CD83 orchestrates immunity towards self and non-self in dendritic cells

Andreas B. Wild, … , Elisabeth Zinser, Alexander Steinkasserer


In-Press Preview

Dendritic cells (DCs) are crucial to balance protective immunity and autoimmune inflammatory processes. Expression of CD83 is a well-established marker for mature DCs although its physiological role is still not completely understood. Using a DC-specific CD83 conditional KO mouse (CD83ΔDC) we provide new insights into the function of CD83 within this cell type. Interestingly, CD83-deficient DCs produced drastically increased IL-2 levels and displayed higher expression of the co-stimulatory molecules CD25 and OX40L, which causes superior induction of antigen-specific T cell responses and compromises Treg suppressive functions. This also directly translates into accelerated immune responses in vivo. Upon Salmonella typhimurium and Listeria monocytogenes infection, CD83ΔDC mice cleared both pathogens more efficiently, and CD83-deficient DCs expressed increased IL-12 levels after bacterial encounter. Using the experimental autoimmune encephalomyelitis (EAE) model, autoimmune inflammation was dramatically aggravated in CD83ΔDC mice, while resolution of inflammation was strongly reduced. This phenotype was associated with increased cell influx into the CNS accompanied by elevated Th17 cell numbers. Concomitantly, CD83ΔDC mice had reduced Treg numbers in peripheral lymphoid organs. In summary, we show that CD83 ablation on DCs results in enhanced immune responses by dysregulating tolerance mechanisms and thereby impairing resolution of inflammation, which also demonstrates high clinical relevance.

Find the latest version:

http://jci.me/126246/pdf
CD83 orchestrates immunity towards self and non-self in dendritic cells

Andreas B. Wild¹, Lena Krzyzak¹, Katrin Peckert¹, Lena Stich¹, Christine Kuhnt¹, Alina Butterhof¹, Christine Seitz¹, Jochen Mattner², Niklas Grüner², Maximilian Gänsbauer², Martin Purtak², Didier Soulat², Thomas H. Winkler³, Lars Nitschke³, Elisabeth Zinser¹ and Alexander Steinkasserer¹

¹ Department of Immune Modulation, Universitätsklinikum Erlangen and Friedrich-Alexander Universität-Erlangen-Nürnberg, Erlangen, Germany. ² Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Universitätsklinikum Erlangen and Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany. ³ Division of Genetics, Department of Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany.

§ Corresponding author:

Prof. Dr. Alexander Steinkasserer, Department of Immune Modulation, University Hospital Erlangen, Hartmannstr. 14, 91052 Erlangen, Germany;
alexander.steinkasserer@uk-erlangen.de
Abstract

Dendritic cells (DCs) are crucial to balance protective immunity and autoimmune inflammatory processes. Expression of CD83 is a well-established marker for mature DCs although its physiological role is still not completely understood. Using a DC-specific CD83 conditional KO mouse (CD83\(^{\Delta DC}\)) we provide new insights into the function of CD83 within this cell type. Interestingly, CD83-deficient DCs produced drastically increased IL-2 levels and displayed higher expression of the co-stimulatory molecules CD25 and OX40L, which causes superior induction of antigen-specific T cell responses and compromises Treg suppressive functions. This also directly translates into accelerated immune responses in vivo. Upon *Salmonella typhimurium* and *Listeria monocytogenes* infection, CD83\(^{\Delta DC}\) mice cleared both pathogens more efficiently, and CD83-deficient DCs expressed increased IL-12 levels after bacterial encounter. Using the experimental autoimmune encephalomyelitis (EAE) model, autoimmune inflammation was dramatically aggravated in CD83\(^{\Delta DC}\) mice, while resolution of inflammation was strongly reduced. This phenotype was associated with increased cell influx into the CNS accompanied by elevated Th17 cell numbers. Concomitantly, CD83\(^{\Delta DC}\) mice had reduced Treg numbers in peripheral lymphoid organs. In summary, we show that CD83 ablation on DCs results in enhanced immune responses by dysregulating tolerance mechanisms and thereby impairing resolution of inflammation, which also demonstrates high clinical relevance.
Introduction

Dendritic cells (DCs) are crucial players in our immune system as they function as professional antigen-presenting cells (APCs) and connect innate and adaptive immune responses. Immature DCs are present in most tissues and are able to sense pathogens via conserved innate immune receptors, including Toll-like receptors (TLRs). Upon activation via these receptors, DCs mature and migrate to lymphoid organs where they prime antigen-specific naive T cells and drive their differentiation into specific effector subsets. These features render DCs as key players not only in physiological immune responses but also in autoimmunity. Presentation of self-antigens can lead to an activation and expansion of self-reactive T cells, provided that mature DCs express stimulatory receptors and cytokines. In contrast, immature DCs insufficiently co-stimulate T cells during antigen-recognition and rather drive them into an anergic or a regulatory phenotype. Thus, the maturation state of DCs significantly affects whether self-reactive T cells are kept in check or get activated, leading to severe autoimmune responses.

One well-characterized marker for this phenotypic transition from immature to mature DCs is the expression of the CD83 molecule. This member of the Ig superfamily is primarily expressed on activated leukocytes including DCs and regulatory T cells (Tregs) as well as on thymic epithelial cells (TECs). A soluble form of CD83 (sCD83), comprised of the extracellular domain, was detected at low levels in sera of healthy individuals and in increased concentrations in patients suffering from hematologic malignancies, and reports suggest B cells as a major source of natural occurring sCD83. Several studies demonstrated profound
immunomodulatory effects of sCD83 in different animal models of autoimmune diseases and transplantation

While sCD83 induces tolerogenic mechanisms, the precise function of membrane-bound CD83 (mCD83) on different cell types was less clear. However, it has been reported that by inhibiting MARCH1-dependent ubiquitination, CD83 stabilizes surface expression of MHC-II and CD86 on DCs and B cells. The same mechanism, but MARCH8-dependent, causes decreased surface MHC-II levels on cortical TECs in CD83−/− mice, resulting in defective positive selection and drastically reduced numbers of peripheral CD4+ T cells. Additionally, our group revealed that expression of CD83 is essential for Treg homeostasis and stability. However, the precise functional relevance of CD83 expression on APCs, such as DCs, is still not properly understood. On human DCs, CD83 expression was shown to correlate with enhanced T cell stimulatory capacity in vitro. In contrast, deletion or overexpression of CD83 on murine APCs exhibits only a minor influence on their T cell activating potential. Since these data were conflicting and CD83-deficiency on APCs has been reported to influence mucosal homeostasis, we were interested to investigate the impact of CD83-deficiency on DCs in the context of intestinal infection/inflammation, but also on neuro-inflammation and the development of neurodegenerative autoimmunity.

Using the experimental-autoimmune-encephalitis (EAE) model we show that specific deletion of CD83 on DCs leads to an aggravated autoimmune neuro-inflammation, which was hallmarked by increased influx of immune cells into the central nervous system CNS and a shift towards encephalitogenic Th17 responses. CD83-deficient DCs display higher levels of co-stimulatory CD25 and OX40L molecules on their surface and secrete more IL-2, which leads to increased induction of antigen-specific T cell proliferation. Additionally, these DCs shape a more inflammatory environment.
upon antigen-specific interaction with T cells. Interestingly, on the other hand, animals with DC-specific CD83 deletion show improved bacterial clearance in acute intestinal infection models, proposing a crucial regulatory function of CD83 for the balance between protective immunity and exacerbated autoimmune responses.

Results

Successful deletion of CD83 on dendritic cells

To ablate CD83 expression in a cell-specific way, we used the previously reported Cre-loxP system for a conditional CD83 knock-out (CD83 cKO)\(^{29}\). Crossing CD83\(^{fl/fl}\) mice with Itgax-Cre mice leads to specific deletion of CD83 expression in CD11c\(^+\) cells, especially in DCs (CD83\(^{ΔDC}\)) (Figure 1A). The successful knock-out of CD83 on DCs was proven on mRNA and on protein level using bone-marrow derived DCs (BMDCs). LPS-treated CD83\(^{ΔDC}\) BMDCs showed a complete abrogation of CD83 mRNA expression when compared to control mice (Figure 1B). On protein level, CD83\(^{fl/fl}\) BMDCs showed a strong increase of CD83 expression after LPS-induced maturation, whereas CD83 expression was lost in either unstimulated or LPS-matured CD83\(^{ΔDC}\) derived BMDCs, as demonstrated by Western blot analyses of whole cell lysates (Figure 1C). Given the central role of DCs in the immune system, we evaluated the impact of DC-specific CD83-deletion on the cellular composition of the spleen of CD83\(^{ΔDC}\) mice. We detected no differences compared to control animals regarding the major cell subpopulations as well as naïve, effector and memory T cells (Figure S1A+B).

Next, we assessed the effect of CD83 deletion on splenic DC subsets. At first, we tested if CD83 ablation altered the distribution of splenic DC subsets. However,
neither the proportions of conventional DCs (cDC1, CD11c⁺CD8α⁺, and cDC2, CD11c⁺CD11b⁺) nor plasmacytoid DCs (pDC, B220⁺SiglecH⁺) were changed in CD83ΔDC mice (Figure S1C). It was previously reported that splenic DCs display only low levels of CD83 but rapidly upregulate its surface display after in vitro stimulation with TLR ligands ⁴. Accordingly, we detected a small proportion of CD83⁺ cells in both cDC subsets of the spleen, which correlated with high expression of MHC-II, while pDCs displayed only minor levels of CD83 (Figure 1D + S1D). In contrast, cDCs from CD83ΔDC mice expressed virtually no CD83. Furthermore, after DC maturation induced by the TLR-ligands CpG DNA and Pam₃CSK₄, hereafter termed TLR-L, CD83 expression was markedly induced in both cDC subsets derived from control animals but not from CD83ΔDC mice (Figure 1D). Interestingly, expression of CD83 was not altered in pDCs when comparing CD83⁻/⁻ and CD83ΔDC mice (Figure S1D). Therefore, we evaluated the deletion efficiency in all splenic DC subsets, using a Cre-reporter mouse strain. We detected nearly 100% reporter gene expression in both cDC1 and cDC2 but a residual portion of plasmacytoid DCs (pDCs) showed no reporter gene expression (Figure S1E), which may account for insufficient deletion in these cells.

CD83 was shown to stabilize the expression of MHC-II on APCs due to blockade of MARCH1-dependent ubiquitination and subsequent degradation ²². Thus, we examined whether DC-specific CD83 deletion would affect the surface expression of MHC-II molecules. Indeed, flow cytometric analyses of splenic DCs revealed that MHC-II expression was significantly reduced in cells derived from CD83ΔDC mice (Figure 1E). The reduction of MHC-II expression was obvious on both cDCs subsets, with the strongest effect on the cDC1 subset whereas cDC2 cells showed a less pronounced decrease. Additionally, CD83ΔDC derived BMDCs displayed reduced
MHC-II levels on their surface (Figure S1E). Thus, using our cell type specific knock-out strategy we successfully deleted CD83 in different DC subsets, which phenotypically leads to diminished MHC-II cell surface expression.

**CD83-deficiency confers an over-activated DC phenotype**

The expression of peptide-loaded MHC-II on DCs is a prerequisite for initiation of antigen-dependent T cell responses, which further depend on sufficient input from co-stimulatory receptors. Thus, we also examined the phenotype of CD83-deficient DCs with regard to co-stimulatory and co-inhibitory molecules. However, neither the co-stimulatory molecules CD80 and CD40 nor the inhibitory receptors PD-L1 and PD-L2 revealed differences between CD83-deficient and control DCs after stimulation with TLR-L, although PD-L2 tended to be less expressed (Figure S2A+B). By contrast, we observed strikingly elevated surface levels of CD25 and OX40L on CD83-deficient BMDCs (Figure 2A + S2C), indicating a more activated phenotype. CD83 also stabilizes surface display of CD86, in a similar way as MHC-II, and accordingly we detected reduced levels of this molecule on CD83-deficient BMDCs after stimulation (Figure S2D). Next, we examined whether this increased up-regulation of co-stimulatory molecules can be attributed to altered TLR-signaling in CD83-deficient DCs. Indeed, increased levels of IRAK1, which is a crucial component of the TLR signaling complex, were observed (Figure 2B+C).

As CD25 expression on DCs is crucial for IL-2 trans-presentation to T cells as an early co-stimulatory signal, we assessed the IL-2 expression of BMDCs after activation. Interestingly, *Il2* mRNA levels are significantly elevated in CD83-deficient BMDCs after treatment with TLR-L (Figure 2D). Additionally, when we analyzed the supernatants of TLR-L stimulated BMDCs after 24 h, we detected highly increased amounts of IL-2 secreted by CD83 cKO BMDCs compared to controls (Figure 2E),
which was even more striking when we took into account the inter-assay variability (Figure S2E). In contrast to IL-2, we detected no differences in the expression of immune-modulatory molecules, such as TGF-β or indoleamine-2,3-dioxygenase 1 (IDO1), in CD83-deficient DCs after stimulation with TLR-L or LPS, respectively (Figure S2F).

In T cells, IL-2 expression is controlled by the transcription factor Nfatc2 and this pathway was also described in DCs as an important route for T cell stimulation. Thus, we were interested if Nfatc2 signaling in BDMCs was modified in CD83ΔDC in comparison to control animals. Interestingly, when BMDCs were stimulated with TLR-L, we detected elevated Nfatc2 levels in the nuclear fraction of BMDCs derived from CD83-deficient mice (Figure 2F). Noteworthy, this effect was already apparent in unstimulated DCs, pointing to an overall more activated phenotype of CD83ΔDC derived BMDCs, consistent with elevated expression of co-stimulatory molecules.

**CD83-deficient DCs induce potent antigen-specific immune responses**

On the one hand, CD83-deficiency on DCs results in degradation of MHC-II and CD86, which play crucial roles in initiating antigen-dependent T cell responses. On the other hand, we demonstrated that CD83-deficient DCs secrete more IL-2 and express higher levels of CD25 and OX40L. Both molecules have been reported to be involved in autoimmune disorders and autoreactive T cell activation. Thus, we evaluated the capacity of CD83ΔDC BMDCs to induce antigen-dependent T cell responses in a co-culture system. To this end, BMDCs were activated with TLR-L, pulsed with MOG35-55 peptide, and subsequently cultivated with CD4+ T cells from 2D2 mice, carrying a transgenic MOG-specific T cell receptor.
Interestingly, cultivation with CD83-deficient DCs induced a pronounced proliferative response of 2D2 T cells as compared to controls (Figure 3A+B). Most strikingly, the addition of a blocking antibody against OX40L to the co-cultures reversed this positive effect on proliferation almost completely (Figure 3C). The antibody did not block the overall T cell response as demonstrated by the non-significant change in proliferation for control DCs but rather affected only CD83-deficient DCs, thus directly linking the altered surface phenotype to improved stimulatory capacity.

Besides the induction of T cell proliferation, DCs critically affect the type of T cell responses by shaping the cytokine milieu during the priming phase and differentiation. Thus, we next examined whether loss of CD83 on DCs would affect the microenvironment of DC-T cell interactions. To this end, we co-cultivated TLR-L activated and MOG\textsubscript{35-55}-pulsed BMDCs together with naïve CD4\textsuperscript{+} T cells, derived from 2D2 mice, and determined the cytokine levels within the supernatants of these co-cultures. Notably, T cells cultivated with CD83-deficient BMDCs secreted considerably higher amounts of IL-17A, IL-6 and TNF-\alpha, while IL-10 was drastically decreased (Figure 3D). Thus, deletion of CD83 on DCs leads to an induction of a pro-inflammatory microenvironment during antigen-specific T cell stimulation. Since IL-6 and TNF-\alpha are cytokines that critically contribute to Th17 polarization and IL-17 secretion was indeed elevated, we reasoned that CD83-deficient DCs favor the development of antigen-specific Th17 responses.

Since we detected reduced amounts of the inhibitory cytokine IL-10, we next questioned whether the increased proliferative response of 2D2 T cells might rely on reduced expansion and/or induction of regulatory T cells in the co-cultures. However, we detected no differences regarding Treg numbers after co-cultivation (Figure S4F). By contrast, when we tested the ability of Tregs to suppress antigen-specific T cell
proliferation in the presence of BMDCs, we observed an impaired suppressive
capacity in co-cultures with CD83-deficient DCs (Figure 3E). These results indicate
that CD83-deficient BMDCs (i) enhance antigen-specific T cell proliferation by
providing stronger co-stimulatory signals and (ii) modulate Treg mediated
suppression. Collectively, we demonstrated that CD83-deletion causes an over-
activated DC phenotype which is characterized by a highly increased potential to
induce antigen-specific T cell proliferation and to shape a Th17-favoring cytokine
milieu. Therefore, we were further interested to elucidate how this would affect
immune responses to foreign and self-antigens.

**Lack of CD83 expression on DCs enhances immune responses to acute
infections**

It has previously been reported that aberrant expression of MHC-II as observed in
March1 

mice, disturbs DC homeostasis, reflected in severely impaired T cell
stimulation and reduced IL-12 production. In sharp contrast, we observed that
CD83-deficient DCs, in which MHC-II ubiquitination and its destabilization is
enhanced, exhibit improved T cell stimulatory capacity (Figure 3B). Thus, we
scrutinized CD83ΔDC DCs with regard to their ability to produce IL-12. Indeed, these
DCs tend to secrete higher IL-12 levels upon TLR stimulation (Figure S3A+B). The
IL-12 family cytokines IL-12 and IL-23 play important roles in the defense against
intracellular bacteria, like Salmonella. Therefore, we analyzed the potential of
CD83-deficient DCs to induce the expression of these cytokines upon bacterial
encounter. To this end, we cultivated BMDCs together with heat-killed Salmonella
typhimurium (HKST) and assessed the expression of Il12b, Il12a and Il23a, which
respectively encode the IL-12p40, IL-12p35 and IL-23p19 subunits. BMDCs derived
from CD83ΔDC mice showed significantly increased Il12b expression levels after
treatment with HKST. Moreover, also \textit{Il12a} and \textit{IL23a} mRNA tended to be elevated in CD83-deficient DCs, indicating enhanced expression of both, IL-12 and IL-23 (Figure 4A). We also tested the ability of BMDCs to secrete IL-12p40 in response to HKST and detected increased levels of this cytokine subunit in BMDC supernatants, thus corroborating our mRNA findings (Figure 4B).

Since IL-12 plays an important role in orchestrating the immune response to intracellular bacteria \textsuperscript{41} and given the fact that CD83-deficient BMDCs produce elevated levels of this cytokine in response to HKST in vitro, we investigated how CD83\textsuperscript{ΔDC} mice respond to bacterial infections. To this end, we infected mice with either \textit{S. typhimurium} or \textit{Listeria monocytogenes} and assessed the bacterial burden in specific organs. Since infection with \textit{S. typhimurium} also leads to the development of colitis, we additionally determined the disease severity in these mice by histopathological analyses of the intestine. Interestingly, CD83\textsuperscript{ΔDC} mice (i) displayed lower levels of colitis and (ii) exhibited a significantly lower bacterial burden in the liver and spleen, suggesting a reduced spreading of the pathogen to distal organs (Figure 4C). Similarly, infection with \textit{L. monocytogenes} resulted in a significantly reduced bacterial spread to the liver in CD83\textsuperscript{ΔDC} mice, compared to controls, whereas the bacterial burden within the spleen of CD83\textsuperscript{ΔDC} mice revealed only a minor reduction (Figure 4D). Interestingly, we also detected increased expression of \textit{Il12b}, \textit{Il12a} and \textit{Il23a} in CD83-deficient DCs after treatment with heat-killed \textit{Staphylococcus aureus} (Figure S3C), suggesting a superior capacity to mount protective responses to bacteria. These data provide clear evidence that a DC-specific CD83-deficiency results in improved immune responses to acute bacterial infections.
**DC-specific CD83 deficiency leads to aggravated autoimmune neuro-inflammation**

Enhanced induction of immune responses is crucial for clearance of pathogens in acute infections but can be deleterious during chronic and autoimmune disorders. Additionally, we observed enhanced T cell stimulatory capacity of CD83 cKO DCs, associated with a Th17-favoring milieu. These data suggest induction of autoimmunity-prone T cell responses by CD83-deficient DCs. Therefore, we investigated how CD83-deficiency on DCs affects the control of immune responses to self-antigens in vivo. We used the MOG$_{35-55}$-induced EAE model, which is a well-established animal model for progressive CNS autoimmunity, leading to paralysis due to inflammation and demyelination-induced axonal damage. Mice were immunized with the MOG$_{35-55}$ peptide and disease symptoms were monitored for up to 30 days. Interestingly, CD83$^{ADC}$ mice succumbed more rapidly to the disease and showed a significantly impaired recovery phase, demonstrated by a long lasting and increased paralyses after the peak of disease, usually observed between day 15 and 18 (Figure 5A). The earlier onset of disease correlated with the infiltration of CD45$^+$ cells into the brain parenchyma (Figure 5B).

Breakdown of the blood-brain-barrier and infiltration of peripheral immune cells into the CNS are hallmarks of EAE. To assess this process in more detail, we isolated cells from the brain and spinal cord of EAE mice at the peak of disease (day 15 after EAE induction) and determined the composition of CD45$^+$ leukocytes via flow cytometry. It is generally accepted that CNS resident immune cells (i.e. microglial cells) can be segregated from infiltrating cells by their respective expression of CD11b and CD45. While resident immune cells display CD11b, but only moderate levels of CD45 on their surface, infiltrating cells are CD45$^{high}$, containing both CD11b$^-$ and CD45$^{high}$.
lymphoid and CD11b\(^+\) myeloid cells (Figure 5C). At the peak of disease, CD83\(^{\Delta DC}\) mice showed a markedly increased influx of peripheral immune cells, paralleled by a reduced proportion of resident cells (Figure 5D).

During the EAE course, the CNS is infiltrated by IL-17 secreting CD4\(^+\) T cells and IL-17 further plays a crucial role in the induction and progression of the disease\(^{45-47}\). As we also observed preferential induction of Th17 responses by CD83-deficient DCs in vitro, we determined the presence of Th17 cells via the expression of IL-17 in CD4\(^+\) T cells in different organs at the peak of disease. When analyzing the CNS of EAE animals, we detected a significantly enriched proportion of IL-17 producing CD4\(^+\) T cells in CD83\(^{\Delta DC}\) mice. Interestingly, elevated numbers of IL-17\(^+\) cells were also present in draining inguinal lymph nodes of CD83\(^{\Delta DC}\) mice at the peak of EAE, and we observed a similar trend for spleen-derived T cells of those animals (Figure 5E + Figure S4A). IFN-\(\gamma\) producing CD4\(^+\) and CD8\(^+\) T cells revealed no difference between CD83\(^{\Delta DC}\) and control mice (Figure S4B+C).

Since the recovery from EAE was significantly impaired in CD83\(^{\Delta DC}\) mice and given the important role of Tregs for resolution of inflammation\(^{48}\), we assessed whether this particular cell subset was affected in CD83\(^{\Delta DC}\) mice. Infiltrating cells from the brain and spinal cord were isolated from EAE mice at the peak of disease and the Treg frequency within the CD4\(^+\) T cell compartment was analyzed. There were no overt differences in the percentages of CD25\(^+\)FoxP3\(^+\) T cells between the CNS of CD83\(^{\Delta DC}\) and control mice. However, when we analyzed the peripheral lymphoid organs, where disease priming is initiated, we observed a striking reduction of the Treg proportion in draining lymph nodes and spleens of CD83\(^{\Delta DC}\) mice (Figure 5F). This effect was apparent only at the peak of disease while Treg numbers in naïve mice and during the initial disease phase (i.e. day 8 after EAE induction) were not
significantly altered in CD83ΔDC mice (Figure S4D+E). Thus, we demonstrate that CD83 deletion on DCs exaggerates development of chronic inflammatory processes paralleled by severely impaired regulatory mechanisms.

**Discussion**

Mature DCs that present self-antigens to autoreactive T cells are critically involved in the development of autoimmune diseases. This maturation process comprises up-regulation of antigen-presenting MHC and co-stimulatory molecules as well as secretion of T cell priming cytokines. One well-characterized marker for DC maturation is the CD83 protein. To unravel the physiological relevance of CD83 expression on DCs we used a conditional knockout system whereby CD83 expression was specifically deleted on both conventional DC subsets as well as on BMDCs. These CD83-deficient DCs exhibit a reduced expression of MHC-II, which is in line with the previously described MARCH-1 opposing effect of CD83 and subsequently higher ubiquitination and turnover of this protein.

Recent reports suggested that aberrant MHC-II expression disturbs DC homeostasis, which is hallmarked by decreased IL-12 production and T cell stimulatory capacity. In contrast, CD83-deficiency, which enhances MHC-II degradation, resulted in increased IL-12 secretion by BMDCs upon challenge with heat-killed Salmonella. Consequently, when infected with *S. typhimurium*, CD83ΔDC mice revealed a strikingly reduced bacterial spreading to peripheral organs and ameliorated gut inflammation. Similar results were obtained upon infection with *L. monocytogenes*, with strongly reduced bacterial burdens in the liver of CD83ΔDC mice. In summary, CD83 on DCs is an important modulator of protective responses against acute infections.
Since exaggerated immune responses are detrimental during chronic inflammatory processes, we investigated the impact of CD83-deletion on DCs using a well-established model for chronic and autoimmune neuro-inflammation, the EAE. Thereby, we show that CD83ΔDC mice succumb to disease more rapidly and display severely an impaired recovery with disturbed resolution of inflammation. This phenotype is characterized by increased inflammatory cell influx into the CNS and by elevated proportions of IL-17 producing T cells at the peak of disease. As we also observed increased IL-17 secretion in DC-T cell co-cultures, we propose that CD83-deficient DCs shape the microenvironment during initiation of the immune response in favor of a Th17 development.

Interestingly, during the course of EAE CD83ΔDC mice displayed reduced Treg numbers in peripheral immune organs but not in the CNS, despite a significantly impaired recovery phase. However, encephalitogenic CNS-infiltrating effector T cells are refractory to Treg-mediated suppression due to their unique pro-inflammatory cytokine profile. Additionally, the same study revealed that MOG-specific Tregs are not generated de novo but expand during EAE from preexisting natural Tregs. Since we observed reduced Treg numbers in the spleen and lymph nodes of CD83ΔDC mice, with coincidently augmented Th17 proportions, we reason that CD83ΔDC mice fail to induce a proper regulatory response in the periphery. Thus, these mice are unable to prevent the generation of further pathogenic Teff cells and consequently exhibit impaired recovery from the autoimmune disease. Collectively, CD83 constitutes a new vital checkpoint which downregulates overshooting immune responses and induces resolution of inflammation.

Paralleled by the superior potential of CD83ΔDC mice to mount pro-inflammatory immune responses in vivo, we observed an over-activated phenotype of CD83-
deficient DCs in vitro. In contrast to decreased expression of MHC-II and CD86 due to lack of MARCH1 inhibition, CD83 cKO DCs display markedly elevated levels of CD25 and OX40L on their surface and produce more IL-2 in response to TLR-L activation. DC-derived IL-2 has been reported to bind to its high affinity receptor CD25 on DCs and is trans-presented to T cells during antigen-recognition, resulting in an enhanced early T cell activation. By contrast, OX40L rather perpetuates T cell proliferation at later stages. Indeed, we observed augmented T cell proliferation upon co-culture with antigen-loaded DCs derived from CD83ΔDC animals. This suggests that elevated OX40L, CD25 and IL-2 expression may jointly compensate for probably compromised antigen-presenting capacity due to low MHC-II and CD86 expression. Noteworthy, the proliferative advantage of T cells activated by CD83 cKO DCs was abolished by blockade of OX40L, underlining the importance of this particular marker for induction of enhanced T cell proliferation. Interestingly, enforced expression of OX40L also aggravates the disease course in EAE mice as seen in CD83ΔDC animals.

Beyond initiation of T cell proliferation, quenching of IL-2 by DC-expressed CD25 has also been shown to regulate T cell differentiation and we noticed an increased propensity of CD83-deficient DCs to induce pro-inflammatory T cell responses in vitro. Thus, expression of IL-2 and CD25 could modulate the outcome of T cell priming by enforcing T effector differentiation.

In addition to a superior capacity to induce antigen-specific T cell proliferation, CD83-deficient DCs impair the suppressive capacity of Tregs. Since Tregs depend on IL-2 secretion by external sources, such as DCs, IL-2 quenching by CD83 cKO DCs, might prevent expansion or induction of these regulatory cells. Interestingly, we indeed observed reduced proportions of Tregs in peripheral lymphoid organs in
CD83ΔDC mice during EAE but not in naïve animals. These data suggest an impaired expansion or survival of Tregs derived from CD83ΔDC mice during disease induction. However, we detected impaired suppressive function rather than defective Treg-expansion in our in vitro culture system. It has been previously reported that Tregs display high levels of OX40 on their surface and enforced expression of OX40L on APCs or agonistic triggering of OX40 disrupts Treg suppressor functions and leads to reduced survival. Indeed, blocking of OX40L on the surface of CD83-deficient DCs reversed the enhanced T cell proliferation, which therefore may rely on mitigated Treg suppression by OX40-OX40L interaction. As a corollary, Treg survival might be impaired during immune responses in vivo.

The over-active phenotype observed in CD83 cKO DCs is also characterized by increased IRAK1 expression levels, which points towards an enhanced TLR signaling capacity. Interestingly, previous reports demonstrated that sCD83 also alters TLR signaling by binding to MD-2 on human monocytes, resulting in a long-lasting degradation of IRAK1 and subsequent induction of tolerogenic mechanisms. Likewise, CD83 on DCs could negatively modulate the TLR pathway either in cis or in trans, which would account for exaggerated inflammatory immune responses in CD83ΔDC animals.

Previous in vitro studies suggested a co-stimulatory effect of CD83 on human DCs but no influence on the stimulatory capacity of murine APCs. Most of these reports focused on the initiation of antigen-specific T cells responses of both human and murine DCs lacking CD83 after maturation periods longer than 24 hours. However, DCs are primed by pathogens to produce IL-12, TNF-α, and IL-2 as early as two hours after stimulation. Thus, secretion of activating and polarizing
cytokines occurs shortly after activation, and assessment of in vitro stimulatory
capacity at later time-points may yield different results.

Here, we demonstrate that CD83-deficient BMDCs express elevated levels of IL-2
and IL-12 early (i.e. 6 h) after stimulation and induce higher antigen-dependent T cell
proliferation and a more pro-inflammatory milieu when co-cultivated shortly after TLR
activation. Thus, we suggest an important modulatory role of CD83 for DC
homeostasis, similarly to its impact on Tregs. Since aberrant expression of MHC-II
disrupts normal DC function and CD83-deficient DCs display lower MHC-II surface
levels, this reduced expression may account for the increased pro-inflammatory
phenotype of DCs derived from CD83^ΔDC^ mice.

In summary, we revealed that CD83 acts as a regulatory checkpoint module
preventing overshooting immune responses, which eventually lead to autoimmunity.
We provided evidence that DC-specific abrogation of CD83 results in intensified
protective antibacterial immune responses but also aggravates autoimmune neuro-
inflammation. Deletion of CD83 confers a more pro-inflammatory phenotype on DCs,
leading to potent induction of antigen-dependent T cell proliferation and Th17
commitment. Additionally, CD83-deficient DCs tend to subvert Treg suppressive
capacity and thus impair the establishment of inflammation-resolving mechanisms.
Finally, these findings may help to find new pathways and therapeutic intervention
strategies to specifically modulate DCs functions for the therapy of autoimmune
disorders such as multiple sclerosis and for the prevention of bacterial infections.

**Material & Methods**

*Animals*
All animals were maintained on a C57BL/6N background. CD83\textsuperscript{fl/fl} mice were generated as described previously \textsuperscript{29} and were crossed with \textit{Itgax}-Cre mice for DC-specific depletion of the \textit{Cd83} gene (CD83\textsuperscript{ΔDC}). \textit{Itgax}-Cre mice were kindly provided by Prof. Jäck (Department of Medicine 3, University Hospital Erlangen). Prof. Krönke (Department of Medicine 3, University Hospital Erlangen) generously provided tdtomato-reporter animals. 2D2 transgenic mice were purchased from Jackson Laboratories.

For most experiments, we used \textit{Itgax}-Cre negative littermates as controls (CD83\textsuperscript{fl/fl}) to minimize the influence of microbiota. However, we included several experiments with mice positive for \textit{Itgax}-Cre but CD83wt (CD83wt\textsuperscript{Cre}) as control animals to rule out the contribution of the Cre-recombinase to our results. In all tested in vitro and in vivo conditions, comparison of CD83\textsuperscript{ΔDC} animals to CD83wt\textsuperscript{Cre} closely resemble results obtained with CD83\textsuperscript{fl/fl} mice (Figure S5).

\textbf{EAE induction}

Mice were immunized subcutaneously with 100 µl of emulsified complete Freund’s adjuvant (CFA, Sigma) supplemented with 10 µg/µl \textit{Mycobacterium tuberculosis} H37Ra (BD) and 100 µg MOG\textsubscript{35-55} peptide (Charité Berlin) and received intraperitoneal injections of 200 ng pertussis toxin (List Biological Laboratories) at the time of immunization and 48 hours later. Mice were monitored every other day for signs of clinical manifestation of EAE and clinical scores were attributed as follows: 0, no disease; 1, tail weakness; 2, hind limb weakness; 3, hind limb paralysis; 4, hind limb paralysis with forelimb weakness; 5, moribund or dead animals.

\textbf{Infection with bacteria}

For infection with \textit{S. typhimurium}, mice were treated with 20mg Streptomycin i.g. 24 hours prior to i.g. infection with 1x10\textsuperscript{7} \textit{S. typhimurium} CFUs. Bacterial counts were
obtained by CFU plating assays using homogenates of the indicated organs on day 4 after infection.

Infection with *L. monocytogenes* 10403S was achieved by intravenous injection of 1.0 x 10^4 CFU per mice. For the determination of bacterial loads of liver and spleen, mice were killed after 3 days. The respective organs were isolated and homogenized in PBS. Serial dilutions of the homogenates were plated on Oxford agar plates and incubated at 37°C for 48 h to allow CFU counting.

*Generation and stimulation of bone marrow derived dendritic cells (BMDCs)*

For generation of BMDCs, both tibiae and femurs were flushed and total BM cells were cultivated for 6-8 days in cell culture dishes in R10 medium containing 10% supernatant of a GM-CSF producing cell line. Fresh medium and GM-CSF was added every three days. Loosely and non-adherent cells were collected and transferred to 24-well plates for stimulations at densities of 0.5-1x10^6 cells per well. For mRNA and phenotype analyses, DCs were treated for 5 hours and 16 hours, respectively, with 0.5 µM CpG ODN2395 and 1 µg/ml Pam3CSK4 (Invivogen). For co-culture experiments, DCs were incubated with CpG OD2395 and Pam3CSK4 together with 2 µg/ml MOG_{35-55} peptide for 3 hours. Unstimulated MOG_{35-55}-loaded DCs served as negative controls. Stimulation with heat-killed *S. typhimurium* (HKST) was performed at a ratio of 10 bacteria per cell. Cytokine levels in supernatants of BMDCs were determined after 16 h stimulation either by CBA (BD and BioLegend) or ELISA (BioLegend) according to manufacturer’s instruction.

*Immunohistochemistry*

For visualization of inflammatory infiltrates, brains and spinal cords from mice (day 1 after EAE induction) were removed, placed in Tissue-Tek® (Sakura) cryoprotective
mold, fixed on dry ice, and stored at −80°C. Sections were sliced sequentially with a thickness of 5 μm with a cryotome (Kryocut CM 2000; Leica). Immunohistological staining was performed using an immunoperoxidase detection system in a wet chamber. Acetone-fixed sections were incubated in PBS. Endogenous peroxidase was blocked by incubating sections in 3% H₂O₂. The primary antibody was a biotinylated anti–mouse CD45.2 antibody (clone 104; Thermo Fisher Scientific). For detection and subsequent visualization, slides were treated with ABC and AEC Kits (Vector Laboratories), mounted in Aqua Tex (Roth), and covered with a coverslip.

**Purification of nuclear and cytoplasmic protein fractions**

Cell pellets were treated with cell lysis buffer (10 mM HEPES pH 7.60, 0.1 mM EDTA, 10 mM KCl, 1 mM DTT, 5% Glycerol, 1x Protease Inhibitor Mix, (Roche) and incubated 15 min on ice. Subsequently, 10% IGEPAL (Sigma) was added and the cell membranes were disrupted by vortexing for 30 s. Cytoplasmic fraction was collected after a centrifugation step (4600 rpm, 5 min, 4°C). The residual nuclei pellet was washed twice in cell lysis buffer and resuspended in nuclear extraction buffer containing 20 mM HEPES pH 7.60, 0.1 mM EDTA, 50 mM KCl, 1 mM DTT, 300 mM NaCl, 10% Glycerol, 1x Protease Inhibitor Mix, (Roche). Afterwards, nuclei were sonicated (40 % amplitude, 45°C, 10 s, 5 min pause) and the supernatant was collected after centrifugation (13000 rpm, 10 min, 4°C). Protein concentrations were determined via BCA according to the manufacturer’s instructions (Thermo Fisher Scientific).

**Western Blot analyses**

Nuclear and cytoplasmic lysates of stimulated BMDCs were denatured in reducing Laemmli buffer at 95°C for 5 min. After protein separation via polyacrylamide gel-electrophoresis and blotting onto a nitrocellulose membrane (GE Healthcare), the
membrane was blocked and subsequently probed with the following primary antibodies: mouse-anti-NFATc2 (25A10.D6.D2, Invitrogen), rabbit-anti-Lamin A/C (2032S, Cell Signaling Technology), mouse-anti-CD83 (F-5, SantaCruz), rabbit-anti-IRAK1 (D51G7, Cell Signaling Technology) or mouse-anti-β-actin (AC-74, Sigma) were diluted 1:1000 in 1x RotiBlock, added to the membranes and incubated overnight at 4°C. Afterwards, membranes were washed and the appropriate HRP-labeled secondary antibody was added. Signals on the blots were detected using ECL Prime Western Blotting Detection Reagent (GE Healthcare).

Isolation of 2D2 CD4+ T cells and co-culture with BMDCs

MOG35-55-specific CD4+ T cells were isolated from spleens and inguinal lymph nodes of 2D2 mice using either total or naïve CD4+ T cell isolation kits (Miltenyi Biotech). For proliferative analyses, T cells were labeled with CellTrace™ Violet Cell (CTV) proliferation dye (Thermo Fisher Scientific) and co-cultivated with MOG-loaded DCs at a T cell:DC ratio of 20:1 for 4 days. Proliferation was quantified using the median fluorescence intensity of CTV of the CD4+ compartment. For the OX40L blocking experiments 10 µg/ml of α-OX40L (RM134L) or isotype-matched control antibody (RTK4530) was added during the cultivation period (both Biolegend). Cytokine analyses were performed after 4 days of co-culturing T cells and DCs at a ratio of 5:1 using a cytometric bead array according to manufacturer’s instructions (BioLegend).

For analysis of Treg suppressive capacity in DC co-cultures, Tregs were isolated from spleens and inguinal lymph nodes of 2D2 mice using CD4+CD25+ regulatory T cell isolation Kit mouse (Miltenyi). CD25+ T cells served as effector T cells (Teff) and were co-cultivated with BMDCs stimulated as described above. Teff cell:DC ratio was adjusted to 20:1 and Tregs were added at decreasing amounts. Suppressive
capacity was calculated from division indices of Treg co-cultures relatively to T_{eff} cell:DC only conditions.

**Isolation of CNS infiltrating cells**

Immune cells within the CNS were isolated by a one-step density centrifugation protocol as described previously \(^6^2\), with modifications. Briefly, mice were perfused with ice-cold PBS by cardiac puncture and brains were collected and placed in RPMI medium on ice. Tissue was homogenized with a tissue dounce grinder set. Myelin was removed by centrifugation of homogenates re-suspended with 37% Percoll\textsuperscript{TM} (Sigma). Cells were collected in R10 medium and either used for restimulation and cytokine analysis or directly for flow cytometry. Resident CNS immune cells (i.e., microglia) were defined as CD11b\textsuperscript{+}CD45\textsuperscript{int}CX3CR1\textsuperscript{+}, whereas the CD45\textsuperscript{hi} population represented infiltrating lymphoid (CD11b\textsuperscript{-}) and myeloid (CD11b\textsuperscript{+}) cells.

**Isolation and restimulation of cells**

Spleens and draining inguinal lymph nodes were collected and grinded between two microscope slides. Cells were passed through a 70 µm cell-strainer and washed with PBS. For spleens, lysis of erythrocytes was performed. Cells were collected in R10 medium for culture or used directly for flow cytometry. For intracellular staining of cytokines cells were re-stimulated with 50 ng/ml PMA and 500 µg/ml ionomycin (Sigma) for 5 h with Golgi-transport inhibitors (Golgi-Plug and Golgi-Stop, BD) added for the last 4 hours.

**Flow cytometry analysis**

Surface staining was performed in PBS containing LIVE/DEAD\textsuperscript{TM} Fixable Aqua Dead Cell Stain (Thermo Fisher Scientific) for 30 min at 4°C. For intracellular staining, cells were fixed and permeabilized and intracellular staining was performed in Permeabilization Reagent (Thermo Fisher Scientific, Cat. 00-5523-00). Antibodies (all
from BioLegend, unless otherwise stated) against the following antigens were used:

CD3 (17A2), CD4 (RM4-5), CD8 (53-6.7), CD11b (M1/70), CD11c (N418), CD25 (PC61), CD45 (I3/2.3), CD83 (Michel19), CD86 (GL-1), FoxP3 (FJK-16s, eBioscience), IFN-γ (XMG1.2), IL-17A (TC11-18H10.1), I-A/I-E-FITC (2G9, BD), I-A/I-E (M5/114.152), OX40L (RM134L). Expression was assessed using a FACSCantoII flow cytometer (BD) and analyzed with FlowJo software version 10 (FlowJo, LLC).

Living leukocytes were gated for further analyses. To compensate for inter-assay variability relative fluorescence intensities were calculated as a ratio of CD83\(^{ΔDC}\) to CD83\(^{fl/fl}\).

**qPCR**

Cells were lysed with RLTPlus buffer (Qiagen) and stored at -80°C until RNA isolation. Total RNA was isolated using the RNeasy Plus Mini Kit (Qiagen) and mRNA was transcribed into cDNA using First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Gene expression was analyzed via qPCR and the SsoAdvanced Universal SYBR Green Supermix on a CFX96 Real-Time System (Biorad) and normalized to the expression of the *Rpl4* reference gene. To compensate for inter-assay variability relative expression levels were calculated as a ratio of CD83\(^{ΔDC}\) to CD83\(^{fl/fl}\). All primer were designed and validated according to the MIQE guidelines. For primer sequences see Table 1

**Statistical analyses**

All statistics were performed with GraphPad Prism 7.0 using the Mann-Whitney U test for non-parametric data or one-way ANOVA. Data are presented as mean values including standard error of the mean (s.e.m.). P-values of \(*p<0.05\), \(**p<0.01\), \(**p<0.001\) and \(***(p<0.0001\) were considered statistically significant.
Study approval

Animal care and all experimental procedures of the present study were performed in accordance with the European Community Standards on the Care and Use of Laboratory Animals and were approved by our institutional ethics committee.

Author Contributions

ABW designed, conducted, and analyzed the majority of experiments, and prepared the manuscript. LK, KP, AB, CK, CS, LS, and EZ performed experiments, analyzed data, and edited the manuscript. JM, NG, MG, MP and DS conducted the infection experiments with living bacteria and edited the manuscript. LN and THW provided scientific insight, supervised some analyses, and edited the manuscript. AS conceived and designed the study, supervised experiments, and prepared the manuscript.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) via the SFB1181 projects B03 (to AS), B06 (to LN), C04 (to JM) and the Graduiertenkolleg 1660 grant B02 to AS. AS was further supported by the DFG project STE432/9-1, DS by the DFG project SO 1149/1-1 and JM by DFG grant MA 2621/4-1. KP was supported by the DFG Project ZI 1225/1-1.

References


(A) Strategy used to generate DC-specific deletion of CD83 (CD83\textsuperscript{ADC}). Mating of CD83\textsuperscript{fl/fl} mice with Itgax-Cre mice leads to deletion of loxP-flanked exons 1 and 2 resulting in ablation of CD83 expression specifically in CD11c\textsuperscript{+} DCs. (B) Assessment of knock-out efficiency on mRNA level. BMDCs were matured with 0.1 µg/ml LPS for 16 h and expression levels of Cd83 were determined via qPCR. Expression levels were normalized to CD83\textsuperscript{fl/fl} BMDCs. (C) Assessment of knock-out efficiency on protein level. BMDCs were stimulated with 0.1 µg/ml LPS for 16 h or left untreated and CD83 expression was analyzed via Western blot of whole cell lysates. GAPDH
was used as loading control. (D) Flow cytometric evaluation of CD83 deletion on splenic DC subsets. Total splenocytes were analyzed either ex vivo or after stimulation with 3.5 µg/ml CpG ODN2395 and 1 µg/ml Pam₃CSK₄ (TLR-L) for 16 h via flow cytometry. FACS data are representative of five mice. (E) Assessment of MHC-II surface expression on cDC on splenic DC subsets. Data represent 4 independent experiments (n=16). Statistical analysis was performed using Mann-Whitney U-test. *p<0.05;***p<0.001; ns = not significant.
Figure 2. CD83-deficiency on DCs leads to enhanced co-stimulatory capacity

(A) Flow cytometry analysis of costimulatory surface markers. BMDCs were stimulated with 3.5 µg/ml CpG ODN2395 and 1 µg/ml Pam3CSK4 (TLR-L) for 16 h and expression of CD86, CD25 and OX40L was assessed on CD11c+MHC-IIhi mature DCs via flow cytometry. FACS data are pooled from six independent experiments and the median fluorescence intensity was normalized to control animals (n=20). (B) Western Blot of cytoplasmic lysates of unstimulated BMDCs and probed against IRAK1. (C) Quantification of IRAK1 amounts in lysates from BMDCs (n=5) (D) Expression of IL-2 after stimulation of DCs. BMDCs were stimulated with TLR-L for 6 h and expression of Il2 mRNA was analyzed via qPCR (n=18-20, pooled from 7 different experiments). (E) Assessment of IL-2 secretion by BMDCs in response to TLR-L via ELISA. BMDCs were stimulated with TLR-L and supernatant was collected after 16 hours (n=19, pooled from 8 different experiments). (F) Assessment of Nfatc2 in nuclear lysates from BMDCs. BMDCs were stimulated with TLR-L for indicated time periods, harvested and sequentially lysed (cytoplasm and nucleus). Nuclear fraction was separated with SDS-PAGE and transferred onto nitrocellulose membranes and probed with Nfatc2. Loading control for nuclear fraction was Lamin A/C. Blot is representative of four experiments. Statistical analysis was performed using Mann-Whitney U-test. *p<0.05; ****p<0.0001; ns = not significant.
Figure 3. Ablation of CD83 boosts DC-mediated pro-inflammatory T cell responses

(A, B) Analysis of stimulatory capacity towards T cells. (A) BMDCs from CD83ΔDC or control mice were activated with 3.5 µg/ml CpG ODN2395 and 1 µg/ml Pam3CSK4 (TLR-L) and pulsed with 2 µg/ml MOG35-55 for 3 h and co-cultivated with CellTrace™Violet (CTV) labelled 2D2 transgenic CD4+ T cells. Proliferation was analyzed after 3 days of co-culture via dye-dilution on a flow cytometer. (B) Quantification of proliferative potential of T cells calculated from the ratio of median fluorescence of CTV. Data are pooled from five different experiments (n=14). (C) Proliferative response of 2D2 T cells to BMDCs in the presence of 10 µg/ml anti-OX40L (α-OX40L) or isotype matched control antibody (n=7, pooled from two independent experiments). (D) Assessment of cytokine secretion in BMDCs-T cell cultures. BMDCs were treated with TLR-L for 16 h and cytokine content in the supernatants were analyzed via cytometric bead array (n=8 from three experiments). (E) Suppressive capacity of Tregs in co-cultures. Tregs and effector T cells (Teff) were cultured at different ratios together with TLR-L activated, MOG-pulsed BMDCs for 4 days (n=6). Statistical analysis was performed using Mann-Whitney U-test or one-way ANOVA with Sidak’s correction for multiple comparisons (only for C). *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns = not significant
Figure 4. Lack of CD83 on dendritic cells enhances immune responses against intracellular bacteria

(A) BMDCs from CD83^ΔDC or control mice were incubated with heat-killed *Salmonella typhimurium* (HKST) at a ratio of 10 bacteria per DC for 6 h and mRNA expression of *Il12a*, *Il12b* and *Il23a* was assessed. Data are pooled from three independent experiments (n=10). (B) Evaluation of IL-12p40 in the supernatants of BMDCs. Cells were stimulated with HKST at a ratio of 10 bacteria per DC for 16 h and supernatants were assessed by ELISA (n=9, from three experiments). (C) Infection of CD83^ΔDC and control mice with *Salmonella typhimurium* (n=9, one representative experiment is shown). Mice were treated with 1.0x10^7 CFU i.g. and sacrificed 4 days later. (D) Infection of CD83^ΔDC and control mice with *Listeria monocytogenes*. Mice were infected intravenously with 1.0x10^4 CFU and were sacrificed 3 days later. Bacterial burden in liver and spleen was assessed by CFU counting of organ homogenates after 48 hours (n=15, pooled from three independent experiments). Statistical analysis was performed using Mann-Whitney U-test. *p<0.05; ***p<0.001; ns = not significant.
Figure 5. CD83-deficiency on DCs leads to exacerbated autoimmune neuro-inflammation

(A) EAE was induced in 8-12 week old female CD83\textsuperscript{ΔDC} or control mice by immunization with MOG\textsubscript{35-55}-peptide. Disease progression was monitored by scoring the level of paralysis for up to 30 days (CD83\textsuperscript{fl/fl} n=16, CD83\textsuperscript{ΔDC} n=21; data are pooled from three independent experiments). (B) Histologic analysis of infiltrating cells into the brain (black arrows). Mice were sacrificed on day 11 after EAE induction and brain sections were obtained from frozen brains. Sections were stained with an anti-CD45 antibody (Clone 104). One representative slide is shown for each group. (C, D) Assessment of CNS infiltration during EAE. At day 15 after EAE induction (peak of disease) total cells from brains and spinal cords were isolated and analyzed via flow cytometry. (C) Total CNS leukocytes (CD45\textsuperscript{+} cells) were classified as resident or infiltrating cells and (D) the respective proportion was analyzed (n=16; data pooled from four independent experiments). (E) Analysis of IL-17 production in CD4\textsuperscript{+} T cells from EAE animals. Single cell suspensions from CNS, draining inguinal lymph nodes (dLNs) or spleens, isolated from mice at EAE peak, were stimulated
with PMA/ionomycin for 5 hours in the presence of Golgi-transport inhibitors and the intracellular amount of IL-17A was determined by flow cytometry (CNS n=11; pooled from three experiments; dLNs/spleen n=16; pooled from four experiments). (D) Assessment of regulatory T cell frequency in EAE mice. Single cell suspensions from CNS, dLNs or spleens, isolated from mice at EAE peak, were analyzed for the frequency of regulatory CD4⁺ T cells (CD25⁺FoxP3⁺) (n=13, pooled from three experiments). Statistical analysis was performed using Mann-Whitney U-test. *p<0.05; **p<0.01; ***p<0.001; ns = not significant