The International Journal of Biochemistry & Cell Biology 44 (2012) 1519–1530

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

Review

Neuromyelitis optica: Aquaporin-4 based pathogenesis mechanisms and new therapies

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ARTICLE INFO

Article history:
Received 6 April 2012
Received in revised form 29 May 2012
Accepted 10 June 2012
Available online 17 June 2012

Keywords:
NMO
Devic's disease
Aquaporin-4
Astrocyte
Complement
Autoimmunity

ABSTRACT

Neuromyelitis optica (NMO) is an autoimmune 'aquaporinopathy' of the central nervous system that causes inflammatory demyelinating lesions primarily in spinal cord and optic nerve, leading to paralysis and blindness. NMO lesions show loss of aquaporin-4 (AQP4), GFAP and myelin, infiltration of granulocytes and macrophages, and perivascular deposition of activated complement. Most patients with NMO are seropositive for immunoglobulin autoantibodies (AQP4-IgG) against AQP4, the principal water channel of astrocytes. There is strong evidence that AQP4-IgG is pathogenic in NMO, probably by a mechanism involving complement-dependent astrocyte cytotoxicity, causing leukocyte infiltration, cytokine release and blood–brain barrier disruption, which leads to oligodendrocyte death, myelin loss and neuron death. Here, we review the evidence for this and alternative proposed NMO pathogenesis mechanisms, such as AQP4-IgG-induced internalization of AQP4 and glutamate transporters, complement-independent cell-mediated cytotoxicity, and AQP4-IgG inhibition of AQP4 water transport function. Based on the initiating pathogenic role of AQP4-IgG binding to astrocyte AQP4 in NMO, selective blocker therapies are under development in which AQP4-targeted monoclonal antibodies or small molecules block binding of AQP4-IgG to astrocytes and consequent downstream pathology.

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Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; AQP, aquaporin; AQP4, aquaporin-4; AQP4-IgG, anti-AQP4 immunoglobulin NMO autoantibody; BN-PAGE, blue-native polyacrylamide gel electrophoresis; CDC, complement-dependent cytotoxicity; CNS, central nervous system; CSF, cerebrospinal fluid; EAAT2, excitatory amino acid transporter 2; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; LDH, lactate dehydrogenase; MAC, membrane attack complex; MBP, myelin basic protein; NK-cell, natural-killer cell; NMDA, N-methyl-D-aspartate; NMO, neuromyelitis optica; OAP, orthogonal arrays of particles; TIRFM, total internal reflection fluorescence microscopy.

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http://dx.doi.org/10.1016/j.biocel.2012.06.013

1. Introduction

The aquaporins (AQPs) are a family of related membrane water channels, most of which, including aquaporin-4 (AQP4), which is the focus of this review, are selective for water transport. Some AQPs, called aquaglyceroporins, also transport glycerol. The water-selective AQPs are involved in a wide range of biological functions including transepithelial fluid transport, cell migration and neuroexcitation (Verkman, 2011, 2012). The aquaglyceroporins are involved in cell proliferation, adipocyte metabolism and epidermal water retention (Hara-Chikuma and Verkman, 2008; Rojek et al., 2008). Loss-of-function mutations in human AQPs cause nephrogenic diabetes insipidus (AQP2) and congenital cataracts (AQP0). The AQPs are assembled in membranes as tetramers in which each ~30 kDa monomer contains six transmembrane helical domains and two short helical segments surrounding cytoplasmic and extracellular vestibules connected by a narrow aqueous pore (Fujiyoshi et al., 2002; Walz et al., 2009). Water-selective AQPs are expressed broadly in mammalian tissues involved in fluid transport, such as epithelial and endothelial cells in kidney, lung, eye and the gastrointestinal tract. The aquaglyceroporins are expressed in adipocytes, epidermal keratinocytes, and various cell types involved in tissue regeneration. Many tumors express AQPs as well.

The focus of this review is on cellular pathogenesis mechanisms of the AQP4-based neuroinflammatory disease, neuromyelitis optica (NMO). NMO was originally thought to be a variant of multiple sclerosis in that both diseases are associated with inflammatory, demyelinating lesions of the central nervous system (CNS). However, there are clear differences in the sites and pathology of lesions, and in their clinical course and response to therapies. NMO primarily affects optic nerve and spinal cord, causing blindness and paralysis, with relatively little brain pathology. The prevalence of NMO is ~2–4 per 100,000 individuals, and ~6–8 times more prevalent in women than in men (Wingerchuk et al., 2007). The incidence of NMO is greater in Asians and blacks than in Caucasians. The median age of onset is 39 years, and 80–90% of NMO patients have relapsing optic neuritis and myelitis rather than a monophasic course (Jarius and Wildemann, 2010). Current NMO therapies include immunosuppression, plasmapheresis and B-cell depletion. The clinical aspects of NMO are discussed in more detail in recent reviews (Fazio et al., 2011; Jarius and Wildemann, 2010; Wingerchuk et al., 2007).

A defining feature of NMO is the presence of serum autoantibodies against AQP4 (called AQP4-IgG or NMO-IgG), which is detected in 60–90% of NMO patients (Jarius and Wildemann, 2010; Lennon et al., 2005). AQP4-IgG seropositivity is highly specific for NMO. AQP4 is expressed on the plasma membrane of astrocytes throughout the CNS, as well as in skeletal muscle and in epithelial cells in kidney, stomach and exocrine glands (Frigeri et al., 1995a,b). Though AQP4-IgG was initially thought to be a serum marker of NMO, perhaps related to astrocyte damage, there is now strong evidence that AQP4-IgG is pathogenic in NMO. AQP4-IgG binding to AQP4 on astrocytes is thought to cause complement-dependent cytotoxicity, leading to leukocyte infiltration, cytokine release and blood–brain barrier breakdown. These initial events lead to oligodendrocyte death, myelin loss and neuron death, and consequent clinical neurological deficit.

Familial NMO occurs in approximately 3% of cases and seems to have a complex genetic basis (Matiello et al., 2010). Previous studies failed to establish significant correlation between polymorphisms in AQP4 sequence and NMO susceptibility (Crane et al., 2011b; Matiello et al., 2011). An increased risk of NMO has been associated with particular human leukocyte antigen alleles in specific populations (Wang et al., 2011; Zephir et al., 2009). It remains largely unknown why patients develop antibodies against AQP4. AQP4 autoimmunity frequently coexists with other autoimmune diseases, and it has been suggested in some NMO patients that AQP4 autoimmunity is initiated as an immune response to tumor antigens (Antoine et al., 2004; Ducray et al., 2007; Pittock and Lennon, 2008). In this review we evaluate potential pathogenic effects of AQP4-IgG binding to AQP4 and their relevance in NMO pathogenesis. How AQP4-IgG causes NMO pathology has important consequences for development of new therapies that target the underlying cause(s) of the disease.

2. AQP4 structure and function

AQP4 was originally cloned in 1994 by our lab based on its homology to known AQPs (Hasegawa et al., 1994). X-ray structure analysis showed that each AQP4 monomer consists of six helical, membrane-spanning domains and two short helical segments surrounding a narrow aqueous pore (Ho et al., 2009), similar to other AQPs. AQP4 monomers assemble in membranes as tetramers, which can further associate in the cell plasma membrane as large aggregates called orthogonal arrays of particles (OAPs). OAPs are seen by freeze-fracture electron microscopy as regular square arrays of intramembrane particles (Landis and Reese, 1974; Rash et al., 1974; Wolburg et al., 2011). Our lab identified AQP4 as the major OAP protein from the appearance of OAPs in AQP4-transfected cells (Yang et al., 1996) and the absence of OAPs in AQP4 knockout mice (Verbavatz et al., 1997). AQP4 is present in two major isoforms: a long (M1) isoform with translation initiation at Met-1, and a short (M23) isoform with translation initiation at Met-23 (Fig. 1A) (Lu et al., 1996; Yang et al., 1995). Both isoforms are expressed in astrocytes, forming heterotetramers. OAP formation is stabilized by intermolecular N-terminus interactions involving residues just downstream of Met-23 (Crane and Verkman, 2009a). Our lab has applied biophysical methods, including quantum dot single particle tracking and super-resolution imaging, to study the structure, dynamics and regulation of AQP4 assembly in OAPs (Crane et al., 2008, 2009; Crane and Verkman, 2009a,b; Jin et al., 2011; Rossi et al., in press).

In the central nervous system, AQP4 is by far the most abundant water channel. AQP4 is concentrated in astrocyte membranes facing blood–brain and brain–CSF interfaces and in the basolateral membrane of ependymal cells (Nielsen et al., 1997; Rash et al., 1998). AQP4 is also expressed in astrocyte-like 'supportive cells' in sensory organs such as retinal Müller cells (Li et al., 2002). AQP4 facilitates movement of water between the blood and brain and the brain and CSF compartments. Knockout mice lacking AQP4 show reduced cytotoxic (cell swelling) brain edema following water intoxication or ischemic stroke (Manley et al., 2000), increased vasogenic (leaky vessel) brain edema in brain tumor (Papadopoulos et al., 2004) or abscess (Bloch et al., 2005), and increased ventricular enlargement in obstructive hydrocephalus (Bloch et al., 2006). Phenotype analysis of knockout mice has also implicated AQP4 involvement in neuroexcitation (Binder et al., 2006; Li et al., 2002; Li and Verkman, 2001; Lu et al., 2008), astrocyte migration (Auguste et al., 2007; Saadoun et al., 2005) and neuroinflammation (Li et al., 2011). Impaired AQP4 water permeability in astrocytes is likely responsible for each of these phenotypes.

3. Cellular consequences of AQP4-IgG binding to AQP4

When bound to their cellular target, antibodies can cause: (i) altered target function; (ii) target internalization, reducing cell surface expression; (iii) complement activation, causing cell death (complement-dependent cytotoxicity, CDC); and/or (iv) activation of effector cells, including natural-killer cells (NK-cells), causing cell death (antibody-dependent cellular cytotoxicity, ADCC). Available evidence implicates (iii) as the primary mechanism involved