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CD83 Expression in CD4+ T Cells Modulates Inflammation and Autoimmunity1

Simone Reinwald,2* Carsten Wiethe,2† Astrid M. Westendorf,*¶ Minka Breloer,‡ Michael Probst-Kepper,§ Bernhard Fleischer,‡ Alexander Steinkasserer,† Jan Buer,¶ and Wiebke Hansen*¶

The transmembrane protein CD83 has been initially described as a maturation marker for dendritic cells. Moreover, there is increasing evidence that CD83 also regulates B cell function, thymic T cell maturation, and peripheral T cell activation. Herein, we show that CD83 expression confers immunosuppressive function to CD4+ T cells. CD83 mRNA is differentially expressed in naturally occurring CD4+CD25+ regulatory T cells, and upon activation these cells rapidly express large amounts of surface CD83. Transduction of naive CD4+CD25- T cells with CD83 encoding retroviruses induces a regulatory phenotype in vitro, which is accompanied by the induction of Foxp3. Functional analysis of CD83-transduced T cells in vivo demonstrates that these CD83+Foxp3+ T cells are able to interfere with the effector phase of severe contact hypersensitivity reaction of the skin. Moreover, adoptive transfer of these cells prevents the paralysis associated with experimental autoimmune encephalomyelitis, suppresses proinflammatory cytokines IFN-γ and IL-17, and increases antinflammatory IL-10 in recipient mice. Taken together, our data provide the first evidence that CD83 expression can contribute to the immunosuppressive function of CD4+ T cells in vivo. The Journal of Immunology, 2008, 180: 5890–5897.

Regulatory T (Treg) cells play essential roles in immune homeostasis and protection against autoimmunity, but also contribute to immunopathology in cancers, infections, and other diseases (1), thereby representing potential candidates for therapeutic interventions to interfere with a broad variety of immunological disorders. Despite many efforts, the molecular bases for Treg cell function have not yet been unraveled. Several studies have suggested a cell contact-dependent mechanism of naturally occurring thymus-derived Treg cells, while peripherally induced Treg cells are supposed to act in a cell contact-independent but cytokine-dependent manner. Most recently, Bopp and coworkers demonstrated that naturally occurring Treg cells and conventional T cells communicate via cell contact-dependent gap junction formation (2). Furthermore, it has been described that different molecules such as CTLA-4 (3), glucocorticoid-induced TNF receptor (4), CD103 (5), G protein-coupled receptor 83 (6), and neuropilin-1 (7) might be involved and/or essential for Treg cell function. However, the precise mechanisms of how and where Treg cell function in vivo and which molecules contribute to its suppressive activity to control a broad variety of immune responses remain elusive. Today, the transcription factor Foxp3 is the only molecule established to be essential for the development and function of Treg cells, thereby representing the Treg cell lineage specification factor (8). Forced expression of Foxp3 in CD4+CD25- nonregulatory T cells by retroviral gene transfer showed acquisition of suppressive activity in vitro and in vivo, indicating that expression of Foxp3 in peripheral T cells may convert naive CD4+CD25- T cells into CD4+CD25+ Treg cells (9, 10).

In the present study we have identified CD83 to be differentially expressed by Treg cells and to be involved in their immunosuppressive capacity. CD83 is a glycosylated Ig-like type 1 transmembrane protein that was initially described as a specific surface marker for activated human dendritic cells (DCs) in the peripheral blood (11). The murine CD83 protein shares an overall homology of 63%, suggesting a possible conserved function. Its expression has been described in the brain and spleen (12) as well as in activated bone marrow-derived DCs (13) and activated B cells (14, 15). Recent studies of mice deficient for CD83 have revealed that CD83 plays a central role in the thymic selection of double-positive thymocytes to single CD4-positive T cells, as these mice show a selective 75–90% reduction in peripheral CD4+ T cells (16, 17). Thus, CD83 expression on thymic epithelial cells seems to represent an additional regulatory component for CD4+ T cell development in the thymus.

During the last few years, there has been increasing evidence for an immunosuppressive function of CD83. A soluble form of CD83 (sCD83) has been detected in normal human serum, which can also be released by cultured DCs and activated B cells (18). This soluble form of CD83 is also present at elevated levels in a number of hematological disorders, including patients with chronic lymphocytic leukemia and mantle cell lymphoma (19).

*Department of Macosal Immunology, Helmholtz Centre for Infection Research, Braunschweig; 1Department of Dermatology, University of Erlangen, Erlangen; 1Department of Immunology, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg; 1Junior Research Group for Xenotransplantation, Department of Visceral and Transplant surgery, Hannover Medical School, Hannover; and 1Institute of Medical Microbiology, University Hospital, Essen, Germany

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2 S.R. and C.W. contributed equally to this work.
3 Address correspondence and reprint requests to Dr. Wiebke Hansen, Institute of Medical Microbiology, University Hospital Essen, Viernsstrasse 179, D-45122 Essen, Germany. E-mail address: wiebke.hansen@uk-essen.de
4 Abbreviations used in this paper: Treg, regulatory T; CD83L, CD83 ligand; CHS, contact hypersensitivity; DC, dendritic cell; DNFβ, 2,4-dinitro-1-fluorobenzene; EAE, experimental autoimmune encephalomyelitis; eGFP, enhanced GFP; IRES, internal ribosomal entry site; mCD83, membrane-bound CD83; MOG, myelin oligodendrocyte glycoprotein; RPS9, ribosomal protein S9; sCD83, soluble CD83; tg, transgenic; WT, wild type.

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Lechmann and colleagues reported that the extracellular Ig domain of human CD83 blocked the DC-mediated proliferation of allogeneic as well as specific CTLs in vitro (20). Additionally, in the experimental autoimmune encephalomyelitis (EAE) mouse model, injections of sCD83 prevented the disease-associated paralysis under prophylactic as well as therapeutic settings in vivo (21). Scholler and coworkers reported that recombinant expression of a CD83-human Ig and CD83-murine Ig fusion protein inhibited DC-mediated T cell stimulation in vitro in a concentration-dependent manner (22). Additionally, they demonstrated that sCD83 down-modulates antitumor immune responses in vivo using the murine P815 tumor model (22). Most recently, Xu and colleagues have shown that a limited course of sCD83 delayed acute cellular rejection of MHC-mismatched mouse skin allografts (23). Further evidence for the inhibitory function of sCD83 isoforms was shown by Dudziak and colleagues (24). They identified four different transcripts of CD83 in unstimulated PBMCs. The largest transcripts encode putative soluble forms and are splice variants of different transcripts of CD83 in unstimulated PBMCs. The largest transcripts encode putative soluble forms and are splice variants of full-length CD83. Interestingly, the smallest CD83 splice product codes for the transmembrane CD83, whereas the smaller CD83 isoforms codes for the transmembrane CD83.

It is well known that viruses target functionally important molecules of the immune system to overcome and/or escape antiviral immune responses. In this respect, Senechal et al. reported that the membrane-bound CD83 surface molecule is a target for human CMV (25). Surprisingly, as a consequence of the human CMV infection, a soluble form of CD83 is shed from the cell surface and subsequently blocks T cell stimulation, thereby resembling the mode of action of recombinantly expressed sCD83. Thus, all these data clearly support an immunoregulatory function of sCD83.

In the present study, we demonstrated that thymus-derived naturally occurring CD4+CD25+ Treg cells expressed elevated levels of CD83 mRNA. Upon activation, these cells rapidly expressed large amounts of surface CD83. Strikingly, we found that forced expression of CD83 in naive CD4+CD25+ T cells converted them into T cells with suppressive capacity in vitro. Moreover, adoptive transfer of CD83-transduced T cells inhibited the effector phase of a severe contact hypersensitivity (CHS) reaction of the skin and strongly reduced paralytic EAE symptoms.

**Materials and Methods**

**Mice**

TCR-HA transgenic mice (26), DO11.10 TCR transgenic mice (27), 2D2 mice (28), BALB/c mice (Harlan), and C57BL/6 mice (Harlan) were housed and bred under specific pathogen-free conditions. The 2D2 mice, which bear a transgenic TCR recognizing myelin oligodendrocyte glycoprotein (MOG)35-55 peptide, were a gift from Dr. V. K. Kuchroo (Harvard University, Boston, MA). All animal experiments were performed in accordance with institutional, state, and federal guidelines.

**Antibodies**

The mAb 6.5 (anti-TCR-HA) was purified from hybridoma supernatant and used in FITC-labeled form. Anti-CD3 (2C11), anti-CD28 (37.51), anti-CD4 (L3T4), anti-TCR, anti-CD25, and anti-CD25 (PC61) were obtained from BD Biosciences; anti-D011.10 TCR (KJ1.26) was from Caltag; and anti-Foxp3 (FJK-16s) and anti-CD83 (Michel17) were from eBioscience, and all were used unlabeled or as FITC, APC, CyChrome, or PE conjugates.

**Cell separation and flow cytometry**

CD4+CD25- T cells were enriched from the whole spleen by negative selection using an autoMACS (Miltenyi Biotec) following the manufacturer’s instructions. For gene expression analysis and proliferation and adoptive transfer experiments, labeled cells were separated using a MoFlo cell sorter (Cytomation), and purity was >97%. Foxp3 staining was performed using the PE-anti-Foxp3 staining kit from eBioscience according to the manufacturer’s recommendations. Flow cytometry analyses were done on a FACSCalibur flow cytometer with CellQuest software (BD Biosciences).

**T cell activation**

Erythrocyte-depleted splenocytes isolated from BALB/c mice were sorted by autoMACS using the CD4+ T cell isolation kit (Miltenyi Biotec) plus additional biotinylated anti-CD25 (1/500) Ab (BD Pharmingen) according to the manufacturer’s recommendations. CD4+CD25+ T cells were stimulated in the presence of 0.75 μg/ml anti-CD3 (plate bound) and 1 μg/ml anti-CD28 mAb for 48 h. Antigen-presenting cells (APCs) were obtained by sorting Treg, CD8+ T cells, and CD4+ T cells from BALB/c mice.
anti-CD28 (soluble) for 40–48 h before retroviral transduction. For CD38 expression analysis, murine CD4<sup>+</sup>CD25<sup>-</sup> and CD4<sup>+</sup>CD25<sup>+</sup> T cells were activated with 1 µg/ml anti-CD3 in the presence of 50 U/ml IL-2.

**Retroviral infection of CD4<sup>+</sup>CD25<sup>−</sup> T cells**

cDNA encoding murine CD83 was amplified by RT-PCR from BALB/c spleen using specific primers (5'-CAT GTC GCA AGG CCT CCA GCT CCT G-3' and 5'-TCT GAT GTG CCC TTG GCT TTG TAA-3'), cloned into pCR2.1 TOPO (Invitrogen), sequenced, and inserted into a murine stem cell virus-based retroviral vector encoding enhanced GFP (eGFP) under control of an internal ribosomal entry site (IRES). This construct, a Foxp3 encoding retroviral vector (6, 9), or the empty control vector was used to stably transfect the ecotropic GPE86 retrovirus packaging cell line. Concentrated and filtered (0.45 µm) retrovirus-containing culture supernatants supplemented with 20 mM HEPES and 8 µg/ml polybrene were used to transduce stimulated CD4<sup>+</sup>CD25<sup>+</sup> T cells by centrifugation at 500 g for 2 h. After 24 h, half of the culture medium was exchanged and 50 U/ml IL-2 added.

**Real-time RT-PCR**

Total RNA was prepared from sorted cell populations using the RNeasy kit (Qiagen) following DNase digestion (Qiagen) and cDNA synthesis by SuperScript II reverse transcriptase and oligo(dT) mixed with random hexamer primers (Invitrogen) following DNase digestion (Qiagen) and cDNA synthesis by SuperScript II reverse transcriptase and oligo(dT) mixed with random hexamer primers (Invitrogen) according to the manufacturers’ recommendations. Real-time RT-PCR was performed in an ABI PRISM cycler (Applied Biosystems) using a SYBR Green PCR kit from Stratagene and specific primers for CD83 (5'-TGCGAGCCCGGAAGA-3' and 5'-TCTGAGTTCCACTGTTCTC'), CTA-4 (5'-GCGTGGGCTGGCTTACACTCATTT-3' and 5'-CTTCCCTGGCTGGCAATTACTT-3'), Foxp3, IL-10, and ribosomal protein S9 (RPS9), as described previously (6).

**Proliferation assay**

Freshly isolated CD4<sup>+</sup>CD25<sup>+</sup> T cells and CD4<sup>+</sup>CD25<sup>−</sup> T cells and sorted control virus or CD83-transduced T cells (1 × 10<sup>5</sup>) were cultured either alone or cocultured with CD4<sup>+</sup>CD25<sup>+</sup> responder T cells at a ratio of 1:1 in the presence of 1 µg/ml anti-CD3 (2C11) and irradiated splenocytes as APCs (4 × 10<sup>6</sup>) for 72 h. In some coculture experiments, a transwell system with 0.2-µm pore size (Nunc) was used or assays were supplemented with 50 µg/ml anti-IL-10 (JES5-2A5; BioSource International) or 50 µg/ml anti-TGF-β1 (1D11; R&D Systems). Proliferation and suppression assays were performed in triplicate in a final volume of 200 µl. Cells were pulsed with 1 µCi/well of [3H]thymidine for the final 8–18 h of the experiment, and [3H]thymidine incorporation was measured by scintillation counting.

**Contact hypersensitivity**

CHS experiments with BALB/c mice were performed as described elsewhere (6, 9). Briefly, mice were sensitized to 2,4-dinitro-1-fluorobenzene (DNFB) on day 0. On day 4, 1 × 10<sup>6</sup> nontransduced, sorted control virus, CD83-transduced or Foxp3-transduced CD4<sup>+</sup>CD25<sup>−</sup> T cells, or sorted CD4<sup>+</sup>CD25<sup>+</sup> Treg cells were injected i.v. into each recipient mouse 24 h before elicitation of CHS responses. After 36 h, CHS responses were determined by the degree of ear swelling of the challenged right ear compared with the nonchallenged left ear.

**Adoptive transfer of MOG-specific retroviral-transduced T cells and induction of EAE**

CD4<sup>+</sup>CD25<sup>+</sup> T cells from 2D2 mice were transduced with CD83 encoding retrovirus or control virus as described above. FACs-sorted virus-transduced MOG-specific CD4<sup>+</sup> T cells (sorted for CD4<sup>+</sup>TCRβ11<sup>+</sup>eGFP<sup>+</sup> cells) were injected i.v. into recipient mice (1.5 × 10<sup>6</sup>/mouse) 1 day before they were immunized with MOG peptide, and induction of EAE was monitored by the degree of clinical symptoms.
FIGURE 3. CD83-transduced T cells CTLA-4, IL-10, and Foxp3 expression in CD83-transduced T cells. A, Real-time RT-PCR analysis for CD83, Foxp3, CTLA-4, and IL-10 was performed using reverse-transcribed RNA isolated from freshly isolated CD4⁺CD25⁺ T cells, CD4⁺CD25⁻ T cells, or sorted eGFP⁺ control vector-transduced (RV-eGFP) or CD83-transduced (RV-CD83) naive T cells 1 wk postinfection. RP59 mRNA expression served as housekeeping gene control. Mean values from at least two independent experiments are shown. B, Foxp3 protein expression was analyzed in CD4⁺CD25⁺ and CD4⁺CD25⁻ T cells isolated from BALB/c mice (upper panel) or sorted eGFP⁺ control virus and CD83-transduced T cells (lower panel) by intracellular FACS staining. One experiment out of three independent analyses with similar results is shown.

EAE induction. EAE induction was conducted essentially as described previously (29). Briefly, mice were injected s.c. with MOG₃₅₋₅₅ peptide (100 μg/mouse) emulsified in CFA (Sigma-Aldrich) further enriched with Mycobacterium tuberculosis H₃₇R₇ (Difco/BD Pharmingen). At days 0 and 2, mice were injected i.p. with pertussis toxin (List/Quadrarchek). Mice were observed daily for clinical signs of disease and scored according to their clinical severity of disease as follows: grade 0, no abnormality; grade 1, limp tail or hindlimb weakness; grade 2, limp tail and hindlimb weakness; grade 3, partial hindlimb paralysis; grade 4, complete hindlimb paralysis; and grade 5, moribund. The data are plotted as the mean daily clinical score for all animals per group.

Measurement of cytokine response
For detection of MOG-specific cytokine responses, 4 × 10⁵ splenocytes depleted of erythrocytes were cultured in vitro in the presence of 20 μM MOG₁₅₋₅₅ peptide. After 72 h, supernatants were collected and cytokine contents were detected using ELISA kits for IFN-γ, IL-10 (BD Pharmin gen), and IL-17 (R&D Systems).

Results
CD4⁺CD25⁺ Treg cells differentially express CD83

Based on the findings that CD83 is expressed by different populations of immune cells and that a soluble form of CD83 is able to suppress immune responses, we examined whether CD83 is expressed by Treg cells and linked to their suppressive function. Therefore, we isolated CD4⁺CD25⁺ Treg cells from wild-type (WT) mice, Ag-specific CD4⁺CD25⁺ Treg cells from OT-II and TCR-HA mice, and analyzed the CD83 mRNA expressions in comparison to their naive CD4⁺CD25⁻ counterparts by real-time RT-PCR. Isolated Treg cells of different origin expressed up to 11-fold higher levels of CD83 mRNA than did naive T cells (Fig. 1A). Next, CD83 expression was investigated on the protein level. Several reports have shown that T cells up-regulate CD83 upon activation. Therefore, we isolated CD8⁰ T cells, CD4⁺CD25⁺ Treg cells, and CD4⁺CD25⁻ T cells from BALB/c mice and stim-ulated them in vitro for different periods before the analysis of the CD83 expression on the separated T cell populations. Despite the high levels of CD83 mRNA in unstimulated CD4⁺CD25⁺ Treg cells, only a small proportion of cells expressed CD83 on their surface similar to unstimulated CD4⁺CD25⁻ T cells (Fig. 1B). Upon activation for 48 h, up to 65% of stimulated CD4⁺CD25⁺ T cells expressed CD83. However, Treg cells up-regulated CD83 surface expression more rapidly and to a higher extent, reaching the maximum of ~85% 48 h after activation (Fig. 1B). In the course of activation we observed a decrease in CD83 expression on both CD4⁺ T cell subsets at 72 h after activation (12 and 2% for CD4⁺CD25⁺ Treg cells and CD4⁺CD25⁻ T cells, respectively). In contrast to both CD4⁺ T cell populations studied, we did not detect significant CD83 surface expression on CD8⁻ T cells, even upon activation in vitro (Fig. 1B). In summary, these data indicate that CD4⁺CD25⁺ Treg cells express high levels of CD83 mRNA and upon activation Treg cells express surface CD83 with greater magnitude and faster kinetics than do CD4⁺CD25⁻ T cells.

CD83 overexpression confers suppressive activity to naive T cells in vitro

To examine the functional role of CD83 in Treg cell biology, we constructed murine stem cell virus-based retroviral vectors encoding CD83 and eGFP under control of an IRES (RV-CD83) to over-express CD83 in naive T cells by retroviral transduction (Fig. 2A). Additionally, an empty control vector was generated that encodes only eGFP (RV-eGFP). Retroviral vectors were stably transduced into GPE86⁺ packaging cells, and virus-containing supernatants were used to infect naive CD4⁺CD25⁻ T cells. One week after infection, eGFP⁺ CD83-transduced and control vector-transduced T cells (~20–30%) were FACS sorted and their proliferative and suppressive capacities were analyzed. Freshly isolated CD4⁺
CD83 and Regulatory T Cells

CD83+ and CD4+CD25− T cells served as controls. As shown in Fig. 2B (upper left panel), CD83-transduced T cells (RV-CD83) exhibited a decreased proliferative activity in comparison to control vector-transduced T cells (RV-eGFP). Furthermore, overexpression of CD83 in naive T cells conferred suppressive capacity to naive CD4+CD25− Treg cells as determined by coculture experiments (Fig. 2B, lower left panel). Similar results were obtained using Ag-specific CD83-transduced T cells. OVA-specific CD4+CD83+ T cells showed significantly reduced proliferative activity and inhibited the proliferation of naive T cells upon stimulation with the cognate Ag (data not shown). To investigate by which mechanism CD83-transduced T cells elicit their suppressive activity in vitro, we performed coculture experiments either by separating the CD83+ T cell population from responder T cells by a semipermeable membrane or by adding IL-10- or TGF-β-neutralizing Abs. As shown in Fig. 2B (upper right panel), CD83-transduced T cells lost their suppressive capacity in the absence of cell-cell contact, whereas the inhibitory function was not affected by neutralizing the cytokines IL-10 and TGF-β (Fig. 2B, lower right panel). Next, we asked whether the IL-2 production in stimulated naive T cells is abrogated in the presence of CD83-transduced T cells. Therefore, we performed coculture experiments using Thy1.1+CD4+CD25− naive T cells as responder T cells. Analysis of IL-2 mRNA expression in re-isolated Thy1.1+ T cells revealed that the presence of CD83-transduced T cells led to a decrease in IL-2 production in responder T cells to a comparable extent as did CD4+CD25− Treg cells (Fig. 2C). Thus, CD83 overexpression in naive CD4+CD25− T cells is accompanied with low proliferative activity and suppressive capacity in a cell-cell contact-dependent manner comparable to naturally occurring CD4+CD25+ Treg cells.

Transduction of naive T cells with CD83 induces Foxp3 expression

Next, we addressed whether CD83 overexpression results in the induction of Treg cell-associated genes, namely Foxp3, CTLA-4, and IL-10. Therefore, real-time RT-PCR analyses of reverse-transcribed mRNA isolated from sorted WT CD4+CD25− and CD4+CD25+ T cells and sorted eGFP+ CD83-transduced and control vector-transduced T cells were conducted. Similar to CD4+CD25+ Treg cells, naive CD4+CD25− T cells transduced with CD83 encoding retrovirus expressed high levels of CD83 mRNA (Fig. 3A, upper left panel). Interestingly, CD83 overexpression resulted in increased Foxp3 and CTLA-4 expression. However, IL-10 expression was unaffected in CD83-transduced T cells in contrast to CD4+CD25+ Treg cells. To confirm these findings, Foxp3 expression in CD83-transduced T cells was analyzed by intracellular staining and compared with the expression level in freshly isolated CD4+CD25+ Treg cells and CD4+CD25− T cells or in control vector-transduced T cells. Similar proportions of Foxp3+ T cells were detected in the freshly isolated CD4+CD25−

FIGURE 4. CD83-transduced CD4+CD25− T cells interfere with the effector phase of severe contact hypersensitivity. BALB/c animals were sensitized to DNFB and i.v. injected with 1 × 106 noninfected (mock), control-vector transduced (RV-eGFP), CD83-transduced (RV-CD83), Foxp3-transduced (RV-Foxp3) CD4+CD25− T cells or freshly isolated CD4+CD25− T cells served as controls. As shown in Fig. 2B (upper left panel), CD83-transduced T cells (RV-CD83) exhibit a decreased proliferative activity in comparison to control vector-transduced T cells (RV-eGFP). Furthermore, overexpression of CD83 in naive T cells conferred suppressive capacity to naive CD4+CD25− Treg cells and ear challenged. As negative control (neg. ctrl.), mice were not sensitized but challenged, and as positive control (pos. ctrl.), mice were sensitized and challenged without injecting any cells. Ear swelling was evaluated 36 h after challenge and is expressed as difference between the challenged right ear and the unchallenged left ear. Data are shown as means ± SD of 5–9 mice in two independent experiments. Student’s t test was used to assess the significance of differences.

FIGURE 5. MOG-specific CD83-transduced CD4+ T cells protect mice from EAE. A, C57BL/6 mice were injected i.v. (day −1) with 1.5 × 106 sorted MOG-specific CD4+ T cells transduced with control vector (RV-eGFP) or CD83-encoding retrovirus (RV-CD83). Control mice were left untreated. One day later EAE was induced and paralysis monitored. Results are represented as average of 3 mice per group. B, At days 30–35 after EAE induction, isolated splenocytes were restimulated in vitro with MOG peptide for 72 h. Harvested cell supernatants were analyzed for MOG-specific secretion of IFN-γ, IL-17, and IL-10 by ELISA, and results represent means ± SEM. Data shown are representative of three independent experiments. Student’s t test was used to assess the significance of differences.
Table I. Specificity of protection from EAE after transfer of MOG-specific CD83+ CD4+ T cellsa

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence</th>
<th>Mean Maximal Score</th>
<th>Mean Day of Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>10/10 (100%)</td>
<td>3.2 ± 0.6</td>
<td>15.0 ± 2.4</td>
</tr>
<tr>
<td>CD4+ CD83+ T cells</td>
<td>3/10 (30%)</td>
<td>0.4 ± 0.2b</td>
<td>18.3 ± 2.1</td>
</tr>
<tr>
<td>eGFPeGFp CD4+ T cells</td>
<td>6/9 (67%)</td>
<td>1.9 ± 1.2a</td>
<td>15.8 ± 2.2</td>
</tr>
</tbody>
</table>

a Mice were injected i.v. (day −1) with sorted MOG-specific CD4+ T cells infected with control vector (eGFp CD4+ T cells) or CD83-encoding retrovirus (CD83+ CD4+ T cells). On day 0, mice were immunized s.c. with MOG35-55 peptide plus pertussis toxin (i.p. on day 0 and day 2). The incidence, mean maximal score (±SE), and mean day of onset of clinical EAE (±SE) are shown.

b Statistically significant when compared with C57BL/6, p < 0.01 by Wilcoxon rank-sum test for mean clinical score.
c Statistically significant when compared with eGFp+ CD4+ T cells; p < 0.05 by Wilcoxon rank-sum test for mean clinical score.
d Not statistically significant when compared with C57BL/6; p > 0.05 by Wilcoxon rank-sum test for mean clinical score.

1 T cells (5%) and control vector-transduced T cells (RV-eGFp) (3%) (Fig. 3B). As expected, most of the isolated CD4+ CD25+ Treg cells expressed Foxp3 (91%). CD83 overexpression in naive T cells induced Foxp3 protein expression in ~17% of CD83-transduced T cells (RV-CD83) (Fig. 3B). These findings demonstrate that forced expression of CD83 in naive T cells results in the induction of Treg cell-associated genes, namely Foxp3 and CTLA-4.

CD83-transduced T cells exhibit suppressive capacity in vivo

It is well known that in vivo processes in which Treg cells interfere with ongoing immune responses are much more complex than those represented by the in vitro situation (30). To study the in vivo regulatory function of CD83-transduced T cells, we examined the capacity of these T cells to inhibit the effector phase of a CHS reaction leading to severe skin inflammation (9, 31). Therefore, WT mice were sensitized to DNFB on the shaved back and noninfected CD4+ CD25+ T cells (mock), control virus (RV-eGFp), CD83-transduced T cells (RV-CD83), Foxp3-transduced T cells (RV-Foxp3), or freshly isolated CD4+ CD25+ Treg cells were injected i.v. before challenge mice with DNFB on the right ear. Ear swelling was assessed as a measure of CHS response. Mice treated with mock-infected naïve CD4+ CD25+ T cells or control virus-infected T cells developed a severe CHS response upon challenge of similar extent as mice without T cell transfer (positive control) (Fig. 4). However, upon adoptive transfer of sorted CD83-transduced T cells mice developed a significantly reduced CHS response to a similar extent as did Foxp3+ CD4+ CD25+ Treg cells and Foxp3-transduced T cells. These data suggest that overexpression of CD83 confers in vivo regulatory function to naïve T cells.

Ag-specific suppression of EAE by CD83-transduced CD4+ T cells

Finally, we addressed the question whether CD83-transduced CD4+ T cells are able to suppress EAE based on a model previously described by Carrier et al. (32). Therefore, MOG-specific CD4+ CD25+ T cells isolated from 2D2 mice, which express a transgenic TCR recognizing MOG35-55 peptide (28), were infected with control vector (RV-eGFp) or CD83-encoding retrovirus (RV-CD83). Transduced MOG-specific CD4+ T cells were transferred into C57BL/6 mice, and EAE was induced 24 h later. Interestingly, adoptive transfer of CD83-transduced MOG-specific CD4+ T cells significantly inhibited the development of EAE compared with mice that received control vector-transduced T cells or control mice (Fig. 5A and Table I). Despite similar time courses of disease, control vector-transduced MOG-specific CD4+ T cells slightly ameliorated the course of EAE when compared with control mice (Table I). However, this reduction was not significant. Furthermore, when reisolated splenocytes were restimulated in vitro with MOG35-55 peptide, transfer of CD83-transduced CD4+ T cells resulted in significantly diminished levels of IFN-γ and IL-17 and increased levels of IL-10 compared with splenocytes reisolated from mice receiving control vector-transduced T cells (RV-eGFp) or control mice (Fig. 5B). These results suggest that forced expression of CD83 in transferred MOG-specific CD4+ T cells contributes to EAE inhibition and down-regulation of immunopathogenic cytokines IL-17 and IFN-γ and up-regulation of IL-10.

Discussion

Treg cells are well-known key players during a variety of immunoregulatory processes. Despite many studies demonstrating that naturally occurring thymus-derived Treg cells act in a cell contact-dependent fashion in vitro, the precise mechanisms of how and where Treg cells function in vivo are not well understood. In the present study we show for the first time that CD83 is involved in the immunosuppressive function of CD4+ T cells both in vitro and in vivo.

It has been demonstrated that CD83 is expressed by different activated immune cells such as DCs, macrophages, and B cells (15, 33), suggesting that CD83 might play an important role in modulating immune responses. However, the role of CD83 in T cell immunology remains unclear. Herein, we have identified CD83 to be differentially expressed by freshly isolated CD4+ CD25+ polyvalent as well as Ag-specific Treg cells. Our real-time RT-PCR analyses of CD83 mRNA expression levels nicely correlate with recently published gene expression profiling data on different Treg cell subsets (8, 34).

Several studies have reported an increased surface expression of CD83 on T cells upon stimulation, which was down-regulated in the course of activation (35, 36). These findings are well in line with our data of CD83 protein expression on stimulated CD4+ T cells, but we did not detect significant CD83 expression on activated CD8+ T cells. Analysis of separated CD4+ CD25+ Treg cells and CD4+ CD25− T cells has clearly revealed that the Treg cell subset within the CD4+ T cell pool is the main source of CD83 expressing CD4+ T cells upon stimulation in vitro (Fig. 1B). However, we also detected an up-regulation of CD83 on stimulated naïve T cells, but to a lower extent and at later time points as on activated Treg cells. One might note a possible discrepancy between the highest CD83 expression on Treg cells 48 h upon stimulation (82%), whereas the inhibitory effect on responder T cells was measured at 72 h. However, suppression of proliferation takes place over a period of 3 days and is determined by thymidine incorporation between 54 and 64–72 h upon stimulation within standard coculture experiments. Thus, molecules that might be involved in the inhibitory function of Treg cells have to be expressed at high levels at time points earlier than 72 h.

Functional studies of CD4+ CD83+ T cells generated by retroviral transduction of freshly isolated CD4+ CD25+ naïve T cells revealed that forced expression of CD83 confers a regulatory phenotype. CD83-transduced T cells exhibited suppressive properties in vitro and were able to interfere with different T cell-mediated immune responses as CHS and EAE in vivo. These results suggest that CD83 expression contributes to the down-regulation of T cell responses and, thereby, CD83 might represent an inhibitory receptor of CD4+ T cells that is up-regulated in the course of activation to keep CD4+ T cell responses in balance, as it was postulated for the role of CD83 on activated B cells (15). Additional expression analysis demonstrated that ~20% of CD83-transduced T cells up-regulate the Treg cell-specific transcriptional regulator Foxp3. This seems to be sufficient for the induction of immunosuppressive function in vitro and in vivo comparable to Foxp3-positive CD4+ CD25+ Treg cells as well as Foxp3-transduced naïve T cells (Figs. 2 and 4). The increased Foxp3 expression in CD83-transduced T cells themselves might suggest a regulation of Foxp3 expression downstream of CD83, which in
turn has been described to induce CTLA-4 expression (37). However, the ligand for CD83 is still unknown, and the lack of conventional signaling motifs in the cytoplasmic tail of CD83 makes it unlikely that CD83 itself is responsible for the induction of Foxp3, but rather that other proteins interacting with CD83 lead to the modulation of T cell responses and Foxp3 induction.

Interestingly, former analysis of CD83+ T cells isolated from CD83 transgenic (tg) mice, expressing CD83 ubiquitously under control of the MHC class I promoter, showed that stimulated CD83+ T cells exhibited an activated phenotype releasing increased amounts of proinflammatory cytokines (36). However, in this study either whole spleen cells were stimulated with anti-CD3 or Ag-specific CD83tg CD8+ T cells were analyzed, which suggested differences in the functional role of CD83 expression on CD4+ and CD8+ T cells. Studies of CD4+ and CD8+ T cell populations isolated from CD83−/− mice contribute to this assumption. CD83 deficiency led to a strong reduction of peripheral CD83 also impaired the development of CD4+ T cells, whereas adoptive transfer of CD83-transduced T cells reversed CD83−/− mice contribute to this assumption. CD83 deficiency led to a strong reduction of peripheral CD83+ T cells, whereas CD8+ T cells were not affected (16). These results are well in line with the lack of CD83 expression on WT CD8+ T cells even upon stimulation (Fig. 1B), suggesting that CD83 does not participate in the function of CD8+ T cells. Similar observations as published for CD83−/− mice were described for CD83 mutant mice (17). The engineered genetic disruption of CD83 also impaired the development of CD4+ T cells, while the CD8− single-positive thymocyte development and numbers were normal (17). This defect in CD4+ T cell development results from modifications of the thymic environment as CD83−/− thymocytes and stem cells developed normally within WT mice (16), suggesting that CD83 expression on thymic epithelial cells contributes to CD4+ but not CD8+ T cell development within the thymus. The discrepancy in the immunosuppressive function of CD83+ T cells generated by retroviral transduction as described in the present study, and the activated phenotype described for CD83+ T cells isolated from CD83tg mice, might also be explained by the ubiquitous CD83 expression within CD83tg mice. The forced expression of CD83 by cells other than T lymphocytes, in particular during thymic T cell maturation, might influence the phenotype of CD4+ T cells, and, furthermore, it could not be excluded that CD83+ DCs and B cells somehow affect CD4+ T cell properties.

Recently, several studies have demonstrated an immunoregulatory function of sCD83. Administration of sCD83 in a murine skin transplantation model delayed skin rejection, and the prolonged graft survival was associated with inhibited allo-reactive T cell proliferation (23). Additionally, it has been shown that sCD83 injections markedly ameliorated the clinical symptoms associated with EAE under prophylactic and therapeutic settings (21). These sCD83-treated mice exhibited a reduced production of IFN-γ (21). Herein, we showed that adoptively transferred Ag-specific CD83-transduced T cells protected mice from the paralysis associated with EAE (Fig. 5A). Several recent findings indicate that the proinflammatory cytokine IL-17 plays a significant role in the development of pathological changes that accompany CNS inflammation in EAE. Although the development of EAE was significantly suppressed in IL-17−/− mice (38), and the administration of neutralizing anti-IL-17 Abs reduced disease incidence and severity (39), IL-17 expression was increased in lymphocytes derived from mice with EAE (40). Congruent with these reports, high amounts of IL-17 and IFN-γ were secreted by splenocytes from EAE-diseased control mice and mice treated with control vector-transduced T cells, whereas adoptive transfer of CD83-transduced T cells resulted in low IL-17 and IFN-γ production. Moreover, we detected a significant increase in IL-10 production by reisolated splenocytes from mice that have received CD83-transduced T cells in contrast to control vector-treated mice and their controls. These findings are well in line with several reports on the role of IL-10 in EAE. Mice with transgenic IL-10 resisted EAE (41), whereas mice with disrupted IL-10 gene were highly EAE susceptible (42, 43). Additionally, CD4+CD25+ lymphocytes have been shown to produce IL-10 and suppress EAE (44, 45), with one report demonstrating that Treg cells secrete the immunosuppressive IL-10 during EAE (46). Thus, one might suggest that CD83-transduced T cells are the main source for IL-10. However, as CD83-transduced T cells did not exhibit an up-regulation in IL-10 mRNA, and their suppressive capacity was not IL-10 dependent in vitro, the exact mode of action of CD83+ T cells in vivo remains to be elucidated.

With regard to the similar effect of sCD83 and CD83-transduced T cells on the course of EAE, one might speculate about secretion and/or shedding of membrane-bound CD83 from adoptively transferred CD83-transduced T cells, resulting in the generation of sCD83, which might be responsible for the observed protection from EAE. However, stimulation of naive T cells in the presence of supernatants from CD83-transduced T cell cultures had no significant effect on the proliferative capacity (data not shown) as it was described for sCD83 (20, 24, 25). Additionally, CD83-transduced T cells and CD4+CD25+ Treg cells lost their in vitro suppressive capacity in the absence of cell-cell contact (Fig. 2B), and most in vitro-stimulated CD4+CD25+ Treg cells expressed membrane-bound CD83 (mCD83) as analyzed by flow cytometry (Fig. 1B). Thus, secretion of sCD83 by immunoregulatory CD4+ T cells seems to be unlikely. Instead, we suggest that Treg cells mediate their suppressive capacity via interaction of mCD83 with its putative ligand.

It is well known that CD83 is strongly expressed by mature DCs, and engagement of its putative ligand by CD83-transduced APCs increases human T cell activation. Inhibition of CD83 surface expression on human DCs by HSV-1 dramatically reduces the capability to induce T cell proliferation (47, 48). Additionally, we have recently shown that inhibition of CD83 expression on DCs by CD83-specific small interfering RNAs leads to a reduced DC-mediated T cell stimulation, indicating that on DCs the membrane-bound form of CD83 acts as a costimulatory molecule (49). Expression of the undefined CD83 ligand (CD83L) has been suggested on DCs (20) and, recently, on activated CD8+ as well as CD4+ T lymphocytes (49). CD83L was expressed on activated T cells only upon stimulation through CD3/CD28 but not CD3 alone, suggesting that CD83L only functions when T cells are activated in the presence of costimulatory signals provided by CD83+ APCs (50). This observation might explain the function of CD83 on Treg cells and the immunosuppressive function of CD83-transduced T cells, respectively. Binding of Treg cells via CD83/CD83L interaction to activated T cells 1) might establish the cell-cell contact described to be important for the immunosuppressive function of Treg cells, and or 2) might interfere with T cell/DC interaction and thereby compete with further T cell stimulation by professional APCs providing costimulatory signals. However, the identification of the CD83L and its intracellular signaling pathway would be helpful to better understand the role of CD83/CD83L interaction in the regulation of different immune responses.

In conclusion, we provide evidence that CD83 is expressed by Treg cells and contributes to their suppressive function in vivo. This highlights the importance of CD83 as a new target for pharmacologic intervention in autoimmune diseases of the CNS and other T cell-mediated inflammatory diseases.

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Disclosures

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References


