Journal of NeuroVirology, 8(suppl. 1): 47–48, 2002
 ISSN 1355–0284/02 $12.00+.00
DOI: 10.1080/13550280290050000

Basic Science Workshop 6

Neuronal dysfunction, signalling & apoptosis

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95

Contribution of HIV-1 Tat and the potential tumor suppressor protein, p73 beta to the regulation of the human Bax gene

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The Bcl-2 family of proteins has been shown to be involved in the inhibition of the apoptosis. On the other hand, some other members of this family of proteins, such as Bax, promote apoptosis. There is evidence that tumor development is associated with the inactivation of Bax concomitant with the over expression of Bcl-2, leading to an overall inhibition of apoptosis. The HIV-1 Tat, a viral protein whose expression can deregulate expression of several important genes was shown to induce apoptosis by up-regulating the endogenous level of Bax protein. In this study we focused our attention on the mechanisms involved in Tat-induced up-regulation of Bax in astrocytic cells. We performed transient tarnsfection assays in the presence of the potential tumor suppressor, p73, which was used because of its ability to up-regulate Bax protein. Results from our transient transfection analysis indicated that Tat stimulates Bax promoter activity in human glioma cells and that this activation is altered in the presence of p73 beta. Western blot analysis demonstrated an increase in the level of Bax protein in Tat-transfected cells, and a decrease in cells co-transfected with Tat and p73 beta expression plasmids. Apoptosis was assessed by TUNEL assay in CFP-Tat-transiently transfected cells in the presence or absence of p73 beta. Apoptosis was shown to be increased in cells transfected with CFP-Tat alone. Interestingly, co-existence of Tat and p73 beta led to a reduced level of apoptosis. In light of the earlier observations pointing at the utility of Tat in protein transduction, a possible therapeutic strategy can be developed to inhibit tumor cell growth.

96

Interaction of immune and central nervous systems during viral infection: a role for Fas and metalloproteinases in apoptosis of neural precursors

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During viral infection, neuroinvasion of the central nervous system (SNC) by immune cells allow efficient elimination of infectious agents and stimulate the process of tissue repair. However immune cells may play key roles in initiating and potentiating detrimental inflammatory reactions, which result in inflammatory CNS diseases such as HTLV-1 associated myelopathy, measles associated encephalitis and HIV associated encephalopath y. In order to understand the pathophysiology of these diseases, we developed paradigms to study 1-neuroinvasion of virus and infected T lymphocytes, 2-virus-induced alteration of glial/neuronal function, 3- the role of inflammatory mediators. Study models consist in cerebral mouse infection with a measles-related virus (CDV), interaction of HTLV-1-infected T cells with primary glial cultures and an *in vitro* model of blood-CSF barrier. Recent evidence from our laboratory indicate that 1-astrocytes and neurones greatly amplify the inflammatory cascade following CDV infection or contact with infected-T cells, through a cytokine-metalloproteinase MMP loop, 2) CDV and HTLV-1 are responsible for neural dysfunctions including alteration of the monoaminergic system, hypothalamic function and astrocytic glutamate catabolism, 3) blood-CSF interface is a potential site for T cell entry. We now report the role of MMP and Fas/Fas ligand system in apoptosis of human multipotent neural precursor induced by HTLV-1 infected T-cells. Blockade of cell death by anti-caspase peptides, detection of active caspase-3, -9, Bid truncation and mitochondrial alteration suggested the mediation of type II Fas. Integration of Fas on neural cell membrane triggered by infected-T cells is a key step. Interferon was not involved, but Fas-mediated apoptosis was modulated by MMPs and the endogenous inhibitors, TIMPs. Infected-T cells also induced death of rat immature oligodendrocytes. These data suggest that Fas-mediated apoptosis in neural precursors may play a significant role in neuroinflammatory diseases following viral infection. They also support a new concept implying MMP and TIMP in the susceptibility of neural precursors to Fas-mediated apotosis.

97

The glycoprotein of attenuated strains of rabies virus strain induces apoptosis

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We reported previously that the attenuated live rabies virus (RV) vaccine strain ERA triggers apoptosis in the human lymphobastoid Jurkat T cell line along with activation of caspase 1, 3, 8 and 9 and cytochrome C. In contrast, infection, with the pathogenic and neurotropic CVS RV strain did not cause apoptosis in Jurkat T cells. To identify which of the two major RV proteins (G and N) is responsible for the triggering of

apoptosis, both G and N of ERA were expressed individually in Jurkat T cells by using the inducible Tet-ON expression system. Induction of RV G expression but not RV N expression resulted in apoptosis indicating that the capacity of a particular RV to trigger apoptosis is largely determined by determinants of G protein. To further examine qualitative aspects of RV G that are associated with the induction of apoptosis and to determine whether observations made in Jurkat T cells take place in neurons, we infected human neuronal cells with recombinant RVs in which G from an attenuated strain was replaced by the G from a virulent strain. This experiment revealed that only recombinant RVs containing the G of a non-pathogenic RV strain, but not of a pathogenic strain, are able to trigger apoptosis in neuronal cells, suggesting that apoptosis is induced by determinants which are only present in the G proteins of non-pathogenic attenuated RV strains. These determinants could be, at least in part, responsible for the unique ability of attenuated RV strains to induce protective immunity.

98

HIV-Tat blocks NGF-induced neuronal differentiation through the STAT-3 pathway

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The HIV-associated dementia (HAD) is a serious disorder of the CNS, which represents a significant risk factor for death due to HIV infection. Among many different pathogenic factors associated with HAD, the transactivating factor of HIV-1 Tat is known to modulate gene expression in a variety of cell types by interacting with different host proteins. The mechanism/s through which Tat protein triggers neuronal dysfunction are not yet completely understood. Rat pheochromocytoma cell line PC12 is a well-established model to study the signal transduction pathways in neuronal differentiation. The nerve growth factor (NGF) binds and activates the TrkA receptors, which in turn activate several substrates, including MAP kinases ERK 1 and ERK 2. The sustained phosphorylation of ERKs is necessary to promote neuronal differentiation in this system. Conversely, PC12 cells over-expressing Tat show transformed phenotype evaluated by colony formation in soft agar and growth in serum-free medium. Upon NGF stimulation, PC12 over-expressing Tat do not extend neuronal processes and proliferate in culture despite of a prompt and sustained activation of MAP kinases indicating that alternative pathway/s are activated downstream of MAP kinases, which results in a positive effect on proliferation and a negative regulation of differentiation. The interference of Tat with one of these pathways has two important consequences: an increased expression of the serine/threonine phosphatase PP2A, and an accelerated degradation, via the proteasome complex, of p35 protein, one of the major players in the NGF-induced differentiation. We also show that, in the presence of Tat, the introduction of a dominant negative form of Stat-3 has two effects: restores the expression of PP2A to the basal levels, and reactivates the differentiation program. In conclusion, our data support a model in which HIV-Tat blocks the NGF induced differentiation in PC12 through the Stat-3 pathway and Stat-3 activation leads to an increased expression of PP2A protein which is likely responsible for the accelerated degradation of p35.