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Intraepithelial cells are the first line of defence against enteric infection

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The role and function of intraepithelial lymphocytes (IELs) within the barrier and mucosal tissue have intrigued scientists for many years. Locality of these cells, within the epithelial layer at the basolateral surface of adjacent epithelial cells, has been a key feature of many studies investigating their functionality. IELs are characterized by the T cell receptor chains, in which the γδ T cells differ from αβ T cells in that they recognise proteins without antigenic processing or MHC molecules. Approximately 50% of small intestinal IELs are γδ T cell receptor cells (TCR) and express multiple TCR variable (V)γ and Vδ regions amongst which Vγ1 and Vγ7 predominate (Proc Natl Acad Sci U S A 1989;**86**:5527-31; Proc Natl Acad Sci U S A 1997;**94**:5761-6; Proc Natl Acad Sci U S A 2004;**101**:5616-21; Infect Immun 2006;**74**:1097-105). In gastrointestinal diseases these cells have been viewed as part of the immunology response to gluten and thereby are part of the criteria for the diagnosis of celiac disease (World J Gastroenterol. 2017; 23(42): 7505–7518). In addition, IELs have been detected at sites of microbial induced damage and described as the first line of defense against pathogens (J Immunol 2005;**175**:8191-9; J Immunol 2005;**175**:1741-50; Gastroenterology 2006;**131**:818-29; Proc Natl Acad Sci U S A 2011;**108**:8743-8748; Gastroenterology 2015;**148**:1417-26). What drives this localisation and recruitment of the IELs into these areas has lead investigations into migration routes and what drives this recruitment. Initially this migration was demonstrated through chemokine gradients via the CCR9/CCL25 axis (J Immunol 210;**185**:5160-5168) with further evidence of migration driven by the molecules such as adhesion and tight junctional proteins which tether the IELs to adjacent epithelial cells (Proc Natl Acad Sci U S A 2012;**109**:7097-102.) and cytokines such as IL-15 (J Immunol 2018 June 8 [Epub ahead of print]).

The authors have used these recent studies as a basis to investigate some of the mechanisms through which IELs respond to gut microbes specifically focusing their distribution from the crypt to villus. It is already known that populations of IEL, particularly the γδ T cell subset in the intestine remain intact in germ free conditions even though these populations fall in peripheral blood. The investigators employed histological clearing techniques to enable 3D deep tissue imaging to be undertaken. TCRγδ^{GFP} mice were used in order to image the distribution of γδ IEL localization along the crypt villus axis of duodenum, jejunum and ileum. Most of the γδ IELs were found in the middle of the villus with lower numbers at the villus tip and crypt. This distribution corresponds with the increased numbers of microbes in this region (Nat Rev Immunol 2010;10:159-169). In germ-free conditions this distribution shifted towards the crypt. Recolonisation of germ-free mice with microbiota from specific pathogen free (SPF) mice or monocolonisation with segmented filamentous bacteria (SFB) or other common commensal bacteria species restored the normal distribution of γδ IELs. The authors argue that these data suggest there is a microbiota-sensing mechanism which determines the distribution of γδ IELs along the crypt villus axis.

The investigators then employed deep (±80 μm) multiphoton intravital microscopy to study the migration patterns of γδ IELs within the intestinal mucosa. They found that γδ IELs migration is restricted to the layer between the epithelial cells and the basement membrane. The area of epithelium covered by γδ IEL migration was reduced in germ-free conditions. Studies with SFB and other commensal bacteria suggested that attachment of the bacterium to the epithelial cell was important for determining γδ IEL migratory behavior. The investigators also found that γδ IELs migrate in both upward and downward directions at a rate of 4 – 6 μm per hour with net migration directed towards the crypt. These migration rates were substantially reduced in germ-free conditions. This net migratory pattern of γδ IELs towards the crypt offsets the upward migratory speed of epithelial cells suggesting that γδ

IELs might be a able to sense epithelial cell proliferation (Proc Natl Acad Sci U S A 1995;92:6147-51, PLoS ONE 11, e0156334). The authors concluded that commensal bacteria direct the migratory behavior of γδ IELs creating a surveillance program that can cover the entire epithelial surface within a few hours.

The investigators then turned their attention to the effect of enteric pathogens on γδ IEL migratory patterns. It was already known that γδ IELs play a critical role in preventing invasion of *Salmonella Enterica* by interacting with occludin in the tight junction (Gastroenterology 2006;131:818829, Gastroenterology 2015;148:1417-26). After infection with *Salmonella Enterica* the γδ IELs remained in the epithelial compartment but the migratory pattern completely changed. The γδ IELs started to migrate between the epithelial cells in the lateral intracellular spaces in a pattern the authors called "flossing" recalling cleaning between teeth with dental floss. This description of IELs movement builds on observations by Edelblum et al (Proc Natl Acad Sci U S A 2012;**109**:7097-102) in which migration of IELs within the epithelial layer was demonstrated to be regulated and driven by the tight junction molecule, occludin. In addition to this movement van Konijnerburg et al determined that *Salmonella* infection also reduced the vertical movement of γδ IELs. Similar results were obtained after infection with *Toxoplasma gondii*. Hotspots of γδ IELs flossing behavior correlated with the presence of Toxoplasma. These changes migratory behavior return to normal after loss of the pathogen from the epithelial surface. The authors conclude that γδ IELs mount a rapid change in migratory behavior enabling appropriate positioning of IELs to counter pathogen invasion.

The mechanisms underlying the changes in γδ IELs behavior were then investigated. Transcriptome analysis of isolated γδ IELs after infection revealed an increase gene associated with bacterial defense responses and adhesion pathways. Analysis of gene changes in epithelial cells isolated with parallel with γδ IELs showed an increase in pathways downstream of MyD88, the adapter protein downstream of Toll-like receptors which sense pathogens. This builds on the findings by Ismail et al (Proc Natl Acad Sci U S A 2011;**108**:8743-8748) that showed that activation of the γδ IELs was dependent on MyD88 with the epithelial cells providing microbial driven signals to the IELs. The authors also found an increase in expression of Wnt/β-Catenin pathways after infection in both γδ IELs and epithelial cells suggesting tissue regeneration and repair responses after infection. These data suggest that the changed in γδ IELs behavior may be triggered by epithelial cells which have responded via MyD88 to luminal pathogens. To investigate this hypothesis the investigators used Villin-^{CreER}Myd88$^{f/f}$ mice in which MyD88 is deleted specifically in intestinal epithelial after exposure of the mice to Tamoxifen. The gene programs in γδ IELs after infection with *Salmonella* were not activated in γδ IELs isolated from Villin-^{CreER}Myd88$^{f/f}$ mice infected with *Salmonella.* Similarly, the increase in "flossing" behavior seen after infection with *Salmonella* or *Toxoplasma* was lost in mice without MyD88. Control experiments eliminated T cell receptors in triggering the increase in flossing behavior after infection with *Salmonella*.

The investigators then studied the metabolic modifications underlying the changes in γδ IELs behavior. Measurement of extracellular acidification and oxygen consumption rates showed an increase in anaerobic glycolysis and oxidative phosphorylation in γδ IELs isolated from mice infected with *Salmonella*. Deletion of MyD88 from the epithelial cells of the mice abolished these metabolic responses confirming that sensing of luminal microbes by the epithelial cells is responsible for these changes in energy production.

To determine if these metabolic responses are required for the changes in migratory behavior of γδ IELs after infection the mTOR pathway, an upstream regulatory pathway of metabolic responses, was blocked with Rapamycin. This substantially reduced the flossing migratory behavior after *Salmonella* infection. Blocking glycolysis with the non-metabolizable glucose analog 2-deoxy-glucose also prevented infection-induced flossing behavior and enhanced bacterial invasion. Conversely metformin which activates glycolysis increased γδ IEL flossing behavior.

Comment

Overall these data show that luminal pathogens triggers a surveillance response in γδ IELs in which the epithelial cells sense the bacteria via a Myd88 mediated mechanism. This increases energy production in γδ IELs which activates migration of γδ IELs enhancing surveillance between epithelial cells and reducing bacterial invasion.

This paper gives important new information on the multifaceted nature of the intestinal barrier that separates the body from its environment and prevents the invasion of microorganisms. Several components of the barrier are now recognized. The epithelial surface of the intestine is covered with two layers of mucus secreted by goblet cells. The outer layer contains commensal bacteria while the inner layer tends not to harbor bacteria but contains the antimicrobial peptides defensins, Cathelicidins and RegIIIα in humans and RegIIIγ in mice. These are secreted by Paneth cells at the base of the crypt in response to intestinal microbes via NF-κB, inflammasome and MyD88 pathways. The inner layer also contains secretory IgA from plasma cells in the lamina propria next to mucosal surfaces (Front Immunol 2012;3:310). A physical barrier is provided by the epithelial cell monolayer lining the intestine and tight junctions between the epithelial cells. Tight junctions are multiprotein complexes at the epithelial apex between the epithelial cells whose permeability is regulated in response inflammatory stimuli including TNF (Cold Spring Harbor Perspect Biol 2018;10: pii: a029314).

It has recently been appreciated that special mechanisms are required to maintain the barrier at the level of the epithelial cell. In health, there is a continuous shedding of epithelial cells from villus tip or colonic surface because of migration of epithelial up the crypt villus axis from stem cells at the base of the crypt (Gastroenterology 2012;143:1389). This "physiological" cell shedding is counter balanced by cell division in the crypt to maintain homeostasis and integrity of the crypt/villus axis. When the epithelial cell is shed, a discontinuity or gap in the villus epithelial monolayer is created, which could compromise the epithelial barrier. However, in the healthy gut, this gap is plugged by redistribution of tight junction proteins to surround the extruding cells. These tight junction proteins include occludin, ZO-1 and the adherens junction protein E-cadherin (Gastroenterology 140:1208-18 Am J Physiol Cell Physiol. 2012; 300:C1404-14). In the inflamed intestine with high concentrations of TNF epithelial shedding rates increase and multiple adjacent cells shed simultaneously. This creates discontinuities or "gaps" in the epithelial monolayer that are too wide to be sealed by the redistribution of tight junctions. This causes the epithelial barrier to fail allowing entry of bacteria and toxins. The "gaps" are also likely to enlarge into the epithelial ulcers characteristic of Inflammatory Bowel disease (PLOS Pathogens 2006; e3. Epub 2006, Gut 2012;61 1146-1153).

This paper provides compelling evidence that γδ IELs patrol the intestinal mucosa just underneath and between epithelial cells to trigger immunological reactions against

microorganisms that have successfully penetrated the epithelial monolayer. There is now abundant evidence that gut microbes play an important role in the pathogenesis of IBD (Clin J Gastro 2018;11:1-10). Given the importance of γδ IELs in bacterial defense this raises the question of whether γδ IELs play a role in IBD. γδ IEL populations in peripheral blood are reduced in Crohn's disease (Dig Dis Sci 2011;56:1613-22). The situation with mucosal γδ IELs in Crohn's disease is less clear with both increased and decreased populations being reported (J Crohn's and Colitis 2017;11:1135-45). The current study emphasizes that the migratory behavior of γδ IELs is critical in their action against invading microorganisms. The presents a major challenge for clinical studies of IBD patients as studying γδ IELs migratory behavior is not possible without in vivo dynamic imaging. Perhaps methods will be developed using confocal or multi-photon colonoscopy (Dig Dis and Sci 2014;59:1344-1346). Another issue is dissecting whether γδ IELs are protective as suggested by their anti-bacterial action or pro-inflammatory as suggested by their capacity to increase IFN-γ secretion by αβ T cells (J Immunol 2013:191;2752-63, Dig Dis and Sci 2014;59:1344-1346).

Clearly, there is much to learn about the role of γδ IELs in human disease. They play a positive role in infectious gastroenteritis, but the situation is much less clear in IBD. Future studies will determine whether γδ IELs biology can be exploited for diagnostic or therapeutic purposes, perhaps by modulating their migratory behavior which the current study has shown to be crucial for their antibacterial action.