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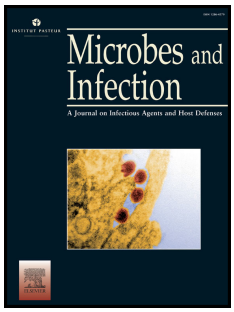
Accepted Manuscript



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PII: S1286-4579(18)30109-6

DOI: 10.1016/j.micinf.2018.05.003

Reference: MICINF 4587

To appear in: *Microbes and Infection*

Received Date: 5 April 2018

Accepted Date: 22 May 2018

Please cite this article as: S. Cording, J. Medvedovic, E. Lecuyer, T. Aychek, G. Eberl, Control of pathogens and microbiota by innate lymphoid cells, *Microbes and Infection* (2018), doi: 10.1016/j.micinf.2018.05.003.

Control of pathogens and microbiota by innate lymphoid cells

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Summary

Innate lymphoid cells (ILCs) are the innate counterpart of T cells. Upon infection or injury, ILCs react promptly to direct the developing immune response to the one most adapted to the threat facing the organism. Therefore, ILCs play an important role early in resistance to infection, but also to maintain homeostasis with the symbiotic microbiota following perturbations induced by diet and pathogens. Such roles of ILCs have been best characterized in the intestine and lung, mucosal sites that are exposed to the environment and are therefore colonized with diverse but specific types of microbes. Understanding the dialogue between pathogens, microbiota and ILCs may lead to new strategies to re-inforce immunity for prevention, vaccination and therapy.

Keywords: Innate lymphoid cells, microbiota, pathogens, homeostasis, mucosal immunity

I. Introduction

ILCs derive from a lymphoid cell precursor common with T cells, with whom they share phenotypes and functions [1,2]. However, T cells are antigen-specific, carry immunological memory, and are selected by their cognate antigen in lymph nodes and Peyer's patches before expansion. Once activated, T cells recirculate through the blood to reach organs where their effector functions are required, eventually committing apoptosis or remaining in tissues as resident memory T (T_{RM}) cells [3]. Nevertheless, the process of T cell selection, activation and recirculation requires several days, leaving the early phase of the immune response without T cell-mediated orchestration of the response best adapted to the type of threat faced by the individual. However, early orchestration is directed by ILCs, which perform similar regulatory functions and provide prompt effector responses to infection and injury. ILCs also play important roles early in life, when the adaptive immune system is not yet in place [1].

An individual faced with intracellular threats, such as tumors, viruses and particular bacteria, responds with type 1 immune responses characterized by the production of type I interferons, the inducer cytokine IL-12, the effector cytokine IFNγ, the release of cytotoxic oxygen radicals and proteins, and the elimination of the transformed or infected cells (Figure 1). Extracellular microbes, such as bacteria and fungi, elicit type 3 responses that are characterized by the release of the inducer cytokine IL-23 and the production of the effector cytokines IL-17 and IL-22, which lead to the reinforcement of mucosal barriers and the recruitment of polymorphonuclear neutrophilic phagocytes that target, ingest and destroy the microbes. In contrast, when faced with large parasites, such as worms, the individual develops type 2 responses that lead to the release of fluids and the production of mucus at mucosal surfaces, and the deposition of collagen to resist parasite penetration. The type 2 inducer cytokines are IL-25, IL-33 and TSLP, which lead to the production of the effector cytokines IL-4, IL-5 and IL-13. Myeloid cells and stromal cells produce the appropriate inducer cytokines in response to a specific type of threat, activating ILCs and T cells to differentiate into ILC1s and Th1, ILC2s and Th2, or ILC3s and Th17, which produce the effector cytokines characteristic of type 1, 2 and 3 immunity [1,4].

Importantly, these immune responses are not only engaged in response to pathogens and tissue injury, but also in response to the symbiotic microbiota [5]. Microbes are present at all mucosal surfaces, as well as within tissues. An estimated 10^{14} bacteria reside in the intestine, together with viruses, fungi, protists and occasional worms, while smaller yet significant collections of microbes are present in the oral cavity, genitourinary system and skin [6,7]. Furthermore, the list of viruses found within our organism is expanding [8], and

bacteria have been identified in lymphoid tissues [9,10] and placenta [11]. As a consequence, the immune system is constantly activated by the presence of these diverse symbiotic microbes. We have therefore argued that the primary function of the immune system is to maintain homeostasis of the host with its microbiota [5], a microbiota that is necessary for digestion, production of metabolites and defense. In contrast, if microbes are associated with injury of the host's cells and tissues, the immune reaction will lead to the elimination of the pathogens.

In this review, we discuss the role of ILCs in the control of the symbiotic or pathogenic microbiota (Figure 2 and Table 1). From recent data, it emerges that ILCs play unique roles in microbiota control, observations that may lead to a new understanding of how chronic inflammatory pathologies emerge when such control is lost, and create new opportunities for prevention and therapy of infectious diseases. We will not discuss the role of NK cells, the oldest member of the ILC family, which has been extensively studied since 40 years and best reviewed elsewhere [12].

II. ILCs in the control of intestinal and hepatic infections

ILC3s are the oldest and best characterized ILC family member (not considering NK cells). In the late 90's, non-B non-T lymphoid cells, termed lymphoid tissue inducer (LTi) cells, have been described that colonize developing secondary lymphoid tissues, the lymph nodes and the Peyer's patches, and shown to be required for their development [13,14]. LTi cells form clusters in fetal lymphoid tissues and the intestinal lamina propria [15], and activate stromal cells to initiate organogenesis [16]. Ten years later, it was shown that LTi cells are part of a larger family of ILC3s that depend on the hormone receptor and transcription factor RORγt [17], and express the type 3 cytokines IL-17, IL-22 and lymphotoxin (LT) [18-21]. Non-LTi ILC3s do not cluster and are not involved in the development of lymphoid tissues. Rather, these cells resemble more freely moving lymphoid effector cells, and together with LTi cells, play a critical role early in defense against enteric pathogens.

Using mouse models that lack all lymphoid cells or only B and T cells, it was reported that ILC3s are required early in the control of intestinal infection by *Proteobacteria*, such as *Citrobacter rodentium*, the murine homologue of human enteropathogenic *Escherichia coli*. The production of IL-22 is paramount for this protective function, and induces the expression of anti-microbial peptides (AMPs) by epithelial cells, such as Reg3γ [18,21-23]. Also involved in increasing epithelial defense is membrane-bound LT, which bind its receptor

LTβR on epithelial cells and induces expression of the neutrophils chemoattractants CXCL1 and CXCL2 [24]. Furthermore, ILC3s are involved in the response to infection by *Salmonella enterica* through the production of IL-17 and IL22, as well as IFNγ [25]. The production of IL-17 by ILC3s and the recruitment of neutrophils are essential in the response to infection by *E.coli K1* and *Klebsiella pneumoniae* and to prevent sepsis [26]. The protective role of ILC3s against pathogens extends to parasites and viruses. Infection by *Toxoplasma gondii* leads to more severe inflammatory pathology in the absence of ILC3s, presumably because of a failure to contain the parasites early [27]. Furthermore, IL-22 production by ILC3s potentiates the activity of IFNλ in epithelial cells and thus increases resistance to rotavirus [28]. However, the pro-inflammatory activity of ILC3s may also lead to pathology through the production of IFNγ in the context of *S. enterica* infection [25], as well as intestinal fibrosis through the expression of IL-17 and IL-22 in the context of *S. typhimurium* infection [29].

ILC2s were first reported in the context of intestinal infection with the helminth *Nippostrongylus brasiliensis*, which evokes a vigorous expansion of "non-B non-T cells" expressing IL-4, IL-5 an IL-13 [30,31]. ILC2 are also involved in defense against *Heligmosomoides polygyrus* [32], *Strongyloides venezuelensis* [33], *Trichinella spiralis* [34] and *T. muris* [35], and are diminished in the blood of children infected with the blood dweller *Schistosoma haematobium* [36]. Both inducer cytokines IL-25 and IL-33 are expressed upon helminth infection and activate ILC2s, noting that ILC2s responding to IL-33 have been suggested to be precursors of ILC2s responding to IL-25 [37]. While IL-33 is expressed by different types of stromal cells, as well as by mast cells [32], the source of IL-25 was only recently identified, in the intestine, as Tuft epithelial cells [38-40]. Interestingly, Tuft cells appear to detect helminth infection through chemosensory signaling via the G-protein coupled receptor Trpm5 [38]. The activity of ILC2s is also promoted, during helminth infection, by the neuropeptide neuromedin U that is expressed by cholinergic neurons in the intestine [41,42].

ILC1s are distinguished from NK cells by their lack of expression of, and requirement for, the transcription factor Eomesodermin [2]. ILC1s provide early protection to the liver from mouse cytomegalovirus infection, producing IFNγ before NK cells are engaged [43], but at the same time prevent the recruitment of NK cells and $CD8^+$ T cells that optimally fight liver infection with adenovirus [44]. Mouse hepatitis virus inoculated orally is also cleared from the intestine by ILC1s, which are activated by IL-15 released from infected stromal cells [45]. Futhermore, ILC1s and their production of IFNγ are engaged in the defense against

bacteria, such as *Clostridium difficile* [46]. Nevertheless, ILC1s and NK cells have largely overlapping function, even though ILC1s are defined as non-cytotoxic. As both cells types react promptly to activators such as IL-12, their relative role is mostly dependent on their tissue distribution before infection.

III. The ILC crosstalk with intestinal microbiota

The large intestinal microbiota has been best characterized, so far, at the level of its bacteriome and of its cross-talk between symbiotic bacteria and ILC3s. In contrast, the interaction between the virome and ILCs remains largely unexplored, and ILC2s are not known to cross-talk with microbes, even though some bacteria have been reported to induce the expansion of ILC2s at the steady state [47]. Therefore, this chapter will discuss mostly our knowledge on the cross-talk between bacterial symbionts and ILC3s.

In 2008, we reported that peptidoglycan released by proliferating Gram$^-$ bacteria in the intestine induce the activation of LTi cells clustered in so-called cryptopatches [15], which are found near the base of crypts in the small intestine [48]. LTi cells in turn activate underlying stromal cells to release chemokines and recruit CCR6$^+$ B cells to form isolated lymphoid follicles (ILFs). ILFs generate microbiota-specific IgA-producing B cells, in a T-cell independent way, and play an important role in intestinal homeostasis [15,49]. The colonizing microbiota is therefore involved in the development of the intestinal immune system. At the same time, it provides a negative feedback on the number and activity of ILC3s, which include LTi cells, by inducing epithelial cells to produce IL-25 [47]. Of note, in both these phenomena, epithelial cells translate the recognition of microbes into signals that regulate ILCs.

Myeloid cells are nevertheless the major relay between microbiota and ILC3s through the production of the type 3 inducer cytokines IL-23 and IL-1β. In the context of *C. rodentium* infection, CX_3CR1^+ macrophages produce higher amounts of these cytokines than do conventional CD103$^+$ DCs, and thus, more efficiently induce the expression by ILC3s of IL-22 [50] and GM-CSF [51]. In addition, CD11b$^+$ DCs, which include CX_3CR1^+ cells, are obligate sources of IL-23 for host survival during *C. rodentium* infection [52], and TNFα produced by CD11b$^+$ DCs during *Helicobacter typhlonius* infection synergizes with IL-23 for the expression of IL-17 by ILC3s [53]. CD11b$^+$ DCs are also activated by microbiota-derived ATP to produce IL-23 during the steady state [54]. Other members of the symbiotic microbiota induce the expression of IL-23 by myeloid cells and as a consequence, IL-22 by ILC3s, such as SFB [55] and *Lactobacilli* species [56]. ILC3s feedback positively on the

production of IL-23 by myeloid cells through their expression of membrane-bound LT and activation of LTβR on DCs [57]. Unexpectedly, intestinal glial cells sense microbiota through a Myd88-dependent pathway and produce neurotrophic factors that activate the Ret receptor on ILC3s and promote their expression of IL-22 [58].

At the steady state, ILC3s are the major source of IL-22 [47]. IL-22, as well as IL-17, induce the production of AMPs by epithelial cells [59], and therefore, are critical for the containment not only of pathogens, but also of the symbiotic microbiota. IL-22 also regulates the fucosylation of epithelial cells, a dietary carbohydrate for many symbionts, and thereby increases resistance to infection by *Salmonella typhimurium* [60] and *C. rodentium* [61]. Furthermore, membrane-bound ($LT\alpha_1\beta_2$) and soluble ($LT\alpha_3$) LT expressed by ILC3s play an important role in the containment of the microbiota [62] through the activation of epithelial cells [24], as well as of DCs and T cells that lead to the production of anti-microbial IgA [63]. The containment of microbiota through the enhancement of epithelial defenses and adaptive immunity must nevertheless be controlled to avoid exaggerate responses to the symbiotic microbiota and consequent pathologic inflammation. To this end, ILCs express MHC class II molecules, as well as the machinery required to process proteins for presentation of peptides onto class II molecules, allowing ILCs to dampen the $CD4^+$ T cell response to microbiota [64] and to SFB in particular [65]. Reciprocally, the presence of $CD4^+$ T cells in the intestinal lamina propria decreases the expression of IL-22 by ILC3s [47,66], presumably through competition for survival and inducing cytokines such as IL-23.

Bacterial symbionts of the *Alcaligenes* genus have been identified that are present in Peyer's patches. ILC3s and IL-22 are critical for the containment of *Alcaligenes*, as the depletion of ILCs using an anti-CD90 antibody leads to the presence of the bacteria in spleen and liver [9]. The depletion experiments were performed in Rag-deficient mice, as well as in mice reconstituted with mature B and T cells, indicating that adaptive immunity is not necessary for the containment of *Alcaligenes*. It remains nevertheless possible that, in normal mice, adaptive immunity develops over time that contributes to the containment of these symbionts through the generation of antibodies. As a matter of fact, *Alcaligenes* promotes the generation of IgA, which contribute to the construction of its niche [10].

IV. Microbe-ILC interactions in the lungs

The impact of ILCs on microbes (and parasites) at mucosal sites other than the intestine has been best studied in the lungs. ILC2s are the dominant ILC subset in the lungs at the steady state, as a consequence of the production of IL-33 and TSLP by the alveolar epithelium [67].

The production of IL-5 and IL-13 by ILC2s promotes the recruitment of eosinophils, the differentiation of monocyte into M2 (or alternatively activated) macrophages, and the differentiation of T cells into Th2 cells [68-70]. ILC2s contribute to the resistance to lung parasites, such as *Strongyloides venezuelensis* [71], *Nippostrongylus brasiliensis* [72,73], *Litomosoides sigmodontis* [74], as well as to tissue repair [75]. However, type 2 immunity induced by ILC2s also leads to increased susceptibility to fungal [76,77] and bacterial infection [78], by mechanisms of immunological cross-regulation [4].

Type 2 immunity promoted by ILC2s plays an important role in the repair phase of immune responses, which occurs, for example, after influenza A virus infection through the production of the EGFR ligand amphiregulin [79]. This repair response is nevertheless inhibited by IFNγ that is induced by the virus infection [80]. Furthermore, the ILC2-initiated repair responses, induced by virus-induced tissue damage, can lead to exacerbated type 2 responses and, as a consequence, asthma. Such an association, and the mechanisms driving this association, have been reported in the context of infection with rhinovirus [81-83] and respiratory syncytial virus [84,85]. Similar pathological consequences are also induced by the fungi *Alternaria alternata* [86] and *Cryptococcus neoformans* [87].

ILC3s also play an important role in the lungs in response to bacterial and fungal infections. IL-22 produced by ILC3s is instrumental in lung immunity to *Streptococcus pneumoniae* [88] and *Pseudomonas aeruginosa* [89]. Interestingly, neonatal colonization of mice with intestinal bacteria induces the influx of IL-22-producing ILC3s into the lung, which confer life-saving resistance to early infection by *Streptococcus pneumoniae* [90].

V. Concluding remarks

We have discussed recent data showing the critical role played by ILC early in the immune responses to pathogens, as these cells can respond promptly to inducer cytokines provided by myeloid and epithelial cells upon infection and injury. Of note, T_{RM} cells, as well as particular subsets of invariant Tαβ or Tγδ cells, can play similar roles as their activation state alleviates the requirement for antigen-specific TCR activation. The prompt activation of ILCs is also important in response to changes in the symbiotic microbiota, in order to maintain homeostasis.

The current knowledge on the role of ILCs in defense and homeostasis has been inferred form mouse models and association studies in human. It is now possible to design strategies to harness the central role of ILCs in homeostasis, defense and immunoregulation for the prevention and therapy of infectious diseases, and of pathological consequences of

231 microbiota dysbiosis. It is also possible that novel vaccination strategies benefit from an early
232 manipulation of ILCs, in order to force the response to specific antigens into the type most
233 adapted to fight the pathogen. The manipulation of ILCs may involve the administration of
234 inducer cytokines, or other molecules, yet to be discovered, that specifically activate subsets
235 of ILCs. Another possibility is the isolation of ILC precursors from the blood of patients, and
236 their expansion *in vitro* into the desired subset before re-administration and induction of type
237 1, 2 or 3 immune responses. We are still in the early days of ILC biology, but also at the
238 exciting transition phase when clinical applications become possible.
239

240 **Table 1**

ILC type	Role	Microbe type	Microbe species	Organ	References
ILC1	+	Bacteria	*Clostridium difficile*	Intestine	[46]
	+	Virus	*Adenovirus*	Liver	[44]
	+		*Mouse hepatitis virus*	Liver	[45]
	+		*Cytomegalovirus*	Liver	[43]
ILC2	–	Bacteria	Streptococcus pneumoniae	Lung	[78]
	–	Virus	*Rhinovirus*	Lung	[81-83]
	–		*Respiratory syncytial virus*	Lung	[84,85]
	+/–		*Influenza*	Lung	[79,80]
	+/–	Fungi	*Cryptococcus neoformans*	Lung	[76,77,87]
	–		*Alternaria altnernata*	Lung	[86]
	+	Parasites	*Trichinella spiralis*	Intestine	[34]
	+		*Trichuris muris*	Intestine	[35]
	+		*Heligmosomoides polygyrus*	Intestine	[32]
	+		*Nippostrongylus brasiliensis*	Intestine/lung	[30,31,72,73]
	+		*Strongyloides venezuelensis*	Intestine/lung	[33,71]
	+		*Litomosoides sigmodontis*	Lung	[74]
	+		*Schistosoma haematobium*	Blood	[36]
ILC3	+	Bacteria	*Commensal microbiota*	Intestine	[15,24,47,49,54,62-64]
	+		*Citrobacter rodentium*	Intestine	[18,21-23,50,52,61]
	+		*Escherichia coli K1*	Intestine	[26]
	+		*SFB*	Intestine	[55,65]
	+		*Lactobacilli*	Intestine	[56]
	+		*Alcaligenes*	Intestine	[9,10]
	+/–		*Salmonella enterica*	Intestine	[25]
	+/–		*Salmonella typhimurium*	Intestine	[29,60]
	+/–		*Helicobacter typhlonius*	Intestine	[53]
	+		*Streptococcus pneumoniae*	Lung	[88,90]
	+		*Pseudomonas aeruginosa*	Lung	[89]
	+		*Klebsiella pneumoniae*	Blood	[26]
	+	Virus	*Rotavirus*	Intestine	[28]
	+	Fungi	*Candida albicans*	Lung	[89]
	+	Parasites	*Toxoplasma gondii*	Intestine	[27]

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Conflict of interests

We declare no conflicts of interests

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Legends to figures

Figure 1. The activation of innate lymphoid cells. The three types of ILCs are promptly activated by distinct types of threats. Such threats are detected by myeloid and non-hematopoietic cells, which express inducer cytokines (in boxes). In reaction to inducer cytokines, ILCs express effector cytokines that both activate defense mechanisms and regulate immunity.

Figure 2. The control of microbiota and pathogens by ILCs. The response of ILCs to symbiotic microbes and pathogens have been extensively described in the intestine and lung, to include bacteria, viruses, fungi and parasites, as well as viruses in the liver. Given their prompt activation, ILCs play a critical role early in the effector response to perturbations in the microbiota and to pathogens, and in the regulation of adaptive immunity. In some cases, however, the reactivity of ILCs contributes to pathology.

Figure 1

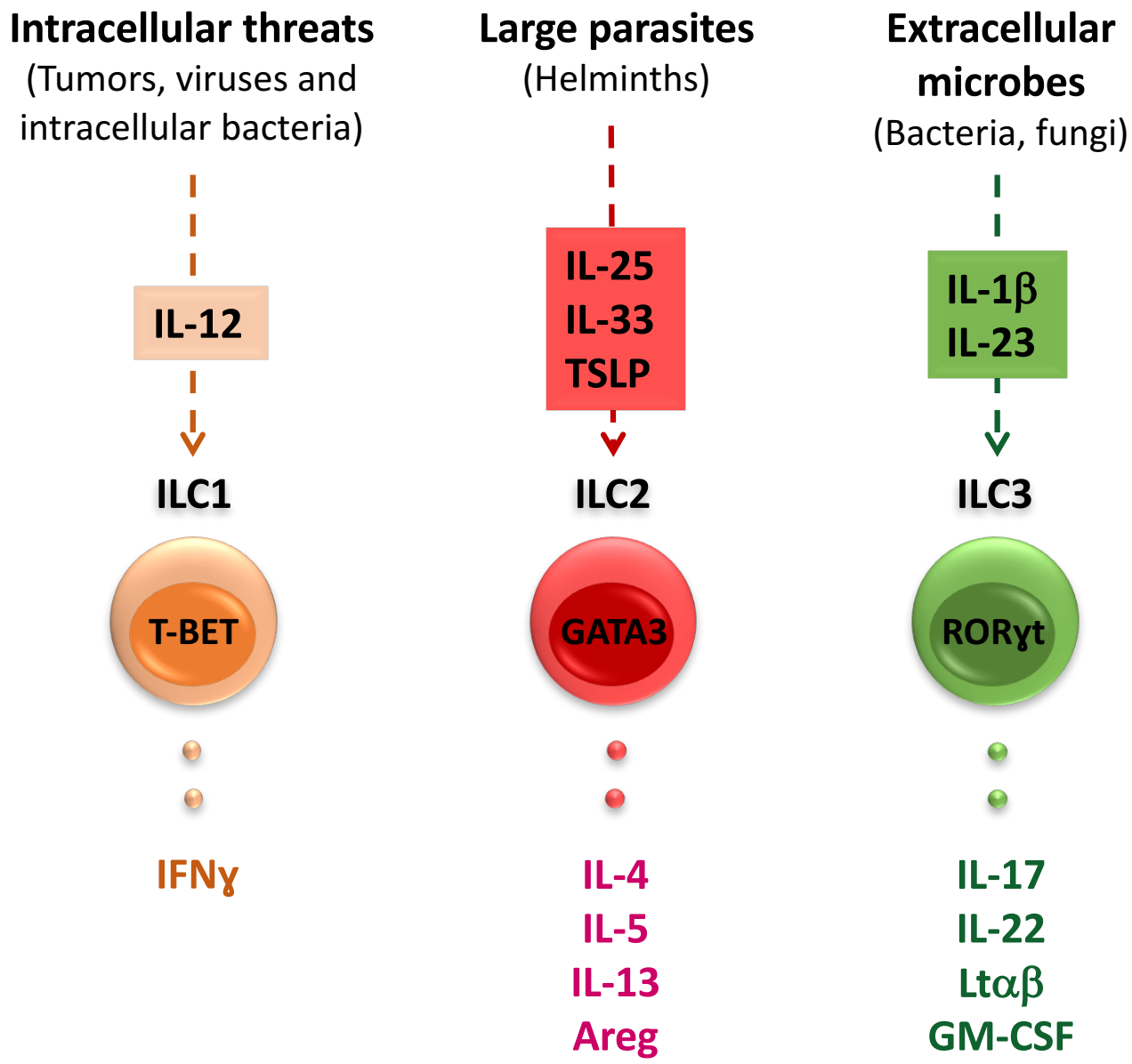
Intracellular threats
(Tumors, viruses and intracellular bacteria)
IL-12
ILC1
T-BET
IFNγ
Large parasites
(Helminths)
IL-25
IL-33
TSLP
ILC2
GATA3
IL-4
IL-5
IL-13
Areg
Extracellular microbes
(Bacteria, fungi)
IL-1β
IL-23
ILC3
RORγt
IL-17
IL-22
Ltαβ
GM-CSF

Figure 2