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Genetic Requirements for the Development and Differentiation of Interleukin-17–Producing γδ T Cells

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Abstract

Most effector T cells are generated in the periphery following an encounter with a foreign antigen and exposure to soluble and membrane-bound mediators. There are, however, some T cell subsets, such as γδ T cells and natural killer T cells, that acquire their effector potential in the thymus before their emigration to the periphery. This developmental preprogramming enables these cells to differentiate rapidly into cytokine-producing effectors during the host immune response. This review focuses on murine interleukin (IL)-17–producing γδ T (γδ-17) cells, which have been shown, through their early production of IL-17, to have a critical role in multiple infectious and autoimmune diseases. Specifically, we discuss what is currently known about the genetic requirements for their generation and compare it with what is known about that of the more extensively studied IL-17–producing helper T (Th17) cells. Based on this comparison, we propose a model for murine γδ-17 development and differentiation.

Keywords

thymus; T cell development; T cell differentiation; IL-17; signal transduction; fate specification

I. INTRODUCTION

Since the discovery of an interleukin (IL)-17-producing subset of murine γδ T (γδ-17) cells just 7 years ago,[1] immunologists have made considerable progress in defining the role of these cells in the immune response. First, it has been shown that this functional subset plays a protective role in host defense to microbes, such as *Mycobacterium tuberculosis*,[2] *Mycobacterium bovis*,[3,4] *Listeria monocytogenes*,[5–8] *Escherichia coli*,[9] and *Candida albicans*.[10] In these infection models, γδ-17 cells produce IL-17 at an early stage in the immune response, and this early IL-17 production is required for the recruitment of neutrophils to the site of infection and subsequent clearance of the pathogen. Significantly, in the *M. bovis* and *L. monocytogenes* models, γδ-17 cells also are required for granuloma formation and optimal αβ T cell effector responses.[3–5,8] Second, γδ-17 cells have a pathogenic role in multiple autoimmune diseases. The early production of IL-17 by γδ T cells contributes to pathogenesis in mouse models of collagen-induced arthritis,[11] colitis,[12] and experimental autoimmune encephalitis (EAE).[13,14] In the colitis model, their mode of action is to promote IL-17 production by colitogenic Th17 cells,[12] whereas in the EAE model, γδ-17 cells not only promote IL-17 production by interleukin-17–producing helper T (Th17) cells, but also inhibit the development of regulatory T cells.[13,14] Last, γδ-17 cells

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have opposing roles in tumor immunity, depending on the tumor model employed. Chemotherapeutic agents that induce tumor cell death elicit a tumor-specific cytotoxic T lymphocyte response that is capable of resolving the tumor.[15,16] Notably, the emergence of these cytotoxic T lymphocytes after chemotherapy is dependent upon the influx, at an earlier time point, of γδ-17 effectors into the tumor bed.[16] However, in multiple transplantable tumor models, γδ-17 cells exert protumor activities by inducing vascularization and subsequent growth of the tumor.[17] Together, these data demonstrate that by rapidly producing IL-17 during an immune response, γδ-17 cells have the ability to control the actions of immune and nonimmune cells alike.

It is interesting that cytokine-producing γδ-17 and Th17 cells are elicited in many of the same inflammatory diseases, but functional γδ-17 cells appear days to weeks before functional Th17 cells.[2,12,18,19] The difference in the kinetics of their appearance may be explained by the fact that peripheral γδ-17 cells are preprogrammed in the thymus to produce IL-17,[18,20] whereas naïve $CD4^+$ T cells are programmed to become Th17 cells in the periphery in the context of an immune response.[21–23] In this review, we compare the current knowledge regarding the genetic requirements for the generation of γδ-17 and Th17 effectors, with the goal of determining whether the respective priming microenvironments dictate the requirements for specific effector fates. This comparison not only identifies signaling pathways that are shared by, and unique to, each IL-17–producing effector but also serves as the basis for a model of γδ-17 development and differentiation.

II. TH17 DIFFERENTIATION PATHWAY

The development of an in vitro culture system in which naïve $CD4^+$ T cells can be induced to differentiate into various types of effectors has aided greatly in the identification of molecules that are sufficient for the generation of Th17 cells. Using such an in vitro culture system, it was shown that transforming growth factor (TGF)-β and IL-6, together with T cell receptor (TCR) engagement, induce the differentiation of naïve $CD4^+$ T cells into Th17 cells.[24–26] More in-depth analyses have revealed that TCR, TGF-β, and IL-6 signaling pathways synergize to induce expression of the transcription factor RORγt,[27] the chemokine receptor CCR6,[28] the cytokine IL-21,[29] and the cytokine receptors IL-1R and IL-23R.[29,30] These phenotypic changes enable Th17 cells to migrate to inflammatory sites[28] and to secrete IL-17 in response to IL-23 and IL-1.[29,30]

Subsequent in vivo studies using genetically modified mice demonstrate that Th17 cell differentiation is more complex than originally thought. First, factors other than the ones previously mentioned also positively regulate Th17 differentiation. These include Notch,[31,32] prostaglandin E_2,[33] sphingosine-1 phosphate,[34,35] B-cell activating factor,[36,37] and the transcription factors c-Rel,[38–40] BATF,[41] interferon regulatory factor-4,[42,43] Runx1,[44] aryl hydrocarbon receptor,[45,46] and Iκbς.[47] Second, it was shown recently that there is a differential requirement for IL-6 in Th17 differentiation, depending on whether naïve T cells are primed in mucosal tissues (IL-6 dependent) or the spleen (IL-6 independent).[48] Notably, this differential requirement for IL-6 in priming Th17 cells in mucosal and secondary lymphoid tissues is because of differences in the properties of their resident dendritic cells (DCs). DC populations from the lamina propria contain a mixture of $CD103^+$ and $CD103^-$ cells, whereas those in the spleen are primarily $CD103^-$. Interestingly, the $CD103^+$ DCs, but not the $CD103^-$ DCs, produce high levels of TGF-β and retinoic acid, which inhibit Th17 differentiation and promote regulatory T cell development in the absence of IL-6.[48] Thus, these data demonstrate that the cytokine requirements for Th17 differentiation differ when naïve $CD4^+$ T cells are primed in mucosal tissues versus secondary lymphoid tissues.[48] As we discuss in Section IV, the idea that the priming microenvironment is able to dictate the cytokine requirements for effector T cell

differentiation will prove to be important in our understanding of γδ-17 development in the thymus.

III. PHENOTYPE OF γδ-17 CELLS

The phenotypes of Th17 and γδ-17 cells are, for the most part, similar (see Table 1 for phenotype of γδ-17 cells). Both express CCR6, IL-23R, CD44, TLR2, and RORγt.[13,27,49–53,56] Interestingly, γδ-17 cells also have been reported to express CD25 (IL-2Rα),[51,52] the C-type lectin receptor dectin-1,[50] the scavenger receptor SCART2,[54] and the Src family kinase, kinase, B lymphoid kinase (Blk).[55] Although Th17 cells express neither CD25 nor dectin-1,[21–23] it is not known whether they express SCART2 or Blk because their expression has not been examined in Th17 cells.

Notably, the vast majority of γδ-17 cells express γδTCRs that use either the Vγ6 or Vγ4 gene segment.[5,11,51] Vγ6$^+$ γδ T cells are only generated early in ontogeny, during the second wave of γδ T cell development in the fetal thymus.[57] Because of the lack of terminal deoxynucleotidyl transferase expression in these cells, the rearranged TCRγ and –δ chains lack nontemplated nucleotide additions and, as a result, lack diversity.[58–62] Around the time of birth, γδ T cells expressing this invariant γδTCR exit the thymus and colonize the lung, tongue, nasal lymphoid tissue, peritoneal cavity, and female reproductive tract.[9,63–66] Vγ4$^+$ γδ T cells, on the other hand, are generated in both the fetal and adult thymus,[57,67] and those generated postnatally express a diverse TCR repertoire.[60,62] Vγ4$^+$ γδ T cells are found not only in secondary lymphoid tissues but also in the lung.[65,68] Because both Vγ6$^+$ and Vγ4$^+$ γδ T cell subsets reside in the lung, it is important to note that their colonization and subsequent residency occur in a sequential fashion, with Vγ6$^+$ γδ T cells being the major γδ T cell population from birth until about 8 to 10 weeks of age; Vγ4$^+$ γδ T cells predominate from that age on.[65]

Despite the extensive number of markers that can be used to identify γδ-17 cells, the vast majority of studies examining the role of specific genes in γδ-17 development, function, or both have enumerated γδ-17 cells based on their ability to produce IL-17 after treatment with phorbol 12-myristate 13-acetate (PMA) and ionomycin (which together simulate antigen receptor signaling by activating protein kinase C and raising intracellular Ca^{2+} levels). However, the use of this functional assay comes with the assumption that protein kinase C– and calcium-regulated signaling pathways mimic all signaling pathways that elicit IL-17 production from γδ-17 cells. As shown in the following example, this assumption is flawed, as it is indeed possible for γδ-17 cells from a mutant mouse to produce wild-type levels of IL-17 after activation with PMA and ionomycin yet produce reduced levels of IL-17 after activation with physiological stimuli. Homozygous knock-in IL-23R green fluorescent protein (GFP) reporter mice, which lack a functional IL-23R, generate γδ-17 cells in numbers equivalent to those in heterozygous IL-23R GFP reporter mice, indicating that this cytokine receptor is not required for γδ-17 development.[7] To assess the functional ability of these cells, γδ-17 cells were activated in vitro with PMA and ionomycin or activated *in vivo* by infection with *L. monocytogenes*. Remarkably, while *in vitro* IL-17 production was found to be comparable between wild type and IL-23R–deficient γδ-17 cells[7]; *in vivo* IL-17 production by γδ-17 cells was markedly reduced in Listeria-infected IL-23R$^{-/-}$ mice compared with Listeria-infected wild-type mice (Fig. 1C, D).[7] These data demonstrate not only that IL-23 is required for the expansion of γδ-17 effectors, but also that the use of PMA and ionomycin to assess γδ-17 cell effector function leads to inconclusive results if not performed in combination with an in vivo functional assay. For this reason, we only review the studies that show unequivocal evidence that a specific gene has a role in γδ-17 development, differentiation, or both.

IV. GENES REQUIRED FOR THE DEVELOPMENT OF γδ-17 CELLS IN THE THYMUS

To date, no in vitro culture system has been developed to identify the factors that play a role in γδ-17 development and differentiation, most likely because a functionally uncommitted γδ thymocyte (which would be comparable to the naïve $CD4^+$ T cell in the in vitro culture system discussed earlier) has yet to be identified. Consequently, we must rely on the use of genetically modified mice to determine whether a specific gene is required for the generation of γδ-17 effectors. In this section and the next, we discuss the genes required for γδ-17 development and differentiation by their associated signaling pathways (see Table 2 for a list of required genes).

A. γδTCR Signaling

Signaling through the αβ TCR is a mandatory step in T helper cell differentiation; however, it is still unclear whether γδTCR signaling, through recognition of self-ligands expressed in the thymus, is required for functionally uncommitted γδ thymocytes to acquire the IL-17 effector fate. This uncertainty stems from the fact that there are lines of evidence both for and against a requirement for γδTCR signaling in the IL-17 effector fate specification. The first line of evidence in favor of a role for γδTCR signaling is that γδ-17 thymocytes, defined by their expression of CCR6, express high levels of CD44,[49–52] an activation marker that is induced on antigen-experienced T cells.[72,73] The second line of evidence is that $CCR6^+$ γδ thymocytes express the zinc finger transcription factor ThPOK at levels significantly higher than those in thymocytes that have recently chosen the γδ lineage.[74] Because ThPOK levels are positively regulated by TCR signaling, these data suggest that elevated levels of ThPOK in γδ-17 thymocytes are the result of γδTCR engagement.[74] The last line of evidence is that γδ-17 thymocytes, but not γδ thymocytes committed to another effector fate, express the Src family kinase Blk,[54] which we recently have shown to be a negative regulator of both TCR and B cell receptor signal transduction.[54,75] In the absence of Blk, γδ-17 development is severely impaired, as evidenced by the significant loss of $Vγ4^+$ γδ-17 cells and the almost complete absence of $Vγ6^+$ γδ-17 cells in the thymus and periphery of $Blk^{-/-}$ mice.[54] Given its role as a negative regulator of antigen receptor signaling, it is conceivable that the expression of Blk in γδ-17 thymocytes serves as a mechanism by which a high γδTCR signaling threshold is set in γδ thymocytes with an affinity for self-ligands. Of course, it cannot be ruled out that Blk, being an Src family kinase, functions in signaling pathways that are coupled to receptors other than the TCR.

The evidence against a role for γδTCR signaling in γδ-17 development is from a study in which the development of T10-/T22-specific γδ T cells was assessed in mice that have extremely low expression levels of T10/T22 (i.e., $β2m^{-/-}$ mice).[18] Interestingly, although it was found that the number of T10-/T22-specific γδ T cells, defined by tetramer staining, is comparable between wild-type and $β2m^{-/-}$ mice, the phenotype of these cells is strikingly different. Specifically, in wild-type mice, the T10-/T22-specific γδ T cell population contained a combination of $CD122^+$ (IL-2Rβ) and $CD122^-$ cells, whereas this population in $β2m^{-/-}$ mice was primarily $CD122^-$.[18] Subsequent functional analysis of wild-type $CD122^+$ and $CD122^-$ T10-/T22-specific γδ T cells revealed that, after TCR stimulation, $CD122^+$ γδ T cells produced interferon (IFN) γ and $CD122^-$ γδ T cells produced IL-17.[18] On the basis of these findings, it was concluded that $CD122^-$ γδ thymocytes, which include γδ-17 thymocytes, did not require ligand for their generation.[18] However, because this study was performed before the identification of γδ-17–specific markers, it is still not known whether the development, function, or both of $CCR6^+$ tetramer-positive γδ thymocytes are indeed normal in $β2m^{-/-}$ mice.

B. TGFβ and IL-6 Signaling

TGFβ signaling is required for thymic development of γδ-17 cells because in the absence of TGFβ1 or Smad3, a component of the TGFβ signaling pathway, the number of IL-17$^+$ γδ thymocytes is reduced drastically relative to that of wild-type mice.[52] Likewise, IL-6 signaling is important because there is a significant decrease in the number of γδ-17 cells, defined by the expression of CCR6, IL-23R and/or CD44 in the thymus, peripheral lymph nodes, spleen, and peritoneal cavity of IL-6$^{-/-}$ mice compared to wild-type mice (Fig. 1A).[14] Interestingly, despite being present in reduced numbers, the γδ-17 cells in IL-6$^{-/-}$ mice are comparable to those in wild-type mice in their expression of RORγt (Fig. 1B)[56] and in their ability to expand and differentiate into IL-17 producers after Listeria infection (Fig. 1C, D). Together, these data suggest that IL-6 does not act directly on functionally uncommitted γδ thymocytes to promote the acquisition of the γδ-17 effector fate, but instead it acts indirectly by regulating the size of the thymic microenvironment that supports γδ-17 development. Consistent with this idea are the findings that (1) IL-6 positively regulates the expression of Delta-like 4, a Notch ligand, on thymic epithelial cell lines[76] and (2) Notch signaling is required for γδ-17 development (see below).[70]

C. Lymphotoxin-β receptor Signaling

Lymphotoxin-β receptor (LTβR) is a member of the tumor necrosis factor receptor superfamily, and signaling through this receptor is dependent on activation of the alternative nuclear factor (NF)-κB pathway.[77] It was shown recently that the generation of IL-17$^+$ γδ thymocytes is severely impaired in mice deficient in components of the LTβR signaling pathway (i.e., LTβR$^{-/-}$, Nfkb2$^{-/-}$, T cell–specific Relb$^{-/-}$).[69] The LTβR ligands that regulate this developmental process are produced by CD4$^+$CD8$^+$ thymocytes, not epithelial cells.[69,78] The mechanism by which the LTβR signaling pathway controls γδ-17 development is by regulating *Rorc* expression in γδ thymocytes committing to the γδ-17 lineage.[69] *Rorc* encodes the transcription factor RORγt, which acts as a master regulator of IL-17–producing cells.[27] Accordingly, RORγt is also required for the development of γδ-17 cells because IL17$^+$ γδ-17 cells are virtually absent from the spleen, lamina propria, and peritoneal cavity of Rorc$^{-/-}$ mice.[27,56,70]

The generation of Th17 cells is not dependent on RelB.[69] Instead, Th17 cell differentiation requires c-Rel, an NF-κB family member belonging to the classical pathway.[39,40] c-Rel is activated by TCR signaling, and it is responsible for inducing *Rorc* expression.[39,40] Therefore, both γδ-17 and Th17 cells utilize NF-κB, albeit through the activation of different receptors, to regulate RORγt expression and drive commitment to the IL-17 effector fate.

It is important to note that LTβR signaling also is required for the generation of IFNγ-producing γδ T (γδ-IFNγ) cells in the thymus.[78] The finding that both γδ-17 and γδ-IFNγ effector lineages are dependent on LTβR signaling suggests that other signaling pathways coordinate with LTβR signaling to direct functionally uncommitted γδ thymocytes to either the IL-17 or IFNγ effector fate. Importantly, for this coordinated signaling to occur, there would have to be physically separate thymic niches that promote and support each effector fate.

D. Notch Signaling

Notch signaling is involved in many cell fate decisions, including those of T helper cell effectors.[79]It has been shown recently that Notch signaling is required for γδ-17 and Th17 development.[31,32,70] In fetal mice that lack Hes-1, a target of the Notch signaling pathway, IL-17$^+$ γδ thymocytes fail to develop.[70] From the Th17 studies, we know that mechanism of action of Notch is to regulate transcriptional activity at the *Rorc* promoter.[31,32]

E. Prostaglandin Signaling

Prostaglandins are lipid mediators that can inhibit or stimulate immune cells.[80] In regard to the generation of IL-17–producing effectors, prostaglandins have been shown to promote both γδ-17 and Th17 development.[33,71] Prostaglandin I_2 regulates γδ-17 development, as evidence by the marked reduction in IL-17$^+$ γδ T cells in the thymus and lung of prostaglandin I_2 receptor-deficient mice compared with wild-type mice.[71] Another prostaglandin, namely prostaglandin E^2, has been shown to play a role in Th17 differentiation. Prostaglandin E_2 promotes Th17 cell function through the upregulation of both IL-23R and IL-1R expression.[33] It is tempting to speculate that prostaglandin I_2 may function in a similar manner in developing γδ-17 thymocytes.

V. GENES REQUIRED FOR THE DIFFERENTIATION OF γδ-17 CELLS IN THE PERIPHERY

A. IL-23R and IL-1R Signaling

IL-23 and IL-1 are produced by innate immune cells, such as DCs, as a consequence of signaling through pattern recognition receptors.[81] Both of these cytokines are crucial for IL-17 production by γδ T cells, as evidenced by the significant reduction in IL-17$^+$ γδ T cells after *L. monocytogenes* infection in IL-23$^{-/-}$ and IL-23R$^{-/-}$ mice (Fig. 2)[6,7] or in IL-1R1$^{-/-}$ mice with EAE.[13] Notably, other IL-17–producing cells, including Th17 cells and natural killer T cells, share the requirement for IL-23 and IL-1 to differentiate into cytokine-producing effectors.[30,82,83]

B. Notch Signaling

Recent evidence suggests that Notch signaling in γδ-17 cells also is required in the periphery to maintain their optimal function. When Hes-1 is conditionally deleted in peripheral γδ-17 cells, there are significantly fewer IL-17$^+$ γδ T cells in these mice than in wild-type mice.[73] This reduction in IL-17–producing γδ T cells may be explained by the ability of Notch signaling to regulate positively both *Rorc* and *IL-17* transcription.[31,32]

C. IL-2/IL-2Rα Signaling

γδ-17 cells express CD25,[51] a surface phenotype shared by regulatory T cells but not Th17 cells.[21–23] The loss of either IL-2 or CD25 has no effect on γδ-17 development because the number of IL-17–producing γδ thymocytes is comparable among CD25$^{-/-}$, IL-2$^{-/-}$, and wild-type mice.[51] Remarkably, their loss does have an effect on γδ-17 cell maintenance because IL-17$^+$ γδ T cells are virtually undetectable in the peritoneal cavity of CD25$^{-/-}$ mice and severely reduced in the peritoneal cavity of IL-2$^{-/-}$ mice.[51] It is not known how IL-2 signaling regulates the maintenance of γδ-17 cells; the cellular source(s) of IL-2, and the mechanism by which CD25 signals in γδ-17 cells, remain unresolved.[51]

VI. A THREE-STAGE MODEL FOR THE DEVELOPMENT AND DIFFERENTIATION OF γδ-17 CELLS

By comparing the genetic requirements for the generation of γδ-17 and Th17 cells, we have found that many of the signaling pathways that regulate their development and function are, in fact, shared. In this section, we build on this comparison to define the stages of γδ-17 development and differentiation and, on the basis of the quantity of supporting data, identify the genes that act at each stage (see Fig. 2).

A. Stage One: Commitment to the γδ-17 Lineage

Both γδ-IFNγ and γδ-17 thymocytes arise from a $CD27^+CD24^+$ γδ thymocyte,[20] which is not committed to any effector lineage.[49] During the first stage of γδ-17 development, these functionally uncommitted cells encounter, in the thymic cortex, LTβR ligands produced by $CD4^+CD8^+$ thymocytes[69,78] and delta-like 4, a Notch1 ligand expressed by cortical thymic epithelial cells.[84] Signaling through both LTβR and Notch leads to the induction of *Rorc* transcription.[31,32,69] In fact, there may be synergy between the NF-κb and Notch signaling pathways in inducing RORγt expression because synergy has been noted between these 2 pathways in other developmental processes.[85,86] These $ROR\gamma T^+$ γδ thymocytes, which we have termed γδ-17 precursors, express no or low levels of CCR6, IL-23R, and CD44 and high levels of CD27 and CD24. Importantly, these γδ-17 precursors can be detected in the thymus of $ROR\gamma t^{gfp/+}$ mice and of wild-type mice using an anti-RORγt antibody (Fig. 3).It is notable that mice that lack expression of Blk possess wild-type numbers of γδ-17 precursors (Fig. 3), suggesting that this kinase is required at a later stage of γδ-17 development in the thymus.

B. Stage Two: Maturation of γδ-17 Precursors into γδ-17 Thymocytes

Within a wild-type thymus, there is a small population of $CCR6^+CD44^{hi}$ γδ thymocytes that produce IL-17 when stimulated in vitro.[49,52] We propose that these mature cells are derived from the pool of γδ-17 precursors in response to factors whose signaling pathways require Blk activity. It also is conceivable that prostaglandin I_2 acts at this stage, if it induces IL-23R and IL-1R expression as prostaglandin E_2 does.[33] Moreover, because these cells are mature, they are probably located in the medulla, where they are positioned to leave the thymus.

C. Stage Three: Differentiation of γδ-17 Cells into IL-17–Producing Effectors

Once γδ-17 cells exit the thymus, they migrate to mucosal and lymphoid tissues, where IL-2/IL-2Rα signaling is required for their homeostasis.[51] During an immune response, IL-23 and IL-1 are produced[81] and delta-like 4 levels are increased,[87,88] all of which act to induce IL-17 production from γδ-17 cells.[6,7,13]

VII. CONCLUDING REMARKS

Through their early production of IL-17, γδ-17 cells play a critical role in controlling the immune response, be it protective, as in the case of microbial infection, or pathogenic, as in the case of autoimmune disease. By reviewing what currently is known about the genetic requirements for γδ-17 cell generation, we have gained a better understanding of the molecules and associated signaling pathways that regulate their development and activation. There is also the realization that more tools are required to study these cells, such as an *in vitro* culture system, not only to identify factors that promote and support their commitment and maturation but also to determine their mechanism of action. Moreover, when investigating the role of a specific gene in γδ-17 development and function, it would be beneficial to use reporter mice that express GFP under the control of the *Rorc* or *Il23r* promoter to track maturation, differentiation, and migration of wild-type and mutant γδ-17 cells under steady-state conditions and during disease states. The knowledge gained will enable the development of vaccines and strategies that promote or suppress γδ-17 cell function.

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ABBREVIATIONS

γδ-17 cells	interleukin-17–producing γδ T cells
Blk	B lymphoid kinase
DCs	dendritic cells
EAE	experimental autoimmune encephalitis
GFP	green fluorescent protein
IFN	interferon
IL	interleukin
LTβR	lymphotoxin-β receptor
NF	nuclear factor
PMA	phorbol 12-myristate 13-acetate
TCR	T cell receptor
TGF	transforming growth factor
Th17 cells	interleukin-17–producing helper T cells

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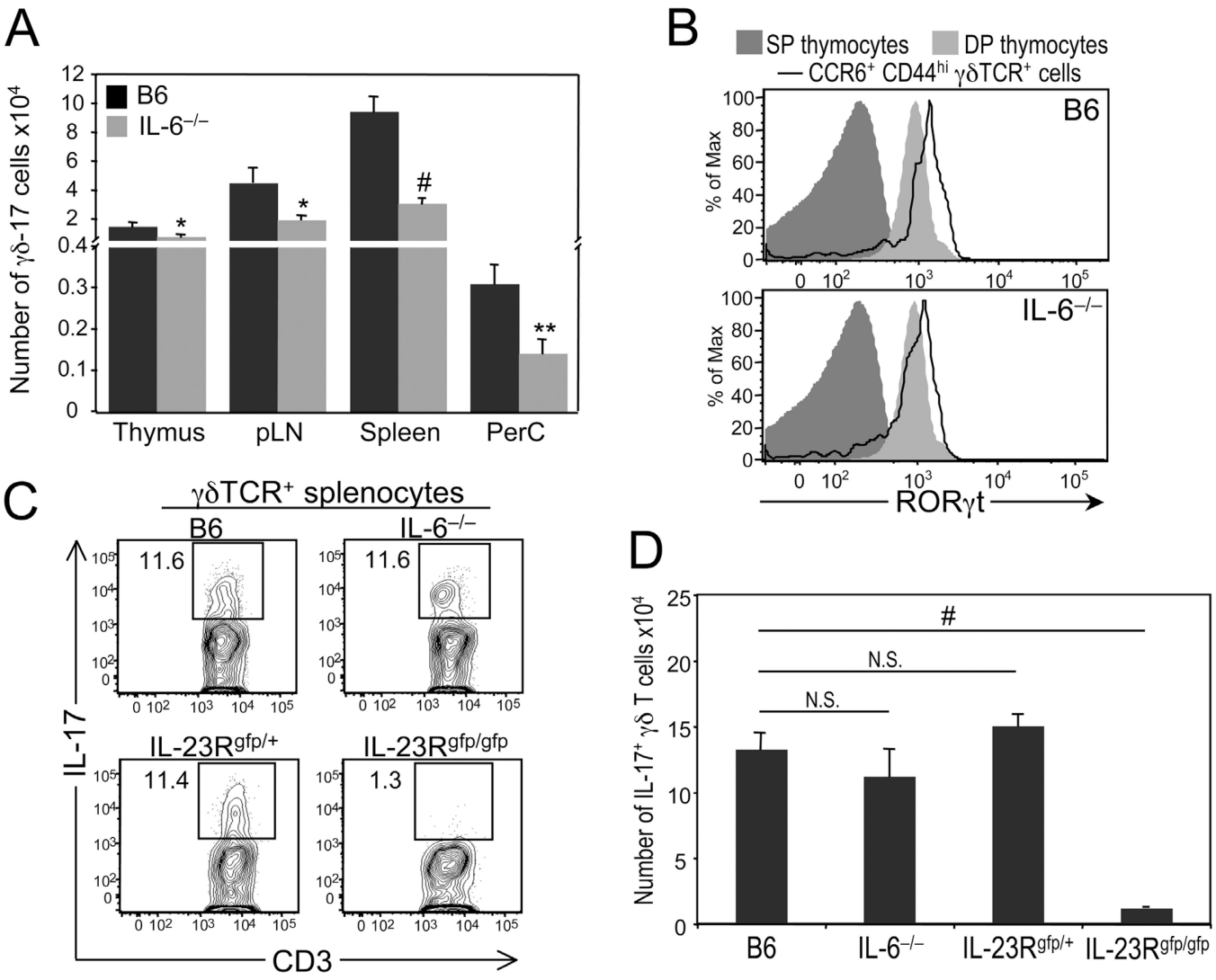

FIGURE 1.

Phenotypic and functional analysis of γδ-17 cells in interleukin (IL)-6–deficient mice. (A) Comparison of the numbers of γδ-17 cells, defined as $CCR6^+$ and $CD44^{hi}$, in the thymus, peripheral lymph nodes (pLN), spleen, and peritoneal cavity (PerC) of 5- to 6-week-old C57BL/6 (B6) and $IL\text{-}6^{-/-}$ mice. *P ≤ 0.05; **P ≤ 0.01; #P ≤ 0.001. (B) Histograms showing representative staining of RORγt in wild-type and IL-6–deficient γδ-17 cells from pLN. RORγt expression levels on $CD4^+$ and $CD8^+$ single-positive (SP) thymocytes are shown as a negative control, whereas those on $CD4^+CD8^+$ double-positive (DP) thymocytes are shown as a positive control. (C) Six-week-old B6, $IL\text{-}6^{-/-}$, $IL\text{-}23R^{gfp/+}$ ($IL\text{-}23R^{+/-}$), and $IL\text{-}23R^{gfp/gfp}$ ($IL\text{-}23R^{-/-}$) were infected intraperitoneally with a 0.3 LD_{50} dose of wild-type Listeria (3×10^5 pfu). Five days after infection, splenocytes were harvested and restimulated *in vitro* with IL-1, IL-23, and Pam_3Cys (a TLR2/1 agonist) for 4 hours in the presence of brefeldin A. Representative dot plots show IL-17 versus CD3 staining in gated γδ T cells. Numbers represent percentage of $IL\text{-}17^+$ γδ T cells. (D) Summary of data presented in C. # P ≤ 0.001. N.S., not significant.

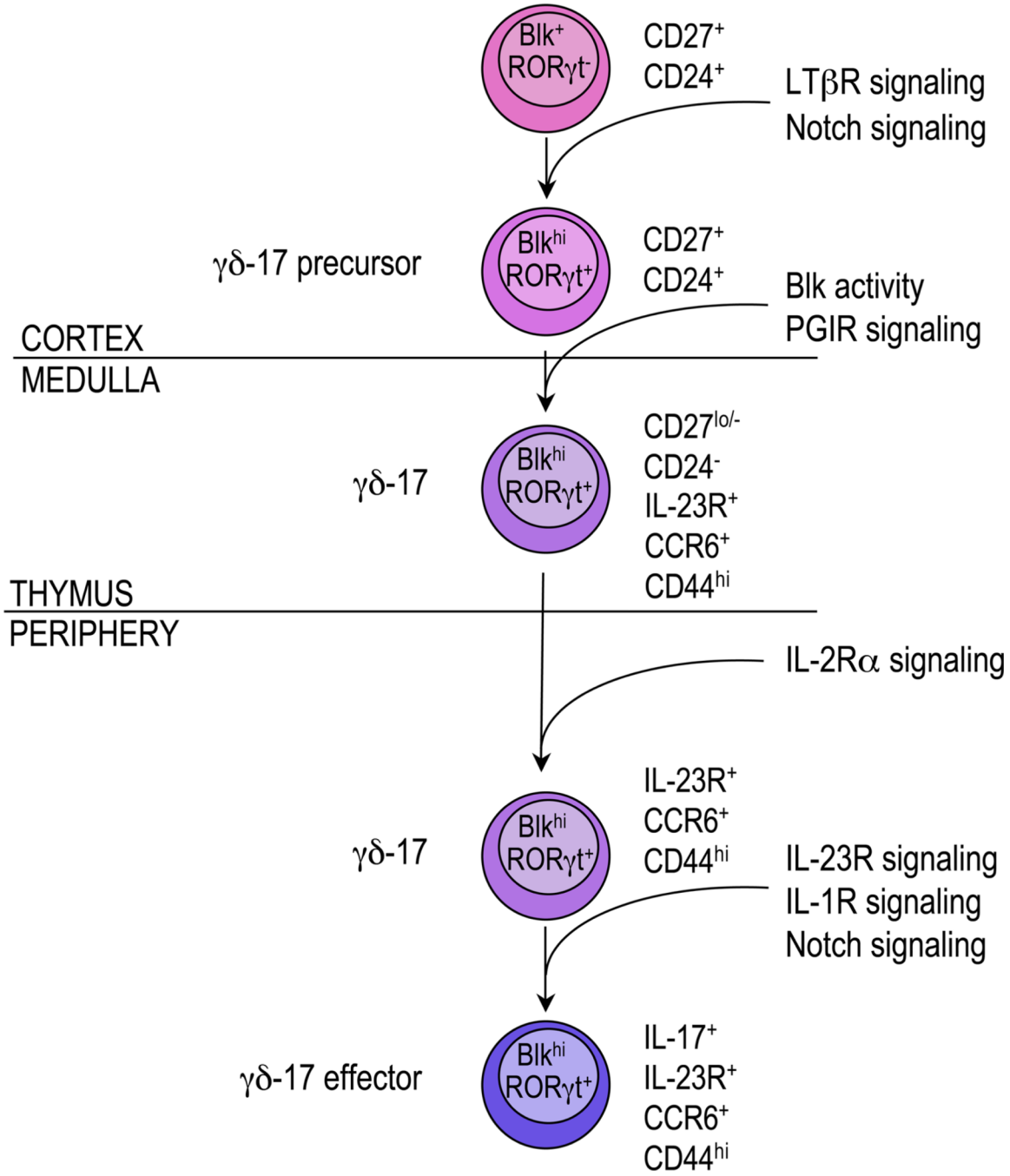

FIGURE 2.

A proposed 3-stage model for the development and differentiation of γδ-17 cells. In the first stage, functionally uncommitted γδ thymocytes commit to the γδ-17 lineage and begin to express RORγt. These RORγT$^+$ γδ thymocytes are referred to as γδ-17 precursors. In the next stage, these γδ-17 precursors mature by acquiring expression of CCR6 and interleukin (IL)-23R and then emigrate to the periphery. At the last stage, peripheral γδ-17 cells differentiate into cytokine-producing effectors. See text for more details. Blk, B lymphoid kinase; LTβR, lymphotoxin-β receptor; PGIR, Prostaglandin I2 receptor.

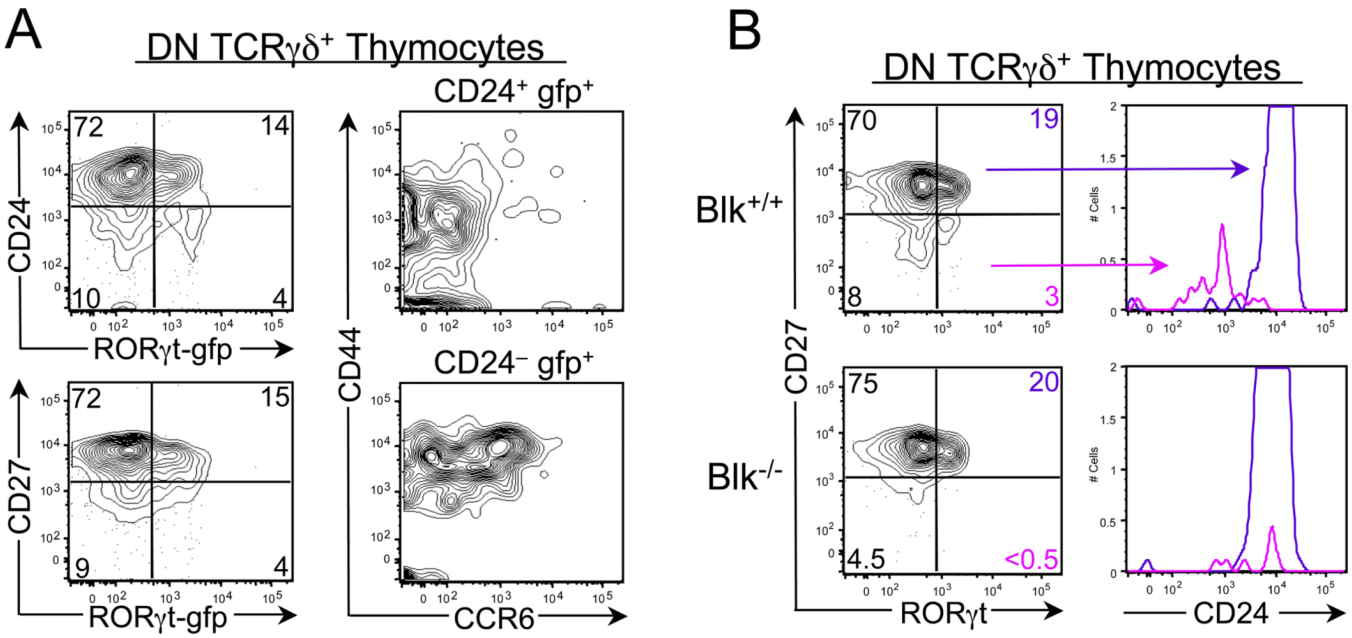

FIGURE 3.

Identification of γδ-17 precursors in the thymus. (A) dot plots show representative CD24 (top left) and CD27 (bottom left) staining versus GFP (representing RORgt expression) on gated DN TCR $\gamma\delta^+$ thymocytes from 3.5-week-old $ROR\gamma t^{gfp/+}$ mice. Numbers represent percentages of cells in each quadrant. Dot plots show representative CD44 versus CCR6 staining on gated $CD24^+gfp^+$ DN $TCR\gamma\delta^+$ thymocytes (top right) and on gated $CD24^{lo}gfp^+$ DN $TCR\gamma\delta^+$ thymocytes (bottom right). (B) Dot plots show representative CD27 versus RORγt staining on gated DN $TCR\gamma\delta^+$ thymocytes from 5-week-old $Blk^{+/+}$ and $Blk^{-/-}$ mice. Numbers represent percentages of cells in each quadrant. Adjacent histograms show CD24 staining on gated $CD27^+ROR\ \gamma t^+$ subset (purple histogram) and on gated CD27-$ROR\gamma t^+$ subset (magenta histogram). Blk, B lymphoid kinase; DN, double negative; GFP, green fluorescent protein; TCR, T cell receptor.

TABLE 1

Phenotype of γδ-17 cells

Markers	References
Surface antigens	
CCR6	Haas et al.;[49] Martin et al.[50]
CD44	Shibata et al.;[51] Haas et al.;[49] Do et al.;[52] Martin et al.[50]
CD25	Shibata et al.;[51] Do et al.[52]
IL-23R	Awasthi et al.;[53] Sutton et al.[13]
SCART2	Kisielow et al.[55]
TLR1/2	Martin et al.[50]
Dectin	Martin et al.[50]
Signaling molecules	
Blk	Laird et al.[56]
Transcription factors	
Rorγt	Ivanov et al.;[27] Lochner et al.[54]

TABLE 2

Genes required for the development and differentiation of γδ–17 cells.

Gene	References
Thymus	
Tgfb1	Do et al.[52]
Smad3	Do et al.[52]
Il6	Petermann et al.[14]
Relb	Powolny-Budnicka et al.[76]
Ltbr	Powolny-Budnicka et al.[76]
Nfkb2	Powolny-Budnicka et al.[76]
Rorc	Ivanov et al.;[27] Lochner et al.;[54] Shibata et al.[74]
Hes1	Shibata et al.[74]
Ptgir	Jaffar et al.[80]
Blk	Laird et al.[56]
Periphery	
Il23	Meeks et al.[6]
Il23r	Riol-Blanco et al.;[7]
Il1r1	Sutton et al.[13]
Il2	Shibata et al.[51]
Il2ra	Shibata et al.[51]
Hes1	Shibata et al.[74]