HHS Public Access

Author manuscript

Nat Rev Immunol. Author manuscript; available in PMC 2024 July 01.

Published in final edited form as:

Nat Rev Immunol. 2024 January ; 24(1): 64–77. doi:10.1038/s41577-023-00906-5.

The emerging family of RORγt⁺ antigen-presenting cells

Jakub Abramson[1,6,†], **Jan Dobeš**[2,6], **Mengze Lyu**[3,4,5,6], **Gregory F. Sonnenberg**[3,4,5,6,†]

[1]Department of Immunology and Regenerative Biology, Weizmann Institute of Science, Rehovot, Israel

[2]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic

[3]Jill Roberts Institute for Research in Inflammatory Bowel Disease, Weill Cornell Medicine, Cornell University, New York, NY, USA

[4]Joan and Sanford I. Weill Department of Medicine, Division of Gastroenterology & Hepatology, Weill Cornell Medicine, Cornell University, New York, NY, USA

[5]Department of Microbiology & Immunology, Weill Cornell Medicine, Cornell University, New York, NY, USA

[6]These authors contributed equally: Jakub Abramson, Jan Dobeš, Mengze Lyu, Gregory F. Sonnenberg

Abstract

Antigen-presenting cells (APCs) are master regulators of the immune response by directly interacting with T cells to orchestrate distinct functional outcomes. Several types of professional APC exist, including conventional dendritic cells, B cells and macrophages, and numerous other cell types have non-classical roles in antigen presentation, such as thymic epithelial cells, endothelial cells and granulocytes. Accumulating evidence indicates the presence of a new family of APCs marked by the lineage-specifying transcription factor retinoic acid receptor-related orphan receptor-γt (RORγt) and demonstrates that these APCs have key roles in shaping immunity, inflammation and tolerance, particularly in the context of host–microorganism interactions. These RORγt⁺ APCs include subsets of group 3 innate lymphoid cells (ILC3s), extrathymic AIRE-expressing cells (eTACs) and potentially other emerging populations. Here, we summarize the major findings that led to the discovery of these RORγt⁺ APCs and their associated functions. We discuss discordance in recent reports and identify gaps in our knowledge in this burgeoning field, which has tremendous potential to advance our understanding of fundamental immune concepts.

† jakub.abramson@weizmann.ac.il; gfsonnenberg@med.cornell.edu.

Author contributions

J.A., J.D., M.L. and G.F.S. are equal contributors and collectively conceived the concepts, wrote the manuscript and prepared the figure drafts.

Competing interests

The authors declare no competing interests.

Introduction

Antigen-presenting cells (APCs) are master regulators of the immune response by displaying antigenic peptides on their cell surface in the context of MHC molecules that are recognized by somatically recombined T cell receptors (TCRs)[1–4]. These cognate interactions result in diverse outcomes for T cells, including clonal deletion, tolerance or differentiation into distinct effector T cell types, depending on the quality or context of the APC. Key contributing factors include the strength and/or duration of TCR–peptide–MHC interactions, the presence of co-stimulatory or co-inhibitory signals, and the delivery or sequestration of cytokine signals[1–6]. Through these complex interactions, APCs are essential for the initiation and regulation of adaptive immune responses, which facilitate protection from pathogens or malignancies, or enforce tolerance to self, microbiota or other innocuous stimuli. Many current biologic therapies aim to augment the specificity or quality of APCs, and a greater understanding of these cell types may hold the key to developing new strategies to combat human disease.

Not all APCs are equal in their functional roles and control over immune responses. Professional APCs **[G]** include conventional dendritic cells (cDCs), B cells and macrophages, which modulate $CD4^+$ T cell responses through their capacity to phagocytose and subsequently to present antigenic peptides on their MHC class II (MHC-II) molecules[2,7]. Other non-professional APCs **[G]** also substantively impact the quality of $CD4^+$ T cell responses, including thymic epithelial cells (TECs), which are required for the development and shaping of the T cell repertoire in the thymus, and various other epithelial cells, endothelial cells and granulocytes, which modulate the quality of $CD4^+$ T cell responses in peripheral tissues[6,8]. Among all types of APC, cDCs are considered to have the primary role in the initiation of T cell responses in secondary lymphoid organs[2,7]. Recently, we and others have defined a new family of APCs that are distinct from these conventional subsets and are marked by high levels of MHC-II and the lineage-specifying transcription factor retinoic acid receptor-related orphan receptor-γt (RORγt). These $ROR\gamma t^+$ APCs **[G]** comprise several molecularly and functionally distinct subsets, including subsets of group 3 innate lymphoid cells (ILC3s), extrathymic autoimmune regulator **[G]** (AIRE)-expressing cells (eTACs) and potentially other emerging populations that resemble DCs. These DC-like populations lack expression of the chemokine receptor CXCR6 or IL-7 receptor (IL-7R; CD127), which defines $MHC\text{-}II^+$ ILC3s, and of AIRE, which defines $ROR\gamma t^+$ eTACs.

Although $ROR\gamma t^+$ APCs have only recently been described, studies have already shown their indispensable role in shaping diverse T cell responses in the periphery, particularly in the context of host–microorganism interactions. Here, we discuss the discovery and functions of this emerging family of $ROR\gamma t^+$ APCs. In outlining the key properties of these cell types, we address discordance in recent reports and identify key gaps in our knowledge that require further investigation in this exciting new field of immunology. For transparency, we indicate those studies that were carried out by authors of this Review.

The discovery of RORγt⁺ APCs

The first identification of a RORγt$^+$ APC occurred only ten years ago, but the field has moved rapidly since and has been supported by fundamental advances in our understanding of RORγt, ILC3 subsets, AIRE and eTACs. Here we provide a brief, historical overview of the discovery of RORγt$^+$ APCs (FIG. 1) and discuss key gaps in our knowledge regarding their lineage relationships, phenotypic markers and developmental ontogeny. Many of these discoveries originated in mice, and although some have already been translated into humans, this remains at the early stages of investigation. Therefore, we also highlight the key differences and/or unknowns between mice and humans.

RORγt: from lymph nodes to type 3 immunity

The *RORC* gene, which was cloned in the 1990s[9–11], encodes two major transcription factor isoforms; whereas the longer RORγ isoform is expressed in many different tissues or cell types, the shorter RORγt isoform was later found to be enriched in thymocytes and to impact T cell survival[12–14]. Deletion of *Rorc* in mice revealed surprisingly extensive effects on adaptive immunity, including a complete lack of secondary lymphoid organs[13,14], and this was also later observed in humans through the study of inborn errors of immunity[15]. A seminal discovery that accounted for this phenotype showed an essential role for RORγt in the development of innate immune cells known as lymphoid tissue inducer (LTi) cells[16] — CD4$^+$CD3$^-$ haematopoietic cells that produce surface-bound lymphotoxin to directly promote secondary lymphoid organogenesis during fetal development[17–19] and support the generation or maturation of tertiary lymphoid structures in the gut following microbiota colonization[20,21].

It is now known that RORγt is not only essential for the development of LTi cells and associated formation of secondary or tertiary lymphoid organs, but also has an indispensable role as a master regulator of type 3 immune responses **[G]**. Seminal studies identified RORγt as a lineage-specifying transcription factor of T helper 17 (T_H17) cells[22], and showed that it is expressed by a unique type of peripherally induced regulatory T (T_{reg}) cells[23–25] and in subsets of innate-like γδ T cells, invariant natural killer T (NKT) cells and mucosal-associated invariant T (MAIT) cells[11,26]. Furthermore, RORγt is required for the development of a broader family of innate lymphocytes, termed group 3 innate lymphoid cells (ILC3s), which include fetal LTi cells and adult LTi-like ILC3s that highly express CCR6, conventional ILC3s that co-express T-bet or natural cytotoxicity receptors such as NKp46, and recently described inflammatory ILC3s derived from the circulation[27–34]. Although the mechanisms are not fully understood, RORγt executes key functions in these diverse types of lymphoid cells to promote their development, preserve their cellular identity or drive their effector programmes.

Intriguingly, in early studies that initially characterized fetal LTi cells, these populations were shown to express MHC-II and have the potential to adopt an APC-like phenotype under certain *in vitro* conditions[19]. This observation and the findings above created a foundation for the discovery of RORγt$^+$ APCs, such that it is now appreciated that expression of MHC-II is a defining feature of several ILC3 subsets (LTi cells, LTi-like ILC3s

and inflammatory ILC3s), types of eTAC and potentially other emerging populations[34–43] (FIG. 2).

Discovery of MHC-II$^+$ LTi-like ILC3s

Beginning in 2010, many groups became interested in the emerging family of ILCs, exploring their development, transcriptional regulation and functional potential. This often necessitated the use of mice lacking adaptive immunity, such as *Rag1*$^{-/-}$ mice, to account for the substantial overlap between T cells and ILCs in terms of marker expression and genetic tools. However, in 2013, the group of Gregory Sonnenberg found that the absence of ILC3s in the presence of adaptive immunity surprisingly resulted in inflammatory T cell responses directed against the intestinal microbiota[35]. This observation was complemented by transcriptional or protein analyses of ILC3s in mice and humans, which revealed an unexpected enrichment in transcripts for MHC-II or other pathways associated with antigen processing and presentation[35]. Indeed, LTi-like ILC3s isolated from mesenteric lymph node or large intestine (and also from many other peripheral tissues) express high levels of MHC-II protein and can take up protein antigens and present processed peptides on MHC-II. This suggests that ILC3s are potent APCs. However, rather than promoting T cell responses, these LTi-like ILC3s were found to have a key role in immune regulation, as *Rorc*^{Cre} (herein referred to as RORγt^{Cre} to reflect isoform specific targeting) -mediated ablation of MHC-II expression by ILC3s resulted in the expansion of T_H17 cell and T_H1 cell populations in the large intestine and the spontaneous onset of chronic inflammation. This is consistent with a lack of key co-stimulatory molecules CD80, CD86 and CD40 on LTi-like ILC3s. Moreover, unfractionated CD4$^+$ T cells from the mice with RORγt^{Cre}-mediated ablation of MHC-II were sufficient to transfer intestinal inflammation to specific-pathogen-free (SPF) *Rag1*$^{-/-}$ mice but not to germ-free *Rag1*$^{-/-}$ recipients. Furthermore, broad-spectrum antibiotics prevented intestinal inflammation in mice lacking MHC-II in the RORγt$^+$ cell compartment and prevented T cells derived from these mice from transferring disease to SPF *Rag1*$^{-/-}$ recipients. Thus, this study defined for the first time a RORγt$^+$ APC population and unexpectedly revealed that MHC-II$^+$ ILC3s educate CD4$^+$ T cell responses to the microbiota, enforcing tolerance and preventing intestinal inflammation[35].

At steady state, MHC-II expression is restricted mainly to the LTi-like ILC3 subset, rather than the conventional NKp46$^+$ or T-bet$^+$ ILC3 subset[35]. This result, as well as the spontaneous expansion of T_H17 cells in the large intestine of mice with a RORγt^{Cre}-mediated ablation of MHC-II, was independently replicated in a subsequent study[44]. There are several other notable distinctions between these two major subsets of ILC3s. Specifically, whereas the LTi-like ILC3s are derived from a unique LTi-cell progenitor, conventional ILC3s are derived from a common helper innate lymphoid cell precursor (CHILP) that is marked by PLZF or ZBTB16 expression[45]. Also, unlike conventional ILC3s, the LTi-like ILC3 subset appears to colonize the intestine prior to birth, is localized in lymphoid clusters, does not require microbiota-derived signals for development or homeostasis[46] and is more stable, such that these cells retain lineage identity following the transient inhibition or deletion of RORγt expression[47,48]. Indeed, emerging studies in humans indicate that LTi-like ILC3s are one of the most abundant haematopoietic cell types

present in the developing gut prior to or shortly after birth[49,50], suggesting that they have key roles in establishing tissue homeostasis and preparing for colonization with microorganisms.

Given that additional RORγt$^+$ APC subsets have been identified recently, it is important to robustly scrutinize and carefully interpret previous findings or conclusions that were attributed to MHC-II$^+$ ILC3s. However, it is equally important to stress that these original studies were not solely reliant on RORγt^{Cre} mice — which are well known to also target gene deletion in double-positive thymocytes, T_H17 cells, some peripherally induced T_{reg} cells, subsets of γδ T cells, invariant NKT cells and MAIT cells — and rather encompassed robust phenotypic and transcriptional profiling, co-cultures of IL-7R$^+$ ILC3s and T cells, imaging strategies and cell-based adoptive transfer approaches[34–36,42,51,52]. Furthermore, the gating strategy used in the above study of MHC-II$^+$ ILC3s[35] involved high IL-7R expression in tissues from adult mice and humans, and often the intestine as a source of these cells. This is important to note, as high IL-7R expression and the presence in adults or large intestinal tissues remains a key distinguishing feature of MHC-II$^+$ ILC3s compared with the other RORγt$^+$ APC subsets discussed in detail below. Therefore, it seems unlikely that the observed phenotypes attributed to MHC-II$^+$ ILC3s in these early studies were the result of major contamination by other RORγt$^+$ APC subsets.

Discovery of RORγt$^+$ APCs expressing AIRE

Subsequent studies expanded the family of RORγt$^+$ APCs to additional cell subsets (FIG. 2), including a rare extrathymic cell population expressing *Aire* that has been reported by several independent studies[37,39–43]. Although all of these studies highlighted some similar markers that characterize the *Aire*$^+$RORγt$^+$ cell subset, different groups named this subset differently as eTACs[42], AIRE$^+$ ILC3-like cells[37,40], Aire-APCs[53], Janus cells[39,41] or a subset of Thetis cells[43] (FIG. 2, Table 1). The variable nomenclature that refers to these *Aire*$^+$RORγt$^+$ APCs introduced considerable confusion in the field concerning their cellular identity, ontogeny and putative functional roles. Here, we refer to these cells collectively as RORγt$^+$ eTACs as this abbreviation has been used for the longest period[54] and well describes a key molecular characteristic of these populations that distinguishes them from LTi-like ILC3s.

The discovery of RORγt$^+$ eTACs stemmed from the study of *AIRE*, which encodes a transcriptional regulator whose loss of function is responsible for autoimmune polyendocrine syndrome type 1 (APS1)[55,56] (BOX 1). Although the expression of AIRE is mainly restricted to a subset of medullary thymic epithelial cells (mTECs), which have a crucial role in the induction of central tolerance by presenting self-antigens to developing thymocytes, early studies indicated that *Aire* transcripts are also clearly detectable in the secondary lymphoid organs[55,57]. However, the identity of such *Aire*-expressing cells outside the thymus remained puzzling and controversial for a relatively long time, as their proper identification and characterization has been technically challenging. Initially, using mice expressing a transgenic green fluorescent protein (GFP) reporter driven by the *Aire* promoter (*Aire*^{GFP}), it was shown that the *Aire*^{GFP} reporter signal is mainly restricted to EpCAM$^+$MHC-II$^+$CD45$^{-/low}$ stromal cells within the lymph node and spleen. Moreover, using immunofluorescence microscopy the authors showed that some of the *Aire*^{GFP}

reporter-positive cells expressed AIRE protein and, similar to its expression in mTECs, AIRE was localized in nuclear speckles[54]. The identity of these eTACs was subsequently revisited in another study from the same group using bone-marrow chimera experiments, which showed that eTACs are not stromal cells but rather have a haematopoietic origin[58]. Moreover, the study demonstrated that the *Aire*^{GFP} reporter signal could be detected in a subset of CD11c^{low}ZBTB46^{+}CCR7^{+} cells, which suggested that eTACs might be a subset of cDCs[58]. It should be noted, however, that both studies characterized the CD11c$^{+/low}$EpCAM^{+} eTACs based on expression of the *Aire*^{GFP} reporter and *Aire* mRNA, rather than AIRE protein.

To further characterize the AIRE-expressing cell population in secondary lymphoid organs, a study co-authored by Jan Dobeš used AIRE-specific antibodies in conjunction with the *Aire*^{GFP} reporter and found that although part of the *Aire*^{GFP} reporter signal was indeed expressed by CD11c^{+} and/or CD11c^{+}EpCAM^{+} cells, the expression of AIRE protein was restricted to a rare lineage-negative population characterized by high expression of MHC-II and RORγt[37]. Therefore, some but not all eTACs express RORγt, and the originally described population of *Aire*^{GFP}-expressing eTACs is, in fact, a heterogeneous group of cells consisting of at least three major subsets — CD11c^{+}RORγt$^{–}$ and CD11c^{+}EpCAM^{+}RORγt$^{–}$ cells (both of which have undetectable AIRE protein and cDC characteristics) and a lineage-negative population characterized by co-expression of RORγt and AIRE protein. Because of the lack of conventional lineage markers and the expression of RORγt, as well as expression of other ILC3-specific genes including *Ccr6, Kit, Id2* and *Il1r1*, this subpopulation of eTACs was originally termed AIRE^{+} ILC3-like cells[37]. Interestingly, whereas AIRE^{+} ILC3-like cells were present in lymph nodes and to a lesser extent in the spleen, they were absent from the Peyer's patches, lamina propria or intraepithelial compartments[37], suggesting that they may be involved in the regulation of immune responses in lymphoid organs draining most peripheral tissues, but not directly in the intestinal mucosa. In addition, RORγt^{+} eTACs express low levels of or no IL-7R. These are clear distinctions from the LTi-like ILC3s described above, which have a high level of expression of IL-7R and are present at both peripheral lymph nodes and mucosal sites.

This original study by Dobeš and others did not provide any lineage tracing data for the AIRE^{+} ILC3-like cells, but it found that these cells have a clear lymphoid morphology[37] and that stimulation of ILC3s with RANKL induced expression of the *Aire*^{GFP} reporter in a fraction of these cells, suggesting that some ILC3s have the capacity to give rise to RORγt^{+} eTACs[37]. Moreover, a recent study from the Sonnenberg laboratory using *Aire*^{Cre}-based lineage tracing also highlighted interconversion potential between LTi-like ILC3s and RORγt^{+} eTACs[42,59] (additional data, not peer reviewed, can be found in the preprint of this study[59]). This, together with the apparent lack of conventional lineage markers and expression of several key ILC3-specifying markers, suggests that RORγt^{+} eTACs share some developmental ontogeny with ILC3s. However, subsequent studies based on bulk RNA sequencing and/or single-cell RNA sequencing (scRNA-seq) showed that RORγt^{+} eTACs have distinct transcriptomes from ILC3s[43,60]. These studies suggested that RORγt^{+} eTACs are composed of several distinct subsets expressing varying levels of *Aire* mRNA (Table 1). However, AIRE protein was detected only in the RORγt^{+} eTAC subset characterized by high expression of the co-stimulatory molecules CD80, CD86 and CD40[40,43]. Importantly,

focusing on transcriptome profiling and chromatin accessibility, these studies show that RORγt$^+$ eTACs have the greatest molecular similarity with CCR7$^+$ migratory cDCs[39,41] rather than ILC3s. In addition, the studies reported some limited overlap between the RORγt$^+$ eTAC-specific gene signature and that of mTECs, which — in addition to *Aire* and genes of the nuclear factor-κB signaling pathway and antigen-presentation machinery — included several stroma-specific transcription factors (such as *Foxj1* and *Tbx3*)[39].

Although RORγt$^+$ eTACs share some transcriptional similarity with cDCs, several recent studies that used different lineage tracing models to better determine their developmental origin suggest that RORγt$^+$ eTACs are likely not part of the cDC lineage. Specifically, *Clec9a*^{Cre}-driven fate mapping, which traces all cDC1s and targets approximately half of the cDC2 subset[61], does not occur in RORγt$^+$ eTACs or ILC3s[42,43,62] (Table 2). These data therefore suggest that RORγt$^+$ eTACs do not share a developmental history with cDC subsets. This conclusion is further supported by lineage tracing using *Itgax*^{Cre}-driven reporter mice (*Itgax* encodes CD11c), which showed detectable reporter signal only in a fraction of RORγt$^+$ eTACs (~5–20%) and LTi-like ILC3s (~5–10%)[42,43] (Table 2). Nonetheless, although most of the RORγt$^+$ eTACs are not labelled by *Itgax*^{Cre}-based fate mapping, they were detected in scRNA-seq experiments based on sorting of fate-mapped cells from *Itgax*^{Cre} mice[41]. Furthermore, RORγt$^+$ eTACs express ZBTB46[39,41,43,58], which was originally assumed to be exclusive to the cDC lineage[63,64]. However, a recent study from the Sonnenberg laboratory showed that ZBTB46 is also robustly expressed in LTi cells and LTi-like ILC3s[62].

Additional lineage tracing models have helped to shed light on the identity of RORγt$^+$ eTACs. Specifically, the use of *Rag1*^{Cre}-based reporters validated that RORγt$^+$ eTACs are, as expected, not derived from progenitors that give rise to the adaptive immune system[43] (Table 2). By contrast, the use of *Il7r*^{Cre}-based fate mapping[65], which is considered to be one of the most reliable reporters of the lymphoid lineage, marked a substantial fraction (~75%) of RORγt$^+$ eTACs[42] (Table 2). Although *Il7r*^{Cre}-based fate mapping marks only a minor fraction of myeloid cells or granulocytes in the spleen and thymus[65], other studies have shown that it can mark certain embryonic yolk sac-derived subsets of the myeloid lineage, including tissue-resident macrophages such as microglia[66]. Therefore, although the *Il7r*^{Cre} reporter signal indicates that RORγt$^+$ eTACs are likely of lymphoid origin, additional lineage tracing or progenitor transfer-based studies are needed to provide more definitive answers. Using a *Rora*^{Cre}-based reporter system, which was previously shown to label most ILC2s and a limited proportion of ILC3s[67], no positive fate mapping was identified in RORγt$^+$ eTACs[43]. In addition, *Il22*^{Cre}-based fate mapping was found to label some of the ILC3 subsets, presumably following activation, but it failed to label RORγt$^+$ eTACs[42,43,62] (Table 2). In conclusion, it still remains unclear whether RORγt$^+$ eTACs constitute an entirely separate lineage of APCs or whether they may represent a developmental branch within the ILC lineage. These and other open questions require better characterization of RORγt$^+$ eTAC progenitors, as well as the transcription factors that control their specification and development.

Although most studies of RORγt$^+$ eTACs were carried out in mice, emerging evidence suggests that a similar population might exist in human lymph nodes[58,68]. Similar to

RORγt$^+$ eTACs in mice, *AIRE*-expressing cells in human lymph nodes were reported to express high levels of molecules associated with antigen presentation (HLA-DR) and co-stimulation (CD80, CD86 and CD40) (Table 1). However, the human eTACs also expressed high levels of IL-7R and CCR7, which are expressed only at very low levels on mouse RORγt$^+$ eTACs. Based on these results, the authors concluded that human eTACs are likely to be a subset of migratory CCR7$^+$ cDCs[68]. Nevertheless, a detailed molecular characterization of these cells in humans remains incomplete and somewhat controversial. For example, whereas one independent study showed that AIRE does not co-localize with the myeloid markers CD11c or CD11b in immunofluorescence analyses of human lymph nodes[58], another study using immunohistochemical analyses showed that AIRE is co-localized with CD11c, CCR7 or CD40[69]. Moreover, although human eTACs share several key features and markers with mouse RORγt$^+$ and RORγt$^-$ eTACs, it remains to be determined whether they actively express RORγt and/or may be derived from RORγt$^+$ precursors.

Discovery of Rorc$^+$ DC-like subsets

It was recently proposed that RORγt is expressed by a subset of CD11b$^+$ cDC2s in the spleen, termed cDC2Bs[38]. However, it should be noted that this conclusion was not fully corroborated by supporting evidence, including no detection of *Rorc* transcripts in the scRNA-seq dataset of this proposed cell type and no demonstrated detection of RORγt protein. Moreover, the study found that RORγt^{Cre} fate mapped only ~0.2% of cDC2s in the spleen of mice[38], suggesting that a RORγt$^+$ cDC population would be exceedingly rare. Indeed, several recent studies, including a study by the same group that proposed the presence of RORγt$^+$ cDC2Bs, have challenged the notion that RORγt is expressed by genuine cDCs[37,42,62]. Instead, these studies highlighted the existence of *Rorc*$^+$ APCs that are developmentally unrelated to cDCs but that share some molecular characteristics. Specifically, based on scRNA-seq analysis of MHC-II$^+$ and *Rorc*-expressing cells isolated from the mesenteric lymph nodes of 2–3-week-old mice, they found that in addition to MHC-II$^+$ LTi-like ILC3s there is a unique though heterogeneous population of *Rorc*$^+$ APCs that the authors collectively termed Thetis cells (TCs). Whereas some subsets of these Thetis cells are analogous to previously described RORγt$^+$ eTACs (TC I and TC III), two other molecularly distinct clusters termed TC II and TC IV were readily distinguished from MHC-II$^+$ ILC3s by the lack of expression of CXCR6 and IL-7R, and from RORγt$^+$ eTACs by the lack of active *Aire* expression (Table 1). Whereas the TC II subset seems to have relatively high transcriptomic similarity to RORγt$^+$ eTACs and shares with them some key molecular characteristics, the TC IV subset has a rather distinct transcriptome and chromatin landscape compared with RORγt$^+$ eTACs, the TC II subset and LTi-like ILC3s. Specifically, the TC IV subset was found to stain for CD11b and CD11c integrin protein, as well as express relatively high levels of various surface marker-encoding genes (including *Cd274*, *Il2ra* and *Itgb8*) that appear to be less robustly expressed by RORγt$^+$ eTACs[43] or ILC3s. Like RORγt$^+$ eTACs, these cells were found to express high levels of the co-stimulatory molecules CD80, CD86 and CD40. The authors proposed that the TC IV subset likely corresponds to their previously described *Rorc*$^+$ cDC2B subset. Nevertheless, unlike a majority of cDC1s and portion of cDC2s, this cell type is not developmentally derived from CLEC9a$^+$ progenitors. Finally, these *Rorc*$^+$ DC-like cells were found to have

transcriptomic and chromatin accessibility profiles with similarities to those of TECs, as had been previously described for the RORγt$^+$ eTACs[39].

It is still not clear whether the TC II and TC IV subsets are part of a larger cell compartment that is developmentally related to RORγt$^+$ eTACs or whether they are separate terminally differentiated entities with defined functional roles and possibly different progenitors. It is also worth noting that whereas cell subsets analogous to TC II have been reported by other independent scRNA-seq studies of RORγt$^+$ cell types (Table 1), there seems to be less consensus about the identity of the TC IV subset, which has not been found in other studies. One possibility for this variability could be that the study identifying TC IV cells used mesenteric lymph nodes of 2–3-week-old mice[43], whereas nearly all other studies profiling RORγt$^+$ cell types have used tissues from adult mice. Another possibility may be that this study[43] used a novel *Rorc*-reporter mouse in which the reporter sequence was inserted downstream of exon 11, which is shared between RORγt and the more broadly expressed RORγ isoform[12–14]. As there was no validation that the TC IV subset expresses the RORγt-specific isoform encoded by *Rorc*, additional investigation is needed, and presently we refer to these cells as *Rorc*$^+$ DC-like cells. Finally, these reports suggest that RORγt$^+$ eTACs and the putative TC IV subset are not only molecularly distinct but may have different functional roles in modulating T cell responses to microorganisms (discussed in more detail below). Elucidating the exact functional roles of these emerging *Rorc*$^+$ DC-like APC subsets that do not express CXCR6, IL-7R or AIRE, as well as their developmental relationships with RORγt$^+$ eTACs and LTi-like ILC3s, will require extensive further investigation.

RORγt$^+$ APCs in tolerance and immunity

It is clear from many studies that RORγt$^+$ APCs have crucial roles in shaping T cell responses with diverse functional outputs, including the peripheral tolerance of microbiota-specific T cells[35,36], instruction of microbiota-specific T cells to FOXP3$^+$RORγt$^+$ T_{reg} cells[41–43], regulation of antibody production[70,71], control of allergen-specific T cells[52], augmentation of antitumour immunity or neuroinflammation[34,51], and the induction of *Candida*-specific T_H17 cells[40]. These reports indicate that RORγt$^+$ APCs are characterized not only by large molecular diversity but also by a substantive diversity of biological functions that stimulate opposing immunological outcomes (FIG. 2). The capacity of RORγt$^+$ APCs to induce both T cell tolerance and T cell activation is not surprising, as it is a hallmark of all types of APC. Specifically, the output of the T cell response is determined by the specific context in which the APC presents a given antigen to cognate T cells. In the absence of proinflammatory triggers and under homeostatic conditions, APCs ‘see’ the antigen as non-dangerous and induce T cell tolerance[72,73] through various mechanisms, including clonal deletion[74,75], induction of T cell anergy[76] and conversion to or proliferation of T_{reg} cells[77]. Under inflammatory conditions, APCs ‘see’ the acquired antigen as dangerous and promote the activation of effector T cell responses and/or their polarization to individual helper subtypes based on the nature of the threat.

MHC-II$^+$ ILC3s

Subsequent research since MHC-II$^+$ LTi-like ILC3s were first identified has highlighted several diverse mechanisms that are used by these cells to shape immune responses in the gastrointestinal tract. For example, in the absence of MHC-II on LTi-like ILC3s, T_H17 cell populations in the gut are expanded, independently of colonization with segmented filamentous bacteria (related to the genus *Clostridium*) or intestinal inflammation[44,78]. Using TCR-transgenic mice responding to a model *Clostridium* flagellin, CBir1, the Sonnenberg laboratory subsequently determined that in the absence of MHC-II on LTi-like ILC3s, CBir1-specific T cells expand in response to microbiota-derived antigen and provoke robust intestinal inflammation. Further *in vitro* co-culture studies with IL-7R$^+$ LTi-like ILC3s, cell-based adoptive transfers and *in vivo* mouse models in which MHC-II expression was restricted to RORγt$^+$ cells showed that ILC3s promote MHC-II-dependent and antigen-dependent apoptosis of CBir1-specific effector T cells in part through the sequestration of pro-survival signals such as IL-2 (Ref.[36]). Intriguingly, expression of MHC-II on LTi-like ILC3s in the large intestine or mesenteric lymph nodes was not affected by inflammatory signals such as interferon-γ, but rather was constitutively high and required promotor IV of the MHC-II transactivator CIITA. This is similar to the pathway that promotes MHC-II expression on thymic epithelium, which led to the concept of ‘intestinal selection’ — which proposes that the education of microbiota-specific effector T cells by MHC-II$^+$ LTi-like ILC3s is required for immune tolerance and intestinal health[36] (FIG. 2). This concept is supported by the finding that human ILC3s from the intestine of individuals with inflammatory bowel disease (IBD) had decreased MHC-II expression relative to non-IBD controls, which correlated with increased T_H17 cells[36].

This tolerogenic role for MHC-II$^+$ LTi-like ILC3s was also found in other contexts and anatomical locations (FIG. 2). Following infection with an enteric pathogen, MHC-II$^+$ LTi-like ILC3s restrict T follicular helper (T_{FH}) cell expansion, in part through PD-L1 signaling, thus reducing the activation of germinal centre B cells and subsequent IgA production against colonic microorganisms[70,71]. During the formation of colorectal tumours, the Sonnenberg laboratory found that MHC-II$^+$ LTi-like ILC3s become dysregulated and exhibit plasticity towards group 1 ILCs. Loss of the tolerogenic LTi-like ILC3s unleashed microbiota-specific T cells to drive inflammation and caused alterations in the composition of the microbiota and resulted in reduced type 1 immunity, subsequently favoring tumour progression or invasion, and resistance to immune checkpoint blockade[51]. Outside of the gut, the Sonnenberg laboratory found that MHC-II$^+$ LTi-like ILC3s are present in the airway of humans and are enriched in the lung-draining lymph nodes. In this location also, LTi-like ILC3s are tolerogenic and restrict both allergen-specific T_H2 cell responses and microorganism-reactive T_H17 cell responses via MHC-II and following exposure of mice to papain or house dust mite extracts, thus protecting from experimental airway inflammation[52].

Nonetheless, not all MHC-II$^+$ ILC3s deliver tolerogenic signals to CD4$^+$ T cells. Using *in vitro* cultures and mice with MHC-II deletion in RORγt$^+$ cells, cytokine-activated, peripheral ILC3-like cells in the spleen were shown to promote T cell responses following experimental immunization[79]. In contrast to the LTi-like ILC3s in the large intestine

and mesenteric lymph nodes, it was found that IL-1β and interferon-γ upregulated the expression of MHC-II and co-stimulatory molecules on these ILC3-like subsets from the spleen or small intestine in mice, whereas microbiota-induced IL-23 was found to downregulate MHC-II expression by these cells[79,80]. TL1A (also known as TNFSF15) also increases the expression of co-stimulatory molecules by ILC3s and their ability to promote T_H1 cell responses[81]. Similarly, IL-1β, IL-18 and transforming growth factor-β (TGFβ) increase expression of MHC-II and co-stimulatory molecules on human ILC3s derived from distinct anatomical locations[82]. For example, ILC3s isolated from the peripheral blood of humans can activate autologous T cells via MHC-II when cultured with cytokines[82]. Tissue-resident ILC3s in humans express similar antigen-presentation machinery to blood ILC3s, but autologous co-culture studies have not yet been carried out to test their ability to activate T cells. It should also be noted that lineage-negative, IL-7R$^+$KIT$^+$ cells in peripheral blood also include circulating ILC precursors as well as mature ILC3s in humans[83].

These studies raise many questions as to what shapes the ability of MHC-II$^+$ ILC3 subsets to promote or inhibit T cell responses, and whether key differences exist between mice and humans. One possibility is that there is unexpected heterogeneity among ILC3s and across distinct tissues. Indeed, a recent study from the Sonnenberg laboratory using the experimental autoimmune encephalomyelitis (EAE) mouse model defined a unique IL-7R$^+$ ILC3 subset in the central nervous system (CNS) characterized by co-expression of T-bet and RORγt, and a lack of CD4 and NKp46 expression. This subset was distinct from LTi-like ILC3s or conventional ILC3s in that the ILC3s in the CNS were derived from the circulation rather than being tissue resident[34]. The authors refer to these cells as ‘inflammatory ILC3s’ to mirror the terminology used to describe ‘inflammatory ILC2s’ that can uniquely enter the circulation[34,84]. Intriguingly, these inflammatory ILC3s in the CNS express MHC-II and the co-stimulatory molecules CD80 and CD86. Co-culture studies and mouse models determined that the inflammatory ILC3s were required to restimulate myelin-specific T cells upon entry into the CNS to promote autoimmune neuroinflammation in a MHC-II-dependent manner[34] (FIG. 2). The inflammatory ILC3s lacked expression of AIRE and deletion of *Aire* in RORγt$^+$ cells did not impact EAE, indicating a distinct functional role for inflammatory ILC3s relative to other RORγt$^+$ APC subsets.

In addition, the Sonnenberg laboratory recently found that the transcriptional repressor ZBTB46, which is highly expressed by LTi-like ILC3s in addition to cDCs, affects whether MHC-II$^+$ ILC3s acquire activating or tolerogenic properties[62]. The ZBTB46 pathway is upregulated during chronic intestinal inflammation in mice and humans, and lineage-specific knockouts indicated that ZBTB46 restricts co-stimulatory pathways on ILC3s and limits their ability to promote T cell effector responses following enteric infection[62]. ZBTB46 is also expressed by RORγt$^+$ eTACs and *Rorc*$^+$ DC-like cells, but a function has not yet been described. Interestingly, genetic variants of *ZBTB46* associate with IBD and multiple sclerosis in humans[85,86]. ZBTB46 expression has been widely used to study the function of cDCs in immunity, inflammation and tolerance[87], and analysis of these data should therefore also consider potential contributions of ZBTB46-expressing RORγt$^+$ APCs. ZBTB46 expression in LTi-like ILC3s is also shaped by the microbiota, RORγt and inflammatory cytokines, suggesting a dynamic ability to regulate this pathway and that it may represent a key molecular determinant of tolerance in LTi-like ILC3s[62].

RORγt+ eTACs

RORγt+ eTACs have been suggested to have the capacity to induce both peripheral tolerance to self-antigens[39,54,58] and antigen-specific T_H17 cells in response to *C. albicans* infection[40]. Specifically, the putative tolerogenic potential of the eTAC compartment is supported by several studies showing that it can mediate deletion of $CD8^+$ T cells[54] and anergy of $CD4^+$ T cells[58] specific for a neo-self-antigen that was expressed by eTACs under the *Aire* promoter in homeostatic conditions. It should, however, be noted that as the $Aire^{GFP}$ reporter is expressed not only in RORγt+ eTACs but also in a fraction of DCs[37], it is difficult to determine which of these subsets makes the main contribution to tolerance induction. In comparison to all other RORγt+ APC subsets, including LTi-like ILC3s, the $AIRE^{hi}$RORγt+ eTAC subset expresses by far the highest levels of co-stimulatory molecules (Table 1), which suggests that these cells are likely to be more immunogenic than tolerogenic.

Another important question relates to whether the expression of AIRE in RORγt+ eTACs has the capacity to promote expression of tissue-restricted antigens **[G]** (TRAs) and whether it is required for the induction of peripheral self-tolerance. It is well established that in mTECs, AIRE facilitates the expression of hundreds of TRA-encoding genes[57], which is required for the elimination of potentially self-reactive T cell clones[88] or their conversion to T_{reg} cells with suppressive function[89,90]. Intuitively, one would assume that AIRE has an analogous role in eTACs. However, several independent studies showed that in both the bulk $Aire^{GFP}$ reporter-positive population and RORγt+ eTACs, AIRE does not seem to promote promiscuous gene expression. Specifically, using microarray analysis[54], bulk RNA sequencing[37] or scRNA-seq[39,42] of RORγt+ eTACs, it was shown that AIRE induces the expression of only several dozen TRA genes in RORγt+ APCs. Similarly, human eTACs were found not to be enriched for the expression of TRA genes[68]. The numbers of AIRE-induced and AIRE-repressed TRA genes were almost the same in the RORγt+ eTACs, suggesting that in these cells AIRE is not the inducer of the promiscuous gene expression programme. Thus, although $Aire^{GFP}$ reporter-positive cells have putative tolerogenic capacity under noninflammatory conditions, they do not seem to produce TRAs in levels and quality comparable to mTECs.

If AIRE does not have the capacity to induce TRA expression in RORγt+ eTACs, is it then required for the induction of peripheral tolerance to self? A possible answer to this important question was in fact partially provided already in a seminal study from 2002 using reciprocal bone-marrow chimeras; whereas transplantation of wild-type bone marrow into $Aire^{-/-}$ recipients resulted in a multi-organ autoimmune phenotype, the reciprocal transplantation of $Aire^{-/-}$ bone marrow into wild-type recipients did not result in any signs of autoimmunity[57]. This suggests that whereas AIRE expression in stromal cells (such as mTECs) is essential for self-tolerance induction, the expression of AIRE in the haematopoietic compartment, including RORγt+ eTACs, seems largely dispensable to prevent autoimmunity. It should, however, be noted that the capacity of thymic AIRE expression to induce self-tolerance is restricted to an early developmental time window[91] and thus it is possible that the use of bone-marrow chimeras in adult mice may not fully address the potential role of AIRE expression in the periphery in the induction of self-tolerance. In addition, recent studies showed that $ROR\gamma t^{Cre}$-dependent ablation of *Aire* in RORγt+ eTACs does not have any

impact on the development of EAE[34] or induction of tolerance to the microbiota[41,42]. More recently, it was shown that ablation of the eTAC compartment resulted in increased resorption of mouse embryos[92]. However, the study did not directly address whether this result was owing to the ablation of RORγt$^+$ eTACs or of AIRE itself.

Collectively, these studies suggest that, under homeostatic conditions, AIRE might have different roles in RORγt$^+$ eTACs and mTECs. Indeed, a more recent study authored by Jan Dobeš and Jakub Abramson showed that RORγt$^+$ eTACs have a strong immunogenic potential, as they were essential for the induction of antigen-specific T_H17 cells in response to *Candida albicans* infection[40] (FIG. 2). The expression of AIRE in these cells was essential for their capacity to induce *Candida*-specific T_H17 cells, as its selective ablation in RORγt$^+$ eTACs using RORγt^{Cre} resulted in a failure to expand *C. albicans*-specific T_H17 cell clones and consequent overgrowth of *C. albicans* in the gastrointestinal tract[40]. The exact molecular mechanisms by which RORγt$^+$ eTACs induce expansion of *Candida*-specific T_H17 cells are still incompletely understood, but likely involve the action of co-stimulatory molecules, as well as high-level expression of various cytokines, such as IL-12p40 (a key component of IL-23 heterodimer) or IL-6, which were previously found to have crucial roles in the induction of T_H17 cells. In addition, it is possible that AIRE may regulate the antigen processing and presentation capacity of RORγt$^+$ eTACs by a similar, yet incompletely understood, mechanism as has been suggested for mTECs[93]. It is worth noting that a similar decrease in *C. albicans*-responsive T_H17 cells was reported in individuals with APS1[40,94], who develop chronic mucocutaneous candidiasis as early as one year of age (BOX 1).

In conclusion, under steady-state conditions, RORγt$^+$ eTACs have, similarly to other types of APC, the capacity to be tolerogenic. However, the importance of AIRE in this process is questionable as it does not induce TRA expression in RORγt$^+$ eTACs and its peripheral deficiency does not seem to result in any obvious autoimmune phenotype. During immune challenge, however, AIRE expression in RORγt$^+$ eTACs is indispensable for mounting an effective *C. albicans*-specific adaptive immune response.

Promoting microbiota-specific T_{reg} cells

Three recent studies identified that RORγt$^+$ APCs, encompassing LTi-like ILC3s, RORγt$^+$ eTACs and *Rorc*$^+$ DC-like cells, have an indispensable role in the generation of microbiota-specific T_{reg} cells that uniquely co-express FOXP3 and RORγt[41–43], which were previously shown to be crucial for maintenance of tolerance to intestinal microorganisms[23–25]. Instrumental in these studies was the development of TCR-transgenic mice specific for *Helicobacter* species, which revealed a key role for these bacteria in inducing the differentiation of antigen-specific RORγt$^+$ T_{reg} cells[95,96]. Originally, it was assumed that the generation of microbiota-specific RORγt$^+$ T_{reg} cells is orchestrated by cDCs, or at least CD11c-expressing cells, but multiple CD11c$^+$ subsets were shown to be redundant in this process[24,95,97]. Surprisingly, the three recent studies reported a paradigm shift that the generation of microbiota-specific RORγt$^+$ T_{reg} cells requires RORγt$^+$ APCs but not cDCs[41–43]. These studies differed in their conclusion as to which RORγt$^+$ APC subset is the main mediator of this process, implicating either MHC-II$^+$ LTi-like ILC3s[42], CD11b$^+$ITGB8$^+$*Rorc*$^+$ DC-like APCs[43] or, potentially, RORγt$^+$ eTACs[41]. Although there

are notable discrepancies between these three reports (BOX 2), many of the major results are congruent. These include the finding that microbiota-specific RORγt$^+$ T_{reg} cells are markedly impaired in mice lacking MHC-II on RORγt$^+$ APCs, a contribution of TGFβ-processing integrins on RORγt$^+$ APCs, and a lack of a requirement for MHC-II on cDCs[41–43]. This induction of microbiota-specific RORγt$^+$ T_{reg} cells by RORγt$^+$ APCs occurs in a key developmental window (2–3 weeks of age for mice) but also following colonization of adult mice with *Helicobacter hepaticus*[41–43]. Furthermore, it may involve CCR7-dependent migration of RORγt$^+$ APCs to the mesenteric lymph nodes, where MHC-II$^+$ LTi-like ILC3s closely associate with RORγt$^+$ T_{reg} cells at interfollicular regions[36,41,42,98]. Finally, in the context of MHC-II$^+$ LTi-like ILC3s, the Sonnenberg laboratory found that these cells are reduced in patients with IBD and that this correlates with reduced RORγt$^+$ T_{reg} cells (and TCR-signaling associated transcripts) in the inflamed intestine of humans[42]. This extends previous findings that ILC3s are inherently dysregulated in the inflamed human intestine[99]. Additional tools and research are required to further dissect the contribution of each RORγt$^+$ APC subset to the induction of microbiota-specific T_{reg} cells in the gut, and it remains possible that all three subsets could contribute in a spatial–temporal manner.

Perspectives and future directions

The past decade of research on ILC3s and recent studies on other emerging RORγt$^+$ APC subsets has yielded many exciting advances. However, study of the functional interactions and lineage relationships among these cell types is still in the early stages, and several confusing points exist including numerous different nomenclatures. We propose a new preliminary nomenclature to add clarity in this field of research (BOX 3), which will likely need to be amended with new discoveries in the future. RORγt$^+$ ILC3s, RORγt$^+$ eTACs and *Rorc*$^+$ DC-like cells share some phenotypic markers but are distinguished by other markers, including IL-7R, CXCR6, CCR6, AIRE, SIGLECG, DPP4, CD11b and CD11c (Table 1, FIG. 2). Future studies therefore need to better define these cell subsets from molecular, functional and developmental perspectives. For example, it will be necessary to decisively determine the identity of RORγt$^+$ eTACs. As these cells are distinct from classical ILC3s and seem to be developmentally unrelated to the cDC lineage, it will be crucial to determine what are their immediate and distal precursors. It is also worth noting that RORγt$^+$ eTACs and *Rorc*$^+$ DC-like cells seem to reach peak levels between days 10 and 20 after birth in mice and are notably absent from the intestine, but the fate and migratory potential of these cells in adult mice is unknown. Data in a recent preprint from the Sonnenberg laboratory (not peer-reviewed) suggest that RORγt$^+$ eTACs and ILC3s have interconversion potential when transferred into lymphocyte-deficient receipients[59], which further highlights the possibility that RORγt$^+$ APCs share lineage relationships and warrants further investigation. New tools to specifically and robustly target each RORγt$^+$ APC subset are urgently needed to clarify their ontogeny, lineage relationships and functional potential.

Moreover, whether the expression of AIRE in RORγt$^+$ eTACs is required for the induction or maintenance of peripheral tolerance to self or microorganisms requires further clarification. Additional studies using different models of autoimmunity with targeted deletion of AIRE will be needed to address this important question in a more comprehensive

manner. Correspondingly, although we have an extensive understanding of how AIRE induces promiscuous gene expression in mTECs, its mechanism of action in RORγt$^+$ eTACs remains largely elusive. It is unclear why AIRE fails to induce promiscuous gene expression in RORγt$^+$ eTACs or how it regulates, at a molecular level, the induction of *Candida*-specific T_H17 cells. Similarly, it will also be important to determine whether RORγt$^+$ eTACs are essential for the induction of adaptive immune responses (or tolerance) to other pathogens.

Finally, additional research into human RORγt$^+$ APCs is extensively needed and raises the possibility to harness these cell types to drive optimal immunity or immune regulation in humans. For example, RORγt expression in human eTACs or *Rorc*$^+$ DC-like cells has not yet been clearly investigated. Answering these questions will lead to a better understanding of how ILC3s and other RORγt$^+$ APCs crosstalk with adaptive immune responses to promote protective T cell responses to pathogens or malignancy, or to induce immune tolerance and prevent inflammatory disorders.

Acknowledgements

We thank members of the Abramson, Dobeš and Sonnenberg Laboratories for discussions and critical reading of the manuscript. G.F.S. is kindly supported by the National Institutes of Health (R01AI143842, R01AI123368, R01AI145989, U01AI095608, R01AI162936, R01AI174468, and R01CA274534), the NIAID Mucosal Immunology Studies Team (MIST), an Investigators in the Pathogenesis of Infectious Disease Award from the Burroughs Wellcome Fund, the Cancer Research Institute, Linda and Glenn Greenberg, the Dalton Family Foundation, and the Roberts Institute for Research in IBD. G.F.S. is a CRI Lloyd J. Old STAR. M.L. is supported by a Crohn's and Colitis Foundation Research Fellowship Award (#935259). J.A. is kindly supported by the Eugene and Marcia Applebaum Professorial Chair, European Research Council (ERC-2016-CoG-724821), IOCB fellowship for sabbatical visit program (RVO 61388963) and Bill and Marika Glied and Family Fund. J.D. is kindly supported by the Czech Science Foundation JUNIOR STAR grant (21-22435M), Czech Science Foundation grant (22-30879S) and by the Charles University PRIMUS grant (Primus/21/MED/003).

Glossary

Professional APCs
Professional antigen-presenting cells (APCs) are a subset of immune cells, mainly conventional dendritic cells, macrophages and B cells, that are specialized in activating naive CD4$^+$ T cell responses through phagocytosis of exogenous antigens, and processing and presentation of antigenic peptides on their MHC class II molecules, together with costimulatory signals.

Non-professional APCs
A subset of cells such as thymic epithelial cells, endothelial cells and granulocytes that modulate the quality of the CD4$^+$ T cell response in peripheral tissues through antigen presentation on MHC class II.

RORγt$^+$ APCs
A newly defined family of antigen-presenting cells that express RORγt and can present antigens to CD4$^+$ T cells through MHC class II and other associated molecules.

Autoimmune regulator

(AIRE). AIRE is a transcriptional regulator initially reported to be expressed by medullary thymic epithelial cells, where AIRE has role in central immune tolerance by inducing the expression of tissue-restricted antigens.

Type 3 immune responses

Type 3 immune responses involve RORγt^+ lymphocytes, including T_H17 cells, that produce the cytokines IL-17 and IL-22 to mediate antimicrobial responses and neutrophil recruitment. Type 3 responses are protective in the case of extracellular bacterial or fungal infections, but if dysregulated, can drive chronic inflammation.

Tissue-restricted antigens

(TRAs). Self-antigens whose coding genes are expressed in less than five different parenchymal tissues (out of approximately 60) based on currently available expression atlases. Expression of these genes may also be restricted to a particular developmental period, be sex-specific or be regulated by complex biochemical pathways.

Table of Contents

Recent studies have revealed a family of antigen-presenting cells (APCs) marked by the transcription factor RORγt that fundamentally shape immunity, inflammation and tolerance. This article reviews heterogeneity among RORγt^+ APCs, their associated functions, and the future promise of this new field.

References

1. Blum JS, Wearsch PA & Cresswell P Pathways of antigen processing. Annu Rev Immunol 31, 443–473 (2013). [PubMed: 23298205]
2. Guermonprez P, Valladeau J, Zitvogel L, Théry C & Amigorena S Antigen presentation and T cell stimulation by dendritic cells. Annu Rev Immunol 20, 621–667 (2002). [PubMed: 11861614]
3. Rossjohn J et al. T cell antigen receptor recognition of antigen-presenting molecules. Annu Rev Immunol 33, 169–200 (2015). [PubMed: 25493333]
4. Pishesha N, Harmand TJ & Ploegh HL A guide to antigen processing and presentation. Nat Rev Immunol 22, 751–764 (2022). [PubMed: 35418563]
5. Huppa JB & Davis MM T-cell-antigen recognition and the immunological synapse. Nat Rev Immunol 3, 973–983 (2003). [PubMed: 14647479]
6. Klein L, Hinterberger M, Wirnsberger G & Kyewski B Antigen presentation in the thymus for positive selection and central tolerance induction. Nat Rev Immunol 9, 833–844 (2009). [PubMed: 19935803]
7. Murphy TL et al. Transcriptional Control of Dendritic Cell Development. Annu Rev Immunol 34, 93–119 (2016). [PubMed: 26735697]
8. Kambayashi T & Laufer TM Atypical MHC class II-expressing antigen-presenting cells: can anything replace a dendritic cell? Nat Rev Immunol 14, 719–730 (2014). [PubMed: 25324123]
9. Hirose T, Smith RJ & Jetten AM ROR gamma: the third member of ROR/RZR orphan receptor subfamily that is highly expressed in skeletal muscle. Biochem Biophys Res Commun 205, 1976–1983 (1994). [PubMed: 7811290]
10. Medvedev A, Yan ZH, Hirose T, Giguère V & Jetten AM Cloning of a cDNA encoding the murine orphan receptor RZR/ROR gamma and characterization of its response element. Gene 181, 199–206 (1996). [PubMed: 8973331]
11. Eberl G RORγt, a multitask nuclear receptor at mucosal surfaces. Mucosal Immunol 10, 27–34 (2017). [PubMed: 27706126]

12. He YW, Deftos ML, Ojala EW & Bevan MJ RORgamma t, a novel isoform of an orphan receptor, negatively regulates Fas ligand expression and IL-2 production in T cells. Immunity 9, 797–806 (1998). [PubMed: 9881970]
13. Sun Z et al. Requirement for RORgamma in thymocyte survival and lymphoid organ development. Science 288, 2369–2373 (2000). [PubMed: 10875923]
14. Kurebayashi S et al. Retinoid-related orphan receptor gamma (RORgamma) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis. Proc Natl Acad Sci U S A 97, 10132–10137 (2000). [PubMed: 10963675]
15. Okada S et al. IMMUNODEFICIENCIES. Impairment of immunity to Candida and Mycobacterium in humans with bi-allelic RORC mutations. Science 349, 606–613 (2015). [PubMed: 26160376]
16. Eberl G et al. An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. Nat Immunol 5, 64–73 (2004). [PubMed: 14691482]
17. Mebius RE, Streeter PR, Michie S, Butcher EC & Weissman IL A developmental switch in lymphocyte homing receptor and endothelial vascular addressin expression regulates lymphocyte homing and permits CD4+ CD3- cells to colonize lymph nodes. Proc Natl Acad Sci U S A 93, 11019–11024 (1996). [PubMed: 8855301]
18. Adachi S, Yoshida H, Kataoka H & Nishikawa S Three distinctive steps in Peyer's patch formation of murine embryo. Int Immunol 9, 507–514 (1997). [PubMed: 9138010]
19. Mebius RE, Rennert P & Weissman IL Developing lymph nodes collect CD4+CD3- LTbeta+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. Immunity 7, 493–504 (1997). [PubMed: 9354470]
20. Eberl G & Littman DR Thymic origin of intestinal alphabeta T cells revealed by fate mapping of RORgammat+ cells. Science 305, 248–251 (2004). [PubMed: 15247480]
21. Bouskra D et al. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. Nature 456, 507–510 (2008). [PubMed: 18987631]
22. Ivanov II et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126, 1121–1133 (2006). [PubMed: 16990136]
23. Lochner M et al. In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORgamma t+ T cells. J Exp Med 205, 1381–1393 (2008). [PubMed: 18504307]
24. Ohnmacht C et al. MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through RORγt+ T cells. Science 349, 989–993 (2015). [PubMed: 26160380]
25. Sefik E et al. MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of RORγ+ regulatory T cells. Science 349, 993–997 (2015). [PubMed: 26272906]
26. Martin B, Hirota K, Cua DJ, Stockinger B & Veldhoen M Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. Immunity 31, 321–330 (2009). [PubMed: 19682928]
27. Satoh-Takayama N et al. Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. Immunity 29, 958–970 (2008). [PubMed: 19084435]
28. Luci C et al. Influence of the transcription factor RORgammat on the development of NKp46+ cell populations in gut and skin. Nat Immunol 10, 75–82 (2009). [PubMed: 19029904]
29. Sanos SL et al. RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. Nat Immunol 10, 83–91 (2009). [PubMed: 19029903]
30. Takatori H et al. Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. J Exp Med 206, 35–41 (2009). [PubMed: 19114665]
31. Cella M et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. Nature 457, 722–725 (2009). [PubMed: 18978771]
32. Buonocore S et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. Nature 464, 1371–1375 (2010). [PubMed: 20393462]
33. Spits H et al. Innate lymphoid cells--a proposal for uniform nomenclature. Nat Rev Immunol 13, 145–149 (2013). [PubMed: 23348417]

34*. Grigg JB et al. Antigen-presenting innate lymphoid cells orchestrate neuroinflammation. Nature 600, 707–712 (2021). [PubMed: 34853467] This publication defines a circulating ILC3 subset in mice and humans, termed inflammatory ILC3s, that promotes inflammatory T cell responses through MHC-II and is essential for autoimmune neuroinflammation.

35*. Hepworth MR et al. Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. Nature 498, 113–117 (2013). [PubMed: 23698371] This publication defines MHC-II$^+$ LTi-like ILC3s in mice and humans as the first described RORγt$^+$ APC subset and identifies unexpected roles for these cells in enforcing CD4$^+$ T cell tolerance to the microbiota.

36. Hepworth MR et al. Immune tolerance. Group 3 innate lymphoid cells mediate intestinal selection of commensal bacteria-specific CD4+ T cells. Science 348, 1031–1035 (2015). [PubMed: 25908663]

37*. Yamano T et al. Aire-expressing ILC3-like cells in the lymph node display potent APC features. J Exp Med 216, 1027–1037 (2019). [PubMed: 30918005] This publication is the first to define RORγt$^+$ eTACs in mouse lymph nodes.

38. Brown CC et al. Transcriptional Basis of Mouse and Human Dendritic Cell Heterogeneity. Cell 179, 846–863.e824 (2019). [PubMed: 31668803]

39*. Wang J et al. Single-cell multiomics defines tolerogenic extrathymic Aire-expressing populations with unique homology to thymic epithelium. Sci Immunol 6, eabl5053 (2021). [PubMed: 34767455] This publication provides the first single-cell analysis focusing on *Aire*-expressing cells in the mouse peripheral immune system.

40*. Dobeš J et al. Extrathymic expression of Aire controls the induction of effective T. Nat Immunol 23, 1098–1108 (2022). [PubMed: 35761088] This publication is the first to define that RORγt$^+$ eTACs promote T_H17 cell responses to *Candida albicans* in mice.

41*. Kedmi R et al. A RORγt+ cell instructs gut microbiota-specific Treg cell differentiation. Nature 610, 737–743 (2022). [PubMed: 36071167] This publication further defines RORγt$^+$ APCs and their ability to migrate to the mesenteric lymph nodes to promote microbiota-specific RORγt$^+$ T_{reg} cells in mice.

42*. Lyu M et al. ILC3s select microbiota-specific regulatory T cells to establish tolerance in the gut. Nature 610, 744–751 (2022). [PubMed: 36071169] This publication defines all RORγt$^+$ APCs in the mesenteric lymph nodes of mice, identifies that MHC-II$^+$ LTi-like ILC3s instruct RORγt$^+$ T_{reg} cells and immune tolerance to the microbiota in mice, and finds that this pathway is altered in human IBD.

43*. Akagbosu B et al. Novel antigen-presenting cell imparts Treg-dependent tolerance to gut microbiota. Nature 610, 752–760 (2022). [PubMed: 36070798] This publication characterized *Rorc*$^+$ DC-like cells in mice and showed that RORγt$^+$ APCs promote the induction of RORγt$^+$ T_{reg} cells in early life.

44. Goto Y et al. Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. Immunity 40, 594–607 (2014). [PubMed: 24684957]

45. Constantinides MG, McDonald BD, Verhoef PA & Bendelac A A committed precursor to innate lymphoid cells. Nature 508, 397–401 (2014). [PubMed: 24509713]

46. Klose CS et al. A T-bet gradient controls the fate and function of CCR6-RORγt+ innate lymphoid cells. Nature 494, 261–265 (2013). [PubMed: 23334414]

47. Withers DR et al. Transient inhibition of ROR-γt therapeutically limits intestinal inflammation by reducing TH17 cells and preserving group 3 innate lymphoid cells. Nat Med 22, 319–323 (2016). [PubMed: 26878233]

48. Fiancette R et al. Reciprocal transcription factor networks govern tissue-resident ILC3 subset function and identity. Nat Immunol 22, 1245–1255 (2021). [PubMed: 34556884]

49. Elmentaite R et al. Cells of the human intestinal tract mapped across space and time. Nature 597, 250–255 (2021). [PubMed: 34497389]

50. Fawkner-Corbett D et al. Spatiotemporal analysis of human intestinal development at single-cell resolution. Cell 184, 810–826 e823 (2021). [PubMed: 33406409]

51. Goc J et al. Dysregulation of ILC3s unleashes progression and immunotherapy resistance in colon cancer. Cell 184, 5015–5030.e5016 (2021). [PubMed: 34407392]

52. Teng F et al. ILC3s control airway inflammation by limiting T cell responses to allergens and microbes. Cell Rep 37, 110051 (2021). [PubMed: 34818549]

53. Huang S et al. Lymph nodes are innervated by a unique population of sensory neurons with immunomodulatory potential. Cell 184, 441–459.e425 (2021). [PubMed: 33333021]

54*. Gardner JM et al. Deletional tolerance mediated by extrathymic Aire-expressing cells. Science 321, 843–847 (2008). [PubMed: 18687966] This publication is the first to identify *Aire*-reporter-positive cells and AIRE protein in mouse lymph nodes.

55. Consortium F-GA An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Nat Genet 17, 399–403 (1997). [PubMed: 9398840]

56. Nagamine K et al. Positional cloning of the APECED gene. Nat Genet 17, 393–398 (1997). [PubMed: 9398839]

57. Anderson MS et al. Projection of an immunological self shadow within the thymus by the aire protein. Science 298, 1395–1401 (2002). [PubMed: 12376594]

58. Gardner JM et al. Extrathymic Aire-expressing cells are a distinct bone marrow-derived population that induce functional inactivation of CD4+ T cells. Immunity 39, 560–572 (2013). [PubMed: 23993652]

59. Lyu M et al. ILC3s select for RORγt+ Tregs and establish tolerance to intestinal microbiota. bioRxiv, 2022.2004.2025.489463 (2022).

60. Wang X et al. Post-Aire maturation of thymic medullary epithelial cells involves selective expression of keratinocyte-specific autoantigens. Front Immunol 3, 19 (2012). [PubMed: 22448160]

61. Salvermoser J et al. Mediated Ablation of Conventional Dendritic Cells Suggests a Lymphoid Path to Generating Dendritic Cells. Front Immunol 9, 699 (2018). [PubMed: 29713321]

62. Zhou W et al. ZBTB46 defines and regulates ILC3s that protect the intestine. Nature 609, 159–165 (2022). [PubMed: 35831503]

63. Satpathy AT et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. J Exp Med 209, 1135–1152 (2012). [PubMed: 22615127]

64. Meredith MM et al. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. J Exp Med 209, 1153–1165 (2012). [PubMed: 22615130]

65. Schlenner SM et al. Fate mapping reveals separate origins of T cells and myeloid lineages in the thymus. Immunity 32, 426–436 (2010). [PubMed: 20303297]

66. Leung GA et al. The lymphoid-associated interleukin 7 receptor (IL7R) regulates tissue-resident macrophage development. Development 146 (2019).

67. Ghaedi M et al. Single-cell analysis of RORα tracer mouse lung reveals ILC progenitors and effector ILC2 subsets. J Exp Med 217 (2020).

68*. Fergusson JR et al. Maturing Human CD127+ CCR7+ PDL1+ Dendritic Cells Express AIRE in the Absence of Tissue Restricted Antigens. Front Immunol 9, 2902 (2018). [PubMed: 30692988] This publication is the first single-cell analysis identifying human cells expressing AIRE in the peripheral immune system.

69. Poliani PL et al. Human peripheral lymphoid tissues contain autoimmune regulator-expressing dendritic cells. Am J Pathol 176, 1104–1112 (2010). [PubMed: 20093495]

70. Melo-Gonzalez F et al. Antigen-presenting ILC3 regulate T cell-dependent IgA responses to colonic mucosal bacteria. J Exp Med 216, 728–742 (2019). [PubMed: 30814299]

71. Wang W et al. The Interaction between Lymphoid Tissue Inducer-Like Cells and T Cells in the Mesenteric Lymph Node Restrains Intestinal Humoral Immunity. Cell Rep 32, 107936 (2020). [PubMed: 32698011]

72. Steinman RM, Hawiger D & Nussenzweig MC Tolerogenic dendritic cells. Annu Rev Immunol 21, 685–711 (2003). [PubMed: 12615891]

73. Liu K et al. Immune tolerance after delivery of dying cells to dendritic cells in situ. J Exp Med 196, 1091–1097 (2002). [PubMed: 12391020]

74. Kurts C, Kosaka H, Carbone FR, Miller JF & Heath WR Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8(+) T cells. J Exp Med 186, 239–245 (1997). [PubMed: 9221753]
75. Probst HC, Lagnel J, Kollias G & van den Broek M Inducible transgenic mice reveal resting dendritic cells as potent inducers of CD8+ T cell tolerance. Immunity 18, 713–720 (2003). [PubMed: 12753747]
76. Hawiger D et al. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J Exp Med 194, 769–779 (2001). [PubMed: 11560993]
77. Curotto de Lafaille MA & Lafaille JJ Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? Immunity 30, 626–635 (2009). [PubMed: 19464985]
78. Ivanov II et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139, 485–498 (2009). [PubMed: 19836068]
79. von Burg N et al. Activated group 3 innate lymphoid cells promote T-cell-mediated immune responses. Proc Natl Acad Sci U S A 111, 12835–12840 (2014). [PubMed: 25136120]
80. Lehmann FM et al. Microbiota-induced tissue signals regulate ILC3-mediated antigen presentation. Nat Commun 11, 1794 (2020). [PubMed: 32286285]
81. Castellanos JG et al. Microbiota-Induced TNF-like Ligand 1A Drives Group 3 Innate Lymphoid Cell-Mediated Barrier Protection and Intestinal T Cell Activation during Colitis. Immunity 49, 1077–1089.e1075 (2018). [PubMed: 30552020]
82. Rao A et al. Cytokines regulate the antigen-presenting characteristics of human circulating and tissue-resident intestinal ILCs. Nat Commun 11, 2049 (2020). [PubMed: 32341343]
83. Lim AI et al. Systemic Human ILC Precursors Provide a Substrate for Tissue ILC Differentiation. Cell 168, 1086–1100 e1010 (2017). [PubMed: 28283063]
84. Huang Y et al. IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential 'inflammatory' type 2 innate lymphoid cells. Nat Immunol 16, 161–169 (2015). [PubMed: 25531830]
85. Anderson CA et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nat Genet 43, 246–252 (2011). [PubMed: 21297633]
86. Lill CM et al. MANBA, CXCR5, SOX8, RPS6KB1 and ZBTB46 are genetic risk loci for multiple sclerosis. Brain 136, 1778–1782 (2013). [PubMed: 23739915]
87. Cabeza-Cabrerizo M, Cardoso A, Minutti CM, Pereira da Costa M & Reis e Sousa C Dendritic Cells Revisited. Annu Rev Immunol 39, 131–166 (2021). [PubMed: 33481643]
88. Liston A, Lesage S, Wilson J, Peltonen L & Goodnow CC Aire regulates negative selection of organ-specific T cells. Nat Immunol 4, 350–354 (2003). [PubMed: 12612579]
89. Aschenbrenner K et al. Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells. Nat Immunol 8, 351–358 (2007). [PubMed: 17322887]
90. Malchow S et al. Aire Enforces Immune Tolerance by Directing Autoreactive T Cells into the Regulatory T Cell Lineage. Immunity 44, 1102–1113 (2016). [PubMed: 27130899]
91. Yang S, Fujikado N, Kolodin D, Benoist C & Mathis D Immune tolerance. Regulatory T cells generated early in life play a distinct role in maintaining self-tolerance. Science 348, 589–594 (2015). [PubMed: 25791085]
92. Gillis-Buck E et al. Extrathymic Aire-expressing cells support maternal-fetal tolerance. Sci Immunol 6, eabf1968 (2021). [PubMed: 34272228]
93. Anderson MS et al. The cellular mechanism of Aire control of T cell tolerance. Immunity 23, 227–239 (2005). [PubMed: 16111640]
94. Kisand K et al. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med 207, 299–308 (2010). [PubMed: 20123959]
95. Xu M et al. c-MAF-dependent regulatory T cells mediate immunological tolerance to a gut pathobiont. Nature 554, 373–377 (2018). [PubMed: 29414937]
96. Russler-Germain EV et al. Gut Helicobacter presentation by multiple dendritic cell subsets enables context-specific regulatory T cell generation. Elife 10, e54792 (2021). [PubMed: 33533717]

97. Chai JN et al. species are potent drivers of colonic T cell responses in homeostasis and inflammation. Sci Immunol 2, eaal5068 (2017). [PubMed: 28733471]

98. Mackley EC et al. CCR7-dependent trafficking of RORγ+ ILCs creates a unique microenvironment within mucosal draining lymph nodes. Nat Commun 6, 5862 (2015). [PubMed: 25575242]

99. Bernink JH et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nat Immunol 14, 221–229 (2013). [PubMed: 23334791]

100. Husebye ES, Anderson MS & Kämpe O Autoimmune Polyendocrine Syndromes. N Engl J Med 378, 2543–2544 (2018).

101. Husebye ES, Perheentupa J, Rautemaa R & Kämpe O Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. J Intern Med 265, 514–529 (2009). [PubMed: 19382991]

102. Abramson J & Husebye ES Autoimmune regulator and self-tolerance - molecular and clinical aspects. Immunol Rev 271, 127–140 (2016). [PubMed: 27088911]

103. Puel A et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med 207, 291–297 (2010). [PubMed: 20123958]

104. Break TJ et al. Aberrant type 1 immunity drives susceptibility to mucosal fungal infections. Science 371, eaay5731 (2021). [PubMed: 33446526]

105. Bruserud Ø et al. Altered Immune Activation and IL-23 Signaling in Response to. Front Immunol 8, 1074 (2017). [PubMed: 28919897]

106. Mathä L et al. Migration of Lung Resident Group 2 Innate Lymphoid Cells Link Allergic Lung Inflammation and Liver Immunity. Front Immunol 12, 679509 (2021). [PubMed: 34305911]

107. Oliphant CJ et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. Immunity 41, 283–295 (2014). [PubMed: 25088770]

Box 1 |

Autoimmune polyendocrine syndrome type 1

Autoimmune polyendocrine syndrome type 1 (APS1; OMIM entry: 240300), also known as autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED), is a rare genetic disorder caused by loss of function in the gene encoding autoimmune regulator (*AIRE*)[100]. Individuals with APS1 characteristically develop at least two clinical components of a diagnostic triad including Addison disease (primary adrenal insufficiency), hypoparathyroidism or chronic mucocutaneous candidiasis. Other components of the disease include enamel hyperplasia, ovarian insufficiency, diarrhea, vitiligo, autoimmune hepatitis or type 1 diabetes and other autoimmune conditions[100–102]. The autoimmune components of this syndrome are readily explained by the deficiency of AIRE in medullary thymic epithelial cells (mTECs), which results in defective presentation of tissue-restricted antigens by mTECs and hence the escape of self-reactive T cells to the periphery. However, the aetiology of chronic mucocutaneous candidiasis remained puzzling until recently, when several different mechanisms have been identified as contributing to this pathology. First, individuals with APS1 develop autoantibodies targeting effector cytokines of the T helper 17 (T_H17) cell response, IL-17 and IL-22, which are crucial for anti-fungal immune responses[94,103]. Second, individuals with APS1 were shown to have high levels of interferon-γ production by T cells in the oral mucosa, leading to excessive epithelial cell death in mucosal tissues[104]. Finally, individuals with APS1 were found to have impaired production of T_H17 cell signature cytokines in response to *Candida albicans*[40,105]. Correspondingly, ablation of *Aire* in RORγt^+ extrathymic AIRE-expressing cells (eTACs) impaired the generation of antigen-specific T_H17 cells in response to *Candida albicans* challenge in mice[40].

Box 2 |

Identity of RORγt$^+$ APCs that promote microbiota-specific T_{reg} cells

Three co-published studies showed an essential role for retinoic acid receptor-related orphan receptor-γt (RORγt)$^+$ antigen-presenting cells (APCs) in promoting RORγt$^+$ microbiota-specific regulatory T (T_{reg}) cells. Here, we discuss the similarities and differences between these reports, as well as their limitations.

A study by Lyu et al. from the group of Gregory Sonnenberg concluded that lymphoid tissue inducer (LTi)-like group 3 innate lymphoid cells (ILC3s) are the major RORγt$^+$ APC subset that promotes the generation of microbiota-specific T_{reg} cells through MHC-II and in part through ITGAV–ITGB3 integrin-mediated processing of transforming growth factor-β (TGFβ)[42]. However, it should be noted that RORγt^{Cre}-mediated ablation of ITGAV results in a modest phenotype relative to MHC-II deletion. Their conclusion was based on co-cultures, single-cell RNA-sequencing (scRNA-seq), imaging and analyses of various mouse models using different Cre drivers. This included *Il22*^{Cre}-driven selective ablation of MHC-II in the ILC3 compartment, which resulted in reduced numbers of RORγt$^+$ T_{reg} cells when exposed to a new microorganism. This was studied both in endogenous T cells and in an antigen-specific manner following colonization of adult mice with *Helicobacter hepaticus*. A gain-of-function study revealed that selective restoration of MHC-II on a limited number of ILC3s, but not RORγt$^+$ extrathymic AIRE-expressing cells (eTACs) or conventional dendritic cells (cDCs), was sufficient to instruct microbiota-specific T_{reg} cell differentiation. Furthermore, the study also showed that RORγt$^+$ eTACs and cDCs are dispensable for the induction of microbiota-specific T_{reg} cells, as no phenotypes were observed in several mouse models selectively targeting these cell types but not ILC3s. All experiments were carried out in adult mice. This study also found altered MHC-II$^+$ ILC3s and RORγt$^+$ T_{reg} cells in the inflamed intestine of patients with inflammatory bowel disease.

A study by Kedmi et al. concluded that a type of RORγt$^+$ APC is essential to induce microbiota-specific T_{reg} cells, through ITGAV–ITGB8 integrin-mediated processing of TGFβ[41]. The paper indicated that this was likely mediated by either MHC-II$^+$ ILC3s or RORγt$^+$ eTACs (termed Janus cells in this study) but did not make a definitive conclusion. Furthermore, the study showed that expression of the chemokine receptor CCR7 on RORγt$^+$ APCs facilitates their homing from the gut to the mesenteric lymph nodes and is required for the subsequent generation of microbiota-specific T_{reg} cells. This was supported by CITE-seq and lineage-specific deletion approaches. Interestingly, RORγt^{Cre}-mediated ablation of *Aire* had no effect on T_{reg} cell generation, suggesting that if RORγt$^+$ eTACs are involved then their expression of *Aire* is dispensable for this process. It is also notable that this study suggested limited expression of ITGB8 on both ILC3s and RORγt$^+$ eTACs. All experiments were carried out in adult mice.

A study by Akagbosu et al. concluded that RORγt$^+$ DC-like cells (termed Thetis cells (TC-IV) in this study and previously referred to as cDC2B) in intestine-draining lymph nodes are essential for the generation of microbiota-specific T_{reg} cells[43]. This conclusion was based on findings that these cell types are present in the mesenteric lymph nodes of

mice at early points of development (2–3 weeks after birth), that they express ITGB8 (a key integrin associated with processing of TGFβ) and that RORγt^{Cre}-mediated deletion of *Itgb8* consequently results in lower numbers of RORγt^{+} T_{reg} cells. Furthermore, this study suggested that MHC-II^{+} ILC3s or RORγt^{+} eTACs were dispensable for the induction of RORγt^{+} T_{reg} cells, as these APCs did not express *Itgb8*, and that MHC-II deletion mediated by *Rora*^{Cre} did not affect RORγt^{+} T_{reg} cell frequencies despite targeting of ILC3s. It should be noted that *Rora*^{Cre} is an atypical way to target ILC3s, as prior studies suggest that it targets a moderate proportion (~40–60%) of ILC3s and does not affect ILC3 homeostasis or secondary lymph node generation when crossed to *Il7r*-floxed mice. Instead, *Rora*^{Cre}*Il7r*$^{flox/flox}$ mice are routinely used as a model to selectively target ILC2s[67,106,107]. Moreover, this paper did not explore antigen-specific interactions with T_{reg} cells and did not explore whether RORγt^{Cre} induces deletion of either MHC-II or ITGB8 on these DC-like cells. This is notable as the dual reporter and creERT2 mouse used to define and characterize these cell types reports fluorescence downstream of exon 11 of the *Rorc* locus, which is shared with RORγt as well as the more common RORγ isoform and thus cannot differentiate between them. Unlike the other two studies, this study analyzed young (2–3-week-old) mice and showed that tamoxifen-induced deletion of MHC-II in *Rorc*$^{+}$ cells in adult mice had a minimal impact on the abundance of RORγt^{+} T_{reg} cells in naive mice a few weeks after weaning.

Box 3 |

Proposed classification of the major RORγt$^+$ APC subsets in mice

The collective body of literature identifies that retinoic acid receptor-related orphan receptor-γt (RORγt)$^+$ antigen-presenting cells (APCs) are essential regulators of immunity, inflammation and tolerance, with involvement in secondary lymphoid tissue formation and the generation of inflammatory or tolerogenic CD4$^+$ T cell responses. However, a major complication in the study of RORγt$^+$ APCs has been the use of distinct nomenclatures to characterize each cell type. For example, RORγt$^+$ extrathymic AIRE-expressing cells (eTACs), AIRE$^+$ group 3 innate lymphoid cell (ILC3)-like cells, AIRE$^+$ APCs, Thetis I cells (TC I) and Janus cells all express CCR6, AIRE and SIGLECG. Also, TC IV cells and the *Rorc*$^+$ cDC2B population seem to represent the same cell subset and are developmentally distinct from conventional dendritic cells (cDCs). This diverse nomenclature adds substantive confusion to the field, particularly when many names are likely referring to the same cell type. Therefore, we propose to provisionally categorize these emerging RORγt$^+$ APCs into three distinct groups, based on their expression of key transcription factors, phenotypic markers and function (FIG. 2, Table 1), until more accurate nomenclature can be established.

The purpose of this nomenclature is to provide clarity to this rapidly emerging field and allow robust discussion of the existing literature. The proposed nomenclature is a starting point that may be modified in the future once additional functional and developmental studies in both mice and humans become publicly available. Of particular importance, we currently have a limited understanding of the phenotype or functionality of RORγt$^+$ eTACs and *Rorc*$^+$ DC-like cells in humans. Although additional research on these cell types in humans will be challenging because of limited tools to study specifically the RORγt isoform of *Rorc* and the need to examine early life lymph nodes, these studies are expected to be transformational as this field continues to move forward and may lead to revision of the proposed nomenclature.

RORγt$^+$ ILC3s

These are currently the best characterized members of the RORγt$^+$ APC family and include tissue-resident LTi-like ILC3s that are found in early life and in adulthood. They are CCR6$^+$, IL-7R$^+$ and CXCR6$^+$, co-express ZBTB46, lack expression of AIRE, are tissue resident at barrier surfaces and lymph nodes, have constitutively high expression of MHC-II, and are tolerogenic in most contexts owing to a lack of co-stimulatory molecules CD80 and CD86, their ability to sequester pro-survival cytokines and their expression of TGFβ-processing integrins. These LTi-like ILC3s orchestrate tolerance to microbiota, allergens and other microorganisms by restraining antigen-specific T helper cells (T_H17, T_H1, T_{FH} and T_H2 cells), as well as instructing the differentiation of microbiota-specific regulatory T (T_{reg}) cells. This group also encompasses circulating 'inflammatory ILC3s' that are IL-7R$^+$, express T-bet and co-stimulatory molecules CD80 and CD86, promote T cell responses in the context of neuroinflammation or vaccination, and are markedly shaped by cytokine signals.

RORγt$^+$ eTACs

Previously AIRE$^+$ ILC3-like cells, AIRE$^+$ APCs, Janus cells, TC I and TC III. They express no or limited IL-7R and lack expression of major lineage markers and CXCR6. Most of these cells co-express AIRE (as a transcript or protein) and ZBTB46, as well as co-stimulatory molecules, including CD40, CD80 and CD86. A minor fraction of these cells (previously TC II or JC3) has very low to no expression of *Aire* transcripts or co-stimulatory molecules. RORγt$^+$ eTACs are more abundant in early life and are mainly present in lymph nodes, whereas they are almost undetectable in the intestine. They promote T cell responses to *Candida albicans*.

Rorc$^+$ DC-like cells

Previously TC IV and cDC2B. These cells are the least well studied so far, lack IL-7R, CXCR6 and AIRE expression, express ZBTB46 and variable levels of ITGB8, CD11b and CD11c, are found in early-life lymph nodes before declining, seem to be absent from the intestine, and have been reported to induce microorganism-specific T_{reg} cells. These cells also express co-stimulatory molecules and, so far, it remains unclear whether they express the RORγt isoform of *Rorc*.

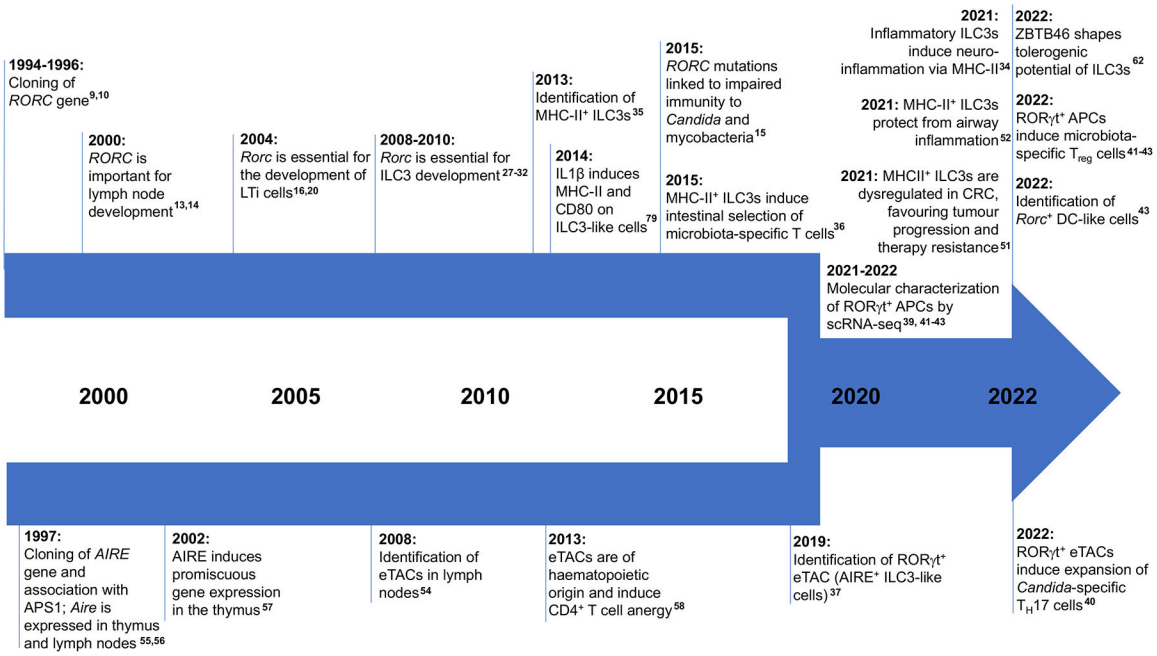

Figure 1 |. A timeline of research leading to the characterization of RORγt$^+$ APCs.
AIRE, autoimmune regulator; APC, antigen-presenting cell; APS1, autoimmune polyendocrine syndrome type 1; CRC, colorectal cancer; DC, dendritic cell; eTAC, extrathymic AIRE-expressing cell; ILC3, group 3 innate lymphoid cell; LTi cell, lymphoid tissue inducer cell; MHC-II, MHC class II; RORγt, retinoic acid receptor-related orphan receptor-γt; scRNA-seq, single-cell RNA-sequencing; T_H17 cell; T helper 17 cell; T_{reg} cell, regulatory T cell.

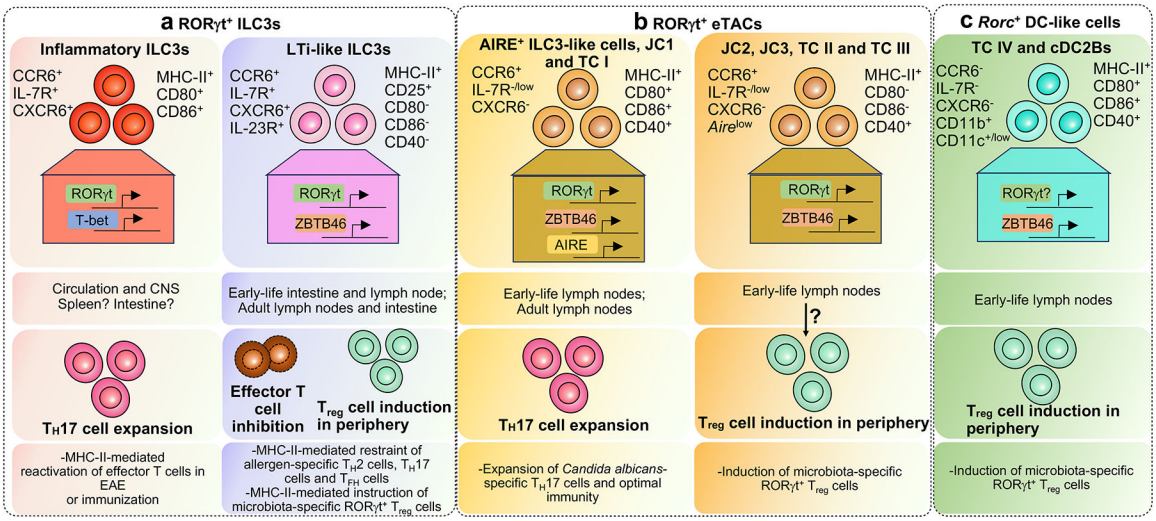

Figure 2 |. The phenotype and function of RORγt$^+$ APCs in mice.

Retinoic acid receptor-related orphan receptor-γt (RORγt)$^+$ antigen-presenting cells (APCs) are a heterogeneous family of cells, composed of many different subsets that differ in their ontogeny, tissue residence, functions and molecular characteristics. Three major subsets are highlighted. **a**, RORγt$^+$ group 3 innate lymphoid cells (ILC3s). This group includes lymphoid tissue inducer (LTi)-like ILC3s, the first type of RORγt$^+$ APC to be discovered, which are defined by the surface markers IL-7 receptor (IL-7R; also known as CD127) and the chemokine receptors CXCR6 and CCR6, by the transcription factor ZBTB46 (as well as RORγt), and by the T cell-impacting molecules MHC class II (MHC-II) and CD25. They lack expression of conventional co-stimulatory molecules such as CD40, CD80 and CD86. LTi-like ILC3s are tissue resident, found in lymph nodes and intestinal lamina propria during early life and adulthood. They co-localize with T cells in the follicular zone of mesenteric lymph nodes to restrain effector T cell responses or instruct the differentiation of regulatory T (T_{reg}) cells specific for intestinal microorganisms through various mechanisms. A separate subset of inflammatory ILC3s is derived from the circulation and can take residence in the central nervous system (CNS), where these cells have a proinflammatory role. Inflammatory ILC3s express the key surface markers IL-7R, CXCR6 and CCR6, the transcription factor T-bet, and the T cell-impacting molecules MHC-II, CD80 and CD86. Inflammatory ILC3s re-stimulate effector T cells that drive autoimmune neuroinflammation. **b**, RORγt$^+$ extrathymic AIRE-expressing cells (eTACs). These cells — which include Janus cells, AIRE$^+$ ILC3-like cells, and group I and III Thetis cells (TC I and TC III) — reside in secondary lymphoid organs and are defined by varying expression levels of *Aire* transcript and/or protein. They are CCR6$^+$CXCR6$^-$, express no or limited IL-7R, express the transcription factor ZBTB46, and are positive for expression of MHC-II. A large fraction of these cells expresses high levels of co-stimulatory molecules such as CD80, CD86 and CD40. RORγt$^+$ eTACs were shown to promote *Candida albicans*-specific T helper 17 (T_H17) cell responses. **c**, *Rorc*$^+$ dendritic cell (DC)-like cells. These are a potential subset of RORγt$^+$ APCs, including TC IV cells and potentially a minor fraction of cDC2Bs, that are found in early life lymph nodes. They express CD11c, CD11b, ZBTB46, MHC-II, CD80, CD86 and CD40, but no IL-7R, CXCR6 or AIRE, and are proposed to drive tolerogenic responses. EAE,

experimental autoimmune encephalomyelitis; T_{FH} cell, T follicular helper cell; T_H2 cell, T helper 2 cell.

Table 1 |

Similarities and differences in key markers across RORγt$^+$ APC subsets in mice

Name of subset	Marker expression											
	AIRE	***Aire***	***Gal***	***H2-Ab1***	***Cd80***	***Cd86***	***Siglecg***	***Ccr7***	***Ccr6***	***Cxcr6***	***Cd11b***	***Itgb8***
RORγt$^+$ILC3s												
LTi-like ILC3[35,36,41,42]	–	–	–	+	–	–	–	+	+	+	–	–/low
Inflammatory ILC3[34]	–	–	–	+	+	+	ND	ND	+	ND	–	ND
RORγt$^+$AIRE^{hi}eTACs												
ILC3-like cell[37,40]	+	+	+	+	+	+	ND	Low/+	+	–	–	–
eTAC1[42]	ND	+	ND	+	ND	ND	+	ND	+	–	–	–
Janus cell (JC)[39]	ND	+	+	+	+	ND	+	Low	+	ND	–	–
JC1[41]	ND	+	+	+	+	+	ND	Low	+	–	–	–
Thetis cell I (TC I)[43]	+	+	ND	+	+	+	+	Low	+	–	–	–
Aire-APC[54]	ND	+	+	+	+	+	+	Low	+	–	–	–
RORγt$^+$AIRE^{low}eTACs												
eTAC2[42]	ND	+	ND	+	ND	ND	–	ND	–	–	–	–
JC2[41]	ND	+	–	+	–	–	ND	Low/+	+	–	–	Low
TC III[43]	Low	+	ND	+	–	–	–	+	–	–	–	–
RORγt$^+$ AIRE$^-$ eTACs?												
JC3[41]	ND	Low/–	–	Mid/+	–	–	ND	+	Low	–	–	Low
TC II[43]	–	–	ND	+	–	–	+	Low	+	–	–	–
***Rorc*$^+$DC-like cells**												
TC IV[43]	–	–	ND	+	–	–	–	+	–	–	+	+
cDC2B[38]	ND	–	ND	+	ND	–	ND	+	–	ND	+	ND

AIRE, autoimmune regulator; APC, antigen presenting cell; DC, dendritic cell; eTAC, extrathymic AIRE-expressing cell; ILC3, group 3 innate lymphoid cell; LTi, lymphoid tissue inducer; ND, not determined; RORγt, retinoic acid receptor-related orphan receptor-γt.

Table 2 |

Available reporter or fate mapping approaches for RORγt$^+$ APCs

Reporter strain	Labelled RORγt$^+$ APC subsets			
	LTi-like ILC3s	RORγt$^+$ eTACs	*Rorc*$^+$ DC-like cells	cDCs
Clec9a^{Cre} ([a])	–	–	5%	+ ([c])
Itgax^{Cre} ([a])	5–10%	5–20%	5–20%	+
Il7r^{Cre} ([a])	+	>75%	ND	<20%
Rag1^{Cre} ([a])	–	–	–	–
RORγt^{Cre} ([a])	+	~50–75%	ND	–
Rora^{Cre} ([a,b])	40–60%	–	–	–
Il22^{Cre} ([a,b])	+ ([a])	–	–	–
Zbtb46^{GFP}	+	+	+	+
Aire^{Cre} ([a])	~20%	~60%	ND	ND
Itgb8^{Tomato}	~0–5%	~1–15%	~40%	ND

[a]Ability to fate map cells depends on the strains being used for gene recombination. For example, Rosa26–STOP–eYFP mutant mice have a lower efficiency than Rosa26–STOP–CAG–TdTomato mice.

[b]*Rora*^{Cre} and *Il22*^{Cre} target ILC3s in a spatial and temporal manner, dependent on microbiota status, age of mice and location. For example, *Il22*^{Cre} efficiently fate maps intestinal ILC3s after weaning but has modest fate mapping in peripheral tissues.

[c]*Clec9a*^{Cre} is more effective at inducing gene recombination in cDC1s, whereas targeting of cDC2s is limited to 50–60% of the population.

APC, antigen presenting cell; cDC, conventional dendritic cell; eTAC, extrathymic AIRE-expressing cell; ILC3, group 3 innate lymphoid cell; LTi, lymphoid tissue inducer; ND, not determined; RORγt, retinoic acid receptor-related orphan receptor-γt.