α-mediated apoptosis and NF-κB activation but the protein's exact mechanism of action is still poorly understood. Since SH protein has been shown to interact with activators of the mitogen-activated protein kinase (MAPK) signal transduction pathway, we addressed the question whether the viral protein manipulates this signaling pathway. We found that MuV SH protein inhibits the UV-induced phosphorylation of the MAP kinase JNK, but not of ERK or p38. In order to gain insights into which signaling pathways that are relevant for apoptosis are affected by SH, an Apoptosis RT^2 Profiler PCR-Array was carried out after infection with SH-deficient recombinant MuV (rMuV). Eight genes were differentially regulated 48 hours post infection when comparing SH-expressing rMuV with SH-deficient rMuV. Among these genes, the expression of pro-apoptotic TNF was down-regulated and the anti-apoptotic gene NAIP was up-regulated. Since TNF gene expression is controlled by members of the MAPK family, we hypothesize that the decrease of TNF is mediated by the interference of SH protein with other proteins of this signaling pathway. The MAPK signaling pathway plays a critical role in many important cellular processes such as cell division, inflammation and apoptosis. Thus, selective manipulation of this pathway provides another viral strategy to evade host defense mechanisms and ensures efficient replication and survival of the virus.

Viral Pathogenesis

Poster-Nr: 078

Abstract-ID: 671

Analysis of the Effects of a Rubella Virus Infection on Primary Endothelial Cells of Adult and of Fetal Origin

Michael Bauer[1], Henriette Geyer[1], Nicole Friedrich[1], Annette Mankertz[1]

[1]Robert Koch Institute, Berlin, Germany

Rubella virus (RV) typically causes only mild symptoms in children and adults. However, maternal infection early in pregnancy can lead to the congenital rubella syndrome (CRS) in infants, the clinical manifestations of which are diverse, ranging from deafness, ocular conditions, and mental retardation to cardiac disease. Approximately 100 000 CRS cases per year are estimated to still occur worldwide. The molecular mechanisms by which RV causes CRS are still unclear.

A prominent feature of histopathology in CRS cases is noninflammatory damage to the endothelium of heart and blood vessels. For this reason, we compared the effects of a RV infection on endothelial cells of fetal and of adult origin, i.e. human umbilical vein endothelial cells (HUVEC) and human saphenous vein endothelial cells (HSaVEC), respectively, as well as on the immortalized cell lines Vero and A549. First we analyzed viral replication and release of RV particles, revealing that release of virions is similarly efficient in all three human cell lines. We also found that RV is able to infect HUVEC and HSaVEC equally efficiently. Interestingly, we discovered that the extent of cell death induced by RV infection is lowest in adult HSaVEC and that dying cells are mostly apoptotic. In contrast, cell death in HUVEC is mostly necrotic. Although wild type rubella strains are substantially more teratogenic than vaccine strains, we found no major differences in the effects caused by HPV-77 (vaccine strain) and by wild type (clinical isolate Würzburg-12) RV in our experiments.

We have shown that there are considerable differences in the extent and type of cell death induced by RV infection between primary endothelial cells of adult and of fetal origin. Since endothelial cells appear to be a main target of fetal RV infection we hypothesize that differentially regulated cell death might be one of the mechanisms contributing to CRS.

Viral Pathogenesis

Poster-Nr: 079

Abstract-ID: 673

Different sets of virulence determinants in highly pathogenic avian influenza viruses suggest several independent evolutionary pathways

Olga Stech[1], Jutta Veits[1], Sayed Abdelwhab[1], Ute Wessels[1], Thomas C. Mettenleiter[1], Jürgen Stech[1]

[1]Institute of Molecular Biology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, 17493 Greifswald – Insel Riems, Germany

Highly pathogenic avian influenza viruses (HPAIV) cause devastating losses in poultry world-wide and raised concerns to ignite a novel pandemic due to repeated zoonotic transmissions to humans. HPAIV have been shown to develop from low-pathogenic precursors of hemagglutinin (HA) serotypes H5 or H7, requiring the acquisition of a polybasic HA cleavage site (HACS). However, the introduction of a polybasic HACS into LPAIV does not necessarily result in high virulence for chicken indicating that, beside the essential polybasic HACS, additional virulence determinants are relevant. Those additional adaptive changes might accumulate already in the low-pathogenic precursors during their circulation in gallinaceous poultry prior to the emergence of an HPAIV. Since this evolutionary process is not well un-derstood, we aimed to unravel the genetic determinants which, in addition to the polybasic HACS, are required for transformation of LPAIV into HPAIV.

To select a minimal gene constellation sufficient for a highly pathogenic virus, we co-transfected plasmids coding for all eight genes from an H5N1 HPAIV and seven, except HA, from an H5N1 LPAIV, and used the supernatant to infect chickens. Shed reassortants carried the HPAIV PB2, NP, HA, NA, and M genes; a reconstituted virus was highly pathogenic and transmissible like the wild-type. A tailored H5N1 reassortant, carrying the LPAIV NA, exhibited 100% lethality both in inoculated and contact chickens. The same lethal phenotype was exhibited by an LPAIV reassortant carrying only the HPAIV HA and NA. However, abolishing the NA stalk deletion led to considerably reduced lethality and no transmission. Conversely, an LPAIV reassortant carrying only the HPAIV HA but the LPAIV NA with engineered stalk deletion displayed 100% lethality both after primary or contact