and nature of IgA responses, thereby affecting the host-mediated selection of microbial communities in the gut. Accordingly, T-MyD88$^{-/-}$ mice display increased susceptibility to colitis. The disease predisposition of T-MyD88$^{-/-}$ mice could be shown to be due to the microbiota dysbiosis, because disease severity was significantly decreased after transfer of fecal microbiota from WT mice. This observation is consistent with a recent report showing that IgA coats colitogenic members of the microbiota and maintains immune homeostasis (Palm et al., 2014). It is noteworthy that Kubinak et al. further demonstrate a significant positive correlation between the relative abundance of GC B cells and the diversity of the mucosal bacterial community. Therefore, a stronger GC and IgA response promotes microbiota diversity, presumably by exploiting targeted bacteria and creating a habitable niche for rare bacterial species (Figure 1).

Several genetically modified mice with defects in the production of IgA in the gut have been used to explore the role of IgA in mucosal immune protection. T-MyD88$^{-/-}$ mice provide a valuable opportunity to understand the previously unexplored role of high-affinity microbiota-specific IgA driven by the microbiota-induced T_{FH} cells in selective control of the mucosa-associated community. It remains unknown where CD4$^+$ T cells encounter TLR ligands, how TLR signaling in CD4$^+$ T cells leads to T_{FH} cell development, and why IgA selectively targets mucosa-associated members. Because most of the IgA induced by T_{FH} cells is specific for microbiota antigens (Kubinak et al., 2015; Kawamoto et al., 2014; Palm et al., 2014), T_{FH} cells generated by the microbiota are also likely to be specific for microbiota antigens. Therefore, dendritic cells (DCs) are likely to play a role in skewing the initial commitment of CD4$^+$ T cells toward the T_{FH} subset. In this context, it has been shown that goblet cells deliver luminal antigen to CD103$^+$ DCs (McDole et al., 2012) and that CD103$^+$ DCs patrol the epithelium and capture bacteria attaching to its surface (Farache et al., 2013). Therefore, DCs localizing to the epithelial layer may sense and capture the mucosa-associated commensals to skew the TCR repertoire and the differentiation of CD4$^+$ T cells toward the T_{FH} subset. In any case, the finding by Kubinak et al. can be used to tailor therapies; for example, oral administration of a mixture of microbiota-specific high-affinity IgAs can be an effective therapy to treat microbiota-driven disease by restoring microbiota diversity.

REFERENCES

Farache, J., Koren, I., Milo, I., Gurevich, I., Kim, K.W., Zigmond, E., Furtado, G.C., Lira, S.A., and Shakhar, G. (2013). Immunity *38*, 581–595.

Ivanov, I.I., and Honda, K. (2012). Cell Host Microbe *12*, 496–508.

Kawamoto, S., Maruya, M., Kato, L.M., Suda, W., Atarashi, K., Doi, Y., Tsutsui, Y., Qin, H., Honda, K., Okada, T., et al. (2014). Immunity *41*, 152–165.

Kubinak, J.L., Petersen, C., Stephens, W.Z., Soto, R., Bake, E., O'Connell, R.M., and Round, J.L. (2015). Cell Host Microbe *17*, this issue, 153–163.

McDole, J.R., Wheeler, L.W., McDonald, K.G., Wang, B., Konjufca, V., Knoop, K.A., Newberry, R.D., and Miller, M.J. (2012). Nature *483*, 345–349.

Nava, G.M., and Stappenbeck, T.S. (2011). Gut Microbes *2*, 99–104.

Palm, N.W., de Zoete, M.R., Cullen, T.W., Barry, N.A., Stefanowski, J., Hao, L., Degnan, P.H., Hu, J., Peter, I., Zhang, W., et al. (2014). Cell *158*, 1000–1010.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., et al.; MetaHIT Consortium (2010). Nature *464*, 59–65.

Hedgehog: Linking Uracil to Innate Defense

Ethan Bier[1,*] **and Victor Nizet**[2,3,*]
[1]Section of Cell and Developmental Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0349, USA
[2]Department of Pediatrics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0687, USA
[3]Skaggs School of Pharmacy & Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0687, USA
*Correspondence: ebier@ucsd.edu (E.B.), vnizet@ucsd.edu (V.N.)
http://dx.doi.org/10.1016/j.chom.2015.01.010

The ability of the gut epithelium to defend against pathogens while tolerating harmless commensal organisms remains an important puzzle. In this issue of *Cell Host & Microbe*, Lee et al. (2015) reveal how pathogen-secreted uracil acts at two steps to induce ROS via the Hedgehog pathway.

Epithelial barriers represent essential lines of defense against tissue invasion by bacterial and viral pathogens. For a sterile barrier such as the alveolar epithelium of the lung, this challenge boils down to keeping the pathogens and their effectors out. In the setting of a diverse natural ecosystem in the gut, the problem is significantly more complicated. Although pathogenic species must be prevented from invading the host or causing local tissue damage, beneficial or symbiotic bacteria comprising the commensal microbiota require tolerance. Thus, both the innate and adaptive (in vertebrates) immune systems must somehow distinguish friend from foe and respond accordingly.

Drosophila has emerged as an important model genetic system for analyzing gut homeostasis. The *Drosophila* gut

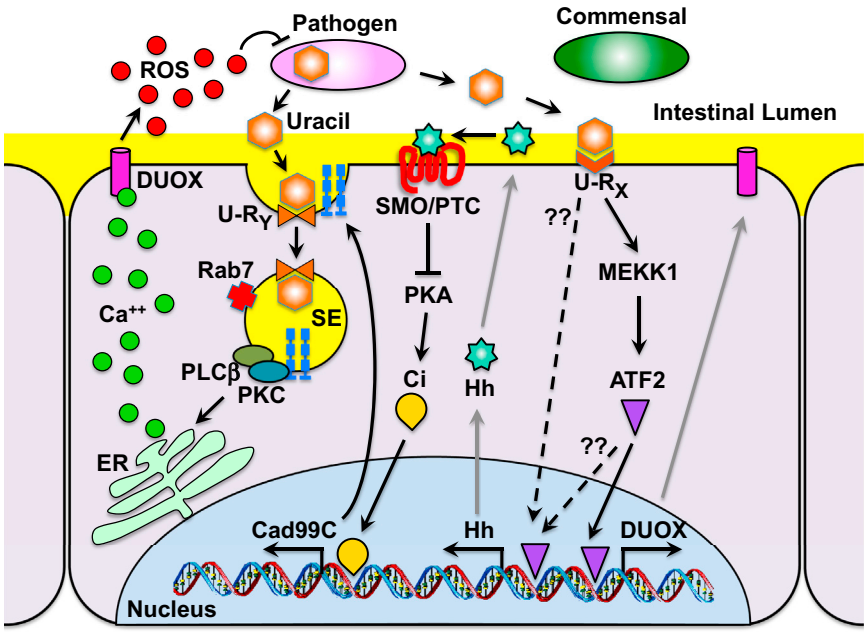

Figure 1. Dual Activities of Uracil in Hedgehog-Mediated Defense against Enteropathogenic Bacteria

Pathogenic (but not commensal) bacteria liberate uracil, which acts via two unknown receptors ($U\text{-}R_X$ and $U\text{-}R_Y$) to induce ROS production to combat infection. Activation of $U\text{-}R_X$ induces Hedgehog (Hh) expression either via the known effectors MEKK1/ATF2 (which induce DOUX expression) or by an alternative pathway. Hh is then secreted from cells and binds to Patched (PTC) to relieve inhibition of Smoothened (SMO) signaling, which in turn inhibits the activity of protein kinase A (PKA) to activate target genes including Cad99C via the transcriptional effector Ci (analogous to Gli proteins in mammals). Activation of the second uracil receptor, $U\text{-}R_Y$, leads to internalization of the receptor complex into signaling endosomes (SE), clustering of Cad99C, activation of PLCβ/PKC signaling, Ca^{2+} release from the endoplasmic reticulum (ER), and DUOX-mediated ROS synthesis to kill the pathogenic bacteria.

has a high degree of morphologic and ultrastructural similarity to the vertebrate intestine, including the presence of functionally distinct domains analogous to the vertebrate small intestine (midgut) and colon (hindgut), as well as functional similarities such as a capacity for stem cell renewal and the generation of reactive oxygen species (ROS) in response to damage (for review, see Buchon et al., 2013; Kim and Lee, 2014). An important advance in understanding intestinal immune homeostasis was made when Lee and colleagues discovered that uracil alerts the *Drosophila* innate immune system to the presence of enteropathogenic bacteria (Lee et al., 2013), triggering production of antibacterial ROS. In that study, the host pathway(s) mediating the uracil signaling response for ROS production and pathogen neutralization were unknown. In this issue, Lee et al. now identify the Hedgehog (Hh) pathway as a primary target and effector of uracil (Lee et al., 2015) (Figure 1).

To identify the mechanism of uracil-mediated ROS activation, the authors performed a comparative transcriptional analysis of uracil-treated versus control intestinal tissue. The Hh signaling pathway scored as the top hit. Indeed, expression of the Hh ligand itself was strongly upregulated in response to uracil treatment, consistent with results of a prior study showing increased Hh expression upon enteric infection (Buchon et al., 2009). Effects of uracil treatment (e.g., ROS production) are blocked by inhibition of Hh signal transduction, while experimental over-activation of the Hh pathway induced ROS even in the absence of uracil treatment.

A critical conclusion of this detailed multipronged analysis is that uracil has two distinct yet synergistic effects that impact epithelial defense. First, uracil induces expression of the Hh ligand via an unknown receptor (Receptor X) to activate Hh signaling and expression of the target gene Cad99, a cadherin family member localized in the plasma membrane. Uracil signaling through a second receptor (Receptor Y, a suspected G protein-coupled receptor) leads to Cad99 clustering, PLCβ/PKC activation, induction of Ca^{2+} release from endoplasmic reticulum (ER) stores, and stimulation of the cell surface DUOX enzyme to produce ROS. The dual action of uracil in this process was elegantly demonstrated when constitutive activation of Hh signaling induced ROS production in conventionally reared flies (exposed to uracil-producing bacteria), but not in flies raised under germ-free conditions. Inhibition of Hh signaling during infection was associated with high host mortality, highlighting the essential role of the pathway. Overexpression of the critical Cad99C moiety in a Hh-deficient background restored formation of the signaling endosome and, consequently, Ca^{2+}-dependent activation of ROS production and resistance to infection.

These discoveries raise several interesting questions for future study. Molecular identification of the two distinct receptors that interact with uracil is of primary interest: Receptor X, involved in inducing Hh expression (and thereby expression of Cad99C), and Receptor Y, involved in transducing Ca^{2+}-activated DUOX activity via the $Rab7^{+}$ signaling endosome. The authors suspect that MEKK1, which is required for ATF2-dependent induction of DUOX gene expression (Ha et al., 2009a, 2009b), lies downstream of Receptor X. In the case of signal transduction via receptor Y, one must both tease apart how uracil-dependent receptor activation leads to clustering of Cad99C and how this stimulates PLCβ/PKC activity. Furthermore, whether Hh expression is induced in the same cells that respond to the ligand or in a distinct cell population remains to be determined. Peptidoglycan, shown by others to also induce Hh signaling (but not Cad99C-dependent endosomal signaling and DUOX activation), could activate Hh expression by the same or a different pathway. The activation of Hh expression may be linked to other known roles of this pathway (or Ca^{2+} signaling) in gut homeostasis, including regulation of stem cell proliferation in the *Drosophila* hindgut (Takashima et al., 2008), an organ that shares similarities in organization with the mammalian crypt-villus axis (Pitsouli and Perrimon, 2008).

It remains to be seen whether ROS production can be compartmentalized to bias killing of pathogenic bacteria and sparing commensal microflora, or whether the inflammatory process is indiscriminate with regard to bacterial class. A recent study found that commensal lactobacilli stimulate stem cell proliferation via ROS produced by another synthetic enzyme, Nox, in both flies and mammals (Jones et al., 2013). Since lactobacilli do not induce DUOX via activation of the signaling endosome, it is intriguing how ROS produced via these two different pathways can lead to alternative cellular responses. Another remaining puzzle is how or why uracil secretion represents an accurate indicator of potentially pathogenic species (Lee et al., 2013), and what mechanisms might be responsible for suppressing uracil secretion in different commensal species (e.g., altered synthesis, transport, metabolism, or sequestration). Certainly additional pathogen signals beyond uracil may feed into the identified pathways, and/or alternative signals produced by commensal bacteria could dampen immune responses at baseline to defend the gut epithelium against ROS damage. These may be productive avenues to build upon the key advances of this work and to investigate in diverse model systems including mammals.

REFERENCES

Buchon, N., Broderick, N.A., Poidevin, M., Pradervand, S., and Lemaitre, B. (2009). Cell Host Microbe *5*, 200–211.

Buchon, N., Broderick, N.A., and Lemaitre, B. (2013). Nat. Rev. Microbiol. *11*, 615–626.

Ha, E.M., Lee, K.A., Park, S.H., Kim, S.H., Nam, H.J., Lee, H.Y., Kang, D., and Lee, W.J. (2009a). Dev. Cell *16*, 386–397.

Ha, E.M., Lee, K.A., Seo, Y.Y., Kim, S.H., Lim, J.H., Oh, B.H., Kim, J., and Lee, W.J. (2009b). Nat. Immunol. *10*, 949–957.

Jones, R.M., Luo, L., Ardita, C.S., Richardson, A.N., Kwon, Y.M., Mercante, J.W., Alam, A., Gates, C.L., Wu, H., Swanson, P.A., et al. (2013). EMBO J. *32*, 3017–3028.

Kim, S.H., and Lee, W.J. (2014). Front Cell Infect Microbiol *3*, 116.

Lee, K.A., Kim, S.H., Kim, E.K., Ha, E.M., You, H., Kim, B., Kim, M.J., Kwon, Y., Ryu, J.H., and Lee, W.J. (2013). Cell *153*, 797–811.

Lee, K.-A., Kim, B., Bhin, J., Kim, D.H., You, H., Kim, E.-K., Kim, S.-H., and Ryu, J.-H. (2015). Cell Host Microbe *17*, this issue, 191–204.

Pitsouli, C., and Perrimon, N. (2008). Nature *454*, 592–593.

Takashima, S., Mkrtchyan, M., Younossi-Hartenstein, A., Merriam, J.R., and Hartenstein, V. (2008). Nature *454*, 651–655.

HSV Cheats the Executioner

Karen L. Mossman[1] and Sandra K. Weller[2,*]
[1]Departments of Biochemistry and Biomedical Sciences and Pathology and Molecular Medicine, Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, ON L8S 4K1, Canada
[2]Department of Molecular Biology and Biophysics, University of Connecticut Health Center, Farmington, CT 06030, USA
*Correspondence: weller@uchc.edu
http://dx.doi.org/10.1016/j.chom.2015.01.013

In this issue of *Cell Host & Microbe*, Huang et al. (2015) and Guo et al. (2015), along with recent work by Wang et al. (2014), reveal that HSV ribonucleotide reductase has opposing activities in either inducing or preventing necroptosis, depending on the host species. This evolutionary twist underscores the importance of selective pressure in virus-host relationships.

The induction of cell death in response to viral pathogens represents a powerful component of the host defense machinery. It is of no surprise that viruses have evolved potent counterdefense mechanisms. The best-studied cell death pathway is apoptosis, characterized by its dependence on caspase activation. Apoptosis has long been recognized as an important antiviral mechanism, by efficiently and ''quietly'' clearing dying cells. Multicellular organisms prevent the spread of viral infections by inducing apoptosis, and viruses, in turn, have evolved mechanisms to block caspase activation, thereby preventing apoptosis (reviewed in Chan et al., 2014). In response, mammalian hosts have evolved a backup mechanism, programmed necrosis or necroptosis, to kill cells and limit viral spread. This defense and counterdefense tug of war reflects a robust evolutionary arms race between hosts and their pathogens that continues to escalate.

Apoptosis and necroptosis pathways are interconnected, and the balance between the two is tightly regulated. Stimulation of the death receptors in the tumor necrosis factor (TNF) family leads to activation of apoptosis via caspase-8, which also prevents necroptosis by cleaving the adaptor proteins receptor-interacting kinase 1 (RIP1) and RIP3. Under conditions in which caspase-8 is inhibited, RIP1 can engage with its partner RIP3 through their RIP homotypic interaction motifs (RHIMs), forming a multiprotein complex termed the necrosome. Here, RIP3 kinase is activated, resulting in the phosphorylation of the mixed lineage kinase domain-like (MLKL) protein, leading to MLKL complex formation and eventual membrane disruption (Figure 1A) (reviewed