from ANDV sero-positive wild-caught rice rats were assessed. RNA-seq analysis, de novo reference-independent assembly and stringent orthology assignments produced 17,756 unique coding and non-coding RNAs. Differential expression analysis of persistently-infected seropositive rice rat spleens revealed 18 differentially expressed transcripts from 16 unique genes. A three-pronged effect on the immune response were observed in 1) suppression of the JAK-STAT pathway at Stat5b and Ccnot1, as well as 2) a bias toward a TH2 response in the enrichment of caspase-1 and 3) stimulation of BCl-1 pathway factors Ppp1cc and MFE. Two of these differentially expressed transcripts, caspase-1 and STAT5b, code for proteins expected to stimulate T helper follicular (TFH) cell development, a phenomenon that has also been described for hantavirus-infected P. maniculatus. Differential expression of a single seropositive rice rat with a higher viral load revealed a robust response of 243 differentially expressed transcripts, suggesting an acute infection. Together, these data help define the fundamental features of the immune response in a hantavirus reservoir host.

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Animation of VZV DNA

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Varicella zoster virus (VZV) is a ubiquitous neurotropic alphaherpesvirus that typically causes childhood varicella (chickenpox) on primary infection and zoster (shingles) after reactivation. During latency most of the ~70 virus genes are transcriptionally silent; however, analysis of latent VZV gene transcription in its natural setting requires analysis of human ganglia removed at autopsy. Recognizing the problems associated with such samples, we have observed that as the post-mortem time interval increases, so do the number of VZV genes transcribed. Based on our data and recent similar findings concerning reactivation of HSV-1, we propose an interesting testable model to describe epigenetic control of neurotropic alphaherpesvirus gene transcription during latency and early reactivation.

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Viralign: A tool for uncovering functional viral elements

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The availability of broad epigenomic profiles of human tissues provides an opportunity to uncover viral sequences and their corresponding functional regulatory elements in otherwise overlooked datasets. We developed Viralign, a throughput screening method to discover and interpret viral functional information in existing short read archive data. Using a comprehensive reference database, Viralign scans sequence data for known viral sequences and generates an alignment report with read information and genome coverage. Viralign analyzes functional datasets for regulatory elements and provides coordinate and visualization files that can be viewed in a genome browser. Additionally, this method searches for potential integration sites and variants by genome assembly. In a pilot study, we performed H3K27me3 ChIP-seq in monocytes of an HHV6 infected individual and compared this to U2OS cells infected with HHV6A and HHV6B and use Viralign to detect HHV6 insertion loci and H3K27me3 enriched regions. The source code as well as additional data for Viralign will be made publicly available.

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Autophagic flux without a block differentiates varicella from herpes simplex virus infection

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Varicella-zoster virus (VZV) is a herpesvirus that causes a characteristic vesicular exanthem in humans with primary infection (varicella) or reactivation (zoster). We have previously observed that vesicular cells are filled with autophagosomes that are easily detectable by confocal microscopy after immunolabeling for the LC3 protein. Through a 3D imaging software program called Imaris we have quantitated autophagosomes as greater than 100 per cell. Similarly, we have assessed autophagy in VZV-infected monolayers after inoculation by the traditional method with infected cells at a ratio of one infected to 8 uninfected cells. Again, autophagosomes are easily detected, but their count is lower than that observed in human skin cells. As an additional control, we enumerated the autophagosomes in the Severe Combined Immuno-Deficient (SCID) Mouse model of VZV infection. In this model, human skin is inserted under the skin of the mouse and subsequently inoculated with VZV-infected cells. Again, autophagy was abundant in the VZV-infected skin and minimal in the mock-infected skin sample. Subsequently, we investigated autophagy following infection with sonically prepared cell free virus in cultured cells. After cell free virus inoculation, autophagy was detected in a majority of infected cells at all time points, but was less than that seen after an infected-cell inoculum. Finally, we investigated VZV-induced autophagic flux by two different methods (radiolabeling proteins and a dual-colored LC3 plasmid); both showed no evidence of a block in autophagy. Overall, therefore, autophagy within a VZV-infected cell was remarkably different from autophagy within an HSV-infected cell, whose genome contains two modifiers of autophagy, ICP34.5 and US11, not present in VZV.

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Recombination, reassortment, and many-to-one genotypes in natural arenavirus infections

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Mutation, recombination, and reassortment generate virus particles with variable genotypes, some of which may be better adapted to infect a new host, resist drug treatment, or escape immune pressure. The arenaviruses are a family of viruses that package a large (L) and small (S) genome segment. Arenaviruses infect mammals and snakes and are associated with fatal disease in both groups of animals. Although recombination and reassortment are well documented in some virus families, neither process has been observed in natural arenavirus infections. In this study, we documented a surprising degree of genetic diversity in arenavirus-infected snakes. Instead of one or two viral species or quasispecies, individual animals harbored complex populations of viral genotypes composed of up to 5 S and 11 L genotypes, which replicated as stable ensembles in culture. S and L segment genotype accumulation was not balanced and a particular S segment genotype dominated, both in individual animals and at a population level. Numerous instances of recombination and
reassortment were detected. Some recombinant segments had unusual organizations with 2 intergenic regions. This genetic fluidity is closer to that observed in influenza viruses than to the relatively placid genetics of mammalian arenavirus. However, the observed imbalance between the S and L segments and the intrahost accumulation and persistence of multiple genotypes is previously undocumented. Overall, this provides an opportunity to study basic mechanisms of viral adaptation and stretches the idea of what it means to be infected by “a virus.”

Roche Research Portfolio: Trusted Performance, Efficient Workflow Solutions
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Along with Roche Pharmaceuticals, Roche Diagnostics is an important part of the foundation that modern healthcare builds upon. Our broad range of innovative diagnostic tests and systems play a pivotal role in the groundbreaking area of integrated healthcare solutions and cover the early detection, targeted screening, evaluation and monitoring of disease. Roche Diagnostics is active in all market segments, from scientific research and clinical laboratory systems to patient self-monitoring.

Preemption, the Virus-Serum-Toxin Act, and the USDA: a case study using iatrogenic abortion due to BoHV-1 vaccines in pregnant cows
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Three major vaccine manufacturers in the United States currently sell multivalent vaccines containing modified live bovine herpesvirus 1 (BoHV-1) for use in pregnant cattle. The first of these products entered the US market in 2003. Yet it has been known since the early 1960s that vaccinal BoHV-1 causes abortion in cattle. The products became popular because they can be used year-round, regardless of pregnancy status in herds. Abortifacient effects have been considered to be minimal, provided initial vaccination is done during the previous 12 months using specific vaccine products and in accordance with label directions. Single nucleotide polymorphisms (SNPs) in BoHV-1 can be used to resolve whether post-vaccination outbreaks of abortion in cattle herds are iatrogenic (Fulton et al.; Vaccine. 2013; 31(11):1471-1479). We tested tissues from 10 abortion episodes (2010–2014) where an apparent association existed between recent use of modified live BoHV-1 and abortion 1–3 months later. Products were used on or off label in individual outbreaks. All 10 episodes had SNP patterns consistent with those of commonly-used modified live BoHV-1 strains (O’Toole et al.; Vet Pathol. 2014, in press). In spite of this, it is likely such products will remain on the market. This is due the absence of meaningful post-marketing surveillance of suspect adverse reactions in animals by the USDA, compounded by the courts’ interpretation of the Virus-Serum-Toxin Act of 1913 [Lynnbrook Farms v. SmithKline Beecham Corp., 79 F.3d 620 (7th Cir.)]. Interesting differences exist between the handling of adverse vaccine reactions in human patients through the National Vaccine Injury Compensation Program (VICP), and similar reactions in animals following use of federally licensed vaccines.

Generating new prions by targeted mutation or segment duplication
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Prions are infectious agents composed entirely of protein. Prion activity results from the conversion of soluble proteins into an insoluble, self-templating amyloid form. Nine different amyloid-based prions have been identified in yeast. All but one contain a glutamine/asparagine (Q/N)-rich region that is responsible for prion activity. Similar Q/N-rich regions are over-represented in eukaryotic genomes. In humans, aggregation-causing mutations in Q/N-rich proteins have been linked to various degenerative diseases, including ALS. Our lab previously developed a prediction algorithm, PAPA (Prion Aggregation Prediction Algorithm), to predict the aggregation propensity of Q/N-rich proteins, and to predict the effects of mutations on aggregation propensity. Here, we tested the ability of PAPA to design mutations to turn non-prion proteins into prions. We identified four yeast Q/N-rich protein fragments that lacked any detectable aggregation or prion activity. In each case, a small number of designed mutations were sufficient to cause these domains to aggregate, and in two cases, to create bona fide prion activity. We then tested whether simply generating tandem repeats of short, aggregation-prone segments within these domains would likewise be sufficient to create...