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A novel mucosal RORγt$^+$NKp46$^+$ cell subset is a source of interleukin-22

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Abstract

Lymphoid tissue-inducer cells are hematopoietic cells essential for the organogenesis of several lymphoid structures during both fetal and adult life, whereas natural killer cells are key effector lymphocytes of the innate immune system. A series of recent reports has identified RORγt$^+$NKp46$^+$ interleukin-22-producing cells in gut and tonsils that share features with both lymphoid tissue-inducer cells and natural killer cells and that may be involved in mucosal immunity and homeostasis.

Introduction and context

The internal surfaces of the body are covered by distinct types of mucosal epithelia. These tissues constitute the first physical and immunological defense against various environmental insults and enclose local immune systems characterized by tissue-specific organization and complexity [1]. Gut-associated lymphoid tissues (GALTs) include lymphoid structures harboring different degrees of organization, such as mesenteric lymph nodes, Peyer's patches, cryptopatches, and isolated lymphoid follicles (ILFs), as well as of a network of immune cells scattered in both epithelium and underlying lamina propria connective tissue [2]. Tonsils belong to the nasopharynx-associated lymphoid tissue (NALT), which shares functional similarities with GALT but differs in its ontogenetic program [3].

Lymphoid tissue-inducer (LTi) cells are hematopoietic cells required for lymphoid organogenesis during both fetal and adult life [4,5]. LTi cell interaction with stromal organizer cells via lymphotoxin beta (LTβ) and tumor necrosis factor signals induces chemokine secretion and adhesion molecule expression upregulation, both crucial events for LTi cell and mature lymphocyte recruitment and subsequent lymphoid tissue organization [4]. In mouse embryo, LTi cell development and function are dependent on the expression of transcription factors such as retinoic acid-related orphan receptor gamma t (RORγt) and Id2 [6,7] as well as of interleukin (IL)-7Rα receptor and LT$\alpha_1\beta_2$ cytokine [4]. In the adult mouse, the LTi cells are found mainly within cryptopatch and ILF gut lymphoid structures, in close contact with dendritic cells [8], as well as in the spleen [9–11].

Natural killer (NK) cells are lymphocytes belonging to the innate immune system that are able to exert two major effector functions, cytotoxicity and cytokine secretion [12]. Therefore, NK cells can limit the spreading of microbial infections as well as of cells undergoing several kinds of cellular stress, and participate in the orchestration of adaptive immune responses. NK cells are ubiquitously expressed in both lymphoid and non-lymphoid organs, where they harbor distinct phenotypic and functional properties, depending on tissue locations [13–15]. An NK cell subset resident in mucosal tissues, such as human tonsils, has been documented [16]. However, the presence and the function of NK cells in GALT have been poorly investigated [17–19].

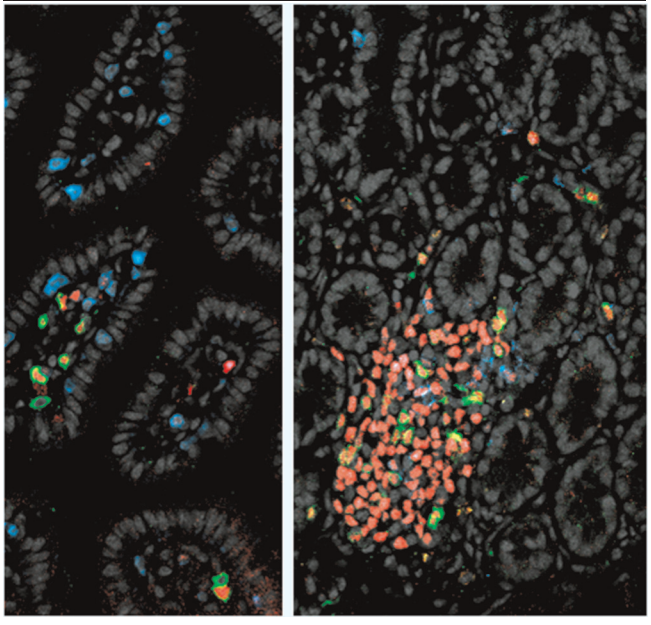
Major recent advances

By using the NK cell marker NKp46 [20], which is the most selective NK cell marker described so far in humans

and mice [21], several groups have recently described a new population of RORγt$^+$NKp46$^+$ IL-22-producing cells in the adult mouse gut [22–25]. Within the mouse gut, RORγt$^+$NKp46$^+$ cells have been identified in at least three different locations. First, RORγt$^+$NKp46$^+$ cells are detectable in variable proportions among RORγt$^+$ cells constituting cryptopatches (Figure 1, right panel) [22,23]. Second, a significant fraction of NKp46$^+$ cells present in the lamina propria also express RORγt, thus revealing the existence of two distinct RORγt$^+$NKp46$^+$ and RORγt$^-$NKp46$^+$ cell subpopulations (Figure 1, left panel) [22–24]. As described below, these two subsets harbor distinct developmental programs as well as phenotypic and functional properties. Finally, RORγt$^+$NKp46$^+$ and RORγt$^-$NKp46$^+$ cells are also found within Peyer's patches, in particular in subepithelial dome and interfollicular areas [22,25].

Interestingly, RORγt$^+$NKp46$^+$ and RORγt$^-$NKp46$^+$ cell subsets have distinct ontogenetic programs. While RORγt$^+$NKp46$^+$ cell development requires both RORγt expression and exposure to commensal flora [22–24], RORγt$^-$NKp46$^+$ cell subset generation is dependent upon IL-2/IL-15 signaling [23,24]. RORγt$^+$NKp46$^+$ cells thus share some developmental requisites with LTi cells, whereas RORγt$^-$NKp46$^+$ cell subset ontogeny appears to be related to that of conventional NK cells. RORγt$^-$NKp46$^+$ cells express the mouse NK cell marker NK1.1 and contain perforin and interferon-gamma (IFN-γ), though at lower levels than in lymphoid NK cells. In contrast, RORγt$^+$NKp46$^+$ cells express low amounts of the NK cell marker NK1.1, are devoid of messages encoding perforin and IFN-γ, but are equipped to secrete IL-22 [22–25]. IL-22, an IL-10 family member, induces epithelial secretion of various antimicrobial products, such as defensins and S100 calcium-binding proteins, and of various molecules involved in epithelial tissue proliferation and homeostasis, thus contributing to greater epithelial resistance to injury after microbial infection of the lung and the gut [26]. Consistent with this protective function of IL-22, mucosal RORγt$^+$NKp46$^+$ cells appear to play a critical role in the defense against the extracellular enteric pathogen *Citrobacter rodentium* [24,25]. Furthermore, IL-22-expressing NK-like cells have been recently shown to contribute to protection from experimental inflammatory bowel disease [27].

Figure 1. Immunofluorescence histology of mouse gut intestine



Immunofluorescence histology of frozen sections of mouse gut intestine stained with anti-NKp46 (green), anti-RORγt (red), and anti-CD3 (blue). Nuclei were counterstained with Sytox (grey). The left panel shows small intestini villi; the right panel shows cryptopatches. Magnification ×40. RORγt, retinoic acid-related orphan receptor gamma t.

A subset of CD56$^+$NKp44$^+$ cells represents the human functional equivalent of the mouse gut RORγt$^+$NKp46$^+$ population. Indeed, these cells reside within human tonsils and intestines, contain mRNA encoding human RORγt, and constitutively produce IL-22, but not IL-17A [25]. Like mouse RORγt$^+$NKp46$^+$ cells, these human IL-22-producing cells express some NK cell-associated markers, including NKp46 (H Spits, personal communication), yet lack perforin and granzyme and are unable to produce IFN-γ. The human mucosal RORγt$^+$CD56$^+$NKp44$^+$ cell subset is possibly the progeny of newly discovered human LTi cells [28]. Indeed, the phenotype of RORγt$^+$CD56$^+$ cells generated *in vitro* from human fetal LTi cells [28] resembles the mucosal RORγt$^+$ cells found in human tonsil and intestines [25]. However, unlike adult cell populations found postnatally, fetal human LTi cells and RORγt$^+$NKp46$^+$ cells produce only very low amounts of IL-22 message yet constitutively express IL-17A [28]. A switch from IL-17 to IL-22 expression has also been reported in mouse LTi cells from birth to adult life [29].

Future directions

Several questions regarding this novel mucosal RORγt$^+$NKp46$^+$ cell subset remain unanswered. First, what are the stimuli that promote the expression of NK cell receptors on these cells? In particular, what is the potential role of commensal flora exposure in inducing the expression of NK cell receptors and their respective ligands? Second, the features shared by both LTi and NK cells in mouse RORγt$^+$NKp46$^+$ and human

RORγt$^+$CD56$^+$NKp44$^+$ cells call for a closer look into their developmental relationships. Finally, the ability to secrete IL-22 highlights the potential contribution of this new cell type to mucosal tissue protection and homeostasis (for example, epithelium repair) upon diverse environmental insults and paves the way for the development of novel strategies to target these functions.

Abbreviations

GALT, gut-associated lymphoid tissue; IFN-γ, interferon-gamma; IL, interleukin; ILF, isolated lymphoid follicle; LTβ, lymphotoxin beta; LTi, lymphoid tissue inducer; NALT, nasopharynx-associated lymphoid tissue; NK, natural killer; RORγt, retinoic acid-related orphan receptor gamma t.

Competing interests

EV is a cofounder and shareholder of Innate Pharma (Marseille, France). The other authors declare that they have no competing interests.

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References

1. Iwasaki A: **Mucosal dendritic cells.** *Annu Rev Immunol* 2007, **25:**381-418.
2. Mowat AM: **Anatomical basis of tolerance and immunity to intestinal antigens.** *Nat Rev Immunol* 2003, **3:**331-41.
3. Kiyono H, Fukuyama S: **NALT-versus Peyer's-patch-mediated mucosal immunity.** *Nat Rev Immunol* 2004, **4:**699-710.
4. Mebius RE: **Organogenesis of lymphoid tissues.** *Nat Rev Immunol* 2003, **3:**292-303.
5. Ivanov II, Diehl GE, Littman DR: **Lymphoid tissue inducer cells in intestinal immunity.** *Curr Top Microbiol Immunol* 2006, **308:**59-82.
6. Eberl G, Marmon S, Sunshine MJ, Rennert PD, Choi Y, Littman DR: **An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells.** *Nat Immunol* 2004, **5:**64-73.
7. Yokota Y, Mansouri A, Mori S, Sugawara S, Adachi S, Nishikawa S, Gruss P: **Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2.** *Nature* 1999, **397:**702-6.
8. Kanamori Y, Ishimaru K, Nanno M, Maki K, Ikuta K, Nariuchi H, Ishikawa H: **Identification of novel lymphoid tissues in murine intestinal mucosa where clusters of c-kit$^+$ IL-7R$^+$ Thy1$^+$ lympho-hemopoietic progenitors develop.** *J Exp Med* 1996, **184:**1449-59.
9. Scandella E, Bolinger B, Lattmann E, Miller S, Favre S, Littman DR, Finke D, Luther SA, Junt T, Ludewig B: **Restoration of lymphoid organ integrity through the interaction of lymphoid tissue-inducer cells with stroma of the T cell zone.** *Nat Immunol* 2008, **9:**667-75.
10. Kim MY, Rossi S, Withers D, McConnell F, Toellner KM, Gaspal F, Jenkinson E, Anderson G, Lane PJ: **Heterogeneity of lymphoid tissue inducer cell populations present in embryonic and adult mouse lymphoid tissues.** *Immunology* 2008, **124:**166-74.
11. Takatori H, Kanno Y, Watford WT, Tato CM, Weiss G, Ivanov II, Littman DR, O'Shea JJ: **Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22.** *J Exp Med* 2009, **206:**35-41.
12. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S: **Functions of natural killer cells.** *Nat Immunol* 2008, **9:**503-10.
13. Hayakawa Y, Huntington ND, Nutt SL, Smyth MJ: **Functional subsets of mouse natural killer cells.** *Immunol Rev* 2006, **214:**47-55.
14. Huntington ND, Vosshenrich CA, Di Santo JP: **Developmental pathways that generate natural-killer-cell diversity in mice and humans.** *Nat Rev Immunol* 2007, **7:**703-14.
15. Caligiuri MA: **Human natural killer cells.** *Blood* 2008, **112:**461-9.
16. Ferlazzo G, Thomas D, Lin SL, Goodman K, Morandi B, Muller WA, Moretta A, Münz C: **The abundant NK cells in human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic.** *J Immunol* 2004, **172:**1455-62.
17. Tagliabue A, Befus AD, Clark DA, Bienenstock J: **Characteristics of natural killer cells in the murine intestinal epithelium and lamina propria.** *J Exp Med* 1982, **155:**1785-96.
18. Eiras P, Leon F, Camarero C, Lombardia M, Roldan E, Bootello A, Roy G: **Intestinal intraepithelial lymphocytes contain a CD3-CD7$^+$ subset expressing natural killer markers and a singular pattern of adhesion molecules.** *Scand J Immunol* 2000, **52:**1-6.
19. Cohavy O, Zhou J, Ware CF, Targan SR: **LIGHT is constitutively expressed on T and NK cells in the human gut and can be induced by CD2-mediated signaling.** *J Immunol* 2005, **174:**646-53.
20. Moretta A, Biassoni R, Bottino C, Mingari MC, Moretta L: **Natural cytotoxicity receptors that trigger human NK-cell-mediated cytolysis.** *Immunol Today* 2000, **21:**228-34.
21. Walzer T, Jaeger S, Chaix J, Vivier E: **Natural killer cells: from CD3(-)NKp46(+) to post-genomics meta-analyses.** *Curr Opin Immunol* 2007, **19:**365-72.
22. Luci C, Reynders A, Ivanov II, Cognet C, Chiche L, Chasson L, Hardwigsen J, Anguiano E, Banchereau J, Chaussabel D, Dalod M, Littman DR, Vivier E, Tomasello E: **Influence of the transcription factor RORgammat on the development of NKp46(+) cell populations in gut and skin.** *Nat Immunol* 2009, **10:**75-82.
23. Sanos SL, Bui VL, Mortha A, Oberle K, Heners C, Johner C, Diefenbach A: **RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46(+) cells.** *Nat Immunol* 2009, **10:**83-91.
24. Satoh-Takayama N, Vosshenrich CA, Lesjean-Pottier S, Sawa S, Lochner M, Rattis F, Mention JJ, Thiam K, Cerf-Bensussan N, Mandelboim O, Eberl G, Di Santo JP: **Microbial flora drives interleukin 22 production in intestinal NKp46$^+$ cells that provide innate mucosal immune defense.** *Immunity* 2008, **29:**958-70.
25. Cella M, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JK, Doherty JM, Mills JC, Colonna M: **A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity.** *Nature* 2009, **457:**722-5.

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26. Ouyang W, Kolls JK, Zheng Y: **The biological functions of T helper 17 cell effector cytokines in inflammation.** *Immunity* 2008, **28:**454-67.
27. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA: **Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease.** *Immunity* 2008, **29:**947-57.

F1000 Factor 3.0 *Recommended*
Evaluated by Rachel Caspi 18 Mar 2009

28. Cupedo T, Crellin NK, Papazian N, Rombouts EJ, Weijer K, Grogan JL, Fibbe WE, Cornelissen JJ, Spits H: **Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC(+) CD127(+) natural killer-like cells.** *Nat Immunol* 2009, **10:**66-74.

F1000 Factor 6.0 *Must Read*
Evaluated by Lars Rogge 21 Jan 2009

29. Ivanov II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR: **Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine.** *Cell Host Microbe* 2008, **4:**337-49.

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