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Antimicrobial Aspects of Inflammatory Resolution in the Mucosa: A Role for Proresolving Mediators

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Mucosal surfaces function as selectively permeable barriers between the host and the outside world. Given their close proximity to microbial Ags, mucosal surfaces have evolved sophisticated mechanisms for maintaining homeostasis and preventing excessive acute inflammatory reactions. The role attributed to epithelial cells was historically limited to serving as a selective barrier; in recent years, numerous findings implicate an active role of the epithelium with proresolving mediators in the maintenance of immunological equilibrium. In this brief review, we highlight new evidence that the epithelium actively contributes to coordination and resolution of inflammation, principally through the generation of anti-inflammatory and proresolusion lipid mediators. These autacoids, derived from ω-6 and ω-3 polyunsaturated fatty acids, are implicated in the initiation, progression, and resolution of acute inflammation and display specific, epithelial-directed actions focused on mucosal homeostasis. We also summarize present knowledge of mechanisms for resolution via regulation of epithelial-derived antimicrobial peptides in response to proresolving lipid mediators. The Journal of Immunology, 2011, 187: 3475–3481.

The resolution of ongoing inflammation was historically considered a passive act of the healing process with dilution of proinflammatory chemical mediators (1) and occurred independent of active biochemical pathways (1, 2). This view has changed in fundamental ways in the past decade. It is now appreciated that uncontrolled inflammation is a unifying component in many diseases, and new evidence indicates that inflammatory resolution is a biosynthetically active process (3). These new findings implicate a tissue decision process wherein acute inflammation, chronic inflammation, or inflammatory resolution hold the answers as to what endogenous mechanisms control the magnitude and duration of the acute response, particularly as they relate to the cardinal signs of inflammation (2, 4). It has now become evident that the resolution program of acute inflammation particularly within mucosal surfaces remains to be uncovered, and that a complete understanding of these critical pathways will undoubtedly direct new therapeutic opportunities.

Inflammation at mucosal surfaces provides a unique setting for which to define resolution pathways. By their nature, mucosal surfaces interact with the environment and thereby the microbial world in which we live. Important in this regard, the microbiota of each mucosal surface is unique. It is estimated, for example, that the skin harbors 182 different bacterial species, whereas the large intestine may support as many as 1220 different bacterial phylotypes (5). Given this diversity of microbiota, it is not surprising that humans have evolved unique mechanisms to counteract regular microbial challenges. Along these same lines, the timely resolution of ongoing local inflammation has evolved to these ever-changing challenges. We are only now beginning to appreciate the unique features and importance of these responses.

In this brief review, we highlight recent discoveries that impact the active resolution of mucosal inflammation. Given their founding role in active resolution mechanisms, we have focused on the unique contributions of specialized proresolving mediators (SPMs), namely, the resolvins, lipid-derived mediators that are agonist dependent, temporally distinct, and functionally carry novel potent mucosa-directed signals (2).

Resolution-based pharmacology: a lesson from aspirin

Resolution of inflammation and return to tissue homeostasis is an exceptionally well-coordinated process. SPMs generated during the resolution phase of ongoing inflammation actively stimulate restoration of tissue homeostasis (3). The first resolvin, known today as resolvin E1 (RvE1), was identified in 1999 as a potent and active initiator of resolution (4).
ordinate, unrestricted, acute inflammation is now acknowledged as an instigating factor, which, when unchecked, contributes to numerous chronic disease states, including cardiovascular disease, metabolic disorders, and cancer. As such, an understanding of the pharmacology of anti-inflammatory and endogenous proresolving has been a significant venture (2).

As a basic feature, cyclooxygenase-2 (COX-2) contributes fundamentally to both inflammation and resolution (6, 7). COX-2 expression is rapidly induced at sites of inflammation and is a key enzyme in the generation of PGs, via its oxygenase and peroxidase activities (7). In brief, after liberation of the ω-6 fatty acid arachidonic acid (AA) from cell membranes via phospholipase A₂, the oxygenase function of COX-2 catalyzes AA to PGG₂ and subsequently to PGH₂ via the peroxidase activity of the enzyme. Nonsteroidal anti-inflammatory drugs lower the amplitude of inflammation and delay resolution (6, 8). Acetylsalicylic acid (ASA, aspirin), stands apart in that it inhibits proinflammatory signals and accelerates resolution (9). ASA irreversibly acetylates COX-2 on serine 516, rendering it incapable of converting AA to PGG₂. In its acetylated state, ASA produces 15R-H(P)ETE and its peroxidase activity remains intact, resulting in formation of 15R-hydroxyeicosatetraenoate. Aside from ASA’s anti-inflammatory action of inhibiting PG synthesis, 15R-hydroxyeicosatetraenoate is a precursor for proresolving 15-epi-lipoxins (10). Such aspirin-triggered lipoxins (ATLs) are more resistant to metabolic inactivation than lipoxins (11) and also assert anti-inflammatory and proresolving activities in a wide range of inflammatory diseases (7, 8). In addition to the arachidonate-derived lipoxins and ATLs, bioactive SPMs are also biosynthesized from the ω-3 polyunsaturated fatty acids (PUFAs). Both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are precursors in the biosynthesis of both aspirin-triggered forms of the E- and D-series resolvins. Of importance, lipoxigenases can initiate the biosynthesis of resolvins (both E- and D-series), as well as protectins and maresins, without ASA treatment (3) (see Fig. 1). These are the main pathways for SPM biosynthesis in the absence of ASA treatment. Other nonsteroidal anti-inflammatory drugs (i.e., indomethacin) can both block the biosynthesis of the aspirin-triggered forms of SPM and lead to enhanced formation of SPMs via the lipoygenase routes involved in the biosynthesis of specific SPMs. The biosynthesis of SPM has recently been reviewed in detail and those interested should see Ref. 12.

**Active resolution: biosynthesis of SPM**

Resolution of acute, self-limited inflammation is distinct, by definition, from anti-inflammatory process (3). Proresolving mediators restrict further infiltration of polymorphonuclear leukocyte (PMN, neutrophil) to sites of acute inflammation and promote resolution via enhanced clearance of apoptotic cells by macrophages (3). Importantly, proresolving mediators stimulate antimicrobial activities of epithelia (13, 14), aiding a return to tissue homeostasis. These are particularly relevant in the eye, lung, and oral epithelial surfaces. For example, RvE1 reduces ocular herpes simplex-induced inflammation (15); protectin D1 reduces ocular epithelial injury (16); resolvin D1 (RvD1), RvE1, and protectin D1 each reduce airway inflammation (17–20); and RvE1 reduces oral inflammation of the periodontium (21) and stimulates the clearance of apoptotic cell from mucosal surfaces (22). The protective role of RvE1 in periodontal disease has been attributed to both diminished inflammation and curtailed osteoclast-dependent destruction of bone (23). In the gastrointestinal tract, RvE1 is protective in murine models of colitis (13, 24–26). Moreover, RvE1 and RvD1 have been recently implicated in the alleviation of inflammatory pain (27). Thus, the potential therapeutic benefits of SPMs are far-reaching.

Much recent attention has been paid to understanding the innate mechanisms involved in the resolution of inflammation at mucosal sites. The best understood are the families of lipid mediators termed the resolvins and the maresins (2). Resolvins have been studied in most detail and are ω-3 PUFAs derived lipid mediators central to activation of the inflammatory resolution program (2, 3). The discovery of resolvins was permitted by using an unbiased systems approach to acute contained self-limited/naturally resolving inflammatory exudates using liquid chromatography–mass spectrometry–mass spectrometry–based lipidomics and earlier knowledge that ω-3 PUFAs are beneficial to a number of cardiovascular and immunoregulatory responses (9). Ensuing studies revealed the existence of novel families of lipid mediators, derived from either EPA (C20:5, 18-series resolvins), as well as DHA (C22:6, 17-series resolvins), which potently and stereoselectively ini-
tiate and enhance the resolution mechanisms in acute inflammation.

Mechanisms of SPM-mediated resolution

To date, an array of SPMs has been identified with potent proresolusion activities; their mechanisms of action are equally diverse. ATL (15-epi-lipoxin) binds to the lipoxin A₄ (LXA₄) receptor (ALX/FPR2; Formyl Peptide Receptor 2), eliciting antagonistic activities on PMN chemotaxis (28). RvE1 binds to and interacts with ChemR23 receptor, resulting in ERK and AKT phosphorylation and subsequent signal transduction via ribosomal protein S6 to enhance macrophage phagocytosis (29). RvE1 also binds to the LTB₄ receptor BLT1 on neutrophils, where it acts as a partial agonist (30). Aside from signal transduction directly affecting leukocyte function, modulation of gene expression in response to SPM has revealed key insight to their mechanism of resolution. LXA₄ and RvE1 induce CCR5 expression on the surface of apoptotic PMN and T cells, resulting in sequestration of CCL3/CCL5 in murine peritonitis, facilitating resolution (31). RvE1 and RvD1 both attenuate PMN transmigration across endothelia (32, 33). Furthermore, RvE1 accelerates the clearance apically adherent PMN from epithelia by enhancing antiadhesive CD55 expression (22). Likewise, ATL induces the expression of an antimicrobial peptide, bacterialidal-permeability enhancing, in epithelial cells (14). Also, resolvin D2 (7,8,9,15-tetrahydroxy-3E,Z,E,E,Z-docosahexaenoic acid) enhances phagocyte killing of microbes, improving survival in cecal ligation puncture-initiated sepsis (34), and RvD1 modulates macrophage responses to LPS-TLR4 signaling, resulting in decreased proinflammatory cytokine release, whereas maintaining IL-10 expression (35).

More recently, RvE1 was discovered to upregulate the expression of intestinal alkaline phosphatase (ALPL), a marker of differentiation with a surprising role in maintenance of bacterial homeostasis (13). Given the proximity of mucosal surfaces to bacterial Ags and the vital role of antimicrobial peptides in host defense, we will discuss the potential role for antimicrobial peptides in the process of resolution.

Antimicrobial peptides in the mucosa

Epithelial cells are uniquely positioned to serve as a direct line of communication between the immune system and the external environment. In their normal state, mucosal surfaces are exposed on the luminal surface to high concentrations of foreign Ags, whereas at the same time, they are intimately associated with the immune system via subepithelial lymphoid tissue (36). Polarized epithelia form a physical selective barrier to allow absorption/secretion whereas preventing entry of pathogens into the body. The mucosal epithelium comprises a heterogeneous population of differentiated epithelia with distinct functions: absorptive enterocytes, mucus-secreting goblet cells, antimicrobial peptide-secreting Paneth cells, and enteroendocrine cells (37).

Antimicrobial peptides are secreted prophylactically by the epithelium into the viscous mucus layer, thus minimizing the instance of epithelium-adhering bacteria. Similarly, Paneth cells secrete antimicrobial peptides (defensins/lectins) maintaining intestinal crypt sterility. Consequently, the epithelium forms an important barrier, preventing the free mixing of luminal antigenic material with the lamina propria, which houses the mucosal immune system (38), and defects in these defensive functions contribute to disease pathogenesis (e.g., loss of function in mucin-2/Paneth cells can contribute to inflammatory bowel disease) (39). Concordantly, antimicrobial peptide generation provides protection for other mucosal epithelial surfaces: lung epithelia produce defensins and LL-37 (40), corneal and conjunctival epithelia express LL-37 (41), and oral epithelia are protected by antimicrobial peptides secreted in saliva (42, 43).

Like many aspects of immunology, the view that the epithelium is merely a physical selective barrier has changed. The epithelium is now viewed as an active player in normal homeostatic mechanisms of mucosal immunity, and in some instances, the epithelium may centrally orchestrate mucosal innate immunity and inflammation (44).

“Classical” antimicrobial peptides

The classically viewed antimicrobial peptides represent a diverse array of small peptides (12–50 aa), containing a positive charge and an amphipathic structure. The most studied antimicrobial peptides to date are cathelicidins and defensins. Cathelicidin (LL-37) is expressed by epithelial cells, neutrophils, monocytes, and macrophages, and can stimulate chemotaxis via the ALX/FPR2 receptor on these cells (45). Posttranslational processing is essential for its antimicrobial activity in vivo (46) and is accomplished by serine proteases such as kallikreins (47) or PMN proteases such as proteinase-3 (48). LL-37 antimicrobial activity was originally thought to neutralize endotoxin because of its cationic/amphipathic capacities to interact with anionic LPS or prevent LPS binding to CD14 (49). Aside from preventing sepsis by interfering with the ability of LPS to stimulate TLR4 signaling, LL-37 have subsequently been demonstrated to directly dampen proinflammatory signaling initiated by LPS (50). Mice deficient in the only known murine cathelicidin (encoded by the gene Cnlp) show significant increases in susceptibility to a number of mucosal infections (51).

Defensins are cationic antimicrobial peptides broadly classified as α- and β-defensins, the former predominantly expressed by PMN and Paneth cells, and the latter by epithelia (52). Similar to LL-37s, α-defensins are activated by proteolytic processing of an inactive precursor (53) and are stored in granules of PMN. In contrast with α-defensins, β-defensins typically have short N-terminal extensions, and all possess some measure of antimicrobial activity in their full-length forms. Defensins have broad antimicrobial actions on Gram-positive and -negative bacteria, and defects in defensin expression have been shown to contribute to a number of mucosal inflammatory diseases, including inflammatory bowel disease and necrotizing enterocolitis (54). β-Defensins are secreted in saliva and are thought to be protective against periodontitis and caries (43). Mutations of the 3′-untranslated region of β-defensin lead to chronic and aggressive periodontitis (55).

Immunomodulatory functions of antimicrobial peptides. Given their name, antimicrobial peptides were originally thought to function merely as “natural antibiotics,” specialized in the killing of bacteria. This bias has hampered discovery of their diverse array of function in immunity and their regulation in host defense. Increasing evidence indicates that aside from their antimicrobial activity, antimicrobial peptides can modulate immune responses by inducing cytokine/chemokine production, inhibiting LPS-
induced proinflammatory cytokine production, promoting wound healing, and modulating the responses of dendritic cells or T cells. As such, antimicrobial peptides may be viewed as bridging the gap between innate and adaptive immunity.

Cathelicidin has immunomodulatory functions; for instance, it is chemotactic to mast cells and PMN via interaction with the ALX/FPR2 receptor (45, 56), which is blocked by the anti-inflammatory LXA₄ stable analog. Cathelicidin stimulates release of the anti-inflammatory PGD₂ from mast cells (57), which as mentioned earlier can prime tissues for resolution by expressing enzymes necessary for resolution. Human β-defensin 2 also possesses immunomodulatory functions and, like LL-37, is known to be chemotactic for mast cells and activated PMN (58). β-Defensin 3 upregulates COX-2 and PGE₂ biosynthesis in gingival fibroblasts (59). β-Defensins antagonize T cell tissue infiltration and promote exfiltration (60, 61). Considering their rapid release in response to “danger signals” and their consequent immunomodulatory activities has led to the concept that antimicrobial peptides can act as early warning signals for infection and the creation of term alarmins (62).

**Antimicrobial peptides and restitution/wound closure.** As part of their proresolving activity, both LL-37 (63, 64) and β-defensin 2 (65) are known to promote epithelial cell migration, necessary for mucosal restitution after physical injury or damage from immune activity. Human β-defensin 2 stimulates migration and proliferation of endothelial cells in wounds, resulting in neovascularization and accelerated wound healing (66). LL-37 has been proposed to initiate tissue remodeling via matrix metalloproteinase activity and promote wound closure via induction of the Snail/Slug transcription factors, necessary for E-cadherin transcription and epithelial adherens junction formation (64).

**“Nonclassical” antimicrobial peptides**

**Bactericidal permeability-increasing protein.** A number of additional mechanisms exist to maintain homeostasis at mucosal surfaces. Among the innate antimicrobial defense molecules of humans is bactericidal permeability-increasing protein (BPI), a 55- to 60-kDa protein originally found in neutrophil azurophilic granules, on the neutrophil cell surface and, to a lesser extent, in specific granules of eosinophils (67). Subsequently, BPI was found to be expressed in epithelial cells (14). Based on an original transcriptional profiling approach to identify novel ATL-regulated genes in intestinal epithelial cells, BPI was found to be expressed in both human and murine epithelial cells of wide origin (oral, pulmonary, and gastrointestinal mucosa), and each was similarly regulated by ATL. Functional studies using a BPI-neutralizing antiserum revealed that surface-localized BPI blocks endotoxin-mediated signaling in epithelia and kills *Salmonella typhimurium*. More recently, molecular studies revealed that epithelial BPI is selectively induced by ATL and prominently regulated by the transcription factors Spi1/3 and C/EBPβ (68). Additional studies in human and murine tissue ex vivo revealed that BPI is diffusely expressed along the crypt-villous axis (14, 68), and that epithelial BPI protein levels decrease along the length of the intestine (69). More recent studies with SPM have revealed the expression of BPI in various mucosal epithelia (67).

As its name infers, BPI selectively exerts multiple antimicrobial actions against Gram-negative bacteria, including cytotoxicity through damage to bacterial inner/outer membranes, neutralization of bacterial LPS (endotoxin), as well as serving as an opsonin for phagocytosis of Gram-negative bacteria by neutrophils (70, 71). The high affinity of BPI for the lipid A region of LPS (72) targets its cytotoxic activity to Gram-negative bacteria. Binding of BPI to the Gram-negative bacterial outer membrane is followed by a time-dependent penetration of the molecule to the bacterial inner membrane where damage results in loss of membrane integrity, dissipation of electrochemical gradients, and bacterial death (73). BPI binds the lipid A region of LPS with high affinity (74, 75), and thereby prevents its interaction with other (proinflammatory) LPS-binding molecules, including LBP and CD14 (76). Because BPI binds the lipid A region common to all LPSs, it is able to neutralize endotoxin from a broad array of Gram-negative pathogens (71). The selective and potent action of BPI against Gram-negative bacteria and their LPS is fully manifest in biologic fluids, including plasma, serum, and whole blood (71, 77). In multiple animal models of Gram-negative sepsis and/or endotoxemia, administration of BPI congeners is associated with improved outcome (78, 79). These studies in epithelia have identified a previously unappreciated “molecular shield” for protection of mucosal surfaces against Gram-negative bacteria and their endotoxin.

**ALPI.** There is much recent interest in ALPI, a 70-kDa, GPI-anchored protein expressed on the apical (luminal) aspect of intestinal epithelial cell (80). In the past, this molecule had been viewed as one of the better epithelial differentiation markers, with little understanding of the true function of this molecule within the mucosa. More recent studies have identified this molecule as a central player in microbial homeostasis (81–83).

A recent microarray screen to identify RvE1-regulated genes in intestinal epithelial cells revealed two important findings. First, these studies revealed the previously unappreciated native expression of the RvE1 receptor ChemR23 on epithelial cells. A screen of various epithelial cell lines revealed prominent expression of ChemR23 on human intestinal epithelial cell lines (T84 and Caco-2). Unique was the pattern of expression on polarized epithelia. This analysis revealed that ChemR23 localizes predominantly to the apical membrane surface, which was somewhat unexpected given that most other G protein-coupled receptors exhibit basolateral expression in polarized epithelia (84). Such membrane distribution of ChemR23 suggested that the localized generation of RvE1 during PMN–epithelial interactions could occur at the apical (luminal) aspect of the tissue. This is an intriguing possibility given that the other known function for RvE1 on mucosal epithelia is to promote the termination and clearance of PMN after transmigration (22), through well-characterized, CD55-dependent mechanisms (85, 86). Thus, the PMN–epithelial interactions that occur within the lumen of the intestine may initiate a proresolving signature to the epithelium during PMN transit through the mucosa.

Second, these microarray studies identified a prominent RvE1-dependent antimicrobial signature within the epithelium, including the induction of BPI and the BPI-like molecule PLUNC (palate, lung, nasal epithelium clone) (13). Also notable was the induction of epithelial ALPI by RvE1. Surface-expressed ALPI was shown to retard Gram-negative bacterial growth and to potentially neutralize LPS through a mechanism involving dephosphorylation of 1,4′-bisphosphorylated glucosamine disaccharide of LPS lipid A (82, 83).
This observation was translated to the murine model dextran sodium sulfate colitis and revealed that induction of ALPI by RvE1 in vivo strongly correlated with the resolution phase of inflammation (Fig. 2). Moreover, inhibition of ALPI activity was shown to increase the severity of colitic disease and abrogate the protective influences of RvE1 (13). Like those defining epithelial expression of BPI (14), these studies provide an example of the critical interface between inflammatory resolution and the importance of antimicrobial mechanisms.

**Conclusions**

Given the close proximity of bacteria to mucosal surfaces, maintenance of tissue homeostasis presents a significant challenge. After successful handling of infiltrating bacteria, the generation of proresolving mediators accelerates the return to homeostasis. This review highlights not only the multifunctional role of antimicrobial peptides in inflammation, but also the interdependent relationship between the induction of antimicrobial peptides and the initiation of resolution path-
ways and the role of resolvins in this process (see Fig. 3). After microbial detection, “alarmins” or “classical” antimicrobial peptides are released by infiltrating immune cells, aiding the killing of bacteria, stimulating neutrophils to generate reactive oxygen species (with inadvertent tissue damage), promoting further release of antimicrobial peptides, and releasing both proinflammatory and anti-inflammatory lipids via COX-2 induction. As such, “classical” antimicrobial peptides could be considered to have both proinflammatory and anti-inflammatory properties, suggesting that antimicrobial peptides prime the inflammatory microenvironment of the mucosal surface for resolution. After generation of SPM, “nonclassical” antimicrobial peptides may accelerate return to homeostasis via continued bacterial killing, inhibition of LPS signaling, and inhibition of “classical” antimicrobial peptide release from leukocytes. As such, it would appear that an interdependent relationship exists between the activity of antimicrobial peptides and the initiation of resolution programs. Along these lines, RvE1 blocks LTB4-stimulated release of LL-37 by human PMN, and LXA4 inhibits proinflammatory actions of LL-37 (45).

Overall, the contribution of microbes to health and disease has provided an elegant lesson in biology. Results from model disease systems and humans allowed the discovery of proresolving mechanisms that are fundamental to our understanding of disease pathogenesis. As summarized in this review, the interdependence of antimicrobial defense mechanisms with inflammatory disease resolution has provided an informative example of how these biochemical pathways yield insight toward a better understanding of tissue function. Ongoing studies of antimicrobial regulation in the mucosa, exemplified by SPM-regulated BPI and ALPI in epithelial intestine, should provide templates for the design of new and effective therapies for inflammatory disease resolution.

Disclosures
S.P.C. and C.N.S. are inventors on patents assigned to Brigham and Women’s Hospital-Partners HealthCare on the composition, uses, and clinical development of anti-inflammatory and proresolving mediators and related compounds. The following are licensed for clinical development: lipoxins to Bayer Health of anti-inflammatory and proresolving mediators and related compounds. S.P.C. and C.N.S. are inventors on patents assigned to Brigham and Women’s Hospital.

References


