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EDITORIAL

Isotype switching: Mouse IgG3 constant region drives increased affinity for polysaccharide antigens

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For many microbes, their polysaccharides (PS) are a critical part of their interaction with the mammalian immune system. Given their lower immunogenicity compared to protein peptides,[1] PS can provide protection against both opsonization and phagocytosis,[2,3] and in many cases actively protects bacteria against elements of both the innate and adaptive immune systems.[4-6] Many bacterial species have traditionally been defined by their capsular polysaccharides (CPS) and polysaccharide O-antigens. These two PS are often highly variable between bacterial strains (e.g.[7]). The biosynthesis of both CPS and O-antigens generally results in long chains (often 10s to 100s of saccharide units) that consist of repeats of a short oligosaccharide.[8] Although sugar moieties are poor substrates for eliciting an efficient adaptive immune response *in vivo*,[9] PS based vaccines have delivered highly effective protection to humans against a range of microbial infections.[10,11]

PS generally have to be administered conjugated to a carrier molecule (usually a protein) in order to engage T cells.[12] Indeed, many of the effective licensed vaccines couple PS to bacterial toxins that generally provide a strong adjuvant effect.[13] In the absence of a carrier, PS stimulate B cells independently by cross-linking antigen receptors. This produces an initial IgM mediated antibody response, which generally switches to an IgG response upon repeated boosting with antigen. The IgG response affords the greater part of the long term immunological memory to the antigen.[14]

The mouse model is used for the large majority of initial vaccination studies. It has a very long track record of success, and the large volume of data available on the murine immune response makes it ideal for comparative studies.[15] However, mice have known immunological differences compared to humans, and such discrepancies have the potential to confound some studies.[16] Mice have different IgG subclasses that respond differently to certain cytokine treatments and different IgG receptors compared to human.[17] Furthermore, mouse B cells tend to only switch antibody classes from IgM to IgG3 when stimulated with T-cell independent antigens. Humans, in contrast, tend to switch to the IgG2 subclass. Although the variable (antigen binding or F_{ab}) regions of the antibodies remain identical, the different constant regions (or F_c) that the subclasses provide have a significant effect not only on antibody avidity[18] but also on affinity.[19,20] The former largely depends on the different capabilities of F_c regions to multimerise *in vivo* and *in vitro*; while the latter depends on the effect F_c regions have on F_{ab}'s structural and conformational properties.[18,20,21] Understanding the nature of this change in avidity and/or affinity is important for understanding the likely impacts that antibody class switching will have on transplanting vaccines between species. Furthermore, it is essential for the use of "humanized" antibodies for passive vaccination, in which there is increasing interest with the rise of multiple antimicrobial resistant bacteria (e.g.,[22]).

In this issue of *Virulence*, Dillon et al.[23] demonstrate that class switching has a profound impact on IgG3 antibodies against the CPS of the globally distributed emerging human pathogen *Burkholderia pseudomallei*.[24-26] Interestingly, the CPS under investigation is an unusual polysaccharide, consisting of a linear homopolymer of a 2-*O*-acetyl-6-deoxy-heptopyranose.[27] As the simplest form of polymer, the potential number of antigen sites is very dense. Indeed, a hexamer conjugated to protein is sufficient to confer protection in a mouse model.[28] Dillon et al. demonstrate that an IgG3 anti-CPS antibody (3C5; composed of Vh6 and IgKV19/28 chains) raised against heat killed *B. pseudomallei* has a strong affinity for

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purified CPS. However, when this antibody switches to other classes, the affinity significantly drops, by an order of magnitude. In contrast, an independent IgG1 class monoclonal (2A5; composed of Vh6 and IgKV21 chains) raised against a conjugate of purified CPS and albumin (T cell dependent antigen) showed very similar affinity when switched to other IgG classes. These effects are mirrored in the activity of the antibodies on ELISA *in vitro*; however, whether the monoclonal will behave similarly under physiological conditions *in vivo* remains to be seen.

These results from Dillon et al. strongly suggest that the mouse IgG3 constant region provides a significant increase in antibody affinity against repetitive PS epitopes. Similar results have been observed with PS from *Streptococcus pyogenes*[29] and *Bacillus anthracis*.[30] Taken together, these results suggest that the constant region of mouse IgG3 acts to polymerize antibodies together to increase the avidity effect. This multimerization has been postulated to be non-covalent in nature.[31,32] However, it is known that human IgG2 (which like murine IgG3 usually responds to PS antigens) forms covalent dimers under some circumstances.[32] It is therefore remotely possible that covalent bonding might also be involved in the multimerization of mouse IgG3.

The above rationale does not preclude the possibility that part of this effect is caused by structural changes in the variable region induced specifically by the IgG3 constant region in 3C5 monoclonals. Indeed, 2 previous studies by the Scharff and Casadevall labs have shown that F_{ab} identical antibodies differing only in isotype class demonstrated differences in antigen specificity and affinity.[20] Further studies investigating whether the F_c region could impose conformational constraints on the F_{ab} to alter its epitope binding structure suggested that F_c regions could indeed do that in 2 independent experimental setups.[19,21] Dillon et al. prepared F_{ab} fragments of their 3C5 IgG3 antibody to address the possibility that the mouse IgG3 constant region causes specific secondary structure changes that affect affinity. These F_{ab} fragments, as expected, showed a further drop in affinity in comparison to the isotype switched antibodies, reflecting the loss of the avidity and /or affinity effect. Dillon et al.'s result still cannot exclude the involvement of the IgG3 F_c from affecting the conformational structure of the epitope binding region. However, the significant alteration in affinity suggests that the F_c multimerization effect provides at least a large part of the affinity advantage of the IgG3 isotype in this case.

These results have significant implications for the use of mouse IgG3 derived antibodies for passive immunization. Because the same effect is not seen in human IgG3, IgG3 derived antibodies are likely to have a significant loss of affinity and/or avidity after being humanized. Furthermore, this effect raises questions for the validity of the mouse model for T cell independent antigens. As the IgG3 subclass is preferred for these antigens in the mouse, likely because of this constant domain linking effect, the same immunological response may not be seen in humans or other model species. Arguably, the F_c class that behaves most like mouse IgG3 is human IgG2, and to a lesser extent human IgG1. Both human isotpyes show a response to PS immunization,[33] and both have been shown to covalently dimerize.[32,34] Accordingly, it would be very interesting in the future to observe the binding effect of a humanized 3C5 using either human IgG2 or IgG1 F_c regions. If both regions prove unsuccessful, it might be necessary to (chemically) modify human F_c regions to mimic the biochemical properties of murine IgG3 before the monoclonals could be utilized for human passive immunization.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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