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

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CD4⁺CD25⁺FOXP3⁺ 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.

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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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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...
informed consent and ethics committee approval. Highly purified CD8+ versus CD4+CD25+CD127hi responder T cells (Tresps) plus CD4+CD25+CD127lo 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 eBioD8R5) from previously enriched CD8+ (CD8 Isolation Kit II; Miltenyi Biotec) and CD4+CD25+ cells (CD4 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 PE (clone RPA-T4), CD4 PerCP (clone SK3), CD4 allophycocyanin (clone SK3), CD6 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), STAT3 pY694 Alexa Fluor 647 (clone 47), CD45RA allophycocyanin (clone HI100), CD45RO PE-Cy7 (clone UCHL1), ILA-DR PE (clone G46-6), IL-10 allophycocyanin (clone JES319F1), and isotype control IgG2a allophycocyanin (clone R35-95) (all from BD Pharmingen); CD127 eFluor 450 (clone eBioD8R5), OX-40 FITC (clone ACT35), ICOS FITC (clone ISA-3), and FOXP3 allophycocyanin (clone SK11), CD45RA allophycocyanin (clone TS2/7), granzyme B allophycocyanin (clone GB11), and TGF-β7-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 Alexa Fluor 647 (clone 47; BD Pharmingen). Proliferation of Tregs and T cells was visualized by 5-ethyl-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+ and CD8+ Tresps or Tregs were labeled with CFSE (0.5 μM in PBS for 10 min at 37°C; Molecular Probes). When CD4+ T cells were used as Tresps, Tregs were labeled with eFluor 670 Cell Proliferation Dye (5 μM in PBS for 10 min at 37°C; eBioscience). A total of 105 Tresps alone, 105 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 1:10 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 First Strand Kit and T cell anergy and immune tolerance Rt2 Profiler PCR array, PAHS-074; SA Biosciences). Changes in cycle threshold (ΔCt values) were calculated using B2M, HPRT-1, RPL3L, and GAPDH as housekeeping genes. Rt2 Profiler PCR Array Data Analysis software (SA Biosciences) 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+ cells. Data are presented as mean ± SD.

Results

Expression of CD127 and CD132 on conventional T cells and Tregs

Surface expression of the high-affinity IL-7Rα-chain (CD127) and IL-7Rγ-chain (CD132) was measured on conventional T cells and Tregs (Fig. 1A, 1B). Compared with the CD19+ B lymphocyte population, which is negative for CD127, all conventional T cell populations with the Milliplex Human Cytokine Immunoassay (IFN-γ, TNF-α, 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+CD25+ 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 First Strand Kit and T cell anergy and immune tolerance Rt2 Profiler PCR array, PAHS-074; SA Biosciences). Changes in cycle threshold (ΔCt values) were calculated using β2M, HPRT-1, RPL3L, and GAPDH as housekeeping genes. Rt2 Profiler PCR Array Data Analysis software (SA Biosciences) 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.

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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+ T cells and Tregs (Fig. 1C). Conistent with their high CD127 expression, IL-7 ABROGATES SUPPRESSIVE ACTIVITY OF Tregs...
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+CD25–CD127+CD127+ Tregs in contact with bead-activated, CFSE-labeled FACS-sorted CD8+ T cells incorporated EdU and induced a low amount of proliferation in naive Tregs but not in memory Tregs (CFSEdim: 7.1 ± 2.3% [naive Tregs] versus 0.7 ± 0.5% [memory Tregs], n = 4, p = 0.009). Culture with Tresps and with bead stimulation also resulted in proliferation of naive Tregs but not memory Tregs (CFSEdim: 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 Tresps 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 (CFSEdim: 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, Tresps, and bead stimulation was abolished when naive Tregs and CD8+ Tresps were cocultured using a Transwell system, suggesting that proximity of the Tresp population was important for the marked naive Treg proliferation (Fig. 2D).
Proliferating naive Tregs retain a FOXP3+IL-10+ phenotype and are enriched in CD45RO+ memory cells (Fig. 2E, MFI FOXP3 in Tregs: 5552±610 [memory Treg RESORTED] versus 2623±586 [naive Treg RESORTED], p<0.0001). After FACS resorting and removal from IL-7, both the proliferating, CD45RO-enriched Tregs and the remaining CFSE-high Tregs fully suppressed the proliferation of Tresps 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 Tresps. 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+ Tresps (contact) or using a Transwell system (n=3). (E) Originally naive Tregs were FACS sorted after 5 d of suppression assay with CD8+ Tresps, anti-CD3/anti-CD28, and 10 ng/ml IL-7 into proliferated memory CD45RO+-enriched (R1, CFSEdim, TregMRESORTED) and naive (R2, CFSEhigh, TregNRESORTED) Tregs, as shown in the representative FACS plot (left panel). FOXP3 expression (upper right panel) and the percentage of CD25+IL-10+ cells of resorted Tregs (lower right panel), as measured by intracellular staining (n=5). (F) TregMRESORTED (○) and TregNRESORTED (▲) were tested in a suppression assay performed with anti-CD3/anti-CD28 stimulation in the absence of IL-7 (mean ± SD) (n=3). **p<0.01, ***p<0.001.

FIGURE 2. Proliferation of Tregs in coculture assay. (A) Confocal imaging of EdU incorporation (white) of Tregs and CFSE-labeled CD8+ Tresps (green) in the absence (medium, upper panels) or presence (lower panels) of IL-7. Scale bars, 5 μm. (B) Proliferation measured as CFSE dilution (% CFSEdim) of CD4+ naive (TregN) or memory (TregM) Tregs stimulated with anti-CD3/anti-CD28 in the absence or presence of CD8+ Tresps and/or IL-7 (n=4). (C) TregN and TregM proliferation in response to anti-CD3/anti-CD28 stimulation in coculture with CD8+ Tresps and IL-7 in the presence or absence of blocking Abs against IL-10, TNF-α, or IFN-γ (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+ Tresps (contact) or using a Transwell system (n=3).
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-α, abrogation of suppressor function of Tregs in the presence of IL-7 could not be restored by blocking the cytokines IL-10, TNF-α, and IFN-γ (Fig. 3B). Also 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 Tresps 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+ Tresp proliferation (Supplemental Fig. 1F), and Treg suppression of allogenic DC stimulated CD4+ and CD8+ 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 Tresps (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+ T cells were not FOXP3− contaminants, it was notable that the memory Treg population remained strongly FOXP3+ 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+ and CD8+ Tresps. 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+ and CD8+ 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+ T cells incorporating EdU. (B) CD4+ 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 (% CFSEdim) 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 (% CFSEdim) of CD4+ (left panels) and CD8+ (right panels) Tresps. 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+: 90 ± 10% [medium] versus −15 ± 11% [IL-7], p = 0.03; GAD65 suppression CD8+: 94 ± 4% [medium] versus −23 ± 23% [IL-7], p = 0.02; proinsulin suppression CD4+: 85 ± 6% [medium] versus 11 ± 31% [IL-7], p = 0.007; proinsulin suppression CD8+: 93 ± 4% [medium] versus 2 ± 31% [IL-7], p = 0.02; TT suppression CD4+: 63 ± 33% [medium] versus 7 ± 28 [IL-7], p = 0.048; TT suppression CD8+: 91 ± 1 [medium] versus −4 ± 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+ Tresp 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% [Tresp+ Tregs] versus 24% [Tresp+IL-7p Tregs], n = 5, p = 0.0006). In contrast, blocking of CD127 on Tresp inhibited their proliferative responsiveness to IL-7, but Treg suppressor function was not restored (proliferation: 48% [CD127Tresp] versus 45% [CD127Tresp+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 pre pulsed 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– pre pulsed Tregs had an impaired suppressive function on un pulsed CD8+ Tresp (suppression: 46% [Tresp+Tregs] versus −3% [Tresp+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 T sup pression 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, 23-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; 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; M-CSF, 2.6-fold; GM-CSF, 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-γ, 2.1-fold), granzyme B (GZMB, 4-fold) and α-1 integrin (ITGA, 5.4-fold). Granzyme B (2.6-fold) and α-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+CD25+CD127low 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 γ-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) FACScanalyzed CD8+ Tresp or CD4+CD25hiCD127low Tregs were pretreated with a blocking Ab against CD127 (bCD127) for 2 h prior to the suppression assay. Proliferation of CD8+ Tresp (%CFSEdim) 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 pre pulsed (IL-7p Treg) with 10 ng/ml of IL-7 for 16 h prior to the suppression assay with CFSE-labeled CD8+ Tresp. Proliferation of CD8+ Tresp was determined after 48 h as the incorporation of EdU and upregulation of CD25 (%EdU･CD25+ of Tresp). Percentage of suppression is indicated for each group (n = 3).
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 downregulates 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, 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, 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, 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, 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, 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, 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, even though there was little IL-2 production detected under these conditions.
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 Tresps 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 Tresps with the anti–IL-7R Ab did not restore Treg suppression in the presence of IL-7. Because the Tresps 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 Tresps. 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 α1 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 Tresps (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, autoreactive 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 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+ 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 autoreactive 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 autoreactive T cell numbers both by reducing the expansion of autoreactive and autoreactive T cells and by restoring the function of Treg populations.

Disclosures
The authors have no financial conflicts of interest.

References


