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Role of CD25$^+$ CD4$^+$ T cells in acute and persistent coronavirus infection of the central nervous system ☆

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ABSTRACT

The influence of CD25$^+$CD4$^+$ regulatory T cells (Treg) on acute and chronic viral infection of the central nervous system (CNS) was examined using a glial tropic murine coronavirus. Treg in the CNS were highest during initial T cell mediated virus control, decreased and then remained relatively stable during persistence. Anti-CD25 treatment did not affect CNS recruitment of inflammatory cells. Viral control was initially delayed; however, neither the kinetics of viral control nor viral persistence were affected. By contrast, the absence of Treg during the acute phase resulted in increased demyelination during viral persistence. These data suggest that CNS inflammation, progression of viral control and viral persistence are relatively independent of CD25$^+$CD4$^+$ Treg. However, their absence during acute infection alters the ability of the host to limit tissue damage.

Introduction

Regulatory T cells (Treg) which express the Foxp3 transcription factor, neuropilin 1 (Nrp-1) and the IL-2 receptor (CD25) comprise ~10% of CD4$^+$ T cells in the naïve animal and play an essential role in regulating the immune response to infection, progression of clinical disease and tissue damage (Langier et al., 2010; Lourenco and La Cava, 2011; Rouse et al., 2006; Rowe et al., 2012). During viral infection the host is challenged to mount an effective anti-viral immune response while minimizing immune mediated damage. Exuberant T cell effector function and tissue damage are regulated by sustained natural Treg (nTreg), induction of antigen specific Foxp3$^+$ Treg (iTreg), secretion of the anti-inflammatory cytokine IL-10 by both Foxp3$^+$ and Foxp3$^-$ T cells, as well as inhibitory ligand receptor interactions (Belkaid, 2007; Curotto de Lafaille and Lafaille, 2009; Langier et al., 2010; Rowe et al., 2012). Treg influence the immune response during a variety of acute viral infections (Rouse et al., 2006; Rowe et al., 2012; Zelinskyy et al., 2009) and are implicated in facilitating persistent infections in both humans and mice (Dittmer et al., 2004; Rowe et al., 2012; Xu et al., 2006). However, their suppressive role and the mechanism(s) of suppression vary depending upon both the pathogen and primary tissue infected. Following mucosal infection by Herpes simplex virus type 2 (HSV-2) Treg facilitate recruitment of virus effectors to the site of infection (Lund et al., 2008). They also enhance the severity of murine hepatitis virus (MHV) induced hepatitis due to their expression of the immunosuppressive cytokine fibrinogen-like protein 2 (Shalev et al., 2009). By contrast, during acute respiratory syncytial virus (RSV) infection of the lung or Herpes simplex virus type 1 (HSV-1) infection of the eye, Treg limit cellular recruitment into the site of infection, diminishing tissue destruction (Lee et al., 2010; Suvas et al., 2004). These findings suggest that Treg play an important role in regulating immunopathology associated with viral infection; however, this anti-inflammatory regulation may also reduce anti-viral activity, leading to delayed clearance and/or viral persistence.

The balance between an effective immune response, limited tissue damage, and establishment of viral persistence is especially critical in the central nervous system (CNS), due to its limited regenerative capacity. Theiler's murine encephalomyelitis virus (TMEV) infection produces an acute encephalitis in mouse strains either susceptible or resistant to chronic CNS infection. Depletion and/or functional inactivation of CD25$^+$ Treg did not affect CNS inflammation or TMEV replication in mice resistant to chronic infection (Richards et al., 2011). The identical depletion strategy resulted in both enhanced inflammation and control of TMEV replication in the CNS of mice susceptible to chronic infection (Richards et al., 2011). By contrast, Treg depletion

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prior to acute infection with a neuronotropic MHV, which also produces chronic demyelination, did not alter CNS inflammation or virus specific T cell responses (Cervantes-Barragan et al., 2012). Following CNS infection with an MHV variant containing a mutation in the immunodominant $CD4^+$ T cell epitope which ameliorates disease, anti-CD25 mediated Treg depletion increased both morbidity and mortality (Anghelina et al., 2009). A beneficial role of Treg was also supported by adoptive transfer of nTreg at a time when CNS infection by a sub-lethal, glial tropic JHM strain of MHV (JHMV) was already established. The increased Treg ameliorated clinical disease and immunopathology without altering viral clearance (Trandem et al., 2010). Nevertheless, the role of $CD25^+$ Treg in the pathogenesis of acute JHMV encephalomyelitis and progression to persistent CNS infection is unclear. To better define the role of $CD25^+$ Treg early during JHMV induced encephalomyelitis and potential consequences on the chronic infection associated with sustained demyelination, the present study examined depletion/functional inactivation of $CD25^+$ cells in wild type (WT) and syngeneic IL-10 reporter mice. The Treg population, composed of both $Nrp1^{hi}$ and $Nrp1^{low}$ Treg, peaked in the CNS during acute infection, declined as virus was controlled, and was retained in the CNS during viral persistence. The absence of $CD25^+CD4^+$ T cells did not influence the composition or extent of the CNS inflammatory cells, including virus-specific $CD8^+$ T cells. However, CD25 depletion transiently impaired infectious virus control in the absence of detectable differences in *ex vivo* cytolytic effector function. The transient delay in virus control correlated with increased tissue damage although viral persistence within the CNS was not altered. These data support the concept that the regulation of the immune response within the CNS by $CD25^+$ T cells during JHMV infection is confined to a temporally narrow window during the initiation and effector phase of the acute inflammatory response.

Results

Kinetics of $CD25^+$ Nrp-1^{hi} nTreg and Nrp-1^{low} iTreg accumulation in the CNS

Prior to initiating anti-CD25 monoclonal antibody (mAb) treatment the kinetics and relative composition of $CD25^+$ and $Foxp3^+CD4^+$ T cells recruited into the CNS were assessed following JHMV infection. CNS accumulation of $CD25^+$ and $Foxp3^+CD4^+$ T cells followed similar patterns throughout infection (Fig. 1A). Total numbers of both $CD25^+CD4^+$ and $Foxp3^+$ T cells peaked at day 7 post infection (p.i.) comprising ~20% of total $CD4^+$ T cells (Fig. 1). These populations declined rapidly by day 10 p.i. and stabilized thereafter (Fig. 1B and C). Importantly, >75% of $CD25^+$ cells expressed $Foxp3^+$ at day 7 p.i. indicating a minority of ~25% non Treg $CD4^+$ effector cells expressed CD25 (Fig. 1A and D). These data show that the vast majority of the $Foxp3^+$ population expressed CD25 (Fig. 1A and D) and this proportion remained stable at 75–80% throughout the infection (Fig. 1A and D).

To distinguish a phenotypic transition of Treg populations, possibly accompanied by differential expression of CD25, nTregs were identified based on high Nrp-1 expression (Weiss et al., 2012; Yadav et al., 2012). At day 7 p.i., $Nrp\text{-}1^{hi}$ nTreg represented the majority (~75%) of $Foxp3^+$ Treg within the CNS (Fig. 2A and B). At day 10 p.i., the frequency of $Nrp\text{-}1^{hi}$ nTreg declined to ~50% and remained stable at all subsequent time points, resulting in an equal proportion of $Nrp\text{-}1^{hi}$ and $Nrp\text{-}1^{low}$ Treg (Fig. 2A and B). Similar to the total $Foxp3^+$ population (Fig. 1D), CD25 expression remained stable at ~75% on both $Nrp\text{-}1^{hi}$ (Fig. 2C) and $Nrp\text{-}1^{low}$ Treg during the course of infection (Fig. 2C and D). These data predicted that the majority of $CD25^+Foxp3^+$ Treg are susceptible to anti-CD25 treatment. Moreover, CD25 treatment at early times during infection was anticipated to primarily target prevailing $Nrp\text{-}1^{hi}$ Treg and only a minor population of $CD25^+$ effector T cells.

Early $CD25^+$ T cell depletion does not alter morbidity or inflammation

The role of $CD25^+$ Treg in JHMV induced sub-lethal encephalomyelitis and viral persistence was thus examined by infection of mice treated with anti-CD25 mAb at day -3, 0, and +3 relative to infection. JHMV induces clinical symptoms associated with encephalitis which transitions to predominantly hind limb paralysis. Severity of clinical symptoms reflects both viral load and the antiviral immune response (Bergmann et al., 2006; Kapil et al., 2009; Weiss and Leibowitz, 2011). Anti-CD25 treatment did not alter disease onset, severity or the progression of clinical symptoms

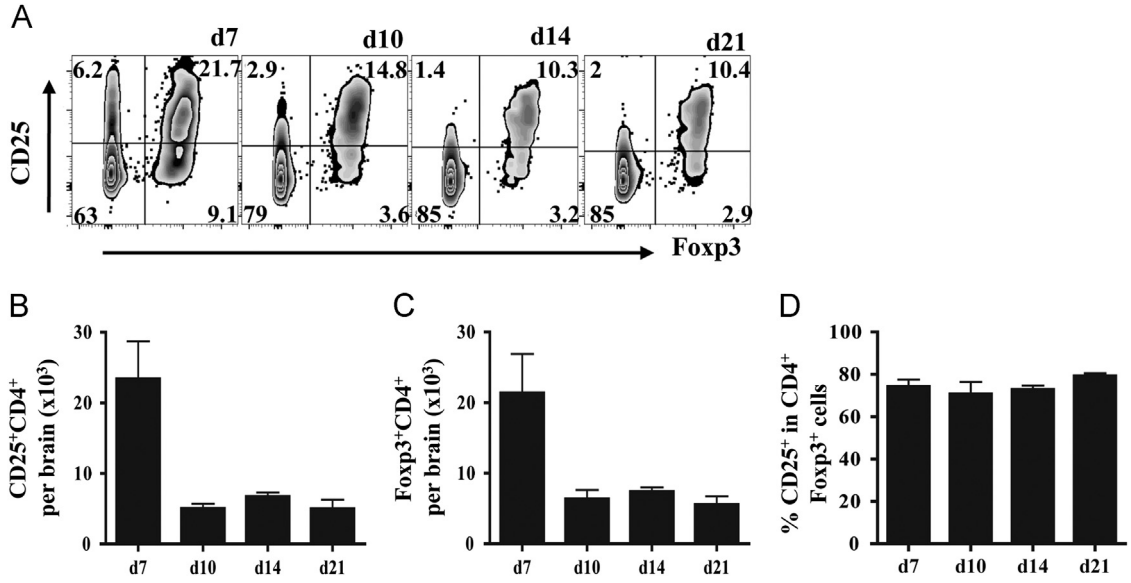

Fig. 1. Recruitment of $CD25^+$ Treg into the CNS. $CD25^+Foxp3^+CD4^+$ T cells in the CNS of infected mice analyzed by flow cytometry. (A) Representative density plots of Foxp3 and CD25 expression, gated on $CD4^+$ T cells. Numbers represent percentages of each population. Bar graphs depict total $CD25^+$ (B) and total $Foxp3^+$ $CD4^+$ T cells (C) recruited into the infected CNS. (D) Frequency of $CD25^+$ within $CD4^+$ $Foxp3^+$ T cells. Data represent mean ± SEM of 6–9 individual mice per time point from at least 2 separate experiments.