ImmunoTools *special* Award 2013

Lorena Usero Redrejo, PhD student

Supervisor: Dr. Carme Roura-Mir

Immunologia Cel.lular, Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra (Cerdanyola del Vallès) Barcelona

Characterization of NKT cells in patients with relapsing-remitting multiple sclerosis

The aim of this project is to study the possible role of NKT cells in the progress or regulation of the autoimmune response in Multiple Sclerosis (MS). We will analyze their presence in active brain lesions from MS patients and generate NKT cell clones to perform phenotypic and antigenic specificity studies.

MS is an inflammatory disease of the CNS characterized by multifocal lesions in the white matter of the brain with immune cells infiltration that is associated with prominent areas of demyelination, axonal damage and glial formation scars. There are two major forms of MS, primary progressive (PP)-MS and Relapsing-Remitting (RR)-MS which later can become secondary progressive (SP)-MS. It has been described that MS begins with the activation of autoreactive $CD4^+$ T cells and their differentiation to Th1 and maybe to Th17 T effector cells. Antibodies, $CD8^+$ T cells and complement or others factors produced by innate immunity cells also contribute to the cell damage. It is thought that a deficient immunoregulation of the Th1 cell-mediated autoimmune attack by Treg and NKT cells could be responsible for the relapsing-remitting phases in MS.

The targets of the autoimmune response are myelin-derived proteins such as MBP, PLP or MOG but also lipid antigens which constitute up to a 70% of the myelin sheath. NKT cells, which express a TCR and NK cell markers, are activated by lipid antigens presented by CD1 molecules. They recognize myelin-derived lipids such as gangliosides, sulfatide, sphingomyelin and other endogenous lipids. There are different subsets of NKT cells, based on CD1 restriction, TCR composition and coreceptors phenotype. It has been shown that MS patients have increased frequency of circulating NKT cells specific for glycosphingolipids, gangliosides, sulfatide or other neutral lipids presented by CD1b or CD1d molecules. CD1 proteins are expressed in several CNS cell types. Reports on the frequency of NKT cells describe a reduction in $V\alpha24J\alpha18^+$ NKT cells in the PBMCs and their detection in brain lesions of MS patients. It has also been observed that in the remitting phase there are transient increases in the number of NKT cells in the cerebrospinal fluid (CSF) and also in PBMCs. In the EAE model it has been shown that the activation of NKT cells with an analogous of α-galactosylceramide (OCH) or the increase of NKT cells number by transgenesis decreased the

severity and delayed the disease onset. This effect was attributed to a shift of the cytokine profile of NKT cells during the different stages as their function was reported to be biased toward T_h2, an immunosuppressive phenotype, in MS patients in remission but not in those with relapse of disease.

A preliminary study carried out in our laboratory showed the expression of the TCR Vα24 and Vβ11 chains by PCR in samples from active brain lesions and CSF from a MS patient, suggesting the presence of NKT cells in the target tissue of the autoimmune response. So the goal of this project is to confirm these results analyzing the presence of NKT cells and the CD1d expression in brain lesions and CSF from a group of RRMS patients. In order to examine a putative pathogenic or regulatory role of NKT cells in RRMS we also will assess their phenotype and soluble factors secretion in CSF and PBMCs from RRMS patients and PBMCs from healthy donors. To do so, we will use a panel of monoclonal antibodies to determine the predominance of the different subsets of $CD3^+Vα24Jα18^+$ NKT cells ($CD4^+$, $CD8^+$, DN). Furthermore, we will characterize their entire phenotype analyzing the expression of different cell markers such as: CD56, CD161, CD94, CD69, CD44, CD40, CD62L, CD45RA, CD45RO, CD25, and other molecules relevant for the NKT cell function (i.e.KIR2DL1, KIR2DL2/D3NKG2D, Granzyme A, Granzyme B). If possible we will measure the expression of HLA-ABC, HLA-DR, CD1a, CD1b, CD1c and CD1d in the CSF and brain tissue samples. As control, we will use labeled isotype matched immunoglobulins. The NKT cells obtained from PBMCs and CSF will be expanded *in vitro* to study their cytokine secretion pattern. We will measure IL-2, IL-4, IL-10, IL-7, IL-13, IL-17A, TNFα, IFNγ, GM-CSF, IL-12p70, TGF-β1 and IL-15 by ELISA. The cytokine profile will help to determine the effector and/or immunoregulatory function of the NKT cells.