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Characterization of blood Microparticles in an EAE model

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Introduction

Microparticles (MP) are membrane fragments shed by activated cells after a variety of stimuli such as stress or inflammatory processes. They play an apparently role in extracellular communication with no direct contact. They also have been shown to contain genetic material, mainly RNA and miRNA, which produce genotypic modifications in the target cell (1). The MP cellular origin can be determinate due to the presence in their membrane of characteristic marker of the cell they come from. It has been proposed that the concentration of some MP can be use as inflammatory state marker (2). In this context our group has focused on MP concentration in diseases with an inflammatory component specifically multiple sclerosis (MS) (3).

MS is an autoimmune demyelinating disease of the central nervous system (CNS) in which neuronal degeneration and inflammatory processes occur simultaneously producing a progressive and disabling clinic. It is widely accepted in the scientific community that Experimental Autoimmune Encephalitis (EAE), a murine model, reflects well some characteristics that occur in the disease (4). This model is characterized by a specific immune activation leading to an increase of leukocytes (CD45 +) that are responsible for the myelin attack.

Due to the relationship between MS inflammatory processes and the increase of MP in MS patients (3), it has been propose that MP are involved in the etiophatology of the disease, with possible applications in diagnosis and monitoring.

Objective

Analysis of concentration and origin on blood MP in the EAE murine model.

Hipothesis

MP are related to the immune processes that occurs in EAE. The characterization of this relationship can gives us information about the role that plays MP in the inflammatory and autoimmune process in MS and open new horizons for research.

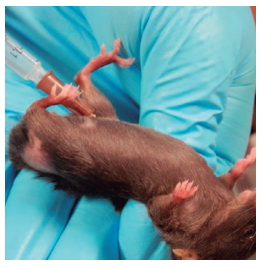
In the EAE group it is expected to found a higher MP count number due to the specific leukocyte activation. In the other hand in the immunized group a lower increased in MP count is expected according to an inespecific immune activation. In control group we do not presume a MP increase.

Methods

Time: days post immunization (dpi) — Beginning of the symptoms: 7-14 dpi

Inmunization (0dpi)

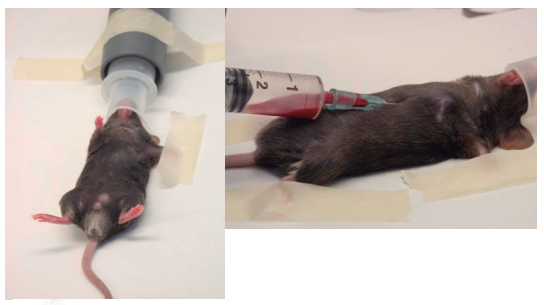
- EAE group: FAC, MOG $_{35-55}$, *Mycobacterium tuberculosis*, pertussis toxin
- Inmunizad group: FAC, ~~M~~OG$_{35-55}$, *Mycobacterium tuberculosis* pertussis toxin
- Control group: no intervention



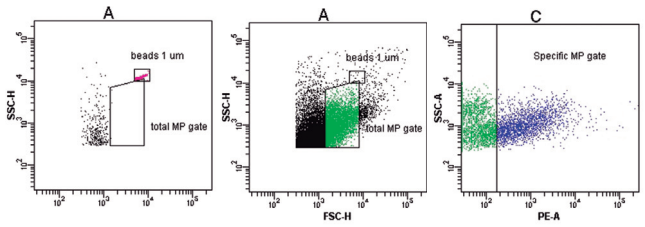
Monitoring: 0-30 dpi (weight and score)

score	Description
0	No neurological signs
1	Tail limp and droopy
2	Mouse doesn´t show fluenty movements
2,5	Mouse is not able to hang on to cage with its hind legs
3	Mouse drags the 2 legs
4	Mouse has paralyzed the entire posterior region
5	Mouse can't move its legs at all
6	Moribund/ Death

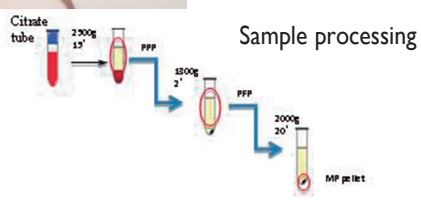
sacrifice and necropsy: 30 dpi



Flow cytometry: Gating strategy

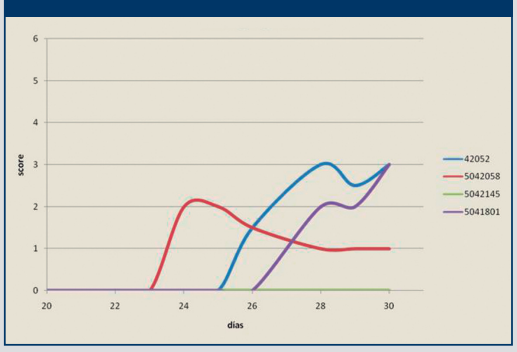


Sample processing

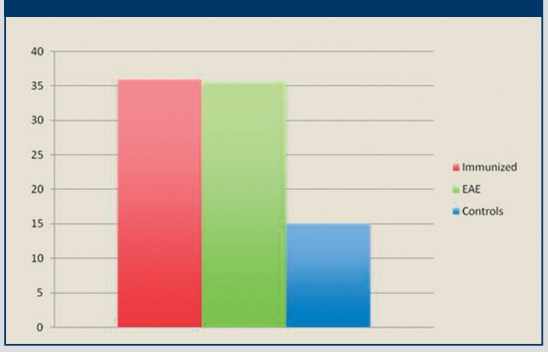


Results

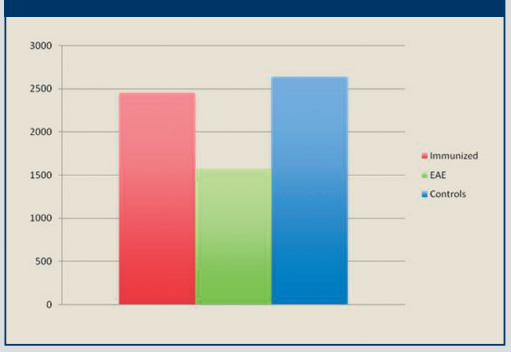
EAE GROUP SCORE



% of CD45 MP FROM Total MP



TOTAL MP



Discussion and conclusions

- **The methodology used for obtening MP is appropriated in this animal model. However the results indicate that one mouse hearth exsanguination is not enough to achieve the necessary blood volume to perform a MP analysis. This required us to carry out a blood pool for each group.**
- **The normal clinical on set in EAE model is on the 7th dpi. However, in our model it was during the 23 th dpi. This may be due to the rigorous asepsis conditions in which the animals are handle and stabulate leading to a delayed immune response in a naïve animals immune system (IS) state (5).**
- **A higher number of CD45+MP was found in the EAE and immunized animals than controls. This demonstrates an immune response in the first two groups. This data corroborate partially the initial hypothesis but is not capable to discern specific from unspecific immune response. We speculate that the similar MP levels in EAE and immunized groups may be due to migration of leucocytes through the blood brain barrier (BBB) into the CNS in EAE group (6), and finally a lower count of leucocyte-derived (CD45+) MP in peripheral blood. Leucocytes from the immunized group are not reduced in number as they do not migrate through an intact BBB. The anatomopathological and cerebrospinal fluid studies pending to do will allow us to confirm this hypothesis.**
- **The CD45+MP study do not reflect EAE status according to the same values in Immunized and EAE groups founded.**
- **The total MP results do not exhibit the same correlation as the CD45+ MP shown. This could be due to changes in MP from other cellular origins (platelet, endothelial) because of variables that are outside the scope of this work.**

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Acknowledgments: Matías Sáenz Cuesta