**T**H**{17}** cells mediate pulmonary collateral priming

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Background: Our laboratory has shown that inhalational sensitization to new antigens is facilitated through an ongoing TH2-polarized inflammation of the lung, a phenomenon we call “collateral priming.”

Objective: We were interested to analyze whether a TH1-polarized pulmonary inflammation also facilitates priming toward new antigens and which cytokine or cytokines are involved.

Methods: TH1-polarized T cells were generated in vitro and transferred into congenic mice. Mice were challenged initially with cognate antigen and an unrelated antigen; consecutively, they received cognate antigen or the secondary antigen. Airway inflammation, antigen-specific IgG2a levels, and airway hyperresponsiveness were assessed to determine the inflammatory phenotype, with antibody blocking studies used to determine cytokine requirements for TH1 collateral priming.

Results: Our experiments revealed that ongoing inflammation of the lung induced by the transfer of TH1-polarized cells also facilitates priming toward new antigens, which results in lymphocytic inflammation of the lung. Interestingly, blocking studies identified IL-17A as a major contributor to this pathology. Accordingly, we could demonstrate for the first time that TH17-polarized cells alone can facilitate priming toward new antigens, inducing lymphocytic airway inflammation and strong airway hyperresponsiveness. Flow cytometric analysis revealed priming of endogenous T cells for IL-17A secretion with a distinct memory/effector phenotype compared to TH1 cells, thus presenting an exciting model to further elucidate differentiation of TH17 cells.

Conclusions: We show that airway inflammation mediated by TH17 cells facilitates sensitization to new antigens and confers increased airway responsiveness in a murine model of polysensitization, suggesting a mechanism involving IL-17A behind the increased risk for allergic sensitization in polysensitized subjects. (J Allergy Clin Immunol 2011;128: 168-77.)

**Key words:** Asthma, IL-17A, TH cell, polysensitization

A wealth of animal data supports the pivotal role of TH2-polarized T cells in the initiation and propagation of allergic airway disease. Yet some murine studies suggest a less unequivocal situation with regard to the role of TH1 polarization in allergic airway disease. Some murine data show a clear-cut effect of TH1-polarized immune responses in inhibiting TH2-polarized airway disease,2,3 and other studies show a significant exacerbation by TH1 cells.4,5

The expansion of the TH1/TH2 dichotomy to include a new distinct cell type, TH17 cells,6 has added more complexity to this question. IL-17A has been shown, in different chronic inflammatory diseases, to underlie effects formerly ascribed to TH1 cytokines.7 In the context of (allergic) airway inflammation, IL-17A has been described to promote different aspects of the disease,5,12 but might also play a protective role.13

Studies in human subjects have generally been less clear cut with regard to the TH1/TH2 polarity of allergic airway disease. Many studies have found that both TH1 and TH2 cytokine levels are increased in the blood and airways of asthmatic patients,1,14-16 with TH1 cytokine levels correlating with disease severity, and similar data have been obtained for IL-17A.17 Additionally, human studies, particularly in childhood, point to a role of TH1-polarized viral infections in the promotion of allergic airway disease.18-20 These data, some of which have been translated into murine models,21 clearly dispute the hygiene hypothesis.22 These controversies show that the immunologic effects of a concomitant airway inflammation on subsequent immune responses to unrelated antigens, such as aeroallergens, remain controversial or unknown. We therefore initially sought to address the question of how a TH1-polarized airway inflammation influences subsequent sensitizations toward neoantigens.

We have recently described a model showing that ongoing TH2-polarized airway inflammation facilitates priming toward secondary unrelated allergens.23 We therefore sought to delineate whether similar mechanisms also promote TH1-polarized airway responses. We show here that ongoing airway inflammation induced by the transfer of TH1-polarized T cells also facilitates pulmonary priming toward a secondary unrelated antigen. Surprisingly, TH1-induced collateral priming was not dependent on IFN-γ but does depend on IL-17A. Analysis of the transferred TH1 cells revealed a small IL-17A-producing population of antigen-specific CD4+ cells, demonstrating that some TH17 cells can escape the in vitro TH1-polarizing conditions. We could further demonstrate that TH17-polarized cells by themselves...
facilitate priming, revealing a novel role for this cell type in the induction of secondary airway inflammation and airway hyperresponsiveness (AHR).

METHODS

Mice

BALB/cJ (wild-type) mice, T-cell receptor–transgenic OT-II mice (C57BL/6-Tg [Tcrctvβ5.2]; C57Bl6 background), and IFN-γ receptor–deficient mice (B6.129S7-Ifnγ1−/−) were purchased from the Jackson Laboratory (Bar Harbor, Me). T-cell receptor–transgenic DO11.10 mice (C57B6J-Tg[DO11.10]10Dlo/J) backcrossed onto an αβ−/− background were bred in our facility. Six- to 10-week-old female mice were used in all experiments. All experimental methods described in this article were performed as approved by the respective institutional animal care and use committees.

Generation of polarized TH cells, adoptive transfer, and in vitro restimulation

CD4+ T and syngeneic T-depleted splenocytes were prepared as described previously.23 CD4+ T cells and antigen-presenting cells (APCs) were cultured with 5 μg/mL OVA peptide 323–339, 25 U/mL recombinant murine IL-2 (Roche, Mannheim, Germany), 5 ng/mL recombinant murine IL-12 (Strathmann Biotec GmbH, Hamburg, Germany), and anti–IL-4 (11B11) to generate TH1 cells. For generation of TH17 cells, CD4+ T cells and APCs were cultured with 5 μg/mL OVA peptide 323–339, 20 ng/mL recombinant murine IL-23 (eBi- science, San Diego, Calif), 2 ng/mL recombinant human TGF-β (Peprotech, Rocky Hill, NJ), 40 ng/mL recombinant murine IL-6 (Milenyi Biotec, Bergisch Gladbach, Germany), anti–IL-4 (11B11), and anti–IFN-γ (XMG1.2). Cells were split 1:2 on day 3 and harvested on day 7.

TH1, TH2, or TH17 cells (5 × 10^6) were injected intravenously into BALB/cJ mice. Purity before injection ranged from 92% to 98% CD4+ KJ1-26+ cells, and an additional aliquot of the cells was retained for in vitro restimulation and analysis by means of ELISA.

Collateral priming protocol

For primary challenge, 24 hours after the transfer of TH1 cells, mice were exposed to either 5 μg of ovalbumin (OVA; grade V; Sigma-Aldrich, St Louis, Mo) and 5 μg of BSA (fraction V; Invitrogen, Carlsbad, Calif) or keyhole limpet hemocyanin (KLH; Sigma-Aldrich) intranasally on days 0 and 1. Secondary challenge was performed with either 5 μg of OVA or BSA or KLH on days 18 and 19. Mice were killed on day 22 (Fig 1, A). For induction of memory, rechallenge was applied on days 74 and 75, with doses as used for secondary challenges.

Analysis of lung function

Mice were anesthetized and intubated orotracheally for lung function measurements, as described previously.24 AHR was assessed based on increases in lung resistance during provocation with increasing doses of aerosolized methacholine defined by means of a feedback-dose control system.25

Antibody treatment

For blocking studies against IFN-γ (clone XMG1.2) or IL-17A (clone 50104.11; R&D Systems, Minneapolis, Minn), mice were treated with 100 μg of anti–IFN-γ intraperitoneally on days −1, 0, 1, 2, and 3 or with 50 μg of anti–IL-17A intraperitoneally and intranasally on days 0, 1, 2, and 3 of the collateral priming protocol (Fig 1, A) 1 hour before primary antigen application.

Analysis of bronchoalveolar lavage fluid

Bronchoalveolar lavage (BAL) inflammatory cells were obtained by means of lavage of the airway lumen with PBS, prepared, stained, and differentiated microscopically, as previously described.26

Determination of serum antibody concentration

Antigen-specific (KLH, BSA, and OVA) antibodies in sera were determined by means of ELISA, as described previously.26

Lymph node and lung cell preparation and restimulation

Mediastinal lymph nodes (mLN) and lungs were harvested on days 4 or 22 of the collateral priming protocol (Fig 1, A) and pooled from each group at the time of death. Single-cell suspensions were obtained as described previously.26 Cells harvested on day 22 were stimulated immediately, whereas cells harvested on day 4 were stimulated after a predetermined 4-day resting period.

Restimulation was performed with 1 μg/mL phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) and 0.75 μg/mL ionomycin (Sigma-Aldrich) for 4 hours (Figs 3, A and B, and 5, A and B, and see Figs E5, A, and E6, A, in this article’s Online Repository at www.jacionline.org), with 5 μg of OVA peptide and freshly isolated APCs (Fig 2, A), 200 μg/mL OVA, BSA, or KLH for 48 hours (Figs 2, B, and 3, C) or with GM-CSF differentiated bone marrow–derived dendritic cells from wild-type mice and 200 μg/mL OVA, KLH, or medium for 48 hours (Fig E6, B). For intracellular cytokine staining, GolgiPlug was added to PMA and ionomycin stimulation, according to the manufacturer’s instructions (BD Biosciences, Mississauga, Ontario, Canada), for 4 hours (Figs 3, A, and 5, A).

Measurement of cytokine production

The cytokines IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-17, IFN-γ, and TNF-α were measured by using Multiplex-based bead technology (Millipore, Temecula, Calif; Fig 2, A and B) or ELISA (R&D Systems; Figs 3, B and C, and 5, B). As per manufacturer’s response, the R&D Systems ELISA used for determination of IL-17 is specific for IL-17A.

Flow cytometric analysis

All staining procedures were performed on ice. Cell surfaces were blocked with anti-FcR (24G2) antibody. The presence of OVA-transgenic T cells was determined with an anti-clonotypic mAb (KJ1-26) antibody, simultaneously identifying CD4+ cells (clone RM4-5, eBi-science), as well as CD44+ (clone IM7, eBi-science) and CD62L+ (clone MEL-14, eBi-science) cells, after fixation and permeabilization and staining for IL-4–producing (clone 11B11), IL-17A–producing (clone TC11-1810.1), and IFN-γ–producing (clone 10G2) cells (all from BD Pharmingen, San Jose, Calif). Cells were analyzed on a LSRII (Becton Dickinson) flow cytometer in association with FlowJo (Treestar, Inc, Ashland, Ore) software.

Determination of statistical significance

Statistical significance was determined by using the Mann-Whitney U test, unless otherwise stated. A P value of less than .05 was considered significant.

Abbreviations used

AHR: Airway hyperresponsiveness

APC: Antigen-presenting cell

BAL: Bronchoalveolar lavage

KLH: Keyhole limpet hemocyanin

LN: Lymph node

mLN: Mediastinal lymph node

OVA: Ovalbumin

PMA: Phorbol 12-myristate 13-acetate

References

24. AHR was assessed based on increases in lung resistance during provocation with increasing doses of aerosolized methacholine defined by means of a feedback-dose control system.

25. Antibody treatment

26. Analysis of bronchoalveolar lavage fluid

27. Determination of serum antibody concentration

28. Lymph node and lung cell preparation and restimulation

29. Measurement of cytokine production

30. Flow cytometric analysis

31. Determination of statistical significance
FIG 1. Protocol and phenotype of $T_{h1}$ collateral priming. A, Collateral priming protocol. i.n., Intranasal; i.v., intravenous. B, BAL pattern of $T_{h1}$ versus $T_{h2}$ (transfer) collateral priming, with OVA and KLH (O+K) applied during the first and OVA (O) or KLH (K) applied during the second challenge phase. C, KLH-specific serum IgG2a levels in $T_{h1}$ collateral priming (representative experiments; n = 3-5 animals per group). Experiments were performed 3 to 7 times. *P < .05.
RESULTS
Phenotype of TH1 collateral priming

To test our hypothesis that collateral priming is a phenomenon that pertains not only to TH2-polarized airway inflammation, we adapted our model for TH2 collateral priming to a TH1-polarized response (Fig 1, A). This model allowed Eisenbarth et al.\(^2\) to determine that an ongoing TH2-polarized airway inflammation facilitates sensitization toward a secondary unrelated antigen.

The collateral priming model consists of the transfer of polarized transgenic DO11.10 T cells and 2 airway challenges. A first airway challenge is performed with the cognate antigen OVA together with a secondary unrelated antigen (BSA or KLH). After the initial antigen-driven inflammation has resolved, we challenged a second time with either cognate antigen or the secondary unrelated antigen (Fig 1, A). Comparing the transfer of TH2-polarized TH cells with the transfer of TH1-polarized TH cells, we observed that a secondary challenge (“second: K”) with an unrelated antigen, KLH, introduced during the first challenge with the cognate antigen OVA (“first: O+K”) facilitates priming toward this antigen, regardless of the polarization (transfer TH1 vs TH2) of the transferred T cells (Fig 1, B). The dose of the secondary antigen alone is not sufficient to induce significant airway inflammation (“first: K, second: K”).

Obviously the composition of the BAL inflammatory influx differs when transferring TH1 versus TH2 cells. TH2 collateral priming induces a typical TH2-polarized airway inflammation, while TH1 collateral priming induces an inflammatory influx that is dominated by lymphocytes and cells of monocytic morphology (Fig 1, B). Transfer of naive transgenic T cells followed by 2 consecutive OVA challenges or transfer of TH1-polarized transgenic T cells and primary challenge with PBS followed by challenge with OVA during secondary challenge did not result in significant airway inflammation (see Fig E1, B, in this article’s Online Repository at www.jacionline.org), demonstrating that activation of the transferred, polarized T-cell population and the ensuing inflammation are necessary for collateral priming to occur.

The increase in levels of KLH-specific IgG2a antibodies in peripheral blood (Fig 1, C) confirmed the induction of a TH1-polarized, systemic KLH-specific antigen response. Finally, rechallenge 8 weeks after initial priming demonstrated that collateral priming induces sustained memory formation (see Fig E1, A).

Independence of TH1 collateral priming from IFN-γ

We carefully examined the cytokine profile of our transferred TH1 cells before transfer and the cytokine profile of mLN cells...
FIG 3. Role of IL-17A in Th1 collateral priming. A and B, Intracellular cytokine analysis (Fig 3, A) and supernatant ELISA levels (Fig 3, B) of IL-17A and IFN-γ production from in vitro polarized Th1 cells (gray, IFN-γ; black, IL-17A). C, Multiplex analysis of IL-17A production as in Fig 2, B. n.d., Not detectable (duplicates). D and E, BAL cells and KLH-specific serum IgG2a levels from mice undergoing the Th1 collateral priming protocol receiving IL-17A–neutralizing antibody (treatment). 1st, First challenge; K, KLH; O, OVA; 2nd, second challenge. n = 5 animals per group. *P < .05. ***P < .001. Experiments were performed 3 to 5 times.
upon restimulation after transfer to identify possible mediators responsible for the Th1 collateral priming phenomenon. As expected, in vitro restimulation of Th1 cells with OVA peptide and APCs elicited a typical Th1 profile: large amounts of IFN-γ; some IL-2, IL-10, and TNF-α; and negligible amounts of IL-4, IL-5, IL-6, and IL-13 compared with cells polarized toward a Th22 phenotype (Fig 2, A). The ex vivo cytokine profile of mLN cells upon in vitro restimulation after an initial intranasal challenge also revealed a typical Th1 profile (Fig 2, B).

In spite of a predominance of IFN-γ, blocking studies with an anti–IFN-γ antibody showed that Th1 collateral priming is independent from the presence of IFN-γ. Administration of anti–IFN-γ antibody during the first antigen challenge did not diminish Th1 collateral priming (Fig 2, C). Similar results were obtained in an OT-II transfer system comparing wild-type with IFN-γ receptor–deficient mice as acceptor mice in the collateral priming protocol (Fig 2, D).

IL-17A is a potent mediator of Th1 transfer–mediated collateral priming

Consecutively, we extended our search for possible mediators of Th1 collateral priming. We included IL-17A in our analysis, considering that in some studies Th17 cells have replaced Th1 cells as principal culprits in certain diseases.7,27

In vitro analyses of Th1-polarized transgenic cells by means of intracellular cytokine staining revealed that among the transgenic T-cell population, we could identify a distinct IL-17A–producing population (Fig 3, A). Although IL-17A–producing T cells represented only a small population compared with IFN-γ–producing cells, considerable amounts of IL-17A were secreted during restimulation, as determined by means of ELISA (Fig 3, B). Additionally, OVA-specific in vitro restimulation of mLN cells after the first antigen challenge revealed significant amounts of IL-17A in the culture supernatants (Fig 3, C). Thus we identified a small population of IL-17A–producing antigen-specific CD4+ cells in our Th1-polarized cultures. These results encouraged us to test whether IL-17A might be a decisive mediator for Th1 collateral priming.

Administering an anti-IL-17A antibody intraperitoneally and intranasally on days 0 to 3 during the first antigen challenge, we observed a significant reduction in Th1 collateral priming, based on a significant reduction in BAL cell counts (Fig 3, D), as well as a significant reduction in KLH-specific IgG2a levels in peripheral blood (Fig 3, E).

IL-17A–secreting cells confer collateral priming in vivo

Because our results revealed IL-17A and not IFN-γ as an important mediator in collateral priming, we sought to investigate whether the effects observed by Th1 cell transfer would be similar when transferring IL-17A–producing Th17 cells. To this end, we performed the collateral priming protocol (Fig 1, A) with in vitro Th17-polarized DO11.10 cells. Analysis of the BAL fluid after the second airway challenge showed that priming toward the unrelated antigen KLH was facilitated by Th17 cell transfer. Th17-polarized collateral priming led to an influx of monocytes and numerous lymphocytes into the airways (Fig 4, A and B), resembling the BAL differential observed in Th1 collateral priming. Systemic sensitization toward the secondary antigen was confirmed by the induction of KLH-specific IgG2a antibodies (Fig 4, C). Finally, Th17 collateral priming led to significant AHR compared to that seen in control mice (Fig 4, D, and see Fig E2 in this article’s Online Repository at www.jacionline.org).

Th17 collateral priming initiates priming of endogenous Th17 cells

Similar to observations by Eisenbarth et al,23 KJ1-26+ CD4+ T cells could not be detected in the lungs after the second challenge (see Fig E3 in this article’s Online Repository at www.jacionline.org), suggesting that priming of endogenous T cells and subsequent Th17 polarization of these cells occurred. Detailed flow cytometric analysis of the cells recruited to the lung after Th17 collateral priming revealed a substantial increase in CD4+ cells, whereas the number of CD8+ cells did not change in comparison with the control group (see Fig E4 in this article’s Online Repository at www.jacionline.org). Intracellular cytokine staining of the CD4+ cells after PMA/ionomycin restimulation revealed induction of endogenous Th17 and Th1 cells in the lung, as shown by a significant production of IL-17A, as well as IFN-γ (Fig 5, A, and see Fig E5 in this article’s Online Repository at www.jacionline.org). Restimulation of lymph node (LN) cells, however, led to significantly lower secretion of either cytokine (Fig 5, A, and see Fig E5). ELISA measurements of IL-17A versus IFN-γ secretion in the supernatants of lung and LN cells (Fig 5, B) confirmed the intracellular cytokine data in that they also revealed significant secretion of IL-17A and IFN-γ in restimulated lung cells, with secretion of these cytokines in restimulated LN cells being less than the detections limits of the ELISAs. Direct comparison of IL-17A and IFN-γ secretion by lung and LN cells that were restimulated either nonspecifically with PMA/ionomycin or specifically with antigens (OVA/KLH in the presence of bone marrow–derived dendritic cells) revealed a similar pattern and confirmed priming toward KLH (see Fig E6 in this article’s Online Repository at www.jacionline.org) because KLH-specific IL-17A, as well as IFN-γ, production could be demonstrated (see Fig E6, B, in this article’s Online Repository at www.jacionline.org).

When examining the phenotype of pulmonary CD4+ cells producing IL-17A or IFN-γ more closely, we observed another interesting difference. Similar to published data in a model of bacterial infection,28 the IL-17A–producing cells in the lung are CD44+ and CD62L+ cells, phenotypically resembling central memory T cells, whereas their IFN-γ counterparts show much higher percentages of CD44+/CD62Linter-low cells, thus resembling effector memory cells (Fig 5, C, and see Fig E5). Moreover, IL-17A–producing lung cells express CD62L in higher amounts compared with the IFN-γ–producing population, regardless of central memory or the effector memory phenotype (Fig 5, D).

DISCUSSION

Our current study extends previous findings demonstrating that an ongoing Th12-polarized pulmonary inflammation facilitates priming toward unrelated antigens.23,26,29,30 We identify IL-17A as the key cytokine to facilitate priming toward new antigens during concomitant Th1-polarized airway inflammation and demonstrate that Th17 cells by themselves can facilitate priming of endogenous T cells for IL-17A production. IL-17A seems to be
a major contributor to both T_{H1} and T_{H17} collateral priming: neutralization of IL-17A in T_{H1} (Fig 3, D) and T_{H17} (see Fig E7 in this article’s Online Repository at www.jacionline.org) collateral priming significantly reduces BAL inflammatory cell influx, suggesting that IL-17A is an important contributor to the collateral priming process under both circumstances. However, neutralization of IL-17A during cell transfer and the first challenge phase did not affect T_{H2} collateral priming (see Fig E7).

Human studies show that exacerbation and chronicity of asthma are linked to T_{H1}-polarized pulmonary inflammation, which often occurs because of viral infections, thus underlining an important role not only for T_{H2}- but also T_{H1}-polarized lymphocytes in asthmatic patients. Our results revealed that collateral priming can be induced through transfer of T_{H1}-polarized cells, possibly modeling the increased risk of allergic sensitization after viral infections seen in children. However, in spite of the importance of IFN-γ as the key T_{H1} cytokine, T_{H1} collateral priming depends on IL-17A. Since the paradigm of a T_{H1}/T_{H2} dichotomy in inflammatory disorders has been revised to include T_{H17} cells, a wealth of data has been generated linking these cells to deleterious effects that were previously thought to be T_{H1}-driven diseases. Additionally, a critical role of
FIG 5. Phenotype of endogenous T<sub>H</sub>17 cells. A and B, Intracellular cytokine staining (Fig 5, A) and ELISA supernatant analysis (Fig 5, B) for IL-17A and IFN-γ secretion from CD4<sup>+</sup> cells of lung/LN cultures from control mice (nil/O+K/O) and mice subjected to collateral priming (Th17/O+K/K). C and D, CD44 and CD62L surface expression (Fig 5, C) and CD62L mean fluorescence intensity (MFI; Fig 5, D) of CD62L<sup>high</sup> and CD62L<sup>inter-low</sup> populations of CD4<sup>+</sup> lung cells producing IFN-γ or IL-17A obtained from lungs of mice subjected to T<sub>H</sub>17 collateral priming. Experiments were performed 3 to 6 times. n.d., Not detected. Fig 5, A and C: For gating strategy, see Fig E5 in this article’s Online Repository.
**Clinical implications: Our findings bring mechanistic knowledge to the phenomena observed when boosting for vaccination and the phenomenon of polysensitization and primary sensitization in asthma, particularly in the context of concomitant viral infections.**

**REFERENCES**

FIG E1. Memory response and control groups for Th1 collateral priming. A, BAL cell numbers of mice that underwent Th1 collateral priming. Mice received Th1-polarized cells and were consecutively challenged with OVA and BSA (O+B) or BSA alone (B) during the first challenge phase and analyzed after the second challenge phase with either BSA (B) or OVA (O) or after a third challenge phase 8 weeks later. B, Comparison of BAL cell numbers from different control mice that were subjected to the collateral priming protocol. naive, Transfer of naive (not polarized) transgenic T cells; ns, not significant; PBS, mice received buffer in the first challenge phase (n = 5 animals per group). Experiments were performed 2 to 3 times. **P > .01.
FIG E2. Invasive lung function after Th17 collateral pulmonary priming. ED100 (A), ED200 (B), and dose-response curve (C) values of mice undergoing Th17 collateral pulmonary priming. 1st, first challenge with OVA and KLH (O+K); 2nd, second challenge with OVA (O) or KLH (K); transfer, Th17 cells or buffer. Fig E2, A and B: Bars represent the effective inhalational dose (ED100 and ED200) of methacholine (MCh) in micrograms calculated from the individual dose-response curves eliciting a 100% or 200% increase in lung resistance (RL) compared with baseline. Fig E2, C: Dose-response curve (methacholine vs lung resistance) of the same mice. Because of the experimental protocol (animals are not provoked further with methacholine once they reach maximal bronchoconstriction, as evidenced by no further increase of bronchoconstriction with higher methacholine dosages), the animal number decreases with increasing methacholine dosages. Fig E2, A and B: n = 8-10. Fig E8, C: n as depicted in graph. *P < .05 and **P < .01, unpaired t test for Th17/O+K/O or Th17/O+K/K versus negative control (nil/O+K/K). The experiment was performed once.
Absence of transferred transgenic T cells after collateral priming protocol. Expression of CD4 and transgenic OVA-specific T-cell receptor (KJ1-26) by IL-17A– and IFN-γ–producing lung cells after unspecific restimulation from control mice (ie, receiving no cells [nil/O+/K/K]) and mice subjected to collateral priming (Th17/O+/K/K). Analysis was performed on day 22 of the collateral priming protocol. Lung cells were pooled from each group at the time of death (n = 5 animals per group). The experiment was performed once. For gating strategy, see Fig E5.
FIG E4. Increase of CD4⁺ cell proportion in lungs after T_{h}17 collateral priming. FACS analysis of CD4⁺ (gray) and CD8⁺ (white) populations among total lung cells from animals that received T_{h}17 cells compared with control mice (nil). All groups were challenged with OVA and KLH (O+K) during the first challenge phase and either OVA (O) or BSA (B) alone during the second challenge phase. Lung cells were pooled from each group at the time of death (n = 3 animals per group). The experiment was performed once.
FIG E5. Gating strategy for intracellular cytokine staining of lung and LN cells. Lymphocytes from lungs or LNs were gated regarding their forward-scatter (FSC) and side-scatter (SSC) properties. CD4+ lymphocytes were analyzed regarding their intracellular IL-17A and IFN-γ expressions. The resulting IL-17A and IFN-γ single-positive populations were further analyzed with regard to CD62L and CD44 surface expression. Experiments were performed 3 times.
FIG E6. Comparison of cytokine release from lung and LN cells after specific and unspecific restimulation. ELISA measurements of IL-17A and IFN-γ production by LN and lung cell cultures from control mice (white, nil/O+K/K) and mice subjected to Th17 collateral priming (black, Th17/O+K/K) at day 22. Cells were restimulated at 4 hours with PMA/ionomycin (A, triplicates) or 48 hours with KLH or OVA, respectively, in the presence of bone marrow-derived dendritic cells, with medium serving as a negative control (B, duplicates). Lung and LN cells were pooled from each group at the time of death (n = 5 animals per group). The experiment was performed once.
FIG E7. Independence of T\textsubscript{n}2 collateral priming from IL-17. BAL differential pattern of collateral priming induced by T\textsubscript{n}17 versus T\textsubscript{n}2 cell transfer subjected to anti–IL-17A antibody (αIL-17A) or matching isotype control treatment during cell transfer and the first challenge phase. OVA and KLH (O\textsuperscript{1}K) were applied during the first challenge phase, and KLH (K) was applied during the second challenge phase. Control mice did not receive cells or antibody (nil; n = 4-10 animals per group). The experiment was performed once. **P > .01. ns, Not significant.
**FIG E8.** Neutrophil influx during the T<sub>h</sub>17 collateral priming protocol. Comparison of neutrophil numbers in BAL fluid from mice that underwent the T<sub>h</sub>17 collateral priming protocol 24 hours after the first (striped), 24 hours after the second (white), and 72 hours after the second (black) challenge phase (n = 3 animals per group). The experiment was performed once.