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TLR-Induced Cytokines Promote Effective Proinflammatory Natural Th17 Cell Responses

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Naive CD4 lymphocytes undergo a polarization process in the periphery to become induced Th17 (iTh17) cells. Using retinoic acid–related orphan receptor γt (RO Rey-t)-gfp mice, we found that RO Rey-t and the transcription factor promyelocytic leukemia zinc finger (PLZF) are valuable new markers to identify the recently described natural Th17 (nTh17) cell population. nTh17 cells are thymically committed to promptly produce large amounts of IL-17 and IL-22. In this study, we show that, in addition to responding to TCR cross-linking, nTh17 cells secrete IL-17 and IL-22 when stimulated with IL-23 plus IL-1β, either in recombiant form or in supernatants from TLR4-activated dendritic cells. This innate-like ability of RO Rey-t* nTh17 cells to respond to TLR4-induced cytokines was not shared by iTh17 cells. The other distinct properties of RO Rey-t+ nTh17 cells are their high expression of PLZF and their absence from lamina propria; iTh17 cells are found therein. RO Rey-t+ nTh17 cells are present in the thymus of germ-free RO Rey-t-gfp and IL-6−/− RO Rey-t-gfp mice, indicating that these cells do not require symbiotic microbiota or IL-6 for their generation. Finally, we found that PLZF−RO Rey-t− nTh17 cells represent one of the primary IL-17–producing innate-like T cell populations in a TLR7 imiquimod model of psoriasis-like disorder, indicating their involvement in this kind of lesion. Collectively, our results reveal RO Rey-t and PLZF as characteristic markers for identifying nTh17 cells and demonstrate one of their novel properties: the ability to respond promptly to TLR-dependent proinflammatory stimuli without TCR engagement, placing them as members of the innate-like T cell family. The Journal of Immunology, 2014, 192: 5635–5642.

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Abbreviations used in this article: BMDC, bone marrow–derived dendritic cell; DC, dendritic cell; GF, germ-free; IMQ, imiquimod; INKT, invariant NKT; INKT17, IL-17–producing INKT; iTh17, induced Th17; LP, lamina propria; MNC, mononuclear cell; nTh17, natural Th17; Pl, PMA plus ionomycin; PLZF, promyelocytic leukemia zinc finger; RO Rey-t, retinoic acid–related orphan receptor γt; WT, wild-type.

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and transcription factors, which render mature cells responsive to a second Ag stimulation. In contrast, some T cells leave the thymus fully differentiated and competent for cytokine secretion without further polarization. The typical representatives of these “nonconventional” T cells are invariant NKT (iNKT) and γδ T cells (3–5). The IL-17–producing iNKT (iNKT17) cell subset originates from a new thymic-differentiation pathway that is dependent on the transcription factor retinoic acid–related orphan receptor γt (RO Rey-t) (3, 4, 6). Similar to iNKT17 cells, CD27− γδ T cells acquire the ability to secrete IL-17 during their thymic development and are distinct from their CD27+ counterparts that produce IFN-γ (3, 5, 7). More recently, a novel IL-17+CD4+ T cell population was identified that, similar to INKT17 and CD27− γδ T cells, acquires the ability to secrete IL-17 during its thymic development (8, 9). These thymic-selected cells are known as natural Th17 (nTh17) cells (8–10).

Natural and induced Th17 (iTh17) cell subsets are distinctly regulated by Akt and mTOR pathways (10, 11), but they share similar functional properties because they both can produce IL-17 and IL-22 following TCR cross-linking. It is generally acknowledged that IL-17 and IL-22 can act in concert to resist bacterial infections, even though they exert potentially distinct roles in autoimmune and allergic responses (12). IL-17 is a key cytokine for psoriasis treatment, whereas IL-22 is important for epithelial cell homeostasis, intestinal defense, tissue repair, and wound healing (12–15). Thus, the characterization of T cell populations that can produce IL-17 alone or associated with IL-22, as well as the identification of stimuli that promote this production, remain important issues.

In this study, we used Rorc(γt)-Gfp Tg mice to phenotypically identify thymic nTh17 cells and demonstrated that they can secrete IL-17 and IL-22 promptly in response to TLR-induced cytokines (i.e., IL-1β and IL-23) in the absence of TCR stimuli. In addition, nTh17 cells express high levels of the transcriptional repressor promyelocytic leukemia zinc finger (PLZF), a marker...
that is highly expressed by innate-like T cells, such as iNKT cells (16). These functional and phenotypic characteristics also were ascribed to peripheral nTh17 cells. Our findings provide new tools to functionally and phenotypically distinguish nTh17 cells from iTh17 cells in the periphery, as well as to better understand the contribution of nTh17 cells to inflammatory responses.

Materials and Methods

Animals
Six- to eight-week-old bacterial artificial chromosome–transgenic Rorc<sup>tg</sup>-Gfp<sup>TG</sup>, IL-6<sup>-/-</sup>Rorc<sup>tg</sup>-Gfp<sup>TG</sup> (17), and C57BL/6j male mice were bred in our facility (Hôpital Enfant Enfants Malades) or purchased from Janvier (Les Genets St. Isle, France). Germ-free (GF) Rorc<sup>tg</sup>-Gfp<sup>TG</sup> mice were obtained from a specific pathogen-free Rorc<sup>tg</sup>-Gfp<sup>TG</sup> colony by aseptic cesarean section, followed by the adoption of the cesarean-derived pups by GF foster mothers. Animal experiments were performed according to the guidelines of the French Institutional Committee.

FACS analysis
Mononuclear cells (MNCs) were stained with CD1d–PBS57-tetramer–allophycocyanin or CD1d–PBS57-tetramer–PE (kindly provided by the National Institutes of Health Tetramer Core Facility), TCRγδ–PE, TCRβ–allophycocyanin or anti-TCR pan–PE, CD4–allophycocyanin or anti-CD8–V500, anti-CD24–PE, anti-CD69–PE, anti-V<sub>gd</sub>–eFluor 450, and anti-CD4–PE–Cy7 (BD Pharmingen, ebioscience, R&D Systems). For intracellular staining, cells were fixed and permeabilized as described (4) and then incubated with anti-IL-17–PerCP-Cy5.5 or allophycocyanin and anti-IL-22–PE or PerCP-eFluor 710 or isotype controls (ebioscience or R&D Systems) and anti-IFN-γ–Alexa Fluor 488 mAb (Invitrogen) and/or anti-PLZF–allophycocyanin (R&D Systems). Cells were then washed and analyzed in a FACSCan II or Fortessa (Becton Dickinson) using FlowJo software.

Enrichment and cell sorting
CD4<sup>+</sup>CD4<sup>+</sup> T cells were enriched by depleting CD8<sup>+</sup>, CD19<sup>+</sup>, CD62<sup>L</sup><sup>-</sup> cells labeled with the corresponding mAbs (BD Pharmingen) and with anti-rat Ig-coated magnetic beads (Bio-Alembads; Ademtech). Depleted populations were stained with CD1d–PBS57-tetramer–allophycocyanin, anti-CD4–allophycocyanin–Cy7, anti-CD8–Pacific Blue, anti-CD4–PE–Cy7, and anti-TCRγδ–PE, and distinct ROR<sub>yt</sub> and ROR<sub>gt</sub> cell subsets were sorted using a FACSaria cell sorter (Becton Dickinson). In others experiments, naïve CD62L<sup>-</sup>CD4<sup>+</sup> T cells were electronically sorted before their differentiation into Th17 cells.

Cell culture and stimulation
Electronically sorted CD4<sup>+</sup>CD4<sup>+</sup> divide–PBS57-tetramer TCRγδ<sup>+</sup> cells were exposed at a concentration of 10<sup>5</sup> cells/mL to coated anti-CD3 and anti-CD28 mAb (BD Pharmingen) for 48 h. IL-17 and IL-22 were measured in supernatants by ELISA (R&D Systems), as described (3, 4). In parallel, T cells were stimulated for 4 h with 10<sup>5</sup> M PMA and 1 µg/ml/ionycin (both from Sigma) in the presence of CD1<sup>t+</sup>-PBS57-tetramer–allophycocyanin, anti-CD4–allophycocyanin–Cy7, anti-CD8–Pacific Blue, anti-CD4–PE–Cy7, and anti-TCRγδ–PE, and distinct ROR<sub>yt</sub> and ROR<sub>gt</sub> cell subsets were sorted using a FACSaria cell sorter (Becton Dickinson). In others experiments, naïve CD62L<sup>-</sup>CD4<sup>+</sup> T cells were electronically sorted before their differentiation into Th17 cells.

Isolation of lamina propria lymphocytes
After cleaning, cutting open lengthwise, and rinsing with PBS, the small intestine was cut into 2-in segments. Epithelial cells were removed by sequential shaking in EDTA-containing PBS solutions. The remaining tissue was digested at 37°C in T cell media containing 1 mg/ml collagenase IV (Life Technologies) and 1 U/ml DNase I (Sigma-Aldrich), and lamina propria (LP) lymphocytes were isolated by a 40/80% Percoll gradient. Isolated cells were incubated for 16 h with supernatants from BMDCs.

Th17 differentiation and stimulation
Electronically sorted naïve CD4<sup>+</sup>CD26<sup>-</sup>CD4<sup>+</sup>CD4<sup>+</sup> T cells were cultured with coated anti-CD3 and anti-CD28 mAbs in Th17 conditions (TGF-β [2 ng/ml], TNF-α [10 ng/ml], IL-1β [10 ng/ml], and IL-6 [20 ng/ml]; all from R&D Systems). Differentiated Th17 cells were rested overnight and further stimulated with anti-CD3 plus anti-CD28 or IL-23 plus IL-1β. Cytokine production was measured in the supernatants.

In vivo TLR stimulation
Mice were injected i.v. with 25 µg LPS or control medium. After 3 h, peripheral lymph node cells were stimulated with PMA plus ionomycin (PI) and stained for surface markers and intracellular cytokine detection. In other experiments, a daily dose of 50 mg imiquimod (5% IMQ cream; Meda AB) or control cream (Vaseline) was applied for 4 d to shaved backs of mice, as described (7).

Adaptive transfer of CD4<sup>+</sup>CD4<sup>+</sup> T cells
Sort naïve CD4<sup>+</sup>CD4<sup>+</sup> splenocytes wild-type (WT) Ly5.2 donors were injected into Rag<sup>-/-</sup>Ly5.1 recipients. After 10 d, thymus, spleen, and lymph nodes cells were incubated for 16 h with supernatants from BMDCs, as previously described, or anti-CD3 plus anti-CD28. Transferred cells were distinguished using anti-Ly5.1–PE (ebioscience), and IL-22 and IL-17 production was assessed by intracellular staining and flow cytometry.

In another set of experiments, sorted TCRβ<sup>+</sup>CD4<sup>+</sup>CD26<sup>-</sup>CD4<sup>+</sup>IL-22<sup>+</sup> ROR<sub>yt</sub> splenic T cells from Rorc<sup>tg</sup>-Gfp<sup>TG</sup> Ly5.2 donors were injected into Rag<sup>-/-</sup>Ly5.1 recipients. Four days later, mice were sensitized by an i.p. injection of 100 µg OVA (grade V, Sigma) emulsified in 0.64 mg alum (Merck). Mice were challenged with 50 mg/ml OVA upon aerosol exposure on three consecutive days (8–10). Twenty-four hours after the last challenge, mice were sacrificed, and samples were collected for further analysis.

Statistical analysis
The nonparametric Mann–Whitney U test was used to calculate the significance levels for all measurements. The p values < 0.05 were considered statistically significant.

Results

ROR<sub>yt</sub> expression and IL-17 production by mature CD4<sup>+</sup> thymocytes
ROR<sub>yt</sub>, the typical transcription factor associated with IL-17 production, is a particularly useful marker to distinguish IL-17–producing iNKT17 cells from IL-4–producing iNKT cells (4, 18). This raised the question of whether ROR<sub>yt</sub> could also identify the recently described thymic natural CD4<sup>+</sup> Th17 cells. To explore this issue, we used Rorc<sup>tg</sup>-Gfp<sup>TG</sup> mice and excluded immature ROR<sub>yt</sub> CD4<sup>+</sup>CD8<sup>-</sup> thymocytes routinely by analyzing only the cells that had already rearranged their TCRβ chain presented on the cell surface and express CD4, but not CD8, together with high levels of the CD44 marker. Knowing that iNKT cells are likely to be present among this TCRβ<sup>-</sup>CD4<sup>+</sup>CD4<sup>+</sup> population, we systematically excluded CD1d–PBS57-tetramer<sup>-</sup> cells from our analysis. In addition, we eliminated TCRβ<sup>+</sup> T cells using anti-TCR pan γδ mAb. We found that up to 0.2% of mature TCRβ<sup>-</sup>TCRγδ<sup>+</sup>CD4<sup>+</sup>CD8<sup>-</sup>CD1d–PBS57-tetramer<sup>-</sup> thymocytes expressed ROR<sub>yt</sub> (Fig. 1A). These results fit with the percentage of thymic nTh17 cells described previously (8, 9). Thymic nTh17 cells are CD24<sup>-</sup> and express CD69 at similar high levels as do recently selected TCRβ<sup>+</sup> thymocytes (Supplemental Fig. 1),
Sorted ex vivo RORγt+ nTh17 thymocytes stimulated with coated anti-CD3 plus anti-CD28 mAbs do not produce any detectable IL-22, but the addition of IL-23 effectively induced the secretion of this cytokine (Fig. 1C). The most striking finding is that RORγt+ nTh17 cells generated IL-22 within only 4 h when IL-23 was provided with PI stimulation (Fig. 1D), clearly showing that thymic RORγt+ nTh17 cells are primed to secrete IL-22. IL-23 also enhanced the frequency of IL-17+ cells among RORγt+ nTh17 cells (Fig. 1D). These findings reveal that thymic nTh17 cells can produce high levels of IL-22 and that the majority of IL-22+ cells coproduce IL-17.

**Innate-like stimulation of thymic RORγt+ nTh17 cells**

Thymic differentiated innate-like T cells, such as iNKT17 and γδ T cells, can secrete IL-17 and IL-22 in response to IL-1β plus IL-23 (19, 20). Fig. 2A shows that this innate-like property also applies to RORγt+ nTh17 thymocytes because they promptly produced IL-17 and IL-22 as early as 4 h after stimulation with IL-23 plus IL-1β.

We addressed the physiological relevance of our results by examining whether endogenous IL-1β and IL-23 could also target RORγt+ nTh17 thymocytes to promote their production of IL-17 and IL-22. Previous reports established that dendritic cells (DCs) secrete IL-1β and IL-23 in response to various pathogen-associated molecular patterns, including TLR agonists (21, 22). Taking advantage of this finding, we stimulated BMDCs with LPS, a TLR4 agonist, and evaluated how the supernatants generated affected IL-17 and IL-22 production by thymic RORγt+ nTh17 cells. As shown in Fig. 2B, the response was quite similar to that induced by exogenous cytokines. Supernatants from DCs activated via the TLR4 pathway lost their ability to induce cytokine production by RORγt+ nTh17 cells in the presence of neutralizing anti–IL-23 plus anti-IL-1β mAbs (Fig. 2C), proving the specific involvement of these two cytokines.

**Peripheral RORγt+ nTh17 T cells produce both IL-17 and IL-22 following IL-1β and IL-23 stimulation**

Having characterized thymic RORγt+ nTh17 cells, we sought to better determine their properties in the periphery. As shown in Supplemental Fig. 2A, CD4+ TCRβ+ T cells expressing similar high levels of CD44 and RORγt as thymic RORγt+ nTh17 lymphocytes also are found in peripheral organs (i.e., spleen or lymph nodes) in the steady-state. RORγt+ nTh17 cells express low levels of PD-1 and high levels of ICOS, CCR6, and IL-23R (Supplemental Fig. 2B), as previously ascribed to nTh17 lymphocytes (9). Similarly, we observed no biased Vβ3 expression (Supplemental Fig. 2C) in the periphery (9).

Further, we asked whether the capacity of RORγt+ nTh17 thymocytes to secrete IL-17 and IL-22 in response to IL-1β plus IL-23 was a particular feature of these cells or whether it was shared by peripheral RORγt+ nTh17 lymphocytes. Lymph node RORγt+CD4+ T cells respond strongly to supernatants from DCs stimulated with a TLR4 agonist by producing IL-17 and IL-22 (Fig. 2D). The latter effect is once again mediated specifically through IL-1β and IL-23 because it was blocked by their respective Abs (Fig. 2E).

Additionally, a TLR4 agonist was injected into mice; this brief in vivo TLR stimulation increased IL-17 production and induced IL-22 production by nTh17 cells in response to both PI or IL-1β.
RORγt+ nTh17 T cells are functionally distinct from iTh17 cells

It might be argued that this innate-like ability to secrete IL-17 and IL-22 upon IL-1β plus IL-23 stimulation is inherent to all types of Th17 cells. However, this is not the case, because Th17 cells differentiated in vitro from naive CD44+CD62L+ T cells and stimulated with IL-1β plus IL-23 generated no detectable IL-17 (Fig. 3A). They are able to secrete IL-17 following anti-CD3 plus anti-CD28 stimulation, showing their functionality (Fig. 3A). These results are in contrast to previous reports showing that, under some conditions, Th17 cells differentiated in vitro were able to produce IL-17 in response to IL-1β plus IL-23 stimulation (23). A likely explanation for this discrepancy are the different in vitro protocols used. To overcome technical in vitro issues and to be as similar as possible to in vivo situations, we tested the ability of naive CD4+RORγt+ T cells to become RORγt+ in vivo and to produce IL-17 and IL-22 in response to IL-1β plus IL-23 stimulation. To address this issue, we first set up experiments using adoptive transfer of sorted naive CD4+CD44+CD62L+Ly5.1+ T cells to Rag−/−Ly5.1+ mice. Ten days later, CD4+Ly5.1+ cells that expressed CD44 were observed in recipient mice (Supplemental Fig. 3A). Under these conditions, a small fraction of transferred cells became IL-17 producers in response to anti-CD3 plus anti-CD28 but not in response to conditioned supernatants from DCs activated via the TLR4 pathway containing IL-1β plus IL-23 (Supplemental Fig. 3B, 3C).

Additionally, we used a protocol capable of inducing in vivo Th17-immune responses. Naive CD4+CD44+CD62L+RORγt+Ly5.1+ T cells were adoptively transferred to Rag−/−Ly5.1+ mice; 3–4 d later recipient animals were immunized and later challenged with OVA. This protocol is used to induce allergic asthma disease, resulting in both Th2 and Th17 cell differentiation. Adoptively transferred CD4+ T cells were found in the lung (Fig. 3B). A fraction of these lymphocytes acquired the expression of RORγt (Fig. 3C) and promptly secreted IL-17 following PI stimulation (Fig. 3C). Nevertheless, these cells were unable to secrete IL-17 in response to IL-1β plus IL-23 stimulation (Fig. 3C) or to express PLZF (Fig. 3D), a characteristic marker of nTh17 cells (see later experiments). RORγt+IL-17+IL-22+ thymocytes were cultured for 16 h in the presence of supernatants from BMDCs, stimulated or not (medium) with TLR4 agonist and with or without anti–IL-1β+ plus anti–IL-23–neutralizing Abs. Representative FACS profiles of IL-17 and IL-22 production among gated nTh17 cells. Data are representative of three independent experiments. (D and E) Freshly isolated lymph node cells were incubated with supernatants from BMDCs that were stimulated or not (medium) with TLR4 agonist and with or without anti–IL-1β+ plus anti–IL-23–neutralizing Abs for 16 h. Representative FACS profiles of IL-17 and IL-22 production by gated lymph node CD1d−PBS57-tetramer+CD4+CD62L+RORγt+Ly5.1+ mice; 3–4 d later recipient animals were immunized and later challenged with OVA. This protocol is used to induce allergic asthma disease, resulting in both Th2 and Th17 cell differentiation. Adoptively transferred CD4+ T cells were found in the lung (Fig. 3B). A fraction of these lymphocytes acquired the expression of RORγt (Fig. 3C) and promptly secreted IL-17 following PI stimulation (Fig. 3C). Nevertheless, these cells were unable to secrete IL-17 in response to IL-1β plus IL-23 stimulation (Fig. 3C) or to express PLZF (Fig. 3D), a characteristic marker of nTh17 cells (see later experiments).
In vitro differentiated iTh17 cells

A

Lung MNC from OVA-treated mice

B

C

D

E

FIGURE 3. iTh17 cells failed to respond to IL-1β plus IL-23 stimulation. (A) Naive electronically sorted CD62L+CD44+CD4+ T cells were differentiated into Th17 cells, as described in Materials and Methods. Th17 cells obtained (5 × 10^5 cells/ml) were stimulated with anti-CD3 plus anti-CD28 (positive control), IL-1β plus IL-23, or medium (negative control) for 72 h. IL-17 was measured in the supernatants. Data represent mean ± SEM from four independent experiments, pooling three mice/experiment. (B and C) Naive electronically sorted CD62L+CD44+CD4+ RORγt Ly5.1+ T cells from Rorc(gtf)-Gfp mice were adoptively transferred to Rag1−/−Ly5.1+ mice and immunized and challenged with OVA, as described in Materials and Methods. Twenty-four hours after the last challenge, lung MNCs were recovered and stimulated in vitro for 4 h. Representative FACS profile of IL-17 and IL-22 production by TCRβ+CD4+Ly5.1+ RORγt+ cells in response to PI stimulation (left panel) or IL-1β plus IL-23 stimulation (right panel). (D) Expression of PLZF (black line) on TCRβ+CD4+Ly5.1+ RORγt+ lung MNCs freshly isolated from OVA-treated mice. Shaded graph represents isotype control. Data are representative of two independent experiments in which three to five individual mice were analyzed. (E) FACS analysis of LP MNCs isolated from Rorc(gtf)-Gfp mice and stimulated with anti-CD3 and anti-CD28 mAb (upper panels) or supernatants from BMDCs stimulated with TLR4 agonist (lower panels). Plots were gated first on CD1d–PBS57-tetramer+ TCRβ+CD4+CD8− and then on CD44+RORγt+ or RORγt− cells. Data are representative of three or four independent experiments, pooling six organs/experiment. Percentages of each subset are indicated in quadrants.
Enhanced numbers of PLZF+ RORγt+ nTh17 cells in acute psoriasiform lesions

In light of the above results, we examined the possible contribution of PLZF+ RORγt+ nTh17 cells in a mouse model of psoriasis-like disorder (27–29). For this, mice were treated epicutaneously with the TLR7 agonist IMQ; the development of acute psoriasiform disorder (27–29). For this, mice were treated epicutaneously with the TLR7 agonist IMQ; the development of acute psoriasiform disorder (27–29).

Discussion

Our findings provide strong evidences that nTh17 cells respond promptly to TLR-derived proinflammatory cytokines (i.e., IL-1β and IL-23) in the absence of TCR stimulation. Moreover, we demonstrated that RORγt associated with PLZF are characteristic markers for identifying nTh17 cells in the thymus and periphery.
from iTh17 cells, and we observed that RORγt+ T cells are abundant in lymph nodes but absent from LP. We detected CD4+CD44highRORγt+TCRgd+ nTh17 cells from lymph node cells stimulated with IL-17 and IL-22 following TLR4 agonist–conditioned supernatants, indicating that they were iTh17 cells. A possible explanation for this discrepancy found no major contribution of IL-6 or microbiota to the development of nTh17 cells. Possible explanations for this discrepancy are the distinct mouse background (B10.BR versus C57BL/6) and experimental protocols used.

nTh17 and iNKT17 cells acquire their ability to secrete IL-17 and IL-22 in the thymus. These particular TCRβ+ T cell populations are functionally very similar, but they are substantially distinct with regard to their thymic development and selection. Indeed, iNKT cells are positively selected by CD1d-expressing CD4+CD8+ thymocytes and recognize glycolipids (36, 37), whereas nTh17 cells are dependent on MHC class II expression on medullary thymic epithelium for their selection and are stimulated by peptides (9). Consequently, iNKT and nTh17 cells have complementary roles based on their ability to promptly produce cytokines in response to distinct Ags. Of note, iNKT17 cells (20), such as nTh17 cells, are capable of producing IL-17 and IL-22 upon indirect TLR activation. In addition to this shared functional property, iNKT17 and nTh17 cells express RORγt, which, to our knowledge, is the most specific marker to distinguish iNKT17 cells from other iNKT cell subsets. Another typical marker of iNKT cells at large is PLZF, a member of the Broad complex, Tramtrack, Bric-à-brac, and zinc finger family that controls a wide range of biological processes (38). PLZF defines not merely the phenotype of iNKT cells, it directs their effector program and is implicated in their proapoptotic propensity (16, 39, 40). Transgenic expression of PLZF during T cell development induced a T cell–intrinsic program leading to an increase in peripheral CD44high expression and effector T cell properties and memory effector phenotype. We showed that cytokine-producing nTh17 cells express high levels of PLZF, which indicates that functional and phenotypic properties are somehow connected and that PLZF has rheostat functions in the generation of nTh17 cells. Taken together, nTh17 and iNKT17 cells require distinct signals for their thymic selection but likely common pathways for acquisition of their ability to promptly produce IL-17 and IL-22. Further studies are required to clarify this point.

In sum, we identified RORγt and PLZF as novel markers to discriminate nTh17 cells. Our findings also reveal a new feature of nTh17 cells: their ability to respond promptly to proinflammatory factors following TLR stimulation and independently to TCR stimuli. Additionally, nTh17 cells are significantly enhanced and constitute a major IL-17–producing T cell population in an IMQ psoriasis-like model, indicating their contribution to this type of lesion. In conclusion, our findings shed new light on nTh17 cell biology and open new possibilities for the use of RORγt and PLZF as markers to better understand this particular T cell population.

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Disclosures
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