lymphocytes. We further detected Ninjurin-1 expression on antigen presenting cells, such as CD11c DCs and F4/80 macrophages within the CNS of MOG-induced EAE-affected C57BL/6 mice. Finally, using a modified Boyden chamber assay, we showed that migration of *ex vivo* CD14 monocytes across the human endothelium is significantly reduced following treatment of BBB-ECs with a competing Ninjurin-1 blocking peptide. Our findings suggest that Ninjurin-1 is a novel adhesion molecule of the CNS endothelium that acts as a regulator of monocyte migration into the brain.

doi:10.1016/j.clim.2009.03.024

OR.18. VLA-4-Dependent Recruitment of Effector Memory CD8+T Lymphocytes into the Central Nervous System

Igal Ifergan[1], Hania Kebir[1], Gabriel Marceau[2], Monique Bernard[1], Pierre Talbot[2], Nathalie Arbour[1], Alexandre Prat[1]. [1]CHUM-Notre-Dame, Montreal, QC, Canada; [2]INRS-Institut Armand-Frappier, Laval, QC, Canada

The presence of $CD8^+$T lymphocytes in infiltrates of the central nervous systems (CNS) has been well documented: clonally expanded $CD8^+$T lymphocytes are present in multiple sclerosis (MS) lesions, as well as in the cerebrospinal fluid (CSF) of MS patients. In human MS, and in its animal model Experimental Autoimmune Encephalomyelitis (EAE), $CD8^+$T lymphocytes are found in active lesions. However the phenotype of migrating $CD8^+$T lymphocytes and the mechanism by which such cells cross the blood-brain barrier (BBB) remains largely unknown. Using CSF obtained from MS patients and spinal cord material from myelin $MOG_{(35-55)}$-induced EAE and from coronavirus-induced encephalitis, we demonstrate that $CD8^+$T lymphocytes are mostly of the effector memory (EM) phenotype ($CD8^+$, $CD62L^-$, $CCR7^-$, $CD28^-$, $GranzymeB^{hi}$). We further show that purified human $CD8^+$TEM lymphocytes transmigrate more readily across human BBB endothelial cells than *ex vivo* un-fractionated CD8 lymphocytes and that BBB endothelium promotes the selective recruitment of $CD8^+$TEM lymphocytes. Furthermore, we provide evidence for an active and selective recruitment of IFN-γ-and IL-17-secreting $CD8^+$lymphocytes by human and mouse BBB endothelium, *in vitro* and *in vivo*. Finally we show that the migration of $CD8^+$T lymphocytes across BBB-ECs is dependent on VLA-4, but independent of ICAM-1/LFA-1, ALCAM and the chemokine MCP-1. Our study thus provides evidence for an active role of the BBB in the recruitment of potentially auto-aggressive IL-17 and IFN-γ secreting CD8 TEM lymphocytes to the CNS, through a VCAM-1/VLA-4 adhesion molecule pathway.

doi:10.1016/j.clim.2009.03.025

OR.19. TNFR1 Controls the Recruitment of Macrophages but Not T Cells in Experimental Autoimmune Uveoretinitis

Ben Raveney, David Copland, Andrew Dick, Lindsay Nicholson. University of Bristol, Bristol, UK

The TNFR1 pathway is essential for the development of experimental autoimmune uveoretinitis (EAU). Macrophages from C57BL/6 wild type (WT) mice with a targeted deletion of TNFR1 (TNFR1 ko) do not produce nitric oxide (NO) in response to stimulation with IFNγ and the mice are resistant to the induction of EAU, although they do develop mild disease. NO is a multifunctional small molecule. It provides a substrate for cytotoxic peroxynitrite generation, which is an important cause of retinal damage, but it also limits T cell proliferation non-destructively. To investigate the role of T cell inactivation in EAU, we purified CD11b+cells from the retinas of WT and TNFR1 ko 24 days after the induction of disease. Antigen presenting cells from the WT, but not TNFR1 ko retinas, inhibited T cell proliferation in an NO dependent fashion. We then analysed TCRζ expression by T cells obtained from the eye at peak disease and showed that this was down-regulated in WT but not TNFR1 ko mice. This raised the question of why an environment more conducive to robust T cell proliferation within the target organ did not result in greater disease in TNFR1 ko mice. To investigate this, we produced mixed bone marrow chimeras from WT and TNFR1 ko. When EAU was induced in these animals, we found that.TNFR1 expression was essential for macrophage, but not T cell trafficking to the target organ. These data show that T cells and macrophages use independent signalling pathways during infiltration of the retina.

doi:10.1016/j.clim.2009.03.026

OR.20. Cellular Migration in Transplantation Studied Using *in vivo* Microscopy

Georges Tocco, Pilhan Kim, Cavit Kant, Charles Lin, Seok-Hyun Yun, Gilles Benichou. Massachusetts General Hospital, Boston, MA

We investigated the trafficking of cells after placement of primarily vascularized (skin and heart) or non-vascularized transplants (skin) in mice, using *in vivo* microscopy. As early as 3 hours after non-vascularized skin transplantation, we observed a massive cellular infiltration of host cells into the graft. The accumulation of host-derived cells was much stronger and faster in primarily vascularized grafts (skin and hearts). The presence of infiltrating MHC-Class-II+ recipient cells was detected in vascularized grafts one day after transplantation while these cells were detected only 4 days after placement of non-vascularized skin grafts. We then examined the migration of donor cells into the recipient. As early as day 1 after placement of vascularized transplants (skin and heart), donor MHC Class-II+cells were found in all lymph nodes (draining and non-draining) and the spleen of the recipients. In contrast, for non-primarily vascularized skin grafts, very few donor cells (4-6 cells) were detected exclusively in the recipient draining lymph nodes and only at 5 days post-transplantation. This finding challenges the current dogma that donor antigen presenting cells (APCs) (passenger leukocytes) initiate the direct pathway of allorecognition by T cells in the recipients' lymph nodes. Indeed direct alloresponses can be recorded 4 days after skin grafting i.e. before donor APCs were found in the recipient's draining lymph nodes. This suggests that direct T cell allorecognition could occur in the