Control of Giardiasis by IL-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. Sahaug, S. Sornes, D. Peirasmaki, S. Svard, N. Langlenad and K. Hanevik, Human memory CD4+ T cell responses against *Giardia lamblia*) highlights the emerging role of IL-17 in immunity against this parasite. Along with recent studies of animal infections with *Giardia*, this work shows that IL-17 appears to be essential for control of this infection 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 examines the cytokine responses of effector memory CD4 T cells in individuals with ongoing and recent infections with *Giardia lamblia*. Several previous studies on human immune responses to this parasite have focused on patients in endemic regions and are complicated by the likelihood of previous *Giardia* infection and/or the presence of co-infections (1-3). 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, the present work uses flow cytometry to focus on cytokine production in response to parasite antigens.
specifically in the CD4+CD197-CD45RA- population of effector memory cells and stratifies subjects by those with infections that were cleared in a shorter time (<8 weeks) compared with those infections which were not rapidly eliminated. By doing so the authors implicated the cytokines IL-17A and TNF in contributing to protective immunity, despite not being the most abundant cytokines detected in culture supernatants.

*Giardia lamblia* (syn. *G. duodenalis* and *G. intestinalis*) is a protozoan parasite that infects humans and most species of mammals (reviewed in (4-6). 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 2-3% in the developed world and 20-30% in the developing world. Infections are initiated by ingestion of infectious cysts found in contaminated food or water and parasites then replicate in the small intestine. Infections can result in sub-clinical 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 sub-clinical 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 (4, 5). The mechanisms involved in both control of the infection and in producing 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 anti-parasite 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 trophozoite of *Giardia* carries 150-200 different VSP genes, but only one 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 (7, 8).
Nevertheless, CD4+ T cell-dependent immune responses have been shown to lead to parasite elimination within a few weeks of infection in most cases (9, 10). While CD4+ T cells are absolutely required for parasite elimination in mouse models of giardiasis, neither IFNγ or IL-4 are necessary (10). Several recent studies have instead examined the role of IL-17 (11-13).

The first study involved experimental *G. lamblia* infection of calves, where 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 transcription factor FoxP3 (11). This was followed shortly by publication of results from mouse infections with the rodent species *G. muris*. These authors found significantly elevated transcript levels for IL-17A in RNA isolated from the duodenum 3 weeks post-infection with *G. muris* cysts. Messenger RNA 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 higher parasite burdens in the small intestine and excreted more cysts than wild-type control mice (12).

A more recent study from the Eckmann group showed by flow cytometry that CD4+ T cells from the lamina propria of the small intestine were producing more IL17A following infection with *G. muris*. Interestingly, they also found elevated IL-17A mRNA in the small intestine of CD4 deficient or RAG deficient mice, suggesting there may be non-T cell sources of IL-17A as well (13). 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 from Dreesen et al., they showed that mice lacking IL-17RA were also defective in control of *G. muris* infections and bone marrow chimeras indicated that the receptor was required on hematopoietic cells to mediate effective anti-parasitic effect. Additional comparisons between WT 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 (14). 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 (relmb), serum amyloid A1 (saa1) and serum amyloid A2 (saa2), all proteins with potential anti-microbial and immunoregulatory functions (15-19).

The paper in the current issue of this journal focuses on a group of *Giardia*-infected individuals in Norway who had acquired infections, presumably 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 expression of IL-17A and TNF in *Giardia* exposed individuals, although the levels of numerous other cytokines including, IFNγ, 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 by CD4+ T cells are a key component of the anti-*Giardia* immune response. Interestingly, while IFNγ is more abundant than IL-17A in supernatants of restimulated cultures from both mouse and...
humans previously infected with *Giardia*, it is IL-17A and not IFNγ that appears to be important for parasite control (Saghaug et al. and (20). This is important for several reasons, most notably that 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 anti-microbial peptide) expression in parasite control (21-25), but the work from Dann et al., suggests that none of these are specifically impaired in the IL-17A deficient mouse (13). They did find reduced levels of β-defensin-1 expression along with other anti-microbial 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 as a correlate to infections which resolve more quickly, suggests that development of a *Giardia* vaccine should focus on formulations which activate this pathway. Dann et al. indicate that Th17 cells do not develop in IL-6 or RORγt deficient mice (13), suggesting that the Th17 response against *Giardia* likely develops in a similar manner to other Th17 responses, requiring IL-6, TGFβ and IL-23 (26). Interestingly, SCID mice and CD4 deficient mice also had elevated levels of IL-17A mRNA in the small intestine following *Giardia* infection, even though these strains cannot eliminate the infections. This suggests 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., GM-CSF or IL-22 (26)) or the role of “Ex-Th17” cells, T cells which no longer express IL-17 and assume characteristics of other Th subsets including Th1, Th2, Treg and TFH cells (27-29). Interestingly, bone marrow chimera studies indicate that IL-17RA expression on hematopoietic cells is more important than on epithelium or stromal cells (13), suggesting that the role of IL-17 on control of *Giardia* infections may involve immune regulatory networks rather than direct activation of epithelial cell effector molecules such as Relmβ.

It will also be important to understand whether Th17 cells have a role in immunopathology associated with *Giardia* infection. As noted above, infection with *Giardia* in 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 (20, 30). These studies have generally implicated CD8+ T cells in mediating this response, but how CD8+ cells are activated during infections with a non-invasive, extracellular microbe is unclear and further examination of immune regulatory pathways involving IL-17 will be important to determining if the IL-17 responses contribute to pathology during giardiasis as well as to protective immunity.

*Giardia* infection has also been correlated to reduced incidence of severe diarrhea in children in the developing world (31, 32). While this may sound paradoxical in regards to the importance of *Giardia* in developmental defects due to chronic diarrhea, the different and 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 (33-35). Moreover, Th17 cells also promote the barrier function of the intestinal epithelium via secretion of IL-22 (36), 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 production of several anti-microbial effector mechanisms including Relmβ, Saa1 and Saa2 that might be effective in reducing pathogen burden due to other enteric pathogens, even if the *Giardia* themselves are not effected 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 (37). It will be interesting to see if the Th17 signature responses, or other specific immune changes, correlate to these long-term sequellae. Importantly, these recent papers demonstrate the power that combinations of animal models and well-designed human studies have on identifying important aspects of human disease. But whether studies of IL-17 will help clarify the reasons for variable outcomes of human infection with this parasite remain to be determined.


