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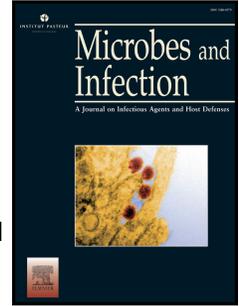


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Control of pathogens and microbiota by innate lymphoid cells

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15 **Summary**

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

25

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

27

28

29 I. Introduction

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

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

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

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

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

77

78 **II. ILCs in the control of intestinal and hepatic infections**

79 ILC3s are the oldest and best characterized ILC family member (not considering NK cells). In
80 the late 90's, non-B non-T lymphoid cells, termed lymphoid tissue inducer (LTi) cells, have
81 been described that colonize developing secondary lymphoid tissues, the lymph nodes and the
82 Peyer's patches, and shown to be required for their development [13,14]. LTi cells form
83 clusters in fetal lymphoid tissues and the intestinal lamina propria [15], and activate stromal
84 cells to initiate organogenesis [16]. Ten years later, it was shown that LTi cells are part of a
85 larger family of ILC3s that depend on the hormone receptor and transcription factor ROR γ t
86 [17], and express the type 3 cytokines IL-17, IL-22 and lymphotoxin (LT) [18-21]. Non-LTi
87 ILC3s do not cluster and are not involved in the development of lymphoid tissues. Rather,
88 these cells resemble more freely moving lymphoid effector cells, and together with LTi cells,
89 play a critical role early in defense against enteric pathogens.

90 Using mouse models that lack all lymphoid cells or only B and T cells, it was reported
91 that ILC3s are required early in the control of intestinal infection by *Proteobacteria*, such as
92 *Citrobacter rodentium*, the murine homologue of human enteropathogenic *Escherichia coli*.
93 The production of IL-22 is paramount for this protective function, and induces the expression
94 of anti-microbial peptides (AMPs) by epithelial cells, such as Reg3 γ [18,21-23]. Also
95 involved in increasing epithelial defense is membrane-bound LT, which bind its receptor

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

108 ILC2s were first reported in the context of intestinal infection with the helminth
109 *Nippostrongylus brasiliensis*, which evokes a vigorous expansion of “non-B non-T cells”
110 expressing IL-4, IL-5 and IL-13 [30,31]. ILC2s are also involved in defense against
111 *Heligmosomoides polygyrus* [32], *Strongyloides venezuelensis* [33], *Trichinella spiralis* [34]
112 and *T. muris* [35], and are diminished in the blood of children infected with the blood dweller
113 *Schistosoma haematobium* [36]. Both inducer cytokines IL-25 and IL-33 are expressed upon
114 helminth infection and activate ILC2s, noting that ILC2s responding to IL-33 have been
115 suggested to be precursors of ILC2s responding to IL-25 [37]. While IL-33 is expressed by
116 different types of stromal cells, as well as by mast cells [32], the source of IL-25 was only
117 recently identified, in the intestine, as Tuft epithelial cells [38-40]. Interestingly, Tuft cells
118 appear to detect helminth infection through chemosensory signaling via the G-protein coupled
119 receptor Trpm5 [38]. The activity of ILC2s is also promoted, during helminth infection, by
120 the neuropeptide neuromedin U that is expressed by cholinergic neurons in the intestine
121 [41,42].

122 ILC1s are distinguished from NK cells by their lack of expression of, and requirement
123 for, the transcription factor Eomesodermin [2]. ILC1s provide early protection to the liver
124 from mouse cytomegalovirus infection, producing IFN γ before NK cells are engaged [43], but
125 at the same time prevent the recruitment of NK cells and CD8⁺ T cells that optimally fight
126 liver infection with adenovirus [44]. Mouse hepatitis virus inoculated orally is also cleared
127 from the intestine by ILC1s, which are activated by IL-15 released from infected stromal cells
128 [45]. Furthermore, ILC1s and their production of IFN γ are engaged in the defense against

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

133

134 **III. The ILC crosstalk with intestinal microbiota**

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

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

152 Myeloid cells are nevertheless the major relay between microbiota and ILC3s through
153 the production of the type 3 inducer cytokines IL-23 and IL-1 β . In the context of *C.*
154 *rodentium* infection, CX₃CR1⁺ macrophages produce higher amounts of these cytokines than
155 do conventional CD103⁺ DCs, and thus, more efficiently induce the expression by ILC3s of
156 IL-22 [50] and GM-CSF [51]. In addition, CD11b⁺ DCs, which include CX₃CR1⁺ cells, are
157 obligate sources of IL-23 for host survival during *C. rodentium* infection [52], and TNF α
158 produced by CD11b⁺ DCs during *Helicobacter typhlonius* infection synergizes with IL-23 for
159 the expression of IL-17 by ILC3s [53]. CD11b⁺ DCs are also activated by microbiota-derived
160 ATP to produce IL-23 during the steady state [54]. Other members of the symbiotic
161 microbiota induce the expression of IL-23 by myeloid cells and as a consequence, IL-22 by
162 ILC3s, such as SFB [55] and *Lactobacilli* species [56]. ILC3s feedback positively on the

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

167 At the steady state, ILC3s are the major source of IL-22 [47]. IL-22, as well as IL-17,
168 induce the production of AMPs by epithelial cells [59], and therefore, are critical for the
169 containment not only of pathogens, but also of the symbiotic microbiota. IL-22 also regulates
170 the fucosylation of epithelial cells, a dietary carbohydrate for many symbionts, and thereby
171 increases resistance to infection by *Salmonella typhimurium* [60] and *C. rodentium* [61].
172 Furthermore, membrane-bound (LT $\alpha_1\beta_2$) and soluble (LT α_3) LT expressed by ILC3s play an
173 important role in the containment of the microbiota [62] through the activation of epithelial
174 cells [24], as well as of DCs and T cells that lead to the production of anti-microbial IgA [63].
175 The containment of microbiota through the enhancement of epithelial defenses and adaptive
176 immunity must nevertheless be controlled to avoid exaggerate responses to the symbiotic
177 microbiota and consequent pathologic inflammation. To this end, ILCs express MHC class II
178 molecules, as well as the machinery required to process proteins for presentation of peptides
179 onto class II molecules, allowing ILCs to dampen the CD4⁺ T cell response to microbiota [64]
180 and to SFB in particular [65]. Reciprocally, the presence of CD4⁺ T cells in the intestinal
181 lamina propria decreases the expression of IL-22 by ILC3s [47,66], presumably through
182 competition for survival and inducing cytokines such as IL-23.

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

192

193 **IV. Microbe-ILC interactions in the lungs**

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

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

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

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

218

219 **V. Concluding remarks**

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

227 The current knowledge on the role of ILCs in defense and homeostasis has been
228 inferred from mouse models and association studies in human. It is now possible to design
229 strategies to harness the central role of ILCs in homeostasis, defense and immunoregulation
230 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	<i>Clostridium difficile</i>	Intestine	[46]
	+	Virus	<i>Adenovirus</i>	Liver	[44]
	+		<i>Mouse hepatitis virus</i>	Liver	[45]
	+		<i>Cytomegalovirus</i>	Liver	[43]
ILC2	-	Bacteria	<i>Streptococcus pneumoniae</i>	Lung	[78]
	-	Virus	<i>Rhinovirus</i>	Lung	[81-83]
	-		<i>Respiratory syncytial virus</i>	Lung	[84,85]
	+/-		<i>Influenza</i>	Lung	[79,80]
	+/-	Fungi	<i>Cryptococcus neoformans</i>	Lung	[76,77,87]
	-		<i>Alternaria alternata</i>	Lung	[86]
	+	Parasites	<i>Trichinella spiralis</i>	Intestine	[34]
	+		<i>Trichuris muris</i>	Intestine	[35]
	+		<i>Heligmosomoides polygyrus</i>	Intestine	[32]
	+		<i>Nippostrongylus brasiliensis</i>	Intestine/lung	[30,31,72,73]
	+		<i>Strongyloides venezuelensis</i>	Intestine/lung	[33,71]
	+		<i>Litomosoides sigmodontis</i>	Lung	[74]
	+		<i>Schistosoma haematobium</i>	Blood	[36]
ILC3	+	Bacteria	<i>Commensal microbiota</i>	Intestine	[15,24,47,49,54,62-64]
	+		<i>Citrobacter rodentium</i>	Intestine	[18,21-23,50,52,61]
	+		<i>Escherichia coli K1</i>	Intestine	[26]
	+		<i>SFB</i>	Intestine	[55,65]
	+		<i>Lactobacilli</i>	Intestine	[56]
	+		<i>Alcaligenes</i>	Intestine	[9,10]
	+/-		<i>Salmonella enterica</i>	Intestine	[25]
	+/-		<i>Salmonella typhimurium</i>	Intestine	[29,60]
	+/-		<i>Helicobacter typhlonius</i>	Intestine	[53]
	+		<i>Streptococcus pneumoniae</i>	Lung	[88,90]
	+		<i>Pseudomonas aeruginosa</i>	Lung	[89]
	+		<i>Klebsiella pneumoniae</i>	Blood	[26]
	+	Virus	<i>Rotavirus</i>	Intestine	[28]
	+	Fungi	<i>Candida albicans</i>	Lung	[89]
	+	Parasites	<i>Toxoplasma gondii</i>	Intestine	[27]

241

242

243 **Conflict of interests**

244 We declare no conflicts of interests

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507 **Legends to figures**

508

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

514

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

521

522

Figure 1

Intracellular threats
(Tumors, viruses and intracellular bacteria)



Large parasites
(Helminths)



Extracellular microbes
(Bacteria, fungi)



Figure 2

