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Coronin 1-Mediated Naive T Cell Survival Is Essential for the Development of Autoimmune Encephalomyelitis

Kerstin Siegmund,* Thomas Zeis,† Gabriele Kunz,* Ton Rolink,† Nicole Schaeren-Wiemers,† and Jean Pieters*

Autoimmune encephalomyelitis is a disease of the CNS that can develop when an initial peripheral inflammatory stimulus is followed by infiltration and reactivation of T lymphocytes in the CNS. We report a crucial role for coronin 1, which is essential for maintenance of the naive T cell pool, for the development of murine experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis. In the absence of coronin 1, immunization with myelin oligoglycoprotein (MOG35–55) peptide largely failed to induce EAE symptoms, despite normal mobilization of leukocyte subsets in the blood, as well as effector cytokine expression comparable with wild-type T cells on polyclonal stimulation. Susceptibility of coronin 1-deficient mice to EAE induction was restored by transfer of wild-type CD4+ T cells, suggesting that the observed resistance of coronin 1-deficient mice to EAE development is T cell intrinsic. Importantly, although coronin 1-deficient regulatory T cells (Tregs) showed a suppressor activity comparable with wild-type Tregs, Treg depletion failed to restore EAE development in coronin 1-deficient animals. These results suggest a hitherto unrecognized role of naive T cells in T cell development of autoimmune encephalomyelitis and reveal coronin 1 as a crucial modulator of EAE induction. The Journal of Immunology, 2011, 186: 000–000.

The immune system has evolved mechanisms to respond to a diverse set of foreign Ags whereas minimizing the risk for autoreactivity. T cells play an important role in ensuring a successful immune response; however, under still ill-defined circumstances, T cells can cause harm to the body’s own constituents and mediate autoimmune pathology. Multiple sclerosis (MS) is an inflammatory demyelinating brain disease, in which autoreactive T cells are thought to play a major role in disease pathogenesis (for review, see Ref. 1). This immune response leads to an inflammation in the CNS, and subsequent demyelination and axonal injury. Experimental autoimmune encephalomyelitis (EAE) is the commonly used animal model to study immunopathogenesis of MS. C57BL/6 mice are immunized with peptides of a CNS-derived autoantigen, for example, myelin oligoglycoprotein (MOG), to induce active EAE (2, 3). In this form of EAE, MOG-specific naive T cells are activated and expanded during the initiation phase of the immune response in the draining lymph node. At later time points, the expanded T cells invade the CNS, where they are reactivated by local and infiltrating APCs (4, 5), and initiate the recruitment of inflammatory cells such as neutrophils and macrophages to induce the effector phase. In this rodent model of EAE, inflammation predominantly targets the spinal cord, resulting in the classic symptoms of ascending flaccid paralysis.

Although Th1 cells were believed to be the main effector cell subset in this particular EAE model, recent work has highlighted the importance of Th17 cells for the pathology of EAE. To date, several studies indicate that Th1, as well as Th17, cells can induce autoimmunity in the CNS and play complementary roles in pathogenesis of EAE (6, 7), whereas Th2 responses are protective (8, 9). In contrast with these effector T cell subsets, regulatory T cells (Tregs) have been implicated in the prevention and resolution of EAE. Increasing the number of Tregs has shown beneficial effects on EAE pathology, whereas decreasing Treg numbers worsened disease outcome (10–13). It thus appears that the balance between different T cell subsets, in particular, Th1, Th17, Th2, and Tregs, may be dictating disease outcome after immunization with myelin-derived peptides.

In healthy mice, the different T cell populations such as naive, memory, and Tregs occupy their own niche in the periphery, and T cell numbers are fairly well maintained. The mechanisms underlying the maintenance of the peripheral T cell pool are incompletely understood. Naive T cell survival in the periphery seems to depend on continuous low-level stimulation of their TCR by self-MHC molecules in secondary lymphoid organs, as well as IL-7 signaling (reviewed in Ref. 14). In the absence of appropriate stimuli, such as in mice lacking MHC expression, the naive T cell pool is greatly diminished and lymphopenia develops, which favors the enrichment of memory-like T cells (15–18). Similarly, ablation of the TCR expression leads to a reduction in peripheral T cells, underscoring that peripheral T cell survival relies on TCR-dependent stimulation.

Recently, coronin 1 (also known as P57 or TACO, for tryptophan aspartate containing coat protein) has been identified as a molecule essential for naive T cell maintenance in the periphery (19–23). Coronin 1, which is a leukocyte-specific molecule of the coronin protein family of WD repeat-containing proteins, is essential for
intracellular calcium mobilization after TCR stimulation and survival of naïve T cells. Mice that lack coronin 1 have strongly reduced naïve T cell numbers in the periphery, whereas thymic cellularity and selection is relatively normal (19–23). Interestingly, despite strongly reduced naïve T cell numbers and the observed in vitro activation defect, coronin 1-deficient mice show relatively normal Ab responses to thymus-dependent and -independent Ags (24).

We show in this article that on immunization with MOG\textsubscript{35–55} peptide, coronin 1-deficient mice are largely resistant to EAE. Failure to induce EAE in the absence of coronin 1 occurred despite the fact that on polyclonal stimulation, coronin 1-deficient CD4\(^+\) T cells expressed effector cytokines comparable with wild-type T cells. The transfer of wild-type CD4\(^+\) T cells before EAE induction could restore susceptibility to EAE. Furthermore, despite normal Treg function in the absence of coronin 1, Tregs were not involved in EAE resistance.

These results suggest an important role for coronin 1 in the development of EAE and furthermore reveal the importance of an intact naïve T cell pool as a prerequisite for autoimmune induction.

Materials and Methods

\textbf{Mice}

Coronin 1-deficient mice were generated in our laboratory as described previously (25) and backcrossed to C57BL/6 for eight generations. The corresponding littermates (knockout and wild-type) were bred in-house as homozygous lines. For the EAE experiments, 8- to 16-wk-old female mice were used. All experiments were approved by the Kantonales Veterinäramt Basel-Stadt.

\textbf{Induction of active EAE}

EAE was induced by active immunization with MOG\textsubscript{35–55} peptide based on established protocols (26, 27). To that end, 8- to 16-wk-old mice were immunized s.c. on the back at day 0 with 200 \(\mu\)g MOG\textsubscript{35–55} peptide (Genscript) emulsified in 100 \(\mu\)l CFA (Sigma) at a final volume of 200 \(\mu\)l. On days 0 and 2, the animals were injected with 250 \(\mu\)g pertussis toxin (Sigma) in 100 \(\mu\)l PBS i.p. In some experiments, wild-type CD4\(^+\) T cells were transferred i.v. to coronin 1-deficient mice 1 d before EAE induction. For the CD4\(^+\) T cell sort, erythrocyte-depleted cell suspensions of lymph node and spleen from naïve wild-type mice were stained with anti-CD4-FITC Ab (clone RM4-5; BioLegend) and subsequently labeled with anti-FITC microbeads (Miltenyi Biotec). Thereafter, the CD4\(^+\) T cells were isolated using LS columns and magnets (Miltenyi Biotec). The sort purity was >95% on reanalysis. For Treg depletion experiments, the mice were treated with 0.5 mg anti-CD25 Ab (PC61, purified according to Ref. 28) i.p. 5 and 3 d before induction of EAE, which corresponds to day 0. Wild-type and coronin 1-deficient mice were treated with 0.5 mg anti-CD25 Ab (PC61) 1 h at room temperature. Slides were mounted with Fluorosave (Calbiochem) and kept at 4°C. Confocal microscopy was performed using a Zeiss LSM 710 microscope with ZEN 2009 software (Carl Zeiss). Leptomeningeal quantification was done using the cellP software (Olympus). Lesion area was determined by F4/80 staining and analyzed using three sections (approximately cervical segment 6, thoracal segment 6, and lumbar segment 1) per animal. Leptomeningeal load was displayed as percentage of whole spinal cord analyzed. The following Abs were used for staining of spinal cord sections: anti-F4/80 (kindly provided by V.H. Perry, University of Southampton) (31), anti-CD4 (clone RM4-5; BioLegend), and polyclonal anti-coronin 1 rabbit antiserum (32). The following secondary Abs were used (all from Jackson ImmunoResearch Laboratories): donkey anti-rabbit DyLight 488 (1:500), donkey anti-rabbit DyLight 549 (1:500), donkey anti-rat Cy3 (1:500), and donkey anti-rat Cy5 (1:500).

\textbf{Treg suppression assay}

T cell subsets were isolated from spleen and peripheral and mesenteric lymph nodes of wild-type and coronin 1-deficient mice. Erythrocyte-depleted cell suspensions were stained with anti-CD25-allophycocyanin, and anti-CD4-Pacific blue (Invitrogen), CD25-PE (clone PC61, BD Biosciences), CD25-PE (clone 7D4; Miltenyi Biotec), IL–4–PE (BioLegend), IL–17A–allophycocyanin (BioLegend), IFN-\(\gamma\) (BioLegend). For Foxp3 detection, the Foxp3-Alexa Fluor 647 flow kit from BioLegend was used and the staining performed according to the manufacturer’s instructions. Before staining, the cells were incubated with the labeled Abs for 15 min on ice in the dark and then washed once with PBS/0.5% BSA. For intracellular staining, cells were first stained for surface Ags as described and then fixed with 2% paraformaldehyde for 20 min at room temperature. After a washing step with PBS/0.5% BSA, the cells were permeabilized using 0.5% saponin (Sigma) in PBS. The sections were incubated with the secondary Abs for 1 h at room temperature. Slides were mounted with Fluorosave (Calbiochem) and kept at 4°C. Confocal microscopy was performed using a Zeiss LSM 710 microscope with ZEN 2009 software (Carl Zeiss). Leptomeningeal quantification was done using the cellP software (Olympus). Lesion area was determined by F4/80 staining and analyzed using three sections (approximately cervical segment 6, thoracal segment 6, and lumbar segment 1) per animal. Leptomeningeal load was displayed as percentage of whole spinal cord analyzed. The following Abs were used for staining of spinal cord sections: anti-F4/80 (kindly provided by V.H. Perry, University of Southampton) (31), anti-CD4 (clone RM4-5; BioLegend), and polyclonal anti-coronin 1 rabbit antiserum (32). The following secondary Abs were used (all from Jackson ImmunoResearch Laboratories): donkey anti-rabbit DyLight 488 (1:500), donkey anti-rabbit DyLight 549 (1:500), donkey anti-rat Cy3 (1:500), and donkey anti-rat Cy5 (1:500).

\textbf{Flow cytometry}

The following Abs were used for staining cells for flow cytometric analyses: anti-CD4 Pacific blue (Invitrogen), CD25-PE-Cy7 (clone PC61, BD Biosciences), CD25-PE (clone 7D4; Miltenyi Biotec), IL–4–PE (BioLegend), IL–17A–allophycocyanin (BioLegend), IFN-\(\gamma\) (BioLegend). For Foxp3 detection, the Foxp3-Alexa Fluor 647 flow kit from BioLegend was used and the staining performed according to the manufacturer’s instructions. Before staining, the cells were incubated with the labeled Abs for 15 min on ice in the dark and then washed once with PBS/0.5% BSA. For intracellular staining, cells were first stained for surface Ags as described and then fixed with 2% paraformaldehyde for 20 min at room temperature. After a washing step with PBS/0.5% BSA, the cells were permeabilized using 0.5% saponin (Sigma) in PBS. The sections were incubated with the secondary Abs for 1 h at room temperature. Slides were mounted with Fluorosave (Calbiochem) and kept at 4°C. Confocal microscopy was performed using a Zeiss LSM 710 microscope with ZEN 2009 software (Carl Zeiss). Leptomeningeal quantification was done using the cellP software (Olympus). Lesion area was determined by F4/80 staining and analyzed using three sections (approximately cervical segment 6, thoracal segment 6, and lumbar segment 1) per animal. Leptomeningeal load was displayed as percentage of whole spinal cord analyzed. The following Abs were used for staining of spinal cord sections: anti-F4/80 (kindly provided by V.H. Perry, University of Southampton) (31), anti-CD4 (clone RM4-5; BioLegend), and polyclonal anti-coronin 1 rabbit antiserum (32). The following secondary Abs were used (all from Jackson ImmunoResearch Laboratories): donkey anti-rabbit DyLight 488 (1:500), donkey anti-rabbit DyLight 549 (1:500), donkey anti-rat Cy3 (1:500), and donkey anti-rat Cy5 (1:500).

\textbf{Immunofluorescence and microscopy}

Spinal cord tissue was removed, embedded in OCT Tissue-Tek (Sakura), and frozen on dry ice. Cryostat sections (10 \(\mu\)m) were air-dried for 30 min and fixed for 30 min in 4% paraformaldehyde for staining with anti-F4/80 Abs (macrophages and microglia), anti-CD4 (T cells), or anti-coronin 1 Abs. Sections were first incubated for 1 h in blocking buffer (5% normal donkey serum, 0.1% Triton X-100, 0.05% Tween 20) followed by incubation with primary Abs in blocking buffer overnight at 4°C. After washing with PBS, the sections were incubated with the secondary Abs for 1 h at room temperature. Slides were mounted with Fluorosave (Calbiochem) and kept at 4°C. Confocal microscopy was performed using a Zeiss LSM 710 microscope with ZEN 2009 software (Carl Zeiss). Leptomeningeal quantification was done using the cellP software (Olympus). Lesion area was determined by F4/80 staining and analyzed using three sections (approximately cervical segment 6, thoracal segment 6, and lumbar segment 1) per animal. Leptomeningeal load was displayed as percentage of whole spinal cord analyzed. The following Abs were used for staining of spinal cord sections: anti-F4/80 (kindly provided by V.H. Perry, University of Southampton) (31), anti-CD4 (clone RM4-5; BioLegend), and polyclonal anti-coronin 1 rabbit antiserum (32). The following secondary Abs were used (all from Jackson ImmunoResearch Laboratories): donkey anti-rabbit DyLight 488 (1:500), donkey anti-rabbit DyLight 549 (1:500), donkey anti-rat Cy3 (1:500), and donkey anti-rat Cy5 (1:500).
Statistical analyses

The data represent the mean (± SD or SEM, indicated in the figure legends); FACS dot plots and microscopic pictures show representative results. The number of mice (n) used per experiment and the number of experiments performed are listed in each figure legend. Statistical significance of the ELISA and flow cytometry results, as well as the EAE day of onset, maximal disease score, and lesion size, was analyzed by Mann-Whitney U statistical test, and the EAE score and weight versus time were analyzed with Wilcoxon signed rank test using GraphPad Prism software (GraphPad).

Results

Role for coronin 1 in the development of EAE

Coronin 1 is a leukocyte-specific regulator of Ca²⁺-dependent signaling processes and is essential for the maintenance of the naive T cell pool in the periphery (19–22). Despite diminished numbers of naive T cells (20), when coronin 1-deficient mice are immunized with a variety of Ags, normal Ab responses are generated (24). To investigate the role of coronin 1 in the pathogenesis of T cell-mediated autoimmunity, we immunized coronin 1-deficient and wild-type C57BL/6 mice with MOG35–55 peptide emulsified in CFA s.c. together with pertussis toxin i.p. leading to induction of active EAE (see Materials and Methods). The development of clinical signs of disease was monitored over several weeks. Initial signs of EAE, starting with tail weakness, were observed 10 d (on average, 11.6 ± 2.7 d) after immunization of wild-type mice while at this time point none of the coronin 1-deficient mice showed any signs of disease (Fig. 1A). Onset and progression of disease were also reflected by weight loss (Fig. 1B). Although by day 17 all wild-type mice had developed full-blown disease with a mean maximal clinical score of 3.1 ± 0.4, only 2 of 32 coronin 1-deficient mice showed clinical signs of EAE. During the entire period of observation, only 13 of 32 coronin 1-deficient mice experienced development of EAE symptoms, which were characterized by a significantly delayed onset of disease (23.6 ± 6 d) and a reduction in disease severity (maximal clinical score, 1.8 ± 0.9) in comparison with wild-type mice (Fig. 1C–E).

Prolonged monitoring of the EAE development up to 60 d revealed a continuous slow-progressing remission in wild-type mice, whereas clinical scores of the coronin 1-deficient mice stayed low also at these late time points after immunization (Supplemental Fig. 1).

To analyze the relative lesion size in spinal cords from immunized wild-type and coronin 1-deficient animals, we stained histological sections with F4/80, a marker for activated microglia/macrophages. Consistent with the aforementioned clinical EAE symptoms, all wild-type mice showed massive inflammatory lesions in the spinal cord; in contrast, only some coronin 1-deficient mice experienced development of inflammatory lesions (Fig. 1F).

Furthermore, also confirming the clinical observations, the lesions of coronin 1-deficient spinal cord were smaller and observed mainly in the lumbar part, which reflects less progressed disease (Fig. 1F). We conclude from these results that coronin 1-deficient mice are largely resistant to EAE induction.

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** EAE pathology in the presence and absence of coronin 1. Coronin 1-deficient (coro1−/−) and wild-type (WT) mice were immunized with MOG35–55 peptide in CFA to induce active EAE. A–E, Disease development and progression were monitored over time using the scoring system described in Materials and Methods. A, Mean clinical score ± SEM of three independent experiments (n = 20). B, Mean body weight ± SEM of two independent experiments (n = 10). C and D, Maximal clinical score and day of disease onset are displayed for every single mouse together with the mean. The numbers included in the graphs represent the mean ± SD calculated only for animals showing signs of EAE (five independent experiments; n_{coro1−/−} = 32; n_{WT} = 33). C, D, Average disease incidence, results of five independent experiments are shown. F, Immunohistological analyses of spinal cord sections (lumbar) at end of the experiment and quantification of lesion sizes (percent of area of the whole spinal cord cross section) of the individual animals (n_{coro1−/−} = 15; n_{WT} = 14). Representative F4/80 staining of wild-type and coronin 1-deficient mice are shown. Scale bar, 500 μm. The corresponding maximal clinical score is displayed.
Immune responses during EAE development in the presence and absence of coronin 1

To monitor immune responses during EAE initiation and progression in wild-type and coronin 1-deficient mice, we took blood samples before and at several time points after immunization. Determination of leukocyte cell counts in the blood after lysis of erythrocytes revealed a strong increase in cellularity in both wild-type and coronin 1-deficient mice upon immunization, whereas nonimmunized mice analyzed in parallel showed no changes in their immune cell composition in blood (Fig. 2). Specifically, immunization led to a comparable increase in leukocyte, granulocyte, and lymphocyte counts (Fig. 2A, 2B), as well as CD4+ T cells (Fig. 2C) in both mouse strains, although overall coronin 1-deficient mice had lower numbers of leukocyte subsets compared with wild-type mice. These results suggest an equal mobilization and activation of immune cells in the presence and absence of coronin 1 as a consequence of immunization, independent of the development of signs of autoimmune encephalomyelitis.

Because on immunization both in the presence and absence of coronin 1 changes in immune cell composition in blood were observed, we addressed whether the resistance to EAE induction in the absence of coronin 1 can be explained by an altered cytokine response. To that end, the ability of coronin 1-deficient CD4+ T cells to produce the Th1-cytokine IFN-γ, the Th17-cytokine IL-17A, and the Th2-cytokine IL-4 was analyzed upon polyclonal stimulation with anti-CD3/CD28 Abs in vitro. Interestingly, coronin 1-deficient mice had equal or even increased numbers of IFN-γ+, IL-17A+, and IL-4+ CD4+ T cells in comparison with wild-type mice (Fig. 2D). Of note is the IL-4-expression that was significantly increased in coronin 1-deficient as compared with wild-type CD4+ T cells, both in naive and immunized wild-type and coronin 1-deficient mice. However, immunization resulted in elevated expression of all analyzed cytokines in both mice strains. Thus, the capability to express cytokines upon polyclonal stimulation ex vivo is similar between coronin 1-deficient and wild-type CD4+ T cells, and therefore cannot explain the resistance of the coronin 1-deficient mice to EAE induction.

We next determined whether the observed resistance of coronin 1-deficient mice to EAE induction was due to defective peripheral T cell responses to the initial immunization with MOG35–55 peptide. MOG-specific immune responses were analyzed ex vivo at different time points after immunization. Cytokine expression, as well as proliferation, was determined at days 6, 12, 16, and 40 after immunization, reflecting time points before disease induction, at time of disease induction, at the peak of clinical signs, and during chronic disease state, respectively. Although coronin 1-deficient mice did not show any signs of EAE up to day 16, some of the wild-type mice had already developed disease symptoms at day

![Figure 2. Immune response of wild-type and coronin 1-deficient mice on EAE induction.](http://www.jimmunol.org)
12, and all of them by day 16 (Fig. 1 and asterisks in Fig. 3A). Polyclonal restimulation in vitro at the indicated time points revealed that the frequency and cell counts of IFN-γ and IL-17A–expressing CD4+ T cells increased in wild-type and in coronin 1-deficient mice on immunization (Supplemental Fig. 2). However, although the cytokine expression peaked in wild-type mice around day 12, the number of cytokine-positive CD4+ T cells of coronin 1-deficient mice increased progressively, indicating different kinetics of cytokine induction between wild-type and coronin 1-deficient mice. The analyses of MOG-specific cytokine expression by ELISA revealed that similar to the results of polyclonal cytokine expression, coronin 1-deficient CD4+ T cells showed delayed MOG-specific cytokine responses. But in contrast with the cytokine expression on polyclonal stimulation, IFN-γ and IL-17A secretion was overall lower in MOG-stimulated splenocyte cultures from coronin 1-deficient mice (Fig. 3A). However, some of the immunized coronin 1-deficient mice secreted IFN-γ and IL-17A on stimulation with MOG at levels as high as wild-type mice, but not in all of those coronin 1-deficient animals was a correlation with the clinical score observed. This is exemplified by an increased IL-17A secretion in two mice without any signs of disease (Fig. 3A). In contrast with the high IFN-4 expression observed on polyclonal stimulation (Fig. 2D, Supplemental Fig. 2), in none of the samples was any MOG-specific IL-4 detected (data not shown).

Splenocytes from immunized wild-type and coronin 1-deficient mice were isolated at different time points after immunization and stimulated ex vivo with either MOG35–55 peptide or anti-CD3 and anti-CD28 Abs, to analyze the MOG-specific and polyclonal proliferative response. None of the mice, neither wild-type nor coronin 1-deficient, showed MOG-induced proliferation when analyzed at day 6 after immunization or if immunized with CFA solely (Fig. 3B and data not shown). Overall, MOG-specific proliferation of coronin 1-deficient splenocytes was greatly reduced in contrast with splenocytes isolated from immunized wild-type mice, which showed a dose-dependent proliferation, which was strongest at onset of EAE (day 12; Fig. 3B). In contrast with the diminished MOG-induced proliferation of coronin 1-deficient splenocytes, proliferation upon polyclonal stimulation was equivalent to that of wild-type splenocytes, especially at later time points after immunization. Interestingly, coronin 1-deficient splenocytes showed less proliferation upon polyclonal stimulation than wild-type cells at days 6 and 12 after immunization, but this difference was not observed anymore at day 16, suggesting that immunization increased the proliferation capacity of coronin 1-deficient T cells to wild-type levels.

**FIGURE 3.** MOG-specific immune responses of wild-type and coronin 1-deficient mice. Active EAE was induced in coronin 1-deficient (coro1−/−) and wild-type (WT) mice, and MOG-specific responses were analyzed ex vivo during EAE development and at peak of the disease (corresponding to days 6, 12, and 16) in one experiment and during chronic disease state at day 40 in two other experiments. A, IFN-γ and IL-17A concentration in the supernatant of MOG35–55 peptide-stimulated splenocytes (50 μg/ml for 48 h) were analyzed by ELISA. Each symbol represents data from one mouse. Animals that experienced development of signs of EAE are labeled with an asterisk. B, Splenocytes of individual mice were stimulated in vitro with increasing concentrations of MOG35–55 peptide or anti-CD3/anti-CD28 Abs. After 72 h of culture, the cells were pulsed with [3H]thymidine and harvested 16 h later for measurement of radioisotope incorporation. Mean cpm ± SD or cpm of individual mice are depicted. n = 5 (days 6, 12, 16); n = 10 (day 40). n.d., not detectable.

**Immune cell infiltration in spinal cords of wild-type and coronin 1-deficient mice upon induction of active EAE**

EAE is initiated by infiltration of MOG-specific CD4+ T cells into the CNS, where they subsequently induce the recruitment of other immune cells. To analyze time-dependent infiltration of immune cells into the spinal cord of wild-type and coronin 1-deficient mice, we induced active EAE as described and analyzed spinal cord sections at different time points thereafter by histology. No infiltration of immune cells was observed before disease onset (day 6), but an increasing number of infiltrating immune cells (F4/80+ macrophages and CD4+ T cells) was detected at days 12 and 16 in the spinal cords of wild-type but not coronin 1-deficient mice (Fig. 4, lesions marked by arrowheads). In contrast, no infiltration was visible in coronin 1-deficient mice at any of those time points.

Coronin 1+ leukocytes were abundantly present in spinal cord lesions of wild-type mice, although the coronin 1 Ab did not stain the remaining area of the spinal cord sections (Fig. 4). Overall, the degree of immune cell infiltration correlated with the degree of disease severity in both mice strains.

**EAE development in coronin 1-deficient mice after transfer of wild-type CD4+ T cells and subsequent immunization with MOG35–55 peptide**

CD4+ T cells isolated from naive wild-type mice were adoptively transferred to coronin 1-deficient mice before immunization with...
MOG35-55 peptide, to analyze whether the resistance of coronin 1-deficient mice toward EAE is T cell intrinsic. The presence of the transferred wild-type CD4+ T cells in the blood of coronin 1-deficient mice was monitored by flow cytometry (Supplemental Fig. 3). Although the adoptive transfer of 1 and 5 million wild-type cells did not and the transfer of 10 million did only slightly change the EAE outcome, the transfer of 20 million wild-type CD4+ T cells restored EAE susceptibility in all coronin 1-deficient recipients (with the exception of one mouse that lost the transferred cells; Supplemental Fig. 3, Fig. 5). The presence of 20 million wild-type CD4+ T cells in coronin 1-deficient mice increased the average disease incidence rate from 43 to 100%, the average maximal clinical score from 1.3 to 2.8, and the average day of onset from 22.7 to 17.8 (Table I). After transfer of wild-type CD4+ T cells, the disease outcome showed a mixed phenotype: four of six mice experienced development of signs of EAE that were equal in severity and day of onset to that of wild-type mice, whereas two recipients experienced development of EAE, which resembled the lower grade disease observed for coronin 1-deficient mice. We conclude from these results that the resistance of coronin 1-deficient mice to EAE induction is caused by a defective T cell response.

**Frequency and functionality of Tregs in the presence and absence of coronin 1**

Development of autoimmune encephalomyelitis depends on the activation, expansion, and migration of myelin-specific CD4+ T cells into the CNS and is controlled by Tregs that suppress autoreactive T cell responses (reviewed by Ref. 33). Given the severely depressed EAE responses in coronin 1-deficient mice, the impact of coronin 1 deficiency on the number and functionality of Tregs was investigated. Flow cytometric analyses revealed an increased frequency of Foxp3+ (CD25+ and CD252) CD4+ T cells in peripheral lymphoid organs of coronin 1-deficient mice, whereas CD25+ CD4+ effector T cell numbers were comparable between wild-type and coronin 1-deficient mice (Fig. 6). However, the total Treg counts were approximately half of that of wild-type mice, likely as a result of the overall reduced CD4+ T cell counts in coronin 1-deficient mice.

To determine the suppressive function of coronin 1-deficient Tregs, we performed an in vitro suppression assay with FACS-
PC61 treatment led to a strong decrease of CD25+CD4+ T cells, depleted Tregs in both wild-type and coronin 1-deficient mice by increased EAE resistance observed for coronin 1-deficient mice, we degree (Fig. 6 depicted for each individual mouse (n = 7 or 8).

sorted CD25+ and CD25−CD4+ T cells. The results revealed that coronin 1-deficient CD25+CD4+ T cells were anergic similar to wild-type CD25+CD4+ T cells and did suppress the proliferation of CD25+CD4+ responder T cells in the cocultures to a similar degree (Fig. 6B). To address the contribution of Tregs to the increased EAE resistance observed for coronin 1-deficient mice, we depleted Tregs in both wild-type and coronin 1-deficient mice by injection of the anti-CD25 Ab PC61 according to the treatment schedule shown in Supplemental Fig. 4. The efficiency of the treatment was monitored by flow cytometry of blood samples before and at two time points after injection of the depleting Ab. PC61 treatment led to a strong decrease of CD25+CD4+ T cells, as well as Foxp3+CD4+ T cells (CD25+ and CD25−), in coronin 1-deficient and wild-type mice that was observed already 1 wk after treatment and became more evident later (Supplemental Fig. 4).

Although Treg depletion resulted in a more severe EAE in wild-type mice compared with mice that were not treated with Abs, it had no effect on disease severity of coronin 1-deficient mice that remained resistant to EAE induction (Fig. 6C). Thus, these results indicate that resistance to EAE induction in the absence of coronin 1 is not due to deregulated Treg response but is more likely caused by a disturbed naive T cell priming.

**Discussion**

Susceptibility to autoimmune encephalomyelitis, as well as MS, requires activation of myelin-specific T cells that have escaped central tolerance (reviewed in Ref. 34). It is believed that those autoreactive T cells are present in the peripheral naive T cell pool in a state of ignorance (35). However, under certain circumstances, these cells can become activated and differentiate into effector T cells that then acquire access to the CNS. The contribution of the naive T cell pool size and composition for the development of EAE is currently not well defined. We show in this study that mice lacking coronin 1, which possess a drastically reduced peripheral naive T cell pool, are highly resistant to induction of EAE as induced by immunization with myelin-derived Ag (MOG). Resistance to EAE development was T cell intrinsic, because transfer of wild-type CD4+ T cells into coronin 1-deficient mice restored disease susceptibility; furthermore, depletion of Tregs had no impact on the resistance to EAE. Strikingly, leukocyte mobilization on immunization, polyclonal T cell responses, and Treg functions in the absence of coronin 1 were comparable with wild-type mice. Together, these results show that coronin 1-deficient mice are resistant to the induction and development of autoimmune encephalomyelitis.

Coronin 1 is a leukocyte-specific protein that in T cells has been implicated in a variety of processes such as migration, cell signaling, and survival (36). Despite its expression in all leukocyte subsets with the exception of Kupffer cells, the major cell type affected by the absence of coronin 1 in vivo is naive T cells in the periphery; in mice lacking coronin 1 expression, peripheral T cell numbers are greatly diminished (19, 20, 23). Coronin 1 is essential for intracellular calcium mobilization downstream of TCR triggering and the consequent translocation of the NFAT into the nucleus (20). This activation defect of naive T cells lacking coronin 1 is reflected by impaired proliferation and cytokine expression upon TCR stimulation in vitro. However, coronin 1-deficient mice are capable of mounting relatively normal Ab responses to T cell-independent, as well as T cell-dependent, Ags (24). The most striking effect of coronin 1 depletion is the apparent increased spontaneous apoptosis of naive T cells ex vivo (19, 20, 23). This observation, together with the resistance of coronin 1-deficient mice to EAE development described in this article, is in accordance with the finding that the extent of T cell death is decisive for autoimmunity (37).

The resistance of coronin 1-deficient mice to EAE induction as described in this article is consistent with a role for coronin 1 in T cell signaling. Indeed, in the absence of coronin 1, MOG-specific T cell responses such as proliferation and cytokine expression after immunization with MOG35–55 peptide are delayed, as well as reduced, strongly suggesting a priming defect in the absence of coronin 1. In addition, several lines of evidence exclude a role for coronin 1 in Ag processing and presentation: 1) the restoration of EAE on transfer of wild-type T cells as shown in this study; 2) the absence of any defect in either macrophage, B cell, and dendritic cell function (3, 24, 25, 38) and the absence of any gross defects in thymic selection, a process that is heavily dependent on Ag processing and presentation (19–21). This observation, together with the resistance of coronin 1-deficient mice to EAE development described in this article, is in accordance with the finding that the extent of T cell death is decisive for autoimmunity (37).

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Disease Incidence (%)</th>
<th>Average Maximum Clinical Score</th>
<th>Average Day of Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>100</td>
<td>3.5 ± 0.0</td>
<td>12.9 ± 2.0</td>
</tr>
<tr>
<td>Coronin 1−/−</td>
<td>43</td>
<td>1.3 ± 0.3</td>
<td>22.7 ± 2.1</td>
</tr>
<tr>
<td>Transferred</td>
<td>100a</td>
<td>2.8 ± 1.0</td>
<td>17.8 ± 6.1</td>
</tr>
</tbody>
</table>

One mouse that lost the transferred cells was excluded from the calculations.
priming can also occur in the spleen or extralymphoid structures such as in liver (39–41).

Our results suggest a more general activation defect in coronin 1-deficient mice, because splenocytes isolated at several time points after immunization with MOG35–55 peptide proliferated poorly upon ex vivo restimulation with the same peptide. This finding implies a diminished in vivo expansion of MOG-specific T cells in coronin 1-deficient mice on immunization, resulting in a low frequency of MOG-specific effector T cells, which can potentially cause disease. In line with the reduced number of MOG-specific effector T cells, cytokine expression on restimulation with MOG35–55 peptide was reduced in the absence of coronin 1. In contrast, proliferation induced by polyclonal stimulation with anti-CD3 and anti-CD28 Abs was less affected, especially if splenocytes were analyzed at later time points after immunization. Thus, it appears as if the threshold for polyclonal activation in vitro is altered by immunization in vivo.

FIGURE 6. Importance of Tregs in wild-type and coronin 1-deficient mice with and without EAE induction. The frequency and suppressive activity of Tregs was analyzed ex vivo, as well as in vivo. A, The frequency and number of CD25 and Foxp3-expressing CD4+ splenocytes was analyzed by flow cytometry. Representative FACS dot plots gated for CD4 are shown. Each symbol represents data from one mouse (n = 8 or 9; two independent experiments). B, The suppressive capacity of CD25+CD4+ T cells from coronin 1-deficient and wild-type mice was analyzed with an in vitro suppression assay. CD25+CD4+ responders were isolated from coronin 1-deficient and wild-type mice. The ratio of responders to Tregs is depicted. Results are expressed as the mean ± SD. [3H]thymidine incorporation from triplicate cultures. One of two experiments is shown. C, CD25+ cells were depleted in vivo by i.p. administration of anti-CD25 Ab (PC61) 3 and 5 d before immunization with MOG35–55 peptide. The control groups were immunized with MOG35–55 peptide but did not receive Abs. Mean clinical score and mean body weight ± SEM, maximal clinical disease score, and the day of disease onset for individual mice are displayed together with the mean and the EAE incidence. n = 7 or 6, for non–PC61-treated wt.
Furthermore, coronin 1-deficient CD4+ T cells did not display a general defect to differentiate into effector T cells, because the cytokine profile of wild-type and coronin 1-deficient CD4+ T cells was comparable after polyclonal stimulation. This is in line with the finding that the memory T cell pool in coronin 1-deficient mice is comparable with wild-type mice (19, 20). However, this does not explain the observed increase of cytokine-expressing CD4+ T cells after immunization in vivo. Interestingly, expression of IL-4, which has been described to protect from EAE development (8, 9), was even higher in coronin 1-deficient mice. However, because no MOG-specific IL-4 secretion was detected, we rule out that the resistance to EAE is caused by immune deviation to a Th2 response. Another possible explanation for the increased resistance of coronin 1-deficient mice to EAE provides the increased Treg/naïve T cell ratio that was observed in the absence of coronin 1. However, because the depletion of Tregs, which showed normal suppressive activity in vitro, did not restore EAE sensitivity, an important contribution of this T cell subset is unlikely. This finding supports the idea that the resistance of coronin 1-deficient mice to EAE development is primarily a consequence of reduced naïve T cell number and/or impaired activation on immunization.

Nevertheless, some coronin 1-deficient mice experienced development of EAE symptoms and displayed MOG-specific IL-17A and IFN-γ secretion, which was, in some cases, as high as observed for wild-type mice. However, in all of these cases, the disease onset was delayed, even if in these few cases disease severity was indistinguishable from wild-type mice. Consistent with the delayed disease onset of the few diseased coronin 1-deficient mice, MOG-specific IL-17A and IFN-γ secretion was delayed. This observation might be explained by the important role of pioneer cells for immune surveillance and for initiating immune responses in peripheral tissues (42). After priming and differentiation in lymphoid tissues, myelin-specific effector T cells must acquire access to the noninflamed CNS, where they act as pioneer T cells to induce disease. Recently, the impact of CCR6+ Th17 pioneer cells for EAE induction has been highlighted by Reboldi et al. (43). Given the strongly reduced naïve T cell pool and the T cell activation defect of coronin 1-deficient mice, the accumulation of threshold numbers of MOG-specific effector T cells that can function as pioneer cells may be severely disturbed. It is well possible that disease induction in those few diseased coronin 1-deficient animals occurred through the migration of only a few pioneer cells in the CNS. Indeed, our results suggest that coronin 1-deficient MOG-reactive T cells once activated and arrived in the CNS have the same potential as wild-type T cells to activate local tissue cells and mediate the recruitment of further immune cells. In agreement with this idea, we found a clear correlation between EAE symptoms and the extent of immune cell infiltration. The hypothesis of a threshold number of MOG-specific T cells is further supported by our observation that transfer of 1 and 5 million wild-type CD4+ T cells from naive mice did not increase EAE susceptibility, whereas 10 million had already a slight effect and 20 million transferred CD4+ T cells did break resistance of coronin 1-deficient mice to EAE induction. In line with this, it has been shown in rodent models of EAE that modulation of the CD4+ T cell pool, which is available for activation by administration of anti-CD4 Abs, either depleting or inhibitory, prevented development of EAE (44–46).

Taken together, the results presented in this article demonstrate, to our knowledge, a hitherto unknown and specific role for the naïve T cell pool 1 in shaping CD4+ T cell responses toward autoantigens and reveal coronin 1 as a crucial molecule in the induction of autoimmune encephalomyelitis.

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Disclosures

Patents related to this work have been filed (WO2007110385A2 and WO2009112542A1) by the University of Basel.

References