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Influence of the Isotype of the Light Chain on the Properties of IgG

Ramon F. Montañó and Sherie L. Morrison

It is widely appreciated that the isotype of the H chain of the Ab molecule influences its functional properties. We have now investigated the contribution of the isotype of the L chain to the structural and functional properties of the Ab molecule. In these studies, the L chain variable region of a murine anti-dansyl Ab was joined to either human \( \kappa \) or \( \lambda \) constant region domains and expressed with mouse-human chimeric H chains of the four human IgG isotypes. The resulting Abs were secreted as fully assembled molecules although, as has been previously observed, IgG4 with either \( \kappa \) or \( \lambda \) chains was also secreted as H\( L\) half-molecules. However, the isotype of the L chain can influence the kinetics of intracellular assembly with IgG1, IgG2, and IgG4A assembling more slowly than their \( \kappa \) counterparts. The isotype of the L chain also influenced the susceptibility of the interchain disulfide bonds to attack by reducing agents with variable effects, depending on the isotype of the H chains. For IgG2, but not for IgG1, \(-3\), and \(-4\), the isotype of the L chain influenced the rate of clearance in mice, with IgG2A having a shorter in vivo half-life than IgG2\( \kappa \). Only slight differences were also observed between \( \lambda \) and \( \kappa \) molecules in their kinetics of binding to and dissociation from the hapten dansyl. These studies demonstrate that the isotype of the L chain has only a slight impact on the structural and functional properties of variable region identical Abs. The Journal of Immunology, 2002, 168: 224–231.

Immunoglobulins are heterodimeric proteins with two H and two L polypeptide chains. H chains are of five different classes: \( \mu \), \( \gamma \), \( \delta \), \( \alpha \), or \( \epsilon \) with four subclasses of \( \gamma \) and two of \( \alpha \) in the human. The L chains come with two different constant regions, \( \kappa \) and \( \lambda \). In the human, there is one \( \kappa \) gene and four highly similar functional \( \lambda \) genes. The ratio of the two types of L chains present in the serum varies from species to species. In mice, the \( \kappa : \lambda \) ratio is 20:1, whereas in humans it is 2:1, and in cattle it is 1:20. Each chain folds into globular, \( \beta \) barrel structures called domains. Both the H and L chains of the Ab molecule are divided into variable and constant regions. The H chain possesses one variable (V\( H \)) and three or four constant (C\( H \)) domains depending on the Ig class, whereas the L chain has only one variable (V\( L \)) and one constant (C\( L \)) domain.

Ab molecules are bifunctional proteins, wherein a specific recognition/ binding capability is combined with “effector” functions to target and eliminate pathogens and foreign molecules. The variable regions comprise the Ab binding site and are responsible for the ability of the Ab molecule to recognize a diverse universe of Ags. It is generally accepted that the effector functions of the Ab such as activation of the complement cascade, mediation of immune phagocytosis, and Ab-dependent cell cytotoxicity are determined by the Fc portion of the molecule. Within the Fc, many of the structural motifs responsible for the recognition of effector elements have been identified (1–4). The different constant regions of the H chain are known to endow the Ab with a broad range of associated functional properties. We have now addressed the question of whether the different L chain constant regions can also contribute to the structural and functional properties of the Ab molecule.

Within the IgG molecule, strong, noncovalent, lateral forces, as well as interchain covalent disulfide bonds, are essential for proper quaternary structure and function of the Ab. Longitudinal interactions between domains in native Ig molecules are minimal (5–7). Within the F(\( ab' \))\( 2 \) portion of the Ab molecule, there are two structural units, V\( L \)-V\( H \) and C\( L \)-C\( H \). The fine structure of the V\( L \)-V\( H \) unit determines the binding specificity of the Ab. The structure of C\( H \)\( 1 \) depends on the isotype of the H chain and the structure of C\( L \) depends on the isotype of the L chain. Although it is widely appreciated that the F(ab\( ' \))\( 2 \) determines the recognition of Ag by the Ab (8), recent evidence has suggested that the structure of the F(ab\( ' \))\( 2 \) may also influence functions normally assumed to be solely dependent on the Fc (9). Indeed, when the inter-H and -L chain disulfide bond pattern of IgG1 was altered to resemble that of IgG4, the ability of the mutant Ab to effect Ab-dependent cell cytotoxicity was abolished, and its ability to activate complement was reduced 15-fold (10).

We have now focused on the C\( L \)-C\( H \)\( 1 \) module within the F(\( ab' \))\( 2 \) and have produced and analyzed Abs with \( \kappa \) or \( \lambda \) C\( L \) associated with C\( H \)\( 1 \) from the four human \( \gamma \) isotypes. All molecules have identical V\( L \)-V\( H \). The isotype of the L chain did not influence the ability of the Abs to activate complement and had only a slight impact on their ability to bind Ags. However, the isotype of the L chain did influence the in vivo half-life of IgG2, a property assumed to be determined by the Fc of the Ab.

Materials and Methods

Construction of chimeric IgG molecules

The mouse anti-dansyl (anti-DNS)\( V \)\( L \) and \( V \)\( H \) genes from the hybridoma 27-44 had previously been joined to human C\( L \) in the expression vector

Abbreviations used in this paper: anti-DNS, anti-dansyl; SH, sulphydryl; FcRn, neonatal FcR.
disulfide bonds in the IgG heavy chain (225). To calculate the amount of alkylating agent required: 10 (nmol DTT: disulfide bond ratio required for each IgG subclass, it was assumed that all cysteines are present as disulfides, and the following equations were used: (μg IgG) × (nmol IgG/μg IgG) × (nmol disulfide/nmol IgG) = nmol IgG disulfide; and nmol IgG disulfide × molar excess factor = nmol of DTT required.

After DTT treatment, iodoacetamide (Sigma-Aldrich) was added to obtain a minimum of 10-fold molar excess over total thiols in solution (including both protein thiol and reducing thiol). The following formula was used to calculate the amount of alkylating agent required: 10 (nmol DTT) × (2 nmol SH/nmol DTT) + (nmol IgG disulfide × (2 nmol SH/disulfide)) = nmol iodoacetamide.

Protein samples (5 μg/sample) were diluted in 0.1 M Tris-Cl, pH 7, incubated in the presence of indicated amounts of DTT at 37°C for 30 min, and then cooled on ice. Iodoacetamide was then added to each sample and further samples were incubated further at 37°C for 60 min in the dark. Finally, samples were placed on ice and were immediately processed for SDS-PAGE analysis.

**Biological clearing experiments**

IgG-secreting transfectomas (~10⁶ cells) were washed twice in methionine-free DMEM (Irvin Scientific, Irvine, CA) supplemented with a nonessential amino acid (Life Technologies, Grand Island, NY) and glutamine (29.2 μg/ml). Cells were labeled overnight at 37°C in 1 ml of DMEM containing 10 μCi of Easy Tag (New England Nuclear, Wilmington, DE). Culture supernatants and cells were processed as described below for subsequent analysis by SDS-PAGE and autoradiography.

For pulse-chase experiments, 10⁴ cells were incubated at 37°C in 50 μCi/ml [³⁵S]methionine (Amersham, Arlington Heights, IL) for 5 min. The pulse was stopped by adding 10 ml of prewarmed IMDM containing a 100-fold excess of unlabeled methionine and 10% horse serum. The material was then split into 10 aliquots of 1 ml each and was incubated for the indicated times at 37°C. Then, 1 ml/sample of ice-cold IMDM was added, and the cells were harvested onto ice and collected by centrifugation. Supernatants were transferred to clean polypropylene tubes and the cell pellets were lysed in 0.5 ml of NDET (1% Nonidet P-40, 0.4% deoxycholate, 66 mM EDTA, 10 mM Tris, pH 7.4) and 0.06 M iodoacetamide. Soluble material was separated by centrifugation and transferred to clean tubes. Secreted and cytoplasmic Ig was immunoprecipitated by incubation with a mixture of rabbit anti-human F(ab')₂, and Fc antisera followed by a preparation of Staphylococcus aureus protein A (IgGorb; The Enzyme Center, Boston, MA). The precipitates were washed twice with NDET containing 0.3% SDS and once with dH₂O, resuspended in sample buffer (25 mM Tris, pH 6.7, 2% SDS, 10% glycerol, and 0.008% bromphenol blue), boiled, and analyzed by SDS-PAGE and autoradiography.

**Metabolic clearance experiments**

Metabolic clearance for each of the recombinant proteins was studied in 8-wk-old, female BALB/c mice (groups of three mice per recombinant protein). One week before administration of radiiodinated IgGs, drinking water was supplemented with 0.1 mg/ml potassium iodide (KI) to block thyroid uptake of radioactive iodine. Radiiodination of IgG was performed using Iodobeads (Pierce) following the manufacturer’s recommendations. Na₂¹²¹I (100 μCi) was incubated for 5 min at room temperature with one iodobead, and then 25 μg of IgG was added. After a 10-min incubation, the iodinated protein was transferred to a new vial to stop the reaction. Radiolabeled IgGs were separated from free iodine by size exclusion chromatography on a 10-nl Sephadex G50 column (Pharmacia, Piscataway, NJ). Labeling efficiency was estimated by determining TCA-precipitable radioactive counts and was consistently greater than 96%. SDS-PAGE and autoradiography of labeled material also confirmed that it was intact (data not shown).

Mice were injected with 5–7 × 10⁷ TCA-precipitable cpm of labeled Ab in 100 μl of PBS by tail-vein injection, and whole-body activity was recorded using a whole-body counter. Counts were registered at the indicated times, corrected for background activity and isotopic decay, and used for production of clearance curves and calculation of Ab half-life.

**Plasmon surface resonance analysis of Ag binding**

Plasmon surface resonance analysis was performed essentially as described (9). Briefly, commercially available IAsys cystes precoated with high m.w. carboxymethylated dextran (Fisons Applied Sensor Technology, Cambridge, U.K.) were used to immobilize the Ag, a DNS-BSA conjugate with a DNS-BSA ratio of 19:1, following the manufacturer’s instructions. The covalently bound DNS-BSA increased the sensorgram response by 5500 arc seconds, indicating that the cystes were coated with ~4 × 10⁵ DNS-BSA molecules. For Ag binding studies, the Ag-coated cuvette was washed with PBS and Tween 20 (PBST; 20 mM NaPO₄, 0.85% NaCl, and 0.05% Tween 20, pH 6.8), and a stable baseline was obtained before addition of Ag-specific Ab. Abs were added to 200 μl of PBST in volumes between 0.5 and 4 μl at time zero and association was observed. When the association curve approximates a plateau, the Ab solution was removed and replaced with PBST, and the dissociation followed with time.

**Direct lysis assays**

Complement (C₃)-mediated hemolysis was performed as described previously (13). Briefly, SRBC (Pocono Rabbit Farm, Canadensis, PA) were coated with DNS-BSA by the chromic chloride method (14) and were resuspended at 0.4% in HBS-BSA (0.01 M HEPES, 0.15 M NaCl, 0.5 mM MgCl₂, 0.15 mM CaCl₂, and 0.1% BSA, pH 7.4). Twenty-five microliters per well of this suspension was added to round-bottom 96-well plates (Costar, Corning, NY). Reombinant IgGs in HBS-BSA at various concentrations were subsequently added in a volume of 50 μl/well and, after mixing, reactions were incubated for 15 min at 4°C. The serum used as a source of C₃ was diluted in HBS-BSA and was added at 50 μl/well. The plates were incubated at 37°C for 60 min and were spun down for 1 min at 3000 × g. Twenty-five microliters of lysate was carefully transferred to a replica plate, and hemolysis was estimated spectrophotometrically by registering the generation of fluorescein blue at 650 nm in an ELISA reader as described (13). Each point was assayed in triplicate, and values obtained were expressed as percentage of lysis compared with a distilled water control.

**FIGURE 1. SDS-PAGE analysis of secreted Igs.** Cells were labeled by growth in [³⁵S]methionine, and the Igs present in the secretions were immunoprecipitated and analyzed by SDS-PAGE and autoradiography without treatment with reducing agents. The positions of fully assembled H₂L₂ molecules and HL half molecules are indicated. This gel is representative of multiple different labels.
Results

Ig assembly is influenced by the isotype of the L chain

As a first step in characterizing the recombinant proteins, secretions from transfectants labeled by growth in the presence of $^{35}$S-methionine were immunoprecipitated with antisera specific for H chain and were analyzed by SDS-PAGE without reducing the disulfide bonds (Fig. 1). All transfectants secreted fully assembled H$_2$L$_2$ molecules. IgG4 with both κ and λ L chains also secreted HL half-molecules. The additional bands migrating smaller than H$_2$L$_2$ are not consistently observed in the secretions and may represent some cell lysis during labeling.

To further characterize the assembly pathways of the different molecules, transfectants were pulsed for 5 min with $^{35}$S-methionine and were then chased with a large excess of cold methionine. Cells were pelleted at varying times after the chase, cytoplasmic lysates were prepared, the Igs were immunoprecipitated, and the precipitates were analyzed by SDS-PAGE without reducing the disulfide bonds (Fig. 2). IgG1, IgG2, and IgG4 Abs with κ L chains were rapidly assembled into H$_2$L$_2$ molecules, with H$_2$L$_2$ assembly virtually complete by 15 min. In contrast, for IgG1λ, IgG2λ, and IgG4λ 30 min after the chase, the majority of the cytoplasmic Igs remained as assembly intermediates. Both the

FIGURE 2. Pulse-chase analysis of intracellular assembly of Abs. IgG-secreting transfectomas were incubated at 37°C in 50 μCi/ml $^{35}$S-methionine for 5 min and then the incorporation of radioactivity was stopped by adding medium with a 100-fold excess of unlabelled methionine and 10% horse serum. Samples were harvested at the indicated minute after the chase, cytoplasmic lysates were prepared, and the Igs were immunoprecipitated. The immunoprecipitates were analyzed by SDS-PAGE and autoradiography without treatment with reducing agents. The positions of assembly intermediates are indicated and the kinetics of intracellular assembly are plotted. It should be noted that the large amount of excess L chain present in IgG4κ (~70% of the total protein) is not indicated.
k- and λ-producing cell lines had free L chain in the cytoplasm. For IgG1 and IgG2, the predominant assembly intermediate was H₂, irrespective of whether the L chains were k or λ. In IgG4, HL appeared to be a more significant assembly intermediate; as noted above, HL half-molecules are present in the secretions of IgG4-producing cell lines. Both IgG3k and IgG3λ assembled slowly, and significant assembly intermediates remained 30 min after the chase. Thus, these studies indicate that the isotype of both the H and L chains can influence the assembly pathway and speed.

Analysis of purified protein

When λ-chain-containing proteins were purified using DNS-affinity chromatography, we found that assembly intermediates were present (Fig. 3A). Although we had routinely observed HL half-molecules in k-chain-containing proteins of the IgG4 isotypes, we had not observed intermediates in assembly for purified proteins of the other isotypes. To obtain homogenous proteins for functional studies, H₂L₂ molecules were isolated by size exclusion on Sepharose columns (Fig. 3B). Reduction of the purified proteins yielded H and L chains of the expected size (Fig. 3C).

Thiol-mediated IgG reduction depends on the isotype of both the H and L chain

The isolation of incomplete Abs following affinity purification of proteins suggested that free thiols present in the medium may be exchanging with the disulfide bonds and breaking them. To more completely investigate this possibility, purified H₂L₂ molecules were treated with varying concentrations of DTT and the cleavage of interchain disulfide bonds were monitored by SDS-PAGE (Fig. 4). IgG1 was the most susceptible to cleavage by DTT, with IgG1λ somewhat more susceptible than IgG1k. At DTT:IgG disulfide ratios as low as 0.8, disulfide bonds are cleaved. For IgG1k, significant HL is seen, whereas for IgG1λ the H-L bonds appear to be broken before the H-H disulfide bond. The disulfide bonds of IgG2, IgG3, and IgG4 are much more resistant to attack by DTT than are those of IgG1. For IgG2k, only at a DTT:IgG disulfide ratio of 100 are significant numbers of interchain disulfides broken; the disulfides of IgG2λ are more susceptible to attack than those of IgG2k and, at this ratio, the Ab is almost completely reduced to H and L chains. In contrast, IgG3k is more susceptible to DTT attack than is IgG3λ, and at a DTT:IgG disulfide ratio of 100 it is virtually completely reduced to H and L. At the same DTT concentration, IgG3k shows only partial cleavage of its interchain disulfide bonds. The disulfides of IgG4λ are slightly more susceptible to cleavage than are those of IgG4k. It is noteworthy that for both IgG4s, the H-H disulfide is broken before the H-L bond and significant amounts of HL half-molecules are observed. Therefore, differences in susceptibility to reducing agents were observed de-
pending on the isotypes of both the H and L chains. However, there was not a consistent difference that depended only on the isotype of the L chain.

**The L chain isotype can influence the in vivo half-life**

The constant region of the H chain is known to influence the in vivo clearance of Ab molecules. To determine whether the isotype of the L chain can also play a role, purified proteins were iodinated and injected into the tail veins of BALB/c mice, and their elimination was followed by whole-body counting over time (Fig. 5). For IgG1, IgG3, and IgG4, the isotype of the L chain did not influence the kinetics of clearance, although the isotype of the H chains did play a role, with IgG4 clearing most rapidly and IgG1 clearing most slowly. However, for IgG2, the isotype of the L chain contributed to the rate of clearance, with IgG2α clearing more rapidly than IgG2κ. Thus, for IgG2, but not for IgG1, -3, and -4, the isotypes of the L chain appear to influence the kinetics of elimination.

**The isotype of the L chain does not influence Ab-mediated cytolysis**

When IgG1 and IgG3 at different concentrations were mixed with DNS-coated SRBC in the presence of human complement, IgG3 was found to be a more potent mediator of cytolysis than IgG1 (Fig. 6). However, the isotype of the L did not influence the ability of the Abs to effect complement-mediated cytolysis. IgG2 and IgG4 do not effectively activate complement and were not tested.

**Kinetics of Ag binding by Abs of different isotype**

To determine the impact of the isotype of the H and L chain on the kinetics of binding and affinity for Ag, proteins at varying concentrations were added to DNS-BSA-coated IAsys biosensor cuvetttes, and the association and dissociation was followed with time (Fig. 7). The $k_a$, $k_d$, and $K_D$ were then calculated (Table I). In general, IgG1, IgG2, and IgG4 with L chains showed an increased $k_a$ and increased $k_d$ relative to the equivalent isotype with IgG1, IgG2, and IgG4 at various concentrations were incubated for 15 min at 4°C with DNS-BSA-coated SRBC in microtiter plates. Human serum diluted 1/80 was then added, and the plates were incubated at 37°C for 60 min and spun down for 1 min at 3000 g. Lysates were carefully transferred to a replica plate and hemolysis was determined spectrophotometrically by registering the generation of fluorescein blue at 630 nm in a ELISA reader. Each point was assayed in triplicate and values obtained were expressed as percentage of lysis compared with a distilled water control. Data are plotted as the mean and SD of values obtained at differing Ab concentrations.

![FIGURE 5](http://www.jimmunol.org/) Ab clearance in mice. Female BALB/c mice (three per recombinant protein) were injected in the tail vein with 5–7 × 10⁵ TCA-precipitable cpm of ¹²⁵I-labeled protein in 100 μl of PBS, and the residual radioactivity was recorded over time using a whole-body counter. The data shown are the average of the three mice with very little variability seen among the different mice. IgG1, IgG3, and IgG4 are shown in the top panel, and IgG2κ and IgG2α are shown in the bottom panel.

![FIGURE 6](http://www.jimmunol.org/) Complement-mediated lysis of DNS-BSA-coated SRBC. Recombinant IgGs at various concentrations were incubated for 15 min at 4°C with DNS-BSA-coated SRBC in microtiter plates. Human serum diluted 1/80 was then added, and the plates were incubated at 37°C for 60 min and spun down for 1 min at 3000 g. Lysates were carefully transferred to a replica plate and hemolysis was determined spectrophotometrically by registering the generation of fluorescein blue at 630 nm in a ELISA reader. Each point was assayed in triplicate and values obtained were expressed as percentage of lysis compared with a distilled water control. Data are plotted as the mean and SD of values obtained at differing Ab concentrations.

![FIGURE 7](http://www.jimmunol.org/) Binding and dissociation of Abs to DNS-BSA immobilized on an IAsys cuvette. Abs at varying concentrations were added to 200 μl of PBST in the cuvette at time zero and association was observed for ~450 s (IgG1, IgG2, and IgG3) or 900 s (IgG4). The Ab solution was then removed and replaced with PBST, and the dissociation was followed with time. Multiple concentrations were examined for each protein.
Table I. Binding constants of Abs to DNS/BSA on a biosensor cuvette

| Anti-DNS Ab | $k_+^a$ | $k_-$ | $K_D$ |
|-------------|---------|-------|-------|
| IgG1κ | $2.1 \times 10^8 \pm 1.1 \times 10^4$ | 0.0031 $\pm$ 0.0003 | $1.46 \times 10^{-4}$ |
| IgG1κ | $1.6 \times 10^8 \pm 0.1 \times 10^4$ | 0.0022 $\pm$ 0.0003 | $1.33 \times 10^{-4}$ |
| IgG2κ | $2.3 \times 10^8 \pm 3.4 \times 10^4$ | 0.0032 $\pm$ 0.0004 | $1.40 \times 10^{-4}$ |
| IgG2κ | $1.6 \times 10^8 \pm 0.4 \times 10^4$ | 0.0012 $\pm$ 0.0004 | $0.77 \times 10^{-4}$ |
| IgG3κ | $0.9 \times 10^8 \pm 2.1 \times 10^4$ | 0.0027 $\pm$ 0.0007 | $3.00 \times 10^{-4}$ |
| IgG3κ | $0.9 \times 10^8 \pm 1.3 \times 10^4$ | 0.0027 $\pm$ 0.0014 | $2.15 \times 10^{-4}$ |
| IgG4κ | $1.9 \times 10^8 \pm 0.4 \times 10^4$ | 0.0063 $\pm$ 0.0007 | $3.26 \times 10^{-4}$ |
| IgG4κ | $0.7 \times 10^8 \pm 1.9 \times 10^4$ | 0.0045 $\pm$ 0.0004 | $6.81 \times 10^{-5}$ |

$k\lambda$ chains. The most profound difference was observed with IgG4, and IgG4κ showed a 2-fold increase in $K_D$ compared with IgG4κ. This increased $K_D$ was also evident in the binding profiles with binding not observed for IgG4κ at concentrations less than 1 μg/ml. For all of these studies, proteins were used that were $H_2L_2$ molecules, as indicated by SDS-PAGE and staining with Coomassie blue. These studies indicate that the isotype of the $L$ chain has at most a slight impact on the equilibrium binding constants of the anti-DNS Abs.

Discussion

The fully assembled Ab molecule is held together by both noncovalent and covalent interactions. Studies of the dissociation and denaturation kinetics of the F(ab')2 of the IgG1κ myeloma Wes showed that the interactions between the H and L chains of F(ab')2 are extremely strong and are maintained at guanidinium hydrochloride conditions sufficient to denature the isolated domains. Although the C regions are primarily responsible for maintaining the association, the V region also contributes significantly to the association of the chains when it is made unimolecular by its connection to the associated C region (15). Examination of the in vitro reassembly of F(ab')2 indicated that the high-affinity association of Fd (V_{H\text{H} C_{1\text{H}}}) and a $k\lambda$ chain derives from a combination of relatively weak interactions involving pairs of domains (V_{H\text{H}V_{L\text{L}}} and C_{1\text{H}1-C_{1\text{L}}}) (6). Interestingly, the binding of one L chain domain to Fd induced a conformational change in the adjacent domain, thereby modifying its reactivity toward the complementary L chain domain.

During the in vivo assembly of the molecule, noncovalent association of the subunits must precede interchain disulfide bond formation because sulfhydryl groups must be brought close together before oxidation. Recent studies have indicated that L chains must be assembled with either a H chain or another L chain to be secreted (16). Covalent assembly of H and L chains proceeds more slowly with $\lambda$ L chain and IgG1, IgG2, and IgG4 H chains, but not with IgG3 H chains. In contrast, IgG3κ is more completely assembled at 30 min than is IgG3κ. IgG3 is distinguished by an extended hinge region that provides extensive segmental flexibility, and this flexibility may make it easier for the free disulfide of the $\lambda$ L and IgG3 H chain to get into close proximity and form a covalent bond. IgG1, IgG2, and IgG4 are less flexible, and it would appear that the formation of the H-L disulfide bond forms more slowly with $\lambda$ than with $k\lambda$ chains, suggesting a different relative position of the disulfides with the different L chains. Sequence comparison of $k\lambda$ and $\lambda$ shows many differences at positions predicted to form the C_{1\text{H}1-C_{1\text{L}}}, and $\lambda$ domains, and this may influence the interactions between the domains during assembly. The susceptibility of the interchain disulfides to attack by reducing agents differs depending on the isotype of both the H and L chain. IgG1 appears to be the most susceptible isotype. Human IgG1 has been shown to have a reactive interchain disulfide that readily undergoes SH-catalyzed disulfide interchange (17), and this may facilitate the cleavage of the interchain disulfides. In addition, it has been shown that in human IgG1, the hinge peptide is fully exposed to solvent (18). Human IgG is a substrate for thioredoxin, a protein with two redox-active cysteine residues that operates with thioredoxin reductase as an NADPH-dependent protein disulfide reductase system. IgG1, IgG3, and IgG4 were readily reduced by thioredoxin into component chains through HL intermediates, whereas IgG2 was not cleaved (19). Consistent with this, the hinge of native IgG2 has been reported to be inaccessible to reaction with mAbs (20). Although we have found IgG2κ to be relatively resistant to DTT-mediated cleavage, in contrast to what was observed with thioredoxin, we found that the isotype of the L chain influences DTT-mediated disulfide bond cleavage with IgG2κ much more sensitive to DTT-mediated reduction than IgG2κ.

It was surprising that changing the isotype of the L chain changed the rate of in vivo clearance of IgG2. The neonatal FcR (FcRn) responsible for transport of Ig across the neonatal rodent intestine has been proposed to play a role in the control of the catabolism of IgG (21–24). FcRn binds to Fc at the interface between the C_{1\text{H}2} and C_{1\text{L}3} domains (25) with residues on the 252–257 and 307–311 loops in C_{1\text{H}2} and at positions 433–436 in C_{1\text{L}3}.

Table II. Sequence comparison of human $\lambda$ and $k\lambda$ chains

| Sequence |
|----------|
| $\lambda$ |
| QPKAAPSVLIFFPaseLQANkAtLGC1sdFYPAGVTVAKDGSF–VKAGVetTTPaK |
| RTVAPSPVIFPPEDDEQKLSQGTA5WCLNNFYPANAVKQHVNDNQSGSQESVTEQD |
| $\kappa$ |
| gSNKYaasSVLSTPQWHSKSYSnCQVTHEG–SVKKTvLAPTEC5 |
| $\kappa$ |
| SDSTYSLD9TITLSKADYKHYVACEvTHQGpSFPsTKSNFNGEC– |

*: Sequence identity is indicated by *. The “*” indicates that one of the following “strong” groups is fully conserved: STA, ATV, SAG, STNK, STPA, SNDEQK, DNEQHK, NEQHRK, FVLM, or HYF. Amino acids making contact with C_{1\text{H}1} on the interface between C_{1\text{H}1} and C_{1\text{L}} in the crystal structure KOL are indicated by small underlined letters (40).
implicated in forming the recognition site (reviewed in Refs. 26 and 27). Three histidine residues present at the C_\text{H}2/C_\text{H}3 interface are responsible, at least in part, for the pH-dependent FeRn/IgG interaction (22). Changing the isotype of the L chain would not be expected to have any affect on the conformation of the C_\text{H}2/C_\text{H}3 interface. Therefore, it seems more likely that something other than the affinity for FeRn is contributing to the differences in serum persistence seen with IgG2\text{\kappa} and IgG2\text{\lambda}. At the present time, it is not clear what that is.

Human isotypes IgG1 and IgG3 are both effective in activating complement. IgG2 will activate only under selected experimental conditions, and IgG4 is inactive (28–30). The second constant domain (C_\text{\kappa}2) plays a critical role in complement activation and forms the binding site for Clq, although different residues have been found to play critical roles in Abs of differing isotypes (1, 31–34). Because we observe no difference in the ability of IgG1 or IgG3 with L chains of different isotypes to activate complement, the L chain isotype does not appear to influence the conformation and accessibility of the C1q binding site within C\text{\kappa}12 of either IgG1 or IgG3.

Using variable-region identical Abs, we have observed both L and H chain-associated differences in binding kinetics. Similar constant-region related differences have been observed by others. Differences in the association rate constants were seen with two clonally related human monoclonal IgA1\kappa and IgG1\kappa Abs with identical variable regions specific for tubulin (35). In this study, the observed differences were seen with the P(ab)\text{\kappa}_2, suggesting that C\text{\kappa}1 played a role in structuring the Ag binding site into a more kinetically competent form. In another study using mouse-human chimeric anti-TAG72, the P(ab)\text{\kappa}_1 from IgG1, -2, and -4 showed identical binding parameters (36). However, different dissociation rate constants were seen for the intact Igs with IgG4 < IgG3 < IgG2 < IgG1. In a study using anti-4-hydroxy-3-nitrophenyl mouse-human chimeric Abs, the same group again saw different kinetic parameters in Abs of different isotypes. In these Abs, the L chain was murine \lambda (37). For these Abs, k_a was in the order IgG4 > IgG2 > IgG3. IgG3 was found to have a 2-fold faster association rate than IgG2 and IgG4; however, this increase in association rate was countered by a >10-fold increase in dissociation rate. In the present study, differences were seen in k_a and k_d depending on the isotype of the L chains; however, Abs with the same H chains showed similar equilibrium binding constants (Table I). The greatest difference observed was the slightly >2-fold differences in equilibrium binding constants seen with IgG4.

It is not clear how the constant region structure of either the L or H chain of the Ab is able to influence Ag binding characteristics. Using murine variable-region identical Abs, IgG3 shows functional affinity correlated with both slower dissociation rate constants and faster association rate constants in comparison to other isotypes (38). However, in the case of murine IgG3, this can be explained by self-association between the constant-region domains (39). Similar self-association has not been observed with any of the Abs used in the present study. Segmental flexibility could also contribute to the ability of the Ab to form multiple interactions with Ag immobilized on a solid support. For the human \gamma constant regions, IgG3 is the most flexible, followed by IgG1 and IgG4, with IgG2 the least flexible (40). Thus, there is no obvious correlation in the present studies between flexibility and the binding parameters.

These studies have shown that the isotype of the L chain can influence some of the properties of the Ab molecule, although it has a minimal impact on most properties. The ability to produce two different L chains arose early during the evolution of the immune system and has been studied in different species; in this study, we have examined only human L chains, and we may find different properties associated with the constant regions of L chains from different species. In addition, we have analyzed only one binding specificity, anti-DNS, a specificity initially associated with a murine \kappa L chain. It will be of interest to expand these studies to include additional binding specificities and Abs that originally contained L chains of differing isotypes. We have also only studied one of the functional \lambda constant regions present in humans. It remains a puzzle as to why there are multiple, closely related, \lambda constant regions in several species, including mouse and man.

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