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Variable Region Domain Exchange Influences the Functional Properties of IgG

Sherie L. Morrison,1,* Stephen B. Porter,1 K. Ryan Trinh,* Letitia A. Wims,* Jerrod Denham,* and Vernon T. Oi‡

In the present study we have characterized a family of anti-dansyl Abs with the variable region of the heavy chain on human C\(\gamma\)\(\kappa\) and the variable region of the light chain on different human \(\gamma\) constant regions (creating inside-out molecules). Although fully assembled molecules were secreted, this variable region exchange slowed the kinetics of Ab assembly. Although the variable region exchange does not lead to a detectable change in the microenvironment of the combining site, it did alter the kinetic parameters of binding to immobilized Ag, slowing both the on and off rates. When effector functions were evaluated, inside-out IgG1 and IgG3 were more effective in complement-mediated cytolysis than their wild-type counterparts. Variable region domain exchange may be one approach to obtaining Abs of identical specificity with altered binding characteristics. Journal of Immunology, 1998, 160: 2802–2808.

Ig molecules of the \(\gamma\) class consist of two heavy (H) and two light (L) chains. The H and L chains fold into 12,000-Da globular \(\beta\) barrel domains with homologous sequences but different functions. There are four domains for the H chain and two for the L chain. H chain and L chain are held together by disulfide bonds and by extremely strong, noncovalent interactions (1). Ig domains other than CH2 interact strongly in a lateral fashion to form modules: \(V_H \cdot V_L\), \(C_L \cdot C_H 1\), and \(C_H 3 \cdot C_H 3\). This lateral pairing buries hydrophobic residues that would be exposed in isolated domains. Longitudinal interactions along the H chain or L chain are weak or nonexistent. Within the IgG1 myeloma protein Kol, there are no nonbonded longitudinal contacts (2).

Although Ab molecules and their domains have been described as pearls on a string with weak longitudinal noncovalent interactions between the domains, but strong lateral interactions (except for CH2), the questions still remain as to whether longitudinal domain interaction exerts any influence and whether the context of the variable regions influences their functional properties. An earlier study (3) in which variable regions from anti-(4-hydroxy-3-nitrophenyl)acetyl (anti-NP) Abs were exchanged between Hlier study (3) in which variable regions from anti-(4-hydroxy-3-nitrophenyl)acetyl (anti-NP) Abs were exchanged between H- and L chain expression vectors (4), transfectants were infected with the H and L chain expression vectors (4), transfectants were selected, and surviving clones were screened for Ab production by ELISA using DNS/BSA-coated plates. DNS/BSA was prepared using dansyl chloride and BSA of RIA grade from Sigma Chemical Co. (St. Louis, MO). The amount of bound chimeric Ab was determined using alkaline phosphatase-conjugated polyclonal goat Ab (Sigma Chemical Co.) against human IgG constant regions existed as SalI-BamHI cloning cassettes (5). Using these cassettes, the IgG constant regions were then joined to \(V_L\), and \(C_\kappa\) was joined to \(V_H\).

Production of transfectedoma proteins

P3X63Ag8.653, an Ig-nonproducing mouse myeloma cell line, was transfected with the H and L chain expression vectors (4), transfectants were selected, and surviving clones were screened for Ab production by ELISA using DNS/BSA-coated plates. DNS/BSA was prepared using dansyl chloride and BSA of RIA grade from Sigma Chemical Co. (St. Louis, MO). The amount of bound chimeric Ab was determined using alkaline phosphatase-conjugated polyclonal goat Ab (Sigma Chemical Co.) against human IgG constant regions. Clones producing large quantities of anti-DNS Ab were expanded and maintained in Iscove’s modified Dulbecco’s medium containing 5% calf serum. Chimeric Abs were purified by DNS-coupled affinity chromatography as described previously (6). The concentrations of purified Abs were determined by a bicinchoninic acid-based protein assay (BCA Protein Assay, Pierce, Rockford, IL). Concentration and purity were confirmed by SDS-PAGE analysis or by chromatography on a Superoxie 12 column (Pharmacia, Piscataway, NJ).

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Materials and Methods

Construction of chimeric IgG molecules

The expressed \(V_\kappa\) and \(V_H\) genes from the mouse anti-dansyl (anti-DNS) hybridoma 27–44 had previously been joined to human \(C_\kappa\) in the pSV184\(\Delta\)neo expression vector and to human IgG heavy chain in the pSV2\(\Delta\)Hag vector, respectively (4). To produce the vectors encoding the \(V_H\) domain proteins of the \(V_H \cdot V_L\) molecules, the EcoRI in the intervening sequence immediately 5' of \(C_\kappa\) in pSV184\(\Delta\)neo was converted to a SalI site using oligonucleotide linkers (Fig. 1). As a result of this manipulation, \(C_\kappa\) and the four human IgG constant regions existed as SalI-BamHI cloning cassettes (5). Using these cassettes, the IgG constant regions were then joined to \(V_L\), and \(C_\kappa\) was joined to \(V_H\).

Abbreviations used in this paper: H, Ig heavy chain; L, Ig light chain; NP, (4-hydroxy-3-nitrophenyl)acetyl; io, inside-out; wt, wild-type; DNS, 1-dimethylamino- naphthalene-5-sulfonyl.

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Biosynthetic labeling

Transfectomas were washed twice in methionine-free DMEM (Irvine Scientific, Irvine, CA) supplemented with nonessential amino acids (Life Technologies, Grand Island, NY) and glutamine (29.2 μg/ml). Cells were labeled in 1 ml of DMEM with 15 μCi of \([\text{\textsuperscript{35}} S]\)methionine (Amersham, Arlington Heights, IL). For labeling of secretions, \([\text{\textsuperscript{35}} S]\)methionine was added to 15 μCi/ml and cells were labeled for 5 min at 37°C. The cuvette was then washed with PBST to begin dissociation. For pulse-chase experiments, \(10^4\) cells were incubated in 50 μCi/ml \([\text{\textsuperscript{35}} S]\)methionine for 5 min, at which time they were diluted to 10 ml in I scove’s modified Dulbecco’s medium containing a 100-fold excess of unlabeled methionine and 10% horse serum. Cells were harvested onto ice at the indicated times and pelletted by centrifugation. For cytoplasmic Ig, the cell pellet was lysed in 0.5 ml of 1% Nonidet P-40, 0.4% deoxycholate, 66 mM EDTA, and 10 mM Tris, pH 7.4; nuclei were pelletted by centrifugation; and the cytoplasmic lysate was transferred to a fresh tube. To immunoprecipitate the cytoplasmic IgG, a mixture of rabbit anti-human Fab and Fc antisera was added followed by precipitation with \(\text{Staphylococcus aureus}\) protein A (IgGsorb, The Enzyme Center, Boston, MA) and washing. The precipitates were resuspended in sample buffer (25 mM Tris, pH 6.7, 2% SDS, 10% glycerol; and 0.008% bromophenol blue), the Abs were eluted from the IgGsorb by boiling, and the samples were analyzed by SDS-PAGE and autoradiography.

Fluorescence emission spectrum

To determine the fluorescence emission spectra of \(\text{\textsuperscript{280}}\)S-bound to the proteins, protein at a concentration of 54 μg/ml was mixed with hapten at a concentration sufficient to saturate the binding sites. The excitation wavelength was 340 nm. The emission spectrum was determined using a spectrofluorometer.

Bioassay analysis of Ag binding

DNS-BSA\(_{\text{a10}}\) was immobilized to a commercially available IAsys cuvette precoated with a high m.w. carboxymethylated dextran (Fisons Applied Technology, Cambridge, U.K.), using the conditions recommended by the supplier. The covalently bound DNS-BSA\(_{\text{a10}}\) increased the sensorgram response by 3500 Arc s. The resonance response increases 163 Arc s/ng/mm\(^2\) of bound protein; therefore, the cuvette’s 18-mm\(^2\) surface was coated with 386 ng or approximately 2.6 \(\times\) 10\(^{-11}\) molecules of DNS-BSA\(_{\text{a10}}\).

For binding studies, the cuvette was washed with PBST (0.02 M NaPO\(_4\) 0.85% NaCl, 0.02% Tween, pH 6.8), and a stable baseline was obtained before each addition of ligand. Ligand was added to 200 μl of PBST in volumes between 1 and 5 μl at time zero, and association was observed. The cuvette was then washed with PBST to begin dissociation. For dissociation in the presence of soluble competitor, after the wash with PBST the signal was allowed to stabilize for 30 to 60 s and then 1 μl of a 38 μg/ml solution of DNS-lysine was added. Dissociation was measured for both 23 and 103 mM added Ag. Any remaining bound ligand was removed with Gentle Elution Buffer (Pierce). Association constants were determined using four to six different Ab concentrations in the 12 to 500 nM range. All calculations were performed using the FASTfit software program (Fisons Applied Biosensor Technology, Cambridge, U.K.). Before performing binding experiments, equal amounts of Ab were run on SDS-PAGE to assure that the relative concentrations were accurate and that the Abs were intact. The gel was dried using Gel Drying Film (Promega, Madison, WI) and scanned using Desk Scan II (Hewlett-Packard, Palo Alto, CA). Band intensities were determined using National Institutes of Health IMAGE software (National Institutes of Health, Bethesda, MD), and the results were used to make any necessary corrections in concentrations.

Complement-mediated hemolysis

SRBC (Pocono Rabbit Farm, Canadensis, PA) were coated with DNS-BSA (0.25 mg/ml DNS-BSA and 5% SRBC in 150 mM NaCl and 0.25 mM CrCl\(_3\), pH 7.0) for 1 h at 30°C and loaded with \([\text{\textsuperscript{51}} Cr]\)sodium chromate (Amersham Corp., Arlington Heights, IL). The free \([\text{\textsuperscript{51}} Cr]\)sodium chromate was removed by washing the cells three times in 10 ml of fresh Gel-HBS buffer (0.01 M HEPES, 0.15 M NaCl, 0.5 mM MgCl\(_2\), 0.15 mM CaCl\(_2\), 0.5% BSA, and 0.1% gelatin, pH 7.4). Chimeric Abs in Gel-HBS at various concentrations were added to round-bottom, 96-well plates (Corning Glass Works, Corning, NY) in a volume of 50 μl. Then, 50 μl of 2% \([\text{\textsuperscript{51}} Cr]\)-loaded SRBC and 10 μl CH\(_50\) units of guinea pig complement (Colorado Serum Co., Denver, CO) preabsorbed against unlabeled SRBC in a volume of 25 μl were added to each well sequentially. The plates were incubated at 37°C for 45 min, unlysed SRBC were pelleted by centrifugation of the plate, and 50 μl of supernatant were counted in a gamma counter. Each point was assayed in triplicate, and the percent lysis was determined.

Results

Assembly and secretion of novel anti-DNS light chain dimers

Expression vectors were constructed with the four IgG constant regions joined to \(\text{\textsuperscript{10}}\)Igns DNS in pSV184\(\Delta\)Hneo and the human C\(_{\kappa}\) joined to \(\text{\textsuperscript{10}}\)Igns DNS in pSV2\(\Delta\)Hgpr\(\text{\textsuperscript{10}}\)DNs (Fig. 1). When only \(\text{\textsuperscript{10}}\)Igns C\(_{\kappa}\) was expressed, it was secreted primarily as nondisulfide-linked light chain (Fig. 2, lanes 1 and 2). When \(\text{\textsuperscript{10}}\)Igns C\(_{\kappa}\) alone was expressed, it remained essentially intracellular (Fig. 2, lanes 3 and 4). However, when \(\text{\textsuperscript{10}}\)Igns C\(_{\kappa}\) and \(\text{\textsuperscript{10}}\)Igns C\(_{\kappa}\) were expressed together, both chains were secreted in approximately equimolar amounts, but most lacked intermolecular disulfide bonds. Both cytoplasmic lyses and secretions from the \(\text{\textsuperscript{10}}\)Igns C\(_{\kappa}\) + \(\text{\textsuperscript{10}}\)Igns C\(_{\kappa}\) transfectants bound disulfyl when assayed by ELISA; cytoplasmic lyses and secretions from cells expressing only \(\text{\textsuperscript{10}}\)Igns C\(_{\kappa}\) or \(\text{\textsuperscript{10}}\)Igns C\(_{\kappa}\) did not bind Ag (data not shown). This suggests that \(\text{\textsuperscript{10}}\)Igns C\(_{\kappa}\) + \(\text{\textsuperscript{10}}\)Igns C\(_{\kappa}\) heterodimers assembled functional DNS binding sites, although it is unclear whether an interchain disulfide bond is required.

Biosynthesis of \(\text{\textsuperscript{10}}\)Ig Ab molecules

Clones expressing \(\text{\textsuperscript{10}}\)Ig joined to \(\text{\textsuperscript{10}}\)IgL joined to the four human \(\gamma\) isotypes were also isolated and characterized. The proteins produced by these transfectomas were designated \(\text{\textsuperscript{10}}\)Ig to
HL half-molecules is a consequence of the IgG3 also assemble using H2 and H2L as intermediates (Fig. 4, than V L C
migrated the proteins produced, io and wt cell lines were grown over-
night in medium containing [35 S]methionine, and the secreted Igs
were immunoprecipitated with DNS-BSA-Sepharose and analyzed
by SDS-PAGE (Fig. 3). IgG1, IgG2, and IgG3 io Abs assemble
and secrete H2L2 heterodimers that bind Ag. Both HL half-molecules
and fully assembled H2L2 that bind Ag are produced by io and wt IgG4. About 10% of wt IgG4 anti-DNS and 30% of io IgG4
anti-DNS consist of HL molecules (Fig. 3A). The presence of the
HL half-molecule is a consequence of the γ4 hinge (7, 8). The
nature of the noncovalent interactions between HL half-molecules
remains unclear. Following reduction, the io heavy chains were
similar in apparent m.w. to their wt counterparts (Fig. 3B); as
shown in Figure 2, V V Ck migrated with a larger apparent m.w.
than V V Ck.

Ig assembly pathways

A pulse-chase experiment was performed to determine whether
switching the variable regions to different constant regions had any
affect on either the kinetics or the pathway of assembly of Ab. In
each case the io Ab-producing cell lines were compared with an
isotype-matched wt Ab-producing cell line. Two to four independent
clones of each io isotype were examined, and within an iso-
type similar assembly patterns were observed. Results from a rep-
resentative clone of each isotype are shown in Figure 4. Several
things are noteworthy. The wt and io IgG1, IgG2, and IgG3 io Abs assemble
and secrete H2L2 intermediates that bind Ag. Both HL half-molecules
and fully assembled H2L2 that bind Ag are produced by io and wt IgG4. About 10% of wt IgG4 anti-DNS and 30% of io IgG4
anti-DNS consist of HL molecules (Fig. 3A). The presence of the
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remains unclear. Following reduction, the io heavy chains were
similar in apparent m.w. to their wt counterparts (Fig. 3B); as
shown in Figure 2, V V Ck migrated with a larger apparent m.w.
than V V Ck.

Ag reactivity

All the proteins containing both V V DNS and V V DNS could be
isolated using Ag. Ag binding was characterized further by mea-
suring the fluorescence emission spectra of bound DNS and by
measuring Ab binding kinetics. Similar fluorescence emission
spectra were obtained for DNS bound by both io and wt proteins
(Fig. 5), indicating that the microenvironment of the hapten in the
combining site was similar in all the proteins.

The kinetics of DNS binding by the engineered Abs were mea-
sured using an IAAsys biosensor. The ka and kd in PBST and PBST
plus DNS-lysine were calculated as described above (Table I). The
Abs were also directly compared by adding equal quantities of the
anti-DNS Abs (5 μg) to the DNS-BSA-coated cuvette (Fig. 6).
The wt and io Abs exhibited different binding kinetics. The wt Abs
showed a more rapid ka than the io Abs. The wt IgG1, IgG2, and
IgG3 approached equilibrium by 300 s; the wt IgG4 required
longer, reflecting its smaller initial ka (Table I). In contrast, the io
Abs did not reach equilibrium during the 1000 s of the experi-
ments. However, when the cuvette was washed with PBST, the io
Abs dissociated more slowly than the wt (Fig. 6), as reflected in a
smaller kd (Table I). Under the conditions of the experiment, re-
binding occurred, and the off-rate represented the competition be-
tween dissociation and rebinding. Addition of DNS-lysine as a
soluble competitor to prevent rebinding increased the kd (Table I),
but again, the io Abs showed a smaller kd than their wt counterparts.
Ab-mediated complement activation

To evaluate the ability of the io Abs to activate the complement cascade, we performed a direct lysis assay using DNS$_80$-BSA-coated SRBCs. Neither io nor wt IgG2 and IgG4 were able to effect lysis (data not shown), and as we had observed previously, wt IgG3 was more effective than wt IgG1 in causing lysis (9, 10). In addition, we found that io IgG1 and IgG3 were significantly more effective than wt IgG1 and IgG3 in activating complement-mediated cytolysis, with io IgG1 as effective as wt IgG3, and io IgG3 causing maximum lysis at a concentration less than one-tenth of that required for wt IgG3 (Fig. 7).

Discussion

These studies confirm that it is possible to create Ab molecules in which the variable regions of the H and L chains are exchanged. Molecules consisting of VH of k and with VL joined to

<table>
<thead>
<tr>
<th>Ab</th>
<th>$k_a$ (M$^{-1}$·s$^{-1}$)</th>
<th>$k_d$ (M$^{-1}$·s$^{-1}$)</th>
<th>$k_o$ (s$^{-1}$)</th>
<th>$k_d$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt IgG1</td>
<td>57,725</td>
<td>39,777</td>
<td>0.007</td>
<td>0.0182</td>
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<tr>
<td>wt IgG2</td>
<td>60,868</td>
<td>42,849</td>
<td>0.005</td>
<td>0.0169</td>
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<tr>
<td>wt IgG3</td>
<td>57,070</td>
<td>36,556</td>
<td>0.005</td>
<td>0.0328</td>
</tr>
<tr>
<td>wt IgG4</td>
<td>13,520</td>
<td>37,518</td>
<td>0.004</td>
<td>0.0211</td>
</tr>
<tr>
<td>io IgG1</td>
<td>22,290</td>
<td>4,697</td>
<td>0.0015</td>
<td>0.0135</td>
</tr>
<tr>
<td>io IgG2</td>
<td>22,731</td>
<td>4,808</td>
<td>0.0014</td>
<td>0.0089</td>
</tr>
<tr>
<td>io IgG3</td>
<td>36,269</td>
<td>5,337</td>
<td>0.0016</td>
<td>0.0119</td>
</tr>
<tr>
<td>io IgG4</td>
<td>22,859</td>
<td>16,478</td>
<td>0.0015</td>
<td>0.0109</td>
</tr>
</tbody>
</table>

* Binding constants were calculated using the FASTfit program as described in Materials and Methods.
C regions of different human γ isotypes were secreted, with covalent assembly occurring between the H and L chains (Fig. 3). The L chain heterodimers of V_H C_k-V_L C_k, although mostly lacking covalent bonds, also were secreted and bound Ag (Fig. 2). Presumably the V_H C_k-V_L C_k form noncovalent heterodimers capable of binding Ag and being secreted. However, L chains consisting of V_H C_k were unable to bind Ag or be secreted. The precise structure of the V_H C_k appears to determine whether it is secreted from cells. Another V_H C_k with the anti-NP variable region was secreted in the absence of an associated L chain (3), while a V_H C_k with a human variable region was not secreted even with the endogenous J558L L chains (11). In several cases failure to secrete L chains can be traced to amino acid sequences present in the variable region (12–14).

Changes in the kinetics of assembly

The change in domain position results in a slowed kinetics of covalent intracellular assembly. In contrast with an earlier study of domain exchanged anti-NP Abs, where L, H, H_L, H_2 L, and H_2 L_2 were present in the secretions (3), only fully assembled H_2 L_2 and free L chain are seen in the secretions of io IgG1, io IgG2, and io IgG3. Like wt IgG4, io IgG4 also secretes HL half-molecules; the formation of half-molecules has been shown to be a consequence of the structure of the γ4 hinge (7, 8).

During in vivo assembly of the molecule, noncovalent association of the subunits must precede interchain disulfide bond formation, since sulphydryl groups must be brought close together before oxidation. Covalent assembly of H chains can begin while they are still nascent chains, and disulfide-linked nascent H chains are present in the murine cell line MOPC-21 that assembles its Ig through an H_2 intermediate (15). Similarly, H-H disulfide bond formation occurs early in the assembly of the io molecules. Strong noncovalent interactions in the Fc also serve to bring H chains into close proximity, and they appear to be dimers at concentrations as low as 10^{-10} M, which would facilitate their covalent assembly (16). The L chain is released from the ribosome before the terminal Cys involved in disulfide bond formation has transited the membrane of the endoplasmic reticulum and is available. Therefore, covalent attachment of L chain must take place after its translation has been completed. Excess L chain has been observed to facilitate Ig assembly and secretion (17), and a variant of the murine cell line MPC-11 which synthesizes less L chain has a delay in the secretion of its Ig, with the predominant intermediate in assembly now H_2 instead of the normally occurring HL. However, for the io Abs, the L chain appears to be present in excess, so the quantity of L chain present should not delay their assembly. In fact, additional transfectants making a large excess of L chain showed similar H chain assembly kinetics (data not shown).
During in vitro reassembly, H-L interaction is accompanied by conformational changes in both subunits (18, 19), and previous studies (20) have suggested that the binding of one κ-chain domain, either V₁ or C_k, to Fd induces a conformational change in the adjacent domain in Fd, modifying its reactivity toward the complementary κ-chain domain. In the io molecules, Fd now consists of V₁ and C₁H₁ and the L chain consists of V_H and C_L. In this altered orientation, the interaction of C₁H₁ and C_L and that of V₁ and V_H may fail to induce the appropriate conformational changes, thereby slowing the covalent assembly process.

Earlier pulse-chase analysis of cells from a patient with a γ₁λ paraprotein had indicated HL half-molecules as an intermediate in the assembly of human IgG1 (21). In contrast, with all the chimeric proteins, both wt and io, H₃ is the predominant assembly intermediate; only wt and io IgG4 show significant quantities of HL molecules, which probably do not represent assembly intermediates but, instead, a final product. This difference from the previous studies may reflect the use of a γ instead of a λ constant region on the L chain. Alternatively, there may be allelotic differences between the γ₁ H chain used in these experiments and the γ₁ H chain present in the patient from which the cells were obtained for the earlier pulse-chase experiments.

**Ag reactivity of io anti-DNS molecules**

Previous studies using anti-NP Abs in which the variable regions had been exchanged between the heavy and light chains had shown no alteration in the functional properties of these Abs and had suggested autonomy of the Ag binding domains (3). Similarly, the io anti-DNS Abs retain the local environment of the hapten binding site as measured by fluorescence enhancement, but, unlike the anti-NP Abs, they have been shown to exhibit altered binding kinetics. The wt 27.44 DNS combining site is believed to bind the dansyl moiety in a lateral position near the rim of the binding pocket (22). In the io molecules, the combining sites may be positioned differently, and this difference in position could alter the structure of the array of Ab molecules on the Ag. This altered positioning could then affect the on and off rates by influencing the ease both of association with immobilized Ags and of rebinding. The constant region of murine Abs has been shown to influence Ag binding (23). At high epitope density, murine IgG3 was seen to have a significantly greater apparent functional affinity than murine IgG1, IgG2b, or F(ab’)₂; these results are consistent with a model for cooperative binding by IgG3 that depends on noncovalent interactions in the variable region, it was possible to change the kinetics of Ag binding by exchanging the variable regions of the H and L chains. The resulting Abs had altered functional properties, including increased efficacy in complement mediated cytolysis. Domain exchange may provide an additional approach to producing Abs with the appropriate constellation of functional properties.

**Changes in complement activation**

Human IgG isotypes differ in their ability to fix complement. IgG1 and IgG3 are most effective; IgG2 is much less effective, functioning under only some assay conditions (6); and IgG4 is unable to bind C1q and activate the complement cascade. The C₁H₂ domain is the binding site for C1q with the exposed β strand of Glu₁³¹⁵, Lys₂³⁰, and Lys₂³² important (24). Amino acid changes within C₁H₂ result in the inability of IgG4 to activate complement (9, 10) and contribute to the differential abilities of the other isotypes to activate complement (6, 7, 25–27). The io IgG1 and io IgG3 molecules were significantly more effective than their wt counterparts in their ability to effect complement-mediated cytolysis. Both io and wt IgG2 and IgG4 remained nonfunctional in this assay. Several aspects of the changed structure in the io Abs could contribute to these differences. The geometric presentation of hapten or antigenic epitopes recognized by Abs can determine their effectiveness in activating complement (28), probably due to the nature of the lattice formed by the Ag-antibody complexes. Switching the domains between the H and L chains in the anti-DNS Abs could alter the position of the binding site relative to the remainder of the molecule. This is possible given the reported position of the combining site at the edge of the variable region (22) (discussed above). A different array of io IgG1 and IgG3 more effective in complement activation could form on the surface of Ag coated-SRBC. It is also possible that the slower off-rate of the io molecules leads to a more stable Ab-C1 complex.

These studies demonstrate that it is possible to alter some of the functional properties of Ab molecules by changing the disposition of the different domains. While maintaining identical amino sequences in the variable region, it was possible to change the kinetics of Ag binding by exchanging the variable regions of the H and L chains. The resulting Abs had altered functional properties, including increased efficacy in complement mediated cytolysis. Domain exchange may provide an additional approach to producing Abs with the appropriate constellation of functional properties.

**References**