IL-6 activated integrated BATF/IRF4 functions in lymphocytes are T-bet-independent and reversed by subcutaneous immunotherapy

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IL-6 plays a central role in supporting pathological T₃₂₂ and T₇₇₇ cell development and inhibiting the protective T regulatory cells in allergic asthma. T₇₇₇ cells have been demonstrated to regulate allergic asthma in general and T-bet-deficiency-induced asthma in particular. Here we found an inverse correlation between T-bet and IL-6 mRNA expression in asthmatic children. Moreover, experimental subcutaneous immunotherapy (SIT) in T-bet(−/−) mice inhibited IL-6, IL-21R and lung T₇₇₇ cells in a setting of asthma. Finally, local delivery of an anti-IL-6R antibody in T-bet(−/−) mice resulted in the resolution of this allergic trait. Noteworthy, BATF, crucial for the immunoglobulin-class-switch and TH2,TH17 development, was found down-regulated in the lungs of T-bet(−/−) mice after SIT and after treatment with anti-IL-6R antibody, indicating a critical role of IL-6 in controlling BATF/IRF4 integrated functions in T₃₂₂, T₇₇₇ cells and B cells also in a T-bet independent fashion in allergic asthma.
**Results**

Here, we found an inverse correlation between *Il-6* and *T-bet* mRNA expression in the peripheral blood mononuclear cells (PBMC) of small children with asthma (Figure 1a and Supplementary Table 1). *T-bet* has been previously reported to be down-regulated in CD4+ T cells in asthmatic children24 and IL-6 was found to be up-regulated in asthmatic patients25–27.

In this study, in a murine model of asthma (Figure 1b), we found a spontaneous significant up-regulation of IL-6 in lung tissue as well as in lung CD4+ T cells from asthmatic *T-bet(−/−)* mice as compared to those isolated from wild type littermates (Figure 1c and d, respectively).

IL-6 up-regulates BATF, a transcription factor involved in both TH17 development and immunoglobulin class switch18,20. We thus next looked at the serum level of IgE of wild-type and *T-bet(−/−)* mice, which is regulated by BATF-JUN-IRF4 and c-maf21–23,34,35, a possible mechanism by which TH17 cells auto-regulate themselves34. When we analyzed *IL-21* and found that this cytokine was up-regulated in *T-bet(−/−)* asthmatic mice after intracellular flow cytometric analysis, we found a significant increase of IgE in the serum of asthmatic *T-bet(−/−)* mice as compared to the wild-type, but no further up-regulation in either wild-type or *T-bet(−/−)* CD4+ T cells after IL-6 stimulation (Figure 2e), indicating that RORγt is controlled by other factors and by *T-bet*29,30.

*T117* cells can be induced by IL-6 in conjunction with *IL-21*28,30 and BATF is known to play an important role in this context21,22,34–36. We then analyzed *IL-21* and found that this cytokine was up-regulated in asthma in wild-type and *T-bet(−/−)* mice (Figure 3a). Similarly, the IL-21R was found up-regulated in the lungs of *T-bet(−/−)* mice both spontaneously and in a setting of asthma (Figure 3b). Interferon regulatory factor 4 is another transcription factor involved in *T117* cell differentiation31,32. Moreover, IL-21 has been shown to induce IRF4 and BATF integrated functions in TH17 cells leading to IL-10 production in these cells19,32. IRF4 has been recently found elevated in sensitized and challenged mice (Figure 3c). We then looked for the TH17 cell signature transcription factor, RAR-related orphan receptor (ROR)γt27,28. RORγt was found to be up-regulated but only in the lungs of naive *T-bet(−/−)* mice and in the lungs of asthmatic wild type mice (Figure 2c).

Because BATF was found to be induced in conjunction with IL-6 in *T-bet(−/−)* mice, we isolated lung CD4+ T cells from naive wild-type and *T-bet(−/−)* mice and stimulated them with IL-6. We found that BATF was induced by IL-6 in *T-bet(−/−)* CD4+ T cells but not in wild-type CD4+ T cells (Figure 2d). However, we observed a significant increase of RORγt in the *T-bet(−/−)* CD4+ T cells compared to the wild-type, but no further up-regulation in either wild-type or *T-bet(−/−)* CD4+ T cells after IL-6 stimulation (Figure 2e), indicating that RORγt is controlled by other factors and by *T-bet*29,30. *T117* cells can be induced by IL-6 in conjunction with *IL-21*28,30 and BATF is known to play an important role in this context21,22. We then analyzed IL-21 and found that this cytokine was up-regulated in asthma in wild-type and *T-bet(−/−)* mice (Figure 3a). Similarly, the IL-21R was found up-regulated in the lungs of *T-bet(−/−)* mice both spontaneously and in a setting of asthma (Figure 3b). Interferon regulatory factor 4 is another transcription factor involved in *T117* cell differentiation31,32. Moreover, IL-21 has been shown to induce IRF4 and BATF integrated functions in TH17 cells leading to IL-10 production in these cells19,32. IRF4 has been recently found elevated in asthmatic patients35. Consistently, we found an up-regulation of *Irf4* mRNA expression in the lung of both wild-type and *T-bet(−/−)* OVA sensitized and challenged mice (Figure 3c). Consistent with our findings at the transcriptional level, we found significantly higher IL-17A protein levels in the supernatants of cultured CD4+ T cells isolated from *T-bet(−/−)* mice after asthma induction as compared to the levels measured in the CD4+ T cell supernatants from wild-type mice (Figure 3d). *T117* cells were shown to produce IL-10 which is regulated by BATF-JUN-IRF4 and c-maf21–23,34,35, a possible mechanism by which *T117* cells auto-regulate themselves34. When we analyzed IL-10 in this context, we found increased levels of this cytokine in CD4+ T cells from *T-bet(−/−)* mice compared to...
wild-type mice (Figure 3e and f). In conclusion, we found an up-regulation of $T_{H17}$ cells expressing both pro-inflammatory and anti-inflammatory cytokines in the lung of asthmatic $T-bet^{-/-}$ mice in a setting of allergic asthma. These cells probably developed via IL-6 and IL-21. $T-bet^{-/-}$ mice have increased CD4$^+$CD25$^+$ effector T cells in their lungs after allergen sensitization but in the absence of allergen challenge. Moreover, IL-2 inhibits T regulatory cell development when given intranasally in experimental asthma. Here, we found that IL-2 was up-regulated in the lungs of $T-bet^{-/-}$ mice as compared to their wild-type littermates (Figure 4a and Supplementary Figure 1). Since $T_{H17}$ cells and Treg cells are controlled reciprocally and high levels of IL-6 induce $T_{H17}$ cells while inhibiting Treg cells, we next analyzed CD4$^+$CD25$^+$Foxp3$^+$ T cells in the lungs of wild-type and $T-bet^{-/-}$ mice. We observed a reduced number of Foxp3$^+$ T cells in both wild-type and especially in T-bet$^{-/-}$ mice after sensitization and challenge with OVA (Figure 4b). This decrease represents a significant proportion of the T regulatory cells that in the lung represent only 5% of the total CD4$^+$ T cells. Moreover, T-bet$^{-/-}$ mice showed significantly lower numbers of Treg cells in the asthma model compared to wild-type mice (Figure 4b). These data indicate that $T_{H17}$ skewing and T regulatory inhibiting conditions are sustained environmental conditions in the lungs of T-bet$^{-/-}$ mice in an experimental asthma model.

A long-term cure for asthma might be the SIT. To investigate a potential role of IL-6 in SIT and in T-bet$^{-/-}$ mice, we applied the protocol of subcutaneous immunotherapy (SIT) in a second murine model of asthma in T-bet$^{-/-}$ mice as shown in Figure 5a. We found that IL-6 mRNA was also significantly induced in this model of allergic asthma in the lung of T-bet$^{-/-}$ mice as compared to non-asthmatic mice (Figure 5b). Moreover, immunotherapy led to a down-regulation of IL-6 mRNA in the lungs of treated mice (Figure 5b). To analyze the role of $T_{H17}$ cells in SIT, we next analyzed the expression of RORgamma mRNA in the lung, which we found increased in asthma and decreased after SIT (Figure 5c). IL-6 induces BATF downstream of the IL-6R pathway. Consistent with a reduction of IL-6, we found that BATF was decreased (Figure 5d) after SIT. IRF4, which acts together with BATF at the IL-10 promoter, was also found down-regulated after immunotherapy (Figure 5e) along with IL-21 and IL-21R (Figure 5f and g). Because the factors inducing $T_{H17}$ cells were down-regulated in immunotherapy in T-bet$^{-/-}$ mice, we looked for IL-17A and IL-10. Both cytokines were found decreased after immunotherapy (Figure 5h and i). Finally, IL-2, a marker of $T_{H17}$ cell differentiation was also found down-regulated (Figure 5j). Considering that we could not find an up-regulation of T regulatory cells in this model, these results indicate a possible role for IL-6 in controlling $T_{H17}$ cells producing IL-17A and IL-10 in asthma that could be inhibited by SIT in the absence of T-bet.

We have previously demonstrated that local blockade of IL-6R in the lung of wild-type mice led to amelioration of the allergic asthmatic reaction. In this study we found that T-bet$^{-/-}$ mice treated with α-IL-6R antibody, as indicated in Figure 6a, had reduced airway inflammation (Figure 6b) and decreased IgE levels in the serum (Figure 6c). IL-6 is known to play an important role in the differentiation of activated B cells into antibody-producing cells. We found here that B cells (CD19$^+$) were decreased after α-IL-6R antibody treatment (Figure 6d), that they express the IL-6R and are induced in the asthma model (Supplementary Figure 2). We also...
observed a down-regulation of CD4+ T cells (Figure 6e) as well as of the Tfh cytokines IL-4, IL-5 and IL-13 after a-IL-6R antibody treatment in T-bet(2/2) mice (Figure 6f, g, h, respectively).

BATF deficient mice have been demonstrated to lack Tfh cells which are important to prime B cell mediated immune responses17. Moreover, BATF has been recently shown to play a central role in inducing TH2 cells reminiscent of the role of IL-617–19. We thus hypothesized that IL-6 controls BATF in allergic asthma. To prove this, we analyzed BATF gene expression in total lung cell cultures isolated from OVA-sensitized T-bet(2/2) mice that were treated with the anti-IL-6R antibody in vitro (Figure 7a). We found that BATF which was induced in T-bet deficient mice (Figure 7b) was significantly reduced after in vitro stimulation with anti-IL-6R antibody treatment in lung cells of T-bet(2/2) mice. Rorc mRNA expression was not changed after anti-IL-6R treatment neither in wild-type, nor in T-bet(2/2) mice (Figure 7c). C-maf (Figure 7d) was found down-regulated only in wild-type mice after administration of the anti-IL-6R antibody. IL-6 induces IL-21 via the transcription factors BATF38 and c-maf40,41. Consistently, we found significantly reduced IL-21 in the lung cells that were in vitro treated with anti-IL-6R antibody. We then asked whether the down-regulation of BATF and IL-21 would result in decreased IL-17A expression. IRF4 has been shown to interact with BATF at the IL-17A promoter. However, we could not observe a downregulation of IRF4 after anti-IL6R antibody treatment in vivo (Figure 8a and b). Similarly, IL-17A was not specifically down-regulated by anti-IL-6R antibody treatment in vivo in T-bet deficient mice (Figure 8c).

It has been recently reported that BATF cooperates with IRF4 and STAT3 in cells stimulated with IL-21 to produce IL-1021–23. We then looked at IL-21R and IL-10 after anti-IL-6R antibody treatment. We
found that T-bet−/− asthmatic mice had decreased IL-21R mRNA and IL-10 mRNA in their lungs after anti-IL-6R antibody treatment (Figure 8d and e). It is thus possible that IL-6 controls the expression of BATF, IL-21 and IL-21R on TH2 and TH17 cells in the lung in a setting of allergic asthma in T-bet−/− mice.

**Discussion**

IL-6 is a pleiotropic cytokine that plays an important role in inflammation and cancer42. It is released by cells of the innate and adaptive immune-response. As it induces the proliferation of T cells as well as B cells its blockade has an impact on immune responses which lead to IgE hyper-production and to the expansion of effector T_{H17} and T_{H2} cells seen in the lungs of asthmatic patients. These findings show the relevant contribution of IL-6 to the pathogenesis of asthma.

The transcription factor T-bet, which is known to transactivate the Ifnγ gene, was found to be down-regulated in asthmatic patients. In this paper, we observed that the PBMC of children with asthma have lower values of T-bet mRNA that coexist with relative higher levels of IL-6 mRNA. Further epigenetic studies are underway in these children to see if T-bet inhibits IL-6 at the promoter level or if pSTAT-3 downstream of IL-6 inhibits T-bet expression or both mechanism are present in asthma. These findings show the relevant contribution of IL-6 to the pathogenesis of asthma.

The experimental design of the murine asthma model with immunotherapy. Mice received 100 μg OVA/Alum intraperitoneally (i.p.), 1000 μg OVA subcutaneous (s.c.) and 2 mg/ml OVA intranasally (i.n.). (b–j) mRNA expression of Il-6 (b), Rorγt (c), Batf (d), Irf4 (e), Il-21 (f), Il-21R (g), Il-17A (h), Il-10 (i) and Il-2 (j) in lung tissue of T-bet−/− mice before and after SIT (n = 3–6 mice per group). Results in this figure are expressed as mean ± s.e.m. Students t test was used to calculate statistical significances in this figure. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.
IRF4 and IL-21R in the lungs of treated asthmatic T-bet(−/−) mice. These findings were accompanied by reduced effector TH17 cells, as well as IL-10 and IL-2 production.

We then asked whether blockade of the IL-6R would recapitulate the results seen after subcutaneous immunotherapy in T-bet deficient mice. We found that local anti-IL-6R antibody treatment had an even higher effect on the immunological markers inhibited by the SIT in the lung of T-bet deficient mice. Specifically, we observed a specific down-regulation of BATF, IL-21/IL-21R and IL-10 after anti-IL-6R antibody treatment. This resulted in a decreased differentiation of naive T cells into TH2 cells. However, the down-regulation of IL-17A after anti-IL-6R antibody treatment was not statistically different from IgG treatment for yet unknown reasons. One possibility could relate to our findings showing that IL-6 induces BATF and not RORct in the CD4+ T cells from T-bet deficient mice.

Similarly to IL-17A also IRF4 was not significantly down-regulated by anti-IL-6R antibody treatment as compared to IgG. This is consistent with an IL-17A inducing role of IRF4 on IL-17A. In fact, it has been previously demonstrated that retrovirus over-expressing IRF4 induce IL-17A. Moreover, IRF4 in conjunction with IL-6 induces RORγt. Thus the residual IRF4 after anti-IL-6R antibody treatment might be responsible for the presence of IL-17A and RORγt. Thus it is possible that IL-17A modulation in asthma in the absence of T-bet requires both IL-6 and probably other not yet described factors that regulate RORγt such as IRF4. Further studies in this direction are underway in our laboratory. The anti-IL-6R treatment also led to a down-regulation of IL-21/IL-21R. IL-21 is an important cytokine that maintains the effector cells and has received attention because of its role in autoimmune diseases. In contrast to IL-21, which is distributed to B, T, NKT cells and DCs, IL-21 is limited and support the effector T cells and NKT cells. Finally we found a down-regulation of TH2 cytokines and IgE serum levels after anti IL-6R antibody treatment. It is possible that the IL-21/IL-21R reflects a decrease in Th2 cells as IL-21 has been described to be a Th2 cytokine.

Taken together, these data demonstrated a multi target immune-regulatory effect of anti-IL-6R antibody immunotherapy that works by inhibiting the main cellular components of allergic asthma in the absence of T-bet. This therapy results in reduction of T112 and markers of TH17 cells, inflammation and IgE production. We found a
Figure 7 | Reduced BATF, c-maf and IL-21 expression after in vitro treatment with α-IL-6R antibody. (a) In vitro treatment with OVA (500 µg/ml) and additionally anti-IL-6R antibody or IgG (15 µg/ml). (b,c) Reduced expression of Batf (b), but not Rorγt (c) after treatment with α-IL-6R antibody. (d) c-maf mRNA expression in total lung cells from untreated, OVA-treated and/or α-IL-6R antibody treated wild-type and T-bet(−/−) mice (n = 3–5 mice per group). (e) Decreased Il-21 mRNA expression in lung CD4+ T cells isolated from wild-type and T-bet(−/−) mice after α-IL-6R antibody treatment. Cells were cultured for 24 h with α-CD3 and α-CD28 (n = 10–16 mice per group). Data are mean ± s.e.m. Students t test was used to calculate statistical significances.* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.

Figure 8 | Decreased IL-21R and IL-10 in T-bet deficient mice treated in vivo with anti-IL-6R antibody. (a) Experimental design of anti-IL-6R antibody treatment in vivo. 100 µg OVA/Alum was administered to the mice intraperitoneally (i.p.) and 2 mg/ml OVA intranasally (i.n.). Some of the mice also received 75 µg of α-IL-6R antibody or IgG. (b) Irf4 mRNA expression in lung tissue of T-bet(−/−) mice (n = 8–9 mice per group). (c) IL-17A measured by ELISA in the supernatants of total lung cell cultures of T-bet(−/−) mice sensitized and challenged in vivo with OVA and treated in vivo with α-IL-6R antibody. (d) Decreased expression of Il-21R mRNA in lung tissue from T-bet deficient mice treated with anti-IL-6R antibody (n = 3–6 mice per group). (d) Decreased IL-10 in the supernatants of total lung cell cultures of T-bet(−/−) mice sensitized with OVA treated in vivo with α-IL-6R antibody. Students t test was used to calculate statistical significances. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. Results are expressed as mean ± s.e.m.
central regulatory role of BATF-IRF4 complex downstream of the IL-6R in mediating this effect because controlling both TH2, TH17 and B cells that is important for the development of new immune-therapeutic strategies for allergic asthma.

**Methods**

**Human studies.** Isolation of PBMCs. Heparinized blood was transferred to a 15 ml sterile tube and diluted with an equal volume of PBS, inverted and carefully stratified on Ficoll-Hypaque. After centrifugation, the layer of peripheral blood mononuclear cells (PBMCs), between plasma and Ficoll, was carefully transferred into a new 15 ml tube. On the one hand the RNA was isolated with a Qiagen DNA/RNA Mini Kit (Qiagen, Hilden, Germany). On the other hand RNA was isolated with an AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany). Quantitative real time PCR was performed as described below. This study was approved by the ethics committee of the University of Munich. (Muenchen, Germany). All mice were as follows: mRORγt (5′-AGC CCT TGC AAC CAC AG-3′), mIL-21R (5′-ACC GCC TGC AAT CGT GCC-3′), mIL12 (5′-GAG GAG GGG GAA AAA AC-3′), mIL-6 (5′-GGG ATT CTT CTC GGA TGG TCC TC-3′), mIL-21R (5′-CTC CCC CCT CCG AGT AGA TCT-3′), m-IL-6 (5′-CTG CTC TCA TCA TCT GCT TG-3′). The mRNA levels of the IL-6 receptor gene HPR (5′-GCC CCA AAA TGG ATT AGG TT-3′, 5′-TTG CGC TCA TCT GAT GT-3′) for human samples. For human samples.

**RNA isolation and quantitative real time-PCR.** Total lung tissue was homogenized and total RNA was then extracted by using Qiagen RNAPure Kit (Qiagen, Hilden, Germany). The resulting template-cDNA was amplified by quantitative real-time PCR using Bio-Rad Laboratories (Bio-Rad Laboratories, Muenchen, Germany). The qPCR was performed with a cycle of 2 min 98°C, 50 cycles at 95°C, 10 s 60°C, followed by 5 s 65°C and 5 s 95°C in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Muenchen, Germany).

**Flow cytometric analysis and intracellular staining.** Isolation of lung CD4⁺ T cells (PBMCs), between plasma and Ficoll, was carefully transferred into a new 15 ml tube. On the one hand the RNA was isolated with a Qiagen DNA/RNA Mini Kit (Qiagen, Hilden, Germany). On the other hand RNA was isolated with an AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany). Quantitative real time PCR was performed as described below. This study was approved by the ethics committee of the University of Munich. (Muenchen, Germany). All mice were as follows: mRORγt (5′-AGC CCT TGC AAC CAC AG-3′), mIL-21R (5′-ACC GCC TGC AAT CGT GCC-3′), mIL12 (5′-GAG GAG GGG GAA AAA AC-3′), mIL-6 (5′-GGG ATT CTT CTC GGA TGG TCC TC-3′), mIL-21R (5′-CTC CCC CCT CCG AGT AGA TCT-3′), m-IL-6 (5′-CTG CTC TCA TCA TCT GCT TG-3′). The mRNA levels of the IL-6 receptor gene HPR (5′-GCC CCA AAA TGG ATT AGG TT-3′, 5′-TTG CGC TCA TCT GAT GT-3′) for human samples. For human samples.

**Statistical analysis.** Differences were evaluated for significance by the Student's two-tailed t test for parametric data. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. Data are given as mean values ± s.e.m.
16. Finotto, S. et al. Local blockade of IL-6R signaling induces lung CD4+ T cell apoptosis in a murine model of asthma via regulatory T cells. *Int Immunol* 19, 685–693 (2007).

17. Betz, B. C. et al. Batf coordinates multiple aspects of B and T cell function required for normal antibody responses. *J Exp Med* 207, 933–942 (2010).

18. Ise, W. et al. The transcription factor BATF controls the global regulators of class-switch recombination in both B cells and T cells. *Nat Immunol* 12, 536–543 (2011).

19. Martinez, G. J. & Dong, C. BATF: bringing (in) another Th17-regulating factor. *J Mol Cell Biol* 6, 66–69 (2009).

20. Schraml, B. U. et al. The AP-1 transcription factor Batf controls TH17 differentiation. *Nature* 460, 405–409 (2009).

21. Ciofani, M. et al. A validated regulatory network for th17 cell specification. *Cell* 151, 289–303 (2012).

22. Glaasmacher, E. et al. A genomic regulatory element that directs assembly and function of immune-specific AP-1–IRF complexes. *Science* 338, 975–980 (2012).

23. Li, P. et al. BATF-JUN is critical for IRF4-mediated transcription in T cells. *Nature* (2012).

24. Munthe-Kaas, M. C. et al. T cell-specific T–box transcription factor haplotype is associated with allergic asthma in children. *J Allergy Clin Immunol* 121, 51–56 (2008).

25. Broide, D. H. et al. Cytokines in symptomatic asthma airways. *J Allergy Clin Immunol* 89, 958–967 (1992).

26. Marini, M., Vittori, E., Hollemberg, J. & Mattoli, S. Expression of the potent inflammatory cytokines, granulocyte-macrophage colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *J Allergy Clin Immunol* 89, 1001–1009 (1992).

27. Yokoyama, A. et al. Circulating interleukin-6 levels in patients with bronchial asthma. *Am J Respir Crit Care Med* 151, 1354–1358 (1995).

28. Zhou, L. et al. IL-6 programs TH(17) cell differentiation by promoting sequential engagement of the TH-1 and IL-17 pathways. *Nat Immunol* 8, 967–974 (2007).

29. Aylja, S. J. & Alcorn, J. F. TH(17) cells in asthma and inflammation. *Biochim Biophys Acta* 1810, 1066–1079 (2011).

30. Ivanov, II, Zhou, L. & Litman, D. R. Transcriptional regulation of Th17 cell differentiation. *Semin Immunol* 19, 409–417 (2007).

31. Brustle, A. et al. The development of inflammatory TH(17) cells requires interferon-regulatory factor 4. *Nat Immunol* 8, 958–966 (2007).

32. Huber, M. et al. IRF4 is essential for IL-21-mediated induction, amplification, and stabilization of the TH17 phenotype. *Proc Natl Acad Sci USA* 105, 20846–20851 (2008).

33. Staudt, V. et al. Interferon-regulatory factor 4 is essential for the developmental program of TH helper 9 cells. *Immunity* 33, 192–202 (2010).

34. Huber, S. et al. TH17 cells express interleukin-10 receptor and are controlled by Foxp3 and Foxp3+ regulatory CD4+ T cells in an interleukin-10-dependent manner. *Immunity* 34, 554–565 (2011).

35. Xu, J. et al. c-Maf regulates IL-10 expression during Th17 polarization. *J Immunol* 182, 6226–6236 (2009).

36. Finotto, S. et al. Asthmatic changes in mice lacking T-bet are mediated by IL-13. *Int Immunol* 17, 993–1007 (2005).

37. Doganci, A. et al. IL-2 receptor beta-chain signaling controls immunosuppressive CD4+ T cells in the draining lymph nodes and lung during allergic airway inflammation in vivo. *J Immunol* 181, 1917–1926 (2008).

38. Ellyard, J. I. & Vinuesa, C. G. A BATF-ling connection between B cells and follicular helper T cells. *Nat Immunol* 12, 519–520 (2011).

39. Yoshizaki, K. et al. Isolation and characterization of B cell differentiation factor (BCDF) secreted from a human B lymphoblastoid cell line. *J Immunol* 132, 2948–2954 (1984).

40. Hiramatsu, Y. et al. c-Maf activates the promoter and enhancer of the IL-21 gene, and TGF-beta inhibits c-Maf-induced IL-21 production in CD4+ T cells. *J Leukoc Biol* 87, 703–712 (2010).

41. Yang, Y., Ochando, J., Yopp, A., Bromberg, J. S. & Ding, Y. IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4 T cell activation. *J Immunol* 174, 2720–2729 (2005).

42. Neurath, M. F. & Finotto, S. IL-6 signaling in autoimmune, chronic inflammation and inflammation-associated cancer. *Cytokine Growth Factor Rev* 22, 83–89 (2011).

43. Mudder, J. et al. IRF4 regulates IL-17A promoter activity and controls RORgammat-dependent TH17 colitis in vivo. *Inflamm Bowel Dis* 17, 1343–1358 (2011).

44. Spolski, R. & Leonard, W. J. Interleukin-21: basic biology and implications for cancer and autoimmunity. *Annu Rev Immunol* 26, 57–79 (2008).

45. Pesce, J. et al. The IL-21 receptor augments Th2 effector function and alternative macrophage activation. *J Clin Invest* 116, 2044–2055 (2006).

46. Tahe, Y. A., van Esch, B. C., Hofman, G. A., Henricks, P. A. & van Oosterhout, A. J. T-alpha, 25-dihydroxvitamin D3 potentiates the beneficial effects of allergen immunotherapy in a mouse model of allergic asthma: role for IL-10 and TGF-beta. *J Immunol* 180, 5211–5221 (2008).

47. Sauer, K. A., Scholtes, P., Karwot, R. & Finotto, S. Isolation of CD4 + T cells from murine lungs: a method to analyze ongoing immune responses in the lung. *Nat Protoc* 1, 2870–2875 (2006).

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**Author contributions**

S.K. and S.F. generated the project. S.F. and S.K. designed the experiments. S.K. performed the experiments. S.F. wrote the manuscript. S.K. and S.F. analyzed the data. H.A.L. and R.R. did the histology and histological analysis. S.R. and C.U. contributed to many experiments. S.M. did the SIT experiments and S.M. and A.G. did the experiments on peripheral blood cell analysis of children. C.R. and T.Z. took care of the clinical part of the study.

**Additional information**

**Supplementary information** accompanies this paper at http://www.nature.com/scientificreports

**Competing financial interests:** The authors declare no competing financial interests.

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