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Glutamate signaling through the kainate receptor enhances human immunoglobulin production

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ABSTRACT

CD23 is implicated as a regulator of IgE synthesis. A soluble form of CD23 (sCD23) is released following cleavage by ADAM10 and enhanced sCD23 is correlated with increased IgE. In the CNS, signaling through the kainate receptor (KAR) increases ADAM10. In B cells, activation of KARs produced a significant increase in ADAM10 and sCD23 release as well as an increase in B cell proliferation and immunoglobulin production. In addition, ADAM10 inhibitors reduce IgE synthesis from *in vitro* cultures of human B cells. Thus, we report for the first time the unique presence of the kainate receptor in B cells and that activation of KARs could serve as a novel mechanism for enhancing B cell activation.

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1. Introduction

CD23 (FCεRII) is a type II integral membrane protein and the low affinity receptor for IgE. Through studies using knock-out and transgenic animals, CD23 has been implicated as a natural, negative regulator of IgE production (Texido et al., 1994; Yu et al., 1994). Although the mechanism remains unclear, interactions with cell surface CD21 as well as direct signaling by the membrane form of CD23 have both been hypothesized to be responsible for IgE modulation. A soluble monomeric form of CD23 (sCD23) is released following proteolytic cleavage by a disintegrin and metalloprotease 10 (ADAM10) (Weskamp et al., 2006). Enhanced CD23 cleavage has been shown to correlate with increased IgE production in both mouse and human (Ford et al., 2006; Saxon et al., 1990). In addition to its effects on allergic disease, sCD23 has been linked to the activation of macrophages, via interaction with CD11b/CD18 or CD11c/CD18, resulting in the release of pro-inflammatory mediators and the onset of inflammatory disease (Lecoanet-Henchoz et al., 1995).

In view of the recent demonstration that ADAM10 is the primary CD23 sheddase, we searched for agents that would modify ADAM10 activity. The overall purpose was to test the hypothesis that ADAM10 modulation would, by virtue being the CD23 sheddase, result in IgE modulation. Ortiz et al., (2005) showed that when a specific type of glutamate receptor, namely the kainate receptor (KAR), was stimulated with its ligand, ADAM10 mRNA increased. KARs are one of three types of multi-subunit, ionotropic glutamate receptors which are named based upon their preferred pharmacological ligand: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), N-methyl-D-aspartic acid (NMDA), and kainic acid (KA). KARs are the most recently identified of the three and have been shown to be widely expressed in the central nervous system (CNS) (Chittajallu et al., 1999; Lerma, 2006), however, little is reported on their presence outside the CNS. Kainic acid, a chemical first isolated from the red algae *Digenea simplex*, is a potent agonist of KARs and is widely used for the generation of epilepsy in laboratory rodent models due to its ability to cause neuro-inflammation following epilepsy induction (Oprica et al., 2006; Engel et al., 2010; Ramsdell and Stafstrom, 2009; Gupta et al., 2009; Zemlyak et al., 2009).

Glutamate, the major excitatory neurotransmitter in the CNS has recently been implicated in a variety of diseases. For example, it has been shown that patients with certain cancers (Eck et al., 1990), human immunodeficiency virus (HIV) (Eck et al., 1989), epilepsy (Rainesalo et al., 2004), autism (Aitken, 2008), and certain autoimmune illnesses such as rheumatoid arthritis (RA) (McNearney et al., 2000), and systemic lupus erythematosus (SLE) (West, 2007) all have elevated levels of glutamate in the periphery. Interestingly, autoimmune disease treatments which include corticosteroid use can also increase peripheral glutamate levels (Borsody and Coco, 2001; Raber, 1998; Eck et al., 1990). While glutamate receptor signaling has been examined in T cells (Ganor et al., 2003a) and macrophages (Boldyrev

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et al., 2004), there are currently no published observations on the effects of glutamatergic stimuli on B cells. We report that human B cells do indeed express the kainate receptor. In keeping with the Ortiz study (Ortiz et al., 2005), KAR activation was found to increased ADAM10 expression and activity, as measured by sCD23 release. A significant increase in B cell proliferation and Ig production was also seen with both purified B cells and PBMC. The implications of this finding for human allergic and autoimmune diseases are discussed.

2. Materials and methods

2.1. Media, reagents, and cell lines

All cells were grown in complete culture medium as indicated (CRPMI-10 or CDMEM-10; RPMI-1640 or Dulbecco's Modified Eagle Medium containing 10% heat inactivated (56 °C, 30 min) fetal bovine serum (Gemini Bio-Products, West Sacramento, CA), 2 mM L-glutamine, 50 μg/mL penicillin, 50 μg/mL streptomycin, 1 mM sodium pyruvate, 50 μg/mL amphotericin B, 50 μM 2-mercaptoethanol and 20 mM HEPES buffer (all from Invitrogen Carlsbad, CA)). All lines are kept in confluent culture under log phase growth in complete culture medium at 37 °C in humidified air with 5% CO_2. Kainic Acid (KA), dimethylsulfoxide (DMSO), L-glutamic acid (Glu), and antagonists (topiramate (TPM), NS102 and NBQX) were all purchased from Sigma (St. Louis, MO). Human IL-21 and mouse anti-human CD40 (clone G28-5) (American Type Culture Collection, (ATCC), Manassas, VA) were generated in our laboratory as previously described (Caven et al., 2005a). rhIL-4 was purchased from R&D Systems (Minneapolis, MN). The ADAM10 selective inhibitor INCB008765 was kindly provided by Incyte Corporation, Wilmington, DE (Fridman et al., 2007; Zhou et al., 2006).

2.2. Human cells

Human tonsils were obtained from routine tonsillectomies at Henrico Doctors Hospital (Richmond, VA) or the VCU Tissue Data Acquisition and Analysis Core (TDAAC). Tonsils were placed in media supplemented with antibiotics and mechanically disrupted using a Seward Stomacher 80 Biomaster Lab Blender (Brinkmann, Westbury, NY) at normal speed for 60 s. To obtain a single cell suspension, the resulting product was underlayed with Ficoll-Hypaque (GE Healthcare Piscataway, NJ). Following centrifugation (20 min at 400×g), the cells at the interface were removed and washed in PBS. To isolate B cells, the tonsilar cells were incubated with a FITC-anti-human IgD (BD Pharmingen San Diego, CA) for 30 min on ice and B cells were isolated by using the Miltenyi anti-FITC Microbeads, per manufacturer's instructions (Miltenyi Biotec Auburn, CA) Final B cell preparations were >95% pure IgD+ by FACS analysis.

Alternatively when whole PBMC was used, buffy coats were obtained from the Virginia Blood Service Center (Richmond, VA). PBMC were isolated by Ficoll gradient density centrifugation. All human studies research was performed in accordance to the Virginia Commonwealth University Institution Review Board per approved protocols.

2.3. RT-PCR and qPCR

RNA was isolated via standard Trizol (Invitrogen) purification protocol and the Access Quick RT-PCR kit (Promega, Madison WI) was used with gene specific primers to examine for the presence of kainate receptor subunits and their transcript variants. Primers were designed using VectorNTI software (Invitrogen). Ready-made primers® for G3PDH and all custom made primers for all sequences were synthesized by Integrated DNA Technologies (Coralville, IA). qPCR experiments were performed by the nucleic acid core in the ABI Prism® 7900 Sequence Detection System (Applied Biosystems, Foster City, Ca) using the TaqMan® One Step PCR Master Mix Reagents Kit. All the samples were tested in triplicate using the following conditions: 48 ºC/30 min; 95 ºC/10 min; and 40 cycles of 95 ºC/15 s and 60 ºC/1 min. The cycle threshold was determined to provide the optimal standard curve values (0.98 to 1.0). The probes and primers for ADAM10 (#Hs00153853_m1) were purchased from ABI. Human beta-actin primers were from the Pre-developed TaqMan®Assay Reagents and were used as endogenous control. Relative ADAM10 mRNA is determined as a relative ratio based upon beta-actin.

2.4. Western blot

Five million cells were lysed in Hepes Buffered Saline (HBS) with 1% NP-40 on ice for 10 min. Nuclei were removed by centrifugation and cytosolic proteins were treated with SDS buffer and heated at 70 ºC for 10 min. Proteins were run on an MES NuPage gel (Invitrogen) and then transferred to nitrocellulose. Blots were stained with a rabbit polyclonal antibody against the human kainate receptor subunit GluK4 (known as *GRIK4* in Genbank) (Chemicon AB5649). Detection was performed with a goat anti-rabbit IgG HRP and chemiluminescence was performed with SuperSignal West Pico Chemiluminescence Substrate (Pierce). To ensure equal loading, Ponceau S (Sigma) staining was performed.

2.5. Cell surface phenotyping

All cells were tested for cell surface antigen expression by direct immunofluorescence and flow cytometric analysis. Briefly, 1×10^6 cells were stained in 100 μl volumes with rabbit anti-human GluK4 (Chemicon) for primary incubation for 30 min at 4 °C. After washing, cells were then stained with a PE labeled goat anti-rabbit IgG (BD Pharmingen San Diego, CA). After 30 min/4 °C and washing, the cells were examined using a Cytomics FC500 Flow Cytometer and data was analyzed using CXP software (Beckman Coulter Fullerton, CA). PI was used to exclude dead cells from the analysis.

2.6. Soluble CD23 release assay

For these studies, the $CD23^+$ human B cell line RPMI8866 was grown in the presence or absence of 5 mM KA or Glu for 24 h in CDMEM10 at a concentration of 1×10^6 cells/mL. For antagonist studies, prior to the addition of KA or Glu cells were pretreated with 50 μM vehicle (DMSO), NBQX, NS102, or TPM for 1 h. After incubation, cell free supernatants were harvested and sCD23 levels determined by ELISA. When primary cells were assayed, cells were cultured with 10 ng/mL IL-4, 1 μg/mL anti-CD40, and 200 ng/mL Il-21 in complete culture media in the presence or absence of 5 mM glutamate. Forty-eight hours later cell free supernatants were harvested for ELISA.

2.7. Culture conditions for immunoglobulin analysis

Primary B cells or PBMC were cultured in the presence of 10 ng/mL IL-4 and 1 μg/mL anti-CD40 in complete culture media in the presence or absence of 5 mM KA or 5 mM Glu. When indicated, 200 ng/mL IL-21 was also added. For antagonism studies, prior to culture primary cells were treated with 10 μM vehicle (DMSO), NBQX, NS102, or TPM. After 14 days of culture, cell free supernatants were analyzed via ELISA for Ig levels. As cells are grown at various densities, line graphs represent Ig production as compared to cell density. When expressed as a bar graph, data represents the cell concentration in which maximum Ig production was observed for the particular isotype displayed.

To determine the effect of ADAM10 inhibition on Ig production, purified human B cells were cultured with 10 ng/mL IL-4, 1 μg/mL anti-CD40, and 200 ng/mL IL-21 in complete culture media in the presence of DMSO as vehicle control or 10 μM ADAM10 specific inhibitor. Five days later, cell free supernatants were harvested for