Short Comunications

MASP-2: New evaluating parameters

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ABSTACT

Introduction: MASP-2 is one of the starters of the lectin pathway, the third complement pathway which components and sequence is now under construction.

Objective: Evaluate the MASp-2 blood-cerebrospinal fluid (CSF) dynamics throughout the behavior of this protein in both compartments and its relation with the flux velocity.

Materials and methods: 56 serum and CSF samples simultaneously taken were studied with or without blood-CSF dysfunction. CSF and serum MASP-2 levels were measured by commercial enzyme-linked immunosorbent assay (ELISA) kit.

Results: A saturation curve-like was obtained when plotting CSF MASP-2 vs. the molecular flux done by its Q albumin values. These empirical results allowed a re-evaluation of the protein characteristics of MASP-2 with a new group of parameters. Those ones can help to describe its properties since the neuroimmunological point of view according to the capacity to diffuse free or in associated forms in the different blood-CSF barriers. The experimental values mimics the enzymatic kinetic curves and serves to name at least new parameters of this molecule CSF/serum diffusion rate Kdacw and saturation MASP-2 concentration, 40, 96 and 26, 78 respectively.

Conclusions: These new parameters can characterize MASP-2 in its diffusion throughout the blood-CSF barrier.

INTRODUCTION

The lectin pathway is one of the activation pathways of the complement system with the classical and alternative one.

This pathways belongs from the invertebrates evolution and it is not requires immunoglobulins to be activated. This is the most complex one and it is considered under construction because new discoveries about it have been reported every year as well as new structures and new evidences of the different components.[1]

Up to now there are five different pattern recognition molecules (PRMs)-mannan-binding lectin (MBL); H-, L- and M-ficolin (also known as Ficolin-3, -2, and -1, respectively); and collectin-kidney1 (CL-K1) -associate with MBL-associated serine proteases (MASP) -1 and -2 to activate complement. In addition, CL-K1 and the related collectin-liver 1 (CL-L1) form heteromers that also associate with MASPs and activate complement.[1,2]

The general characteristic of these starters is done because they cannot initiate by themselves the enzymatic activity of the complement cascade and they should employ other structures with enzymatic properties.

One of these structures is MASP-2. It is a serin protease as well as MASP-1 and its functions and the relationship between both proteases was changing in the same way as they have been better studied in their characteristics and associations.[2,3]

Although MASP-2 is able to autoactivate, a role ofMASP-1 in activating MASP-2 suggested an intercomplex activation mechanism as opposed to the intracomplex mechanism of C1. A recent study demonstrated that MASP-1 is the exclusive activator of MASP-2 under physiological conditions.[2]

These enzymes maintain in the biological fluids an equilibrium between activated and non-activated forms, between associated to a starter and the free non-associated in the environment.

MASP-2 it is found in blood as well as other proteins like immunoglobulins can be synthesized in cerebrospinal fluid (CSF) or to diffuse into from blood.

The objective of this paper is to evaluate the MASp-2 blood/cerebrospinal fluid (CSF) dynamics throughout the behavior of this protein in both compartments and its relation with

the flux velocity.

METHODS

56 serum and CSF paired samples obtained simultaneously were studied.

Albumin in serum and CSF was quantificated by nephelometric assay using Behring Nephelometer-Analyzer from Siemens (Marburg. Germany).

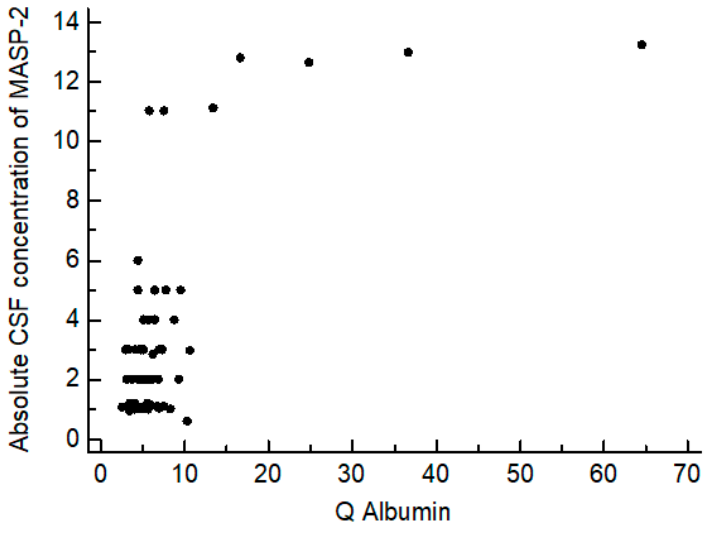
CSF and serum MASP-2 levels were measured with commercial enzyme-linked immunosorbent assay (ELISA) kits (Hycult Biotech, Uden, the Netherlands).

Statistical package MedCalc version 13.3.3.0 and GraphPad Prism version 5.01 was used.

RESULTS

When the different MASP-2 concentration in CSF was plotted against their respective value of Q albumin of the patient it is possible to find a typical curve that reveals a process that it can be found in nature in the enzymatic curves type Michaelis-Menten.

This curve demonstrated that the increment of Q albumin evidence an increment value of CSF MASP2 values as it shown at Figure 1.



Q albumin is a measure of the molecular flow between blood and CSF. The saturation-like curve allows to introduce the values of Q albumin and Q MASP-2 in a kinetic-like curve and to determine the constants of the equation as well as an enzymatic analysis as it appears in Figure 2.

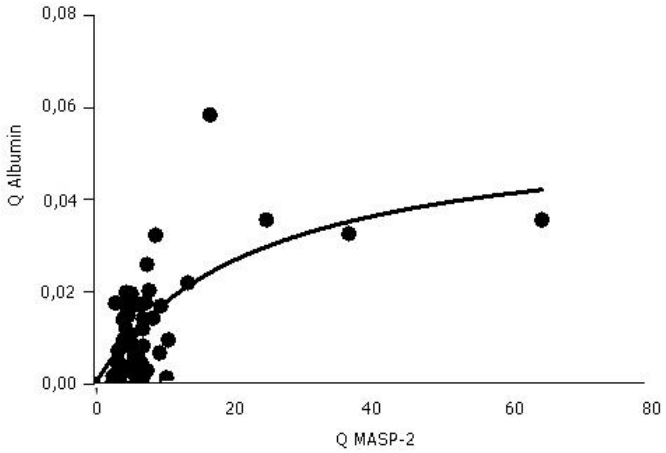


Fig. 2. - Kinetic-like curve obtained by the plotting of Q albumin vs. Q MASP-2.

In order to determine the new parameter that characterize MASP-2 and its diffusion from blood to CSF it was necessary to perform the nonlinear regression to find the best-fit values of the parameters. You can`t really interpret the best-fit values without knowing how precise they are, and it is possible to see as standard errors and confidence intervals inn Table.

Table. Best-fit values. Q albumin vs. Q MASP-2

Qmax	0.05681
Kdacw	22.60
Std. Error	
Qmax	0.01376
Kdacw	8.382
95 % Confidence Intervals	
Qmax	0.02922 to 0.08441
Kdacw	5.783 to 39.42
Goodness of Fit	
Degrees of Freedom	54
R^2	0.4116
Absolute Sum of Squares	0.003758
Sy.x	0.008342
Number of point analyzed	56

The new parameters obtained by the employ of the enzymatic kinetics analysis was the constant Kdacw (similar than Km in kinetics analysis) and the maximum molecular flow done by the corresponding to the maximum Q MASP2 values. Kdacw and saturation Q MASP-2 are 22.60 and 0.05681 respectively.

DISCUSSION

The saturation curve-like when plotting CSF MASP-2 against the Q albumin values allowed a re-evaluation of the protein characteristics of MASP-2 with a new group of variables and its improvement. The experimental values mimic the enzymatic kinetic curves (4) and serves to name at least new parameters of this molecule CSF/serum diffusion rate:

This new parameters can help to describe its properties since the neuroimmunological point of view. Notice that MASP-2 can diffuse from blood to CSF in different forms: free or in associated forms according the blood-CSF barrier.

The Kdacw values can be calculated equal as the Km constant in the kinetics curves and it means the value of Q Albumin or CSF/serum diffusion rate when the semi-maximum values of Q MASP-2 we can find in CSF. The Q MASP-2 maximum is the natural and optimal relationship between MASP2 in CSF transferred from blood and the MASP2 found in blood, and it will be considered their maximum diffusion rate. Over these values, there is possible to find intrathecal MASP-2 synthesis.

It is possible to associate these constant values with other characteristics in the lectin complement pathway. This new parameters can characterize MASP-2 in its diffusion throughout the blood-CSF barrier.

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