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Chemotaxis of T-cells after infection of human choroid plexus papilloma cells with *Echovirus 30* in an *in vitro* model of the blood–cerebrospinal fluid barrier

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ABSTRACT

Enterovirus is the most common pathogen causing viral meningitis especially in children. Besides the blood–brain barrier (BBB) the choroid plexus, which forms the blood–cerebrospinal-fluid (CSF) barrier (BCSFB), was shown to be involved in the pathogenesis of enteroviral meningitis. In a human *in vitro* model of the BCSFB consisting of human choroid plexus papilloma cells (HIBCPP), the permissiveness of plexus epithelial cells for *Echovirus 30* (EV30) was analyzed by immunoblotting and quantitative real-time PCR (Q-PCR). HIBCPP could be directly infected by EV30 from the apical as well as from the physiological relevant basolateral side. During an infection period of 5 h no alterations of barrier function and cell viability could be observed. Analysis of the cytokine/chemokine-profile following enteroviral infection with a cytometric bead array (CBA) and Q-PCR revealed an enhanced secretion of PanGRO (CXCL1, CXCL2 and CXCL3), IL8 and CCL5. Q-PCR showed a significant upregulation of CXCL1, CXCL2 and CXCL3 in a time dependant manner. However, there was only a minor effect of HIBCPP-infection with EV30 on transepithelial T lymphocyte migration with or without the chemoattractant CXCL12. Moreover, CXCL3 did not significantly enhance T cell migrations. Therefore additional factors must be involved for the *in vivo* reported enhanced T cell migration into the CNS in the context of enteroviral meningitis. As HIBCPP are permissive for infection with EV30, they constitute a valuable human *in vitro* model to study viral infection at the BCSFB.

1. Introduction

Despite the increasing number of vaccines against meningitis causing agents, the disease remains an infectious disease, which is associated with a high rate of morbidity in children (Kim, 2003). Depending on the infectious agent, two major forms are distinguished, the viral and the bacterial meningitis. With an incidence of 12–688/100.000/year, viral meningitis is almost three-fold as frequent as the bacterial meningitis (Giorgi Rossi et al., 2009; Rantakallio et al., 1986). Even though the acute phase of viral meningitis is often mild, the long-term consequences as reactivation of a viral infection within the central nervous system (CNS) in the elderly or immunocompromised host are unknown. Enteroviruses and especially *Echovirus 30* (EV30), family *Picornaviridae*, genus *Enterovirus*, species *Human enterovirus B* are the most common pathogens identified in viral meningitis (Chadwick, 2005; Roth et al., 2007). Echoviruses are highly infectious and preferentially target infants and young children, whereas

Abbreviations: BBB, blood–brain barrier; BCSFB, blood–cerebrospinal fluid barrier; CAR, coxsackie adenovirus receptor; CBA, cytometric bead array; CNS, central nervous system; CPE, cytopathic effect; CSF, cerebrospinal fluid; CVB3, *coxsackievirus B 3*, family *Picornaviridae*, genus *Enterovirus*, species *Human enterovirus B*; DAF, decay acceleration factor; ECL, enhanced chemiluminescence detection system; EV30, *echovirus 30* (EV30), family *Picornaviridae*, genus *Enterovirus*, species *Human enterovirus B*; HBMEC, human brain microvascular endothelial cells; HIBCPP, human choroid plexus papilloma cells; HIV, human immunodeficiency virus; HTLV, human T cell leukaemia virus; IL, interleukin; LPS, lipopolysaccharide; MDM, monocyte derived macrophages; MOCK, mokula; MOI, multiplicity of infection; MS, multiple sclerosis; PCR, polymerase chain reaction; PMN, polymorphonuclear granulocytes; Q-PCR, quantitative real time polymerase chain reaction; RIPA, radio immuno precipitation assay; RKI, "Robert Koch" Institute; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; SD, standard deviation; TEER, transepithelial electrical resistance; TBE, tick borne encephalitis.

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coxsackie virus B3 (CVB3) primarily affects neonates (Rhoades et al., 2011). Murine models provide large evidence for the importance of the choroid plexus epithelium, which is a key component of the blood–cerebrospinal fluid barrier (BCSFB) in the pathogenesis of enteroviral meningitis (Tabor-Godwin et al., 2010; Feuer et al., 2003). Only recently, a human cell line of choroid plexus epithelial cells providing an *in vitro* model of the BCSFB was established and characterized (Ishiwata et al., 2005; Schwerk et al., 2012).

It has become clear that the CNS is considerably more than an immune privileged site (Engelhardt et al., 2001) disposing of a large pool of complex cellular and humoral defense mechanisms during CNS infection and inflammation (Ransohoff et al., 2003). However, the patterns of host reactions to bacterial and viral infection of the CNS are poorly understood. The knowledge about the different pathomechanisms initiated by viral and bacterial pathogens at the blood–brain barrier(BBB) and the blood–cerebrospinal-fluid barrier (BCSFB) seems of major importance for the understanding of the differences in the clinical course and outcome. Comparing the results of the lumbar punctures in viral and bacterial meningitis suggests that polymorphonuclear granulocytes (PMN) dominate in bacterial, whereas lymphocytes (especially T lymphocytes) are associated with viral infections of the CNS (Lucht et al., 1992). In the context of viral infection, it has been shown that the BCSFB supports trafficking of T cells in response to infection (Meeker et al., 2012).

There is little knowledge about the chemokines involved in the phenomenon of T cell entry across the blood–brain barrier (BBB) and BCSFB into the CNS. T cell chemotaxis is triggered by stimulation with IL2 and IL8 and TNFα (Escotte et al., 2006). The strongest known chemoattractant for T lymphocytes is CXCL12 (previously named SDF-1; stromal derived factor 1) (Ara et al., 2003; Askari et al., 2003; Bleul et al., 1996). In the acute phase of multiple sclerosis (MS), CXCL12 has been shown to be enhanced within the cerebrospinal fluid (CSF) (Krumbholz et al., 2006). Human brain microvascular endothelial cells (HBMEC) secrete CXCL12 after stimulation with TNFα and INFγ (Liu and Dorovini-Zis, 2009). Only recently, it has been shown in an *in vitro* model of the BBB consisting of HBMEC that CXCL12 stimulates the transmigration of $CD4^+$ and $CD8^+$ T lymphocytes across the BBB (Man et al., 2012). Within the CNS, CXCL12 is expressed in astrocytes (Ohtani et al., 1998; Zheng et al., 1999; Rostasy et al., 2003) and to a lesser extent in neurons (Ohtani et al., 1998; Rostasy et al., 2003; Gleichmann et al., 2001), oligodendroglial cells (Gleichmann et al., 2001), microglia (Ohtani et al., 1998), monocyte derived macrophages (MDM) (Zheng et al., 1999) and endothelial cells (Liu and Dorovini-Zis, 2009). Interestingly, in the context of infection with human immunodeficiency virus (HIV) or stimulation with lipopolysaccharides (LPS), CXCL12 expression within MDM is down regulated, whereas CXCL12 expression is upregulated in astrocytes when exposed to HIV-infected MDM conditioned media (Zheng et al., 1999).

Moreover, distinct patterns of chemokine expression have been linked with several different viral CNS infections (Hosking and Lane, 2010; Piqueras et al., 2006). In the context of enteroviral infection *in vitro* secretion of CCL5, IL8 and CCL2 by airway epithelial cells (Renois et al., 2010) and *in vivo* enhancement of IL6, IL8 and IFNγ within the CSF have been reported (Sato et al., 2003).

Currently, at the BCSFB the regulation of chemokine secretion, leukocyte influx into the CNS and the interplay between pathogens and leukocytes has not been studied in detail yet. To gain knowledge about the impact of enteroviral infection in the context of the BCSFB, its effect on cytokine expression and secretion as well as on transmigration of naïve T lymphocytes was investigated using a recently established *in vitro* model of the BCSFB based on a human choroid plexus papilloma cell line (HIBCPP) (Ishiwata et al., 2005; Schwerk et al., 2012). As CVB3 plays an important role as enteroviral pathogen during pregnancy and in newborns (Rhoades et al., 2011), investigations on CNS infection with neonatal animal models are often performed with CVB3 (Feuer et al., 2003, 2005). In contrast, EV30 majorly affects infants and young children (Rhoades et al., 2011). Consequently, we chose EV30 for the investigation of the effect of enteroviral infection on the BCSFB in our differentiated polarized epithelial cell culture model of the BCSFB.

2. Material and methods

2.1. Virus preparations and titration

The wild-type strain EV30 was obtained from the reference centre for enterovirus, the "Robert-Koch" Institute RKI (Berlin, Germany). The strain used is the so-called "Bastianni-strain" that represents a prototype of enterovirus and has been isolated of a patient with aseptic meningitis in 1958 (Plager and Decher, 1963). Confluent monolayers of RD-cells were infected with 100 μl of EV30 until cytopathic effect (CPE) higher 90% was reached. The virus-cell suspension was frozen at −20 °C over night, then centrifuged for 15 min at 4 °C and 4000 rpm, aliquoted and stored at −80 °C until used. For control experiments, Mokula (MOCK) of virus was produced with confluent RD-cells that underwent the equal procedures as the virus-infected cells. After portioning, MOCK was stored at −80 °C. The virus titers were determined by titration on 96-well on RD-cells. Cell-lysis was determined every 12 h and the titers were calculated according to the REED and Muench formula (Reed and Muench, 1938).

2.2. Cultivation of HIBCPP and measurement of transepithelial electical resistance (TEER)

HIBCPP were cultured and seeded on cell culture inserts (Greiner Bio-one, Germany; pore diameter 3.0 μm, pore density 2.0×10^6 pores/cm^2, 0.33 cm^2) as previously described (Schwerk et al., 2012). In brief, HIBCPP were seeded on cell culture inserts, which were flipped over and placed in a medium-flooded 12-well plate (for the inverted cell culture model) or HIBCPP were seeded into the upper filter well (for the standard cell culture model) (Tenenbaum et al., 2009). With an epithelial tissue voltohmmeter (Millipore, Germany) transepithelial elecrical resistance (TEER) was measured every day. When TEER was higher than 90 Ω × cm^2, cell culture was continued in HIBCPP-medium containing 1% serum. 1 or 2 days later, as TEER reached values above 300 Ω × cm^2, cells were used in the experiments.

2.3. Infection of HIBCPP with virus, measurement of the transepithelial electrical resistance (TEER) and determination of permeability

For the infection experiments, HIBCPP grown on cell culture inserts with TEER above 300 Ω × cm^2 were used. Experiments were performed in RPMI-1640 medium containing 10% fetal-calf-serum (FCS). When performing the infection experiments, virus was added to the upper compartment of the cell culture inserts in the multiplicity of infection (MOI) 10. For control experiments, either cell culture medium or MOCK of virus was added. At the beginning and at the end of each experiment, TEER was measured. As previously described, high TEER values correlate with low paracellular permeability (Schwerk et al., 2012). To confirm these findings in the enteroviral infection experiments, paracellular permeability was determined by the measurement of the passage of a Dextran-TexasRed (Invitrogen, Germany) tracer solution (100 mg/ml) from the upper to the lower compartment of cell culture inserts in a Tecan Infinite M200 Multiwell reader (Tecan, Switzerland, Magellan V6.6 software).