The Innate Immune System in the Gastrointestinal Tract: Role of Intraepithelial Lymphocytes and Lamina Propria Innate Lymphoid Cells in Intestinal Inflammation

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Background: The gastrointestinal tract harbors the largest microbiota load in the human body, hence maintaining a delicate balance between immunity against invading pathogens and tolerance toward commensal. Such immune equilibrium, or intestinal homeostasis, is conducted by a tight regulation and cooperation of the different branches of the immune system, including the innate and the adaptive immune system. However, several factors affect this delicate equilibrium, ultimately leading to gastrointestinal disorders including inflammatory bowel disease. Therefore, here we decided to review the currently available information about innate immunity lymphocyte subsets playing a role in intestinal inflammation.

Results: Intestinal innate lymphocytes are composed of intraepithelial lymphocytes (IELs) and lamina propria innate lymphoid cells (ILCs). While IELs can be divided into natural or induced, ILCs can be classified into type 1, 2, or 3, resembling, respectively, the properties of TH1, TH2, or TH17 adaptive lymphocytes. Noteworthy, the phenotype and function of both IELs and ILCs are disrupted under inflammatory conditions, where they help to exacerbate intestinal immune responses.

Conclusions: The modulation of both IELs and ILCs to control intestinal inflammatory responses represents a major challenge, as they provide tight regulation among the epithelium, the microbiota, and the adaptive immune system. An improved understanding of the innate immunity mechanisms involved in gastrointestinal inflammation would therefore aid in the diagnosis and further treatment of gastrointestinal inflammatory disorders.

Key Words: gastrointestinal tract, inflammatory bowel disease, innate immune system, innate lymphoid cells, intraepithelial lymphocytes

INTRODUCTION

The immune system from the gastrointestinal (GI) tract displays unique characteristics as it can be divided into inductive and effector sites. Among the latter, the epithelial layer and the lamina propria (LP) underneath are the main effector sites, located at the forefront of the luminal content. Most studies of the GI immune system have traditionally focused on the characterization of antigen-specific adaptive immune responses, as they are essential to maintain the mechanism of immune homeostasis by inducing the mechanisms of immune tolerance toward nutrients/commensals, at the same time that they maintain the capacity to trigger active immune responses against invading pathogens. However, the innate immune system provides the first line of defense in virtually all immune responses. Nevertheless, the study of innate immune responses in the GI tract has been traditionally limited to the characterization of intraepithelial lymphocytes (IELs), although the discovery of innate lymphoid cells (ILCs) has provided a new dimension to the implications of the innate immune system in the GI tract. In this review, we will summarize what is currently known about human IELs/ILCs in the context of intestinal inflammation.

THE INNATE IMMUNE SYSTEM IN THE GASTROINTESTINAL TRACT

The intestinal luminal surface is covered by a single layer of intestinal epithelial cells (IECs), which are organized following a well-structured villae. Indeed, IECs also possess the microscopic structure of finger-like villi, providing the gut with the greatest epithelial surface area of the human body,

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allowing for efficient nutrient absorption. Nevertheless, such a surface is also exposed to potential harmful luminal content, including antigens and microbes. Therefore, a competent and discriminating epithelial layer is crucial to maintaining immune tolerance within the gut.

Traditionally, bacteria have been treated from a disease perspective. However, in the last years, commensal bacteria have turned out to be essential to maintain epithelial barrier integrity. In fact, enteric microbes are responsible for several immune[1] and metabolic functions, including shaping of the mucosal innate immunity, but also generation of immunomodulatory short-chain fatty acids (SCFAs) after degradation of nondigestible dietary fiber and production of essential vitamins.[2] The secretion of specific molecules by members of the commensal microbiota also benefits the intestinal barrier functions.[3] Additionally, commensal bacteria play numerous functions in the gut as they protect against enteric pathogens by occupying specific niches and producing and inducing antimicrobial factors.[4–7] Moreover, it has been recently described that commensals control the enterocytes' turnover rate and their regenerative functions,[8–11] as proven by the use of germ-free, gnotobiotic, and conventionally raised animal models.[12–14] Indeed, commensals can also interact and modulate the outcome of immune responses elicited by cells from the innate immune system located not just between the enterocytes, as is the case of the IELs, but also in the LP underneath the IECs, where innate lymphoid cells are enriched, as we discuss below (Fig. 1).

Intraepithelial Lymphocytes

IELs are located within the epithelial cells of the GI tract. This unique location in the interface between the lumen and the lamina propria is essential to controlling the integrity of the epithelium, which is continuously exposed to antigens and potential pathogens from the gut lumen. IELs are potent, rapidly activated cytolytic and immunoregulatory effectors that can protect host tissues from infection, cell transformation, and uncontrolled infiltration by systemic cells.[15] On average, there are about 10–20 IELs per 100 villus enterocytes in the small intestine in humans.[16] Given the great intestinal epithelia surface area, IELs comprise a considerable fraction of the total body's T cells.[15] IELs belong to both the T-cell receptor (TCR) $\alpha\beta^+$ (TCR$\alpha\beta^+$) and TCR$\gamma\delta^+$ lineages.[17–19] In human duodenum, TCR$\gamma\delta^+$ IELs range from 2% to 10% under healthy conditions and are dramatically increased to 15% to 60% in CeD.[20, 21] $CD4^+$ IELs are scarce, especially in the small intestine.[22, 23] Nonetheless, in other mammals, the IEL compartment presents different features compared with those in humans; for instance, the murine large intestine harbors primarily $\alpha\beta$T cells expressing CD4 or CD8$\alpha\beta$,[24, 25] similar to those in the systemic circulation. Interestingly, IELs are differently distributed in the epithelium of the small and large intestine,

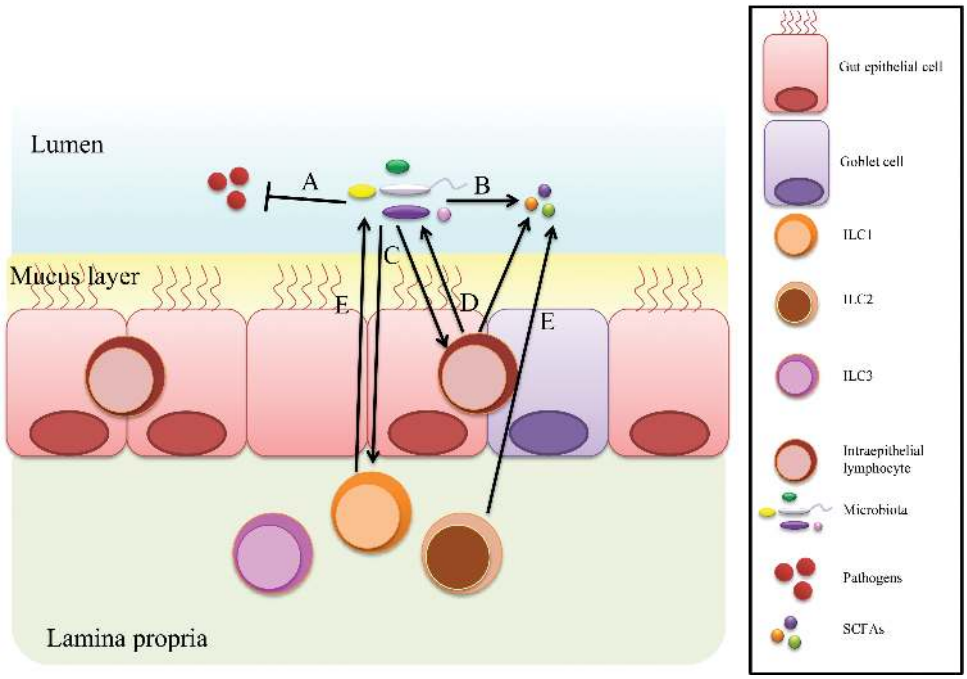

FIGURE 1. Interactions between the microbiota and the innate lymphocytes. The microbiota prevents pathogens from occupying specific niches, hence protecting the integrity of the intestinal epithelium (A). Moreover, through the production of SCFAs, the epithelial integrity is also maintained (B). Additionally, the microbiota modulates the immune system development and immune function elicited not only by IELs, but also by cells located in the lamina propria, such as ILCs (C). IELs recognize harmless antigens including dietary antigens, such as SCFAs, and commensal microbiota (D). Similarly, ILCs interact extensively with both the microbiota and derived metabolites (E).

probably influenced by their different digestive functions and physiological conditions[26] but also by the microbiota composition and abundance.[27, 28] Under physiological conditions, IELs can be divided in 2 major subpopulations based on the mechanisms by which they are activated and on the antigens that they recognize. Thus, they can be divided into the so-called "natural" IELs, previously known as "type b" IELs; and the "induced" IELs, which previously were named as "type a" IELs.[19] As natural IELs do not depend on exogenous antigen-driven differentiation, they are the first type of antigen-experienced T cells to populate the gut, even before birth.[29] Precursors for natural IELs go through an "alternative" self-antigen-based thymic maturation process, resulting in the functional differentiation of mature CD4 and CD8αβ double-negative, TCRγδ- expressing, or TCRαβ-expressing T cells that directly migrate to the intestinal epithelium.[30–32] On the contrary, induced IELs derive from conventional CD4+ or CD8αβ+ TCRαβ+ T cells and are selected in the thymus. After a positive selection, mature thymocytes leave the thymus and reach the periphery as conventional naïve CD4+ or CD8αβ+ TCRαβ+ T cells. These naïve lymphocytes respond to cognate antigens by maturating into antigen-experienced cells. Although natural IELs are tuned mainly to self-antigens, including dietary antigens and commensal microbiota, induced IELs are predominantly shaped by non-self-antigens. Therefore, induced IELs increase with age in response to exposure to exogenous antigens,[33–36] whereas natural IELs remain constant, becoming a minor IEL population in adult age.[26] In the murine and human small intestine, ~50% are natural IELs and ~50% are induced IELs, whereas in the large intestine ~100% are induced IELs both in humans and mice.[37]

Despite the previously mentioned differences within the IELs, they all share common characteristics that differentiate them from conventional T cells; for instance, 99% of IELs in the human duodenum express CD103 (also known as the αE integrin),[38] which interacts with E-cadherin on IECs,[20, 39] hence maintaining them in the epithelial layer. However, CD103 decreases distally, being present in less than 90% of ileum IELs and around 70% of colonic IELs.[40] Moreover, a significant proportion of lamina propria lymphocytes also express CD103.[40] Besides, most IELs, especially those in the small intestine, express CD8αα homodimers, a hallmark of their activated phenotype.[30, 31, 41, 42] Furthermore, the majority of IELs contain abundant cytoplasmic granules for cytotoxic activity, and they can express effector cytokines, such as interferon-γ (IFNγ) and interleukin (IL-) 2, IL-4, or IL-17.[43–50] Additionally, IELs express both activating and inhibitory types of innate natural killer (NK) cell receptors, such as the NKG2D and NKG2A, respectively, which are related to cell-stress sensing.[20, 39, 41, 43, 51, 52] Furthermore, IELs are antigen-experienced cells that typically express activation markers, such as CD44 and CD69.[53]

Besides conventional TCRγδ$^+$ and TCRαβ$^+$ T cells, the intraepithelial compartment contains a large heterogeneous group of CD3-CD7+ IELs, most of which do not fulfill NK or ILC phenotypes, and whose role and functions are not fully understood. These CD3-CD7+ subsets have been mostly neglected in the study of the human colon and IBD, and information available is mostly limited to the human duodenum in the context of celiac disease and their role in celiac refractoriness.[54, 55] Due the presence of these CD3-CD7+ subsets through the entire intestine length[56] and given their clinical relevance in refractory celiac disease, they deserve to be mentioned. A summary of the different IEL phenotypes found in humans and mice, both in the small and large intestine, can be found in Table 1.

As previously mentioned, natural IELs recognize harmless antigens including dietary antigens and commensal microbiota. In fact, studies performed in "germ-free" and "antigen-free" mice have shown that IEL populations are reduced in an antigen-deprived environment, demonstrating that both the microbiota and dietary antigens play a crucial role in the establishment of the IEL repertoire.[57–59] Similar results have been obtained in mice fed with an amino acid–based, protein-free diet.[60] However, the small intestine, which harbors a lower amount of commensal bacteria, contains at least 10 times more IELs than the colon.[53] Several studies have shown the influence of the microbiota in the homeostasis of IELs. NOD2 pattern recognition receptor is expressed by antigen-presenting cells and intestinal epithelial cells. Its activation induces the production and secretion of IL-15, promoting the survival and maintenance of TCRαβ+CD8αα+ IELs. Moreover, the homeostasis of TCRαβ+CD8αα+ IELs is influenced by the normal gut microbiota, as shown in mice lacking NOD2, which contain reduced numbers of this subtype of IELs.[61]

Lamina Propria and Epithelial Innate Lymphoid Cells

As opposed to the IEL compartment, the LP has been traditionally associated with CD4$^+$ T cells, which mediate the effects of the adaptive immune system. Nevertheless, it has now become evident that the LP also carries a large population of innate lymphocytes in the shape of ILCs. One of the reasons why the study of LP-ILCs has been traditionally neglected relies on the large heterogeneity displayed between the different types of ILCs, but also because of differences found between tissues and individuals.[62] For instance, the GI mucosa and skin tissues contain high frequencies of ILCs, whereas nonmucosal and lung tissues are poor in this kind of cells. Moreover, ILCs at the intestinal and respiratory mucosa exhibit an important role as regulators of the epithelial barrier. Hence, immune and epithelial cells interact intensively with both the microbiota and its derived metabolites. Therefore, ILCs are strategically located in nonlymphoid tissue contributing to regulating the epithelium integrity and keeping the homeostasis at the time that they maintain the capacity of mounting pro-inflammatory responses.[63] These data are consistent with the role that ILCs

TABLE 1: Small and Large Intestine IELs Subsets in Mouse and Human

		Human		Mouse	
Small intestine	Natural IELs	TCRγδ+ (+) CD4–CD8– CD8αα+	TCRαβ+ (+) CD4–CD8– CD8αα+	TCRγδ+ (+++): CD4–CD8– CD8αα+	TCRαβ+ (+++) CD4–CD8– CD8αα+
	Induced IELs		TCRαβ+ (+++) CD8αβ+ CD8αα+ CD4+ CD8αα+ CD8αβ+ CD4+		TCRαβ+ (+++) CD8αβ+ CD8αα+ CD4+ CD8αα+ CD8αβ+ CD4+
Large intestine	Natural IELs	TCRγδ+ (+) CD4–CD8– CD8αα+	TCRαβ (++) CD4–CD8– CD8αα+	TCRγδ+ (+) CD4–CD8– CD8αα+	TCRαβ (++) CD4–CD8– CD8αα+
	Induced IELs		TCRαβ+ (++) CD8αβ+ CD8αα+ CD4+ CD8αα+ CD8αβ+ CD4+		TCRαβ+ (++) CD8αβ+ CD8αα+ CD4+ CD8αα+ CD8αβ+ CD4+

Modified from Cheroutre et al.[26] Plus symbols represent frequency of expression, from low (+) to high (+++).

play in human barrier surface immunity.[64, 65] Nevertheless, and despite the role of ILCs in tissue homeostasis, ILC deficiency has no apparent clinical effects over years of follow-up, suggesting that ILCs have redundant function with the adaptive immune system in humans.[66]

Regarding ILCs' composition, they englobe a heterogeneous population of innate immune cells, including not just classical "cytotoxic" NK cells, but also other cell types that can be categorized into 3 different groups—ILCs1, ILCs2, and ILCs3—on the basis of their expression of cytokine and transcription factors,[64, 67–69] which are phenotypically and functionally associated with classical T helper (T_H) cells—T_H1, T_H2, and T_H 17, respectively (Table 2). ILCs are involved in host defense against infection, metabolic homeostasis, and tissue repair, although they can also contribute to chronic inflammatory diseases, such as asthma or colitis.[64, 67, 70, 71] All ILC subsets are derived from ILC precursors (ILCPs) and lack of antigen receptors such as TCR or B-cell receptor (BCR), they and can be activated by cytokines.[72] As previously mentioned, ILCs can be subdivided into 3 main groups analogous to the main subsets of T helper lymphocytes that present characteristic features. However, despite this segregation, recent studies have shown plasticity within and between these cells due to environmental pressure.[67] Hence, both circulating and tissue-resident ILCPs are the "cellular substrate" for ILC differentiation in situ in response to local environmental signals,[73] where ILC differentiation will occur based on the specific requirements to replenish steady-state losses in response to infection and/or inflammation in any given tissue.[73]

Group 1 innate lymphoid cells

Group 1 ILCs include NK cells, which are considered the innate counterpart to cytotoxic $CD8^+$ T cells, lamina propria (LP) ILC1, and intraepithelial (ie) ILC1-like cells present within the epithelium in mucosal tissues (Table 2). All ILC1 subsets are responsive to inflammatory cytokines such as IL-15, IL-12, and IL-18.[74] When activated, they share the production of IFN-γ, tumor-necrosis factor alpha (TNF-α), and cytotoxic granules. In most of the cases, the expression of the marker NKp46 is also present in ILC1s cells.[75] Indeed, both populations display similarities that are not shared with the rest of ILCs as intestinal CD127+ ILC1s are dependent on IL-15 but not on IL-7, as the rest of the ILCs are.[76] However, there are several features that distinguish NK and ILC1s, as the latter express CD127 (IL-7Rα) and depend on the T-box transcription factor (T-bet), whereas NK cells typically depend on the transcriptional factor Eomes.[77] Also, ieILCs1 (but not NK cells) have been related to Crohn's disease (CrD), were they are expanded and contribute to pathology in the anti-CD40-induced colitis model in mice.[78]

Due the lack of a specific marker to detect ILC1—in contrast to ILC2 and ILC3, which are defined in the lin-CD127+CD161+ gate by the presence of CRTH2+ and ckit+, respectively—their characterization is difficult. Recent studies on ILCs have shown, using t-distributed stochastic neighbor embedding analysis, that no ILC1s are detected as previously defined ($CD127^+CRTH2^-$ c-Kit^- $NKp44^-$), as other markers expressed by this type of cells are not compatible with the current definition of ILC1s. Simoni et al. maintain that these cells are in fact contaminating cells, as supported

TABLE 2: Human ILCs Subsets, Phenotype, and Role in the Human Gastrointestinal Tract Both Under Homeostatic Conditions and in the Presence of Inflammation

Type	Phenotype	Adaptive Equivalence	Phenotype	Transcription Factors	Secreted Factors	Stimulation	Role on Intestinal Homeostasis	Role on Intestinal Disease	References
ILCs1	NK cells	CD8+ T-cells	IL12-R, NCR, IL-15R, IL18R, CD94	Eomes, T-bet	IFN-γ, TNFα, perforin, granzyme	IL-15, IL-12, IL-18	Protection against bacterial infection	Increased in Crohn's disease; association with anti-CD40-induced colitis	74, 75, 76, 77, 78, 97, 108, 109
	LP ILC1	Th1	IL-12R, CD127, NCR	T-bet	IFN-γ, TNFα				
	ie, ILC1		IL-12R, NCR, CD103, CD94, IL-15R	T-bet, Nfil3					
ILCs2	Natural helper cells	Th2	IL-25R, IL-33R, CD127	GATA3	IL-4, IL-5, IL-6, IL-9, IL-13, Amphiregulin	IEC-derived IL-33, IL-25, TSLP53, PGD, ICOSL, TCR	Immune response to helminths; eosinophil recruitment; produce factors for epithelial barrier repairing; role of IL-22BP during colon tumorigenesis	Fibrosis in Crohn's disease; human colitis-associated neoplasia	63, 67, 82, 83, 87, 88, 89, 113
ILCs3	LTi cells	Th17	IL-23R, IL1R, CD127, CCR6	RORγt, AHR	IL-17A, IL-22, TNF, LTa1b1	IL-23, IL-1β, TL1A, PGE2, AHR ligand	Formation of secondary lymphoid organs during embiogenesis; effector role in innate immunity	LTi cells are decreased in Crohn's disease	91, 92, 93, 94, 95, 96
	NCR+ILC3		IL-23R, NCR, IL-1R, CCR6, CD127	RORγt	IL-22		Establishment of gut microbial environment; tissue homeostasis; GM-CSF-mediated recruitment and activation of inflammatory monocytes	Enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) colitis; source of innate IL-17 in patients with IBD	63, 65, 69, 90, 98, 107, 108, 112, 114
	NCR-ILC3		IL-23R, IL-1R, CCR6, CD127	RORγt	IFN-γ, IL-17, IL-22				

also in other studies, where it was shown that tonsil ILC1 cells expressed transcripts coding for rearranged TCR, and several other T-cell-specific genes.[62] Similar observations about T-cell-contaminating ILC1s have also been made using a CD5 antibody,[66, 79] hence suggesting that ILC1s' supposed plasticity could indeed be also explained by the presence of contaminating cells,[80] an issue that would also be supported by the bimodal expression of T-bet.[81] These hints, together with technical limitations,[82, 83] could explain why contaminating cells have been characterized as ILC1s.[62] However, this finding generates controversy as CD5 and CD4, markers traditionally associated with T cells, have been found to be present in peripheral blood ILC1.[84] Additionally, Nagasawa et al. found CD5+ ILCs in the human thymus and cord blood that were described as immature ILCs with the capacity to differentiate into mature cytokine-secreting ILCs.[85] Moreover, using Simoni's data and alternative clustering, Bernink found an ILC1 cluster with lower T-bet levels of that of the NK cells,[86] suggesting that Simoni's conclusions are not sufficiently supported by their data. Thus, this is an unresolved debate, and further research would be needed to clarify this issue.

Group 2 innate lymphoid cells

Group 2 ILCs include natural helper cells, nuocytes, innate helper 2 cells, and multipotent progenitor type 2 cells (Table 2). All these cells share the production of cytokines characteristic of the T_H2 subset of helper T cells (IL-5, IL-6, IL-9, and IL-13) and are activated upon IEC-derived IL-33, IL-25, or TSLP.[67] Group 2 ILCs are responsible for the immune response to helminths and lung diseases such as asthma.[87] Similarly, they produce factors for epithelial barrier repair.[82, 83] Within the adipose tissue, ILC2s play a role in metabolic homeostasis controlling eosinophilic activation, which in the end promotes insulin sensitivity.[88, 89] It has been also described that ILC2s receive signals from the enteric nervous system as the neuropeptide VIP—which is secreted by enteric neurons following a circadian rhythm—activates ILC2s, hence suggesting that ILC2 recruitment follows the circadian clock.[63]

Group 3 innate lymphoid cells

ILC3s represent a highly diverse population that results from the variable expression of numerous markers, such as CD56, ICOS, and the NCRs (Table 2). Group 3 ILCs are capable of producing the cytokines IL17A and/or IL-22. ILC3s include lymphoid tissue–inducer (LTi) cells and natural cytotoxicity receptor–positive (NCR+) and NCR− ILC3s. Although the former are necessary for the development of lymph nodes, Peyer's patches, and ectopic lymphoid structure[90]; the latter play a role in the establishment of the gut microbial environment.[65]

LTi cells are crucial for the formation of secondary lymphoid organs during embryogenesis, and they produce lymphotoxin (LTa1b1) and TNF-α, thus stimulating the mesenchymal cell production of chemokines and adhesion molecules essential for lymphoid organogenesis.[91] LTi cells are relatively rare in adult tissues, probably due the reduced need of adult tissue to form new lymphoid structures. However, in the intestine, where this need may be higher, the amount of LTi cells is considerable, and they are particularly associated with intestinal cryptopatches and isolated lymphoid follicles.[92, 93] Interestingly, it has been shown that LTi cells that retain RORγt expression maintain their potential to act as inducer cells and maintain a capacity to sustain epithelial cell integrity through the production of IL-22, whereas LTi cells that lose RORγt expression tend to function as NK-like cells, producing IFN-γ, and are capable of cytotoxic function.[94, 95]

Apart from this function, LTi cells may play an effector role in innate immunity, as has been suggested due their capability of producing IL-17A and IL-22 upon stimulation.[96]

A recent study by Simoni et al.[62] accurately identified and characterized ILCs by mass cytometry across healthy and inflamed tissue types. According to their analysis, ILC3 can be divided into 2 main subsets: NKp44– ILC3 and NKp44+ ILC3 cells. In humans, ILC3s express Toll-like receptors, which, upon engagement with their corresponding ligands, mediate ILC3 activation.[97] Finally, ILC2s and ILC3s interact with the nervous system, suggesting a role in tissue homeostasis.[98]

A summary of the markers associated with the different ILC subsets in mice and humans can be found in Table 3.

Plasticity among ILC subsets

To add further complexity to ILC function, these subsets are dynamic as ILC2 and ILC3 cells can be converted into ILC1 by the upregulation of T-bet and the downregulation of GATA-3 and RORγt, respectively, coupled in both cases with the acquisition of producing IFN-γ[77] in an IL-12- and IL-1βhi-dependent manner in the case of ILC2, and in an IL-12- and IL-15-dependent manner in the case of ILC3, both in humans and mice.[77, 99] Moreover, this conversion process is bidirectional, as CD127+ ILC1s are converted to ILC3s in the presence of IL-1β and IL-23 in a process that can be further enhanced by retinoic acid (RA),[80] whereas ILC2-derived ILC1s can indeed revert to ILC2s in the presence of IL-4.[100] Similarly, in mice, ILC2 can convert into ILC3 in the presence of TFG-β and IL-6.[99] Nonetheless, such conversions between ILC types do not seem to be stochastic as ILC1s are increased in the inflamed intestinal mucosa in CrD patients,[81, 101] due to the detriment of ILC3s, likely due to ILC3s transdifferentiation into ILC1s (Fig. 2).[77]

Interestingly, it has been recently published that differences in the distribution of ILC subsets exist throughout the gut.[102] In this study, it was suggested that compartment-specific concentration of IL-7 may modulate the intestinal ILC pool. This fact was supported by the finding of a positive correlation between mRNA levels of IL-7 and the frequency of ILC3s. However, further research would be needed to clarify the mechanisms that orchestrate the distribution of ILCs along the gut under healthy and disease conditions.

TABLE 3: Phenotypes of ILCs Subsets in Mouse and Human

	ILC1		ILC2	ILC3		
	ILC1	NK		LTi Cells	NCR-	NCR+
Human	T-bet, NKp46, NK1.1, CD90, CD94, CD127, IFN-γ, TNF-α, TRAIL	Eomes, T-bet, NKp46, NK1.1, CD49b, CD11b, CD90, CD94, Ly49, IFN-γ, TNF-α	GATA-3hi, RORα, IL-33R, IL-17RB, TSLPR, CD25, CD127, Sca-1, CD90, CD117, ICOS, KLRG1, IL-4+/-, IL-5, IL-9, IL-13, AREG+/-	RORγt, AhR, CD25, CD117, CD127, CD161+/-, IL-1, IL-22, IL-23R, CCR6, CCR7, CXCR5, LTα, LTβ	RORγt, AhR, CCR6+/-, CD4+/-, CD25, CD90, CD117, CD127, IL-17, IL-22	RORγt, T-bet, AhR, NKp46, CD90, CD117, CD127, IL-22, IFNγ+/-
Mouse	T-bet, NKp46, NK1.1, CD90, CD94, CD117, CD161, CD56+/-, CD127+/-, IFN-γ, TNF-α	Eomes, T-bet, NKp30, NKp44, NKp46, CD56, KIR, CD94, IFN-γ, TNF-α,	GATA-3hi, IL-33R, IL-17RB, TSLPR, NKp30+/-, CRTH2, CD117+/-, CD25, CD127, ICOS, IL-4, IL-5, L-9, IL-13, AREG+/-	RORγt, AhR, CD4, CD25, CD90, CD117, CD127, IL-17, IL-22, IL-23R, CCR6, CCR7, CXCR5, LTα, LTβ	RORγt, AhR, CCR6, CD161, CD117, CD127, IL-17, IL-22	RORγt, T-bet, AhR, NKp30, NKp44, NKp46, CCR6, CD117, CD127, CD161, IL-22, IFNγ+/-

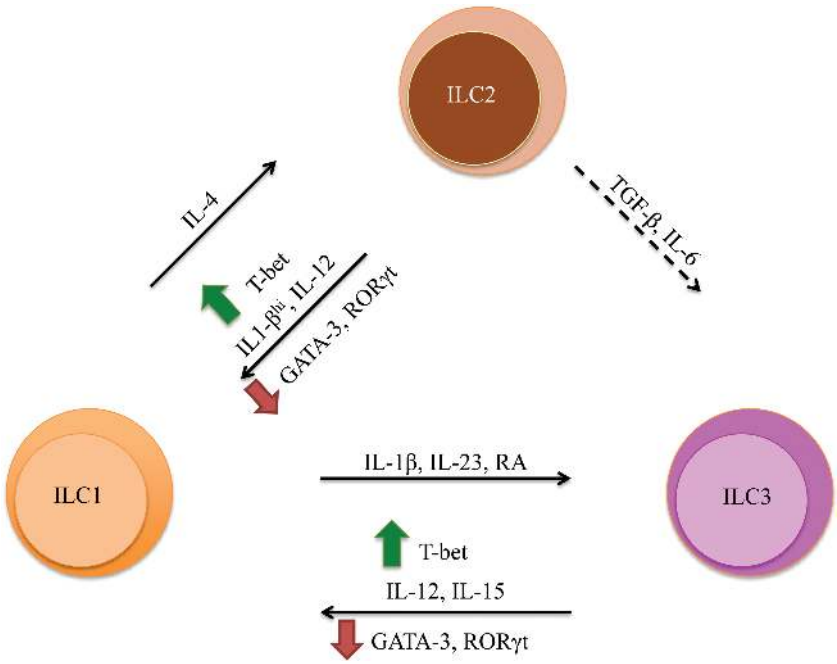

FIGURE 2. Plasticity among ILC subsets. By stimulation with different cytokines, growth factors, or metabolites, ILCs may undergo reversible transdifferentiation. Dashed line: applied for mice only. Abbreviation: TGF-β, transforming growth factor beta.

MURINE MODELS OF INNATE LYMPHOID CELLS AND INTRAEPITHELIAL LYMPHOCYTES

Several murine models have tried to unravel the role of both ILCs and IELs in the context of GI inflammation. In a study conducted by Song et al.,[65] mice selectively lacking either NKp46$^+$ILC3s or all ILC3s were generated and further crossed with T-cell-deficient mice to investigate the specific function of NKp46$^+$ILC3s. With this approach, they demonstrated that

NKp46$^+$ILC3s have a unique role in granulocyte-macrophage colony-stimulating factor-mediated recruitment and activation of inflammatory monocytes during innate intestinal inflammation, although they are redundant for clearance of *Clostridium rodentium*, independently of T cells. To study the role of CD8$^+$ T cells in inflammatory bowel disease (IBD), Nancey et al. induced murine colitis using 2,4- dinitrobenzene sulfonic acid (DNBS), which modifies self-antigens, hence triggering the priming of CD8+ T cells. Such primed cells were recruited to the colon, where they drove the lysis of colonic IECs, hence proving that CD8$^+$ T cells were the initiators of the inflammatory response.[103] In another model of spontaneous colitis induced by deletion of phosphoinositide-dependent protein kinase 1 (PDK1) in T cells, TCRγδ$^+$ IELs were shown to be responsible for colitis induction, likely dependent on IL-17 secretion.[104] Other studies using murine models have also shown the role of TCRγδ$^+$ IELs in the inmunopathology of IBD.[105–107]

Referred to the role of ILC in the presence of GI inflammation, the specific contribution of ILC1s in infectious diseases against microorganisms was deciphered by using mice lacking T-bet. Thus, although CD127$^+$ intestinal ILCs1 were able to protect mice against *Toxoplasma gondii*, mice that lacked ILC1s were unable to control the infection.[97] Similarly, T-bet-deficient NK cells were unable to migrate and failed in controlling *T. gondii* infection, suggesting that NK cells could also be implicated in this mechanism.[97] In other study, using RORγt fate-mapping mice, Klose et al. deciphered a role for ILC1s, which were derived from RORγt$^+$ ILC3s, in the protection of the epithelial barrier against *Salmonella enterica*.[108] T-bet-deficient ILCs are also implicated in the development of colitis in Tbx21$^{-/-}$Rag2$^{-/-}$ mice, indicating that ILC1s and NK play a role in the protection against colitis in murine models.[109] Indeed, ILCs are also implicated in murine models of inflammatory diseases because IFN-γ- and IL-17A-producing ILCs are involved in *Helicobacter hepaticus*–induced colitis.[110]

Referring to the role of ILC2s on GI immune responses, their contribution was discovered using mouse models in which ILC2s are transferred, deleted genetically, or ablated temporally.[111–113] Huber et al. generated *Il22bp*$^{-/-}$ mice and used a colitis-associated colon cancer model to resemble the pathology of human colitis-associated neoplasia to analyze the role of IL-22BP during tumorigenesis in the colon. In mice lacking the IL-22 soluble receptor IL-22BP, tumor development was strongly accelerated, and the number and size of the tumors increased compared with wild-type mice, demonstrating that IL-22 and its receptor IL-22BP are crucial in the regulation of intestinal tissue repair and tumorigenesis in the colon.[114]

As for ILC3s, they are known to be a key source of IL-22. Hence, ILC3-derived IL-22 at early stages of GI infection mediates resistance to *Clostridium rodentium* infection,[97] whereas at later time points both T- and B-cell-derived IL-22 are responsible for *C. rodentium* infection resistance. By using an *IL22*$^{-/-}$ mouse model, the implication of ILC3s in the protection against rotaviral infection was also evidenced. This susceptibility was further reverted, and the infection cleared, by the exogenous administration of IL-22,[107, 115] which is crucial to keeping the integrity of the epithelial barrier. However, the commensal microbiota induces the release of IL-25 from IECs, which subsequently acts on CD11c$^+$ cells to limit ILC3-derived IL-22 secretion. Thus, IL-25 administration intensifies the effect of dextran sulfate sodium (DSS) in a colitis model.[63] IL-22 has also been used to diminish the immunopathology in a murine model of *C. rodentium* infection or DSS-induced colitis with defects in the NF-κβ signaling pathway.[113] Similarly, in a model of colitis-associated cancer after DSS administration, it was shown that IL-22 reduces epithelial damage and inflammation-associated cancer in the acute phase but has detrimental effects in the recovery phase.[114] The role of IL-22 in tumor development has also been studied in ApcMin mice, which spontaneously develop colon cancer. In this model, mice deficient in IL-22 had fewer tumors, whereas mice deficient in IL-22-binding protein developed more colon tumors.[114] In bacteria-induced colon cancer, an accumulation of IL-17$^+$IL-22$^+$ colonic ILCs has been shown. However, in a mouse model, depletion of IL-17 together with IL-22 was sufficient to block the development of invasive colon cancer.[110] However, in another study conducted by Chan et al.,[111] it was demonstrated that IL-17-producing ILC3s could contribute to the development of tumorigenesis in the mouse gut via the IL-23/IL-17 signaling pathway during chronic GI infection. Moreover, IL-17 could also induce tumor growth through angiogenesis.[112] Together, and given the dual role of IL-22 in the development and progression of tumors, it seems obvious that further research is necessary to better understand the mechanisms by which ILCs interact with malignant cells.

INTRAEPITHELIAL LYMPHOCYTES AND INNATE LYMPHOID CELLS IN HUMAN GASTROINTESTINAL DISORDERS

The pro-inflammatory cytokine IL-15 is overexpressed by IEC in patients with celiac disease (CeD), leading to a cytotoxic response by CD8αβ$^+$-induced IELs through the NKG2D–DAP10 signaling pathway.[115] In these patients, the most characteristic feature is the permanent increase of TCRγδ IEL, which is irrespective of diet and disease severity. However, their role in disease development is obscure. They have been shown to be present at all stages of disease, even in patients in long-term dietary treatment.[116–118] In some studies, it has been suggested that TCR-γδ+ IELs may have regulatory or even protective functions in celiac disease patients.[20]

Referring to IBD, which can be divided into CrD and ulcerative colitis (UC), its pathogenesis is thought to be the consequence of an aberrant CD4$^+$ T-cell response directed against the intestinal microbiota (for excellent reviews on this topic, see [119, 120]). Despite their similarities, UC and CrD represent immunologically different diseases with distinct effector CD4+ T-cell types involved,[121–124] something not surprising as

both diseases display differences in type, location, and distribution of inflammation, and in the associated symptoms and related complications.

In the context of ILCs' contribution to IBD, the deregulation of ROR γt-dependent ILCs, together with the production of IL-17 and IL-22, is related to IBD pathogenesis.[125] Hence, IFNγ-producing ILC1s are associated with CrD, where they also block IL-22 production, leading to the development of IBD.[81] In a similar manner, in patients with CrD, a decrease in the expression of MHCII on mucosal ILC3s compared with healthy intestinal tissue has been reported. This fact presents an inverse correlation with frequencies of pro-inflammatory colonic T_H17 cells, and the amount of circulating IgG associated with commensal bacteria. As a consequence, ILC3-mediated "intestinal selection" has been proposed as a potential therapeutic target in IBD.[126] Interestingly, ILC3s have also been found to secrete GM-CSF, which can recruit myeloid cells and further promote intestinal inflammation.[127] ILC3 has been shown to be able to move within intestinal tissue when activated. Thus, these 2 mechanisms may contribute to the induction and progression of inflammation throughout the gut.

On the contrary, UC seems to be mediated by ILC2s, as those patients display increased production of mucosal IL-4, IL-5, and IL-13 (all of them related to a type 2 immune phenotype), although further studies are needed to clarify the exact role of ILC2s in UC.[128, 129]

Nevertheless, LP-ILC is not the only innate lymphocytes contributing to GI inflammation, as IELs also play a crucial role in other GI diseases besides IBD, such as CeD, or the less prevalent lymphocytic gastritis (LyG). Both diseases are often diagnosed together. In fact, 45% of LyG cases are concomitant to CeD. CeD and LyG are characterized by an increase in $CD8^+$ IELs in both the duodenum and stomach, respectively. It has been recently described that in LyG, the molecular mechanisms triggering massive $CD8^+$ infiltration are shared indeed with CeD, being the NKG2D system involved in both pathologies.[52] In CeD, TCR-activated $CD8\alpha\beta^+TCR\alpha\beta^+$-induced IELs cause severe villous atrophy by targeting IECs that express stress-induced MHC class I polypeptide-related sequence (MIC) antigens, in an NKG2D-dependent fashion.[115, 130] In LyG, besides the NKG2D receptor, the ligand MICA and the pro-inflammatory cytokine IL-15 were also upregulated in stomach corpus biopsies corresponding to LyG patients. Similarly, these molecular players were induced in vitro when gastric epithelial cells were stimulated with SCFAs and several strains of *Propionibacterium acnes*, a bacterium found to be associated with LyG when compared with *Helicobacter pylori* gastritis biopsies or those corresponding to healthy controls by 16S rRNA microbiota comparative analysis.[52]

CONCLUDING REMARKS

IELs harbor a unique location within the epithelium that provides the first line of defense against pathogens, while protecting the integrity of the mucosal barrier, hence maintaining a homeostatic environment. However, certain environmental conditions may disturb this homeostasis, triggering the induction of a pro-inflammatory response by IELs that might lead to detrimental GI pathologies. Similarly, ILCs are involved in a myriad of diseases states, while maintaining an important relationship with the gut microbiota (Fig. 1). Therefore, nowadays it represents a challenge to modulate the function of both IELs and ILCs to treat GI inflammatory diseases, as they keep a tight connection with the epithelium, the microbiota, and other immune cells.

Understanding the mechanisms and the diverse signaling pathways that control the different communication networks among the distinct players of the immune system, including cells, metabolites, and signaling molecules, may provide new insights for the diagnosis of GI disorders and for the development of potential therapies to either prevent or treat GI inflammatory diseases.

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