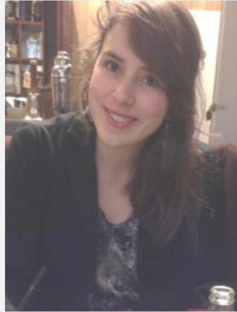
ImmunoTools *special* Award 2014



Maxime De Laere, PhD student

Supervisors: Prof. Dr. Zwi Berneman & Dr. Nathalie Cools

Laboratory of Experimental Hematology (Vaxinfectio)
Faculty of Medicine and Health Sciences
University of Antwerp, Wilrijk (Antwerp)

Dendritic cell recruitment to the central nervous system: moving targets to treat multiple sclerosis

Background

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). This demyelinating neurodegenerative disorder is considered to be a T cell-mediated autoimmune disease, in which autoreactive T cells specific for myelin-derived antigens cause inflammation and tissue damage in the CNS. Emerging evidence indicates that this auto-immune reaction is, at least in part, driven by dendritic cells (DC), a subtype of antigen-presenting cells with the unique capacity to stimulate and polarize T cells, thus playing a crucial role in maintaining the homeostasis between tolerance and immunity. Recent research in our laboratory has indeed shown an altered frequency and phenotype of DC in the peripheral blood of MS patients as compared to healthy controls[1]. Besides aberrant expression of maturation markers, circulating DC of MS patients also demonstrate an increased expression of migration markers, such as chemokine receptors CCR5 and CCR7. In addition, others have reported increased levels of a variety of chemokines, including CCL3, CCL5 and CCL19, in parenchymal lesions and cerebrospinal fluid (CSF) of MS patients[2–4]. Together with our results, this suggests an increased migration of DC from the bloodstream to the CNS. DC and DC transmigration consequently represent interesting targets for the development of new therapeutic strategies for MS. However, the phenotypic and functional characteristics of these pathogenic transmigrating DC have not been characterized completely, neither has the process of DC transmigration been studied in detail in the specific context of MS.

Aims

Current project focuses on the transmigratory capacity of DC isolated from the blood of MS patients. Using an in vitro blood-brain barrier (BBB) model, we aim at studying the transmigration of these DC in response to a variety of chemokines and at identifying the role of different adhesion molecules, integrins and matrixmetalloproteinases in the transmigration of these cells through the blood-brain barrier. In addition to studying the transmigration of pathogenic DC isolated from the blood of MS patients, the transmigratory capacity of in vitro generated tolerogenic DC (tolDC) will also be analyzed. TolDC-based therapies are currently one of the most promising immunotherapeutic strategies for auto-immune diseases such as MS. For such treatment to be effective, migration of the injected DC to lymph nodes and to the site of inflammation, which in case of MS is the CNS, is a prerequisite.

Methods implementing **ImmunoTools** reagents (**in bold**)

The in vitro BBB model consists of human astrocytes and human umbilical vein endothelial cells (HUVECs) co-cultured on opposing sides of a 3µm pore-size tissue culture insert insert. DC transmigration through this bilayer is stimulated by different chemokines (**CCL2, CCL3, CCL5, CCL7, CCL8, CCL18, CXCL10, CXCL12a, CXCL12b**), coated onto the apical side of the HUVEC layer and added to the lower well compartment. Twenty-four hours after adding the DC to the upper compartment, 2 different DC subsets can be isolated, namely migrated DC, isolated from the lower compartment, and non-migrated DC, recovered from the upper compartment. The phenotype, gene expression profile and T cell-stimulatory capacity of these different DC subsets is subsequently analyzed using flow cytometry, RT-PCR-array and DC/T-cell co-cultures, respectively. Multiparametric flow cytometric analysis allows us to differentiate between conventional DC (**CD11c**) and plasmacytoid DC and to analyze the degree of maturation and activation state of the DC (**HLA-DR, CD40, CD80, CD86**) as well as the expression of migration-associated markers, such as **CD62L**.

TolDC will be differentiated in vitro from CD14+ monocytes in presence of **IL-4** and **GM-CSF**, as described earlier[5]. In addition, immunomodulatory agents such as IL-10, TGF-β and VitD3 will be added to confer a stable immunosuppressive phenotype to the cultured DC. Chemokine-dependent migration of these tolDC will be studied using the in vitro BBB model.

In conclusion, by studying the migratory capacity of pathogenic MS-specific DC and of in vitro generated tolDC, we aim at further unraveling the role of these cells in the pathogenesis of MS and at investigating and exploiting the use of tolDC as a potential treatment for MS. The reagents offered by ImmunoTools will be of great value for realizing this project.

1. Thewissen, K. *et al.* Circulating dendritic cells of multiple sclerosis patients are proinflammatory and their frequency is correlated with MS-associated genetic risk factors. *Mult. Scler., Manuscript accepted for publication*
2. Balashov, K. E., Rottman, J. B., Weiner, H. L. & Hancock, W. W. CCR5+ and CXCR3+ T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. *Proc. Natl. Acad. Sci. USA* **96,** 6873–6878 (1999).
3. Boven, L. A., Montagne, L., Nottet, H. S. L. M. & De Groot, C. J. A. Macrophage inflammatory protein-1alpha (MIP-1alpha), MIP-1beta , and RANTES mRNA semiquantification and protein expression in active demyelinating multiple sclerosis (MS) lesions. *Clin. Exp. Immunol.* **122,** 257–263 (2000).
4. Krumbholz, M. *et al.* CCL19 is constitutively expressed in the CNS, up-regulated in neuroinflammation, active and also inactive multiple sclerosis lesions. *J. Neuroimmunol.* **190,** 72–79 (2007).
5. Anguille, S. *et al.* Short-term cultured, interleukin-15 differentiated dendritic cells have potent immunostimulatory properties. *J. Transl. Med.* **7,** 109 (2009).

ImmunoTools *special* AWARD for **Maxime De Laere** includes 17 reagents

FITC - conjugated anti-human CD86, HLA-DR,

PE - conjugated anti-human CD11c, CD80,

APC -conjugated anti-human CD40, CD62L,

recombinant human cytokines rh GM-CSF, rh IL-4, rh IP-10/CXCL10, rh MCP1/CCL2, rh MCP2/CCL8, rh MCP3/CCL7, rh MIP-1α/CCL3, rh MIP-4/CCL18, rh RANTES/CCL5, rh SDF-1α/CXCL12a, rh SDF-1β /CXCL12b, DETAILS