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Regulatory T Cells Selectively Preserve Immune Privilege of Self-Antigens during Viral Central Nervous System Infection

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Regulatory T cells (Tregs) are important for the attenuation of immune reactions. During viral CNS infections, however, an indiscriminate maintenance of CNS immune privilege through Treg-mediated negative regulation could prevent autoimmune sequelae but impair the control of viral replication. We analyzed in this study the impact of Tregs on the development of acute viral encephalomyelitis, T cell-mediated antiviral protection, and prevention of CNS autoimmunity following intranasal infection with the gliatropic mouse hepatitis virus strain A59. To assess the contribution of Tregs in vivo, we specifically depleted CD4+ Foxp3+ T cells in a diphtheria toxin-dependent manner. We found that depletion of Tregs had no impact on viral distribution and clearance and did not significantly alter virus-specific CD4+ and CD8+ T cell responses. However, Treg depletion led to a more severe CNS inflammation associated with neuronal damage. Dissection of the underlying immunopathological mechanisms revealed the elaborate Treg-dependent regulation of self-reactive CD4+ T cell proliferation within the CNS-draining lymph node and downturning of CXCR3 expression on T cells. Taken together, these results suggest that Tregs preserve CNS immune privilege through selective control of CNS-specific Th cells while keeping protective antiviral immunity fully operative. The Journal of Immunology, 2012, 188: 000–000.

Viral infections have been implicated in a variety of autoimmune diseases, including immune-mediated demyelinating disorders such as multiple sclerosis (1, 2). A number of viruses, including EBV (3), human herpes virus-6 (4), human coronavirus OC-43 (5), and human coronavirus 229E (6), have been found in the CNS of multiple sclerosis patients. Furthermore, durable immune responses against these viruses have been detected in affected individuals (4, 7, 8). Infection with neurotropic viruses may not only induce specific antiviral immune responses in the CNS, but also most likely predispose to a stereotyped immunopathological tissue response that precipitates de novo immune reactions against genuine self-antigens (2, 9). Thus, damage control mechanisms in organs with poor regenerative capacity, such as the CNS, have to guarantee efficient control of the pathogen and, at the same time, should attenuate immunopathological bystander and autoimmune reactions. In other words, dedicated regulatory circuits should establish a relative immune privilege in the CNS (10) that shields self-determinants from overshooting immune responses.

The delicate balance between T cell-mediated immunopathology/autoimmunity and pathogen control is maintained by different layers of negative regulatory processes including T cell-intrinsic inhibitory signaling pathways (11) or, more global, T cell-extrinsic processes such as distinct regulatory cell populations (12, 13). Regulatory T cells (Tregs) are a subset of CD4+ T cells that can attenuate T cell proliferation and/or function (14). Tregs are characterized by the expression of particular surface molecules including the IL-2Rα (CD25), CTLA-4, and glucocorticoid-induced TNFR and the transcription factor Foxp3 (15). The multiple mechanisms employed by Tregs to down regulate T cell function include secretion of suppressive cytokines or expression of ligands for co-inhibitory molecules that either directly act on T cells or inhibit the function of APCs (14, 16). The general immunosuppressive function and hence protective role of Tregs has been demonstrated in various settings of experimental autoimmune diseases such as experimental autoimmune encephalomyelitis (17) or transplant rejection (18). In viral infections, Tregs can attenuate virus-mediated tissue damage through improved innate (19) or CD8+ T cell-mediated (20) virus control. However, in other viral infections, the tradeoff for Treg-reduced immunopathology and attenuated disease severity can be a delay in viral clearance and global attenuation of antiviral T cell responses (21, 22), indicating that the function of Tregs in viral infections is context dependent. It is likely that differential Treg-mediated control of T cell reactivity during viral infection is particularly important in organs in which infection-associated immunopathology and initiation of autoimmune reactions would be most detrimental.

To assess the importance of Tregs during viral CNS infection and clarify the role of Tregs in the initiation of CNS-specific T
cell responses, we used the mouse hepatitis virus (MHV) as a model infection. MHV belongs to the family of coronaviridae and permits studies of natural host–pathogen interactions. CNS infection with MHV causes acute encephalomyelitis and can lead to a chronic disease with demyelination and ascending paralysis (23). To specifically deplete Tregs in vivo, we employed DEREG mice that express the human diphertheria toxin (DT) receptor and the enhanced GFP (eGFP) under the control of the Foxp3 promoter (24). We found that depletion of Tregs did neither alter viral distribution and clearance kinetics, nor did it significantly promote the virus-specific CD4+ and CD8+ T cell response. However, Treg-depleted mice showed a more severe CNS pathology that was due to increased recruitment of T cells that did not respond to the known MHV epitopes. Mechanistic dissection of the Treg-controlled inflammation during coronaviral CNS infection revealed that Tregs control the inflammatory processes mainly in the CNS-draining lymph node by suppressing the proliferation of self-reactive Th cells and by global downturning of the expression of CXCR3 on both CD8+ and CD4+ T cells in secondary lymphoid organs. Taken together, this study shows that during viral CNS infection, Tregs can selectively control infection-associated autoimmune immune responses while efficient antiviral immunity is maintained.

Materials and Methods

Ethics statement

Experiments were performed in accordance with federal and cantonal guidelines under permission numbers SG09/83 and SG09/87 following review and approval by the Cantonal Veterinary Office (St. Gallen, Switzerland).

Mice and Treg depletion

C57BL/6 (B6) mice were purchased from Charles River Laboratories (Sulzfeld, Germany). Myeloid oligodendrocyte glycoprotein (MOG35-55)-specific CD4+ TCR-transgenic mice expressing the allelic marker Thy1.1 (2D2 × Thy1.1) were described previously (25). Depletion of Treg mice (DEREG) was described previously (24). 2D2 and DEREG mice were crossed to obtain double-transgenic 2D2 × DEREG mice. CXCR3−/− mice were obtained from the Institut für Labortierkunde (University of Zürich). All mice were maintained in individually ventilated cages and used between 6 and 9 wk of age. To deplete Treg cells, DEREG mice were injected with DT (List Biological Laboratories). Control mice (B6) received the same DT treatment as DEREG mice. Mice were infected intranasally (i.n.) with 500 pfu of the MHV strain A59 via the i.n. route was used to grant the virus access to the CNS. The ensuing acute encephalomyelitis was assayed on day 8 postinfection (p.i.). Control mice (B6) received the same DT treatment as DEREG mice. 2D2 × DEREG donor mice were injected with 1 μg DT 1 d prior to cell isolation.

Virus infections and determination of virus titers

Mice were infected intranasally (i.n.) with 5 × 105 pfu or i.p. with 50 pfu MHV A59 as previously described (26). Mice were sacrificed at the indicated time points, and organs were stored at −70°C until further analysis. MHV titers were determined by standard plaque assay using L929 cells (27). L929 were purchased from the European Collection of Cell Cultures.

Histology

For histological analysis, mice were sacrificed, immediately perfused with PBS (PBS, pH 7.5), and organs were fixed in 4% formaldehyde for at least 12 h and embedded in paraffin. Five-micrometer-thick sections were stained with H&E. Images were acquired using a Leica DMRa microscope (Leica Microsystems) and processed using Adobe Photoshop (Adobe Systems). For immunohistochemical analysis, mice were sacrificed and immediately perfused with PBS, followed by PBS/4% parafomaldehyde (pH 7.5). Organs were further fixed in PBS/4% parafomaldehyde for at least 12 h and embedded in 4% low-melting agarose (USB). Twenty-micrometer sections were cut using a vibratom (Leica VT 1200S; Leica Microsystems). Free floating sections were permeabilized in PBS/1% Triton X-100 (Sigma-Aldrich) and blocked with PBS/0.1% Triton X-100 and 1% normal goat serum (Braunschwig). Sections were further incubated with anti-human CD3 (1:50; AbD Serotec), followed by a Dylight 647-labeled anti-rat secondary Ab (Jackson ImmunoResearch Laboratories). Nuclei were stained with DAPI (Sigma-Aldrich), and sections were mounted with fluorescence mounting solution (DakoCytomation). Images were acquired with Zeiss LSM210 microscope (Zeiss) and processed using ZEN software (Zeiss) and Adobe Photoshop (Adobe Systems). Vessel widths were determined by measuring the perpendicular diameter of cross-sectioned infiltrated vessels including the infiltrates using the IM1000 software (Leica Microsystems).

Cell isolation and flow cytometry

Mice were sacrificed and immediately perfused with PBS. Single-cell suspensions from spleen and cervical lymph nodes (CLNs) were prepared by mechanical disruption of the organs. Brains were mechanically disrupted, and the leukocytes were further enriched using a 70–30% Percoll gradient (GE Healthcare) and centrifugation for 25 min at 800 × g. Analysis of MHV-specific CD8+ T cell responses was performed using PE-conjugated MHV S598/H-2Kb tetramers (Sanquin, Amsterdam, The Netherlands). For surface staining, the following mAbs were used: PE-labeled anti-CD8, anti-CD4 (BioLegend), alloglycocytein-labeled anti-Thy1.1 (eBioscience), anti-CD8, anti-CD4, anti-CXCR3 and, anti-Ly5.1 (BioLegend). 7-Aminoactinomycin D (Calbiochem) was used to discriminate dead cells in flow cytometric analysis. For peptide-specific cytokine production, 107 splenocytes were restimulated with S598 or M133 peptides in the presence of brefeldin A (5 μg/ml) for 5 h at 37°C. Cells were stimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml; both purchased from Sigma-Aldrich) as positive control or left untreated as a negative control. For intracellular staining, restimulated cells were surface stained and fixed with Cytotox-Cytoperm (BD Biosciences) for 20 min. Fixed cells were incubated at 4°C for 40 min with permeabilization buffer (2% FCS/0.5% saponin/PBS) containing anti–IFN-γ, anti–IL-4, anti–IL-17, or anti–IL-10 mAb (BD Biosciences). Foxp3 staining was performed with the mouse regulatory T cell staining kit (eBioscience) using the Foxp3 PE (FIK-16) Ab. Samples were analyzed by flow cytometry using a FACSCalibur (BD Biosciences); data were analyzed using Cell Quest (BD Biosciences) and FlowJo software (Tree Star). Both M133 (TVYRPPIEDYHHTLT) and S598 (RCQIFANI) peptides were purchased from Neosystem (Strasbourg, France). For adoptive transfer experiments, single-cell suspensions from spleens of Thy1.1, 2D2 × Thy1.1, or 2D2 × DEREG × Thy1.1 mice were stained with CFSE (Molecular Probes, Leiden, The Netherlands), and 1.5 × 107 CFSE-labeled splenocytes were transferred i.v. to the recipient mice.

Statistical analysis

All statistical analyses were performed with Prism 4.0 (GraphPad). Data were analyzed with the nonpaired Student t test. A p value of <0.05 was considered significant.

Results

MHV-induced CNS inflammation is aggravated in the absence of Tregs

Access of lymphocytes to the CNS is tightly controlled under homeostatic conditions (28). During infection with the neurotropic MHV, activated CD4+ and CD8+ T cells gain access to the CNS and contribute to viral control (23). In this study, infection with MHV strain A59 via the i.n. route was used to grant the virus access to the CNS. The ensuing acute encephalomyelitis was associated with a significant accumulation of Foxp3+ Tregs that constituted ∼7% of the CNS-infiltrating CD4+ T cells at day 8 p.i. (Fig 1A). Because DEREG mice express the eGFP/DTR receptor transgene only in the CD4+ T cell compartment (Supplemental Fig. 1), administration of DT to MHV-infected mice permitted efficient and specific ablation of Tregs in the CNS and secondary lymphoid organs such as CLNs and spleen, as shown by the almost complete loss of the eGFP signal (Fig 1B). To assess whether Tregs modulate the magnitude of acute MHV-induced CNS inflammation, B6 and DEREG mice were i.n. infected with MHV, treated with DT, and acute CNS inflammation was assessed on day 8 p.i. In situ analysis revealed significantly increased perivascular (Fig 2A, 2B) and intraparenchymal inflammation (Fig 2C) in MHV-infected DEREG mice whereby the majority of the CNS-infiltrating cells expressed the T cell marker.
However, DT treatment of DEREG mice did not enhance infiltration with other immune cells such as macrophages, dendritic cells, or neutrophils (data not shown). Furthermore, Treg-depleted mice showed accumulation of extravascular erythrocytes (Fig. 2D, arrows in *top panel*) and increased numbers of apoptotic neurons (Fig. 2D, arrows in *bottom panel*, 2E) in areas of high MHV replication such as the hypothalamus. These data indicate that the absence of Tregs during MHV CNS infection exacerbated the T cell-dominated inflammatory response in the CNS.

**Impact of Tregs on antiviral immunity**

Because the lack of Tregs improved access of T cells to the CNS during MHV infection, we tested whether viral replication and distribution would be affected by the altered immune cell distribution. Interestingly, viral peak titers on day 6 p.i. and the kinetics of viral clearance were not different in DT-treated B6 and DEREG mice (Fig. 3A). To assess whether the presence of Tregs could result in more efficient viral clearance from particular regions of the CNS, we determined viral titers on days 6 and 8 in the olfactory bulb, hippocampal region, cortex, cerebellum, and spinal cord. As shown in Fig. 3B and 3C, Treg depletion did not result in an altered distribution of the virus, suggesting that the enhanced T cell infiltration in the absence of Tregs did not significantly affect the host’s ability to cope with the viral infection.

To further delineate to which extent Tregs impact on the antiviral T cell response against MHV, we assessed the CD8+ T cell response against the S598 epitope (29) and the CD4+ T cell response against the M133 epitope (30) in the presence and absence of Tregs. Although the overall T cell density in the absence of Tregs was increased (Fig. 2C), the relative abundance of virus-specific T cells in the CNS was reduced in Treg-depleted mice (Fig. 4A–C). Interestingly, the expected phenotype, namely increased antiviral T cell responses, was only observed in the spleen, where M133-specific CD4+ T cells were increased in the absence of Tregs, whereas similar numbers of M133-specific CD4+ T cells were found in the CNS of B6 and DEREG mice (Fig. 4C, *left panel*). As described previously (24), a residual population of 1 to 2% of Foxp3+CD4+ T cells could not be depleted in DEREG mice (Fig. 4C, *right panel*). Nevertheless, enumeration of the total
numbers of CNS-infiltrating cells revealed that recruitment of virus-specific CD8+ and CD4+ T cells to the CNS was not affected by the Treg depletion (Fig. 4D, 4E), whereas the numbers of CD8+ (Fig. 4D) and CD4+ T cells (Fig. 4E) that were not specific for the known viral epitopes were significantly increased. To assess whether T cells that do not recognize the known MHV epitopes would be under Treg control, we assessed T cell proliferation using BrdU incorporation and determined the expression of the activation markers CD69 and CD44 on the different T cell populations. We found that the absence of Tregs permitted increased T cell proliferation in CLNs (Supplemental Fig. 2A, 2B). However, both antiviral CD8+ (Supplemental Fig. 2C) and CD4+ T cells (Supplemental Fig. 2D) were exempt from the increased BrdU incorporation, indicating that mainly cells with other specificities were controlled by Tregs. Furthermore, tetramer-negative CD8+ T cells in CLNs showed significantly increased CD44 expression (Supplemental Fig. 2F), indicating that Tregs controlled both proliferation and activation of T cells that did not respond to the known MHV epitopes.

To exclude that the observed effects were a consequence of Foxp3 promoter activity-dependent depletion in non-Tregs, we generated mixed bone marrow chimeras using mice with the scurfy mutation (31) as donors. Again, DT-treated DEREG + scurfy→B6 chimeras did not show an increased recruitment of virus-specific CD8+ T cells into the CNS compared with B6 + scurfy→B6 chimeras, but a significantly elevated fraction of cells that did not bind the MHV-specific tetramer (Supplemental Fig. 3). Taken together, these data suggest that the aggravated CNS inflammation in Treg-depleted mice during MHV infection was not due to alterations in the antiviral T cell response, but rather a consequence of activation or differential recruitment of virus-unrelated T cells.

**Tregs efficiently attenuate CNS infiltration by self-reactive CD4+ T cells**

To assess whether MHV infection is able to induce the activation and infiltration of truly self-reactive T cells into the CNS, B6 mice were infected with MHV, and, at the peak of infection at day 6 p.i., CFSE-labeled MOG peptide (MOG35-55)-specific CD4+ T cells from 2D2-transgenic mice (25) were adoptively transferred into infected recipients. As shown in Fig. 5A (top panel), proliferating MOG-specific CD4+ T cells could be detected in CLNs and CNS of MHV-infected mice on day 10 p.i., whereas nontransgenic CD4+ T cells were not activated (Fig. 5A, middle panel), hence excluding inflammation-driven bystander activation. Importantly, i.p. MHV infection, which restricts viral replication to spleen, liver, and lung (32), did not drive 2D2 cells into the CNS (Fig. 5A, bottom panel) indicating that MHV infection drives autoreactive T cells into the CNS only when the cytopathic virus replicates in this compartment. Moreover, although MHV infection in the CNS was cleared around day 12 p.i. (Fig. 3), MOG-specific CD4+ T cells prevailed in the CNS for a prolonged period of time (Fig. 5B). Thus, MHV infection precipitated not only the activation of MOG-specific CD4+ T cells and facilitated their access to the infected tissue, but also fostered the persistence of autoreactive T cells in this vulnerable organ.

To assess whether and to which extent Tregs control the proliferation and infiltration of autoreactive T cells to the CNS, MHV-infected B6 and DEREG mice were treated with DT at days 5, 7, 9, and 11 p.i., and at day 6 p.i., CFSE-labeled splenocytes from 2D2×DEREG mice (treated with DT to eliminate Tregs from the donor population) were adoptively transferred into the infected mice. At day 12 p.i., activation and migration of MOG-specific CD4+ T cells in CNS, CLNs, and spleen was assessed. Treg depletion had a significant effect on both 2D2 T cell activation and their migration into the CNS, with the most pronounced effect on CD4+ T cell activation in the CLNs (Fig. 6A, 6B). However, the absence of Tregs did not significantly alter the differentiation pattern of CNS-infiltrating MOG-specific CD4+ T cells because IFN-γ was the dominant cytokine produced both in the absence and in the presence of Tregs (Fig. 6C). Taken together, these data indicate that Tregs can efficiently restrict the proliferation of CNS-specific CD4+ T cells and that this activity is exerted mainly within the CNS-draining lymph node.

To assess whether Tregs impact on the migration of self-reactive CD4+ T cells from the CNS-draining lymph nodes to the CNS, we first analyzed the expression of chemokine receptors on CNS-infiltrating T cells during MHV infection. Although CCR6 (33) has been implicated in the migration of CD4+ T cells to the CNS, this receptor was not expressed on CD4+ or CD8+ T cells in the CNS during MHV infection (data not shown). Because previous reports have shown that CXCL10 (i.e., one of the ligands of CXCR3) is upregulated in the CNS of MHV-infected mice and critically regulates MHV-induced pathology (34, 35), we assessed whether Tregs have an impact on the expression of CXCR3 on CD8+ and CD4+ T cells during MHV infection. Indeed, ablation of Tregs resulted in a pronounced upregulation of CXCR3 on both CD4+ (Fig. 7A, 7B) and CD8+ T cells (Fig. 7C, 7D) in spleen and CNS-draining lymph nodes. It is noteworthy that CXCR3 expression in CNS-infiltrating T cells was not significantly affected...
by the depletion of Tregs (Fig. 7A–D), suggesting that Tregs selectively down-tune CXCR3 expression on activated T cells within secondary lymphoid organs. The importance of CXCR3-mediated T cell migration during MHV infection is illustrated by the significantly reduced accumulation of T cells within the CNS in the absence of this chemokine receptor (Fig. 7E–H), indicating that, under these conditions, Tregs selectively impact on homing of effector T cells to control virus-mediated CNS immunopathology.

Discussion

Because the CNS is a vital organ with poor regenerative capacity, immune privilege preservation for self-antigens is essential to limit inflammation-associated damage. This study reveals the elaborate regulation of CNS immune privilege by Tregs via two distinct mechanisms: first, by attenuating the proliferation of self-reactive CD8+ T cells within the CNS-draining lymph node, and second, by down-tuning the migration efficacy of T cells to the CNS by pre-empting exaggerated CXCR3 expression on T cells within secondary lymphoid organs.

CNS immune privilege can be regarded as a selective advantage in the protection from immunopathological/autoimmune damage (10). The efficient self-protection from overshooting inflammatory reactions has been attributed to the segregation of the CNS parenchyma from peripheral circuits through the blood–brain barrier. A recent conceptual revision suggests that the blood–brain barrier should be referred to as a capillary barrier for soluble substances, whereas control over cellular neuroinflammatory reactions is maintained by dedicated cell populations at different locations (28). For example, perivascular APCs can regulate access of activated T cells from the vasculature to the CNS parenchyma (36). The present study reveals that Tregs contribute to preservation of CNS immune privilege during viral infection by acting on the activation of self-reactive T cells within the CNS-draining lymph node.

The importance of Tregs for the maintenance of immune privilege has been demonstrated for other tissues and inflammatory settings including transplant rejection (reviewed in Ref. 18) and chronic parasite infection (reviewed in Ref. 37). It is particularly interesting that Tregs can even facilitate immune privilege of Leishmania Ags during skin infection by attenuating inflammatory reactions. Such limited antimicrobial protection permits establishment of the chronic low-level persistence of the pathogen and facilitates long-term protection through the maintenance of T and B cell memory (38). Hence, in this particular setting of chronic pathogen infection, Treg-mediated functional immune privilege (37) can secure coexistence of host and pathogen.

In viral infections, the impact of Tregs on the host–pathogen relationship is strongly context dependent and most likely geared...
toward the optimal net effect for host survival. For example, Treg depletion during respiratory syncytial virus infection can lead to a lag in antiviral T cell recruitment to infected lungs and hence results in transiently elevated viral titers in this organ. However, the overall effect of Treg depletion in this system is, despite a delayed clearance of the virus, a reduced inflammatory lung disease (20). Likewise, during cytopathic HSV-2 infection, Tregs are essential to instruct innate immune cells to migrate to the inflamed mucosal surface and thereby to control the pathogen more efficiently (19). A more classical role of Tregs in terms of global immune suppression has been demonstrated for acute and chronic retroviral infection in which Treg depletion results in...
higher T cell responses and improved control of the virus (21, 39). We present in this paper a third scenario for the impact of Tregs on virus–host interaction: the absence of a significant modulation of antiviral immunity. We observed almost indistinguishable viral distribution patterns, clearance kinetics, and virus-specific CD8+ and CD4+ T cell responses in MHV-infected Treg-depleted DEREG mice when compared with B6 mice. Similar findings were obtained in a recent study by Trandem et al. (40) using adoptive transfer of Tregs into RAG-deficient hosts together with intracranial infection with an attenuated MHV JHM mutant. Although CD4+Foxp3+ T cell-reconstituted RAG-deficient mice showed no altered viral replication pattern compared with mice reconstituted with CD4+Foxp3+ T cells, enumeration of total CD8+ and CD4+ T cells (G) and S598 tetramer-positive CD8+ and M133-responsive IFN-γ+ CD4+ T cells (H) in the CLNs. Pooled data with mean ± SEM from one out of two independent experiments with four mice per group. (E–H) Data represent the mean ± SEM from one out of two independent experiments with four mice per group. Statistical analysis was performed using the Student t test (*p < 0.05, **p < 0.01, ***p < 0.001).

During MHV infection, virus-specific CD4+ T cells can acquire a Treg phenotype (42). Thus, the interpretation that Tregs can selectively contribute to immune privilege of CNS self-antigens during viral infection whereas the initiation of protective T cell responses remains unaltered raises the question of how such selectivity is achieved. We can offer two possible explanations: first, Ag dose has been shown to be an important determinant for Treg differentiation in which high Ag doses favor the induction of effector T cells, whereas low Ag doses lead to increased Treg differentiation (43). Second, it has been shown that the inflammatory environment, in particular the levels of the proinflammatory cytokine IL-6, critically influence the balance of Treg versus T effector differentiation (44, 45). Interestingly, DC–T cell interaction in the presence of high Ag doses favors the production of IL-6 and hence the suppression of Treg induction (43). Furthermore, IL-6 is one of the strongly induced proinflammatory cytokines in secondary lymphoid organs during acute MHV infection (46). It is therefore possible that Tregs exert their negative regulatory effects preferably under low Ag dose/low inflammation conditions such as late release of self-antigens during cytopathic virus infection. Conversely, the activation (or action) of Tregs under high Ag dose/high inflammation conditions within secondary lymphoid organs.

FIGURE 7. Tregs control CNS migration of T cells through the regulation of CXCR3. (A–D) B6 and DEREG mice were infected i.n. with $5 \times 10^4$ PFU MHV on day 0 and treated on days −1, 1, 3, 5, and 7 with DT. Flow cytometric analysis of CXCR3 expression was performed on day 8 p.i. CXCR3 expression on CD4+ (A, B) and CD8+ (C, D) T cells in CNS, CLNs, and spleens of B6 and DEREG mice. (A and C) Representative histograms from B6 (red lines) and DEREG (black lines) mice. Filled plots represent staining with the isotype control Ab. Quantification of CXCR3+ CD4+ (B) and CD8+ (D) T cells in CNS, CLNs, and spleens of B6 and DEREG mice. (E–H) Lack of CXCR3 expression severely impairs migration of CD4+ and CD8+ T cells into the CNS during MHV infection. B6 and CXCR3−/− mice were infected with $5 \times 10^4$ PFU MHV i.n. Lymphocytes of the brain and CLNs were isolated and analyzed at day 8 p.i. (E) Enumeration of CNS-infiltrating CD8+ and CD4+ T cells and (F) S598 tetramer-positive CD8+ and M133-responsive IFN-γ+ CD4+ T cells. Enumeration of total CD8+ and CD4+ T cells (G) and S598 tetramer-positive CD8+ and M133-responsive IFN-γ+ CD4+ T cells (H) in the CLNs. Pooled data with mean ± SEM from two (B) and three (D) independent experiments with four mice per group. (E–H) Data represent the mean ± SEM from one out of two independent experiments with four mice per group. Statistical analysis was performed using the Student t test (*p < 0.05, **p < 0.01, ***p < 0.001).
during acute MHV infection is kept in check by IL-6 or other proinflammatory mediators.

In summary, this study reveals a novel immune privilege paradigm for Treg function; namely, the ability of these cells to selectively attenuate CNS-specific Th cells while keeping antiviral immunity fully operational.

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Disclosures

The authors have no financial interests of interest.

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