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The Differential Ability of Human IgG1 and IgG4 to Activate Complement Is Determined by the COOH-terminal Sequence of the Cα2 Domain

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Summary

Using domain switch chimeric antibodies, we confirm the important role of Cα2 in complement activation. In addition, we demonstrate that the structures responsible for the differential ability of human IgG1 and IgG4 to activate complement are located at the COOH-terminal part (from residue 292 to 340) of the Cα2 domain. The amino acids in Cα2 that might be involved in complement interaction are discussed. While Cα3 contributes to efficient complement activation, Cα3 from IgG2 and Cα3 IgG3 are equally effective.

The ability to activate the complement cascade and thereby remove pathogenic agents is one of the most important effector functions of the antibody molecule. IgM and IgG are the only isotypes that activate the classical complement pathway. The four human IgG isotypes have very similar amino acid sequences, but differ markedly in their ability to activate complement. IgG3 and IgG1 are effective in activating complement, IgG2 fixes complement poorly, and IgG4 appears completely deficient in the ability to activate complement (1, 2). An unresolved question is what amino acid differences determine this isotype-specific complement activation.

The hinge region shows the most sequence variation among the IgG isotypes (2, 3). The hinge serves to both covalently link the two heavy chains and to endow the antibody molecule with segmental flexibility. We and others have shown that a hinge region is essential for Clq binding and complement activation (3, 4). However, the flexibility of the hinge does not directly correlate with proficiency in complement activation, and replacing the rigid hinge of IgG4 with the flexible hinge of IgG3 did not result in an IgG4 molecule that can activate complement (3). Therefore, while the hinge region may modulate the ability of the IgG molecule to activate complement, it does not appear to determine the isotype-specific differences.

Studies using antibody fragments suggested that the Cα2 domain plays an important role in complement activation. Fab (IgG depleted of Cα3) and Cα2 fragments bind C1 and activate complement, while Fab and Cα3 fragments did not show any activity (5–7). Moreover, aglycosylated IgG lacking carbohydrate in Cα2 is unable to activate complement (8, 9). While these experiments indicate that Cα2 is primarily responsible for C1 binding and complement activation, Cα2 is not the only structure required. IgG that lacks Cα3 is only 50% as efficient in its ability to activate complement as intact IgG (5, 7). Moreover, while Glu 318, Lys 320, and Lys 322 in Cα2 have been shown in vitro mutagenesis to be important for complement activation (10), these residues are conserved among isotypes that do and do not activate complement, and thus could not determine the isotype-specific differences.

In this study, we have used domain exchange molecules to further investigate the residues responsible for the isotype-specific variation in complement activation. We confirm that Cα2 appears to be the domain important for complement activation. Furthermore, we provide evidence that the COOH-terminal part (residues 292–340) of the Cα2 domain contains the residues responsible for the isotype-specific differences in complement activation.

Materials and Methods

Construction of Chimeric IgG Molecules. The expressed Vλ and Vκ genes from the mouse antidansyl (DNS) hybridoma 27–44 were joined to human Cλ in the pSV2AHneo expression vector and to human IgG Cα in the pSV2ΔHgpt vector, respectively (11). As shown in Fig. 1, a novel PvuII site was generated between exons in the IgG2 and IgG3 constant region genes such that each gene contains one PvuII site. The Cα1 and Cα3 domain switch constant region genes were produced using clones with the appropriate PvuII sites. A PvuII site located between the hinge and Cα2 exons and a SacI site between the Cα2 and Cα3 exons were used to generate the Cα2 domain switch genes. IgG1 and IgG4 domain switch genes were generated using a SacI site located within the Cα2 exon. The domain switch heavy chain genes were cloned into the pSV2ΔHgpt expression vector and transfected with their specific
Figure 1. Schematic diagram of the domain switch chimeric antibodies. Up arrows (t) represent SacI sites and down arrows (1) represent PvuⅠsites, which were introduced by using oligonucleotide linkers. All heavy chain constant regions were joined to the murine antidansyl variable region. Mutants with exon exchanges are given a four-digit name: the first digit refers to the subclass of the C1 domains, and the second, third, and fourth digits indicate the subclasses of the hinge, C2, and C3 domains, respectively. WT indicates the wild-type gene.

light chain gene into the Ig nonproducing cell line, P3X63 Ag8.653, by either protoplast fusion or electroporation (12). The clones were selected in medium containing the antibiotic G418 and screened for antibody production by ELISA. Chimeric antidansyl antibodies were purified by affinity chromatography.

Complement-mediated Hemolysis. SRBC were coated with DNS/BSA and incubated with "Cr-sodium chromate (Amersham Corp., Arlington Heights, IL). The free "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, 0.15 mM CaCl2, 0.5 mM MgCl2, 0.1% gelatin, pH 7.4). Twofold serial dilutions of chimeric antibodies in Gel-HBS buffer were added to the round-bottomed, 96-well plate (Corning Glass Works, Corning, NY) in a volume of 50 μl. 50 μl of 2 U CHs of guinea pig complement (Colorado Serum Co., Denver, CO) 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 supernatant was counted in a gamma counter. The percentage of cell lysis was determined.

Complement Consumption Assay. To assay complement consumption, 8 μg of chimeric antibody was incubated with increasing amounts of DNS/BSA and 2 U CHs of complement at 37°C for 45 min. "Cr-loaded hemolysin-sensitized SRBC were then added and incubated for another 45 min. After centrifugation, the amount of released "Cr was determined. Antibody only, antigen only, buffer, and water controls were included in the experiments. The percentage of complement consumption was calculated as: 100x [(1 - cpm of Ag + Ab + complement)/(cpm of Ab + complement)].

Results

A set of mouse-human chimeric antibodies having identical antigen-combining sites and light chain but with constant region domains switched between IgG2 and IgG3 or IgG1 and IgG4 were generated as described above. Fig. 1 is a schematic diagram of the genetic structures of the antibody constant regions.

To locate the domain responsible for complement activation, domains were changed between IgG2 and IgG3, and...
The human IgG molecules differ markedly in their ability to activate complement in spite of having very similar amino acid sequences. The important role of the C₂ domain of IgG in complement activation was supported by earlier antibody fragmentation studies (5–7). The contribution by C₃ was also indicated with the finding that C₃ could stabilize the conformation of C₁ and protect it from attack by the C₁ inhibitor (13). The studies reported here show that it is the C₂ but not C₃ that determines the human IgG isotype-specific differences in complement activation. Moreover, our studies now locate the important residues to the COOH terminus of the C₂ domain between residues 292 and 340. Within that region, IgG₁ differs from IgG₄ at only four residues: 296 (Tyr vs. Phe), 327 (Ala vs. Gly), 330 (Ala vs. Ser), and 331 (Pro vs. Ser). The locations of these amino acids in the three-dimensional structure of IgG₁ Fc are shown in Fig. 4. Using the nomenclature of Beale and Feinstein (15), residues 330 and 331 are located on the Fy₃ β strand and fold into proximity with the previously identified 318-320-322 residues (10). Therefore, these residues together may provide the binding site for C₁q. The side chain of residue 327 is mostly buried inside the molecule and is probably not directly involved in C₁q binding. However, the greater flexibility afforded by the Gly residue in IgG₄ may change the conformation of the nearby C₁q binding site and thus affect complement activation. Residue 296 is located on the surface of X face. It seems unlikely that a C₁q molecule can bind the proposed binding site on Y face and residue 296 simultaneously. However, residue 296 in the other C₂ is quite accessible, as shown in Fig. 4, and might contribute to the C₁q binding site. The fact that both C₂ domains are required for C₁ activation gives indirect support for this possibility (5).

Noteworthy is the fact that the IgG(4-4-4/1-1) molecule is slightly deficient in its ability to activate complement relative to wild-type IgG₁. A significant difference between the two molecules is in the hinge region. It is quite possible that the rigid hinge of IgG₄ impedes access to the C₁q binding site thus decreasing the effectiveness of the molecule. We have observed a similar modulating effect of the hinge region on FcyRI binding (Canfield, S. M., and S. L. Morrison, manuscript submitted for publication). Alternatively, additional amino acid variation between the two isotypes within the C₂ NH₂ terminal to the exchange point may further influence complement activation.
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