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Strategy for anti-aquaporin-4 auto-antibody identification and quantification using a new cell-based assay

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Abstract NMO-IgG is a specific biomarker of neuromyelitis optica (NMO) that targets the aquaporin-4 (AQP4) water channel protein. The current gold standard for NMO-IgG identification is indirect immunofluorescence (IIF). Our aim in this study was to develop a new quantitative cell-based assay (CBA) and to propose a rational strategy for anti-AQP4 Ab identification and quantification. We observed an excellent correlation between the CBA and IIF for NMO-IgG/anti-AQP4 detection. The CBA appeared more sensitive than IIF but on the other hand, IIF allows the simultaneous detection of various auto-Abs, underlining the complementarity between both methods. In conclusion, we propose to use IIF for the screening of patients at diagnosis in order to identify auto-Abs targeting the central nervous system. A highly sensitive, AQP4 specific and quantitative assay such as our CBA could be used thereafter to specifically identify the target of the Ab and to monitor its serum concentration under treatment.

Abbreviations: NMO, neuromyelitis optica; NMOSD, NMO spectrum disorders; LETM, longitudinally extensive transverse myelitis; MS, multiple sclerosis; CIS, clinically isolated syndrome; AQP4, aquaporin-4; CNS, central nervous system; BBB, blood–brain barrier; CBA, cell-based assay; AU, arbitrary unit.

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1. Introduction

Neuromyelitis optica (NMO) is a severe inflammatory demyelinating disease of the central nervous system (CNS) that affects mainly the optic nerves and the spinal cord [1]. In 2004, a highly disease-specific autoantibody (Ab) named NMO-IgG was discovered in NMO and NMO related diseases, i.e. relapsing optic neuritis and longitudinally extensive transverse myelitis (LETM) [2]. The target antigen of NMO-IgG was identified as aquaporin-4 (AQP4), the main water channel protein in the CNS, mainly expressed on astrocyte end-feet at the blood–brain barrier (BBB) and the brain-cerebrospinal fluid barrier [3]. Owing to their very high specificity in NMO, NMO-IgG auto-Abs are generally absent in other demyelinating diseases of the CNS, including the classical form of multiple sclerosis (MS), and allow an early discrimination between MS and NMO [2,4]. Consequently, NMO-IgG has been included in the revised diagnostic criteria for NMO [5].

During the few last years, several effector functions have been described for anti-AQP4 Abs in vitro [6,7]. More recently, the passive transfer of the disease in rodents by injection of immunoglobulin G (IgG) from NMO patients definitively demonstrated the pathogenic role of anti-AQP4 Abs in the CNS [8–10]. Accordingly, anti-AQP4 Ab serum concentrations were found to correlate with disease activity and response to treatment, further supporting the usefulness of NMO-IgG/anti-AQP4 Ab as a biomarker for the diagnosis and follow-up of NMO patients [11,12].

The current gold standard for NMO-IgG identification is still indirect immunofluorescence (IIF) on sections of CNS tissues but several AQP4-specific assays have been developed in order to specifically identify and quantify anti-AQP4 Abs [13]. Our aim in this study was to develop a new cell-based assay (CBA) and to compare this AQP4-specific and quantitative test to IIF in order to propose a rational strategy for NMO-IgG/anti-AQP4 Ab monitoring in NMO patients.

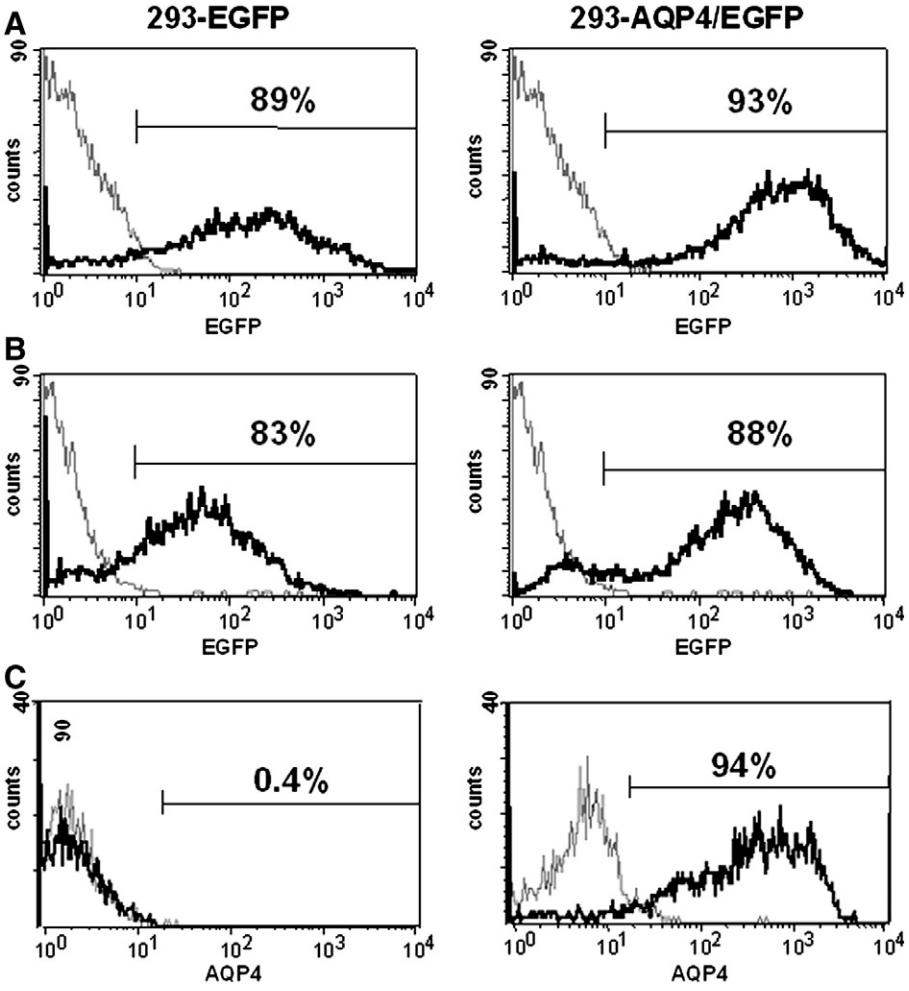

Figure 1 293-AQP4/EGFP cell line stably expresses AQP4. HEK-293T human cells were transduced with an HIV-1-based vector harboring either human *AQP4* and *EGFP* genes (293-AQP4/EGFP cells) or the *EGFP* gene alone as a negative control (293-EGFP cells). (A) Representative FACS analyses showing EGFP expression measured 48 h later to evaluate transduction efficiency. Grey histograms represent EGFP expression in non transduced 293T cells. (B) The same analysis was performed 1 month after transduction to test the stability of transgenes expression over time. (C) Expression of AQP4 by 293-AQP4/EGFP cells. 293-EGFP (left) and 293-AQP4/EGFP cells (right) were labeled with a rabbit anti-AQP4 Ab after cell permeabilization and analyzed by flow cytometry. Grey histograms represent the isotype control values.