



ADDENDUM

Innate lymphoid cells link gut microbes with mucosal T cell immunity

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ABSTRACT

Despite continuous exposure to trillions of microbes, the intestinal immune system protects the mucosa by balancing barrier protection, tolerance, and immunity. As both sentinel and effector, the mucosal innate immune system plays a central role in coordinating these responses. By integrating signals from the intestinal microbiota, mononuclear phagocytes (MNPs) serve as a critical link in regulating effector functions of group 3 innate lymphoid cells (ILC3s). Our recent work identified the role for MNP production of the IBD-linked protein TNF-like ligand 1A (TL1A) in modulating microbial regulation of ILC3 barrier immunity. These findings highlight a broader role for ILC3s in local control of T cell immunity and their potential role in the pathogenesis and treatment of inflammatory disease.

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Introduction

Group 3 innate lymphoid cells (ILC3s) have emerged as a central regulator in barrier immunity.^{1,2} In response to inflammatory stimuli, including IL-1b and IL-23, ILC3s produce the cytokine IL-22 which promotes epithelial cell proliferation and healing (Figure 1).³ Pleiotropic effects of IL-22 reinforce barrier immunity by inducing anti-microbial peptide production and maintaining microbial homeostasis. In addition, emerging data has identified a broader role for ILC3s in modulating mucosal immunity. The mechanisms guiding this response, including the impact of gut microbiota and IBD-associated genetic pathways, are an active area of research with important diagnostic and therapeutic potential.

Intestinal mononuclear phagocytes (MNPs) act as sentinels for the intestinal immune system. CX3CR1 + MNPs can form transepithelial dendrites and sample the intestinal microbiota in an active process regulated by the microbiota.⁴ In the setting of dysbiosis, these largely tissue-resident MNPs can upregulate CCR7 and migrate to lymphatics carrying antigens from non-invasive luminal microbes.⁵ During inflammation, however, these MNPs expand and co-localize with ILC3s in the tissue.^{6–8} Following infection with *Citrobacter rodentium*, CX3CR1+ MNPs are required

for mucosal protection via ILC3 production of IL-22.^{7,8} Although genetic deletion models in mice revealed that CD103+ dendritic cells (DCs) were dispensable,⁹ a coordinated response with conventional DCs may be required for protective ILC3 immunity.¹⁰ Thus, the MNP-ILC3 interface creates a functional unit linking microbial signals with ILC3 effector functions, but the mechanisms regulating their interactions are not clearly defined. In our recent paper, we defined a key role for MNP-derived TL1A in regulating ILC3 mucosal immunity. This addendum aims to contextualize the key mechanistic findings of this study in their contribution to inflammatory bowel disease (IBD) and mucosal immunology.

TL1A and innate lymphoid cells in IBD pathogenesis

To date, genome-wide association studies have linked over 200 genes with IBD, and many of these genes are implicated in immunoregulatory functions.¹¹ One of the first and strongest IBD-linked polymorphisms discovered occurs in the Tumor Necrosis Factor Superfamily member 15 (*TNFSF15*) locus, which shares remarkable homology with *TNFA*.¹² *TNFSF15* variants span ethnic cohorts and are associated with more aggressive IBD. *TNFSF15* encodes

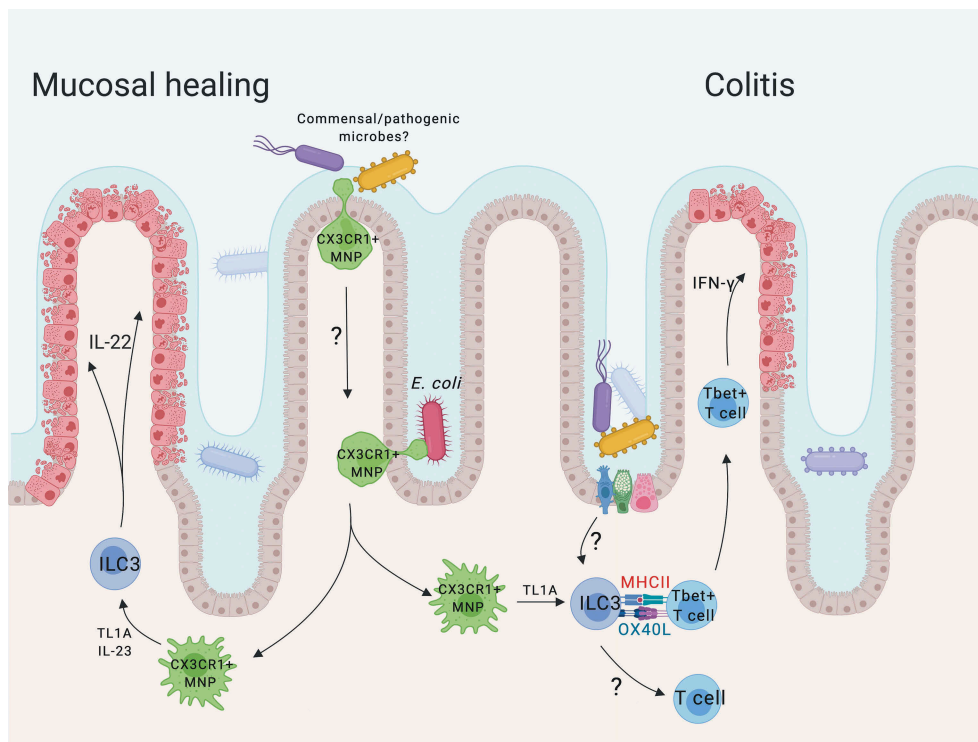


Figure 1. Microbial induction of TL1A regulates ILC3 barrier immunity. Mucosal-associated microbiota are sensed by intestinal CX3CR1+ MNPs and induce expression of cytokines and TL1A. TL1A enhances ILC3 production of effector cytokines, including IL-22, promoting mucosal healing associated with acute colitis. TL1A also induces MHCII+ ILC3 expression of the co-stimulatory molecule OX40L which can support mucosal inflammatory T cells. The broader impact of ILC3 expression of OX40L in regulating microbe-specific mucosal T cell immunity remains to be determined.

for the protein TNF-like ligand 1A (TL1A). Although the mechanism of how TL1A contributes to IBD pathogenesis may be pleiotropic, its function has been linked with intestinal immunomodulation. Previous reports have shown a role for TL1A overexpression in exacerbating T cell- and ILC2-dependent disease, as well as fibrosis;^{13–15} however, recent data using mouse models with genetic deficiencies in TL1A or its monogamous receptor, called death receptor 3 (DR3), have revealed a potential protective role for TL1A during acute colitis.¹⁶ This duality could reflect multiple functions of TL1A under homeostatic or inflammatory conditions, which we reasoned could be explained by additional cellular effectors downstream of TL1A.

Indeed, our previous work, as well as others, identified TL1A as a potentiator of ILC3 cytokine production *in vitro*;^{7,17} however, the role for TL1A in regulating ILC3 function *in vivo* was not known. To address this question, we generated a novel mouse model with an ILC3-specific deletion of DR3.¹⁸ While deletion of DR3 did not impact intestinal ILC3 numbers or subsets during homeostasis,

our results show that DR3-deficient ILC3s produce less IL-22 and are unable to reduce the severity of chemical or infectious colitis. This finding offers critical additional insight into the function of TL1A during acute colitis. Moreover, the protective role for TL1A in acute colitis offers a potential explanation for the strong evolutionary conservation of this genetic polymorphism which, in the context of either chronic inflammation or additional genetic risk, could negatively impact disease. Coupling of human tissue immunophenotyping with genotypic analysis will help offer critical insight into the impact of IBD-linked genetics on the contribution of ILC3 immunity.

MNP production of TL1A links IBD-associated gut microbes to ILC immunity

Previous work by our laboratory and others identified CX3CR1+ MNPs as critical regulators of ILC3 barrier immunity.^{7,8} However, the exact mechanisms of how CX3CR1+ MNPs regulate ILC3 function are not well defined. Our recent

publication identified CX3CR1+ MNPs as the primary producers of intestinal TL1A in both mice and humans;¹⁸ furthermore, IBD patients with active intestinal inflammation had higher CX3CR1+ MNP TL1A production than healthy controls, but the factors regulating this induction were unknown. While previous work identified bacteria and immune complexes as robust inducers of TL1A *in vitro*,¹⁹ our *in vivo* mouse models identified gut microbes, and in particular adherent, mucosal-associated microbes, as critical for the induction of MNP-derived TL1A.

Using mice with a specific deletion of TL1A in CD11c+ CX3CR1+ MNPs, we demonstrated a critical requirement for MNP production of TL1A in regulating ILC3s *in vivo*. The mechanisms guiding MNP-ILC3 cellular interactions in the intestines require further study and there are several possibilities to consider. First, in contrast to the structure of the lymph nodes, which maximize antigen presentation to a variety of stochastically arranged T cell receptors, the mechanism by which MNPs and ILC3s interact is less clear. A recent study demonstrated that constitutive production of CXCL16 by MNPs in the small intestine functionally recruit CXCR6+ NKp46+ ILC3s to the lamina propria, enabling rapid and productive interactions. Likewise, TL1A signaling induces ILC3 production of GM-CSF, as well as other chemokines, which may be critical in promoting these MNP-ILC3 interactions. Second, microbial regulation may offer a mechanism for local organization of this response. Although mucosal adherence and MYD88 are required for MNP production of TL1A, it is not clear yet which types and/or strains of bacteria are most efficient in this process and whether this regulation will be different in the small intestine compared to the colon. A recent study revealed novel antigen access by CX3CR1+ MNPs in the colon through goblet cell-associated passages.²⁰ The role for these and other points of regulated access to the mucosal-associated microbiota may impact these cellular interactions. Finally, the requirement for direct interaction between MNPs and ILC3s needs to be assessed. Membrane bound TL1A can be cleaved by the TNF α converting enzyme (TACE) to produce soluble TL1A. While recombinant soluble TL1A is sufficient to activate ILC3s *in vitro*, the

role for TACE and direct interaction of MNPs and ILC3s *in vivo* needs to be assessed.

TL1A tunes IL-23 signaling in ILC3s

While TL1A stimulation alone can induce IL-22 production by ILC3s, our recent paper shows that TL1A synergizes with IL-23 stimulation to exponentially increase IL-22 production.¹⁸ In addition to *TNFSF15*, polymorphisms in *IL23R* are highly associated with IBD.²¹ The functional synergy in ILC3s may reflect the convergence of this genetic risk. Their respective signaling pathways in ILC3s appear to be unique, as TL1A activation of IL-22 requires p38-MAPK signaling and does not induce STAT3 phosphorylation directly. Reports in human macrophages shows that TL1A synergizes with NOD2 ligand MDP through non-canonical activation of IL-1 β , and subsequently works in an autocrine fashion to enhance inflammatory cytokine production.²² In ILC3s, we showed that the synergy between TL1A and IL-23 is IL-1R- and MYD88-independent, but the mechanism by which TL1A tunes IL-23-induced transcription remains undefined. Furthermore, while some target gene expression is enhanced, TL1A independently activates or antagonizes the expression of others. Technical advances in sequencing technology to assess single-cell resolution and genomic accessibility may provide insight into the transcriptional mechanisms of this regulation. A mechanistic understanding of the synergy between these two highly linked IBD pathways will help identify novel therapeutic strategies.

ILC3s as regulators of mucosal T cell immunity

Data continue to emerge illustrating a role for ILC3s in regulating local T cell immunity directly and indirectly via cytokine production. Indirectly, ILC3-derived IL-22 induces epithelial cells to secrete the acute phase reactant SAA, which promotes IL-17 production by lamina propria T cells.²³ In addition, GM-CSF production by ILC3s recruits local macrophages and supports polarization of regulatory T cells.²⁴ Directly, MNP derived IL-1 β induces ILC3 production of IL-2 in the small intestine, which can support regulatory T cells.²⁵ During

IBD, ILC3 production of IL-2 is reduced, resulting in the diminished regulatory effect of ILC3s.

In addition to cytokine production, ILC3 expression of MHCII can shape antigen-specific T cell responses in the intestine.²⁶ Although splenic ILC3s can express conventional B7 co-stimulatory molecules, intestinal ILC3s do not.²⁷ While this lack of ‘signal 2’ may limit T cell responses during homeostasis,²⁶ our recent study showed that TL1A induced during colitis drives expression of the co-stimulatory molecule OX40L on intestinal MHCII+ ILC3s. Expanding on previous work which showed that lymphoid tissue inducer cell expression of OX40L was critical for maintaining T cell memory in secondary lymphoid tissue,²⁸ our recent work shows that both TL1A and OX40L ligand are required for ILC3s to support inflammatory colonic T cell responses in a transfer T cell colitis model (Figure 1).¹⁸

This work collectively highlights two key questions that will need to be addressed in understanding the impact of ILC3 regulation of mucosal T cell immunity to gut microbes. First, does local regulation of ILC3s enable tissue-specific regulation of T cell effectors? These mucosal mechanisms described above may have less of an impact on systemic infection or vaccine immunity and subsequently may provide a portal for selective targeting of intestinal immunity. Second, how broadly do ILC3s regulate microbe-specific mucosal T cell immunity? OX40L, for example, is also required for the effective generation of regulatory T cells to suppress transfer T cell colitis²⁹ and emerging data suggest that OX40L expression by ILC3s may provide this signal for regulatory T cells.³⁰ Selective genetic deletion models coupled with antigen-specific T cell reagents are needed to define the potential contribution of ILC3s in this local immune regulation.

Conclusions

Our recent data identify the importance of MNP-production of TL1A in linking gut microbes to ILC3 immunity. With stereotypical changes noted in the microbiome of patients with IBD, it will be important to understand the microbial characteristics that contribute to TL1A-dependent immunity – both the protective acute response and the inflammatory chronic response. In addition, the synergy between TL1A and

IL-23 highlights the importance of the MNP-ILC3 interface in IBD. Therapies designed to target this interface may enable selective mucosal regulation. Finally, the role for ILC3s and ILC3 expression of OX40L in regulating the inflammatory response in IBD still needs to be determined. Pharmacologic targeting of the MNP-ILC3 axis coupled with diagnostic genetic and microbiome stratification has the potential to guide therapeutic strategies for IBD.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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