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The interplay of CD4+ and CD8+ T cells targeting autoantigens is responsible for the progression of a number of autoimmune diseases, including type 1 diabetes mellitus (T1D). Understanding the molecular mechanisms that regulate T cell activation is crucial for designing effective therapies for autoimmune diseases. We probed a panel of Abs with T cell-modulating activity and identified a mAb specific for the H chain of CD98 (CD98hc) that was able to suppress T cell proliferation. The anti-CD98hc mAb also inhibited Ag-specific proliferation and the acquisition of effector function by CD4+ and CD8+ T cells in vitro and in vivo. Injection of the anti-CD98hc mAb completely prevented the onset of cyclophosphamide-induced diabetes in NOD mice. Treatment of diabetic NOD mice with anti-CD98hc reversed the diabetic state to normal levels, coincident with decreased proliferation of CD4+ T cells. Furthermore, treatment of diabetic NOD mice with CD98hc small interfering RNA resolved T1D. These data indicate that strategies targeting CD98hc might have clinical application for treating T1D and other T cell-mediated autoimmune diseases. The Journal of Immunology, 2012, 188: 2227–2234.

Both innate and adaptive immune responses control T cell responses to foreign and autoantigens (1, 2). The activation of innate immune influences APCs to express T cell costimulatory molecules, which are essential for effective T cell-mediated adaptive immune responses. A variety of costimulatory receptors expressed on T cells can positively or negatively regulate the proliferation, functional differentiation, and survival of T cells (3–6). A number of reports investigating the experimental manipulation of these molecular interactions have demonstrated effective modulation of T cell function, and this has led investigators to generate panels of reagents, such as mAbs or recombinant soluble ligands, that target each costimulatory receptor/ligand pair, to establish strategies for treating T cell-mediated autoimmune diseases.

Type 1 diabetes mellitus (T1D) is a progressive autoimmune disease characterized by mononuclear cell infiltration into pancreatic islets, and the subsequent destruction of insulin-producing β cells (2, 7). NOD mice recapitulate many features of T1D pathogenesis in humans (8, 9). Many studies using these mice have demonstrated that the early stages of the diabetogenic response involve effector T cells targeting β cell Ags, including glutamic acid decarboxylase 65, insulin, islet amyloid polypeptide, chromogranin A, islet-specific glucose-6-phosphatase catalytic subunit-related protein, and islet antigen 2 (10). Insulitis or islet inflammation is detectable in NOD mice at ~4 wk of age (7, 8, 11). Overt diabetes is typically diagnosed in NOD mice older than 12 wk of age, at a time when ~90% of the β cell mass has been destroyed.

CD98/4F2 is a disulfide-linked complex composed of a glycosylated H chain of CD98 (CD98hc) and a nonglycosylated L chain of CD98 (12). CD98hc is a type II transmembrane glycoprotein consisting of 529 aa, with many proposed biological functions, such as an amino acid transport when paired with the L chain of CD98, and a regulator of integrin-mediated cell adhesion, in addition to its demonstrated role in regulating fusion (12–14). It has been reported that an Ab specific for mouse CD98hc is able to suppress the Con A-mediated activation of mouse T cells (15). Furthermore, a recent article has indicated that genetic deletion of CD98hc in T cells negatively impacts T cell clonal expansion and suppresses the development of experimental acute encephalomyelitis and T1D induced by adoptively transferring pathogenic T cells (16). However, it remains unclear if CD98hc will be a good target molecule for treating spontaneously progressive autoimmune diseases.

In this study, we systematically investigated molecules that modulate T cell function by establishing a panel of mAbs that recognize spleen cells and have the ability to modulate T cell function. One Ab that highly suppressed T cell proliferation recognized CD98hc. The anti-CD98hc mAb suppressed proliferation and the functional differentiation of both CD4+ and CD8+ T cells. This Ab resolved T1D in NOD mice by inhibiting T cell responses. Furthermore, treatment of NOD mice with small interfering RNA (siRNA) against CD98hc also suppressed the progression of T1D in NOD mice. These data indicate that CD98hc contributes to the development of T1D and suggest that therapies targeting CD98hc, such as mAbs and siRNA, might be used clinically for preventing or treating T1D and other T cell-mediated autoimmune diseases.

Materials and Methods

Mice

Female Wistar rats and C57BL/6, BALB/c, NOD, and NOD/SCID mice were purchased from Japan SLC (Hamamatsu). OT-I or OT-II TCR transgenic mice were purchased from Taconic. All mice and rats were maintained under specific pathogen-free conditions in the animal research center of the University of Tokushima, and all experiments were performed in accordance with institutional guidelines for animal care and use. Diabetes incidence in NOD mice was monitored by weekly measurement of urine...
glucose concentrations and venous blood glucose concentrations in non-fasting mice. Mice with blood glucose concentrations >250 mg/dl were considered diabetic. T1D developed in >80% of female NOD mice between 20 and 25 wk of age in our colony.

Establishment and selection of hybridomas

A total of 2 × 10^7 BALB/c mouse spleen cells were injected i.p. into Wistar rats three times at 10-d intervals. Total spleen cells from Wistar rats 5 d after the last immunization were fused with SP2 hybridoma parent cells (ATCC). Cell supernatants were selected for further study. NOD mice were stimulated with pancreatic Ags (20% of total volume) for 5 d after the last immunization were fused with SP2 hybridoma parent cells (ATCC). Cell supernatants were selected for further study. BDF1 mice were immunized with OVA emulsified in CFA and were treated with anti-OVA or control IgG (200 µg/mouse) on days 1, 2, 5, and 8. The day of OVA immunization was designated day 0. C57BL/6 mice received CFSE-labeled T cells from OT-I or OT-II TCR transgenic mice and were immunized with OVA protein (100 µg) or OVA peptide (257–264; 75 µg) emulsified in CFA immediately after T cell transfer. Recipient mice were treated with anti-OVA or control IgG (200 µg/mouse) on days 1 and 2. The day of OVA immunization was designated day 0.

Flow cytometry

Lymphocytes obtained from spleen, pancreatic lymph nodes, and pancreas were stained with fluorochrome-conjugated Abs to CD4, CD8, TCRVβ2, and TCRVβ5 (eBioscience). In some experiments, cells were stimulated with 250 ng/ml PMA (Sigma-Aldrich) and 1 µg/ml ionomycin (Sigma-Aldrich) for 5 h in the presence of monensin, followed by surface staining for CD4 or CD8 and intracellular staining for granzyme B, IFN-γ, or IL-17 (eBioscience). 7-Aminoactinomycin D (Sigma-Aldrich) was used to exclude dead cells. CD25+CD4+Foxp3+ cells were identified using a mouse regulatory T cell staining kit from eBioscience according to the manufacturer’s instructions. Peripheral mononuclear cells from mice were stained with the I-142 mAb, followed by PE-conjugated anti-rat IgG (Jackson ImmunoResearch Laboratories). Data were collected on a FACSCanto II (BD Biosciences) and analyzed using FlowJo software (Tree Star).

T cell proliferation

Cells were stimulated with soluble or plate-bound anti-CD3 mAb (1 µg/ml; eBioscience) in the presence of I-143 or a control rat IgG (10 µg/ml; Jackson ImmunoResearch Laboratories) for 3 d. IL-12 (10 ng/ml; eBioscience) and anti–IL-4 (1 µg/ml) or TGF-β (1 ng/ml; Roche), IL-6 (10 ng/ml; Miltenyi Biotec), and anti–INF-γ (4 µg/ml) were added to the cultures for Th1- or Th17-skewing conditions, respectively. Pancreata from diabetic NOD mice were dissected by scissors into small pieces in ice-cold PBS, homogenized, and sonicated with a Sonifire sonicator (Branson Ultrasonics) for 10 min. After centrifugation at 15,000 rpm for 20 min, the supernatants were used as a source of pancreatic Ags. Spleen cells from NOD mice were stimulated with pancreatic Ags (20% of total volume) for 3 d, and [3H]-thymidine uptake by the spleen cells was determined during the final 16 h of culture. Spleenocytes were resuspended at a concentration of 1 × 10^7 cells/ml in serum-free DMEM at 37°C. An equal volume of CFSE stock (5 mM in DMSO) in 37°C serum-free DMEM was added to the cell preparation and incubated for 5 min at 37°C. CFSE labeling was quenched by adding an equal volume of heat-inactivated FCS. CFSE-labeled lymph node cells from OT-I or OT-II TCR transgenic mice were adoptively transferred into C57BL/6 mice. For T cell stimulation, spleenocytes were stimulated with soluble anti-CD3 mAb (1 µg/ml). Cells were incubated for 2 d, harvested, and stained for flow cytometry with anti-CD8, anti-CD4, and 7-aminactinomycin D.

ELISA

OVA (100 µg) emulsified in CFA was injected at the tail base of C57BL/6 mice. Serum samples were obtained from peripheral blood. The anti-OVA IgM and IgG titers were measured by mouse anti-OVA IgM and mouse anti-OVA IgG kits (Alpha Diagnostic International) according to the manufacturer’s protocols.

Transfection

A total of 293T cells were transfected with mouse full-length CD98hc cDNA in pcDNA3.1 using GeneJuice (Merck), and stable cells expressing CD98hc were obtained after Zeocin treatment.

Immunoprecipitation

Spleen cells from C57BL/6 mice were lysed with lysis buffer. After centrifugation, the supernatants were incubated with I-142 for 2 h at 4°C and further incubated with protein G agarose for 1 h at 4°C. After washing the protein G agarose with lysis buffer, boiled immunoprecipitants in sample buffer were subjected to SDS-PAGE. Gels were stained with a silver staining kit (Invitrogen).

Isolation of cells

Total T cells were purified from spleen cells by incubating with anti-FcR and were depleted of FcR-positive cells using anti-rat IgG MicroBeads (Miltenyi Biotec). CD4+ or CD8+ T cells were purified from total spleen or lymph node cells by incubating with anti-CD4 (GXK1.5) or anti-CD8 mAb (53-6.72), followed by positive selection of CD4+ or CD8+ cells with anti-IgG MicroBeads (Miltenyi Biotec). CD4+ T cells were isolated from single-cell suspensions of spleens using the CD4+ T Cell Isolation Kit II. In each case, purity of the cell separation was verified to be ≥95% by immunofluorescence and FACS analysis.

Ab treatment

NOD mice 12 wk old were injected i.p. with cyclophosphamide (200 mg/kg body weight) two times at 7-d intervals. The day of the second cyclophosphamide injection was designated day 0. Anti-CD98hc mAb (100 µg/mouse) was injected on days 0, 3, 6, 9, 12, and 15. In some experiments, diabetic mice showing glycosuria and fasting glycemia >250 mg/dl were given anti-CD98hc (100 µg/mouse) or control IgG three times every other day, beginning the day the mice developed diabetes. Remission was defined by normal glycemia and the absence of glycosuria. C57BL/6 mice were immunized with OVA emulsified in CFA and were treated with anti-CD98hc or control IgG (100 µg/mouse) on days 1, 2, 5, and 8. The day of OVA immunization was designated day 0. C57BL/6 mice received CFSE-labeled T cells from OT-I or OT-II TCR transgenic mice and were immunized with OVA protein (100 µg) or OVA peptide (257–264; 75 µg) emulsified in CFA immediately after T cell transfer. Recipient mice were treated with anti-CD98hc or control IgG (200 µg/mouse) on days 1 and 2. The day of OVA immunization was designated day 0.

siRNA treatment

A mixture of three siRNAs against mouse CD98hc or negative control siRNA without any target sequence (4 nmol per mouse; B-Bridge) (Supplemental Table I) was mixed with AteloGene Systemic (Koken), according to the manufacturer’s protocol. The siRNA transfection mix was injected i.p. into NOD/SCID mice that had received total T cells from diabetic NOD mice. The siRNA was administered on days 2, 5, and 8 (the day when T cells were transferred was designated day 0).

Histopathology

Tissues were fixed in buffered 10% formalin and embedded in paraffin, and then 8-µm sections were stained with HE&E and scored for immune infiltrates. For autoimmune pancreatitis, 0 = no pancreatitis; 1 = periductal infiltrates and focal acinar infiltrates with limited tissue destruction; 2 = periductal infiltrates and moderate acinar infiltrates (<50% of acinar tissue destroyed in one or more pancreatic lobes); and 3 = widespread periductal and acinar infiltrates (>50% of acinar tissue in several pancreatic lobes destroyed). The number of islets in 10 fields was counted, and the mean ± SE was calculated.

Statistical analysis

The p values were derived using the two-tailed Student t test. Statistical differences in survival rates between two groups with TID development were analyzed by the Kaplan–Meier log-rank test. Differences were considered significant when p < 0.05.

Results

Establishment of mAbs that modulate T cell proliferation

To identify molecules that control T cell function, we first immunized Wistar rats with BALB/c spleen cells and established >200 hybridoma cell lines that recognized mouse spleen cells. We selected 43 hybridoma cell lines producing mAbs that were able to augment or inhibit T cell proliferation in an MLR assay. One hybridoma cell line was selected from the 43 because the mAb (cell line I-142) produced by this cell line was the best at inhibiting T cell proliferation in an MLR assay (data not shown).

We first determined whether the I-142 mAb directly suppressed T cell proliferation induced by TCR/CD3-mediated stimulation. I-142 addition suppressed T cell proliferation when total spleen cells were stimulated with soluble anti-CD3 mAb (Fig. 1A) or when purified CD4+ or CD8+ T cells (Fig. 1B) were stimulated with plate-bound anti-CD3 mAb. I-142 inhibited Th1 and Th17 differentiation in CD4+ T cells (Fig. 1C) and suppressed granzyme B-producing cytotoxic T cell differentiation in CD8+ T cells (Fig. 1D). We next ascertained whether I-142 could suppress T cell-mediated immune responses in vivo. I-142 (100 µg) was injected...
four times into mice immunized with OVA protein emulsified in CFA. We observed that I-142 inhibited the production of OVA-specific IgM and IgG, indicating that the I-142 mAb is able to suppress Ag-specific Ab production (Fig. 1E).

**I-142 mAb recognizes CD98hc**

We next identified the protein recognized by I-142. Cell lysates from total spleen cells were immunoprecipitated with the I-142 mAb and subjected to SDS-PAGE. Silver staining showed a specific band around 75 kDa (Fig. 2A). Liquid chromatography–mass spectrometry analysis of this band revealed that this band corresponds to the H chain of CD98 (CD98hc) (data not shown). 293T cells transfected with control vector or vector encoding the cDNA of mouse CD98hc were stained with I-142 to confirm that I-142 mAb recognizes CD98hc (Fig. 2B). Positive staining was observed in 293T cells overexpressing CD98hc, whereas 293T cells transfected

**FIGURE 2.** I-142 recognizes the H chain of CD98. (A) Cell lysates from total spleen cells were immunoprecipitated with control rat IgG or the I-142 mAb. The immunoprecipitants were subjected to SDS-PAGE, and gels were silver stained. (B) Mouse CD98hc cDNA was transfected into 293T cells. Control vector-transfected 293T cells (293/mock) or mouse CD98hc cDNA-transfected 293T cells (293/mCD98hc) were stained with I-142, followed by PE-conjugated anti-rat IgG, and analyzed by flow cytometry. Green or pink lines indicate the cells stained with secondary Ab alone or I-142 and secondary Ab, respectively. The data shown in this figure are representative of at least three experiments.
with control vector were not stained by the I-142 mAb (Fig. 2B). Taken together, these data indicate that I-142 recognizes mouse CD98hc, although we cannot deny the possibility that I-142 recognizes CD98hc as a complex with other molecules.

Anti-CD98hc suppresses CD4\(^+\) and CD8\(^+\) T cell proliferation in vitro and in vivo

We determined whether or not the I-142 mAb could inhibit T cell proliferation and effector functions in vivo. CD4\(^+\) T cells from OT-II TCR transgenic mice (A) or CD8\(^+\) T cells from OT-I TCR transgenic mice (B) were transferred into C57BL/6 mice (n = 5 per group) that were subsequently immunized with OVA protein (OT-II) or OVA peptide (OT-I) in CFA and treated with control rat IgG or the I-142 mAb. CFSE dilution in CD4\(^+\)TCR\(\alpha^+\)V\(\beta^+\) or CD8\(^+\)TCR\(\alpha^+\)V\(\beta^+\) 4 d after immunization was evaluated by flow cytometry. The number indicates percent of proliferating cells. Data are shown as the mean ± S.D. The data shown in this figure are representative of at least three experiments. \(^*p < 0.05\).

I-142 administration prevents the progression of T1D in NOD mice

To examine whether blockade of CD98 with I-142 suppresses T cell-mediated autoimmune pathological states, we made use of NOD mice because they develop T1D owing to aberrant effector functions of both CD4\(^+\) and CD8\(^+\) T cells. We first tested whether I-142 has a protective role for T1D in NOD mice by administering the I-142 mAb into NOD mice that had been injected with cyclophosphamide. Cyclophosphamide induces T1D in ∼70% of NOD mice in our colony. Of interest, all mice treated with I-142 remained free of disease (Fig. 4A). Histopathological analysis showed that most of the islets in control diabetic mice exhibited intrainsulitis (Fig. 4B), whereas the majority of islets in treated mice with OVA peptide emulsified by CFA and treated with control IgG or I-142. Treatment with I-142 reduced cell division (Fig. 3B). These data demonstrate that the I-142 mAb is capable of suppressing both CD4\(^+\) and CD8\(^+\) T cell division in vivo.

FIGURE 3. Anti-CD98hc inhibits T cell proliferation in vivo. CFSE-labeled CD4\(^+\) T cells from OT-II TCR transgenic mice (A) or CD8\(^+\) T cells from OT-I TCR transgenic mice (B) were transferred into C57BL/6 mice (n = 5 per group) that were subsequently immunized with OVA protein (OT-II) or OVA peptide (OT-I) in CFA and treated with control rat IgG or the I-142 mAb. CFSE dilution in CD4\(^+\)TCR\(\alpha^+\)V\(\beta^+\) or CD8\(^+\)TCR\(\alpha^+\)V\(\beta^+\) 4 d after immunization was evaluated by flow cytometry. The number indicates percent of proliferating cells. Data are shown as the mean ± S.D. The data shown in this figure are representative of at least three experiments. \(^*p < 0.05\).

FIGURE 4. Anti-CD98hc prevents development of T1D. (A) NOD mice (n = 6 per group) were injected with cyclophosphamide twice at 14-d intervals. The final injection of cyclophosphamide is day 0. I-142 (100 µg per mouse) was injected on days 0, 3, 6, 9, 12, and 15. The development of T1D was evaluated by measuring urine and blood glucose levels. (B) The histological section at day 10 was stained with H&E. Scale bars, 100 µm. As the negative control, the pancreata from cyclophosphamide-untreated age-matched NOD mice were used (Non-CPM). (C) Histological data at day 10 were scored. (D) The number of islets in four fields at day 10 was calculated. Data are shown as the mean ± S.D. The data shown in this figure are representative of at least three experiments. \(^*p < 0.05\).
mice were not inflamed (Fig. 4B) or had slight peri-insulitis (Fig. 4B, 4C). The total number of islets was larger in the I-142–treated group than in the control IgG-treated group, although the number was less than that in cyclophosphamide-untreated mice (Fig. 4D). These data suggest that the I-142 mAb protects the islets from damage, which helps maintain their number. Such results indicate that the I-142 mAb is able to protect against T1D when administered during an early stage of the disease.

As increased numbers of regulatory T cells are able to inhibit the development of T1D in NOD mice (17), we tested the effect of I-142 on the number of Foxp3+ CD4+ T cells in cyclophosphamide-treated NOD mice. The data shown in this figure are representative of at least three experiments.

FIGURE 5. The I-142 mAb does not induce regulatory T cells. (A) NOD mice were injected with cyclophosphamide twice at 14-d intervals (n = 5 per group). The final injection of cyclophosphamide is day 0. I-142 (100 µg per mouse) was injected on days 0, 2, 4, and 6. CD4+CD25+Foxp3+ cells in the spleen, pancreas, or pancreatic lymph nodes in control IgG- or I-142–treated mice were examined by flow cytometry on day 10. Data are shown as the mean ± S.D. (B) NOD mice were injected with cyclophosphamide twice at 14-d intervals. The final injection of cyclophosphamide is day 0. I-142 (100 µg per mouse) was injected on days 0, 2, 4, and 6. Total spleen cells from the NOD mice on day 10 were transferred into NOD mice (n = 5 per group) that had been treated with cyclophosphamide twice at 14-d intervals. Cells were transferred 6 d after cyclophosphamide injection of recipient mice. The data shown in this figure are representative of at least three experiments.

FIGURE 6. Anti-CD98hc resolves T1D. After female NOD mice developed T1D, mice (n = 10 per group) were treated with control IgG or I-142 (100 µg per mouse). Each Ab was injected on days 0, 3, 5, and 7 (the day on which mice developed T1D was designated day 0). The restoration to normoglycemia was evaluated by monitoring urine and blood glucose levels. (A) This panel shows the representative course of blood glucose for one mouse, each treated with control rat IgG or I-142. (B) Effective treatment is defined by a blood glucose level <250 mg/dl just after the final Ab treatment and thereafter normoglycemia for >14 d. Data are shown as the mean ± S.D. **p < 0.01. (C and D) Histological sections after treatment with control IgG or I-142 were stained with H&E 10 d after initial treatment. Scale bars, 100 µm. Insulitis scores were calculated as described in Materials and Methods. (E) Total islets were counted 10 d after the final treatment. Data are shown as the mean ± S.D. **p < 0.01. (F) Total T cells from pancreatic lymph nodes were obtained from NOD mice treated with I-142 or control rat IgG 2 d after the final Ab treatment and were stimulated with pancreatic Ags for 3 d. T cell proliferation in pancreatic lymph nodes was measured by incorporation of [3H]-thymidine. As the negative control, lymph node cells from rat IgG-treated mice were cultured without any exogenous Ags (No Antigen). Data are shown as the mean ± S.D. *p < 0.05.
pancreatic lymph nodes, and pancreas was comparable between control Ig- and I-142–treated mice (Fig. 5A). To further examine whether treatment with I-142 induces the differentiation of cells with T cell suppressive activity, regardless of Foxp3+ expression, we transferred total spleen cells from both cyclophosphamide- and I-142–treated mice into cyclophosphamide-treated NOD mice and monitored blood glucose levels. We did not observe any inhibitory effect of the spleen cells from I-142–treated mice compared with control IgG-treated mice (Fig. 5B). These data suggest that I-142 is directly suppressive for effector T cells in vivo and does not induce the differentiation of cells with suppressive activity.

**Anti-CD98hc inhibits the progression of TID**

We evaluated if the I-142 mAb is able to reverse TID during the advanced hyperglycemic stage. Accordingly, blood glucose levels were monitored beginning at 15 wk, and mice displaying hyperglycemia between the ages of 20 and 30 wk were injected with I-142 or control IgG every 3 d. We found that >80% of the I-142–treated mice were protected from diabetes throughout the third treatment (Fig. 6A, 6B). To examine whether the restoration of normoglycemia by I-142 treatment involved interfering with autoantigen-specific T cell proliferation and effector T cell differentiation. We assessed T cell proliferation and cytokine secretion in T cells from NOD mice treated with I-142 or control IgG. T cells proliferated against pancreatic Ags more vigorously in control IgG-treated NOD mice than in I-142 mAb-treated NOD mice (Fig. 6F). These data indicate that I-142 suppressed diabetogenic T cell proliferation.

**Anti-CD98hc inhibits the progression of TID independent of B cell suppression**

Previous articles have indicated that B cells are involved in TID in NOD mice (18). As CD98 deficiency in B cells perturbs the differentiation of Ab-producing cells (19), we investigated whether T cells are involved in the anti-CD98hc mAb-mediated suppression of TID in NOD mice. We injected NOD/SCID mice with total T cells from diabetic NOD mice and treated the mice with I-142. NOD/SCID mice injected with T cells from diabetic NOD mice began to develop diabetes 2 wk after transfer of the cells. Treatment with I-142 suppressed the development of diabetes (Fig. 7). These data indicate that I-142 is able to suppress TID through direct inhibition of T cells.

**CD98 siRNA inhibit the progression of TID**

To determine if inhibition of CD98 function is involved in suppression of TID, we treated NOD/SCID mice adoptively transferred with T cells from diabetic NOD mice with siRNA against CD98hc. The siRNA against CD98hc or control siRNA was injected together with atelocollagen into NOD/SCID mice that had been adoptively transferred with diabetogenic T cells. CD98hc expression on PBMCs was evaluated. The expression of CD98hc was efficiently downregulated by treatment with siRNA against CD98hc, but not control siRNA (Fig. 8A). Intraperitoneal injection of siRNA against CD98hc prevented the development of TID (Fig. 8B). Even in mice that developed TID, the development was delayed by treatment with CD98hcsiRNA. These data indicate that downregulation of CD98hc in vivo is able to suppress TID development in NOD mice.

**Discussion**

We identified a mAb that recognizes CD98hc by probing a panel of mAbs that modulate T cell proliferation. The I-142 mAb exhibits significantly greater number of total islets than did the control IgG-treated mice (Fig. 6E). Overall, treatment with I-142 led to a significant increase in the number of noninflamed islets.

Because TID in NOD mice is caused by aberrant T cell responses, we sought to determine whether the restoration of normoglycemia by I-142 treatment involved interfering with autoantigen-specific T cell proliferation and effector T cell differentiation. We assessed T cell proliferation and cytokine secretion in T cells from NOD mice treated with I-142 or control IgG. T cells proliferated against pancreatic Ags more vigorously in control IgG-treated NOD mice than in I-142 mAb-treated NOD mice (Fig. 6F). These data indicate that I-142 suppressed diabetogenic T cell proliferation.

**FIGURE 7.** Anti-CD98hc resolves TID independent of B cells. Total T cells (1×10⁶) from diabetic NOD mice (n = 4) were transferred into NOD/SCID mice (n = 10 per group) that were treated with I-142 or control IgG (100 µg per mouse). Ab was injected 0, 3, 6, 9, 12, and 15 d after T cell transfer, and the development of diabetes was monitored by measuring blood glucose. Five mice in each group were used in this experiment. The experiments in this figure are representative of three independent experiments. *p < 0.05.

**FIGURE 8.** Treatment with CD98hc siRNA suppresses TID development. Total T cells from diabetic NOD mice were injected into NOD/SCID mice (n = 10 per group). The mice were treated with CD98hc siRNA (4 nmol per mouse) or control siRNA on days 2, 5, and 8 (the day on which T cells were transferred is designated day 0). (A) Expression of CD98hc on peripheral mononuclear cells with forward light scatter/side light scatter gate was evaluated by staining cells with I-142, followed by anti-rat IgG for flow cytometry on day 2. Shadow, secondary Ab alone; dotted line, control siRNA; bold line, CD98hc siRNA. (B) The development of TID was evaluated by measuring blood glucose levels. Ten mice in each group were used in the experiments. The experiments in this figure are representative of three independent experiments. *p < 0.05.
a strong inhibitory effect for both CD4+ and CD8+ T cell proliferation and functional differentiation. Injection of I-142 in cyclophosphamide-induced NOD mice completely prevented the onset of T1D. Treatment of diabetic NOD mice with I-142 restored the diabetic state to a normal level by suppressing T cell responses. Furthermore, treatment with siRNA against CD98hc inhibited the development of T1D. These data indicate that blocking CD98hc with an Ab or siRNA may have clinical applications for the treatment of T1D and other T cell-mediated autoimmune diseases in humans.

CD98hc has a variety of functions, including amino acid transport and participation in integrin signaling (12, 20, 21). Previous articles have indicated that CD98 induces aggregation in human T cells, which is blocked by soluble β1 integrin Abs (22), and an anti-CD98 mAb suppresses Con A-mediated murine T cell proliferation (15). Recently, Cantor et al. (16) reported that deletion of the mouse CD98hc gene disturbs T cell function, suggesting that CD98hc controls Ag-specific T cell proliferation. To ascertain whether the effect of I-142 administration on the resolution of T1D in NOD mice is attributed to the inhibition of CD98hc function, we downregulated CD98hc expression by injecting CD98hc siRNA. Treatment with CD98hc siRNA suppressed T1D development induced by diabeticogenic T cells transferred into NOD/SCID mice. This treatment reduced the surface expression of CD98hc on PBMCs. Although we did not address which cells are the actual target cells for this treatment in vivo, this approach would also be useful in treating T1D by inhibiting CD98hc function. Taken together, these data indicate that inhibition of CD98hc function in vivo is able to suppress T1D development in NOD mice, which suggests that the effect of the I-142 mAb on development of T1D is attributable to the inhibition of CD98hc function. Indeed, our anti-CD98hc mAb was able to suppress Ag-specific proliferation and effector functions of both CD4+ and CD8+ T cells in vitro and in vivo. A previous study using CD98hc-deficient T cells showed reduced T cell proliferation, but no defect in acquiring effector functions (16). This article indicated that defective T cell proliferation in CD98hc-deficient T cells is attributed to lack of the integrin-binding domain of CD98hc (16). Therefore, the suppressive effect of I-142 on T cell effector functions in our studies might suggest that the I-142 mAb influences other functions of CD98hc besides integrin activity. For instance, we cannot rule out the possibility that I-142 recognizes complexes of CD98hc with other unidentified CD98hc-associated molecules because I-142 does not recognize CD98hc in Western blots (data not shown). It is possible that I-142 also affects the function of such associated molecules. Therefore, it would be important to test whether I-142 affects T cell functions, using CD98hc-deficient T cells. In any case, these data suggest that I-142 administration or inhibition of CD98hc with siRNA would be a good strategy for suppressing the development or progression of T1D, which would at least partly be attributed to the inhibition of T cell proliferation.

Multiple factors might contribute to the therapeutic effect of the I-142 mAb on T1D because CD98hc is expressed in various tissues in addition to the immune system (14, 23). Even in immune cells, CD98hc is expressed on B cells, dendritic cells, and macrophages (data not shown). Nevertheless, as I-142 suppressed the proliferation of both CD4+ and CD8+ T cells in vitro and in vivo, the therapeutic effect of I-142 on T1D, at least partly, would likely depend on suppressing T cell proliferation. Indeed, we have evidence that deletion of the CD98hc gene in T cells suppresses T cell-mediated pathological responses in vivo (data not shown). This finding leads to the intriguing possibility that the I-142 mAb interacts with cells located in the pancreas, including islet β cells, and that these cells prevent damage to β cells. In any case, it would be important to establish NOD mice lacking the CD98hc gene in T cells, or other types of cells, to directly analyze the major target cells for the I-142 mAb in terms of inhibiting T1D development.

Currently, considerable interest has arisen in the potential of immunotherapy for treating T1D. The pathways that break down immunological tolerance against β cells are unclear but include a significant contribution by T cells, through either CTL killing or cytokine release. Several attempts to treat T1D using Abs have been reported (18, 24, 25). Anti-CD3 mAb has shown therapeutic potential in NOD mice by modulating regulatory T cells (26), and the efficacy of anti-CD3 mAb to treat human T1D is currently in clinical trials (27). In contrast, treatment with I-142 did not affect the number of CD4+Foxp3+ regulatory T cells, and did not induce suppressive activity to inhibit T1D in NOD mice, which suggests that anti-CD3 and anti-CD98 treatments use different mechanisms to suppress T1D in NOD mice. Therefore, it is important to determine the best schedules for treatment with each Ab in relation to the disease stages of T1D. In addition, because CD98hc is expressed on various types of cells, anti-CD98hc mAb treatment might induce unappreciated adverse effects. Nevertheless, we have not observed any macroscopic or microscopic alterations in mice after >10 injections of anti-CD98hc mAb. Furthermore, in future it would be important to examine if I-142 tolerizes T cells or how long the effect of I-142 lasts after initial treatment. In any case, it will be necessary to carefully apply anti-CD98hc mAb to develop therapeutic strategies for T1D.

In conclusion, these data indicate that anti-CD98hc mAb has a potent ability to resolve T1D in NOD mice. The I-142 mAb is able to suppress both CD4+ and CD8+ T cell proliferation and functional differentiation. The inhibition of CD98hc by siRNA in vivo also exhibited a therapeutic effect on T1D in NOD mice. These data show that CD98hc is crucial for the progression of T1D in NOD mice and suggest that inhibition of CD98hc function could be a useful strategy for the treatment of T cell-mediated autoimmune diseases, including T1D.

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Disclosures
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