Identification of the Fcγ receptor class I binding site in human IgG through the use of recombinant IgG1/IgG2 hybrid and point-mutated antibodies

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ABSTRACT To characterize the region on human IgG1 responsible for its high-affinity interaction with the human Fcγ receptor class I (FcγRI), we have analyzed the binding properties of a series of genetically engineered chimeric anti-dinitrophenyl antibodies with identical murine antibody combining sites and hybrid IgG1/IgG2 human constant (C) regions. In addition, we have investigated a panel of reciprocally point-mutated IgG1 and IgG2 chimeric antibodies to identify the amino acid residues that confer cytophilic properties to human IgG1. Our data unambiguously indicate that cytophilic activity of IgG1 is an intrinsic property of its heavy-chain C region 2 (CH2) domain. We report that the entire sequence spanning residues 234–237 (LLGGP) is required to restore full binding activity to IgG2 and IgG4 and that individual amino acid substitutions failed to render IgG2 active. Nevertheless, the reciprocal single point mutations in IgG1 either significantly lowered its activity or abolished it completely. Finally, we observed that an IgG2 antibody containing the entire ELLGGP sequence (residues 233–238) was more active than wild-type IgG1. This finding suggests that in addition to the primary contact site identified in the N terminus of the γ1 CH2 domain, secondary sites involving residues from the C-terminal half of the domain may also contribute to the IgG1–FcγRI interaction.

Fcγ receptors for human IgGs (Fcγ,R) form a family of integral membrane proteins that specifically bind to immunoglobulin Fc regions. The interaction of Fcγ,Rs with IgG triggers classical host effector functions such as phagocytosis of immune complexes (1), antibody-dependent cell-mediated cytotoxicity (2–4), and the release of inflammatory mediators (5).

The classes of human Fcγ,R have been defined on the basis of their reactivity with monoclonal antibodies (6). The high-affinity FcγRI (CD64) is a 72-kDa glycoprotein expressed on mononuclear phagocytes (7) and interferon γ-activated neutrophils (2). FcγRI displays a hierarchy of affinities for monovalent human IgG subclasses. IgG1 and IgG3 bind with high affinity (Kd 10–10–10 M–1), whereas IgG4 is 10-fold less cytophilic and IgG2 is devoid of any significant binding activity (8).

Several indirect approaches suggest that the primary site that mediates the IgG1–FcγRI interaction resides within the heavy-chain constant region 2 (CH2) domain. Monoclonal antibodies directed against a CH2 domain N-terminal epitope including the isotypic-specific residue K274, inhibited the IgG1–FcγRI interaction (9). Furthermore, the CH2 domain-deleted IgG1 myeloma protein TIM failed to bind FcγRI (10). Results obtained with isolated CH2 domains remain controversial since dimeric, but not monomeric, domains possess cytophilic activity (11, 12). In the context of a hybrid molecule, however, only a single cytophilic H chain is required for binding activity (13). More recently, the functional analysis of a panel of genetically engineered human IgG1/IgE hybrid antibodies indicated that the FcγRI binding site was essentially a property of the IgG1 CH2 domain (14).

Several observations suggest that the CH2 N terminus forms at least part of the IgG1 FcRI recognition site. Comparative primary sequence analysis revealed that an LLGGP motif spanning residues 234–238 is conserved in all cytophilic IgGs (15). Site-directed mutagenesis of E235 in noncytophilic mouse IgG2b to L235 produced the LLGGP motif on an IgG2b background, rendering this molecule fully active (16). This was not predicted to be the only critical residue in the human site since IgG1 and IgG4 are identical at this position, yet IgG4 is 10-fold less active. Finally, whereas IgG1 Fc fragments obtained by digestion with papain retain cytophilic activity, those obtained with thermolysin, which cleaves between residues 234 and 235, do not (11). This indicates that FcγRI binding activity requires the presence of residues N-proximal to L235 but not that of the hinge disulfide bonds, since reduction and alkylation of IgG1 has no significant effect on its binding affinity for FcγRI (15).

We have engineered a panel of chimeric IgG1/IgG2 hybrid molecules and a set of reciprocally point-mutated IgG1 and IgG2 antibodies to identify the region(s) and ultimately the amino acids that are essential for IgG1–FcγRI recognition and are sufficient to restore full activity to IgG2. We report that IgG2 molecules containing only an IgG1 CH2 domain are as cytophilic as wild-type IgG1 and that the amino acids that are critical for this activity are L234, L235, and G237.

MATERIALS AND METHODS

Cell Lines. The murine myeloma λ-chain-producing mutant cell line MOPC 315.26 was maintained as described (17). U937 cells were grown in RPMI 1640 medium supplemented with 2% fetal calf serum, 2 mM glutamine, and antibiotics.

Plasmid Constructs. The pSV2neoVH315 mammalian expression vector has been described (18). The 3.2-kilobase HindIII/BamHI IgG1 and IgG2 CH2 gene fragments were kindly provided by L. Hood (California Institute of Technology). All molecular cloning techniques were performed according to Sambrook et al. (19). The human IgG1, IgG2, and IgG4 C region genes were cloned into pEMBL-19, where all

Abbreviations: C, constant; H, H chain; FcγRI, high-affinity Fcγ receptor class I.

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subsequent genetic manipulations were done. Oligonucleotide-directed mutagenesis was performed by the Eckstein method (20) (Amersham) to introduce unique XbaI I and EcoRI sites in the hinge-C\textsubscript{H}2 and C\textsubscript{H}2-C\textsubscript{H}3 introns, respectively, and to substitute amino acids. Hybrid IgGl/IgG2, IgG2/IgGl, and IgGl/IgG4 genes were constructed by reciprocally exchanging exons excised with the appropriate restriction enzymes. The transfer of native and modified IgGl, IgG2, and IgG4 genes into the pSV2neoV\textsubscript{H}315 expression vector was performed as described (18). The resulting expression vector encodes an entire H chain composed of a human IgG C region and the murine MOPC 315 H-chain variable region (V\textsubscript{H}) domain. The identity of each construct was confirmed in both pEMBL-19 and in pSV2neoV\textsubscript{H}315 by restriction map analysis and DNA sequencing.

Electroporation. The pSV2neoV\textsubscript{H}315 IgGl, IgG2, and IgG4 H-chain constructs were transfected into the \(\lambda\)-chain-producing cell line MOPC 315.26 by electroporation according to the method of Baker et al. (21). The electroporated cells were grown and selected in G418 (GIBCO) (17). Antibody production was quantified by IgG-specific capture ELISA, using myeloma human IgGl and IgG2 as standards.

Assembly of Chimeric Molecules. The assembly of the chimeric antibodies was evaluated by SDS/polyacrylamide gradient (4–15%) gel electrophoresis under nonreducing conditions. Culture supernatants containing \(\approx 5 \mu\)g of chimeric immunoglobulin were incubated with 30 \(\mu\)l of a 10% suspension of formalin-treated \textit{Staphylococcus aureus} bacteria for 1 hr at 4°C. The pellets were washed six times in phosphate-buffered saline (PBS) containing 0.5% Triton X-100, 0.25% deoxycholate, 0.5% SDS, 10 mM EDTA, and 2 mM phenylmethylsulfonyl fluoride and then boiled for 3 min in 2X sample buffer containing 4% SDS and 1 mM iodoacetamide to prevent disulfide interchange.

Preparation of Culture Supernatants for Binding Inhibition Assays. Culture supernatants were concentrated 10-fold using Centriprep-30 microconcentrators (Amicon). The concentrated culture supernatants were then subjected to ultracentrifugation for 30 min at 100,000 \(\times g\) at 4°C to remove potential IgG aggregates. The final concentration of chimeric antibodies was assessed by triplicate ELISA determinations.

Binding Inhibition Studies. Purified human IgGl myeloma protein was radioiodinated using iodobeads (Pierce) to a specific activity of \(1 \times 10^{6} \text{cpm/\mu g}\). Bound \(^{125}\text{I}\) was separated from free material by gel filtration through a Biogel P4 spin column. IgGl concentration was measured spectrophotometrically at 280 nm using an extinction coefficient \(E_{280}^{\text{cM}}\) of 1.4. U937 cells were washed once in PBS containing 0.02% sodium azide and bovine serum albumin (1 mg/ml) and resuspended in the same buffer. Direct binding and inhibition assays were carried out in 96-well U-bottom microtiter plates essentially as described by Raychaudhuri et al. (22). In brief, for the inhibition assay, \(2.5 \times 10^{6}\) U937 cells were incubated per well with 10 nM radioiodlated IgGl and increasing concentrations of chimeric inhibitor for 2 hr at room temperature and then spun through a dibutyl phthalate oil cushion; cellular pellets were assayed for bound radioactivity. Nonspecific binding was determined from the residual bound radioactivity in the presence of 10 \(\mu\)M unlabeled IgGl and never exceeded 2% of total binding. All values were corrected for nonspecific binding. The association constants of the chimeric antibodies were estimated from the displacement of the inhibition curve (at 50%) obtained with chimeric inhibitor relative to that obtained with myeloma IgGl for which the \(K_{d}\) was determined in a direct binding assay (23). Three independent determinations were obtained in triplicate for each chimeric species.

RESULTS

Construction, Expression, and \(F_{c},\)RI Binding Properties of Hybrid IgGl and IgG2 Antibodies. The role of individual domains in the cytophilic activity of human IgG isotypes was analyzed by exon-shuffling experiments. The panel of hybrid genes is depicted schematically in Fig. 1. An IgGl molecule with an IgG4 hinge was also constructed (data not shown). Hybrid molecules are described by four digit numbers indicating the subclass origin of the C\textsubscript{H}1, hinge, C\textsubscript{H}2, and C\textsubscript{H}3 exons, respectively.

Chimeric immunoglobulins from clones secreting in the range of 5–10 \(\mu\)g of IgG per ml were affinity isolated with \textit{S. aureus} cells and analyzed by SDS/PAGE. Nonreduced SDS/PAGE revealed that all molecules migrated as a single band with an apparent molecular mass of 150 kDa corresponding to a covalently assembled H\textsubscript{L}H\textsubscript{L} molecule that could be dissociated into its constituent H and L chains upon reduction (data not shown). Representative electrophoretic patterns obtained with wild-type chimeric IgGl and IgG2 and hybrid IgGl-1-2-1, IgG2-2-1-2 molecules are shown in Fig. 2.\textsuperscript{\textdagger}

The binding properties of the exon-shuffled antibodies were assessed by competitive inhibition assays. To validate the use of chimeric IgGs as inhibitors, we confirmed that the cytophilic activity of the IgGl chimera was indistinguishable from that of myeloma IgGl. Representative binding inhibition curves obtained for these molecules are depicted in Fig. 3A. The curves demonstrate a typical sigmoidal shape and are superimposable. This indicated that the \(K_{d}\) value for the chimeric IgGl molecule was the same as that of myeloma IgGl, which was determined in an independent direct binding assay.

\textsuperscript{\textdagger}Example of nomenclature for hybrid molecules: IgGl-1-2-1 refers to C\textsubscript{H}1, hinge, and C\textsubscript{H}3 exons of IgGl origin, and a C\textsubscript{H}2 exon of IgG2 origin.
experiment to be $4.30 \times 10^6 \text{ M}^{-1}$. A similar experiment confirmed that chimeric IgG2 was devoid of any cytophilic activity at molar concentrations up to $10^{-7} \text{ M}$ (Fig. 3A). Myeloma IgG2 displayed a 30-fold lower activity than did IgG1, probably reflecting a small amount of contaminating cytophilic isotypes in the IgG2 preparation.

To assess the relative contribution of the Fc C region domains and the genetic hinge, combinations of domain and hinge exons were reciprocally exchanged (Fig. 1). Table 1 summarizes the FcRI binding properties of these molecules as assessed by competitive inhibition assays. Neither the hinge nor the C\textsubscript{H}2 domain was shown to contribute significantly to the IgG–FcRI interaction, since grafting of an IgG1 hinge or C\textsubscript{H}3 domain on an IgG2 background did not restore measurable activity to the IgG2 subclass within the range of concentrations tested. Conversely, IgG1 hybrid molecules containing either an IgG2 hinge or C\textsubscript{H}3 domain retained full cytophilic activity. In marked contrast, the insertion of an IgG1 C\textsubscript{H}2 domain conferred full cytophilic properties to IgG2 (Fig. 3B). Indeed, the IgG2-2-1-2 hybrid immunoglobulin was found to bind to the U937 FcRI with an affinity ($K_a = 3.77 \times 10^6 \pm 0.86 \text{ M}^{-1}$) comparable to that of wild-type IgG1, whereas the reciprocal hybrid molecule IgG1-1-2-1 was totally inactive. All molecules containing an IgG1 C\textsubscript{H}2 domain were active regardless of the isotypic origin of the hinge or other \(\gamma\)-chain C domains. Conversely, all hybrids containing an IgG2 C\textsubscript{H}2 domain were functionally inactive. In addition, we also observed that the replacement of an IgG1 hinge with its IgG4 counterpart did not affect the activity of the IgG1 chimera (Table 1).

**Table 1. Apparent $K_a$ values of hybrid IgG–U937 FcRI interactions**

<table>
<thead>
<tr>
<th>IgG species</th>
<th>$K_a \times 10^{-8} \text{ M}^{-1}$</th>
<th>SD. $\times 10^{-8} \text{ M}^{-1}$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1 (myeloma)</td>
<td>4.30*</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>IgG1-1-1-1</td>
<td>4.30</td>
<td>0.49</td>
<td>3</td>
</tr>
<tr>
<td>IgG2-2-2-2</td>
<td>$&lt;0.05$</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>IgG1-1-1-2</td>
<td>5.04</td>
<td>0.46</td>
<td>3</td>
</tr>
<tr>
<td>IgG2-2-2-1</td>
<td>$&lt;0.05$</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>IgG1-1-2-1</td>
<td>$&lt;0.05$</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>IgG2-2-1-2</td>
<td>3.77</td>
<td>0.86</td>
<td>3</td>
</tr>
<tr>
<td>IgG1-2-1-1</td>
<td>3.51</td>
<td>0.40</td>
<td>3</td>
</tr>
<tr>
<td>IgG2-1-2-2</td>
<td>$&lt;0.05$</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>IgG1-1-2-2</td>
<td>$&lt;0.05$</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>IgG2-2-2-1</td>
<td>3.51</td>
<td>0.13</td>
<td>3</td>
</tr>
<tr>
<td>IgG2-1-2-2</td>
<td>6.83</td>
<td>1.57</td>
<td>3</td>
</tr>
<tr>
<td>IgG1-4-1-1</td>
<td>3.45</td>
<td>—</td>
<td>1</td>
</tr>
</tbody>
</table>

*This $K_a$ value was obtained by Scatchard analysis of data from a direct binding experiment. All other apparent $K_a$ values were obtained from binding inhibition experiments as described in Materials and Methods and are expressed as the means from independent determinations ($n$) performed in triplicate.

**Construction, Expression, and FcRI Binding Properties of IgG1, IgG2, and IgG4 C\textsubscript{H}2 Domain Point Mutants.** The existence of striking primary sequence differences in the lower hinge region of IgG1 and IgG2 C\textsubscript{H}2 domains (Table 2) and the indirect evidence that this region may be structurally important for FcRI recognition led us to concentrate on residues 233–238 (Eu numbering) for more detailed analyses. Individual IgG1 residues E233, L234, and L235 of the ELLGGP sequence were systematically substituted with the corresponding IgG2 residues P233, V234, and A235, respectively. In addition, IgG1 G237 was deleted, since there is no corresponding residue at position 237 in IgG2. A similar mutagenesis strategy was also used to modify the N-terminal region of the IgG2 C\textsubscript{H}2 domain. Residues P233, V234, and L235 of the IgG2 PVAG–P sequence were replaced with the corresponding IgG1 residues E, L, and L, respectively. In addition, a glycine residue was inserted at position 237. Multiple substitutions were also performed such that in all, a panel of 12 IgG1 and IgG2 analogues with mutated C\textsubscript{H}2 domains were generated. The amino acid sequences of these variants, and an additional IgG4 F234 to L234 mutant molecule, are shown in Table 2. SDS/PAGE analysis revealed covalent H2L2 assembly in all cases. The electrophoretic patterns for three critical mutants are shown in Fig. 2. The relative binding activities of the mutated molecules obtained from two independent transfections were compared in binding inhibition assays at two different concentrations of chimeric inhibitor (2.3 and 6.7 nM) and equimolar concentra-

**Fig. 2. SDS/PAGE analysis of hybrid and point-mutated chimeric IgGs.** Samples were analyzed as described. IgG1 M, IgG1 R, and IgG2 R, IgG1 myeloma and recombinant IgG1 and IgG2 proteins, respectively. Immunoprecipitated supernatant from mock-transfected MOPC 315.26 cells (Medium) shows a faint 150-kDa band likely corresponding to bovine IgG.
The genetic hinges of IgG1 (15 residues) and IgG2 (12 residues), which include the upper and core regions of the “functional hinge” differ by three amino acids in length and by their primary sequences. However, reciprocal shuffling of hinge regions between IgG1 and IgG2 subclasses and the grafting of an IgG4 hinge onto an IgG1 background did not affect the Fc,RI binding properties of the parent molecules. These findings strongly suggest that neither the length of the upper hinge, which restricts segmental flexibility (24), nor the amino acid sequence of the core hinge modulates cytophilic activity. In addition, it has been shown that this effector function is not affected by reduction of the hinge disulfide bridges (15). However, it requires the presence of a spacer between the Fab arms and the Fc region since the hinge-deleted IgG1 paraprotein Dob does not bind to Fc,RI on monocytes (10).

Reciprocal exchange of C_4H3 domains, which differ by only one conservative substitution, did not affect the binding properties of the native molecules. Our results are in agreement with the findings that noncovalent pFc' dimers (10) and monoclonal anti-C_4H3 antibodies are unable to inhibit the binding of IgG1 to the monocyte Fc,RI (9).

Reciprocal shuffling of C_4H2 domains between the IgG1 and IgG2 subclasses unambiguously revealed that the Fc,RI binding site is an intrinsic property of the IgG1 C_4H2 domain, since all IgG2 hybrid molecules containing an IgG1 C_4H2 domain are as active as wild-type IgG1. Furthermore, cytophilic activity was abolished in all IgG1 hybrids containing an IgG2 C_4H2 domain. These results are consistent with indirect evidence that the C_4H2 domain is the primary site for Fc,RI binding.

**Fig. 4.** Inhibition of 125I-IgG1 binding to U937 Fc,RI receptors by recombinant native and point-mutated IgG1, IgG2, and IgG4 molecules. (A) Bar graph representation of an experiment in which the concentrations of both 125I-IgG1 and recombinant inhibitor were kept constant at 6.67 nM. The inhibitor used in the assay is indicated at the base of its corresponding bar. WT, wild type. (B) Binding inhibition curves using 10 nM 125I-IgG1 and increasing concentrations of the following inhibitors: wild-type recombinant IgG1 (a), IgG1/EVLGGP (b), IgG2/PLLGGP (c), and IgG2/ELLGGP (d).

**Table 2.** Apparent $K_a$ values of the Fc,RI-IgG interaction obtained with IgG1 and IgG2 point mutants

<table>
<thead>
<tr>
<th>IgG background (amino acids)</th>
<th>IgG1 WT APELLGGP</th>
<th>IgG2 WT APFVAGP</th>
<th>IgG1 APELLGGP</th>
<th>IgG2 APELLGGP</th>
<th>IgG1 APELLGGP</th>
<th>IgG2 APELLGGP</th>
<th>IgG1 APELLGGP</th>
<th>IgG2 APELLGGP</th>
<th>IgG1 APELLGGP</th>
<th>IgG2 APELLGGP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence</td>
<td>$K_a \times 10^{-8}$ M$^{-1}$</td>
<td>$\pm 10^{-8}$ M$^{-1}$</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG1 WT APELLGGP</td>
<td>4.30</td>
<td>0.49</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG2 WT APFVAGP</td>
<td>5.17</td>
<td>0.39</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG1 APELLGGP</td>
<td>16.5</td>
<td>5.51</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG2 APELLGGP</td>
<td>3.44</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IgG4 WT APELLGGP</td>
<td>0.04</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
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</table>

Underlined letters indicate point mutations. All apparent $K_a$ values were obtained from binding inhibition experiments and are expressed as the means of independent determinations (n) performed in triplicate. WT, wild type.
Covalent IgG1 C_β_2 domain dimers retain 85% of the cytophilic activity of the parent molecule (11), whereas the C_β_2 domain-deleted paraprotein IgG1 TIM is devoid of activity (10). Furthermore, monoclonal anti-C_β_2 antibodies inhibit the IgG1-Fc,RI interaction (9). Recently, reciprocal domain and hinge exchange experiments between human IgG1 and IgE showed that both the IgG1 C_β_2 and C_β_3 domains were required to restore full Fc,RI binding activity to IgE (14). Based on free energy calculations, it was concluded that the IgG1 C_β_2 domain contributes ~75% of the free energy gain associated with the IgG1-Fc,RI interaction.

Several lines of evidence suggest that the activity of the Fc,RI binding site depends on its conformation. Granting an IgG1 C_β_2 domain onto an IgE background did not restore Fc,RI binding activity to IgE (14) despite its free energy contribution to the Fc,RI interaction. The affinity of bispecific mouse IgG2a/IgG2b hybrid antibodies suggests that only one cytophilic H chain is required for the IgG1-Fc,RI interaction (13). However, the findings that only dimeric isolated C_β_2 domains are cytophilic (12) and that the C_β_3 domain-deleted IgG1 paraprotein SIZ fails to bind Fc,RI (10) infer that quaternary interactions between the domains are required to stabilize the active conformation of the IgG1 effector site. Furthermore, inactive aglycosylated IgG1 displays significantly enhanced Fc,RI and Fc,RIIa binding activity and cytophilic activity (25). Since these enzymes cleave within the C_β_2 domain, these findings suggest that carbohydrates maintain its conformation and stabilize the Fc,RI binding site (8).

The most striking primary sequence differences between IgG1 and IgG2 C_β_2 domains are clustered in the lower hinge region (residues 233–238) encoded by the C_β_2 exon. A conserved LLGGP motif between positions 234 and 238 is found in all cytophilic IgG subclasses. The functional importance of the crystallographically defined “upper region” of the C_β_2 domain is supported by the finding that inactive aglycosylated IgG3 Fc fragments show structural perturbations, as assessed by 1H NMR, in the vicinity of the H268 reporter group located in the spatial proximity of the lower hinge (25). In addition, full Fc,RI binding activity was restored to the inactive mouse IgG2b subclass by reconstructing the LLGGP motif with a single L for E substitution at position 235 (16). Moreover, reciprocal reports (26, 27) showed that the binding activity of human IgG3 was lost in a panel of single point mutants where L234, L235, and G237 were replaced with A residues. Together, these observations led us to engineer a panel of IgG1 and IgG2 chimeras containing single and sequential point mutations in this region. Full binding activity was restored to an IgG2 molecule in which G was inserted at position 237, and V234 and A235 were replaced with their IgG1 counterparts L234 and L235 (IgG2/PLLGGP), respectively. Interestingly, within the limits of the binding-inhibition assay, none of the individual mutations could restore even partial activity to IgG2, although the single reciprocal mutations in IgG1 significantly diminished or abolished it completely. Our results confirm the prediction that the LLGGP motif is essential for Fc,RI recognition (15). Indeed, an IgG4 mutant containing the ELLGGP motif was found to be as active as wild-type IgG1. Since only a single H chain is required for the Fc,RI interaction (13), it is likely that at least the “docking” portion of the binding site is located on a loop or a single flexible strand at the N terminus of the C_β_2 domain. Unfortunately, the protein segment containing the LLGGP sequence yielded no electron density in the x-ray crystallographic structure of human IgG1 Fc (28).

Interestingly, grafting of the ELLGGP motif onto IgG2 resulted in a molecule 4-fold more active than native IgG1. Although both the PLLGGP and the ELLGGP motifs restored activity to IgG2, only the ELLGGP sequence conferred full binding activity to IgG1. To rationalize these observations, it is necessary to propose the existence of "secondary" IgG2 Fc,RI binding sites, which are either nonexistent in IgG1 or are more active than their IgG1 counterparts. These sites probably reside in the IgG2 C_β_2 domain, since none of the shuffled IgG1 constructs displayed enhanced binding activity. IgG1 and IgG2 C_β_2 domains diverge at five residues in their C-terminal halves. Whether these divergent residues contribute to a “secondary” interaction site between C_β_2 and Fc,RI remains to be investigated.

We would like to thank Dr. Leroy Hood for providing the original y1, y2, and y4 C region gene segments and Cathy Horne for engineering the EcoRI site in the C_β_2-C_β_3 introns. We are also grateful to Dr. Aline Rinfret for providing the pSV2neoVg315 expression vector and William Bradley for synthesizing the oligonucleotides used in this study. We also thank Dr. AlexMarks for donating the MOPC 315.26 variant cell line. This work was supported by Grant MT-4259 from the Medical Research Council of Canada. D.E.I is the recipient of a Medical Research Council Grant (MT-7081) and M.S.C is the recipient of an Ontario Graduate Scholarship.