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CCAAT/Enhancer-Binding Protein α Negatively Regulates IFN-γ Expression in T Cells

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Humoral immunity, including Ab switching and somatic hypermutation, is critically regulated by CD4⁺ T cells. T follicular helper (Tfh) cells have been recently shown to be a distinct T cell subset important in germinal center reactions. The transcriptional regulation of Tfh cell development and function has not been well understood. In this study, we report that C/EBPα, a basic region/leucine zipper transcription factor, is highly expressed in Tfh cells. Cebpa-deficient CD4⁺ T cells exhibit enhanced IFN-γ expression in vitro and in vivo. T cell–specific Cebpa knockout mice, although not defective in Tfh cell generation, produce significantly increased levels of IgG2a/b and IgG3 following immunization with a protein Ag. Moreover, C/EBPα binds to the Ifng gene and inhibits T-bet–driven Ifng transcription in a DNA binding–dependent manner. Our study thus demonstrates that C/EBPα restricts IFN-γ expression in T cells to allow proper class switching by B cells. The Journal of Immunology, 2014, 193: 000–000.

Antibody production is a major defense mechanism by the immune system. To generate effective abs against various pathogens, B cells need to receive cognate help from CD4⁺ T cells, especially in the germinal center (GC), in which somatic hypermutation and class-switch recombination take place (1). Class-switch recombination, by generating different isotypes of Ig that vary in binding to Fc receptors, half-lives, and activation of the complement system as well as tissue localization (2), is necessary for optimal humoral immunity. Both Th1 and Th2 cells have been shown to regulate class-switching: IL-4 is able to promote B cell proliferation and class switching, especially to IgE and IgG1, whereas IFN-γ regulates IgG2 and IgG3 Ab production. T follicular helper (Tfh) cells, which produce substantial amounts of IL-21 and IL-4, promote the production of isotype-switched, high-affinity Abs in the GC (3–7).

Th cell differentiation is programmed by lineage-specific master transcription factors (8). T-bet, encoded by Tbx21, is an essential factor to induce IFN-γ and Th1 differentiation. GATA3 is a master regulator for Th2 differentiation. Retinoic acid–related orphan receptor (ROR)-γ and ROR-α are critical transcription factors for inducing IL-17 and Th17 differentiation. B cell lymphoma 6 (Bcl6) has been shown to be a critical transcription factor that regulates the generation of Tfh cells (9–11). Bcl6 does not appear to regulate early Tfh cell generation (7), and our new data indicate that Ascl2 initiates CXCR5 expression in T cells and their migration to the B cell follicles (12).

In T-dependent responses, B cells make cognate interaction with activated CD4⁺ T cells at the T/B border and differentiate along either extrafollicular or follicular pathways. Importantly, note that this initial T/B interaction initiates the process of isotype switching toward IgG1 and IgG2a in B cells (13). Although extrafollicular Ab produced by short-lived plasma cells is of relatively low affinity, this early Ab can be important for fast protection against virus infection (14–16). IgG2a-producing B cells are enriched in the extrafollicular region and are relatively low in frequencies in GCs of normal mice, where IgG1⁺ cells prevail, possibly because IFN-γ expression is downregulated in Tfh cells (14, 17). However, IgG2a production is dramatically increased in a lupus model (Roquin−/−) in an IFN-γ–dependent manner (18). In several other mouse lupus models, including MRL/lpr mice, among the IgG isotypes elevated in the mice, IgG2a is most dramatically increased in a T cell–dependent manner and is closely linked to lupus pathogenesis (19, 20). Heightened IgG2a production may thus have implications in autoimmunity. However, mechanisms whereby Ag-specific CD4⁺ T cells regulate class switching toward IgG2 in GCs and extrafollicular regions have not fully been understood.

C/EBPα is the founding member of a family of basic region (BR)/leucine zipper transcription factor. C/EBP family proteins share highly homologous C-terminal dimerization (leucine zipper) domains and DNA binding (BR) motifs but differ in their N-terminal transcription-activation domains and bind DNA either as homo- or heterodimers (21). C/EBPα has been reported to play critical roles in lineage-specific gene regulation in several cell types during hepatic, adipogenic, granulocytic, skin, lung, and placenta development (22). However, roles of C/EBPα in T cell differentiation remain unknown.

In this study, we report that C/EBPα is highly expressed in Tfh cells compared with other Th subsets. C/EBPα suppressed Th1
and Th17 differentiation in vitro. The deficiency of Cebpa in T cells resulted in enhanced IFN-γ expression in vivo and increased Ag-specific IgG2a/b and IgG3 production. Furthermore, CEBPα binds to the Ifng gene in Th cells and suppresses T-bet-mediated Ifng gene transcription. Taken together, CEBPα expressed in T cells plays a crucial role in negative regulation of IgG2 and IgG3 Ab responses in vivo by controlling IFN-γ production. This study provides a new mechanism whereby appropriate T cell function is regulated in humoral immunity.

Materials and Methods

Mice

Cebpa TII (23) and Cd4-cre transgenic (Tg) mice (24) were provided by The Jackson Laboratory (Bar Harbor, ME) and by Dr. C.B. Wilson (Department of Immunology, University of Washington, Seattle, WA). T cell-specific Cebpa conditional knockout (KO) mice were produced by breeding Cebpa TII mice with Cd4-cre Tg mice. Screening of Cebpa conditional KO mice was carried out as previously described (23, 24). Mice 6–10 wk of age were used in experiments following protocols approved by the MD Anderson Cancer Center Institutional Animal Care and Use Committee.

Th cell differentiation and stimulation of activated T cells

CD44+CD62LhiCd4− naïve CD4+ T cells from lymph nodes and spleens of mice were purified by FACS sorting. For Th cell differentiation, naïve CD4+ T cells were stimulated with plate-bound anti-CD3 (0.5 μg/ml; 2C11, Bio X Cell) plus anti-CD28 (0.5 μg/ml; 37.51, Bio X Cell) in the presence of neutralizing Abs (10 μg/ml anti–IL-4 [1B11, Bio X Cell], 10 μg/ml anti–IFN-γ [XMG 1.2, Bio X Cell], and anti–TGF-β [1D11, Bio X Cell]) or with polarizing cytokines for Th0; 10 ng/ml IL-2, 5 ng/ml TGF-β, and anti–IL-4 for Th1; 50 U/ml human IL-2, 5 ng/ml TGF-β, and anti–IFN-γ and anti–IL-4 for inducible regulatory T cells (iTregs); 20 ng/ml IL-2, anti–IFN-γ, anti–IL-4, and anti–TGF-β for Th17-like cells. For stimulation with peptide-loaded APCs, FACS-sorted naïve CD4+ T cells were cultured with irradiated splenocytes in the presence of 10 μg/ml OT-II peptide (chicken OVA peptide 323–339). After 4 d of culture, cells were washed and restimulated with plate-bound anti-CD3 (0.5 μg/ml) for 4 h and cells were then collected for RNA extraction. For cytokine measurement by ELISA, culture supernatants were collected at 24 h. For intracellular cytokine staining, cells were restimulated with 500 ng/ml ionomycin and 50 ng/ml PMA in supernatants were collected at 24 h. For intracellular cytokine analysis, cells were spin-infected with retrovirus expressing CEBPα or control empty vector. Four days after activation, GFP+ or Ihc2+ cells were sorted by flow cytometry and gene expression was assessed by quantitative real-time PCR. For cytokine measurement, ELISA and intracellular cytokine staining were performed as described above. For proliferation assay, Vybrant DyeCycle violet stain ( Molecular Probes) was used as an indicator for cell division.

Quantitative RT-PCR

Total RNA extracted using TRIzol reagent (Invitrogen) was used to generate cDNA by PCR. Mutant Cebpa lacking the BR (287–247 aa) was cloned by PCR and expressed in vitro transcribed Cebpa RNA reverse transcriptase (Invitrogen). For quantification of cytokine, cDNA samples were amplified in iQ SYBR Green supermix (Bio-Rad Laboratories). The data were normalized to an Actb reference. The primer pairs for analysis were previously described (17). Primer pairs were as follows: Cebpa (endos plus exo), forward, 5′-TGGTTGAAGTTACAGTGGTAC-3′, reverse, 5′-CCTTGACAAGAGGAGCTCTCA-3′; Cebpa (endo), forward, 5′-ACAATCGATCTACCCAGAG-3′, reverse, 5′-CACTAGACGACACCATGC-3′.

Reporter assay

293T cells were transfected with pGFP-RV-T-bet (25) together with luciferase construct containing the human Ifng promoter (26). We generated luciferase mutant constructs (Δ 56 to 48 and Δ 51 to 48) by PCR. Total amount of plasmid DNA remained constant in each sample. These cells were further transfected with various amount of pGFP-RV-Cebpa or pGFP-RV-ΔIR Cebpa. Firefly and Renilla luciferase activity was measured with a Dual-Luciferase reporter system (Promega). Data were normalized by the activity of Renilla luciferase.

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were done as described (27, 28) with anti-H3K36me3 Ab (Upstate Biotechnology), anti-H3K27me3 (Upstate Biotechnology), anti–RNA polymerase II (Santa Cruz Biotechnology), anti–C/EBPβ, or anti–H3K4me3 antibodies. For analysis of Cebpa expression, draining lymph node (dLN) cells and splenocytes from immunized mice were measured with ELISA. Serum samples were added in a 3-fold serial dilution onto plates precoated with OVA, KLH, or NP-BSA (NP-BSA for high affinity and NP26-BSA for low affinity). Ag-specific Abs were detected with HRP-conjugated goat anti-mouse IgM or rat anti-mouse IgG Abs (SouthernBiotech). The relative affinity of anti-NP Abs was estimated by calculating a NA/AP ratio (NA/NP26-BSA/(NA+NP26-BSA)). For analysis of Tfh, cells were stimulated as triplicates with 0, 5, 20, and 100 μg/ml KLH or 0, 4, 20, and 100 μg/ml OVA. After 4 d of restimulation, measurement of IFN-γ and IL-17 was carried out by ELISA. For intracellular cytokine staining, dLN cells and splenocytes from immunized mice were restimulated with 0 and 100 μg/ml KLH or 0 and 250 μg/ml OVA for 24 h. PMA and ionomycin or KLH-loaded APCs were used for restimulation of ex vivo PD-1−Th (CD4+, PD-1−, and CXCR5−), extratollicular T (Tef, CD4+, PD-1+, and CXCR5+), and non-Th (CD4+, CD4+ and CXCR5+) cells to detect intracellular IFN-γ. In the last 5 h, GolgiStop (BD Biosciences) was added, and IFN-γ–producing cells were detected in the CD4+ population, described above. To measure mRNA expression or for Western blotting, CD4+ T cell subpopulations were purified by FACS sorting from dLNs on the basis of their specific markers. For mRNA measurement, cells were restimulated with anti-CD3 Ab for 4 h. Anti-C/EBPα Ab (C-18) was purchased from Santa Cruz Biotechnology for Western blotting.
**Immunohistochemical analysis**

Inguinal lymph nodes were isolated from either wild-type (WT) or Cebpa KO mice 7 d after immunization with KLH in CFA and embedded in OCT and frozen with isopentane. Staining of peanut agglutinin (PNA), CD4, IgG2a, and IgG1 Abs were purchased from BD Biosciences. Cultured cells were applied on glass slides by cytospin (Thermo Scientific) and then fixed and permeabilized. Anti-hemagglutinin Ab (6E2) (Cell Signaling Technology) was used for staining.

**Statistical analysis**

All data are represented as means ± SD, and statistical analysis was performed with the Student t test. Differences were recognized as significant with a p value <0.05.

**Results**

**C/EBPα is highly expressed in Tfh cells**

In the gene expression microarray analysis, Cebpa mRNA expression was higher in CXCR5+ Tfh cells than in Th1, Th2, and Th17 cells (17). We thus confirmed Cebpa mRNA expression in Th1, Th2, Th17, iTregs, and Tfh-like cells by real-time RT-PCR analysis. We cultured naive CD4+ T cells isolated from OT-II Tg mice under Th1, Th2, Th17, and iTReg as well as Tfh-like promoting culture conditions with OVA peptide–loading irradiated APCs. The expression of Cebpa mRNA was highly induced in Tfh-like cells but not in other Th cell lineages upon restimulation on day 5 (Fig. 1A). IL-6 or IL-21 alone was not sufficient in upregulating Cebpa mRNA expression in purified T cells (data not shown), suggesting that a collective signal from APCs and cytokines is required for its regulation.

![Image](https://www.jimmunol.org/)

**FIGURE 1.** CEBPα is highly expressed in Tfh cells. (A) CD4+ T cells isolated from OT-II TCR Tg mice were stimulated with OVA peptide-loaded APCs under Th1, Th2, Th17, iTReg, and Tfh cell–like skewing conditions for 5 d. The cultured cells were restimulated with anti-CD3 Ab. Then, Cebpa mRNA expression was measured by quantitative PCR analysis. Data were normalized to β-actin. The expression in naive CD4+ T cells was determined as one. (B) B6 mice (n = 3) were immunized with KLH/CFA. After 7 d, CXCR5+ Tfh and CXCR5+ non–Tfh CD4+ cells were isolated from CD4+CD44+ dLN and analyzed. Tfh cells expressed higher levels of mRNA for Cxcr5, Bcl6, and Cebpa than did non–Tfh cells (Fig. 1B). In addition to the mRNA analysis, the expression of C/EBPα in naive T, non–Tfh, and Tfh cells was further confirmed at the protein level (Fig. 1C). Tfh cells, which regulate extrafollicular Ab response, have been identified as PD-1+CXCR5+ CD4+ T cells outside of follicles (14). Tfh cells but not PD-1 and CXCR5 double-negative cells expressed mRNA for Cebpa and Bcl6, although their expression levels were less than those of PD-1+ Tfh cells (Fig. 1D). These data indicate that C/EBPα is highly expressed both in Tef and PD-1+ Tfh cells in vivo.

C/EBPα negatively regulates IFN-γ expression as well as IgG2a/b and IgG3 Ab response in vivo

Because C/EBPα is highly expressed in Tfh cells (Fig. 1), we speculated that C/EBPα may regulate the generation or function of Tfh cells. To examine this, we crossed Cebpa Tg mice with Cd4-Cre Tg mice. Staining for CD4 and CD8 in the thymus and for CD62L and CD44 in peripheral CD4+ T cells did not show any abnormality of T cell subsets in the absence of Cebpa. The frequency of CD4+Foxp3+CD25+ Tregs was not changed, indicating no obvious defect in T cell development as a result of Cebpa deficiency (Supplemental Fig. 1A). When we evaluated Cebpa gene deletion at genomic and mRNA levels, Cebpa was preferentially disrupted in CD4+ T cells but not in B cells and dendritic cells (Supplemental Fig. 1B). When we measured the expression of Cebpb and Cebpd in naive, Tef, and PD-1+ Tfh cells isolated from Cebpa Tg (WT) and Cebpa Tg/Cd4-Cre Tg (KO) mice immunized with KLH in CFA, we did not find they were changed in the absence of Cebpa (Supplemental Fig. 1C), indicating that the functional defects caused by Cebpa deficiency in T cells may not be compensated by increased expression of C/EBPβ and C/EBPδ in vivo.

To examine the function of CEBPα in T cells, WT and Cebpa KO mice were immunized with KLH plus CFA. After 7 d, GC B cells were detected by staining for GL7 and Fas as well as CXCR5 and Bcl6 in B200+ dLN cells. CXCR5 with BTLA or Bcl6 staining was used to identify Tfh cells in dLNs. GC B cells and Tfh cells developed normally in Cebpa-deficient mice (Fig. 2A, Supplemental Fig. 1D). Additionally, we analyzed the localization of GC B cells and Tfh cells either in WT and KO mice by immunohistochemical staining with PNA (brown) and anti-CD4 (blue). There was no significant difference between the two groups of mice (Supplemental Fig. 1E), suggesting that the KO T cells were able to migrate properly and induce GC reactions. The expression of Tfh-related genes such as Bcl6, Cxcr5, Il21r, Il6ra, Il6st, Icos, Il22, Sap, Cdh40lg, and Il4 was further analyzed by real-time RT-PCR in Tfh cells isolated from immunized WT and Cebpa KO mice. Whereas C/EBPα expression in Tfh cells isolated from KO mice was completely eliminated, the expression levels of the Tfh-related genes were found to be comparable in Cebpa-deficient and -sufficient Tfh cells (Supplemental Fig. 1F and data not shown), indicating that C/EBPα is not necessary for the development of Tfh cells and their migration and/or localization.

We further analyzed KLH-specific Ab production and found IgG2a/b and IgG3 production in sera of immunized Cebpa KO mice to be significantly enhanced (~2 to 3-fold at 135, 405, and 1215 dilution for IgG2a, at 5, 15, 45, and 135 dilution for IgG2b, and at 10, 30, 90, 270, and 810 for IgG3) compared with that of WT mice, whereas the production of other Ab isotypes such as IgM and IgG1 was similar between WT and Cebpa KO mice.
C/EBPα function in T cells

To understand the mechanism underlying the enhanced IgG2 and IgG3 production in KO mice, splenocytes from immunized WT and Cebpa KO mice were stimulated with various concentrations of KLH protein. IL-2 expression was measured by ELISA on day 1 and incorporation of [3H]thymidine on day 3 of restimulation. Cebpa KO T cells did not show any difference of IL-2 production or proliferation when compared with WT cells (Supplemental Fig. 3A). Because the class switching of IgG2 and IgG3 is regulated by IFN-γ (30–33), IFN-γ and IL-17 production by KLH-specific T cells in immunized mice was detected by ELISA and intracellular cytokine staining (ICS). In response to KLH restimulation, Cebpa KO CD4+ T cells in dLNs expressed higher levels of IFN-γ and IL-17 than did WT cells by ELISA and ICS (Fig. 3A, 3B). Taken together, C/EBPα appears to negatively regulate IFN-γ and IL-17 expression in Ag-specific T cell responses in vivo.

We next asked which T cell populations contributed to the enhanced IFN-γ expression in Cebpa KO mice. Thh, Tef, and non–Tfh cells were isolated from WT or Cebpa KO mice after immunization and stimulated with PMA plus ionomycin or KLH-loaded APCs. The frequencies of IFN-γ Thh, IFN-γ Tef, and IFN-γ non–Tfh cells were increased 2- to 3-fold in the KO mice compared with those in WT mice upon stimulation (Fig. 3C). These data indicate that IFN-γ expression in effector CD4+ T cells localized both in outside and inside of follicles was enhanced in the absence of Cebpa and that the suppressive activity of C/EBPα in IFN-γ expression might not be restricted to Tfh cells.

To further confirm the suppressive role of C/EBPα in IFN-γ expression in vivo, we immunized WT and Cebpa KO mice with OVA in aluminum sulfate, which induces Th2-biased immune response. Although the generation of GC B cells, Tfh cells, and Tef cells was not affected (Fig. 4A), OVA-specific IgG2a/b production was enhanced in the absence of C/EBPα in T cells (Fig. 4B). Furthermore, upon restimulation with OVA, Cebpa KO CD4+ T cells in spleen expressed higher levels of IFN-γ than WT cells by ELISA and ICS (Fig. 4C). These results further emphasize the importance of suppressive activity of C/EBPα in IFN-γ expression in T cells.

C/EBPα inhibits Th1 and Th17 cell differentiation

The results from the above in vivo experiment indicate that C/EBPα is not required for Tfh cell generation but may negatively regulate their IFN-γ expression. To further confirm the role of C/EBPα in Th cell differentiation, naive CD4+ T cells were isolated from WT and the KO mice by FACs sorting and stimulated with anti-CD3 and CD28 Abs under Th1, Th2, and Th17 skewing condition. After 4 d, cells were restimulated and cytokine expression was detected by ELISA and ICS. Under Th1 and Th17 conditions, Cebpa KO CD4+ T cells exhibited enhanced amounts of IFN-γ and IL-17, respectively. In contrast, loss of C/EBPα did not affect IL-4 production under Th2 condition (Fig. 5A). Similar results were obtained by ELISA assay (Fig. 5B). To further address whether C/EBPα inhibits Th cell differentiation, the expression of master transcription factors were measured by quantitative RT-PCR. Thx21 and Gata3 mRNA expression in Th1 or Th2 cells, respectively, was not changed by
thermore, the retroviral introduction of C/EBPα suppressed IFN-γ expression in WT and Cebpa KO CD4+ T cells cultured under Th1 conditions to the same levels (Supplemental Fig. 3D). Overexpression of C/EBPα in the Th17 condition also resulted in the suppression of Th17-related genes such as Il17a, Il17f, and Rorc (Fig. 6B, lower). These results indicate that C/EBPα functions to suppress both Th1 and Th17 cell differentiation.

C/EBPα expression was significantly enhanced in Cebpa-deficient T cells, whereas T-bet expression was not affected, and C/EBPα overexpression inhibits IFN-γ but not T-bet expression. Based on these results, we speculated that C/EBPα may antagonize T-bet function in activating Ifng transcription. To confirm the requirement of DNA binding by C/EBPα in suppression of IFN-γ, we deleted the DNA binding motif of C/EBPα and retrovirally introduced WT and mutant C/EBPα into CD4+ T cells cultured in Th1-skewing condition, finding that the exogenous expression level of mutant C/EBPα was almost equal to that of WT. Overexpression of mutant C/EBPα failed to suppress IFN-γ expression at both the protein and mRNA levels, whereas WT CEBPα efficiently suppressed Th1 differentiation (Fig. 7A). To verify C/EBPα binding in the Ifng locus in vivo, we performed ChIP analysis with anti-C/EBPα Ab in Th cells isolated from immunized B6 mice by cell sorting. The possible regulatory regions of the Ifng gene have been previously identified as CNS (34, 35). We found that C/EBPα binding exist in each CNS such as Ifng promoter, CNS-55, CNS-34, CNS-22, CNS1 (CNS-5), and CNS2 (CNS+17) but not to the genomic region of H19 imprinting control region (H19 ICR), whereas the binding was not observed in WT non–Tfh cells and Cebpa KO Tfh cells (Fig. 7B). This result confirms that C/EBPα directly bind to the Ifng gene locus in ex vivo Tfh cells. We next examined whether C/EBPα suppresses the transcription of the Ifng regulated by T-bet. We carried out a reporter assay using the Ifng promoter (26), which has been reported to contain two conserved putative binding sites for T-bet (Supplemental Fig. 4A). In line with a previous report (36), our data suggest that the T-bet recognition site possibly overlaps with the C/EBPα site. Alternatively, the DNA binding mutant of C/EBPα failed to suppress Ifng promoter activity (Fig. 7C, lower). Of note, the failure of the suppression of Ifng promoter activity and IFN-γ expression by the Δ BR mutant was not due to lack of nuclear localization (Supplemental Fig. 4B). Furthermore, overexpression of C/EBPα resulted in reduced accumulation of RNA polymerase on Ifng promoter without affecting histone modifications (Supplemental Fig. 4C). These data suggest that C/EBPα suppresses IFN-γ expression at the transcription level.

Bcl6 has been reported to be a suppressor of IFN-γ expression (10). We finally examined whether Bcl6 and C/EBPα cooperatively suppress IFN-γ expression. Naive OT-2 CD4+ T cells were stimulated under Th1 skewing condition and infected with Bcl6 and/or C/EBPα. After 4 d of culture, doubly infected cells were sorted and restimulated. Single induction of either Bcl6 or C/EBPα significantly suppressed IFN-γ production. In the dual overexpression, C/EBPα and Bcl6 more potently suppressed IFN-γ expression, although TNF-α expression was not affected by the overexpression (Fig. 7D). These data indicate that C/EBPα suppresses T-bet-mediated Ifng gene transcription in collaboration with Bcl6.

FIGURE 3. Enhanced IFN-γ and IL-17 production in T cells from immunized Cebpa KO mice. (A) Total dLN cells were isolated from WT (n = 4) and Cebpa KO mice (n = 4) after immunization. Then, cells were stimulated with indicated concentrations of KLH. IFN-γ and IL-17 were measured by ELISA after 7 or 5 d of treatment, respectively. (B) The cells isolated in (A) were stimulated with 0 or 100 μg/ml KLH. After 24 h, IFN-γ and IL-17 were detected in CD4+ cells by ICS (upper). Frequency of IFN-γ- and IL-17+ cells was shown by combining three experiments (lower). Data are the means of three independent experiments. (C) PD-1hi Tfh, Tef, and non–Tfh cells were isolated from WT (n = 4) and Cebpa KO mice (n = 4) after 7 d of immunization. IFN-γ expression was detected in Tfh, Tef, and non–Tfh cells stimulated with PMA and ionomycin or KLH-loaded APCs by ICS (left). Frequency of these IFN-γ-expressing T cell populations was shown by combining results from two independent experiments (lower). *p < 0.05, **p < 0.01.
Discussion

C/EBPα has been reported to regulate multiple biological events, including differentiation of myeloid lineage, cell proliferation, metabolism, and leukemogenesis. However, a role of C/EBPα in T cells remains unclear. In this study, we report that C/EBPα is highly expressed by Tfh cells. Although not required for Tfh cell differentiation, C/EBPα is a potent suppressor of Th1 and Th17 differentiation. Following KLH CFA immunization, we found an augmented IgG2a/b and IgG3 production in Th1 and Th17 differentiation. In Th17 skewing condition, ectopic expression of C/EBPα could not induce expression of these genes (data not shown). Histological analysis also indicated normal migration of Cebpa-deficient T cells into GCs. One of the crucial roles of Tfh cells is facilitating affinity maturation of Abs in B cells. We observed normal affinity maturation in the KO mice upon NP-KLH immunization. These data suggest that Cebpa-deficient T cells developed normally into Tfh cells and provided similar help to B cells as do WT T cells. This idea is further supported by the observation that the frequency of Bcl6+ GC B cells and their expression level in T cell–specific Cebpa KO mice were comparable to those in WT mice after immunization.

Alternatively, production of IgG2a/b and IgG3 was enhanced in inside and outside of follicles of immunized Cebpa KO mice. Consistent with this, increased IFN-γ expression was observed in PD-1hi Tfh, Tef, and non–Tfh cells isolated from Cebpa KO mice, although we did not observe the developmental defect of Tef cells in the KO mice after immunization (data not shown), indicating that C/EBPα may contribute to proper Ab production by suppressing IFN-γ in T cells both outside and inside of follicles. These results also suggest a suppressive effect of C/EBPα on IFN-γ expression in various CD4+ T cell subpopulations, despite higher expression of C/EBPα in Tfh cells.

C/EBPα was found to be a strong suppressor of Th1 and Th17 differentiation. In Th17 skewing condition, ectopic expression of C/EBPα suppressed the expression of Rorc as well as of Il17. Consistent with this observation, IL-17 and ROR-γ expression was enhanced in Cebpa KO T cells cultured under Th17 skewing condition. In contrast, in Th1 culture, IFN-γ was a strong inducer of Rorc expression in WT Tfh cells.

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expression but not T-bet was inhibited by enforced expression of C/EBPα. Additionally, Cebpa KO T cells exhibited an enhanced activity to express IFN-γ but not T-bet. These findings suggest that C/EBPα is able to suppress Th17 differentiation by suppressing the expression of a key transcription factor. Meanwhile, whereas T-bet expression is not affected by C/EBPα, T-bet–mediated IFN-γ transcription is inhibited by C/EBPα. This idea is supported by our observations that C/EBPα bound to Ifng promoter as well as other regulatory CNS regions in Tfh cells generated in vivo and that ΔBR C/EBPα, which cannot bind to its target sequence, failed to repress IFN-γ expression as well as Ifng promoter activity induced by T-bet. Furthermore, C/EBPα suppressed DNA polymerase II recruitment on Ifng promoter, without affecting histone modifications. Taken together, C/EBPα suppresses IFN-γ expression at the transcriptional level. Cebpβ expression was not affected in the absence of Cebpa, and Cebpd expression was modestly increased in Cebpa KO naïve, Th1, and Th2 cells but not in Th17 cells, iTregs, and Thh-like cells (data not shown). Therefore, C/EBPβ or C/EBPδ may not compensate for Cebpa deficiency. In our previous report, Bcl6 was demonstrated to be a crucial suppressor of IFN-γ production as well (10). Indeed, in our double overexpression experiment, C/EBPα and Bcl6 had additive effects in suppressing IFN-γ expression in Th1 cells. Thus, these observations indicate that C/EBPα plays a crucial role in antagonizing IFN-γ at the transcription level in a coordinated way with Bcl6 during Tfh differentiation. Additionally, although Th1 cells and Th17 cells in vitro and non–Tfh cells in vivo express lower levels of C/EBPα than do Tfh cells, it could still suppress IFN-γ and IL-17.

Our data show that Tfh cells express higher levels of C/EBPα than do other Th cell subsets both in vitro and in vivo, and thus...
C/EBPa is thought to be a novel useful marker to identify Tfh cells among CD4+ T cell subsets. Interestingly, human Tfh cells also highly express C/EBPa (37). Further analysis of regulatory mechanisms governing C/EBPa during Tfh cell commitment may reveal novel regulation of this differentiation process.

Tfh cells are a newly identified T cell subset whose regulation is not well understood. Bcl6 has been previously shown to be a key transcription factor not only governing Tfh cell development, but also antagonizing the alternate T cell differentiation processes. In this study, our results have demonstrated C/EBPa, as the second transcription factor selectively expressed in Tfh cells, in regulation of a proper Ab response by suppressing IFN-γ expression.

Our observations may aid a better understanding of humoral and pathogenesis of autoimmune disorders.

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Disclosures
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