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## IL-7 Abrogates Suppressive Activity of Human CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> Regulatory T Cells and Allows Expansion of Alloreactive and Autoreactive T Cells

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# IL-7 Abrogates Suppressive Activity of Human CD4<sup>+</sup>CD25<sup>+</sup> FOXP3<sup>+</sup> Regulatory T Cells and Allows Expansion of Alloreactive and Autoreactive T Cells

Anne-Kristin Heninger,<sup>1</sup> Anke Theil,<sup>1</sup> Carmen Wilhelm, Cathleen Petzold, Nicole Huebel, Karsten Kretschmer, Ezio Bonifacio, and Paolo Monti

CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> regulatory T cells (Tregs) control the activation and expansion of alloreactive and autoreactive T cell clones. Because uncontrolled activation and expansion of autoreactive T cells occur in an IL-7-rich environment, we explored the possibility that IL-7 may affect the function of Treg. We show that the functional high-affinity IL-7R is expressed on both naive and memory Tregs, and exposure to IL-7 results in STAT-5 phosphorylation. Naive, but not memory, Tregs proliferated greatly and acquired a memory phenotype in the setting of a suppression assay when IL-7 was present. Importantly, the presence of IL-7 abrogated the capacity of Tregs to suppress proliferation of conventional T cells in response to TCR activators, including alloantigens and autoantigens. Removal of IL-7 restored the suppressive function of Tregs. Preblocking of the IL-7R on the Tregs also restored suppressor function, indicating that IL-7 directly affected Treg function. Thus, prolonged periods of homeostatic expansion can temporarily release natural regulatory brakes on T cells, thereby providing an additional mechanism for activating and expanding alloreactive and autoreactive T cells. *The Journal of Immunology*, 2012, 189: 5649–5658.

The homeostatic cytokine IL-7 is involved in the survival and expansion of T cells, including alloreactive and autoreactive clones. In the steady state, the immune system relies on low concentrations of IL-7 to regulate T cell homeostasis and preserve T cell repertoire diversity. However, during lymphopenia, an IL-7-rich environment provides a milieu for expansion and activation of T cells. This mechanism of homeostatic expansion was demonstrated to exist in various conditions, such as T cell responses to tumors (1), graft-versus-host disease (2), and autoimmunity (3).

With respect to autoimmunity, low-affinity interactions with self-Ag-MHC complexes that are necessary for T cell expansion during lymphopenia (4) and the development of properties that are remarkably similar to Ag expanded effector and memory cells (5)

were shown to give selective advantage to autoreactive T cells during IL-7-mediated homeostatic proliferation. A chronic state of lymphopenia (6), exogenous immune-suppression (7), and exogenous administration of IL-7 were all shown to exacerbate destruction of pancreatic  $\beta$  cells in mouse models of diabetes (8). In humans, single-nucleotide polymorphisms of IL-7R $\alpha$  are associated with an increased risk for developing type 1 diabetes (9) and multiple sclerosis (10). In the context of islet transplantation, increased IL-7 that occurs posttransplant contributes to an expansion and activation of autoreactive memory T cells (3).

IL-7-mediated expansion of autoreactive T cells and other cells was thought to occur via a direct effect on cells that express the IL-7R complex at moderate to high levels. These include the T effector cells, but not the CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> regulatory T cells (Tregs), which are distinguished by low expression of IL-7R $\alpha$  (11, 12). Studies in animal models and humans discovered an impairment of the Treg compartment during lymphopenia (7, 13), but a direct link between IL-7 and Treg has not been addressed. In the current study, we asked whether IL-7 has an effect on Treg homeostasis and suppressive function. We studied the expression of the high-affinity IL-7R on Treg subpopulations, responsiveness of Tregs to IL-7, as well as the gene-expression profile of activated Tregs in the presence of IL-7. Finally, we determined the effect of IL-7 on the Treg-suppressive capacity of T cells under different stimulating conditions that included allogeneic cells and diabetes-relevant autoantigens. Our findings demonstrate that an IL-7-rich environment abrogates Treg suppressor function and promotes the activation and expansion of T effector cells, alloreactive and autoreactive T cells, and Tregs, all of which become fully functional cell populations. Therefore, we suggest that environments in which IL-7–IL-7R signaling is enhanced favor alloreactive and autoreactive T cell expansion by releasing T cells from the inhibitory network of Tregs.

## Materials and Methods

### Cell isolation and purification

Sodium-heparinized peripheral venous blood samples were kindly provided by the Deutsches Rotes Kreuz Blutspendedienst Ost Dresden with

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The sequences presented in this article have been submitted to the Gene Expression Omnibus database (<http://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE40755.

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The online version of this article contains supplemental material.

Abbreviations used in this article: DC, dendritic cell; EdU, 5-ethynyl-2'-deoxyuridine; MFI, median fluorescence intensity; Treg, regulatory T cell; Tresp, responder T cell; TT, tetanus toxoid.

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informed consent and ethics committee approval. Highly purified CD8<sup>+</sup> versus CD4<sup>+</sup>CD25<sup>-</sup>CD127<sup>hi</sup> responder T cells (Tresps) plus CD4<sup>+</sup>CD25<sup>hi</sup>CD127<sup>low</sup> T cells (Tregs) were isolated using the FACS Aria II cell sorter (Beckton Dickinson), according to the expression of CD8 (clone SK1) or CD4/CD25/CD127 (clone SK3, clone M-A251, clone eBioDDR5) from previously enriched CD8<sup>+</sup> (CD8<sup>+</sup> Isolation Kit II; Miltenyi Biotec) and CD4<sup>+</sup>CD25<sup>+</sup> cells (CD4<sup>+</sup> Isolation Kit II; Miltenyi Biotec), followed by CD25 MicroBeads II). An effect of Abs used for sorting on IL-7 signaling was assessed by pSTAT5 expression (Supplemental Fig. 1B). Naive and memory subsets of Tregs were FACS sorted according to CD45RA expression. The purity of sorted Tregs (>98%) was confirmed by intracellular FOXP3 staining (Supplemental Fig. 1C).

#### Cell staining and FACS analysis

Cells were stained using the following mAbs: CD3 allophycocyanin (clone UCHT1), CD4 PB (clone RPA-T4), CD4 PerCP (clone SK3), CD4 allophycocyanin (clone SK3), CD8 allophycocyanin-Cy7 (clone SK1), CD127 PE (clone M21), CD25 PE (clone M-A251), CD28 PE (clone CD28.2), CD31 PE (clone WM59), CD49d PE (clone L25), CD62L (clone SK11), CD69 PE (clone L78), CD95 allophycocyanin (clone DX2), CTLA-4 allophycocyanin (clone BNI3), CD132 biotin (clone TUGh4), AKT pT308 PE (clone J1-223.371), STAT5 pY694 Alexa Fluor 647 (clone 47), CD45RA allophycocyanin (clone HI100), CD45RO PE-Cy7 (clone UCHL1), HLA-DR PE (clone G46-6), IL-10 allophycocyanin (clone JES319F1), and isotype control rat IgG<sub>2a</sub> allophycocyanin (clone R35-95) (all from BD Pharmingen); CD127 eFluor 450 (clone eBioDDR5), OX-40 FITC (clone ACT35), ICOS FITC (clone ISA-3), and FOXP3 allophycocyanin (clone PCH101) (all from eBioscience); FOXP3 Alexa Fluor 488 (clone 259D), CD49a allophycocyanin (clone TS2/7), granzyme B allophycocyanin (clone GB11), and TGF- $\beta$ -LAP PerCP (clone BG/hLAP) (all from BioLegend); CD39 allophycocyanin (clone MZ18-23C8; Miltenyi Biotec); and GITR allophycocyanin (clone FAB689A; R&D Systems). 7-Aminoactinomycin D (BD Pharmingen) was used to exclude dead cells. Cells were acquired on a Becton Dickinson LSR-II flow cytometer with FACS Diva software and analyzed using FlowJo software version 7.6.1 (TreeStar).

#### Confocal microscopy

FACS-sorted Tregs or T cells were allowed to settle onto a 12-mm poly-L-lysine-coated coverslip (Biocoat; BD Pharmingen), fixed in 3% formaldehyde, permeabilized with methanol, and blocked in 0.25% fish skin gelatin. Cells were subsequently stained with anti-phospho-STAT5pY694 AF647 (clone 47; BD Pharmingen). Proliferation of Tregs and T cells was visualized by 5-ethynyl-2'-deoxyuridine (EdU) incorporation, according to the manufacturer's instructions (Click-It EdU; Invitrogen). All images were acquired with a Leica SP5 inverse laser scanning confocal microscope.

#### T cell-suppression assays

CD4<sup>+</sup> and CD8<sup>+</sup> Tresps or Tregs were labeled with CFSE (0.5  $\mu$ M in PBS for 10 min at 37°C; Molecular Probes). When CD4<sup>+</sup> T cells were used as Tresps, Tregs were labeled with eFluor 670 Cell Proliferation Dye (5  $\mu$ M in PBS for 10 min at 37°C; eBioscience). A total of 10<sup>5</sup> Tresps alone, 10<sup>5</sup> allogenic Tregs alone, or a mixture of both at a 1:1 ratio, unless otherwise indicated, was stimulated with anti-CD3/anti-CD28-coupled beads (Invitrogen-Dynal) at a 1:50 bead/T cell ratio, in the presence or absence of 10 ng/ml recombinant human IL-7 (R&D Systems) in 96-well plates. After 5 d, proliferation of Tresps and Tregs was measured as CFSE dilution. For Ag-specific suppression assays, CFSE-labeled whole PBMC were mixed 1:1 with eFluor 670 Cell Proliferation Dye-labeled allogenic Tregs and stimulated with tetanus toxoid (1  $\mu$ g/ml; Sanofi Pasteur MSD), GAD65 (5  $\mu$ g/ml; Diamyd Medical), proinsulin (10  $\mu$ g/ml; Lilly), or allogenic dendritic cells (DCs) (1:10 DC/PBMC ratio). Blocking CD127 on T cells was achieved by preincubation with 20  $\mu$ g/ml monoclonal anti-CD127 Ab (clone R-34-34; Dendritics) for 2 h at 37°C. Blocking of cytokines was performed by daily addition of anti-IL-2 Ab (1  $\mu$ g/ml; R&D Systems), anti-IL-10 plus anti-IL-10R (1  $\mu$ g/ml each; BD Pharmingen), anti-IFN- $\gamma$  (500 ng/ml; R&D Systems), or anti-TNF- $\alpha$  plus anti-TNF-TNFSF1A (500 ng/ml; R&D Systems). For Transwell experiments, 10<sup>5</sup> CFSE-labeled naive Tregs were seeded in the upper well and 10<sup>5</sup> CFSE-labeled CD8<sup>+</sup> Tresps were seeded in the bottom well of a Transwell chamber (0.4- $\mu$ m-pore polycarbonate membrane; Corning Costar). Upper and lower compartments were stimulated with anti-CD3/CD28 beads (at a 1:100 bead/T cell ratio) in the presence or absence of 10 ng/ml IL-7.

#### EdU-proliferation assay

Short-term proliferation assays were performed by staining with the Click-iT EdU Pacific Blue Kit (Invitrogen), according to the manufacturer's protocol.

#### Cytokine analysis

Culture supernatants from suppression assays were collected on day 2 or 5, and cytokines were measured in a multiplex format using the Luminex 200 platform (Luminex) with the Milliplex Human Cytokine Immunoassay (IFN- $\gamma$ , TNF- $\alpha$ , IL-2, IL-4, IL-5, IL-10, and IL-17; Millipore), according to the manufacturer's protocol. All samples were measured in technical duplicates.

#### PCR array

CD4<sup>+</sup>CD25<sup>+</sup> naive and memory Tregs (each obtained from two donors) were isolated from fresh PBMC, as described above, and stimulated separately with anti-CD3/anti-CD28-coupled beads (Invitrogen-Dynal) at a 1:10 bead/T cell ratio, treated or not with 10 ng/ml recombinant human IL-7 (R&D Systems), for 16 h. Subsequently, cell lysates were prepared separately from the two donors and pooled, and RNA was extracted using the RNeasy mini kit (QIAGEN). cDNA synthesis and PCR array were performed following the manufacturer's instructions (RT<sup>2</sup> First Strand Kit and T cell anergy and immune tolerance RT<sup>2</sup> Profiler PCR array, PAHS-074; SABiosciences). Changes in cycle threshold ( $\Delta$ ct values) were calculated using *B2M*, *HPRT-1*, *RPL13*, and *GADPH* as housekeeping genes. RT<sup>2</sup> Profiler PCR Array Data Analysis software (SABiosciences) was used for further analysis and generation of heat maps. Complete PCR array data are available at <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE40755>.

#### Statistical analysis

The two-tailed paired Student *t* test was used for all comparisons. Expression of CD127, CD132, CD25, and FOXP3 on T cell subsets was assessed as median fluorescence intensity (MFI). T cell responsiveness to IL-7 was assessed as MFI of pSTAT5. Proliferation was assessed by CFSE dilution or EdU<sup>+</sup> cells. Data are presented as mean  $\pm$  SD.

## Results

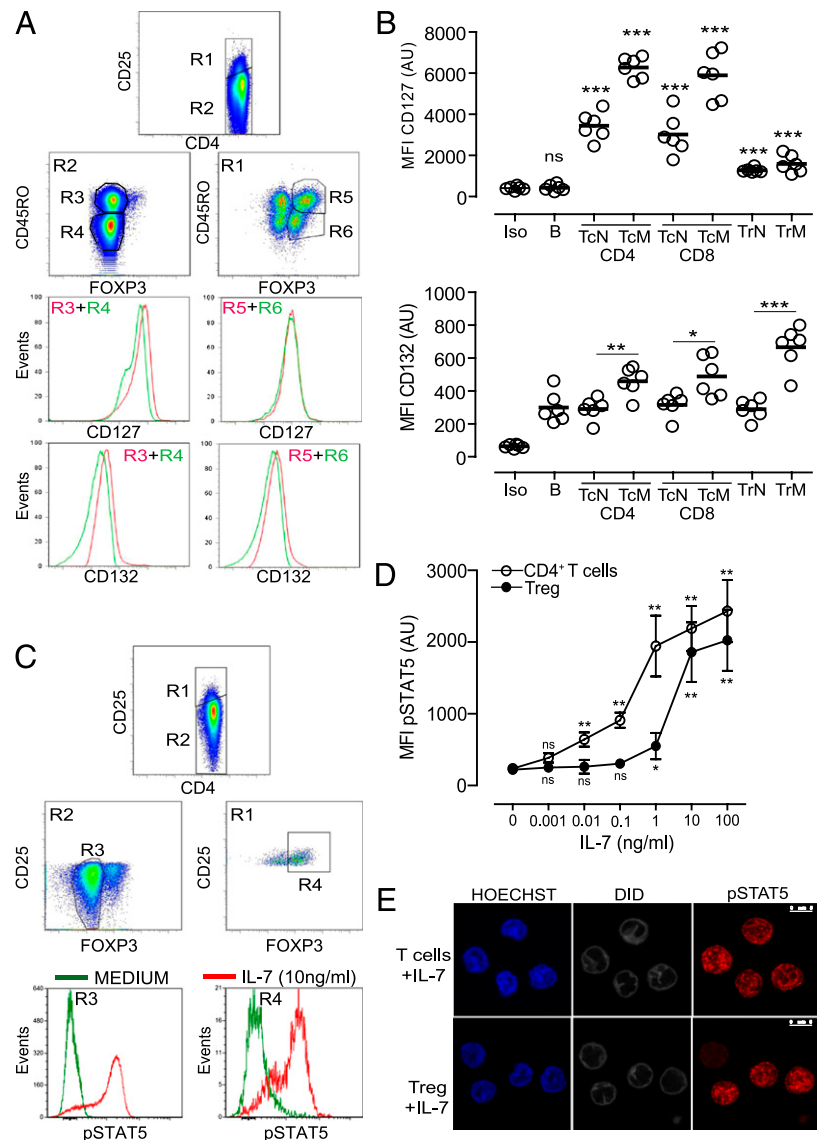
#### Expression of CD127 and CD132 on conventional T cells and Tregs

Surface expression of the high-affinity IL-7R $\alpha$ -chain (CD127) and IL-7R $\gamma$ -chain (CD132) was measured on conventional T cells and Tregs (Fig. 1A, 1B). Compared with the CD19<sup>+</sup> B lymphocyte population, which is negative for CD127, all conventional T cell and Treg subsets had significant surface expression of CD127. The CD45RO<sup>+</sup> subset of conventional CD4<sup>+</sup> (MFI, 6279  $\pm$  513) and CD8<sup>+</sup> (MFI, 5894  $\pm$  1144) T cells had the highest expression ( $p$  < 0.001, versus CD45RO<sup>-</sup> subsets). CD127 expression on Tregs was low (naive Tregs, 1282  $\pm$  115; memory Tregs, 1591  $\pm$  432), but it was significantly higher than on B cells (391  $\pm$  23,  $p$  < 0.001). CD132 expression on Tregs was comparable to that found on conventional T cells. Again, expression of CD132 was highest on the CD45RO<sup>+</sup> subsets of T conventional cells and Tregs. As shown previously (14), memory Tregs had the highest surface expression of CD25 (MFI: 1206  $\pm$  182 [naive] versus 1749  $\pm$  133 [memory],  $p$  = 0.004) and FOXP3 (MFI: 1807  $\pm$  445 [naive] versus 3635  $\pm$  909 [memory],  $p$  = 0.0003) (Supplemental Fig. 1A).

#### Conventional T cell and Treg responsiveness to IL-7

IL-7R signaling depends on the activation of Jak3 and Jak1, phosphorylation of STAT5 and AKT, and the nuclear translocation of STAT5 dimers. Having excluded that Abs CD8, CD25, and CD127 used for T cell subset isolation have an effect on STAT5 signaling in our experimental setting (Supplemental Fig. 1B), we studied STAT5 phosphorylation in different T cell subsets. pSTAT5 was induced upon stimulation with IL-7 in resting CD4<sup>+</sup> T cells and Tregs (Fig. 1C). Consistent with their high CD127 expression,

**FIGURE 1.** Expression of IL-7R and responsiveness to IL-7 in T cells and Tregs. **(A)** Expression of CD127 and CD132 on gated CD4<sup>+</sup>CD25<sup>hi</sup>CD45RO<sup>+</sup>FOXP3<sup>+</sup> memory Tregs (R1 and R5), CD4<sup>+</sup>CD25<sup>high</sup>CD45RO<sup>+</sup>FOXP3<sup>+</sup> naive Tregs (R1 and R6), CD4<sup>+</sup>CD25<sup>low</sup>CD45RO<sup>+</sup>FOXP3<sup>+</sup> memory conventional T cells (R2 and R3), and CD4<sup>+</sup>CD25<sup>low</sup>CD45RO<sup>+</sup>FOXP3<sup>+</sup> naive conventional T cells (R2 and R4). Plots show one representative donor. **(B)** CD127 and CD132 expression (MFI) in B cells (B), CD4<sup>+</sup> naive (CD4 TcN), CD4<sup>+</sup> memory (CD4 TcM), CD8<sup>+</sup> naive (CD8 TcN), CD8<sup>+</sup> memory (CD8 TcM), Treg naive (TrN), and Treg memory (TrM) cells from six donors. **(C)** PBMCs stimulated or not with 10 ng/ml IL-7 were gated into CD4<sup>+</sup>CD25<sup>hi</sup>FOXP3<sup>+</sup> Tregs (R1 and R4) and CD4<sup>+</sup>CD25<sup>low</sup>FOXP3<sup>+</sup> T cells (R2 and R3). pSTAT5 (lower panels) was measured on unstimulated (green) and stimulated (red) cells. Plots show one representative donor. **(D)** Dose-response effect of IL-7 on pSTAT5 in conventional CD4<sup>+</sup>CD25<sup>low</sup>FOXP3<sup>+</sup> T cells (○) and CD4<sup>+</sup>CD25<sup>high</sup>FOXP3<sup>+</sup> Tregs (●). Mean ± SD of four donors. **(E)** Intracellular localization of pSTAT5 in FACS-sorted CD4<sup>+</sup>CD25<sup>low</sup>CD127<sup>high</sup> T cells (upper panels) and CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup> Tregs (lower panels) stimulated with IL-7. Nuclei were stained with Hoechst (left panels, blue), and cell and intracellular membranes were stained with dialkylcarbocyanine (DID; middle panels, white). pSTAT5 was detected with a specific anti-p(Y694)STAT5 Alexa Fluor 647 Ab (right panels, red). Scale bars, 5 μm. The *p* values indicate comparison with no IL-7. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001. ns, *p* > 0.05.



conventional T cells were highly sensitive to IL-7 (15), with IL-7 concentrations as low as 1 pg/ml yielding pSTAT5 above background, with the maximal pSTAT5 signal observed at 1–10 ng/ml (Fig. 1D). Tregs were less sensitive, with pSTAT5 becoming detectable when cells were exposed to IL-7 concentrations of 1 ng/ml and reaching maximal levels at 10 ng/ml. Upon IL-7 stimulation, nuclear translocation of pSTAT5 was observed in both Tregs and conventional T cells (Fig. 1E).

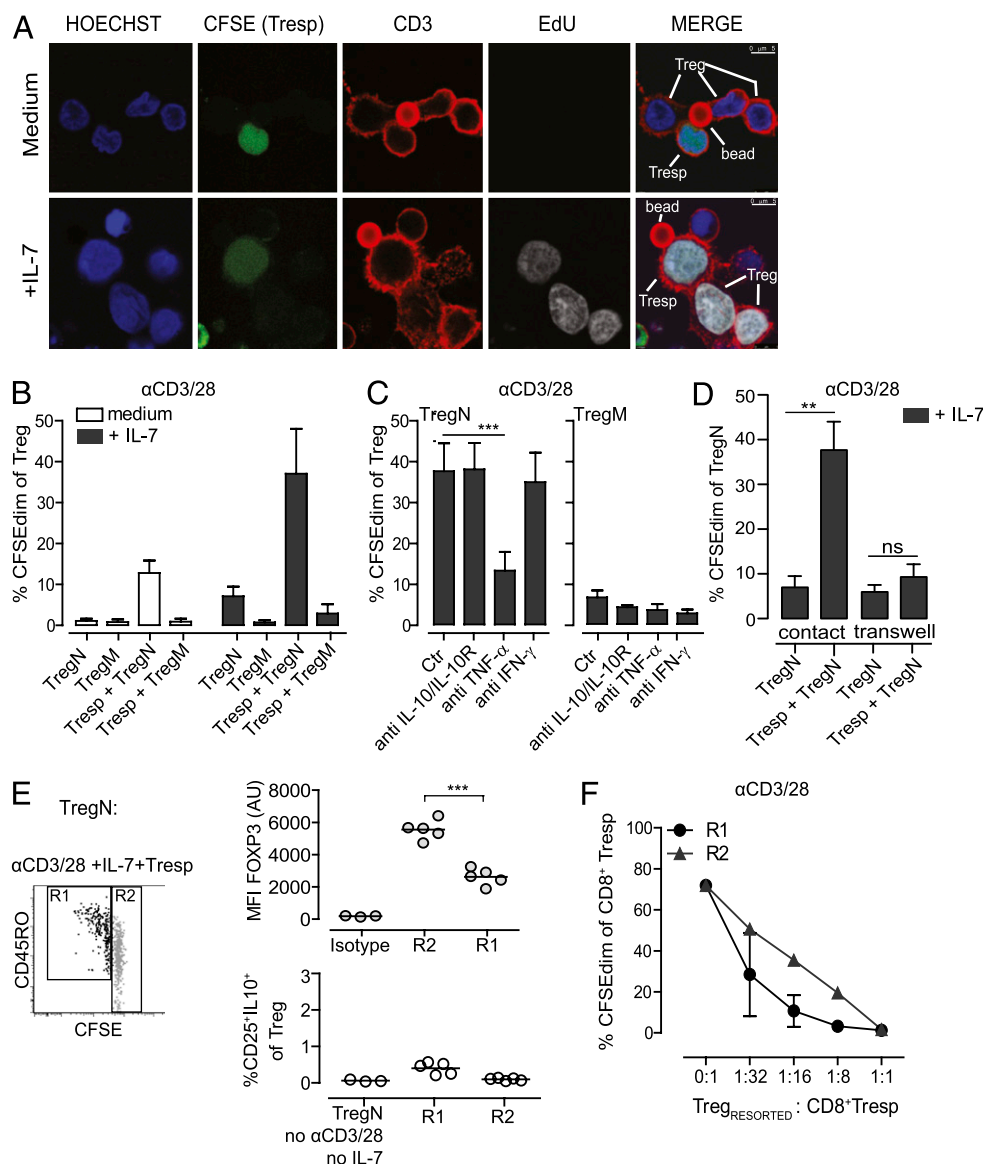
#### IL-7 induces proliferation of naive, but not memory, Tregs

Tregs are anergic cells *in vitro*, and they proliferate only in response to strong signals through the TCR and the addition of high doses of IL-2 (16). Because Tregs exhibited IL-7-mediated pSTAT5 signaling, we examined their ability to proliferate in the presence of IL-7. As demonstrated by microscopy (Fig. 2A), FACS-sorted CD4<sup>+</sup>CD25<sup>hi</sup>CD127<sup>low</sup> Tregs in contact with bead-activated, CFSE-labeled FACS-sorted CD8<sup>+</sup> T cells incorporated EdU and increased cell size only when IL-7 was present in the culture medium. Using CFSE-labeled Tregs, neither naive Tregs nor memory Tregs proliferated when cultured alone with anti-CD3/anti-CD28-coated bead stimulation (CFSE<sup>dim</sup>: 1.1 ± 0.5% [naive Tregs] versus 0.8 ± 0.6% [memory Tregs], *n* = 4, Fig. 2B). However, the presence of IL-7 in addition to bead stimulation

induced a low amount of proliferation in naive Tregs but not in memory Tregs (CFSE<sup>dim</sup>: 7.1 ± 2.3% [naive Tregs] versus 0.7 ± 0.5% [memory Tregs], *n* = 4, *p* = 0.009). Culture with Tregs and with bead stimulation also resulted in proliferation of naive Tregs but not memory Tregs (CFSE<sup>dim</sup>: 12.8 ± 3.1% [naive Tregs] versus 0.9 ± 0.7% [memory Tregs], *n* = 4, *p* = 0.0003). Treg proliferation was greatest when naive Tregs were cocultured with Tregs and bead stimulation in the presence of IL-7. Under these conditions, we consistently observed proliferation of naive Tregs, whereas memory Tregs displayed a low proliferation rate (CFSE<sup>dim</sup>: 37.0 ± 11.0% [naive Tregs] versus 2.9 ± 2.3% [memory Tregs], *n* = 4, *p* = 0.0009).

It was recently suggested that TNF-α was able to induce Treg expansion (17). Blocking experiments confirmed that TNF-α contributed to Treg proliferation in the cocultures (Fig. 2C). This was significant for naive Tregs, but memory Tregs showed low proliferation under all conditions. No effect on Treg proliferation was observed after blocking IL-10 or IFN-γ, which are also abundant in Tresp cultures. Of note, the enhanced proliferation of naive Tregs in the presence of IL-7, Tregs, and bead stimulation was abolished when naive Tregs and CD8<sup>+</sup> Tregs were cocultured using a Transwell system, suggesting that proximity of the Tresp population was important for the marked naive Treg proliferation (Fig. 2D).





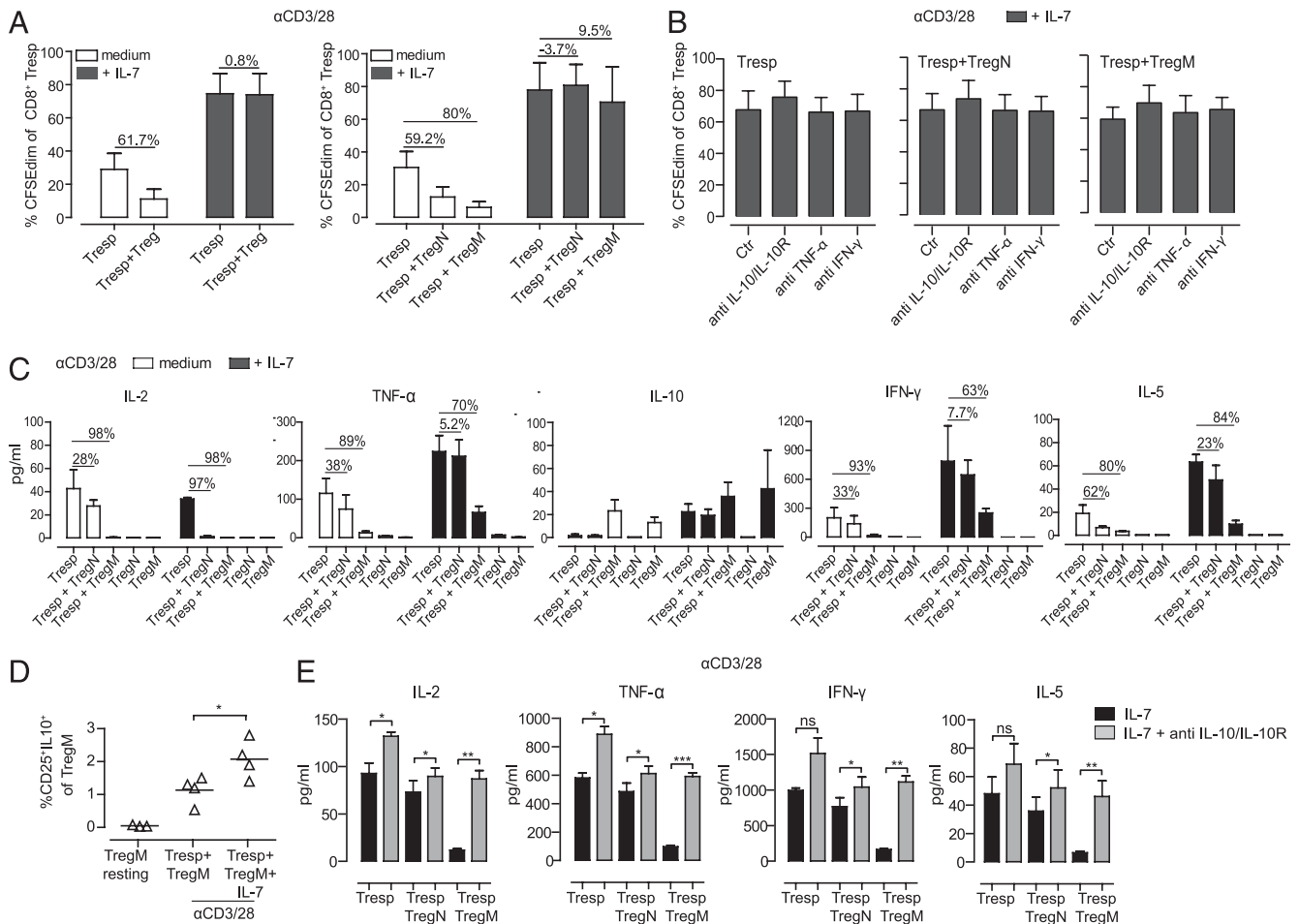
**FIGURE 2.** Proliferation of Tregs in coculture assay. **(A)** Confocal imaging of EdU incorporation (white) of Tregs and CFSE-labeled CD8<sup>+</sup> Tregs (green) in the absence (medium, upper panels) or presence (lower panels) of IL-7. Scale bars, 5  $\mu$ m. **(B)** Proliferation measured as CFSE dilution (% CFSE<sup>dim</sup>) of CD4<sup>+</sup> naive (TregN) or memory (TregM) Tregs stimulated with anti-CD3/anti-CD28 in the absence or presence of CD8<sup>+</sup> Tregs and/or IL-7 ( $n = 4$ ). **(C)** TregN and TregM proliferation in response to anti-CD3/anti-CD28 stimulation in coculture with CD8<sup>+</sup> Tregs and IL-7 in the presence or absence of blocking Abs against IL-10, TNF- $\alpha$ , or IFN- $\gamma$  ( $n = 3$ ). **(D)** Proliferation of TregN stimulated with anti-CD3/anti-CD28 beads in the presence of IL-7 was measured following cell-cell contact with anti-CD3/anti-CD28 bead-stimulated CD8<sup>+</sup> Tregs (contact) or using a Transwell system ( $n = 3$ ). **(E)** Originally naive Tregs were FACS sorted after 5 d of suppression assay with CD8<sup>+</sup> Tregs, anti-CD3/anti-CD28, and 10 ng/ml IL-7 into proliferated memory CD45RO<sup>+</sup>-enriched (R1, CFSE<sup>dim</sup>, TregM<sub>RESORTED</sub>) and naive (R2, CFSE<sup>high</sup>, TregN<sub>RESORTED</sub>) Tregs, as shown in the representative FACS plot (left panel). FOXP3 expression (upper right panel) and the percentage of CD25<sup>+</sup>IL-10<sup>+</sup> cells of resorted Tregs (lower right panel), as measured by intracellular staining ( $n = 5$ ). **(F)** TregM<sub>RESORTED</sub> (●) and TregN<sub>RESORTED</sub> (▲) were tested in a suppression assay performed with anti-CD3/anti-CD28 stimulation in the absence of IL-7 (mean  $\pm$  SD) ( $n = 3$ ). \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Proliferating naive Tregs retain a FOXP3<sup>+</sup>IL-10<sup>+</sup> phenotype and are enriched in CD45RO<sup>+</sup> memory cells (Fig. 2E, MFI FOXP3 in Tregs: 5552  $\pm$  610 [memory Treg<sub>RESORTED</sub>] versus 2623  $\pm$  586 [naive Treg<sub>RESORTED</sub>],  $p < 0.0001$ ). After FACS resorting and removal from IL-7, both the proliferating, CD45RO-enriched Tregs and the remaining CFSE<sup>high</sup> Tregs fully suppressed the proliferation of Tregs in a second suppression assay performed in the absence of IL-7 (Fig. 2F).

#### Treg suppressor capacity is impaired in the presence of IL-7

An important function of Tregs is their ability to suppress proliferation of Tregs. Our findings that normally anergic Tregs

proliferate in the presence of IL-7 prompted us to examine whether suppressor function was affected while Tregs were exposed to IL-7 (Fig. 3A). In the absence of IL-7, Tregs efficiently suppressed anti-CD3/anti-CD28 bead-stimulated CFSE-labeled CD8<sup>+</sup> Treg proliferation. In contrast, and consistent with the microscopy images (Fig. 2A), in the presence of IL-7 we observed complete abrogation of Treg suppressive capacity (Fig. 3A, left panel; suppression: 62  $\pm$  17% [medium] versus 1  $\pm$  9% [IL-7],  $n = 6$ ,  $p = 0.0001$ ). This phenomenon was observed for both naive Tregs (suppression: 59  $\pm$  11% [medium] versus -4  $\pm$  5% [IL-7],  $n = 4$ ,  $p = 0.004$ ) and memory Tregs (suppression: 80  $\pm$  9% [medium] versus 9  $\pm$  14% [IL-7],  $n = 4$ ,  $p = 0.002$ ), although some



**FIGURE 3.** CD8<sup>+</sup> Tresp proliferation and cytokine production in coculture assays. **(A)** Proliferation of CD8<sup>+</sup> Tresp to anti-CD3/anti-CD28 stimulation in the presence of total Tregs (left panel,  $n = 6$ ) and separated naive Tregs (TregN) or memory Tregs (TregM) in the absence (white bar) or presence (black bars) of IL-7 (10 ng/ml) (right panel,  $n = 4$ ). Suppression is given (above the columns) as the percentage reduction in proliferation in the presence of Tregs compared with the absence of Tregs. **(B)** Proliferation of CD8<sup>+</sup> Tresp with anti-CD3/anti-CD28 stimulation and IL-7 cultured alone (left panel) or with TregN (middle panel) or TregM (right panel) in the presence of blocking Abs to IL-10, TNF- $\alpha$ , and IFN- $\gamma$  ( $n = 3$ ). **(C)** CD8<sup>+</sup> Tresp, TregN, and TregM were cultured alone or in coculture (suppression assay) with anti-CD3/anti-CD28 stimulation and in the presence or absence of IL-7. Supernatants were collected after 5 d. Concentration (mean  $\pm$  SD) of IL-2, TNF- $\alpha$ , IL-10, IFN- $\gamma$ , and IL-5 from culture supernatants is shown ( $n = 3$ ). The percentage of suppression of cytokine production is indicated above the columns. **(D)** Percentage of CD25<sup>+</sup>IL-10<sup>+</sup> TregM ( $n = 4$ ) after 5 d of suppression assay in coculture with CD8<sup>+</sup> Tresp and anti-CD3/anti-CD28 stimulation in the absence or presence of 10 ng/ml IL-7. **(E)** CD8<sup>+</sup> Tresp were cultured alone or with TregN or TregM with anti-CD3/anti-CD28 stimulation and 10 ng/ml IL-7 and in the presence of a combination of anti-IL-10/anti-IL-10R-blocking Abs. Supernatants were collected after 5 d. Concentration of IL-2, TNF- $\alpha$ , IFN- $\gamma$ , and IL-5 (mean  $\pm$  SD) in supernatants with (gray bars) and without (black bars) the addition of anti-IL-10/anti-IL-10R-blocking Abs ( $n = 4$ ). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . ns,  $p > 0.05$ .

suppressor function remained for memory Tregs in the presence of IL-7 (Fig. 3A, right panel). Unlike Treg proliferation, which was inhibited by blocking TNF- $\alpha$ , abrogation of suppressor function of Tregs in the presence of IL-7 could not be restored by blocking the cytokines IL-10, TNF- $\alpha$ , and IFN- $\gamma$  (Fig. 3B). As observed for pSTAT5 (Fig. 1D), suppressor activity was already affected at an IL-7 concentration of 0.1 ng/ml in naive Tregs and at 1 ng/ml in memory Tregs and was maximally affected at 10 ng/ml in both Treg subsets (Supplemental Fig. 1D). Because the addition of IL-7 to beads and Tresp markedly increased Tresp proliferation, and it is known that the strength of TCR stimulation can affect both Treg suppressive capacity and Tresp sensitivity to Treg suppression (18), the suppression assay was also performed in the presence of lower bead concentrations (Supplemental Fig. 1E). The addition of IL-7 abrogated suppression by Tregs even at a low bead concentration, indicating that the effect was not due to oversaturation of the proliferation signal for normal Treg function. Importantly, IL-7 also abrogated Treg suppression of bead-stimulated CD4<sup>+</sup> Tresp proliferation (Sup-

plemental Fig. 1F), and Treg suppression of allogeneic DC stimulated CD4<sup>+</sup> and CD8<sup>+</sup> Tresp proliferation (Supplemental Fig. 1G).

The Treg/Tresp ratio can also affect the efficiency of Treg suppression. Moreover, it was reported that strong TCR stimulation leads to killing of Tregs via granzyme B production from conventional T cells (19). Because IL-7 affects T cell proliferation and apoptosis, we addressed these possibilities by examining Treg and Tresp numbers (Supplemental Fig. 2A) and apoptosis (Supplemental Fig. 2B) at the end of the suppression assay with or without IL-7. No evidence of decreased Treg numbers or increased Treg cell death was observed in the presence of IL-7 for 5 d, suggesting that the abrogation of suppression in the presence of IL-7 was not due to the loss of Tregs.

*IL-7 impairs naive Treg suppression of cytokine production, but IL-10 production by memory Tregs allows for continued suppression of cytokines in an IL-7 environment*

We next determined the effect of IL-7 on the ability of Tregs to suppress effector cytokine production of Tresp (Fig. 3C). Naive

Tregs inhibited Tresp cytokine production, and this capacity was impaired by the presence of IL-7. Memory Tregs also strongly suppressed cytokine production, but this capacity was maintained in the presence of IL-7, suggesting that, at least for the memory Treg subset, suppression of proliferation and cytokine production may be uncoupled and rely on multiple mechanisms. Potentially relevant to this observation, memory Treg cultures, but not naive Treg cultures, contained IL-10; although not substantial, IL-10 production was more pronounced in the presence of IL-7 (Fig. 3C). IL-10-producing cells within the memory Treg cultures were few but were more abundant after exposure to IL-7 (Fig. 3D). Although we were unable to formally demonstrate that the IL-10-producing CD4<sup>+</sup> T cells were not FOXP3<sup>+</sup> contaminants, it was notable that the memory Treg population remained strongly FOXP3<sup>+</sup> after culture (Supplemental Fig. 2C). Selective blocking of IL-10 in Tresp/Treg cocultures using Abs against IL-10 and IL-10R was sufficient to demonstrate the impairment of cytokine suppression in the memory Treg subset as well (Fig. 3E). Thus, we conclude that IL-7 abrogates Treg suppression of Tresp proliferation and cytokine production and that, distinct from naive Tregs, cultures containing memory Tregs additionally suppress cytokine production via IL-10.

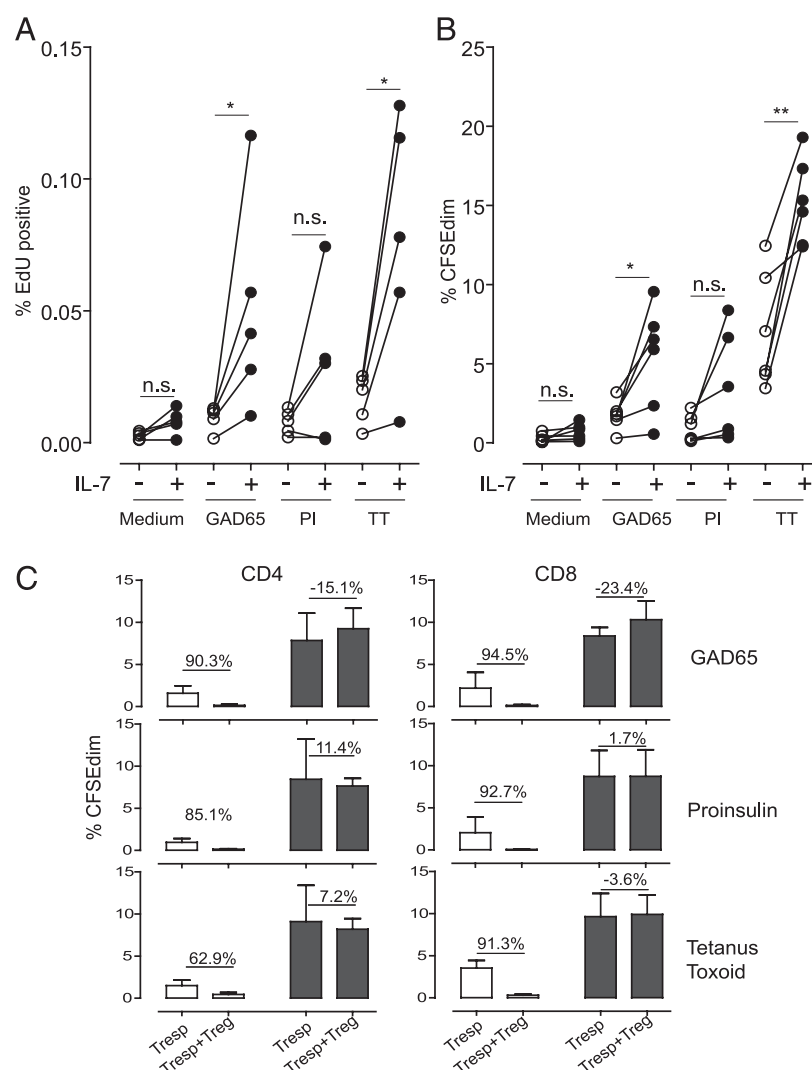
Cytokine production was also informative with respect to potential mechanisms of the IL-7-mediated abrogation of suppression. Lack of suppression was not mediated by overproduction of IL-2, because there was no measurable IL-2 in the cultures at the

end of the 5-d culture period in the presence of Tregs in both conditions with or without IL-7 (Fig. 3C). Similar results were obtained by measuring IL-2 concentration at day 2 of culture (data not shown). Moreover, the addition of anti-IL-2 to the culture did not rescue suppression by naive or memory Tregs (Supplemental Fig. 1H).

#### IL-7 abrogates Treg-mediated suppression of T cell response to Ags

Our findings of IL-7-mediated abrogation of suppressive capacity were observed when strong TCR stimulation was provided by anti-CD3/anti-CD28 beads or allogeneic DCs. To determine the potential relevance of these findings to physiological and clinical conditions, we performed suppression assays with naturally processed Ags and APCs. The type 1 diabetes-relevant Ags, GAD65 and proinsulin, as well as the vaccine Ag tetanus toxoid (TT) were loaded onto autologous monocytes and presented to CD4<sup>+</sup> and CD8<sup>+</sup> Tresp. The addition of 10 ng/ml IL-7 increased activation, as measured by EdU incorporation by T cells within 48 h (Fig. 4A, Supplemental Fig. 2D), and increased proliferation, as measured by CFSE dilution of T cells after 5 d (Fig. 4B, Supplemental Fig. 2E), in the majority of the subjects tested. Background response in the presence of autologous monocytes without Ag was only marginally and nonsignificantly increased by IL-7. Addition of Tregs in the absence of IL-7 efficiently suppressed CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses to GAD65, proinsulin, and TT (Fig. 4C).

**FIGURE 4.** Effect of IL-7 on GAD65-, proinsulin-, and TT-stimulated T cell responses. **(A)** PBMCs were stimulated with GAD65 (65-kDa isoform of glutamic acid decarboxylase), proinsulin (PI), or TT in the absence (○) or presence (●) of IL-7 (10 ng/ml). Activated precursor frequency was measured after 48 h as the percentage of CD4<sup>+</sup> T cells incorporating EdU. **(B)** CD4<sup>+</sup> T cell proliferation (CFSE dilution) over 5 d in PBMCs stimulated with GAD65, proinsulin (PI), or TT was measured as the percentage of CFSE-diluting T cells (% CFSE<sup>dim</sup>) in the absence (○) or presence (●) of IL-7 (10 ng/ml). **(C)** Ag-specific suppression assay was performed by adding FACS-sorted eFluor 670 Cell Proliferation Dye-labeled Tregs, at a 1:1 ratio, to CFSE-labeled PBMCs stimulated with GAD65, proinsulin, or TT in the absence (white bars) or presence (black bars) of IL-7 (10 ng/ml). Proliferation was measured as the percentage of CFSE dilution and CD25 upregulation (% CFSE<sup>dim</sup>) of CD4<sup>+</sup> (left panels) and CD8<sup>+</sup> (right panels) Tresp. The percentage of suppression is indicated for each group ( $n = 3$ ). \* $p < 0.05$ , \*\* $p < 0.01$ , n.s.,  $p > 0.05$ .



However, suppression of responses by Tregs was severely reduced in the presence of IL-7 (GAD65 suppression CD4<sup>+</sup>:  $90 \pm 10\%$  [medium] versus  $-15 \pm 11\%$  [IL-7],  $p = 0.03$ ; GAD65 suppression CD8<sup>+</sup>:  $94 \pm 4\%$  [medium] versus  $-23 \pm 23\%$  [IL-7],  $p = 0.02$ ; proinsulin suppression CD4<sup>+</sup>:  $85 \pm 6\%$  [medium] versus  $11 \pm 31\%$  [IL-7],  $p = 0.007$ ; proinsulin suppression CD8<sup>+</sup>:  $93 \pm 4\%$  [medium] versus  $2 \pm 31\%$  [IL-7],  $p = 0.02$ ; TT suppression CD4<sup>+</sup>:  $63 \pm 33\%$  [medium] versus  $7 \pm 28$  [IL-7],  $p = 0.048$ ; TT suppression CD8<sup>+</sup>:  $91 \pm 1$  [medium] versus  $-4 \pm 5$  [IL-7],  $p = 0.001$ ).

#### Abrogation of suppressive function is by a direct effect of IL-7 on Tregs

The mechanism by which IL-7 abrogated the ability of Tregs to suppress Tresp proliferation could include direct effects on Tregs and/or Tregs. To address this, Tregs or CD8<sup>+</sup> Tregs were treated with an anti-CD127-blocking Ab prior to suppression assay in the presence of IL-7 (Fig. 5A). Blocking CD127 on Tregs restored suppressor function (proliferation: 66% [Tresps+Tregs] versus 24% [Tresps+bCD127Tregs],  $n = 5$ ,  $p = 0.0006$ ). In contrast, blocking of CD127 on Tregs inhibited their proliferative responsiveness to IL-7, but Treg suppressor function was not restored (proliferation: 48% [bCD127Tresps] versus 45% [bCD127Tresps+Tregs],  $n = 5$ ,  $p = 0.2$ ). These data suggest that a major mechanism by which IL-7 abrogates suppression is by direct effects on Treg. To corroborate this, Tregs were prepulsed with IL-7 for 16 h prior to plating in a short-term suppression assay using EdU incorporation as a readout (Fig. 5B). Consistent with the blocking experiments, IL-7-prepulsed Tregs had an impaired suppressive function on non-pulsed CD8<sup>+</sup> Tregs (suppression: 46% [Tresps+Tregs] versus -3% [Tresps+IL-7p Tregs],  $n = 3$ ,  $p = 0.06$ ).

#### Gene-expression analysis of Tregs treated with IL-7

To further analyze the potential mechanism of IL-7-mediated abrogation of Treg suppressor function, we examined the effect of IL-7 on the expression of 84 genes involved in Treg suppression and T cell anergy (Fig. 6, Supplemental Fig. 3A, Supplemental Table I). To evaluate gene expression in conditions similar to those of the suppression assay, Tregs were activated with anti-CD3/anti-CD28 beads. First, we compared gene expression in bead-stimulated naive Tregs versus memory Tregs in the absence of IL-7 (Fig. 6A). Differences were observed in the expression of genes related to the genetic signature of Tregs, including FOXP3, CTLA-4, and PD-1 (memory versus naive Tregs: FOXP3, 2.5-fold; CTLA4, 3.2-fold; PD1, 5.0-fold), as well as an increased activation of genes of some cytokines (IL-5, 213-fold; IL-13, 25-fold; IL-10, 9.5-fold; IL-31, 8.9-fold; MCSF, 2.6-fold; GMCSF, 8.4-fold) and downmodulation of the IL-2 gene (2.2-fold). Treatment of bead-stimulated naive Tregs with IL-7 (Fig. 6B)

caused upregulation of cytokine genes (IL-5, 7.3-fold; IL-13, 2.6-fold; IL-2, 2.0-fold; IL-6, 5.0-fold; IFN- $\gamma$ , 2.1-fold), granzyme B (GZMB, 4-fold) and  $\alpha$ -1 integrin (ITGA, 5.4-fold). Granzyme B (2.6-fold) and  $\alpha$ -1 integrin (2.46-fold) were also upregulated by IL-7 in bead-stimulated memory Tregs (Fig. 6C). Moreover, selectively for memory Tregs, IL-7 induced an 8.3-fold downregulation of the EOMES gene. Protein-expression changes in freshly sorted CD4<sup>+</sup>CD25<sup>hi</sup>CD127<sup>low</sup> Tregs after a 40-h exposure to IL-7 with anti-CD3/anti-CD28 are shown in Supplemental Fig. 3C. IL-7 increased the protein expression of FOXP3, CD25, GITR, CD49d, OX40, and granzyme B and decreased the expression of CD127 and CD62L.

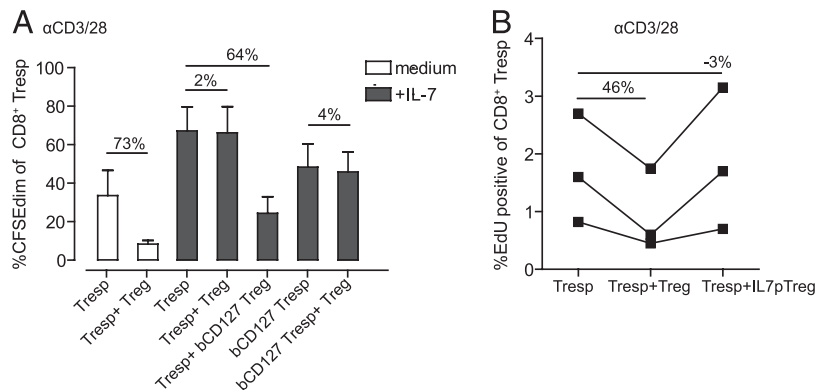
## Discussion

The presence of alloreactive and autoreactive clones in the T cell repertoire is determined by thymus-dependent pathways, but activation and expansion of naive clonal populations are regulated in peripheral lymphoid organs by Ag encounter, homeostatic mechanisms, and the inhibitory network of Tregs (20, 21). In the current study, we showed that the homeostatic cytokine IL-7 directly interferes with an important regulatory checkpoint of autoimmunity and releases T cells from the inhibitory network of Tregs.

The homeostatic cytokine IL-7 plays a nonredundant role in the survival and maintenance of the peripheral T cell pool in the steady state (22). When T cell loss occurs during infections, radio/chemotherapy, and immunosuppression, there is a vigorous cytokine-dependent T cell expansion in the periphery. Although it is recognized that IL-7 promotes survival and expansion of conventional T cells (23), the low Treg expression of CD127, which is a crucial part of the high-affinity IL-7R, left doubts as to whether Tregs were responsive to IL-7. Our findings unequivocally show that all components of a functional high-affinity IL-7R are expressed on both naive and memory Tregs and that exposure to IL-7 leads to concentration-dependent IL-7R signaling. Consistent with CD127 surface expression data, higher concentrations of IL-7 were required for Tregs than for conventional T cell IL-7R signaling.

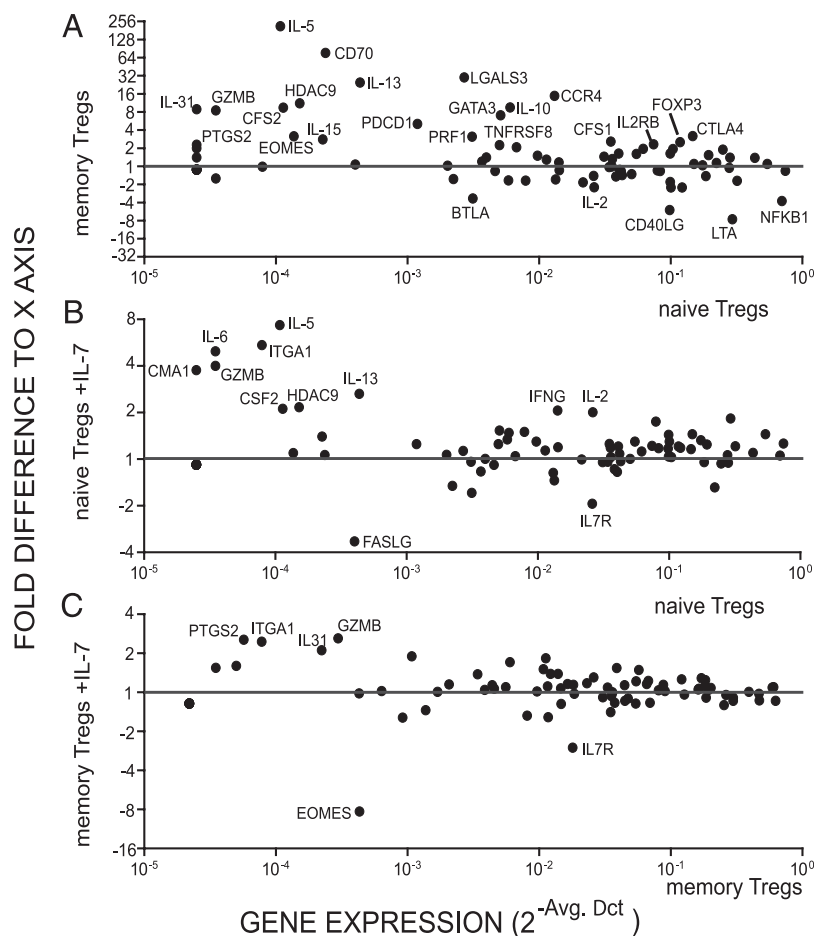
Peripheral homeostasis of conventional T cells and Tregs is regulated by different  $\gamma$ -chain cytokine-signal requirements. With respect to IL-2, a two-threshold model was proposed, in which background concentrations of IL-2 are necessary for Treg survival, whereas conventional T cells require high IL-2 concentrations to receive sufficient signal strength to proliferate (24). Our findings for IL-7 are consistent with a two-threshold mechanism for IL-7, which is reciprocal to that of IL-2 with respect to conventional T cells and Tregs. In the model, conventional T cells express abundant CD127, resulting in maintenance of IL-7 signaling at

**FIGURE 5.** IL-7 has a direct effect on Treg suppressive capacity. **(A)** FACS-sorted CFSE-labeled CD8<sup>+</sup> Tresp or CD4<sup>+</sup>CD25<sup>hi</sup>CD127<sup>low</sup> Tregs were pretreated with a blocking Ab against CD127 (bCD127) for 2 h prior to the suppression assay. Proliferation of CD8<sup>+</sup> Tresp (%CFSE<sup>dim</sup>) in response to anti-CD3/anti-CD28 stimulation in the absence (white bars) or presence (black bars) of IL-7 (10 ng/ml). Percentage of suppression is indicated for each group ( $n = 5$ ). **(B)** Tregs were left untreated or were prepulsed (IL-7pTreg) with 10 ng/ml of IL-7 for 16 h prior to the suppression assay with CFSE-labeled CD8<sup>+</sup> Tresp. Proliferation of CD8<sup>+</sup> Tresp was determined after 48 h as the incorporation of EdU and upregulation of CD25 (%EdU<sup>+</sup>CD25<sup>+</sup> of Tresp). Percentage of suppression is indicated for each group ( $n = 3$ ).





**FIGURE 6.** Changes in gene-expression analysis of anti-CD3/anti-CD28-activated Tregs upon IL-7 treatment, as measured by quantitative PCR array. Graphs show actual gene expression ( $2^{-\text{avg.}\Delta\text{ct}}$ , x-axis) relative to housekeeping genes ( $2^{-\Delta\Delta\text{ct}}$ ) versus the fold difference of the compared group (y-axis). **(A)** Bead-stimulated naive Tregs (x-axis) versus bead-stimulated memory Tregs (y-axis). **(B)** Bead-stimulated naive Tregs (x-axis) versus bead-stimulated naive Tregs treated with IL-7 (y-axis). **(C)** Bead-stimulated memory Tregs (x-axis) versus bead-stimulated memory Tregs treated with IL-7 (y-axis). In all cases, the cell population used for gene-expression analysis was the originally sorted cells after 16 h of incubation under the stated conditions. The genes with >2-fold difference are shown ( $n = 1$ ).



physiological IL-7 concentrations. In contrast, Tregs express low amounts of CD127 and are likely to achieve relevant IL-7 signaling only in IL-7-rich environments. Another notable difference between IL-2 and IL-7 environments is that IL-2 promotes surface expression of its specific receptor chain, whereas IL-7 down-regulates the expression of CD127. Finally, within conventional T cells, the relative availability of CD25 or CD127 influences the ability of the specific cytokine ligand to cause signaling, again consistent with a reciprocal relationship between IL-2- and IL-7-signaling pathways (25).

One hallmark of Tregs, in particular memory Tregs, is their anergy. Overcoming anergy in Tregs is achieved via a combination of strong TCR and IL-2 signaling (16, 26, 27). Of interest, IL-7 plus strong TCR activation was able to induce low, but significant, proliferation rates in naive Tregs; this was markedly enhanced in the presence of conventional T cells, even though there was little IL-2 production detected under these conditions. Tresp production of TNF- $\alpha$ , as recently suggested (17), appeared largely responsible for additional naive Treg proliferation observed in the presence of conventional T cells in our model. Transwell experiments suggested that TNF- $\alpha$  and/or other mechanisms supporting naive Treg proliferation require close proximity or contact with activated conventional T cells. Novel was our finding that TNF- $\alpha$  has no or only minimal effect on memory Tregs. Memory Tregs, but not naive Tregs, were also refractory to proliferative effects of IL-7. A number of the substantial gene-expression differences observed between memory and naive Tregs support this. This strict anergy of memory Tregs to IL-7 may also explain the relative reduction in Tregs observed in some clinical settings characterized by lymphopenia (13). Thus, it appears that memory conversion of Tregs is associated with enhanced

control of proliferation in favor of increased suppressor function, as found in this study and reported previously (14).

In the presence of IL-7, Tregs lost their ability to suppress proliferation of conventional T cells. Both IL-7 and IL-15 were shown to reverse suppression by Tregs (28, 29). Moreover, it was reported that when IL-7 is used in combination with IL-2 for long-term expansion of Tregs, the expanded population showed increased proliferation and reduced suppressive capacity (30). Although for the naive Tregs, both proliferation and cytokine production of conventional T cells were affected, we found that in the presence of IL-7, suppression of cytokine production, including the production of IL-2, remained intact for the memory Treg subset. Previous data imply a direct relationship between suppression of cytokine and proliferation (31), and some suggest that suppression of proliferation is caused, at least in part, by suppression and usage of IL-2 by Tregs (32, 33). Our finding that IL-7 completely abrogates suppression of proliferation, even in the incomplete suppression of effector cytokine production, casts doubt on this. According to our data, memory Tregs have additional mechanisms of suppression to those used by naive Tregs. One of these appears to be via IL-10 production, which was predominantly found in the cultures that included the memory Treg subset. Blocking IL-10 signaling in Treg/Tresp cocultures was sufficient to synchronize IL-7 abrogation of suppression on proliferation and cytokine production for both the naive and memory Treg subsets. However, we did not formally show that the IL-10-producing cells were FOXP3 $^{+}$ , and we conclude that IL-10 production by memory Tregs or contaminating CD4 $^{+}$  non-Tregs contribute to suppression independently of other mechanisms in our cocultures.

The observations with respect to cytokine production and cytokine blocking also indicate that IL-7 does not use the more obvious methods of reversal of suppression, such as increasing IL-2 concentration (34, 35). Indeed, abrogation of suppression was observed without an increase in IL-2 and in the presence of anti-IL-2 Ab. An excess of proliferation by the Tregs is also unlikely to be the mechanism, because IL-7-mediated abrogation of suppression even was seen with low anti-CD3/anti-CD28 bead concentrations, with allogeneic DCs as TCR stimulation, and with autoantigen-specific T cell responses. Moreover, mechanisms of Treg killing, such as granzyme B, would not be supported by the healthy Treg numbers and survival observed at the end of the cultures. Instead, and uniquely, we observed a direct effect of IL-7 on Tregs. Tregs that were rendered insensitive to IL-7 by preincubation with an anti-IL-7R Ab were fully functional in the presence of IL-7. In contrast, preincubation of the Tregs with the anti-IL-7R Ab did not restore Treg suppression in the presence of IL-7. Because the Tregs and Tregs have markedly different CD127 expression, we are hesitant to interpret these findings as excluding a contribution of the effects of IL-7 on Tregs. Nevertheless, we suggest that, collectively, the findings strongly support the notion that IL-7/IL-7R signaling in Tregs impairs their suppressor function.

In relation to potential mechanisms involved in the IL-7-mediated abrogation of suppression, we performed preliminary experiments assessing the gene-expression profiles of TCR-stimulated memory and naive Tregs in the presence and absence of IL-7. Although requiring confirmation, the addition of IL-7 altered the expression of multiple genes within the restricted panel examined. Despite this, most changes were modest, and no obvious changes could be linked to the loss of suppressor function. Upregulation of granzyme B and  $\alpha 1$  integrin (CD49a) and downregulation of IL-7R were consistent between memory and naive Tregs. Granzyme A and B in Tregs are relevant to Treg suppressor function (36, 37). However, the effect of IL-7 on granzyme B expression would be expected to increase, rather than decrease, this function, and protein analysis cast doubt on a mechanistic role for granzyme B. Integrin costimulation of T effector cells may play a role with regard to Tregs (38); again, this was not investigated with respect to Treg expression and could not be confirmed at the protein level.

IL-7-mediated Treg proliferation while suppressive function is abrogated could have advantages for the immune system. For example, in situations of severe reduction of the T cell compartment, abrogation of Treg suppressive function may facilitate and accelerate T cell proliferation to restore immunocompetence. Once steady state is achieved, reduction of IL-7 concentration would restore Treg suppressive capacity. However, we hypothesize that when increased IL-7 is prolonged, compromised Treg function increases the risk of expanding autoreactive T cells, or in the case of graft-versus-host disease and transplantation, alloreactive T cells. Consistent with this, IL-7 concentration is increased in the synovial fluid of patients with rheumatoid arthritis (39), the cerebrospinal fluid of patients with multiple sclerosis (10), and the salivary glands of patients with Sjögren's syndrome (40). Moreover, the major sources of IL-7 are stromal cell populations hosted in bone marrow niches (41) that house Tregs (42) and that support extensive proliferation of bone marrow resident mature T cells, including diabetogenic (43) and colitogenic (44) T cells.

We showed that IL-7 can unmask autoreactivity to diabetes-associated autoantigens, similar to what other investigators noted when Tregs were removed (45). In vivo, increased concentrations of IL-7 posttransplantation are associated with expansion of

autoreactive CD8<sup>+</sup> T cells (3). In animal models, exogenous administration of IL-7 is a potent inducer of autoimmune diabetes and is associated with a reduction in Treg suppressive activity (8); conversely, blocking IL-7R can reverse diabetes in NOD mice (46, 47). Therefore, we suggest that prolonged increased availability of IL-7 can result in expansion of autoreactive T cells both by direct effects on survival and proliferation of conventional T cells, as well as by releasing the suppression provided by Tregs. We further suggest that the balance of IL-7 versus IL-2 signaling may influence the size of the autoreactive T cell pool and note that genes of the IL-2RA and IL-7R provide susceptibility to autoimmune disease, including type 1 diabetes (9, 48). Polymorphisms in the IL-7R gene associated with the risk for developing type 1 diabetes influence the ratio of membrane-bound and a released soluble form of IL-7R in T cells. This may affect the sensitivity of T cells to IL-7 by affecting surface expression of IL-7R and/or the bioavailability of circulating IL-7 through the binding of soluble IL-7R to IL-7. Such mechanisms in Treg suppressive function and proliferation have not been clarified and need to be investigated further. Finally, our findings also have direct relevance to the control of alloreactive T cells, especially in lymphopenic conditions as seen in graft-versus-host disease. Thus, targeting the IL-7/IL-7R axis may provide an avenue to control autoreactive and alloreactive T cell numbers both by reducing the expansion of autoreactive and alloreactive T cells and by restoring the function of Treg populations.

## Disclosures

The authors have no financial conflicts of interest.

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