Immunoglobulin isotype influences affinity and specificity

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For almost half a century the Ig molecule has been considered a bifunctional molecule consisting of two independent regions, a variable (V) region responsible for specificity and affinity, and a constant (C) region responsible for such effector functions as complement activation and interaction with Fc receptors. The origin of this view dates back to the Nobel Prize-winning work of Rodney R. Porter, who used proteolytic digestion in the late 1950s to cleave the Ab molecule into fragments that eventually were known as the antigen (Ag) binding fragment, or Fab, and the Fc domain, so named because it could be easily crystallized (1). Two decades later, the elucidation of the mechanism for the generation of Ab diversity supported this concept by showing that distinct genes encoding V and C regions were rearranged to express the Ig molecule (2). Additional evidence for the independence between V and C region roles came from the demonstration that isotype switching produced new effector functions, while preserving antibody specificity. Furthermore, studies done by Oi et al. (3), using fluorescent labels, failed to provide any evidence of interaction between the two regions. Consistent with this view, X-ray crystallographic studies of Fab fragments showed that V region sequences were separated from the first domain of the C region (CH1) by long polypeptide chains that lacked ordered structure and seemed to insulate the V regions from the C regions, while tethering the two regions into one molecule (4). However, this neat view of one molecule with two independent functional regions is at odds with several observations in the literature, and recent studies, including the study by Tudor et al. in PNAS (5), suggest that the basic model of Ig structure-function needs to be reconsidered and revised.

C Region Can Affect V Region

Evidence that the C region can affect V region structure and translate into differences in affinity and/or specificity has been accumulating for some time. In 1991, Kato et al. (6) labeled specific residues with ^{13}C in three murine Fab isotypes—IgG1, IgG2a, and IgG2b—and used NMR to study their positions before and after Ag binding. The results revealed significant differences between these Fabs upon Ag binding in the positions of two conserved residues in the light chain constant (CL) domain. These investigators also deleted the entire first domain of the heavy chain (CH1) from an IgG2a Fab and found significantly altered Ag binding. They suggested this was due to a conformational change in the CL domain upon losing its heavy chain binding partner, which translated into a conformational change in the heavy chain (VH) and light chain (VL) domains (6).

Two years later, a comparison of binding characteristics of a family of isotype switched Abs to a microbial polysaccharide suggested that changing the isotype altered Ab–Ag binding kinetics and thermodynamic parameters despite the presence of identical V region protein sequences (7). Several years later, IgG1 and IgA mAbs, as well as their Fabs, all with identical V regions binding the same epitope, were found to manifest differences in affinity (8). These observations failed to change the existing dogma, possibly because the field was reluctant to abandon a cherished paradigm without additional data. Alternatively, it is possible that a contribution from avidity differences was not unequivocally ruled out. However, in the past decade, a series of studies done with a family of V region identical isotype switched Abs to *Cryptococcus neoformans* polysaccharide using monovalent peptide mimetics provided strong evidence for C-mediated effects on V region function as manifested by isotype-related differences in specificity and affinity (9–12). In addition, two other groups, including the report by Tudor et al. (5), described additional examples in which Abs expressing identical V region sequences manifested altered specificity and/or affinity (13).

More focus on the role of Ig isotype may be critical in increasing the efficacy and application of immunoglobulins in developing therapeutics and diagnostics.

Given that five independent groups have now reported that C region can affect V region affinity and/or specificity (5, 7, 8, 10, 13), it is worthwhile to consider the profound implications of this phenomenon for humoral immunity. The ability of the C region to influence specificity could help explain isotype restriction for certain Ab responses, such as the preference for IgM/IgG3 and IgG2a in murine responses to polysaccharide Ags and viruses, respectively (14, 15). For example, a higher affinity or novel specificity found in an isotype switched B cell could lead to preferential binding and clonal expansion. In addition, the Ab idiotype (Id) for V region identical mAbs has been shown to be affected by the choice of C region, suggesting an explanation for isotype restriction in anti-Id responses (16). The fact that isotype switching can alter the affinity and/or specificity of an Ab implies that primary and secondary responses could originate from different B-cell populations and that isotype switching could lead to loss of reactivity for the original epitope while gaining novel specificity. In this regard, it is noteworthy that isotype switching of mAbs to fungal polysaccharide conferred reactivity with self-Ags (12), potentially implicating this phenomenon in the generation of autoimmunity, whereby autoreactive Abs also express isotype restriction (17). C region-mediated changes on V region structure could explain the phenomenon of isotype restriction of Id responses and the immunogenicity and tolerance of certain Ids (18). The realization that the C region can influence V region affinity and/or specificity has important implications for the engineering of Ab molecules and the choice of isotype in therapeutic Abs. It may also need to be considered in creating more effective vaccines.

The mechanism by which C region affects V region affinity and/or specificity is currently unknown. Given that C region can affect the Id, a plausible explanation is that differing C regions allosterically impose constraints on V region structure, paratope, and flexibility to differing degrees, upon binding Ag. Indirect evidence for this mechanism comes from circular dichroism studies showing the

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existence of a structural linkage between the V and C domains that can affect V region conformation upon binding Ag (19). It is noteworthy that not all isotype changes result in V region changes (20), and it is conceivable that the susceptibility of the V region to structural modification by the attached C region is a function of the V region used and/or somatic mutations that either facilitate or inhibit the transfer of structural information from C to V. Clearly, an entirely new facet of Ig structure is now apparent, and there is suddenly a tremendous amount of work to be done to better understand this critically important host defense molecule.

C Region Can Affect Viral Neutralization

In PNAS, Tudor et al. (5) explore the influence of the C region of two human mAbs to HIV gp41: an IgG1 and a monomeric IgA2, on Ag binding, HIV-1 transcytosis, $CD4^+$ T-cell infection and epitope mapping. The IgA2 bound native Ag tighter than the IgG1 and blocked HIV-1 transcytosis at much lower concentrations than IgG1. However, the IgG1 was more efficient in blocking HIV-1 transfer from LCs to $CD4^+$ T cells. Perhaps unexpectedly for Abs with similar specificity, the mAbs in combination synergistically blocked HIV-1 transfer to autologous T cells and reduced $CD4^+$ T-cell infection. Finally, epitope mapping studies showed that the 2F5 IgG1 recognized an additional epitope to that of the IgA2 on gp41. Because both the 2F5 IgG1 and IgA2 mAbs have three identical V (VL, CL, and VH) region domains, the difference in epitope mapping and Ag binding must originate from the CH domains. These studies suggest separate roles as well as combinatorial roles for the IgG and IgA response to HIV-1 infection. In this case, differences in C regions complement each other regarding the response to HIV-1 infection, transcytosis, and neutralization. This comparison of antiviral activity of two mAb isotypes shows that activity is regulated and influenced by the C region. Mucosal transmission is an important entry pathway for HIV-1 infection, and this study has important implications for vaccine design, suggesting the need to elicit both mucosal IgA responses to HIV-1 infection as well as IgG responses, because the synergistic effects of this combination are very powerful.

Finally, we note that the finding that the C region can affect V region affinity and/or specificity also has esthetic implications because the previously divided Ig molecule has now been made structurally whole again, and the mechanism of class switch recombination is reconciled with somatic mutation, because both impact the generation of Ab diversity and rely on the same molecular machinery. This has very important implications for the creation of more effective vaccines and immunotherapies for cancers and inflammatory and autoimmune diseases. More focus on the role of Ig isotype may be critical in increasing the efficacy and application of immunoglobulins in developing therapeutics and diagnostics.

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