The protection of A2aR on BBB permeability from Th1 cytokines in MS

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INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease of the CNS characterized by a CD4+ Th1 lymphocyte-mediated autoimmune response, inflammatory cell infiltration and demyelination in the CNS, and progressive and recurrent impairment. Th1 cytokines are key factors in the regulation of inflammatory responses correlated with the loss of blood brain barrier (BBB) integrity. It has been reported that adenosine acting at its receptors, is a potent endogenous regulator of inflammation. Most notably, adenosine prevents the increase in vascular permeability and thus promotes endothelial barrier function. Here we investigated the effects of A2aR manipulation on Th1 cytokines.

OBJECTIVE

To investigate the effects of A2a receptor (A2aR) on the brain endothelium and BBB integrity in MS.

DESIGN/METHODS

MS brain tissue obtained from UCLA were used to detected A2aR expression in MS lesion. We examined the distribution of F-actin and the expression of tight junction proteins in cultured endothelial cells (bEnd.3) treated with Th1 cytokines or Th1 cytokines plus specific A2aR agonist (CGS21680). C57BL/6 mice were immunized by myelin oligodendrocyte glycoprotein (MOG_{35-55}) to induce experimental autoimmune encephalomyelitis (EAE) and then given CGS21680 by i.p. daily. Neurological impairment was evaluated using disease scores. Blood-brain barrier (BBB) permeability was measured by the content of fluorescent tracers, sodium fluoride (Na-F) in CNS after i.p. injection and FITC-dextran in CNS after i.v. injection.

RESULTS

A2aR is expressed by endothelium in human MS and mice EAE lesion

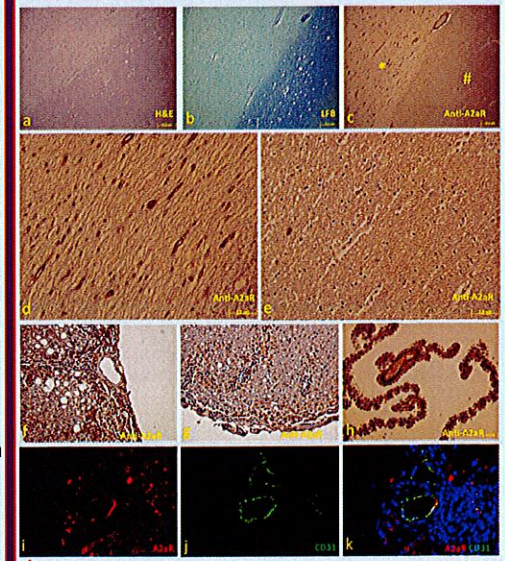


Fig1. a-b: MS lesion in human brain white matter (a: HE staining, b: Luxol Fast Blue Staining c-e: A2aR was expressed by endothelium in de-myelinated area (anti-A2aR immunohistochemistry staining) f-h: in additional to immune cells, endothelium in EAE lesion also expressed A2aR (anti-A2aR immunohistochemistry staining). i-k double fluorescence staining showed that in EAE lesion, CD31 positive endothelium were also A2aR positive.

Fig3. FITC phalloidin staining of F-actin in bEnd.3 endothelium illustrated Th1 cytokines could decrease the circumferential actin in association with a dramatic increase in stress fiber formation, particularly in the central regions of the cells at variable degree. These changes were partially inhibited by CGS21680.

a1:control;a2:IL1β(10ng/ml);a3:TNFα(10ng/ml);a4:IFNγ(10ng/ml);b1:CGS21680(100μM);b2:IL1β(10ng/ml)+CGS21680(100μM);b3:TNFα(10ng/ml)+CGS21680(100μM);b4:IFNγ(10ng/ml)+CGS 21680(100μM).

RESULTS

A2aR specific agonist prevents endothelial cells from injury caused by Th1 cytokines in vitro.

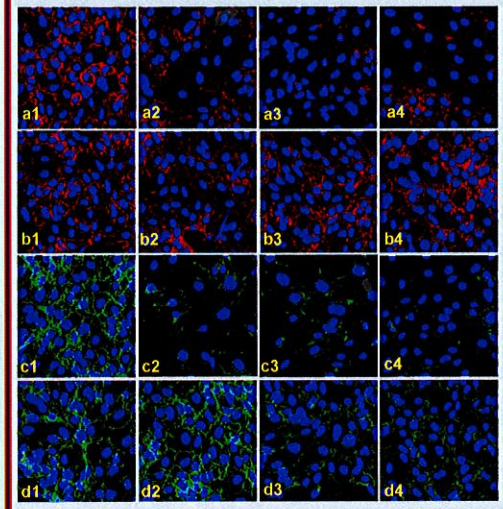
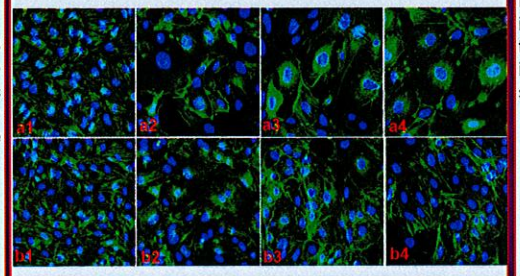


Fig2. The expression of tight junction proteins zo-1 (a1-b4) and claudin5 (c1-d4) were decreased in bEnd.3 cells treatment with th1 cytokines. The effects was prevented by CGS administration. a1, c1: control; a2, c2: IL1β(10ng/ml);a3,c3:TNFα(10ng/ml);a4,c4:IFNγ(10ng/ml);b1,d1:CGS21680(100μM);b2,d2:IL1β(10ng/ml)+CGS21680(100μM);b3,d3:TNFα(10ng/ml)+CGS21680(100μM);b4,d4:IFNγ(10ng/ml)+CGS 21680(100μM).



RESULTS

A2aR specific agonist CGS21680(100ng/kg,i.p.) ameliorates neuro-inflammation and neuro-behavioral deficits in vivo, and decreases the BBB permeability in EAE

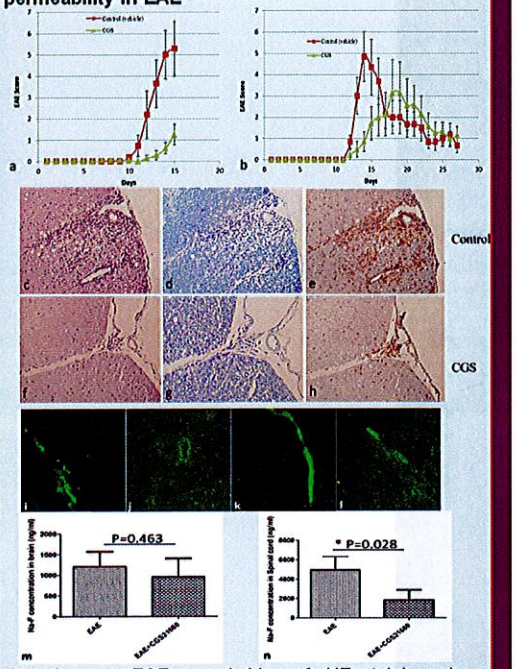


Fig4. Average EAE score(a,b) ; c,f: HE staining; d,g: Luxol Fast Blue staining; e,h: anti-CD45 immunohistochemistry. FITC-dextran as tracer to study the BBB permeability(i Naïve ; j EAE, k: CGS control, i:CGS+EAE).Na-F concentration in EAE brain(m) and spinal cord(n).

CONCLUSIONS

Activation of the A2aR exerts a strong protection on BBB from increased permeability caused by Th1 cytokines. Our results indicated that A2aR agonists could represent a novel therapeutic tool for MS treatment.