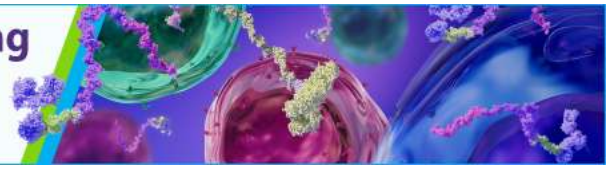

The Journal of Immunology

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Cutting Edge

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Cutting Edge: IL-27 Induces the Transcription Factor c-Maf, Cytokine IL-21, and the Costimulatory Receptor ICOS that Coordinately Act Together to Promote Differentiation of IL-10-Producing Tr1 Cells[1]

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IL-27 has recently been identified as a differentiation factor for the generation of IL-10-producing regulatory type 1 (Tr1) T cells. However, how IL-27 induces the expansion of Tr1 cells has not been elucidated. In this study we demonstrate that IL-27 drives the expansion and differentiation of IL-10-producing murine Tr1 cells by inducing three key elements: the transcription factor c-Maf, the cytokine IL-21, and the costimulatory receptor ICOS. IL-27-driven c-Maf expression transactivates IL-21 production, which acts as an autocrine growth factor for the expansion and/or maintenance of IL-27-induced Tr1 cells. ICOS further promotes IL-27-driven Tr1 cells. Each of those elements is essential, because loss of c-Maf, IL-21-signaling, or ICOS decreases the frequency of IL-27-induced differentiation of IL-10-producing Tr1 cells. ***The Journal of Immunology,*** **2009, 183: 797–801.**

Interleukin-27, a member of the IL-12/IL-23 heterodimeric family of cytokines produced by APCs, is composed of two chains, IL-27p28 and EBV-induced gene 3 (1). Activated T cells and NK cells have the highest expression of IL-27R, which is composed of two chains, a specific IL-27Ra chain (WSX-1 or TCCR) and a signaling chain, gp130, that it shares with IL-6R (1). Initial studies have suggested that, similarly as IL-12, IL-27 induces the expansion of proinflammatory Th1 cells by activating the STAT-1-mediated T-bet pathway (1). However, analysis of the IL-27Ra$^{-/-}$ (WSX-1$^{-/-}$) mice infected with various pathogens resulted in clearance of the parasites with exaggerated T cell responses and enhanced proinflammatory cytokine production (1). Furthermore, IL-27Ra$^{-/-}$ mice developed severe experimental autoimmune encephalomyelitis with enhanced Th17 responses (1), and treatment with rIL-27 suppressed disease and decreased the frequency of Th17 cells (1). These paradoxical observations led to the hypothesis that IL-27 may not be necessary for the generation of proinflammatory T cells (Th1 or Th17), but rather play a crucial role in regulating T cell responses. Subsequently, three groups, including ours, reported that IL-27 not only inhibited the generation of Th17 cells but also induced differentiation of IL-10-producing regulatory T cell type 1 (Tr1)[3] from naive T cells (2–4).

Tr1 cells are a subset of T cells that have strong immunosuppressive properties, predominantly produce IL-10 with variable amounts of IFN-γ, but do not express Forkhead box 3 (Foxp3) (5). Adoptive transfer of Tr1 cells has been shown to suppress autoimmunity, colitis, graft-vs-host disease, and tissue inflammation (6). Although initial studies suggested that Tr1 cells are induced by repetitive antigenic stimulation of T cells in the presence of IL-10 (6), T cells differentiated in the presence of IL-10 could not be propagated long term in culture. The identification of IL-27 as a differentiating factor for the generation of Tr1 cells provided a means by which they could be grown in large numbers and facilitated their functional analysis. However, the molecular mechanisms by which IL-27 mediates the generation and/or expansion of Tr1 cells are not well understood. Thus, we analyzed the expression of various key cytokines and transcription factors induced by IL-27. Our results show that IL-27 is a potent inducer of three essential elements: the transcription factor c-Maf, the cytokine IL-21, and the costimulatory

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[3] Abbreviations used in this paper: Tr1, regulatory T cell type 1; Foxp3, Forkhead box P3; TFh, T follicular helper cell; WT, wild type.

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receptor ICOS, which coordinately work together to promote differentiation of Tr1 cells.

Materials and Methods

Mice and reagents

IL-10-enhanced GFP reporter mice (Vert-X), *Foxp3.gfp* "knock-in" mice, ICOS$^{-/-}$ mice on C57BL/6 background, were generated as described (7, 8). WSX-1$^{-/-}$ mice on C57BL/6 background were obtained from C. Saris (Amgen, Thousand Oaks, CA), and IL-21R-deficient mice on NOD background (9) from N. Sarvetnick (Scripps Research Institute, La Jolla, CA). c-Maf$^{-/-}$ mice on an N5 BALB/c background have been described (10). Mice were housed in conventional, pathogen-free facilities at the Harvard Institute of Medicine (Boston, MA). All experiments were undertaken in accordance with guidelines from the Committee on Animals at Harvard Medical School (Boston, MA).

T cell differentiation and proliferation in vitro

Naive CD4$^+$ T cells (CD4$^+$CD62L^{high}CD25$^-$), pooled from both spleen and lymph nodes, or memory cells (CD4$^+$CD44$^+$), obtained from lymph nodes, were purified by flow cytometry and stimulated with plate-bound Ab against CD3 (145-2C11; 1 μg ml^{-1}) and CD28 (PV-1; 1 μg ml^{-1}). Cells were cultured as previously described (8). Monoclonal anti-TGF-β1 Ab (10 μg ml^{-1}), mouse IL-21 (80 ng ml^{-1}), mouse IL-27 (25 ng ml^{-1}), and anti-mouse IL-21 Ab (25 μg ml^{-1}) were all purchased from R&D Systems. Proliferation assay was performed as previously described (8).

Measurement of cytokines

Secreted cytokines were measured after 48 h by cytometric bead array (BD Biosciences) or ELISA. Intracellular cytokine staining was performed by as previously described (8).

Quantitative real-time PCR

RNA was extracted with RNeasy mini kits (Qiagen) and analyzed by real-time PCR according to the manufacturer's instructions (Applied Biosystems). The following primers/probe mixtures were purchased from Applied Biosystems: IL-10 (catalog no. Mm 00439615_g1); ICOS (catalog no Mm004497600_m1); c-Maf (catalog no Mm 02581355_S1); IL-21 (catalog no. Mm00517640_m1); and IL-21R (catalog no. Mm00600319_M1).

Luciferase assay

HEK 293T cells (10^5) were cotransfected with pGL3-IL-21-Luc reporter plasmid and *Renilla* luciferase reporter plasmid (pRL-TK) and vector pcDNA3.1, pcDNA3.1(hemagglutinin-c-Maf), pcDNA3.1(T-bet), or pcDNA3.1(human GATA3). Cells were collected 24 h post-transfection and IL-21 promoter activities were analyzed using Dual-Glo luciferase assay system (Promega) according to the manufacturer's instructions. The luciferase activities were normalized against the *Renilla* luciferase activity.

Fetal thymic organ culture

Thymi from c-Maf$^{-/-}$ fetuses were removed on embryonic day 16.5 and individual lobes were cultured for 7 days. Genotyping was performed using DNA isolated from the fetal limbs. Thymocytes were recovered on day 7 of culture after collagenase digestion. CD4$^+$CD8$^-$CD25$^-$ cells were sorted and cultured for 4 days on anti-CD3 (2 μg ml^{-1})- and anti-CD28 (2 μg ml^{-1})-coated plates.

Statistics

Statistical analysis was performed using an unpaired Student's *t* test.

Results and Discussion

Our previous studies showed that TGF-β and IL-27 acted synergistically to generate Tr1 cells. However, under such culture conditions low Foxp3 expression was also induced by TGF-β. Using IL-10-enhanced GFP reporter mice (Vert-X), we developed culture conditions under which IL-27 alone could induce Tr1 cells. These in vitro derived Tr1 cells were as suppressive as natural Foxp3$^+$ regulatory T (Treg) cells in inhibiting T cell proliferation in vitro (supplemental Fig. 1).[4]

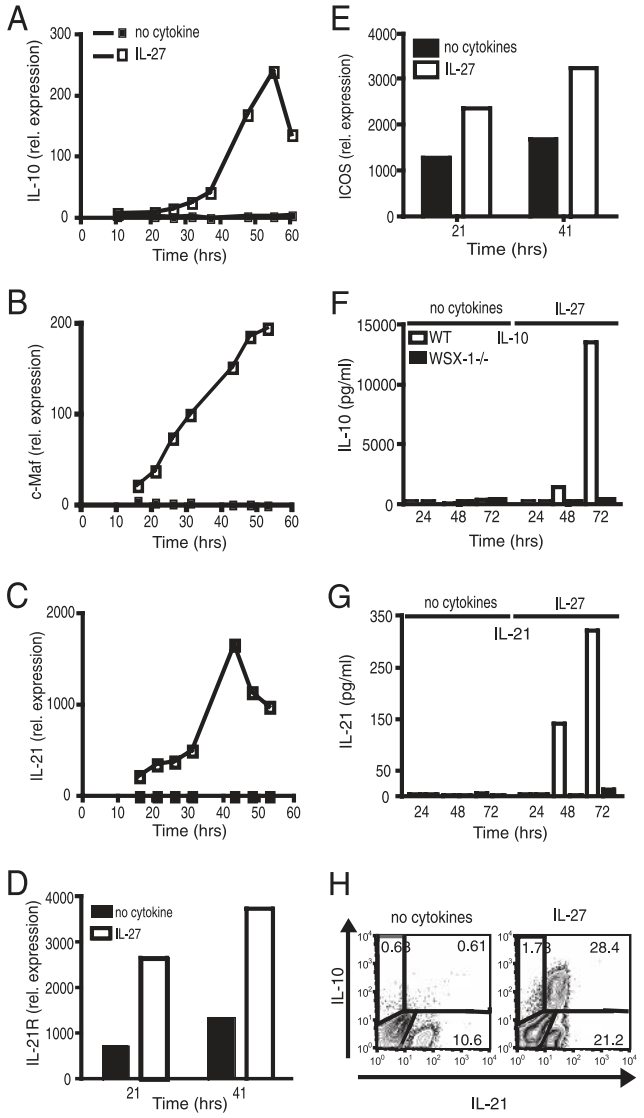

FIGURE 1. IL-27 induces c-Maf, IL-21, IL-21R, and ICOS. *A–E*, RNA isolated from naive CD4$^+$CD62L^{high}CD25$^-$ cells cultured with IL-27 (open squares) or without IL-27 (closed squares) were subjected to real-time PCR relative to the expression (rel. expression) of mRNA encoding β-actin ($2^{-\Delta C_T} \times 100{,}000$; where C_T is cycle threshold) to examine the expression of cytokines at different time points following activation. Real-time PCR of IL-10 (*A*), c-Maf (*B*), IL-21 (*C*), IL-21R (*D*), and ICOS (*E*) induction by IL-27 is shown. *F* and *G*, IL-10 and IL-21 production was measured by cytokine bead array as induced by IL-27 in WT (open bars) and IL-27Ra$^{-/-}$ (WSX-1$^{-/-}$) (filled bars) CD4$^+$CD62L^{high}CD25$^-$ cells. *H*, Intracellular cytokine staining of IL-10 and IL-21 by T cells following activation in the presence of IL-27.

To understand the molecular mechanisms by which IL-27 induces and expands Tr1 cells, we analyzed the expression of genes up-regulated by IL-27 at multiple time points following T cell activation in the presence of IL-27. As expected, IL-27 induced IL-10 mRNA expression that peaked 48 h postactivation (Fig. 1*A*). Interestingly, IL-27 induced the expression of the transcription factor c-Maf, at early time points, and this expression progressively increased over time (Fig. 1*B*). Consistent

[4] The online version of this article contains supplemental material.

with our previous observation that c-Maf regulates IL-21 expression in Th17 cells (8), we observed that IL-27 also induced IL-21 mRNA and that IL-10 and IL-21 showed similar kinetics of mRNA expression (Fig. 1, *A* and *C*). Although IL-21R expression was low in unactivated T and B cells, TCR-driven activation up-regulated this expression and IL-27 further up-regulated IL-21R expression in activated T cells (Fig. 1*D*). We (8) and others (11) have shown that the c-Maf transcription factor is downstream of ICOS, and because IL-10-producing T cells were first shown to be preferentially costimulated by ICOS (12), we examined the expression of ICOS mRNA and observed that the addition of IL-27 indeed induced higher ICOS expression than T cell activation without IL-27 (Fig. 1*E*). Thus, IL-27, in addition to inducing IL-10 production, induced c-Maf, IL-21, IL-21R, and ICOS expression. At the protein level, T cells activated in the presence of IL-27 produced both IL-10 and IL-21, thus confirming mRNA expression (Fig. 1, *F* and *G*). Besides the mRNA expression, we also observed an increase in ICOS expression induced by IL-27 at the protein level (data not shown). Furthermore, IL-27Ra$^{-/-}$ (WSX-1$^{-/-}$) mice produced no detectable IL-10 or IL-21 and did not show an increased ICOS expression upon activation, indicating that IL-10 and IL-21 production and increased ICOS expression were specifically induced by IL-27 (Fig. 1, *F* and *G*, and data not shown). To analyze the cells that produce both IL-10 and IL-21, we undertook intracellular cytokine staining for IL-10 and IL-21 after 3 days of culture in vitro with IL-27 and found that the IL-10-producing cells also produced IL-21 (Fig. 1*H*).

Because IL-21 belongs to the IL-2 cytokine family and uses the common γ-chain receptor, we hypothesized that IL-27-driven IL-21 production from T cells may be an autocrine growth factor for the generation of Tr1 cells. To test this, we first added a neutralizing IL-21 Ab in the presence of IL-27 and found that blocking IL-21 reduced the frequency of IL-10-producing T cells significantly by >75% (Fig. 2*A*) and IL-10 cytokine production in the culture supernatants by >50% (Fig. 2*B*). Further addition of IL-21 together with IL-27 increased IL-10 production, but this increase with exogenous IL-21 was modest (Fig. 2*B*). These data raised the issue of whether IL-21 could directly expand IL-10-producing T cells. However, activation of T cells from mice lacking IL-27Ra (WSX-1) signaling in the presence of IL-21 and IL-27 did not expand Tr1 cells (supplemental Fig. 2). We further confirmed the role of IL-21 in the expansion of Tr1 cells using CD4$^+$ T cells from IL-21R-deficient mice. Loss of IL-21 signaling resulted in the inhibition of IL-27-driven generation of IL-10-producing T cells by >75% (Fig. 2*C*) and IL-10 cytokine production in the culture supernatants by over 90% (Fig. 2*D*). However, loss of IL-21 signaling had no effect on IL-27-driven IFN-γ production (data not shown). Furthermore, IL-21R-deficient CD4$^+$ T cells stimulated with IL-27 expressed lower levels of c-Maf and IL-21, as determined by real-time PCR (Fig. 2*E*). These data suggest that IL-21 may be an important growth factor induced by IL-27 to expand Tr1 cells without affecting the expansion of IFN-γ-producing cells. Because IL-27 not only induces IL-21 production but also induces IL-21R expression, these data suggest that IL-27-mediated IL-21R up-regulation might be required for IL-21 to expand Tr1 cells.

To study the relevance of IL-21 in expanding Tr1 cells in vivo, we examined the frequency of IL-10-producing Tr1 cells generated in vivo in IL-21R$^{-/-}$ mice. We found that the frac-

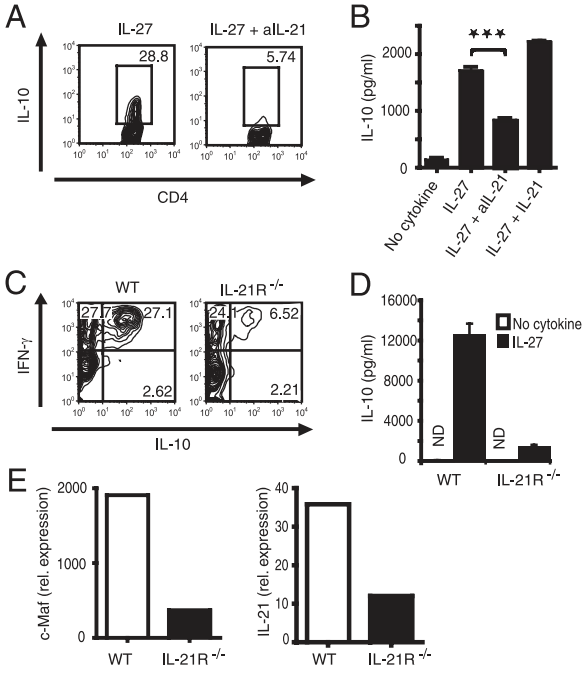

FIGURE 2. IL-21 is necessary for IL-10 production in Tr1 cells. *A*, IL-10.GFP expression as analyzed by flow cytometry in naive T cells activated in the presence of IL-27 for 72 h with or without the addition of neutralizing anti-IL-21 (aIL-21) Ab. *B*, ELISA to detect IL-10 production in the supernatant of naive T cells differentiated with IL-27 and anti-IL-21 or IL-21 (mean and SD; ***, $p = 0.0003$). *C*, IL-10 and IFN-γ production by WT and IL-21R$^{-/-}$ naive T cells activated in the presence of IL-27 for 72 h as determined by intracellular cytokine staining and analysis by flow cytometry. *D*, Supernatants from IL-27 differentiated naive T cells from WT and IL-21R$^{-/-}$ mice analyzed by IL-10 cytokine ELISA (mean and SD); ND, Not detected. *E*, Real-time PCR analysis of c-Maf and IL-21 in WT and IL-21R$^{-/-}$ CD4$^+$ cells stimulated with IL-27; rel. expression, Relative expression.

tion of IL-10-producing CD4$^+$CD44$^+$ memory T cells was significantly reduced in IL-21R$^{-/-}$ mice, which showed only 10% as many IL-10-producing T cells compared with wild-type (WT) mice (Fig. 3*A*). In contrast, the frequency of IFN-γ producers was similar in WT and IL-21R$^{-/-}$ cells. To further determine whether IL-27 could correct the defect in Tr1 cell development, CD4$^+$CD44$^+$ memory T cells were activated in the presence of IL-27, but IL-21R$^{-/-}$ mice continued to show a significant reduction in IL-10-producing T cells (data not shown). CD4$^+$CD44$^+$ T cells purified from IL-21R$^{-/-}$ mice also showed a lower expression of c-Maf and IL-21 mRNA (Fig. 3*B*), highlighting the importance of this amplification loop in generating Tr1 cells. In addition to the induction of c-Maf, IL-21, and IL-21R, IL-27 also enhanced the expression of ICOS. Because IL-10-producing T cells were first shown to be preferentially costimulated by ICOS (12), we analyzed the effect of a lack of ICOS signaling on the induction of Tr1 cells by IL-27. In vitro differentiation of T cells from ICOS-deficient mice demonstrated that IL-10 production by ICOS$^{-/-}$ T cells was similar to that by WT T cells at 48 h, but by 72 h there was a significant defect in IL-10 production induced by IL-27, as determined by cytometric bead array (Fig. 3*C*) and by intracellular staining (data not shown). In the plate-bound Ab system used here, the ICOS ligand (ICOS-L) is most likely provided by the CD4$^+$ T cells, because T cells can express ICOS-L when activated (13). When Tr1 cells were differentiated from

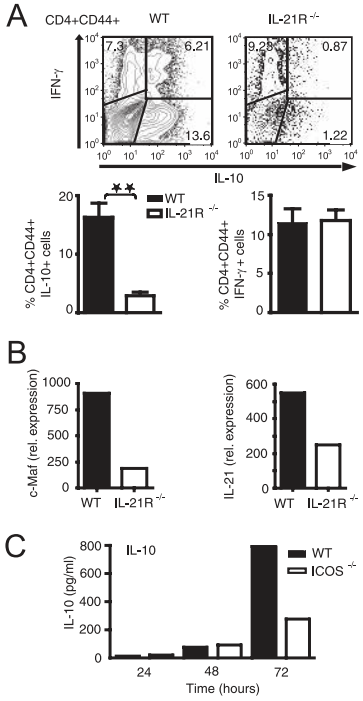

FIGURE 3. Memory $CD4^+CD44^+$ cells from IL-$21R^{-/-}$ mice are defective in IL-10 production in vivo. *A*, IL-10 and IFN-γ production as detected by intracellular cytokine staining in memory $CD4^+CD44^+$ T cells after 72 h of in vitro activation ($n = 5$ mice per group). IL-$21R^{-/-}$ $CD4^+CD44^+$ T cells from lymph nodes are defective in IL-10 production (**, $p = 0.0077$) but not for IFN-γ as compared with WT controls. *B*, Real-time PCR analysis of IL-21 and c-Maf in WT and IL-$21R^{-/-}$ $CD4^+CD44^+$ T cells cultured in vitro for 48 h; rel. expression, Relative expression. *C*, IL-10 production induced by IL-27 in WT (filled bars) and $ICOS^{-/-}$ (open bars) $CD4^+CD62L^+$ $CD25^-$ T cells as measured by cytokine bead array.

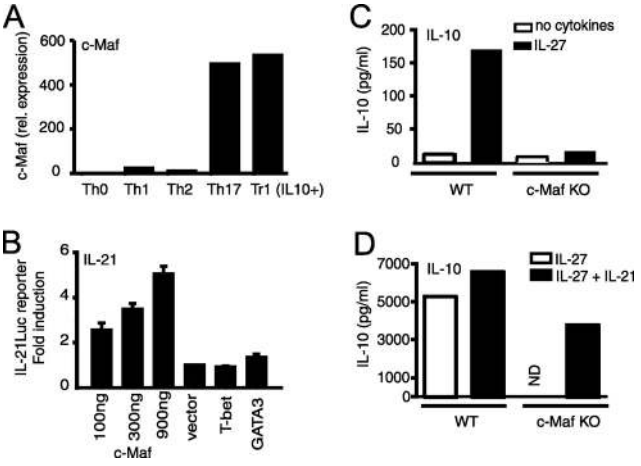

FIGURE 4. IL-27 induces c-Maf, which transactivates IL-21. *A*, Relative expression (rel. expression) of c-Maf in Th0, Th1, Th2, and Th17 cells as detected by real-time PCR. *B*, c-Maf transactivates the IL-21 promoter in HEK293 T cells as detected by cotransfection of the IL-21-luciferase reporter plasmid (IL-21Luc reporter) with c-Maf expression vector (c-Maf) transfected at three different plasmid concentrations (100, 300 or 900 ng), the control empty expression vector (vector; 900 ng), the T-bet vector (900 ng), or the GATA3 (900 ng) vector. Promoter activity was quantified by a luciferase assay 24 h post-transfection. The promoter-luciferase activity observed by transfection of the empty expression vector was normalized to 1. *C* and *D*, IL-10 production induced by IL-27 (*C*) or IL-27 plus IL-21 (*D*) in WT and c-$Maf^{-/-}$ $CD4^+CD8^-CD25^-$ T cells after 4 days in culture, as detected by cytokine bead array. ND, Not detectable; KO, knockout.

$ICOS^{-/-}$ mice, it was clear that $ICOS^{-/-}$ mice had a defect in sustaining growth/expansion of Tr1 cells in vitro.

Our results clearly demonstrated that IL-27 induces expression of c-Maf and that expressions of c-Maf and IL-21 mRNA appeared to be coexpressed in differentiating Tr1 cells under various differentiation conditions (Figs. 1, 2*E*, and 3*B*). Therefore, we compared c-Maf expression in T cell subsets (Th0, Th1, Th2, Th17, and Tr1), and observed that Tr1 cells had ~500-fold higher expression than Th1 or Th2 cells (Fig. 4*A*). We have observed that c-Maf and IL-21 mRNA were coexpressed by Tr1 cells, suggesting that c-Maf may be a transcription factor for IL-21, which in turn expands Tr1 cells. Our analysis of the IL-21 promoter revealed four putative conserved binding sites located 1070 bp (half MARE), 370 bp (v-MARE), 260 bp (half MARE) and 200 bp (v-MARE) upstream of the transcriptional start site (where MARE is Maf recognition element and v-MARE is v-Maf recognition element). To test this, an IL-21 promoter-luciferase reporter construct was cotransfected with a c-Maf expression plasmid into HEK 293T cells. Interestingly, c-Maf could transactivate IL-21 promoter-luciferase in a dose-dependent manner, but the transcription factors T-bet and GATA3, which are involved in Th1 and Th2 differentiation, could not (Fig. 4*B*), suggesting that c-Maf may expand Tr1 cells by inducing IL-21 production. Indeed, we have previously shown that c-Maf-deficient mice have a defect in IL-21 production (8). To address whether c-Maf-deficient mice have a defect in the IL-27-mediated IL-10 and IFN-γ production, we activated c-Maf-deficient T cells in the presence of IL-27 and analyzed the expression of IL-10 and IFN-γ. IL-27 was not able to induce either IL-10 or IL-21 production in c-Maf-deficient $CD4^+$ T cells (Fig. 4*C* and data not shown), but the IL-27-mediated IFN-γ response was not affected (data not shown). As c-Maf has been described to directly transactivate the IL-10 promoter (14, 15), we further added IL-21 to IL-27-activated c-Maf-deficient $CD4^+$ T cells and showed that exogenous IL-21 can partially rescue IL-10 production in c-$Maf^{-/-}$ T cells (Fig. 4*D*), highlighting the importance of IL-21 transactivation by c-Maf.

These data clearly show that IL-27 induces c-Maf, a transcription factor previously identified in Th2 cells, to transactivate IL-21, which then drives the expansion of Tr1 cells. Therefore, similar to Th17 and T follicular helper (TFh) cells, Tr1 cells express c-Maf and IL-21 and use IL-21 for autocrine growth and expansion. It is interesting to note that three different T cell subsets, Tr1, Th17, and TFh cells, which express c-Maf and IL-21, also produce IL-10, albeit at different levels (8, 16, 17). IL-21 acts as an autocrine growth/differentiation factor for all three subsets of T cells. It stands to reason that IL-21, which belongs to the family of IL-2 growth factors and uses the common γ-chain for signaling, may act as an expansion/growth factor for cells that do not produce IL-2. Consistent with this idea, loss of IL-21 or IL-21 signaling results in a defect in all the three T cell subsets, Th17, TFh, and Tr1 (8, 16). Our data are consistent with a recent study showing that IL-21 mediates its inhibitory effects by inducing IL-10 production (18). Similar to IL-6, which induces IL-21 by inducing phospho-STAT-3, IL-27 also induces phospho-STAT-3 and IL-21, most likely due to the fact that IL-6 and IL-27 both share the gp130 chain for signaling.

IL-27-enhanced expression of ICOS is of interest because ICOS was initially shown to costimulate IL-10-producing T cells (12). We and others have shown that ICOS/ICOS ligand interaction induces c-Maf expression (8, 11) which may further enhance stable IL-21 production from developing Tr1 cells. Like Th17 and TFh cells, ICOS appears to be crucial in maintaining IL-27-driven Tr1 cells. These results are supported by the observation that $ICOS^{-/-}$ mice indeed have a defect in IL-10-producing T cells and therefore develop more severe autoimmunity (8, 19). IL-27 also up-regulates ICOS for maintenance and survival of Tr1 cells.

In summary, we have demonstrated that IL-27 drives the expansion and differentiation of Tr1 cells by inducing expression of three key elements: the transcription factor c-Maf, the growth factor IL-21, and the costimulatory receptor ICOS, which coordinately act to mediate Tr1 differentiation (supplemental Fig. 3). Loss of any of these factors results in a defective IL-27-driven, IL-10-producing Tr1 cells.

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Disclosures

The authors have no financial conflict of interest.

References

1. Stumhofer, J. S., and C. A. Hunter. 2008. Advances in understanding the anti-inflammatory properties of IL-27. *Immunol. Lett.* 117: 123–130.
2. Awasthi, A., Y. Carrier, J. P. Peron, E. Bettelli, M. Kamanaka, R. A. Flavell, V. K. Kuchroo, M. Oukka, and H. L. Weiner. 2007. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat. Immunol.* 8: 1380–1389.
3. Stumhofer, J. S., J. S. Silver, A. Laurence, P. M. Porrett, T. H. Harris, L. A. Turka, M. Ernst, C. J. Saris, J. J. O'Shea, and C. A. Hunter. 2007. Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nat. Immunol* 8: 1363–1371.
4. Fitzgerald, D. C., G. X. Zhang, M. El-Behi, Z. Fonseca-Kelly, H. Li, S. Yu, C. J. Saris, B. Gran, B. Ciric, and A. Rostami. 2007. Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. *Nat. Immunol.* 8: 1372–1379.
5. Vieira, P. L., J. R. Christensen, S. Minaee, E. J. O'Neill, F. J. Barrat, A. Boonstra, T. Barthlott, B. Stockinger, D. C. Wraith, and A. O'Garra. 2004. IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring $CD4^+CD25^+$ regulatory T cells. *J. Immunol.* 172: 5986–5993.
6. Roncarolo, M. G., S. Gregori, M. Battaglia, R. Bacchetta, K. Fleischhauer, and M. K. Levings. 2006. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol. Rev.* 212: 28–50.
7. Sun, J., R. Madan, C. L. Karp, and T. J. Braciale. 2009. Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. *Nat. Med.* 15: 277–284.
8. Bauquet, A. T., H. Jin, A. M. Paterson, M. Mitsdoerffer, I. C. Ho, A. H. Sharpe, and V. K. Kuchroo. 2009. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. *Nat. Immunol.* 10: 167–175.
9. Datta, S., and N. E. Sarvetnick. 2008 IL-21 limits peripheral lymphocyte numbers through T cell homeostatic mechanisms. *PLoS ONE* 3: e3118.
10. Kim, J. I., I. C. Ho, M. J. Grusby, and L. H. Glimcher. 1999. The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. *Immunity.* 10: 745–751.
11. Nurieva, R. I., J. Duong, H. Kishikawa, U. Dianzani, J. M. Rojo, I. Ho, R. A. Flavell, and C. Dong. 2003. Transcriptional regulation of Th2 differentiation by inducible costimulator. *Immunity* 18: 801–811.
12. Hutloff, A., A. M. Dittrich, K. C. Beier, B. Eljaschewitsch, R. Kraft, I. Anagnostopoulos, and R. A. Kroczek. 1999. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* 397: 263–266.
13. Ling, V., P. W. Wu, H. F. Finnerty, K. M. Bean, V. Spaulding, L. A. Fouser, J. P. Leonard, S. E. Hunter, R. Zollner, J. L. Thomas, et al. 2000. Cutting edge: identification of GL50, a novel B7-like protein that functionally binds to ICOS receptor. *J. Immunol.* 164: 1653–1657.
14. Cao, S., J. Liu, L. Song, and X. Ma. 2005. The protooncogene c-Maf is an essential transcription factor for IL-10 gene expression in macrophages. *J. Immunol.* 174: 3484–3492.
15. Xu, J., Y. Yang, G. Qiu, G. Lal, Z. Wu, D. E. Levy, J. C. Ochando, J. S. Bromberg, and Y. Ding. 2009. c-Maf regulates IL-10 expression during Th17 polarization. *J. Immunol.* 182: 6226–6236.
16. Nurieva, R., X. O. Yang, G. Martinez, Y. Zhang, A. D. Panopoulos, L. Ma, K. Schluns, Q. Tian, S. S. Watowich, A. M. Jetten, and C. Dong. 2007. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* 448: 480–483.
17. Zhou, L., I. I. Ivanov, R. Spolski, R. Min, K. Shenderov, T. Egawa, D. E. Levy, W. J. Leonard, and D. R. Littman. 2007. IL-6 programs T_H-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat. Immunol.* 8: 967–974.
18. Spolski, R., H. P. Kim, W. Zhu, D. E. Levy, and W. J. Leonard. 2009. IL-21 mediates suppressive effects via its induction of IL-10. *J. Immunol.* 182: 2859–2867.
19. Dong, C., A. E. Juedes, U. A. Temann, S. Shresta, J. P. Allison, N. H. Ruddle, and R. A. Flavell. 2001. ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature* 409: 97–101.