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Th17 cells mediate pulmonary collateral priming

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Abstract

Background—Our laboratory has shown earlier 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 towards new antigens and which cytokine(s) would be 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 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 an ongoing inflammation of the lung induced by the transfer of Th1-polarized cells also facilitates priming towards new antigens which results in a 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 towards 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.

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Conclusions—We show that airway inflammation mediated by Th17 cells facilitates sensitization to new antigens and confers increased airway responsiveness in a mouse model of polysensitization suggesting a mechanism involving IL-17A behind the increased risk for allergic sensitization in polysensitized individuals.

Keywords

Asthma; IL-17A; T helper cell; polysensitization

Introduction

A wealth of animal data supports the pivotal role of Th2-polarized T cells in the initiation and propagation of allergic airway disease.¹ Yet, some mouse studies suggest a less unequivocal situation with regards to the role of Th1 polarization in allergic airway disease: Some mouse data show a clear-cut effect of Th1-polarized immune responses in inhibiting Th2-polarized airway disease,^{2,3} 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, the Th17 cells⁶ 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^{8–12} but might also play a protective role.¹³

Studies in humans have generally been less clear-cut with regards to the Th1/Th2 polarity of allergic airway disease. Many studies have found that both Th1 and Th2 cytokines are elevated in the blood and airways of asthma patients^{14–16} with Th1 cytokines correlating with disease severity and similar data has been obtained for IL-17A¹⁷. 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 mouse models²¹ clearly dispute the “hygiene hypothesis”.²² These controversies show that the immunological 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 how a Th1-polarized airway inflammation influences subsequent sensitizations towards neoantigens.

We have recently described a model which shows that an ongoing Th2-polarized airway inflammation facilitates priming towards secondary, unrelated allergens.²³ We therefore sought to delineate whether similar mechanisms also promote Th1-polarized airway responses. We show here that an ongoing airway inflammation induced by the transfer of Th1-polarized T cells also facilitates pulmonary priming towards a secondary unrelated antigen. Surprisingly, Th1-induced collateral priming was not dependent on IFN- γ , but depends 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 AHR.

Methods

Mice

BALB/cJ (WT), TCR-transgenic OT-II mice (C57BL/6-Tg (Tcr α Tcr β)425Cbn/J, C57/Bl6 background) and IFN- γ receptor-deficient mice (B6.129S7-Ifn γ 1tm1Agt/, C57/Bl6

background) were purchased from The Jackson Laboratory. TCR-transgenic DO11.10 mice (C.Cg-Tg(DO11.10)10Dlo/J) backcrossed onto an $\alpha\beta^{-/-}$ background were bred in our facility. Six- to 10-wk-old female mice were used in all experiments. All experimental methods described in this manuscript were performed as approved by the respective Institutional Animal Care and Use Committee.

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

CD4⁺ T and syngeneic T-depleted splenocytes were prepared as described previously.²³ To generate Th1 cells CD4⁺ T cells and APCs were cultured with 5 μ g/ml pOVA323–339, 25 U/ml recombinant murine IL-2 (Roche), 5 ng/ml recombinant murine IL-12 (Strathmann Biotec GmbH), and anti-IL-4 (11B11). For generation of Th17 cells CD4⁺ T cells and APCs were cultured with 5 μ g/ml pOVA323–339, 20ng/ml recombinant murine IL-23 (eBioscience), 2ng/ml recombinant human TGF- β (Peprotech), 40ng/ml recombinant murine IL-6 (Milenyi Biotec), anti-IL-4 (11B11) and anti-IFN- γ (XMG1.2). Cells were split 1:2 on day 3 and harvested on day 7.

5×10^6 Th2, Th1 or Th17 cells were injected i.v. into BALB/cJ mice. Purity before injection ranged from 92% to 98% CD4⁺KJ1-26⁺ and an additional aliquot of the cells was retained for in vitro restimulation and analysis by ELISA.

Collateral priming protocol

For primary challenge, 24h after the transfer of Th cells, mice were exposed to either 5 μ g of OVA (grade V; Sigma-Aldrich) and 5 μ g of BSA (fraction V; Invitrogen) or KLH (Sigma-Aldrich) intranasally on days 0 and 1. Secondary challenge was performed with either 5 μ g of OVA or BSA or KLH on days 18 and 19. Mice were sacrificed on day 22 (Figure 1A). For induction of memory rechallenge was applied on days 74 and 75 with doses as used for secondary challenge.

Analysis of lung function

Mice were anesthetized and intubated orotracheally for lung function measurements as described previously.²⁴ AHR was assessed by increases in lung resistance under provocation with increasing doses of aerosolized methacholine (MCh) 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) mice were treated with 100 μ g anti-IFN- γ intraperitoneally on day –1, 0, 1, 2, and 3 or with 50 μ g anti-IL-17A intraperitoneally and intranasally on day 0, 1, 2, and 3 of collateral priming protocol (Figure 1A), one hour before primary antigen application.

Analysis of BAL

BAL inflammatory cells were obtained by lavage of the airway lumen with PBS, prepared, stained and differentiated microscopically as previously described²⁶.

Determination of serum Ab concentration

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

Lymph node (LN) and lung cell preparation and restimulation

Mediastinal LNs and lungs were harvested on day 4 or 22 of collateral priming protocol (Figure 1A) and pooled from each group at time of sacrifice. Single cell suspensions were obtained as described previously.²⁶ Cells harvested on day 22 were stimulated immediately while cells harvested on day 4 were stimulated after a pre-determined four day resting period. Restimulation was performed with 1 µg/ml PMA (Sigma-Aldrich) and 0.75 µg/ml Ionomycin (Sigma-Aldrich) (Figures 3B, 5B, Online Repository Figure E5A) for 4h, with 5 µg pOVA and freshly isolated APC (Figure 2A) or 200 µg/ml OVA, BSA or KLH for 48h (Figure 2B and 3C) or with GM-CSF differentiated BMDC from wt mice and 200 µg/ml OVA, KLH or Medium for 48h (Online Repository Figure E5B). For intracellular cytokine staining GolgiPlug was added to PMA and Ionomycin stimulation according to manufacturer's instructions (BD Biosciences) for 4 h (Figures 3A, 5A).

Measurement of cytokine production

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

Flow cytometry 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, eBioscience) as well as CD44⁺ (clone IM7, eBioscience) and CD62L⁺ (clone MEL-14, eBioscience) cells after fixation and permeabilization and staining for IL-4 (clone 11B11), IL-17A (clone TC11-1810.1) and IFN-γ producing cells (clone XMG1.2) (all BD Pharmingen). Cells were analyzed on a LSR II (Becton Dickinson) flow cytometer in association with FlowJo (Treestar) software.

Determination of statistical significance

Statistical significance was determined using the Mann-Whitney U test, unless otherwise stated. A $p < 0.05$ was considered to be significant. Unless indicated otherwise, five mice were used for each condition studied in an individual experiment. Each treatment condition was repeated at least three times.

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 priming²³ to a Th1-polarized response (Figure 1A). This model allowed Eisenbarth et al. to determine that an ongoing Th2-polarized airway inflammation facilitates sensitization towards a secondary unrelated antigen.²³

The collateral priming model consists in the transfer of polarized transgenic DO11.10 T cells and two 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 challenge a second time with either cognate antigen or the secondary, unrelated antigen (Figure 1A). Comparing the transfer of Th2-polarized Th cells to the transfer of Th1-polarized Th cells, we observed that a secondary challenge (2nd: "K") with an unrelated antigen, KLH, introduced during the first challenge

with the cognate antigen OVA (“1st: O+K”) facilitates priming towards this antigen, regardless of the polarization (transfer Th1 vs. Th2) of the transferred T cells (Figure 1B). The dose of the secondary antigen alone is not sufficient to induce significant airway inflammation (“1st: K, 2nd: K”).

Obviously the composition of the BAL inflammatory influx differs when transferring Th1 vs. Th2 cells. While Th2 collateral priming induces a typical Th2-polarized airway inflammation, Th1 collateral priming induces an inflammatory influx that is dominated by lymphocytes and cells of monocytic morphology (Figure 1B). Transfer of naïve transgenic T cells followed by two 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 (Figure E1B in the, Online Repository) demonstrating that activation of the transferred polarized T cell population and the ensuing inflammation are necessary for collateral priming to occur.

The elevation of KLH-specific IgG2a antibodies in peripheral blood (Figure 1C)—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 Figure E1A in the, Online Repository).

Independence of Th1 collateral priming from IFN- γ

We carefully examined the cytokine profile of our transferred Th1 cells before transfer, as well as the cytokine profile of mLN cells upon restimulation after transfer, in order to identify possible mediators responsible for the Th1 collateral priming phenomenon. As—expected in vitro restimulation of Th1 cells with OVA peptide and APC elicited a typical Th1 profile: large amounts of IFN- γ , some IL-2, -10 and TNF- α and negligible amounts of IL-4, -5, -6 and -13 compared to cells polarized towards a Th2 phenotype (Figure 2A). The ex vivo cytokine profile of mLN cells upon in vitro restimulation after an initial intranasal challenge also revealed a typical Th1 profile (Figure 2B).

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 (Figure 2C). Similar results were obtained in an OT-II transfer system comparing WT to IFN- γ receptor deficient mice as acceptor mice in the collateral priming protocol (Figure 2D).

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 (Figure 3A). Although IL-17A producing T cells represented only a small population compared to IFN- γ -producing cells, considerable amounts of IL-17A were secreted during restimulation, as determined by ELISA measurements (Figure 3B). Additionally, OVA-specific in vitro restimulation of the mLN cells after the first antigen challenge revealed significant amounts of IL-17A in the culture supernatants (Figure 3C). 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 an important mediator for Th1 collateral priming.

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

IL-17A secreting cells confer collateral priming in vivo

Since 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 (Figure 1A) with in vitro Th17 polarized DO11.10 cells. Analysis of the BAL after the second airway challenge showed that priming towards 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 (Figure 4A and 4B), resembling the BAL differential observed in Th1 collateral priming. Systemic sensitization towards the secondary antigen was confirmed by the induction of KLH-specific IgG2a antibodies (Figure 4C). Finally, Th17 collateral priming led to significant AHR compared to control mice (Figure 4D, Figure E8, in the Online Repository).

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 Figure E2, in the Online Repository), 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 to the control group (see Figure E3 in the Online Repository). Intracellular cytokine staining of the CD4+ cells upon 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 (Figure 5A). Restimulation of LN cells, however led to significantly lower secretion of either cytokine (Figure 5A). ELISA measurements of IL-17A vs. IFN- γ secretion in supernatants of lung and of LN cells (Figure 5B) 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 below the detections limits of the ELISAs. Direct comparison of IL-17A and IFN- γ secretion by lung and LN cells that were restimulated either unspecifically with PMA/Ionomycin or specifically with antigens (OVA/KLH in the presence of BMDC) revealed a similar pattern and confirmed priming towards KLH (see Figure E5, in the Online Repository), since KLH-specific IL-17A as well as IFN- γ production could be demonstrated (see Figure E5B, in the Online Repository). 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 infection²⁸, the IL-17A producing cells in the lung are CD44+ and CD62L+, phenotypically resembling central memory T cells while their IFN- γ + counterparts show much higher percentages of CD44+/CD62L- cells thus resembling effector memory cells (Figure 5C). Moreover, IL-17A producing lung cells express CD62L in higher amounts compared to the IFN- γ producing populations, regardless of central memory or the effector memory phenotype (Figure 5D).

Discussion

Our current study extends previous findings demonstrating that an ongoing Th2 polarized pulmonary inflammation facilitates priming towards unrelated antigens.^{23,26,29,30} We identify IL-17A as the key cytokine to facilitate priming towards new antigens during a

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 major contributor to both Th1 and Th17 collateral priming: Neutralization of IL-17A in Th1 (Figure 3D) and Th17 (see Figure E7, in the Online Repository) 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 first challenge phase did not affect Th2 collateral priming (see Figure E7, in the Online Repository).

Human studies show that exacerbation and chronicity of asthma are linked to Th1 polarized pulmonary inflammation often occurring due to viral infections,^{18–20,31} thus underlining an important role not only for Th2 but also Th1 polarized lymphocytes in asthma. Our results revealed that collateral priming can be induced through transfer of Th1 polarized cells, possibly modelling the increased risk of allergic sensitization after viral infections seen in children.^{18–20} However, in spite of the importance of IFN- γ as the key Th1 cytokine, Th1 collateral priming depends on IL-17A. Since the paradigm of a Th1/Th2 dichotomy in inflammatory disorders has been revised to include Th17 cells, a wealth of data has been generated linking these cells to deleterious effects that were previously thought to be Th1-driven diseases.^{7,27,32,33} Additionally, a critical role of Th17 cells in host defense, including viral infections, has been demonstrated.^{34,35} In allergic airway disease IL-17A levels correlate with disease severity and the influx of neutrophils.^{36–38} Rodent models of allergic airway inflammation revealed a role for IL-17A in the recruitment of neutrophils as well as eosinophils^{10,39} and complement factor C3a as important regulator of IL-23/Th17-axis in severe asthma.⁴⁰ Our studies confirm the crucial role of Th17 in pulmonary priming for airway inflammation and reactivity and thus underline the need to address these cells in more detail when searching for new interventional strategies in allergic airway disease.

The development of an IL-17A producing subpopulation of CD4⁺ under Th1 polarizing conditions was surprising since IFN- γ is described to counterbalance Th17 polarization.⁴¹ However, in light of *in vivo* circumstances where developing Th17 cells might encounter IFN- γ and other cytokines favoring or opposing Th17 cell development, the presence of a minor IL-17A producing population that is not susceptible to IFN- γ suppression is not inconceivable. In fact, our own *in vivo* data on cytokine secretion by lung and LN cells after the secondary challenge in Th17 collateral priming (Figure 5A and 5B) showed secretion of IL-17A and large amounts of IFN- γ by distinct populations of lung cells, suggesting that Th17 cells can escape the suppressive effects of IFN- γ during their development. Indeed, studies have suggested that under certain circumstances, the presence of IL-17A might even be important for recruitment of Th1 cells in bacterial infections.⁴²

Since IL-17A – in contrast to IL-4, which was identified as crucial cytokine for Th2 collateral priming – is not directly acting as a T-cell differentiation factor, the mechanism behind IL-17A-mediated collateral priming might be more indirect. Various cell types including bronchial epithelial cells and fibroblasts are described to secrete chemokines and cytokines (e.g. Th17 differentiation factor IL-6) and upregulate leukocyte adhesion molecules like ICAM-1 in response to IL-17A stimulation.⁴³ Together with the presence of other inflammatory cells during the first challenge phase, for instance macrophages⁴⁴ which under inflammatory conditions have been described to be sufficient for Th17 polarization of naïve T-cells, this might provide a milieu sufficient for Th17 differentiation.

Contrary to some studies which point towards a pivotal role of IL-17A in neutrophilia,^{10,34,45} we found a lymphocytic influx into the BAL on day 22 after Th17 collateral priming with few neutrophils. This discrepancy might depend on different protocols for the induction of airway inflammation, in particular with regards to the amount of antigen used.

Higher amounts of antigen invariably increase the amount antigen-contaminating LPS which dose-dependently induces neutrophilia.⁴⁶ Additionally, analysis of BAL cells at different time points after challenges revealed that neutrophils appear in the lung 24h after the first challenge phase (d4) as well as 24h after the second challenge phase (d20), but decrease in number with time as is seen at 72h after the second challenge (d22), suggesting that early neutrophilia might be missed in our protocol (see Figure E4, in the Online Repository). Recent clinical data also suggest a role for IL-17A (and IL-17F) in COPD and asthma, but shows no correlation with neutrophilic airway inflammation.⁴⁷

Several studies have shown a seminal role for the IFN- γ /IL-12 axis in the induction of AHR,⁴⁸⁻⁴⁹ whereas other studies demonstrate contribution of the Th17/IL-23 axis and neutrophil recruitment in conferring AHR.³⁹⁻⁴⁵ However, a recent study describes conversion of Th17 into IFN- γ -producers in vivo as a prerequisite for AHR,⁵⁰ which constitutes a combination of both axes and is conceivable in the light of potential Th17 cell plasticity.⁵¹

Additional Th plasticity has been observed in allergic airway disease where a subset of IL-17A secreting Th2 cells has been detected at increased levels in asthmatic patients and pro-inflammatory cytokine stimulation was shown to induce IL-17A secretion from classical Th2 cells.⁵² Our studies addressed the role of IL-17A vs. IFN- γ during the collateral priming process where we did observe a role for IL-17A but not for IFN- γ (Figure 3D and 3E). However,, at the present time we can neither distinguish between a singular contribution of IL-17A vs. IFN- γ towards the induction of AHR nor exclude a contribution of IL-17A-producing Th2 cells to collateral priming.

Our findings concerning the role of IL-17A in pulmonary priming might be particularly critical with regards to steroid resistant asthma, which has been described to be mediated by Th17 cells.¹² Extrapolating our data, patients suffering from this form of asthma would be at a particular risk for developing new sensitizations.

Evolutionarily, collateral priming might have evolved to ensure that during an ongoing immune response bystander cells with different antigen-specificity could be more easily recruited to become polarized effector cells. At least partially, we draw upon this effect when boosting for vaccination to enhance a response against a given pathogen. However, in the context of allergic responses this beneficial effect can have deleterious consequences as any lung inflammation, regardless of its origin (allergic, viral, environmental) or polarization will increase the risk of de-novo sensitization towards unrelated, harmless antigens. Ultimately this process leads to polysensitization, a subtype of allergic disease with a much worse clinical course which is immensely more difficult to treat⁵³⁻⁵⁵ and thus needs to become a focus of future research directions.

Clinical Implication

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.

Capsule summary

We show that airway inflammation mediated by Th17 cells facilitates sensitization to new antigens and confers increased airway inflammation and airway responsiveness in a mouse model of polysensitization.

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Abbreviations used

AHR	airway hyperresponsiveness
APC	antigen presenting cells
BAL	bronchoalveolar lavage
BSA	bovine serum albumin
IFN	interferon IL; interleukin
i.n.	intranasal
i.v.	intravenous
KLH	keyhole limpet hemocyanin
KO	knock out
LN	lymph nodes
MCh	methacholine
mLN	mediastinal LN
OVA	ovalbumin
RL	lung resistance
Th	T helper
TLR	toll like receptor
TLR4d	TLR4 defective
Wt	wildtype

References

1. Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. *J Clin Invest.* 2008; 118:3546–3556. [PubMed: 18982161]
2. Erb KJ, Holloway JW, Sobeck A, Moll H, Le GG. Infection of mice with *Mycobacterium bovis*-*Bacillus Calmette-Guerin* (BCG) suppresses allergen-induced airway eosinophilia. *J Exp Med.* 1998; 187:561–569. [PubMed: 9463406]
3. Wohlleben G, Muller J, Tatsch U, Hambrecht C, Herz U, Renz H, et al. Influenza A virus infection inhibits the efficient recruitment of Th2 cells into the airways and the development of airway eosinophilia. *J Immunol.* 2003; 170:4601–4611. [PubMed: 12707338]
4. Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. *J Clin Invest.* 1999; 103:175–183. [PubMed: 9916129]
5. Randolph DA, Stephens R, Carruthers CJ, Chaplin DD. Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J Clin Invest.* 1999; 104:1021–1029. [PubMed: 10525040]
6. Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol.* 2007; 25:821–852. [PubMed: 17201677]

7. Steinman L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat Med.* 2007; 13:139–145. [PubMed: 17290272]
8. Laan M, Cui ZH, Hoshino H, Lotvall J, Sjostrand M, Gruenert DC, et al. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J Immunol.* 1999; 162:2347–2352. [PubMed: 9973514]
9. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, et al. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity.* 2002; 17:375–387. [PubMed: 12354389]
10. Hellings PW, Kasran A, Liu Z, Vandekerckhove P, Wuyts A, Overbergh L, et al. Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. *Am J Respir Cell Mol Biol.* 2003; 28:42–50. [PubMed: 12495931]
11. Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, Wu R. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem.* 2003; 278:17036–17043. [PubMed: 12624114]
12. McKinley L, Alcorn JF, Peterson A, Dupont RB, Kapadia S, Logar A, et al. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol.* 2008; 181:4089–4097. [PubMed: 18768865]
13. Schnyder-Candrian S, Togbe D, Couillin I, Mercier I, Brombacher F, Quesniaux V, et al. Interleukin-17 is a negative regulator of established allergic asthma. *J Exp Med.* 2006; 203:2715–2725. [PubMed: 17101734]
14. Cho SH, Stanciu LA, Holgate ST, Johnston SL. Increased interleukin-4, interleukin-5, and interferon-gamma in airway CD4+ and CD8+ T cells in atopic asthma. *Am J Respir Crit Care Med.* 2005; 171:224–230. [PubMed: 15502111]
15. Krug N, Madden J, Redington AE, Lackie P, Djukanovic R, Schauer U, et al. T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am J Respir Cell Mol Biol.* 1996; 14:319–326. [PubMed: 8600935]
16. ten Hacken NH, Oosterhoff Y, Kauffman HF, Guevarra L, Satoh T, Tollerud DJ, et al. Elevated serum interferon-gamma in atopic asthma correlates with increased airways responsiveness and circadian peak expiratory flow variation. *Eur Respir J.* 1998; 11:312–316. [PubMed: 9551730]
17. Wang YH, Liu YJ. The IL-17 cytokine family and their role in allergic inflammation. *Curr Opin Immunol.* 2008; 20:697–702. [PubMed: 18832032]
18. Wright AL, Taussig LM, Ray CG, Harrison HR, Holberg CJ. The Tucson Children's Respiratory Study. II. Lower respiratory tract illness in the first year of life. *Am J Epidemiol.* 1989; 129:1232–1246. [PubMed: 2729259]
19. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med.* 2000; 161:1501–1507. [PubMed: 10806145]
20. Illi S, von ME, Lau S, Bergmann R, Niggemann B, Sommerfeld C, et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ.* 2001; 322:390–395. [PubMed: 11179155]
21. Dahl ME, Dabbagh K, Liggitt D, Kim S, Lewis DB. Viral-induced T helper type 1 responses enhance allergic disease by effects on lung dendritic cells. *Nat Immunol.* 2004; 5:337–343. [PubMed: 14973436]
22. Strachan DP. Hay fever, hygiene, and household size. *BMJ.* 1989; 299:1259–1260. [PubMed: 2513902]
23. Eisenbarth SC, Zhadkevich A, Ranney P, Herrick CA, Bottomly K. IL-4-dependent Th2 collateral priming to inhaled antigens independent of Toll-like receptor 4 and myeloid differentiation factor 88. *J Immunol.* 2004; 172:4527–4534. [PubMed: 15034070]
24. Glaab T, Mitzner W, Braun A, Ernst H, Korolewicz R, Hohlfeld JM, et al. Repetitive measurements of pulmonary mechanics to inhaled cholinergic challenge in spontaneously breathing mice. *J Appl Physiol.* 2004; 97:1104–1111. [PubMed: 15121749]
25. Hoymann HG. New developments in lung function measurements in rodents. *Exp Toxicol Pathol.* 2006; 57(Suppl 2):5–11. [PubMed: 16618537]

26. Dittrich AM, Chen HC, Xu L, Ranney P, Connolly S, Yarovsky TO, et al. A new mechanism for inhalational priming: IL-4 bypasses innate immune signals. *J Immunol.* 2008; 181:7307–7315. [PubMed: 18981153]
27. Furuzawa-Carballeda J, Vargas-Rojas MI, Cabral AR. Autoimmune inflammation from the Th17 perspective. *Autoimmun Rev.* 2007; 6:169–175. [PubMed: 17289553]
28. Pepper M, Linehan JL, Pagan AJ, Zell T, Dileepan T, Cleary PP, et al. Different routes of bacterial infection induce long-lived TH1 memory cells and short-lived TH17 cells. *Nat Immunol.* 2010; 11:83–89. [PubMed: 19935657]
29. Kheradmand F, Kiss A, Xu J, Lee SH, Kolattukudy PE, Corry DB. A protease-activated pathway underlying Th cell type 2 activation and allergic lung disease. *J Immunol.* 2002; 169:5904–5911. [PubMed: 12421974]
30. Hurst SD, Seymour BW, Muchamuel T, Kurup VP, Coffman RL. Modulation of inhaled antigen-induced IgE tolerance by ongoing Th2 responses in the lung. *J Immunol.* 2001; 166:4922–4930. [PubMed: 11290770]
31. Mallia P, Johnston SL. How viral infections cause exacerbation of airway diseases. *Chest.* 2006; 130:1203–1210. [PubMed: 17035457]
32. Nembrini C, Marsland BJ, Kopf M. IL-17-producing T cells in lung immunity and inflammation. *J Allergy Clin Immunol.* 2009; 123:986–994. [PubMed: 19410688]
33. Tesmer LA, Lundy SK, Sarkar S, Fox DA. Th17 cells in human disease. *Immunol Rev.* 2008; 223:87–113. [PubMed: 18613831]
34. Dubin PJ, Kolls JK. Th17 cytokines and mucosal immunity. *Immunol Rev.* 2008; 226:160–171. [PubMed: 19161423]
35. Khader SA, Gaffen SL, Kolls JK. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. *Mucosal Immunol.* 2009; 2:403–411. [PubMed: 19587639]
36. Zhao Y, Yang J, Gao YD, Guo W. Th17 Immunity in Patients with Allergic Asthma. *Int Arch Allergy Immunol.* 2009; 151:297–307. [PubMed: 19844129]
37. Bullens DM, Truyen E, Coteur L, Dilissen E, Hellings PW, Dupont LJ, et al. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir Res.* 2006; 7:135. [PubMed: 17083726]
38. Barczyk A, Pierzchala W, Sozanska E. Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir Med.* 2003; 97:726–733. [PubMed: 12814161]
39. Wakashin H, Hirose K, Maezawa Y, Kagami S, Suto A, Watanabe N, et al. IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med.* 2008; 178:1023–1032. [PubMed: 18787221]
40. Lajoie S, Lewkowich IP, Suzuki Y, Clark JR, Sproles AA, Dienger K, et al. Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma. *Nat Immunol.* 2010; 11:928–935. [PubMed: 20802484]
41. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol.* 2005; 6:1123–1132. [PubMed: 16200070]
42. Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, Cilley GE, et al. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat Immunol.* 2007; 8:369–377. [PubMed: 17351619]
43. Oboki K, Ohno T, Saito H, Nakae S. Th17 and allergy. *Allergol Int.* 2008; 57:121–134. [PubMed: 18427165]
44. Egan PJ, van NA, Campbell IK, Wicks IP. Promotion of the local differentiation of murine Th17 cells by synovial macrophages during acute inflammatory arthritis. *Arthritis Rheum.* 2008; 58:3720–3729. [PubMed: 19035489]
45. Wilson RH, Whitehead GS, Nakano H, Free ME, Kolls JK, Cook DN. Allergic Sensitization Through the Airway Primes Th17-dependent Neutrophilia and Airway Hyperresponsiveness. *Am J Respir Crit Care Med.* 2009; 180:720–730. [PubMed: 19661246]

46. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med*. 2002; 196:1645–1651. [PubMed: 12486107]
47. Doe C, Bafadhel M, Siddiqui S, Desai D, Mistry V, Rugman P, et al. Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. *Chest*. 2010; 138:1140–1147. [PubMed: 20538817]
48. Kim YS, Choi SJ, Choi JP, Jeon SG, Oh SY, Lee BJ, et al. IL-12-STAT4-IFN-gamma axis is a key downstream pathway in the development of IL-13-mediated asthma phenotypes in a Th2 type asthma model. *Exp Mol Med*. 2010
49. Hessel EM, Van Oosterhout AJ, Van AI, Van EB, Hofman G, Van LH, et al. Development of airway hyperresponsiveness is dependent on interferon-gamma and independent of eosinophil infiltration. *Am J Respir Cell Mol Biol*. 1997; 16:325–334. [PubMed: 9070618]
50. Ashino S, Wakita D, Shiohama Y, Iwakura Y, Chamoto K, Ohkuri T, et al. A T(h)17-polarized cell population that has infiltrated the lung requires cells that convert to IFN- γ production in order to induce airway hyperresponsiveness. *Int Immunol*. 2010; 22:503–513. [PubMed: 20501609]
51. Lee YK, Turner H, Maynard CL, Oliver JR, Chen D, Elson CO, et al. Late developmental plasticity in the T helper 17 lineage. *Immunity*. 2009; 30:92–107. [PubMed: 19119024]
52. Wang YH, Voo KS, Liu B, Chen CY, Uygungil B, Spoede W, et al. A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. *J Exp Med*. 2010; 207:2479–2491. [PubMed: 20921287]
53. Akerman M, Valentine-Maher S, Rao M, Tanningco G, Khan R, Tuysugoglu G, et al. Allergen sensitivity and asthma severity at an inner city asthma center. *J Asthma*. 2003; 40:55–62. [PubMed: 12699212]
54. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TA. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. *J Allergy Clin Immunol*. 1989; 83:875–882. [PubMed: 2715548]
55. Kjellman B, Gustafsson PM. Asthma severity, allergy and lung function during young middle life in subjects with asthma in childhood. *Respir Med*. 2002; 96:716–724. [PubMed: 12243318]

Figure 1. Protocol and phenotype of Th1 collateral priming
A, Collateral priming protocol. B, BAL pattern of Th1 vs. Th2 (transfer) collateral priming, with OVA and KLH (O+K) during the first (1st) and OVA or KLH (O, K) applied during the second (2nd) challenge phase. C, KLH specific serum IgG2a in Th1 collateral priming (Representative experiments; n=3–5 animals/group; experiments performed 3–7 times, * p<0.05).

Figure 2. Cytokines profiles of Th1 collateral priming and independence from IFN- γ

A, Cytokine profiles of *in vitro* polarized Th1 (white) and Th2 (black) cells (duplicates, experiment performed 17 times) and B, LN cultures obtained at day 4 of Th1 collateral priming (black: IFN- γ , grey: IL-5, white: IL-13; mean values of duplicates, experiment performed 5 times). C, BAL cells from mice undergoing Th1 collateral priming protocol receiving IFN- γ -neutralizing antibody or isotype (treatment) or D, deficient for IFN- γ receptor vs. wt mice (acceptor). 1st: first challenge; 2nd: second challenge; O: OVA, B: BSA; experiments performed 3–4 times; ns: not significant

Figure 3. Role of IL-17A in Th1 collateral priming
A, Intracellular cytokine analysis and B, supernatant ELISA levels of IL-17A and IFN- γ production from in vitro polarized Th1 cells (IFN- γ (grey), IL-17A (black duplicates). C, Multiplex analysis of IL-17A production as in Figure 2B (n.d.: not detectable; duplicates). D,E BAL cells and KLH-specific serum IgG2a from mice undergoing Th1 collateral priming protocol receiving IL-17A-neutralizing antibody (treatment). 1st: first challenge; 2nd: second challenge; O: OVA, K: KLH; n=5 animals/group *p<0.05; ***p<0.001; A–E: Experiments performed 3–5 times.

Figure 4. Th17 cells mediated collateral priming
A, BAL differential, B, lung H&E staining, C, KLH-specific serum IgG2a and D, invasive lung function measurements of mice subjected to Th17-collateral priming; control mice received either no cells or only KLH (K) in the first (1st) and second (2nd) challenge phase. B, magnification 100×, D, bars represent the effective inhalational dose (ED₁₅₀) of MCh in µg eliciting a 150% increase in lung resistance (RL) compared to baseline. (D: n=9–10 *p<0.05 (unpaired student's t-test); A, C: n=3–10 *p<0.05 **p<0.01 ***p<0.001; experiments performed 8 times)

Figure 5. Phenotype of endogenous Th17 cells

A, Intracellular cytokine staining and B, ELISA supernatant analysis for IL-17A and IFN- γ secretion from CD4⁺ cells of lung/LN cultures from control (nil/O+K/O) and mice subjected to collateral priming (Th17/O+K/K). C, CD44 and CD62L surface expression-, and D, CD62L mean fluorescence intensity (MFI) of CD62L^{high} and CD62L^{inter-low} populations of CD4⁺ lung cells producing IFN γ or IL-17A, obtained from lungs of mice subjected to Th17 collateral priming. Experiments performed 3–6 times; n.d.: not detected; A,C: for gating strategy see Fig. E6 in Online Repository.