COMMENT

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Comment on "Enterocyte–innate lymphoid cell crosstalk drives early IFNg-mediated control of Cryptosporidium"

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The parasite *Cryptosporidia* infects and completes its entire life cycle within the intestinal epithelium. It has been long appreciated that interferon gamma is critical for protection, but the source of this cytokine, the mechanism of induction and the cell site of action have been unknown. Here Gullicksrud et al.[1] demonstrate in a mouse model that interferon gamma regulation and protection center on the infected epithelial cell. The work is of importance for the fundamental advances it provides on mucosal defense from intracellular parasitism, as well as providing a foundation for the advancement of vaccination against this cause of diarrhea, malnutrition and death of infants in low and middle income countries.

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The article by Gullicksrud et al.[1] answers not only an important question for the cryptosporidiosis field, but for mucosal immunologists in general. The role of the intestinal epithelial cells in the innate control of enteric infections is as fascinating as it is important.

There is no cell in the body that is more evolutionarily adapted to respond to the microbial world than the intestinal epithelial cell or enterocyte[2]. This single-cell layer serves as the sole barrier between us and the bacteria, viruses and parasites in our gut. Recently the scientific community has come to the appreciation that the gut is more than a PVC pipe with one end the esophagus and the other the colon, but in fact a finely tuned organ of the immune system. Immunologic functions of the epithelial lining of the gut include early innate recognition of enteropathogens by pattern recognition receptors, secretion of mucins and antimicrobial peptides to defend the gut barrier, autophagy to clear invasive microbes, luminal translocation of secretory IgA antibodies, and reactive oxygen production.

Pertinent to this article, intestinal epithelial cell communication with the innate and acquired immune system is critical in the maintenance of homeostasis in the intestine. One needs to go no further than to understand the role of the gut epithelial cytokine alarmins IL-25 and IL-33 in innate defense against *Clostridioides difficile* diarrhea, to appreciate their exquisite maintenance of the balance of inflammation vs homeostasis[3,4].

The intestinal epithelium is even more central with the human parasite *Cryptosporidium* as this is the cell that not only responds but is the cell where the parasite invades, grows, and replicates (Fig. 1). To take a step back, *Cryptosporidium* is an apicomplexan parasite as are malaria and toxoplasmosis, with the difference that the entire life cycle occurs in the small intestine epithelium. Where this article breaks new ground is in its discovery of epithelial–immune crosstalk leading to intracellular immunity.

It had long been appreciated that gamma interferon is key to innate immunity to cryptosporidiosis, even to the extent that the workhorse animal model was an interferon-gamma knockout mouse[5]. What had been unknown until this article was the source of, and mechanism of action of, interferon gamma.

Gullicksrud et al.[1] observed an increase in interferon gamma upon infection of mice with *Cryptosporidium parvum*. Neutralization of interferon gamma increased parasite number, even in a $Rag2^{-/-}$ mouse (lacking B and T cells), indicating that there was an innate immune system source of interferon gamma. They then went on to test the importance of interferon gamma in mice that additionally lack the common gamma chain of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 and thereby lack innate lymphoid cells ($Rag2^{-/-}Il2rg^{-/-}$ mice). These mice were even more susceptible to cryptosporidiosis, indicating both innate and acquired immunity participate in parasite clearance.

Innate producers of gamma interferon include NK cells, ILC1s, and ILC3s. In infected mice, there was a substantial increase in gamma interferon producing ILC1. Since it was known in other systems that IL-12 and IL-18 can synergistically induce ILC1 to produce interferon gamma, this was tested in the presence of cryptosporidia infection. Both $IL\text{-}12^{-/-}$ and $IL\text{-}18^{-/-}$ mice had increased susceptibility, and an epithelial-lineage specific knockout of IL-18 was used to demonstrate that intestinal epithelial cells were one source of IL-18 during cryptosporidia infection. Validating this, the use of mAbs to simultaneously blockade IL-12 and IL-18 also increased parasite burden. With the recent appreciation of the importance of the microbiome in cryptosporidiosis[6], it will be important to assess its role in epithelial cell production of IL-18.

A picture is thus emerging of intestinal epithelial cell production of IL-18 synergizing with IL-12 (perhaps from dendritic cells) to induce ILC1 to produce interferon gamma, with the IL-18 arising from inflammasome activation by the parasite. What remained to be determined was where the interferon gamma acts. STAT1 is the major STAT for interferon-gamma signaling, and $STAT1^{-/-}$ mice were more susceptible to cryptosporidiosis. While cell type-

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specific deletion of STAT1 from dendritic cells or macrophages was not found to increase the number of parasites infecting mice, deletion of STAT1 from the intestinal epithelial cells (using a tamoxifen-inducible villin-cre recombinase) parasite oocyst shedding was increased. Thus it seems likely that interferon gamma is acting to protect at least in part by actions on epithelial cells.

Gullicksrud et al. thus demonstrate that the intestinal epithelium activates and responds to interferon-gamma. Innate lymphoid cells type 1 (ILC1) are a source of interferon gamma, and that induction of its expression during cryptosporidiosis was due to the synergistic action on ILC1 of IL-18 from the epithelium and IL-12. The site of action for interferon gamma was shown to likely be back at the intestinal epithelium, as conditional knockout of STAT1, the major STAT protein activated by interferon gamma, in the epithelium led to an increase in parasite number (Fig. 2).

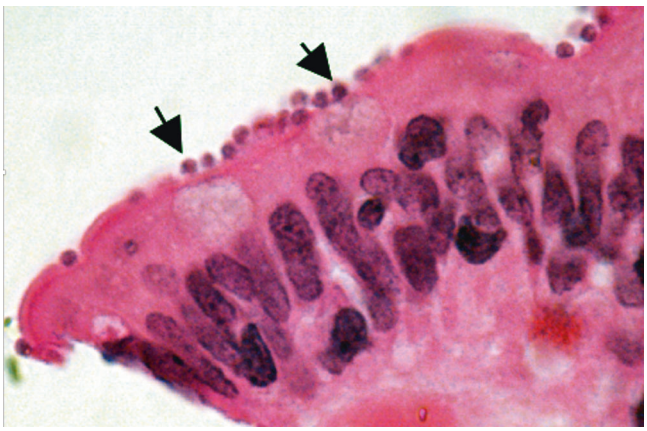

Fig. 1 Cryptosporidia-infected human small intestine. Arrowheads point to the intracellular parasites in the intestinal epithelial cells.

Acquired immunity exists to cryptosporidiosis: IgA against sporozoite proteins is associated with resistance to the second infection in children[7], and the overall incidence of infection declines with age. Immunity to cryptosporidiosis is associated with both class I and class II alleles, supporting the role of both CD4 and CD8 T cells[8]. It is thus of interest that one of the interferon gamma inducible epithelial genes discovered by Gullicksrud et al.[1] was the class II transactivator that enhances class I and induces MHC class II gene expression. One could envision that the anti-parasite activity of interferon gamma is greater in vivo than in vitro for this ability to promote recognition of infected cells via up-regulation of class I and II. There is thus potentially a direct connection of innate to acquired immunity acting through the cryptosporidia-infected intestinal epithelial cells.

The clinical importance of this work derives from the contribution of cryptosporidiosis to the health of children in low and middle income countries. Cryptosporidiosis is most important as a cause of diarrhea and malnutrition in the critical 1st year of life when most deaths due to diarrheal disease occur. It is a common and currently untreatable infection: in our Bangladesh cohort cryptosporidiosis diarrhea and infection occurred in 12% and 29% respectively of infants in the 1st year of life. We discovered that diarrhea due to *Cryptosporidium* (as well as *Campylobacter* but no other enteropathogens) in the first year of life was independently associated with the future development of malnutrition[9]. We also found that asymptomatic infection with both *Cryptosporidium hominis* and *Cryptosporidium meleagridis* from birth to age 2 years was associated with the development of malnutrition. Children with cryptosporidiosis (either asymptomatic or diarrhea) had an odds ratio of 2.69 (95% CI 1.17–6.15) for being malnourished (severely stunted, HAZ < −3) at age 2 years[9]. Therefore prevention

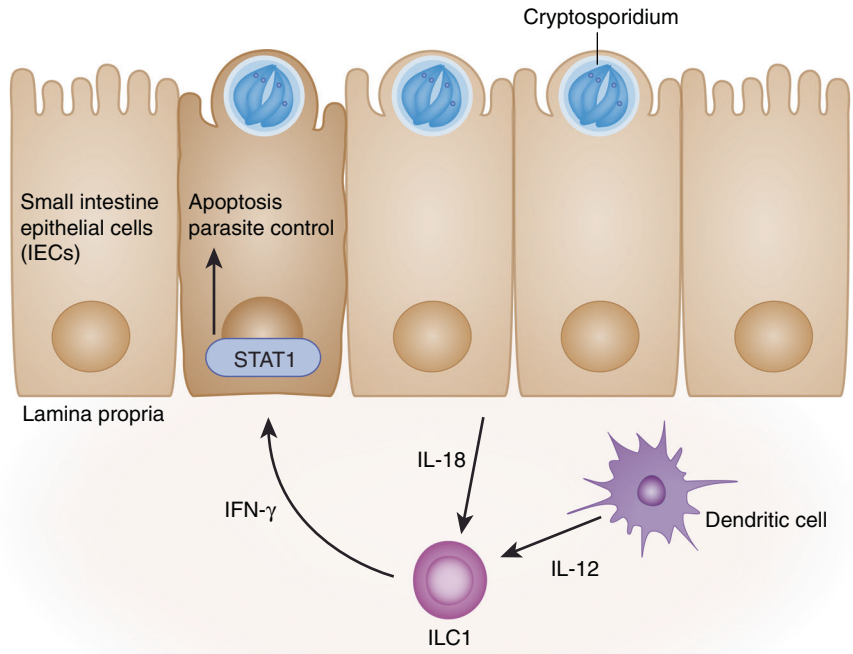

Fig. 2 Model for interferon gamma production and protection during cryptosporidiosis. IL-18 produced by the intestinal epithelial cells works synergistically with IL-12 from dendritic cells to induce innate lymphoid cells type 1 to produce gamma interferon. Gamma interferon activates STAT1 within the IECs to clear the parasitic infection, perhaps in part by upregulating antigen presentation via class I and II to allow acquired immune responses to be active.

of cryptosporidia-associated malnutrition might be an added benefit of vaccination.

Gullicksrud et al.[1] therefore by giving us a picture for how the innate immune system acts to protect at the level of the cryptosporidia-infected epithelial cell provides insights into the design of vaccine and therapeutics. Thus a critical barrier to progress in child health is beginning to be breached.

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AUTHOR CONTRIBUTIONS

W.A.P. and A.N.D. co-wrote the article.

COMPETING INTERESTS

W.A.P. is a consultant for TechLab, Inc which makes diagnostic tests for cryptosporidiosis. A.N.D. declares no conflicts.

ADDITIONAL INFORMATION

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