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COMMENTARY

Impaired Innate Immunity at Birth: Deficiency of Bactericidal/Permeability-Increasing Protein (BPI) in the Neutrophils of Newborns

Commentary on the article by Nupponen *et al.* on page 670

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Innate immunity is comprised of cellular and humoral factors that provide rapid protection against microbial invasion. The past 2 decades have seen a rapid expansion of our understanding of the innate immune system on a molecular level. Host receptors for microbes and their surface components have been defined, intracellular signaling pathways have been elucidated, and effector molecules have been isolated and characterized (1). Despite its meaning (*i.e.* "present at birth") and its importance in the context of impairment in acquired immunity at birth, relatively few studies have addressed innate immunity in newborns.

A key cellular effector of innate immunity is the neutrophil whose cytoplasmic granules are replete with antimicrobial proteins and peptides (2–4). Among the antimicrobial proteins of humans is the bactericidal/permeability-increasing protein (BPI), a cationic ~50 kD protein found in the primary (azurophilic) granules of neutrophils that was discovered and isolated by Elsbach and Weiss working at New York University in the late 1970s (5, 6). BPI possesses high affinity toward the lipid A region of the lipopolysaccharides (LPS or "endotoxin") that comprise the outer leaflet of the Gram-negative bacterial outer membrane (7). Binding of BPI to the lipid A moiety of LPS targets BPI's cytotoxic activity (manifest against many, but not all, species of Gram-negative bacteria (8, 9);), opsonizes such bacteria for enhanced phagocytosis (10); and neutralizes the inflammatory (endotoxic) effects of LPS (11).

BPI's endotoxin-neutralizing activity, based on BPI's ability to bind LPS thereby preventing interaction of LPS with host receptors, is particularly potent (at nanomolecular levels), is evident against both isolated LPS and whole Gram-negative bacteria, and is manifest in biologic fluids, including whole blood (8). Of note, BPI's endotoxin-neutralizing activity is opposite to that of its structural homolog, LPS-binding protein (LBP), a liver-derived plasma constituent that enhances endotoxin's activity by delivering LPS to its cellular receptors (12). It is this potent endotoxin neutralizing activity of BPI that has rendered it an attractive candidate for pharmaceutical development as a novel anti-infective agent (13). Recombinant N-terminal fragments of BPI (such as $rBPI_{21}$ and $rBPI_{23}$) have demonstrated safety without immunogenicity in phase I human trials (14) and neutralized endotoxin-induced cytokine responses and physiologic changes in human volunteers (15, 16). In a recently completed phase III trial in children with fulminant meningococcal sepsis, administration of $rBPI_{21}$ was associated with improved clinical outcomes (17). However, the mortality rate in this study was substantially lower than expected and, thus, the trial was underpowered to detect significant differences in survival, the primary outcome variable. As a result, approval of $rBPI_{21}$ as a novel drug awaits "further information" (18).

Although impairment in the antibacterial function of newborn neutrophils has been noted for some time (19), it is only recently that BPI has been studied in newborns. The genesis of work on BPI in newborns can be traced to a study by Qing *et al.* demonstrating that human cord blood-derived neutrophils lack a ~50 kD membrane protein that binds the lipid A region of LPS (20). The localization (BPI can be found on the cell membrane (21);), molecular weight, and lipid A binding properties of this deficient membrane protein were similar to those of BPI (and to that of the LPS receptor, CD14), prompting our laboratory to directly measure intracellular neutrophil BPI content of adults and newborns. Employing detergent extraction of neutrophils and BPI-specific Western Blotting, we found that the neutrophils of newborns have, on average, 3- to 4-fold less intracellular BPI than neutrophils of adults (22). This diminished BPI level correlated with diminished activity of neutrophil sulfuric acid extracts against the BPI-sensitive bacterium *E. coli* K1/r. Deficiency of BPI was apparently selective as the relative levels of two other primary granule constituents, myeloperoxidase (MPO) and the defensin peptides, were indistinguishable.

Approaching the question from the standpoint of neutrophil degranulation, Nupponen and colleagues have now

studied the ability of neutrophils from newborns and adults to release BPI to the extracellular space (23). They find that when stimulated with the secretagogue phorbol myristate acetate (PMA), the neutrophils of *preterm* newborns release significantly less BPI per cell than adult neutrophils. No differences were found in the ability of newborn and adult neutrophils to release MPO, suggesting a selective deficiency in BPI release by the neutrophils of preterm infants.

In an apparent discordance with our study demonstrating lower intracellular pools of BPI in neutrophils of full term newborns relative to those of adults (22), Nupponen *et al.* find no difference in the amounts of BPI released from the neutrophils of full term newborns and those of adults. However, our study examined intracellular BPI content whereas Nupponen's focused on BPI released by PMA stimulation. Thus, the use of these distinct methodologies may have provided somewhat different information. Of note, BPI possesses a highly hydrophobic C-terminal half that is apparently tightly associated with the primary granule membrane (24). Consistent with this, the majority of cell-associated BPI remains intracellular even in the presence of a strong secretagogue (25) (and unpublished observations). One possible explanation for the apparent discrepancy between our studies could therefore be the presence of distinct sub-populations of intracellular BPI subject to different degrees of extracellular release. Regardless of these differences, what is most consistent and remarkable among the Nupponen study, our study, and, most likely, the Qing study is the consistently lower BPI content in the neutrophils of (preterm) newborns when compared with adults. Taken together, these three studies suggest an age-dependent maturation in the ability of human neutrophils to mobilize BPI to sites of infection.

Why should the expression of BPI be developmentally regulated? One possibility is that the need to quench endotoxin activity may vary with age, perhaps due to a similar age-dependent variation in the sensitivity of humans to the inflammatory effects of endotoxin (26). Little is known about the regulation of BPI gene expression. Of note, a recent study demonstrates novel expression of BPI by human mucosal epithelial cells that is inducible by lipoxins (endogenous anti-inflammatory lipids induced by aspirin *in vivo*) (27). Whether endogenous lipid mediators may play a role in the developmental expression of BPI is another question that can now be addressed.

Lastly, these emerging data may have important clinical consequences. For example, the ability of recombinant proteins such as $rBPI_{21}$ to enhance antibacterial activity of human cord blood and to block bacterial endotoxic activity in cord blood (*viz*., tumor necrosis factor release (9);) raises the possibility that supplementing the relatively low endogenous BPI stores of newborns (*e.g.* by i.v. administration of $rBPI_{21}$) may provide clinical benefit. A population that might particularly benefit from such intervention would be very low birthweight premature infants who are at high risk for Gram-negative sepsis (28, 29) and/or other conditions associated with endotoxemia, such as necrotizing enterocolitis (30–32).

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