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Finding a new way to calm the storm

Glen McGillivray and Lauren O. Bakaletz*

The Research Institute at Nationwide Children's Hospital, Center for Microbial Pathogenesis, The Ohio State University College of Medicine, Columbus, Ohio 43205

The inflammatory response is a critical mechanism used by multicellular organisms to protect themselves from cellular damage caused by microorganisms or other stimuli. In a dysregulated state, inflammation can also contribute to the chronicity of debilitating conditions such as cystic fibrosis, Crohn's disease, and asthma.¹ In a recent study, Pingel *et al.* have provided evidence of a novel mechanism used by the host to dampen the inflammatory response observed during periodontal disease (Fig. 1) that can result due to the presence of pro-inflammatory bacterial molecules. This dampening process is likely to contribute to maintaining tissue homeostasis² and might further lend itself to manipulation in order to better treat this painful, debilitating, and unsightly condition.

Both the host, and microbes that colonize its mucosal surfaces, have developed remarkable strategies to control and limit the inflammatory response, each to their own benefit (Fig. 2). *Porphyromonas gingivalis* is a predominant etiological agent of periodontal disease, a chronic polymicrobial inflammatory disease of the gingiva and surrounding tissue that ultimately leads to tooth loss. This microorganism expresses an impressive array of proteases, destructive enzymes, and adhesins such as the hemagglutinin B (HagB) protein.³ In addition, *P. gingivalis* utilizes several means to evade the immune response such as production of lipopolysaccharide (LPS) that is significantly less inflammatory than LPS of other microorganisms of the oral cavity.³

Inflammation is induced when stimuli such as bacterial LPS, lipotechoic acid, or muramyl dipetide binds to host cell receptors, which subsequently initiates a series of signal-transducing events that culminate in the increased expression of pro-inflammatory molecules, such as the lipid-derived leukotrienes and specific cytokines. At several key points in this process, mechanisms are employed by the host to maintain tight control of inflammation and thereby avoid a dysregulated response. Inflammation can be modulated by affecting the expression of genes that encode inflammatory mediators and altering the stability of encoded mRNAs for some of these molecules, among others.¹ In addition, the host can also produce negative regulators of cytosolic signal transduction proteins, control the amount and location of surface receptors that engage inflammatory stimuli, and secrete molecules that bind stimulants before they engage signal-transducing receptors.⁴ As an example of the latter mechanism, a small number of human antimicrobial peptides (APs) can bind and neutralize bacterial LPS, thus dampening the inflammatory response at the source of the stimulus.⁵

Most APs are small (18 – 45 amino acids), cationic proteins of the innate immune system that are classified into two major families, the defensins and the cathelicidins. These proteins are produced by epithelial cells of the respiratory and oral mucosa and typically have anti-

Corresponding author information: Center for Microbial Pathogenesis, The Research Institute at Nationwide Children's Hospital, Rm. W591, The Ohio State University College of Medicine, 700 Children's Drive, Columbus, OH 43205-2696, Phone: (614) 722-2915, Fax: (614) 722-2818, Lauren.Bakaletz@nationwidechildrens.org.

viral, anti-fungal, and/or anti-bacterial properties.⁵ It has been suggested that APs be renamed 'host defense peptides' which summarizes the growing appreciation for the diverse and multi-functional activities that these proteins possess beyond their microbicidal properties. For instance, human beta-defensin-3 (hBD-3) is chemotactic for, and activates, specific subsets of monocytes and dendritic cells, professional antigen-presenting cells whose activities provide a bridge between the innate and adaptive arms of the immune system.⁶

Now, Pingel *et al.* have uncovered evidence that hBD-3 possesses a novel activity whereby by targeting and binding to a potent bacterial activator of the inflammatory response, it can abrogate this effect.² The authors demonstrated by biosensor analysis that hBD-3 bound to a recombinant form of the *P. gingivalis* non-fimbrial adhesin HagB (rHagB). Neither human beta-defensin-1 nor human beta-defensin-2 interacted with this adhesin, which showed that the ability to bind rHagB was not a general property shared indiscriminately by other APs of this family. It will be interesting to uncover the mechanism behind the strong selective binding to rHagB by hBD-3 specifically, given the extensive structural similarities among the numerous human defensins.

What is the effect of rHagB alone, or the interaction between rHagB and hBD-3, on the inflammatory response of cells cultured *in vitro*? In the cited study, both oral keratinocytes and myeloid dendritic cells increased expression of pro-inflammatory cytokines in the presence of rHagB compared to cells treated with PBS alone. In contrast, out of a panel of 22 cytokines tested, dendritic cells incubated in the presence of both rHagB and hBD-3 produced significantly reduced amounts of the pro-inflammatory cytokines IL-6, IL-8, and TNF- α . To begin to unravel the molecular pathways responsible for this dampened inflammatory response, the authors demonstrated that expression of the extracellular signal-regulated kinases (ERK 1/2), but not p38 or c-Jun N-terminal kinases, was diminished in dendritic cells incubated with hBD-3 and rHagB when compared to cells incubated with rHagB alone. An exciting question that remains is what is the ultimate fate of the hBD-3-rHagB 'complexes'? And, if they are to be degraded, what are the cellular pathways involved in this process?

While the study by Pingel *et al.* has been discussed primarily from the standpoint of host-derived benefits gained from maintaining tight control of inflammation, for normal flora (and even those that can behave as opportunistic pathogens when conditions are optimal for them to do so), keeping the host inflammatory response at bay can have distinct advantages.² Once a disease process is initiated, members of the normal bacterial flora, which are generally well-tolerated by their mammalian hosts, are now targeted for elimination as part of the immune response. To avoid immune-mediated clearance, bacteria have evolved countermeasures for immune evasion. One recent example is the ability of nontypeable *Haemophilus influenzae* (another commensal opportunist) to decorate the endotoxin contained in its outer membrane with phosphorylcholine stolen from its host. Such decoration not only decreases the early inflammatory response, but also delays clearance of this bacterium from the airway.⁷⁻⁹

Collectively, the data presented in the study by Pingel *et al.* also provide evidence that dysregulation of hBD-3 could be associated with the severe inflammation observed in periodontal disease and, conversely, that therapeutic intervention via exogenous delivery of hBD-3 may provide a benefit to those who suffer from this chronic infection. Important litmus tests for this hypothesis will be to determine if hBD-3 can reduce the inflammatory response of dendritic cells incubated with *P. gingivalis* that express native HagB, and also to assess the ability of this AP to reduce HagB-induced inflammation in animal models of periodontal disease.

Literature Cited

1. Han J, Ulevitch RJ. Limiting inflammatory responses during activation of innate immunity. *Nat Immunol.* 2005; 6:1198–205. [PubMed: 16369559]
2. Pingel LC, Kohlgraf KG, Hansen CJ, Eastman CG, Dietrich DE, Burnell K, et al. Human beta-defensin 3 binds to hemagglutinin B (rHagB), a non-fimbrial adhesin from *Porphyromonas gingivalis*, and attenuates a pro-inflammatory cytokine response. *Immunology and Cell Biology.* 2008 doi:xxxxxxx.
3. Holt SC, Kesavalu L, Walker S, Genco CA. Virulence factors of *Porphyromonas gingivalis*. *Periodontol 2000.* 1999; 20:168–238. [PubMed: 10522227]
4. Abreu MT, Fukata M, Arditi M. TLR signaling in the gut in health and disease. *J Immunol.* 2005; 174:4453–60. [PubMed: 15814663]
5. Lehrer RI. Primate defensins. *Nat Rev Microbiol.* 2004; 2:727–38. [PubMed: 15372083]
6. Dhople V, Krukemeyer A, Ramamoorthy A. The human beta-defensin-3, an antibacterial peptide with multiple biological functions. *Biochim Biophys Acta.* 2006; 1758:1499–512. [PubMed: 16978580]
7. West-Barnette S, Rockel A, Swords WE. Biofilm growth increases phosphorylcholine content and decreases potency of nontypeable *Haemophilus influenzae* endotoxins. *Infect Immun.* 2006; 74:1828–36. [PubMed: 16495557]
8. Hong W, Mason K, Jurcisek J, Novotny L, Bakaletz LO, Swords WE. Phosphorylcholine decreases early inflammation and promotes the establishment of stable biofilm communities of nontypeable *Haemophilus influenzae* strain 86-028NP in a chinchilla model of otitis media. *Infect Immun.* 2007; 75:958–65. [PubMed: 17130253]
9. Pang B, Winn D, Johnson R, Hong W, West-Barnette S, Kock N, et al. Lipooligosaccharides containing phosphorylcholine delay pulmonary clearance of nontypeable *Haemophilus influenzae*. *Infect Immun.* 2008; 76:2037–43. [PubMed: 18347044]

Figure 1.
Advanced periodontitis
<http://www.enexus.com/gumdisease/>

Figure 2.

List of mechanisms used by the host and bacteria to dampen the inflammatory response. The highlighted text emphasizes the host strategy used to control inflammation as discussed in the manuscript by Pingel *et al.*.