Acute inflammation: endogenous cannabinoids mellow the harsh proinflammatory environment

Andrew S. Neish

Department of Pathology, Emory University School of Medicine, Atlanta, Georgia, USA.

Under normal conditions, there is a paucity of neutrophils within the intestinal mucosa; however, these innate immune cells rapidly infiltrate the mucosa in response to infection and are critical for pathogen control. Unfortunately, these cells can cause extensive damage to the intestine if the initial inflammatory influx is not resolved. Factors that promote resolution of inflammation are of great interest, as they have therapeutic potential for limiting uncontrolled inflammatory damage. In this issue of the *JCI*, Szabady et al. demonstrate that the multidrug resistance transporter P-glycoprotein (P-gp) secretes endocannabinoids into the intestinal lumen that counteract the proinflammatory actions of the eicosanoid hepoxilin A3, which is secreted into the lumen by the efflux pump MRP2 and serves as a potent neutrophil chemoattractant. Moreover, the antiinflammatory actions of P-gp–secreted endocannabinoids were mediated by peripheral cannabinoid receptor CB2 on neutrophils. Together, the results of this study identify an important mechanism by which endogenous endocannabinoids facilitate the resolution of inflammation; this mechanism has potential to be therapeutically exploited.

The search for inflammation-suppressing biolipids

A striking histological characteristic of a healthy gut mucosa is the general absence of neutrophils; however, these acute inflammatory cells are capable of rapid influx into the mucosa and are necessary for controlling infection. This innate immune process depends upon the classical sequence of neutrophil recruitment: adhesion, extravasation, chemotaxis, and terminal transepithelial migration of assembled neutrophils from the basement membrane of the epithelial monolayer to the apical surface, where they are positioned to intercept luminal pathogens. While neutrophil influx into the intestinal lumen is a critical response to enteric infection that mediates the phagocytosis and killing of bacteria, this influx is also universally recognized as a double-edged sword, as the products of neutrophil degranulation and oxidative burst tightly associate with collateral cellular and macromolecular damage (1). Chronic neutrophilic inflammation in the gut, such as that seen in ulcerative colitis and certain enteric infections, can result in scarring, functional alterations, and increased risk of neoplasia. Thus, it is clear that the mucosa requires fine-tuned strategies to rapidly summon acute inflammatory cells when needed and to counteract proinflammatory signaling to rapidly clear inflammatory cells and stimulate restitution. Understandably, mechanisms involved in the resolution of inflammation are a current topic of intense interest, given the obvious potential of these pathways for therapeutic exploitation.

An important area of investigation is the identification and characterization of numerous bioactive lipid compounds, often eicosanoid mediators, which can play varying roles in inflammatory processes. For example, the lipid chemoattractant hepoxilin A_3 (HxA_3) is an eicosanoid that is secreted from intestinal epithelial cells by the apically restricted efflux pump multidrug resistance protein 2 (MRP2) and that mediates terminal translocation of recruited neutrophils across the epithelial monolayer (2). Conversely, lipid mediators, including lipoxin A_4 and the resolvins, also possess important antiinflammatory and restitutive functions (3). Thus, lipid mediators represent a fruitful source of potentially novel inflammatory-modulating agents.

In this issue, Szabady et al. describe a bioactive lipid-mediated mechanism that serves to counterbalance activation of the HxA_3/MRP2 proinflammatory pathway (4). Interestingly, the antiinflammatory mediators identified are endocannabinoids (ECs), which are part of a biochemical system that is gaining increased attention as both an endogenous signaling pathway and as a potential mechanism for therapeutic properties imputed to exogenous phytocannabinoids (5). Szabady and colleagues sought to discover inflammation-suppressing molecules by evaluating the P-glycoprotein–dependent (P-gp–dependent) secreted lipidome of unstimulated epithelial monolayers. P-gp is a multidrug transporter known to efflux small hydrophobic compounds that is encoded on the *Mdr1* gene. Mice lacking the *Mdr1a* gene develop spontaneous colitis (6), and polymorphisms in the human *MDR1* gene are known risk alleles for inflammatory bowel disease (IBD) (7); therefore, compounds secreted by this pathway may play a role in antiinflammatory and/or homeostatic functions.

Szabady et al. found that apical supernatants from quiescent epithelia cells suppressed HxA_3-simulated neutrophil translocation in vitro. The molecule associated with this activity was characterized to be less than 1 kB, lipophilic, and absent in *Mdr1*-deficient cells. Furthermore, screening for activity across a panel of GPCRs detected significant agonist activity for the peripheral cannabinoid receptor CB2.

▶ **Related Article: p. 4044**

Conflict of interest: The author has declared that no conflict of interest exists.
Reference information: *J Clin Invest*. 2018;128(9):3750–3751. https://doi.org/10.1172/JCI122885.

Finally, mass spectral analysis detected anandamine or arachidonoylethanolamine (AEA), which belongs to the chemical family of *N*-acyl ethanolamine (NAE), a group that includes many ECs.

Enter the cannabinoids

Phytocannabinoids are a family that includes at least 100 related terpenoid-like compounds produced by the flowering bodies of plants of the genus *Cannabis*. *Cannabis*-produced compounds include the well-known major psychoactive component of the plant, Δ-9-tetrahydrocannabinol (THC), and cannabidiol (CBD), a compound that has attracted interest for its promising antiseizure and antiinflammatory activity (5, 8–10). Phytocannabinoids mediate their effects via the classical cannabinoid receptors CB1 and CB2 and likely act on other receptors as well. CB1 and CB2 are seven-transmembrane GPCRs that are linked to cAMP and MAPK cascades. CB1 is predominantly expressed in the CNS, while CB2 is predominantly expressed on immune cells, including microglial cells of the CNS (9). The discovery of the cannabinoid receptors spurred the quest to identify endogenous agonists that might phenocopy the effect of the phytocannabinoids. Thus, ECs are operationally defined by their ability to stimulate the CB1 and CB2 receptors. Collectively, these ligands and receptors as well as complex enzymatic and transporter apparatus involved in their synthesis, secretion, and degradation make up the EC system.

Szabady and colleagues used several approaches to validate the antiinflammatory effects mediated by quiescent epithelial cell supernatants. First, purified NAE compounds suppressed neutrophil migration in vitro; however, these compounds unexpectedly did not act as direct CB2 receptor agonists. Second, epithelial cells from P-gp–deficient mice (*Mdra-1*$^{-/-}$) lacked inhibitory activity and detectable NAE secretion, suggesting a role for this efflux pump in secretion of the lipid effector molecules. Third, CB2-deficient mice (*Cnr2*$^{-/-}$) showed markedly increased inflammation and emigrated neutrophils in models of experimental colitis. Finally, compared with that of WT neutrophils, *Cnr2*-null neutrophil migration was less impaired in response to ECs. Overall, Szabady et al. posit that constitutive secretion of NAE class ECs via the P-gp efflux pump acts to prevent the final translocation of neutrophils into the lumen, thus working as a break or counterweight to HxA_3/MDR-mediated inflammation.

Future directions

The results of this study lead to several questions that remain to be answered. ECs are thought to be rapidly and enzymatically produced during inflammation, similarly to eicosanoids, and are subsequently rapidly hydrolyzed, implying short-term autocrine or paracrine activity. This rapid generation and turnover are consistent with antiinflammatory activity in localized tissue microenvironments, such as the subepithelial mucosa. Continuous secretion may represent a mechanism of homeostasis, while induced secretion in the later stages of inflammation may represent a prorestitutive function. Do other, nongastrointestinal mucosal barriers deploy this system? What are the actual CB2-dependent effects on neutrophil biology that render them in an immobile (couch locked?) state? How is the EC system regulated? Are P-gp expression and/or NAE biosynthesis altered in germ-free mice, or more importantly, by specific stains of commensal bacteria or candidate probiotics?

From a therapeutic standpoint, an obvious application would be further discovery and development of other endogenous cannabinoids and possible synthetic agonists that could be employed as topical therapeutics. Additionally, the study by Szabady and colleagues provides a timely mechanistic justification for further medicinal evaluation of phytocannabinoids (11). Taken together, these findings illustrate the value in continued exploration of natural products as potential therapeutics as well as the startling degree of shared functionality of bioactive molecules across the plant and animal kingdoms.

Address correspondence to: Andrew S. Neish, 105-A Whitehead Bldg., Department of Pathology, Emory University School of Medicine, Atlanta, Georgia 30322, USA. Phone: 404.727.8545; Email: aneish@emory.edu.

1. Fournier BM, Parkos CA. The role of neutrophils during intestinal inflammation. *Mucosal Immunol*. 2012;5(4):354–366.
2. Mrsny RJ, et al. Identification of hepoxilin A3 in inflammatory events: a required role in neutrophil migration across intestinal epithelia. *Proc Natl Acad Sci U S A*. 2004;101(19):7421–7426.
3. Bannenberg G, Serhan CN. Specialized pro-resolving lipid mediators in the inflammatory response: an update. *Biochim Biophys Acta*. 2010;1801(12):1260–1273.
4. Szabady RL, et al. Intestinal P-glycoprotein exports endocannabinoids to prevent inflammation and maintain homeostasis. *J Clin Invest*. 2018;128(9):4044–4056.
5. Cabral GA, Rogers TJ, Lichtman AH. Turning over a new leaf: cannabinoid and endocannabinoid modulation of immune function. *J Neuroimmune Pharmacol*. 2015;10(2):193–203.
6. Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. *J Immunol*. 1998;161(10):5733–5744.
7. Ho GT, Gaya DR, Satsangi J. Multidrug resistance (MDR1) gene in inflammatory bowel disease: a key player? *Inflamm Bowel Dis*. 2005;11(11):1013–1019.
8. Tanasescu R, Constantinescu CS. Cannabinoids and the immune system: an overview. *Immunobiology*. 2010;215(8):588–597.
9. Turcotte C, Blanchet MR, Laviolette M, Flamand N. The CB2 receptor and its role as a regulator of inflammation. *Cell Mol Life Sci*. 2016;73(23):4449–4470.
10. Storr MA, Keenan CM, Zhang H, Patel KD, Makriyannis A, Sharkey KA. Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. *Inflamm Bowel Dis*. 2009;15(11):1678–1685.
11. Schicho R, Storr M. Cannabis finds its way into treatment of Crohn's disease. *Pharmacology*. 2014;93(1–2):1–3.