Commentary

Modulating Phagocyte Activation: The Pros and Cons of *Helicobacter pylori* Virulence Factors

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Helicobacter pylori (Hp) is a highly successful human pathogen that has colonized the gastric mucosa of approximately half of the world's population. Infection with this gram-negative bacterium induces a state of chronic inflammation that does not resolve the underlying infection and often leads to gastric or duodenal ulcers or more rarely to gastric adenocarcinoma or mucosa-associated lymphoid tissue (MALT) lymphoma. A characteristic feature of Hp-induced inflammation is the massive recruitment of phagocytes (particularly neutrophils) to the gastric mucosa, and it is generally believed that the ensuing tissue damage is due to the combined effects of bacterial factors and host inflammatory mediators. Multiple bacterial virulence factors are known to modulate Hp-induced inflammation, including LPS, PicB, urease, and the vacuolating cytotoxin VacA (1). Of particular interest in this regard is a recently identified virulence factor called Hp neutrophil-activating protein (HP-NAP). HP-NAP is a 150-kD dodecameric iron-binding protein that promotes adhesion of PMNs to endothelial cells (2, 3). In this issue, Satin et al. demonstrate that purified recombinant HP-NAP is a highly antigenic protein that stimulates phagocyte chemotaxis, NADPH oxidase assembly, and production of reactive oxygen species (ROS) (4). Interestingly, all Hp strains examined thus far carry the *napA* gene; however, protein expression is variable and the mechanism by which *napA* expression is regulated is unknown (2). That not all Hp produce HP-NAP is significant, as Hp strains have generally been divided into two groups (5). Type I strains are associated with phagocyte infiltration and tissue damage and are commonly found in persons with ulcer disease. In contrast, type II strains induce much less inflammation and are associated with asymptomatic infection. Moreover, our laboratory has recently demonstrated that type I Hp resist phagocytic killing and persist inside macrophages, whereas type II Hp do not (6). Collectively, the available data suggest that Hp has evolved a unique ability to stimulate certain phagocyte functions while inhibiting others. Here, the features of Hp virulence factors that individually and collectively contribute to the ability of Hp to activate phagocytes yet avoid clearance by the host immune response are discussed.

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Phagocyte Recruitment in Response to Hp

In response to chemotactic agents, mononuclear phagocytes and PMNs upregulate surface adhesion receptors and are recruited from the bloodstream to sites of infection. During the course of a normal infection, PMNs are recruited early, macrophages are more abundant at later times, and phagocyte numbers decline upon elimination of the invading microbe. The fact that neutrophils consistently outnumber macrophages in the Hp-infected stomach suggests that Hp induces a state of "chronic acute inflammation." Moreover, the available data suggest that phagocyte recruitment is directly modulated by HP-NAP and factors secreted by the Type IV organelle (see below) encoded by the *cag* pathogenicity island (*cag* PAI) (1, 2, 4, 7, 8).

HP-NAP was originally identified as a component of Hp outer membranes that stimulated PMN attachment to endothelial cells (2). Satin et al. now demonstrate that purified HP-NAP induces adhesion and chemotaxis of both mononuclear phagocytes and PMNs by upregulating adhesion receptors of the β2 integrin family (4). The biochemical data indicate that HP-NAP oligomers are found both in the cytosol and on the bacterial surface; however, HP-NAP lacks NH_2- or COOH-terminal signals that would confer export via the general *sec*-dependent pathway or an ATP-binding cassette transporter (Type II and Type I secretion, respectively) (9). Consequently, it is not known how HP-NAP reaches the outer membrane. Significantly, Hp urease, another oligomeric virulence factor located in the cytosol and on the bacterial surface, is thought to be adsorbed onto the bacterial surface after "altruistic lysis" of neighboring organisms (10). Moreover, surface urease is essential for colonization because it generates ammonia to buffer Hp as it passes through the highly acidic gastric lumen (10). Although altruistic lysis appears to be a somewhat arbitrary mechanism for targeting essential proteins to the outer membrane, it is tempting to speculate that the

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unique surface properties of Hp support binding and retention of released HP-NAP and urease, and that this is important for bacterial persistence and ulcer formation. Although mutants lacking HP-NAP have not yet been characterized, the potency of this protein suggests that it is a key player in mediating phagocyte recruitment during Hp infection. In this regard, it will be of interest to determine whether HP-NAP is chemotactic for murine phagocytes, since PMN recruitment to the stomach is much less pronounced in mouse models of Hp infection (11).

One distinguishing feature of type I Hp is the presence of the *cag* PAI that encodes a Type IV secretion system (1, 8). Like Type III secretion systems, Type IV organelles are found in virulent strains of some gram-negative bacteria, and these large complexes of cytosolic, inner membrane, and outer membrane proteins function as contact-dependent molecular syringes to transport proteins directly into host cells. Tight binding of type I Hp to the epithelium induces synthesis and secretion of the neutrophil chemotactic agent IL-8 via a nuclear factor (NF)-κB–dependent mechanism, and Hp strains with mutations in PAI genes such as *picB/cagE* are impaired in their ability to induce inflammation (12). Thus, it is likely that contact-dependent secretion of one or more factors transported by the Type IV apparatus directly promotes PMN activation and migration to the infected stomach.

At least two scenarios can be envisioned to explain the dramatic difference in the ability of type I and type II Hp to stimulate phagocyte migration to the gastric mucosa. In one scenario, HP-NAP is responsible for the basal phagocyte recruitment to the gastric mucosa observed with all strains of Hp, whereas signals provided by the Type IV secretion apparatus recruit additional PMNs after colonization by type I organisms. In a second scenario, HP-NAP and the *cag* PAI may be coordinately expressed by type I Hp to maximize phagocyte influx, whereas other factors common to all Hp (such as LPS and urease) may regulate phagocyte recruitment induced by type II organisms. Additional scenarios that allow for different levels of HP-NAP expression are also possible.

Phagocyte Activation

Activated phagocytes exhibit an increased capacity to ingest and kill microorganisms. In part, phagocyte activation stimulates assembly of the NADPH oxidase complex and the respiratory burst. One key feature of the NADPH oxidase is that the enzyme is unassembled and inactive in resting cells. Upon stimulation, the cytosolic components $p47^{phox}$, $p67^{phox}$, $p40^{phox}$, and rac2 translocate to the membrane and bind cytochrome b558 ($gp91^{phox}/p22^{phox}$). Oxidase activation is triggered by receptor–ligand interactions, and the nature of the receptor engaged determines the site of oxidant production (13). Specifically, soluble stimuli such as FMLP and PMA promote oxidase assembly at the plasma membrane and rapid release of superoxide into the extracellular milieu. In contrast, phagocytic particles engaging Fcγ receptors (FcRs) stimulate oxidase assembly on forming phagosomes, and the subsequent concentration of superoxide and hydrogen peroxide inside the phagocytic vacuole maximizes toxicity to the ingested microbe and minimizes damage to host tissues.

A key feature of some but not all strains of Hp is their ability to bind to and activate PMNs and stimulate rapid and robust production of ROS comparable to that induced by PMA (14, 15). Although the site of NADPH oxidase assembly was not determined in these studies, the speed and magnitude of the response suggest that the oxidase was targeted to the plasma membrane rather than the phagosome. Consistent with this hypothesis, a growing body of evidence suggests that type I Hp delay their entry into phagocytes (see below), and this delay may be essential for Hp to target NADPH oxidase subunits to the cell surface. In the current work, Satin et al. (4) clearly show that purified HP-NAP is sufficient to induce oxidase assembly at the plasma membrane and a strong respiratory burst in both monocytes and PMNs. Interestingly, surface urease also plays a key role in modulating the respiratory burst; and type I Hp with surface urease bind PMNs and induce a strong respiratory burst by a mechanism that is independent of ammonia production, whereas isogenic *ureB* mutants do not (16). Collectively, the data suggest that coordinate adsorption of HP-NAP and urease on the surface of type I Hp is essential for these organisms to induce NADPH oxidase assembly at the plasma membrane, extracellular release of ROS, and ultimately ulcer formation. Although host tissue is damaged, Hp is protected from the toxic effects of ROS by superoxide dismutase and catalase (1).

In contrast, other strains of Hp bind poorly to PMNs and do not stimulate production of ROS (14, 15). Binding and phagocytosis of these less virulent organisms are enhanced after exposure to serum opsonins. However, opsonization is not sufficient to induce robust release of ROS from PMNs, and these strains induce a weak and delayed respiratory burst that likely reflects oxidase assembly on the phagosome membrane (14, 15). Significantly, type II Hp bind poorly to PMNs in the absence of opsonins (6), but whether these less stimulatory strains are exclusively type II Hp remains to be determined.

Although the combined effects of the *cag* PAI, urease, and HP-NAP may suggest that Hp infection induces global phagocyte activation, the effects of Hp LPS (endotoxin) argue against this hypothesis. In contrast to LPS derived from *Escherichia coli* or *Salmonella*, Hp LPS is at least 1,000-fold less potent (17). Therefore, macrophage activation and production of cytokines such as IL-1, IL-6, and TNF-α are limited during Hp infection (17). This may be explained in part by the unusual structure of Hp LPS and its impaired ability to bind LPS binding protein and CD14 (18). Consequently, we would argue that Hp uses its virulence factors to specifically stimulate the respiratory burst at a site of infection where levels of phagocyte-activating cytokines are otherwise limiting.

Modulation of Phagocytosis and the Intraphagosome Environment

The goal of phagocytes is to ingest and kill invading microbes, and killing usually occurs as a result of the com-

bined effects of ROS, phagosome acidification, and lysosomal proteases. Phagocytosis is triggered when specific receptors on the phagocyte bind ligands on the microbe surface. Importantly, the receptors engaged during phagocytosis and subsequent signaling events modulate induction of the respiratory burst, phagosome–lysosome fusion, and consequently, the fate of the ingested microorganism. One hallmark of intracellular pathogens is their ability to control their fate inside phagocytes. On the other hand, the ability of extracellular pathogens to modify the intraphagosomal environment is less clear. Therefore, it is significant that at least some strains of Hp resist phagocytic killing by both macrophages and PMNs (6, 15, 19). Moreover, we hypothesize that avoidance of phagocytic killing is intimately linked to the ability of Hp to delay their entry into phagocytes and that this altered rate of uptake is also essential for NAP-induced activation of plasma membrane NADPH oxidase.

Although the receptor(s) that mediate ingestion of Hp have not yet been identified, data from several laboratories suggest that proteins on the surface of some strains of Hp allow these organisms to regulate their uptake into phagocytes. First, as noted above, individual strains of Hp differ in their ability to bind to PMNs although the reasons for these differences have not been defined (6, 20). Second, tight binding of Hp to PMNs correlates with abundant surface urease, retarded phagocytosis, and strong respiratory burst (16). Third, urease prevents deposition of serum opsonins onto Hp, thereby precluding rapid ingestion after engagement of FcRs and CR3 (21). Fourth, opsonins reduce the amount of ROS produced by activating strains of Hp (14, 15). Fifth, unopsonized type I and type II Hp bind to macrophages; however, type I organisms exhibit delayed phagocytosis that is sensitive to chloramphenicol, whereas type II Hp are rapidly ingested (6). Sixth, delayed phagocytosis is linked to intracellular survival, since type I Hp persist inside macrophages within a novel vacuole called a megasome, whereas rapidly ingested type II strains do not (6). It is unlikely that NAP-mediated diversion of the NADPH oxidase away from the phagosome is required for intraphagosomal survival of Hp; however, we believe that delayed ingestion of Hp is essential for both NAP-mediated signaling and subsequent modification of the phagosome. Consequently, ROS-induced tissue damage and persistence of Hp are inherently linked, and both may be essential for Hp pathogenesis.

Conclusions

Hp is a highly successful human pathogen that persists in the gastric mucosa. In this niche, bacteria usually thrive for many years in spite of the host immune response. A growing body of evidence suggests that Hp utilizes a broad array of virulence factors to modulate its interactions with both epithelial cells and phagocytes. Of particular interest in this regard is the ability of type I Hp to both modulate its entry into phagocytes and at the same time induce extensive tis-

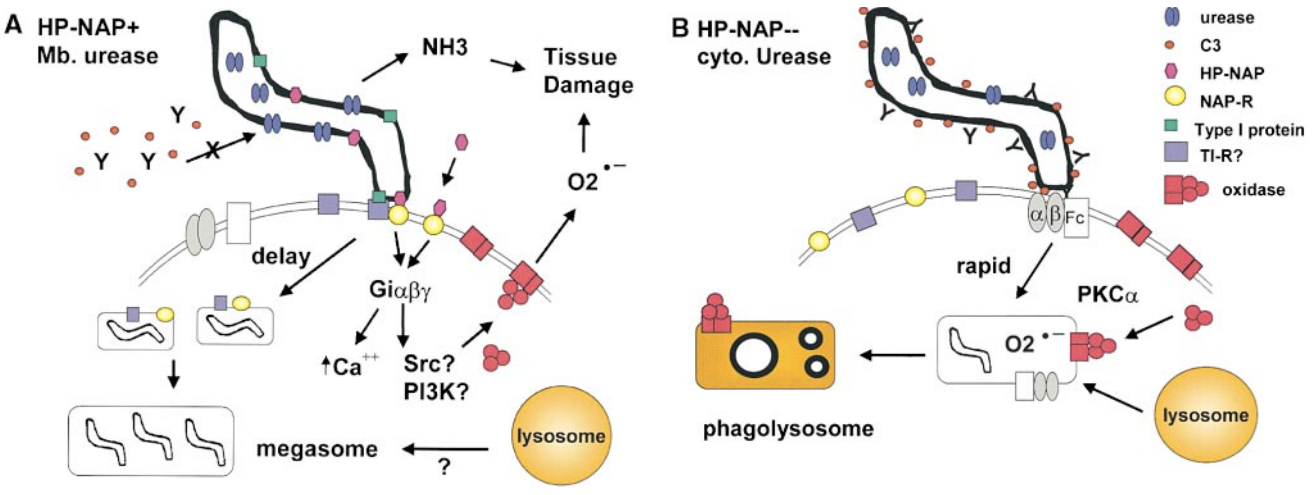

Figure 1. Modulation of Hp–phagocyte interactions by HP-NAP, urease, and other virulence factors. (A) Type I/HP-NAP$^+$/surface urease$^+$ organisms. Surface urease (blue dimers) prevents opsonization with C3 and antibodies (red circles and Ys), thereby preventing phagocytosis via FcRs (white rectangles) and CR3 (gray dimers). An unidentified protein (green squares) allows Hp to bind tightly to the phagocyte via an unidentified receptor (light purple squares). Signaling from this receptor induces phagocytosis after a delay of several minutes. Meanwhile, HP-NAP (pink hexagons), and perhaps free NAP released after bacterial lysis, engage a G protein–coupled receptor (yellow circles) triggering translocation of p47/p67/p47^{phox} (dark pink circles) to the plasma membrane by a Src and phosphatidylinositol 3-kinase–dependent mechanism (reference 4). Ammonia generated by urease and superoxide generated by the assembled NADPH oxidase (dark pink) damage host tissue. After ingestion and phagosome–phagosome fusion, Hp persist inside megasomes that resist fusion with lysosomes (reference 6). Mb., membrane. (B) Type II/HP-NAP$^-$/surface urease$^-$ organisms. In the absence of surface urease and NAP, Hp is rapidly phagocytosed after opsonization with C3 and IgG, and does not stimulate oxidase assembly at the plasma membrane. Activation of protein kinase Cα (PKCα) downstream of FcRs and CR3 stimulates NADPH oxidase assembly on the phagosome and subsequent phagosome–lysosome fusion (references 22, 23). Megasomes do not form, and Hp is killed and digested in phagolysosomes. cyto., cytoplasm.

sue damage by specifically activating the NADPH oxidase. Although all of the players have not yet been identified, the available data clearly indicate a key role for HP-NAP, urease, and other factors associated with type I organisms in Hp-induced tissue damage and persistence. A possible model that integrates the effects of these virulence factors is shown in Fig. 1.

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