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Anti-aquaporin-4 auto-antibodies orchestrate the pathogenesis in neuromyelitis optica ☆

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ABSTRACT

NMO-IgG, the auto-antibody specific to the aquaporin-4 (AQP4) water channel associated with the autoimmune inflammatory disease neuromyelitis optica (NMO), is considered to be an accurate serum biomarker and is thought to be an important contributor to NMO pathology. In this review, we summarize recent evidences from our group and others indicating that NMO-IgG can be implicated at several levels in the immuno-pathology of NMO. NMO-IgG/anti-AQP4 antibodies may compromise the integrity of the blood–brain barrier and consequently facilitate and enhance the perivascular inflammation characteristic of NMO. Lastly, NMO-IgG can induce astrocyte injury which may lead to the accumulation of excitatory/toxic molecules and accordingly damage oligodendrocytes and compromise myelin integrity.

Contents

1. Introduction

Neuromyelitis optica (NMO or Devic's disease) is a severe autoimmune inflammatory demyelinating disease of the central nervous system (CNS) which predominantly affects the spinal cord and the optic nerves [1]. The recent description of NMO-IgG, a highly disease-specific autoantibody found in NMO and NMO related diseases (i.e. relapsing optic neuritis and longitudinally extensive transverse myelitis) but absent in the classical form of multiple sclerosis (MS), convincingly demonstrated that NMO is a specific disease and not just a subtype of MS [2]. The prominent role of humoral mechanisms in mediating the necrotizing lesions in NMO was already suspected before the description of NMO-IgG. In contrast to prototypical MS, NMO active lesions displayed perivascular immunoglobulin deposition, complement activation and polymorphonuclear (neutrophils and eosinophils) infiltration supporting the importance of antibody mediated pathogenesis in NMO [3].

The target antigen of NMO-IgG was identified as aquaporin-4 (AQP4), the main water channel protein in the CNS, expressed on astrocyte end-feet at the blood–brain barrier (BBB) and the brain–cerebrospinal fluid barrier [4]. A series of clinical and pathologic observations suggested that anti-AQP4 plays a central role in the physiopathology of NMO [5,6]. In this review, we will summarize the

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latest advances describing novel anti-AQP4 Ab effector functions indicative of a central role for anti-AQP4 Ab in NMO pathogenesis and defining NMO as an auto-immune channelopathy. The description of novel pathogenic mechanisms is important to better understand the molecular mechanisms contributing to the formation of specific pathological features described in NMO lesions.

2. Role of anti-AQP4 antibodies in the break down of the blood–brain barrier (BBB)

The vasculocentric pattern of NMO patient sera immunoreactivity observed by indirect immunofluorescence (IIF) clearly indicates that NMO-IgG primarily target the BBB [2]. Using transfected cell lines, it has been shown that the epitope recognized by NMO-IgG is located in the extracellular domain of the AQP4 molecule [7]. We confirmed by confocal microscopy and flow cytometry that primary human astrocytes express the AQP4 and are specifically recognized by NMO-IgG [8]. Using an *in vitro* model of the BBB in which primary human fetal astrocytes (HFAs) were grown above a monolayer of human BBB-derived endothelial cells (BBB-ECs), we demonstrated that NMO-IgG binding to astrocytes induces AQP4 internalization and disrupts AQP4 polarized expression at the interface between astrocyte end-feet and endothelial cells [8]. Following NMO-IgG binding, AQP4 is rapidly translocated from the surface to early endosome antigen 1 (EEA1) containing early endosomal vesicles with probable subsequent degradation [7,9]. AQP4 plays a crucial role in the regulation of water fluxes in the CNS. Zhou and colleagues demonstrated that the absence of AQP4 expression alters the neurovascular unit integrity leading to tight junction opening and swollen perivascular astrocytic endfeet resulting in BBB hyperpermeability [10]. The exact relevance of AQP4 internalization remains to be determined but it is thought that AQP4 redistribution impacts on water homeostasis in the CNS and may favor edema [9]. Accordingly, using our *in vitro* model of the BBB we showed that the disruption of the polarized expression of AQP4 induced by NMO-IgG is associated with a significant increase in BBB permeability [8]. This specific and direct effect of NMO-IgG on the BBB may account for the prominent edematous features of NMO lesions. In addition, complement-mediated inflammation and astrocyte-directed cytotoxicity described below provide additional mechanisms that may further alter the astro-endothelial synapse and contribute to the break down of the BBB.

3. Anti-AQP4 antibodies mediate the perivascular inflammatory infiltration

In addition to compromising the integrity of the BBB, NMO-IgG bound to astrocytes can serve as a potent complement activator and lead to the release of anaphylatoxins namely C3a and C5a. Indeed, NMO-IgG are predominantly IgG1, an isotype that is a strong complement activator and the tetrameric structure of AQP4 molecules favors the oligomerization of bound IgG which is required for complement classical pathway activation. Anaphylatoxins are implicated in several deleterious immune responses (e.g. the inflammatory processes in hypersensitivity diseases like asthma) and their potent chemoattractant effect results in massive influxes and activation of granulocytes that can perturb the homeostasis of the affected organ [11].

In NMO lesions, terminal complement products associated with immunoglobulins are readily observed along with numerous neutrophil and eosinophil granulocytes comprising the inflammatory infiltrate [1]. In order to evaluate the chemoattractant potential of NMO-IgG binding to astrocytes, we used our astrocyte/BBB-EC co-culture BBB model. HFAs pre-incubated with NMO-IgG positive sera supplemented with fresh serum as a source of complement induced a significant increase in granulocyte migration through BBB-ECs. This was not the case with control sera. We were able to inhibit the chemoattraction induced by NMO-IgG by heat-inactivating the serum suggesting that complement chemoattraction is important for granulocyte recruitment to areas of NMO-IgG binding. The exact contribution of anaphylatoxins and/or other chemotactic factors released after complement activation remains to be defined. Following their migration through the BBB, granulocytes showed increased levels of surface CD107a, a protein restricted to lysosomes and transiently present on the cell surface following degranulation but this was not prevented by complement inactivation. Taken together, these data suggest a two step process whereby granulocytes that are first chemoattracted by NMO-IgG-induced complement activation, are then activated and degranulate following interaction between their Fc gamma receptor and the Fc portion of astrocyte-bound NMO-IgG. Experiments are underway to evaluate whether this degranulation is toxic towards the astrocytes or any of the components of the BBB.

These data indicate that astrocyte-bound NMO-IgG are sufficient to recapitulate the vasculocentric granulocyte infiltration and complement activation products deposition associated with NMO lesions.

4. Anti-AQP4 antibodies induce astrocyte injury and demyelination

As described above, binding of NMO-IgG to AQP4 can induce complement activation and can affect astrocyte physiology resulting in increased BBB permeability and inflammatory cellular infiltration. NMO-IgG may also contribute to NMO lesion formation by impacting on glial cell survival. In an *in vitro* killing assay using NMO-IgG, purified natural killer (NK) cells and primary cultures of HFAs we showed that NMO-IgG can induce a dose dependent NK cell degranulation and antibody dependent cellular cytotoxicity (ADCC) resulting in significant astrocyte killing [8]. The ability of NMO-IgG to induce ADCC was correlated with their ability to bind AQP4 expressed by astrocytes. Although the role of NK cells, which were used as a proof of concept, remains to be defined in NMO, several other cell type present in NMO lesions, such as neutrophils, macrophages or microglia cells, express Fc receptor and are capable of ADCC.

Hinson et al. recently described another mechanism by which NMO-IgG could induce astrocyte injury and potentially contribute to demyelination. Using AQP4-transfected nonneural cells and rat primary astrocytes, they demonstrated that NMO-IgG binding to AQP4 initiates complement activation and subsequently plasma membrane damage [7,9]. Interestingly, rat primary astrocytes appeared relatively resistant to NMO-IgG -complement-dependent cytotoxicity (CDC) compared to AQP4-transfected human embryonic kidney (HEK)-293 cells. Accordingly, we were not able to detect complement dependent astrocyte death in our *in vitro* studies using primary human astrocytes (unpublished data). We hypothesize that this is due to the strong expression of complement regulatory proteins (i.e. CD46, CD55, CD59) by human astrocytes [12] and the question remains as to the importance of the formation of the membrane attack complex (MAC) in demyelinating diseases [13]. These direct and indirect astrocyte-directed effector functions of NMO-IgG are in agreement with NMO lesion pathology where a loss of AQP4 and GFAP immunoreactivity can occur in the absence of, and probably before, demyelination. Hinson et al. further demonstrated that AQP4 internalization perturbs astrocytes-controlled glutamate homeostasis by limiting glutamate uptake by astrocytes [9]. AQP4 and the Na+ -dependent excitatory amino acid transporter (EAAT2) are found as a complex at the plasma membrane and NMO-IgG binding to AQP4 induces the internalization of both AQP4 and EAAT2 resulting in impaired glutamate uptake by astrocytes. Immunocytochemical staining indicated that AQP4-deficient NMO spinal cord lesions also exhibit a marked reduction in EAAT2 expression [9]. Glutamate homeostasis is critical for proper CNS functions and even though astrocytes are thought to be resistant to extracellular