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Brief Conclusive Report

Hepatocytes induce Foxp3$^+$ regulatory T cells by Notch signaling

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ABSTRACT

The liver plays a pivotal role in maintaining immunological tolerance, although the exact molecular mechanism is still largely unknown. The induction of systemic tolerance by liver resident APCs has been attributed to peripheral deletion and to the induction of T_{regs}. HCs, the parenchymal cells in the liver, could function as nonprofessional APCs and interact and establish cell–cell contact with T lymphocytes. We hypothesized that HCs from healthy or regenerated livers may contribute to induction of functional T_{regs}. Here, we show that murine HCs induced Foxp3$^+$ T_{regs} within CD4$^+$ T cells in vitro, which increased in the presence of TGF-β. Interestingly, a further Foxp3$^+$ T_{reg} expansion was observed if HCs were isolated from regenerated livers. Additionally, the induction of Foxp3$^+$ T_{regs} was associated with the Notch signaling pathway, as the ability of HCs to enhance Foxp3 was abolished by γ-secretase inhibition. Furthermore, HC-iT_{regs} showed ability to suppress the proliferative response of CD4$^+$ T cells to anti-CD3 stimulation in vitro. Thus, HCs may play a pivotal role in the induction of tolerance via Notch-mediated conversion of CD4$^+$ T cells into Foxp3$^+$ T_{regs} upon TCR stimulation. ***J. Leukoc. Biol.*** **96: 571–577; 2014.**

Introduction

The liver is a site of complex immune activity and provides protection against pathogens, transformed liver cells, and metastatic cells, while at the same time, tolerating harmless self and foreign antigens [1]. Over the last decades, the contribution of the liver to the induction of peripheral and systemic tolerance was investigated intensively. The tolerance effect of the liver was first described in the late 1960s by demonstrating long-term acceptance of liver allografts without immunosuppression in several species, such as mouse, rat, and pig [2]. The induction and maintenance of tolerance in the liver are mediated by several mechanisms, including the immunosuppressive cytokine expression, induction of T cell unresponsiveness, and antigen-specific T_{reg} induction [3]. Liver dendritic cells, KCs, LSECs, and HSCs are reported to be capable of presenting antigen to CD4$^+$ T cells, suggesting that a subset of hepatic cells may be directly or indirectly responsible for the differentiation of T_{regs} (reviewed in refs. [4, 5]). HCs, representing the largest cell population in the liver, also function as APCs and contribute to T cell activation and immune regulation in the liver. HCs interact and establish cell–cell contact with T lymphocytes through fenestrations in the LSECs [6] and constitutively express MHC class I molecules. In addition, in response to IFN-γ exposure and also in patients with various forms of chronic liver diseases, expression of MHC class II molecules in HCs has been demonstrated [7]. Recently, it was shown that HCs promote apoptosis in postactivated CD8$^+$ T cells, which in turn, leads to induction of functional tolerance in vitro [8]. Therefore, the current evidence suggests that HCs might contribute to hepatic immune tolerance. Nonetheless, the role of HCs to induce T_{regs} and to contribute to the liver tolerance is still unclear.

The Notch signaling pathway has been shown to control cell-fate decisions in naive T cells in the thymus and the periphery [9]. In mammals, there are four Notch receptors—Notch1–Notch4—and five Notch ligands—Jagged-1 and -2 and Dll1, -3, and -4 [10]. The Notch ligands of the Jagged family have been attributed to T_{reg} differentiation and suppression of autoimmune diseases [11]. Overexpression of Jagged-1 by APCs and Jagged-2-expressing hematopoietic progenitor cells was shown to induce T_{regs} [12–14]. Recently, it was shown that the induction of Foxp3$^+$ T_{regs} is indeed cooperatively regulated by Notch signaling and TGF-β [15]. Notably, liver inflammation is controlled by the immune regulatory cytokine TGF-β [16], which also plays a crucial role in T_{reg} conversion [17]. Moreover, Notch signaling is activated during liver regeneration [18, 19] and inflammation [20], and mutations in Notch signaling result in liver disease associated with the Alagille syn-

Abbreviations: DAPT=N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester, DEREG=depletion of regulatory T cells, Dll=delta-like ligand, Foxp3=forkhead box protein P3, GITR=glucocorticoid-induced TNFR, HC=hepatocyte, HSC=hepatic stellate cell, iT_{reg}=induced T regulatory cell, KC=Kupffer cell, LSEC=liver sinusoidal endothelial cell, NICD=Notch intracellular domain, nT_{reg}=naturally occurring regulatory T cell, T_{reg}=regulatory T cell, WT=wild-type

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drome [21], as a result of impaired liver-repair mechanisms [22]. Therefore, Notch-dependent generation of T_{regs} in the liver may contribute to limiting inflammatory processes and maintaining liver tolerance. Here, we show that HCs in the presence of TGF-β induce the expression of Foxp3 in $CD4^+$ T cells, which are dependent on Notch signaling. Additionally, we observed that HCs from regenerated livers promote enhanced TGF-β-mediated induction of functional $Foxp3^+$ T_{regs}. Taken together, our findings suggest that Notch-dependent generation of T_{regs} by HCs might be one of the mechanisms of inducing and maintaining immunological tolerance in the liver.

MATERIALS AND METHODS

Mice

Male DEREG mice that express GFP under control of the Foxp3 promoter, CD45.1 and sex/age-matched (8–10 weeks old) C57BL/6 WT mice were bred in the animal facilities of the University Medical Center Hamburg-Eppendorf (Hamburg, Germany). Animals received humane care according to guidelines of the National Institute of Health in Germany. Experiments were approved by the Institutional Review Board, "Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz" (Hamburg, Germany).

Animal treatment and HC preparation

Con A (8 mg/kg; Sigma-Aldrich, Munich) or saline as control was administered i.v. Eight days after injection, mice were killed, and primary HCs were isolated as described previously [23]. In brief, the liver was perfused with Ca^+/Mg^+-free HBSS, supplemented with 50 mM HEPES and 500 μM EGTA, digested with 0.004% Liberase (w/v) in situ, and removed, and liver capsules were gently disrupted in $NaHCO_3$-free HBSS with 10 mM HEPES and 0.2% BSA. Subsequently, single-cell suspensions were gently pressed through a 10-μm nylon mesh, and the HCs were allowed to settle by gravity for 15 min. Parenchymal cells were separated from nonparenchymal cells by 90% Percoll gradient centrifugation.

Cell purification

Spleens were passed through 100-μm nylon meshes, resulting in single-cell suspensions. T cells were isolated using the $CD4^+$ T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer's instructions. To isolate splenic $CD4^+CD25^-$ T cells, splenocytes were sorted using a combination of MACS and FACS (BD FACSAria), as described previously [24], to achieve highest purity of $CD4^+CD25^-$ responder T cells (~98%).

In vitro coculture

For in vitro studies, HCs were isolated from WT mice, 8 days upon saline or Con A treatment. HCs were cocultured with splenic $CD4^+$ T cells from DEREG mice (triplicates) in flat-bottom, 24-well culture plates (Nalge Nunc International, Schwerte, Germany) in complete William's E Medium (Gibco Life Technologies, Darmstadt, Germany). Cells were cultured in the presence of plate-bound αCD3 mAb (5 μg/mL; BioLegend, San Diego, CA, USA) for 48 h. If required, 4 ng/mL TGF-β (R&D Systems, Abingdon, UK) was added to the culture medium. Notch signaling was blocked by γ-secretase inhibitor DAPT (20μM; Sigma-Aldrich, Munich, Germany), dissolved in DMSO (Sigma-Aldrich). The final concentration of DMSO in cocultures was 0.7%. DMSO (0.7%) was used as a control as well. Notch1 signaling was blocked by neutralizing αNotch1 mAb (Clone A6, 20 μg/mL; Thermo Fisher, Waltham, MA, USA) with mouse IgG2b isotype (BioLegend) as a control.

Determination of cytokines

Sandwich ELISA for IL-2 was performed using Microlon 96-well, high-binding, flat-bottom microtiter plates (Greiner Bio-One, Frickenhausen, Germany). IL-2 antibody was purchased from BioLegend. Streptavidin-peroxidase and the tetramethylbenzidine Substrate Reagent Set were purchased from R&D Systems and BD PharMingen (Heidelberg, Germany), respectively, and used according to the manufacturer's instructions.

Flow cytometric analysis

Leukocytes were stained using a standard protocol, including Fc blocking steps. The used anti-mouse mAb are summarized in Supplemental Table 1. Data were recorded using the BD LSR II system and analyzed after gating out dead cells using BD FACSDiva software.

Immunohistochemistry

Formalin-fixed, paraffin-embedded liver tissue was used for immunohistochemical staining of Jagged-1, as described previously [25]. Primary antibody to Jagged-1 (1:250, ab7771; Abcam, Cambridge, UK) was incubated overnight at 4°C. Antigen Unmasking Solution (Vector Laboratories, Peterborough, UK) was used for antigen retrieval. Visualization was performed with ZytoChem Plus AP Polymer Kit (Zytomed Systems, Berlin, Germany) and AP-Permanent Red Kit (Zytomed Systems), according to the manufacturer's instructions. The slides were counterstained with hematoxylin. Staining with secondary antibody only, i.e., without primary antibody, was proven to be negative (not shown).

Suppression assay

$CD4^+CD25^-$ responder T cells were isolated from CD45.1 mice and labeled with cell proliferation dye eFluor 670 (eBioscience, Frankfurt, Germany). HC-preactivated T_{regs} were reisolated by FACS sorting via GFP.

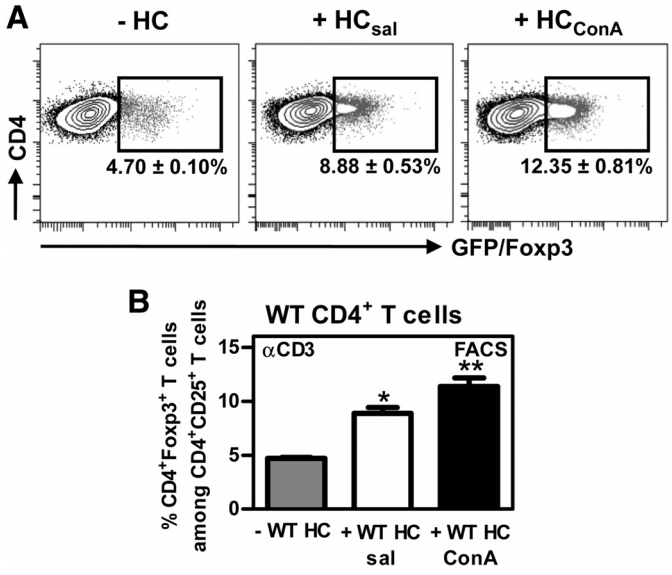

Figure 1. HCs increase the frequency of $CD4^+Foxp3^+$ T_{regs}. Splenocytes were isolated from DEREG mice, and total $CD4^+$ T cells were purified by MACS sorting. HCs were isolated from WT mice 8 days after saline or (sal) Con A administration. Subsequently, HCs and T cells were cocultured in the presence of αCD3 mAb. After 48 h, T cells were harvested to analyze the frequency of $CD4^+Foxp3^+$ cells among $CD4^+CD25^+$ cells by flow cytometry. Contour plots (A) and quantifications (B) are representative of two independent experiments (n=3). *$P \leq 0.05$; **$P \leq 0.01$.

FACS-sorted CD4$^+$CD25^{high} nT$_{regs}$ were used as control. Subsequently, responder T cells were cocultured with HC-preactivated T$_{regs}$ at different ratios in 96-well, U-bottom plates (Nalge Nunc International) in the presence of αCD3 mAb (5 μg/mL). After 72 h, cells were harvested and stained for surface markers and with viability dye propidium iodide (BioLegend). Proliferation of living CD45.1 responder T cells was assessed by flow cytometry. Percentage of inhibition was calculated using proliferation index with respect to baseline percentage in the absence of T$_{regs}$.

Statistical analysis

Data are presented as mean values ± SEM. Results were analyzed using unpaired, two-tailed Student's *t*-test or one-way ANOVA with Bonferroni's post hoc test. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

HCs increase the frequency of Foxp3$^+$ T cells

Recently, it has been shown that liver resident cells, e.g., HSCs, are able to expand and induce allogenic Foxp3$^+$ T$_{regs}$ [26, 27]. Therefore, we initially analyzed the capacity of HCs to modify responder T cells to convert them into T$_{regs}$. With the use of DEREG mice [28], we observed a frequency of CD4$^+$GFP$^+$ T$_{regs}$ among CD4$^+$CD25$^+$ cells of ~5% in cultures of T cells alone, reflecting the percentage of nT$_{regs}$. In the presence of HCs, frequencies of CD4$^+$Foxp3$^+$ T$_{regs}$ were slightly but significantly increased to ~9%, which was even more obvious with HCs isolated from inflamed and subsequently regenerated livers, 8 days after Con A treatment (~12%; **Fig. 1A** and **B**). The up-regulation of Foxp3 in total CD4$^+$ T cells in the presence of HCs indicated an activation and expansion of the T$_{reg}$ population.

TGF-β potentiates induction of Foxp3$^+$ T$_{regs}$ by HCs

Many cell types in human and rodent can produce and respond to TGF-β. In T cells, TGF-β induces Foxp3, a transcription factor essential for programming and developing T$_{regs}$ [29]. As TGF-β is generally induced in response to liver inflammation and damage, which was also observed upon Con A administration [30], we investigated whether the up-regulation of Foxp3 in CD4$^+$ T cells in the presence of HCs could be potentiated by TGF-β. For this purpose, we sorted CD4$^+$GFP$^-$ T cells from DEREG mice and cultured them with HCs in the presence or absence of TGF-β for 48 h. As shown in **Fig. 2A** and **B**, addition of TGF-β to the cocultures induced an increase of T$_{reg}$ frequency to >20% in the presence of naive HCs and a significantly higher increase to ~32% of T$_{regs}$ in the presence of HCs from Con A-pretreated mice. We analyzed further the surface expression of established T$_{reg}$ markers to characterize the described T$_{reg}$ population in more de-

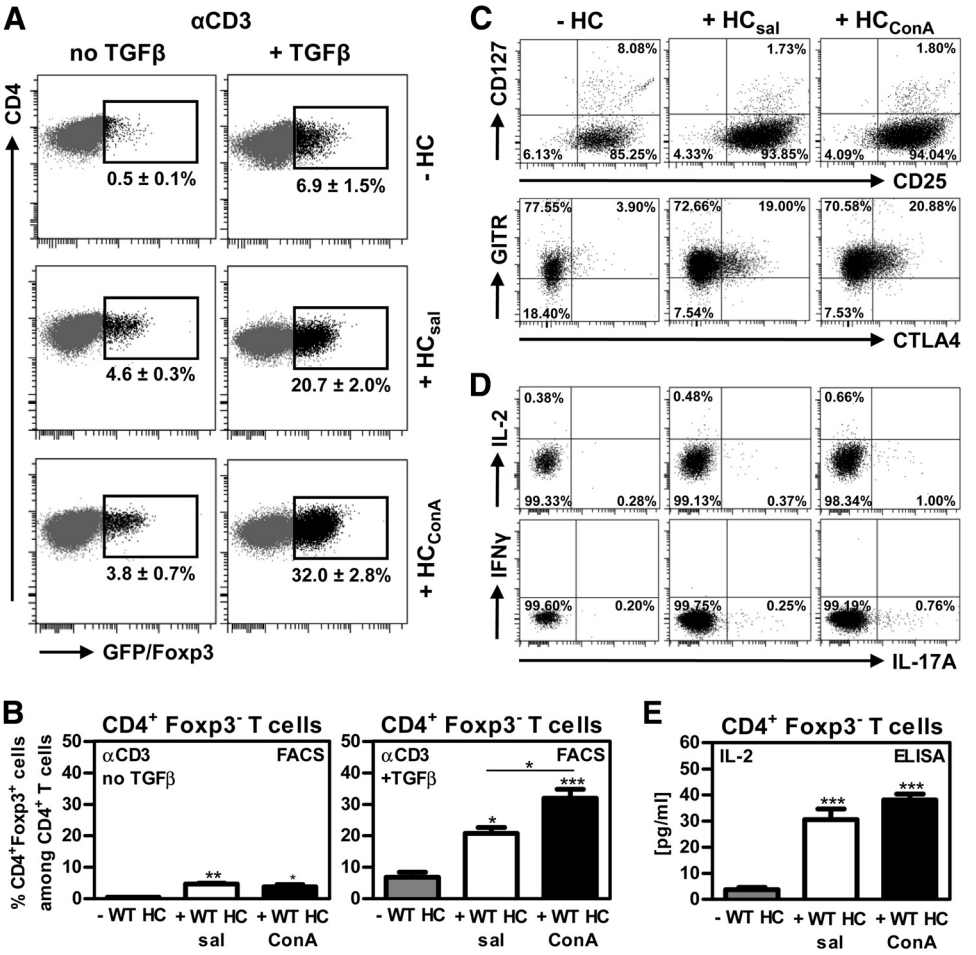

Figure 2. HCs promote induction of CD4$^+$Foxp3$^+$ T$_{regs}$ in the presence of TGF-β. Sorted CD4$^+$Foxp3$^-$ responder T cells from DEREG mice were stimulated with αCD3 mAb and cultured without HCs or with saline- or Con A-primed HCs for 48 h. TGF-β was added as indicated. Representative dot plots (A) and quantification (B) of CD4$^+$Foxp3$^+$ T$_{regs}$ among CD4$^+$ T cells are depicted. (C) Representative dot plots of CD25, CD127, GITR, and CTLA4 surface expression of CD4$^+$Foxp3$^+$ T cells after cultivation with or without HCs. (D) T cells were intracellulary stained and analyzed for IL-2, IFN-γ, and IL-17 expression upon cocultivation. Cells were gated on CD4$^+$Foxp3$^+$ T cells. All gates were set according to isotype controls. (E) IL-2 release by CD4$^+$ T cells was analyzed via ELISA. The data represent the average of two independent experiments (n=6). *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

tail. As expected, >95% of CD4$^+$Foxp3$^+$ T cells coexpressed CD25 and lack CD127 expression. FACS analysis revealed that the majority (~92%) of HC-primed Foxp3$^+$ T cells was also positive for the well-known T_{reg} marker GITR. Additionally, CTLA4 expression was strongly up-regulated by T_{regs} isolated from HC cocultures compared with T_{regs} generated by TGF-β alone (20% vs. 4%; Fig. 2C).

The conversion of CD4$^+$Foxp3$^-$ T cells into CD4$^+$Foxp3$^+$ T_{regs} generally requires IL-2 and TGF-β, as well as αCD28 costimulation [17]. The IL-2 levels in the cocultures with HCs from saline and Con A-pretreated mice were increased significantly compared with the control group (Fig. 2E), and additional signals produced by HCs in the cocultures may have stimulated CD4$^+$Foxp3$^-$ T cells to convert to Foxp3$^+$ T_{regs}. Nevertheless, increased IL-2 levels might also argue for a potential role of HCs to enhance effector T cell activity. To address this possibility, we measured Th1 and Th17 cytokines in Foxp3$^+$ T cells. As shown in Fig. 2D, expression of IL-2, IFN-γ, and IL-17 was completely absent in Foxp3$^+$ cells even in the presence of HCs, suggesting a putative role for IL-2 in the differentiation of conventional T cells into Foxp3$^+$ T_{regs}. We demonstrate that TGF-β-dependent priming of CD4$^+$Foxp3$^-$ T cells by HCs resulted in de novo generation of Foxp3$^+$ T_{regs} in the absence of αCD28 costimulation and exogenous IL-2. This is in line with other reports showing that liver-resident conventional, as well as unconventional, APCs induce tolerance in naive T cells [27, 31] and might argue for a synergistic effect of HCs and other liver-resident APCs capable of releasing TGF-β, e.g., HSCs [32] and KCs [33].

Notch signaling contributes to TGF-β-mediated T_{reg} induction by HCs

It has been shown that Notch1 and TGF-β cooperatively regulate Foxp3 expression [15]. Moreover, HCs express rather Jagged ligands than Dll ligands, and Jagged has been implicated in Notch-dependent differentiation of naive T cells into T_{regs} [11]. Those findings prompted us to study expression of Notch ligands on HCs and the functional consequence of Notch signaling on Foxp3$^+$ T_{reg} generation in the coculture system. Immunohistochemical analysis showed expression of Jagged-1 in HCs, confirming findings from a previous study that demonstrated Jagged-1 expression in HCs [34]. Jagged-1 expression was even stronger in HCs isolated from regenerated livers and was expressed predominantly in areas surrounding blood vessels (**Fig. 3**). This distribution of Jagged-1 might favor the interaction with T cells entering the liver via the blood flow. Moreover, mRNA analysis revealed that expression of Jagged-1, but not other Notch ligands, such as Jagged-2, as well as Dll1, -3, and -4, was increased in HCs isolated from Con A-pretreated mice compared with HCs isolated from controls (data not shown), suggesting that HCs might induce T_{regs} by Jagged-1-mediated Notch activation. We thus examined the expression of NICD, which functions as a transcription factor in the nucleus and Notch-specific target gene *hes-1* that were increased significantly in HC-primed T cells (Supplemental Fig. 1), indicating a functional impact of Notch signaling on HC-mediated de novo generation of T_{regs}. Published evidence indicated that αCD3 stimulation up-regulated Notch1

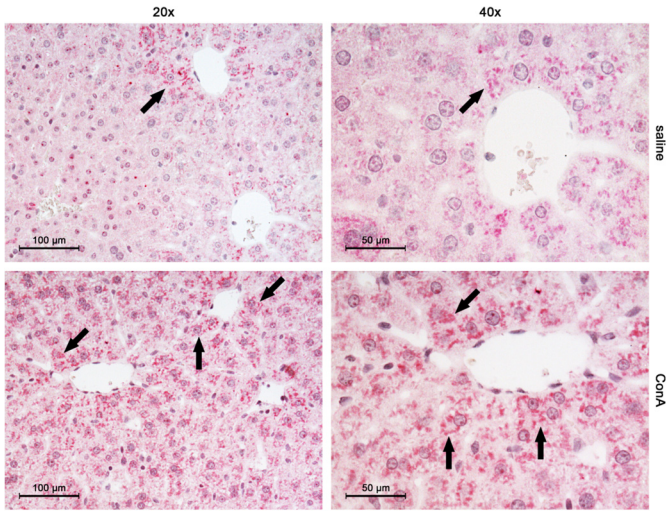

Figure 3. Jagged-1 expression is increased in regenerated HCs. Jagged-1 protein expression and distribution in livers, 8 days upon saline or Con A treatment, were determined by immunohistochemistry. Jagged-1 was present predominantly in HCs surrounding blood vessels. Strong staining (arrows) was observed in regenerated livers, 8 days upon Con A treatment. Healthy liver (upper); original magnifications at 20× (left) and 40× (right). Regenerated liver (lower); original magnifications at 20× (left) and 40× (right). The slides were counterstained with hematoxylin. Data are representative of two independent experiments (n=3). *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

surface expression in CD4$^+$ T cells [35]. Therefore, it seems that in contrast to alterations in Notch signaling, we failed to observe a further up-regulation of Notch1 surface expression following coculture with HCs compared with control (data not shown) in the presence of αCD3 stimulation. We used the γ-secretase inhibitor DAPT, which blocks Notch signaling by inhibition of proteolytic cleavage of the transmembrane domain of Notch, thereby inhibiting the release of NICD. Incubation of cells with DAPT led to a significant inhibition of de novo generation of Foxp3$^+$ T cells in the presence of HCs and TGF-β (**Fig. 4A** and **B**). Flow cytometric analysis revealed that the DAPT concentration used in the experiments was not toxic to CD4$^+$ T cells, as counterstaining with propidium iodide showed no impaired cell viability compared with the control (Fig. 4C). Likewise, measurement of HC toxicity by lactate dehydrogenase release revealed that DAPT was also not toxic to these cells (data not shown). Moreover, we used a Notch1-neutralizing antibody to address off-target effects of DAPT. Foxp3 mRNA expression was reduced significantly by ~53% in HC cocultures compared with the control. It should be noted that DAPT abrogated Foxp3 mRNA expression by ~82%, most likely as a result of complete inhibition of the Notch axis (Supplemental Fig. 2). Overall, these results indicate that Notch receptors expressed by CD4$^+$ T cells and their ligands, in particular, Jagged-1, expressed on HCs, may cooperate with TGF-β signaling to generate Foxp3$^+$ T_{regs}. Interestingly, Del Papa et al. [36] recently described Notch1/Jagged-1 involvement in human mesenchymal stem cell-mediated iT_{reg} generation, independent of TGF-β and TCR stimulation. Therefore,

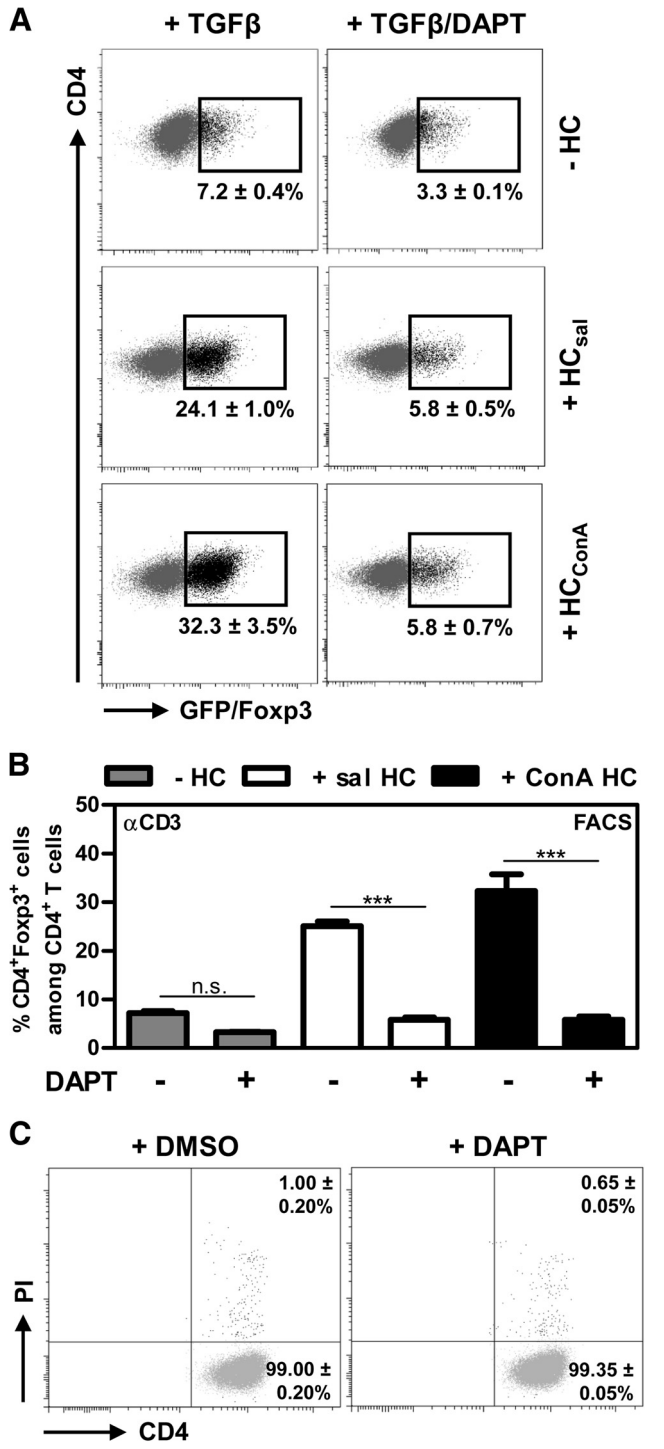

Figure 4. Notch signaling contributes to T_{reg} induction by HCs. CD4$^+$Foxp3$^-$ T cells were purified from DEREG mice by FACS sorting and cocultured with HCs, isolated from saline- or Con A-treated mice in the presence of αCD3 mAb and TGF-β for 48 h. Notch signaling was blocked by addition of γ-secretase inhibitor DAPT into the coculture. Representative dot plots (A) and quantification (B) of CD4$^+$Foxp3$^+$ T_{regs} among CD4$^+$ T cells are shown. (C) Toxicity of DAPT on CD4$^+$ T cells was monitored by FACS analysis using propidium iodide (PI) staining. Representative dot plots depict the frequency of CD4$^+$PI$^+$ cells upon cocultivation. ***$P \leq 0.001$.

it seems that cells with a certain capacity for self renewal in both humans and rodents trigger the Notch axis to favor de novo generation of T_{regs} contributing to immunological tolerance during regeneration.

HC-iT_{regs} suppress proliferation of naive CD4$^+$CD25$^-$ responder T cells

It has been observed previously that the functional ability of in vitro-expanded T_{regs} can be evaluated in proliferation assays [37]. As we demonstrated that HCs promoted TGF-β-dependent de novo induction of Foxp3$^+$ T_{regs} via Notch signaling, we assessed the suppressive capacity of HC-iT_{regs} to evaluate their regulatory function. Freshly isolated, congenic CD4$^+$CD25$^-$ responder T cells were stimulated with αCD3 in the presence of nT_{regs} or HC-induced Foxp3$^+$ T_{regs} at differ-

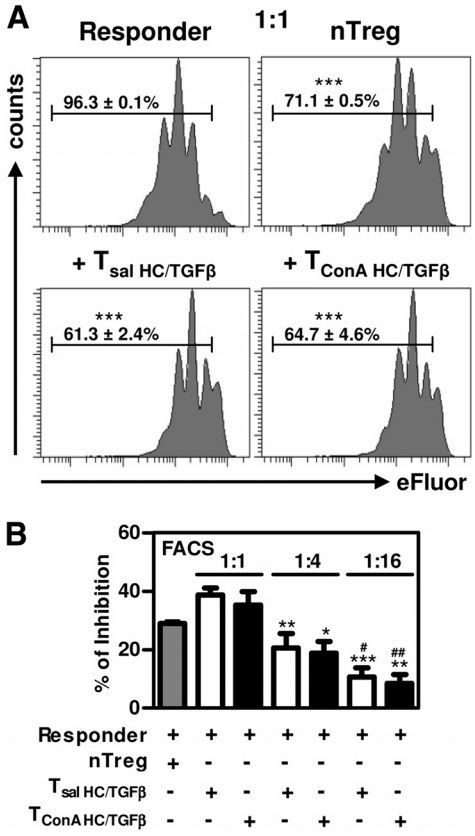

Figure 5. HC-iT_{regs} exhibit potent suppressive activity. eFluor-labeled CD4$^+$CD45.1$^+$CD25$^-$ responder T cells were stimulated with αCD3 mAb in the presence of nT_{regs}, T_{sal} HC/TGF-β, or $T_{Con\ A}$ HC/TGF-β at different T_{reg}:responder ratios. After 72 h, T cells were harvested, and proliferation of living CD45.1$^+$ responder T cells was assessed by the decreased intensity of eFluor staining by flow cytometry. Representative histograms (numbers indicate percentages of divided cells; ***$P \leq 0.001$, significant from baseline suppression in the absence of T_{regs}; A) and bar graph depicting cell proliferation (B) from one of two independent experiments are shown (n=3). Asterisks show significance from ratio 1:1 of T_{sal} HC/TGF-β or $T_{Con\ A}$ HC/TGF-β; number signs are significant from nT_{reg}. Every value is referred to the baseline inhibition, generated in the absence of T_{reg}. #,*$P \leq 0.05$; ##,**$P \leq 0.01$; ***$P \leq 0.001$.

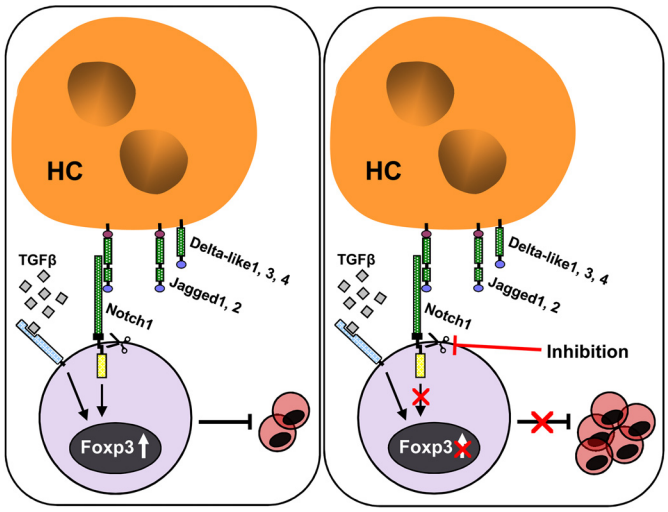

Figure 6. Schematic illustration of the putative mechanism. The secretion of immunoregulatory cytokines, such as TGF-β, is elevated in the inflamed liver. Under these conditions, HCs interact with the $CD4^+$ T cell and induce $Foxp3^+$ T_{regs} in naive T cells, using Notch signaling to abolish immune activation and maintain tolerance (left). Down-modulation and/or inhibition of Notch signaling inhibit Foxp3 induction in $CD4^+$ T cells by HCs and enhance expansion of effector T cells (right).

ent ratios. After 72 h, proliferation was assessed via flow cytometry. Under these conditions, HC-iT_{regs} caused an increased suppression of responsive $CD4^+$ T cell proliferation compared with nT_{reg}. A significant inhibition of ~30% of T cell proliferation was detected in the coculture of 1:1 nT_{regs} and responder cells, whereas T_{regs} harvested from both HC cocultures significantly suppressed proliferation of responder cells by ~40% at the highest ratio of 1:1 (**Fig. 5A**). These data showed similar suppressive potential of HC-expanded T_{regs} regardless the in vivo treatment that mice received before HC isolation. Titration of T_{regs} indicated less-suppressive activities that again supports the notion of a regulatory capacity of HC-iT_{regs} (Fig. 5B).

It is well established that T_{regs} suppress liver inflammation and autoimmune liver diseases [38]. Moreover, it was suggested that ectopic expression of autoantigens in HCs culminates in the conversion of conventional T cells into $Foxp3^+$ T_{regs} in the presence of TGF-β in vivo [39]. Nevertheless, the molecular mechanism driving T_{reg} induction in the liver remains elusive, and there is still no profound knowledge about the interaction of HCs and $CD4^+$ T cells. Here, our findings indicate that HCs indeed interact with $CD4^+$ T cells, which might lead to the induction of Foxp3 and de novo generation of T_{regs}. This de novo generation of $Foxp3^+$ T_{regs} depended on Notch signaling mediated by Jagged-1, expressed on HCs and exogenous TGF-β, which in vivo, might be released by other liver resident APCs, such as HSCs and KCs upon liver inflammation. Our findings demonstrate a putative mechanism of how $Foxp3^+$ T_{regs} might be generated within the liver (**Fig. 6**) and how HCs might contribute to liver tolerance. Overall, Notch signaling in the liver could be a potential target to shift liver immunity to prevent autoimmune diseases or to potentiate immune response toward harmful antigens. In conclusion, expansion of T_{regs} by HCs could be of value for ex vivo expansion of $Foxp3^+$ T cells for cellular immune therapy.

AUTHORSHIP

S.B. performed experiments, analyzed data, and wrote the manuscript. A.E. and B.C. assisted in experimental design and/or contributed technical knowledge. K.K. contributed to writing. G.T. designed the research, supervised the experiments, and contributed to writing of the manuscript.

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DISCLOSURES

The authors declare no conflict of interest.

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KEY WORDS:
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