Experimental and Molecular Pathology 91 (2011) 335–345

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

C5b-9-activated, $K_v1.3$ channels mediate oligodendrocyte cell cycle activation and dedifferentiation

Cosmin A. Tegla [a,c], Cornelia Cudrici [a], Monika Rozycka [a], Katerina Soloviova [a], Takahiro Ito [a], Anil K. Singh [a], Aamer Khan [a], Philippe Azimzadeh [a], Maria Andrian-Albescu [a], Anver Khan [a], Florin Niculescu [b], Violeta Rus [b], Susan I.V. Judge [a,c], Horea Rus [a,c,d,*]

[a] Department of Neurology, University of Maryland School of Medicine, Baltimore MD, USA
[b] Department of Medicine, Division of Rheumatology and Clinical Immunology, University of Maryland School of Medicine, Baltimore MD, USA
[c] Research Service, Veterans Administration Maryland Health Care System, Baltimore MD, USA
[d] Veterans Administration, Multiple Sclerosis Center of Excellence, Baltimore MD, USA

ARTICLE INFO

Article history:
Received 30 March 2011
Available online 22 April 2011

Keywords:
Voltage-gated potassium channels
Oligodendrocyte
Complement activation
C5b-9
Multiple sclerosis

ABSTRACT

Voltage-gated potassium (K_v) channels play an important role in the regulation of growth factor-induced cell proliferation. We have previously shown that cell cycle activation is induced in oligodendrocytes (OLGs) by complement C5b-9, but the role of K_v channels in these cells had not been investigated. Differentiated OLGs were found to express $K_v1.4$ channels, but little $K_v1.3$. Exposure of OLGs to C5b-9 modulated $K_v1.3$ functional channels and increased protein expression, whereas C5b6 had no effect. Pretreatment with the recombinant scorpion toxin rOsK-1, a highly selective $K_v1.3$ inhibitor, blocked the expression of $K_v1.3$ induced by C5b-9. rOsK-1 inhibited Akt phosphorylation and activation by C5b-9 but had no effect on ERK1 activation. These data strongly suggest a role for $K_v1.3$ in controlling the Akt activation induced by C5b-9. Since Akt plays a major role in C5b-9-induced cell cycle activation, we also investigated the effect of inhibiting $K_v1.3$ channels on DNA synthesis. rOsK-1 significantly inhibited the DNA synthesis induced by C5b-9 in OLG, indicating that $K_v1.3$ plays an important role in the C5b-9-induced cell cycle. In addition, C5b-9-mediated myelin basic protein and proteolipid protein mRNA decay was completely abrogated by inhibition of $K_v1.3$ expression. In the brains of multiple sclerosis patients, C5b-9 co-localized with $NG2^+$ OLG progenitor cells that expressed $K_v1.3$ channels. Taken together, these data suggest that $K_v1.3$ channels play an important role in controlling C5b-9-induced cell cycle activation and OLG dedifferentiation, both in vitro and in vivo.

Introduction

Oligodendrocytes (OLGs) myelinate the axons of the central nervous system (CNS) and undergo apoptosis during development (Tang et al., 2001; Trapp et al., 1997). In the developing CNS, OLGs are selectively rescued from apoptosis by survival signals provided by axonal contact and growth factors (Barres and Raff, 1999; Billon et al., 2002). A correlation has been demonstrated between the expression of the delayed, outward-rectifying voltage-gated K_v channels and the proliferative potential of OLG-lineage cells. Proliferating OLG progenitor cells (OPCs) display large K_v currents, whereas postmitotic OLGs do not express such currents (Attali et al., 1997; Chittajallu et al., 2002). However, few studies have attempted to identify the cellular mechanisms responsible for these K_v channel changes in OPCs and mature OLGs (Vautier et al., 2004). Several K_v1 (KCNA) family members ($K_v1.1$–1.6) have been described in immature OLGs on both the mRNA and protein levels. Significant levels of $K_v1.2$, $K_v1.3$, $K_v1.4$, $K_v1.5$, and $K_v1.6$ have been found, with $K_v1.3$ and $K_v1.5$ being up-regulated under the influence of proliferation-stimulating growth factors (Attali et al., 1997; Chittajallu et al., 2002). $K_v1.3$ is the main KCNA family member involved in the regulation of proliferation and differentiation of oligodendroglial cells (Attali et al., 1997; Chittajallu et al., 2002). Currents passing through $K_v1.3$-containing channels play an important role in the G_1/S transition of proliferating OPCs. Inhibition of K_v channels also causes an accumulation of the cyclin-dependent kinase inhibitors p27 and p21 as well as G1 arrest in OPCs (Ghiani et al., 1999). In addition, K_v channel blockage has been shown to impair remyelination in a cuprizone model of demyelination (Bacia et al., 2004).

Complement activation and the subsequent assembly of the terminal complement complex (C5b-9, composed of the C5b, C6, C7, C8, and C9 proteins) play a significant role in the pathogenesis of a variety of CNS diseases, including multiple sclerosis (MS; reviewed in (Rus et al., 2006)). By forming pores in the plasma membrane, C5b-9 causes cell death and induces apoptosis (Cragg et al., 2000;

* Corresponding author at: University of Maryland, School of Medicine, Department of Neurology, 655 W Baltimore St, BRB 12-033, Baltimore, MD 21201, USA. Fax: +1 410 706 0186.
E-mail address: hrus@umaryland.edu (H. Rus).

0014-4800/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexmp.2011.04.006

Papadimitriou et al., 1994, 1991). However, like other nucleated cells, OLGs can survive limited C5b-9 complement attack through the protection provided by complement-inhibitory proteins and by the elimination of membranes carrying C5b-9 complexes (Carney et al., 1985; Scolding et al., 1989). We have shown that C5b-9 at sublytic doses inhibits the mitochondrial pathway of apoptosis (Soane et al., 2001, 1999) and Fas-mediated apoptosis by regulating caspase-8 processing (Cudrici et al., 2006). C5b-9 activates the cell cycle in OLGs (Rus et al., 1997, 1996), and this induction of the S phase of the cell cycle is c-Jun-dependent (Rus et al., 1996). These C5b-9 pro-survival effects are mediated by the activation of the ERK1 and phosphatidylinositol 3-kinase (PI3K)/Akt pathways, a process that is Gi protein-dependent (Rus et al., 1997, 1996; Soane et al., 2001).

To date, the role of inflammation in modulating $K_v1.3$ expression by OLGs during demyelination has not been evaluated. Complement activation and C5b-9 deposition are hallmarks of the most frequently pathological form of MS, the type II pattern (Lucchinetti et al., 2000). However, it is not clear at present whether K_v channels are involved in the OLG cell cycle activation mediated by C5b-9 or how K_v channel expression might affect the signaling pathways involved in cell activation.

In the present report, we have demonstrated that $K_v1.3$ is involved in the cell cycle activation induced by C5b-9. Since both the ERK and Akt pathways are known to be induced by C5b-9, we assessed the possible role of $K_v1.3$ in the activation of these kinases. Inhibition of $K_v1.3$ by rOsK-1 significantly reduced Akt phosphorylation and activation by C5b-9 but had no effect on ERK1 activation. In addition, we found that C5b-9-mediated myelin basic protein (MBP) and proteolipid protein (PLP) mRNA down-regulation was completely abrogated by inhibition of $K_v1.3$ expression. In the brains of patients with MS, C5b-9 was co-localized with $NG2^+$ cells that expressed $K_v1.3$ channels. In conclusion, our data suggest that $K_v1.3$ channels play an important role in controlling cell cycle activation by affecting the C5b-9-mediated activation of Akt and may also have a significant role in OLG dedifferentiation.

Material and methods

Brain tissue

Frozen brain tissue specimens were obtained at autopsy from 6 patients with a definitive diagnosis of MS from the Human Brain and Spinal Fluid Resource Center, Veterans Affairs West Los Angeles Health Care Center. Active lesions contained abundant infiltrates consisting of T cells and macrophages with detectable myelin degradation products. Inflammation was restricted to the lesion margins in chronic active lesions. Regions of normal appearing white matter (NAWM) and normal appearing gray matter lesions (NAGM) that lacked macroscopic or histological evidence of demyelination were also used. The samples were derived from patients between the ages of 30 and 62 with a mean age of 50. Four healthy control samples were obtained from Cooperative Human Tissue Network, Charlottesville, VA.

Primary cultures of OLG progenitor cells and OLGs

Neonatal OLGs were purified from the brains of 1-day-old Sprague–Dawley rats as previously described (Soane et al., 2001). After removal of the meninges, the brain was minced and sequentially passed through nylon meshes. The dissociated cell suspension was plated onto 75-cm^2 plates in DMEM/Ham's F-12 medium containing 10% fetal serum bovine (FBS). OPCs were separated from the astrocyte monolayer by shaking overnight at 200 rpm on a rotary shaker. The OPC cell suspension was collected and resuspended in defined medium containing serum-free DMEM/Ham's F-12, transferrin (500 ng/ml; Sigma Chemical Co., St. Louis, MO), insulin (75 ng/ml; Sigma), bFGF (75 μg/ml; Peprotech Inc, Rocky Hill, NJ) and 1 mM sodium pyruvate (Sigma). The cells were differentiated at 37 °C for 52 h. Over 85% of the cells expressed myelin basic protein (MBP), proteolipid protein (PLP), and galactocerebroside (GC). Fewer than 3% of the cells were negative for MBP and were astrocytes or OPC in varying stages of differentiation.

Membrane assembly of sublytic C5b-9 using terminal complement proteins

Purified human complement proteins C5b6, C7, C8, and C9 were obtained from Quidel (San Diego, CA) and Advanced Research Technologies (San Diego, CA). In brief, OLGs were incubated at 37 °C with 18 U of C5b6 and 10 μg/ml each of C7, C8, and C9 in a final volume of 2 ml for the indicated periods of time. In some experiments, normal human serum (NHS) pooled from several healthy donors was used as a source of serum complement. Rabbit antiserum recognizing GC was used to sensitize rat OLG; the cells were then incubated with NHS, heat-inactivated NHS (HI-NHS) or NHS containing K76 COONa (Otsuka Pharmaceuticals Co, Tokyo, Japan) at a final dilution of 1/10 for various time periods. As previously described, K76 prevents C5b-9 assembly in serum by binding to C5 (Niculescu et al., 1997; Rus et al., 1996). The concentrations of complement proteins used in this study were sublytic for OLG, as determined by staining cells with the vital dye trypan blue and measuring release of cytoplasmic lactate dehydrogenase as an indicator of cell death (Niculescu et al., 1997).

Electrophysiological studies

Cells were voltage-clamped using the whole-cell variation patch-clamp technique as previously described. Data acquisition and analysis was performed using an AxoPatch 200B patch clamp amplifier, Digidata 1332A analog-to-digital converter, and pClamp 8.2 software (Molecular Devices Corp., Sunnyvale, CA). Pipettes with a tip resistance of 2 to 3 MΩ were made from borosilicate glass (World Precision Instruments, New Haven, CT) on a micropipette puller (Model P-2000; Sutter Instrument Company, Novato, CA) and heat-polished. The pipette was filled with a previously filtered solution composed of (in mMol/l): 2 $NaCl_2$, 145 KCl, 1 $MgCl_2$, 5 EGTA, and 10 HEPES (the pH was adjusted to 7.3 with potassium hydroxide). The standard bath solution contained (in mMol/l): 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES (pH adjusted to 7.4 with NaOH). The osmolarity of the solutions was adjusted with D-glucose to 300 and 305 mOsm, respectively. Currents were low-pass-filtered at 2 or 5 kHz and sampled at 5 or 10 kHz. Data analysis was performed using Clampfit (version 8.2 of pClamp) and SigmaPlot 8.0 (SYSTAT Software, Point Richmond, CA). The pipette offset current was zeroed immediately before contacting the cell membrane. Only standard whole-cell experiments with access resistance <10 MΩ and membrane resistance >400 MΩ were included in this study. The membrane capacitances of the analyzed cells varied between 10 and 40 pF. Linear capacitance and leakage currents were subtracted online using a standard P/4 protocol. K_v currents were elicited by 500-ms pulses stepped from a holding potential of -60 to $+60$ mV, preceded by a conditioning pulse of -110 mV for 200 ms. Resting potential V_R (zero-current potential) was determined from current–voltage recordings, measured during the first few minutes after achieving the whole-cell recording conformation. The threshold for activation V_A was estimated from voltage–current relationship. After the whole-cell configuration was established and control measurements were made, serum C5b-9 was added directly to the experimental chamber. After 5–8 min of incubation, the measurements were repeated. The time of incubation was established in separate experiments on 10 cells (data not shown). Because of the cell-to cell variability, the evaluation of the effect of C5b-9 on the outward currents in OLGs was done for each cell independently. Statistical analysis comparing the chord conductance for control cells and that treated for cells with serum C5b-9 was performed using t-tests for paired observations. The statistical significance of an