Grasping the nettle: A bacterial invasin that targets immunoglobulin variable domains

DOI 10.1074/jbc.H118.002949

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Edited by Ursula Jakob

In a new paper, the protein InvD from *Yersinia pseudotuberculosis*, a zoonotic pathogen, is shown to assist late-stage invasion of intestinal epithelia. Remarkably, InvD acts by binding the Fab region of IgG or IgA. It straddles adjacent light-chain and heavy-chain variable domains, but its binding is different from that of antigens in that complementarity-determining regions do not participate. Structure determination revealed that its Fab-interacting domain adopts an immunoglobulin-like fold, fused to the preceding immunoglobulin-like domain and carried on a long stalk anchored to the bacterial outer membrane. Possible roles of this unusual host–pathogen interaction include avoidance of clearance from the intestine by secretory IgA.

Pathogens must devote significant resources to the challenge of colonizing their hosts, despite an array of passive and active immune safeguards. The concept of an “arms race” between would-be invader and defender nicely conveys the notion of a continuous struggle that pits a rapidly evolving simple organism against a much more slowly evolving and sophisticated one. Nowhere is this contest more fascinating than in the gut, where potentially dangerous intruders must be treated differently from legions of commensal bacteria. Studies of microbial immune evasion have revealed the wealth of strategies deployed by bacteria. These studies also yielded powerful biochemical tools, the promise of new antibiotics and vaccines, and a deeper understanding of host immunity.

There appears to be no facet of the immune system that cannot be blocked or hijacked. Immunoglobulins (Igs), the “guided missiles” of acquired immunity, are no exception; they can be captured by some bacteria and effectively disarmed. Disarmament is commonly achieved through binding to the Fc regions of Igs (Fig. 1). Normally, Igs use their Fab “arms” to bind antigens, after which their Fc “stems” trigger host responses such as phagocytosis and activation of complement. By binding to Fcs, microorganisms likely block access to the host proteins needed to trigger defenses. Note that B-cell receptors (BCRs) contain Igs bound to cells via their Fcs (Fig. 1).

In a new paper, Sadana et al. (1) describe the identification and 3D structure of another bacterial protein that binds IgG to assist colonization of the host, but that binds to Fab rather than Fc. InvD is one of many virulence factors produced by the zoonotic pathogen *Yersinia pseudotuberculosis*. Closely related to the plague-causing *Yersinia pestis*, and transmitted via the oral-fecal route, *Y. pseudotuberculosis* bacteria is able to invade and colonize its host by penetrating the epithelial cell layer of the intestine. It causes diseases in humans ranging from diarrhea and enteroocolitis to sequelae such as reactive arthritis and iritis.

InvD belongs to a subfamily of invasins (encoded by *InvA-InvE*) that consists of a bacterial outer-membrane anchor, a chain of bacterial Ig-like (Blg) domains, and a nonconserved C-terminal adhesion domain (AD). In a mouse model, the authors established that InvD expression favors bacterial viability in the intestine. Bacteria express InvD throughout the intestinal tract, but not in lymphoid tissue, and InvD is resistant to proteolysis. Expression of InvD is maximal at 37 °C and during the acute stage of infection. Taking these observations together suggested that InvD is important for late-stage invasion events such as colonization of the intestinal tract.

In pursuit of a ligand for the AD of InvD, the authors experimentally excluded as candidates several carbohydrates and lipids and all the proteins displayed on human epithelial-2 cells. They then noticed that InvD pulled down IgG and IgA by binding them with a *Kd* of 1–10 μM. Deletion of the AD eliminated binding. Critically, binding occurred only to Fab fragments and not to Fc fragments (Fig. 1). Moreover, InvD binds to mouse B-cells, presumably via Fabs of membrane-bound Igs within BCRs, but not to other immune cells.

Whereabouts in the Fab does InvD bind? The authors focused on the two variable (V) domains, one from each of the light and heavy chains (Fig. 1). V domains contain segments of very high-sequence variation, called complementarity-determining regions (CDRs), which recognize epitopes (Fig. 1). Beyond the CDRs, there is more conservation among V domains, allowing their classification as VH1, VH2 etc. for heavy-chain, and VK1, VK2 etc. for light-chain V domains. Using phage display, the authors scanned multiple versions of an engineered protein in which these two domains are linked (a single-chain (sc)Fv (Fig. 1)). They found preferential binding of a VH3/VK1 combination (although not all VH3/VK1 combinations bound to InvD), but no preference for any CDR sequence. VH3 and VK1 are the most abundant families in the human germ line repertoire. The authors therefore provided strong evidence that InvD binds via its AD to a common class of IgGs and IgAs, but does not form a canonical antibody–antigen complex. InvD thus joins a select group of V domain binders that...
includes HIV gp120 (2), protein L from Peptostreptococcus spp. (PpL) (3) and SpA from Staphylococcus aureus (that binds to sites in Fc and Fab) (4). Whether or not the constant domains of the Fab also contribute to binding is not addressed directly by the authors.

In further work the authors solved the crystal structure of the AD along with the two Blg domains preceding it. Unlike the ADs of other invasins, the InvD AD has an Ig-like fold but with extensive variations and insertions. The AD seems unstable alone and likely forms a “superdomain” with the tightly associated adjacent Blg domain. An obvious next step would be to solve a structure of its complex with a VH3/VK1 Ig or scFv.

What advantage does this unusual interaction afford the bacterium? Several explanations can be offered. InvD (like other V domain binders such as gp120, PpL, and SpA) might act as a “superantigen,” binding to a subpopulation of B-cells via their BCRs (Fig. 1) with consequences such as B-cell proliferation, activation, or apoptosis (5). The AD is likely projected 70–80 nm clear of the bacterial surface by an extended but flexible stalk of 12 Blg domains. This suggests that the AD could intercept incoming VH3/VK1 Igs and keep them at a distance from the bacterial membrane. Here they might serve as decoys, perhaps triggering “futile” complement activation that depletes complement proteins. It is, however, in the intestine that InvD is most highly expressed, and, here, dimeric secretory IgAs (SlgAs) (Fig. 1) provide a first line of defense. SlgA-mediated agglutination of bacterial cells causes entrapment in mucus and/or clearance via peristalsis (6). AD-captured IgA molecules might be prone to cross-linking locally between adjacent, appropriately spaced InvD molecules harmlessly on the same cell rather than cross-linking different cells. Alternatively, InvD-mediated binding to IgA could enhance translocation of Yersinia across the epithelial barrier. We await with interest the outcome of future efforts to provide the full story behind this intriguing example of a bacterial hijack of host immunity.

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