Follicular regulatory T cells control humoral and allergic immunity by restraining early B cell responses

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Follicular regulatory T (TFR) cells have specialized roles in modulating follicular helper T (TFH) cell activation of B cells. However, the precise role of TFR cells in controlling antibody responses to foreign antigens and autoantigens in vivo is still unclear due to a lack of specific tools. A TFR cell-deleter mouse was developed that selectively deletes TFR cells, facilitating temporal studies. TFR cells were found to regulate early, but not late, germinal center (GC) responses to control antigen-specific antibody and B cell memory. Deletion of TFR cells also resulted in increased self-reactive immunoglobulin (Ig) G and IgE. The increased IgE levels led us to interrogate the role of TFR cells in house dust mite models. TFR cells were found to control TFR13 cell-induced IgE. In vivo, loss of TFR cells increased house-dust-mite-specific IgE and lung inflammation. Thus, TFR cells control IgG and IgE responses to vaccines, allergens and autoantigens, and exert critical immunoregulatory functions before GC formation.

Tfh cells migrate to B cell follicles to stimulate antibody production by B cells in the GC reaction. The GC reaction results in somatic hypermutation, affinity maturation and class-switch recombination, although these processes may also occur outside GCs. Tfh cells provide essential costimulation (through the inducible costimulatory molecule ICOS and CD40L) and cytokines (such as interleukin (IL)-21 and IL-4) to help promote B cell responses. Tfh cells possess a degree of phenotypic plasticity that can be altered by the inflammatory milieu, causing Tfh cells to produce cytokines typically made by T helper (Th)1, Th2 and Th17 cells. Tfh cells are thought to be distinct from Th2 cells because Th2 cells can produce both IL-4 and IL-13 and express the transcription factor Bcl6. The role of Tfh cells in controlling antibody responses to foreign antigens and autoantigens in vivo is still unclear due to a lack of specific tools. A TFR cell-deleter mouse was developed that selectively deletes TFR cells, facilitating temporal studies. TFR cells were found to regulate early, but not late, germinal center (GC) responses to control antigen-specific antibody and B cell memory. Deletion of TFR cells also resulted in increased self-reactive immunoglobulin (Ig) G and IgE. The increased IgE levels led us to interrogate the role of TFR cells in house dust mite models. TFR cells were found to control TFR13 cell-induced IgE. In vivo, loss of TFR cells increased house-dust-mite-specific IgE and lung inflammation. Thus, TFR cells control IgG and IgE responses to vaccines, allergens and autoantigens, and exert critical immunoregulatory functions before GC formation.

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Tfh cells inhibit Th2 cell-mediated B cell responses. In vitro assays have shown that Tfh cells can inhibit antibody secretion, class-switch recombination and somatic hypermutation through metabolic reprogramming and epigenetic remodeling of B cells. In addition, Tfh cells can suppress Tfh cell production of effector cytokines such as IL-4 and IL-21 in vitro, while maintaining the Tfh cell transcriptional program. The role of Tfh cells in controlling Tfh cell-mediated B cell responses in vivo is less clear. Adoptive transfer studies into lymphopenic mice have shown that Tfh cells inhibit antigen-specific IgG levels. However, studies using bone marrow chimera and/or genetic models, in which the transcription factor Bcl6 was deleted in FoxP3+ cells, have suggested that Tfh cells regulate non-antigen-specific B cell responses but do not substantially affect GC B cells or antigen-specific IgG levels; however, results have been inconsistent. Moreover, IL-10 produced by Tfh cells can promote, rather than inhibit, plasma cell formation. One explanation for the variability between studies may be due to the models used because Bcl6 can be expressed on Tfh cell subsets other than Tfh cells, Bcl6 might not be completely necessary for development of all Tfh cells, and compensatory effects may rescue Tfh cell deletion in non-inducible systems.

To determine the precise role of Tfh cells in controlling B cell responses a Tfh cell-deleter mouse model was developed to inducibly delete Tfh cells in intact hosts at specific time points during immune responses. It was demonstrated that Tfh cells potently regulate antigen-specific and memory IgG levels early during responses before GC formation. Using a Tfh cell-like HDM challenge model, it was found that Tfh cells can regulate IL-13 production by Tfh cells and control IgE responses. Deletion of Tfh cells in vivo during HDM sensitization resulted in increased HDM-specific IgE and lung inflammation. Taken together, these data demonstrate that Tfh cells are key regulators of humoral and allergic immunity by controlling early GC responses.

Results

Development of a specific and inducible Tfh cell-deleter mouse model. To study the role of Tfh cells during immune responses in vivo, we created a mouse model to perturb Tfh cells in an inducible manner. To achieve this, a mouse containing a Ccr5RES-LoxP-STOP-LoxP-DTR allele knocked into the Ccr5 locus was generated, which was crossed to a FoxP3RES-CreYFP allele-containing

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mouse to generate a Cxcr5^{RES-LoxP-STOP-LoxP-DTR} Foxp3^{RES-CreYFP} strain, referred to as the T_{reg}^{DTR} strain (where DTR is diphtheria toxin receptor) (Fig. 1a). In T_{reg}^{DTR} mice, FoxP3-expressing cells produce Cre recombinase which excises the stop cassette in the Cxcr5^{RES-LoxP-STOP-LoxP-DTR} allele, resulting in an active Cxcr5^{RES-DTR} allele and, hence, DTR expression under the control of Cxcr5. Therefore, only cells expressing both FoxP3 and Cxcr5, such as T_{reg} cells, express DTR on the cell surface, making them susceptible to deletion with diphtheria toxin (DT). DTR expression was evaluated on T_{reg} cells and CXCR5^{+} Treg cells from wild-type (WT) FoxP3, Cre/WT mice, gated on Cre-derived or WT-derived FoxP3 alleles. Column graphs represent the mean with error bars indicating s.e.m. The P value indicates a two-tailed Student’s t-test. Data are from individual experiments and represent two (b-d,g) or four (e,f) independent experiments with similar results.

Fig. 1 | Development of a T_{reg} cell-specific deleter model. a, Schematic diagram of the T_{reg}^{DTR} strain. Allele details (left) and schematic of events leading to T_{reg} cell-specific DTR expression (right) are shown. b, DTR expression on T_{reg} cells (left), CXCR5^{+} T_{reg} cells (middle) or T_{reg} cells (right) from control (Foxp3^{WT}), Foxp3^{DTR} or T_{reg}^{DTR} mice. c, DTR expression on CXCR5-negative, CXCR5-medium or CXCR5-high T_{reg} cells from T_{reg}^{DTR} mice. d, Quantification of T_{reg} cells from T_{reg}^{DTR} (Foxp3^{WT-Cxcr5^{RES-LoxP-STOP-LoxP-DTR}}/WT) or control (Foxp3^{WT-Cxcr5^{RES-LoxP-STOP-LoxP-DTR}}/WT) mice that were immunized 7 d previously and received DT 2, 4 and 6 d after immunization. e, Quantification of B cells from T_{reg}^{DTR} or control mice (Foxp3^{WT-Cxcr5^{RES-LoxP-STOP-LoxP-DTR}}/WT) that were immunized 7 d previously and received DT 2, 4 and 6 d after immunization. f, Quantification of T_{reg} cells, CXCR5^{+} T_{reg} and T_{reg} cells from mice as in e, g. Quantification of T_{reg} cells (by assessing CXCR5^{+} T_{reg} cells) from Foxp3^{WT-Cxcr5^{RES-LoxP-STOP-LoxP-DTR}}/WT control (Foxp3^{WT-Cxcr5^{RES-LoxP-STOP-LoxP-DTR}}/WT) mice, gated on Cre-derived or WT-derived FoxP3 alleles. Column graphs represent the mean with error bars indicating s.e.m. The P value indicates a two-tailed Student’s t-test. Data are from individual experiments and represent two (b-d,g) or four (e,f) independent experiments with similar results.
To determine the efficiency of Tfr cell deletion, mice were immunized with (4-hydroxy-3-nitrophenyl)acetyl-ovalbumin (NP-OVA) and (4-hydroxy-3-nitrophenyl)acetyl-fusion-ovalbumin (NP-OVA/FP) and assessed for elevated Ki67 expression in NP-OVA-specific antibodies when Tfr cells were deleted post-GC formation was due to the stage of GC, and not the total duration of Tfr cell deletion, because pre-GC deletion strategies have a phenotype as early as day 5 after Tfr cell deletion, and deletion of Tfr cells after GC formation does not result in a phenotype, even at day 26 (see Supplementary Fig. 2f). These data demonstrate that Tfr cells can regulate GC B cell development and antigen-specific antibody responses early, before GC formation, and have less regulatory control after GCs have been initiated.

Tfr cells potentiate regulation of late GC formation. Previous data suggest that Tfr cells can be limited by cytokines produced in GCs. Moreover, Tfr cells seem to be less frequent in large, developed GCs (data not shown). These findings suggest that Tfr cells might regulate B cell responses most potently before mature GCs form. To assess the role of Tfr cells before GC initiation, Tfr–DTR or control (Foxp3<sup>cre</sup>/LoxP-STOP-LoxP-DTR<sup>foxp3</sup>) mice were immunized with NP-OVA and Tfr cells were deleted on days 5–9 with administration of DT, and B cell responses at day 21 were assessed. Tfr cells were markedly attenuated, even 12 d after the last DT injection (Fig. 2a). There were minor, but notable, increases in the frequency of Tfr cells compared with control mice. The CD19<sup>+</sup>GL7<sup>+</sup>FAS<sup>+</sup> GC B cell frequency was approximately twofold higher in Tfr–DTR mice compared with control mice, demonstrating that Tfr cells potentiate regulation of initial GC formation (Fig. 2b). In addition, naive B cells, gated as CD38<sup>+</sup>IgG1<sup>+</sup> B cells, were slightly attenuated in Tfr–DTR mice. CD138<sup>+</sup> plasma cells, IgG1<sup>+</sup> class-switched B cells and IgG1<sup>+</sup>CD38<sup>+</sup> memory-like B cells were also increased in Tfr–DTR mice, suggesting that Tfr cells regulate many arms of B cell effector responses (Fig. 2b,c). Tfr cell have previously been shown to cause metabolic reprogramming, including inhibition of glycolysis, in B cells. It was found that Tfr cells were deleted from Tfr–DTR but not from either Cxcr5<sup>cre</sup>/LoxP-STOP-LoxP-DTR<sup>foxp3</sup> or Foxp3<sup>cre</sup>/LoxP-STOP-LoxP-DTR<sup>foxp3</sup> control mice (Fig. 1d–f). Deletion of Tfr cells was selective in Tfr–DTR mice because B cells, Tfr cells, total Cxcr5<sup>+</sup>Treg cells or activated Ki67<sup>+</sup> Treg cells were not deleted in Tfr–DTR mice (Fig. 1e,f and see Supplementary Fig. 1c). Moreover, deletion of Tfr cells in Tfr–DTR mice resulted in the loss of Foxp3<sup>+</sup> cells within individual GCs (see Supplementary Fig. 1d). To determine whether deletion of Tfr cells was cell intrinsic, Cxcr5<sup>Cre</sup>/LoxP-STOP-LoxP-DTR<sup>foxp3</sup>/lox<sup>foxp3</sup> cells in which only ~50% of the Tfr cells will express DTR on the surface) were assessed for preferential deletion of Tfr–DTR mice, suggesting that Tfr cells were also increased in Tfr–DTR mice, which supports the idea that Tfr cells regulate autoreactive IgG and IgE antibodies.

Next, we assessed whether Tfr cells can regulate autoreactive antibodies. Tfr cells were deleted in Tfr–DTR mice before GC formation (at days 5–9), and the sera analyzed at day 21 with autoantigen protein arrays. Autoreactive IgG was increased for a third of the autoantigens in Tfr–DTR mice compared with control mice using a stringent cutoff (Mann–Whitney U-test, P < 0.01) (Fig. 3a). In most cases, control mice had antibodies that recognize autoantigens, but levels were higher in the Tfr–DTR mice. However, in one example, there was a substantial signal for anti-histone H1 autoantibodies in Tfr–DTR mice, but no detectable signal in control mice (Fig. 3a). These data demonstrate that Tfr cells can regulate levels and formation of autoreactive IgG antibodies.

Next, it was determined whether any of the substantial amounts of IgE in the Tfr cell-deleted mice were specific for autoantigens. Such autoreactive antibodies could be pathogenic, because patients with systemic lupus erythematosus (SLE) can generate autoreactive IgE responses, and autoreactive IgE can exacerbate disease in mouse models of lupus. Fifteen autoantigens were recognized to a higher degree by IgE in Tfr–DTR compared with control mice (Mann–Whitney U-test, P < 0.01) (Fig. 3b). These autoantigens include La/SSB and Ro-52/SSA, which are targets for autoreactive IgE responses in SLE patients; anti-SSB and -SSA IgE may be indicators of immune complex-mediated disease. In addition, complement had higher IgE autoantibody scores in Tfr–DTR compared with control mice. When a less stringent cutoff of P < 0.05 was used, anti-β2-microglobulin and anti-GP2 IgE were present in Tfr–DTR mice, but not in control mice (data not shown). Using the stringent P < 0.01 cutoff, there was evidence of increases in IgG and IgE targeting the same eight autoantigens, including Ro-52/SSA, MPO, CENP-B, PL-7, TTG and M2 (Fig. 3c,d). Some of these IgG and IgE autoantibodies, such as anti-Ro-52/SSA, are increased in SLE patients.

Next, it was determined whether Tfr cells can regulate initial activation and class-switch recombination of autoreactive B cells. Tfr cell-mediated, antigen-specific, autoreactive B cell, class-switch recombination assay was developed. Myelinating oligodendrocyte glycoprotein (MOG) was chosen as the model autoantigen because MOG immunization generates functional Tfh and Tfr cells, MOG-specific B cells cause a Devic-like disease in experimental autoimmune encephalomyelitis models and B cell depletion has a large
Fig. 2 | T<sub>reg</sub> cells potently regulate early GC formation. a, Quantification of T<sub>reg</sub> (gated as CD4<sup>+</sup>ICOS<sup>+</sup> CXCR5<sup>+</sup>FoxP3<sup>+</sup>CD19<sup>+</sup>) and T<sub>n</sub> (gated as CD4<sup>+</sup>ICOS<sup>-</sup>CXCR5<sup>+</sup>FoxP3<sup>-</sup>CD19<sup>-</sup>) cells from dLNs of T<sub>reg</sub>-DTR (Foxp3<sup>Cre</sup>ScidC<sup>S<i>+</i></sup>DLN<sup>DT</sup>F<sup>+</sup>) or control (Foxp3<sup>Cre</sup>ScidC<sup>S<i>+</i></sup>DLN<sup>DT</sup>F<sup>-</sup>) mice 21 d after immunization. DT was administered on days 5, 7 and 9 to delete T<sub>reg</sub> cells before GC initiation. b, Quantification of GC B cells (gated as CD19<sup>+</sup>GL7<sup>+</sup>FAS<sup>+</sup>) and naive B cells (gated as CD38<sup>+</sup>IgG1<sup>+</sup>) from dLNs at day 21 after immunization as in a. c, Quantification of plasma cells (gated as CD138<sup>+</sup>), class-switched B cells (gated as CD19<sup>+</sup>IgG1<sup>+</sup>CD38<sup>-</sup>) and memory-like B cells (gated as CD19<sup>+</sup>IgG1<sup>+</sup>CD38<sup>-</sup>) at day 21 after immunization as in a. d, Glut1 expression on B cells from mice as in a. Representative histogram is shown on the left and quantification on the right. MFI, mean fluorescence intensity. e, Quantification of total IgG (far left), NP-specific IgG (middle left), total IgE (middle right) and total IgA (far right) analyzed from serum of mice as in a. f, Quantification of T<sub>reg</sub> (gated as CD4<sup>+</sup>ICOS<sup>-</sup>CXCR5<sup>-</sup>FoxP3<sup>-</sup>CD19<sup>-</sup>) and T<sub>n</sub> (gated as CD4<sup>+</sup>ICOS<sup>-</sup>CXCR5<sup>+</sup>FoxP3<sup>+</sup>CD19<sup>+</sup>) cells from dLNs of T<sub>reg</sub>-DTR (Foxp3<sup>Cre</sup>ScidC<sup>S<i>+</i></sup>DLN<sup>DT</sup>F<sup>+</sup>) or control (Foxp3<sup>Cre</sup>ScidC<sup>S<i>+</i></sup>DLN<sup>DT</sup>F<sup>-</sup>) mice at day 21 after immunization. DT was administered on days 10, 12 and 14 to delete T<sub>reg</sub> cells after GC formation. g, Quantification of GC B cells (gated as CD19<sup>+</sup>GL7<sup>+</sup>FAS<sup>+</sup>) and plasma cells (CD138<sup>+</sup>) from dLNs at day 21 after immunization as in f, h, Quantification of total IgG (left) and NP-specific IgG (right). Column graphs represent the mean with error bars indicating s.e.m. The P value indicates a two-tailed Student’s t-test. Data are either combined results from four (a–c) or three (f–h) independent experiments, or from an individual experiment that represents two independent experiments (d).
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therapeutic benefit in multiple sclerosis\(^3\). For this assay, TFH and TFR cells were sorted from MOG\(^{35-55}\)-immunized Foxp3\(^{GFP}\) mice and cultured with B cells isolated from naive IgHMOG mice in the presence of recombinant MOG (rMOG) (Fig. 3e). TFH cells could stimulate IgH\(^{MOG}\) B cells to expand and undergo class-switch recombination (Fig. 3f,g). Importantly, TFR cells could potently suppress IgH\(^{MOG}\) B
TFR cells regulate antibody memory responses. Next, we determined whether TFR cells regulate memory B cell responses. To do this, Tfr–DTR or control (Foxp3<sup>−/−</sup>CXCR5<sup>−/−</sup>) mice were immunized with NP-OVA containing a mild adjuvant, MF59-like Addavax, and given DT from day 5 to day 9. At day 30, mice were boosted intraperitoneally with NP-OVA. Increases in GC B cells were found in TFR–DTR compared with control mice after rechallenge (Fig. 4b). However, it is important to note that this assay cannot distinguish GC B cells that form from memory B cells or naive B cells. In contrast to GC B cells, there were no significant increases in TFH cell frequency or expression of Ki67 in TFR–DTR mice after rechallenge (Fig. 4d). Taken together, these data indicate that TFR cells restrain the quantity, but promote the affinity, of antigen-specific antibody during memory responses by regulating early GCs.

HDM antigen generates distinct populations of T<sub>H</sub> and T<sub>F</sub> cells. The finding that T<sub>F</sub> cells compete in mice immunized with NP-OVA suggested that T<sub>F</sub> cells may be able to regulate T<sub>H</sub>2-like responses. Therefore, it was next determined whether HDM exposure, a T<sub>H</sub>2-like response, generated distinct populations of T<sub>F</sub> and T<sub>H</sub> cells. C57BL/6 mice were challenged with HDM intranasally every 2 d and mediastinal lymph nodes were assessed on day 7. Unimmunized mice had a very small ICOS<sup>+</sup>CXCR5<sup>+</sup>CD4<sup>+</sup> population made up of ~60% TFR cells, a ratio that is typical in basal states<sup>13</sup> (Fig. 5a). In comparison, HDM-exposed mice developed a substantially larger population of ICOS<sup>+</sup>CXCR5<sup>+</sup>CD4<sup>+</sup> cells in which TFR cells were only ~30%. Moreover, a subpopulation of T<sub>H</sub> and T<sub>F</sub> cells was...
Fig. 5 | HDM antigen generates distinct populations of T<sub>Tfh</sub> and T<sub>Tfr</sub> cells. **a**, Quantification of T<sub>Tfh</sub> and T<sub>Tfr</sub> cells in response to HDM challenge. WT mice were either challenged or not challenged (control) with HDM intranasally on days 0, 2, 4 and 6. The dLNs were harvested on day 7. The gating strategy to identify T<sub>Tfh</sub> and T<sub>Tfr</sub> cells (left), total numbers of T<sub>Tfh</sub> and T<sub>Tfr</sub> cells (middle), and gating strategy for ‘GC’ T<sub>Tfh</sub> and T<sub>Tfr</sub> cells (right) is shown. **b**, PCA showing the relationship between transcriptional profiles of T<sub>Tfh</sub> (CD4<sup>+</sup>ICOS<sup>+</sup>CXCR5<sup>+</sup>FoxP3<sup>−</sup>CD19<sup>−</sup>) and T<sub>Tfr</sub> (CD4<sup>+</sup>ICOS<sup>+</sup>CXCR5<sup>+</sup>FoxP3<sup>+</sup>CD19<sup>−</sup>) cells generated in response to NP-OVA (subcutaneous) or HDM (intranasal) challenge in Foxp3<sup>−/−</sup> mice. PC, principal component. **c**, GSEA comparing T<sub>Tfh</sub> cells generated in response to NP-OVA or HDM for T<sub>Tfh</sub> or T<sub>Tfr</sub> signatures (GSE14308). NES, normalized enrichment score. **d**, GSEA comparing T<sub>Tfh</sub> cells generated in response to NP-OVA or HDM for T<sub>Tfh</sub> or T<sub>Tfr</sub> signatures. **e**, Venn diagram demonstrating the overlap of differentially expressed genes (P < 0.05) between NP-OVA and HDM components for T<sub>Tfh</sub> and T<sub>Tfr</sub> cells. **f**, Heatmap showing the 39 commonly differentially expressed genes in T<sub>Tfh</sub> and T<sub>Tfr</sub> cells in NP-OVA versus HDM challenge as in **e, g**. Heatmap of common follicular T cell and T<sub>Tfh</sub>, T<sub>Tfr</sub> genes in T<sub>Tfh</sub> and T<sub>Tfr</sub> cells generated in response to NP-OVA or HDM challenge. **h**, IL-13 production by HDM T<sub>Tfr</sub> cells. Intracellular staining was performed on HDM-treated mice as in a. FMO, stain without anti-IL-13 antibody. Column graphs represent the mean with error bars indicating s.e.m. The P value indicates a two-tailed Student’s t-test. Data either represent three independent experiments (a, h) or are combined data from two independent experiments (b-g).
found, which assumed a GC-like phenotype, suggesting proper T FH and T FR cell effector differentiation.

To determine whether after HDM exposure T FH and T FR cells transcriptionally resemble T FH and T FR cells, transcriptional analysis was performed. Foxp3R3ES-GFP mice were immunized with NP-OVA (emulsified in Freund’s complete adjuvant) subcutaneously on day 0 or HDM intranasally on days 0, 2, 4, and 6, and T FH (gated as CD4+ICOS+CXCR5+Foxp3−CD19+), T FR (gated as CD4+ICOS+CXCR5+Foxp3+CD19+), or T conventional (T con, gated as CD4+ICOS+CXCR5-Foxp3+CD19+) cells were sorted on day 7 and RNA sequencing (RNA-seq) transcriptional analysis was performed. Using principal component analysis (PCA), most follicular T cell populations separated from each other; however, HDM T FH cells clustered closer to T con cells than other cells (Fig. 5b). Next, it was determined whether T FH cells from HDM-challenged mice transcriptionally resemble T FH cells or whether they take on a T 2-like phenotype. OVA- and HDM-specific T FH cells had a similar enrichment for T FH genes and HDM T FH cells did not have enrichment for T 2 genes (Fig. 5c). Similarly, T FR cells from both OVA and HDM challenges had strong enrichment for T FR genes (Fig. 5d).

Although T FH and T FR cells from HDM-challenged mice had intact transcriptional programs, differentially expressed genes were found between these cells and their OVA challenge counterparts. There were 374 genes differentially expressed (P < 0.05) between OVA and HDM T FH cells, and 665 genes differentially expressed (P < 0.05) between OVA and HDM T FR cells, with 39 genes being differentially expressed in both T FH and T FR cells (Fig. 5e). When these 39 genes were assessed in more detail, a subset of genes was found expressed in HDM T FH and T FR cells, but not OVA populations, such as Gfi1 (T FH cells: P = 0.0130; T FR cells: P = 0.0424), which has a role in stabilizing T 2 cells (Fig. 5f). Genes commonly expressed in T FR and T FR cells were also evaluated. Some genes, such as Icos and Id2, seemed to be expressed less in HDM T FH cells compared with OVA T FH cells, although only Id2 was statistically significant (P = 0.00004) (Fig. 5g). A low, but positive, transcript for Il33 was found in HDM T FH cells that was not present in OVA T FH cells. In addition, HDM T FH cells expressed more Gata3. To assess whether a subset of T FH cells could produce IL-13, intracellular cytokine staining was performed and a small proportion of T FR cells was found that produced IL-13 (Fig. 5b). Taken together, these data demonstrate that T FR and T FR cells from HDM challenge have T FH and T FR transcriptional programs, but also some distinct transcriptional characteristics, such as an IL-13 transcript in HDM T FR cells. As HDM T FH cells have an intact T 2, but not a T 2, program yet express IL-13, these cells are referred to as ‘T 2-like’ cells.

T FR cells regulate T FH13 cell-mediated IgE responses to HDM in vivo. As it was found that T FH13-like cells from HDM-challenged mice expressed IL-13, and T FR cell-deleted mice had elevated levels of autoreactive IgE, it was hypothesized that T FR cells may regulate IL-13 and IgE responses in the context of T 2-like allergic responses. To test this hypothesis, an in vitro suppression assay was developed in which T FH13-like cells mediate class switching of B cells to IgE in response to HDM antigen. HDM was given intranasally to Foxp3R3ES-GFP mice every 2 d and, on day 7, total B, T FH (gated as CD4+ICOS+CXCR5+Foxp3−CD19+), or T FR (gated as CD4+ICOS+CXCR5+Foxp3+CD19+) cells were sorted from mediastinal lymph nodes. Cells were cultured together along with HDM for 6 d (Fig. 6a). T FR cells inhibited T FH cell proliferation and were still present at the end of the culture (Fig. 6b). It was found that cultures containing T FH13 and B cells contained large amounts of T 2-like cytokines, including IL-5, IL-13 and IL-4, all of which were suppressed by the addition of T FR cells (Fig. 6c,d). In addition, T FR cells suppressed the frequency of T FH13 cells (gated as CD4+CXCR5−) expressing IL-13 protein (Fig. 6e). T FH13 cells stimulated B cells to undergo class switching to IgG1 and, to a lesser extent, IgE (Fig. 6f). Importantly, addition of T FR cells resulted in near-complete reduction in IgE+B cells and a substantial reduction in IgG1+B cells (Fig. 6f,g). No evidence was found of class switching to IgE when HDM was omitted from the wells, or when similar cultures were performed using cells from NP-OVA immunization (see Supplementary Fig. 4; data not shown). Although HDM T FH cells suppressed class switching of NP-OVA B cells to IgG1, NP-OVA T FR cells may suppress T FH13 cell-mediated IgE class switching of B cells less potently than HDM T FR cells (see Supplementary Fig. 4).

To determine whether T FR cells prevent class switching to IgE, suppress already class-switched IgE+B cells, or both, levels of GL7, a GC B cell-expressed molecule that is attenuated on B cells during class switching, were assessed. It was found that IgE+B cells had lower expression of GL7 in the presence of T FR cells, suggesting that IgE+ class-switched B cells are less activated (Fig. 6h). Protein levels of IgE and IgG1 were also analyzed within switched B cells. Both IgE- and IgG1-expressing B cells had lower expression of IgE and IgG1, respectively, if T FR cells were present, although this did not reach statistical significance for IgE (Fig. 6i). To determine whether T FH13 cell cytokines were essential for full IgE responses, IL-13- or IL-4-blocking antibodies were added to cultures and the levels of IgE were assessed. T FH13 cells stimulated large amounts of IgE secretion, which was strongly attenuated when IL-13- or IL-4-blocking antibodies were added, demonstrating that cytokines produced by T FH13 cells stimulate IgE (Fig. 6i). Importantly, IgE and IgG production was substantially suppressed by the presence of T FR cells (Fig. 6j). Taken together, these data demonstrate that T FR cells can suppress T FH1-cell-mediated IL-13 and IgE responses in vitro.

T FR cells regulate antigen-specific IgE responses in vivo. Next the role of T FR cells was assessed in allergic immunity in vivo. For this, a HDM sensitization and challenge model was used that results in antigen-specific IgE responses and IL-13-dependent eosinophilic lung inflammation. T FH–DTR or control (Foxp3R3ES-Cxcr5R5fox) mice were sensitized with HDM and given DT to delete T FR cells. On day 7 mice with HDM were challenged (Fig. 7a). Robust deletion of T FR cells was found in T FH–DTR mice as a percentage of both total CXCR5+CX4+ cells and total CD4+ cells (Fig. 7b). No evidence was found of deletion of activated T FH cells in T FH–DTR mice (see Supplementary Fig. 5a). Deletion of T FR cells did not result in altered frequencies of T FH cells, GC B cells, IgG1+B cells, IgE+B cells or plasma cells (Fig. 7c). In addition, deletion of T FR cells did not alter the relative class switching to IgG or IgE in GC B cells (Fig. 7d). However, when plasma cells were assessed, it was found that deletion of T FR cells resulted in small increases in IgE plasma cells (Fig. 7e). Moreover, T FH–DTR mice had substantially higher levels of total and HDM-specific IgE compared with control mice (Fig. 7f). These data demonstrate that T FR cells can control HDM-specific IgE responses in vivo.

To determine whether deletion of T FR cells results in altered lung inflammation, bronchoalveolar lavage fluid was analyzed and increases in eosinophils found (Fig. 7g). Histological analysis of lungs showed increased inflammation consisting of cell infiltration to the airway/vessel walls and alveolar parenchyma in T FH–DTR cells-deleted mice compared with control mice (Fig. 7h). It was found that the immune cell infiltrate in the lungs of T FR cell-deleted mice was positive for Gr1 or SiglecF, suggesting the presence of granulocytes and eosinophils, respectively (Fig. 7i and see Supplementary Fig. 5b). Taken together, these data demonstrate that T FR cells regulate HDM-specific IgE responses in vivo and control immune cell infiltration during HDM sensitization and challenge.

Discussion

The precise role of T FR cells in modulating B cell responses has been elusive due to the lack of specific mouse models to study T FR cells.
Fig. 6 | T<sub>FH</sub> cells regulate T<sub>FH</sub>13 cell-mediated IgE responses in vitro. a, Schematic of experimental design for an in vitro HDM suppression assay. Total B, T<sub>FH</sub> and T<sub>FR</sub> cells were purified from the dLNs of mice that received HDM on day 0, 2, 4 and 6, and were added to culture wells along with HDM for 6 d. b, Quantification of total T<sub>FH</sub> cells (left) and the percentage of T<sub>FH</sub> cells (FoxP3<sup>+</sup> of CD4<sup>+</sup>CD19<sup>-</sup> cells) (right) from cultures as in a. c, Quantification of cytokines in culture supernatants from cultures as in a. Cytokines listed in red have levels notably lower in cultures containing T<sub>FR</sub> cells compared with cytokines in culture supernatants from cultures as in a. GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; TNF, tumor necrosis factor. d, Column graphs of IL-5, IL-4 and IL-13 from the data in a. e, Intracellular cytokine staining of IL-13 in T<sub>FH</sub> cells from cultures as in a. f, Analysis of class switching to IgG1 and IgE in cultures as in a. Representative gating (left, pregated on CD19<sup>+</sup>IgA<sup>-</sup>CD4<sup>-</sup> cells), IgE<sup>+</sup> B cell quantification (middle) and IgG1<sup>+</sup> B cell quantification (right) are shown. g, Counts of total IgE<sup>+</sup> (left) and IgG1<sup>+</sup> (right) B cells expressed as relative counts from experiments as in a. h, Analysis of class switching to IgG1 and IgE in cultures as in a. i, Expression of IgG on IgE<sup>+</sup> B cells (left) and IgG1 on IgG1<sup>+</sup> B cells are shown from cultures as in a. j, Levels of IgE (left) and IgG (right) in culture supernatants from cultures as in a. Anti-IL-13 (all-13) or anti-IL-4 (all-4) was added to the indicated wells. Column graphs represent the mean with error bars indicating s.e.m. The P value indicates a two-tailed Student’s t-test (b–i, j, right) or one-way analysis of variance with Tukey’s correction (j, left). Data are from individual experiments and represent three independent experiments.
Fig. 7 | T= cells regulate HDM-specific IgE responses in vivo. a, Schematic of HDM sensitization-and-challenge model to induce lung inflammation. T=–DTR (Foxp3<sup>Cre</sup>ΔCD244<sup>DTR/DT</sup>) or control (Foxp3<sup>Cre</sup>ΔCD244<sup>WT/WT</sup>) mice received HDM sensitization (sens.) intranasally (i.n.) at day 0, followed by DT administration at days 0, 2, 4 and 6. Mice were challenged with HDM on days 7–11 and harvested on day 15. b, Analysis of T= cells from dLNs of HDM-challenged mice as in a. Representative gating (left) and quantification (right) are shown. c, Quantification of T<sub>fh</sub>, GC B cells (CD19<sup>+</sup>GL7<sup>+</sup>), total IgG<sup>+</sup> B cells (CD19<sup>+</sup>IGE<sup>+</sup>), total IgG<sup>+</sup> B cells (CD19<sup>+</sup>IGE<sup>+</sup>), and total plasma cells (CD138<sup>+</sup>) from dLNs of HDM-challenged mice as in a. d, Quantification of IgG1 and IgE expression in GC B cells (CD19<sup>+</sup>GL7<sup>+</sup>IGE<sup>+</sup>). e, Quantification of IgG1 and IgE expression in plasma cells (CD138<sup>+</sup>). f, Quantification of total IgE or HDM-specific IgE from HDM-challenged mice as in a (n = 30, control; n = 22, T=–DTR). AU, arbitrary units. g, Quantification of eosinophils (Eos, left). h, Immunofluorescence micrographs of lungs stained for actin, SiglecF, Gr1, and I-A. Scale bars, 100 μm. Column graphs represent the mean with error bars indicating s.e.m. The P value indicates a two-tailed Student’s t-test (b–e) or a Mann–Whitney U-test (f–h). Data are from individual experiments and represent three independent experiments (b, c left), are combined data from four independent experiments (c right, d–g) or are from one experiment (h, i).
In the present study, a new T<sub>FR</sub>-DTR mouse strain was developed to study T<sub>FR</sub> cells at distinct stages of immune responses. It was found that T<sub>FR</sub> cells potently regulate foreign and self-reactive IgG responses, especially before initial GC development. Surprisingly, a population of IL-13-expressing, T<sub>FR</sub>13-like cells was found during HDm challenge, and T<sub>FR</sub> cells were found to potently regulate these cells to control IL-13- and HDm-specific IgE responses. Taken together, these data indicate that T<sub>FR</sub> cells have a dynamic role in controlling many types of B cell responses to foreign and self-reactive antigens, particularly before initial GC formation.

Although T<sub>FR</sub> cells can be found inside and outside of GCs, there has been a debate in the field as to where and when T<sub>FR</sub> cells regulate B cells. T<sub>FR</sub> cells are less frequently found in GCs compared with the B cell follicle, and cytokines produced in high levels in GCs, such as IL-21, can inhibit T<sub>FR</sub> cells. Based on these observations, it has been suggested that T<sub>FR</sub> cells do not substantially regulate B cell responses within GCs. However, T<sub>FR</sub> cells can regulate GC B cells in vitro. In the present study, it has been demonstrated that T<sub>FR</sub> cells potently regulate antibody responses before, but not after, GC formation. Although the experiments in this study cannot eliminate the possibility of T<sub>FR</sub> cells having roles within very early GCs, the lack of a phenotype in mature GCs suggests that T<sub>FR</sub> cells regulate GC development. However, it is important to note that T<sub>FR</sub> cells may have more subtle roles in GCs such as facilitating GC resolution and promoting immune homeostasis.

It was also found that T<sub>FR</sub> cells regulate memory B cell responses. It was proposed that they do this by preventing GC formation where some memory B cells originate. However, it is possible that non-GC memory B cells may also be regulated by T<sub>FR</sub> cells in the B cell follicle. As reactivation of memory B cells may require secondary GCs, T<sub>FR</sub> cells probably have two roles in modulating B cell memory: regulation of memory B cell formation and regulation of secondary GCs during memory B cell reactivation. In addition, it was found that T<sub>FR</sub> cells promote antibody affinity during memory, suggesting that T<sub>FR</sub> cells can modulate not only the quantity of antigen-specific antibody, but also the quality of the antibody. Previous studies found that deletion of Bcl6 in all T<sub>FR</sub> cell subsets from birth resulted in increased autoreactive antibodies, but these antibodies develop only after months. It was found that deletion of T<sub>FR</sub> cells resulted in increases in a variety of autoreactive IgG antibodies and, surprisingly, autoreactive IgE antibodies. Autoreactive IgE has been found in autoimmune diseases such as SLE and has been suggested to enhance autoimmune pathology; it has also been found in models of epithelial damage.

The role of T<sub>FH</sub> and T<sub>FR</sub> cells in T<sub>H</sub>2-like immunity has been unclear. Although T<sub>FH</sub> cells are not thought to make IL-13, T<sub>FH</sub> cells have been implicated in controlling IgE responses to HDm. Likewise, attenuated percentages of T<sub>FR</sub> cells correlate with worse allergy in patients. Evidence was found of a small frequency of IL-13-producing T<sub>FR</sub> cells in vivo after HDm administration. Using an in vitro HDm IgE assay, it was shown that these T<sub>FR</sub> cells could produce large amounts of IL-13, IL-5, and IL-4, and potentially stimulate B cell IgE responses. These T<sub>FR</sub> cells have been referred as T<sub>FR</sub>13-like cells to distinguish them from previously described T<sub>FR</sub>2 cells. Both IL-13 and IgE responses were potently suppressed by T<sub>FR</sub> cells. Interestingly, deletion of T<sub>FR</sub> cells in vivo during HDm sensitization/challenge resulted in higher levels of antigen-specific IgE and increased lung inflammation. High-affinity IgE responses occur preferentially through sequential switching of IgG1 to IgE. As there was such a profound increase in IgE serum levels and alterations in IgE+, but not IgG1+, plasma cells, these data suggest that T<sub>FR</sub> cells probably limit IgE plasma cell responses rather than sequential switching. However, more in-depth studies are necessary to fully determine the role of T<sub>FR</sub> cells in sequential switching. Taken together, these results demonstrate that T<sub>FR</sub> cells can have roles in T<sub>H</sub>2-like responses to HDm and that these responses are controlled by T<sub>FR</sub> cells. Therefore, T<sub>FR</sub> cells are likely to have roles in regulating allergic inflammation and immunity to helminth infections, and modulation of T<sub>FR</sub> cells may help to control these responses.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41590-019-0472-4.

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**References**

1. Crotty, S. Follicular helper CD4 T cells (TFH). *Annu. Rev. Immunol.* 29, 621–663 (2011).
2. Victoria, G. D. & Nussenzweig, M. C. Germinal centers. *Annu. Rev. Immunol.* 30, 429–457 (2012).
3. Crotty, S. T follicular helper cell differentiation, function, and roles in disease. *Immunity* 41, 529–542 (2014).
4. Maceiras, A. R., Fonseca, V. R., Agua-Doce, A. & Graca, L. T follicular regulatory T cells. *Annu. Rev. Immunol.* 34, 335–368 (2016).
5. Cannons, J. L., Lu, K. T. & Schwartzberg, P. L. T follicular helper cell diversity and plasticity. *Trends Immunol.* 34, 200–207 (2013).
6. Weinstein, J. S. et al. TFH cells progressively differentiate to regulate the germinal center response. *Nat. Immunol.* 17, 1197–1205 (2016).
7. Lutjke, K. et al. The development and fate of follicular helper T cells defined by an IL-21 reporter mouse. *Nat. Immunol.* 13, 491–498 (2012).
8. Liang, H. E. et al. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nat. Immunol.* 13, 58–66 (2011).
9. Ballastros-Tato, A. et al. T follicular helper cell plasticity shapes pathogenic T helper 2 cell-mediated immunity to inhaled house dust mite. *Immunity* 44, 239–273 (2016).
10. Coquet, J. M. et al. Interleukin-21-producing CD4<sup>+</sup> T cells promote type 2 immunity to house dust mites. *Immunity* 43, 318–330 (2015).
11. Noval Rivas, M. & Chatila, T. A. Regulatory T cells in allergic diseases. *J. Allergy Clin. Immunol.* 138, 639–652 (2016).
12. Curotto de Lafaille, M. A. et al. Adaptive Foxp3<sup>+</sup> regulatory T cell-dependent and -independent control of allergic inflammation. *Nat. Immunol.* 29, 114–126 (2008).
13. Sage, P. T. & Sharpe, A. H. T follicular regulatory cells. *Immunol. Rev.* 271, 246–259 (2016).
14. Maceiras, A. R., Fonseca, V. R., Agua-Doce, A. & Graca, L. T follicular regulatory cells in mice and men. *Immunology* 152, 25–35 (2017).
15. Sage, P. T. et al. Suppression by TFR cells leads to durable and selective inhibition of B cell effector function. *Nat. Immunol.* 17, 1436–1446 (2016).
16. Sage, P. T., Paterson, A. M., Lovitch, S. B. & Sharpe, A. H. The co-inhibitory receptor cTla-4 controls B cell responses by modulating T follicular helper, T follicular regulatory, and T regulatory cells. *Immunity* 41, 1026–1039 (2014).
17. Sage, P. T., Alvarez, D., Godic, J., von Andrian, U. H. & Sharpe, A. H. Circulating T follicular regulatory and helper cells have memory-like properties. *J. Clin. Invest.* 124, 5191–5204 (2014).
18. Sage, P. T., Francisco, L. M., Carman, C. V. & Sharpe, A. H. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. *Nat. Immunol.* 14, 152–161 (2013).
19. Wollenberg, J. et al. Regulation of the germinal center reaction by Foxp3<sup>+</sup> follicular regulatory T cells. *Immunity* 187, 4535–4560 (2011).
20. Wu, H. et al. Follicular regulatory T cells repress cytokine production by follicular helper T cells and optimize IgG responses in mice. *Eur. J. Immunol.* 46, 1152–1161 (2016).
21. Linterman, M. A. et al. Foxp3<sup>+</sup> follicular regulatory T cells control the germinal center response. *Nat. Med.* 17, 975–982 (2011).
22. Fu, W. et al. Deficiency in T follicular regulatory cells promotes autoimmunity. *J. Exp. Med.* 215, 815–825 (2018).
23. Laidlaw, B. J. et al. Interleukin-10 from CD4<sup>+</sup> follicular regulatory T cells promotes the germinal center response. *Sci. Immunol.* 2, eaan4767 (2017).
24. Kim, J. M., Rasmussen, J. P. & Rudensky, A. Y. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat. Immunol.* 8, 191–197 (2007).
25. Hou, S. et al. FoxP3 and Ezh2 regulate TFR cell suppressive function and cytokine production in human lymph nodes. *J. Exp. Med.* 215, 1531–1542 (2018).
26. Dema, B. et al. Immunoglobulin E plays an immunoregulatory role in lupus. *J. Exp. Med.* 211, 2159–2168 (2014).
28. Dema, B. et al. Autoreactive IgE is prevalent in systemic lupus erythematosus and is associated with increased disease activity and nephritis. *PLoS ONE* **9**, e90424 (2014).

29. Sage, P. T. et al. Dendritic cell PD-L1 limits autoimmunity and follicular T cell differentiation and function. *J. Immunol.* **200**, 2592–2602 (2018).

30. Bettelli, E., Baeten, D., Jager, A., Sobel, R. A. & Kuchroo, V. K. Myelin oligodendrocyte glycoprotein-specific T and B cells cooperate to induce a Devic-like disease in mice. *J. Clin. Invest.* **116**, 2393–2402 (2006).

31. Krishnamoorthy, G., Lassmann, H., Wekerle, H. & Holz, A. Spontaneous opticospinal encephalomyelitis in a double-transgenic mouse model of autoimmune T cell/B cell cooperation. *J. Clin. Invest.* **116**, 2385–2392 (2006).

32. Mulero, P., Midaglia, L. & Montalban, X. Ocrelizumab: a new milestone in multiple sclerosis therapy. *Ther. Adv. Neurol. Disord.* **11**, 1756286418773025 (2018).

33. McHeyzer-Williams, L. J., Milpied, P. J., Okitsu, S. L. & McHeyzer-Williams, M. G. Class-switched memory B cells remodel BCRs within secondary germinal centers. *Nat. Immunol.* **16**, 296–305 (2015).

34. Zhu, J., Jankovic, D., Grinberg, A., Guo, L. & Paul, W. E. Gfi-1 plays an important role in IL-2-mediated Th2 cell expansion. *Proc. Natl Acad. Sci. USA* **103**, 18214–18219 (2006).

35. Tomlinson, K. L., Davies, G. C., Sutton, D. J. & Palframan, R. T. Neutralisation of interleukin-13 in mice prevents airway pathology caused by chronic exposure to house dust mite. *PLoS ONE* **5**, e13136 (2010).

36. Botta, D. et al. Dynamic regulation of T follicular regulatory cell responses by interleukin 2 during influenza infection. *Nat. Immunol.* **18**, 1249–1260 (2017).

37. Crawford, G. et al. Epithelial damage and tissue gammadelta T cells promote a unique tumor-protective IgE response. *Nat. Immunol.* **19**, 859–870 (2018).

38. Yao, Y. et al. Allergen immunotherapy improves defective follicular regulatory T cells in patients with allergic rhinitis. *J. Allergy Clin. Immunol.* **144**, 118–128 (2019).

39. He, J. S. et al. IgG1 memory B cells keep the memory of IgE responses. *Nat. Commun.* **8**, 641 (2017).

40. Xiong, H., Dolpady, J., Wabl, M., Curotto de Lafaille, M. A. & Lafaille, J. J. Sequential class switching is required for the generation of high affinity IgE antibodies. *J. Exp. Med.* **209**, 353–364 (2012).

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**Author contributions**

R.L.C, J.D., M.T.M. and P.T.S. performed the experiments. R.L.C, A.D., S.B.L. and P.T.S. analyzed the data. B.R.B., V.K.K. and A.H.S provided key technical help and reagents. P.T.S. conceived of the project and wrote the manuscript. All the authors edited the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Methods

Mice. Studies of Foxp3\textsuperscript{GFP-Cre} mice on the C57Bl/6 background have been published previously\textsuperscript{17}. Foxp3\textsuperscript{GFP-Cre}\textsuperscript{+}\textsuperscript{LOX-STOP} and Foxp3\textsuperscript{GFP-Cre}\textsuperscript{+}\textsuperscript{MOG35–55} mice on the C57Bl/6 background were from Jackson Laboratories. IgG1-PBS mice were a kind gift from Hartmut Wekerle\textsuperscript{18}. Cxcr5\textsuperscript{GFP-Cre}\textsuperscript{+}\textsuperscript{LOX-STOP} and Cxcr5\textsuperscript{GFP-Cre}\textsuperscript{+}\textsuperscript{M1/70} knockin mice were generated by constructing a targeting vector in which an IRES-Frt.PGKNeo-Frt.LOX-P-STOP.LOX-P-hsgef sequence was placed directly downstream of the stop sequence of Cxcr5. The targeting vector was introduced into C57Bl/6 embryonic stem cells by electroporation, and the resulting neo-resistant embryonic stem cells were screened for homologous recombination. Positive clones were microinjected into albino-B6 blastocysts and implanted into pseudopregnant female mice to generate chimeras. Germline transmission was achieved and mice were bred to Flp-carrying mice to remove the NEO cassette\textsuperscript{19}. The resulting mice were then bred to Foxp3\textsuperscript{GFP-Cre} mice to generate the T\textsubscript{FH}–DTR colony. Mouse progeny were routinely screened for leakiness of the Foxp3\textsuperscript{GFP-Cre} allele by flow cytometry. All mice were used according to Brigham and Women's Hospital Animal Care and Use Committee and National Institutes of Health guidelines.

Immunization. Mice were immunized with 100 μg NP-OVA (Biosearch Technologies) or 100 μg MOG35–55 (UCLA Biopolymer Facility) emulsified in incomplete Freund's adjuvant subcutaneously in the mouse flanks as previously described\textsuperscript{20,21}, unless otherwise noted. For memory studies, mice were immunized with 100 μg NP-OVA mixed with Addavax MF59-like adjuvant (InvivoGen) subcutaneously in one flank. Then, 30 d later mice received a boost of 100 μg NP-OVA in PBS intraperitoneally. For allergy studies, mice were sensitized with 10 μg HDM (Greer Labs) in PBS intranasally and then challenged with 10 μg HDM in PBS intranasally on days 7, 8, 9, 10 and 11, followed by harvesting at day 15. In some cases mice received 1 μg PT in PBS intraperitoneally to delete T\textsubscript{FH} cells at indicated timepoints.

Antibodies. The following antibodies were used for surface staining at 4 °C for 30 min: anti-CD4 (Biolegend, 1:200, RM4-5), anti-ICOS (Biolegend, 1:200, 15F9), anti-CD19 (Biolegend, 1:200, 6D5), anti-p-1 (1:200, RMP1-30), anti-CXCR5 biotin (BD Biosciences, 1:100, 2G8), anti-GL7 (BD Biosciences, 1:200, GL-7), anti-HB-EGF/DTR (R&D Systems, 1:200, AF-259-NA), anti-CX3D8 (Biolegend, 1:200, 90), anti-CD138 (Biolegend, 1:200, 281-2), anti-IA (Biolegend, 1:200, M5/114.15.2), anti-SiglecF (BD Biosciences, 1:200, E50-2440), anti-CD5a (Biolegend, 1:200, 2G3.67), anti-CD11c (Biolegend, 1:200, N418) and anti-CD11b (Biolegend, 1:200, M1/70). For CXCR5 detection, streptavidin-BV421 (Biolegend, 1:400, 405225) was used at 4 °C. In some cases, anti-IgE (BD Biosciences, 1:200, R35-72) was included to block IgE bound to cell surfaces. For intracellular staining, samples were fixed with the Foxp3 Fix/Perm buffer set according to the manufacturer's instructions, stained with 10 μg/ml of HDM, and resuspended in PBS supplemented with 1% fetal bovine serum and 1 mM m filters. Cells and supernatants were collected for flow cytometric and ELISA. For total IgG and IgE levels in serum, Maxisorp (Nunc) plates were coated with HDM-specific antibody ELISAs, Maxisorp plates were coated with NP-BSA (Biosearch Technologies), followed by the secondary reagents above. Anti-HDM IgE kits (Chondrex) were used for HDM-specific antibody quantification.

Autoantigen arrays. Autoantibody reactivities against a panel of autoantigens were measured using an autoantigen microarray platform developed by the University of Texas Southwestern Medical Center. Genepix Pro v6.0 software was used to analyze IgG or IgE fluorescence. The antibody score was generated through the following equation: logₐ (net fluorescence intensity x signal-to-noise ratio + 1). The Mann–Whitney U-test was used to determine statistical significance.

RNA-seq. RNA-seq was performed as described previously\textsuperscript{22}. Briefly, RNA was isolated using MyOne Silane Dynabeads (Thermo Fisher Scientific). RNA was fragmented, and barcoded using 8-basepair barcodes together with standard Illumina adaptors. Primers were removed using Agencourt AMPure XP bead cleanup (Beckman Coulter/Agence) and samples were amplified with 14 PCR cycles. Libraries were gel purified and quantified using a Qubit high-sensitivity DNA kit (Invitrogen) and library quality was confirmed using Tapestation high-sensitivity DNA DNA tapes (Agilent Technologies). RNA-seq reactions were sequenced on an Illumina NextSeq sequencer (Illumina) according to the manufacturer's instructions, sequencing 50-basepair reads. Analysis was performed using the CLC Genomics Workbench v8.0.1 RNA-seq analysis software package (Qiagen). Briefly, reads were aligned (mismatch cost = 2, insertion cost = 3, deletion cost = 3, length fraction = 0.8, similarity fraction = 0.8) to the mouse genome and differential expression analysis was performed (total count filter cutoff = 5.0). Results were normalized to reads per million. Gene-e (Broad Institute) was used to generate heatmaps. The datasets generated during the current study are available on the Gene Expression Omnibus (GSE134153) and are available from the corresponding author on reasonable request.

Gene set enrichment analysis. RNA-seq data were compared with T\textsubscript{FH} signatures\textsuperscript{23}, T\textsubscript{FH} signatures\textsuperscript{24} or T\textsubscript{FH} signatures\textsuperscript{25} using default settings in gene set enrichment analysis (GSEA) software (Broad Institute).

Histology. Paraffin-embedded lung sections were stained with hematoxylin and eosin or periodic acid–Schiff. Images were taken on an Olympus BX41 microscope and Olympus DP26 camera and images obtained using Olympus CellSens v1.1 software. Lung inflammation was scored as: no inflammation: 0; perivascular/ peribronchial inflammation without infiltration of airway/vessel walls: 1; infiltration of airway/vessel walls without extension into alveolar parenchyma: 2; destruction of alveolar parenchyma: 3.

Statistics. Most statistical tests were performed using Prism v6.0 (GraphPad) using the two-tailed, unpaired, Student's t-test for normalized data, or the Mann–Whitney U-test for non-normal data, as indicated. Statistics for RNA-seq were performed using CLC Genomics Workbench (Qiagen). Statistics for gene set enrichment was performed at the GSEA (Broad Institute). All measurements were taken from distinct samples, except for memory experiments in which the same mice were bled before and after boost.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The data that support the findings of this study are available from the corresponding author upon request. Transcriptomic data have been deposited in the Gene Expression Omnibus with the accession code GSE134153.

References

41. Bettelli, E. et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 441, 235–238 (2006).
42. Litzenburger, T. et al. B lymphocytes producing demyelinating autoantibodies: development and function in gene-targeted transgenic mice. J. Exp. Med. 188, 169–180 (1998).
43. Sage, P. T. & Sharpe, A. H. In vitro assay to sensitively measure TFR suppressive capacity and TFR stimulation of B cell responses. Methods Mol. Biol. 1291, 151–160 (2015).
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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

✔ n/a □ Confirmed

☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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☐ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection |
|-----------------|
| BD FACSDiva software, Softmax Pro, Illumina NextSeq Software Suite, Olympus CellSens v1.1, Nikon Elements Acquisition Software AR 5.02, Genepix Pro v6.0 |

| Data analysis |
|---------------|
| Flowjo v9, Graphpad Prism v7, Softmax Pro, CLC Genomics v9, Imagej v. 2.0.0, GSEA v3.0 |

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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- A list of figures that have associated raw data
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Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences
Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size  No sample size calculations were performed. Sample sizes were chosen based on empirical data, previous publications and availability of mice.

Data exclusions  Analysis of mice included QC steps during flow cytometric analysis, samples which had inconsistencies in genotype confirmation or showed evidence of leesiness of alleles (pre-established to be a B cell frequency below 40%) were not analyzed further.

Replication  All experiments were performed multiple independent times with separate mice. Littermates were used for experimental and control mice within experiments. All attempts at replication were successful, no experiment could not be replicated.

Randomization  Randomization is not relevant, mendelian genetics determined experimental and control groups. All controls were from littersmates.

Blinding  Histology was scored in a blind manner. Other experiments were not performed blinded because the same analysis gates and cutoffs were used for all samples regardless of group.

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Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your studies. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a                             | Involved in the study |
| □ Antibodies                    | □ ChIP-seq |
| □ Eukaryotic cell lines         | □ Flow cytometry |
| □ Palaeontology                 | □ MRI-based neuroimaging |
| □ Animals and other organisms   |         |
| □ Human research participants   |         |
| □ Clinical data                 |         |

**Antibodies**

Antibodies used

- Antibodies. The following antibodies were used for surface staining at 4°C for 30 minutes: anti-CD4 (Biolegend, 1:200, RM4-5, 100540, Lot# B2691856), anti-iCOS (Biolegend, 1:200, 1595, 10706, Lot# B259751), anti-CD19 (Biolegend, 1:200, 6D5, 115530, Lot# B276004), anti-PD-1 (Biolegend, 1:200, RMPI-31, 109110, Lot# B63885), anti-CKCR5 (BD Biosciences, 1:100, 2G8, 551960, Lot# B285670), anti-IL-7 (BD Biosciences, 1:200, 5L-7, 553666, Lot# B261880), anti-FAS (BD Biosciences, 1:200, Jo2, 553653, Lot# 9039631), anti-H8-EGF/DTK (R&D Systems, 1:200, AF-259-NA, AF-259-NA, Lot# PX1017051), anti-CD38 (Biolegend, 1:200, 90, 10720, Lot# B240225), anti-CD138 (Biolegend, 1:200, 281-2, 142506, Lot# B268275), anti-IA (Biolegend, 1:200, M5/114.15.2, 107620, Lot# B252427), anti-SiglecF (BD Biosciences, 1:200, E50-2440, 565527, Lot# 7248417), anti-CD8a (Biolegend, 1:200, 53-6.7, 107714, Lot# B247008), anti-CD11c (Biolegend, 1:200, N418, 117310, Lot# B262130), anti-CD11b (Biolegend, 1:200, 12.101, Lot# B253922). For further CKCR5 detection, streptavidin-BV421 (Biolegend, 1:400, 405225, Lot# B274677) was used at 4°C. In some cases, anti-iGF (BD Biosciences, 1:200, R35-72, 553413, Lot# 3081842) was included to block iGf bound to cell surfaces. For intracellular staining, samples were fixed with the Foxp3 Fix/Perm buffer set according to the manufacturer’s instructions [eBioscience]. Samples were then intracellularly stained with anti-iGF [BD Biosciences, 1:200, AR8-1, 55083, Lot# 9939865], anti-iGF [BD Biosciences, 1:200, R35-72, 564207, Lot# 7349053], anti-FoxP3 [eBioscience, 1:200, FJK-165, 2002121, Lot# 2011698], or anti-Ki67 [BD Biosciences, 1:100, 855, 561126, Lot# 8194601], anti-GLUT1 (abcam, EPR3915, ab355020, Lot# GR51090-3), anti-l-13 (eBioscience, 1:200, eblob13A, 2020-01-31, Lot# 4323739). In some cases, a donkey anti-goat A647 secondary was used [Invitrogen, 1:400, A4147, Lot# 872645]. For blocking desired cytokines in vitro, the following antibodies were added: anti-IL-4 (Biolegend, 11B11, 504121, Lot# B242087), and anti-IL-13 (R&D Systems, 38213, MAB413, Lot# AEQ1280301). For immunofluorescence the following antibodies were used: anti-actin, anti-GF [Biolegend, RR6-8C5, 108408, Lot# B200797], anti-SiglecF (BD Biosciences, ES02440, 562680, Lot# 8008651), and anti-IA as previously shown.

Validation

All antibodies used were commercially available, passed manufacturer’s quality controls, have been validated for indicated applications, and have been previously published using specific applications.

**Animals and other organisms**

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research.

Laboratory animals

- Foxp3RES-GFP, Foxp3RES-CreYFP, Foxp3DTDr, IgHMOG, Ccr5RES-IoxP-STOP-IoxP-DTR mice on the C57Bl/6 background were
Flow Cytometry

Plots

- The axis labels state the marker and fluorochrome used (e.g., CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Draining lymph nodes were passed through 70 micron filters and resuspended in PBS supplemented with 1% FBS with 1mM EDTA.

Instrument
BD LSRII, BD FACS Symphony, BD FACS Canto, BD ARIA II

Software
BD FACS DIVA (collection), FlowJo (Analysis)

Cell population abundance
Post sort analysis was done by re-running collection tubes on cell sorter.

Gating strategy
All samples were pregated on SSA-SSH singlets, FSC-SSA for lymphocyte population. Gating strategies are included in figures.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.