Immune regulation of epithelial cell function: Implications for GI pathologies

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\textbf{A B S T R A C T}

The mammalian immune system is a complex and dynamic network that recognizes, responds, and adapts to numerous foreign and self-molecules. CD4\textsuperscript{+} T cells orchestrate adaptive immune responses and upon stimulation by antigen, naïve CD4\textsuperscript{+} T cells proliferate and differentiate into various T cell subsets including T helper (Th) 1, Th2, and Th17 effector cells, and T regulatory cells (Treg). Each of the T cell subsets is characterized by distinct profiles of cytokines and carries out distinct and sometimes opposing activities. Initiated by IL-12 released from dendritic cells, the development of Th1 cells is the typical host response against the invasion of intracellular pathogens such as bacteria or viruses. Th1 cells deliver cell-mediated immunity through their secreted cytokines such as IFN-\(\gamma\), TNF-\(\alpha\), IL-1\(\beta\), IL-2, and IL-12, and are responsible for the clearance of intracellular pathogens. Th2 cells are initiated by IL-4 and develop in response to allergens or the invasion of extracellular pathogens. Th2 cytokines include IL-4, IL-5, and IL-13, and are particularly important for allergic responses and the clearance of parasites.

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1. Introduction

The divergent Th1/Th2 lineages, with their polarized cytokine profiles and counter-regulatory abilities, are the classical paradigm to explain orchestration of the host response to pathogens and establishment of memory responses (Mosmann & Coffman, 1989). Recent studies questioned this strict division with the discovery of the Th17 subset, a third T effector cell lineage that is distinct from the Th1 and Th2 lineages (Harrington et al., 2005). First associated with autoimmune diseases including inflammatory bowel disease (IBD) (Bamias, Nyce, De La Rue, & Cominelli, 2005b; Cua & Kastelein, 2006; Murphy et al., 2003; Weaver, Hatton, Mangan, & Harrington, 2007), Th17 cells also participate in antimicrobial immunity and inflammatory pathologies (Bettelli, Korn, Oukka, & Kuchroo, 2008). Their differentiation requires TGF-\(\beta\) in concert with other inflammatory mediators such as IL-1, IL-6, IL-21, or IL-23 (Manel, Unutmaz, & Littman, 2008; Volpe et al., 2008). Both Th1 cytokines and most members of the Th17 family, especially IL-17A and IL-17F, are involved in the pathogenesis of IBD (Fujino et al., 2003; Nielsen, Kirman, Rudiger, Hendel, & Vainer, 2003). In contrast, IL-17E (IL-25) promotes Th2 responses and inhibits Th1 and Th17 cytokine responses (Fort et al., 2001; Kleinschek et al., 2007; Owyang et al., 2006). The differentiation of CD4\textsuperscript{+} T cells into Tregulatory cells (Tregs) plays a critical role in maintaining immunological tolerance to self-antigens or suppressing excessive immune responses (Vignali, Collison, & Workman, 2008). The two

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most well-deﬁned populations of Tregs are the forkhead box protein 3 (Foxp3)+ Tregs and the interleukin-10 (IL-10)-producing type 1 Tregs (Tr1 cells). The reciprocal regulation among the effector and regulatory T cells enables the delicate balance between the immunologic responses and therefore, is critical to overall human and animal health.

More than one third of the world’s population are infected with gastrointestinal roundworms including those within the genera Ascaris, Trichuris, and Strongyloides. In general, most enteric nematodes induce an elevation of Th2 cytokines, including IL-4 and IL-13, that are linked to protective immunity against worm infection (Elliott et al., 2003; Summers et al., 2003). The rising prevalence of autoimmune diseases including diabetes, IBD, and celiac disease, is mirrored by the decreasing incidence of helminth infection and this has been attributed, in part, to the polarity of Th cell responses. This relationship between the epidemiology of these diseases forms the basis for the “hygiene hypothesis” that links improvements in hygienic measures to an increase in autoimmune and allergic diseases (Weinstock et al., 2002). Although this concept does not address the influence of other factors such as environmental pollutants or changes in diet, it does support the importance of polarized Th cell cytokine profiles (Weinstock et al., 2002). The clinical success of therapies directed at targeting Th2 cytokines provides an alternate therapeutic approach to the treatment of IBD, especially Crohn’s disease, than that offered by the current anti-inﬂammatory and anti-immune regimens (Elliott et al., 2003; Summers et al., 2003). The protective eﬀects of nematode infection were attributed to the well-documented ability of Th2 cytokines to downregulate pro-inﬂammatory Th1 cytokine production (Sutton et al., 2008) or to promote the development of Treg (Setiawan et al., 2007). As one of the major regulatory mechanisms in the immune system is control of the balance of cytokines, these autoimmune GI pathologies are considered excellent targets for immunomodulatory therapies.

1.1. Immune mediated changes in gut function

An important function of the intestinal epithelium is to control intraluminal ﬂuid by regulation of tissue permeability, absorption of nutrients or ions, and secretion. Contractions of smooth muscle are necessary for mixing contents with digestive secretions, increasing exposure to the absorptive surface, and propelling contents along the GI tract. Worm expulsion is dependent on intact Th2 response, with IL-4 and IL-13 binding to receptors on both hematopoietic and non-hematopoietic cells, which are uniquely linked to STAT6 signaling (Urban et al., 2000). The importance of the immune regulatory Th2 cytokines, IL-4 and IL-13, in parasite expulsion is reinforced by several studies investigating the role of these cytokines in parasite-induced alterations in intestinal mucosal function (Akiho, Blennerhassett, Deng, & Collins, 2002; Finkelman, Wynn, Donaldson, & Urban, 1999; Madden et al., 2002, 2004; Zhao et al., 2002, 2003). IL-4 and IL-13 bind to receptors located on the cell surface of immune and structural cells in the gut including epithelial cells, smooth muscle cells, and neurons. The biological activity of IL-13 is attributed to its binding to the type II IL-4R that is composed of IL-13Rα1 in a heterodimeric complex with IL-4Rα, which is linked to activation of STAT6 (Zurawski et al., 1995). There is a constitutive expression of the type II IL-4R on epithelial cells indicating that IL-4/IL-13 can exert direct eﬀects on epithelial cells through STAT6-dependent genes and that expression of these receptor components changes during infection, thereby inﬂuencing the eﬀects of the Th2 cytokines (Morrison et al., 2006, 2009). Enteric nematode infection alters epithelial function through multiple eﬀects that shift the balance of ion and ﬂuid ﬂow towards the gut lumen. In contrast, the Th1 proﬁle, featuring high levels of IL-12 or IFN-γ, promotes worm survival in the small intestine or colon. The Th1 cytokines, IL-1β and TNF-α, are elevated early in the inﬂammatory process and induce a general hypo-responsiveness of mucosal function. IL-17 also increases permeability of epithelial cell lines (Kinugasa, Sakaguchi, Gu, & Reinecker, 2000) and it is possible that some of the inﬂammation-induced changes in gut function in IBD patients and animal models of IBD may be attributed to members of the Th17 family.

1.1.1. Mucosal barrier function

A key function of the intestinal epithelium is to act as a physical barrier between the external and internal milieu. A major function of the epithelial cells is regulation of mucosal barrier function. When this is breached, the host responds by restricting access of pathogens to the surface epithelium and activating appropriate local innate and adaptive immune responses. Control of intestinal permeability is critical as changes in function that allow passage of intraluminal bacteria across the mucosal barrier in large numbers trigger innate immune responses. The regulation of intestinal permeability is emerging as a key factor in the pathogenesis of several autoimmune diseases that aﬀect the GI tract including diabetes, celiac disease, and IBD (Fasano & Shea-Donohue, 2005). Tight junctions are one of the hallmarks of absorptive and secretory epithelia. As a functional barrier between apical and basolateral compartments, tight junctions selectively regulate the passive diﬀusion of ions and small water-soluble solutes through the paracellular pathway, compensating for any gradients generated by transcellular pathways (Diamond, 1978). Tight junctions are dynamic complexes and are able to adapt to a variety of physiologic (Madara & Pappenheimer, 1987) and pathologic (Milks, Conyers, & Cramer, 1986) events. Many of the proteins that make up the tight junction strands have been identiﬁed and the most important are occludin (Furuse et al., 1993), members of the claudin family (Furuse, Fujita, Hiroaki, Fujimoto, & Tsukita, 1998), a group of at least 20 tissue-speciﬁc proteins, and ZO-1–3. “Leaky” gut, which is emerging as a deﬁning feature of a number of Th1 inﬂammatory pathologies such as IBD, celiac disease, and diabetes, is linked to changes in tight junction protein expression and arrangement contraction of the perijunctional ring, and apoptosis (Bruewer et al., 2003). The pro-inﬂammatory cytokines, TNF-α and IFN-γ (Wang et al., 2005), are implicated in the impaired mucosal barrier in IBD (Murphy et al., 2003).

The consequences of nematode infection on barrier function are not as well-characterized, but infection induces a stereotypic STAT6-dependent increase in intestinal permeability, demonstrating the importance of IL-4 and IL-13. In addition, recent studies have linked the inappropriate upregulation of IL-13 and increase in intestinal permeability to the development of the chronic inﬂammation in experimental models of colitis (Bamias et al., 2005a; Fichtner-Feigl et al., 2007). Of interest is that the eﬀects of IL-13 are observed in SCID mice showing that they are independent of T and B cells (Elfrey et al., 2006). In contrast to the eﬀects of microbial pathogens on tight junctions, nematode infection increased expression of claudin 1, but had no eﬀect on claudin 2, suggesting that changes in tight junction protein expression are not the major factor in the Th2-induced decrease in intestinal permeability (Shea-Donohue, unpublished data). Potential players in the infection-induced changes in permeability are mast cells. Nematode infection is characterized by a mastocytosis that is dependent on IL-3, IL-9, and IL-10, as well as IL-4 signaling through STAT6-activated pathways (Katona, Urban, Finkelman, Gause, & Madden, 1995; Madden et al., 1991). IL-4 in the presence of stem cell factor, markedly increases mast cell proliferation and promotes mast cell release of Th2 cytokines such as IL-3, IL-5, and IL-13 (Bischoff, Sellge, Manns, & Lorentz, 2001). Of
interest is that IL-4-mediated increases in intestinal permeability were absent in mast cell-deficient mice (Shea-Donohue, unpublished data). Recent evidence also implicates changes in mast cell activity in the protective effects of nematode infection in a murine model of colitis (Sutton et al., 2008).

1.1.2. Epithelial cell secretion and absorption

A second critical function of the intestinal epithelium is the regulation of nutrients, ions and fluid transport. In the gut, carbohydrates are broken down into monosaccharides (glucose, fructose and galactose) prior to absorption by small intestinal cells. Glucose absorption in intestinal cells occurs at the brush border membrane primarily through the unidirectional Na+-linked glucose co-transporter, (SGLT-1). Ussing chamber measurement of glucose absorption is correlated uniquely with SGLT-1 activity since the current measured is linked to the movement of sodium. This transporter is expressed highly in the small intestine and is remarkably stable. The expression of SGLT-1 is restricted to the intestine and kidney and its activity is linked to presence of luminal contents, specifically the carbohydrate content (Dyer et al., 2009).

The physiological importance of this transporter is that co-transport of sodium with glucose and other nutrients is the major driving force behind fluid absorption after a meal. It is also the basis for oral rehydration therapy because bacterial toxins, such as cholera toxin, do not affect SGLT-1. There are also a number of facilitative glucose transporters. Dietary fructose enters through the facilitated glucose transporter, GLUT-5, on the apical membrane and both glucose and fructose exit the cell via the facilitative glucose transporter, GLUT-2. This transporter was thought initially to be localized only to the basolateral membrane, however, it was discovered later that high concentrations of luminal glucose (above 30 mM) induce insertion of GLUT-2 into the apical membrane and that the effect of high glucose is mimicked by low levels of artificial sugars (Gouyon et al., 2003; Kellett & Helliwell, 2000; Mace et al., 2007). Apical GLUT-2 provides a cooperative mechanism whereby glucose absorption can be matched to dietary intake. Of interest is that the apical expression of GLUT-2 and GLUT-5 is up-regulated in experimental models of diabetes as well as in diabetic patients (Corpe et al., 1996; Kellett, Brot-Laroche, Mace, & Leturque, 2008).

Nematode infection is described as eliciting a “weep and sweep” response in the small intestine (Anthony, Rutitzky, Urban, Stadecker, & Gause, 2007); however, the data do not entirely support this. Infection-induced upregulation of Th2 cytokines induces a STAT6-dependent stereotyped inhibition of epithelial secretion in response to secretagogues as well as an inhibition of sodium-linked glucose absorption (Madden et al., 2002, 2004; Shea-Donohue et al., 2001). Several of the Th1 cytokines also decrease epithelial cell secretion (Resta-Lenert & Barrett, 2006), which is considered to be a key anti-bacterial defense mechanism. These infection-mediated effects on secretion and absorption are mimicked by exogenous administration of the Th2 cytokines, IL-4 and IL-13 and they are also STAT6-dependent. Body weights in infected mice are comparable to uninfected mice indicating that there is sufficient glucose transport through other mechanisms. The increase in intraluminal fluid (“weep”) therefore, is due to decreased absorption of fluid rather than increased secretion. Along with changes in smooth muscle contractility, changes in epithelial cell function facilitate worm expulsion.

1.2. Role of intestinal epithelial cells in the innate immune response

The intestinal epithelium is a critical component of the innate immune response and plays a key role in host defense against luminal pathogens. Indeed, in responses to enteric pathogens, epithelial cells can rapidly reprogram their cellular gene expression to better withstand their effects. In addition to genes that may affect the pathogen directly, epithelial cells also express a number of other factors such as heat shock proteins, trefoil factors, and other molecules. An important epithelial cell-generated immune molecule is the cytokine, thyrom immunon Aceptor protein (TSLP). Under steady-state conditions, TSLP is produced constitutively by the intestinal epithelium (Rimoldi et al., 2005). TSLP is proposed to play a key role in the crosstalk between epithelial and dendritic cells by conditioning of dendritic cells to induce noninflammatory Th2 responses (Rimoldi et al., 2005). In addition, TSLP is low in autoimmune inflammatory pathologies such as Crohn’s disease (Rimoldi et al., 2005). Nematode infection increases the production of Th2 cytokines, which augment the expansion and function of TSLP- dendritic cell-activated Th2 memory cells (Omori & Ziegler, 2007; Wang et al., 2007).

Recent studies indicate that IL-25 (IL-17E) is critical to the development of the Th2 response. Of equal importance is that IL-25 also inhibits pro-inflammatory Th1 and Th17 cytokine responses, as it was seen that IL-25−/− animals develop severe inflammation during nematode infection or are highly susceptible to experimental autoimmune encephalomyelitis with elevated expression of Th1 and Th17 cytokines (Kleinschek et al., 2007; Zaph et al., 2008). The mechanisms by which IL-25 activation of immune cells are poorly understood. The receptor for IL-25 consists of IL-17RB, which binds IL-25 and is a 56-kDa single transmembrane protein expressed abundantly in kidney, intestine, and other peripheral organs (Lee et al., 2001), and IL-17RA, a receptor subunit for IL-17A, which appears to be necessary for the biological activity of IL-25 in the lung (Rickel et al., 2008) and is expressed in both structural and immune cells (Yao et al., 1995). Both receptor subunits are expressed in the entire cross section of intestine implying that IL-25 may act both on immune cells to influence gut immunity and epithelial cells to impact gut function. It is also intriguing that IL-17A and IL-25 have opposite biological activities in many aspects, but share the same receptor subunit. During nematode infection, IL-17RB is up-regulated whereas IL-17RA is down-regulated suggesting the presence of different mechanisms of immune control. Of interest is that IL-25 receptors are expressed also on antigen presenting cells such as dendritic cell and macrophages (Gratchev et al., 2004). Other reports show that IL-25-induced release of TSLP from lung epithelial cells activated mucosal dendritic cells, which then induced differentiation of Th2 cells which express IL-25R (Wang et al., 2007).

IL-25 is highly expressed throughout the gut and recent data showed that epithelial cells are the major source of IL-25 (Zhao, et al. unpublished). Thus, IL-25 production by epithelial cells may have a more direct role in activation of dendritic cells in the lamina propria than previously recognized. As a major immunomodulatory cytokine in gut mucosa, IL-25 itself is also influenced by various immune mediators. In a Th2-dominant environment such as nematode infection, IL-25 is up-regulated through a mechanism involving IL-4/IL-13-activated STAT6 signaling pathway (Zhao, et al. unpublished). On the other hand, IL-25 is down-regulated in Th1-dominant pathologies, such as intestinal inflammation (Caruso et al., 2009). Epithelial-derived IL-25 plays a conspicuous role in infection-induced changes in epithelial cell function. Exogenous administration of IL-25 to mice induces characteristic changes in epithelial function including hypo-secretion in response to various secretagogues, reduced glucose absorption, and increased permeability (Zhao et al. unpublished), a common feature seen in nematode infection or exogenous administration of IL-4/IL-13 (Madden et al., 2002, 2004; Shea-Donohue et al., 2001; Zhao et al., 2003, 2006). Further evidence on the role of IL-25 came from the studies showing that the stereotypic hyposecretory effects of nematode infection-induced in WT mice are attenuated in IL-25−/−.
mice (Zhao et al. unpublished). The mechanism by which IL-25 regulates epithelial cell function remains unexplored. Based on the fact that IL-25 increases production of Th2 cytokines IL-4 and IL-13, both of which have effects on gut epithelial function, it is plausible that these two downstream cytokines mediate, at least in part, the effects of IL-25.

2. Conclusions

Polarized cytokine profiles serve to orchestrate changes in gut function that promote clearance of pathogens. In this way, nematode infection up-regulates Th2 cytokines leading to stereotypic changes in gut function that facilitate worm expulsion. Both immune and epithelial cells express receptors for IL-4/IL-13, as well as STAT6, which are important in the changes in epithelial permeability that enhance the exposure of immune cells to worm antigens that lead to the development of the Th2 responses. The increase in intraluminal fluid limits interaction of the worms with the mucosa and likely is involved in the timing of the clearance process. The discovery of IL-25 adds another dimension to the importance of epithelial cells that play critical role in the initiation, amplification, and maintenance of the Th2-mediated protective immunity. Given the proposed roles of IL-25 in the prevention of a number of autoimmune pro-inflammatory pathologies that alter function changes in the ability of epithelial cells to function as immune cells, the expression and distribution IL-4/IL-13/IL-25 receptors, and the level of expression of STAT6-dependent genes are likely to play key roles in the therapeutic actions of nematode infection (Fig. 1).

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References


