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The interdependence of antibody C and V regions on specificity and affinity

Significant implications for the engineering of therapeutic antibodies

Joshua D Nosanchuk

Departments of Medicine and Microbiology & Immunology; Albert Einstein College of Medicine; Bronx, NY USA

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Since Paul Ehrlich coined the term "antibody" in 1891,[1] the study of these molecules has led to remarkable scientific discoveries that have revolutionized our therapeutic approach to patients. However, we clearly have much left to learn about the structure-function of these remarkable glycoproteins. A rich and exciting emerging area of investigation is breaking down a long-held belief that the variable (V) and constant (C) regions composing an immunoglobulin molecule are functionally independent.

What is the evidence for their historic consideration as distinct functional entities? RR Porter's 1959 paper applying papain to the characterization of immunoglobulin was the first to separate the molecule into what we know refer to as Fab and Fc domains[2] and this work, in part, led to his co-receipt of the 1972 Nobel Prize for Medicine. Subsequent to these findings, additional genetic and functional studies supported the concept of independence of function between the V and C regions, especially the fact that the specificity of an antibody was not seen as being impacted by isotype switching.

What is the evidence that the C region can influence affinity and specificity? This remarkable story was initiated by a 1991 paper by Kato and colleagues that applied ^{13}C NMR to the study of antibody switch variants to dansyl (5-dimethylaminonaphthalene-1-sulfonyl).[3] Most significantly, their data on antigen binding by switch variants with or without C region deletions strongly suggested that alterations in the C region impacted the conformation of both the heavy (VH) and light (VL) domains in the V region. In a 1993 paper assessing the role of heavy chain constant (CH) domains in isotype switch antibodies to N-acetyl-glucosamine (GlcNAc) residues in polysaccharide from group A streptococcus, Cooper and colleagues carefully described that IgG3 antibody bound more efficiently than IgG1 or IgG2a antibodies with identical V regions.[4] Along this line, in 2003, Michaelsen and colleagues described V region homologous isotype variable antibodies to *Neisseria meningitidis* with different binding activities that translated to significant differences in antibacterial potency.[5] Isotype has also been found to impact specificity, affinity, and antimicrobial activity in V region identical antibodies against fungi[6] and, most recently, HIV.[7] In studies on tubulin binding, Pritsch and coworkers identified four different isotype antibodies from a lymphoma patient with identical V regions that bound the same epitope, but were significantly different in affinity.[8] Hence, there is strong evidence that affinity and specificity can be significantly impacted by the C region.

However, the most extensive evidence for the C region impacting antibody affinity and specificity comes from Casadevall's group in a collection of papers clearly demonstrating that antibody interactions with the polysaccharide of *Cryptococcus neoformans* are affected by the C region.[9-14] Moreover, this work has provided a key mechanistic insight into the impact of isotype switched, V region identical antibodies. They applied NMR spectroscopy and fluorescence emission spectroscopy to probe the binding of a panel of antibodies to ^{15}N labeled peptides memetics to prove that the C region can alter the paratope and impact specificity.[15] In another study using small angle X-ray scattering, they demonstrated that isotype switch antibodies have significantly different domain orientations, which could affect antigen binding.[10] Independently, Correa and colleagues similarly found structural differences between V region identical, human IgA and IgG antibodies by crystallographic analyses.[16] The Casadevall group also found that C regions of DNA-binding antibodies impacted specificity and affected the secondary structure of the antibodies.[17,18]

The paper by Hubbard and colleagues is notable in that they extend the impact of the C region to chimeric mouse–human engineered antibodies to complex, multivalent antigens.[19] This group recently characterized affinity and protection efficacies of isotype switch variants of F26G3,[20] a murine IgG3 antibody to poly-glutamic acid (PGA) from the capsule of *Bacillus anthracis*.[21] In addition to certain toxins, PGA is an essential virulence factor of *B. anthracis*. In generating murine isotype switch variants, the Nevada group determined that altering the IgG3 to IgG1, IgG2a, or IgG2b

Correspondence to: Joshua D Nosanchuk; Email: josh.nosanchuk@einstein.yu.edu
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changed antibody binding, affinity, and protective efficacy.[20] Subsequently, in order to develop F26G3 for therapeutic use, chimeric IgG subclasses were engineered and characterized.[19] Significantly, the affinity of each isotype chimeric to PGA was reduced 9- to 20-fold compared with F26G3 and the pattern of binding to intact capsule was also significantly altered. There is remarkably little previously published demonstrating the impact of human C regions on the biological activities of chimeric antibodies to multivalent antigens such as PGA; however, the report on these antibodies to *B. anthracis* are consistent with that reported for chimerics to *C. neoformans* polysaccharide[13] in 2007 and to tumor-associated glycoprotein 72 (TAG72) in 1996.[22] Hence, there is sufficient data to consider that there is indeed a dynamic cooperative interplay between the C and V regions in regards to biological functions such as affinity and specificity.

It is thus essential that there be increased focus on the function of specific C regions in developing antibody therapeutics. This is further supported, for example, by Beehouwer and coworkers demonstration that V region identical human antibodies of different isotypes have significant differences in biological activity, particularly protective efficacy, against *C. neoformans*.[23] The areas of potential research are rich and varied, and include questions such as what are the key residues in the C region that affect V region biology, whether the V region influences C region biology (such as Fc engagement with receptors or complement activation), which portions of an antibody are especially important for protecting against or inducing autoimmunity, and does the C region influence isotype restriction? Given the interest in antibody-based therapeutics such questions are indeed important and their pursuit will surely result in exciting new fundamental and highly translational information.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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