Role of innate lymphocytes in infection and inflammation

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Innate lymphocytes exhibit various effector functions such as limiting the expansion of microorganisms including viruses, bacteria, and parasites; unlike T and B cells, innate lymphocytes act without antigen-specific receptors. In addition to two prototypic innate lymphocyte populations, namely classical natural killer (cNK) cells and lymphoid tissue inducer (LTI) cells, multiple types of innate lymphocytes have been identified during the last decade. These include thymic natural killer (NK) cells, NK receptor-positive LTI cells, LTI cells producing IL-17 and IL-22 and Th2-type innate lymphocytes such as natural helper (NH) cells and nuocytes. The transcription factor Id2 appears to be essential for the differentiation of all innate lymphocytes, suggesting the presence of a common progenitor population. Like helper T cell subsets in adaptive immune responses, NK cells, LTI cells, and Th2-type innate lymphocytes play distinct roles in innate immune responses by producing Th1, Th17, and Th2 cytokines, respectively (Figure 1). Cooperation between those innate lymphocytes and antigen-specific T and B cells is likely important in protective immunity against various microbes. Here we review the properties of various innate lymphocytes and their effector functions.

CONVENTIONAL NK CELLS
CD3+NKp46+ NK cells comprise one of the classical innate lymphocyte populations (Walzer et al., 2007). However, recent studies have revealed that these cells contain both CD127+ (IL-7Rα) and CD127− cells (Satoh-Takayama et al., 2008; Luci et al., 2009; Sanos et al., 2009; Table 1). CD127−NKp46+ cells are cNK cells, which produce interferon-γ (IFN-γ) and exhibit strong cytotoxicity (Trinchieri, 1989). cNK cells differentiate from lymphoid progenitor cells and the development of cNK cells requires the transcription factor E4BP4/NF-IL-3 (Gascoyne et al., 2009; Kaminzono et al., 2009) and the cytokine IL-15 (Kennedy et al., 2000; Cooper et al., 2001; Ranson et al., 2003; Vossenhenrich et al., 2005). cNK cells play an important role in innate immune responses against viral infection, and are particularly important in fighting herpes virus infections. cNK cells limit the propagation of viruses prior to the onset of adaptive immune responses associated with...
the expansion of virus-specific CD8\(^+\) cytotoxic T lymphocytes (Biron et al., 1999; Arase and Lanier, 2004). cNK cells recognize ligands that are induced on target cells by viral infection or cellular stresses using specific receptors such as CD94/NKG2D and exhibit cytotoxic activity via a variety of effector molecules such as perforin, granzyme, Fas-L, and TRAIL (Trinchieri, 1989; Biron et al., 1999; Arase and Lanier, 2004). NK cells differentiating in the thymus (thymic NK cells) are unique in that they express CD127 (IL-7R\(\alpha\)) and GATA3 unlike cNK cells. Thymic NK cells produce IFN-\(\gamma\) and GM-CSF at much higher levels than cNK cells and their differentiation depends on the cytokine IL-7 and the transcription factor GATA3 (Vosshenrich et al., 2006; Ribeiro et al., 2010).

**ROR\(^{g+}\) INNATE LYMPHOCYTES**

Lymphoid tissue inducer cells were originally defined as Lin\(^-\)CD4\(^+\)CD117\(^+\) (c-Kit\(^+\)) CD127\(^+\)CD25\(^+\)IL-2R\(\alpha\)) CD90(Thy-1\(^+\)) cells (Mebius et al., 2001) but it was later shown that CD4\(^-\) LTi cells are abundantly observed in newborn mice (Sawa et al., 2010). LTI cells originate from Lin\(^-\)CD4\(^-\)CD117\(^{int}\)Sca-1\(^+\)CD127\(^+\) lymphoid progenitor cells in the fetal liver (Mebius et al., 2001). Their differentiation is dependent on the transcription factors ROR\(^{g+}\) and Id2, and IL-7 regulates their survival and activity (Mebius et al., 1997; Yokota et al., 1999; Sun et al., 2000; Eberl et al., 2004; Meier et al., 2007). LTI cells are present in the anlagen of both lymph nodes and Peyer’s patches during fetal development (Mebius, 2003). Lymph nodes and Peyer’s patches are missing in the absence of ROR\(^{g+}\) (Kurebayashi et al., 2000; Sun et al., 2000). Adult CD4\(^+\) LTI cells promote the maturation of tertiary lymphoid tissues after birth and the restoration of secondary lymphoid tissues after viral infection (Scandella et al., 2008; Tsuji et al., 2008). It is intriguing that the differentiation of adult, but not fetal. LTI is dependent on Notch signaling (Possot et al., 2011).

Earlier studies have shown that a population of CD4\(^+\) LTI cells generates lytic NK1.1\(^+\) cells after cultivation with IL-2 (Mebius et al., 1997; Yoshida et al., 2001), suggesting that LTI cells are progenitors of NK cells. It was later reported that, in humans, “immature NK cells” expressing CD127 and ROR\(^{g+}\) develop into NK cells (Cupedo et al., 2009; Hughes et al., 2009). However, a recent report showed that tonsillar Lin\(^-\)CD117\(^+\)CD127\(^+\) cells maintain CD117 but do not acquire perforin or granzyme B expression after cultivation with irradiated PBMC, PHA, and IL-2 (Crellin et al., 2010a). On the other hand, tonsillar Lin\(^-\)CD117\(^+\)CD127\(^-\) cells lose CD117 expression and acquire perforin, granzyme B, and several NK receptors. It is possible that the immature NK cells described previously (Freud et al., 2006; Cupedo et al., 2009; Hughes et al., 2009) contained both CD127\(^+\) LTI and immature cNK cells. Recent fate mapping analysis in mouse confirmed that cNK cells develop independently of LTI cells (Satoh-Takayama et al., 2010; Vonarbourg et al., 2010). In addition, E4BP4/NFIL3 is required for the differentiation of mouse cNK cells but there

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| Characteristic | cNK | Thymic NK | NKR\(^{g+}\) LTI | NKR\(^{g-}\) LTI |
|---------------|-----|-----------|-----------------|-----------------|
| **SURFACE PHENOTYPE** |     |           |                 |                 |
| CD3           | –   | –         | –               | –               |
| NKp46         | +   | +         | +               | +               |
| CD90          | +   | +         | +               | +               |
| CD127         | –   | +         | +               | +               |
| Characteristic transcription factor | E4BP4 | GATA3 | ROR\(^{g+}\) | ROR\(^{g-}\) |
| Cytokines     | IFN\(\gamma\) | IFN\(\gamma\), GM-CSF | IL-17, IL-22 | IL-17, IL-22 |
| Localization  | All lymphoid tissues | Thymus | Intestine | Intestine |

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is currently no report of a deficiency of LTi-related cells in the absence of E4BP4/NFIL3.

Recent studies have shown the presence of Lin−CD127+ RORγ+ NKP46+ innate lymphocytes sharing the characteristics of both NK and L Ti cells (NKR+ L Ti cells, Table 1). Because NKR+ L Ti produce IL-22, these cells are sometimes called NK22 (Cella et al., 2009), NCR22 (Satoh-Takayama et al., 2010), or ILC22 (Spits and Di Santo, 2011). Although these cells express NKP46, they exhibit little cytotoxic activity. Most NKR+ L Ti cells are present in the intestine while NKR+ cNK cells are found in all lymphoid tissues (Reynders et al., 2011; Table 1). Approximately 90% of NKP46+ cells in the lamina propria of small intestine are NKR+ L Ti expressing CD127 and only 10% are cNK cells, both of which are absent in Id2−/− mice without affecting T cells (Satoh-Takayama et al., 2008). CD127+ NKP46+ cells are increased in IL-15 transgenic mice and CD127+ NKP46− cells are decreased in IL-7−/− mice while total NKP46+ cells were unchanged in both cases (Satoh-Takayama et al., 2008), indicating the dependence of cNK cells on IL-15 and NKR+ L Ti cells on IL-7.

NKR+ L Ti cells are characterized by the expression of RORγ and IL-22 and their differentiation is dependent on RORγ while RORγ is not required for the differentiation of cNK cells (Satoh-Takayama et al., 2008; Luci et al., 2009; Sanos et al., 2009). RORγ fate mapping analysis using rorc-Cre mice (Lochner et al., 2008), in which Cre recombinase is inserted into the rorc locus encoding RORγ, that were further crossed with loxP-flanked YFP Rosa26 (Rosa-YFP) mice (Srinivas et al., 2001) revealed that YFP+ cells that have previously expressed RORγ (herein RORγfm+ cells) were either L Ti cells (NKR− L Ti cells) or NKR+ L Ti cells and no cNK cells in the bone marrow, spleen, liver, or lymph nodes were RORγfm+ (Satoh-Takayama et al., 2010; Vonarbourg et al., 2010; Figure 2). RORγfm+ cells are composed of both NKP46+ and NKP46− populations and NKP46− RORγfm+ cells are identical to LTi cells. LTi cells express CD25 and CCR6 but NKR+ L Ti cells do not express CD25 or CCR6. NKR+ L Ti cells are CD4fm+, indicating that these cells are derived from CD4+ RORγ+ L Ti cells (Figure 2). Notably, RORγ− NKR+ L Ti cells produce IFNy as cNK cells (Satoh-Takayama et al., 2010; Vonarbourg et al., 2010; Table 1). RORγ− NKR+ L Ti cells and thymic NK cells share some characteristics but their cytokine production profiles are different (Table 1). In the human, the CD3− CD127+NKP44+ cell population expresses RORγ and is a counterpart of the NKR+ L Ti population in mouse (Cella et al., 2009; Cupedo et al., 2009; Crellin et al., 2010a).

Adult L Ti cells provide effector functions by producing IL-17 and IL-22 (Cupedo et al., 2009; Takatori et al., 2009). L Ti cells producing high levels of these cytokines are CD4− and are sometimes called ILC17 (Spits and Di Santo, 2011). Production of IL-17 and IL-22 is further enhanced in vitro upon stimulation with IL-23 (Cella et al., 2009; Buonocore et al., 2010). L Ti cells also produce IL-17 and IL-22 in vivo in response to zymosan (Takatori et al., 2009) or flagellin (Van Maele et al., 2010) but L Ti cells do not directly respond to microbe-derived substances. Other cells such as dendritic cells and macrophages likely mediate the effects of zymosan and flagellin.

NKR+ L Ti cells are mainly present in lamina propria of the intestinal mucosa and are important in innate immune responses against Citrobacter rodentium (Satoh-Takayama et al., 2008; Zheng et al., 2008; Luci et al., 2009; Sanos et al., 2009). IL-22 is an important cytokine in the activation and defense of epithelial cells (Zeneewicz et al., 2008; Wolk et al., 2010). Because IL-22 induces the production of antimicrobial peptides such as β-defensin psoriasin, calgranulin A, calgranulin B, RegIIIβ, and RegIIIγ by epithelial cells (Wolk et al., 2004, 2006; Zheng et al., 2008), IL-22 is important for protection against C. rodentium (Zheng et al., 2008; Sonnenberg et al., 2011), Klebsiella pneumoniae (Happel et al., 2005; Auja

![FIGURE 2](image-url)
et al., 2008), and Candida albicans (De Luca et al., 2010). Thus, NKR\(^+\)LTi cells play a major role in the innate immune responses against those microbes through the production of IL-22.

Lymphoid tissue inducer-related cells are also involved in the pathophysiology of some forms of inflammatory colitis (Buono-core et al., 2010; Vonarbourg et al., 2010). Inflammatory colitis induced by administration of anti-CD40 mAb is not induced in \(\gamma c^{-/-}\)rag2\(^{-/-}\) mice lacking all innate lymphocytes but adoptive transfer of NKR\(^+\)LTi cells restores the induction of colitis. Intriguingly, IL-23-responsive IFN-\(\gamma\)-producing innate lymphocytes were found in the lamina propria of inflammatory bowel disease patients (Takayama et al., 2010), implying the involvement of innate lymphocytes in human diseases. Intestinal flora does not affect the number of LTi cells but affects the function of LTi cells (Sawa et al., 2011). Expression of IL-22 but not IL-17 is higher in germ-free mice than in SPF mice and antibiotic treatment in SPF mice increased the expression of IL-22. IL-22 expression was higher in il25\(^{-/-}\)LTi cells and administration of IL-25 suppressed IL-22 expression in wild type LTi cells, suggesting that epithelial cell-derived IL-25 suppressed IL-22 production (Sawa et al., 2011). The effect of IL-25 is indirect because LTi cells do not express IL-25 receptors (IL-17RB). It is likely that IL-17RB\(^+\) dendritic cells suppress IL-22 production in a contact-dependent manner.

**Th2 Cytokine-Producing Innate Lymphocytes**

The third subset of innate lymphocytes identified most recently is the ROR\(\gamma\)-independent Lin\(^{-}\)CD90\(^+\)CD127\(^+\)GATA3\(^+\) innate lymphocyte subset, which is Id2- and IL-7-dependent (Figure 2). This population is capable of producing Th2 cytokines, most notably IL-5 and IL-13, but not IL-4. This Th2 cytokine-producing subset was first reported in 2010 in named NH cells (Morod et al., 2010), MPP\(\text{type}^2\) (Saenz et al., 2010), nuocytes (Neill et al., 2010), or innate helper type 2 (Ih2) cells (Price et al., 2010). Among these reports, NH cells were identified in naïve animals while all other cell types were observed upon cytokine administration or helminth infection in cytokine-GFP reporter mice. Although their relationships are currently unclear, NH cells, nuocytes, and Ih2 cells share similar characteristics (Table 2). MPP\(\text{type}^2\) cells seem to be of myeloid lineage. It remains to be determined how many cell types are present in this subset.

Th2 activity is important for anti-helminth immunity (McKenzie et al., 1998; Urban et al., 1998; Perrigoue et al., 2008; Maizels et al., 2009). In addition, Th2 reactions are involved in the pathophysiology of various allergic diseases including asthma (Grüng et al., 1998; Walter et al., 2001; Larché et al., 2003; Holgate and Polosa, 2008). Although the antigen-specific Th2 response plays a central role both in protective immunity against helminths and antigen-specific allergic responses, accumulating evidence indicates the involvement of innate immune cells in the early phase of Th2 responses.

Recent studies shed light on the role of epithelial cells in regulating Th2 responses. Epithelial cell-derived thymic stromal lymphopoietin (TSLP), IL-25 and IL-33 have been implicated in regulating Th2-type innate immune responses (Fort et al., 2001; Soumelis et al., 2002; Schmitz et al., 2005; Zaph et al., 2007). These cytokines induce Th2-type cytokines from various cells (Min et al., 2004; Gessner et al., 2005; Vochringer et al., 2006; Saenz et al., 2008; Sokol et al., 2008; Terashima et al., 2008). TSLP was originally identified from thymic stromal cells and thought to support the growth and differentiation of T and B cells but is now considered to be a Th2-inducing cytokine (Eisenbarth et al., 2002). TSLP acts to induce dendritic cells capable of differentiating naïve CD4\(^{+}\) T cells to Th2 cells (Soumelis et al., 2002; Ying et al., 2005). IL-33 induces the production of Th2-type cytokines from mast cells (Ho et al., 2007), basophils (Kondo et al., 2008), eosinophils (Perrigoue et al., 2009), CD4\(^+\) T cells (Louten et al., 2011), CD8\(^+\) T cells (Yang et al., 2011a), Tregs (Brunner et al., 2011), and CD11b\(^+\) innate cells (Turnquist et al., 2011). IL-25 activates NKT cells (Terashima et al., 2008) and CD11\(\alpha\) non-T, non-B cells (Fallon et al., 2006; Perrigoue et al., 2009) and induces proallergic type 2 responses (Angkasekwinai et al., 2007). Administration of IL-25 and IL-33 induces rapid production of Th2-type cytokines such as IL-5 and IL-13 in mice independently of T or B cells (Fort et al., 2001; Hurst et al., 2002; Humphreys et al., 2008), suggesting that Th2 cytokine-producing innate lymphocytes, i.e., Th2-type innate lymphocytes, are regulated by those epithelial cell-derived

### Table 2 | Comparison between Th2-type innate lymphocytes.

| Characteristics | Cells |
|-----------------|-------|
| | NH cell | Nuocyte/Ih2 | Innate IL-9 producer | MPP\(\text{type}^2\) |
| | | | | |
| **SURFACE PHENOTYPE** | | | | |
| CD45 | + | + | + | |
| Sca-1 | + | + | +/− | + | + |
| CD90 | + | + | + | |
| CD34 | − | − | +/− | +/− |
| CD44 | + | + | + | |
| FoxR1 | − | − | +/− | +/− |
| IL-17RB | + | + | + | |
| CD127 | + | + | − | − | +/− |
| ICOS | + | + | + | |
| CD117 | + | +/− | − | + | + |
| T1/ST2 | + | +/− | + | +/− | +/− |
| CD25 | + | − | +/− | +/− |
| MHC class II | − | + | +/− | +* | +/− |
| **LOCALIZATION** | | | | |
| FALC | + | − | | |
| Lymph nodes | − | + | + | + |
| Lung | +** | +*** | +*** | |

*After cultivation with IL-3 and SCF.
**Mostly after administration of papain or IL-33, and helminth infection.
***These cells were induced by IL-25 in vivo.
****IL-33 alone induces only moderate proliferation.
cytokines. CD117+ non-T, non-B cells were not induced in il25−/− mice upon helminth infection (Fallon et al., 2006), indicating that these cells do not respond to IL-33.

Natural helper cells were discovered in adipose tissues and are present in lymphoid clusters termed fat-associated lymphoid clusters or FALC (Moro et al., 2010). In addition to CD90 and CD127, NH cells isolated from FALC express CD117, Sca-1, CD25, CD44, CD45, CD69, αβ7, ICOS, IL-17RB (IL-25R), and T1/ST2 (IL-33R) but lack MHC class II, Flt3, and conventional lineage markers. NH cells were also observed in the bone marrow (Brickshawana et al., 2011 and our unpublished observation) although the expression level of CD117 is very low in the bone marrow. NH cells were also observed in the lung of naïve mice although the numbers are small (Monticelli et al., 2011; Halim et al., 2012; Ikutani et al., 2012). IL-7 supports the survival of NH cells and IL-2 induces their proliferation (Moro et al., 2010). Neither FALC NH cells nor lung NH cells differentiate under myeloid differentiation conditions. NH cells do not differentiate to T cells upon culture on OP1 stromal cells expressing delta-like 1 or upon intrathymic transfer. NH cells isolated from FALC express CD117, Sca-1, CD25, CD44, CD45, CD69, αβ7, ICOS, IL-17RB (IL-25R), and T1/ST2 (IL-33R) but lack MHC class II, Flt3, and conventional lineage markers. NH cells were also observed in the bone marrow (Brickshawana et al., 2011 and our unpublished observation) although the expression level of CD117 is very low in the bone marrow. NH cells were also observed in the lung of naïve mice although the numbers are small (Monticelli et al., 2011; Halim et al., 2012; Ikutani et al., 2012). IL-7 supports the survival of NH cells and IL-2 induces their proliferation (Moro et al., 2010). Neither FALC NH cells nor lung NH cells differentiate under myeloid differentiation conditions. NH cells do not differentiate to T cells upon culture on OP1 stromal cells expressing delta-like 1 or upon intrathymic transfer (Moro et al., 2010; Yang et al., 2011b).

In terms of transcription factors involved in the differentiation of lymphocytes, NH cells express Il2 and Gata3 but lack RORγ. FALC and NH cells are present in rorc (RORγ)-deficient mice, indicating that Tli are not involved in FALC formation or in the differentiation of NH cells. Notable characteristics of NH cells are their abilities to constitutively produce IL-5, IL-6, and IL-13 and to support IgA production from B cells which play important roles in an early phase of anti-helminth immune responses. Similarly, a combination of IL-2 and IL-25 (IL-2 + IL-25) also induces large amounts of IL-5 and IL-13 from NH cells.

Natural helper cells play an important role in the innate immune response against helminth infection (Koyasu et al., 2010). *Nippostrongylus brasiliensis* is a model for the migratory pathway of the human hookworm, which infects subcutaneously, enters vasculature, migrates to the lungs, molts, penetrates the alveoli, and ascends the trachea, where the worms are swallowed to complete maturation in the small intestine (Hotez et al., 2004). Adult worms are cleared after 8–10 days by a Th2 response (Urban et al., 1998). Among Th2 cytokines, IL-13 is critical for goblet cell hyperplasia, which is important for anti-helminth Th2 immunity (Urban et al., 1998; Mohrs et al., 2005). *il4−/−* mice show lower Th2 response but are able to expel the worms (Kopf et al., 1993) but *il13−/−* mice are impaired in goblet cell hyperplasia and are unable to expel the worms (Urban et al., 1998; McKenzie et al., 1999). IL-13, in addition to IL-5, is important in inducing eosinophilia (Wynn, 2003). Mice deficient for both γc and rag−2 lack NH cells in addition to T, B, and NK cells. γc−/−rag−2−/− mice produce neither IL-5 nor IL-13 and are impaired in goblet cell hyperplasia upon *N. brasiliensis* infection. Administration of IL-33 fails to induce IL-5 and IL-13 production in γc−/−rag−2−/− mice. Adoptive transfer of NH cells isolated from FALC restored goblet cell hyperplasia along with the production of IL-5 and IL-13 (Moro et al., 2010). NH cells likely contribute to the early production of IL-5 and IL-13 upon *N. brasiliensis* infection, which induces eosinophilia and goblet cell hyperplasia (Figure 3B).

Natural helper cells are present in rag2−/− and nu/nu mice but absent in id2−/−, γc−/−, il7−/−, and cd127−/− mice (Moro et al., 2010; Yang et al., 2011b). As expected, NH cells are derived from common lymphoid progenitors (CLPs) and lymphoid-primed multipotent progenitors (LMPP), the latter of which retain some
myeloid potential (Igarashi et al., 2002; Welner et al., 2009). In rag1-cre mice crossed with Rosa-YFP mice, more than half of NH cells are Rag1−/− (Yang et al., 2011b), indicating the close relationship between NH cells and T/B lymphocytes. Cell transfer analysis showed that NH cells were generated from hematopoietic stem cells, LMPPs, and CLPs (Yang et al., 2011b). LMPPs were more efficient at generating NH cells than CLPs, which is similar to the case of T cells (Schwarz et al., 2007; Serwold et al., 2009). Lymphoid progenitor-derived NH cells express a high level of GATA3 as expected. Again, they expressed il4 mRNA but no IL-4 protein was detected.

Neill et al. (2010) generated il13-eGFP reporter mice and demonstrated that administration of IL-25 or IL-33 induced a GFP+ population in the mesenteric lymph nodes (~3% of total cells in mesenteric lymph nodes), ~80% of which were cells of unknown lineage. Only a small number of GFP+ cells were present in the spleen, mesenteric lymph nodes, and bone marrow of naive mice (~0.2%) but administration of IL-25 or IL-33 significantly increased the number of GFP+ cells in these organs with the exception of bone marrow. GFP+ cells were named nuocytes as the 13th letter of the Greek alphabet is nu. IL-25-elicited nuocytes are present in the mesenteric lymph nodes, intestinal lamina propria, spleen and express CD117, CD45, CD127, CD90, ICOS, MHC class II, CD44, CD49d, ICAM-1, CD117RB, T1/ST2, and CCR9. Nuocytes express MHC class II but not CD25, which is different from naïve NH cells that express CD25 but not MHC class II (Table 2; Moro et al., 2010; Neill et al., 2010). IL-33-elicited GFP+ cells were also observed in mesenteric lymph nodes, spleen, and intestinal lamina propria. The authors then considered CD3−CD19−FcγRI−CD11b−IL-17RB+ cells to be nuocytes and reported that induction of nuocytes was unaffected in rag2−/−, Wsh/sh, Jk18−/−, and rorc−/− mice but impaired in roar−/− mice (Neill et al., 2010; Wong et al., 2012). In vitro culture of 106/ml nuocytes prepared from spleens of IL-25-treated wild type mice produced microgram levels of IL-5 and IL-13 in response to IL-7+IL-33 (Neill et al., 2010). Unlike NH cells, nuocytes are reported to produce IL-4 (Wong et al., 2012). In contrast to nuocytes, NH cells are not found in lymph nodes and, in fact, do not express the chemokine receptors necessary for lymphocyte recruitment to the lymph node even with activation (our unpublished observations), which further discriminates NH cells from nuocytes. Because NH cells produce a large amount of IL-13 in response to IL-33, it is likely that IL-33-elicited GFP+ cells contain both NH cells and nuocytes while the majority of IL-25-elicited GFP+ cells are nuocytes.

Nippostrongyulus brasiliensis infection also induced GFP+ cells in the intestinal lamina propria of il13-eGFP reporter mice (Neill et al., 2010). Il17rb−/− mice expelled N. brasiliensis more slowly than wild type mice and worm expulsion was severely impaired in il17rb−/−il11rb−/− mice lacking both IL-17RB and T1/ST2. The lack of either IL-17RB or T1/ST2 in il13-eGFP reporter mice greatly reduced the appearance of GFP+ cells in mesenteric lymph nodes. Il17rb−/− mice exhibited impaired IL-25-triggered eosinophilia in the peritoneal cavity and impaired goblet cell hyperplasia in the intestine. Lin−CD45+ICOS+ cells sorted from splenocytes of IL-25-treated wild type mice were defined as nuocytes and adoptive transfer of such nuocytes restored both eosinophilia and goblet cell hyperplasia. Worm expulsion is dependent on T cells and rag2−/− mice cannot expel worms even after transfer of wild type nuocytes. Nuocyte numbers in mesenteric lymph nodes increased in rag2−/− mice on day 4 after infection of rag2−/− mice but rapidly decreased on day 6, suggesting that the expansion of nuocytes is T and/or B cell-dependent.

Intriguingly, two groups (Price et al., 2010; Saenz et al., 2010) have reported the induction of different types of GFP+ cells in the same il4-eGFP reporter mice (4-get mice; Mohrs et al., 2001) upon IL-25 or IL-33 administration. Saenz et al. (2010) observed that administration of IL-25 to 4-get mice increased a CD117+ non-T, non-B population ~60-fold in mesenteric lymph nodes and that this was associated with the increase of il4, il5, and il13 mRNAs in the large intestine (Saenz et al., 2010). GFP+ cells in the Peyers patches and mesenteric lymph nodes were CD117int compared to mast cells and were present in Wsh/sh mice. Intraperitoneal administration of IL-25 did not induce GFP+ cells in the spleen or bone marrow or increase the frequency of NH cells in the mesenteric (Saenz et al., 2010). The CD117int population did not express gata3, junt, c-maf, stat6, or t1/st2 mRNAs. N. brasiliensis infection also induced a CD117int population in mesenteric lymph nodes in an IL-17RA- and IL-17RB-dependent manner. Administration of anti-IL-25 antibodies reduced the number of cells in the CD117int population after N. brasiliensis infection. The CD117int population contains both GFP+ and GFP− cells; GFP+ cells were Lin−T1/ST2lo/−CD127hl/− and GFP− cells were Lin−T1/ST2lo/−CD127−, indicating that there are at least two CD117int populations induced by IL-25 and N. brasiliensis infection.

Il25−/− mice produced only low levels of Th2-type cytokines and parasite-specific IgG1 upon Trichurus muris infection and were susceptible to T. muris infection. Adoptive transfer of IL-25-elicited wild type CD117int cells restored the immune response and protective immunity against T. muris in il25−/− mice, indicating a role for this population in anti-helminth immunity. In an in vitro culture system, GFP+ cells gave rise to mast cells in the presence of SCF and IL-3. GFP− cells became mast cells, basophils, and macrophages in the presence of SCF and IL-3 with or without OP9 stromal cells. Because of such myeloid potential, it is unlikely that these cells were of lymphoid lineage and thus were named MPPtype2 for multipotent progenitor cell type 2 (Saenz et al., 2010). Such myeloid differentiation potential and the lack of gata3 expression discriminate MPPtype2 from NH cells.

Price et al. (2010) reported that the 4-get mouse has Lin−CD117−CD90+GFP+ cells in multiple tissues including liver, mesenteric lymph nodes, spleen, and bone marrow. This population, named lh2 cells, was present in rag2−/−, Wsh/sh, Δdblghata mice but absent in yc−/− mice. Lh2 cells seem closer to nuocytes than to NH cells because lh2 cells and nuocytes are present in lymph nodes, while, as mentioned above, NH cells were not present in lymph nodes. Mice in which YFP-Cre was inserted into the il13 coding region (YetCre13 mice) that were further crossed with Rosa-YFP mice have Lin−CD117−IL-13hm+ cells, which were similar to the GFP+ cells observed in 4-get mice. It is intriguing that GFP+ cells in 4-get mice do not produce IL-4 at the protein level, which indicates that reporter expression does not always correlate with the expression of the original gene product.
Administration of 500 ng of IL-25 or IL-33 for four consecutive days to 4-get mice increased GFP⁺ cells in mesenteric lymph nodes, spleen, liver, and peritoneum; this was associated with an increase in eosinophils. Administration of IL-25 to mice obtained from YetCre13 mice crossed with Rosa-flx-Stop-diophtheria toxin α mice and 4-get mice greatly reduced Lin⁻CD117⁺GFP⁺ cells and eosinophils in mesenteric lymph nodes, spleen, liver, and peritoneum. N. brasiliensis infection also increased this population in mesenteric lymph nodes, spleen, and lung. rag2⁻/⁻ but not γc⁻/⁻ rag2⁻/⁻ mice were able to clear N. brasiliensis after administration of IL-25 for four consecutive days. Adoptive transfer of Lin⁻CD117⁺GFP⁺ cells from YetCre13 mice crossed with 4-get mice into γc⁻/⁻ rag2⁻/⁻ mice did not result in clearance of the worms but administration IL-25 in addition to the transfer of Lin⁻CD117⁺GFP⁺ cells resulted in eosinophilia and clearance of worms. N. brasiliensis infection of YetCre13 mice or another reporter mouse strain in which human CD4 coding sequences were inserted into the il3 locus induced YFP⁺ or human CD4⁺ cells, respectively, in the lung (Liang et al., 2012). Induction of YFP⁺ cells and eosinophilia were impaired in YetCre13 mice crossed with gata3floxflox−/− mice, suggesting that sustained expression of IL-13 requires GATA3 (Liang et al., 2012).

**TH2-TYPE INNATE LYMPHOCYTES AND ALLERGIC INFLAMMATION**

Significant numbers of NH cells are induced in the lung upon influenza A virus infection (Chang et al., 2011; Monticelli et al., 2011). Influenza virus infection induces IL-33 in the lung and causes airway hypersensitivity (Chang et al., 2011; Le Goffic et al., 2011). il3floxflox⁻/⁻ mice did not exhibit H3N1 influenza A virus infection-induced airway hypersensitivity while OVA-induced airway hypersensitivity was unaffected (Chang et al., 2011). However, H3N1 infection as well as intranasal administration of IL-33 induced Lin⁻CD117⁺Sca-1⁺T1/T2⁺ NH cells in the lung and bronchoalveolar lavage fluid and this was also observed in rag2⁻/⁻ mice. NH cells purified as Lin⁻T1/T2⁺ cells from IL-33-treated mice isolated from IL-33-treated mice did not exhibit IL-4, IFN-γ, or IL17A after cultivation with IL-2, IL-33, or IL-2 + IL-33. il3⁻/⁻ mice did not develop airway hypersensitivity or cellular infiltration upon H3N1 infection, but both responses were restored by adoptive transfer of NH cells from rag2⁻/⁻ but not il3⁻/⁻ mice. Administration of anti-CD90 mAb depleted NH cells in the lung and impaired airway hypersensitivity upon H3N1 infection (Chang et al., 2011), indicating that NH cells are responsible for airway hypersensitivity induced by H3N1 influenza virus infection. There is a caveat, however, in that anti-CD90 mAb also depletes NK cells in rag2⁻/⁻ mice. In addition, NH cells play a major role in airway hypersensitivity induced by glycolipid antigen (Kim et al., 2012) or papain (Halim et al., 2012). Similarly, nuocytes were reported to play a role in airway hypersensitivity induced by intranasal administration of IL-25 or IL-33 in il4/Il13 dual reporter mice (Barlow et al., 2011).

Monticelli et al. (2011) and Halim et al. (2012) reported the presence of a small number of NH cells in the lung of naive wild type and rag1⁻/⁻ mice. A mouse has 2–3 × 10⁴ lung NH cells which represent 0.4–1% of total live cells in the lung. Lung NH cells produced IL-5 and IL-13 when cultured with a combination of IL-2, IL-7, and IL-33. They produced minimal amounts of IL-17 and IL-22 in response to IL-23, confirming the difference between Th2-type innate lymphocytes and RORγt⁺ Tii-related Th17-type innate lymphocytes (Monticelli et al., 2011). Lung NH cells express Id2 but not RORγt as do FALT NH cells, and id2⁻/⁻ mice lack lung NH cells as expected.

Increases in the number of lung NH cells were also observed upon intranasal infection with H1N1 influenza A virus (Monticelli et al., 2011). Depletion of NH cells in the lung by intraperitoneal injection of anti-CD90 mAb did not affect viral load but impaired lung functions such as blood oxygen saturation level and epithelial integrity. H1N1 infection-induced eosinophilia in the bronchoalveolar lavage fluid and the eosinophilia was reduced by anti-CD90 mAb treatment. Adoptive transfer of Lin⁻CD25⁺CD90⁺T1/T2⁺ NH cells isolated from the lung of CD90.1 (Thy-1.1) wild type mice into CD90.2 (Thy-1.2) congenic mice treated with anti-CD90.2 mAb restored the epithelial responses of the recipient mice. Administration of anti-T1/T2 mAb reduced NH cell numbers and greatly impaired lung function upon H1N1 infection. Administration of IL-13 to anti-CD90 mAb-treated mice infected with H1N1 restored goblet cell hyperplasia but did not improve lung function or virus-induced morbidity and mortality, suggesting the presence of a factor(s) other than IL-13 involved in the maintenance of epithelial integrity. Among the cytokines produced by NH cells, amphiregulin was identified as a critical factor (Monticelli et al., 2011). Amphiregulin is involved in the remodeling and repair of epithelial injury (Doloinay et al., 2006; Enomoto et al., 2009; Fukumoto et al., 2010). Purified lung NH cells produce amphiregulin in response to IL-2 + IL-7, and IL-33 further enhanced amphiregulin production induced by IL-2 + IL-7. Administration of amphiregulin restored lung function and epithelial integrity in anti-CD90-treated mice infected with H1N1 without inducing IL-5 or IL-13 (Monticelli et al., 2011), indicating that amphiregulin produced by IL-33-activated NH cells plays a critical role in the process of repairing epithelial integrity after influenza virus infection.

**PROTEASE-INDUCED ALLERGIC RESPONSES AND IL-9 PRODUCING CELLS**

IL-9, another Th2 cytokine, is highly expressed in the lungs of asthmatic patients (Shimbara et al., 2000; Erpenbeck et al., 2003) and anti-IL-9 mAb is currently in clinical trial for atopic diseases (White et al., 2009). In mice, overexpression of IL-9 in the lung induces an asthma-like phenotype in an IL-13-dependent manner (Temann et al., 1998, 2002, 2007) and blocking of IL-9 reduces airway hypersensitivity (Kung et al., 2001; Cheng et al., 2002). IL-9 induces mucous production, goblet cell hyperplasia, and other features of airway remodeling (Townsend et al., 2000; Kearley et al., 2011), effects shared by IL-13 via goblet cell hyperplasia (Wynn, 2003) and IL-5 via eosinophilia (Cho et al., 2004). These results collectively suggest the presence of an IL-9/IL-13 axis in the lung. On the other hand, IL-9 may not play a critical role in some forms of asthmatic diseases because the absence of IL-9 shows little effect on antigen-induced pulmonary inflammation or airway hypersensitivity (McMillan et al., 2002)

Papain can cause occupational asthma (Novey et al., 1979) and has been used in allergic models because it has a strong
adjuvant activity in the induction of asthma-like symptoms and airway hypersensitivity (Oboki et al., 2010; Wang et al., 2010). Halim et al. (2012) demonstrated that NH cells play a critical role in protease allergen-induced airway inflammation. NH cells induced by papain do not respond to IL-33 alone and require IL-2 or IL-7 along with IL-33 to produce IL-5 and IL-13 (Halim et al., 2012). Papain-induced transient production of IL-9 in the lung (Tan et al., 2010), raising the possible involvement of an IL-9/IL-13 axis in airway hypersensitivity induced by innate lymphocytes. To examine the source of IL-9, IL-9 fate mapping mice were generated by crossing il9-Cre mice and rosa-YFP mice (Wilhelm et al., 2011). Intranasal challenge with papain induces Th2-type responses with production of IL-4, IL-5, IL-9, and IL-13 and the appearance of IL-9+ cells in bronchoalveolar lavage fluid, most of which are Lin−/CD45+/CD90+ cells. These cells did not express CD117 or CD127 but expressed heterogeneous levels of Sca-1, CD25, T1/ST2, and MHC class II, implying that these cells are distinct from NH cells or nuocytes, although it is possible that papain stimulates the surface phenotype of these cells. Stimulation with PMA and ionomycin in vitro induced the production of IL-5 and IL-13 but not IL-4 or IL-9 by IL-9+ cells. Papain-induced Lin−CD90+CD25+IL-9+ cells responded to IL-2 but not to IL-25, IL-33, or TSLP to produce IL-9. In addition, TLR ligands did not induce IL-9 production by these cells. Intranasal administration of IL-33 also induced IL-9+ cells, which were similar to those induced by papain.

Papain-induced IL-9 production in the lung was absent in γc−/−rag2−/− mice, suggesting that innate lymphocytes are the source of IL-9. In fact, intratracheal transfer of Lin−CD90+ cells isolated from papain-challenged mice into γc−/−rag2−/− mice followed by intranasal challenge with papain restored the production of IL-5, IL-9, and IL-13 (Wilhelm et al., 2011). Papain-treated mice produced IL-4, IL-5, IL-6, IL-9, and IL-13 in the lung but administration of anti-IL-2 mAb reduced only IL-9 production. Papain-treated rag1−/− mice produced lower amounts of IL-9 than wild type mice but produced IL-9 at a level similar to wild type mice when IL-2 was intranasally administered after papain challenge. Intranasal administration of IL-2 alone showed no effect. Papain-induced IL-9 producing cells were mostly Lin−CD90+ cells, which also produce IL-13. These results collectively indicate that innate lymphocytes cooperate with T and/or B cells and that T and/or B cell-derived IL-2 plays a positive role in IL-9 production by innate lymphocytes.

Th2-TYPE INNATE LYMPHOCYTES IN HUMANS

Th2-type innate lymphocytes are also observed in humans. The presence of innate lymphocytes similar to NH cells has been reported in human gut, lung, mesentery, and blood (Moro et al., 2010; Mjösberg et al., 2011; Monticelli et al., 2011). FALC and Lin−CD117+CD127+ cells were observed in human mesentery (Moro et al., 2010). Human lung and bronchoalveolar lavage fluid also have a similar Lin−/CD25+CD127+T1/ST2+ population (Monticelli et al., 2011). CRTH2 was identified as a valuable marker for human NH cells (Mjösberg et al., 2011). CD45−CD127+CRTH2+ cells produced IL-13 in response to IL-2 + IL-25 and IL-2 + IL-33. CD45−CD127+CRTH2+ cells expressed a low level of RORγt, which is different from mouse NH cells. It should be noted that RORγt+ LTI cells are able to produce IL-5 and IL-13 in addition to IL-22 in response to TLR2 ligands (Crellin et al., 2010b), implying that NH cells and LTI-related cells share some characteristics in humans. Peripheral blood contains Lin−CD45+CD127+CRTH2+ cells expressing CD7, CD161, CD62L, and CD25 but lacking NKp44 and MHC class II. Lin−CD45+CD127+ cells produce a variety of cytokines including TNFα, IFN-γ, IL-22, IL-17, IL-13, and IL-2 but only Lin−CD45+CD127+CRTH2+ cells produce IL-13. Thus, human LTI and Th2-type innate lymphocytes share similar characteristics.

Rhinosinusitis patients with nasal polyps have more il5 and il13 mRNAs than those without nasal polyps (Van Bruggen et al., 2008). Nasal tissue from the nasal polyps of patients with chronic rhinosinusitis has an increased number of Lin−CD45+CD127+CD161+CRTH2+ cells, which do not express RORγt (Mjösberg et al., 2011). Treatment of lamina propria cells from ulcerative colitis patients with anti-CD161 reduced IL-5 and IL-13 expression (Fuss et al., 2004) and it was proposed that the depletion of NKT cells resulted in the reduction of Th2 cytokines (Fuss et al., 2004). However, it is also possible that the loss of Lin−CD45+CD127+CRTH2+ cells is the cause of reduced Th2 cytokine production because these cells also express CD161.

PERSPECTIVES

In this article we have summarized current knowledge regarding various innate lymphocyte subsets by focusing on the most recently identified Th2-type innate lymphocyte subsets. As discussed here, Th2-type innate lymphocytes seem to comprise more than one type of cells. There are differences between NH cells and nuocytes/Ih2 cells in migration to lymph nodes, surface phenotypes, and cytokine responsiveness. It is likely that there are at least two distinct populations within Th2-type innate lymphocytes but it is also possible that there is plasticity within Th2-type innate lymphocytes as is true for RORγt+ LTI-related innate lymphocytes. It should be noted that, in many experiments, cells are detected by reporter gene expression after treatment of mice with cytokines, viruses, and papain. There is a caveat that such treatment changes the naive stage surface phenotypes of cells and it is critical to analyze innate lymphocytes in naive mice and examine the changes in characteristics after activation.

Innate immune cells of myeloid origin express a variety of toll-like receptors and lectins that respond to microbe-derived substances (Kawai and Akira, 2010). In contrast, cNK and RORγt+ LTI-related cells express a limited number of toll-like receptors (Crellin et al., 2010b). It is intriguing that Th2-type innate lymphocytes do not respond to toll-like receptor ligands but respond only to cytokines. It will be of interest to determine whether Th2-type innate lymphocytes directly respond to microbe-derived substances.

Distinct types of Th cells are involved in the pathophysiology of various diseases such as inflammatory, allergic, and autoimmune diseases. It is likely that innate lymphocytes are involved in the pathophysiology of various diseases via the production of distinct sets of cytokines (Figure 1). Th2-type innate lymphocytes are...
likely involved in innate immune responses resulting in the pathophysiology of allergic diseases such as asthma. It will be of interest to study how innate lymphocytes are involved in disease onset and progression and whether perturbation of innate lymphocytes can lead to new treatments for human diseases. Finally, the presence of NH cells in FALC localized in adipose tissue suggests that NH cells have regulatory functions in the homeostasis of adipose tissues, which will be an important subject for future studies.

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