Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Eur J Immunol. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Eur J Immunol. 2015 August ; 45(8): 2335–2342. doi:10.1002/eji.201445213.

Expression and Function of The TNF-Superfamily Receptor DR3 in Human Group 3 Innate Lymphoid Cells

Yong-Oon Ahn[1,3], **Matthew A. Weeres**[1], **Marie-Luise Neulen**[1], **Jahyang Choi**[3], **Seong-Ho Kang**[3], **Dae Seog Heo**[3,4], **Rachel Bergerson**[1], **Bruce R. Blazar**[1], **Jeffrey S. Miller**[2], and **Michael R. Verneris**[1]

[1]Department of Pediatrics, Division of Blood and Marrow Transplantation University of Minnesota, USA

[2]Department of Medicine, Division of Blood and Marrow Transplantation University of Minnesota, USA

[3]Cancer Research Institute, Seoul National University College of Medicine and Hospital Seoul, Korea

[4]Department of Internal Medicine, Seoul National University College of Medicine and Hospital Seoul, Korea

Abstract

Death receptor 3 (DR3, TNFRSF25) is expressed by activated lymphocytes and signaling by its ligand, TL1A, enhances cytokine expression and proliferation. Recent studies show that DR3 is also present on murine type 2 innate lymphoid cells (ILC2s). Here, we show that DR3 is expressed by IL-22 producing human group 3 innate lymphoid cells (ILC3s). Stimulation of ILC3 cells with exogenous TL1A alone had no impact on cytokine production or proliferation. Addition of TL1A to IL-1β + IL-23 significantly enhanced the amount IL-22 produced by ILC3 cells as well as the percentage IL-22 and IL-8 producing cells. Addition of TL1A to IL-1β + IL-23 also augmented ILC3 proliferation in short term (5 day assays). Mechanistically, this occurred through the up-regulation of CD25 and responsiveness to IL-2 stimulation. The combination of TL1A, IL-1β+ IL-23 and IL-2 expanded ILC3 cells (39.3 fold) while IL-1β+ IL-23 did not increase proliferation above controls. After two weeks of expansion, ILC3 cells maintained their phenotype, transcription factor expression and function (IL-22 production). These findings identify DR3 as a costimulatory molecule on ILC3 cells that can be exploited for ex vivo expansion and clinical use.

Introduction

The tumor necrosis factor (TNF) superfamily-associated receptors and ligands mediate a variety of essential activities within the immune system. Death Receptor 3 (DR3 or TNFRSF25) is a member of this family which bears the greatest homology to TNF. The only ligand for DR3 is TNF-like protein 1A (TL1A, TNFSF15) which shows restricted expression, mainly in the gastrointestinal tract and is produced by macrophage and dendritic

Correspondence: Verneris@umn.edu.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

cells at inflammatory sites[1, 2] or in response to FcγR signaling[3]. DR3 is expressed by a variety of lymphocytes including T, NK and NKT cells where it modulates activation. For instance, in peripheral T cells, DR3 expression is increased upon T cell receptor ligation and DR3:TL1A interaction lead to proliferation and inflammatory cytokine production [4]. In experimental models, DR3 signaling also augments antiviral immune T cell responses[5]. Following IL-12/18-activation DR3 is induced on NK cells and TL1A enhances IFN-γ production [6] and cytotoxicity [7]. Tregs constitutively express DR3, and agonist antibodies induce *in vivo* Treg expansion, amplifying IL-2 responsiveness [8, 9].

A Th2 and/or Th17-dependent pathological role of DR3 and TL1A interaction is also clear. Murine NKT cells constitutively express DR3 and in allergic pulmonary inflammatory models, TL1A costimulates IL-5 and IL-13 production [10]. TL1A transgenic mice develop IL-13-dependent small intestine inflammation [11, 12], and in other models TL1A blockade attenuates chronic colitis by modulating Th1 and Th17 cells [13]. In Th17-dependent autoimmune diseases, DR3 mediated signaling worsens pathology [14]. Whether TL1A:DR3 interactions drive Th17 T cell differentiation per se is controversial. In $TL1A^{-/-}$ mice experimental allergic encephalomyelitis was attenuated due to a reduction in Th17 T cells; suggesting a role for TL1A in Th17 polarization or expansion[14]. However, other studies show that Th17 cell polarization does not require TL1A signaling [15] and that these interactions (DR3:TL1A) can inhibit the differentiation of naïve T cells into Th17 cells [16]. Therefore, DR3 may regulate the function and proliferation of fully committed Th17 cells.

Innate lymphoid cells (ILCs) are Id2-precursor derived lymphoid cells that lack rearranged antigen receptors [17, 18]. Like T helper cells, ILCs can be subdivided based on transcription factor and cytokine expression which dictates function. ILC1 cells express T-bet and produce inflammatory cytokines such as IFN-γ upon activation. ILC2 cells are characterized by GATA3 expression and production of IL-5 and IL-13 in response to parasitic infections. ILC3 cells express the RAR-related orphan receptor γt (ROR-γt) transcription factor and produce IL-22 and/or IL-17A upon stimulation with IL-1β and IL-23. In humans, there are a number of different ILC3 subtypes including lymphoid tissue inducer (LTi) cells, found in fetal tissues and IL-22-producing innate lymphoid cells, present in adult secondary lymphoid tissues [18]. Fetal ILC3 cells orchestrate SLT organogenesis during fetal life. In adult life, ILC3 cells are thought to contribute to the regeneration or maintenance of injured SLTs as well as the maintenance of mucosal integrity through IL-22 production [19, 20]. ILC3 rarely circulate in the peripheral blood and thus, studies of human ILC3 cells have been mainly performed on tissues obtained at the time of surgery for other pathological conditions. We previously reported a strategy to generate ILC3 cells *in vitro* from $CD34^{+}$ cells [21]. In humans, ILC3 cells have a phenotype that overlaps with stage III natural killer (NK) cell progenitors [22]. Using this developmental system we demonstrated that ILC3 and conventional NK (cNK) cells have distinct phenotypes and developmental cytokine requirements. More specifically, cNK cells express CD7, CD94 and LFA-1 and require IL-15 for their differentiation, while in contrast, ILC3 cells lack these and instead require IL-7 and SCF for development [23]. Upon activation with IL-1β and IL-23, ILC3 cells produce IL22, GM-CSF and IL-8 and express TNF-superfamily members (OX40L, and BAFF) [23].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Recently, two studies show that murine ILC2 cells also express DR3. Stimulation of mouse ILC2 cells with TL1A led to cytokine production (IL-5 and IL-13), proliferation, exacerbation of experimental, Th2-driven allergic lung disease and augmentation of function (i.e., eradication of infectious pathogens). These studies also indicate that human ILC2 cells also express and respond to ligation of DR3 [24, 25]. Prior studies in mice, show that fetal $CD4^+CD3^-$ LTi cells (a subset of ILC3 cells) express DR3 [26], however the functional consequence of DR3 signaling was not investigated. Recently, DR3 was found to be expressed on murine and human ILC3 cells [2]. In this study, we show that DR3 is expressed by human ILC3 cells and that DR3:TL1A signaling significantly increased cytokine production and cell proliferation. Mechanistically, this occurs through the upregulation of CD25 and responsiveness to IL-2 stimulation. TL1A expanded ILC3 cells maintain their phenotype and function. Collectively, these studies show that DR3:TL1A axis costimulates ILC3 cells and may be exploited to expand ILC3 cells for clinical use.

Materials and Methods

Generation and purification of ILC3 and NK cells from $CD34^+$ precursor cells

ILC3 and cNK cells were generated from $CD34^+$ cells as previously described by us [21, 23]. Briefly, umbilical cord blood-derived $CD34^+$ cells were isolated (Miltenyi Biotec) and cocultued with irradiated murine stromal line EL08.1D2 cells. Recombinant human IL-3 (for the first week), IL-7, IL-15, FLT3L, and SCF were added weekly with fresh media via demidepletion. At D21-28, ILC3 cells and cNK cells are separated based on their phenotypes using negative selection [23].

Flow cytometry

Antibodies against IL-22, IL-8, and CD117 were purchased from ebioscience, and all other antibodies used in this study are from BD Biosciences. Intracellular IL-22 and IL-8 staining was performed after stimulation with rhIL-1β and IL-23 (10ng/ml each, from R&D systems) and/or TL1A (20ng/ml, from PeproTech) for 6 hours in the presence of monemsin (GolgiStop from BD Biosciences). Intracellular staining was performed with cytofix/cytoperm (from BD). To detect phosphorylated proteins, isolated ILC3 and cNK cells were allowed to rest for 2 hours without cytokines, then stimulated with rhTL1A or IL-1β and IL-23 or both for 15 minutes. After this, phosphorylated proteins were detected by FACS. To detect DR3 in ILC3 and cNK cells, cells were incubated with biotinylated antibody against DR3 or control biotinylated goat IgG (from R&D Systems, BAF943 and BAF108, respectively) followed by incubation with streptavidin-PE (Biolegned).

IL-22 ELISA

IL-22 production was also measured using ELISA (R&D Systems). MACS-purified ILC3 cells were seeded in a 96-well plate in 200ul of fresh B0 media. Cells were stimulated with rhIL-1β (10ng/ml), rhIL-23 (10ng/ml) and/or rhTL1A (20ng/ml) for 16 hrs. Media was harvested and IL-22 was measured according to manufacturer's specifications, differences were determined using paired t-test.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Cell proliferation assay

ILC3 cells were isolated from d21-28 cultures as previously described [21, 23] and resuspended with fresh media and stimulated with IL-1β and IL-23 (10ng/ml each) and/or TL1A (20ng/ml) in the presence of rhIL-15 (10ng/ml) overnight as a survival factor. After 16hrs, cells were harvested, washed and resuspended with fresh media and further cultured with 1,000IU/ml of rhIL-2 (R&D systems) for an additional two weeks. Total cell number was determined by trypan blue exclusion, and phenotype was analyzed by FACS. In some experiments, cells were stained with CFSE (carboxyfluorescein diacetate, succinimidyl ester, Molecular Probes) after overnight stimulation, and then cultured with IL-2. CFSE dilution was measured after 5 days. The percentage of cells that divided was calculated using Flowjo software.

Isolation of ILC3 cells from primary human tonsillar cells

De-identified surgically resected human tonsillar tissues were finely cut to small fragments, transferred on a 70μm strainer and rendered to a single cell suspension using a syringe plunger. Mononuclear cells were harvested after ficoll sedimentation, and $CD3^+$, $CD14^+$, and $CD19^+$ cells were depleted by magnetic bead depletion. Cells were washed, resuspended with media containing 10% AB- sera; but no other cytokines and rested overnight. For surface staining of DR3, ILC3 cells were identified as previously described [27]. Briefly, mononuclear cells were stained with the following antibodies directed toward lineage antigens (CD3, CD19, CD94, CD1a, CD11c, CD123, and BDCA2, CD14, FcεR1, and CD34), CD127, CD117, NKp44, and CRTH2. For PCR experiments, ILC3 cells were isolated by FACS sorting using a combination of NKp44, CD94, LFA-1, CD3, CD14 and CD19. RNA was extracted from ILC3 cells for quantitative RT-PCR (qRT-PCR).

qRT-PCR

Expression levels of ROR-γt, AhR, EOMES, and DR3 were measured in purified ILC3 cells, cNK cells, and expanded ILC3 cells as previously described [23]. Predesigned TaqMan Gene Expression Assays primer/probe mix Hs00600930_g1 (Applied Biosystems) was used for measuring DR3 (targeting exon 3–4). Transcripts were analyzed by the ΔΔCt method and normalized to 18S rRNA.

Statistical Analysis

Statistical analyses were performed using the paired Student's t-test.

Results

ILC3 cells constitutively express DR3

Human ILC3 cells and cNK cells were generated from $CD34^+$ cells as we previously described [21]. These two populations can be discriminated using FACS since ILC3 cells are $CD56^+LFA\text{-}1^-CD94^-CD7^-$, while cNK cells are $CD56^+LFA\text{-}1^+CD94^+CD7^{+/-}$ [23]. Based on the differential expression of LFA-1, HSC-derived ILC3 cells and cNK cells were purified using magnetic bead selection and their specificity was confirmed using qPCR (Fig. 1A). ILC3 cells expressed high levels of RORγt and AhR, while cNK cells lacked these

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

transcription factors and instead, expressed EOMES (Fig. 1B). Also shown in Figure 1B is that DR3 mRNA is constitutively expressed by resting ILC3 cells and the levels are significantly higher compared to resting cNK cells (500 fold, n=3 $p<0.001$). To confirm these findings, we also purified ILC3 and cNK cells from tonsillar tissues and found similar findings (Fig. 1C). Next, surface expression was demonstrated. As shown in figure 1D, ILC3 cells expressed high levels of surface DR3 while resting cNK cells did not. Again, similar findings were observed in ILC3 cells in tonsillar tissues (Fig. 1E).

DR3 ligation enhances IL-1β and IL-23-induced cytokine production

Next we tested the effect of DR3:TL1A interactions on cytokine production by ILC3 cells. When added alone, rhTL1A did not trigger IL-22 production. However, TL1A combined with IL-1β and IL-23 lead to a significant enhancement in IL-22 production by ILC3 cells (Fig. 2A). The expression of both IL-22 and IL-8 was tested using intracellular cytokine staining and a higher percentage of cells produced these cytokines when TL1A was added to IL-1β and IL-23 (Fig. 2B). These results were consistent across a series of donors for both cytokines (Fig. 2C). Previous studies show that ILC3 cells also secrete IFN-γ in response to IL-23 [28], and that TL1A is an inducer of IFN-γ production in IL-12/18 activated human NK cells [6]. However, IFN-γ was not detected when human ILC3 cells were treated with rhTL1A alone or in combination with IL-1β and IL-23 (data not shown). Prior studies also demonstrate that TL1A drives T cell mediated pathology in EAE [14], and IBD [13], in part through a Th17-axis. However, freshly isolated or expanded ILC3 cells did not produce IL-17A by qPCR, ELISA and intracellular cytokine staining when stimulated with IL-1β IL-23 and TL1A (data not shown). Therefore, while TL1A has a pathogenic role in inflammatory diseases, it may also have a protective role related to ILC3 function in mucosal immunity.

IL-1β IL-23 and TL1A induced CD25 Expression on ILC3s

Prior studies show that DR3 signaling leads to the proliferation of a variety of different T cell subsets[9, 14] through the up-regulation of the IL-2 receptor α-chain (CD25) and enhanced IL-2 responsiveness[4]. Therefore, we investigated whether similar events occur in ILC3 cells. As shown in Figure 2D, resting ILC3 cells ($CD56^{+}LFA\text{-}1^{-}$) have detectable levels of CD25, while this cytokine receptor is nearly absent for cNK cells ($CD56^{+}LFA\text{-}1^{+}$). To test the impact of IL-1β and IL-23 on CD25 expression, ILC3 cells were stimulated these cytokines and CD25 expression was assessed by FACS. CD25 was moderately increased after 16 hrs of stimulation with IL-1β and IL-23 (Fig. 2E). Stimulation of ILC3 cells with TL1A alone did not increase CD25 expression (Figure 2E), while the combination of IL-1β IL-23 and TL1A resulted in the highest increases CD25 as measured by MFI (Figure 2E).

TL1A costimulates IL-1β and IL-23 induced ILC3 proliferation

Even though IL-2 is not necessary for ILC3 generation from HSCs [23] the above findings led us to speculate that IL-2 may have an important role in the proliferation of mature, activated ILC3 cells. To test whether DR3 and IL-2 drove ILC3 cell proliferation, cells were cultured in media containing +/− TL1A and +/− IL-1β and IL-23. After 16 hours of stimulation the cells were washed, labeled with CFSE and then cultured in media containing rhIL-2 (1000 IU/ml) for 5 days. A representative donor is shown in Fig. 2E, the addition of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

TL1A did not significantly influence ILC3 the dilution of CSFE in IL-2 containing media. In contrast, ILC3 cells that were stimulated with IL-1β and IL-23 proliferated and this was further augmented when TL1A was added to these conditions. These results were consistent in ILC3 cells generated from a number of different donors (n=7, p=0.001, Fig. 4B). Interestingly, ILC3 cell proliferation was not observed when IL-1β IL-23 and TL1A were added to cultures containing IL-7 and/or SCF only (not shown) further supporting the assertion that IL-2R upregulation is the main mechanism for these findings. Therefore, TL1A enhancement of IL-1β and IL-23-induced proliferation depends on CD25 up-regulation and increased IL-2 responsiveness.

Characteristics of ILC3 cells expanded with IL-1β IL-23 and TL1A

While the above findings show that the addition of TL1A to IL-1β and IL-23 increase cell proliferation, it does not provide any insight into whether the cells actually expand under these conditions. To determine whether the stimulation-induced CD25 upregulation could be used to drive ILC3 cell expansion, ILC3 cells were pre-stimulated as above (IL-15 +/−TL1A +/−IL-1β and IL-23), washed after 16 hours and then cultured in IL-2 (1000 IU/ml) containing media. Fold expansion was determined after 14 days. Expansion was not different for cells pre-stimulated in IL-15 compared to those that were pre-stimulated with IL-15, IL-1β and IL-23 (Fig. 3A). In contrast, cells pre-stimulated with IL-15, TL1A, IL-1β and IL-23 showed significantly higher expansion than either IL-15 alone (39.3±16.5 vs. 22.5±14.6, p=0.004) or those treated with IL-15, IL-1β and IL-23 (39.3±16.5 vs. 13.9±8.5, p=0.004, Fig. 3A). Expanded ILC3 cells maintained their phenotype ($CD56^{+}CD117^{high}CD94^{-}CD7^{-}LFA\text{-}1^{-}NKp44^{+}$, Fig. 3B) and transcription factor RORγt and AhR expression (Fig. 3C). Even though surface IL-1R1 expression in ILC3 cells was transiently down-regulated by exogenous IL-1β, after 14 days the expression was recovered (not shown). Finally, 14 day *in vitro* expanded ILC3 cells able to produce IL-22 in response to IL-1β and IL-23 stimulation (Fig. 3D).

Discussion

Here we demonstrate the expression of DR3 on HSC-derived and freshly isolated human ILC3 cells from tonsils. Even though resting ILC3 cells express DR3 (as evidenced by qPCR, FACS) stimulation of ILC3 cells with the only known ligand TL1A, alone did not drive cytokine production or proliferation. However, TL1A stimulation of ILC3 cells in the presence of IL-1β and IL-23 potently augments cytokine production (IL-22 and IL-8), proliferation and expansion. Prior studies show that IL-1β and IL-23 activate and expand ILC3 cells[29, 30] and thus, our studies extend these findings by demonstrating that short-term TL1A stimulation potently cooperates with IL-1β and IL-23, supporting the conclusion that DR3 functions as a costimulatory molecule on ILC3 cells. Interestingly, the percentage of cytokine producing ILC3 cells was significantly increased following the addition of TL1A to IL-1β and IL-23. Such findings suggest that ILC3 cells have differing thresholds for activation and that TL1A reduces this threshold.

Mechanistically, we demonstrate that TL1A functions to upregulate the IL-2R (CD25) and the responsiveness of ILC3 cells to IL-2 stimulation. While resting ILC3 cells express

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

CD25, the combination of TL1A, IL-1β and IL-23 significantly augmented CD25 expression. Treatment with these cytokines (TL1A, IL-1β and IL-23) resulted in higher percentages of ILC3 cells undergoing IL-2 induced proliferation (CSFE dilution) in a short-term (5 day) assay. Given that the CSFE dilution assay is not a good measure of cell expansion, additional experiments were performed and showed significantly higher numbers of ILC3 cells after 14 days of culture. Interestingly, pre-stimulation of ILC3s with IL-1β and IL-23 (i.e., no TL1A) showed short-term proliferation in the presence of IL-2, but after 14 days the expansion was no different than the controls. Considering that ILC3 cell hold promise as a cellular therapy for inflammatory bowel disease, these findings suggest that IL-1β and IL-23 are likely not sufficient to expand ILC3 cells for clinical use with the conditions described here.

TL1A has been associated with the worsening of autoimmune diseases in a number of experimental models, perhaps by driving either Th1 or Th17 genetic programs [6, 7, 13, 14]. However, TL1A did not induce IFN-γ or IL-17 production in human ILC3 cells, even when cultured in IL-15, which was used as a survival factor but has the potential to skew cells to a more inflammatory state. For IFN-γ or IL-17 to be produced, one might expect a loss of key transcription factors (ROR-γτ and AHR) and the acquisition of other transcription factors (T-bet), as has been shown for IL-12 treated ILC3 cells [31]. However, under the conditions tested, we did not observe TL1A (in the presence of IL-1β and IL-23) to change the transcription factor expression or cytokine production in expanded human ILC3 cells

Recently, TL1A has been shown to be expressed by murine and human ILC2 cells[24, 25]. In these studies, TL1A has a similar function as described here (augmenting activation induced cytokine production and proliferation). While ILC2 cells may protect again pulmonary pathogens, they also seem to play a major role in the induction or propagation of reactive airways disease. Thus, it should be determined whether clinical targeting of DR3 might be a useful target for the treatment of reactive airways disease.

Recently, Longman and coworkers show that CX3CR1+ mononuclear phagocytes are in close contact with ILC3 cells in the murine intestine. They go onto show that these phagocytes cells are the main source of IL-23 following an infectious challenge or in severe colitis [2]. CX3CR1+ mononuclear phagocytes also produce TL1A in response to stimulation with bacterial products (LPS or CpGs) and that in both mice and humans DR3:TL1A interactions drive IL-22 production. Our results support and extend these findings. Taken together, these findings show that TL1A alone has little function in resting cells, but in combination with IL-1β and IL-23, it significantly augments activation induced ILC3 cytokine production (IL-22 and IL-8), proliferation and expansion. Mechanistically, TL1A induces CD25 expression and the responsiveness to IL-2 stimulation. These data support the notion that the DR3:TL1A axis is costimulatory in ILC3 cells and suggests that TL1A may be used for ex vivo expansion of ILC3 cells for clinical use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Acknowledgments

This work was supported by American Cancer Society (MRV), Children's Cancer Research Fund (MRV), P01 CA65493 (JSM; BRB), P01 111412 (MRV and JSM) and R01 HL55417 (JSM), CA72669 (BRB), grant ASBMT New Investigators Award (YOA), and PO1067493 (JSM). This study was also supported by a grant from the National R&D Program for Cancer Control (1420140, YOA), and the Innovative Research Institute for Cell Therapy (A062260, DSH), Ministry of Health and Welfare, Republic of Korea.

References

1. Bamias G, Mishina M, Nyce M, Ross WG, Kollias G, Rivera-Nieves J, Pizarro TT, Cominelli F. Role of TL1A and its receptor DR3 in two models of chronic murine ileitis. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103:8441–8446. [PubMed: 16698931]
2. Longman RS, Diehl GE, Victorio DA, Huh JR, Galan C, Miraldi ER, Swaminath A, Bonneau R, Scherl EJ, Littman DR. CX3CR1+ mononuclear phagocytes support colitis-associated innate lymphoid cell production of IL-22. J Exp Med. 2014
3. Prehn JL, Thomas LS, Landers CJ, Yu QT, Michelsen KS, Targan SR. The T cell costimulator TL1A is induced by FcgammaR signaling in human monocytes and dendritic cells. Journal of immunology (Baltimore, Md : 1950). 2007; 178:4033–4038.
4. Migone TS, Zhang J, Luo X, Zhuang L, Chen C, Hu B, Hong JS, Perry JW, Chen SF, Zhou JX, Cho YH, Ullrich S, Kanakaraj P, Carrell J, Boyd E, Olsen HS, Hu G, Pukac L, Liu D, Ni J, Kim S, Gentz R, Feng P, Moore PA, Ruben SM, Wei P. TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. Immunity. 2002; 16:479–492. [PubMed: 11911831]
5. Twohig JP, Marsden M, Cuff SM, Ferdinand JR, Gallimore AM, Perks WV. Al-Shamkhani, A., Humphreys, I. R. and Wang, E. C., The death receptor 3/TL1A pathway is essential for efficient development of antiviral CD4(+) and CD8(+) T-cell immunity. FASEB J. 2012; 26:3575–3586. [PubMed: 22593543]
6. Papadakis KA, Prehn JL, Landers C, Han Q, Luo X, Cha SC, Wei P, Targan SR. TL1A synergizes with IL-12 and IL-18 to enhance IFN-gamma production in human T cells and NK cells. J Immunol. 2004; 172:7002–7007. [PubMed: 15153521]
7. Heidemann SC, Chavez V, Landers CJ, Kucharzik T, Prehn JL, Targan SR. TL1A selectively enhances IL-12/IL-18-induced NK cell cytotoxicity against NK-resistant tumor targets. J Clin Immunol. 2010; 30:531–538. [PubMed: 20349123]
8. Schreiber TH, Wolf D, Tsai MS, Chirinos J, Deyev VV, Gonzalez L, Malek TR, Levy RB, Podack ER. Therapeutic Treg expansion in mice by TNFRSF25 prevents allergic lung inflammation. J Clin Invest. 2010; 120:3629–3640. [PubMed: 20890040]
9. Wolf D, Schreiber TH, Tryphonopoulos P, Li S, Tzakis AG, Ruiz P, Podack ER. Tregs expanded in vivo by TNFRSF25 agonists promote cardiac allograft survival. Transplantation. 2012; 94:569–574. [PubMed: 22902792]
10. Fang L, Adkins B, Deyev V, Podack ER. Essential role of TNF receptor superfamily 25 (TNFRSF25) in the development of allergic lung inflammation. J Exp Med. 2008; 205:1037–1048. [PubMed: 18411341]
11. Meylan F, Song YJ, Fuss I, Villarreal S, Kahle E, Malm IJ, Acharya K, Ramos HL, Lo L, Mentink-Kane MM, Wynn TA, Migone TS, Strober W, Siegel RM. The TNF-family cytokine TL1A drives IL-13-dependent small intestinal inflammation. Mucosal Immunol. 2011; 4:172–185. [PubMed: 20980995]
12. Taraban VY, Slebioda TJ, Willoughby JE, Buchan SL, James S, Sheth B, Smyth NR, Thomas GJ, Wang EC, Al-Shamkhani A. Sustained TL1A expression modulates effector and regulatory T-cell responses and drives intestinal goblet cell hyperplasia. Mucosal Immunol. 2011; 4:186–196. [PubMed: 20962771]
13. Takedatsu H, Michelsen KS, Wei B, Landers CJ, Thomas LS, Dhall D, Braun J, Targan SR. TL1A (TNFSF15) regulates the development of chronic colitis by modulating both T-helper 1 and T-helper 17 activation. Gastroenterology. 2008; 135:552–567. [PubMed: 18598698]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

14. Pappu BP, Borodovsky A, Zheng TS, Yang X, Wu P, Dong X, Weng S, Browning B, Scott ML, Ma L, Su L, Tian Q, Schneider P, Flavell RA, Dong C, Burkly LC. TL1A-DR3 interaction regulates Th17 cell function and Th17-mediated autoimmune disease. J Exp Med. 2008; 205:1049–1062. [PubMed: 18411337]
15. Meylan F, Davidson TS, Kahle E, Kinder M, Acharya K, Jankovic D, Bundoc V, Hodges M, Shevach EM, Keane-Myers A, Wang EC, Siegel RM. The TNF-family receptor DR3 is essential for diverse T cell-mediated inflammatory diseases. Immunity. 2008; 29:79–89. [PubMed: 18571443]
16. Jones GW, Stumhofer JS, Foster T, Twohig JP, Hertzog P, Topley N, Williams AS, Hunter CA, Jenkins BJ, Wang EC, Jones SA. Naive and activated T cells display differential responsiveness to TL1A that affects Th17 generation, maintenance, and proliferation. FASEB J. 2011; 25:409–419. [PubMed: 20826539]
17. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. Nat Immunol. 2011; 12:21–27. [PubMed: 21113163]
18. Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. Annual Review of Immunology. 2012; 30:647–675.
19. Eberl G, Marmon S, Sunshine MJ, Rennert PD, Choi Y, Littman DR. An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. Nat Immunol. 2004; 5:64–73. [PubMed: 14691482]
20. Scandella E, Bolinger B, Lattmann E, Miller S, Favre S, Littman DR, Finke D, Luther SA, Junt T, Ludewig B. Restoration of lymphoid organ integrity through the interaction of lymphoid tissue-inducer cells with stroma of the T cell zone. Nat Immunol. 2008; 9:667–675. [PubMed: 18425132]
21. Tang Q, Ahn YO, Southern P, Blazar BR, Miller JS, Verneris MR. Development of IL-22-producing NK lineage cells from umbilical cord blood hematopoietic stem cells in the absence of secondary lymphoid tissue. Blood. 2011; 117:4052–4055. [PubMed: 21310921]
22. Hughes T, Becknell B, McClory S, Briercheck E, Freud AG, Zhang X, Mao H, Nuovo G, Yu J, Caligiuri MA. Stage 3 immature human natural killer cells found in secondary lymphoid tissue constitutively and selectively express the TH 17 cytokine interleukin-22. Blood. 2009; 113:4008–4010. [PubMed: 19244159]
23. Ahn YO, Blazar BR, Miller JS, Verneris MR. Lineage relationships of human interleukin-22-producing CD56+ RORgammat+ innate lymphoid cells and conventional natural killer cells. Blood. 2013; 121:2234–2243. [PubMed: 23327921]
24. Meylan F, Hawley ET, Barron L, Barlow JL, Penumetcha P, Pelletier M, Sciume G, Richard AC, Hayes ET, Gomez-Rodriguez J, Chen X, Paul WE, Wynn TA, McKenzie AN, Siegel RM. The TNF-family cytokine TL1A promotes allergic immunopathology through group 2 innate lymphoid cells. Mucosal Immunol. 2013
25. Yu X, Pappu R, Ramirez-Carrozzi V, Ota N, Caplazi P, Zhang J, Yan D, Xu M, Lee WP, Grogan JL. TNF superfamily member TL1A elicits type 2 innate lymphoid cells at mucosal barriers. Mucosal Immunol. 2013
26. Kim MY, Toellner KM, White A, McConnell FM, Gaspal FM, Parnell SM, Jenkinson E, Anderson G, Lane PJ. Neonatal and adult CD4+ CD3− cells share similar gene expression profile, and neonatal cells up-regulate OX40 ligand in response to TL1A (TNFSF15). J Immunol. 2006; 177:3074–3081. [PubMed: 16920944]
27. Hazenberg MD, Spits H. Human innate lymphoid cells. Blood. 2014; 124:700–709. [PubMed: 24778151]
28. Buonocore S, Ahern PP, Uhlig HH, Ivanov II, Littman DR, Maloy KJ, Powrie F. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. Nature. 2010; 464:1371–1375. [PubMed: 20393462]
29. Hughes T, Becknell B, Freud AG, McClory S, Briercheck E, Yu J, Mao C, Giovenzana C, Nuovo G, Wei L, Zhang X, Gavrilin MA, Wewers MD, Caligiuri MA. Interleukin-1beta selectively expands and sustains interleukin-22+ immature human natural killer cells in secondary lymphoid tissue. Immunity. 2010; 32:803–814. [PubMed: 20620944]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

30. Cella M, Otero K, Colonna M. Expansion of human NK-22 cells with IL-7, IL-2, and IL-1beta reveals intrinsic functional plasticity. Proc Natl Acad Sci U S A. 2010; 107:10961–10966. [PubMed: 20534450]

31. Bernink JH, Peters CP, Munneke M, te Velde AA, Meijer SL, Weijer K, Hreggvidsdottir HS, Heinsbroek SE, Legrand N, Buskens CJ, Bemelman WA, Mjosberg JM, Spits H. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nat Immunol. 2013; 14:221–229. [PubMed: 23334791]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

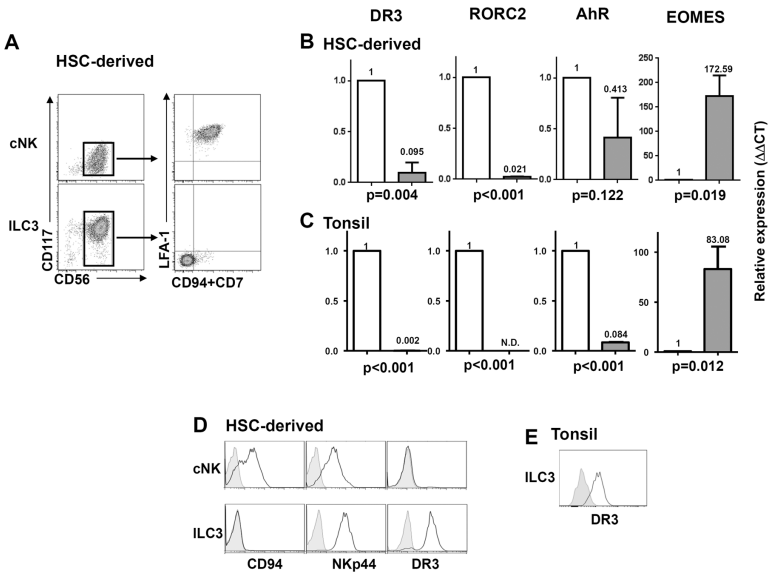

Figure 1. DR3 is expressed only in ILC3 cells, but not cNK cells, and stimulation of ILC3 cells with TL1A results in down-stream signaling

(A) Phenotype of purified cNK cells and ILC3 cells from d21 HSC differentiation cultures. (B, C) Expression of DR3, RORC2, AhR, and EOMES in ILC3 (white bars) and cNK cells (gray bars) purified from HSC-derived (B, n=3) and freshly isolated tonsil (C, n=3) by qPCR. All graphs indicate means; error bars denote SD. (D) Surface expression of DR3 on HSC-derived ILC3 cells and cNK cells from HSC-derived cells (at D21). Shown is a representative FACS plot of cells from more than five donors after gating on $CD56^{+}CD117^{high}LFA\text{-}1^{-}$ ILC3 cells or $CD56^{+}CD117^{low}LFA\text{-}1^{+}$ cNK cells (as in figure 1A). The expression of CD94, NKp44 are also shown as staining controls. Filled histograms are control IgG. (E) Expression of DR3 (open histogram) or isotype control (closed histogram) in ILC3 cells from tonsil. See methods for antibody and gating path.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

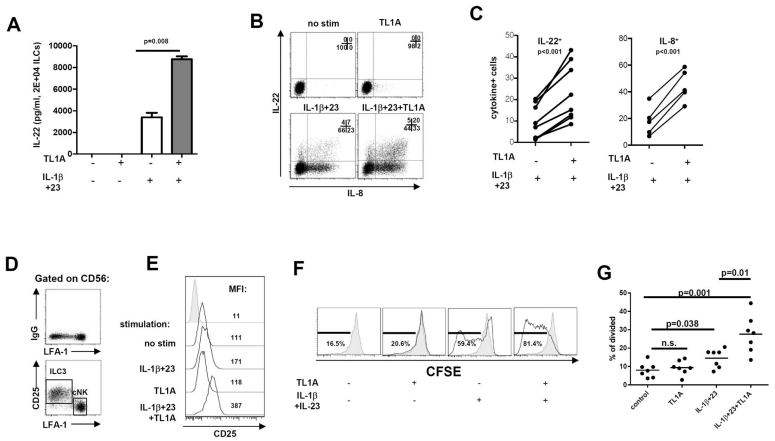

Figure 2. TL1A enhances ILC3 cytokine production IL-1β and IL-23 and drives IL-2 dependent proliferation

(A) ELISA for IL-22 in HSC-derived ILC3 cells at rest, or stimulation with TL1A alone, stimulation, IL-1β and IL-23 or the combination of the two (n=3). All graphs indicate means; error bars denote SD. (B) A representative donor showing IL-22 and IL-8 production in ILC3 cells at rest, or stimulation with TL1A alone, stimulation, IL-1β and IL-23 or the combination of the two. (C) Cumulative data showing the increase in the percentage of cytokine producing cells. Each line represents an individual ILC3 donor (n=8 for IL-22 and n=5 for IL-8). (D) Expression of CD25 in resting, HSC-derived ILC3 ($CD56^{+}LFA\text{-}1^{-}$) and cNK cells ($CD56^{+}LFA\text{-}1^{+}$). (E) Influence of IL-1β and IL-23, TL1A, and the combination (IL-1β and IL-23, TL1A) on CD25 expression on ILC3 cells. Cells were harvested and resuspended in media with IL-15 only (no stim), IL-1β and IL-23 and/or TL1A for 16 hours. Filled gray histogram is mouse IgG antibody and open histograms denote CD25 expression. MFI is listed to the left for each condition. Same results were obtained more than five donors, and representative results from one donor were shown. (F) TL1A induces proliferation of IL-1β and IL-23 stimulated ILC3 cells. Results from a representative donor where purified ILC3 cells were cultured with IL-15 alone as a survival factor (gray histograms), or stimulated with IL-1β and IL-23 +/− TL1A overnight (open histograms). The cells were washed (to remove the cytokines), stained with CFSE and then cultured with rhIL-2 (1,000 U/ml) for 5 days and analyzed. (G) Cumulative data showing the percentage of proliferating ILC3 cells at D5 (n=7 unique donors).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

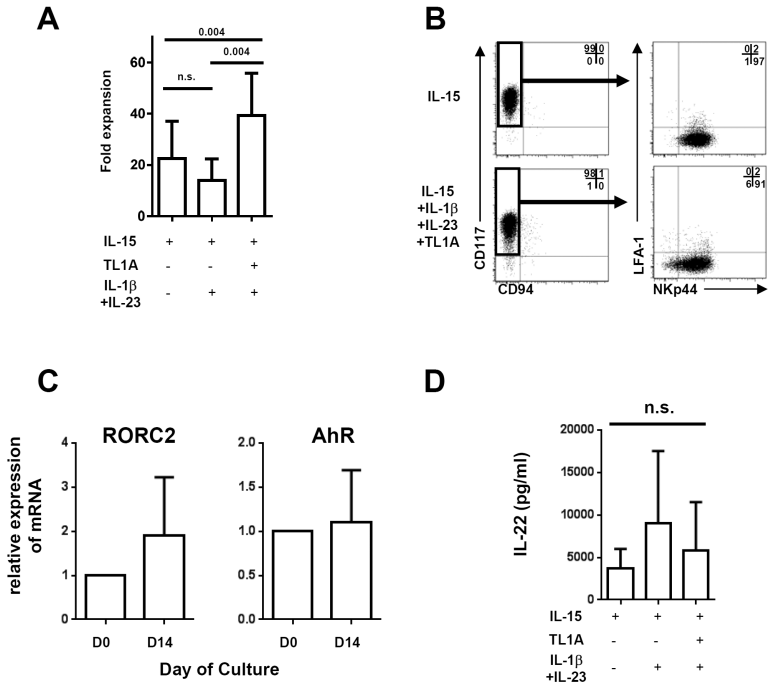

Figure 3. Fold expansion, phenotype, transcription factor expression and function of expanded ILC3 cells

(A) Fold expansion when ILC3 cells were cultured for 14 days after stimulation (n=7). (B) Phenotype of ILC3 cells stimulated with either IL-1β and IL-23+TL1A or IL-15 cells after 14 days expansion with IL-2. Representative data are shown >5 donors. (C) No significant differences of RORC2 and AhR mRNA expression between before and after expansion with IL-2 (left). (C) Expanded cells produce IL-22 after stimulation with IL-1β+23 (n=3). All graphs indicate means; error bars denote SD.