# Concentration and Activity of the Soluble Form of the Interleukin-7 Receptor α in Type 1 Diabetes Identifies an Interplay Between Hyperglycemia and Immune Function

Paolo Monti,[1] Cristina Brigatti,[1] Miriam Krasmann,[2] Anette G. Ziegler,[2] and Ezio Bonifacio[3]

Soluble interleukin-7 (IL-7) receptor α (sCD127) is implicated in the pathogenesis of autoimmune diseases. We show that serum sCD127 concentrations are increased at the onset of type 1 diabetes (T1D; $n = 390$) as compared with concentrations in age-matched islet autoantibody–negative first-degree relatives of patients ($n = 392$; $P = 0.00001$). sCD127 concentration in patients was influenced by islet autoantibody status ($P = 0.003$) and genotype of the rs6897932 single nucleotide polymorphism within the *IL-7RA* gene ($P = 0.006$). Release of sCD127 in vitro was strongly upregulated by activation of T lymphocytes and affected by exposure to cytokines. sCD127 bound IL-7 and was antagonistic to IL-7 signaling and IL-7–mediated T-cell proliferation, suggesting a regulatory feedback mechanism on T-cell expansion. Remarkably, high glucose led to a glycated form of sCD127 that was ineffective as an IL-7 antagonist. The finding of glycated sCD127 in the circulation of patients at onset of T1D suggested that physiological regulation of IL-7–mediated T-cell survival and expansion by sCD127 may be compromised in T1D. The findings indicate that genetic, immunologic, and metabolic factors contribute to a dysregulation of the IL-7/IL-7 receptor pathway in T1D and identify a novel hyperglycemia-mediated interference of immune regulatory networks. *Diabetes* 62:2500–2508, 2013

The homeostatic cytokine interleukin-7 (IL-7) interacts with the IL-7 receptor (IL-7R) α chain (CD127) and the $g_c$ receptor (CD132) on the T-cell surface, forming a complex crucial to several signaling cascades involved in the survival and proliferation of T cells, including autoreactive T-cell clones. The IL-7/IL-7R pathway has been implicated in the expansion of autoimmunity and alloimmunity. In type 1 diabetes (T1D), increased concentrations of IL-7 are found postislet transplantation and are associated with homeostatic proliferation of T cells (1). Elevated IL-7 concentrations have also been reported in the synovial fluid of patients with rheumatoid arthritis (2), in the serum and cerebrospinal fluid of patients with multiple sclerosis (3), and in the serum of patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (4). Moreover, IL-7 accelerates diabetes onset in the nonobese diabetic mouse (5), while blockade of the IL-7R can reverse diabetes in the same model (6,7).

As with many cytokine receptors, a soluble form of the IL-7R α chain (sCD127) has been identified (8). It is derived both from alternative splicing and by release of membrane-bound sCD127. The production of sCD127 is in part determined by regulation of transcription and mRNA processing, especially splicing, and this is affected by polymorphisms within the *CD127* gene. Four common *CD127* haplotypes have been described (9). Haplotype 2 is identified by a T allele in exon 6 (rs6897932). This single nucleotide polymorphism (SNP) is associated with reduced exon splicing and lower production of mRNA encoding soluble IL-7Ra in healthy control individuals. In contrast, transcripts that skip exon 6 (C allele of rs6897932) confer susceptibility to T1D and encode sCD127, potentially altering the production of sCD127 and IL-7R signaling in T cells (10).

Little is known with respect to how membrane versus sCD127 affects T-cell responses. Soluble forms of receptors can be antagonistic, such as tumor necrosis factor (TNF) receptor I and II (11) and the IL-6R subunit gp130 (12), or agonistic, such as the soluble IL-15 receptor α, where precomplexed IL-15/soluble IL-15 receptor α increases natural killer and CD8+ T-cell proliferation up to 50 times compared with IL-15 alone (13). In the current study, we asked whether sCD127 functions as an antagonistic or agonistic molecule in IL-7 signaling and whether it is dysregulated in patients with T1D. The findings show that sCD127 acts as an antagonist, is increased by immune activation, and affected by T1D-relevant CD127 polymorphisms. Its concentration is increased at onset of T1D, consistent with immune activation. Remarkably, however, sCD127 is glycated in patients, and the glycated form is ineffective as an antagonist of IL-7–mediated T-cell expansion. These findings show the complexity of cytokine signaling and associations within a disease process and unveil a novel effect of high blood glucose that affects immune function.

## RESEARCH DESIGN AND METHODS

**Subjects.** Serum samples were collected at disease onset from 390 patients with diabetes aged <20 years (213 males; mean age ± SD, 9.9 ± 4.4 years) participating in the DiMelli study that recruits the majority of new-onset patients with diabetes aged <20 years in Bavaria, Germany (14), and from 392 islet autoantibody–negative first-degree relatives (213 males; mean age ± SD, 9.9 ± 4.4 years) participating in the German TEENDIAB study (15). Among the 390 patients, 349 were positive for at least one islet autoantibody against GAD65, IA-2, or ZnT8. Genotyping for the rs6897932 SNP within the *IL-7Ra* gene was performed using the MassARRAY system with iPLEX chemistry (Sequenom, San Diego, CA). Serum concentration of sCD127 was also tested in samples from islet autoantibody–negative ($n = 317$, 169 female; median age 11.2 years; range, 7.3–16.0 years) and –positive first-degree relatives of patients with T1D ($n = 317$, 158 female; 138 with one, 179 with at least two islet autoantibodies; median age 11.2 years; range, 7.3–16.0 years).

**sCD127 binding to IL-7.** ELISA was used to measure sCD127 binding to IL-7. High-binding polystyrene 96-well plates were coated overnight with sCD127 (100 ng/mL; R&D Systems) and/or sCD132 (1 ng/mL; R&D Systems) in PBS,

washed, blocked with PBS-2% BSA, and incubated with increasing concentrations (0.125–2,048 pg/mL) of biotinylated IL-7 (R&D Systems) for 2 h. Detection of bound IL-7 was performed by incubation of plates with streptavidin-horseradish peroxidase (HRP; R&D Systems) followed by addition of tetramethylbenzidine substrate solution (R&D Systems) and colorimetric determination of sCD127-bound IL-7.

**sCD127 effects on IL-7 signaling and T-cell proliferation.** Peripheral blood mononuclear cells (PBMCs) were incubated for 20 min with IL-7 (10 ng/mL) at 37°C in RPMI 1640 supplemented with 5% FCS in the absence or presence of 100 ng/mL of human CD127 (R&D Systems). PBMCs were fixed with 5% formaldehyde, stained with anti-CD4 Pacific blue (clone RPA-T4; BD Biosciences), permeabilized with methanol, and stained with anti-phosphorylated (p)Y694 signal transducer and activator of transcription 5 (STAT5) Alexa Fluor 647 (clone 47; BD Biosciences) and anti-pT308 Akt phycoerythrin (clone J1-223.371; BD Biosciences). For antigen-specific and polyclonal T-cell proliferation, PBMCs were stained with 5- (and 6-)carboxyfluorescein diacetate succinimidyl ester (CFSE) and incubated with antigens GAD65 and tetanus toxoid (TT) (16), or with anti-CD3/CD28–coated beads at a 1:100 bead/cell ratio, in the absence or presence of IL-7 (1 ng/mL) and varying concentrations of sCD127. After 5 days, PBMCs were stained with anti-CD4, and the percentage of proliferating cells (CFSE$^{dim}$) was determined on gated CD4$^+$ T cells by flow cytometry.

**Regulation of membrane-bound and sCD127.** PBMCs were cultured for 24 h in RPMI 1640 supplemented with 5% FCS with the following stimuli: anti-CD3/CD28–coated beads (1:100 bead/cell ratio; Dynal), IL-2 (100 U/mL; BioSource International), IL-7 (10 ng/mL; R&D Systems), interferon-γ (IFN-γ; 10 ng/mL; BioSource International), IL-4 (10 ng/mL; PeproTech), IL-17 (10 ng/mL; R&D Systems), TNF-α (10 ng/mL; R&D Systems), IL-10 (10 ng/mL; R&D Systems), IL-12 (10 ng/mL; PeproTech), rapamycin (sirolimus, 10 ng/mL; Sigma-Aldrich), FK506 (tacrolimus, 10 ng/mL; Fujizawa Pharmaceutical), mycophenolate mofetil (MMF, 1 µg/mL; Roche), daclizumab (1 µg/mL; Roche), high glucose (33.3 mmol/L; Sigma-Aldrich), and insulin (100 U/mL; Sigma-Aldrich). PBMCs were subsequently stained with anti-CD4 Pacific blue (clone RPA-T4; BD Biosciences), anti-CD8 allophycocyanin-Cy7 (clone SK1; BD Biosciences), and anti-CD127 phycoerythrin (clone M21; BD Biosciences), and surface expression of CD127 in gated CD4$^+$ and CD8$^+$ T cells was analyzed by flow cytometry. Supernatant from PBMC cultures was taken to measure sCD127 concentration.

**sCD127 ELISA.** sCD127 was measured in serum and cell-culture supernatant using a sandwich ELISA. High-binding polystyrene 96-well plates were coated overnight in PBS with 50 mg/mL of monoclonal mouse anti-human CD127 antibody (clone 40131; R&D Systems), washed, and the plate blocked with PBS–2% BSA for 3 h. After washing, 50 µL of serum diluted 1:5 in PBS–0.1% BSA or undiluted culture supernatant was added to the wells for 2 h at room temperature. Plates were washed and incubated with 5 µg/mL biotinylated polyclonal goat anti-human CD127 (R&D Systems) for 1 h, washed, streptavidin-HRP added, washed, and developed with tetramethylbenzidine substrate solution and colorimetric measurement.

**Bioinformatic analysis for prediction of sCD127 glycation sites and experimental glycation of sCD127.** The mRNA sequence of IL-7Ra/CD127 (*Homo sapiens*; accession number NP_002176.2) was subjected to computer-assisted analysis to predict the presence of potential glycation sites using an online algorithm (NetGlycate 1.0 Server, http://www.cbs.dtu.dk/services/NetGlycate/).

Recombinant human sCD127 (R&D Systems) was experimentally glycated by incubation for 7 days in the dark at 37°C in the presence of 100 mmol/L D-glucose (Sigma-Aldrich) or PBS as control. sCD127 glycation was evaluated by nontryptophan fluorescence as previously reported (17). Aliquots (200 ng) of native, glucose-treated, and PBS-treated sCD127 were electrophoresed by 12% SDS-PAGE under denaturing conditions and electroblotted onto 0.22-µm nitrocellulose membranes (Bio-Rad).

**Detection of glycated sCD127 in serum samples.** Sera were incubated with anti-mouse immunoglobulin G (IgG) 1–coupled beads coated with mouse IgG1 anti-human sCD127 (clone 40131; R&D Systems) to immobilize serum sCD127 on beads, as we previously described (18). Beads were washed and incubated with a recombinant receptor for advanced glycation end products (RAGE)/human IgG protein chimera (R&D Systems) that recognizes and binds to glycated proteins. RAGE detection was performed by incubation with goat anti-human IgG fluorescein isothiocyanate (Sigma-Aldrich).

**Statistical analysis.** Expression of CD127, pSTAT5, and pAKT was assessed as median fluorescence intensity (MFI). To evaluate proliferative T-cell responses, the percentage of CD4$^+$CFSE$^{dim}$ cells was calculated. IL-7 binding to sCD127 was assessed as arbitrary units of optical density. Data are presented as mean ± SD, and Student $t$ test was used for comparisons. sCD127 concentration in serum samples was calculated in nanograms per milliliter according to a standard curve. Comparisons of sCD127 and glycated sCD127 concentrations between patients and control subjects and according to CD127 genotype were performed by linear regression analysis and included age and sex as covariates. A two-tailed $P$ value <0.05 was considered significant.

## RESULTS

**sCD127 binds to IL-7.** IL-7 interacts with the IL-7Ra chain (CD127) and the γ$_c$ (CD132) for signaling on the T-cell surface, and both CD127 and CD132 have a circulating soluble form. We therefore asked whether sCD127 is able to bind to IL-7 and whether CD132 is required for IL-7 binding. We used ELISA (Fig. 1A), in which plastic is coated with sCD127, sCD132, and in combination, and IL-7 binding is detected by streptavidin-biotin HRP. Typical in vivo concentrations of sCD127 (100 ng/mL) and sCD132 (1 ng/mL) were used. IL-7 binding to sCD127 was detectable from an IL-7 concentration of 1 pg/mL, concentrations that are found in the circulation of immunocompetent subjects (Fig. 1B). Binding increased to 32 pg/mL, which is an IL-7 concentration seen in immunosuppressed subjects, and was saturated at 256 pg/mL. IL-7 did not bind to sCD132, and the presence of sCD132 did not influence the binding of IL-7 to sCD127.

**sCD127 inhibits IL-7 signaling and T-cell proliferation.** We next sought to determine whether sCD127 is agonistic or antagonistic with respect to the biological effect of IL-7 on T-cell signaling and proliferation (Fig. 2). Stimulation with IL-7 induced high amounts of pSTAT5 in CD4$^+$ and CD8$^+$ T cells (MFI mean ± SD, CD4$^+$: 2,265 ± 506; CD8$^+$: 3,095 ± 647) and pAKT (MFI mean ± SD, CD4$^+$: 880 ± 149; CD8$^+$: 1,086 ± 181), and this was markedly reduced in T cells when sCD127 was added to the culture (soluble STAT5 MFI mean ± SD, CD4$^+$: 519 ± 174, $P < 0.0001$; CD8$^+$: 1,112 ± 309, $P < 0.0001$; pAKT mean ± SD, CD4$^+$: 585 ± 131, $P < 0.0001$; CD8$^+$: 775 ± 108, $P < 0.0001$). The inclusion of IL-7 (1 ng/mL) to PBMCs cultured with GAD65, TT, or anti-CD3/CD28 beads increased CD4$^+$ T-cell proliferation, and this was reversed by the addition of sCD127 for both healthy control subjects and patients with T1D (percent CD4$^+$CFSE$^{dim}$ mean ± SD; medium + IL-7: 0.51 ± 0.25 vs. medium + IL-7 + sCD127: 0.34 ± 0.21, $P = 0.45$; GAD65 + IL-7: 6.83 ± 2.15 vs. GAD65 + IL-7 + sCD127: 4.15 ± 1.32, $P < 0.0001$; TT + IL-7: 8.02 ± 3.45 vs. TT + IL-7 + sCD127: 5.05 ± 2.23, $P < 0.0001$; anti-CD3/CD28 + IL-7:
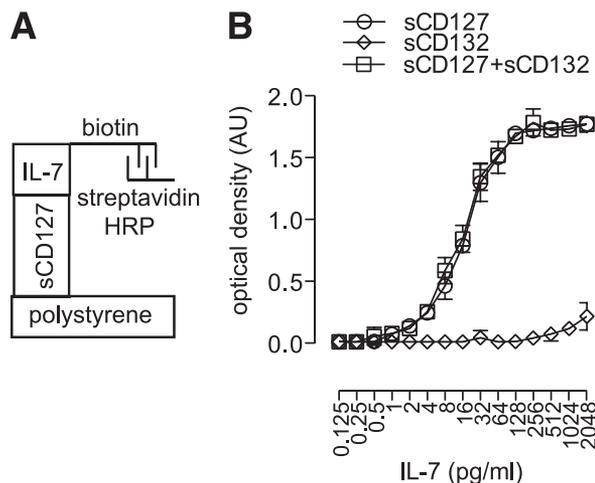


FIG. 1. sCD127 binding to IL-7. *A*: sCD127 was immobilized on a high-binding polystyrene surface to detect binding of biotinylated IL-7 by colorimetric determination with streptavidin-HRP as shown schematically. *B*: IL-7 binding (optical density, *y*-axis) over an IL-7 concentration range from 0.125–2,048 ng/mL (*x*-axis) to sCD127 (100 ng/mL; circles), sCD132 (1 ng/mL; diamonds), and sCD127 + sCD132 (100 ng/mL + 1 ng/mL; squares). Error bars represent mean ± SD from *n* = 3 experiments. AU, arbitrary units.
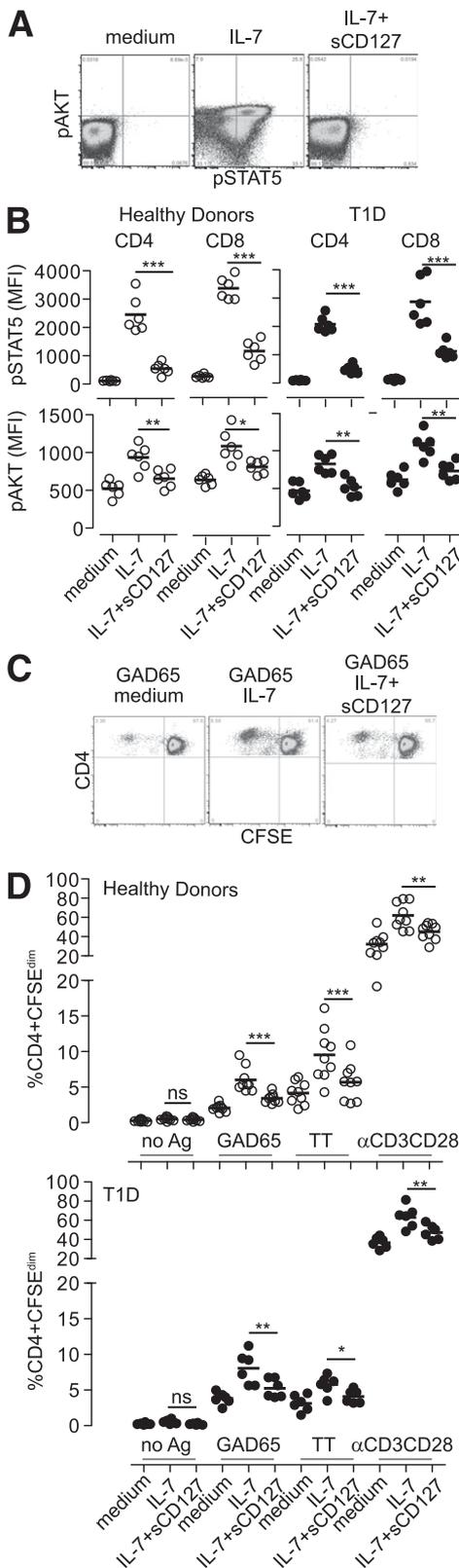
FIG. 2. sCD127 inhibits IL-7 signaling and IL-7–mediated T-cell proliferation. *A*: Representative fluorescence-activated cell sorting (FACS) plot showing pSTAT5 and pAkt of gated CD4$^+$ T cells from PBMCs cultured with IL-7 (1 ng/mL) in the absence or presence of sCD127 (100 ng/mL). *B*: MFI (*y*-axis) of pSTAT5 and pAkt in CD4$^+$ and CD8$^+$ T cells from PBMCs from healthy subjects and patients with T1D cultured with medium, IL-7 (1 ng/mL), and IL-7 + sCD127 (100 ng/mL); *n* = 6 samples. *C*: Representative FACS plot showing proliferation of gated CD4$^+$ T cells from CFSE-labeled PBMCs cultured for 5 days with GAD65 (5 μg/mL) and IL-7 (1 ng/mL) in the presence or absence of sCD127 (100 ng/mL). *D*:

62.69 ± 12.45 vs. anti-CD3/CD28 + IL-7 + sCD127: 46.18 ± 8.02, *P* < 0.0001; Fig. 2*C* and *D*). These data indicate that sCD127 is an antagonist soluble cytokine receptor.

**Membrane-bound and sCD127 are regulated by immune stimuli.** The primary source(s) of sCD127 has not been determined but may be the result of alternative splicing, receptor shedding, and receptor cleavage. Antigenic stimulation of PBMCs induces downregulation of membrane-bound CD127 in the proliferating subset and a correspondent release of sCD127 in the culture supernatant proportional to the percentage of proliferating cells (Fig. 3*A* and *B*). We aimed to clarify whether active immune processes and immunomodulation can influence both the expression of CD127 on the surface of T cells and shedding/cleavage of sCD127. PBMCs from healthy control subjects (*n* = 12) and patients with T1D (*n* = 6) were cultured in the presence of immune stimuli: anti-CD3/CD28–coated beads (T-cell receptor [TCR]/costimulation triggering), IL-2 (T-cell proliferation), IL-7 (T-cell survival/homeostatic proliferation), IFN-γ (Th1), IL-4 (Th2), IL-17 (Th17), TNF-α (inflammation), IL-12 (dendritic cells), IL-10 (immune regulation); immunosuppressive drugs: rapamycin, FK506, anti-CD25 daclizumab; or metabolic stimuli: high glucose and insulin (Fig. 3*C* and *D*). CD127 was highly expressed in both CD4$^+$ (MFI; control subjects, 5,550 ± 1,029; patients, 5,453 ± 1,039; *P* = 0.89) and CD8$^+$ (MFI; control subjects, 5,741 ± 881; patients, 6,266 ± 867; *P* = 0.29) T cells, and only low concentrations of sCD127 were present in the culture supernatant in unstimulated conditions (ng/mL; control subjects, 1.7 ± 0.5; patients, 1.9 ± 0.7; *P* = 0.45). T-cell activation with anti-CD3/CD28 downregulated membrane-bound CD127 (CD4$^+$ MFI: 3,995 ± 840, *P* = 0.0005; CD8$^+$ MFI: 3,468 ± 1,179, *P* = 0.0003) and increased the concentration of sCD127 in the supernatant (ng/mL; 19.1 ± 8.9; *P* = 0.0008). A similar effect was observed with IL-2 (40 IU/mL) on surface expression of CD127 (CD4$^+$ MFI: 3,847 ± 1,076, *P* = 0.0003; CD8$^+$ MFI: 3,468 ± 1,179, *P* = 0.0004) and sCD127 in the culture supernatant (ng/mL: 10.6 ± 5.7; *P* = 0.014). The addition of IL-7 (1 ng/mL) strongly downregulated CD127 on the cell surface of T cells (CD4$^+$ MFI: 845 ± 389, *P* < 0.0001; CD8$^+$ MFI: 1,722 ± 736, *P* < 0.0001), but sCD127 concentration in the culture supernatant remained unchanged (ng/mL; 3.0 ± 1.87; *P* = 0.47), suggesting a mechanism of receptor internalization rather that shedding, as we have previously described (18). Other cytokines had minor or no effects on CD127 surface expression, but generally increased sCD127 concentration in the supernatant, the strongest effect seen with the noncommon γ chain using cytokine IL-10 (ng/mL; IFN-γ: 1.7 ± 0.6, *P* = 0.88; IL-4: 5.1 ± 2.2, *P* = 0.009; IL-17: 3.4 ± 2.1, *P* = 0.038; TNF-α: 3.1 ± 2, *P* = 0.041; IL-12: 4.3 ± 2.6, *P* = 0.07; and IL-10: 8.4 ± 4.3, *P* = 0.002).

No effect on surface CD127 and sCD127 was observed by the addition of the immunosuppressive drugs rapamycin (10 ng/mL), FK506 (5 ng/mL), mycophenolate mofetil (MMF) (1 mg/mL) and daclizumab (1 μg/mL) or by the addition of high glucose (33.3 mmol/L) or insulin (40 IU/mL).

Proliferation of gated CD4$^+$ T cells (%CD4$^+$CFSE$^{dim}$, *y*-axis) from healthy subjects and patients with T1D stimulated with medium, GAD65 (5 mg/mL), TT (40LFU), and anti-CD3/CD28–coated beads (αIFN; 1:100 bead/T cell ratio) in medium, IL-7 (1 ng/mL), and IL-7 + sCD127 (100 ng/mL); *n* = 9 samples. *P* < 0.05, **P* < 0.01, ***P* < 0.001. Lines in *B* and *D* represent means.
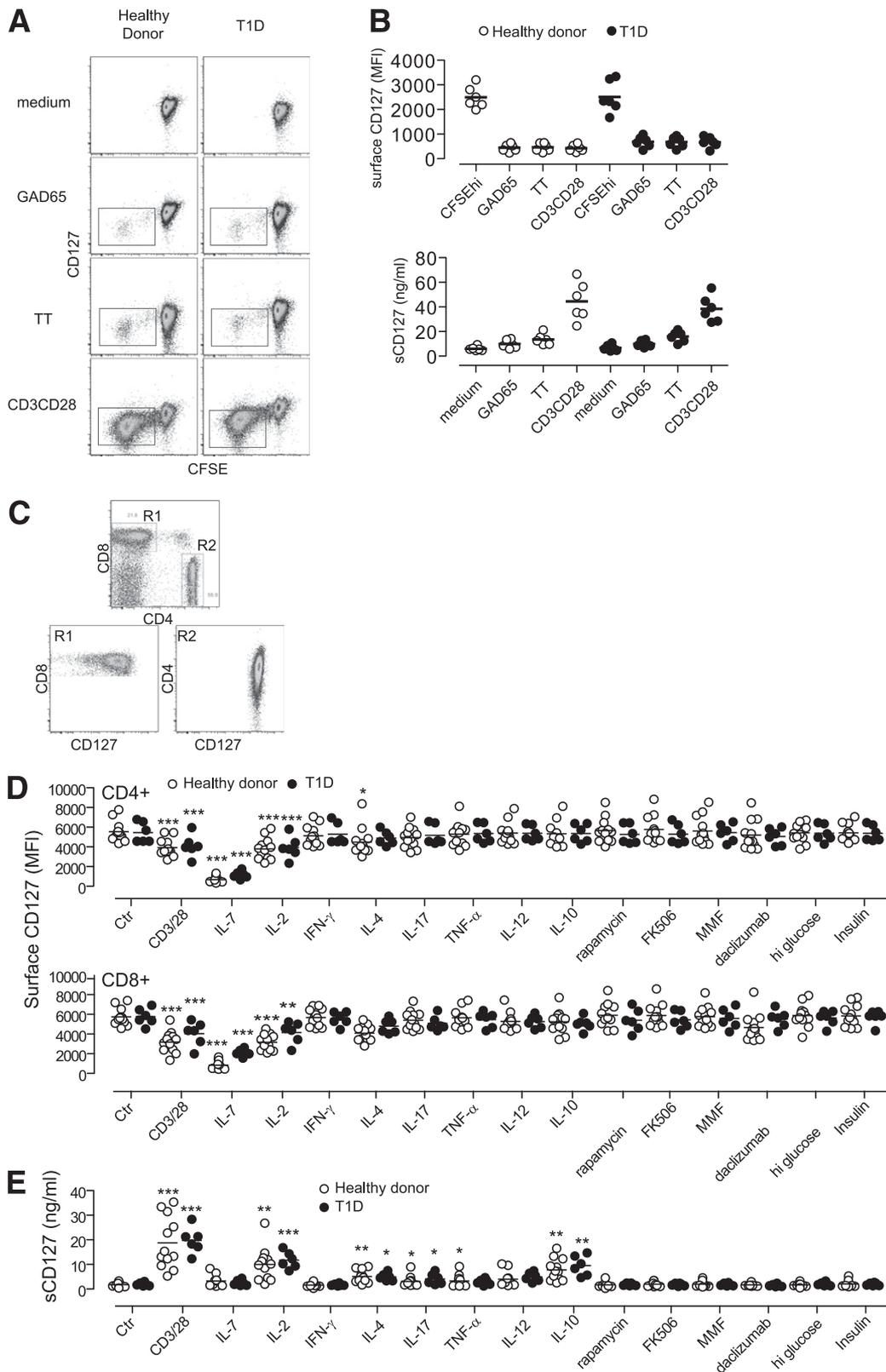
FIG. 3. Regulation of surface expression of CD127 and release of sCD127. *A*: Membrane expression of CD127 in gated CD4$^+$CFSE$^{dim}$ T cells after 5 days proliferation in response to GAD65, TT and anti-CD3/CD28. Representative fluorescence-activated cell sorting (FACS) plots are shown. *A*: Membrane (*top*) and soluble (*bottom*) CD127 in gated CD4$^+$CFSE$^{dim}$ T cells from healthy subjects (*n* = 6) and patients with T1D (*n* = 6). *C*: CD127 expression in gated CD4$^+$ and CD8$^+$ T cells. Representative FACS plot of unstimulated cells is shown. *D*: Surface expression (MFI, *y*-axis) of CD127 on CD4$^+$ T cells (*top*) and CD8$^+$ T cells (*bottom*) from healthy subjects (open circles, *n* = 12) and patients with T1D (filled circles, *n* = 6): different donors after 48-h incubation with medium, immune stimuli, immune-suppressive drugs, and metabolic stimuli. Statistical significance is indicated on the top of each condition (*$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001; not significant, $P$ > 0.05). *E*: sCD127 (ng/mL) in the culture supernatant from *D*. Lines in *D* and *E* indicate mean values for each condition. Ctr, control; MMF, mycophenolate mofetil.

**Circulating sCD127 is increased in patients with autoimmune diabetes.** Since sCD127 was observed in culture supernatants after TCR stimulation, we asked whether sCD127 concentration was increased in autoimmunity (Fig. 4). The mean concentration of sCD127 was significantly higher in patients with diabetes diagnosed before age 20 years ($85.1 \pm 48.6$ ng/mL; $n = 390$) as compared with age- and sex-matched antibody-negative relatives ($70 \pm 46.6$ ng/mL; $P = 0.00001$; Fig. 4A). Moreover, patients who were islet autoantibody–positive had significantly higher sCD127 ($90.0 \pm 49.7$) as compared with patients who were islet autoantibody–negative ($69.7 \pm 40.0$; $P = 0.031$; Fig. 4B). No difference in sCD127 concentration was observed in patients with one autoantibody as compared with patients with multiple islet autoantibodies. Circulating sCD127 concentration was not associated with sex or age, and among the patients, there was no association between sCD127 concentrations and HLA genotype, C-peptide concentration, and $HbA_{1c}$ (data not shown). The release of sCD127 as a spliced variant is determined by polymorphisms of the IL-7R gene (10), and accordingly, sCD127 concentrations in patients with diabetes were associated with the genotype of the rs6897932 SNP (Fig. 4C). Patients homozygous for the T1D risk allele C had higher circulating sCD127 (CC genotype, $99.6 \pm 55.8$; $n = 149$) as compared with heterozygous patients (CT, $71.6 \pm 32.5$; $n = 98$; $P = 10^{-6}$) and patients without the risk allele (TT, $45.7 \pm 22.4$; $n = 13$; $P = 10^{-7}$). The data suggest that autoimmunity and IL-7R genotype are likely to influence the serum concentration of sCD127.

We next examined the prediabetes period. Serum concentration of sCD127 was similar in islet autoantibody–positive first-degree relatives of patients with T1D ($75.2 \pm 47.9$ ng/mL; $n = 318$) and age-matched islet autoantibody–negative relatives ($70.5 \pm 51.5$ ng/mL; $n = 318$; $P = 0.15$; Fig. 4D).

**Glycation of sCD127.** Unlike other autoimmune disorders, T1D is characterized by high blood glucose. This is relevant to a number of biochemical processes since it leads to nonenzymatic glycation of circulating proteins, and this can influence protein structure and function. We therefore asked whether sCD127 can undergo glycation and whether this could affect its binding capacity to IL-7 (19). The sequence of the *IL-7Ra/CD127* (*Homo sapiens*; accession number NP_002176.2) was analyzed for potential glycation sites. Lysine residues in the extracellular domain of CD127 potentially involved in sCD127 protein glycation included K81, K97, K119, K157, K161, K187, K204, and K214 (Fig. 5A and B). Recombinant sCD127 was therefore experimentally glycated by incubation with D-glucose and the presence of glycation products assessed by spectrofluorimetric analysis to measure the nontryptophan fluorescence as a known glycation marker (Fig. 5C). A significant increase of glycation fluorescence was recorded for sCD127 incubated with glucose (GsCD127) as compared with sCD127 incubated with PBS, indicating that sCD127 was sugar-modified under the experimental conditions. Western blot analysis suggested that the experimental glycation did not cause significant protein alterations (Fig. 5D). As an iso-osmotic control, sCD127 was also incubated with the nonreducing sugar mannitol (20), which did not produce glycation products nor structural or functional modifications (not shown).

**sCD127 binding to IL-7 is reduced by experimental glycation.** To determine whether sCD127 glycation influences its binding capacity to IL-7, experimentally glycated
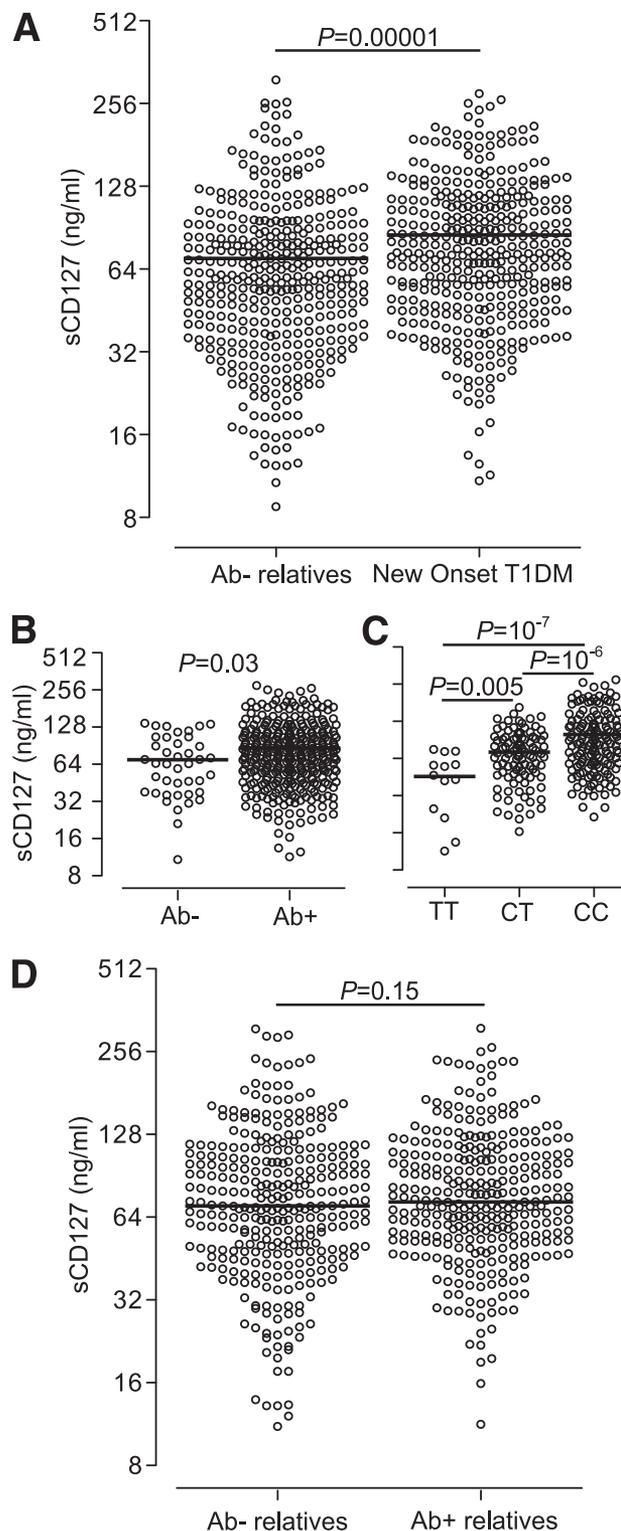


FIG. 4. Circulating sCD127 in new-onset patients with T1D. *A*: sCD127 concentration (ng/mL; *y*-axis) in serum samples from islet autoantibody–negative first-degree relatives of patients with T1D ($n = 392$) and patients with new-onset T1D ($n = 390$). *B*: sCD127 concentration (ng/mL) in patients with new-onset T1D stratified according to the presence of islet autoantibodies against GAD65, IA-2, or ZnT8 (autoantibody-positive [Ab⁺]; $n = 349$) and the absence of islet autoantibodies (autoantibody-negative [Ab⁻]; $n = 41$). *C*: sCD127 concentration (ng/mL) in patients with T1D stratified for genotypes of the rs6897932 SNP within the IL-7Rα gene. The C allele has been previously associated with T1D. *D*: sCD127 concentration (ng/mL; *y*-axis) in serum samples from islet autoantibody–negative first-degree relatives of patients with T1D ($n = 318$) and islet autoantibody–positive first-degree relatives of patients with T1D ($n = 318$).

**A**

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MTILGTTFGMVFSLLQVVSGESGYAQNGDLEDAELDDYSFSCYSQLEVNG  #50
SQHSLTCAFEDPDVNITNLEFEICGALVEVKCLNFRKLQEIYFIETKKFL  #100
LIGKSNICVKVGEKSLTCKKIDLTTIVKPEAPFDLSVVYREGANDFVVTF  #150
NTSHLQKKYVKVLMHDVAYRQEKDENKWTHVNLSSTKLTLLQRKLQPAAM  #200
YEIKVRSIPDHYFKGFWSEWSPSYYFRTPEINNSSGEMDPILLTISILSF  #250
FSVALLVILACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNP  #300
ESFLDCQIHRVDDIQARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCP  #350
SEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHV  #400
YQDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQPILTSLGSNQEEAYV  #450
TMSSFYQNQ                                           #500
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**B**

**C**

**D**

FIG. 5. Experimental glycation of sCD127. *A*: **Bioinformatic analysis of potential glycation sites within the primary structure of the extracellular CD127 domain (underlined). The lysine residues (K) in boldface indicate potential glycation sites.** *B*: **Glycation potential of lysine residues (1 max; −1 min) within the entire primary sequence of CD127.** *C*: **Nontryptophan advanced glycation end product fluorescence of native sCD127 and GsCD127. Spectra were collected at an excitation wavelength of 370 nm, and the reported traces show the fluorescence emissions.** *D*: **SDS-PAGE analysis of sCD127 incubated for 10 days with PBS or D-glucose (D-Glu), in comparison with native sCD127 (nat). AU, arbitrary units.**

(from 1 to 100 mmol/L of D-glucose) sCD127 (GsCD127) was used in the IL-7–binding ELISA. Experimental glycation of sCD127 progressively reduced its binding capacity to IL-7 with a significant decrease observed at a D-glucose concentration of 20 mmol/L and further decrease at 50 and 100 mmol/L (Fig. 6A). In contrast to sCD127, addition of GsCD127 poorly inhibited IL-7–induced STAT5 and AKT phosphorylation, suggesting an impaired function of the glycated receptor (pSTAT5 MFI CD4$^+$; IL-7 + sCD127 = 519 ± 174 vs. IL-7 + GsCD127 = 1,790 ± 536, $P = 0.003$; CD8$^+$; IL-7 + sCD127 = 1,112 ± 309 vs. IL-7 + GsCD127 = 1,864 ± 559; $P = 0.003$; pAKT MFI CD4$^+$, IL-7 + sCD127 = 585 ± 131 vs. IL-7 + GsCD127 = 830 ± 163, $P < 0.0001$; CD8$^+$; IL-7 + sCD127 = 775 ± 108 vs. IL-7 + GsCD127 = 957 ± 139, $P = 0.03$; Fig. 6B). Finally, addition of sCD127 significantly reduced the proliferative effect of IL-7, whereas the addition of GsCD127 only minimally affected

IL-7–assisted proliferation (CD4$^+$CFSE$^{dim}$: GAD65 + IL-7 + sCD127 = 3.43 ± 0.67 vs. GAD65 + IL-7 + GsCD127 = 4.99 ± 1.5; $n = 9$, $P = 0.037$; TT responsiveness, TT + IL-7 + sCD127 = 5.69 ± 2.65 vs. TT + IL-7 + GsCD127 = 8.01 ± 2.85; $n = 9$, $P < 0.0001$; Fig. 6C). These data suggest that glycation of sCD127 impairs its function of IL-7 binding.

**Glycated sCD127 and membrane-bound CD127 in patients with T1D.** We subsequently asked whether glycated sCD127 is present in the circulation of patients with T1D. Glycated proteins are engaged by RAGE, a transmembrane receptor of the Ig superfamily belonging to the pattern recognition receptors. We developed a flow cytometric assay in which the glycated form of the sCD127 protein is detected by its capacity to bind to RAGE (Fig. 6D) and searched for GsCD127 in a subset of the patients with diabetes ($n = 26$; age, 9.7 ± 4.5 years; all islet autoantibody–positive) and antibody-negative relatives ($n = 35$; age, 9.1 ± 4.5 years). Serum amounts of GsCD127 were increased in patients with diabetes (MFI, 219.3 ± 62.3; $n = 26$) as compared with those in islet autoantibody–negative relative subjects (MFI, 170.5 ± 49.2; $P = 0.0011$; Fig. 6E). GsCD127 concentration was not associated with age ($P = 0.92$) or sCD127 concentration ($P = 0.29$) and remained significantly associated with T1D after adjustment for these covariates ($P = 0.002$). Within the patients, GsCD127 concentration was higher in those with the rs6897932 SNP CC genotype ($P = 0.04$), but there was no relationship with HbA$_{1c}$ ($P = 0.5$) or autoantibody status ($P = 0.6$). In order to evaluate glycation of the membrane-bound CD127, we induced sCD127 release by stimulation of T cells from healthy subjects and patients with anti-CD3/CD28 and measured RAGE binding (Fig. 6G). Glycation was increased in sCD127 released from T cells from patients with T1D as compared with T cells from healthy subjects after 24 h (GsCD127 MFI: healthy subjects, 97.5 ± 17.4 vs. T1D, 129.3 ± 24.3; $P = 0.027$). sCD127 released at later time points in culture after 3 and 7 days displayed similar glycation levels.

## DISCUSSION

Alterations of the soluble form of IL-7Rα has been associated with the pathogenesis of autoimmune diseases (3,21). In this study, we report that sCD127 is antagonistic to IL-7 signaling and that its concentration is affected by immune activation and genetic polymorphisms in the *IL-7Rα* gene. Of particular interest, the concentration of sCD127 was increased in patients with T1D. However, the potential beneficial effects of downregulating IL-7–mediated T-cell pathogenesis by sCD127 may be diminished in patients by the presence of a glycosylated low-activity form of the soluble receptor.

Relevant to T1D was the increased concentration of sCD127 in the circulation at disease onset. The findings were generated in a relatively large cohort of patients with young age (<20 years) and an age-matched cohort of islet autoantibody–negative first-degree relatives. Moreover, within patients, sCD127 concentrations were only increased in the islet autoantibody–positive subset consistent with a relationship to autoimmune diabetes. There was a clear relationship between sCD127 concentration and CD127 genotype of the patients similar to what was reported for control subjects and patients with multiple sclerosis (22). However, it is unlikely that the increased concentration observed in patients was solely due to this relationship since the association of CD127 genotype and
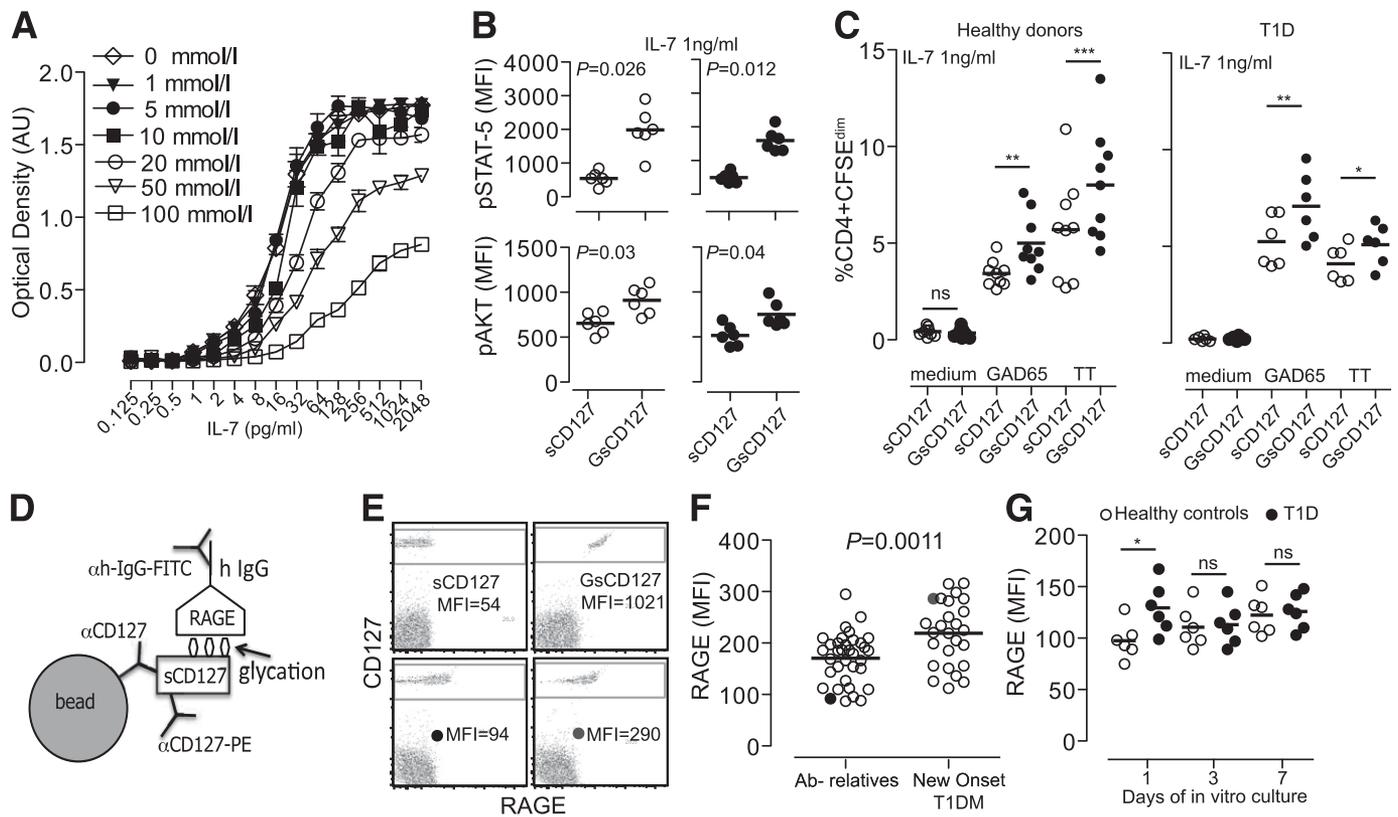
FIG. 6. Glycation affects sCD127 function and glycated sCD127 in serum. *A*: Binding of IL-7 (*y*-axis) at increasing concentrations (*x*-axis) to sCD127 after experimental glycation with increasing concentrations of D-glucose as indicated by the different symbols. Error bars represent mean ± SD from four experiments. Substantially reduced binding was observed by exposure to glucose concentrations >10 mmol/L. *B*: STAT5 and Akt phosphorylation in gated CD4$^+$ T cells from PBMCs treated with IL-7 (1 ng/mL) in the presence of sCD127 and GsCD127 obtained by exposure to 100 mmol/L glucose. *C*: CD4$^+$ T-cell proliferation (%CD4$^+$CFSE$^{dim}$; *y*-axis) from PBMCs treated with IL-7 (1 ng/mL) in the presence of sCD127 (open circles) or GsCD127 (filled circles); *n* = 9 samples. Lines represent mean values. *D*: Detection of glycosylated sCD127 in serum samples by capturing the sCD127 on flow cytometric beads followed by detection of glycosylated form with RAGE–human IgG chimeric protein and anti-human IgG antibody as described in the upper schematic diagram. Fluorescence intensity of RAGE was measured by flow cytometry on sCD127-coated beads in comparison with uncoated beads as shown in the lower fluorescence-activated cell sorting (FACS) plot. *E*: FACS plots for glycated sCD127 quantification of recombinant human sCD127 (*top left*; MFI = 54), GsCD127 (*top right*; MFI = 1021), a control serum with low glycated sCD127 reading (*bottom left*; MFI = 94, indicated as filled circle in *F*), and a patient serum with high glycated sCD127 reading (*bottom right*; MFI = 290, indicated as filled circle in *F*). *F*: Relative amount of glycated sCD127 (MFI; *y*-axis) in serum samples from islet autoantibody–negative first-degree relatives; *n* = 35) and patients with new-onset T1D (*n* = 26). Lines represent means. *G*: Relative amount of glycated sCD127 in culture supernatant collected at days 1, 3, and 7 poststimulation with CD3/CD28 from healthy subjects (*n* = 6) and patients with T1D (*n* = 6). *P < 0.05; **P < 0.01; ***P < 0.001. AU, arbitrary units.

T1D is modest. Although genotyping data were unavailable in the control subjects to confirm this, the minor allele frequency within the patient cohort was 24%, which is similar to that in 2,298 German islet autoantibody–negative first-degree relatives (25%, data not shown) and what is reported in Caucasian control subjects (21).

We predict that the increased sCD127 concentration in patients represents T-cell activation and inflammation at diabetes onset, since we did not observe increases in islet autoantibody–positive relatives. T-cell activation and expansion via TCR engagement, costimulation, and IL-2 downregulated membrane expression of CD127 induced substantial release of sCD127 in the culture medium. These findings are consistent with shedding of membrane-bound CD127 in these settings. In comparison, the homeostatic cytokine IL-7 markedly downregulated the expression of CD127 on the cell surface with only modest release of sCD127, consistent with our previous findings that IL-7 leads to internalization of CD127 (18). It is perhaps noteworthy that among the non–γ-chain cytokines, the suppressive cytokine IL-10 was the most powerful inducer of sCD127, whereas the Th1 cytokine IFN-γ had no influence. Altogether, the in

vitro data indicate that T-cell activation and inflammation are likely to augment sCD127 concentrations.

The IL-7/IL-7R axis plays an important role in the survival and expansion of T cells, including autoreactive clones. IL-7 accelerates diabetes onset in the nonobese diabetic mouse model (5), and modulation of IL-7 concentration or biological function in NOD mice by blockade of the IL-7R with monoclonal antibodies was shown to reverse diabetes (6,7). IL-7 is constitutively produced by stromal cells, and its circulating concentration is apparently determined by consumption by IL-7R–positive T cells (23). In line with a previous report in the context of HIV infection (24), we show that circulating sCD127 can bind to IL-7 and strongly inhibit its bioactivity. Thus, sCD127 represents an endogenous regulator of IL-7 bioactivity and a candidate molecule for therapeutically targeting IL-7. The affinity of CD127 for IL-7 ($K_d = 10^{-8}$ M) is considerably lower than the CD127/CD132 complex ($K_d = 10^{-11}$ M) on the T-cell surface (25). However, we found serum concentrations of sCD127 (70–80 ng/mL) >10,000-fold higher than its ligand IL-7 (5 pg/mL) in steady-state conditions (26) and >1,000-fold higher when IL-7 is elevated in

lymphopenic conditions (1). We therefore hypothesize that endogenous sCD127 may bind a significant fraction of circulating IL-7. In our hands, sCD132, which was reported to exist in a soluble circulating form at high concentration (1 ng/mL) (24), did not bind to IL-7 and did not contribute to sCD127/IL-7 interaction.

The increased sCD127 concentration observed at onset of T1D should assist the regulation of immune activation and T-cell expansion. We remained intrigued, however, that T1D onset is notionally a highly inflammatory state in which immune regulation appears to be ineffective. Hyperglycemia is the hallmark of T1D and is itself a mediator of inflammation (27). One mechanism by which it mediates inflammation is the production of advanced glycation end products that have proinflammatory properties through engagement of pattern recognition receptors such as RAGE (28). Nonenzymatic glycation occurs when proteins are exposed to high glucose. In patients with diabetes, glycation has been reported for proteins with long half-lives such as hemoglobin, but also for proteins with short half-lives such as insulin (29). Bioinformatic analysis showed that sCD127 is rich in lysine residues as potential sites of glycation. Consistent with this, we were able to show that sCD127 can be glycated in vitro and found that serum from patients at onset of T1D contained increased levels of a glycated form of sCD127. Importantly, we found that glycation of sCD127 almost completely abolished its ability to bind IL-7 and prevent IL-7 biological activity. These novel findings indicate that hyperglycemia may have previously undiscovered effects on physiological immune-regulatory mechanisms and that potential glycation of proteins in patients with diabetes should be considered in pathogenesis and therapy.

We and others have hypothesized that altered T-cell homeostasis can contribute to the expansion of autoreactive T cells in different autoimmune diseases. We now show that IL-7 biological activity can be regulated by sCD127, but this regulation may be compromised in patients with hyperglycemia. In addition to demonstrating the complexity of cytokine signaling, the findings unveil a novel interplay between metabolic and immunological processes in T1D.

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