human subjects TdT can introduce random N nucleotides to either replace some or all of the lost *V*κ or *J*κ nucleotides or to add to the original germline sequence.[15] Each codon created by N addition increases the potential diversity of the repertoire 20-fold. Thus the initial diversification of the κ repertoire is focused at the *VJ* junction that defines the light chain CDR3, or CDR-L3.

The λ locus

The λ locus, which is located on chromosome 22q11.2, contains 4 functional *C*λ exons, each of which is associated with its own *J*λ (Fig 3). *V*λ genes are arranged in 3 distinct clusters, each containing members of different *V*λ families.[16] Depending on the individual haplotype, there are approximately 30 to 36 potentially functional *V*λ gene segments and an equal number of pseudogenes.

During early B-cell development, H chains form a complex with unconventional λ light chains, known as surrogate or pseudo-light chains (ΨLC), to form a pre–B-cell receptor. The genes encoding the ΨLC proteins λ14.1 (λ5) and V_{preB} are located within the λ light chain locus on chromosome 22. Together, these 2 genes create a product with considerable homology to conventional λ light chains. A critical difference between these unconventional *ΨLC* genes and other L chains is that *λ14.1* and V_{preB} gene rearrangement is not required for *ΨLC* expression. The region of the *ΨLC* gene that corresponds to CDR-L3 covers CDR-H3 in the pre–B-cell receptor, allowing the pre–B cell to avoid antigen-specific selection.[17]

The H chain locus

The H chain locus, which is located on chromosome 14q32.33, is considerably more complex than the light chain clusters. The approximately 80 V_H gene segments near the telomere of the long arm of chromosome 14 can be grouped into 7 different families of related gene segments.[18] Of these, approximately 39 are

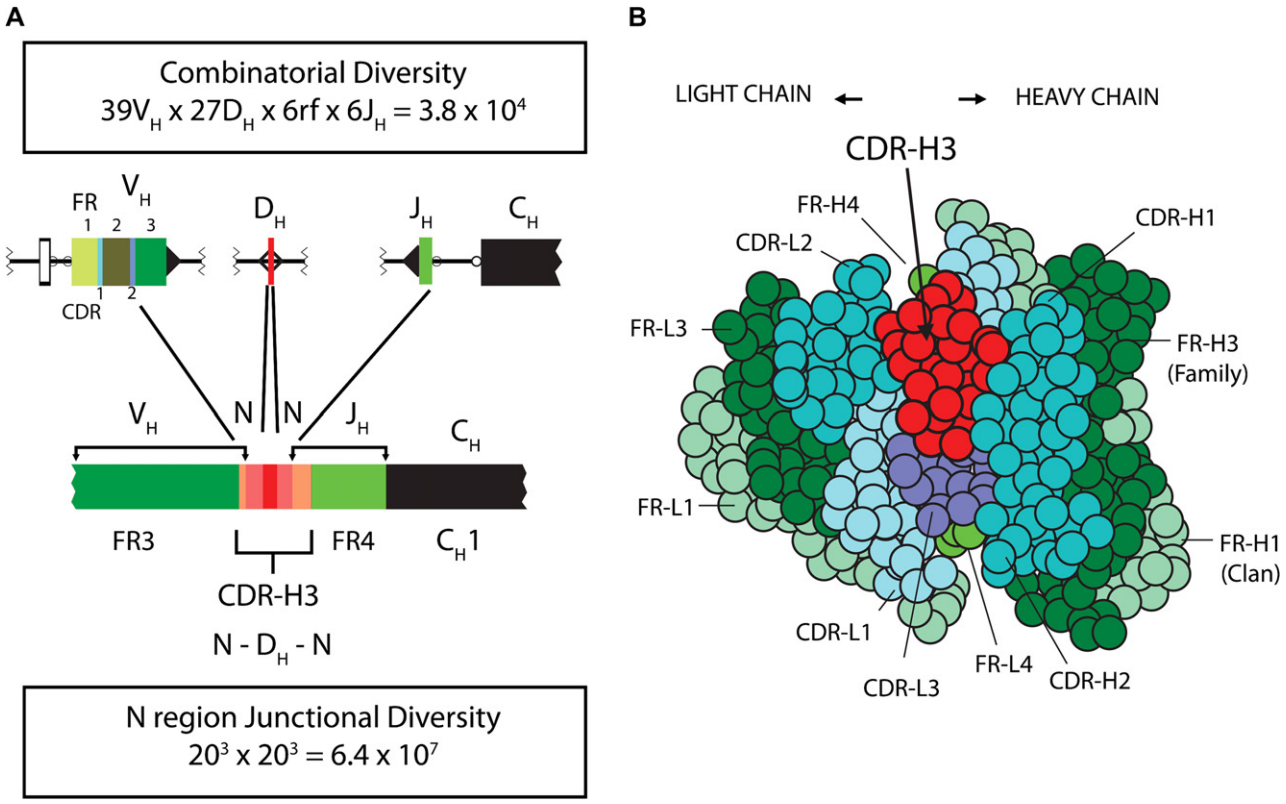

FIG 4. The antigen-binding site is the product of a nested gradient of diversity. **A**, H chain rearrangement can yield as many as 38,000 different VDJ combinations. The addition of 9 N nucleotides on either side of the D gene segment can yield up to 64,000,000 different CDR-H3 junctional sequences. **B**, The view is looking into the binding site as an antigen would see the antigen-binding site. This site is created by the juxtaposition of the 3 CDRs of the H chain and the 3 CDRs of the light chain. The V_H domain is on the right side. The central location of CDR-H3, which because of N addition is the focus for repertoire diversity, is readily apparent.

functional. Adjacent to the most centromeric V_H, *V6-1*, are 27 D_H (D for diversity) gene segments (Fig 3)[19] and 6 J_H gene segments. Each V_H and J_H gene segment is associated with a 2-turn *RSS*, which prevents direct $V \rightarrow J$ joining. A pair of 1-turn *RSS*s flanks each D_H segment. Recombination begins with the joining of a D_H to a J_H gene segment, followed by the joining of a V_H element to the amino-terminal end of the *DJ* intermediate. The V_H gene segment contains FR1, FR2, and FR3; CDR1 and CDR2; and the amino-terminal portion of CDR3. The D_H gene segment forms the middle of CDR3, and the J_H element contains the carboxy terminus of CDR3 and FR4 in its entirety (Fig 1). Random assortment of one of approximately 39 active V_H and one of 27 D_H gene segments with one of the 6 J_H gene segments can generate more than 10^4 different *VDJ* combinations (Fig 4).

Although combinatorial joining of individual *V*, *D*, and *J* gene segments maximizes germline-encoded diversity, the junctional diversity created by *VDJ* joining is the major source of variation in the preimmune repertoire (Fig 4). First, D_H gene segments can rearrange by either inversion or deletion, and each D_H gene segment can be spliced and translated in each of the 3 potential reading frames. This gives each D_H gene segment the potential to encode 6 different peptide fragments.

Second, the rearrangement process proceeds through a step that creates a hairpin ligation between the 5′ and 3′ termini of the rearranging gene segment. Nicking to resolve the hairpin structure leaves a 3′ overhang that creates a palindromic extension, termed a P junction, that can add germline-encoded nucleotides.

Third, the terminus of each rearranging gene segment can undergo a loss of 1 to several nucleotides during the recombination process.

Fourth, TdT can add numerous N nucleotides at random to replace or add to the original germline sequence. N nucleotides can be inserted between the *V* and *D* segments, as well as between the *D* and *J* segments. The imprecision of the joining process and variation in the extent of N addition permits generation of CDR-H3s of varying length and structure. As a result, more than 10^7 different H chain *VDJ* junctions, or CDR-H3s, can be generated at the time of gene segment rearrangement. Taken as a whole, somatic variation in CDR3, combinatorial rearrangement of individual gene segments, and combinatorial association between different L and H chains can yield a potential preimmune antibody repertoire of greater than 10^{16} different immunoglobulins.

Class-switch recombination

Located downstream of the VDJ loci are 9 functional C_H genes (Fig 3).[20] These constant genes consist of a series of exons, each encoding a separate domain, hinge, or terminus. All C_H genes can undergo alternative splicing to generate 2 different types of carboxy termini: either a membrane terminus that anchors immunoglobulin on the B-lymphocyte surface or a secreted terminus that occurs in the soluble form of the immunoglobulin. With the exception of $C_H1\delta$, each C_HI constant region is preceded by both an exon that cannot be translated (an *I* exon) and a region of repetitive DNA

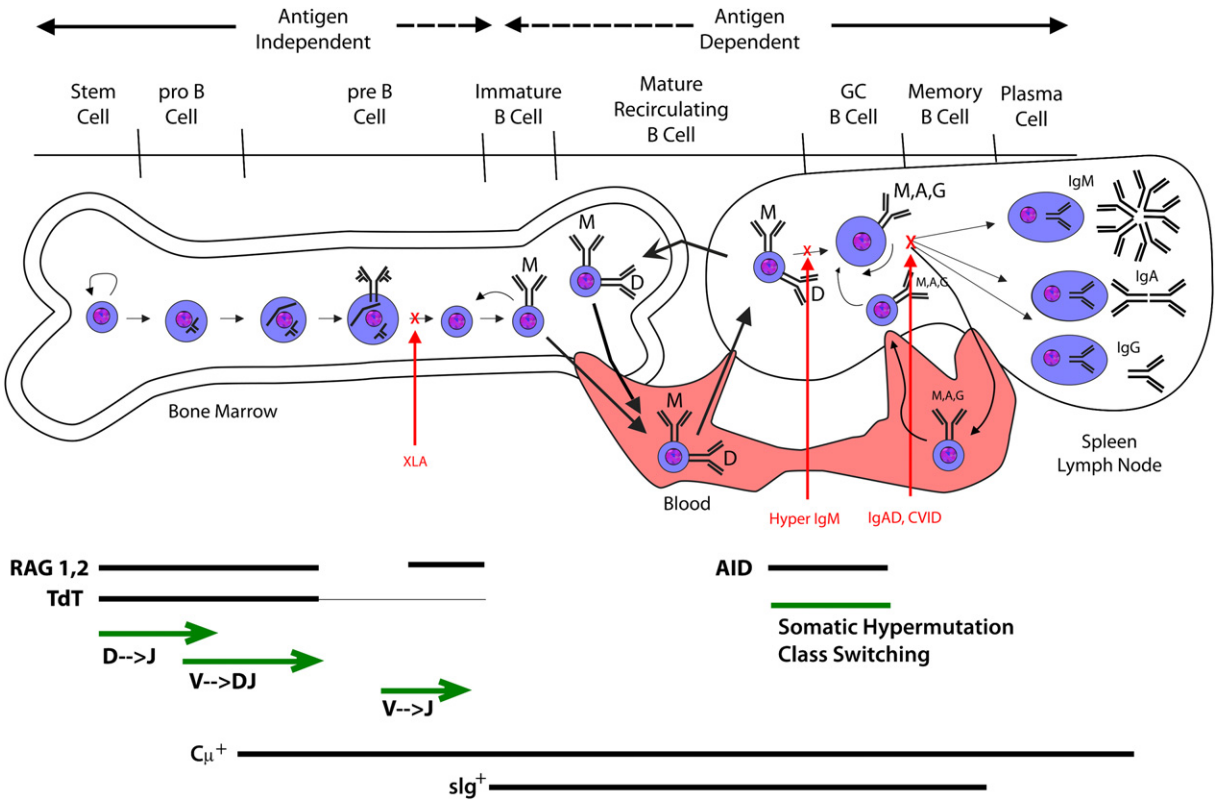

FIG 5. Immunoglobulin diversification and B-cell development. B-cell development as a function of immunoglobulin rearrangement and modification is shown. After birth, B-cell development begins in the bone marrow and is independent of antigen stimulation. The pre–B cell is defined by the presence of cytoplasmic μ protein ($C\mu^+$). With development, the fate of the B cell becomes increasingly dependent on its response to antigen. Immature B cells leave the bone marrow and begin to express IgD. They recirculate through the blood, the secondary lymphoid organs, and the bone marrow. Encounter with cognate antigen can cause the cell to become a memory B cell or a plasma cell. Patients with X-linked agammaglobulinemia *(XLA)* lack Bruton tyrosine kinase function and have difficulty making immature B cells and IgM. Patients with hyper-IgM syndrome *(Hyper IgM)* are unable to class-switch. Patients with selective IgA deficiency *(IgAD)* or common variable immune deficiency *(CVID)* can class-switch but have difficulty becoming plasma cells or memory B cells.

termed the switch. Cocktails of cytokine signals transmitted by T cells or other extracellular influences variably activate the I exon, initiating transcription and thus activating the gene. Through recombination between the $C\mu$ switch region and one of the switch regions of the 7 other H chain constant regions (a process termed class-switching or class-switch recombination [CSR]), the same VDJ heavy chain variable domain can be juxtaposed to any of the H chain classes.[20] This enables the B cell to tailor both the receptor and the effector ends of the antibody molecule to meet a specific need.

Somatic hypermutation

A final mechanism of immunoglobulin diversity is engaged only after exposure to antigen. With T-cell help, the variable domain genes of germinal center lymphocytes undergo somatic hypermutation (SHM) at a rate of up to 10^{-3} changes per base pair per cell cycle. SHM is correlated with transcription of the locus, and in human subjects 2 separate mechanisms are involved: the first mechanism targets mutation hot spots with the *RGYW* (purine/G/pyrimidine/A) motif,[21] and the second mechanism incorporates an error-prone DNA synthesis that can lead to a nucleotide mismatch between the original template and the mutated DNA strand.[22] Other species use gene conversion between functional and nonfunctional V sequences to introduce additional somatic diversity. SHM allows affinity maturation of the antibody repertoire in response to repeated immunization or exposure to antigen.

Activation-induced cytidine deaminase

Activation-induced cytidine deaminase (AID) plays a key role in both CSR and SHM.[11,23] AID is a single-strand DNA cytidine deaminase that can be expressed in activated germinal center B cells.[24] Transcription of an immunoglobulin *V* domain or of the switch region upstream of the C_H1 domain opens the DNA helix to generate single-strand DNA that can then be deaminated by AID to form mismatched dU/dG DNA base pairs. The base excision repair protein uracil DNA glycosylase removes the mismatched dU base, creating an abasic site. Differential repair of the lesion leads to either SHM or CSR. The mismatch repair proteins MSH2 and MSH6 can also bind and process the dU:dG mismatch. Deficiencies of AID and uracil DNA glycosylase underlie some forms of the hyper-IgM syndrome.

Generation of immunoglobulin diversity occurs at defined stages of B-cell development

Creation of immunoglobulin diversity is hierarchical. In pro–B cells $D_H \rightarrow J_H$ joining precedes $V_H \rightarrow DJ_H$ rearrangement, and $V_L \rightarrow J_L$ joining takes place at the late pre–B-cell stage. Production of a properly functioning B-cell receptor is essential for development beyond the pre–B-cell stage. For example, function-loss mutations in RAG1/2 and DNA-dependent protein kinase (DNA-PKcs and Ku 70/80) preclude B-cell development, as well as T-cell development, leading to severe combined immune deficiency. In frame, functional VDJ_H rearrangement allows the pro–B cell to produce μ H chains, most of which are retained in the endoplasmic reticulum. The appearance of cytoplasmic μ H chains defines the pre–B cell.

Pre–B cells whose μ H chains can associate V_{preB} and λ14.1 (λ5), which together form the surrogate light chain (ΨLC), begin to express a pre–B-cell receptor. Its appearance turns off RAG1 and RAG2, preventing further H chain rearrangement (allelic exclusion). This is followed by 4 to 6 cycles of cell division.[25] Late pre–B daughter cells reactivate RAG1 and RAG2 and begin to undergo $V_L \rightarrow J_L$ rearrangement. Successful production of a complete κ or λ light chain permits expression of conventional IgM on the cell surface (sIgM), which identifies the immature B cell. Immature B cells that have successfully produced an acceptable IgM B-cell receptor extend transcription of the H chain locus to include the $C\delta$ exons downstream of $C\mu$. Alternative splicing permits co-production of IgM and IgD. These now newly mature IgM^+IgD^+ B cells enter the blood and migrate to the periphery, where they form the majority of the B-cell pool in the spleen and the other secondary lymphoid organs. The IgM and IgD on each of these cells share the same variable domains.

The lifespan of mature B cells expressing surface IgM and IgD appears entirely dependent on antigen selection. After leaving the bone marrow, unstimulated cells live only days or a few weeks. As originally postulated by Burnet's "clonal selection" theory, B cells are rescued from apoptosis by their response to a cognate antigen. The reaction to antigen leads to activation, which might then be followed by diversification. The nature of the activation process is critical. T cell–independent stimulation of B cells induces differentiation into short-lived plasma cells with limited class switching. T-dependent stimulation adds additional layers of diversification, including SHM of the variable domains, which permits affinity maturation, class-switching to the entire array of classes available, and differentiation into the long-lived memory B-cell pool or into the long-lived plasma cell population.

H CHAIN C DOMAIN STRUCTURE AND FUNCTION

In general, the C domain of the H chain defines effector function, whereas the paired V domains of the antibody confer antigenic specificity. The H chain constant domain is generally defined as C_H1-C_H2-C_H3 (IgG, IgA, and IgD), with an additional domain (C_H4) for IgM and IgE. As described above, the C_H1 domain is located within the F(ab) region, whereas the remaining C_H domains (C_H2-C_H3 or C_H2-C_H4) comprise the Fc fragment. This Fc fragment defines the isotype and subclass of the immunoglobulin. Despite amino acid differences between the isotypes and subclasses, each C_H region folds into a fairly constant structure consisting of a 3-strand/4-strand β sheet pinned together by an intrachain disulfide bond. The Fc fragment mediates effector function by binding to the Fc receptor (FcR) on effector cells or activating other immune mediators, such as complement.[26] For this reason, changes in the Fc region can significantly affect the end result of an antibody-antigen interaction. The Fc region can also affect the affinity or kinetics of binding of the antibody by the Fv region and thus influence antigen recognition or binding.[27]

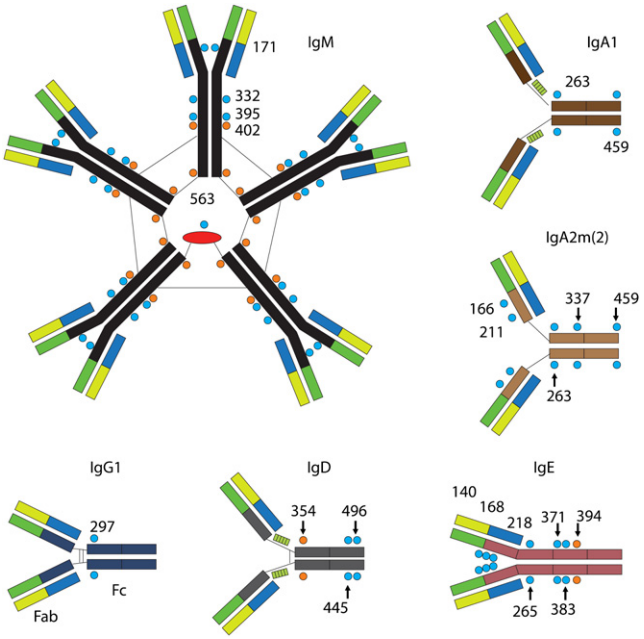

FIG 6. Structural and glycosylation properties of immunoglobulins. Depiction of the structure and glycosylation sites (indicated by amino acid location) for human IgM, IgG, IgD, IgE, IgA2, and IgA2. Adapted from Arnold et al.[28]

Role of glycosylation

Immunoglobulins are glycoproteins, and the glycans associated especially with the Fc domain of immunoglobulins have been shown to affect antibody function. The extent of glycosylation varies by isotype (Fig 6).[28] For IgG molecules, there is an N-linked glycosylation site located at Asn297 on each of the 3 C_H2 domains. The core of this complex biantennary type of sugar is a heptasaccharide consisting of N-acetylglucosamine and mannose. Variation in glycosylation is seen between IgG molecules, as well as within the 3 sites on the same molecule because of differences in terminal sialic acid, galactose, N-acetylglucosamine, and fucosylation of the core. These differences can lead to as many as 32 possible glycosylation patterns. The glycans at this site interact with a hydrophobic pocket on the Fc domain that stabilizes the immunoglobulin structure.[29,30] At a similar site in the C_H2 domain of IgD, Asn354, mutations that prevent glycosylation are associated with the loss of IgD production, suggesting that glycans in the C_H2 domain can be essential for immunoglobulin stability.

Glycans on immunoglobulins profoundly influence binding to FcRs on effector cells, as well as immune mediators. When IgG sequences are mutated such that glycosylation is eliminated, there is reduced or no binding of the aglycosylated IgG to FcγR. This led to the suggestion that the N-glycan at Asn297 was critical for the engagement of IgG with FcγR. This is in contrast to engagement between IgA and IgE and FcαR and FcεR,

TABLE I. Properties of immunoglobulin isotypes/subclasses

	Serum (%)	Structure	Complement fixation	Opsonizing	Cross-placenta	Other functions	FcR
IgG	75	Monomer	+	+++	+	For all IgG subclasses:	FcγR
IgG1	67% IgG	Monomer	Yes	Yes	+	Secondary response	I, II, III
IgG2	22% IgG	Monomer	Yes	Yes	+	Neutralize toxins and	II
IgG3	7% IgG	Monomer	Yes	Yes	+	virus	I, II, III
IgG4	4% IgG	Monomer	No	No	+		I, II
IgM	10	Pentamer	+++	+	−	Primary response	
IgA	15	Monomer, dimer	−	−	−	Mucosal response	FcαR (CD89)
IgA1		Monomer, dimer	−	−	−		
IgA2		Monomer, dimer	−	−	−		
IgD	<0.5	Monomer	−	−	−	Homeostasis	FcδR
IgE	<0.01	Monomer	−	−	−	Allergy	FcϵR I, II

respectively, where it had been shown that glycans were not required for interaction. Subsequently, it was shown that point mutations could be introduced to the C_H domains that would permit binding to FcγR in the absence of glycosylation.[31] However, the observation that the Asn297 site is conserved across evolution and that IgG is subjected to posttranslational modifications at this site suggests that glycosylation at this position makes a significant contribution to antibody function *in vivo*.

A key effector function for IgG antibodies is antibody-dependent cellular cytotoxicity (ADCC) in which antibody-coated antigens activate effector cells, such as natural killer cells or monocytes, to destroy the antibody-coated target by binding of the complex to the FcγR. The ADCC activity was shown to be significantly dependent on the glycan composition of the IgG and, furthermore, the net result of binding to activating and inhibitory FcγR. A number of engineered cell lines, as well as glycosidase inhibitors, are available to direct the sugar composition of glycans on an immunoglobulin. Through these studies, it was demonstrated that ADCC activity increases after a reduction in the fucose content of an antibody.[32,33]

Complement-dependent cytotoxicity is another effector function of IgG that is dependent on the binding of C1q to the Fc domain. Glycosylation also plays a role in complement-dependent cytotoxicity, requiring the presence of a complex structure containing at least 2 N-acetylglucosamines with multiple galactoses and sialic acids.

There are also experiments of nature in which aberrantly glycosylated immunoglobulins are associated with detrimental effects. For example, higher than normal levels of IgG lacking sialic acid or galactose are found in patients with a number of autoimmune diseases, rheumatoid arthritis in particular. N-glycans terminate with N-acetylglucosamine, which can activate the complement cascade through mannose-binding lectin and create an inflammatory state.[34] Removal of the majority of the secondary glycan structure with Endo-S (1 N-acetylglucosamine, a terminal fucose, or both remains with the core sugar) has been shown to reduce the pathogenesis and proinflammatory properties of autoantibodies in murine models.[35] This is not limited to IgG because a reduction in terminal galactose on IgA has been associated with decreased clearance of IgA from the circulation, with the subsequent development of nephropathy.

Intravenous immunoglobulin can be used in selected circumstances to ameliorate inflammatory diseases. The anti-inflammatory activity of intravenous immunoglobulin has shown to be associated with sialic acid in a 2,6 linkage to a terminal galactose on IgG. Recently, a receptor specific to this sialyated Fc has been identified on myeloid cells.[36] Engagement of this receptor with sialyated IgG might upregulate inhibitory FcγR to reduce inflammation by means of IgG/activating FcγR engagement.

Immunoglobulin glycosylation can also alter other antibody functions. For example, it has been shown that an anti-HIV antibody that fails to neutralize acquires neutralization activity when expressed in a cell line that results in posttranslational modification of an antibody with a marked increase in sialic acid, fucose, and N-acetylglucosamine levels.[37] It is hypothesized that the glycan interactions between the antibody and virus interfere with the normal infection process.

O-linked glycans also play a pivotal role in the immune response. There are several potential O-linked sites in the hinge region of IgD and IgA antibodies, which serve to protect the hinge from proteases, bind bacteria, or both. Understanding the effect of differential glycosylation on immunoglobulin function is contributing to the design of more effective immunotherapies through either engineered passive immunotherapy[4] or *in vivo* treatment with glycan modifiers.[38]

Heavy chain isotypes

Early in B-cell development, productively rearranged variable domains (V_H and V_L) are expressed in association with the μ heavy chain to produce IgM and then IgD by means of alternative splicing. Later during development and in response to antigenic stimulation and cytokine regulation, these variable domains can associate with the other isotypes (IgG, IgA, and IgE) in a controlled process; that is, isotype switching does not occur merely by chance. The C_H genes for each isotype are aligned in the same transcriptional orientation on human chromosome 14. Isotypes differ in a number of properties, including size, complement fixation, FcR binding, and isotype response to antigen. The choice of isotype is dependent on the antigen itself and the signaling pathways that are activated, as well as the local microenvironment, as summarized in Table I.

IgM. IgM is the first immunoglobulin expressed during B-cell development. Naive B cells express monomeric IgM on their surface and associate with CD79a and CD79b, polypeptide chains that participate in IgM cell signaling. On maturation and antigenic stimulation, multimeric (usually pentameric and rarely hexameric) IgM, in which single IgM units link to each other by disulfide bonds in the C_H4 region, is secreted (Figure 5). The pentamer also contains a polypeptide chain, the J-chain, which is bound to 2 of the monomers by means of a disulfide bond. The

J-chain facilitates secretion at mucosal surfaces (see below). Generally, although monomeric IgM molecules have low affinity because of their immaturity, high avidity can be attained by means of multimeric interactions between the pentameric secreted antibody and the antigen, especially if that antigen contains multiple repeating epitopes itself. IgM functions by opsonizing (coating) antigen for destruction and fixing complement. The pentameric nature of the antibody renders it very efficient in this process.

IgM antibodies are associated with a primary immune response and are frequently used to diagnose acute exposure to an immunogen or pathogen. Given that IgM is expressed early in B-cell development, the μ heavy chain associates with V_H and V_L regions that have not undergone much somatic mutation in response to antigen. As a result, IgM antibodies tend to be more polyreactive than other isotypes, which allows IgM-bearing B cells to respond quickly to a variety of antigens. These relatively low-affinity IgM antibodies are also called natural antibodies. Some of these natural antibodies not only participate as a first line of defense but also play a role in immunoregulation.[39] Natural antibodies might react with autoantigens but are rarely responsible for autoimmune disease or pathogenesis. Pathogenic autoantibodies tend to be drawn from the somatically mutated, high-affinity IgG population.

IgD. Circulating IgD is found at very low levels in the serum, with a short serum half-life, which can be attributed to the sensitivity of the molecule, with the hinge region in particular, to proteolysis. The function of circulating IgD is unclear because it is not known to participate in the major antibody effector mechanisms. Circulating IgD can react with specific bacterial proteins, such as the IgD-binding protein of *Moraxella catarrhalis*, independently of the variable regions of the antibody.[40] The binding of these bacterial proteins to the constant region of IgD results in B-cell stimulation and activation.

Although the membrane-bound form of IgD has been more extensively studied, even here its function remains poorly understood. Similar to IgM, membrane-bound IgD is associated with CD79a and CD79b for signaling. IgD is expressed on the membranes of B cells when they leave the bone marrow and populate secondary lymphoid organs. Most IgD^+ B cells also coexpress IgM, and both participate in B-cell receptor signaling through CD79a and CD79b. IgD can replace IgM and *vice versa* on IgD^+IgM^+ B cells. It has been proposed that membrane-bound IgD regulates B-cell fate at specific developmental stages through changes in activation status.[41]

IgG. IgG is the predominant isotype found in the body. It has the longest serum half-life of all immunoglobulin isotypes. It is also the most extensively studied class of immunoglobulins. Based on structural, antigenic, and functional differences in the constant region of the heavy chain, C_H1 and C_H3 in particular, 4 IgG subclasses (IgG1, IgG2, IgG3, and IgG4) were identified. These IgG subclasses were numbered in reference to the rank order (IgG1 > IgG2 > IgG3 > IgG4) of the serum levels of these antibodies in the blood of healthy subjects living in an affluent western European environment. The differences in the C_H domains affect antibody flexibility and functional affinity, some of which facilitate cooperative interactions with multivalent antigens. The mobility or flexibility of the F(ab) and Fv portions of the antibody are primarily controlled by the C_H1 domain and hinge region. The IgG subclasses exhibit different functional activities. Activation of the complement cascade is an important means of clearance of opsonized pathogens. Although IgG4 is the only subclass that fails to fix complement, affinity for C1q, which is the first component of the complement pathway and binds to the C_H2 domain of IgG, differs between members of the other 3 IgG subclasses (IgG3 > IgG1 > IgG2). There are also defined differences in the affinity to the 3 classes of FcγR (I, II, and III). IgG1 and IgG3 bind to all 3 FcγR classes. IgG4 binds only FcγRII and FcγRIII, although this binding is significantly weaker than that of IgG1. IgG2 binds only to FcγRII.

There are also similarities within the subclasses, such as transplacental transport and participation in the secondary immune response. Within the secondary antibody response, there is skewing in the predominant subclass that is induced. For example, IgG1 and IgG3 antibodies are generally induced in response to protein antigens, whereas IgG2 and IgG4 antibodies are associated with polysaccharide antigens. The response to a given antigen can also result in a skewed IgG subclass response, and this is frequently a source of investigation as to correlates of protection or for the design of vaccines.

Specific subclasses can be associated with individual disease processes. For example, in patients with pemphigus vulgaris, a mucocutaneous blistering disease, IgG4 antibodies to desmoglein 3 are pathogenic,[42,43] whereas first-degree relatives with IgG1 autoantibodies to the same protein show no evidence of the disease.

IgG antibodies also contribute directly to an immune response, including neutralization of toxins and viruses. Here again, IgG subclass affects the outcome of this interaction. In patients with HIV, it has been shown that IgG3 antibodies can be more effective at neutralizing virus than IgG1 antibodies, presumably through an increase in antibody flexibility, improving antibody access or inducing changes in the oligomer structure of the virus.[44,45]

IgA. IgA serum levels tend to be higher than IgM levels but considerably lower than IgG levels. Conversely, IgA levels are much higher than IgG levels at mucosal surfaces and in secretions, including saliva and breast milk.[46] In particular, IgA can contribute up to 50% of the protein in colostrum, the "first milk" given to the neonate by the mother. Although generally a monomer in the serum, IgA at the mucosa, termed secretory IgA (sIgA), is a dimer (sometimes trimer and tetramer) associated with a J-chain and another polypeptide chain, the secretory component (SC; discussed below). Similar to IgM, the C_H3 domains of IgA have short tailpieces to which the J-chain binds through disulfide bonds, whereas the SC is disulfide bonded to one of the C_H2 domains of the dimer. There are 2 subclasses of IgA, IgA1 and IgA2, with structures that differ mainly in their hinge regions. IgA1 has a longer hinge region with a duplicated stretch of amino acids that is lacking in IgA2. This elongated hinge region increases the sensitivity of IgA1 to bacterial proteases in spite of partial protection by glycans. Such increased protection against protease digestion might explain why IgA2 predominates in many mucosal secretions, such as the genital tract, whereas more than 90% of serum IgA is in the form of IgA1.

IgA is critical at protecting mucosal surfaces from toxins, viruses, and bacteria by means of direct neutralization or prevention of binding to the mucosal surface. Intracellular IgA might also be important in preventing bacterial or viral infection, pathogenesis, or both. The polymeric nature of sIgA might be particularly important. For example, polymeric IgA (pIgA) is more effective than monomeric IgA at preventing *Clostridium difficile* toxin A–induced damage to epithelial cells.[47] Although complement fixation by IgA does not appear to be a major effector mechanism at the mucosal surface, the IgA receptor is expressed

on neutrophils, which might be activated to mediate ADCC locally. As described above, specific bacteria can be trapped by the glycans on IgA. Finally, it has been proposed that sIgA might also act as a potentiator of the immune response in intestinal tissue by means of uptake of antigen to dendritic cells.[48]

IgE. Although it is present at the lowest serum concentration and has the shortest half-life, IgE is a very potent immunoglobulin. It is associated with hypersensitivity and allergic reactions, as well as the response to parasitic worm infections. IgE binds with extremely high affinity to FcεRI, which is expressed on mast cells, basophils, Langerhans cells, and eosinophils. Circulating IgE upregulates FcεR expression on these cells. The combination of strong binding and upregulation of FcεR expression contributes to the remarkable potency of this immunoglobulin.

Recently, there has been the development of anti-IgE antibodies as therapy for allergy and asthma.[49] Antibodies are designed to target free IgE, as well as B cells with membrane-bound IgE, but not IgE bound to FcεR because the latter would stimulate degranulation and the release of inflammatory mediators. IgE has a much lower affinity for FcεRII, or CD23, which is expressed both on the same cells as FcεRI and on B cells, natural killer cells, and platelets.

Higher-order structures

The J-chain. The J-chain is a relatively conserved, 15- to 16-kd polypeptide (137 amino acids) incorporated into pIgA or polymeric IgM in the antibody-producing cell during the secretory pathway. There are 6 cysteine residues for intrachain disulfide bonds plus the 2 cysteines for attachment to the IgA or IgM tailpiece. There is a single N-linked glycan that contributes approximately 8% of the mass to the molecule. This glycan is critical to association with monomeric IgA. Although the J-chain is produced by B cells, it is not necessarily produced by all B cells. It appears that J-chain expression might be restricted to those areas, such as the lamina propria, in which mucosal antibody is important, as opposed to B cells in the distal bone marrow. Free J-chain is not found outside the cell and is only found as part of the polymeric immunoglobulin complex. It has been shown that the J-chain is essential for polymerization and secretion of IgA. In contrast, pentameric IgM requires the J-chain for secretion (but not formation), and hexameric IgM does not require the J-chain at all.

Dimers, pentamers, and hexamers. Polymeric immunoglobulin is generally more effective than monomeric immunoglobulin in terms of binding to FcR on the cell surface. As described above, IgA and IgM molecules have the capacity to be naturally expressed as multimeric antibodies. Both immunoglobulins have a short tailpiece (18 amino acids) in the C_H3 domain, with a penultimate cysteine residue to which the J-chain forms a disulfide bond with one of the monomers, with the other forming a tailpiece-to-tailpiece disulfide bond. Typically dimeric structures are formed for IgA, and pentameric structures are formed for IgM.

FCRS

FcγR

FcRs for immunoglobulin link the humoral immune compartment to the cellular immune compartment. The net result of binding of immunoglobulin to receptor is a function of the receptor, the cell on which it is expressed, and any ancillary signals. Tight regulation of binding to the FcR is necessary to maintain a healthy immune system.

The most extensively studied FcRs are the IgG-binding receptors, termed FcγR. In human subjects 3 classes of FcγR have been identified: I, II, and III. FcγRII and FcγRIII each have 2 isoforms, A and B. These FcγRs are expressed, to varying degrees, on many hematopoietic cells, as well as other cells, such as endothelial cells. T cells have proved to be a stark exception. The FcγRs differ in their binding affinity to IgG, with FcγRI showing the highest affinity, whereas FcγRII and FcγRIII bind with lower affinity. For that reason, only FcγRI binds monomeric IgG, whereas the other 2 receptors bind aggregated IgG or immune complexes. Of note, FcγRI has 3 extracellular domains, whereas FcγRII and FcγRIII have only 2 extracellular domains.

As described above, there are differences in binding of IgG subclasses to FcγR. There are also differences in the signaling pathway that is associated with each FcγR. FcγRI, FcγRIIA, and FcγRIIIA all transduce an activating signal when IgG binds. However, FcγRIIB transmits an inhibitory signal, and no signal is associated with binding to FcγRIIIB. Although the other FcγRs are typical transmembrane proteins, FcγRIIIB lacks this feature and instead is attached by glycophosphatidylinositol tail. The end result of the interaction of antibody and antigen with FcγR tends to be a balancing act between inhibitory and stimulatory activities and a complex function of the IgG subclass, the particular FcγR bound, and the cells expressing the FcγR.[50]

The neonatal FcR

There is another FcγR, the neonatal Fc receptor (FcRn), which was originally shown to mediate the transcytosis of maternal IgG to the neonate. Subsequently, it was determined that the FcRn is also responsible for the regulation of serum IgG levels. IgG binds to FcRn in the acidic environment of the endosomes, which protects it from destruction by lysosomes. The IgG is recycled to the surface and released into circulation by the pH change. The FcRn is saturable, and once IgG levels exceed a threshold, it is degraded by the lysosomes. Whereas the C_H3 domain of IgG Fc binds to FcγR, it is the C_H2-C_H3 region that binds to FcRn. Binding is thus independent of the sugar moiety, which is attached to the C_H2 domain. It should also be noted that binding to FcRn is strictly pH dependent, whereas this is not the case with FcγR. Mutagenesis studies have demonstrated that mutations in the Fc region can increase or decrease interactions with FcRn. For example, mutations at positions 250 and 428 of IgG1 resulted in an increase in serum half-life for the single mutant M428L and the double mutant T250Q/M428L.[51] Others have shown that a single mutation of human IgG1, N434A, and a triple mutant, T307A/E380A/N434A, also show an enhanced half-life when tested in human FcRn transgenic mice.[52] That affinity for FcRn can be increased, resulting in increased immunoglobulin half-life, suggests that improved therapeutics might be designed to decrease dosing.

FcεR

The FcRs for IgE are also relatively well studied, especially in terms of the development of therapeutic anti-IgE antibodies for the treatment of allergy and asthma, as described above. It is the C_H3 domain of IgE that binds to FcεRI and CD23; however, there

are distinct differences in binding. FcεRI captures both C_H3 domains of IgE because of the unique shape of the IgE molecule. On the other hand, CD23 consists of a trimer on the cell surface, and 2 heads of this trimer must separately contact a C_H3 domain of IgE for strong binding.

FcαR

The FcR for IgA, CD89, is expressed on myeloid cells, including PMNs, monocytes, and a population of dendritic cells. There are 5 exons, including 2 extracellular domains, EC1 and EC2, each of which encodes a single immunoglobulin-like domain. IgA binds to membrane-distal EC1 in contrast to the usual binding of IgG to the membrane-proximal extracellular domain of FcγR. Multiple splice variants have been demonstrated, and whereas full-length CD89 binds pIgA with higher affinity than serum IgA, there is no difference in binding to truncated CD89. Signaling through the FcαR is accomplished through the FcR γ-chain which contains an immunoreceptor tyrosine-based activation motif signaling motif. Not all FcαRs associate with γ-chain, resulting in "γ-less" FcRs that endocytose bound IgA to early endosomes and then recycle IgA back to the cell surface. Cross-linking of FcαR with an associated γ-chain results in the activation of a number of signaling molecules in the lipid rafts, calcium release, and induction of nicotinamide adenine dinucleotide phosphate oxidase activity. Outside of endocytosis, the biologic and cellular functions of PMNs after FcαR stimulation are dependent on tyrosine kinase activity of the associated γ-chain. Cross-linking of FcαR has also been shown to induce effector functions, such as phagocytosis and ADCC.

FcδR

The FcR for IgD is less well understood. A receptor for IgD has been reported to be present on human CD4 and CD8 T cells. Its expression is upregulated by mitogenic stimulation of the T cells. Binding of IgD to this putative FcδR is mediated by glycans on the IgD surface and might not necessarily be a function of a defined FcδR. Binding of IgD to receptors with putative FcδR activity on T cells has been proposed to serve as a bridge for stimulation of IgD-expressing B cells or as antigen presentation by the B cells to the T cells, but this remains controversial.

IMMUNOGLOBULIN TRANSPORT

The transport of polymeric immunoglobulin into mucosal secretions is a function of the polymeric immunoglobulin receptor (pIgR). This receptor is found on the basolateral surface of epithelial cells lining the mucosal surface. Membrane-bound pIgR consists of 5 immunoglobulin-like domains (extracellular portion) with a transmembrane and cytoplasmic domain. pIgA (with the J-chain) binds to the pIgR on the epithelial cell. It is then internalized and transcytosed to the apical cell membrane. The extracellular portion of the pIgR is cleaved to form the SC and covalently associates with the pIgA. The complex of pIgA with SC forms sIgA. The SC forms a disulfide link with Cys311 in Cα2 of one of the monomers of the pIgA. Although the SC is not physically associated with the J-chain of the pIgA, the J-chain is required for SC to associate with pIgA. SC is not covalently linked to pentameric IgM but rather associates noncovalently with pentameric IgM because of excess free SC.

Extensive analysis of the glycosylation patterns of the components of sIgA has predicted a model in which most of the molecule is covered in glycans, with the exception of the F(ab) or antigen-binding sites. In this manner sIgA participates in both the adaptive (antigen binding) and innate (adhesion caused by glycans) arms of the immune system. Although the SC does not have a direct role in the biologic activity of sIgA, it does confer some protection from proteolytic cleavage after secretion and anchors the sIgA to mucus lining the epithelium. Moreover, as a result of covalent binding of SC to pIgA, sIgA is the most stable immunoglobulin in secretions.

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REFERENCES

1. Williams AF, Barclay AN. The immunoglobulin superfamily—domains for cell surface recognition. Annu Rev Immunol 1988;6:381-405.
2. Harpaz Y, Chothia C. Many of the immunoglobulin superfamily domains in cell adhesion molecules and surface receptors belong to a new structural set which is close to that containing variable domains. J Mol Biol 1994;238:528-39.
3. Torres RM, Imboden J, Schroeder HW Jr. Antigen receptor genes, gene products, and co-receptors. In: Rich RR, Fleisher TA, Shearer WT, Schroeder HW Jr, Frew AJ, Weyand CM, editors. Clinical immunology: principles and practice. 3rd ed London: Mosby Elsevier; 2008. p. 53-77.
4. Smith KA, Nelson PN, Warren P, Astley SJ, Murray PG, Greenman J. Demystified…recombinant antibodies. J Clin Pathol 2004;57:912-7.
5. Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, et al. Naturally occurring antibodies devoid of light chains. Nature 1993;363:446-8.
6. Roux KH, Greenberg AS, Greene L, Strelets L, Avila D, McKinney EC, et al. Structural analysis of the nurse shark (new) antigen receptor (NAR): molecular convergence of NAR and unusual mammalian immunoglobulins. Proc Natl Acad Sci U S A 1998;95:11804-9.
7. Vincke C, Loris R, Saerens D, Martinez-Rodriguez S, Muyldermans S, Conrath K. General strategy to humanize a camelid single-domain antibody and identification of a universal humanized nanobody scaffold. J Biol Chem 2009;284:3273-84.
8. Jazwinska EC, Dunckley H, Propert DN, Gatenby PA, Serjeantson SW. GM typing by immunoglobulin heavy chain gene RFLP analysis. Am J Hum Genet 1988;43:175-81.
9. Leder P. The genetics of antibody diversity. Sci Am 1982;246:102-15.
10. Tonegawa S. Somatic generation of antibody diversity. Nature 1983;302:575-81.
11. Dudley DD, Chaudhuri J, Bassing CH, Alt FW. Mechanism and control of V(D)J recombination versus class switch recombination: similarities and differences. Adv Immunol 2005;86:43-112.
12. Zachau HG. The human immunoglobulin kappa genes. In: Honjo T, Alt FW, Rabbitts PH, editors. Immunoglobulin genes. 2nd ed London: Academic Press; 1995. p. 173.
13. Brodeur PH, Riblet RJ. The immunoglobulin heavy chain variable region (IgH-V) locus in the mouse. I. One hundred Igh-V genes comprise seven families of homologous genes. Eur J Immunol 1984;14:922-30.
14. Kirkham PM, Schroeder HW Jr. Antibody structure and the evolution of immunoglobulin V gene segments. Semin Immunol 1994;6:347-60.
15. Lee SK, Bridges SL Jr, Koopman WJ, Schroeder HW Jr. The immunoglobulin kappa light chain repertoire expressed in the synovium of a patient with rheumatoid arthritis. Arthritis Rheum 1992;35:905-13.
16. Kawasaki K, Minoshima S, Nakato E, Shibuya K, Shintani A, Schmeits JL, et al. One-megabase sequence analysis of the human immunoglobulin lambda gene locus. PCR Methods Appl 1997;7:250-61.
17. Bankovich AJ, Raunser S, Juo ZS, Walz T, Davis MM, Garcia KC. Structural insight into pre-B cell receptor function. Science 2007;316:291-4.
18. Matsuda F, Ishii K, Bourvagnet P, Kuma K, Hayashida H, Miyata T, et al. The complete nucleotide sequence of the human immunoglobulin heavy chain variable region locus. J Exp Med 1998;188:2151-62.
19. Corbett SJ, Tomlinson IM, Sonnhammer EL, Buck D, Winter G. Sequence of the human immunoglobulin diversity (D) segment locus: a systematic analysis provides no evidence for the use of DIR segments, inverted D segments, "minor" D segments or D-D recombination. J Mol Biol 1997;270:587-97.
20. Honjo T. Immunoglobulin genes. Annu Rev Immunol 1983;1:499-528.
21. Dorner T, Foster SJ, Farner NL, Lipsky PE. Somatic hypermutation of human immunoglobulin heavy chain genes: targeting of RGYW motifs on both DNA strands. Eur J Immunol 1998;28:3384-96.

22. Rada C, Ehrenstein MR, Neuberger MS, Milstein C. Hot spot focusing of somatic hypermutation in MSH2-deficient mice suggests two stages of mutational targeting. Immunity 1998;9:135-41.
23. Neuberger MS. Antibody diversification by somatic mutation: from Burnet onwards. Immunol Cell Biol 2008;86:124-32.
24. Muramatsu M, Nagaoka H, Shinkura R, Begum NA, Honjo T. Discovery of activation-induced cytidine deaminase, the engraver of antibody memory. Adv Immunol 2007;94:1-36.
25. Matthias P, Rolink AG. Transcriptional networks in developing and mature B cells. Nat Rev Immunol 2005;5:497-508.
26. Normansell DE. Human immunoglobulin subclasses. Diagn Clin Immunol 1987;5: 115-28.
27. Torres M, Casadevall A. The immunoglobulin constant region contributes to affinity and specificity. Trends Immunol 2008;29:91-7.
28. Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol 2007;25:21-50.
29. Liu H, Bulseco G-G, Sun J. Effect of posttranslational modifications on the thermal stability of a recombinant monoclonal antibody. Immunol Lett 2006;106:144-53.
30. Siberil S, de Romeuf C, Bihoreau N, Fernandez N, Meterreau JL, Regenman A, et al. Selection of a human anti-RhD monoclonal antibody for therapeutic use: impact of IgG glycosylation on activating and inhibitory Fc gamma R functions. Clin Immunol 2006;118:170-9.
31. Sazinsky SL, Ott RG, Silver NW, Tidor B, Ravetch JV, Wittrup KD. Aglycosylated immunoglobulin G1 variants productively engage activating Fc receptors. Proc Natl Acad Sci U S A 2008;105:20167-72.
32. Kanda Y, Yamada T, Mori K, Okazaki A, Inoue M, Kitajima-Miyama K, et al. Comparison of biological activity among nonfucosylated therapeutic IgG1 antibodies with three different N-linked Fc oligosaccharides: the high mannose, hybrid and complex types. Glycobiology 2006;17:104-18.
33. Natsume A, Wakitani M, Yamane-Ohnuki N, Shoji-Hosaka E, Niwa R, Uchida K, et al. Fucose removal from complex-type oligosaccharide enhances the antibody-dependent cellular cytotoxicity of single-gene-encoded antibody comprising a single-chain antibody linked the antibody constant region. J Immunol Methods 2005; 306:93-103.
34. Arnold JN, Dwek RA, Rudd PM, Sim RB. Mannan binding lectin and its interaction with immunoglobulins in health and in disease. Immunol Lett 2006;106:103-10.
35. Albert H, Collin M, Dudziak D, Ravetch JV, Nimmerjahn F. In vivo enzymatic modulation of IgG glycosylation inhibits autoimmune disease in an IgG subclass-dependent manner. Proc Natl Acad Sci U S A 2009;105:15005-9.
36. Anthony RM, Wermeling F, Karlsson MC, Ravetch JV. Identification of a receptor required for the anti-inflammatory activity of IVIG. Proc Natl Acad Sci U S A 2008;105:19571-8.
37. Miranda LR, Duval M, Doherty H, Seaman MS, Posner MR, Cavacini LA. The neutralization properties of a HIV-specific antibody are markedly altered by glycosylation events outside the antigen-binding domain. J Immunol 2007;178:7132-8.
38. Collin M, Shannon O, Bjorck L. IgG glycan hydrolysis by a bacterial enzyme as a therapy against autoimmune conditions. Proc Natl Acad Sci U S A 2008;105: 4265-70.
39. Boes M. Role of natural and immune IgM antibodies in immune responses. Mol Immunol 2000;37:1141-9.
40. Riesbeck K, Nordstrom T. Structure and immunological action of the human pathogen *Moraxella catarrhalis* IgD-binding protein. Crit Rev Immunol 2006;26: 353-76.
41. Geisberger R, Lamers M, Achatz G. The riddle of the dual expression of IgM and IgD. Immunology 2006;118:429-37.
42. Parlowsky T, Welzel J, Amagai M, Zillikens D, Wygold T. Neonatal pemphigus vulgaris: IgG4 autoantibodies to desmoglein 3 induce skin blisters in newborns. J Am Acad Dermatol 2003;48:623-5.
43. Yeh SW, Cavacini LA, Bhol KC, Lin MS, Kumar M, Duval M, et al. Pathogenic human monoclonal antibody against desmoglein 3. J Immunol 2006; 120:68-75.
44. Cavacini LA, Kuhrt D, Duval M, Mayer K, Posner M. Binding and neutralization activity of IgG1 and IgG3 from serum of HIV infected individuals. AIDS Res Hum Retroviruses 2003;19:785-92.
45. Scharf O, Golding H, King LR, Eller N, Frazier D, Golding B, et al. Immunoglobin G3 from polyclonal human immunodeficiency virus (HIV) immune globulin is more potent than other subclasses in neutralizing HIV type 1. J Virol 2001;75: 6558-65.
46. Woof JM, Mestecky J. Mucosal immunoglobulins. Immunol Rev 2005;206:64-82.
47. Stubbe H, Berdoz J, Kraehenbuhl JP, Corthesy B. Polymeric IgA is superior to monomeric IgA and IgG carrying the same variable domain in preventing *Clostridium difficile* toxin A damaging of T84 monolayers. J Immunol 2000;164:1952-60.
48. Corthesy B. Roundtrip ticket for secretory IgA: role in mucosal homeostasis? J Immunol 2007;178:27-32.
49. Chang TW, Wu PC, Hsu CL, Hung AF. Anti-IgE antibodies for the treatment of IgE-mediated allergic diseases. Adv Immunol 2007;93:63-119.
50. Nimmerjahn F, Ravetch JV. FC-receptors are regulators of immunity. Adv Immunol 2007;96:179-204.
51. Hinton PR, Johlfs MG, Xiong JM, Hanestad K, Ong KC, Bullock C, et al. Engineered human IgG antibodies with longer serum half-lives in primates. J Biol Chem 2004;279:6213-6.
52. Petkova SB, Akilesh S, Sproule TJ, Christianson GJ, Al Khabbaz H, Brown AC, et al. Enhanced half-life of genetically engineered human IgG1 antibodies in a humanized FcRn mouse model: potential application in humorally mediated autoimmune disease. Int Immunol 2006;18:1759-69.