AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical and Vaccine Immunology

COMMENTARY

Control of Giardiasis by Interleukin-17 in Humans and Mice—Are the Questions All Answered?

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For years, studies of the immune response to *Giardia lamblia* infection focused on the production of IgA by infected hosts and antigenic variation by the parasite to escape destruction by this IgA. A new study by Hanevik and colleagues (C. S. Saghaug, S. Sørnes, D. Peirasmaki, S. Svärd, N. Langeland, and K. Hanevik, Clin Vaccine Immunol 23:11–18, 2016, http://dx.doi.org/10.1128/CVI.00419-15) highlights the emerging role of interleukin-17 (IL-17) in immunity to this parasite. Along with recent studies of *Giardia* infections of animals, this work shows that IL-17 appears to be essential for the control of these infections and to be a key factor linking cellular and humoral immune responses.

The article by Saghaug et al. in this issue of *Clinical and Vaccine Immunology* (1) examines the cytokine responses of effector memory CD4 T cells in individuals with ongoing and recent infections with *Giardia lamblia*. Several previous studies of human immune responses to this parasite have focused on patients in regions where giardiasis is endemic and are complicated by the likelihood of previous *Giardia* infection and/or the presence of coinfections (2–4). Other studies have utilized animal models of giardiasis that allow detailed analysis of immune responses but are unable to correlate these to real clinical outcomes. However, Saghaug et al. used flow cytometry to focus on cytokine production in response to parasite antigens specifically in the $CD4^+$ $CD197^-$ $CD45RA^-$ population of effector memory cells and stratified subjects by those with infections that were cleared in a shorter time (<8 weeks) and those with infections that were not rapidly eliminated. By doing so, they implicated the cytokines interleukin-17A (IL-17A) and tumor necrosis factor (TNF) in contributing to protective immunity, despite not being the most abundant cytokines detected in culture supernatants.

G. lamblia (synonyms, *G. duodenalis* and *G. intestinalis*) is a protozoan parasite that infects humans and most species of mammals (reviewed in references 5 to 7). It is the most commonly diagnosed parasitic cause of diarrhea in humans in North America and is ubiquitous in the developing world. Prevalence rates are reported to be 2 to 3% in the developed world and 20 to 30% in the developing world. Infections are initiated by the ingestion of infectious cysts found in contaminated food or water, and parasites then replicate in the small intestine. Infections can result in subclinical disease with patients exhibiting little, if any, sign of the infection or can produce severe cramps, nausea, and diarrhea. Nutrient malabsorption can occur in patients with subclinical disease, as well as those experiencing acute symptoms, and *Giardia* infection has been correlated to physical and cognitive developmental defects in children in several studies (5, 6). The mechanisms involved in both control of the infection and the production of symptoms are not well understood, although immune responses are considered important for both.

Giardia infections in humans and animal models are characterized by abundant production of antiparasite IgA. Much of this antibody is directed against isoforms of the variant-specific surface protein (VSP), a cysteine-rich protein that coats the parasite surface. Each *Giardia* trophozoite carries 150 to 200 different VSP-encoding genes, but only 1 is expressed on the parasite surface at a time. Switch variants are selected *in vivo* by IgA, and the ability to switch VSP expression allows the parasite to evade this element of host immunity (8, 9). Nevertheless, $CD4^+$ T-cell-dependent immune responses have been shown to lead to parasite elimination within a few weeks of infection in most cases (10, 11). While $CD4^+$ T cells are absolutely required for parasite elimination in mouse models of giardiasis, neither gamma interferon (IFN-γ) nor interleukin-4 (IL-4) is necessary (11). Several recent studies have instead examined the role of IL-17 (12–14).

The first study involved experimental *G. lamblia* infection of calves, where the level of mRNA for IL-17 was shown to be increased in $CD4^+$ T cells isolated from peripheral blood after *ex vivo* restimulation with parasite extracts. Interestingly, the only other transcript that significantly increased following infection was the regulatory T cell (Treg) transcription factor FoxP3 (12). This was followed shortly by the publication of results of mouse infections with the rodent species *G. muris*. Those authors found significantly elevated IL-17A transcript levels in RNA isolated from the duodenum 3 weeks postinfection with *G. muris* cysts. mRNA for the transcription factor RORγt, which is commonly associated with Th17 cells, was also elevated following infection. More importantly, mice lacking the IL-17RA subunit of the IL-17 receptor had greater parasite burdens in the small intestine and excreted more cysts than wild-type control mice (13).

In a more recent study, the Eckmann group showed by flow cytometry that $CD4^+$ T cells from the lamina propria of the small intestine were producing more IL-17A following infection with *G. muris*. Interestingly, they also found elevated IL-17A mRNA levels in the small intestines of CD4-deficient or RAG-deficient mice, suggesting that there may be non-T-cell

Accepted manuscript posted online 18 November 2015

Citation Singer SM. 2016. Control of giardiasis by interleukin-17 in humans and mice—are the questions all answered? Clin Vaccine Immunol 23:2–5. doi:10.1128/CVI.00648-15.

Editor: P. P. Wilkins

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sources of IL-17A as well (14). Infections in mice lacking IL-17A and in bone marrow chimeras demonstrated that IL-17A production by hematopoietic cells was required for efficient control of *G. muris* and also *G. lamblia*. Similar to the previous paper by Dreesen et al., they showed that mice lacking IL-17RA were also defective in the control of *G. muris* infections and bone marrow chimeras indicated that the receptor was required on hematopoietic cells to mediate an effective antiparasitic effect. Additional comparisons of wild-type and IL-17A-deficient animals found that mice lacking IL-17A had significantly reduced fecal IgA levels both before and after infection, consistent with a role for IL-17 in inducing expression of the poly-Ig receptor that transports IgA from the serum into the intestinal lumen (15). IL-17A-deficient mice also had lower levels of mRNA encoding β-defensin-1, and IL-17RA-deficient mice had lower levels of mRNAs for resistin-like molecule β (RELM-β), serum amyloid A1 (Saa1), and serum amyloid A2 (Saa2), all proteins with potential antimicrobial and immunoregulatory functions (16–20).

The study by Saghaug et al. reported in this issue (1) focused on a group of *Giardia*-infected individuals in Norway who had presumably acquired infections while traveling abroad. Using flow cytometry, the authors found increases in IL-17A and TNF production by $CD4^+$ $CD197^-$ $CD45RA^-$ T cells after restimulation with *Giardia* extracts, indicative of a memory T cell response. Analysis of supernatants from restimulated cultures of mononuclear cells confirmed the expression of IL-17A and TNF in *Giardia*-exposed individuals, although the levels of numerous other cytokines, including IFN-γ, IL-13, IL-10, and IL-1β, were also significantly elevated in these supernatants.

Together, these publications make a reasonably strong case that IL-17 responses of $CD4^+$ T cells are a key component of the anti-*Giardia* immune response. Interestingly, while IFN-γ is more abundant than IL-17A in supernatants of restimulated cultures of cells from both mice and humans previously infected with *Giardia*, it is IL-17A and not IFN-γ that appears to be important for parasite control (1, 21). This is important for several reasons, most notably that it will allow more-focused research into how immune responses shape the variable outcomes of *Giardia* infection. While $CD4^+$ T cell responses have been known to play a central role in *Giardia* control, the effector mechanisms actually responsible for parasite elimination are unknown. As mentioned above, IgA certainly plays a role but antigenic variation by the parasite provides a means of immune escape and may require other mechanisms. Mouse studies have implicated mast cell responses, intestinal hypermotility, nitric oxide production, and α-defensin (an antimicrobial peptide) expression in parasite control (22–26), but the work of Dann et al. suggests that none of these is specifically impaired in the IL-17A-deficient mouse (14). They did find reduced expression levels of β-defensin-1 along with other antimicrobial peptides (RELM-β, Saa1, and Saa2), as well as reduced transport of IgA into the intestinal lumen in the absence of IL-17A or its receptor IL-17RA, but additional studies are needed to determine which of these mechanisms (or others) are primarily responsible for parasite elimination.

Identification of IL-17-producing effector memory T cells as a correlate to infections that resolve more quickly suggests that the development of a *Giardia* vaccine should focus on formulations that activate this pathway. Dann et al. indicated that Th17 cells do not develop in IL-6- or RORγt-deficient mice (14), suggesting that the Th17 response to *Giardia* likely develops in a manner similar to that of other Th17 responses, requiring IL-6, transforming growth factor β, and IL-23 (27). Interestingly, SCID mice and CD4-deficient mice also had elevated levels of IL-17A mRNA in their small intestines following *Giardia* infection, even though these strains cannot eliminate the infections. This suggests that other cells can produce IL-17 in this situation, e.g., innate lymphocytes, but also that products of Th17 cells in addition to IL-17 may be important for effective immunity. Additional work looking at other characteristic Th17 cytokines (e.g., granulocyte-macrophage stimulating factor or IL-22 [27]) or the role of "Ex-Th17" cells, T cells that no longer express IL-17 and assume characteristics of other Th subsets, including Th1, Th2, Treg, and T_{FH} cells (28–30), is needed. Interestingly, bone marrow chimera studies indicate that IL-17RA expression on hematopoietic cells is more important than that on epithelium or stromal cells (14), suggesting that the role of IL-17 in the control of *Giardia* infections may involve immune regulatory networks rather than direct activation of epithelial cell effector molecules such as RELM-β.

It will also be important to understand whether Th17 cells have a role in immunopathology associated with *Giardia* infection. As noted above, *Giardia* infection of children is correlated to defects in physical and cognitive development. The mechanisms involved in these defects are poorly understood, although immune responses have been implicated in contributing to nutrient malabsorption in mouse models (21, 31). These studies have generally implicated $CD8^+$ T cells in mediating this response, but how $CD8^+$ cells are activated during infections with a noninvasive, extracellular microbe is unclear and further examination of immune regulatory pathways involving IL-17 will be important in determining if the IL-17 responses contribute to pathology during giardiasis, as well as to protective immunity.

Giardia infection has also been correlated to a reduced incidence of severe diarrhea in children in the developing world (32, 33). While this may sound paradoxical with regard to the importance of *Giardia* infection in developmental defects due to chronic diarrhea, the specific definitions of diarrhea used when performing these analyses are quite different. IL-17 has been shown to directly upregulate the barrier function of the intestinal tract (34–36). Moreover, Th17 cells also promote the barrier function of the intestinal epithelium via secretion of IL-22 (37), and the generation of Th17 cells in *Giardia* infection may therefore reduce the severity of diarrhea associated with other enteric pathogens. The IL-17 response also upregulates the production of several antimicrobial effector mechanisms, including RELM-β, Saa1, and Saa2, that might be effective in reducing burdens due to other enteric pathogens, even if *Giardia* itself is not affected by them.

Finally, *Giardia* infection has also been correlated to the development of chronic fatigue syndrome and irritable bowel syndrome in patients years after their *Giardia* infections have been eliminated (38). It will be interesting to see if the Th17 signature responses, or other specific immune changes, correlate to these long-term sequelae. Importantly, these recent papers demonstrate the power that combinations of animal models and well-designed human studies have in identifying important aspects of human disease. However, whether studies of IL-17 will help clarify the reasons for the variable outcomes of human infections with this parasite remains to be determined.

FUNDING INFORMATION

HHS | NIH | National Institute of Allergy and Infectious Diseases (NIAID) provided funding to Steven M. Singer under grant number AI109591-01A1.

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