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A ‘natural’ way to provide innate mucosal immunity

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Summary

The mucosal barrier comprises a layered defense system including physio-chemical and immunological strategies to contain commensal microflora while protecting the host against potential pathogens. In contrast to the clearly established and well-characterized role for the adaptive immune system in intestinal defense, our knowledge on innate immune mechanisms that operate in the gut is much less defined. The recent identification of novel innate lymphoid cells (ILC), including ‘NK-like’ cells that naturally produce IL-22 and appear to play a role in intestinal defense, demonstrate an unexpected and increasing complexity in mucosal innate immunity.

Introduction

Homeostasis between the intestinal microbiota and the immune system is required for efficient energy and metabolite extraction from food, protection from pathogenic microbes, degradation of xenobiotics and maintenance of a competent epithelial barrier [1,2]. This equilibrium necessitates a constant dialogue mediated by pattern recognition receptors (PRRs) on epithelial and immune cells and pathogen-associated molecular patterns expressed by a complex ($\sim 10^{14}$ bacteria and 10^3 species) microbiota [3,4]. The intestinal immune system is a dynamic structure that comprises of a vast network of lymphoid tissues, lymphoid cells and dendritic cells (DCs) within the epithelium and lamina propria (LP). Lymphocytes reinforce the mucosal barrier via IgA production and epithelial cell activation resulting in production of mucus and anti-bacterial peptides. In the LP, DCs capture microbial antigens and drive pro-inflammatory Th17 cells and regulatory T cell responses [5,6]. Distinct bacterial taxa induce the development of lymphoid tissues [7], while Th17 and Treg cell differentiation shape the bacterial community demonstrating the reciprocal impact of the intestinal immune system on symbiotic microbiota [8,9,10].

In addition to the aforementioned adaptive immune component, innate lymphocytes including natural killer (NK) cells [11,12] and mucosal-associated invariant T (MAIT) cells [13] are also found in the intestine. While the function of MAIT cells has not yet been fully defined [14], it is known that NK cells can rapidly detect and then destroy stressed, transformed or infected target cells [15]. As NK cells can be found in lymphoid and non-lymphoid tissues and circulate in the blood and lymph [16], they may have a role in systemic immunosurveillance against various pathogens and tumors. NK cells are also capable of prompt secretion of several types of cytokines (including IFN- γ and TNF- α) that play an important role in the activation of DC and macrophages, thereby increasing their antigen-processing and anti-microbial activity, respectively. Chemokine secretion by NK cells can

influence inflammatory responses and immune defense through recruitment of hematopoietic effector cells [17].

The aforementioned biological functions of NK cells fit squarely within the realm of ‘T-helper 1-like’ (pro-inflammatory) activities of the immune response, supporting the long-held view that NK cells represent a homogeneous cohort of Th1-polarized innate lymphocytes. Still, evidence has accumulated suggesting that NK cells may exhibit phenotypic and functional ‘diversity’ [18,19] that operates at the level of cell activation (attributes of resting, primed and chronically stimulated NK cells differ; [20,21,22-24] as well as at the level of different tissue environments. NK cells that are found in the thymus, lymph node, liver, pancreas and uterus have markedly different cell surface phenotypes and biological functions [25-27,28,29]. Thus tissue microenvironments may condition the ultimate biological effector program of NK cell subsets.

A family of natural cytotoxicity receptors (NCR) has been characterized that appear to play important roles in NK cell target recognition [30]. NKp46 (encoded at the *Ncr1* locus in mice) is highly conserved NCR in mammals and has been proposed as an NK cell-specific marker [31]. The analysis of NKp46⁺ cells in the intestinal mucosa demonstrated that NKp46 also delineates a subset of innate lymphoid cells (ILC) that differ from classical NK cells. This review will update our current knowledge on IL-22-producing NKp46⁺ cells as well as other novel ‘natural’ immune defense pathways that operate at mucosal surfaces.

NKp46 identifies a novel subset of ‘non-NK’ cells in the murine intestine: NCR22 cells

Whereas NK cells have been documented in the intestinal mucosa [11,12], their developmental pathways and biological roles are not fully understood. NK cells, by virtue of their rapid cytokine response, might play an important role in intestinal immunity by interfacing with intestinal DC to regulate immune responses. Alternatively, NK cells may

eliminate stressed or infected cells within the intestinal lamina propria (LP) or epithelium. In this way, intestinal NK cells could contribute to the maintenance of epithelial homeostasis.

Recently, several independent groups have characterized human and murine mucosal lymphocytes expressing natural cytotoxicity receptor family members [32,33,34,35,36]. In the mouse, NKp46 had been previously shown to be highly and specifically expressed in immature and mature NK cells in the mouse [31,37] and ‘knock-in’ mice bearing a GFP reporter at the *Ncr1* locus [38] were used in one study to identify these cells in the gut [32]. Unlike splenic NK cells that were relatively homogeneous in NK1.1 expression, intestinal NKp46⁺ cells clearly comprised distinct subsets, including a large population of CD127⁺NK1.1⁻ cells that lacked many of the markers of mature NK cells (Ly49 family members, CD11b, CD27, NKG2D). Moreover, this subset of NKp46⁺ cells in the intestine lacked perforin, natural cytotoxicity and did not transcribe IFN- γ , and thus bore little functional resemblance to classical NK cells found in the bone marrow or spleen [32,35,36]. In contrast, these unusual gut NKp46⁺ cells expressed the nuclear hormone receptor retinoic acid receptor-related orphan receptor gamma t (ROR γ t, encoded at the *Rorc* locus) that plays an essential role in the development of Lymphoid Tissue-inducer (LTi) cells [39] and in the differentiation of Th17 cells [5]. Further studies demonstrated that intestinal NKp46⁺ cells produced substantial amounts of interleukin (IL)-22, but little IL-17A [32,33,35], thereby distinguishing them from Th17 T cell subsets [5,6,40,41]. Nevertheless, IL-22⁺NKp46⁺ cells required *Rorc* for their development [32,35,36] and their homeostasis was dependent on the presence of microbial flora, as germ-free mice showed a strong decrease in the absolute numbers of NKp46⁺ cells that express *Rorc* and *Il22* [32,35]. While commensal microbe segmented filamentous bacterium (SFB) induces intestinal Th17 T cells [8,9], its role in regulating innate IL-22 production in the gut is unknown. In contrast, development of NK1.1⁺ NKp46⁺ intestinal cells was unaffected by

Rorc deficiency and these cells developed normally in the absence of microbial flora. Taken together, these observations identify a novel subset of intestinal mucosal NKp46⁺ cells that can be clearly distinguished from ‘classical’ NK cells in terms of phenotype, function, transcription factor dependency and interactions with microbial flora (Figure 1).

Studies by the Colonna [●●33] and Spits [●●34] laboratories using human fetal and adult lymphoid tissue identified novel innate lymphocyte subsets characterized by CD127 (IL-7R α), CD56 and NKp44 expression with strong IL-22 production in response to IL-23. ‘NK22’ cells in humans [●●33], like their murine counterparts, express *Rorc* [●●33,●●34], and the aryl hydrocarbon receptor (*Ahr*) shown to be critical in the regulation of IL-22 production [42]. As the mouse intestinal cells express NKp46 and show robust production of IL-22 following IL-23 stimulation [43], we have designated these as ‘NCR22’ cells [●●44], a term that could also apply to the human counterparts. While a standard nomenclature for IL-22-producing innate cells has yet to be defined, we would caution the use of ‘NK’ for these cells that thus far have not been shown to exert natural killing.

Soon after their discovery, the question whether IL-22-producing NKp46⁺ cells were more related (developmentally and functionally) to NK cells or to LTi cells was raised [45,46]. In the next sections, we will examine recent data that impacts on this debate as well as outstanding issues in NCR22 biology that are the subject of ongoing investigation.

Relationship of NCR22 cells to classical NK cells

In addition to the phenotypic and functional differences between NK cells and NCR22 cells, several aspects of the developmental process suggested that these two cell types were distinct (Figure 1). Unlike intestinal NK1.1⁺ cells, NCR22 cells were unaffected by the absence of IL-15 signaling [●●32,●●35]. Still, since immature NK cell precursors are also IL-15-independent [47] and NCR22 have attributes of immature NK cells, it was possible that

NCR22 cells expressing *Rorc* might represent precursors to more mature classical NK cells. Moreover, previous studies demonstrated that fetal *Rorc*⁺ LTi cells could develop into lytic NK1.1⁺ NK cells following culture *in vitro* [48], while immature NK cells in the secondary lymphoid tissue of humans and mice were shown to express *Rorc* [49,50].

A recent series of papers addressed the developmental relationship of human and mouse *Rorc*-expressing NKp46⁺ cells to classical NK cells [●●44,●●51]. Our group showed that NCR22 cells shared a developmental relationship with NK cells and LTi cells by expressing and requiring the transcriptional repressor Id2 for normal development and homeostasis [●●44,52]. We further demonstrated that IL-7 was critically required for NCR22 development. Finally, we used a fate-mapping approach to follow the destiny of *Rorc*-expressing cells and found that the vast majority of classical NK cells (in the bone marrow, liver, lymph node, spleen and intestine) were not descendants of *Rorc*⁺ precursors [●●44]. In an independent study from the Spits' laboratory [●●51], human lineage-negative *RORC*⁺ precursors in fetal and adult lymphoid tissues were shown to acquire CD56 and NCR expression, but failed to develop functional characteristics of mature NK cells (these cells remained IL-22⁺ and lacked perforin and granzyme B expression). Collectively, these studies clearly exclude a major role for *Rorc*-expressing hematopoietic precursors as developmental intermediates in the differentiation of classical NK cells, and reinforce the notion that NCR22 and NK cells derive from distinct developmental pathways. Along these lines, it has been recently shown that the transcription factor E4bp4/Nfil3 is a critical determinant of NK cell development [●●53,●●54] and might provide a 'signature' to dissect classical NK cell differentiation [55]. While E4bp4/Nfil3-deficient mice do not appear to have defects in formation of secondary lymphoid tissue (suggesting normal LTi function), it is not yet reported whether NCR22 differentiation in these mice is affected.

Relationship of NCR22 cells to Lymphoid Tissue inducer (LTi) cells

LTi cells are classically defined as CD3⁻CD4⁺ hematopoietic cells that promote the formation of lymphoid tissues (including LN, PP and intestinal isolated lymphoid follicle, ILF) via a cross-talk with stromal cells that results in the recruitment of B and T lymphocytes to functionally distinct zones [56]. Several signals have been identified that are critical to this process. LTi cells from *Rorc*-deficient mice are not generated and stromal cells in lymphoid tissue anlagen from *Rorc*^{-/-} mice fail to express VCAM and ICAM resulting in abortive lymphoid tissue genesis [39]. LTi cells express CD127 and IL-7 is required for the activation of LTi cells to express membrane-bound lymphotoxin LT α 1 β 2 heterotrimer that activates stromal cells through the LT β R [57]. Chemokine/chemokine receptor interactions (operating through CCR7 and CXCR5) also play a critical role in LTi localization and subsequent lymphoid tissue development [57,58].

While LTi and intestinal NCR22 cells share *Rorc* and *Il7* dependency for their development (Figure 1), the chemokine/chemokine receptor interactions that dictate tissue localization of NCR22 cells are not yet identified. Moreover, there has been some debate about where NCR22 cells localize as two groups identified these cells in cryptopatches (CP) using NKp46 antibodies [35,36], while another group found Ncr1-GFP expressing cells in the LP and in ILFs, but not in CP [32]. One possible explanation might involve the distinction between CP and ILF (that represent a continuum) [59]. However, their localization in the LP might be more consistent with their role in IL-22 production to stimulate epithelial function. Nevertheless, LT β R signals appear dispensable for the development of NCR22 cells (Satoh-Takayama, unpublished), whereas these signals are critical for lymphoid tissue organogenesis suggesting that CP/ILF structures are not obligatory for NCR22 differentiation.

Do LTi and NCR22 cells subserve similar biological functions? Like NCR22 cells, both human and mouse LTi cells have been shown to express IL-22 and also IL-17

[●●34,58,●60]. Nevertheless, IL-17 and IL-22 are not required for the development of lymphoid tissues, suggesting other roles for IL-17/IL-22-producing LTi and NCR22 cells, including inflammation and immunity to infection (see below). The analysis of lymphoid tissue induction *in vivo* is technically challenging, although elegant studies have shown *bona fide* lymphoid tissue organogenesis following transfer of purified LTi cells [61]. *In vitro* surrogates of this process include assays that measure induction of VCAM and ICAM expression on stromal cells using co-culture systems. While human CD56⁺IL-22⁺ cells show activity in this assay [●●34,●●51], it remains to be shown that murine NCR22 cells have LTi-like activity *in vitro* or *in vivo*. In the absence of demonstrable LTi activity, the use of the term ‘NKR-LTi’ [●●35] could be cautioned. Furthermore, since only a fraction of LTi or NCR22 cells produce IL-22 after stimulation, it is not clear whether IL-22 production and LTi-like activity are exerted by the same cell or by functionally distinct subsets within LTi and NCR22 cell populations being analyzed. Defining the functional relationship of IL-22 producing NKp46⁺ cells to classical LTi cells remains an area of further investigation.

Roles for NCR22 cells in intestinal immune defense and tissue homeostasis

IL-22 was initially identified as a member of the IL-10 family and is expressed by Th17 and Th22 T cells, NK cells, NKT cells and $\gamma\delta$ T cells subsets [62]. IL-22 exhibits both anti-inflammatory as well as pro-inflammatory properties, depending on the tissue context [63]. A protective role for IL-22 in immune defense has been demonstrated at mucosal surfaces using lung and intestinal infection models [●●64,●●65]. While the cellular source of mucosal IL-22 remains a matter of debate, IL-22 production is retained in Rag-deficient mice consistent with an innate immune cell origin [●●64]. Considering the prompt production of IL-22 by NCR22 cells, we hypothesized that intestinal NCR22 cells were involved in immune defense against the pathogen *Citrobacter rodentium*. Using Rag-deficient mice lacking the γ_c

chain, we found that the complete absence of NCR22 cells was correlated with accentuated susceptibility to *C. rodentium* [●●32]. In contrast, mice lacking classical NK cells showed partial susceptibility [●●33,43] consistent with a minor role for NK1.1⁺ cells in defense against this pathogen. Surprisingly, Ncr1-deficient mice showed no increased susceptibility to *C. rodentium* [43] suggesting that Ncr1 ligand-driven signals (if present) are not essential for the anti-microbial response. In a separate set of studies, the Flavell lab demonstrated that intestinal IL-22 production from innate cells also was required for immune protection in the dextran sulfate-induced (DSS) colitis model [●●66]. In both *C. rodentium* and DSS models, increased IL-23 production (likely via PRR-triggered DCs) was upstream of the enhanced IL-22 production. The likely cellular target of IL-22 was the intestinal epithelium, since IL-22 was shown to stimulate epithelial cells to promote secretion of anti-microbial proteins (β -defensins, RegIII family members and lipocalin 2) that reinforce mucosal barrier function [●●64,●●65]. Accordingly, RegIII β and RegIII γ transcripts are strongly reduced in epithelial cells from mice lacking intestinal NKp46⁺ cells [●●32,●●35], and while these mice still maintain the capacity to restrict entry of commensal microflora, their susceptibility to pathogenic micro-organisms is accentuated. In this way, intestinal NKp46⁺ cells provide a form of ‘pre-emptive’ immune defense that operates to strengthen the epithelial barrier.

Since IL-22 has also been shown to have pro-inflammatory properties [63], it is possible that NCR22 cells may be involved in intestinal pathologies under certain conditions. Infection by *Toxoplasma gondii* in mice causes an ileitis that is associated with increased local IL-22 production [●67]. While CD4⁺ T cells appear to be the major IL-22 producers in this context, the residual IL-22 production in Rag-deficient mice infected with *T. gondii* suggests additional potential pathological roles for NCR22 cells or LTi cells. Recently, innate IL-23-responsive colitis-promoting cells that appear hardwired for IL-17 production have been identified [●●68]. These *Rorc*-dependent cells appear functionally distinct from NCR22

cells, but share several phenotypic (CD127, CD90) and developmental characteristics (Figure 1). Whether these innate cells represent unique lineages or differentiation states within a single innate cell lineage is unknown.

Roles for NCR22 or NCR22-like cells at other tissue sites

These observations suggest that an intestinal ‘niche’ conditions the differentiation of diverse NKp46⁺ cell subsets that are important for mucosal immunity. An obvious next question is whether NKp46⁺ cells are present at other mucosal sites or in other tissues under steady-state conditions (where they might be involved in tissue homeostasis) or recruited to these sites following infection or inflammation. Sites where IL-22 has been shown to play an important role in immune defense or in inflammation (such as liver, skin, and lung) could be considered. Increased epithelial turnover that is a hallmark of certain skin disorders, including psoriasis [69] may be driven by local hyper-secretion of IL-22. While NCR22 cells are poorly represented in normal skin [●●36], inflammation may result in recruitment of NCR22 cells. Subsequent IL-23 stimulation could then lead to increased IL-22 production and exacerbation of epithelial pathology. As IL-22 polymorphisms have been associated with susceptibility to certain types of intestinal cancer [70], it is interesting to consider the role for NCR22 cells in the initiation and progression of intestinal tumors.

Conclusions

The identification of NCR22 cells that are ‘naturally’ programmed for IL-22 production identifies a novel cellular immune defense mechanism in the gut. In addition to classical NK cells (IFN- γ ⁺), LTi cells (IL-17⁺/IL-22⁺) and Thy1⁺ cells (IL-17⁺/IFN- γ ⁺) [●●68], NCR22 constitute a novel emerging family of innate lymphoid cells (ILC) that have prompt cytokine production capacity and unique roles in immune defense, especially at mucosal

surfaces. Very recently, two additional ILCs were identified in mice: IL-13-producing cells in ‘fat associated lymphoid clusters’ (FALC), and IL-13 and IL-5-producing ‘nuocytes’ (both of these cell types are implicated in immune protection against *Nippostrongylus brasiliensis*) [71,72]. Collectively, these novel ILC demonstrate a remarkable range of functional capacities (Figure 1) that resemble that of differentiated T cell subsets. Understanding the mechanisms governing innate diversity may provide evidence for an evolutionary relationship to those operating in T cells.

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References

1. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. **Host-bacterial mutualism in the human intestine.** *Science* 2005, **307**:1915-1920.
2. Round JL, Mazmanian SK. **The gut microbiota shapes intestinal immune responses during health and disease.** *Nat Rev Immunol* 2009, **9**:313-323.
3. Akira S, Uematsu S, Takeuchi O. **Pathogen recognition and innate immunity.** *Cell* 2006, **124**:783-801.
4. Sansonetti PJ. **War and peace at mucosal surfaces.** *Nat Rev Immunol* 2004, **4**:953-964.
5. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. **The orphan nuclear receptor ROR γ t directs the differentiation program of proinflammatory IL-17⁺ T helper cells.** *Cell* 2006, **126**:1121-1133.
6. Lochner M, Peduto L, Cherrier M, Sawa S, Langa F, Varona R, Riethmacher D, Sitahar M, Di Santo JP, Eberl G. **In vivo equilibrium of proinflammatory IL-17⁺ and regulatory IL-10⁺ Foxp3⁺ ROR γ t⁺ T cells.** *J Exp Med* 2008, **205**:1381-1393.
- 7. Bouskra D, Brézillon C, Bérard M, Werts C, Varona R, Boneca IG, Eberl G. **Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis.** *Nature* 2008, **456**:507-510.

Demonstration that peptidoglycan from gram-negative commensals play an important role in regulating isolated lymphoid follicle (ILF) formation in the gut.

- 8. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, et al. **Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria.** *Cell* 2009, **139**:485-98.

A single commensal microbe – segmented filamentous bacterium – is shown to be

sufficient in coordinating the differentiation of Th17 cells in the intestine (see also ●●9).

●●9. Gaboriau-Routhiau V, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, et al. **The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses.** *Immunity* 2009, **31**:677-689.

See annotation ●●8.

●●10. Mazmanian SK, Round JL, Kasper DL. **A microbial symbiosis factor prevents intestinal inflammatory disease.** *Nature* 2008, **453**:620-625.

Polysaccharide A from the commensal *Bacteroides fragilis* regulates inflammation induced by the pathogen *Helicobacter hepaticus*.

11. Leon F, Roldan E, Sanchez L, Camarero C, Bootello A, Roy G. **Human small-intestinal epithelium contains functional natural killer lymphocytes.** *Gastroenterology* 2003, **125**:345-356.

12. 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**:1785-1796.

13. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, Affaticati P, Gilfillan S, Lantz O. **Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1.** *Nature* 2003, **422**:164-9.

14. Treiner E, Lantz O. **CD1d- and MR1-restricted invariant T cells : of mice and man.** *Curr Opin Immunol* 2006, **18**:519-26.

15. Di Santo JP. **Natural killer cell developmental pathways: a question of balance.** *Annu Rev Immunol* 2006, **24**:257-86.

16. Grégoire C, Chasson L, Luci C, Tomasello E, Geissmann F, Vivier E, Walzer T. **The trafficking of natural killer cells.** *Immunol Rev* 2007, **220**:169-82.

17. Lodoen MB, Lanier LL. **Natural killer cells as an initial defense against pathogens.** *Curr Opin Immunol* 2006, **18**:391-8.

18. Di Santo JP. **Natural killer cells: diversity in search of a niche.** *Nat Immunol* 2008, **9**:473-5.

19. Di Santo JP. **Functionally distinct NK-cell subsets: developmental origins and biological implications.** *Eur J Immunol* 2008, **38**:2948-51.

●20. Maroof A, Beattie L, Zubairi S, Svensson M, Stager S, Kaye PM. **Posttranscriptional regulation of II10 gene expression allows natural killer cells to express immunoregulatory function.** *Immunity* 2008, **29**:295-305.

Demonstration that chronic stimulation of NK cells can lead to IL-10 production that provides immunoregulatory function during infection (see also ●●21).

●21. Lee SH, Kim KS, Fodil-Cornu N, Vidal SM, Biron CA. **Activating receptors promote NK cell expansion for maintenance, IL-10 production, and CD8 T cell regulation during viral infection.** *J Exp Med* 2009, **206**:2235-51.

See annotation ●●20.

22. Vosshenrich CA, Lesjean-Pottier S, Hasan M, Richard-Le Goff O, Corcuff E, Mandelboim O, Di Santo JP. **CD11cIb220+ interferon-producing killer dendritic cells are activated natural killer cells.** *J Exp Med* 2007, **204**:2569-78.

23. Lucas M, Schachterle W, Oberle K, Aichele P, Diefenbach A. **Dendritic cells prime natural killer cells by trans-presenting interleukin 15.** *Immunity* 2007, **26**:503-17.

24. Huntington ND, Tabarias H, Fairfax K, Brady J, Hayakawa Y, Degli-Esposti MA, Smyth MJ, Tarlinton DM, Nutt SL. **NK cell maturation and peripheral homeostasis is associated with KLRG1 up-regulation.** *J Immunol* 2007, **178**:4764-70.

25. Vosshenrich CA, García-Ojeda ME, Samson-Villéger SI, Pasqualetto V, Enault L, Richard-Le Goff O, Corcuff E, Guy-Grand D, Rocha B, Cumano A, et al. **A thymic pathway**

of mouse natural killer cell development characterized by expression of GATA-3 and CD127. *Nat Immunol* 2006, **7**:1217-24.

26. 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* 2003, **172**:1455-X.

27. Takeda K, Cretney E, Hayakawa Y, Ota T, Akiba H, Ogasawara K, Yagita H, Kinoshita K, Okumura K, Smyth MJ. **TRAIL identifies immature natural killer cells in newborn mice and adult mouse liver.** *Blood* 2005, **105**:2082-9.

•28. Brauner H, Elemans M, Lemos S, Broberger C, Holmberg D, Flodström-Tullberg M, Kärre K, Höglund P. **Distinct phenotype and function of NK cells in the pancreas of nonobese diabetic mice.** *J Immunol* 2010, **184**:2272-80.

Additional evidence for organ-specific diversification of NK cell phenotype and functions (see also ••29).

•29. Yadi H, Burke S, Madeja Z, Hemberger M, Moffett A, Colucci F. **Unique receptor repertoire in mouse uterine NK cells.** *J Immunol* 2008, **181**:6140-7.

See annotation ••29.

30. Moretta L, Moretta A. **Unravelling natural killer cell function: triggering and inhibitory human NK receptors.** *EMBO J* 2004, **23**:255-9.

31. Walzer T, Bléry M, Chaix J, Fuseri N, Chasson L, Robbins SH, Jaeger S, André P, Gauthier L, Daniel L, et al. **Identification, activation, and selective in vivo ablation of mouse NK cells via NKp46.** *Proc Natl Acad Sci U S A* 2007, **104**:3384-9.

••32. Satoh-Takayama N, Vosshenrich CA, Lesjean-Pottier S, Sawa S, Lochner M, Rattis F, Mention JJ, Thiam K, Cerf-Bensussan N, Mandelboim O et al. **Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense.** *Immunity* 2008, **29**:958-70.

Characterization of a novel innate IL-22 producing cell in the gut that expressed the natural cytotoxicity receptor NKp46. Unlike classical NK cells, the NKp46⁺IL-22⁺ cells require the transcription factor *Rorc* for their development. (see also ●●35,●●36)

●●33. 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.

Discovery of NKp44⁺ IL-22 producing human ‘NK-like’ cells that are enriched in mucosal lymphoid tissue and ‘cross-talk’ with epithelial cells.

●●34. 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.

Discovery of IL-22 and IL-17-producing innate LTi-like cells in fetal and adult lymphoid tissues that can give rise to NK-like IL-22-producing cells.

●●35. 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.

See annotation ●●32.

●●36. Luci C, Reynders A, Ivanov II, Cognet C, Chiche L, Chasson L, Hardwigsen J, Anguiano E, Banchereau J, Chaussabel D et al. **Influence of the transcription factor RORgammat on the development of NKp46⁺ cell populations in gut and skin.** *Nat Immunol* 2009, **10**:75-82.

See annotation ●●32.

37. Biassoni R, Pessino A, Bottino C, Pende D, Moretta L, Moretta A. **The murine homologue of the human NKp46, a triggering receptor involved in the induction of**

natural cytotoxicity. *Eur J Immunol* 1999, **29**:1014-1020.

38. Gazit R, Gruda R, Elboim M, Arnon TI, Katz G, Achdout H, Hanna J, Qimron U, Landau G, et al. **Lethal influenza infection in the absence of the natural killer cell receptor gene *Ncr1*.** *Nat Immunol* 2006, **7**:517-23.

39. 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.

40. Michel ML, Keller AC, Paget C, Fujio M, Trottein F, Savage PB, Wong CH, Schneider E, Dy M, Leite-de-Moraes MC. **Identification of an IL-17-producing NK1.1(neg) iNKT cell population involved in airway neutrophilia.** *J Exp Med* 2007, **204**:995-1001.

41. Michel ML, Mendes-da-Cruz D, Keller AC, Lochner M, Schneider E, Dy M, Eberl G, Leite-de-Moraes MC. **Critical role of ROR-gammat in a new thymic pathway leading to IL-17-producing invariant NKT cell differentiation.** *Proc Natl Acad Sci USA* 2008, **105**:19845-50.

42. Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B. **The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins.** *Nature* 2008, **453**:106-9.

43. Satoh-Takayama N, Dumoutier L, Lesjean-Pottier S, Ribeiro VS, Mandelboim O, Renauld JC, Vosshenrich CA, Di Santo JP. **The natural cytotoxicity receptor NKp46 is dispensable for IL-22-mediated innate intestinal immune defense against *Citrobacter rodentium*.** *J Immunol* 2009, **183**:6579-87.

●●44. Satoh-Takayama N, Lesjean-Pottier S, Vieira P, Sawa S, Eberl G, Vosshenrich CA, Di Santo JP. **IL-7 and IL-15 independently program the differentiation of intestinal CD3-NKp46+ cell subsets from Id2-dependent precursors.** *J Exp Med* 2010, **207**:273-80.

Evidence that mouse NK cells and IL-22-producing NKp46⁺ cells (here designated NCR22 cells) represent two distinct lineages of Id2-dependent innate lymphoid cells.

45. Colonna M. **Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity.** *Immunity* 2009, **31**:15-23.
46. Malmberg KJ, Ljunggren HG. **Spotlight on IL-22-producing NK cell receptor-expressing mucosal lymphocytes.** *Nat Immunol* 2009, **10**:11-12.
47. Vosshenrich CA, Ranson T, Samson SI, Corcuff E, Colucci F, Rosmaraki EE, Di Santo JP. **Roles for common cytokine receptor gamma-chain-dependent cytokines in the generation, differentiation, and maturation of NK cell precursors and peripheral NK cells in vivo.** *J Immunol* 2005, **174**:1213-21.
48. Mebius RE, Rennert P, Weissman IL. **Developing lymph nodes collect CD4⁺CD3⁻LTbeta⁺ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells.** *Immunity* 1997, **7**:493-504.
49. Chiossone L, Chaix J, Fuseri N, Roth C, Vivier E, Walzer T. **Maturation of mouse NK cells is a 4-stage developmental program.** *Blood* 2009, **113**:5488-96.
50. Hughes T, Becknell B, McClory S, Briercheck E, Freud AG, Zhang X, Mao H, Nuovo G, Yu J et al. **Stage 3 immature human natural killer cells found in secondary lymphoid tissue constitutively and selectively express the TH 17 cytokine interleukin-22.** *Blood* 2009, **113**:4008-10.
- 51. Crellin NK, Trifari S, Kaplan CD, Cupedo T, Spits H. **Human NKp44+IL-22+ cells and LTi-like cells constitute a stable RORC⁺ lineage distinct from conventional natural killer cells.** *J Exp Med* 2010, **207**:281-90.

Evidence that human NK cells and IL-22-producing NKp46⁺ cells represent two distinct lineages of innate lymphoid cells.

52. 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**:702-706.

••53. Gascoyne DM, Long E, Veiga-Fernandes H, de Boer J, Williams O, Seddon B, Coles M, Kioussis D, Brady HJ. **The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development.** *Nat Immunol* 2009, **10**:1118-24.

Evidence that the transcription factor Nfil3/E4bp4 is a critical determinant of ‘classical’ NK cell development. (see also ••54).

••54. Kamizono S, Duncan GS, Seidel MG, Morimoto A, Hamada K, Grosveld G, Akashi K, Lind EF, Haight JP, Ohashi PS, et al. **Nfil3/E4bp4 is required for the development and maturation of NK cells in vivo.** *J Exp Med* 2009, **20**/2977-86.

See annotation ••53.

55. Di Santo, J.P. **A defining factor for natural killer cell development.** *Nat Immunol* 2009, **10**:1051-2.

56. Mebius RE. **Organogenesis of lymphoid tissues.** *Nat Rev Immunol* 2003, **3**:292-303.

57. Luther SA, Ansel KM, Cyster JG. **Overlapping roles of CXCL13, interleukin 7 receptor alpha, and CCR7 ligands in lymph node development.** *J Exp Med* 2003, **197**:1191-8.

58. Marchesi F, Martin AP, Thirunarayanan N, Devany E, Mayer L, Grisotto MG, Furtado GC, Lira SA. **CXCL13 expression in the gut promotes accumulation of IL-22-producing lymphoid tissue-inducer cells, and formation of isolated lymphoid follicles.** *Mucosal Immunol* 2009, **2**:486-94.

59. Eberl G, Sawa S. **Opening the crypt: current facts and hypotheses on the function of cryptopatches.** *Trends Immunol* 2010, **31**:50-5.

•60. 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.

Evidence that peripheral splenic LT α -like cells (CD3⁻CD4⁺) in adult lymphoid tissues provide an innate source of IL-17 and IL-22.

61. Tsuji M, Suzuki K, Kitamura H, Maruya M, Kinoshita K, Ivanov II, Itoh K, Littman DR, Fagarasan S. **Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut.** *Immunity* 2008, **29**:261-71.

62. Nagem RA, Ferreira Júnior JR, Dumoutier L, Renauld JC, Polikarpov I. **Interleukin-22 and its crystal structure.** *Vitam Horm* 2006, **74**:77-103.

63. Sanjabi S, Zenewicz LA, Kamanaka M, Flavell RA. **Anti-inflammatory and pro-inflammatory roles of TGF-beta, IL-10, and IL-22 in immunity and autoimmunity.** *Curr Opin Pharmacol.* 2009, **9**:447-53.

••64. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, Abbas AR, Modrusan Z, Ghilardi N, de Sauvage FJ, et al. **Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens.** *Nat Med* 2008, **14**:282-9.

Demonstration that IL-22 plays an essential protective role in innate host defense in the intestine and lung using gram-negative infection models in mice. (see also ••65).

••65. Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, Reinhart TA, McAllister F, Edeal J, Gaus K, et al. **IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia.** *Nat Med* 2008, **14**:275-81.

See annotation ••64.

••66. 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.

Using the dextran-sulfate colitis model, discovery of innate IL-22 cell source that

plays a critical role in host response via reinforcing the intestinal barrier.

•67. Muñoz M, Heimesaat MM, Danker K, Struck D, Lohmann U, Plickert R, Bereswill S, Fischer A, Dunay IR, Wolk K, et al. **Interleukin (IL)-23 mediates *Toxoplasma gondii*-induced immunopathology in the gut via matrixmetalloproteinase-2 and IL-22 but independent of IL-17.** *J Exp Med* 2009, **206**:3047-59.

A pathological role for IL-22 in the gut following infection with *Toxoplasma gondii*.

••68. Buonocore S, Ahern PP, Uhlig HH, Ivanov II, Littman DR, Maloy KJ, Powrie F. **Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology.** *Nature* 2010 (in press).

A novel Thy1⁺ ILC that promotes intestinal pathology through IL-17 and IFN- γ production following pathogen encounter.

69. Fitch E, Harper E, Skorcheva I, Kurtz SE, Blauvelt A. **Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines.** *Curr Rheumatol Rep* 2007, **9**:461-7.

70. Thompson CL, Plummer SJ, Tucker TC, Casey G, Li L. **Interleukin-22 genetic polymorphisms and risk of colon cancer.** *Cancer Causes Control*. 2010 (in press).

••71. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, Furusawa J, Ohtani M, Fujii H, Koyasu S. **Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)/Sca-1(+) lymphoid cells.** *Nature* 2010, **463**:540-4.

A novel ILC producing ‘Th2-like’ cytokines (IL-5, IL-13) and critical in the innate immune response against intestinal parasites. (see also ••72).

••72. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, Bucks C, Kane CM, Fallon PG, Pannell R, et al. **Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity.** *Nature* 2010 (in press).

See annotation ••71.

Figure 1. **Diversity of Innate Lymphoid Cells.** Several distinct innate lymphoid cells (ILC) have been recently identified that have diverse roles in innate immunity. NK cells were the first described ILC, followed by IL-22-producing NKp46+ cells (NCR22) and lymphoid tissue inducer (LTi) cells. ILC development relies on distinct cytokine and transcription factors as indicated. NCR22 cells require interactions with commensal flora for their homeostasis. See main text for further details.

Diversity of Innate Lymphoid Cells

