VOLUME 203, ISSUE 2 25 OCTOBER 2008 ISSN 0165-5728

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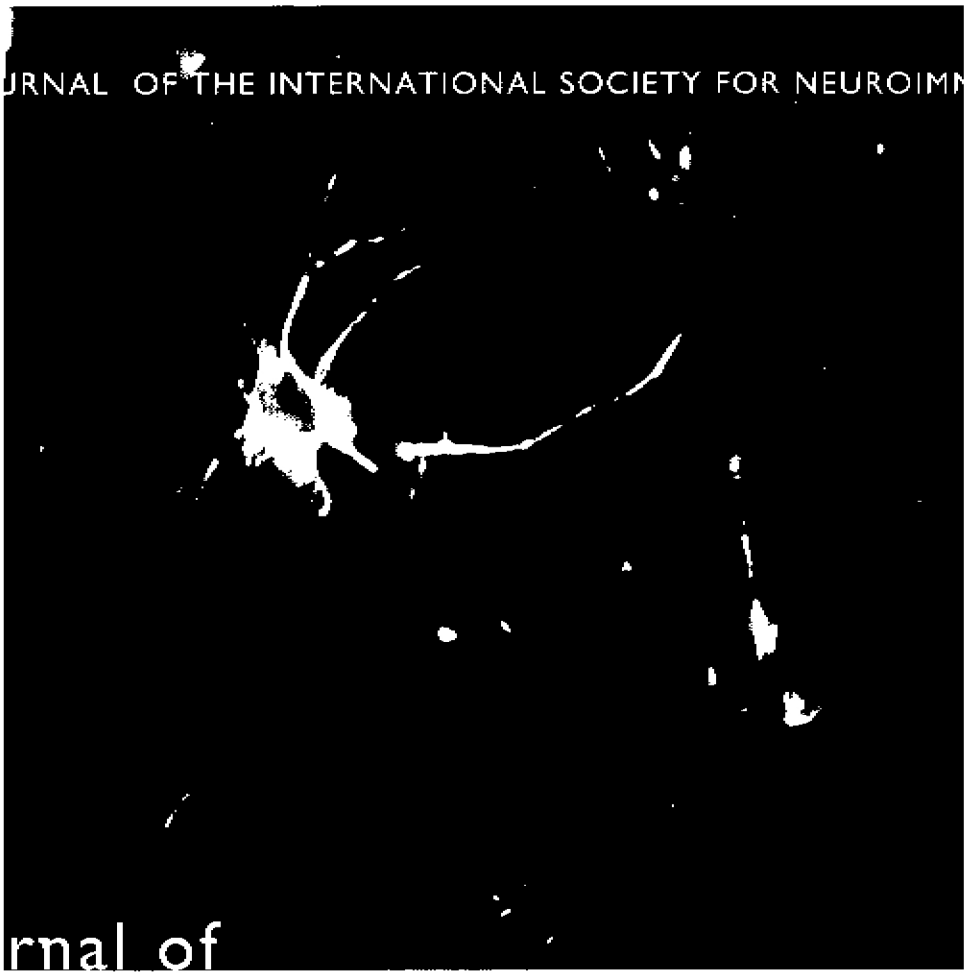

Neuroimmunology

9th International Congress of Neuroimmunology

TMBP-GFP cells increased during the next 24 h. The vast majority of T cells moved continuously at the vessel surface. A small proportion of the T cells (5%), however, were arrested in contact with perivascular phagocytes. On day 2.5 p.t. the TMBP-GFP cells started to distribute throughout the entire meningeal areas and to immigrate into the CNS parenchyma along the perivascular Virchow–Robin spaces. An increasing part of the T cells (25%) were now forming long lasting contacts to perivascular/meningeal phagocytes. From day 3 p.t. on, the T cells invaded the parenchyma adjacent to the meninges and CNS vessels and within the next 24 h they diffusely distributed throughout the entire CNS tissue. Within the CNS parenchyma 65% of the T cells were constantly moving whereas 35% were stationary.

2-Photon microscopy was complemented by studies of the gene expression profiles of TMBP-GFP cells on their way into the CNS tissue. TMBP-GFP cells residing in the spleen and circulating in blood displayed a "migratory" phenotype with low activation markers and upregulation of genes relevant for cell migration. Importantly, upon entry into the meningeal, TMBP-GFP cells were strongly activated producing high amounts of pro-inflammatory cytokines. Further activation occurred following entry into the parenchyma.

We present data indicating that autoaggressive T cells enter their target organ via the meninges where they recognize endogenous autoantigen presented by resident meningeal/perivascular phagocytes.

50 — Mechanisms by which toll like receptors exacerbate or modulate disease severity in experimental autoimmune encephalomyelitis

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Multiple Sclerosis (MS) is an immune mediated demyelinating disease of the central nervous system (CNS). We aim to find mechanisms by which toll like receptors (TLRs) exacerbate or suppress CNS inflammatory demyelination. Both effects have been observed in experimental autoimmune encephalomyelitis (EAE) an animal model of MS-TLRs 2 and 9 in exacerbating disease, TLR4 in delaying onset and TLR3 in reducing severity. Naturally occurring CD4+CD25+ regulatory T cells (Tregs) can suppress autoimmune reactions and were recently found to express TLRs.

We are in the process of studying the effect of TLR3 on Treg suppression in the mouse by co-culturing CD4+CD25-responder cells with different ratios of Tregs with or without poly(I:C). Preliminary studies indicate that TLR3 ligand poly(I:C) increases the suppressive ability of Tregs in C57BL/6 mice and this is a potential mechanism by which EAE disease severity is reduced after poly(I:C) administration. We also determined which cytokines and chemokines are induced by TLR stimuli in murine splenocytes. The inflammatory chemokine MCP-1 was unregulated by TLR stimuli-Pam3CSK (TLR1/2), HKLM (TLR2), LPS (TLR4), Flagellin (TLR5), follistatin-like 1 (TLR6) and ssRNA40 (TLR7). The anti-inflammatory cytokine IL-10 was unregulated by the same stimuli and also by poly(I:C) (TLR3) and CpG ODN1826 (TLR9). TLR9 also induced increased production of IL12p70. All TLR stimuli also induced increased T cell proliferation. The response of human monocyte derived dendritic cells (MDDCs) to TLR stimuli in terms of maturation and cytokine and chemokine production was also studied. MCP-1 was unregulated by TLR stimuli poly(I:C) and LPS. The inflammatory cytokines IL-6 and TNF-a were unregulated by these two stimuli and also by Pam3CSK4 and HKLM. The effects of TLR stimuli on MDDCs in MS patients are in progress. We are also in the process of determining the effect of a range of TLR stimuli on the clinical and pathological outcome of EAE.

TLR stimuli alter the regulatory ability of Tregs and this may contribute to disease exacerbation or suppression in EAE. TLR stimuli lead to the production of cytokines and chemokines that may regulate autoimmune diseases and there is potential to aid immunotherapy by targeting these TLRs and the cytokines produced.

51 — A flow cytometric approach in the characterization of actively induced EAE in Lewis rat

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Actively induced Experimental Autoimmune Encephalomyelitis (EAE) is a well reproducible model for portraying the acute phase of multiple sclerosis as well as good model to develop new promising treatments. Both lymph node and spinal cord (s.c.) have been widely described by means of molecular biology and immunohistochemistry. The aim of this study was to characterize both spleen and s.c. lymphocyte populations and to highlight possible useful markers to be monitored during new therapeutic treatments. Thus at the onset (day 10 after EAE induction) and at the peak of the disease (day 14 after EAE induction) we investigated CD4, CD8 and encephalitogenic TCRVb8.2+ T cells prevalence as well as the expression of adhesion molecules CD49d (VLA-4) and CD11a (LFA-1).

EAE was actively induced by subcutaneously inoculation of 50 μg guinea pig Myelin Basic Protein (gifted by P. Riccio) in complete Freund's adjuvant with inactivated Mycobacterium tuberculosis. The spleen and s.c. were dissected from both healthy (controls) and EAE-induced animals, cells were collected, stained with different combinations of conjugated antibodies and acquired using a flow cytometer.

In the spleen of EAE animal we reported a significant decrease in the mean absolute cell number on day 14, together with the presence of a CD45+ population other than lymphocytes as soon as day 10. Furthermore we observed an increase in CD4 and decrease in CD8 T cells compared to the controls on day 14, while no changes were reported on day 10. Moreover no alteration in the TCRVb8.2+ lymphocytes percentage as well as in CD49d (VLA-4) and CD11a (LFA-1) expression on CD4 and CD8 T cells was never observed. In the spinal cord no infiltrating lymphocytes were detected until day 14. Therefore the results on s.c. were compared to those obtained in the spleen on day 14. Thus CD4 lymphocytes percentage was higher in the s.c., while CD8 lymphocytes prevalence decreased. We also observed an increase in CD4 TCRVb8.2+ T cells percentage together with a significant increase in the expression of CD49d (VLA-4) which was more accentuated for CD4 than for CD8 T cells.

Our results confirm that EAE in Lewis rats is a CD4 T cell mediated disease in which the expression of TCR Vb8.2 and adhesion molecules is probably regulated mainly in the target organ. We show that multiparametric flow cytometry can be an informative method to describe immune system cells during the EAE ongoing.

Supported by a MIUR 2006 grant (prot. 2006064219-002).

52 — MMP-12 is beneficial in a model of ms through modulation of cytokines, chemokines and osteopontin

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While the elevation of several members of the matrix metalloproteinase (MMP) family is thought to promote the pathophysiology of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), the rise of particular MMP members may have contrary beneficial functions during disease processes. MMP-12 mRNA levels rise significantly in the spinal cord in EAE-afflicted mice (Weaver et al., FASEB J 19:1668–1670, 2005) and