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Epithelial V-like antigen regulates permeability of the blood–CSF barrier

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ABSTRACT

Epithelial V-like antigen (EVA), a CD3-binding immunoglobulin-like protein, regulates embryonic thymic development. Here we demonstrate that EVA is expressed in choroid plexus from mature immune competent and lymphocyte-deficient (RAG−/−) mice. Choroid plexus epithelial cells from RAG−/− mice demonstrated reduced junctional integrity and enhanced permeability that was associated with decreased expression of *E*-cadherin and EVA mRNA as compared to wild-type mice. Following iv infusion of an anti-CD3 antibody (145-2C11) that also binds EVA, expression of *E*-cadherin and EVA mRNA approached levels seen in wild-type mice. Immuno-fluorescent staining for cadherin also revealed decreased expression in untreated RAG−/− mice that could be increased by 145-2C11 treatment. Expression of mouse EVA in HEK-293 cells followed by challenge with 145-2C11 resulted in increased cytosolic calcium that was not seen in control cells. These results suggest that EVA expressed in choroid plexus cells may regulate the permeability of the blood–CSF barrier.

T lymphocytes and other immune cells traffic through the neuroprotective barriers continually in the absence of inflammation to protect the host from severe infections. A steady state number of T lymphocytes is maintained within the CNS to perform this immune surveillance [1]. How this steady state is maintained is unclear. Our prior work and that of others demonstrated that immune surveillance of the brain by T lymphocytes occurs primarily through the blood–CSF barrier rather than the blood-endothelial barrier at the blood–brain barrier (BBB) [2–5]. Transmigration of T lymphocytes through the blood–CSF barrier requires binding of the T cell to P-selectin expressed on the choroid plexus epithelium, and the junctional integrity between the epithelial cells forms the blood–CSF barrier [3–5]. Based on these data, we hypothesized that regulation of steady state numbers of CNS T lymphocytes occurs at the level of the blood–CSF barrier through modification of the junctions between choroid plexus epithelial cells.

Here we analyzed the choroid plexus in wild-type and lymphocyte-deficient mice (RAG−/−). The goal was to determine whether or not the blood–CSF barrier was more permissive in lymphocyte-deficient animals in order to permit enhanced immune surveillance of the CNS and maintain a constant level of T lymphocytes within the CNS. Consistent with this hypothesis, histologic analysis of the choroid plexus epithelium from RAG−/− mice revealed a reduction in the area of junctional contacts between cells that was associated with enhanced permeability and decreased cadherin expression.

In addition, to characterize a potential mechanism to regulate this gating process, we demonstrate differential expression of epithelial V-like antigen (EVA) in choroid plexus from wild-type and immune-deficient mice. EVA is an immunoglobulin-like adhesion molecule that is important in embryonic thymic development [6–7]. It has structural homology to the subunits of CD3, the T lymphocyte marker, and binds a monoclonal antibody raised against mouse CD3 (145-2C11) [6–7]. Here administration of 145-2C11 *in vivo* resulted in normalization of EVA and cadherin expression in RAG−/− mice closer to wild-type levels and led to partial restoration of choroid plexus morphology. *In vitro* activation of EVA by 145-2C11 caused calcium mobilization. These results suggest a novel mechanism to regulate the integrity of the blood–CSF barrier.

Materials and methods

Animals. C57BL6/J wild-type and RAG-1-deficient mice were purchased from Jackson Laboratories and used at 6–8 weeks of age. All mice were maintained in sterile and pathogen-free conditions. All animal studies were reviewed and approved by the Yale University School of Medicine Institutional Animal Care and Review Committee (IACUC). For *in vivo* treatment with 145-2C11, mice were injected with 50 μg of antibody 24 h prior to sacrifice.

Permeability assays. Evans Blue dye (0.1 ml of a 0.5% wt/vol solution in PBS) was injected intravenously into wild-type and RAG-1−/− C57BL6/J mice [8]. One hour later, mice were anesthetized with

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ketamine/xylazine, and intracardiac perfusion was performed with ice cold PBS. Brains were isolated, the choroid plexus was microdissected, and dye was extracted from choroid plexus samples in formamide (5 μl/mg of tissue) for 3 days at room temperature. Absorbance at 650 nm was measured to determine dye concentration.

Immunocytochemistry. Brain frozen sections (7 μm) were either stained with hematoxylin or processed for immunocytochemistry according to standard protocols using bovine serum albumin (BSA), goat serum and Triton X-100 as preincubation blockers [9]. Antibodies were diluted in BSA and Triton X-100 solution and added to the sections for 2 h. Sections were washed and incubated with secondary antibody for an additional 1 h. Primary antibodies were rabbit anti-pan-cadherin (Abcam) and mouse anti-β-tubulin (Invitrogen), and secondary antibodies were Alexa fluorophore conjugated goat antibodies (Invitrogen). Alexa-546 phalloidin was obtained from Invitrogen. Fluorescent images were obtained on a Zeiss Axiovert 200 fluorescent microscope with either a 20× or 40× objective (Zeiss). Quantitative analysis of cadherin staining was performed with Axiovision 4.6.3 Automeasurement.

RT-PCR and real time analysis. Total RNA was isolated from microdissected choroid plexus tissue by guanidine isothyocyanate lysis and solubilization followed by phenol–chloroform extraction. Samples were then cleaned by column purification (Rneasy; Qiagen). Reverse transcription was performed with Superscript II (Gibco). Quantitative PCR was performed using TaqMan primers (FAM-labeled) commercially obtained (Applied Biosystems), and samples were run on a SmartCycler (Cepheid). Samples were normalized to GAPDH C_t values for each experiment. Each experiment was performed at least three times from separate RNA preparations.

Cells and transfections. HEK-293 cells were maintained in DMEM supplemented with 10% FBS and glutamine. Mouse EVA-1 cDNA was obtained from Origene in the expression vector, pCMV6-Kan/Neo. Transfections into HEK-293 cells were performed using Lipofectamine (Invitrogen).

Calcium flux assays. For calcium flux experiments, HEK-293 cells were labeled with 1 μM Fluo-4 for 30 min. at room temperature. Cells were washed three times with HBSS and then analyzed on an LS-50b fluorometer (Perkin Elmer). Excitation was at 494 nm and emission at 516 nm.

Statistics. Comparisons between groups were made using a Student's T test (unpaired, unequal variance) with a $P < 0.05$

Table 1
Real time PCR analysis of EVA and *E*-cadherin expression from microdissected choroid plexus

Condition	EVA (C_t ± SEM)	*n*	*E*-Cadherin (C_t ± SEM)	*n*	GAPDH (C_t)
Wild-type untreated	26.81 ± 0.35*	9	26.24 ± 0.13*	6	20.0
Wild-type 2C11	27.38 ± 0.20	6	27.02 ± 0.19	6	20.0
RAG−/− untreated	29.55 ± 0.13	9	29.18 ± 0.03	6	20.0
RAG−/− 2C11	28.18 ± 0.20**	6	27.88 ± 0.06*	6	20.0

Real time PCR analysis was performed as described in Materials and methods. C_t values were normalized using GAPDH expression as a control ($C_t = 20.0$). For EVA expression, $^{*}P < 0.0001$ and $^{**}P = 0.0003$ as compared to the RAG−/− untreated condition. For cadherin expression, $^{*}P < 0.0001$ for both conditions as compared to the RAG−/− untreated condition. C_t: threshold cycle.

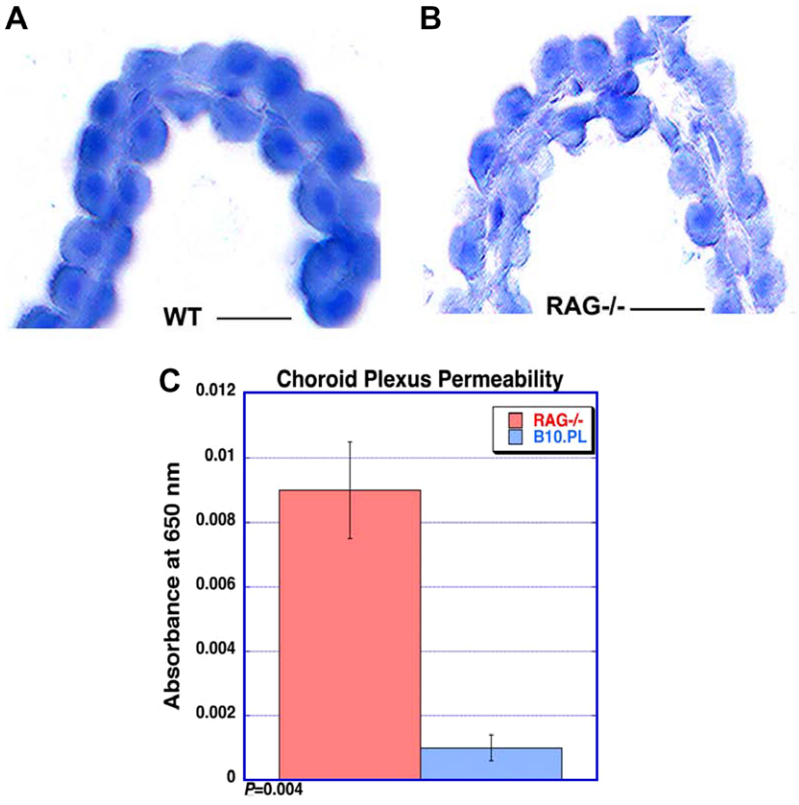

Fig. 1. Altered choroid plexus morphology and increased permeability in lymphocyte-deficient mice. (A,B) The choroid plexus in wild-type C57BL6/J wild-type (A) as compared to lymphocyte-deficient RAG−/− mice (B) demonstrated distinct differences in morphology as determined by hematoxylin staining. The outer epithelial layer is more ordered and continuous in the wild-type as compared to knockout animal (frozen section; hematoxylin). There was no evidence of any inflammatory infiltrate in either sample. (C) Permeability of microdissected choroid plexus was analyzed using the Evans Blue technique. Consistent with the altered morphology observed by histologic analysis, there was substantially greater linkage of intravascular Evans Blue dye into the choroid plexus structure in RAG−/− mice as compared to wild-type controls. As normalized to tissue weight, the OD650 was 0.009 ± 0.0015 for RAG−/− and 0.001 ± 0.0004 for wild-type ($n = 5$; $P = 0.004$). Scale bar, 20 μm.