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Role of group 3 innate lymphoid cells in antibody production

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Abstract

Innate lymphoid cells (ILCs) constitute a heterogeneous family of effector lymphocytes of the innate immune system that mediate lymphoid organogenesis, tissue repair, immunity and inflammation. The initial view that ILCs exert their protective functions solely during the innate phase of an immune response has been recently challenged by evidence indicating that ILCs shape adaptive immunity by establishing both contact-dependent and contact-independent interactions with multiple hematopoietic and non-hematopoietic cells, including B cells. Some of these interactions enhance antibody responses both systemically and at mucosal sites of entry.

Introduction

Innate lymphoid cells (ILCs) include developmentally related groups of helper-like cells of the innate immune system that functionally mirror well-defined subsets of CD4⁺ T helper (T_H) cells [1–2]. Some of the effector molecules expressed by ILCs are known modulators of adaptive antibody responses emerging from T cell-dependent (TD) or T cell-independent (TI) pathways of B cell activation. This review discusses how group 3 ILC modulates homeostasis and antibody production in systemic and mucosal lymphoid tissues.

Phenotype and function of ILCs

Multiple subsets of ILCs emerge from a common lymphoid progenitor through a developmental pathway initiated by bone marrow or fetal liver stem cells. This pathway is dictated by signals from common cytokine receptor γ -chain and various transcription factors, including ID2, nuclear factor interleukin-3 regulated (NFIL3) and GATA3 [3–6].

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Despite their phenotypic and functional heterogeneity, ILCs share multiple properties, including lymphoid morphology, absence of common lineage-specific molecules, and lack of somatically recombined antigen receptors [1–2].

Besides cytotoxic natural killer (NK) cells, ILCs include three groups of helper-like innate cells characterized by the expression of distinct sets of transcription factors and cytokines [3,7]. Similar to type 1 T_H (T_H1) cells, group 1 ILCs (ILC1) depend on the transcription factor T-bet and secrete interferon (IFN)- γ and tumor necrosis factor (TNF) in response to interleukin-12 (IL-12) [5,8–9]. In contrast, ILC2 require the transcription factors GATA3, ROR- α and TCF1 [10–12] and release IL-5 and IL-13 in response to IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), thus resembling T_H2 cells [1,13–14]. Finally, ILC3 are highly dependent on the transcription factor ROR γ t and secrete IL-22 and IL-17A in response to IL-23 and IL-1 β , therefore mimicking T_H22 and T_H17 cells [15–17]. Metabolites of dietary vitamin A, including retinoic acid (RA), further contribute to the development and homeostasis of ILC3 [18].

ILCs secrete effector cytokines during the innate phase of an immune response, prior to the initiation of adaptive immunity [1]. ILC1 provide protection against viruses, intracellular bacteria and tumors and play an important role in inflammation, whereas ILC2 enhance immunity against nematodes and contribute to allergic inflammation [2]. Finally, ILC3 include lymphoid tissue inducer (LTi) cells, which mediate lymphoid organogenesis, as well as natural cytotoxicity receptor (NCR)⁺ ILC3 and NCR[−] ILC3, which promote epithelial integrity and immune responses against extracellular bacteria [1–2,7]. These responses may entail the induction of protective antibodies by systemic and mucosal B cells of the adaptive immune system.

Role of ILC3 in lymphoid organ development

ILC3 form a heterogeneous family of developmentally related lymphoid populations that rely on the cytokine IL-7 and the transcription factor ROR γ t for their differentiation [15,19–21]. LTi cells are prototypic members of the ILC3 family [22]. These cells were first described some 20 years ago as fetal CD4⁺CD3[−] lymphocytes inhabiting the anlagen of mouse lymph nodes and embryonic Peyer's patches (PPs) [23]. Subsequent studies demonstrated that LTi cells are essential for the development of lymphoid organs during fetal life [24].

Lymphoid tissue organogenesis involves a specialized subset of stromal cells (SCs) that express elevated levels of LT β receptor (LT β R) [22]. These SCs are referred to as lymphoid tissue organizer (LTo) cells and become strongly activated in response to engagement of LT β R by transmembrane lymphotoxin $\alpha_1\beta_2$ (LT $\alpha_1\beta_2$) from LTi cells [22]. Signals from LT β R stimulate LTo cell up-regulation of vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), mucosal addressin cell adhesion molecule (MAdCAM) and receptor activator of NF- κ B ligand (RANKL) as well as LTo cell release of chemokines such as CXCL13, CCL19, CCL20 [22]. Such activation-related events promote the recruitment and spatial organization of B and T cells. Recently, a population of

CD4⁺CD127⁺RORC⁺ ILCs with LT_i-like function was identified in developing lymph nodes and mesentery from humans during the first trimester of gestation [25].

Immediately after birth, LT_i cells form primitive lymphoid clusters, termed cryptopatches, in the lamina propria (LP) of both small and large intestinal segments [26]. These LT_i cells retain lymphoid tissue-inducing activity and indeed promote the induction of more organized lymphoid structures, called isolated lymphoid follicles (ILFs), in response to postnatal colonization by intestinal bacteria [27–28].

ILC3 with LT_i-like function have also been identified in adult secondary lymphoid organs different from ILFs, including peripheral lymph nodes, spleen and PPs from the small intestine [29–30]. These ILC3 facilitate the segregation of B and T cell areas and the generation of optimal immune responses by interacting with SCs, including marginal reticular cells (MRCs) [31]. Similar ILC3-SC interactions foster the reparation of the lymphoid microenvironment after infection with lymphocytic choriomeningitis virus [32]. Finally, ILC3 cooperate with B cells to induce the development of follicular dendritic cells (FDCs) from ubiquitous perivascular precursors [33]. These SCs specialize in providing a structural scaffold to lymphoid follicles and in trapping antigen during immune responses[33].

Role of ILC3 in homeostasis and immunity

Besides promoting the development, maintenance and repair of peripheral lymphoid tissues, ILC3 maintain gut homeostasis in two ways: by preserving the integrity of intestinal epithelial cells (IECs) and by segregating commensal bacteria in the intestinal lumen [15,17,34–36]. These effects largely rely on ILC3 secretion of powerful IECstimulating cytokines, namely IL-22 [17,35–36]. Indeed, depletion of ILC3 causes systemic inflammation by inducing mucosal translocation and peripheral dissemination of intestinal bacteria predominantly belonging to the *Alcaligenes* genus [36]. Similarly, loss of IL-22 from ILC3 triggers intestinal inflammation by causing outgrowth of segmented filamentous bacteria (SFB), a mouse-specific group of commensals that stimulate the expansion of pro-inflammatory T_H17 cells [37].

Mucosal ILC3 release IL-22 upon exposure to IL-23 from dendritic cells (DCs) and macrophages [15,38]. This response contributes to the segregation of commensal bacteria in the intestinal lumen by inducing IEC release of β -defensins, RegIII β , RegIII γ and other antimicrobial proteins through a transcriptional program dictated by signal transducer and activator of transcription 3 (STAT3) [15,38–39]. IL-22 further reinforces the gut barrier by stimulating IEC production of MUC1, a transmembrane mucin that forms a protective glycocalyx on the gut epithelium [40]. Together with LT, IL-22 also stimulates extensive fucosylation of the glycoproteins that form the glycocalyx, a key requirement for host-microbiota symbiosis [41–42].

Besides promoting gut homeostasis, IL-22 from ILC3 provides a first line of defence against enteric pathogens. Indeed, loss of IL-22 exacerbates the invasion of the large intestine by *Citrobacter rodentium*, a murine pathogen used as model for human attaching and effacing infections [17,39]. Also in this case, the protective effect of IL-22 is largely dependent on

IEC release of antimicrobial peptides, suggesting that ILC3 use similar signaling programs to control intestinal commensals and pathogens.

Role of ILC3 in systemic antibody responses

Growing evidence indicates that, besides mediating innate immunity, ILC3 regulate adaptive immune responses (Figure 1), including antibody production by B cells [1,29,43–44]. After recognizing antigen through transmembrane immunoglobulin M (IgM) and IgD antibodies generally referred to as B cell receptor (BCR), mature naïve B cells usually follow a TD pathway to generate humoral immunity [45]. This pathway involves cognate interactions of B cells with effector CD4⁺ T follicular helper (T_{FH}) cells positioned at the outer edge of the lymphoid follicle, also known as T-B border [45]. At this stage, B cells receive robust antigenic signals and multiple T_{FH} cells co-stimulatory signals, including signals from the TNF family member CD40 ligand (CD40L) and the cytokines IL-4 and IL-21 [46–47]. Antigen-activated B cells emerging from this initial cognate interaction progress along two distinct maturation pathways to generate a primary antibody response [48].

In the extrafollicular pathway, B cells differentiate into short-lived plasmablasts that secrete IgM with low affinity for antigen [48]. In the follicular pathway, B cells migrate to the center of the follicle and further interact with T_{FH} cells to initiate the germinal center (GC) reaction [47]. In this reaction, B cells undergo massive clonal expansion combined with somatic hypermutation (SHM), a DNA-editing process required for antigen-driven selection of Ig mutants with higher affinity for antigen [48]. GC B cells also undergo class switch recombination (CSR) from IgM to IgG, IgA or IgE, a DNA-modifying process that allows antibodies to modulate their effector functions without changing antigen specificity [48–49]. Both SHM and CSR require activation cytidine deaminase (AID), a DNA-modifying enzyme induced by B cells in response to CD40L and cytokines [48]. Ultimately, GC B cells differentiate into long-lived memory B cells, which re-circulate, or antibody-secreting plasma cells, which home to the bone marrow [49].

Secondary antibody responses to previously encountered antigens involve the activation of memory B cells by antigen-primed CD4⁺ T cells, which may include memory T_{FH} cells [46]. Recent mouse studies indicate that ILC3 strategically located at follicular sites of lymphocyte entry promote the survival of memory CD4⁺ T cells to enhance secondary IgG responses, including affinity maturation [30–31,50]. This effect involves ILC3 expression of OX40 ligand (OX40L) and CD30 ligand (CD30L), two TNF family members that engage OX40 and CD30 receptors on memory CD4⁺ T cells [30,50]. Thus, ILC3 may shape not only the magnitude, but also the affinity and duration of secondary TD antibody responses by controlling the survival of memory CD4⁺ T cells.

Additional mouse studies show that splenic NCR⁺ ILC3 internalize antigen and, in the presence of IL-1 β , express the antigen-presenting molecule major histocompatibility complex-II (MHC-II), the T cell-costimulatory molecules CD80 and CD86, and the T cell-activating cytokines IL-2, IFN- γ and TNF [44]. These changes allow ILC3 to present processed antigen to CD4⁺ T cells, which thereafter undergo expansion and initiate TD antibody production [44]. Indeed, selective deletion of MHC-II in ILC3 impairs specific IgG

responses to a protein antigen combined with CpG-DNA, an adjuvant that stimulates the Toll-like receptor (TLR) family member TLR9 [44]. When activated by cytokines or TLR ligands, also human ILC3 release IL-2 and up-regulate the expression of leukocyte antigen-II (HLA-II), the human equivalent of MHC-II [15,25,51–53]. These human findings further corroborate the involvement of ILC3 in T cell responses.

Besides enhancing TD antibody production against protein antigens, ILC3 help TI responses against carbohydrate antigens [29]. These antigens extensively cross-link BCRs on innate-like B cells inhabiting extrafollicular areas, including splenic marginal zone (MZ) B cells [54]. Strategically positioned between the immune system and the circulation, MZ B cells are poised to mount explosive responses against blood-borne TI antigens such as polysaccharides and microbial TLR ligands [54]. In addition to antigenic BCR and TLR signals, MZ B cells receive co-stimulatory signals from DCs, macrophages, neutrophils and inflammatory monocytes [54–55]. After sensing microbes through TLRs, scavenger receptors and other pattern recognition receptors, these myeloid cells of the innate immune system release B cell-stimulating cytokines such as IL-6, IL-10 and CXCL10 as well as B cell-activating factor of the TNF family (BAFF) and its homologue a proliferation-inducing ligand (APRIL), two CD40L-related molecules that stimulate MZ B cell production of low-affinity antibodies in the absence of help from T_{FH} cells [55–59].

In humans, ILC3 inhabiting the MZ and perfollicular zone of the spleen closely interact with MRCs expressing MAdCAM-1 [29]. Activation of MRCs by TNF and LT from ILC3 up-regulates MRC expression of ILC3-targeting survival factors, including IL-7 [29]. In the presence of IL-1 β and IL-23 from local DCs and macrophages, ILC3 express BAFF, CD40L and Delta-like 1 (DLL1), a NOTCH2 ligand involved in the differentiation of MZ B cells [29,54]. In mice, splenic ILC3 express APRIL along with DLL1 and depletion of ILC3 hampers MZ B cell production of antibodies to TI antigens [29]. In both humans and mice, ILC3 further help MZ B cells by releasing the cytokine GM-CSF, which co-opts neutrophils expressing BAFF and APRIL [29]. Thus, ILC3 cooperate with multiple cells of the innate and adaptive immune systems to enhance antibody responses developing along TD and TI pathways.

Role of ILC3 in mucosal antibody responses

Host-commensal mutualism involves intestinal release of massive amounts of non-inflammatory IgA antibodies [60]. Intestinal IgA predominantly emerges from a TD pathway that unfolds in the follicles of PPs, a compartment of the gut-associated lymphoid tissue that develops during fetal life in response to LT_i cells [60]. After postnatal intestinal colonization by commensal bacteria, PPs develop a GC reaction involving cognate interaction of follicular B cells with T_{FH} cells that emerge from tolerogenic T regulatory (Treg) cells [61–64]. Similar to Treg cells, PP-associated T_{FH} cells express the IgA-inducing cytokine transforming growth factor- β (TGF- β 1) along with CD40L and IL-21 [61–64].

Growing evidence indicates that ILC3 modulate intestinal immunity independently of their lymphoid tissue-inducing function. For instance, ILC3 have been shown to constrain the proliferation of commensal-specific CD4⁺ T cells through an MHC-IIrestricted mechanism

[43]. Indeed, deletion of MHC-II in ILC3 triggers an intestinal inflammatory process characterized by dysregulated CD4⁺ T cell and IgG responses to commensals [43]. Considering the critical role played by Treg cells in IgA-mediated host-microbiota mutualism [63], future studies may need to address whether ILC3 induce Treg cells to finely tune IgA production and affinity maturation in PPs.

Recent findings show that intestinal ILC3 induce additional IgA through a TD pathway that takes place in the non-organized lymphoid tissue of the gut LP [65]. In this pathway, ILC3 release soluble LT α ₃ to promote homing of CD40L-expressing T_H cells to the LP [65].

IgA can also emerge from a TI pathway that unfolds in the organized lymphoid tissue of ILFs [62]. Unlike PPs, ILFs develop after birth from cryptopatches through a microbiota-driven process involving the activation of SCs by microbial TLR ligands as well as transmembrane LT α ₁ β 2 and soluble TNF from ILC3 [28]. These signals stimulate SC release of the chemokines CXCL13, CCL19 and CCL20, which recruit B cells and DCs to ILFs [28]. ILC3 further cooperate with SCs to stimulate DC and macrophage production of TNF as well as matrix metalloprotease 9 (MMP9) and MMP13, two enzymes that generate active TGF- β 1 from a latent precursor expressed by DCs, macrophages and SCs [28]. In the presence of TLR ligands from the microbiota, TGF- β 1 induces IgA CSR and production in B cells [28]. This TI pathway further involves DC, macrophage and SC production of BAFF in response to TLR ligands from the microbiota as well as LT α ₁ β 2 from ILC3 [28]. Besides TNF and LT α ₁ β 2, ILC3 express additional TNF family members such as receptor activator of NF- κ B ligand (RANKL) and LIGHT, which may further enhance DC survival and activation in ILFs, including BAFF secretion [28].

More IgA originates from a TI pathway that takes place in the LP and requires the activation of B cells by TGF- β 1, BAFF, APRIL, IL-6, RA and nitric oxide from various cell types, including DCs, SCs and IECs [60]. Recent studies indicate that transmembrane LT α ₁ β 2 on ILC3 enhances TI IgA production in the LP by stimulating the release of nitric oxide from a subset of CD11c⁺ DCs expressing the enzyme inducible nitric oxide synthase [65]. In the presence of nitric oxide, these DCs upregulate the expression of BAFF and APRIL and thereafter induce IgA CSR and production in LP B cells [65–66]. Intestinal ILC3 could further enhance IgA responses in the LP by secreting GM-CSF upon exposure to IL-1 β from macrophages. Indeed, GM-CSF stimulates CD103⁺CD11b⁺ DCs to release RA [67], a tolerogenic metabolite of dietary vitamin A that cooperates with TGF- β 1 to induce IgA production [68].

Conclusions

A hallmark of ILC3 relates to their expression of the TNF family members LT, TNF, OX40L, CD30L, LIGHT, TRANCE, BAFF, APRIL and CD40L [28–29,50]. All of these molecules play an essential role in lymphoid organogenesis, B-T segregation and B or T cell activation, diversification, differentiation and intestinal homing. Activated ILC3 also release IL-2, IL-6, IL-10, IL-17 and IP-10 [44], which contribute to the initiation or modulation of T and B cell responses. Furthermore, activated ILC3 internalize and process antigen and express the T cell-co-stimulatory molecules MHC-II, CD80 and CD86 [44]. These

remarkable features place ILC3 in a gray area that blurs the conventional boundaries between CD4⁺ T cells and DCs. Given their dual functional nature, ILC3 might represent ancestral inducer-effector cells capable to orchestrate both primitive and evolutionarily evolved pathways specialized in antibody production.

ILC3 may have acquired the ability to induce extrafollicular TI antibody responses in the LP prior to the inception of cognate CD4⁺ T cell-DC/B cell interactions in organized lymphoid tissues. Consistent with this possibility, lampreys, cartilaginous fishes and teleosts mount complex systemic and mucosal antibody responses in the absence of lymphoid follicles [69–70]. With the emergence of organized lymphoid architectures, ILC3 may have learned how to stimulate follicular TI antibody responses in ILFs. Finally, the appearance of highly evolved CD4⁺ T cells may have forced ILC3 to divert their ancestral taste for B cells to finely tune sophisticated TD antibody responses, including affinity maturation and secondary antibody production.

Some of these considerations are highly speculative. Less speculative is the prediction that the next few years will see a surge of new studies dissecting the complexity of the mechanisms by which ILCs initiate and regulate antibody responses, both systemically and at mucosal sites of entry.

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Highlights

- ILCs are helper lymphocytes of the innate immune system
- ILC3 shape the development of lymphoid organs
- ILC3 regulate adaptive immunity, including the activation of T and B lymphocytes
- ILC3 enhance T-dependent and T independent antibody responses by B cells
- ILC3 promote systemic IgM and IgG production as well as mucosal IgA production

Figure 1. Regulation of systemic antibody production by ILC3

(a) Geography of splenic ILC3, MZ B cells, neutrophils (also termed N_{BH} cells), MRCs, and T cells. FO, follicle; RP, red pulp; PALS, periaarterial lymphoid sheath; PB, plasmablast; PC, plasma cell. (b) ILC3 promote TI antibody responses by stimulating MZ B cells through a mechanism that may involve BAFF (in humans), APRIL (in mice), CD40L (in humans) and DLL1. ILC3 further enhance TI antibody production by co-opting N_{BH} cells through the release of GM-CSF. In addition to activating MZ B cells, ILC3-stimulated N_{BH} cells enhance the generation and survival of MZ B cell-derived PCs through a mechanism implicating APRIL (in humans) and BAFF (in mice). These effects likely involve MRCs, as ILC3 activate MRCs through LT and TNF, which in turn stimulate MRC production of ILC3 survival and activation factors, including IL-7. (c) ILC3 promote primary TD antibody responses after up-regulating MHC-II and T cell co-stimulatory molecules in response to IL-1 β . These ILC3 induce expansion and activation of antigen-specific CD4⁺ T cells by functioning as antigen-presenting cells. (d) ILC3 promote secondary TD antibody responses by enhancing the survival of memory CD4⁺ T cells through contact-dependent OX40-OX40L and CD30-CD30L interactions. These ILC3 are positioned at the T-B border.