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HIV gp41 modulates expression of cytokines and chemokine receptors by astrocytes

Cornelia Speith1, Barbara Joestl1, Maria Barowa1,2, Manfred P. Dietrich1
1Institute for Hygiene and Social Medicine and Ludwig Boltzmann-Institute for AIDS Research, Innsbruck, Austria; 2present address: University of Southern California, Los Angeles, USA

The human immunodeficiency virus HIV-1 induces a complex of neurological symptoms (AIDS dementia complex; ADC) in 20-30% of HIV-infected patients. We investigated a possible role of HIV transmembrane protein gp41 in the development of ADC.

Induction of the astrocyte cell line L87 with gp41 induced time- and dose-dependently more than twofold upregulation of IL-10 secretion. Thus, gp41 via induction of anti-inflammation effects may inhibit the activity of brain-infiltrating T-cells and favor the spreading of HIV in the brain. Usage of inhibitors for different signal transduction pathways indicated the same transduction cascade for the modulation of IL-10 production in astrocytes as in monocytes with participation of cAMP/Pde5/cyclase pathway and activation of p70S6K.

We also investigated the biological consequence of enhanced IL-10 production on monocytes and astrocytes. In both cell types upregulation of IL-10 went in parallel with an enhanced expression of the chemokine receptor and HIV-1 coreceptor CCR5. Expression of the chemokine receptor CXCR4 was upregulated or only slightly upregulated. A possible role for gp41 in modulation of brain-specific host defense, cell migration and infectivity by HIV is discussed on the basis of these results.

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Subclones of a human neural cell line, SVG, differentially synthesizes cytokines and neurotrophins.

A.W. Phillips1,2, N. Merkel1, B. Curtin1 and E.O. Major2
1Department of Biology, Howard University, Washington DC, USA 20059
2LMNN, NINDS, NIH, Bethesda MD, USA 20892

The SVG cell line is a heterogeneous population of cells of neural origin established from human fetal brain cells and immortalized with the SV-40 large T (SV-40-T) gene product. Immunocytochemistry indicates 100% of cells are positive for the SV-40-T protein. SVG cells were co-cultured with human fetal brain cells to achieve fused heterokaryotypic cells. Clones of these cells were established and characterized. Here we report that there is a difference between select subclones and the SVG cell line. Immunoassay of media conditioned by the clones shows the cells secrete several cytokines including, BDNF, NGF, VEGF and PDGF. Furthermore, the data suggest that there are differences in the levels of cytokines secreted between the clones. Notably, there is not much fluctuation in levels of BDNF secretion among the subclones but only half the subclones secrete PDGF. Because some neurotrophic viruses such as JCV and HIV-1 display different tropism for glial or neuronal cells, these clones may provide a valuable substrate to dissect differences in viral susceptibility.

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Unraveling the complexity of the PrP receptor.

Laschñas, C.1, Peyrin, J.-M.1, Haik, S.1, Simonneau, S.1, Deslys, J.-P.1, Dormont, D.1 and Weiss, S.1, CEA, Service de Neurologie (DSV/DRM), CRSSA, 60-68, av. du Général Leclerc, BP 62, 92265 Fontenay aux Roses Cedex, France and 2Laboratorium für Molekulares Biologie-Genzentrum Institut für Biochemie der LMU, Feodor-Lynen-Str.25, 81377 München, Germany

In recent years, several candidate molecules have been proposed as receptors for the prion protein (PrP) which is central in the pathogenesis of transmissible spongiform encephalopathies. Lagendar sulfate proteoglycans (HSPGs), an unidentified 66 kDa cell surface molecule and the 37 kDa laminin receptor precursor (LRP). We show the LRP dependent binding of PrP at the surface of neuronal and non-neuronal cells using LRP antibodies. Hyperexpression of PrP in Hela cells did not increase the cell binding of exogenous PrP. Cell binding is similar in PrP(+)/- and PrP(0) primary neurons, demonstrating that PrP does not act as a coreceptor for LRP. Using antibodies covering the whole PrP sequence, we identified two LRP binding sites on PrP: PrP(144 to 179) binding directly to LRP, and PrP(53 to 93) binding to LRP only in the presence of a third interaction partner which we identified as HSPGs. Adding HSPGs to CHO cells deficient in heparan sulfate synthesis restored the capacity of PrP to bind to LRP through PrP(53 to 93). Using overlay assays on membrane enriched cell fractions we show that recombinant PrP binds to two proteins of 37 kDa and 67 kDa, both of which being recognized by polyclonal and monoclonal LRP antibodies. We conclude that LRP and the mature form of the laminin receptor (the 67 kDa LR) are specific receptors for PrP at the cell surface, and that HSPGs constitute an active interaction partner stabilizing the binding of part of the PrP molecule to its receptor.

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**P126**

37 kDa LRP acts as the cell surface receptor for the prion protein.

Gauschnecz, S.1, Hartt, C.1, Lecht, C.1, Rieger, R.1, Ryley, M. L.1, Lasnitzki, C.1,2, and Weiss, S.1,1Laboratorium für Molekulares Biologie-Genzentrum Institut für Biochemie der LMU, Feodor-Lynen-Str.25, 81377 München, Germany and 2CEA, Service de Neurologie (DSV/DRM), CRSSA, 60-68, av. du Général Leclerc, BP 62, 92265 Fontenay aux Roses Cedex, France

Recently, the 37 kDa laminin receptor precursor (LRP) was identified as an interactor for the prion protein (PrP) (1, for reviews 2 and 3). LRP and PrP co-localize on the surface of N2a cells and Semliki Forest Virus (SFV) RNA transfected BHK cells hyperexpressing PrP and LRP. The LRP dependent binding of recombinant and authentic PrP to N2a cells was demonstrated by cell binding assays. Hyperexpression of LRP on the surface of BHK cells resulted in a dose dependent binding of exogenous PrP. Trypsin treatment of the cell surface of N2a cells showed the LRP dependent internalization of PrP. In contrast, in wild-type LRP, the expression of a LRP mutant lacking the transmembrane domain (aa68 to aa101) termed LRP(68-101) led to secretion of the mutant from BHK cells and completely abolished PrP binding and internalization, confirming that LRP is required for both processes. Mapping analysis in the yeast two hybrid system identified an interaction domain on PrP for LRP termed PrP(68-101). We also show that LRP appears monomorphic by size exclusion chromatography, fails to interact with itself in the yeast two hybrid system and is present as a 37 kDa protein on the surface of N2a and BHK cells while a higher molecular weight form has been described which may result from a heterodimerization of LRP. Application of an LRP specific antibody to N2a cells abolished the generation of PrP(68-101) demonstrating that LRP is essential for prion replication.
Abstracts

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Homodimerization of the prion protein. Hundt, C., Gauczyński, S. and Weiss, S., Laboratorium für Molekulare Biologie-Genezentrum-Institut für Biochemie der LMU, Födor-Lyven Str.25, 81377 München, Germany

Dimer formation of prion proteins (for review see 1) has been observed in cultured cells, in brains of hamsters suffering from scrapie (2) and within the PrP oligomerization process (3). Highly purified FLAG tagged prion proteins from hamster, man and cattle expressed in the baculovirus system appeared to be dimeric under native conditions. The octarepeat region of the prion protein (aa55 to aa93) and PrP80-230 represent PrP/PrP interaction domains identified by a yeast two hybrid analysis. Additional octarepeats as observed in patients suffering from familial CJD reduced or abolished the PrP/PrP interaction in the yeast two hybrid system whereas the polymorphism Met29Val, the GSS mutation Pro102Leu and the FFI mutation Asp178Asn had no influence on the PrP/PrP interaction. Behavioural PrP/PrP interactions were further observed in SFV-RNA transfected BHK cells co-expressing differently tagged hamster prion proteins. We hypothesize that PrP dimers might play an important role in the PrP oligomerization process involving the conversion of PrP(Sc) to PrP(Sc).


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RNA Aptamers directed against purified PrPSc preparations (PPPs) recognize specifically PrPSc in brain homogenates and reduce PrPSc propagation in Scn2a cells. Lamézas, C., and Weiss, S., iCEA, Service de Neurovirologie (DSV/DRM), CRSSA, 60-68, av. du Général Leclerc, BP.6, 92265 Fontenay-aux-Roses Cedex, France and 2Laboratorium für Molekulare Biologie Genezentrum-Institut für Biochemie der LMU München, Födor-Lyven-Strasse 25, D-81377 Munich, Germany

 Antibodies failed for a long time to distinguish between the prion protein isoforms PrPSc and PrPSc. Recently, however, an antibody has been identified, which recognizes PrPSc (1). Simultaneously, RNA aptamers have been reported which recognize recombinant PrPSc and authentic PrPSc from brain homogenates of mouse, hamster and cattle (2). Purified PrPSc preparations (PPPs) represent immobile aggregates consisting mainly of PrPSc (3). Employing a complex RNA library (2) and PPPs prepared from scrapie infected hamster brains (4), we selected RNA aptamers specifically interacting with PPPs. The selected RNA aptamer PPP-I recognized specifically PrPSc/BSE/CJD from hamster, cattle and man suffering from scrapie, BSE and CJD respectively. The digoxigenin labelled PPP-I aptamer specifically recognized PrPSc from hamster brains in dot blot analyses. The PPP-I aptamer reduced the amount of PrPSc in Scn2a cells suggesting that it interferes with the PrPSc generation process. The PPP-I aptamer might be a powerful tool in diagnosis and therapy of transmissible spongiform encephalopathies.


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Transcellular and paracellular pathways of HIV neuroinvasion and heart invasion and cell signaling. Falila, M.F., Gualova, C., Berger O., Graves, M.C., Pepik, W., Pushkarsky, T., Bukinsky, M., and Brena, A. *UCLA Medicine and Neurology, Los Angeles, California, USA. John Hopkins Oncology, Baltimore, Maryland, USA. *Flower Institute, Mahasset, New York, USA. *Leukocyte Biology, Baylor College of Medicine, Houston, Texas, USA.

Our hypothesis has been that HIV-1 invades across brain microvascular endothelial cells (BMVEC) and coronary artery endothelial cells (CAEC) by transcellular and paracellular routes or piggy-back in mononuclear cells but not by replication in endothelia. Background: Previous studies in BMVEC showed HIV infection ranging from productive to latent, but molecular techniques were not used. Methods: We observed using microscopic (immunofluorescence and transmission electron microscopy), immunologic (ELISA) and molecular (HIV DNA PCR with the RUS, pol and 2LTR primers and HIV RNA PCR (AmpliCor)) techniques that HIV-1 (macrophage-tropic and lymphotropic strain) did not productively infect CAEC or BMVEC. CAEC were obtained commercially and BMVEC were derived from temporal lobe excisional tissues. Both BMVEC and CAEC displayed CXCR4 but BMVEC displayed a greater density of CCR3 compared to CAEC both in vivo and in vitro. BMVEC formed a tight monolayer on a porous membrane that was used as a blood-brain barrier (BBB) model. Results: HIV DNA PCR showed reverse transcription in BMVEC of only RUS, whereas in CAEC also of pol. The exposure of BMVEC and CAEC to purified HIV-1NL4-3 resulted in rapid activation of interleukin activated protein kinase (MAPK) cascade involving MEF, ERK, JNK, p38. Pertussis toxin blocked ERK and JNK activation, indicating a role for a G-protein-coupled receptor. HIV-1R5-FL induced interleukin-6 secretion by endothelia in prolonged fashion. Exposure of CAEC to HIV-1 resulted in severe cytoplasmic vacuolization. The kinetics of viral invasion in the blood-brain barrier (BBB) model constructed with BMVEC showed greatly increased permeability to HIV-1 24 h after infection but no change in immun permeability. These results suggest that HIV-1 could remodel the endothelia into a vascular, spongy barrier that allows virus invasion by transcytosis through these vacuoles. The abused drug cocaine (as well as tumor necrosis factor-a) increased endothelial permeability to HIV and well as the virus, suggesting that the cocaine could potentiate virus invasion by a paracellular route. Cocaine also enhanced adhesion molecule expression and monocyte transmigration across the BBB, and could potentiate HIV invasion by these mechanisms. Conclusions: Taken together, the results indicate that HIV-1 may penetrate into the brain and the myocardium by both transcytosis and paracellular transport.

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