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SUMMARY

Innate lymphoid cells (ILCs) are emerging as a family of effectors and regulators of innate immunity and tissue remodeling. Interleukin 22 (IL-22) and IL-17−producing ILCs, which depend on RORγt, express CD127 and CD161. We describe another Lin^- $CD127^+CD161^+$ ILC population found in humans that express the chemoattractant receptor CRTH2. These cells responded *in vitro* to IL-2 plus IL-25 and IL-33 by producing IL-13. $CRTH2^+$ ILCs were found in fetal and adult lung and gut. In fetal gut these cells expressed IL-13 but not IL-17 or IL-22. $CRTH2^+$ ILCs were enriched in nasal polyps of chronic rhinosinusitis, a typical type 2 inflammatory disease. These data identify a unique human ILC type, which provides an innate source of T_H2 cytokines.

INTRODUCTION

Innate lymphoid cells represent an emerging family of cell types that appear to play crucial roles in tissue remodeling and in innate immunity against pathogenic and non-pathogenic micro organisms[1-3]. These cells are characterized by a lymphoid morphology and an absence of recombination activating gene (RAG)−dependent receptors. Natural killer (NK) cells and Lymphoid Tissue inducer (LTi) cells are the prototypic members of this family. Whereas NK cells have a crucial innate defensive role against viral infections, in particular against herpes viruses, LTi cells are essential for the formation of lymph nodes during embryonic development. Initially LTi cells were not considered to have a function in the immune response. However, the recent discovery in mouse and humans that LTi cells are able to produce interleukin 17 (IL-17) and IL-22 (refs. [4-6]), cytokines that are known to mediate immunity against microbes, has led researchers to reconsider the functions of these cells in immune responses. More recently, cells were discovered that show characteristics of both NK and LTi cells. Like LTi cells these cells are of lymphoid origin, express the IL-7Rα chain (CD127) and the transcription factor RORγt but they also share with NK cells the expression of NKp46 and, in humans, NKp44 and CD56 (refs. [7-10]). These NK receptor−expressing cells are mostly found at mucosal sites both in mice and humans and produce IL-22. Innate lymphoid cells (ILCs) dedicated to the production of IL-17 and interferon-γ (IFN-γ), either exclusively or, in combination, have been described both in mice and humans[11-13]. IL-22-producing ILCs were shown in several systems to be involved in responses against bacteria. For instance, IL-22−producing ILCs are essential for the innate immune response against the enteric

bacterium *Citrobacter rodentium* in the gut[14] and *Klebsiella pneumoniae* in the lung[15]. It seems therefore that the ILC family comprises cells that share a number of characteristics but may have different effector functions mediated by distinct cytokines and dependent on their anatomical localization[3].

Recently, another Id2-dependent non−T, non−B cell population which produces type 2 cytokines was discovered in the mouse. These cells have been designated natural helper lymphocytes (NHLs)[16], multi-potent progenitor type 2 (MPP2) cells[17], nuocytes[18] and innate helper (IH) type 2 cells[19]. Although differences exist between these cell types, they have in common the production of T_H2 cytokines, most notably IL-5 and IL-13 in response to the IL-17 family member IL-25 (IL-17E) and the IL-1 family member IL-33. The innate lymphocyte−derived T_H2 cytokines mediate eosinophilia and goblet cell hyperplasia, both of which are critical for anti-helminth reactions. These type 2 ILC populations may be involved in disease since their signature cytokines IL-5 and IL-13 also play a role in pathophysiology of type 2 immunity−associated diseases such as asthma and allergic diarrhea.

Like LTi cells and RORγt$^+$ ILCs[9,20], type 2 ILCs are dependent on the gamma common cytokine receptor (γc) and IL-7[16,21]. Type 2 ILCs depend on Id2[16] similar to LTi and IL-17− and IL-22−producing ILCs[22,23], but in contrast to those cells type 2 ILCs seem to be independent of RORγt[16]. The common reliance of NK cells, LTi cells and various ILC populations on Id2 and γc cytokines support the contention that these cell types have a common origin[3].

A human type 2 ILC population comparable to the one found in mice has not yet been described, although innate cells producing IL-13 have been previously documented.

Previously it was shown that cord blood $CD34^+$ progenitor cells cultured in the presence of IL-2, can develop into a non−T, non−B cell type that expresses CD161 but lacks CD56 (ref. [24]). These cells produced IL-13 but not IFN-γ, and contained an IL-5–producing subset. However, an equivalent cell type was not identified *in vivo*. More recently, we demonstrated that lineage (T, B and monocytes)–negative (Lin^-) $CD127^+CD117^+$ cells from tonsil are able to produce IL-13 following stimulation with TLR2 ligand and IL-2 (ref. [13]). These IL-13−producing human ILCs expressed *RORC* transcripts and protein and co-expressed IL-22. Cloned lines were established from these cells which mostly co-expressed IL-22 and IL-13, although some clones were identified that produced IL-13 and IL-5 but not IL-22 (ref. [13]). These latter clones still expressed *RORC* transcripts, in contrast to mouse type 2 ILCs. We also found that ILCs from the tonsil when stimulated with IL-23 and IL-2 produced IL-22 but not IL-13 whereas when these ILCs were simulated with IL-2 and TLR2 agonists they do produce IL-13 (ref. [13]) suggesting that tonsil ILCs are plastic and produce different cytokines dependent on the stimulus given to those cells. It is, however, also possible that tonsil ILCs contain two subsets, one of which produces IL-13 and does not respond to IL-23. Obviously a marker discriminating IL-13-producing from IL-22 –producing ILCs would allow for addressing this issue.

Here we describe a human Lin^-CD127^+ ILC population that is characterized by the expression of the T_H2 marker Chemoattractant receptor−homologous molecule expressed on T_H2 lymphocytes (CRTH2). These ILCs were found in lung, gut and nasal tissues as well as in peripheral blood and expressed the NK cell marker CD161 and CD7, had a lymphoid size and lacked the mast cell and basophil markers FcεR1 and IL-3R. Like mouse natural helper lymphocytes, nuocytes and type 2 innate helper lymphocytes,

these human CRTH2$^+$ cells responded to IL-25 and IL-33 by producing IL-13 and IL-5 and we therefore suggest that these cells represent the human equivalent of mouse type 2 ILCs.

RESULTS

The fetal gut contain a lin$^-$CRTH2$^+$CD127$^+$ population

In the mouse type 2 ILCs have been found in the intestine. In search of a human cell population that may represent the equivalent of the type 2 ILCs described in the mouse we performed an extensive analysis of cells in both fetal and adult intestines, focusing on cells that were Lin$^-$ and IL-7Rα$^+$. Thus, we stained single cell suspensions derived from intestinal tissues with a cocktail of lineage specific antibodies, CD45 and a collection of antibodies, including the antibody against CRTH2 which is known to be expressed on T_H2 cells and myeloid cells associated with type 2 immune responses such as basophils and mast cells. Unexpectedly, we found the highest proportion of Lin$^-$CRTH2$^+$ lymphoid cells in the fetal gut and therefore decided to perform an extensive analysis of all ILC populations within this organ. Within the Lin$^-$ population, we could clearly distinguish cells with high and cells with intermediate expression of CD45 (CD45^{high} versus CD45^{dim}) (**Fig. 1a**). Analyzing these two populations for CD117 (c-Kit) and CD127 (IL-7Rα) expression we observed that the great majority (>90%) of the CD45^{dim} cells co-expressed CD117 and CD127 and most likely contain LTi[4]. By contrast, only around 30% of the CD45^{high} cells expressed CD127. Most of the CD45$^+$CD127$^-$ cells were CD56$^+$perforin$^+$ and are therefore identifiable as NK cells (data not shown). We then

gated on $CD127^+$ cells and analyzed the $CD45^{high}$ Lin^- cells within that gate and compared these with $CD127^+CD45^{dim}$ cells. Both populations expressed CCR6, CCR4, the lymphoid marker CD7 and CD161, which are also expressed on NK and LTi cells (**Fig. 1b**). Thus the $CD45^{high}CD127^+$ cells most likely represent an ILC population. The $CD45^{dim}$ cells, like LTi cells in the mesenteric lymph nodes, expressed IL-1βR and contained two populations, one that expressed NKp44 and the other lacking this antigen (**Fig. 1b**). By contrast, the fetal gut $CD45^{high}$ cells all lacked NKp44 and expressed low amounts of IL-1βR (**Fig. 1b**). The great majority of these $CD45^{high}CD127^+$ cells were clearly positive for the type 2 marker CRTH2 (**Fig. 1c**). To assess whether these $CRTH2^+$ cells were not mast cells or basophils, that also express $CRTH2^+$, we analyzed the $CRTH2^+$ ILCs for the presence of the IL-3 receptor (CD123) and the FcεRI which are normally highly expressed on those cell types. The $CRTH2^+$ lymphoid cells did not bind IL-3R− nor FcεRI−specific antibodies (**Fig. 1c**), indicating that these cells are distinct from both mast cells and basophils.

Fetal LTi cells and postnatal IL-17− and IL-22−producing ILCs express and depend on the transcription factor RORγt, whereas type 2 ILCs in the mouse have been shown to be negative for this transcription factor. Therefore we analyzed the expression of RORγt on the various ILC populations. Since this antibody has a high non-specific binding activity we used the binding to NK cells, which do not express RORγt as assessed by real-time PCR analysis, as background. $CD45^{dim}CD127^+$ ILCs were RORγt positive whereas NK cells ($CD45^+CD127^-CD56^+$ cells) were negative for this factor (**Fig. 1d**). The $CRTH2^+$ cells expressed more RORγt protein than NK cells but this expression was much lower than on $CD45^{dim}CD127^+$ ILCs (**Fig. 1d**). PCR analysis confirmed that the $CRTH2^+$ ILCs

expressed less *RORC* transcripts than CD45^{dim}CD127^{+} ILCs, but still more than NK cells (**Fig. 1d**). Thus, a CRTH2^{+} ILC population, distinct from RORγt^{+} ILC, mast cells and basophils can be found in fetal gut.

Fetal gut CRTH2^{+} ILCs respond to IL-25 and IL-33

The signature cytokines of human and mouse RORγt^{+} ILCs are IL-17 and IL-22 (reviewed in [3]) whereas mouse type 2 ILCs secrete IL-13 (refs. [16,18,19]). We therefore analyzed the expression of transcripts for these cytokines and IL-5 in the various ILC populations isolated from the fetal gut. Whereas CD45^{dim}CD127^{+}NKp44^{+} expressed IL-22 and some IL-17 transcripts they did not express IL-13 (**Fig. 2**). By contrast, CRTH2^{+} ILCs clearly expressed IL-13 but no IL-17 or IL-22 transcripts. Neither the CRTH2^{+} population nor the other ILC populations in the gut expressed IL-5 *ex vivo* (data not shown). Since CRTH2^{+} cells express CD25 and IL-13, we stimulated freshly isolated fetal gut CRTH2^{+} cells, with IL-2, and the combination IL-2 plus IL-25 or IL-2 plus IL-33. IL-2 in combination with IL-25 or IL-33 clearly stimulated production of IL-13 protein (**Fig. 3**).

To investigate whether the CRTH2^{+} population represents a stable cell type we established cell lines starting from fetal gut CRTH2^{+} cells by using a technique that was also successfully applied to generate lines of NK cells[25] and IL-17− and IL-22−producing ILCs[13,26]. Robustly growing cultures could be generated and the cells showed a stable expression of CRTH2 and CD127 (**Fig. 4a**). Further, these cell lines lacked expression of CD3ε and FcεR1 (**Fig. 4a**) as well as CD123 and CD94 (data not shown), demonstrating the stability of the cell lines and their inability to differentiate towards the basophil, mast

cell, T cell or NK cell lineages (**Fig. 4a**). *In vitro* expanded CRTH2$^+$ ILCs expressed intracellular IL-13 but no IL-17 following activation with the polyclonal activator phorbol 12-myristate 13-acetate (PMA) and ionomycin whereas a fraction of the IL-13−producing cells also produced IL-22 (**Fig. 4b**). As previously reported for IL-22−producing NKp44$^+$ ILCs, CRTH2$^+$ ILC cell lines expressed aryl hydrocarbon receptor (*AHR*) (**Fig. 4c**). However, CRTH2$^+$ ILCs did not respond to IL-23 (**Fig. 4d**), in contrast to NKp44$^+$ ILCs isolated from inflamed tonsils (**Fig. 4e**), to elicit production of IL-22 or expression of *IL17* (data not shown), consistent with the lack of the *IL23R* (**Fig. 4f**).

CRTH2$^+$ ILC cell lines clearly expressed *ST2* (also known as *IL1RL1*, subunit of IL33R), *IL17RB* (subunit of IL25R) and *IL17RA* (common subunit of IL25R and IL17R) transcripts (**Fig. 4f**). Consistent with the expression of *ST2*, the majority of the cell lines clearly responded to IL-2 in combination with IL-33 and IL-25 plus IL-33 (**Fig. 4g**) whereas response to IL-25 was less pronounced and not seen in all cell lines (**Fig. 4g** and **Supplementary Fig. 1**). The responses of individual cell lines are shown in **Supplementary Fig 1**.

The CRTH2$^+$ ILC cell lines never showed any IL-17 expression (data not shown) but in contrast to the *ex vivo* isolated cells, the cell lines did express IL-5 transcripts (data not shown). Thus, we have identified a stable Lin$^-$ CRTH2$^+$ CD127$^+$ population that expresses IL-13 but not IL-17 or IL-22 transcripts *ex vivo* and respond *in vitro* to IL-25 and IL-33 by producing IL-13.

CRTH2$^+$ ILCs are enriched in chronic rhinosinusitis

To gain more insights into the possible functions of CRTH2$^+$ ILCs, we performed an extensive analysis of the presence of Lin$^-$CRTH2$^+$CD127$^+$ in a variety of tissues. These cells were located in mucosal tissues at different ontogenic stages as they were found in both fetal and adult gut and lung (**Fig. 5a** and **Table 1**).

Given the production of type 2 cytokines and the responsiveness to IL-25 and IL-33, we analyzed the presence of CRTH2$^+$ cells in chronically inflamed airway tissues, specifically nasal polyps of patients with chronic rhinosinusitis (CRS). This disease is characterized by the presence of very high local titers of IgE and high numbers of eosinophils that may be driven by the eosinophil growth factor IL-5 and further supported by IL-13. IL-5 and IL-13 transcripts are indeed elevated in these patients compared to chronic rhinosinusitis patients without nasal polyps[27]. Indeed, nose polyps contained relatively high proportions of CRTH2$^+$CD127$^+$CD161$^+$ ILCs when compared to non-inflamed nose tissue from healthy, non-allergic donors (**Fig. 5b** and **Table 1**). These data support the hypothesis that CRTH2$^+$ cells are type 2 ILCs and that they can contribute to type 2 mediated disease. In contrast to CRTH2$^+$ ILCs in peripheral blood, which express low amounts of RORγt, the nose polyp–residing CRTH2$^+$ cells did not seem to express any RORγt protein (**Fig. 5d**), an observation that associates these cells to the mouse natural helper cells which were reported to be completely negative for RORγt[16].

CRTH2$^+$CCR6$^+$ ILCs are present in peripheral blood

That the CRTH2$^+$ ILCs are present in many tissues may mean that they differentiate in these organs from a circulating precursor or that mature CRTH2$^+$ ILCs may be circulating. To address this issue we analyzed adult human peripheral blood for the

presence of $Lin^{-}CRTH2^{+}$ and other ILCs. For this analysis we depleted peripheral blood mononuclear cells (PBMCs) of the majority of T (with anti-CD3), B (anti-CD19) cells and monocytes (anti-CD14). When gating on Lin^{-} cells, a population of $CD127^{+}$ cells, that is distinct from CD56−expressing NK cells were clearly present (**Fig. 6a**). Within the $Lin^{-}CD127^{+}$ population, we could distinguish subsets of $CD117^{+}$ and $CD117^{-}$ cells.

To investigate how the peripheral $Lin^{-}CRTH2^{+}$ cells may be related to the cells we found in other human tissues, we analyzed the expression of comprehensive panel of markers. $CD127^{+}CD117^{+}$ cells abundantly expressed the lymphoid markers CD7, CD161, CD25 and CD62L (**Fig. 6b**). They were negative for NKp44, thus suggesting they are different from the previously described human IL-22−producing $NKp44^{+}$ cells, and expressed low amounts of HLA-DR. The $CD127^{+}CD117^{-}$ population was very similar to the $CD127^{+}CD117^{+}$ population with respect to these markers, although some, such as CD25 and CD161, clearly was present in a bimodal distribution (**Fig. 6b**), suggesting that this population is heterogeneous. Neither $CD127^{+}CD117^{+}$ nor $CD127^{+}CD117^{-}$ cells expressed CD34 (results not shown), indicating that these cells populations do not contain immature hematopoietic progenitors. Both the $CD127^{+}CD117^{+}$ and the $CD127^{+}CD117^{-}$ populations expressed CCR6 and CRTH2 (**Fig. 6c**), two receptors also found on fetal gut type 2 ILCs (**Fig. 1b**). By contrast, CRTH2 was not present on neither $CD56^{high}$ nor $CD56^{+}$ NK cells (**Supplementary Fig. 2**). The expression of RORγt protein on $CRTH2^{+}$ cells was lower than that expressed in T_H17 cells but by comparison to NK cells, which lack RORγt, the $CRTH2^{+}$ cells expressed some RORγt (**Fig. 6d**), which is in agreement with the observation in fetal gut $CRTH2^{+}$ cells. Altogether, these data

indicated that human peripheral blood contains a cell population that shares the main phenotypic features of tissue resident type 2 ILCs.

Blood CRTH2$^+$ ILCs respond to IL-25 and IL-33

Lin$^-$CD127$^+$CD117$^+$ circulating cells stimulated with PMA plus ionomycin produced a wide range of cytokines, including IL-2, IL-13, tumor necrosis factor (TNF) and low amounts of IL-22 and IL-17 (**Supplementary Fig. 3**), suggesting that these cells are functionally heterogeneous. Indeed, when we stimulated freshly isolated cells with IL-2 only, or a combination of IL-2 plus IL-25 or IL-2 plus IL-33, IL-13 was induced only in the CRTH2$^+$ but not in the CRTH2$^-$ subset (**Fig. 7**). To determine the stability of these cells, CRTH2$^+$ cell lines were generated from these cells. All expanded cells expressed CRTH2 and CD127 (**Fig. 8a**) and weakly expressed *RORC* transcripts (**Fig. 8b**) similar to *ex vivo* isolated CRTH2$^+$ ILCs, confirming the stability of this phenotype. The cultured cells expressed large amounts of IL-13 when stimulated with the polyclonal stimulus ionomycin plus PMA but did not express IL-17 (**Fig. 8c**). Furthermore these cell lines expressed *ST2*, *IL17RB* and *IL17RA* (**Fig. 8d**). CD117$^-$ cells expressed more *ST2* and *IL17RB* as compared to CD117$^+$ cells, fitting with the higher responsiveness of CD117$^-$ ILC cell lines to IL-25 and IL-33 (**Supplementary Fig. 4**). As seen for fetal gut, most blood CRTH2$^+$ ILC cell lines responded to IL-25 but the response was more pronounced to IL-33, and combinations of these two cytokines (**Fig 8e**). The responses of individual cell lines are shown in **Supplementary Fig. 4**. IL-22 was expressed in a minority of the PMA plus ionomycin−stimulated cells but all IL-22 producing cells also produced IL-13 (**Fig. 8c**). The CRTH2$^+$ ILCs expressed *AHR*, a transcription factor

associated with IL-22 production (**Fig. 8f**). However, stimulation with IL-23 or IL-1β did not further enhance IL-22 secretion (**Fig. 8g**), in contrast to what was seen for tonsil NKp44^{+} cells (**Fig. 4e**). Taken together our data indicate that part of the CRTH2^{+} cells from peripheral blood has the ability to produce IL-22, in contrast to the freshly isolated CRTH2^{+} ILCs from fetal gut.

DISCUSSION

Here we describe CRTH2+ innate lymphoid cells that are distinct from basophils and mast cells.. Lin^{-}CRTH2^{+} ILCs are present in intestinal tissue, during the fetal stage, and persist in adults. *Ex vivo* isolated fetal gut CRTH2^{+} ILCs expressed IL-13 but not IL-17 nor IL-22. *In vitro,* these cells responded to IL-25 and IL-33 by producing the type 2 cytokine IL-13. Our data suggest therefore that the CRTH2^{+} ILCs described here are similar to a non−B, non−T, γc-dependent IL-25−responsive cell type that was identified previously[21] and more recently rediscovered by several other groups, who named these cells nuocytes[18], natural helper lymphocytes (NHL)[16] and innate helper 2 cells (ih2)[19], respectively.

In the mouse it was shown that the type 2 ILCs play essential roles in the innate response against helminthes and are responsible for goblet cell hyperplasia and eosinophilia[16,18,19]. Given these features it is possible that type 2 ILCs are involved in the pathophysiology of type 2 immunity−driven inflammatory diseases also in other tissues, for example the airways. Indeed, we find type 2 ILCs in human healthy lung. Further, IL-13−producing ILCs were recently shown to mediate influenza-induced airway hyper−reactivity[28], supporting a crucial role for type 2 ILCs in IL-13−IL-33 axis driven airway

inflammation. In this respect our observation of relatively high proportions of type 2 ILCs in nasal polyps of rhinosinusitis patients is relevant as this disease is characterized by eosinophilia, most likely caused by IL-5. Furthermore, IL-5 and IL-13 transcripts are elevated in these patients compared to chronic rhinosinusitis patients without nasal polyps[27].

Since CRTH2$^+$ ILCs are also localized in the gut, it is conceivable that these cells may play a role in chronic gut inflammations with an IL-13−linked etiology, such as ulcerative colitis (UC)[29]. Interestingly, anti-CD161 treatment of lamina propria (LP) cells from UC patients resulted in a 90% reduction in amounts of IL-5 and IL-13 (ref [30]). It was concluded that the CD161$^+$ NKT cells were the main producers, however it may be possible that type 2 ILCs that also express CD161 contribute to IL-13 production in UC tissues. Future work analyzing the function of CRTH2$^+$ cells in other type 2 immunity−mediated inflammatory diseases such as asthma, allergic diarrhea and atopic skin disorders should reveal the possible role of these cells in type 2 immune−mediated inflammatory diseases possibly providing new avenues for therapeutic intervention directed at these cells.

The presence of these cells in fetal tissues in particular in fetal gut raises the question whether these cells are involved in tissue generation. Perhaps fetal type 2 ILCs and LTi cells exert their tissue generating activity in different tissues during embryonic development. Studies in the mouse should be done to test this possibility. For instance identification of cell surface markers specific for type 2 ILCs in the mouse may allow for demonstrating the effects of specific deletion of those cells during development. LTi cells

have also been implicated in regeneration of lymphoid tissue after acute viral infections[31] and it is possible that type 2 ILCs also have such a function as well.

The presence of CRTH2$^+$ cells in a variety of tissues raised the question where these are derived from circulating mature CRTH2$^+$ ILCs. Addressing this issue we observed that IL-25 and IL-33−responsive CRTH2$^+$ cells with similar phenotypes as those found in the tissues were present in the peripheral blood. However, the peripheral blood type 2 ILCs did not express IL-13 or other cytokine transcripts *ex vivo* in contrast to what we observed in fetal gut, indicating they are in a non-activated state. The majority of CRTH2$^+$ peripheral blood cells co-expressed CCR6 which may be instrumental in homing of these cells to the tissues. It is possible that type 2 ILC homing in different tissues get activated *in situ* and therefore adopt slightly different features such as expression of IL-13 and perhaps certain cell surface receptors which might explain why type 2 ILCs found in different tissues in the mouse are not identical to each other[32]. Further supporting this idea, we observed a clear-cut IL-13 producing profile of gut tissue type 2 ILCs whereas a minority of the circulating CRTH2$^+$ ILCs also produced some IL-22, which was not regulated by IL-1β or IL-23, further supporting that the circulating ILC population is distinct from the previously described IL-22 producing ILCs but have more functional plasticity than tissue resident type 2 ILCs.

Both fetal gut and blood type 2 ILCs clearly responded to IL-25 and even more pronounced to IL-33, consistent with observations in mice[16,18]. Accordingly, type 2 ILCs specifically expressed transcripts for the receptors for IL-25 (*IL17RA* and *IL17RB*) and IL-33 (*ST2* also known as *IL1RL1*).

Compared to NK cells that do not express transcripts of *RORC* nor RORγt protein, CRTH2$^+$ type 2 ILCs express some RORγt as determined by flow cytometry and analysis of *RORC* transcripts. This observation was unexpected since mouse type 2 ILCs were reported to be negative for RORC transcripts[16]. In contrast to fetal LTi cells and post natal IL-17 or IL-22−producing ILCs, type 2 ILCs are also independent of RORγt for their development in the mouse[16]. The expression of RORγt in human CRTH2$^+$ cells does not necessarily mean that these cells depend on this transcription factor. It is possible that type 2 ILCs and IL-17, IL-22−producing ILCs have a common RORγt$^+$ precursor; whereas differentiation of the common precursor to IL-17, IL-22−producing ILCs requires RORγt, this might not be the case for type 2 ILCs. An analogous situation exists in the T cell system. RORγt is expressed on all double positive thymocytes[33]. Although RORγt is required for optimal survival of DP thymocytes, mature T_H cells do develop in RORγt deficient mice except for T_H17 cells[34]. Published evidence exists to suggest that RORγt expression is not always stable[35]; using RORγt reporter mice these researchers observed that downregulation of RORγt was associated with a functional shift from IL-22 to IFN-γ production. Perhaps an analogous situation exists for CRTH2$^+$ ILCs in that they may develop from RORγγt$^+$ ILCs whereby downregulation of RORγt and IL-22 production parallels upregulation of CRTH2 and IL-13. The question whether or not IL-17, IL-22−producing ILCs and type 2 ILCs derive from a common RORγt$^+$ precursor should be addressed in mouse models with cell fate mapping experiments.

In summary we describe here a unique human ILC population which is responsive to IL-25 and IL-33 and produces high amounts of IL-13. Compared to what we observed in lung, gut and healthy nasal tissues relatively high proportions of CRTH2$^+$ cells are

present in inflamed nasal polyps of chronic rhinosinusitis, a type 2 immune mediated inflammatory disease[27]. These cells may therefore be the human equivalent of cells recently found in the mouse which were termed natural helper cells, nuocytes or innate helper cell type 2.

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Author contributions

J.M.M. designed the study, did experiments, analyzed the data and wrote the manuscript; S.T. designed the study, did experiments, analyzed the data and wrote the manuscript; N.K.C. did experiments and analyzed the data; C.P.P. did experiments, provided and processed gut tissue; C.M.V.D. provided inflamed and non-inflamed nasal tissue; B.P. provided and processed lung tissue; W.J.F. provided inflamed and non-inflamed nasal tissue; T.C. designed the study; H.S. designed the study and wrote the manuscript

Figure legends

Figure 1 Lineage negative lymphocytes in the fetal gut contain a CRTH2$^+$CD127$^+$ ILC population.

(**a**) Flow cytometry analysis of two distinct ILC populations in the fetal gut. Lin (CD1a, CD3, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA2, FcεR1) negative cells were gated as 1) CD45^{dim} or 2) CD45^{high} and analyzed for expression of CD127 and CD117. (**b**) Flow cytometry characterization of Lin$^-$CD127$^+$CD45^{dim} (grey line) and Lin$^-$CD127$^+$CD45^{high} (black line) as compared to isotype control (shaded light grey). (**c**) Flow cytometry analysis of CRTH2, CD117, CD123 (IL-3R) and FcεR1 expression on CD45^{high}Lin$^-$CD127$^+$ cells (black line) and peripheral blood monocytes or basophils in (light grey thin line). Isotypes are shown in shaded light grey. (**d**) Flow cytometry analysis of RORγt protein expression in CD56^{dim} peripheral blood NK cells (dashed grey line), fetal gut CD45^{high}Lin$^-$CD127$^+$CRTH2$^+$ ILCs (black solid line) and CD45^{dim}Lin$^-$CD127$^+$NKp44$^+$ ILCs (grey solid line). Data are representative of at least 5 fetal donors. Right panel in (**d**) shows *RORC* mRNA expression in fetal gut CRTH2$^+$ ILC (gated CD45^{high}Lin$^-$CD127$^+$) as compared to fetal gut NKp44$^+$ ILCs, NKp44$^-$ ILCs (gated CD45^{dim}Lin$^-$CD127$^+$CD117$^+$), conventional NK cells (cNK, CD45^{high}CD127$^-$CD56$^+$), tonsil NKp44$^+$ ILCs and fetal mesenteric lymph node (MLN) NKp44$^-$ ILCs. Data are shown as median and range (n=2-5).

Figure 2 CRTH2$^+$ fetal gut ILCs express *IL13* transcripts ex vivo.

Fetal gut CRTH2$^+$ ILCs (gated CD45^{high}Lin$^-$CD127$^+$) were purified by flow cytometric sorting and analyzed with real-time PCR for expression of *IL13*, *IL22*, *IL17*, *IFNG* and *TNF* transcripts as compared to the expression in fetal gut NKp44$^+$ ILCs, NKp44$^-$ ILCs (gated CD45^{dim}Lin$^-$CD127$^+$CD117$^+$), conventional NK (cNK, CD45^{high}Lin$^-$CD127$^-$ CD56$^+$) cells, tonsil NKp44$^+$ ILCs and fetal mesenteric lymph node (MLN) NKp44$^-$ ILCs. Lineage cocktail included CD1a, CD3, CD4, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA2 and FcεR1. Data are shown as median and range and were obtained from 2-5 donors.

Figure 3 CRTH2$^+$ fetal gut ILCs respond to IL-25 and IL-33 with production of IL-13 protein in vitro.

(**a**) In experiment 1, fetal gut CD45^{high}Lin$^-$CD127$^+$CRTH2$^+$ ILCs were sorted by flow cytometry and cultured for 4 days with IL-2 (10 U/mL) or a combination of IL-2 and IL-25 (10 U/mL and 50 ng/mL, respectively). (**b**) In experiment 2, CD45^{high}Lin$^-$ CD127$^+$CRTH2$^+$ ILCs were sorted also on basis of CD117 and stimulated as in a. (**c**) Stimulation of fetal gut CD117$^+$CRTH2$^+$ and CD117$^-$CRTH2$^+$ ILCs with IL-2 or a combination of IL-2 and IL-33 (10 U/mL and 50 ng/mL, respectively). Supernatants were analyzed for IL-13 protein by ELISA. Concentrations were normalized for 2000 cells/200 μL in a 96-plate well. Lineage cocktail for flow cytometry sorting included CD1a, CD3, CD4, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA2 and FcεR1.

Figure 4 Analysis of stable cell lines generated from CRTH2$^+$ fetal gut ILCs.

(**a**) Flow cytometric analysis of expanded fetal gut $CD45^{high}Lin^-$ (CD1a, CD3, CD4, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA2, FcεR1) $CD127^+CD117^+CRTH2^+$ ILC cell lines (black lines, isotype controls in shaded light grey). (**b**) Flow cytometry analysis of PMA−Ionomycin stimulated fetal gut $CRTH2^+$ ILC cell lines (black lines, unstimulated cells in shaded light grey) and $CD56^+$ conventional NK cell line (grey lines) stained for intracellular IL-13, IL-22 and IL-17. Far right plot shows PMA−ionomycin stimulated fetal gut $CD127^+CD117^+CRTH2^+$ ILC cell line. Data are representative of 2-3 fetal donors. (**c**) Expression of *AHR* mRNA in fetal gut $CRTH2^+$ ILC, blood $CD56^{dim}$ conventional NK cells (cNK) and tonsil $NKp44^+$ ILC cell lines (data shown as median and range, n=3-4). (**d**) IL-22 secretion from fetal gut $CRTH2^+$ ILC or (**e**) tonsil $NKp44^+$ ILC cell lines following stimulation with IL-1β (50 ng/mL), IL-23 (50 ng/mL) and combinations of these (data shown as mean and standard error, n=3-4). (**f**) Expression of *ST2*, *IL17RB*, *IL17RA* and *IL23R* mRNA in fetal gut $CRTH2^+$ ILC, blood $CD56^{dim}$ conventional NK cell (cNK) and tonsil $NKp44^+$ ILC cell lines (data shown as median and range, n=3-5). (**g**) IL-13 response of fetal gut $CRTH2^+$ ILCs to IL-25, IL-33 and combinations of these (n=3).

Figure 5 $CRTH2^+$ ILCs are distributed in several fetal and adult tissues and are enriched in nose polyps of chronic rhinosinusitis (CRS) patients.

(**a**) Mononuclear cells were isolated from fetal gut, fetal lung, adult gut and adult lung, stained for ILCs and analyzed by flow cytometry. ILCs were gated as $CD45^+Lin^-$(CD1a, CD3, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA2, FcεR1) $CD127^+$. Expression of CD117 and CRTH2 is shown as percentages of the Lin^-CD127^+ gate. Data

are representative of 2-5 donors. (**b**) Shows CRTH2$^+$ ILCs in healthy control (HC) nasal tissue (left dot plot) and chronic rhinosinusitis (CRS) nasal polyps (right dot plot). Differences in CRTH2$^+$ ILC frequencies were calculated using Mann-Whitney two-tailed test. ILCs were defined as CD45$^+$Lin$^-$ (CD1a, CD3, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA2, FcεR1) CD127$^+$ and analyzed for expression of (**c**) CD161 and (**d**) RORγt. The figures in c-d are representative of 4 CRS patients.

Figure 6 CRTH2$^+$CD127$^+$CCR6$^+$ innate lymphoid cells are present in peripheral blood.

(**a**) Flow cytometric analysis of peripheral blood cells that were pre-depleted from the majority of T, B cells and monocytes; gating shows the presence of a Lin$^-$CD127$^+$CD117$^+$ and a Lin$^-$CD127$^+$CD117$^-$ populations (right plot); (**b**) flow cytometric analysis of the indicated markers in the Lin$^-$CD127$^+$CD117$^+$ and a Lin$^-$CD127$^+$CD117$^-$ populations, gated as in (**a**); Shaded grey indicate isotype control Ab; (**c**) Flow cytometric analysis of CCR6 versus CRTH2 expression in Lin$^-$CD127$^+$CD117$^+$ and a Lin$^-$CD127$^+$CD117$^-$ cells, gated as in a; (**d**) Expression of RORγt protein in lin$^-$CD127$^+$CRTH2$^+$ ILCs (black line), conventional CD56$^+$ NK cells (dashed grey line) and T_H17 (CD3$^+$CD4$^+$CCR4$^+$CCR6$^+$) cells (solid grey line). All plots are representative of at least 3 independent donors.

Figure 7 CRTH2$^+$ peripheral blood ILCs respond to IL-25 and IL-33 with production of IL-13 protein.

PBMC were depleted from T (CD3), B (CD19), NK (CD16) cells and monocytes (CD14) using magnetic beads. $Lin^-CD127^+CRTH2^+$ and $Lin^-CD127^+CRTH2^-$ cells were sorted and cultured for 4 days with (**a**) IL-2 (10 U/mL) or a combination of IL-2+IL-25 (10 U/mL+50 ng/mL) and (**b**) IL-2 (10 U/mL) or a combination of IL-2+IL-33 (10 U/mL+50 ng/mL). Supernatants were analyzed for IL-13 protein by ELISA. Concentrations were normalized for 2000 cells/200 μL. Data shown as mean and standard error (n=2-6). Lineage cocktail used for sorting included CD1a, CD3, CD4, CD11c, CD14, CD19, CD34, CD56, CD94, CD123, TCRαβ, TCRγδ, BDCA2 and FcεR1.

Figure 8 Stable cell lines can be generated from $CRTH2^+$ peripheral blood ILCs.

(**a**) Flow cytometry analysis of peripheral blood Lin^- (CD1a, CD3, CD4, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA2, FcεR1) $CD117^+CD127^+CRTH2^+$ ILC cell lines (black lines, isotype controls in shaded light grey) (**b**) *RORC* mRNA expression in $CRTH2^+$ cell lines, freshly isolated fetal gut $CRTH2^+$ ILCs, $CD56^+$ NK cell (cNK) and $NKp44^+$ ILCs. (**c**) Flow cytometry analysis of PMA−Ionomycin stimulated $CD117^+CD127^+CRTH2^+$ ILC cell lines (black lines, unstimulated cells in shaded light grey) and $CD56^{dim}$ NK cell lines (grey lines) stained for intracellular IL-13, IL-22 and IL-17. Far right plot shows PMA−ionomycin stimulated $CRTH2^+$ ILC cell line. Data are representative of 3 donors. (**d**) Expression of *ST2*, *IL17RB*, *IL17RA* and *IL23R* mRNA in blood $CRTH2^+$ ILCs, blood $CD56^{dim}$ conventional NK cell (cNK) and tonsil $NKp44^+$ ILC cell lines (data shown as median and range, n=1-5). (**e**) IL-13 response of $CRTH2^+$ ILCs to the indicated cytokines (n=3). (**f**) Expression of *AHR* mRNA in $CRTH2^+$ ILC, blood $CD56^{dim}$ conventional NK cells (cNK) and tonsil $NKp44^+$ ILC cell lines (data shown as

median and range, n=2-5). (**g**) IL-22 secretion from CRTH2^{+} ILC cell lines upon stimulation with the indicated cytokines (data shown as mean and standard error, n=2).

Table I. Tissue distribution of $CD45^+Lin^-CD127^+CRTH2^+$ ILCs

Tissue	$CD45^+Lin^-CD127^+CRTH2^+$ % of $CD45^+$ (range)
Fetal gut (*n*=5)	0.5-2.3
Fetal lung (*n* =2)	0.2-0.3
Adult ileum (*n* =5)	0.01-0.1
Adult lung (*n* =3)	0.02-0.08
Adult blood (*n* =3)	0.01-0.03
Adult inflamed nose polyp (*n* =4)	0.1-3.6
Adult non-inflamed nose tissue (*n* =4)	0-0.1

Supplementary table I. Sequences of real-time PCR primers designed in house.

mRNA	**Primer**	**Sequences (5′-3′)**
18S rRNA	Forward primer	AAT CTG GAG CTG GCC TTT CA
	Reverse primer	CTG GAA GAT CTG CAG CCT TT
IL5	Forward primer	AGC TGC CTA CGT GTA TGC CA
	Reverse primer	CAG GAA CAG GAA TCC TCA GA
IL13	Forward primer	ATT GCT CTC ACT TGC CTT GG
	Reverse primer	GTC AGG TTG ATG CTC CAT ACC
*TNF**	Forward primer	TGC TTG TTC CTC AGC CTC TT
	Reverse primer	TGG GCT ACA GGC TTG TCA CT
*IL17RA***	Forward primer	ATC CTG CTG GTG GGC TCC GT
	Reverse primer	ACG TAG AGG GGG TGG TCG GC
IL17RB	Forward primer	CCA ACA CAG CAC TAT CAT CG
	Reverse primer	ATA TGG AGT CAG CTG CAC CG
IL1RL1 (*ST2*)	Forward primer	ATG TTC TGG ATT GAG GCC AC
	Reverse primer	GAC TAC ATC TTC TCC AGG TAG CAT
IL23R	Forward primer	AAC AAC AGC TCG GCT TTG GT
	Reverse primer	GGA ATA TCT GGC GGA TAT CC
AHR	Forward primer	CTT AGG CTC AGC GTC AGT TA
	Reverse primer	GTA AGT TCA GGC CTT CTC TG
RORC	Forward primer	AAT CTG GAG CTG GCC TTT CA
	Reverse primer	CTG GAA GAT CTG CAG CCT TT

* Kindly provided by M. Vondenhoff at the Academic Medical Center, Amsterdam
** Kindly provided by A. te Velde at the Academic Medical Center, Amsterdam

METHODS

Fetal and adult tissues

Human fetal tissues were obtained from elective abortions at the Stichting Bloemenhove clinic in Heemstede, the Netherlands, upon on the receipt of informed consents. The use of human abortion tissues was approved by the Medical Ethical Commission of the Academic Medical Center, Amsterdam. Gestational age was determined by ultrasonic measurement of the diameter of the skull or femur and ranged from 14-17 weeks.

Adult non-inflamed nose conchae tissue was obtained from healthy individuals. Inflamed nose polyps were from chronic rhinosinusitis patients. Adult lung tissues were obtained after informed consents from patients undergoing lung tumor surgery, where the tissues were obtained at a clear distance from the tumor. Collection of lung and nose tissue was approved by the Medical Ethical Commission of the Academic Medical Center, Amsterdam. Inflamed ileum tissue was collected from Crohn's disease patients undergoing resection surgery. Non-inflamed ileum was obtained from patients undergoing colon tumor resection surgery from which ileum tissue was collected at a clear distance from the tumor. Both inflamed and non-inflamed ileum tissue was obtained as residual material after clinical procedures according to the ethical guidelines at the Academic Medical Center (AMC), Amsterdam, the Netherlands.

Buffy coats were provided by the blood bank at Sanquin, Amsterdam or, alternatively, from the Blood Centers of the Pacific (CA, USA) after written informed consent.

Isolation of cells

All solid tissues were rinsed of connective tissue, fat and muscle. Intestinal tissues were also cleared from meconium after which the adult ileum tissue was incubated with dithiothreitol (154 μg/ml), 0.1% β-mercaptoethanol and 5 mM EDTA for elimination of epithelial cells and mucus. The tissues were cut into fine pieces and digested for 30-45 min at 37 °C with Liberase TM (125 μg/ml) and DNase I (200 μg/ml) (both from Roche). Lung cells were isolated by incubating the tissue with 50 U/ml DNAse type I (Sigma-Aldrich) and collagenase type I 300 U/ml (Worthington). The cell suspensions were filtered through a 70-μm nylon mesh or equivalent and mononuclear cells were isolated using Ficoll Paque Plus (GE Healthcare). PBMC were isolated on Lymphoprep (Nycomed) or Ficoll-Paque.

Flow cytometric analysis and sorting

The following anti-human antibodies were used (clone name within brackets):

Fluorescein isothiocyanate (FITC) - conjugated anti-CD1a (HI149), CD3 (OKT3), CD11c (3.9), CD94 (DX22), CD123 (6H6), FcER1α (AER-37), phycoerythrin (PE) - anti-CD7 (CD7-6B7), CD94 (HP-3D9), CD161 (HP-3G10), KIR3DL1 (DX9), perforin (DG9), anti-NKp44 (P44-8), peridinin chlorophyll protein–cyanine 5.5 –conjugated anti-CD117 (104D2), Alexa Fluor 647 (AF647)–conjugated anti-CD25 (BC96), NKp46 (9E2), NKp44 (P44-8), NKp30 (P30-15), Granzyme B (GB11), CCR7 (TG8/CCR7), CCR6 (TG7/CCR6), allophycocyanin (APC)-conjugated anti-NKG2D (1D11), Alexa Fluor 700–conjugated anti-CD56 (HCD56), (all from Biolegend), biotinylated anti-human CCR6 (11A9) in combination with streptavidin Horizon V450 (BD), FITC-

conjugated anti-CD4 (RPA-T4), CD14 (MφP9), CD16 (3G8), CD19 (HIB19), CD34 (581), CD56 (NCAM16.2), TCRαβ (IP26), TCRγδ (B1), PE-conjugated anti-CD16 (3G8), CCR4 (1G1), CXCR3 (IC6/CXCR3), AF647-conjugated anti-CRTH2 (CD294; BM16), CXCR5 (RF8B2), APC-cyanine 7 (Cy7) anti-CD45 (2D1) and isotypes Alexa Fluor 700 (MOPC-21), PE, APC, PECy7 (X40) (all from Beckton Dickinson), APC-conjugated anti-CD4 (S3.5) (Invitrogen), PE-conjugated anti-CD45RA (ALB11), PE-cyanine 7 (Cy7) anti-human CD127 (R34.34) (Beckman Coulter), PE-conjugated anti-RORγt (AFKJS-9) (eBioscience), PE-conjugated anti-IL-1R1 (goat polyclonal) (R&D), FITC-conjugated anti-human BDCA2 (CD303; AC144; Milenyi).

For flow cytometric phenotype analysis, data were acquired on an LSRFortessa or LSRII (BD) and analyzed with FlowJo software (TreeStar, Inc.).

For flow cytometry sorting, PBMC were depleted of T, B, NK cells and monocytes by labeling with FITC-conjugated anti-CD3, CD14, CD16 and CD19 antibodies (described above) plus anti-FITC microbeads (Miltenyi) or the corresponding EasySep antibodies plus beads (StemCell Technologies). $Lin^{-}CD127^{+}CRTH2^{+}$ ILCs from fetal gut and peripheral blood were sorted on a FACSAria (BD) to ≥ 98% purity.

Establishment of $CD127^{+}CRTH2^{+}$ cell lines and analysis of cytokine production

$Lin^{-}CD127^{+}CRTH2^{+}$ ILCs and NK cells were expanded with irradiated allogeneic PBMC (25 Gy), irradiated JY EBV-transformed B cells (50 Gy), 1 μg/ml PHA (Oxoid) and 100 U/ml IL-2 (Novartis) in Yssel's Medium (AMC or Genentech, in-house made) supplemented with 1% Human AB Serum. Fresh and expanded $Lin^{-}CD127^{+}CRTH2^{+}$ ILCs and NK cells were stimulated for 3-4 days with IL-2 (10 U/ml, Novartis), IL-25 (50

ng/ml; R&D Systems), IL-33 (50 ng/ml; R&D Systems) and combinations of these cytokines. Fresh cells were stimulated with PMA plus ionomycin for 24 h. Expanded cells were stimulated with IL-2, IL-1β (Miltenyi), IL-23 (R&D) or combinations of these cytokines for 3-4 days. Supernatants were analyzed for presence of IL-13 and IL-22 with ELISA (Sanquin and Genentech-Roche in-house or R&D, respectively). Multiple cytokine detection was performed in some experiments using Luminex (Biorad).

Intracellular cytokine staining

E*x vivo* expanded cell lines were stimulated for 6 h with 10 ng/ml PMA (Sigma) and 500 nM ionomycin (Merck) in the presence of Golgiplug (BD) or 5 □g/ml BFA for the final 4 hours of culture. Cell permeabilization, staining, and subsequent washings were performed using the Cytofix/cytoperm kit (BD). The following antibodies were used: APC-conjugated anti-IL-13 (JES10-5A2, BioLegend), APC-conjugated IL-17 (BL168, BioLegend) and PE or Alexa647–conjugated anti-IL-22 (142928, R&D or 3F11, Genentech-Roche[36], respectively), anti-IFN-γ (B27. BD Bioscience) or anti-TNF (MAb11, BD Bioscience). Data were acquired on an LSRFortessa or LSRII (BD) and analyzed with FlowJo software (Tree Star, Inc.).

Quantitative real-time PCR

Total RNA was extracted using the RNeasy micro kit (QIAGEN) or the Nucleospin RNA XS kit (Macherey-Nagel) according to the manufacturer's instructions. Complementary DNA was produced using the high-capacity cDNA archive kit (Applied Biosystems). PCR was performed using SYBR Green I master mix (Roche) on a LightCycler 480

Instrument II (Roche). In house designed primers are given in **Supplementary Table 1**. Primers for *IL17*, *IL22* and *IFNG* were used as previously published[4]. The LinRegPCR software[37,38] was used for quantification of expression. All samples were normalized using 18S rRNA expression and expressed in arbitrary units.

Statistical analysis

Differences in nasal tissue $CRTH2^+$ ILC frequencies between healthy controls and chronic rhinosinusitis patients were calculated using two-tailed Mann-Whitney U-test.

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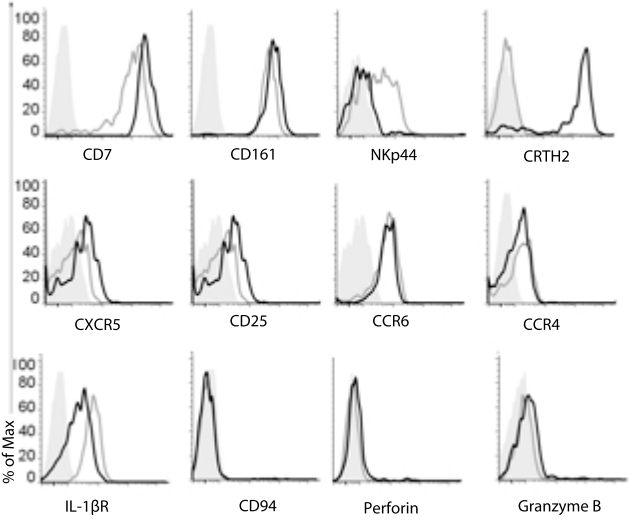
Lineage
CD45
1
2
1) CD45dim
2
90
1
7
CD117
CD127
2) CD45hi
1
29
66
4
CD117
CD127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80
60
40
20
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CD161
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CRTH2
CXCR5
CD25
CCR6
CCR4
IL-1βR
CD94
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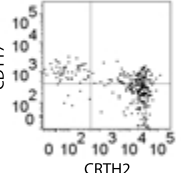
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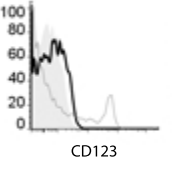
10^4
10^3
10^2
10^1
10^0
0 10^2 10^3 10^4 10^5
CRTH2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60
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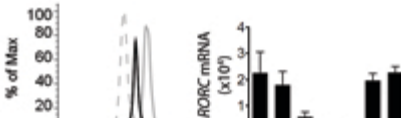
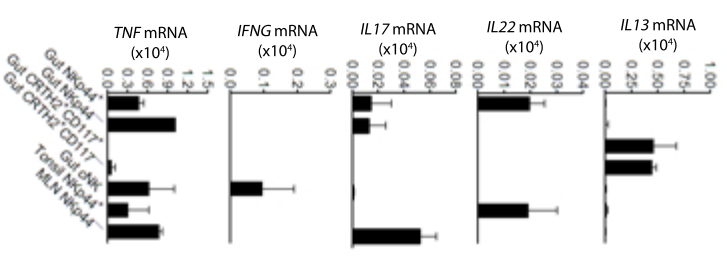
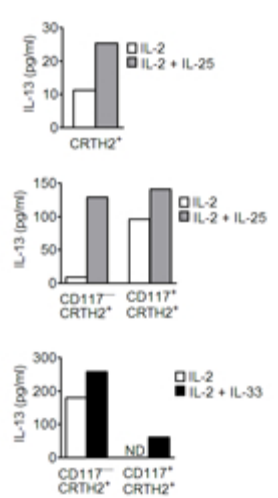
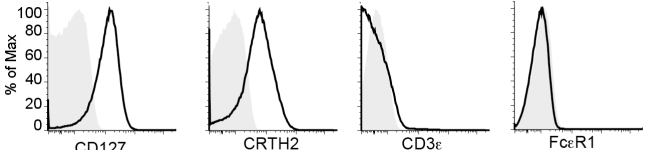
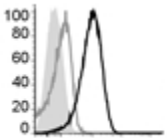
20
0
CD123
FcεR1

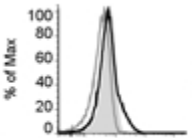

100
80
60
40
20
0
% of Max
10¹ 10² 10³ 10⁴
RORγt
RORC mRNA (x10⁴)
4
3
2
1
0
Gut NKp44⁺
Gut NKp44⁻
Gut CRTH2⁺CD117⁺
Gut CRTH2⁻CD117⁺
Gut cNK
Tonsil NKp44⁺
MLN NKp44⁻

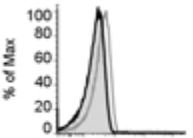

TNF mRNA (x10⁴)
IFNG mRNA (x10⁴)
IL17 mRNA (x10⁴)
IL22 mRNA (x10⁴)
IL13 mRNA (x10⁴)
Gut NKp44⁻
Gut NKp44⁺
Gut CRTH2⁻ CD117⁺
Gut CRTH2⁺ CD117⁺
Gut cNK
Tonsil NKp44⁺
MLN NKp44⁺
0.0
0.3
0.6
0.9
1.2
1.5
0.0
0.1
0.2
0.3
0.00
0.02
0.04
0.06
0.08
0.00
0.01
0.02
0.03
0.04
0.00
0.25
0.50
0.75
1.00

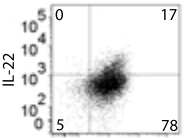

IL-13 (pg/ml)
30
20
10
0
IL-2
IL-2 + IL-25
CRTH2+
IL-13 (pg/ml)
150
100
50
0
IL-2
IL-2 + IL-25
CD117−
CRTH2+
CD117+
CRTH2+
IL-13 (pg/ml)
300
200
100
0
IL-2
IL-2 + IL-33
ND
CD117−
CRTH2+
CD117+
CRTH2+

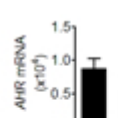

% of Max
100
80
60
40
20
0
CD127
CRTH2
CD3ε
FcεR1


% of Max
100
80
60
40
20
0
0 10^2 10^3 10^4 10^5
IL-13


% of Max
100
80
60
40
20
0
0 10^2 10^3 10^4 10^5
IL-22

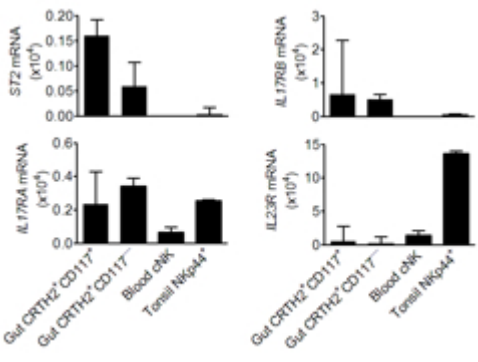

% of Max
100
80
60
40
20
0
0 10^2 10^3 10^4 10^5
IL-17


IL-22
10^5
10^4
10^3
10^2
0
0
17
5
78
0 10^2 10^3 10^4 10^5
IL-13

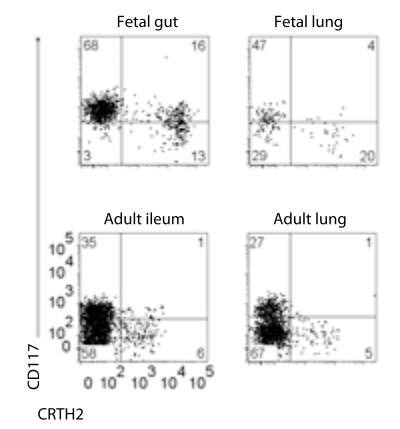

AHR mRNA
(x10⁴)
1.5
1.0
0.5
0.0
Gut CRTH2⁺ CD117⁺
Gut CRTH2⁺ CD117⁻
Blood cNK
Tonsil NKp44⁺

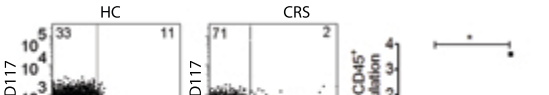

IL-22 (pg/ml)
400
300
200
100
0
IL-2
IL-1β
IL-23
IL-2 + IL-1β
IL-2 + IL-23
IL-1β + IL-23
IL-2 + IL-1β + IL-23

IL-22 (ng/ml)
4
3
2
1
0
IL-2
IL-1β
IL-23
IL-2 + IL-1β
IL-2 + IL-23
IL-1β + IL-23
IL-2 + IL-1β + IL-23


ST2 mRNA (x10⁴)
0.20
0.15
0.10
0.05
0.00
IL17RB mRNA (x10⁴)
3
2
1
0
IL17RA mRNA (x10⁴)
0.6
0.4
0.2
0.0
IL23R mRNA (x10⁴)
15
10
5
0
Gut CRTH2⁺ CD117⁺
Gut CRTH2⁺ CD117⁻
Blood cNK
Tonsil NKp44⁺
Gut CRTH2⁺ CD117⁺
Gut CRTH2⁺ CD117⁻
Blood cNK
Tonsil NKp44⁺


IL-13 (ng/ml)
4
3
2
1
0
IL-2
IL-25
IL-33
IL-2 + IL-25
IL-2 + IL-33
IL-25 + IL-33
IL-2 + IL-25 + IL-33

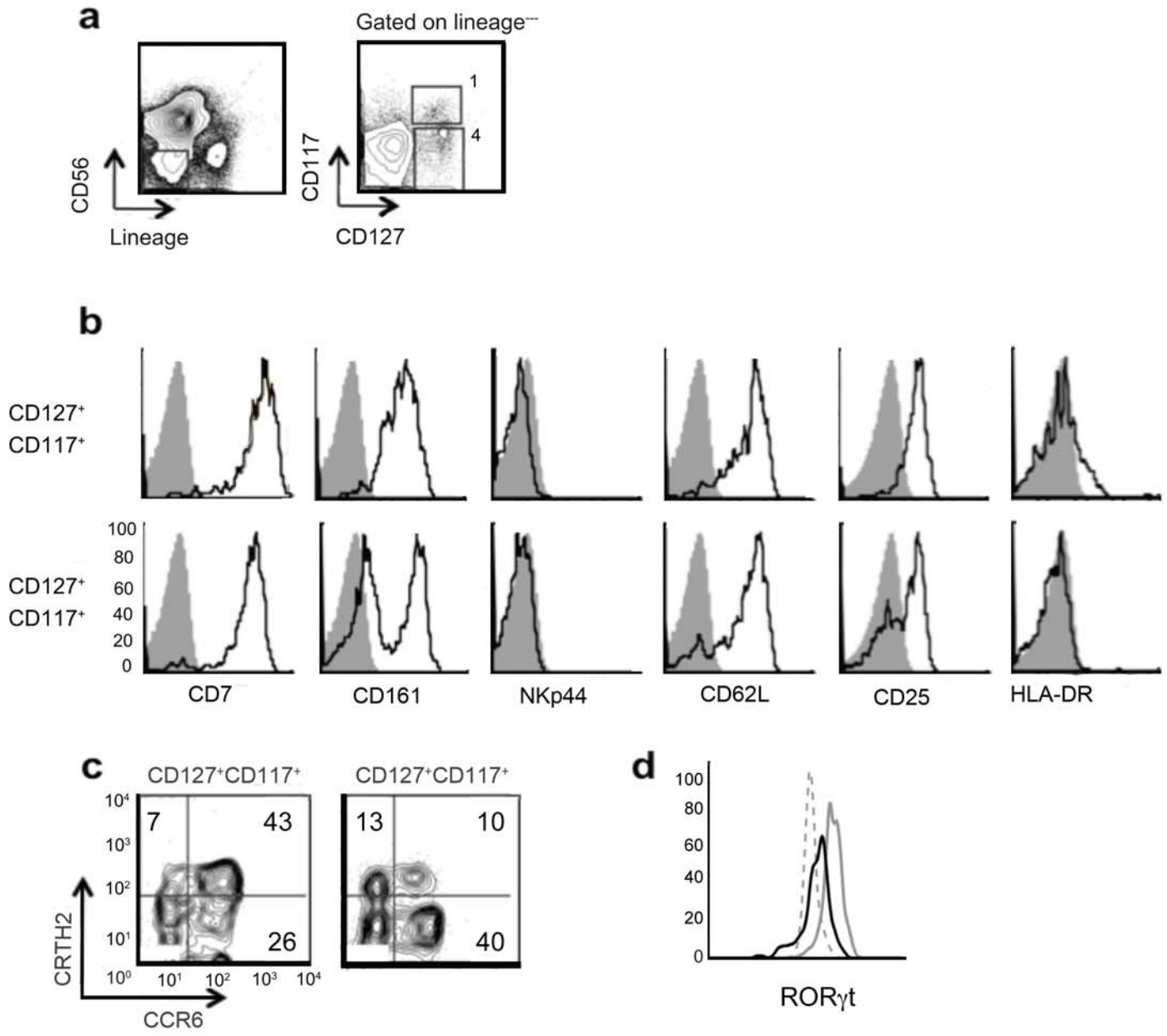

Fetal gut
68
16
3
13
Fetal lung
47
4
29
20
Adult ileum
35
1
58
6
Adult lung
27
1
67
5
CD117
CRTH2
0
10^2
10^3
10^4
10^5


HC
CRS
CD117
CRTH2
33
11
24
32
71
2
25
2
% of CD45+ population
HC
CRS
*

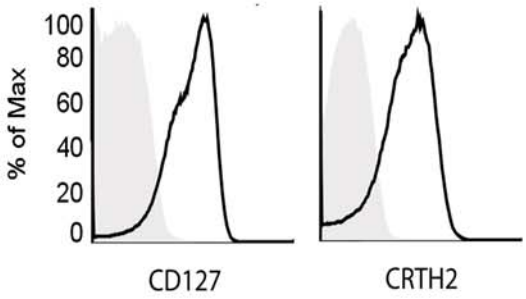
CD161

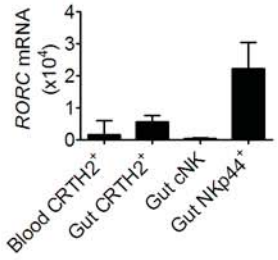
100
80
60
40
20
0
% of Max
RORγt
Nose polyp CRTH2+ ILC
Fetal gut CRTH2+ ILC
Peripheral cNK
Fetal gut NKp44+ ILC

Figure 6

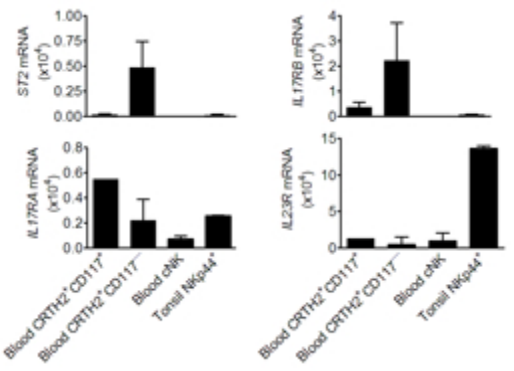

a
Gated on lineage⁻⁻⁻
1
4
CD56
Lineage
CD117
CD127
b
CD127⁺
CD117⁺
CD127⁺
CD117⁺
100
80
60
40
20
0
CD7
CD161
NKp44
CD62L
CD25
HLA-DR
c
CD127⁺CD117⁺
CD127⁺CD117⁺
7
43
26
13
10
40
CRTH2
CCR6
d
100
80
60
40
20
0
RORγt

IL-13 (pg/ml)
80
60
40
20
0
IL-2
IL-2 + IL-25
CD117⁻ CRTH2⁺
CD117⁺ CRTH2⁺
CD117⁺ CRTH2⁻
IL-13 (pg/ml)
200
150
100
50
0
IL-2
IL-2 + IL-33
CD117⁻ CRTH2⁺
CD117⁺ CRTH2⁻

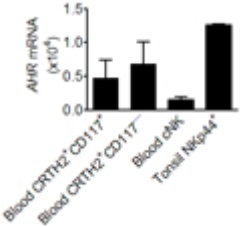

100
80
60
40
20
0
% of Max
CD127
CRTH2

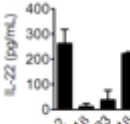

RORC mRNA (x10^-4)
4
3
2
1
0
Blood CRTH2+
Gut CRTH2+
Gut cNK
Gut NKp44+


% of Max
100
80
60
40
20
0
IL-13
IL-22
IL-17
IL-22
IL-13
0
29
4
67
10^5
10^4
10^3
10^2
0
0 10^2 10^3 10^4 10^5


ST2 mRNA (x10⁴)
1.00
0.75
0.50
0.25
0.00
IL17RB mRNA (x10⁴)
4
3
2
1
0
IL17RA mRNA (x10⁴)
0.8
0.6
0.4
0.2
0.0
IL23R mRNA (x10⁴)
15
10
5
0
Blood CRTH2+ CD117+
Blood CRTH2+ CD117−
Blood cNK
Tonsil NKp44+


IL-13 (ng/ml)
15
10
5
0
IL-2
IL-25
IL-33
IL-2 + IL-25
IL-2 + IL-33
IL-25 + IL-33
IL-2 + IL-25 + IL-33


AHR mRNA (x10⁻³)
1.5
1.0
0.5
0.0
Blood CRTH2+ CD117+
Blood CRTH2+ CD117−
Blood cNK
Tonsil NKp44+


IL-22 (pg/mL)
400
300
200
100
0
IL-2
IL-1β
IL-23
IL-2 + IL-1β
IL-2 + IL-23
IL-1β + IL-23
IL-2 + IL-1β + IL-23