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Expression of the Mouse Hepatitis Virus Receptor by Central Nervous System Microglia

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Detection of the mouse hepatitis virus receptor within the central nervous system (CNS) has been elusive. Receptor expression on microglia was reduced during acute infection and restored following immune-mediated virus control. Receptor down regulation was independent of neutrophils, NK cells, gamma interferon, or perforin. Infection of mice devoid of distinct inflammatory cells revealed $CD4^+$ T cells as the major cell type influencing receptor expression by microglia. In addition to demonstrating receptor expression on CNS resident cells, these data suggest that transient receptor down regulation on microglia aids in establishing persistence in the CNS by assisting virus infection of other glial cell types.

Interactions between the mouse hepatitis virus (MHV) spike (S) protein and the virus receptor influences tropism, spread, and pathogenesis (8, 17). The primary MHV receptor is the four-immunoglobulin isoform of CEACAM1a (1, 4). Low-affinity binding to related molecules and receptor-independent infection in vitro have also been described (5, 7, 9, 25–27). Despite limited in vitro receptor usage by the neurotropic JHM strain of MHV (JHMV) (5, 25, 27), central nervous system (CNS) ependymal cells, microglia, oligodendroglia, astrocytes, and neurons are infected in vivo (22). Low levels of MHV receptor mRNA are expressed by the CNS (5, 24). However, no receptor was identified by a direct binding assay (23), and only CNS endothelial cells, but not glial cells, expressed the receptor by immunohistochemistry (10). The absence of CNS expression led to proposals of alternate lower-affinity receptors (5) or a receptor-independent mechanism of virus infection (15). To resolve this issue, CEACAM1a expression on $CD45^{low}$ microglia isolated from the CNS of adult mice was examined. CNS mononuclear cells were isolated from homogenates by using a Percoll step gradient as described previously (2, 3). CNS-derived cells were labeled with phycoerythrin, fluorescein isothiocyanate, or allophycocyanin-labeled monoclonal antibodies (MAb) specific for CD45 (30-F11), CD4 (RM4-5), CD8 (53-6.7), MHC class II I-A/I-E (2G9), Ly-6G (1A8), CD11b (M1/70) (BD PharMingen, San Diego, Calif.), and F4/80 (Serotec, Raleigh, N.C.). Receptor expression was detected by using biotinylated MAb CC-1 (23) and phycoerythrin-conjugated avidin. Biotinylated immunoglobulin G1 (BD PharMingen) was used as an isotype control. Cells were analyzed on a FACS Calibur flow cytometer (Becton Dickinson, San Jose, Calif.), using Cellquest software. CEACAM1a was expressed by microglia from naïve susceptible C57BL/6 and BALB/c mice but not by microglia from resistant SJL mice (National Cancer Institute, Fredrick, Md.) (Fig. 1).

Microglia from BALB/c mice infected intracerebrally with 500 PFU of the J.2.2v-1 MAb-derived variant of JHMV (8) were examined at various times postinfection (p.i.). Whereas receptor expression was unaltered at 5 days p.i, expression decreased at 7 days p.i. based on mean fluorescence intensity (MFI) (Fig. 2A). The majority of microglia regained CEACAM1a expression by day 14 p.i. (Fig. 2A), and this expression returned to uninfected levels by day 30 p.i. (data not shown) concomitantly with virus clearance and partial resolution of inflammation. Similarly, microglia from C57BL/6 mice infected with 250 PFU of JHMV also exhibited reduced receptor expression by 7 days p.i. (Fig. 2B). Although down regulation was protracted in infected C57BL/6 mice compared to BALB/c mice (Fig. 2A and B), receptor was reexpressed at naïve levels by day 30 p.i. (Fig. 2B). These results suggest that either virus or inflammation regulates microglia receptor expression. Intracellular S protein-receptor interaction (18) or extracellular progeny could inhibit receptor detection. Sequestration appears unlikely, as only a small fraction of microglia are infected (22). To distinguish between a viral effect and inflammation, receptor expression was investigated during uncontrolled virus replication in mice immunosuppressed by irradiation (850 rad) 24 h prior to infection (16, 28) and in immunodeficient SCID mice (3). At day 7 p.i., receptor expression on microglia from infected irradiated BALB/c mice (Fig. 3A) and C57BL/6 mice (data not shown) was equivalent to that on microglia from naïve mice. No loss in receptor expression was noted in a limited number of survivors at day 9 p.i. (data not shown). $CD45^{high}$ inflammatory cells (Fig. 3D) were below the detection level (16, 28). Unaltered receptor expression despite uncontrolled virus replication (16, 28) indicates that neither intracellular trapping, extracellular binding of viral particles, nor soluble S protein masks receptor detection.

CNS infection induces the recruitment of innate and adaptive immune components (2, 3, 29). SCID mice were infected with 500 PFU of JHMV to determine whether neutrophils, NK

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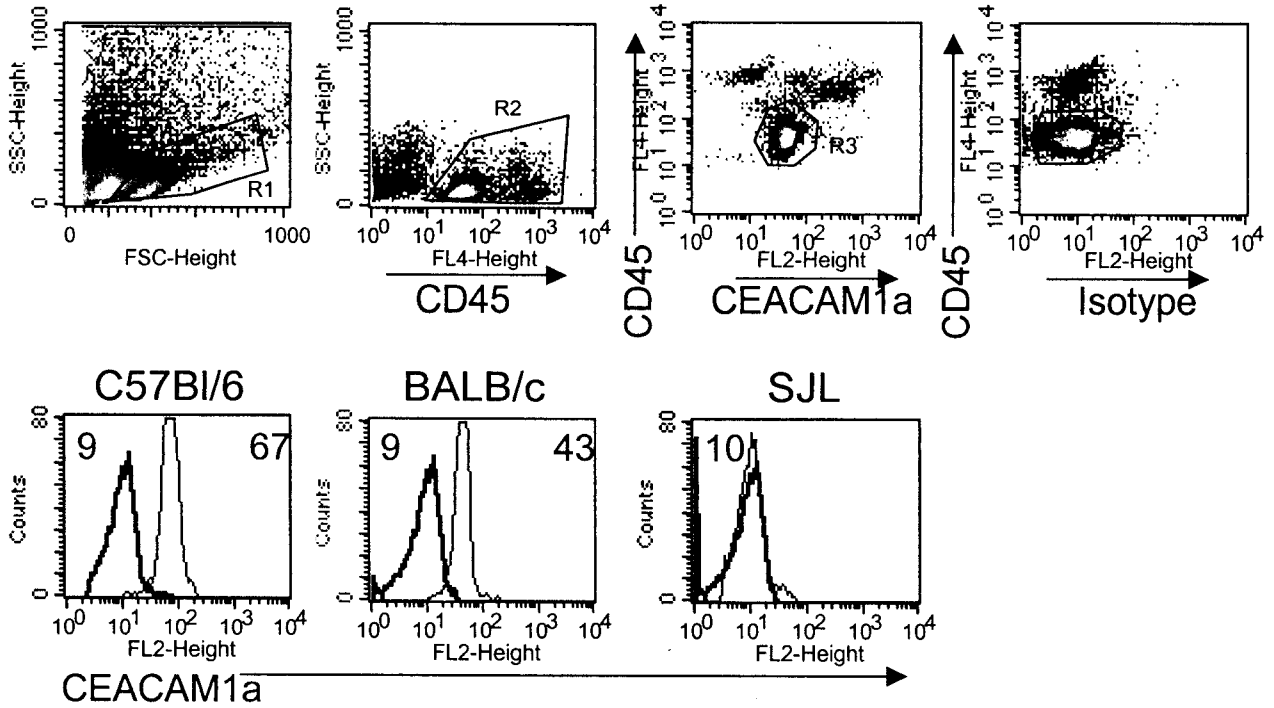


FIG. 1. CEACAM1a expression on microglia from adult mice. Single-cell brain suspensions from naïve mice were stained with anti-CD45, anti-CEACAM1a, or isotype control MAb. In the top row are representative density plots showing forward-side scatter and staining patterns. R1 indicates the live cell gate used for analysis. CD45 expression differentiates $CD45^{low}$ microglia and $CD45^{high}$ infiltrating cells (combined in R2). R3 and R4 highlight $CD45^{low}$ microglia stained with CC-1 or isotype control MAb. Shown in the bottom row is CEACAM1a expression (light lines) on microglia from C57Bl/6 and BALB/c mice but not SJL mice (R3). Dark lines represent isotype control MAb (R4); numbers are MFI values for CEACAM1a (right) and the isotype control (left).

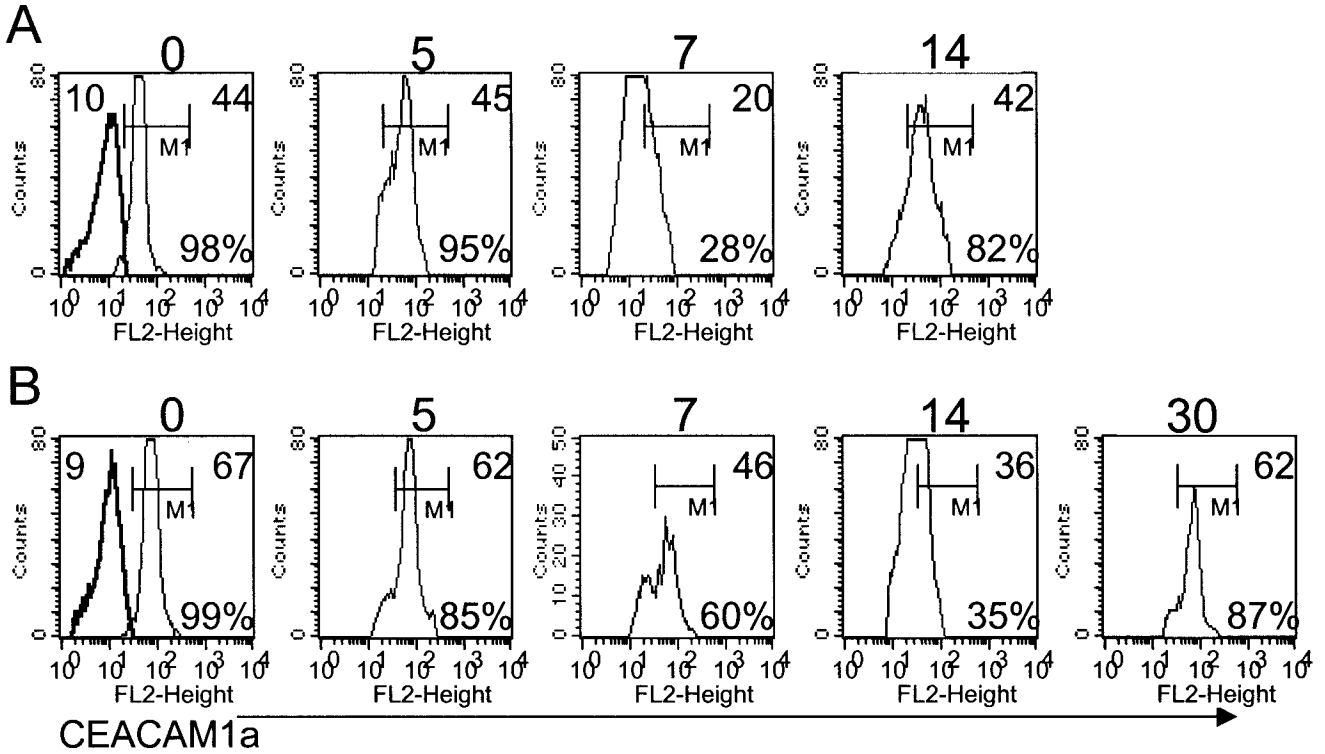
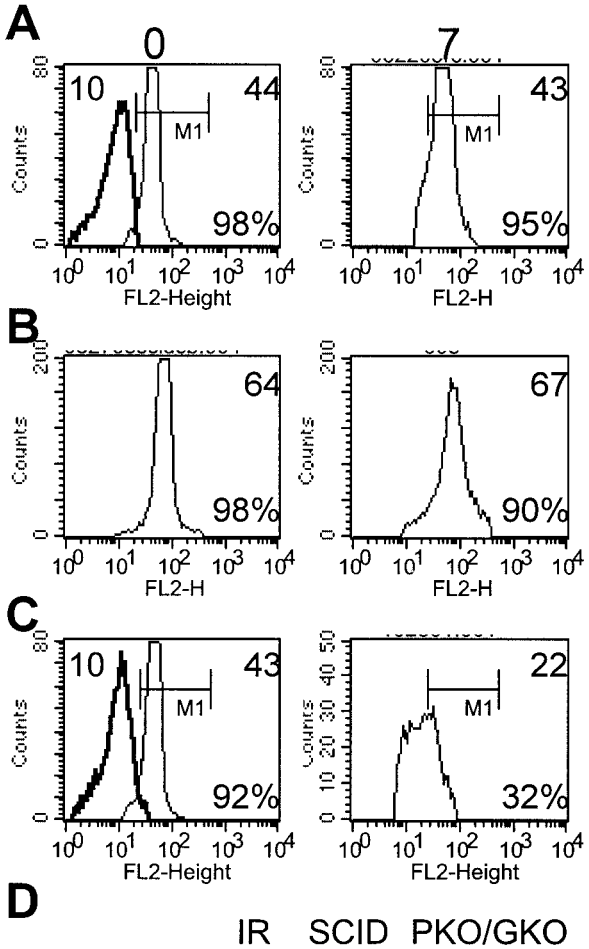


FIG. 2. CEACAM1a expression on microglia is down regulated following JHMV infection. $CD45^{low}$ microglia from naïve mice compared with those from infected BALB/c (A) or C57Bl/6 (B) mice at days 5, 7, 14 and 30 p.i. were analyzed for CEACAM1a expression. The number above each histogram is the number of days p.i. Regions and gates are described in Fig. 1. Numbers in the upper parts of the histograms are MFI values for MAb CC1 (right) and the isotype control (left). Percentages of microglia positive for CEACAM1a expression are given in the bottom parts of the histograms. The data presented are representative of three to five experiments.



	IR	SCID	PKO/GKO
CD45^{hi}	3	32	65
CD8^{+}	0	0	27
CD4^{+}	0	0	10
Ly-6G^{+}	0	10	21
NK^{+}	0	5	1
F480^{+}	3	18	6

FIG. 3. CEACAM1a expression on microglia from immunodeficient mice. (A to C) CD45^{low} microglia isolated from naïve (left) or infected irradiated BALB/c (A), SCID (B), or PKO/GKO (C) mice at day 7 p.i. (right) stained for CEACAM1a (light line) or the isotype control (dark line). Regions and gates are described in Fig. 1. Numbers in the upper parts of the histograms are MFI values for CEACAM1a (right) or the isotype control (left). Percentages of microglia expressing CEACAM1a are given in the bottom right parts of the histograms. (D) Percentages of CD45^{high} infiltrating cells and distinct phenotypic populations in irradiated BALB/c (IR), SCID, and PKO/GKO mice at 7 days p.i. The data presented are representative of three to five experiments.

cells, or macrophages regulate receptor expression (Fig. 3D). Microglia from uninfected and infected SCID mice exhibited no difference in receptor expression at 7 days p.i. (Fig. 3B) or at 10 days p.i. (data not shown). These data confirm that decreased receptor expression is not associated with virus replication and suggest that neutrophils, matrix metalloprotease-9 secretion (29), NK cells, and macrophages are not sufficient to influence receptor expression. To test gamma interferon (IFN-γ) secretion and perforin-mediated cytolysis as potential mediators, mice deficient in both functions (PKO/GKO mice) (2) were infected with 500 PFU of JHMV. Receptor expression on microglia from infected PKO/GKO mice was decreased at day 7 p.i. (Fig. 3C), indicating that neither of these anti-viral effector mechanisms are sufficient to mediate decreased receptor expression.

The fact that receptor expression in infected SCID mice was distinct from that in infected PKO/GKO mice suggested a link between T-cell infiltration and reduced receptor levels. Mice were therefore depleted of CD4^{+} T cells (MAb GK1.5) or CD8^{+} T cells (MAb 2.43) by MAb treatment on days −1, +1, and + 3 relative to infection (Fig. 4A). Controls received anti-β-galactosidase MAb GL113. The majority of microglia isolated from infected CD4^{+}-T-cell-deficient mice at day 7 p.i. retained receptor expression compared to controls (Fig. 4B). Receptor loss was less severe on microglia from CD8^{+}-T-cell-depleted mice (Fig. 4B), despite CD4^{+}-T-cell accumulation in the CNS similar to that for infected controls (data not shown). CD45^{high} CD11b^{+}/class II^{+} macrophages from the CNS of CD4^{+}-T-cell-deficient mice also retained receptor expression at 7 days p.i., while a minority of macrophages within the CNS of CD8-deficient mice exhibited decreased expression (Fig. 4C). These data indicate that CD4^{+} T cells and, to a lesser extent, CD8^{+} T cells influence the loss of receptor expression on both microglia and infiltrating macrophages.

Regulation of receptor expression by T cells appears to be a novel method by which immune responses influence the course of viral infection in an immune-privileged site via inhibition of attachment and entry. While protective, cytotoxic mechanisms in the CNS also induce immune-mediated pathology (3), decreased microglial susceptibility may thus be a mechanism to avoid overt immune pathology. The mechanism(s) by which CD4^{+} T cells regulate receptor expression on microglia is unclear but is likely to involve soluble factors, although the possibility of a reversible conformational change cannot be ruled out. JHMV infection results in the release of proinflammatory cytokines, chemokines, and matrix metalloproteinases (13, 16, 28). Analysis of IFN-γ-deficient mice excluded the possibility of a role for IFN-γ in receptor regulation in vivo, despite contradicting in vitro studies (21). IFN-α/β mRNA is also increased in the CNS of immunodeficient mice (unpublished observation), suggesting no correlation with receptor down regulation. However, unlike many potential proinflammatory cytokines that are decreased prior to diminished receptor expression (16), RANTES is maintained until JHMV infection and inflammation resolve (13), correlating with the transient down regulation of CEACAM1a on microglia. Reduced receptor expression occurs as infectious virus declines and thus may contribute to the limiting of virus spread, cytopathology, and potential glial cell-mediated damage. Viral persistence in vitro is associated with both reduced cytopathology (19, 20) and reduced receptor expression (6, 19), while increased expression enhances virus-induced cytopathology (18). Receptor down regulation in vivo appears to be widespread, although only a few microglia or macrophages are infected (22). Down regulation may be specific for only microglia and macrophages, thereby facilitating the infection of other glial cell types not responsive to receptor down regulation. Alter-

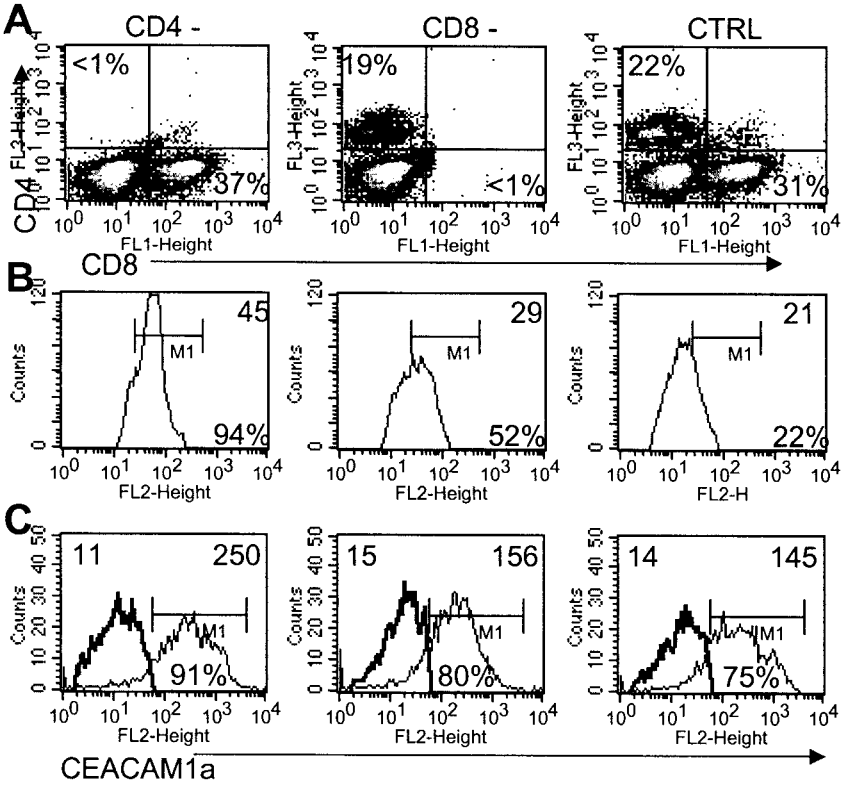


FIG. 4. CEACAM1a expression on microglia and macrophages is regulated by $CD4^+$ T cells. Mice were treated with anti-CD4, anti-CD8, or control MAb. CNS cells from infected CD4-depleted (CD4−; left), CD8-depleted (CD8−; middle), or control (CTRL; right) mice confirm the absence of specific cell types (A). Percentages of $CD4^+$ or $CD8^+$ T cells within the CNS infiltrates are indicated. $CD45^{low}$ microglia (B) or $CD45^{high}$ infiltrating $CD11b^+$/class II^+ macrophages (C) at day 7 p.i. were analyzed for CEACAM1a expression. In panels B and C, numbers in the upper parts of the histograms are MFI values and percentages in the bottom right parts of the histograms are those of cells expressing CEACAM1a. The data presented represent one of three similar experiments.

natively, reduced expression may modulate lymphocyte function. CEACAM1a signaling increases T-cell chemotaxis and cytokine secretion (12, 14) as well as B-cell activation (11). Thus, reduced receptor on CNS resident antigen-presenting cells may reduce T-cell recruitment, cytokine secretion, and possibly cytolysis in an attempt to limit immune-mediated pathology.

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