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Abstract 13

- Granulocytes in actively induced Lewis rat EAE -

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Experimental Autoimmune Encephalomyelitis (EAE) is an inflammatory T cell mediated disease of the central nervous system. An acute form of EAE can be actively induced in Lewis rat by the inoculation of Myelin Basic Protein (MBP) resuspended in complete Freud's adjuvant (CFA).
Together with the important role of the spleen in EAE development, at the peak of actively induced EAE in Lewis rats (day 14 after immunization) we have recently described a spleen total cell number decrease with a parallel large presence of a CD3-CD45+ cells. Moreover, a population sharing similar immunophenotype and physical characteristics has been observed at the same time point in the spinal cord (s.c.).
We hypothesized this populations could be mainly composed by granulocytes in both the organs because of its high side scatter (SSc) value observed with the flow cytometer. We strongly suspected a role of the Mycobacterium Tuberculosis in the development of this population in the spleen, while in the s.c. it could also include microglia cells.
The aims of the present work were to investigate the effect of CFA -/+ MBP on the spleen, to elucidate the origin of this leukocyte population and to characterize it on day 14 both in the spleen and the s.c. of CFA and CFA + MBP (EAE) treated Lewis rats.
We subcutaneously inoculated animals with CFA -/+ 50µg MBP (gift by Prof Riccio) while the controls were represented by untreated animals. On day 14 the spleen and s.c. were dissected. The spleen was weighted and a portion was fixed for histological analysis. The cells from both spleen and s.c. were collected, stained with different combination of antibodies (anti CD3, HIS48, CD11b, CD45) and acquired using a FACSCanto flow cytometer (FACS).
We have previously described a decrease in the absolute cell count on day 14. Therefore we first investigated spleen cellular density calculating it as the ratio between the absolute cell number and the spleen weight. We showed a significant cellular density decrease in the spleen of CFA and EAE animals in comparison to the controls.
By means of FASC, in the same samples we also observed that CFA -/+ MBP induced a significant increase in the ratio between pan-granulocyte positive (HIS48+) and CD3+ cells. Moreover, the higher SSc population was mainly constituted by HIS48+CD11b+ cells.
Besides cellular analysis Hematoxylin-Eosin staining suggested a lymphoid hypoplasia of the periarteriolar lymphoid sheath and a myeloid hyperplasia in the red pulp.
On the other hand, even though the CFA and EAE rats seemed to have similar clinical score, we could recovery infiltrating cells only from the s.c. of the EAE animals. The flow cytometric analysis demonstrated the presence of both HIS48+ and CD3+ cells and a decrease in the HIS48+/ CD3+ ratio when compared with the EAE spleen. We also demonstrated that the higher SSc population was mainly constituted of HIS48+CD11b+ cells, with a CD45 fluorescence intensity comparable to that of lymphocyte population.
We can argue CFA was responsible for changes affecting both cellular density and the increase in the granulocyte population in the spleen, while it could not affected s.c leukocytes homing by itself. CFA is essential for EAE induction in Lewis rat and this could be also due to the innate immune response it evocates.
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