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CD98hc regulates the development of experimental colitis by controlling effector and regulatory CD4$^+$ T cells

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ABSTRACT

CD4$^+$ T cell activation is controlled by signaling through the T cell receptor in addition to various co-receptors, and is also affected by their interactions with effector and regulatory T cells in the micro-environment. Inflammatory bowel diseases (IBD) are caused by the persistent activation and expansion of auto-aggressive CD4$^+$ T cells that attack intestinal epithelial cells. However, the molecular basis for the persistent activation of CD4$^+$ T cells in IBD remains unclear. In this study, we investigated how the CD98 heavy chain (CD98hc, *Slc3a2*) affected the development of colitis in an experimental animal model. Transferring CD98hc-deficient CD4$^+$CD25$^-$ T cells into *Rag2*$^{-/-}$ mice did not cause colitis accompanied by increasing Foxp3$^+$ inducible regulatory T cells. By comparison, CD98hc-deficient naturally occurring regulatory T cells (nTregs) had a decreased capability to suppress colitis induced by CD4$^+$CD25$^-$ T cells, although CD98hc-deficient mice did not have a defect in the development of nTregs. Blocking CD98hc with an anti-CD98 blocking antibody prevented the development of colitis. Our results indicate that CD98hc regulates the expansion of autoimmune CD4$^+$ T cells in addition to controlling nTregs functions, which suggests the CD98hc as an important target molecule for establishing strategies for treating colitis.

1. Introduction

Inflammatory bowel diseases (IBD) are chronic inflammatory disorders of the gastrointestinal tract that are characterized by persistent mucosal T cell-mediated inflammation [1–4]. Increasing evidence suggests that dysregulation of mucosal CD4$^+$ T cells is involved in the pathogenesis of IBD. CD4$^+$ T cell dysregulation can be attributed, in part, to the reduced function of naturally occurring regulatory T cells that express CD4, CD25, and Foxp3 (nTreg) [2,3]. Numerous studies have demonstrated the capability of nTregs to prevent T cell-mediated immune responses, and that nTreg-mediated suppression requires direct cell–cell contact with responder T cells and antigen-presenting cells [5]. Regarding the roles of nTregs in colitis, *Rag2*$^{-/-}$ mice that receive CD4$^+$CD25$^-$ T cells develop severe colitis, which is inhibited by co-transferring nTregs [2,3]. This highlights the crucial role of nTregs in the onset or progression of colitis. However, the underlying molecular mechanisms that cause and maintain the inflammatory responses in IBD remain unclear. Recent studies identified inducible regulatory T cells (iTregs). This is a cell population that is distinct from nTregs and can control various autoimmune responses and immune responses to infection [6–9]. iTregs also express Foxp3 and can be induced *in vitro* by stimulating CD4$^+$CD25$^-$Foxp3$^-$ cells in the presence of IL-2 and TGF-β [10]. Adoptive transfer of naive CD4$^+$ T cells into lymphopenic mice results in autoimmune symptoms with a spontaneous late recovery. This recovery is regulated by the generation of iTregs, which does not occur in the absence of IL-2 [11], thus establishing the necessity of IL-2 for generating iTregs.

T cell activation is controlled both by signals transmitted through T cell receptor (TCR) and those transmitted through co-stimulatory molecules [12–14]. CD98 heavy chain (CD98hc, *Slc3a2*) is linked to the CD98 light chain and this complex regulates amino acid transport and integrin-mediated signaling [15,16]. Ours and other groups have revealed that CD98hc is crucial for T cell proliferation [17,18]. We demonstrated that blocking CD98hc with an anti-CD98hc antibody suppressed the development of type 1 diabetes [17]. Other studies using CD98hc-deficient mice have shown reduced proliferation by B cells [19] and T cells [18,20],

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which indicated that CD98hc contributed to both T and B cell proliferation. These data demonstrate that signaling through CD98hc is important for T cell proliferation and that blocking CD98hc might be useful for treating T cell-mediated inflammatory responses.

To determine the roles of CD98hc in the progression of IBD, we investigated if the absence of CD98hc on $CD4^+$ T cells affected the progression or severity of colitis. We found that deleting CD98hc in T cells completely suppressed the development of $CD4^+CD25^-$ T cell-induced colitis. Furthermore, CD98hc-deficient T cells have a defect in the differentiation toward effector cells and still be able to differentiate into inducible $Foxp3^+$ regulatory T cells (iTreg). In contrast, nTregs in CD98hc-deficient mice had a reduced capability to suppress colitis. These results indicate that CD98hc is crucial for expansion of autoimmune $CD4^+$ T cells in addition to regulating nTregs, which suggests CD98hc as an important target molecule for establishing strategies for treating colitis.

2. Materials and methods

2.1. Mice

Six- to eight-week-old C57BL/6 (B6), BALB/c, *Rag2*$^{-/-}$ under a B6 background and C.B-17 SCID mice were purchased from Japan SLC (Hamamatsu, Japan) or Taconic (Germantown, NY, USA). B6 Thy1.1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). *Slc3A2*$^{flox/flox}$ mice [20] were crossed with CD4-*Cre* transgenic mice. All mice were maintained under specific pathogen-free conditions in the animal facilities at the University of Tokushima, Japan. All experiments were approved by the ethics committee on animal care at the University of Tokushima and performed in accordance with institutional guidelines for animal care at the University of Tokushima.

2.2. Flow cytometry

Fluorochrome-conjugated monoclonal antibodies specific for mouse CD4, Thy1.1, Thy1.2, IFN-γ, TNF-α, and IL-17 were purchased from Biolegend (San Diego, CA). $CD4^+Foxp3^+$ cells were identified using a mouse regulatory T cell staining kit (eBioscience). All cell samples were resuspended in PBS staining buffer containing 2% FCS and 0.01% NaN_3 (Sigma), and pre-incubated for 15 min at 4 °C with 2.4G2 supernatant to block Fc receptors, then washed and stained with a specific mAb for 20 min at 4 °C. 7-amino actinomycin D (7AAD; Sigma–Aldrich) was used to exclude dead cells. For intracellular staining, cells were fixed with 4% paraformaldehyde (Wako, Japan) and permeabilized with buffer containing 0.1% saponin. Data were acquired with a FACSCantoII (BD Biosciences, NJ, USA) and analyzed using FACSDiva (BD Biosciences) or FlowJo (Tree Star, OR, USA) software.

2.3. Adoptive T cell transfer

$CD4^+$ T cells were purified from the spleens of C57BL/6 wild type or CD98hc$^{-/-}$ mice using an anti-CD4 MACS magnetic separation system (Miltenyi Biotec, Auburn, CA, USA) according to the manufacturer's instructions. $CD4^+CD25^+$ T cells and $CD4^+CD25^-$ T cells were isolated from among $CD4^+$ T cells using a CD25 microbeads kit (Miltenyi Biotec). All cell populations were confirmed to be >95% pure by flow cytometry. To induce colitis, *Rag2*$^{-/-}$ or C.B-17 SCID mice were injected intraperitoneally (i.p.) with 200 μl of PBS containing 5×10^5 $CD4^+CD25^-$ T cells. For Treg co-transfer experiments, *Rag2*$^{-/-}$ mice were given i.p. injections of 5×10^5 $CD4^+CD25^-$ T cells along with 5×10^5wild type or CD98hc-deficient $CD4^+CD25^+$ T cells.

2.4. Antibody treatment

Mice were i.p. administered 250 μg of control IgG or 250 μg of an anti-CD98hc Ab that was started at day 0 and continued every 3 days (total of 5 times). Rat IgG (Sigma–Aldrich) was used as control IgG.

2.5. Disease monitoring and clinical scores

Mice were weighed daily and monitored for signs of soft stool and diarrhea. Clinical scores were assessed by gross appearance (no hunching or wasting = 0; mild to moderate hunching or wasting = 1; and severe hunching or wasting = 2), stool consistency (well formed pellets = 0; soft or loose stool = 1; and liquid stool = 2), and one additional point was added for the presence of any gross blood. These scores were summed for each mouse with total clinical scores ranging from 0 to 5.

2.6. Histological analysis

Colon sections were fixed in 4% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). The extent of inflammation was scored in a blinded manner on a scale of 0–3 for each of 3 criteria: cell infiltration, goblet cell depletion, and crypt damage. These scores were summed for each mouse with total histological scores ranging from 0 to 9.

2.7. Preparation of lamina propria (LP) cells

Colon segments were treated for 30 min at 37 °C with PBS containing 10% FCS, 20 mM HEPES, 100 U/ml of penicillin, 10 μg/ml of polymyxin B (Calbiochem), 100 μg/ml of streptomycin, 1 mM sodium pyruvate, and 10 mM EDTA to remove epithelial cells and then washed with PBS. Colon segments were cut into very small pieces and digested for 45–90 min with stirring at 37 °C with 400 Mandl units/ml of collagenase D (Roche) and 10 μg/ml of DNase I (Roche) in RPMI 1640 medium plus 10% FCS. EDTA (final concentration: 10 mM) was added and cell suspensions were incubated for 5 additional min at 37 °C. After enzyme treatment, cells were subjected to density-gradient centrifugation in 40–75% Percoll (Amersham Bioscience). Cells at the interface were used in assays as LP lymphocytes.

2.8. iTregs assay

Naïve $CD4^+CD25^-$ T cells from wild type or CD98hc$^{-/-}$ mice were stimulated with an anti-CD3 antibody (2 μg/ml) bound to culture plate wells and soluble anti-CD28 antibody (2 μg/ml) in the presence of TGF-β (5 ng/ml). Cells were cultured for 3 days and then assessed by flow cytometry.

2.9. Treg suppression assay

Naïve $CD4^+CD25^-$ T cells from Thy1.1 mice labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) were cultured in 96-well plates for 3 days along with different proportions of Tregs from Thy1.2 wild type or CD98hc$^{-/-}$ mice and in the presence of an anti-CD3 antibody (1 μg/ml) and mitomycin-treated splenocytes. The suppressive capability of Tregs was determined by assessing the proliferation of $CD4^+CD25^-$ T cells based on CFSE dilution.

2.10. Elisa

Serum concentrations of IFN-γ, TNF-α, and IL-17 were determined using standard ELISA kits (eBioscience) according to the manufacturer's protocols.

2.11. Statistical analysis

Statistical comparisons were made by unpaired, two-tailed Student's *t*-tests. Differences were considered significant for $p < 0.05$.

3. Results

3.1. CD98hc-deficient $CD4^+CD25^-$ cells have decreased capability to induce colitis

To investigate the role of CD98hc in the progression of colitis, we used a colitis model in which $CD4^+CD25^-$ T cells from C57BL/6 mice were adoptively transferred into $Rag2^{-/-}$ mice. $Rag2^{-/-}$ mice with transferred control $CD4^+CD25^-$ T cells exhibited progressive weight loss beginning at 4 weeks after T cell transfer (Fig. 1A). In contrast, $Rag2^{-/-}$ mice with transferred $CD4^+CD25^-$ T cells from $Slc3a2^{flox/flox}$ mice crossed with CD4-*Cre* transgenic mice ($CD98hc^{-/-}$) appeared to be healthy and actually had an increase in body weight (Fig 1A). The clinical scores were clearly different between $Rag2^{-/-}$ mice with transferred $CD4^+CD25^-$ T cells from control and $CD98hc^{-/-}$ mice (Fig. 1A). Histological examinations demonstrated prominent epithelial hyperplasia with glandular elongation and massive infiltrations of mononuclear cells in the lamina propria (LP) of $Rag2^{-/-}$ mice with transferred wild type $CD4^+CD25^-$ T cells (Fig. 1B). In contrast, these changes were largely abrogated in the LP of $Rag2^{-/-}$ mice with transferred $CD98hc^{-/-}$ $CD4^+CD25^-$ T cells. These differences were confirmed based on the histological scores of multiple colon sections (Fig. 1B).

There were more total spleen cells in mice with transferred wild type $CD4^+CD25^-$ T cell than those with transferred $CD98hc^{-/-}$ cells (Fig. 2A). The serum concentrations of both interferon (IFN)-γ and IL-17 were increased in mice with transferred wild type $CD4^+CD25^-$ T cells compared to mice that received $CD98hc^{-/-}$ $CD4^+CD25^-$ T cells (Fig. 2B). We also quantified the absolute numbers of immune cells in the LP. There were fewer LP $CD4^+$ T cells in the colons of $Rag2^{-/-}$ mice with transferred $CD98hc^{-/-}$ $CD4^+CD25^-$ T cells compared to $Rag2^{-/-}$ mice that received wild type $CD4^+CD25^-$ T cells (Fig. 2C).

We next examined cytokine production by LP $CD4^+$ T cells. LP $CD4^+$ T cells from $Rag2^{-/-}$ mice with transferred $CD98hc^{-/-}$ $CD4^+CD25^-$ T cells had significantly fewer IL-17 producing cells than $Rag2^{-/-}$ mice with transferred wild type $CD4^+CD25^-$ T cells (Fig. 2D). However, the frequencies of IFN-γ- and TNF-α-producing cells were comparable between these two groups (Fig. 2D).

3.2. CD98hc deficiency affects iTregs differentiation but not nTreg development

We next investigated for the presence of $Foxp3^+$ induced regulatory T cells (iTregs) in the LP of $Rag2^{-/-}$ mice with transferred $CD98hc^{-/-}$ or control $CD4^+CD25^-$ T cells.

Flow cytometry analysis showed that about 10% of $CD3^+CD4^+$ T cells isolated from $Rag2^{-/-}$ mice with transferred $CD98hc^{-/-}$ $CD4^+$ $CD25^-$ T cells had differentiated into $Foxp3^+$ cells, whereas control cells were about 1% $Foxp3^+$ (Fig. 3A). We assessed the differentiation of iTregs *in vitro* by culturing $CD4^+$ T cells under iTregs-polarizing conditions (Fig. 3B). $CD98hc^{-/-}$ $CD4^+$ T cells produced a similar percentage of $Foxp3^+$ cells compared with wild type $CD4^+$ T cells (Fig. 3B).

We then assessed the numbers of nTregs in $CD98hc^{-/-}$ mice and found that they had a frequency of nTregs in the spleen comparable to that in $CD98hc^{+/+}$ mice (Fig. 3C). Total spleen cell numbers were also comparable between $CD98hc^{-/-}$ and control mice (data not shown). We then assessed the *in vitro* suppressive capability of nTregs harvested from $CD98hc^{-/-}$ mice. $CD4^+CD25^-$ T cells from C57BL/6 Thy1.1 mice were cultured in the presence of nTregs from

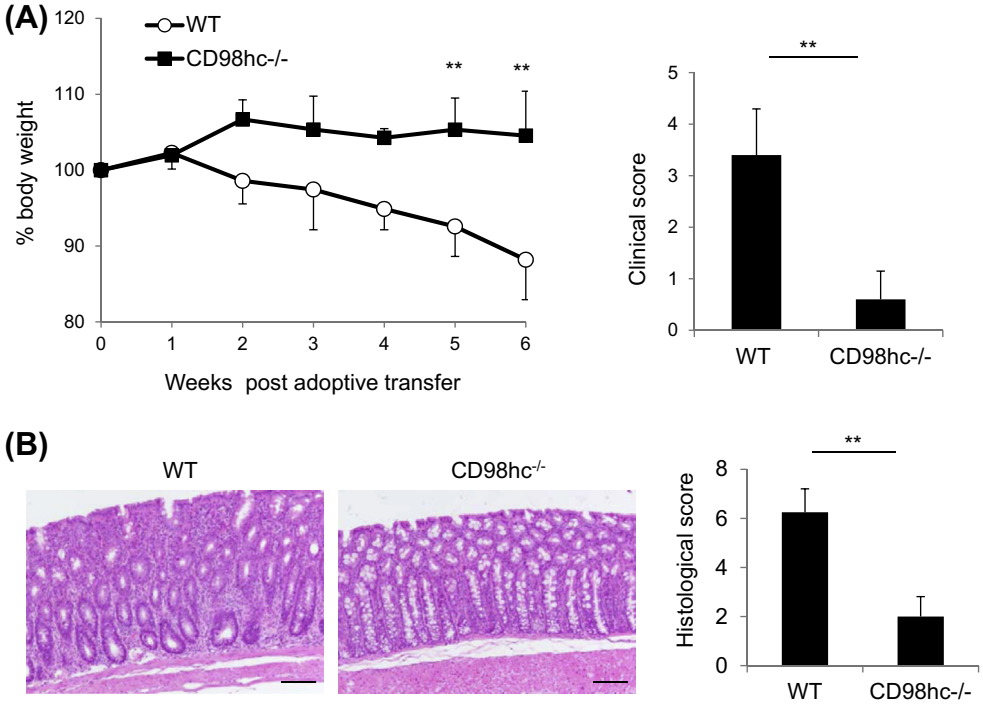

Fig. 1. CD98hc-deficient $CD25^-CD4^+$ T cells have decreased capability to induce colitis. $Rag2^{-/-}$ mice were adoptively transferred with $CD25^-CD4^+$ T cells (5×10^5) from C57BL/6 wild type (WT; $CD98hc^{+/+}$) or $CD98hc^{-/-}$ mice. (A) Changes in body weight are expressed as the percentages of original weight (left panel). Open circles: WT; closed squares: $CD98hc^{-/-}$. Clinical scores were determined at 6 weeks after T cell transfer (right panel); $N = 6$ per group. Results are means ± SD's. $^{**}p < 0.01$. (B) Hematoxylin and eosin (HE) staining of colon sections from recipient mice at 6 weeks after adoptive transfer. Scale bar = 100 μm. Histological scoring was done for multiple colon sections (right panel); $N = 6$ per group. Results are means ± SD's. $^{**}p < 0.01$. The results in this figure are representative of three independent experiments.

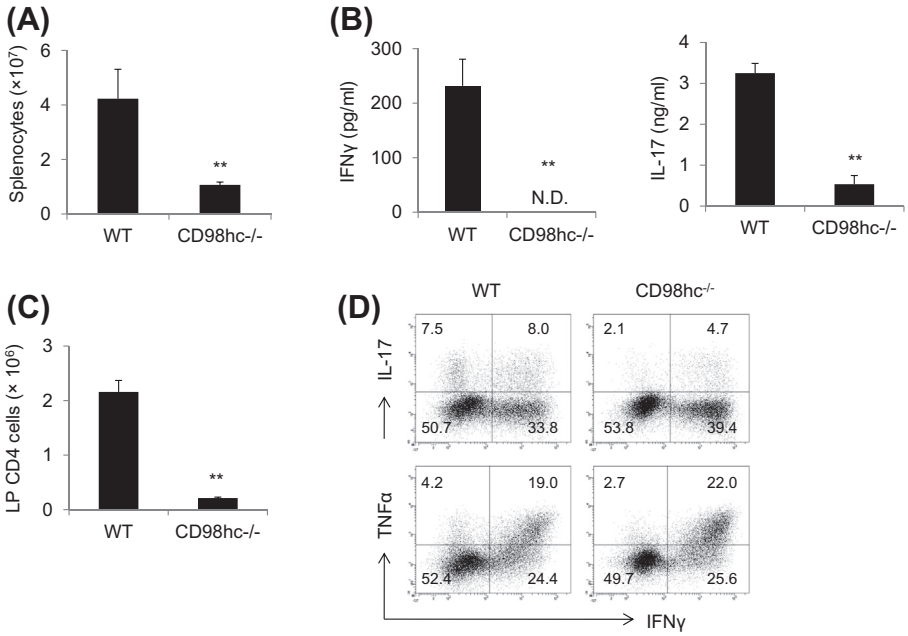

Fig. 2. CD98hc deficient CD25$^-$CD4$^+$ T cells have decreased capability to induce colitis. *Rag2*$^{-/-}$ mice were adoptively transferred with CD25$^-$CD4$^+$ T cells from C57BL/6 wild type or CD98hc$^{-/-}$ mice and assessed at 6 weeks after transfer. (A) Absolute numbers of total splenocytes. (B) Serum levels of IL-17 and IFN-γ. N.D.: not detected. (C) Absolute numbers of CD4$^+$ T cells in the LP; $N = 6$ per group. Results are means ± SD's. $^{**}p < 0.01$. (D) Flow cytometry analysis for IFN-γ, TNF-α, and IL-17 gated on CD4$^+$LP cells after restimulation for 5 h with PMA, ionomycin, and monensin. The results in this figure are representative of 3 independent experiments.

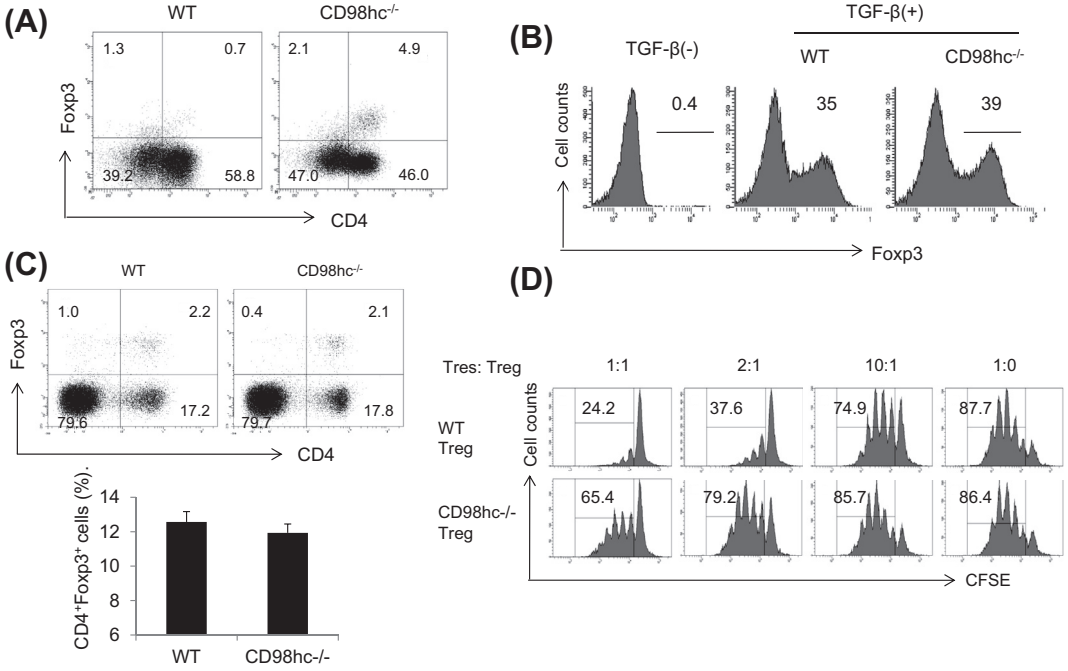

Fig. 3. CD98hc-deficient CD4$^+$CD25$^-$ T cells tend to differentiate into iTregs *in vivo*. (A) Foxp3 expression gated on CD4$^+$ cells in the LP of *Rag2*$^{-/-}$ recipients at 6 weeks after adoptive transfer of either C57BL/6 wild type or CD98hc-deficient CD25$^-$CD4$^+$ T cells. Numbers indicate the percentages of Foxp3$^+$ cells. (B) Naïve CD25$^-$CD4$^+$ T cells from wild type or CD98hc-deficient mice were cultured for 72 h under iTregs-skewing conditions. Numbers above the lines indicate percentages of Foxp3$^+$ cells. (C) Flow cytometry profiles of pooled spleen cells ($N = 3$) from 8-week-old wild type or CD98hc$^{-/-}$ mice showing Foxp3 expression in the CD4$^+$ T cell fraction. Numbers indicate the percentages Foxp3$^+$ cells. Relative Foxp3$^+$ T cell percentages among CD4$^+$ T cells are shown as means ± SD's. (D) *In vitro* suppressive activity of Tregs, as assessed by the proliferation of CFSE labeled naïve CD25$^-$CD4$^+$ T cells from C57BL/6 Thy1.1 mice stimulated with anti-CD3 plus antigen-presenting cells in the presence of varying ratios of Tregs sorted from C57BL/6 Thy1.2 wild type or CD98hc$^{-/-}$ mice. CFSE dilution gated on Thy1.1$^+$ cells was analyzed. The results in this figure are representative of 3 independent experiments.

CD98hc$^{-/-}$ or control mice (Thy1.2) at different ratios after which T cell proliferation was examined. CD98hc$^{-/-}$ nTregs exhibited a significantly reduced suppressive activity compared to control nTregs (Fig. 3D). These results indicated that nTregs from CD98hc$^{-/-}$ mice were less capable of suppressing T cell proliferation *in vitro*.

3.3. CD98hc deficiency affects nTreg function in vivo

To assess the contribution of CD98hc to nTreg function *in vivo*, we adoptively transferred *Rag2*$^{-/-}$ mice with wild type CD4$^+$CD25$^-$ T cells along with CD4$^+$CD25$^+$ nTregs from WT or CD98hc$^{-/-}$ mice.

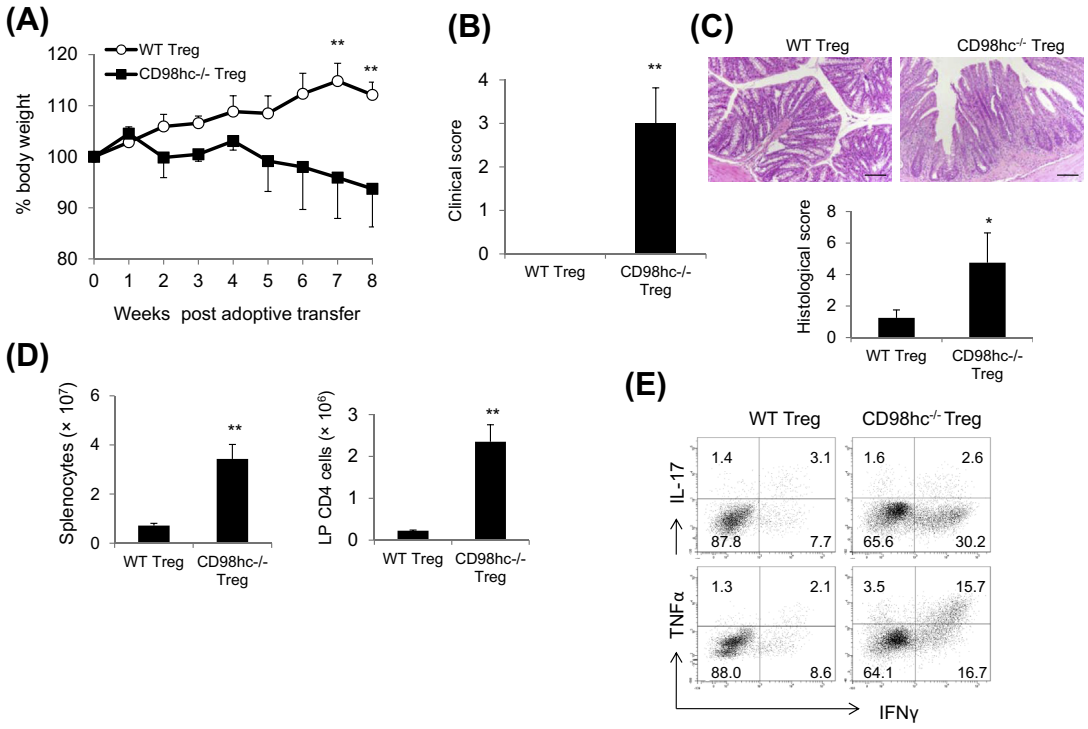

Fig. 4. CD98hc-deficient $CD4^+CD25^+$Tregs have decreased capability to suppress colitis. *Rag2*$^{-/-}$ mice were adoptively transferred with wild type C57BL/6 $CD4^+CD25^-$ T cells (5×10^5) along with C57BL/6 wild type or CD98hc-deficient $CD4^+CD25^+$ nTregs (5×10^5). (A) Changes in body weight are expressed as the percentages of the original weight. Open circles: wild type nTregs; closed squares: CD98hc$^{-/-}$ nTregs. (B) Clinical scores were determined at 8 weeks after T cell transfer. (C) Hematoxylin and eosin (H&E) staining of colon sections from recipient mice at 8 weeks after adoptive transfer. Scale bar = 100 μm. Histological scoring was done for multiple colon sections ($^*p < 0.05$); $N = 6$ per group. (D) Absolute numbers of total splenocytes and absolute numbers of $CD4^+$ T cells in the LP; $N = 6$ per group. Results are means ± SD's. $^*p < 0.05$, $^{**}p < 0.01$. (E) Flow cytometry analysis for the expressions of IFN-γ, TNF-α, and IL-17 gated on $CD4^+$LP cells after restimulation for 5 h with PMA, ionomycin, and monensin. The results in this figure are representative of 3 independent experiments.

Rag2$^{-/-}$ mice with transferred $CD4^+CD25^-$ T cells and control $CD4^+CD25^+$ T cells did not exhibit any weight loss (Fig. 4A). In contrast, *Rag2*$^{-/-}$ mice with transferred $CD4^+CD25^-$ T cells and CD98hc$^{-/-}$ $CD4^+CD25^+$ nTregs showed gradual weight loss. Colitis assessments based on clinical scores showed a clear difference between *Rag2*$^{-/-}$ mice with transferred wild type and CD98hc$^{-/-}$ $CD4^+CD25^+$ nTregs (Fig. 4B).

Histological examinations demonstrated epithelial hyperplasia and massive infiltrations of mononuclear cells into the LP of *Rag2*$^{-/-}$ mice with transferred CD98hc$^{-/-}$ $CD4^+CD25^+$ nTregs, whereas these changes were mostly absent in mice that received control $CD4^+CD25^+$ nTregs (Fig. 4C). These differences were confirmed based on the histological scores of multiple colon sections (Fig. 4C).

We quantified the absolute numbers of splenocytes and immune cells in the LP and found increased numbers of spleen cells and LP $CD4^+$ T cells in the colon tissues of *Rag2*$^{-/-}$ mice that received CD98hc$^{-/-}$ $CD4^+CD25^+$ nTregs compared with those that received wild type nTregs (Fig. 4D). We also examined cytokine production by LP-resident $CD4^+$ T cells. LP $CD4^+$ T cells from *Rag2*$^{-/-}$ mice with transferred $CD4^+CD25^-$ T cells and CD98hc$^{-/-}$ nTregs contained significantly more IFN-γ- and TNF-α but not IL-17$^+$ producing cells than did *Rag2*$^{-/-}$ mice with transferred $CD4^+CD25^-$ T cells and control nTregs (Fig. 4E).

To evaluate if blocking CD98hc with an anti-CD98hc blocking antibody could suppress the development of colitis, we treated C.B-17 SCID mice (that had received $CD4^+CD25^-$ T cells from wild type BALB/c mice) with an anti-CD98hc antibody. This treatment was begun on day 0 (when T cells were transferred) and continued every 3 days (total of 5 times). Treating mice with the anti-CD98hc mAb significantly ameliorated the development of colitis, as shown by a gradual weight gain, in contrast to the weight loss by mice treated with control IgG (Supplementary Fig. 1A). The clinical scores were also milder for anti-CD98hc-treated mice compared to control IgG-treated mice (Supplementary Fig. 1B). Colon sections from mice treated with the anti-CD98hc mAb had reduced mononuclear cell infiltration and less epithelial cell hyperplasia as compared to control IgG-treated mice (Supplementary Fig. 1C). These differences were confirmed based on the histological scores of multiple colon sections (Supplementary Fig. 1C).

4. Discussion

CD98hc plays an important role in T cell proliferation, and blocking CD98hc inhibits effector T cell proliferation [17,18]. In this study, we investigated if CD98hc on $CD4^+$ T cells played a role in the development of experimental colitis. We found that colitis was not induced in *Rag2*$^{-/-}$ mice after adoptive transfer of CD98hc$^{-/-}$ $CD4^+CD25^-$ T cells. *Rag2*$^{-/-}$ mice that received CD98hc-deficient $CD4^+CD25^-$ T cells had a significantly reduced expansion of their T cell compartment compared to mice that received wild type $CD4^+CD25^-$ T cells. Transferring CD98hc deficient $CD4^+CD25^-$ T cells into *Rag2*$^{-/-}$ mice did not cause colitis, accompanied by an increased frequency of iTregs. These results suggested that CD98hc regulates the expansion of effector colitogenic $CD4^+$ T cells, which contributes to the development or progression of colitis.

Regarding a mechanism for weak colitis by CD98hc-deficient $CD4^+$ T cells, our results suggest that weak colitis by transferring CD98hc-deficient $CD4^+CD25^-$ T cells would be attributable to the intrinsic low proliferative capability of CD98hc-deficient $CD4^+$ T

cells. We also found that CD98hc$^{-/-}$ CD4^{+}CD25^{-} T cells that were transferred into *Rag2*$^{-/-}$ mice differentiated into Foxp3^{+} iTregs, although total iTregs numbers appeared to be similar between control CD4^{+}CD25^{-} T cell and CD98hc-deficient CD4^{+}CD25^{-} T cell transfer groups. The roles of iTreg in the suppression of colitis need to be addressed in the future studies.

We and another group demonstrated that CD98hc was crucial for CD4^{+} T cell expansion [17,18]. Our current results also suggested that the proliferation of colitogenic CD4^{+} T cells *in vivo* was reduced due to CD98hc deficiency. Although the proliferation of B cells governed by CD98hc is regulated by the Erk1/2 signaling pathway [19], the molecular mechanism by which CD98hc regulates CD4^{+} T cell expansion remains unclear. One study showed that the integrin-binding domain of CD98 was sufficient for full clonal expansion of T cells [18], which suggests the crucial role for adhesive signaling in CD98hc-mediated T cell proliferation. We also found that the absence of CD98hc on T cells abrogated nTreg function. These results indicated that nTregs used CD98hc as a critical switch for determining suppressive functions. The molecular basis of how CD98hc controls nTreg function remains unclear. One possibility is that the absence or inhibition of CD98hc in T cells suppresses T cell proliferation [17,18], which may result in dysregulated *in vivo* expansion of nTregs. Although CD98hc controls both integrin signaling and amino acid transport, a previous study showed that integrin signaling through CD98hc was crucial for T cell proliferation [18]. Thus, integrin signaling might also be involved in the expansion of nTregs *in vivo*. In addition, although CD98hc-deficient nTregs lack suppressive functions, CD98hc-deficient mice do not spontaneously develop colitis (data not shown). That would be explained by the low intrinsic proliferative activity of CD98hc deficient CD4^{+} effector T cells. Taken together, these results suggest that CD98hc is a key molecule for balancing effector T cells and nTregs and thus is important for maintaining immune tolerance.

As we here demonstrated strong suppressive activity for experimental colitis by anti-CD98hc antibody, our results suggest that CD98hc may be a target molecule for establishing therapies for autoimmune disorders. However, previous studies showed that CD98hc also regulated conventional T cell proliferation and the subsequent acquisition of T cell effector functions [17,18]. Therefore, it will be important to establish systems to suppress or activate CD98hc in a cell type-specific manner as a possible therapeutic strategy for manipulating immunity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.144.

References

[1] D.K. Podolsky, Inflammatory bowel disease, N. Engl. J. Med. 347 (2002) 417–429.
[2] A. Izcue, J.L. Coombes, F. Powrie, Regulatory lymphocytes and intestinal inflammation, Annu. Rev. Immunol. 27 (2009) 313–338.
[3] A. Kaser, S. Zeissig, R.S. Blumberg, Inflammatory bowel disease, Annu. Rev. Immunol. 28 (2010) 573–621.
[4] J.H. Buckner, Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases, Nat. Rev. Immunol. 10 (2010) 849–859.
[5] S. Sakaguchi, M. Miyara, C.M. Costantino, D.A. Hafler, FOXP3+ regulatory T cells in the human immune system, Nat. Rev. Immunol. 10 (2010) 490–500.
[6] A.M. Bilate, J.J. Lafaille, Induced CD4+Foxp3+ regulatory T cells in immune tolerance, Annu. Rev. Immunol. 30 (2012) 733–758.
[7] J. Zhu, W.E. Paul, Peripheral CD4+ T-cell differentiation regulated by networks of cytokines and transcription factors, Immunol. Rev. 238 (2010) 247–262.
[8] L. Zhou, M.M. Chong, D.R. Littman, Plasticity of CD4+ T cell lineage differentiation, Immunity 30 (2009) 646–655.
[9] M.A. Curotto de Lafaille, J.J. Lafaille, Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor?, Immunity 30 (2009) 626–635.
[10] D.A. Horwitz, S.G. Zheng, J.D. Gray, Natural and TGF-beta-induced Foxp3(+)CD4(+) CD25(+) regulatory T cells are not mirror images of each other, Trends Immunol. 29 (2008) 429–435.
[11] B. Knoechel, J. Lohr, E. Kahn, J.A. Bluestone, A.K. Abbas, Sequential development of interleukin 2-dependent effector and regulatory T cells in response to endogenous systemic antigen, J. Exp. Med. 202 (2005) 1375–1386.
[12] R.N. Germain, I. Stefanova, The dynamics of T cell receptor signaling: complex orchestration and the key roles of tempo and cooperation, Annu. Rev. Immunol. 17 (1999) 467–522.
[13] F. Castellino, R.N. Germain, Cooperation between CD4+ and CD8+ T cells: when, where, and how, Annu. Rev. Immunol. 24 (2006) 519–540.
[14] M.G. Rudolph, R.L. Stanfield, I.A. Wilson, How TCRs bind MHCs, peptides, and coreceptors, Annu. Rev. Immunol. 24 (2006) 419–466.
[15] J.C. Porter, N. Hogg, Integrins take partners: cross-talk between integrins and other membrane receptors, Trends Cell Biol. 8 (1998) 390–396.
[16] J.M. Cantor, M.H. Ginsberg, D.M. Rose, Integrin-associated proteins as potential therapeutic targets, Immunol. Rev. 223 (2008) 236–251.
[17] G. Lian, H. Arimochi, A. Kitamura, J. Nishida, S. Li, K. Kishihara, Y. Maekawa, K. Yasutomo, Manipulation of CD98 resolves type 1 diabetes in nonobese diabetic mice, J. Immunol. 188 (2012) 2227–2234.
[18] J. Cantor, M. Slepak, N. Ege, J.T. Chang, M.H. Ginsberg, Loss of T cell CD98 H chain specifically ablates T cell clonal expansion and protects from autoimmunity, J. Immunol. 187 (2011) 851–860.
[19] J. Cantor, C.D. Browne, R. Ruppert, C.C. Feral, R. Fassler, R.C. Rickert, M.H. Ginsberg, CD98hc facilitates B cell proliferation and adaptive humoral immunity, Nat. Immunol. 10 (2009) 412–419.
[20] Z. Liu, J. Hou, J. Chen, H. Tsumura, M. Ito, Y. Ito, X. Hu, X.K. Li, Deletion of CD98 heavy chain in T cells results in cardiac allograft acceptance by increasing regulatory T cells, Transplantation 93 (2012) 1116–1124.