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Kevwords

Keyword 1:Autoimmune Demyelinaäon
Keyword 2: Neuromyelitis Optica (NMO)

TItle

Generation of aquaporin-4 autoantibodies from B cells of patients with Neuromyelitis Optica: towards precision medicine

Body

Introduction

Neuromyelitis optica is an antibody-mediated disease, commonly associated with autoantibodies directed against the astrocytic water channel aquaporin-4 (AQP4). These antibodies can often persist for many years, even decades.

AQP4-antibodies are higher inthe periphery ttlan inCSF. The peripheral B-cell subset secreting AQP4-antibodies and the conditions which modulate their production are poorly understood although one study suggested plasmablasts are the source of AQP4-antibodies.

Methods

We studied unfractionated PBMCs and sorted plasmablast populations from 12 patients with widely-varying serum levels of AQP4-antibodies (1:20 – 1:12800) to investigate ttle source of AQP4-antibodies. All patients had been treated with immunotherapies prior to sampling. Culture conditions utilised selected combinations of CD40L, IL-2 (50 ng/ml), IL-1 (1 ng/ml), TNFo (1 ng/ml), BAFF (200 ng/ml), IL-21 (50 ng/ml), IL-6 (10 ng/ml) and APRIL (300 ng/ml). gG levels were measured by EUSA. AQP4-antibodies were determined by live cell based assay, followed-by flow cytometric quantification.

Results

In vitro culture of patient plasmablasts (CD3-CD14-CD19+CD27++CD38++) generated modest quantities ofIgG, but no AQP4-antibodies. The lack of AQP4-antibodies was also observed from unfractionated PBMCs cultured under plasmablast-maintainance conditions (IL-6+/-APRIL). By contrast, conditions which promoted B-cell proliferation (IL-2, CD40L,TNFo, IL-21) generated large quantities of gG (mean 12µg/ml, QR 1.8-19),and often high levels of AQP4-antibodies. The number of plasmablasts and total-IgG generated per well *in vitro* showed little variation across the wide-range of supernatant AQP4-antibody levels, suggesting the baseline frequency of AQP4-specific B-cells determined the AQP4-antibody levels in culture. Furthermore, ttle AQP4-antibody supernatant levels correlated well with the serum levels from the 12 patients (Spearman's r=0.81,p = 0.0023).

Conclusions

AQP4-antibodies can be derived in large quantities from circulating B cells rattler than circulating plasmablasts. The robust correlatIon between *in vitro* AQP4-antibody levels and serum AQP4-levels suggests a mechanism of action for CD20 depletion and does not implicate long-lived CD20-negative cells in the pattlogenesis of NMO.

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