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Preview

Friend or Foe? Turning a Host Defense Protein Into a Pathogen's Accomplice

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Cystatins are cysteine protease inhibitors that are at the front-line of defense against pathogens that secrete proteases as virulence factors. In this issue, Vincents et al. (2008) reveal how the bacterial protease IdeS from *Streptococcus pyogenes* hijacks normal cystatin C function to convert it into a cofactor that enhances proteolytic destruction of host-defense antibodies.

The human pathogen *Streptococcus pyogenes* (group A streptococcus) is a Gram-positive bacterium that is the etiological agent of diverse diseases ranging from scarlet fever to life-threatening conditions such as necrotizing fascitis. Successful infection by *S. pyogenes* depends upon its ability to counteract the host's immune responses. In particular, recognition of the bacteria by specific IgG antibodies results in its rapid elimination from human blood via complement activation and circulating innate immune cells.

Antibodies are therefore critical mediators of the humoral immune response that prime bacterial pathogens for elimination from the host. In response to these defense mechanisms, *S. pyogenes* has evolved multiple virulence determinants that permit the evasion of antibody-mediated immune responses. For example, *S. pyogenes* coats its surface with M-protein, a cell wall-anchored protein that binds the Fc region of antibodies, thereby blocking recognition by immune cells. In addition, the bacterium secretes the IgG-inactivating cysteine proteases SpeB and IdeS (von Pawel-Rammingen and Bjorck, 2003). Whereas SpeB is expressed late in infection and has activity against many substrates, IdeS is produced early in infection and exhibits exclusive specificity for the human IgG subtypes. Although both SpeB and IdeS cleave IgG at the same site to generate two stable Fab fragments and one Fc fragment, the expression pattern and substrate specificity of IdeS suggest that it is the major factor that protects *S. pyogenes* from immune targeting by IgG antibodies (von Pawel-Rammingen and Bjorck, 2003).

Consistent with this hypothesis, IdeS has been shown to reduce Fc-mediated phagocytic killing and improve survival of *S. pyogenes* in human blood (von Pawel-Rammingen et al., 2002; Lei et al., 2001). Furthermore, patients with *S. pyogenes* infections produce antibodies specific for IdeS that are capable of neutralizing its catalytic function (Lei et al., 2001; Akesson et al., 2006). Consequently, developing inhibitors of the IgG endopeptidase activity of IdeS may have therapeutic potential. A possible mode of inhibiting IdeS activity was suggested by the recent X-ray crystal structures of IdeS (Wenig et al., 2004; Agniswamy et al., 2006), which revealed that it adopts a canonical papain-like fold despite a lack of sequence homology with papain-like proteases. This observation suggests that cystatins, endogenous proteinaceous inhibitors that reversibly bind to the active site cleft of cysteine proteases, might serve as host defense factors that block the degradation of IgG by IdeS.

Cystatins have long been recognized as important mediators of innate immunity and have been shown to reduce the growth of a variety of viral, parasitic, and bacterial pathogens that deploy cysteine proteases as virulence factors (Dubin, 2005). However, in this issue of *Chemistry & Biology*, Vincents et al. (2008) find that *S. pyogenes* has devised an elegant solution to this host defense mechanism by creating a protease that can convert an inhibitor into a cofactor that stimulates rather than reduces its activity. The authors find that although most cystatins failed to significantly inhibit IgG cleavage by IdeS, human cystatin C unexpectedly enhanced IdeS function both in vitro and in human samples. This observation appears to be the first example of a protease inhibitor dually functioning as an inhibitor and activator and represents yet another mechanism by which bacterial pathogens co-opt host cell proteins to enhance their virulence.

Although determining the precise mechanism by which cystatin C promotes IdeS activity requires further experimentation, Vincents et al. (2008) present evidence indicating that cystatin C alters the conformational state and/or stoichiometry of IdeS. The cofactor effect of cystatin C was observed primarily at low concentrations of IdeS, and SPR experiments suggest that cystatin C improves IdeS dimerization, a process that has previously been reported to enhance protease activity (Agniswamy et al., 2006). Based on these observations, the authors propose a model in which binding of IdeS to cystatin C serves as a mechanism to compensate for the reduction in IdeS activity that occurs upon radial diffusion of the protease into bodily fluids (Figure 1). Stimulation of IdeS activity by cystatin C at these remote sites releases Fc fragments that could prime circulating neutrophils at a distance from *S. pyogenes* (Söderberg and von Pawel-Rammingen, 2008).

The identification of mutations in IdeS that reduce its interaction with cystatin C but that fail to disrupt IdeS catalytic function might allow this model to be tested in vivo. It would also be interesting to examine whether cystatin C binding to IdeS counteracts the neutralizing effect of antibodies specific for IdeS. Regardless, delineating the interfaces on both IdeS and cystatin C that mediate these interactions will undoubtedly provide

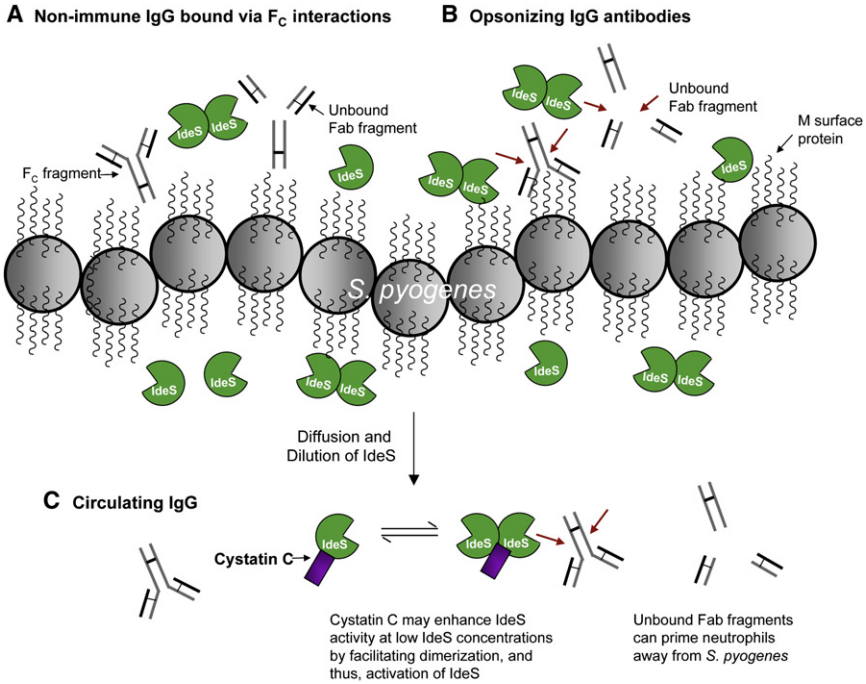

Figure 1. IdeS Inactivates IgG Antibodies in Mutiple Cellular Contexts
(A) On the surface of *S. pyogenes*, IdeS cleaves IgG bound to cell wall-anchored M and M-like protein through its Fc region, releasing unbound Fab fragments from the bacterial surface.
(B) IdeS inactivates opsonizing IgG antibodies bound to the bacterial surface proteins by cleaving the hinge region of IgG subtypes. As IdeS diffuses away from *S. pyogenes*, the dilution effect results in lowered IdeS activity, since IdeS functions optimally as a dimer and thus is concentration dependent.
(C) At remote sites, IdeS binding to cystatin C, the most abundant cystatin in the human body, induces a conformational change that enhances IdeS activity, allowing IdeS to cleave circulating IgG more efficiently. Inactivation of circulating IgG by IdeS may stimulate immune responses at a distance from *S. pyogenes*, since released Fc fragments have been shown to prime circulating neutrophils (Söderberg and von Pawel-Rammingen, 2008).

insight into how cystatin C can function both as a protease inhibitor and cofactor, and how IdeS functions as an endopeptidase specific for IgG. Intriguingly, both IdeS and cystatin C have been demonstrated to have multiple protein binding sites. In addition to recruiting cystatin C, IdeS binds IgG through an exosite interaction (Vincents et al., 2004) and integrins through its RGD motif (Lei et al., 2001). Similarly, cystatin C has been shown to form a ternary complex with papain and legumain; cystatin C binding to papain does not affect its interaction with legumain (Alvarez-Fernandez et al., 1999).

Since IdeS has proven resistant to most small molecule inhibitors that target its active site, the findings of Vincents et al. (2008) suggest that inhibitors that disrupt the interaction of IdeS with cystatin C or with itself might be a more effective therapeutic strategy. While it is still not clear whether cystatins may generally act as cofactors for other protease targets, this unexpected discovery should prompt closer scrutiny of the relationship between proteinaceous protease inhibitors and their target proteases.

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