Basic Science Workshop 4

Viral infections & autoimmune disease

Chairpersons: M. Brahic (Paris, FR)
P. Kennedy (Glasgow, UK)

61

Expression of mRNA encoding an NKG2D ligand, RAE-1, and effects of syngenic Natural Killer cells on neurons in primary culture

E. Backström, B. Chambers, K. Kristensson, H.-G. Ljunggren
Karolinska Institute (Stockholm, SE)

Natural Killer (NK) cells are important components of the innate and early induced immune response in the defense against a number of microbial infections and against tumors. Their function is regulated by engagement of activating and inhibitory receptors. This raises an interesting question concerning the nervous system, since neurons normally lack the expression of the inhibitory MHC class I molecules. The protein encoded by retionic acid early inducible gene-1 (RAE-1), was recently identified as a ligand for the activating receptor NKG2D, that unlike other activating receptors is expressed on all NK cells. In order to determine the molecular mechanisms by which NK cells may kill a population of neurons, mouse dorsal root ganglia (DRG) and hippocampal neurons were exposed to syngenic IL-2-activated NK cells. The DRG neurons were rapidly killed while the hippocampus neurons were found to be resistant. Hippocampal neurons from beta2-microglobulin deficient mice were also resistant, which shows that resistance is not due to expression of inhibitory MHC class I molecules on the target cells. mRNA encoding RAE-1 was strongly expressed in the susceptible DRG neurons, but only at low levels in the resistant hippocampal neurons from the central nervous system. This study shows that high expression of mRNA encoding RAE-1 in neurons parallels susceptibility to NK cells and that lack of inhibitory MHC class I molecules in the nervous system is not enough for eliciting an NK cell-mediated attack.

62

Clonal expansion of CD8+ T cells in the cerebrospinal fluid of multiple sclerosis patients

M. Jacobsen, D. Zhou, S. Cepok, F. Vogel, N. Sommer, B. Hemmer
Clinical Neuroimmunology Group (Marburg, D)

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS). Although the aetiology of MS is still unknown, it is widely believed that T cells play a central role in its pathogenesis. Recent studies demonstrated oligoclonal expansion of CD8+ T cells in MS lesions and cerebrospinal fluid (CSF) of MS patients. We identified accumulation of CD8+

T cells with identical or highly similar T-cell receptor variable—beta (TCRBV) and—alpha chains in the CSF of 5 out of 5 patients with multiple sclerosis. In none of the patients we found clonal expansion of CD4+ T cells. Further phenotypic analysis of *in vivo* expanded CD8+ T cells demonstrated, that these cells belong to a memory T cell population and are characterised by the secretion of both Th1 and Th2 cytokines (Tc0 phenotype). Using flow cytometry for TCRBV expression and clone-specific rt-PCR we demonstrate the occurrence and persistence of the CNS expanded clonotypes in the peripheral blood of the MS patients. The frequency of the clonotypes in the peripheral blood varied during the disease course. Our findings support the role of CD8+ T cells in the pathogenesis of MS and favour the idea of an ongoing immune response in the peripheral lymphoid system of MS patients. Although the target antigens of these CD8+ T cells are as yet unknown, viral antigens are among the candidates.

63

Induction of autoreactive CD8+ cytotoxic T-cells during Theiler's Murine encephalomyelitis virus infection: implications for autoimmunity

I. Tsunoda, R.S. Fujinami
University of Utah (Salt Lake City, USA)

Theiler's murine encephalomyelitis virus (TMEV) belongs to the family Picornaviridae and certain strains of virus cause demyelinating disease in infected mice. Although immune responses are involved in demyelination, the effector mechanisms leading to demyelination are not well defined. We hypothesize that autoreactive cytotoxic cells contribute to the central nervous system pathology. We tested whether an autoreactive cell in SJL/J mice induced by TMEV infection mediated killing of syngenic target cells. Spleen cells from TMEV infected mice were stimulated *in vitro* with TMEV infected antigen presenting cells and the stimulated lymphocytes used as effector cells. The effector cells differed from conventional cytotoxic T cells in that these cells could kill both TMEV infected as well as uninfected syngenic or semisyngenic cell lines. However, these effector cells could not kill an allogenic cell line. The TMEV induced autoreactive cells differed from conventional natural killer (NK) cells or lymphokine-activated killer (LAK) cells, in that they could not kill NK cell sensitive or NK cell resistant cell lines. To demonstrate that these effector cells were specific for TMEV infection, SJL/J mice were infected with vaccinia virus. Induction of autoreactive cells was not observed. Cytotoxicity required direct cell-to-cell contact,

and was mediated by a Fas-FasL pathway, and not by a perforin route. The killer cells were CD3 + CD4 – CD8+. Intracerebral adoptive transfer of the effector cells into naïve mice caused meningitis and perivascular cuffing not only in the brain parenchyma, but also in the spinal cord lesions, which was not evident in control mice. This is the first report demonstrating that TMEV infection can induce autoreactive cytotoxic cells that can induce central nervous system pathology.

64

Demyelination is mediated by gamma-delta T cells in mice infected with a neurotropic coronavirus

A. Dandekar, S. Perlman

University of Iowa (Iowa City, USA)

Infection of mice with mouse hepatitis virus, strain JHM (MHV) results in acute and chronic demyelination with many similarities to the human disease multiple sclerosis (MS). This pathological process is largely T cell-mediated and MHV infection of mice lacking B and T cells does not result in demyelination. In apparent contradiction to these results, robust demyelination was detected in MHV-infected young nude (athymic) mice. Herein, we show that demyelination in nude mice is mediated by gamma-delta T cells. These cells, but not conventional CD4 or CD8 alpha-beta T cells, were detected in the central nervous system (CNS) of MHV-infected nude mice and their depletion with neutralizing antibody resulted in an 80% reduction in demyelination. These results show, for the first time, that gamma-delta T cells can substitute for alpha-beta T cells in a virus model of demyelination and further support a pathologic role for gamma-delta T cells in patients with MS.