Review

The immunoglobulin constant region contributes to affinity and specificity

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A central dogma in immunology is that antibody specificity is solely the result of variable (V)-region interactions with an antigen. However, this view is not tenable in light of numerous reports that constant heavy (C_H) domains can affect binding affinity and specificity and V-region structure. Kinetic and thermodynamic proof for the occurrence of this phenomenon is now available. C_H-region effects on affinity and specificity suggest new mechanisms for generating antibody diversity and polyreactivity (multispecificity) that impact current views on idiotype regulation, autoimmunity, and B cell selection and change our understanding of vaccine responses.

Introduction: the classical view of antibody function

The problem of immunoglobulin (Ig) generation of diversity was solved three decades ago when it was determined that the enormous capacity of the immune system to recognize foreign antigens (Ags) was achieved through combinatorial diversity of variable (V) regions and Ig somatic mutation [1]. Since then, immunological dogma has posited that specificity and affinity are properties conferred by the V region solely, whereas the constant (C) region conferred antibody (Ab) isotype and effector functions such as complement activation, Fc receptor binding, avidity, and serum half-life [2] (Box 1). By this paradigm, Ag binding and effector functions were viewed as resulting from distinct structural features associated with distal regions of the Ig molecule (Figure 1). Thus, structural differences in the Ab-binding site (paratope) accounted for the unique binding specificity and the ability of Abs to recognize an almost unlimited number of epitopes [3]. At the other end of the molecule, the Fc domain (Figure 1) was assumed to have effector functions without participating in the Ag-binding affinity and/or specificity. Nevertheless, it was accepted that C_H domains contributed to the apparent affinity through avidity effects. According to the classical view, B cell isotype switching could change the avidity and effector functions of an Ab without changing Ag specificity [4]. This tidy concept has been challenged by several studies showing that identical V regions can manifest differences in the magnitude of binding to Ag, fine specificity, and idiotypic (Id) recognition [5–8]. Our group has shown that Abs to fungus *Cryptococcus neoformans* of different isotype, but identical V region, have different binding patterns [8–10] (Figure 2), suggesting that the Ab-binding characteristics are not solely determined by the V region. The isotype of the light (L) chain might also have an impact on the structural and functional properties of V-region-identical Abs, although, in this article, we will focus on the effect of the heavy (H) chain on binding affinity and specificity, given the paucity of data on L-chain effects.

Affinity and Ab-Ag interactions

Affinity between an Ab and its Ag can be viewed as a measure of the strength of electrostatic and hydrophobic interactions [11,12]. Affinity can also be divided into intrinsic and functional affinity [11]. Intrinsic affinity applies to monovalent interactions formed during the binding of a paratope to an epitope (monovalent interaction) [11], and it relates to the equilibrium association constant (K_A) that characterizes the interaction between the Ab-binding site and its epitope [11]. Intrinsic affinity can depend on temperature, pressure, pH, ionic strength, the concentration of molecules in solution, and the presence of structures that confine the interacting species, which are not necessarily inherent to the epitope or paratope or paratope-epitope pair [11]. Functional affinity refers to multivalent interactions involving the binding of two or more Ab paratopes on an Ag [3].

The interaction between an Ab and its Ag involves diffusion-limited orientation of the two interacting molecules that is driven by nonspecific, long-range electrostatic forces to form a hydrophobically associated intermediate complex [13,14]. This is followed by a docking step that involves the formation of specific contacts and noncovalent bonds [13,14]. Consequently, the interactions that take place during Ab-Ag binding would cause structural differences at the molecular level. Furthermore, the structural differences created by the Ab binding to Ag on the final complex might have implications for the immune response [14–17].

Glossary

Affinity: measure of the binding strength for a monovalent epitope.
Antibody (Ab) plasticity: refers to the ability of an Ab to change its molecular conformation, by modifying the electrostatic and hydrophobic interactions at the antigen-binding site and its surroundings.
Avidity: overall binding intensity between an Ab and a multivalent antigen presenting multiple epitopes.
Desolvation: the release of chemically bound water.
Fine specificity: refers to differences of binding between Abs with the same specificity.
Mimicry: a compound that mimics structural characteristics such as conformation, charges, and steric aspects of a protein.
Polyreactivity: ability of an Ab to recognize unrelated self- and foreign antigens.
Specificity: ability of an Ab to recognize a specific epitope.

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Box 1. The classical view: C_H domains determine Ab isotype and effector functions

B cells can express a new isotype by linking different C_H regions to the same V_H by using class switch recombination (CSR). The C_H domains determine the five main Ig isotypes: IgM, IgD, IgG, IgE, and IgA. In humans, the IgG class is subdivided into four subclasses: IgG1, IgG2, IgG3, and IgG4, whereas, in mice, the four subclasses are known as IgG1, IgG2a, IgG2b, and IgG3. In humans, IgA has two subclasses, known as IgA_1 and IgA_2. To have a complete Ab molecule, the heavy (H) chain associates with one of the two types of light (L) chain, the lambda (λ) or the kappa (κ) chains (Figure 1).

The Fc region activates the complement system, determines serum half-life, and binds Fc receptors expressed on the surface of phagocytic cells, such as neutrophils and macrophages. Differences in valency and isotype are reflected in Ab avidity and the interaction with Fc receptors. The C_H domains affect Ab half-life either by conformational changes of the C_{H2}-C_{H3} domains, altered proteolysis, or receptor interaction [68].

A characteristic of all IgGs is the C_{H2} glycosylation at Asn297 [69], which is necessary for complement activation and interactions with Fc receptors [70,71]. Glycosylation can affect the conformation of some Ab molecules by increasing or decreasing the stability of amino acid residues in the Ag-binding site [72]. It is notable that C_{H2}-domain-associated oligosaccharides play an essential role in IgG1-mediated Ab effector function [73–75]. Another characteristic of the Ab molecule is the hinge, which joins the Fab and Fc regions and provides inter-H-chain stability through disulfide bonds and segmental flexibility [29,76]. It is believed that the flexibility of the hinge allows Abs to adopt a wide variety of angles, thus permitting the binding to distant epitopes [77,78]. IgM and IgE do not have a hinge domain, instead they have an extra C_H domain, and these molecules are considered to be less flexible than the other Ab classes. Particularly, the existence of a hinge region with its inherent flexibility has been one of the reasons why structural differences in C_H domains have not been thought to affect the V region.

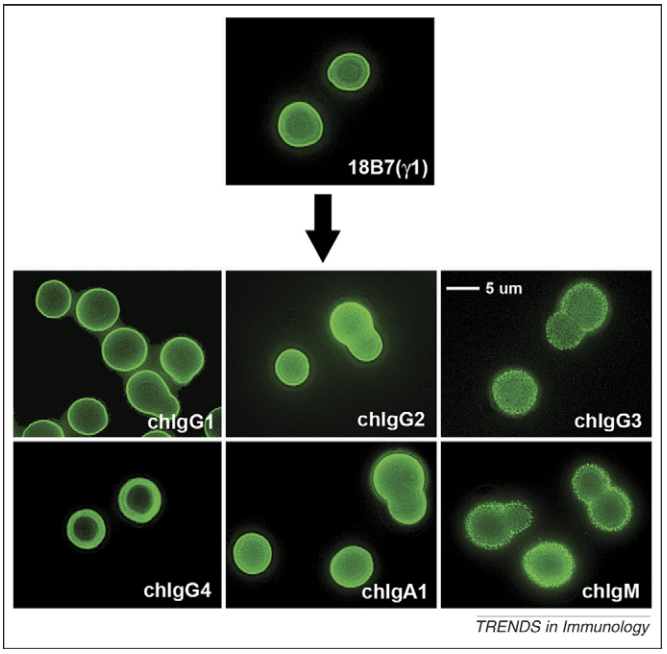

Figure 2. Demonstration of how isotype can affect Ab fine specificity. The figure shows the immunofluorescence patterns of the fungus *Cryptococcus neoformans* bound with murine and chimeric mAbs to the capsular polysaccharide. The V regions of anti-*C. neoformans* capsular polysaccharide murine mAb 18B7 (IgG1) were cloned into expression vectors for each one of the human C_H and the C_L chains to generate complete mouse-human chimeric Abs. Two of the mouse-human chimeric Abs, IgG3 and IgM, showed a punctuate immunofluorescence pattern when bound to *C. neoformans* cells, which differ from the paternal murine mAb despite having identical V regions [9]. The punctuate pattern is not the result of aggregation or a secondary Ab effect, because IgG1 derived from IgM gave a similar pattern, and the immunoflourescence pattern with primary- and secondary-labeled antibodies was similar. Copyright 2002, The American Association of Immunologists, Inc.

Solubility, pH, and the effect of side chain rearrangement in the Ab-Ag interaction

Macromolecular assembly, such as Ab-Ag binding, is facilitated by the same hydrophobic and electrostatic interactions that stabilize the native folded state of proteins. A major contribution to the free energy of protein folding arises from the hydrophobic interactions with solvent [18]. In addition, water molecules at the binding interface contribute to the stability of the Ag-Ab complex by filling "empty" cavities and/or by forming hydrogen bonds [19,20]. Abs must be able to recognize and bind polar

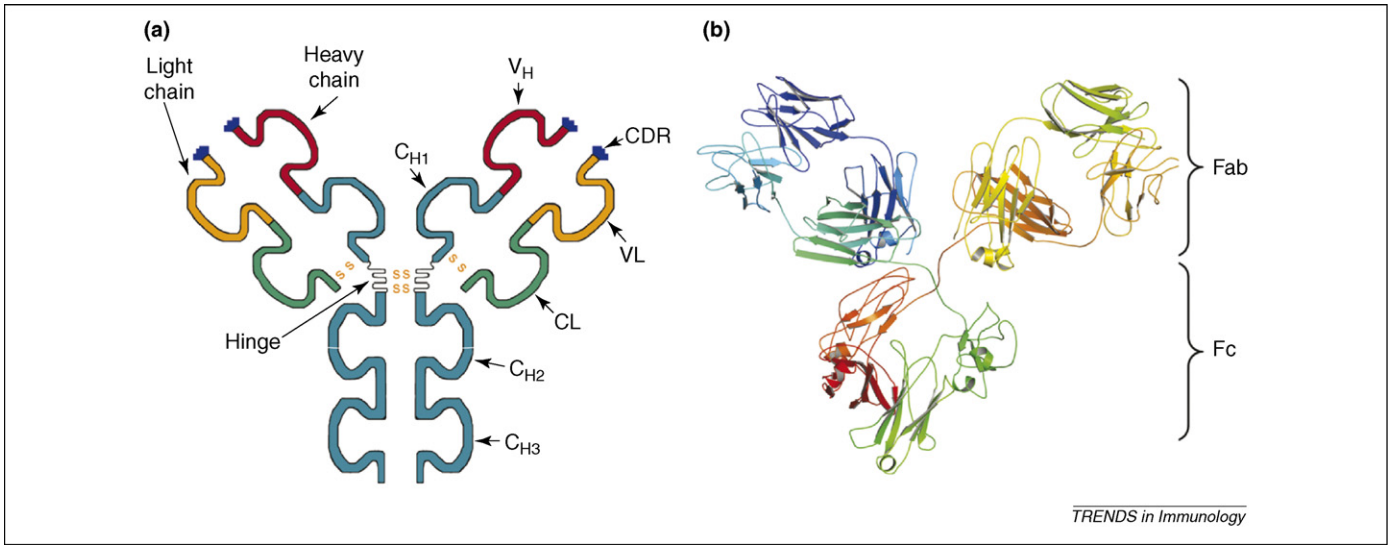

Figure 1. Illustration of an Ab molecule. **(a)** Abs are formed by two heavy (H) chains and two light (L) chains, containing ~450 amino acids and 250 amino acids, respectively. Each H and L chain is composed of a variable (V) domain and a constant (C) domain. The Fab portion contains the V_H and C_{H1} domains and the complete L chain. The Fc portion contains the C_{H2} and C_{H3} domains and is responsible for the functional differences between isotypes. The N terminus of the H and L chains has three segments or loops, the complementary-determining regions (CDR), which are highly variable in length and sequence. The CDRs recognize and bind a specific molecule(s) or structure(s) on the surface of an Ag, termed the epitope. **(b)** Homology model of an Ab molecule (courtesy of Dr. Narcis Fernadez-Fuentes).

components given that epitopes lie on the solvent-exposed surface of Ags. Environmental variations, such as pH and ionic strength, can lead to the redistribution of conformational states and affect the binding of certain isotypes to their Ags [10,21]. The release of water from the Ab-Ag bond during the process of desolvation also plays a key role in determining the effect of electrostatics by counteracting the favorable electrostatic interactions formed between the Ab and its Ag [22].

Mutations of paratope and epitope residues have demonstrated that only a small subset of residues dominates the energetics of some Ab-Ag interactions, leading to the concept of functional residues based on their energetic contributions [23]. Structural evidence has suggested that Abs with high affinity manifest a "lock and key" type of binding, involving a higher amount of polar and charged interactions [16–18,22] that contribute to the rigidity of the Ag-binding site and to a higher binding energy for the Ab-Ag interaction [16,18,22,23]. By contrast, more crossreactive Abs show fewer electrostatic contributions to Ab-Ag binding and an increase in hydrophobicity [24], thus allowing for greater molecular flexibility [25]. Flexible areas have more intermolecular interactions, via polar and charged residues, in comparison to the rigid regions [17,18]. The regions of highest flexibility and rigidity are both located at the binding interface, but they occur at different locations at the binding interface [17]. Away from the binding site are regions of intermediate flexibility that could offer points of conformational adjustment after the initial Ab-Ag encounter [17]. This flexibility concept is different from the one conferred by the hinge. Hinge bending motions involve large movements of the different Ab domains that alter the structure of the Ag-binding interface [26], whereas areas of molecular flexibility made up of electrostatic and hydrophobic interactions maintain the Ab-Ag-binding interface.

The view that Ab-Ag binding is the product of a flexible union is now supported by considerable evidence. Structural variation of elements distant from the binding site can have substantial influence on the functional affinity and specificity, particularly for multivalent interactions [27], by affecting the molecular flexibility of the Ag-binding site [3]. Furthermore, an alteration in the paratope, when there is binding at a different epitope or to a different epitope of a multivalent Ag, does not necessarily occur through contact residues on the binding interface with the Ag [11,12,16]. Amino acid residues that do not interact with the Ag or that are far from the binding site can produce structural differences, at the molecular level, in the whole body of the Ab molecule that induce subtle changes in the binding activity [3]. The change in Ab structure upon binding to its Ag might be sufficient to account for changes in affinity; thus, gross changes in molecular shape are not required [28]. In this regard, small, stereochemically significant movements of Ab side chains upon Ag binding have been demonstrated by X-ray crystallography [20]. Possible examples of non-typical members of the Ag-binding site are amino acid residues in the C_H domains that influence segmental flexibility [29], inter-Ab associations [30], and amino acid residues in the V region that are not contact residues but determine or influence the canonical conformations of the complementarity-determining regions (CDRs) [31].

Thus, the alterations in specificity and affinity observed for monoclonal antibodies (mAbs) could be a result of electrostatic and hydrophobic interactions resulting from differences in the C_H domains' microenvironment, such as pH, ionic strength, etc., that affect the Ag-binding site. Many studies support the idea that a limited number of V-region differences [32–34] and non-contact residues or residues that do not directly contribute to Ag binding can significantly change Ab conformation and function [23,33,35]. Because differences in binding have been observed with Abs that share the same V region, changes in paratope conformation might well be attributed to changes in the microenvironment of the binding site owing to the associated C_H.

The mechanism for this effect could involve modifying the amount of electrostatic and hydrophobic interactions present at the C_{H1} domain (Figure 1), thus turning the Ag-binding site into a more flexible or rigid form [18,36], without a "gross" conformational change. In fact, non-contact residues or residues located at sites distant from the Ag-binding site modulate conformation and function [37–39]. Thus, C_H domains' structural differences between Ab classes and subclasses can influence the interaction of the V regions with Ag (Box 2). For instance, anti-tubulin human IgG1 and IgA Abs sharing identical V-region sequences bind Ag with significant differences in affinities [40]. These differences were also observed at the monovalent Fab level, indicating that the C_{H1} domain might play a role in modulating the binding affinity of these complexes. When the same V region was cloned into expression vectors to express complete recombinant IgM, IgA, and IgG1 molecules, IgM and IgA had similar affinity, whereas the recombinant IgG1 showed a lower affinity than the recombinant IgA, but the same affinity as the parental human IgG1 [41]. Similar observations have been made by our group: V-region-identical Abs and Fabs to *C. neoformans* show different functional affinities for a peptide mimetic [10] that are not a result of avidity. Consequently, conformational signaling through the V_H-C_{H1} interface would impose structural and/or kinetic constraints in the Ab-combining site [41] (Table 1).

Mimicry and Ag-binding site plasticity

Ab plasticity might be interpreted as an indication of the adaptability of the binding site [42]. Ab-combining site plasticity might facilitate Ab promiscuity, i.e. Abs capable of recognizing more than one Ag (crossreactive Abs) [16,42]. The ability of certain Abs to converge onto common epitope determinants with similar affinities, even after class switch recombination (CSR), could provide these

Table 1. Summary of key observations demonstrating that C_H domains affect affinity and specificity

Observation	Refs
Specificity differences between Abs with identical V regions but different isotypes.	[6,5,63,81]
Differences in affinity and kinetic constants of Abs with identical V regions.	[10,27]
Conformational signaling through the V_H-C_{H1} domains.	[40,41]
Isotype restriction of anti-Id Abs.	[54]

Box 2. Effect of C_H domains on Ab affinity and specificity

Evidence has demonstrated that Ab isotype can influence Ab binding (Table 1). Chimeric mAbs (chIgG1, chIgG3, and chIgM) to a meningococcal antigen with identical V regions revealed slightly different binding activities [63]. This mAb set was shown to have differences in their functional properties, such as bactericidal activity, respiratory-burst-associated opsonophagocytosis, and complement deposition, which might influence their protective effects against meningococcal disease [63]. Paradoxically, pentameric chIgM did not have a higher binding affinity, which was expected because of its higher avidity, suggesting that polyvalency alone is not enough to increase binding. In addition, the *in vitro* antibacterial activity of chIgG1 was superior to that of chIgM [63]. Differences in binding specificity and epitope density have also been shown in mAbs generated against the GlcNAc component of group A streptococci cell wall polysaccharide [79]. These mAbs shared identical V regions, but demonstrated variations in specificity [5]. Our group previously showed that isotype can influence the binding and idiotypic properties of a set of V-region-identical mouse-human chimeric Abs and two families of mouse mAbs [8,80] to the capsular polysaccharide of *Cryptococcus neoformans.* In addition, the mouse family of IgGs has also shown differences in their kinetic and thermodynamic properties of binding [10]. Differences in Ab reactivity of V-region-identical Abs have also been seen with an anti-HIV Ab to the V3 loop, where changing an IgG2 to an IgG1 enhanced the binding reactivity of this Ab toward infected cells or to the virion, whereas the IgG3 variant has less reactivity [81].

Differences in binding affinity associated with isotype have also been demonstrated with hinge-deleted and intact mAbs. Binding affinity of an intact IgG1 to a monovalent peptide was better than the hinge-deleted IgG1, whereas binding to a multivalent form of the peptide, of the hinge-deleted and intact IgG1, was similar [82]. Intact IgG4 did not bind as well to monovalent and multivalent peptide as did IgG1 [82]. Surprisingly, hinge-deleted IgG4 showed better binding than intact IgG4 and was similar to the IgG1 Abs in binding affinity. These results also revealed that Abs with the same specificity and different isotype have differences in binding monovalent and multivalent Ags. Future studies with Ab class switch variants could provide useful information regarding the role of isotype in mediating protection against pathogens as well as in autoimmune diseases.

Abs with a rapid way to increase recognition of Ags and consequently increase their overall recognition potential. Furthermore, V-region somatic mutation could modify the permissiveness of the V region to structural influences emanating from the C_H region, because one can imagine that some mutations make the V region more permissive to C_H-region constraints, whereas others enhance rigidity and fix certain conformations.

Ab paratope plasticity could allow for a convergence of specificities that confer Abs with the ability to recognize a common or distinct subset of antigenic determinants [42]. We have suggested [10] that mAb polyspecificity against multivalent Ags could be a consequence of binding site plasticity conferred by long-range interactions from the C_H domains, rather than only by mimicry at the level of the epitope [43]. The ability of an Ab to change specificity could provide a way to manage the continuous genetic variation of pathogens. By manipulating the Ag-binding site, an Ab can move from a polyreactive Ab to a highly specific one [44]. Alternatively, it might be possible that certain V-C combinations provide binding site plasticity that can keep up with antigenic variation without a loss in Ag binding. Hence, the isotype restriction observed with certain Ab responses might reflect structural constraints conferred on the V region by a C_H-region interaction. This phenomenon could account for the isotype restriction observed with *Plasmodium falciparum* Ags, in which the response is skewed toward the IgG2b subclass, an unusual isotype in Ab responses to protein Ags [45]. Thus, rather than having to generate a new Ab with a different specificity, simple isotype switching combined with plasticity of the binding site can generate a "fit" to the Ag. This can be of particular importance for multivalent Ags, such as polysaccharides, nucleic acids, and proteins, in which Abs with the same V region and a different C_H region might recognize slightly different epitopes according to microenvironment variations induced by the associated C_H chain. This, in turn, would increase the heterogeneity of the Ab-combining site and might be important for the development of Ab crossreactivity and Id networks, which consequently would have a major role in the immunoregulation of the humoral response.

Ab polyreactivity, idiotype, and immunoregulation of the humoral response

Most natural Abs produced by humans and mice are polyreactive. These Abs bind to numerous Ags with low to moderate affinity [46]. Naturally occurring Abs have a crucial role in early protection against a variety of pathogens [46] and act by expanding the Ag-binding capacity of the Ab repertoire. In addition, these Abs can serve as a template for high-affinity auto-Abs formed during autoimmune diseases [47]. The frequency with which polyreactive Abs are generated by B cells is a function of isotype and the structures formed by the combination of the V-C domains [46]. Polyreactivity can arise from the conformational flexibility of the Ag-binding site [16,42] of the Ab molecule and its ability to recognize different Ags in the same conformation [48]. In this regard, X-ray crystallography has shown that an Ab with high affinity to one Ag can modify its conformation to bind related Ags with moderate affinity [43].

B cells producing polyreactive Abs can be involved in the production of natural Abs and/or contribute to the production of auto-Abs [49]. Polyreactive B cells fail to stimulate Ag-specific T cells because they lack sufficient expression of the costimulatory molecules B7–1 and B7–2 [50], which could be the most probable explanations for the contribution toward immunological tolerance [49]. Hence, the potential B cell repertoire would have multiple functions: high-affinity Abs to protect against foreign invaders and low-affinity polyreactive Abs, like those found in autoimmune diseases, to protect against autoimmune diseases by contributing to immunological tolerance. The recruitment of B cells expressing low-affinity, polyreactive Abs might also reinforce self-tolerance in a system constantly renewing B lymphocytes [51], but the persistence of autoantibody-producing B cells could explain why these Abs are detected in diseases that involve tissue destruction [52].

When an Ig is used as an Ag, it will be treated like any other foreign protein and will elicit an Ab response. Abs that recognize unique sequences on the Ab paratope are known as anti-Id Abs. These anti-Id Abs can competitively bind the combining site of specific Abs. The anti-Id network

is an autoimmune process that assists in the immune response against infectious pathogens [53] by eliciting auto-anti-Id Abs that can regulate the immune response at different levels, such as interacting with different subsets of T cells [53]. Patterns of Ig idiotype expression and differences in idiotype reactivity associated with isotype have been described in a variety of well-studied Abs [54,55]. Furthermore, certain isotypes can control the immune response by losing immunogenicity when switching to a particular isotype, a mechanism that is associated with the acquisition of Id-specific tolerogenicity [54]. An example is the anti-Id Abs against the well-characterized IgA T15 idiotype. These anti-Id Abs only recognize Abs of the IgA isotype expressing the T15 idiotype [7]. In another example, the ability of mouse-human chimeric (ch) Abs to bind to the CDR of an anti-ICAM-1 IgG2a mAb has been evaluated to determine the anti-Id responses of Abs. ChIgG1 is able to compete with the parental IgG2a mAb, whereas chIgG2 and chIgG4 Abs show decreased competitive abilities even though they have identical V regions [56]. Therefore, these results suggest that the C_H region contributes to the formation of anti-Id determinants.

Immunological implications of an effect of isotype on affinity and specificity

The ability of the C_H region to affect Ab affinity and specificity suggests an additional mechanism for generating immune diversity. As such, the course of the secondary immune response could be affected by the generation of Abs with identical V regions but different isotypes, and could give origin to a stronger or weaker memory response depending on isotype. Furthermore, the primary and secondary responses might come from different B cells [57,58], especially if isotype switching from IgM to IgG leads to loss of binding, whereas some B cells expressing IgG made in response to another antigen might react with the antigen of interest and be stimulated to proliferate. Given that isotype could affect specificity, Ag binding to the B cell receptor could directly trigger clonal expansion of isotype-restricted Ag-specific cells. In this regard, the IgM and IgG2 (or IgG3 in mice) predominance in antipolysaccharide responses [30], the IgG1 predominance in pathogenic autoimmune Abs [59], and the IgG2a predominance in neutralizing Abs to viruses might reflect isotype-related effects on specificity [60] that, when combined with local stimuli that promote B cell switching to certain isotypes, result in isotype-restricted Ab responses.

Ag recognition by an Ab and subsequent affinity maturation involve a process in which physiological discrimination and the physical properties of these two molecules intersect. Crystallographic studies have shown that germline mAb36–35, identified in the context of an immune response against p-azophenylarsonate, can bind a set of dodecapetides without changing the V-region conformational state [48]. Germline Abs can recognize multiple Ags by having greater intrinsic flexibility, a mechanism that is supported by thermodynamic data [61]. Thus, the primary response has an amplified recognition potential that also ensures Abs with a high degree of specificity.

Isotype choice in immunotherapeutics

The ability of mAbs to recognize an Ag with high specificity has made them attractive for their use as therapeutic reagents. Therapeutic mouse monoclonal Abs can elicit human anti-mouse Ab (HAMA) responses, which can impede their clinical use. HAMA are predominant against the Fc portion of an Ab and are characterized by immune complex formation and hypersensitivity reactions [62]. Thus, construction of murine Abs with human C regions (chimeric Abs) and/or complete human mAbs have become very important for research and the development of therapeutic leads, and address the problem of immune reactions. Because Ab isotype can have an effect on functional affinity and specificity of binding [8,10,40,5,63] (Box 2), a crucial aspect in the generation of mouse-human chimeric and human mAbs is the choice of isotype. Thus, when selecting the isotype for the desired effector functions, one should also consider the possibility of changes in fine specificity and affinity. To understand mAb behavior and to design mAbs that are effective as therapeutic reagents, we need to understand better the interaction between the V and C domains and the effects on binding affinity and specificity.

A holistic view of the Ig molecule

In contrast to the current paradigm that views Ab molecules as being composed of two noninteracting and largely independent domains, we propose that the Ig molecule be considered as a whole entity with regards to binding and effector functions. There is now considerable evidence from various laboratories that the C_H domains can affect specificity and affinity independent of avidity. However, it is also possible that V-region structure affects C_H-region effector functions. In this regard, there is the unexplained observation of intra-subclass differences in complement activation and Ab half-life in patients with autoimmune diseases [64]. Similarly, murine IgG1s vary greatly in their ability to activate complement [59], a finding that is difficult to explain if complement activation is solely the purview of C_H-region function. Another poorly understood phenomenon is the V- and C-region restriction observed in certain Ab responses. For example, Ab responses to polysaccharide Ags are notoriously restricted in both V- and C-region usage [65], and they preferentially use 5′-V regions attached to IgM and IgG3 C regions. Perhaps this restriction is a reflection of structural constraints that allow certain V regions [66,67] to bind antigen preferentially in the context of certain C regions. Similarly, the predominance of the IgG2 isotype among viral-neutralizing Abs could reflect structural constraints imposed by the C region. Finally, pathogenic murine autoimmune Abs are often of the IgG2a isotype, suggesting that isotype restriction could reflect a propensity of the C_H region to confer self-reactivity. At the very least, the observation that the C_H region can alter specificity and affinity demands a re-examination of our view on the relationship between Ab structure and function, whereby conformational effects resulting from C_H-region constraints on V-region structure affect antigen specificity and binding and V-region constraints extend to the C_H region to affect certain effector functions (Box 3).

Box 3. Outstanding questions

Are class switch recombination and VDJ recombination linked to produce Abs of a particular isotype?
How does isotype alter the idiotypic response?
Does the V-region type affect C_H-region function?
What are the crucial residues that are permissive for the transmission of structural effects from the C_H region to the V region and vice versa?
If C_H-region type affects specificity, how does one separate the effects of changed specificity from different C_H-conferred effector functions when assessing the relative efficacy of different Ab isotypes against microbes and tumors?

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