Innate Lymphoid Cells (ILCs) are immune cells typically found on mucosal surfaces and in secondary lymphoid organs where they regulate the immune response to pathogens. Despite their key role in the immune response, there are still fundamental gaps in our understanding of ILCs. Here we report a human ILC population present in the follicles of tonsils and lymph nodes termed follicular regulatory ILCs (ILC_{FR}) that to our knowledge has not been previously identified. ILC_{FR} have a distinct phenotype and transcriptional program when compared to other defined ILCs. Surprisingly, ILC_{FR} inhibit the ability of follicular helper T (Tfh) cells to provide B cell help. The localization of ILC_{FR} to the germinal centers suggests these cells may interfere with germinal center B cell (GC-B) and germinal center Tfh cell (GC-Tfh) interactions through the production of transforming growth factor beta (TGF-β). Intriguingly, under conditions of impaired GC-Tfh-GC-B cell interactions, such as human immunodeficiency virus (HIV) infection, the frequency of these cells is increased. Overall, we predict a role for ILC_{FR} in regulating GC-Tfh-GC-B cell interactions and propose they expand in chronic inflammatory conditions.

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**Results**

A newly identified innate lymphoid cell exists in human tonsils. We examined by flow cytometry tonsillar mononuclear cells (TMNCs) from healthy individuals for the presence of ILCs and observed a unique population within this family of innate cells. These ILCs are phenotypically identifiable by examining a lineage negative population (CD11b−CD11c−CD14−CD16−CD14~) gated separately for Lin markers CD19+CD3− that are also CD616−CD45+CD127−CD74+CXCR5+ (Fig. 1a). These ILCs expressed similar amounts of common ILC markers when compared to ILC3 positive controls including IL-2Ry, CD77, IL12RB1, and CCR6, but lacked or exhibited very low expression of markers for ILC1 (Nkp44, CD56, Tbet), ILC2 (KLRG1, GATA3), and ILC3 (Nkp44) or other leukocyte lineage markers16−18 shown by flow cytometry (Fig. 1c). ILCFR lack the surface protein expression of IL7RA, a common surface marker for ILCs, which has recently been shown to have a redundant signaling role to IL15RA as ILCs persist in the small intestinal lamina propria (siLP) of adult and neonatal Il7ra KO mice. IL-15 sustains wild-type and Il7ra KO ILC survival in vitro and promotes IL-7R deficiency19. This indicates that not all ILCs require expression of IL7RA, which is further evidenced by discovery of a CD127 low ILC population in human.20 Importantly, these ILCs simultaneously expressed unique markers including ID3 and CD74 (Fig. 1a, c). It has also been shown that ID3 and CD74 are expressed on a transcript level by murine intestinal regulatory ILCs21. Intriguingly, CD74 itself functions in a multifaceted role as part of the invariant chain of MHC II but also as the receptor for the inflammatory cytokine macrophage migration inhibitory factor (MIF). It has been shown that CD74 can act as a decoy receptor, which binds and neutralizes circulating MIF in the serum22. Hence, CD74 provides a potential newly identified mechanism for the negative regulation of MIF signal transduction and may be critically involved in controlling the pro-inflammatory actions of this cytokine. Another highly distinctive feature of ILCFR is their similar expression levels of CXCR5 (Fig. 2c) when compared to GC-Tfh, gated as CD4+CD3+CD45RA−CXCR5hiPD1hi (Supplementary Fig. 1a) and GC-B cells, gated as CD19+CD38+IgD−CD3i19 (Supplementary Fig. 1b). This similar expression level of CXCR5 RNA transcript suggests close physical proximity to other germinal center resident cells, and the ability to enter the germinal center follicles23. It is worth noting that ILCFR lacked CD25, CD4, and FoxP3 expression (Figs. 1c, 2c) the distinguishing markers of T regulatory cells (CD4+CD25+CD45RA−CXCR5−PD1−CD25+CD127+) (Supplementary Fig. 1c, 2d). ILCFR also lacked classical ILC2/Tfh markers RORA, GATA3 (Figs. 1c, 2c, 2g), ICOS, IL2RA, KLRG1 (Figs. 1c, 2f) and do not express transcripts of known Tfh/ILC2 cytokines IL-13, IL-5, or ILC2 cytokine IL-10 (Fig. 2h). Additionally, the other three ILC subsets type 1, 2, and 3, were identified in TMNCs based on established gating strategies7 (Fig. 1a). Notably, ILC3 and ILCFR subsets were found to be the two most dominant tissue resident ILC subpopulations with a statistically significant increase in frequency of ILCFR over ILC2s. (Fig. 1b). All three other ILC subsets have varying expression of CXCR5 but markedly less Bcl6 expression suggesting location outside of the germinal center follicles themselves. Additionally, the other three subsets of ILCs also lack ID3 and CD74 expression allowing distinction from ILCFR (Fig. 2c, g). Collectively, this new Lin−CD161−CD45+CD127−CD74+CXCR5+ ILC +CXCR5+ cell population is present in human tonsils and displays distinct surface markers and transcription factors from ILC1s, ILC2s, ILC3s, and Tregs.

**Discussion**

**Innate lymphoid cells (ILCs)** are located at many pathogen barriers including mucosal surfaces and secondary lymphoid organs (SLOs) where they can both sustain the integrity of the organ and promote lymphoid organogenesis itself. ILCs are defined by the lack of a rearranged antigen receptor and other lineage surface markers and are subdivided into three groups: group 1 ILCs are similar to Type 1 helper T cells (Th1) and are characterized by their ability to secrete interferon gamma (IFN-γ) and respond to interleukin-12 (IL-12), IL-15, and IL-18; group 2 ILCs are analogous to Type 2 helper T cells (Th2) and produce the same cytokines including IL-5, IL-9, and IL-13 while responding to IL-25 and IL-33 stimulation; and group 3 ILCs which are similar to Type 17 and 22 helper T cells (Th17/22) and responding to IL-25 and IL-33 stimulation; and group 3 ILCs are able to produce IFN-γ which are similar to Type 17 and 22 helper T cells (Th17/22) and are produced by the same cytokines including IL-5, IL-9, and IL-13 while responding to IL-25 and IL-33 stimulation; and group 3 ILCs which are similar to Type 17 and 22 helper T cells (Th17/22) and are able to produce IFN-γ, IL-17 and IL-22 after stimulation by IL-1β and IL-23. A newly described ILC2 population (ILC2o) has recently been identified in mice that has regulatory properties due to their IL-10 production in response to IL-33 stimulation. ILC2o also express the transcriptional regulatory factor ID34. These cell types and the cytokines they secrete play an important role in regulating adaptive Th1, Th2, and type Th17/22 cell responses by promoting protective immunity and homeostasis5,6. Recent work has shown that circulating ILCs are irreversibly depleted in HIV infection and can be rescued upon viral control with antiretroviral (ART) therapy7, only if started during acute infection. These innate cells may be a crucial component leading to the adaptive CD4+ T cell immune cell dysfunction seen in late stages of HIV infection.

Germinal centers, found in SLOs, are critical for the success of humoral immunity and constitute a major target in the design of vaccines and immunotherapies. Specifically, germinal centers constitute the sites where long-lived memory B and plasma cells are generated8–10. They are also the sites where antibodies are generated, after B cells undergo affinity maturation and isotype switching8–10. This process is dependent on the interactions of a subset of CD4+ T cells called follicular helper T cells (Thf) and GC-B cells. The GC-Thf/GC-B cell interactions are tightly regulated by receptor/ligand interactions including CD40L/CD40 and ICOS/ICOSL as well as secretion of cytokines that can mediate GC-Thf and GC-B cell help, such as IL-21, IL-4, IL-6, and IL-10. All cells entering the B cell follicles (the dark zone) respond to CXCL13 gradients via upregulation of their CXCR5 receptor and modulation of CCR7 expression, which is required for their entry into the germinal center13,14. Within the germinal center, activated Thf cells produce Interleukin-21 (IL-21), and express co-activation markers such as CD40L and ICOS, to allow for appropriate interaction and activation of the GC-B cells. Currently, there are fundamental gaps in understanding the cellular and molecular mechanisms during immune dysregulation and irregularities in germinal centers.

In this work, we describe a unique ILC population found in human tonsils and lymph nodes within the germinal center follicles. We find that they function in a regulatory manner, and as such, we name this newly identified cell population follicular regulatory ILCs (ILCFR), consistent with their location and function. These cells suppress the adaptive germinal center interaction between GC-Thf and GC-B cells via production of TGF-β in vitro, resulting in a decreased production of IgG as well as germinal center-mediated helper cytokines. These cells are expanded in chronic viral infection and may play a role in the immune dysregulation observed in these chronic inflammatory states.
transcriptional profiles. We isolated ILC FR ex vivo from eight healthy human tonsils as defined by Lin−CD161−CD45 +CD127loCD74+CXCR5+. We also sorted six other cell subsets including ILC1s, ILC2s, ILC3s, GC-B cells, GC-Tfh cells, and Tregs as described in methods. Multidimensional scale (MDS) analysis of the seven subsets revealed that ILC FR are transcriptionally distinct from other ILCs as well as from other T cell and B cell subsets (Fig. 2a). Our sorted population of ILC3s were comprised of additional specific subtypes (LTi or NCR+ ILC3s), hence the MDS analysis shows as expected, two clusters of ILC3s. One subset that is more similar to ILC1s, the NCR+ ILC3s, long established as transcriptionally similar to ILC1s with the ability to be reprogrammed into ILC1s with considerable cytotoxic capacity25. The other cluster consists of lymphoid tissue inducer like ILC3s, which are more distinct and groups closer to ILC2s26. ILC2s however, expressed unique transcriptional profiles distinct from ILCFR. ILC1 or NCR+ ILC3s. ILCFR and other ILCs (ILC2 and ILC3s (LTi)) expressed multiple B cell associated, and antigen presenting cell associated transcripts (Supplementary Fig. 2c). This may be due to the ability to present antigen, as ILCFR do not express CD19 surface protein as proven by Flow cytometric analysis and RNA seq analysis (Figs. 1a, 2f). Overall, the MDS plot analysis confirmed the unique transcriptional profile of ILCFR when compared to other ILCs, Tregs, GC-Tih, and GC-B cells.

We additionally performed Venn diagram analysis to identify differentially expressed genes (DEGs) both upregulated (left) and downregulated (right) that are common or unique between (ILC FR) vs (ILC1,2,3), (ILC FR) vs (GC-Tih), and (ILC FR) vs (Treg) (Fig. 2b). We found that ILC FR have 426 upregulated unique DEGs with increased expression, such as SMAD5 and NOTCH2 compared to Tregs alone; and 700 genes that are downregulated such as IL1R2, IL10RA, IL15RA, IL17A, CCR5. When ILC FR were compared to ILC1,2,3s, this newly identified ILC subset has 1268 upregulated genes, such as IL21R, IL-21, IL10 and ID3; and 1320 downregulated genes including EOMES, FASLG, TGFBR2, IL17RE, RORC, and NCAM1. We also compared ILC FR to Tregs and ILC1,2,3s simultaneously, which showed that ILC FR have 399 upregulated genes over ILC1,2,3s and Tregs (ILCFR vs ILC123 and ILCFR vs Tregs) (Fig. 2b left) including genes such as BCL6 and TGFBI, and 466 downregulated genes when compared to other ILCs or Tregs (Fig. 2b right), which include IL23R, IL-22, TGFBR3, IL1R1, and IL2RA. Moreover ILC FR lack the expression of classical transcription factors necessary for the development of other ILC subsets and Tregs namely RORC (encoding for RORγt), important for ILC3 development, TBX21 (encoding for Tbet) for ILC1 development27,28, GATA3 for ILC2 subset development28, and FOXP3 for Tregs24 (Fig. 2c, e), while sharing many common ILC genes including IL2RG, CD7, CCR6, and low expression of IL-7R (encoding for CD127) (Fig. 2d). Canonical Tregs were used for comparison in vitro and for sequencing instead of the recently described germinal center follicular regulatory T cells (Tfr) due to inconsistent suppressive functioning in our hands of Tfr in vitro. Additionally, they express unique identifying transcription factors such as ID3 and SOX4, but also ID2 and the common gamma chain (γc chain), which are required for ILC development16,17 (Fig. 2e–g). Of note was the increased expression of SOX4 in ILC FR, which can be upregulated via TGF-β signaling through SMAD3, and could be one mechanism by which ILC FR are inhibiting the GC-Tih and GC-B cell interaction. Furthermore,
ILC FR express intermediate levels of the transcription factor NFIL3, which plays a critical role in the development of mucosal tissue-associated innate lymphocytes. Taken together, these results identified that the ILC FR subset is transcriptionally unique with distinct gene expression profiles when compared to other ILC subsets, and adaptive regulatory cells in the human tonsil. We contend that these cells represent a newly identified innate lymphoid cell subset.

Examining cytokine receptor expression ex vivo, from sequencing human tonsillar ILC FR indicated constitutive expression of IL10RB, IL2RG, and IL21R, suggesting these cells respond to IL-10, IL-2, and IL-21 signaling (Fig. 2f). On the other hand, cytokine transcriptome analysis of this cell population indicated intermediate levels of TGFB2, TGFB3 and low IL10 and TGFB1 transcript expression profiles (Fig. 2h) suggesting that these cells might function though the TGF-β pathway. Intriguingly, there were high expression levels of IL-8 and its receptor CXCR2, which are involved in cell migration towards immune sites. Similarly, ILC FR expressed high gene transcripts of CCR7 which is necessary, much like CXCR5, for recruitment into secondary lymphoid organs and germinal centers. CCR7 has been shown to be important for keeping the GC-Tfh and GC-B cells in close proximity to promote interactions that are required for efficient antibody responses. Of additional interest, ILC FR expressed high levels of IDO1, an intracellular enzyme, which has been shown to lead to immune tolerance by the regulation of natural killer (NK) cells, T cells and Tregs. The immune modulatory actions of IDO1 mainly result from starving the cellular microenvironment of tryptophan, and this reduction causes immunosuppressive effects. Note, ILC FR showed upregulated transcript levels of many inflammatory cytokine surface receptors (CD74, IFNAR1 and 2, IFNGR1 and 2, IL12R, IL17R) suggesting that ILC FR have the ability to sense inflammatory microenvironments and respond (Fig. 2f).

RNA-sequencing-based pathway analysis further showed ILC FR are immunosuppressive and unique compared to other ILCs (Supplementary Fig. 2a) and Tregs (Supplementary Fig. 2b). ILC FR had decreased gene expression in the pathways for CD40 signaling, GATA3 signaling, IL-2 and IL-7 signaling, TCR signaling, and 41BB signaling when compared to Treg and ILC1, 2, and 3; all important for immune cell activation, differentiation and survival (Supplementary Fig. 2a, 2b). These results tell us that human tonsillar ILC FR express genes that suggest they have regulatory capacity.

ILC FR localize into the germinal center. To demonstrate the localization of ILC FR in situ within intact human tonsil tissue, we performed imaging analyses that allowed for the simultaneous detection of CD4, CD19, Ki67, CD8, JOPRO-1 (nucleus), CD74 and ID3 surface and intracellular markers for identification of ILC FR within human tonsillar follicular areas. The images were further analyzed by histocytometry. For visualization purposes, CD19+ B cells were stained in blue, Ki67 (proliferating cells) in cyan, ID3 in red, JOPRO-1 (cell nucleus marker) in gray.
CD74 in green, CD4 (T cells) in yellow, and CD8 (T cells) in magenta to delineate separate cell populations at various magnifications. (Fig. 3d–g). B cell germinal center follicles were defined as areas displaying a high density of CD19 (blue). In line with previous reports, our imaging analysis showed that tonsils are highly populated with B cell follicles (blue (germinal centers)) and have T cell areas surrounding them (yellow)36 (Fig. 3b). Additionally, the CD19−CD4−CD8− cells were analyzed in combination with the surface marker CD74 and transcription factor ID3, to specifically identify ILCFR (red dots) which, as described above, are marked by the absence of CD8, CD4, and CD19 with positivity for ID3 in the nucleoplasm and CD74 on the cell surface (Fig. 3a–g, Supplementary Fig. 3a-b, 4a-d). We saw that these cells were able to be visualized within the human tonsils. Four additional human tonsils were analyzed, and imaging show similar ILCFR localization trends (Supplementary Figs. 3, 4) revealing positioning that was either directly within the germinal center follicular CD19+ B cell areas themselves (in blue, Fig. 3a, b, Supplementary Fig. 3c) or in the T cell zone just outside the B cell follicles (yellow, Fig.3b, Supplementary Fig. 4b). Isotype controls were also used to confirm ID3 staining (Supplementary Fig. 4e). While the majority of ILCFR were located outside of the B cell follicle, around 22% were within the germinal center itself (Fig. 3c, Supplementary Fig. 3c-d). Germinal centers are extremely dynamic microenvironments and this cytologic in situ snapshot suggests ILCFR are able to modulate their receptor expression and exist both within and bordering germinal centers. This data highlights the uniqueness of ILCFR in that they can indeed be located within the B cell area of germinal center follicles and are separate from B cells, CD4+ and CD8+ T cells.
**ILC FR disrupt the GC-Tfh–GC-B cell interaction.** In an effort to decipher how ILC FR have the potential to modulate GC-Tfh-GC-B interactions, we assessed their function using an in vitro coculture assay\(^{37,38}\). Specifically, we determined whether ILC FR could interfere with Tfh-dependent B cell responses. Tonsillar GC-Tfh cells (CD4\(^+\)CD3\(^+\)CD45RA\(^-\)CXCR5\(^+\)PDI\(^+\))\(^{23,37}\), were cultured with autologous GC-B cells (CD19\(^+\)IgM\(^-\)IgD\(^-\)CD319lo)\(^{37}\) in the presence of the inflammatory super antigen staphylococcal enterotoxin B (SEB), as previously reported, which recapitulates an inflammatory reaction between the GC-Tfh and cognate GC-B cell\(^{38,40}\). These cells were cultured for five days with or without the addition of ILC FR (Lin\(^-\)CD45\(^+\)CD127loCD161\(^+\)CD74\(^+\)CXCR5\(^+\)) (Supplementary Fig. 5) in a ratio of 30:30:1 (GC-Tfh: GC-B: ILC FR). The stringent nature of the gating strategy used to sort ILC FR was included to emphasize ILC FR are non-T, non-B, non-lineage (macrophage/DC/monocyte/NK/neutrophil) ILCs and to demonstrate the purity of the sorted ILC FR cell population. With ILC FR themselves being so rare in patients, purity was assessed by using extremely stringent gating and the reproducibility of sorting results (Supplementary Fig. 5). We have previously shown that five days of co-culture results in peak IgG production from TMNC\(^{37,40}\). The addition of low numbers of our ILC FR (1000 cells) to co-cultures of GC-Tfh and GC-B cells significantly (\(p < 0.05\)) reduced the quantity of IgG produced by GC-B cells, as measured in the co-culture supernatant when compared to GC-Tfh and GC-B cells cultured in the absence of ILC FR (Fig. 4a). The reduction in IgG levels also demonstrates ILC FR are not a B cell population. Further analysis of co-culture supernatants revealed a significant decrease in secretion of IL-21 by GC-Tfh cells in the presence of ILC FR (\(p < 0.05\)), but not Tregs (Fig. 4b). We also observed a significant decrease (\(p < 0.01\)) in sCD40L in the supernatant, when ILC FR, but not Tregs, were added to the coculture of GC-Tfh and GC-B cells (Fig. 4c), demonstrating that this ILC FR subset is able to attenuate GC-Tfh/GC-B cell activation. This decrease in sCD40L could be due to decreased production or increased binding to CD40 on ILC FR to remove it from the microenvironment. Further studies to assess this mechanism should be undertaken. As ILC FR can be found in the follicular areas of the germinal centers, a highly regulated cellular area, indicating they may have an important role to play within the germinal center itself. Collectively, these data strongly demonstrate that ILC FR contribute to disrupting GC-Tfh and GC-B cells interactions, events that occur mainly in the light zone of the germinal center where we have determined follicular ILC FR are present (Fig. 3d–e). Additionally, ILC FR show specific suppressive activity as there were no significant changes in other chemokines/ cytokines including BLR (CXCL13), IL-6, IL-8, IL-17, IL-22, MCP-1, IFN-γ, IP-10, MIP-1α, MIP-1β, or GM-CSF in the coculture supernatants after five days with GC-Tfh and GC-B cells with or without Tregs or ILC FR (Supplementary Fig. 6a-k, respectively). Furthermore, there were no significant changes in co-stimulatory surface markers expression on either GC-Tfh (PD-1, ICOS, CD40L) (Supplementary Fig. 7a, 7c, 7e, respectively) or GC-B cells (PD-1, ICOSL, CD40) (Supplementary Fig. 7b, 7d, 7f, respectively) after the five days in co-culture. Of note, there was...
very little IgG production detected in wells supplemented with 30,000 GC-Tfh or 30,000 GC-B alone in the presence of SEB (Supplementary Fig. 6o). This is due to the necessity of the GC-Tfh/GC-B cell interaction for B-cell-derived IgG production. Of note, it has been previously established that ILC3s participate in enhancing T-cell-mediated immune responses. In an attempt to evaluate their biological effect on GC-Tfh/GC-B cell interactions, we co-cultured in vitro ILC3s with GC-Tfh and GC-B cells. Interestingly, ILC3s showed the opposite effect of ILCFR when co-cultured with the GC-Tfh and GC-B cells, leading to significant increases in IgG production ($p < 0.05$) from GC-B cells (Fig. 4d).

Given that ILC3s are located outside of the germinal center and express low Bcl6 levels (Fig. 2c), these observations suggest ILC3 may exert an effect on the GC-Tfh/GC-B cell interaction facilitating higher B-cell-derived IgG production. Thus, this newly described ILCFR population is functionally distinct from ILC3s, demonstrating strong suppressive activity on antibody production during the adaptive immune response. These results indicate that ILCFR disrupt the interactions between GC-Tfh and GC-B cells, resulting in the production of less sCD40L and IL-21 by GC-Tfh cells and decreased IgG production by GC-B cells.

**ILCFR Produce TGF-β disrupting GC-Tfh/GC-B interactions.** To further elucidate the mechanism behind the suppressive effects of ILCFR observed in our in vitro co-cultures, we examined whether regulatory components were involved in the disruption of interactions between GC-Tfh and GC-B cells. Based on previous findings demonstrating that intestinal ILC210 exerted their regulatory effects via IL-10 production, we performed ELISA and Luminex assays to assess IL-10 production. Interestingly, ELISA, IL-10 was not increased when GC-Tfh and GC-B cells were co-cultured with ILCFR (Supplementary Fig. 8a), also confirmed by Luminex (Supplementary Fig. 8b) as previously reported. This led us to examine other potentially suppressive cytokines such as TGF-β in these co-culture supernatants. Strikingly, when compared to the positive control cultures of GC-Tfh and GC-B cells alone, levels of all three subtypes of TGF-β were significantly increased in the presence of ILCFR. Indeed, there were significant increases in TGF-β1, 2, and 3 compared to co-cultures without ILCFR ($p < 0.0001$) (Fig. 5a) and cultures with Tregs ($p < 0.0001$) (Supplementary Fig. 6l). Furthermore, stimulation of ILCFR with 1 μg/ml of SEB for 24 h also confirmed that ILCFR are able to produce TGF-β upon activation (Supplementary Fig. 6m, 6n). ILCFR appear to upregulate the expression of the TGF-β1 pathway specifically upon activation as they express low levels of TGF-β1 RNA transcript in their baseline inactivated state (Fig. 2h). We then determined whether the presence of TGF-β was indispensable for the suppression of the GC-Tfh cell helper program by assessing the effect of TGF-β neutralization with antibody 1D11. 1D11 is a humanized form of monoclonal antibody that neutralizes the three major active TGF-β isoforms (TGF-β1, -2, and -3), and does not bind other ligands in the TGF-β superfamily, such as multifunctional growth factors activin or bone morphogenetic proteins (BMPs). When blocking antibody was added daily to co-cultures, we observed significant restoration of GC-Tfh-cell-dependent B cell IgG production (average of 1500 ng/mL increase in IgG and $p$-value < 0.05) in the presence of ILCFR. These levels were above those seen in wells with GC-Tfh and GC-B cells only due to complete loss of this suppressive cytokine, and this contrasted with lack of effect upon addition of the isotype control to co-cultures of GC-Tfh and GC-B cells with ILCFR (Fig. 5b). As there were increased levels of TGF-β in the GC-Tfh-GC-B cell control wells, further mechanistic pathway studies blocking other signaling proteins (SMADs) within the TGFβ pathway are warranted. We also determined which subtype of TGF-β was most important for ILCFR-mediated suppression of the germinal center reaction, and whether the suppressive cytokine alone was able to disrupt the GC-Tfh-GC-B cell interaction. Supplementing the GC-Tfh-GC-B co-culture with 3000 pg/ml (amount determined in co-cultures after five days with GC-Tfh, GC-B, and ILCFR in the presence of SEB) of exogenous rhTGF-β1, caused a significant reduction in IgG levels ($p < 0.05$; 23.5-fold decrease), suggesting modulation of GC-Tfh cell function (Fig. 5c), even more significantly than cultures of GC-Tfh and GC-B cells with ILCFR. These data demonstrate that TGFβ1 alone is sufficient to recapitulate the suppression observed when ILCFR are present. This effect was not due to compound-related toxicity, as shown by viability measurement after co-culture assays using flow cytometry (Supplementary Fig. 7g, 7h). Cytokine analysis of co-culture supernatants at day 5 demonstrated the effect of TGF-β on IL-21 production by GC-Tfh cells. Blocking TGF-β caused levels of IL-21 to increase to levels higher than those observed in cultures with GC-Tfh and GC-B cells with ILCFR plus isotype controls ($p < 0.01$; Fig. 5d). However, comparing ILCFR plus isotype wells to the GC-Tfh and GC-B cell positive control showed no significant difference in IL-21 or IgG levels (Fig. 5b, d). This highlights the importance of the TGF-β pathway in modulating the interactions of ILCFR with GC-Tfh and GC-B cells. TGF-β blockade, however, had no effect on sCD40L secretion (Fig. 5e). Additionally, supplementation of rhTGF-β1 to cultures of GC-Tfh and GC-B cells showed the most significant inhibition of IL-21 ($p < 0.05$; Fig. 5f) and sCD40L levels in the culture supernatants ($p < 0.01$; Fig. 5g), which indicates TGF-β1 is able to most effectively suppress GC-Tfh cell help and subsequent GC-B cell function. Interestingly, RNAseq data showed that ex vivo sorted GC-Tfh cells expressed higher level of TGFRI and TGFRII compared to GC-B cells (Table 1), suggesting that GC-Tfh cells may be preferentially impacted by TGF-β secretion from ILCFR in the co-culture. To determine whether IL-10 produced by ILCFR could also interfere with GC-Tfh-GC-B cell interaction, we performed IL-10 neutralization experiments in the co-culture assays. Addition of an IL-10 neutralizing antibody in co-cultures containing ILCFR failed to recover IgG production (Fig. 5h). Furthermore, there was no recovery of sCD40L (Fig. 5i) or IL-21 levels (Fig. 5i) with IL-10 neutralization. Statistically significant increases in IgG levels were only observed when TGF-β neutralization was present (Fig. 5h). Based on these findings, our data indicates that TGF-β production from these newly described ILCFR are responsible for the majority of the suppression observed of the GC-Tfh-GC-B cell interaction.

**ILCFR are expanded during chronic HIV infection.** We examined the dynamics of ILCFR in the context of HIV infection. The hallmark of HIV infection include a persistent systemic inflammatory state and immune dysfunction as well as overwhelming TGF-β-induced fibrosis of lymph nodes that is only partially restored by antiretroviral therapy. Thus, we hypothesized that ILCFR may be altered in this disease state. In fact, we and others have previously shown that GC-Tfh cells from HIV+ lymph nodes are functionally impaired in their interactions with GC-B cells despite their increase in cell numbers. This defect in GC-Tfh cells could contribute to the altered quality of anti-HIV antibody responses during infection. However, the underlying mechanisms of this cellular dysfunction remain poorly understood. We found a significant increase in frequencies of ILCFR among lymph nodes cells of HIV+ compared to HIV− individuals ($p < 0.05$; Fig. 6a, b), whereas there was no change in overall numbers of CD45+ lymph node cells (Fig. 6c). Additionally, expression of Bcl6, a transcription factor required for germinal center entry, was significantly higher ($p < 0.01$) among
Fig. 5 ILCFR-derived TGF-β attenuates the interaction of GC-Tfh and GC-B cells and reverses with blockade. a Production of TGF-β in the supernatants of 5-day co-culture: 30,000 GC-Tfh with 30,000 autologous GC-B cells (black) plus or minus addition of 1000 ILCFR (red) plus SEB measured by Luminex (n = 12 biologically independent tonsils per group, 3 independent experiments) (Paired, two-tailed parametric t-test; ****p < 0.0001; df = 11, β1: 95% CI: (1352 to 2048), t = 10.76, β2: 95% CI: (32.68 to 69.68), t = 6.09, β3: 95% CI: (9.947 to 15.43), t = 10.18; Mean ± SD). b IgG production in the supernatants of 5-day co-culture with or without blocking of TGF-β with 1ug/mL TGF-β neutralizing antibody (gray) or 1ug/mL mouse IgG isotype control (red) measured by ELISA (n = 7 biologically independent tonsils per group, 3 independent experiments) (Ordinary one-way ANOVA with multiple comparisons; F = 8.380; *p = 0.0420; Mean ± SD). c IgG production in the supernatants of 5-day co-culture plus or minus 3000 pg/mL of recombinant human TGF-β1 (blue) or 1ug/mL mouse IgG isotype control (red) measured by ELISA (n = 10 tonsils per group, 4 independent experiments) (Ordinary one-way ANOVA with multiple comparisons; F = 3.658; *p = 0.0309; Mean ± SD). d Production of IL-21 and sCD40L in supernatants of 5-day co-culture with or without ILCFR plus or minus TGF-β neutralizing antibody (gray) or isotype control (red) analyzed by 28 plex Luminex (n = 10 biologically independent tonsils per group, 4 independent experiments) (Ordinary one-way ANOVA with multiple comparisons; IL-21: F = 3.654; *p = 0.0394; sCD40L: F = 5.206; *p = 0.0127; Mean ± SD). e Production of IL-21 and g sCD40L in supernatants of 5-day co-culture with or without ILCFR plus or minus recombinant human TGF-β1 (blue) or 1ug/mL mouse IgG isotype control (red) measured by ELISA (n = 10 tonsils per group, 4 independent experiments) (Ordinary one-way ANOVA with multiple comparisons; IL-21: F = 3.654; *p = 0.0394; sCD40L: F = 5.206; *p = 0.0127; Mean ± SD). f Production of IL-10 and IL-21 in supernatants of 5-day co-culture with or without IL-10 blocking antibody (teal), or both IL-10 and TGF-β blocking antibodies (gray), or isotype control (red) measured by ELISA. (Ordinary one-way ANOVA with multiple comparisons; F = 43.22; Thh+B vs. IL-10 and TGF-β block: *p = 0.0325; Thh+B vs. isotype: *p = 0.0197; IL-10 and TGF-β block vs. isotype: *p = 0.0114; IL-10 and TGF-β block: *p = 0.0175; Mean ± SD). g Production of sCD40L and i IL-21 in the supernatants of 5-day co-culture plus or minus 1000 ILCFR with or without IL-10 blocking antibody (teal), or both IL-10 and TGF-β blocking antibodies (gray), or isotype control (red) measured by ELISA. (n = 4 biologically independent tonsils per group, 2 independent experiments).

| Cytokine Receptor expression on sorted human tonsillar cell subsets. |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|                    | ILC1                | ILC2                | ILC3                | ILCFR               | GC-Tfh              |
| IL-7R               | 403.6734            | 237.6888            | 398.1677            | 70.2166              | 31.5694             |
| IL15RA              | 4.173651            | 5.759125            | 4.736666            | 4.838582             | 0.902477            |
| IL-15               | 1.220763            | 1.341808            | 0.879143            | 4.18531              | 1.736381            |
| TGFBR1              | 9.13155             | 5.63533             | 8.35876             | 3.86731              | 15.65845            |
| TGFBR2             | 64.4227             | 45.61228            | 68.3044             | 36.08439             | 45.3508             |
| RPKM values were used to show IL-15 expression, TGF-βR, IL-7R, and IL-15R receptor expression on tonsillar sorted subtypes with this table. |
the expanded ILC FR population in HIV+ compared to HIV− individuals (Fig. 6d, e), suggesting that ILC FR are not only expanded in HIV infection, but are also more likely to be located within the germinal center follicle compared to in the absence of infection. Taken together, these data indicate that this ILC FR cell population may be contribute to the immune dysfunction associated with chronic HIV infection.

Discussion

Here we describe an ILC population that we termed follicular regulatory innate lymphoid cells (ILC FR), which excitingly appear to play an important role in disrupting the interactions between GC-Tfh and GC-B cells within germinal centers. We demonstrate that ILC FR are present and functional in human tonsils and lymph nodes where we showed that they were localized within the germinal center follicle and are uniquely identified by CD74, and ID3 expression. While these cells express some B cell RNA transcripts such as CD19 and EBFI, they are not B cells, T cells or ILC1s, ILC2s, or ILC3s based on RNA seq and MDS plot analysis. ILC FR are negative for CD19 protein expression via flow cytometric experiments and were gated stringently as CD19 negative in addition to other cell lineage markers. Furthermore, we see decreased IgG in T cell–B cell co-cultures in the presence of ILC FR. ILC FR can be observed between B cells and ILCs on the MDS plot, closer to ILC2s and ILC3s, and they demonstrate more expression of common markers and transcription factors with ILCs (ID2, CD7, CCR6, IL2RG, IL12RB1) than they do B cells or T cells. Additionally, while ILC FR share some low levels of common ILC1 transcripts (Tbet), or dendritic cells, combining extensive gene transcript analysis for all markers for each lineage and functional assays of cytokine and activation shows that ILC FR are in fact unique from these cell populations. Furthermore, these cells are not ILC2s, as they lack GATA3, KLRL1, and other ILC2 necessary markers. Furthermore, in contrast to the newly described ILC210 cells, which employ the IL-10 program for immunosuppression, ILC FR produce robust amounts of TGF-β upon activation, and can suppress GC-Tfh cell-dependent IgG production from GC-B cells, thus disrupting their interaction. Co-culture of GC-Tfh and GC-B cells with ILC FR also leads to decreased IL-21 and sCD40L production in vitro, both known to be necessary for germinal center formation and antibody secretion. Further studies into whether ILC FR directly contact the GC-Tfh or GC-B cells to exert their immunosuppressive effects are warranted. RNA-sequencing data and functional analysis suggests that ILC FR likely sense inflammatory microenvironments through inflammatory cytokine receptors (CD74, IFNGR1 and 2, IFNAR1 and 2, IL17RA), are activated, and begin to secrete TGF-β to exert their immunosuppressive effects. Hence, in chronic states where there are high levels of inflammatory cytokines (such as chronic HIV), ILC FR may be expanded in attempts to produce more TGF-β. Overall, this data supports the hypothesis that these novel cells are needed T,B and ILC subsets, and are involved in disrupting T cell–B cell interactions in the germinal center, which are critical for an efficient humoral response in our hands. It is possible that ILC FR serve to balance the T cell–B cell interaction in the germinal center by limiting the IL-21 and sCD40L pathways, crucial for germinal center formation and maintenance. Overall, our findings identify a unique innate lymphoid subset with distinct phenotype, function and location. Our data further demonstrate that these cells can regulate initiation and development of immune responses in health and disease.

It has also been established that germinal center reactions are impaired in chronic viral infections and autoimmune diseases.23,48 The hallmarks of HIV-1 pathology include immunodeficiency, lymphoid tissue destruction, gut barrier breakdown, and systemic immune activation.44 These features are only slightly reversed by viral suppressive long-term antiretroviral therapy.49 The cause of the progressive depletion of CD4+ T cells in HIV-infected...
individuals is one of the most fundamental and controversial issues in HIV/acquired immunodeficiency syndrome (AIDS) research. Additionally, previous studies have suggested that lymph node fibrosis following HIV infection is an important mechanism underlying the pathogenesis of HIV/AIDS due to the necessity of healthy lymph node tissue for maintenance of immune homeostasis as well as the survival, proliferation, and differentiation of lymphocytes. Furthermore, TGF-β1 is considered to serve a central role in inducing fibroblasts to synthesize collagen and thereby induce fibrosis. As our data showed a 3-fold increase in the frequency of ILC2p in chronic HIV-1 infection, we hypothesize that this increase in ILC2p may lead to an increase in the amount of TGF-β1 in the germinal center and eliciting fibrotic reactions. Therefore, ILC2p may be an important source of the TGF-β1 found in secondary lymphoid organs in HIV infection.

Finally, the tumor microenvironment, as with the immunological milieu of chronic infection, contains a multitude of suppressive mechanisms that allow tumors to escape immune surveillance similar to virally infected cells in chronic viral infections. Both of these chronic inflammatory states lead to dysfunctional adaptive T cell responses. Therefore, expanding our ILC2 characterization into cancer models will be an interesting natural next step based on the compelling chronic HIV infection data. Many cancers have inactivating mutations in their TGF-β receptor or SMAD complex pathways, discontinuing the tumor suppressive effects of TGF-β signaling especially in cells already harboring oncogenic mutations (prenatal). Furthermore, the presence of tumor-infiltrating lymphocytes coincides with the highest levels of TGF-β secretion and is thus a suspected source of the accumulation of TGF-β1 in the tumor microenvironment. While the specific cells producing TGF-β remain elusive, we propose that with the understanding gained about ILC2p, these cells may be an as yet unidentified source of this suppressive cytokine that can lead to cancer progression and immune dysregulation. ILC2p are trafficked to sites of inflammation such as those seen in chronic HIV-infected lymph nodes, are activated by inflammatory microenvironments, produce large amounts of TGF-β1, expand in frequency, and nonspecifically suppress the chronic inflammatory state seen in viral infections and cancer microenvironments. We believe these newly identified ILC2p could play a key role in the dysfunction seen in both viral and tumor microenvironments.

In conclusion, ILC2p are activated by inflammatory environments and exert their suppressive function on GC-Tfh and GC-B cells interactions. They disrupt interaction of GC-Tfh and GC-B cells in the germinal center by secreting large amounts of TGF-β1 which leads to decreased sCD40L, IL-21, and IgG production. This loss of IgG production illustrates the inability of GC-Tfh cells to appropriately help the GC-B cells respond to antigen. This could lead to diminished vaccine responses and reactions to infection. The expansion of this suppressive cell population observed in chronic HIV infection could also help explain the immune dysfunction and collagen deposition (fibrosis) that is hallmarks of this disease. Given that tumor microenvironments and autoimmune diseases are also characterized by a defective adaptive immune response, there is perhaps a role for ILC2p in these disease contexts. Further studies are needed to investigate the role that ILC2p may play in many diseases of immunologic dysfunction and how targeting them could serve as a novel therapeutic intervention.

**Methods**

**Lead contact.** Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Elias Haddad (eh336@drexel.edu).

**Human samples.** Blood samples and tonsils obtained from donors with repeated chronic tonsillar infections but were uninfected at time of tonsil removal at both Martin’s Primary Health System for Il-10 and Foxplong for Children (Pennsylvania). Tonsils used for experiments were provided by patients aged 2–45, and were digested, made into single cell TMNC suspensions and frozen in bovine serum albumin (Sigma) plus 10% DMSO (VWR) for cryopreservation in liquid nitrogen. The Institutional Review Boards at the relevant institutions approved all procedures, and all participants provided signed informed consent.

**Phenotypic analysis of germinal center.** Tonsil cells from adult donors or HIV+ lymph nodes were incubated with fluorochrome-conjugated antibodies. The following fluorochrome-conjugated antihuman antibodies were used: CD3 (Clone: HIT3a, Cat: 303024), CD4 (Clone: RPA-T4, Cat: 300520), CD25 (Clone: BC6, Cat: 356410), CD11c (Clone: N418, Cat: 329924), CD161 (Clone: 2A2E1, Cat: 329907), CD319 (Clone: 162.1, Cat: 331806), CD62 (Clone: BA5b, Cat: 302706), ICOS (Clone: C93.8A, Cat: 313506), CD11c (Clone: BU15, Cat: 337110) CD11b (Clone: LM2, Cat: 393110), CD16 (Clone: 3G8, Cat: 302062), CD14 (Clone: M5E2, Cat: 301822), CD161 (HPRG, Cat: 3G9, Cat: 339916), NKp44 (Clone: 325110), CRTH2 (Clone: 353404), IL-10 (Clone: JESI-9D7, Cat: 601420), TGF-β (Clone: TW4-2F8, Cat: 349610), CD40 (Clone: A5D4, Cat: 334308), CD40L (Clone: 24-3J, Cat: 310810), IL2RG (Clone: TGUM2, Cat: 132305), CD7 (Clone: CD7-6B7, Cat: 343118), CD74 (Clone: LN2, Cat: 326186), CD56 (Clone: S1.1H11, Cat: 362504), KLRG1 (Clone: CA207, Cat: 369154), CD45RA (Clone: 2H4LDH11LDB9, Cat: IM2711U) from Beckman Coulter, FoxP3 (Clone: eBioFoxP3, Cat: 559879), ID3 (Clone: Q21-559, Cat: 563081) were from BD Biosciences and CRTH2 (Clone: RF8 B2, Cat: 356910), IgD (Clone: IA6-2, Cat: 348226) and Bcl6 (Clone: L50-823, Cat: 560068), CD249/CRTH2 (Clone: BM16, Cat: 563501), Tbet (Clone: 04-46, Cat: 561268), GATA3 (Clone: L50-823, Cat: 560668), CD19 (Clone: HIB1B, Cat: 557921), CXCR5 (Clone: RGF8 B2, Cat: 356910), IgD (Clone: IA6-2, Cat: 348226) and Bcl6 (Clone: K12-91, Cat: 561522) ICOSL (Clone: CD33/B7-H2, Cat: 303940), CD39 (Clone: 39C, Cat: 560239), CD45 (Clone: 2D1, Cat: 560178), CD117 (Clone: YB5, Cat: 559879), CD127 (Clone: A019D5, Cat: 351380), ID3 (Clone: S20-778, Cat: 564564), ROTYR (Clone: Q21-559, Cat: 563081) were from BD Biosciences and CD45RA (Clone: 2H4LDH11LDB9, Cat: IM2711U) from Beckman Coulter, FoxP3 (Clone: PCH101, Cat: 53-4776-42) from Bioscientific. LIVE/DEAD Fixable Cell Stain (Life Technologies, Cat: L34957) was used to gate on live cells. Cells were phenotyped as follows: ILC1s were defined as LineageCD45RA−CD127+CD11c−; ILC2s defined as LineageCD45RA−CD127−CD11c−; ILC3s defined as LineageCD45RA−CD127−CD11c+; ILC7s defined as LineageCD45RA−CD127+CD11c+; ILC SRCs defined as LineageCD45RA−CD127+CD11c−; ILC17s defined as LineageCD45RA−CD127−CD11c−. Tonsil cells from adult donors or HIV+ tonsils were thawed and then incubated with 100 ng/mL of staphylococcal enterotoxin B (SEB) (Toxin Technology) with or without 100 ng/mL of staphylococcal enterotoxin B (SEB) (Toxin Technology) in RPMI 1640 (Corning) with 10% fetal bovine serum (Access Biologics) and 1% penicillin/streptomycin (Gibco) for 6 h before the staining protocol outlined above was employed or for 24 h before TGF-β1 production was assessed.

**Functional analysis of germinal center.** Tonsil cells from healthy donors were stimulated with 1 μg/mL of staphylococcal enterotoxin B (SEB) (Toxin Technology) with or without 100 ng/mL of staphylococcal enterotoxin B (SEB) (Toxin Technology) in RPMI 1640 (Corning) with 10% fetal bovine serum (Access Biologics) and 1% penicillin/streptomycin (Gibco) for 6 h before the staining protocol outlined above was employed or for 24 h before TGF-β1 production was assessed.

**Co-culture assay.** Tonsils from donors were thawed and then incubated with fluorochrome-conjugated antibodies for 15 min at 4 °C in the dark. Samples were sorted on a BD FACSAria.

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supplemented wells, 3000 pg/mL of rhTGF-β1 was once added at day 0 to the experimental wells or 1ug/mL mouse IgG isotype control. Cells were kept for 5 days (tonsil) or co-culture followed by flow cytometry analysis. Supernatant and cells were collected at day 5 for subsequent analysis.

**Total IgG and IL-10 ELISA.** For human samples, total IgG was measured by ELISA on culture supernatant as previously described. Total IgG and IL-10 ELISA were performed on a Bio-Plex 200 System (using bead regions defined in the Bio-Rad protocol) and analyzed with the Bio-Plex Manager 6.1 software from Bio-Rad.

**Cytokine and chemokine analysis.** Supernatants collected from tonsils in coculture were analyzed for chemokine/cytokine levels using Bio-Plex Pro magnetic bead assays (Bio-Rad, Hercules, CA USA). The following human chemokine premixed panels was used: BLC (CXCL13), sCD40L (CD154), MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), SDF-1 (CXCL12), MIP-3β (CCL19), MIP-3α (CCL20), GM-CSF, IP-10 (CXCL10), Fractalkine (CX3CL1), IL-1β, IL-6, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-21, IL-22, IL-23, TNF-α, IFN-α, IFN-γ and TGF-β1, TGF-β2, TGF-β3. The manufacturer’s protocol was followed. Data were acquired on a Bio-Plex 200 System (using bead regions defined in the Bio-Rad protocol) and analyzed with the Bio-Plex Manager 6.1 software from Bio-Rad.

**Gene expression analysis.** Healthy human donor tonsil cells (from seven biologically distinct humans) were directly sorted into cold RLT buffer (QIAGEN) supplemented with 1% beta-mercaptoethanol (BM) (Sigma) and quickly stored at −80°C. The seven cell populations were sorted to 2000–5000 cells each and included GC-Th, GC-B, Treg, ILC1, ILC2, ILC3, and ILCP described phenotypically above. Total RNA was isolated using the RNasy Micro Kit (Qiagen) following recommended procedures, with on-column DNase treatment. Total RNA was normalized prior to oligo-dT capture and cDNA synthesis with SMART-Seq v4 (Takara). RNA libraries were generated using the Nextera XT DNA Library Prep Kit (Illumina). All sample quality assessment was performed on a 5300 Fragment Analyzer System (Agilent) and quantified using a Qubit 3.0 fluorometer (Life Technologies). Medium depth sequencing (>16 million reads per sample) was performed on a NextSeq 550 System (Illumina) using two High Output flow cells each with a 75 base pair, Paired End run.

Demultiplexed fast-q paired end reads adapters of length less than 36 and average phred quality score of less than 30 were trimmed and filtered using the skewer software. Alignment was performed with HISAT2 to the Homo sapiens NCBI reference genome assembly version GRCh38 and sorted with SAMtools. The aligned reads were counted and assigned gene meta-information using the featureCounts software, and analysis was conducted using the R programming language and various packages from the Bioconductor suite. Normalization, weighting, and subsequent differential expression was performed using limma and gene set enrichment analysis was performed using GSEA pathway enrichment sets from MsigDB.

**Immunohistocytometry antibodies.** Tissues were stained with the following antibodies: Primary/Conjugated antibodies: CD74 (Rabbit polyclonal, Abcam, #ab47722), CD8 (Mouse IgG2b, clone: 4B11, Thermo Scientific, #MA1-80231), D3 (Mouse IgG1, clone: OTI8B3, abcam, ab236505), K67 Brilliant Violet 480 (BD Horizon, clone: B56, 566172), CD19 Alexa Fluor 647 (Biolegend, clone: A17136C, 396304), CD4 Alexa Fluor 700 (R&D systems, goat polyclonal, FAB8156N). Secondary antibodies: Donkey anti- Rabbit IgG Brilliant Violet 421 (Biolegend, clone: polyG06, 406410), Goat anti-Mouse IgG2b Alexia Fluor 488 (Life Technologies, A21141), Goat anti-Mouse IgG1 Alexa Fluor 594 (Life Technologies, A21125), Mouse IgG1 kappa isotype control clone B11/6, abcam, ab91353.

**Multiplexed confocal imaging.** Paraffin tissue blocks were cut into 5-micron-thick sections and subsequently mounted onto slides (Leica-Apex Superior Adhesive Slides). Deparaffinization/rehydration was achieved through sequential Xylene-H2O-H2O-H2O solutions. Antigen retrieval via heating/permanganation (110 degrees Celsius/ 5–6 PSI/ 15 min) of the slides was performed with Reveal Decloaker (Biocare Medical) in a decloaking chamber (Biocare Medical). After
cooling down the slides, the tissues were blocked/permeabilized with a Phospho Buffered Solution (PBS)/Bovine Serum Albumin (BSA)/Triton-X solution. Titrated amounts of primary antibodies were then added for overnight staining at 4 °C. Next day, slides were washed in PBS before being incubated with titrated amounts of appropriate secondary antibodies for 2 h, which was followed by conjugated antibodies. A final washing step with PBS was then performed, and then the nuclear stain Jopro-1 was applied (ThermoFisher Scientific). Slide were subsequently mounted with a glass coverslip using Fluoromount G (Southern Biotech).

Confocal images were obtained using a Nikon (A1+) confocal system operated through the NIS-elements AR software. A ×40 (NA 1.3) objective was used to scan the tissues. Multiple ×40 field of views and Z stack slices generated from each tissue were stitched together via the NIS-elements AR software. Pixel density of each tissue field of views ranged from 452 × 1467 to 4720 × 1472 with Imaris software version 9.5.0 (Bitplane). Histocytometry analysis was performed on a Leica SP8 confocal microscope with a ×40 objective (NA 1.30) at 1% zoom. Images were visualized using the software Imaris version 9.6.0 (Bitplane). Staining produced a nuclear pattern consistent with transcription factor localization as previously described (https://www.proteinatlas.org/ENSG00000117348-Il3/antibody).

**Quantitative imaging analysis (histocytometry).** Confocal images were analyzed with Imaris software version 9.5.0 (Bitplane). Histocytometry analysis was performed to generate quantitative data from the images. Briefly, 3-dimensional segmented surfaces (based on nuclear signal) of splivoor corrected images were generated with Imaris via the Surface Creation module. Average voxel intensities for all channels, as well as volume and sphericity of the 3-dimensional surfaces generated from histocytometry were exported in Microsoft Excel format. After conversion of the files to comma separated value (.CSV) files, the data were imported into Flowjo (version 10) for further analysis/quantification. The results of the histocytometry analysis were expressed as frequencies.

**Statistics and reproducibility.** All flow cytometry, co-culture, ELISA and Luminex data were analyzed using GraphPad Prism v7. Paired Student’s t-test (Wilcoxon) was used when comparing two groups. The Pairwise multiple t-test and non-parametric one-way ANOVA test was used when comparing more than two groups to each other. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. N numbers ranged from 4–17 biological replicates/individual patient tonsils per group and included at least two independent experiments per assay.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Sequence and gene expression data are available in the Gene Expression Omnibus (accession number GSE168407). All other data are available in the main text or supplementary materials.

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