Secreted Bacterial Vesicles as Good Samaritans

Meta J. Kuehn[1,*]
[1]Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, USA
*Correspondence: meta.kuehn@duke.edu
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Bacteriodes fragilis and polysaccharide A capsular antigen (PSA) produced by this commensal bacteria can mediate immune tolerance in the gastrointestinal (GI) tract. When looking for naturally secreted forms of PSA, Shen et al. (2012) suprisingly found that PSA is packaged in outer membrane vesicles (OMVs), bacterial blebs with a disreputable past.

Polysaccharide A capsular antigen (PSA) produced by the commensal *Bacterioides fragilis* has immunomodulatory effects and treatment with *B. fragilis* or PSA alone can induce immune tolerance, a condition critical to preventing inflammatory bowel disease (IBD) and Crohn's disease (Mazmanian et al., 2008; Round and Mazmanian, 2010). In this issue, Mazmanian and colleagues extend these findings and show that outer membrane vesicles (OMVs) derived from *B. fragilis* are the delivery device by which PSA is transported to dendritic cells (DCs) to favorably modulate the immune system (Shen et al., 2012). They further delineate the key messengers in the process—TLR2-mediated OMV-PSA recognition and Gadd45α signaling in DCs, production of the immunoregulatory cytokine IL-10 by DCs and Tregs (T regulatory cells), and the consequent prevention of T helper cell proliferation and production of inflammatory mediators (Figure 1). The authors not only demonstrate these processes using in vitro assays but also show results using an animal model of experimental colitis that are impressively consistent with this pathway.

OMVs are well on their way to becoming widely recognized for being ubiquitously produced by pathogens and nonpathogens alike (Deatherage and Cookson, 2012; Kulp and Kuehn, 2010). However, OMVs have not always enjoyed serious or benevolent consideration. For some time they were deemed as products of bacterial lysis or death. Shortcomings in mechanistic details have plagued the OMV field, only now emerging as a bona fide secretion mechanism (McBroom et al., 2006). OMV production captures both the insoluble outermost membrane and soluble portions of lumenal content; thus, in the case of Gram-negative

bacteria, the vesicles are spherical portions of outer membrane with lumenal periplasmic content. Additionally, based on the organisms for which they have been studied and the molecules they transport, OMVs are typically considered virulence factors (Ellis and Kuehn, 2010). In cases when bacterial toxins are attached to the outer bacterial membrane, such as for cytolysin A (ClyA) in uropathogenic *Escherichia coli*, hemolysin (HlyA) in extraintestinal pathogenic *E. coli,* or heat-labile enterotoxin (LT) in enterotoxigenic *E. coli*, these toxins are also associated with the external OMV surface. Other notable OMV cargo include peptidoglycan, flagellin, lipopolysaccharide (LPS), and CpG DNA, pathogen-associated molecular patterns (PAMPs) that can significantly activate inflammatory immune responses. Therefore, by describing their benefit to the host in reducing harmful inflammation, Shen et al. introduce a new paradigm to the field.

Shen et al. discuss several questions evoked by their study. Notably, what are the details of the internal signaling pathway that result in IL-10 activation by PSA[+] OMVs? They have begun to address this using microarray results and identify differences in transcriptional profiles of specific genes in TLR2+/+ and −/− bone marrow-derived DCs that have been activated with either PSA[+] OMVs or ΔPSA[−] OMVs. They also raise the question of how the signals between commensals and immune cells as well as those between immunomodulatory cells are communicated. They speculate that OMV-mediated delivery is likely beneficial for long-distance delivery, a process discussed in more detail in a recent review (Kulp and Kuehn, 2010), and may play a role in host-commensal

communication. Answers regarding the communication between DCs, Tregs, and CD4^{+} Tcells will also further our understanding of tolerance versus adaptive immunity. Finally, they question whether OMV content besides PSA might contribute to the process.

In the case of beneficial PSA[+] OMV signaling, it will be important to understand why *B. fragilis* OMVs also harbor polysaccharide B (PSB) and not polysaccharide G (PSG). To date, there are ever-increasing examples of enriched/excluded OMV cargo (Kulp and Kuehn, 2010), but little is known about the genetically controlled mechanics of this process. Assessing a seemingly basic question of whether OMVs harbor PSA on their interior or exterior might begin the process of understanding the selectivity. Or, perhaps it is something physical about the type of polysaccharide cargo that induces OMV production. Data presented in this study showed no difference in the amount of OMV production for PSA[+] and ΔPSA bacteria, demonstrating that PSA is not responsible. Addressing these questions will further OMV research because they address some poorly understood aspects of OMV-mediated secretion.

If *B. fragilis* makes OMVs during normal growth in a healthy gut, and the gut houses (at least) thousands of types of bacteria that likely produce OMVs, how is the immune system discerning responses to the large cast of immunomodulatory components associated with those OMVs? Do DCs, which are expected to take up OMVs indiscriminately, only respond to those made by *B. fragilis*? In addition to OMV content, it should also be considered that native packaging of the cargo matters. For instance, does a mixture of ΔPSA OMVs

supplemented with purified PSA signal along the same pathways as PSA⁺ OMVs? This is not an idle question, since it was not the case for experiments comparing epithelial monolayer production of the proinflammatory cytokines TNFα and IL-6 in response to ΔLT OMVs supplemented with LT versus LT⁺ OMVs (Chutkan and Kuehn, 2011). In other words, the manner by which bacterial factors are associated with OMVs impacts host cell signaling. In a complex milieu of OMVs, secreted bacterial factors, and secreted host factors, it is not surprising that the immune system is sensitive to not only what is being presented but how it is being presented.

It should also be mentioned here that eukaryotic cells produce membrane vesicles (MVs) and exosomes, which are relatives of OMVs (Deatherage and Cookson, 2012). As with their bacterial counterparts, these eukaryote-derived vesicles have been observed for decades; however, their mechanism of production and function are only now being elucidated. Their roles in intercellular communication and immunobiology show unsurprising parallels with OMV-mediated signaling.

In sum, Shen et al. have uncovered the physiological benefit of the OMV-

Figure 1. Model Summarizing the Promotion of Tolerance and the Suppression of a Damaging Inflammatory Response by *B. fragilis* OMVs in the GI Tract
B. fragilis produces polysaccharide A capsular antigen (PSA)-containing OMVs that are recognized by dendritic cells (DCs) through TLR2. Gadd45α signaling and IL-10 production in DCs is required to induce T regulatory cell (Treg) responses, notably IL-10 production, that suppress CD4 T cell-mediated inflammatory responses. Due to these immunmodulatory effects, PSA-containing OMVs can prevent experimental colitis and thus have therapeutic potential for inflammatory bowel disease (IBD) and Crohn's disease. See text and (Shen et al., 2012) for additional details.

captured capsular material produced by a commensal gut organism. Recent discoveries comparing diseased and healthy microbiome states and the role of the microbiome in gastrointestinal (GI) tract development (Chung et al., 2012; Kau et al., 2011) have raised a multitude of questions regarding the physical gap between bacterial symbionts and the immunology of the GI tract. This study by the Mazmanian group of how *B. fragilis* activates an anti-inflamm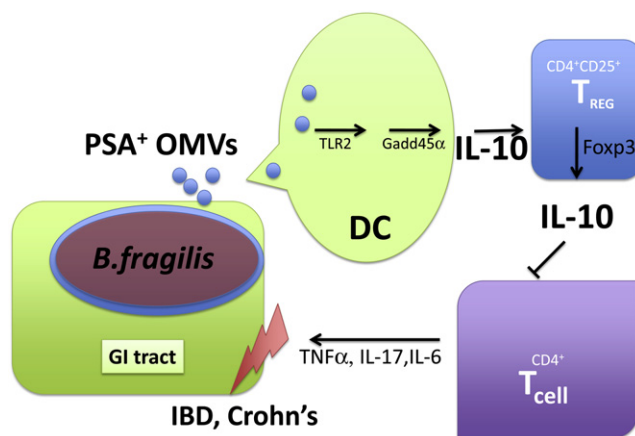atory program in the gut is a successful and satisfying example of how to approach the identification of the molecular basis by which mammals overcome this gap. As a bonus, their findings could generate solutions for individuals suffering from IBD, Crohn's, and colitis.

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