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Molecular Epidemiology of Influenza A Viruses

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Influenza viruses have a segmented genome. By double infection of an organism new strains with replaced surface antigens and responsible for pandemics are created. Avian strains do not spread in the human population and vice versa. We show that the viral nucleoprotein (NP) plays a major role in species specificity, and that pigs represent “mixing vessels” for the double infection. From 25 NP sequences a consensus sequence of 30 amino acids is extracted, which defines an avian and a human NP subtype. The NPs of pig influenza viruses belong either to the one or to the other subtype, while the NPs of all the other mammalian strains are of the avian subtype. According to a phylogenetic tree it is estimated, that a common ancestor has existed about 1900.

Effect of Bromelain on the Glycoprotein of Influenza C Virus

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The surface glycoprotein of influenza C/Johannesburg/1/66 can be isolated in a soluble and biological active form by treatment of virions with the protease bromelain. N-terminal amino acid sequence analysis of the C-terminal, hydrophobic peptide, which remains in the viral membrane showed, that the cleavage site for bromelain is at serine 176 of the smaller subunit. The molecular weight of the membrane anchoring peptide is therefore 4 kDa. A second cleavage at the C-terminus of the smaller subunit by bromelain may produce a ragged C-terminal end of the molecule, since the smaller subunit is separated as a double
band in SDS-PAGE. The difference in molecular weight is approximately 1–1.5 kDa, determined by SDS-PAGE. As a third effect of bromelain the uncleave precursor glycoprotein is cleaved into the subunits gp 65 and gp 30. The N-terminus of the bromelain cleaved gp 30 possesses an additional arginine residue, compared to the in vivo cleaved glycoprotein. The effect of this arginine residue on the fusion activity of the glycoprotein is being studied at present.

Masking of an Antigenic Epitope of the Hemagglutinin of Influenza Virus by Carbohydrate

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Carbohydrate analysis has revealed that the hemagglutinins (HA) of the Rostock and the Dutch strain of fowl plague virus have 7 and 6 oligosaccharide side chains, respectively. As shown by nucleotide sequence asparagine¹⁴⁹ located in the antigenic epitope B on the tip of the HA-spike is not glycosylated in the Dutch strain, because serine¹⁵¹ has been exchanged for alanine. Monoclonal antibodies raised against the subtype H7 and a rabbit antiserum against a synthetic peptide comprising amino acids 143 through 162 demonstrate a shielding effect of the sugar at asparagine¹⁴⁹ for the Rostock strain but not for the Dutch strain, when they were analysed by Western blotting, ELISA and immunoprecipitation. Enzymatic digestion of the carbohydrate side chains removes the differential characteristics of binding antibodies between both strains.

Molecular Characterization of an Attenuated Rabies Virus

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Since 1983 the rabies virus strain SAD B19 Tübingen is used as a life vaccine in the field trial on the oral immunization of foxes in several European countries. Until now no case of rabies caused by the vaccine strain has been found. – In order to characterize the genome of this attenuated virus, cDNA to the genomic RNA was synthesized and cloned in Lambda gt 10. Deletion clones were constructed in pEMBL-phagemids and sequenced by the chain-terminating inhibitor method. Analysis of nucleotide sequence as well as deduced amino acid sequence revealed a high degree of homology to the previously published nucleotide sequence of the pathogenic rabies virus strain PV in both coding and noncoding regions. In addition, distinct differences could be observed. The significance of these findings with regard to virulence is discussed.
Studies on the Structure of Marburg Virus Genom

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Marburg virus belongs to a new family of negative stranded RNA viruses, called Filoviridae. It was first isolated in 1967 during an outbreak of hemorrhagic fever among laboratory workers in Marburg, Germany, who had been exposed to tissues of African green monkeys (Cercopithecus aethiops) imported from Uganda. Further episodes of Marburg virus were reported from South Africa, Zimbabwe and Kenya. The virus is indigenous to Africa and causes a severe, febrile hemorrhagic disease. – Marburg virus contains seven proteins, L (180kd), GP (140kd), NP (96kd), VP 40 (38kd), VP35 (32kd), VP 30 (28kd) and VP 24 (24kd), and a single strand of negative RNA with a mol. wt. of approximately $4.2 \times 10^6$. We could identify five different mRNA species coding for the five viral proteins NP, VP40, VP35, VP30 and VP 24. The mRNA species coding for L and GP have not been identified yet by in vitro translation. We have obtained large cDNA libraries from virion RNA and mRNA and have sequenced parts of the genes coding for GP and VP40. A part of GP, comprising about 400 amino acids, has been expressed in E. coli as a fusion protein with β-galactosidase.

Molecular Cloning and Nucleotide Sequence Analysis of the Genome of Nephropathia Epidemica Virus Strain Hällnäs B1

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Nephropathia epidemica is a mild form of Hemorrhagic Fever with Renal Syndrome (HFRS) which is caused by several closely related Hantavirus strains (e. g. Hällnäs B1 and Puumala virus). The viruses are endemic in bank voles in Europe and epidemiological surveys revealed that about 3% of the human population have antibodies against these viruses. The genome of these viruses is composed of three RNA segments (L, M, and S). The genomic M segment is coding for two major envelope glycoproteins (G1 and G2). In order to identify the antigenic epitopes the M segment of the genomic RNA of Nephropathia epidemica virus strain Hällnäs B1 was characterized by molecular cloning and nucleotide sequence analysis. A lambda gt10 cDNA library was established using total cellular RNA isolated from infected Vero E6 cells. The cDNA was synthesized by random priming. To detect the specific sequences of the M segment the cDNA library (600,000 recombinant phages) was screened using a probe of the Hantaan M segment cDNA which had been cloned previously. Under low stringency hybridization conditions 50 recombinants were detected, isolated, and used for the determination of their nucleotide sequences. The nucleotide sequence and the resulting amino acid sequences of the coding regions were compared to the corresponding data of Hantaan virus strain 76–118. – BMFT project 0318973A.
New Member of the Protein-Tyrosine-Kinase Family: The src and lck Related Proto-Oncogene, c-tkl

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C-tkl, which has previously been isolated from a chicken spleen cDNA library is a new member of the src-family of proto-oncogenes. It codes for a protein, which has most significant homology to lck. The new gene is therefore referred to as c-tkl for cellular tyrosine kinase related to lck. – C-tkl was inserted into the genome of a baculovirus. Extracts from insect cells infected with tkl-recombinant baculovirus were incubated with antibodies raised against pS1\(^{ctkl}\) and a protein kinase assay was performed. Under these experimental conditions P51\(^{ctkl}\) shows autophosphorylation activity. – Furthermore we examined the expression of c-tkl related genes in human tissues and corresponding tumors. We found tkl-specific transcripts in lymphocytes, spleen, colon and stomach, which are 3.8 and 11.5 kb in length.

The v-fms Oncogene Product as Receptor for the Growth Hormone CSF-1

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The v-fms oncogene of the McDonough strain of feline sarcoma virus encodes a tyrosine kinase which is closely related to the c-fms-specified receptor for macrophage colony stimulating factor (CSF-1). We have cloned and sequenced the c-DNA of the feline CSF-1 gene. The sequence revealed an amino acid homology of 83 and 88% to the corresponding murine and human sequences. – To study the interaction between CSF-1 and its receptor in a cell system free of other mammalian factors, we have expressed the v-fms gene in chicken embryo fibroblasts. These cells did not show a transformed morphology. Addition of CSF-1 to the growth medium, however, induced the morphological changes typical for transformation after two days. These data suggest that v-fms-mediated transformation occurs via an autocrine loop mechanism.

Phosphorylation of SU40 T-AG and p53 in SU40 Transformed and Transformation-Defective Cell Lines

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Phosphorylation of SU40 large T-Rg and the cellular onco-protein p53 was examined in SU40 transformed and transformation-defective cells. T-Rg of transformation negative
revertants (*wt-REV2*) exhibited reduced phosphorylation of Ser123 as compared to transformed SU-52.2 cells. Fusion of *wt-REV2* with rat embryonal fibroblasts (*REF-52.2*) resulted in transformed cells (*wt-REV2 fus*) with T-Ag phosphorylation like SU-52.2. Furthermore, we examined transformed (*PT10-t*) and transformation-defective (*PT10-nt*) cell lines, both containing the second exon of T-AG. *PT10-t* expressed one major T-Ag (T1), phosphorylated like wt T-Ag, and one minor T-Ag (T2), missing in phosphorylation of Ser106 and with reduced phosphorylation of Ser123. *PT10-nt* exhibited only T2 with incomplete phosphorylation. – Phosphorylation of p53 was different in SU-52.2 and *wt-REV2* and not reversible by fusion of *wt-REV2*. Experiments with *PT10-t* and *PT10-nt* are in progress. – In summary, we assume a defect of at least one cellular kinase in transformation-defective cells, which can partially be complemented by fusion with *REF-52.2* cells.

**Regulation of the p53 Oncoprotein in Non-Transformed and Transformed Cells**

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Regulation of the p53 oncoprotein in non-transformed cells. p53 has been characterised as a nuclear oncoprotein. It has immortalising properties in primary cells and transforming capabilities in cooperation with the activated H-ras gene. In many cases the activation of nuclear oncogenes appears to arise due to an elevation in the content of the oncoprotein. This elevation may be attributed either to a higher level of expression in the gene products or to a higher stability of the oncoprotein. We studied the metabolic stability of the p53 oncoprotein in various immortalised, as well as SV40 and other transformed cell lines. Pulse-chase experiments have shown that p53 has a low stability in non-transformed cells. In contrast, p53 showed a higher stability in transformed cells, which was elevated even further by its association with the SV40 T antigen as well as with a protein of the heat shock family. Stabilization seems to be correlated with self-aggregation of p53 as well as with the formation of high molecular weight heterooligomers between p53 and T antigen or p53 and the 70KDa heat shock protein.

**Differential Interactions of Subclasses of SV40 T Antigen within Control Regions on the SV40 DNA**

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Differential interactions of subclasses of SV40 T antigen within control regions on the SV40 DNA. SV40 is a DNA tumorvirus that codes for the viral protein. SV40 T antigen, which is involved in the regulation of viral replication, SV40 early and late transcription. To regulate these processes T antigen binds to three separate regions on the SV40 DNA termed site I, II or III. These individual binding sites were prepared either by the specific synthesis of oligonucleotides or digestion of the SV40 DNA with appropriate restriction enzymes and then coupled to streptavidin-biotin affinity columns. Using these affinity columns, we were
able to determine which structural subclasses of T antigen, derived from a cell extract after SV40 infection, were in fact able to bind to the different binding regions. We report here, that newly synthesized T antigen bound to the binding sites I and II, whereas "old" T antigen bound exclusively to the binding site I. In contrast, T antigen in complex with the oncoprotein p53 was unable to bind to any of the three sites. Elution of proteins bound to the binding sites I, II and III results in monomeric T antigen. Exceptionally highly oligomeric forms of T antigen were detected after incubation of T antigen with the binding site I.

Epstein Barr Virus as a Model for Persistent Virus Infections and Strategies for Control of Related Diseases

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EBV enters the human body via the oral route and shows primary replication predominantly in the tonsils. From there the virus is spread throughout the body via lymphoid cells. We have to assume that a widespread spectrum of cells, including the epithelial lineage becomes latently infected. The immune system controls the excessive proliferation of EBV-genome positive peripheral B-lymphocytes and prevents production of EBV from such lymphocytes and also from other celltypes of the body. The complete lytic cycle of EBV replication in healthy individuals is restricted to a few selective sites, such as the parotid glands. Saliva remains a lifelong source of EBV. In persons with an impaired immune system EBV is lytically produced in additional sites of the human body causing diseases such as hairy leukoplakia in AIDS patients. – For control by the immune system information on EB-viral and cellular components of the infected cells are essential. EBV-genome positive malignant lymphoma cells have either a reduced or no production of an at most truncated version of a viral membrane antigen, which is a major component of the lymphocyte detected membrane antigen complex. Furthermore, the malignant cells have an altered expression of the antigen presenting HLA class I molecules. Both alterations render lymphoma cells less recognizable by the immune system. As a consequence immunological approaches seem inappropriate to control BL cells. However, prevention or delay of EBV infection by vaccination may considerably reduce the risk for development of EBV-related malignancies. Chemotherapy or agents altering antigen presentation in lymphoma cells have to be used to control EBV related neoplastic diseases. – Excessive activation of EBV replication is responsible for fulminant infections and for severe complications mainly in immunosuppressed persons, such as transplant recipients or AIDS patients. Chemotherapy seems to be useful in controlling these diseases. The use of immunoglobulins is not widely evaluated but may be useful for a variety of EBV related disorders. – Various therapeutic approaches will be discussed with respect to their potential to control the wide spectrum of EBV related diseases.
DNA Replication in HSV-Infected Cells

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Herpes simplex viruses (HSV) possess at least seven genes, the products of which are essential for the replication of HSV-1 DNA (Chalberg, Proc.Natl.Acad.Sci.USA 83 (1986) 9094. In HSV-infected cells, not only herpesviral DNA is replicated but also DNA sequences without any relationship to herpesviruses are replicated under certain circumstances. This process of heterologous gene amplification is dependent on the expression of early genes and is closely associated with the synthesis of HSV DNA. Gene amplification is not a consequence of HSV DNA replication, but a simultaneous process. By the use of defined DNA-negative temperature-sensitive mutants, hsR,tsS,tsX (Matz,Subak-Sharpe, and Preston, J.Gen.Virol. 64 (1983) 2261) we have shown that amplification of heterologous DNA sequences (SV40, either integrated in the host cell genome or alternatively in a recombinant plasmid) does not require previous replication of the herpesvirus genome and thus is independent of the expression of late viral genes. – It is, in principle, possible that early HSV gene functions may exert damaging (mutagenic) influences upon the host cell genome in the absence of lytic virus multiplication. The biological relevance of this phenomenon needs to be investigated. – This work was supported by Deutsche Forschungsgemeinschaft, SFB 31 Tumorentstehung und -Entwicklung.

The Role of a New Class of HSV-Genes Detected within the BamHI DNA Fragment B (0.738 to 0.809 mu) of the Viral Genome (HSV-1) in the Molecular Mechanisms of Virulence and Latency

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The investigations on the molecular mechanisms of the virulence and latency of herpes simplex virus type 1 (HSV-1) led to the identification of a new class of viral genes, which are located within the BamHI DNA fragment B (0.738 to 0.809 mu) of the HSV-1 genome. These genes play a functional role in the determination of the virulence, latency and organotropism of HSV-1. A 1.5 kb RNA transcript could be shown to be involved in those mechanisms which control the determination of the target organ for latent virus. Alterations or deletions of this gene which lead to changes of the 1.5 kb RNA transcript could be correlated to the latency phenotype of HSV-1. HSV-1 strains which do not transcribe the 1.5 kb RNA persist as latent virus in the spleen and no more in the ganglia of latently infected tree shrews. The characterization of this 1.5 kb RNA transcript revealed that the transcription occurs at an early time after infection (4 h p.i.). Furthermore it was found that the 1.5 kb transcript is spliced. – DFG project Da 142/1–4.
Enterovirus-Induced Cardiomyopathy

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To date, there is firm evidence from in situ nucleic acid hybridization that enterovirus infections of the human heart result in a significant number of patients presenting with clinical symptoms and signs of myocarditis and/or dilated cardiomyopathy. In patients with dilated cardiomyopathy, the most dramatic manifestation of myocarditis, the incidence of enterovirus infections was found to be approximately 30 percent. Moreover, enterovirus RNA was not only found to be present at an early stage of clinically obvious myocardi- tis, but also in chronic dilated cardiomyopathy, indicating persistence of the virus in the human heart. The concept of enterovirus persistence in chronic dilated cardiomyopathy is further substantiated by the presence of enterovirus RNA in follow-up biopsies of patients with ongoing disease. In addition, antisera raised against bacterially synthesized Coxsackievirus B3 proteins are described, which reveal a broad spectrum of cross-reactivity within the enteroviruses. The combination of these antisera with the in situ hybridization approach will allow the question of whether restricted virus replication is implicated in persistent forms of enterovirus-induced cardiomyopathy to be resolved.

Molecular Basis of Viral Antigens: Linkage of Sequential and Conformational Epitopes of Neutralizing Monoclonal Antibodies Against Poliovirus, Type 1

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Neutralizing monoclonal antibodies against sequential epitopes were obtained by a combined in vivo/in vitro immunization protocol. These epitopes involved residues 97–101 of VP1 and residues 164–170 of VP2. Cross-neutralization tests of resistant mutants revealed a linkage of both sequential epitopes to new conformational epitopes. Insight into the structure of the conformational epitopes was obtained by one mutant in each case, where a point mutation leading to an amino acid exchange had occurred in a neighbouring loop of the sequential epitope. Thus the conformational epitope of VP1 is formed by residues of loop 93–104 and residues 141–152. In the case of VP2 the epitope is formed by residues 127–185 in a double loop. The combination of our antibodies will be a very useful tool for monitoring structural changes at these sites during virus morphogenesis or during virus cell interactions.
Suppression of Hematopoiesis in Human Long-Term Marrow Cultures by Hepatitis A Virus

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In order to develop a pathogenetic model for the hematological changes seen in hepatitis A infections, we studied the effect of HAV (strain GBM, generously supplied by Dr. Flehmig) on human long-term bone-marrow cultures (LTBMC). Inoculation of LTBMCs resulted in a dose and duration-of-culture-dependent reduction of granulocyte-/macrophage progenitors (CFU-GM), measured by a semisolid methylcellulose assay. Persistence of virus over many weeks in a subpopulation of the supportive LTBMC-stromal-cells was demonstrated by an immunocytochemical (APAAP) technique. In addition sequential estimations of TCID₅₀ revealed replication of HAV in primary bone-marrow cultures. As measurements of known soluble hematopoetic inhibitors such as IFN-γ, TNF-α in culture supernatants revealed no differences between infected and control cultures, a direct effect of HAV on hematopoietic progenitor cells seems probable.

Hog Cholera Virus-Characterization of Specific Antiserum and Identification of cDNA Clones

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A highly specific antiserum was raised against the pestivirus inducing hog cholera (hog cholera virus, HCV). Using immunoprecipitation and SDS-PAGE, this antiserum served for comparison of HCV-induced proteins with those from a related and better characterized pestivirus, bovine viral diarrhoea virus (BVDV). In addition to immunological relationships, the apparent molecular weights of some proteins induced by both viruses were quite similar. HCV genomic RNA was found to be about 12 kb in length, comparable to BVDV RNA. cDNA was synthesized starting from RNA isolated from partially purified virions and cloned in lambda-gt11. Screening with the antiserum resulted in identification of several positive clones. Partial sequencing of one HCV-derived cDNA clone revealed a high degree of homology to a portion of the BVDV sequence.
Characterization of the Thymidine Kinase Gene of Fish Lymphocystis Disease Virus

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Fish Lymphocystis Disease Virus (FLDV) is a member of the family Iridoviridae that causes a widely distributed disease in pleuronectides, characterized by papilloma like lesions. A thymidine kinase (TK) activity has been reported for two members of the family Iridoviridae namely Frog Virus 3 and FLDV. The identification of the TK gene locus in FLDV gene was performed by transformation of 3T3 TK negative to 3T3 TK positive cells using the complete gene library of the viral genome (1). The TK gene locus was mapped on a EcoRI/HindIII DNA fragment (4.7 kbp) ranging from 0.669 to 0.718 map units. For further characterization of this gene the DNA nucleotide sequence was analysed. An open reading frame could be detected on the lower DNA strand that codes for 318 amino acid residues. A consensus sequence deduced from TK genes of vaccinia virus, shope fibroma virus, mouse and human showed homology to a region at the 3' end of FLDV TK gene. The characterization of the FLDV TK gene product is in progress now. – DFG project Da 142/2–4.

Reference
1. Scholz, J., A. Rösen-Wolff, M. Touray, R. Schnitzler, and G. Darai: Virus Res. 9 (1988) 63–72.

Structural Properties and the Coding Capacity of the Repetitive DNA Sequences of the Genome of Insect Iridescent Virus Type 6

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The genome of Insect Iridescent Virus Type 6 – Chilo Iridescent Virus (CIV) – was shown to be circularly permuted and terminally redundant. A defined gene library of the CIV DNA (209 kbp) was used for identification of structural properties of the viral genome. Hybridization experiments and heteroduplex mapping were performed. A complex of repetitive DNA elements was detected which maps at the coordinates 0.920 to 0.944 mu (PvuII DNA fragment L (5.06 kbp), a part of EcoRI DNA fragment C, 13.5 kbp; 0.909 to 0.974 mu) and at the coordinates 0.535 to 0.548 mu (2.6 kbp) and 0.571 to 0.582 mu (2.3 kbp), both are terminal regions of the EcoRI DNA fragment H (9.8 kbp; 0.535 to 0.582 mu). The DNA nucleotide sequence of these three regions was determined which revealed the presence of a cluster of tandem DNA repetitions in a cascade formation within the PvuII DNA fragment L. An inverted repetition was found to be located in the right terminus of the EcoRI DNA fragment H between the coordinates 0.571 and 0.582 of the viral genome. Analysis of the coding capacity of the DNA sequences revealed the presence of five ORFs (118 to 333 amino acid residues). The analysis of the amino acid sequences of the largest ORF revealed that the deduced amino acid sequence of the putative gene product contained two repeti-
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TRI (three domains of 50 amino acid residues) and TR2 (two domains of 74 amino acid residues). Sequences of 43 amino acid residues of ORF (160 to 202 AA) were homologous within the majority of ORFs. The following consensus sequence was found in all ORFs: -(MANL(X)6IGSSST(X)6L(X)1LGS(X)1LQISG(X)26(X)1VN-). — Although the classical, canonical and non-canonical transcriptional start signals were detectable, polyadenylation signals were not observed. — DFG project DA 142/2–4.

Duplication of Enhancer Sequences in HPV 6 from Atypically Localized Condylomata acuminata

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Human papillomavirus (HPV) 6 usually induces tumors of the genital mucosa. An HPV 6 related DNA was detected in an extrachromosomal state in atypically located condylomata of the mamilla and was molecularly cloned. The cloned DNA showed typical HPV 6 DNA fragment patterns except for a 0.24kb larger PstI B fragment. The identity of the cloned HPV DNA with the viral DNA in the biopsy was confirmed by comparative restriction analysis. Sequencing revealed an exact 235bp duplication encompassing nucleotides 7681 to 7896. Within the duplication, there is a 20bp insertion, shown by Rando et al. to possess enhancer activity (Virology 155, (1986) 545–556). A HinfI-PstI fragment, containing the whole duplication, showed 7-fold increase in CAT-expression when compared to the monomeric sequence. This may be important for the unusual tissue tropism.

Silencer Activity in the Genome of the Human Papillomavirus 8

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Human papillomavirus 8 (HPV 8) induces macular skin lesions in patients with Epidermodysplasia verruciformis which develop frequently into carcinomas. A clinically inapparent latent infection of the normal population must be expected. To analyse transcription control signals we cloned HPV 8 sequences into an enhancerdependent CAT-vector and tested for transient expression in mouse fibroblasts. Thereby we identified a cisacting negative regulatory element at the transition from the non-coding to the early region. A corresponding 240 bp fragment was cloned downstream from the SV40 enhancer/promoter driven cat-gene and reduced cat-gene expression to 30%. Insertion of a control fragment did not influence cat-gene expression. Repression occurred at the transcriptional level as confirmed by RNase protection experiments.
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The 3'-Terminal Hairpin of ADV DNA Binds Specifically to Empty ADV Particles

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We have previously shown by transfer of gel-purified proteins to a nitrocellulose membrane and probing with labelled DNA ("South-Western blotting") that the ADV capsid protein VP1 binds specifically to a 720 base pair ADV RF DNA fragment which includes the 3'-terminal palindrome (Willwand and Kaaden: Virology 166 (1988) 52–57). New experiments demonstrated the involvement of 3'-terminal palindromic sequences in the interaction. Furthermore, gel mobility shift assays showed that this site binds to empty ADV capsids. Complexes were built predominantly with a conformationally distinct DNA form having a covalently closed (turnaround) hairpin. Thus, the secondary structure of the palindrome is important in the specific recognition of ADV DNA by the viral capsid. We suppose that this recognition serves a role in selecting de novo synthesized viral DNA strands for their subsequent packaging into pre-formed empty capsids.

Protease-Like Sequence in Hepatitis B Virus Core Antigen is Functionally Different from Retroviral Proteases, and not Required for Generation of e-Antigen

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Mammalian hepadnaviruses contain in their core gene products a sequence resembling the active-site residues of aspartic acid and retroviral proteases. It was postulated that the hepadnaviral protease-like sequence, too, is part of a protease. As substrate, the core protein itself was suggested. By self-cleavage it would give rise to e-antigen (HBeAg), a secretory core gene product found in the serum of HBV-infected individuals. – We have experimentally tested this hypothesis by replacing the putatively essential aspartate, and additionally the following Thr-residue. Transient expression of the mutant virus genomes in HepG2 cells demonstrated that biosynthesis of secreted HBeAg was completely independent of an intact protease-like sequence. Activity of the viral polymerase, though markedly reduced in the double mutants, was only slightly diminished in the single mutants. We conclude that a hepadnaviral protease, if existent at all, is functionally different from aspartic and retroviral proteases.
Expression of the Pol Open Reading Frame of the Hepatitis B Virus Genome in Escherichia coli

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The pol open reading frame of the hepatitis B virus (HBV) was expressed in *E. coli* as a β-gal fusion protein characterized by the presence of the collagenase susceptible site of human pro-α-collagen between HBV-pol and the β-gal moiety. The pol fusion protein was subjected to different experimental procedures: 1) Pol specific antibodies were predominantly found in sera of chronic HBsAg carriers. 2) The pol fusion protein bound to nucleic acids. In case of HBV RNA containing the DR1 and downstream located sequences binding seemed to take place in a specific manner. 3) Crude lysates partially purified by DE52 chromatography showed an up to sevenfold higher DNA polymerase activity on gapped DNA than similarly treated negative lysates. An additional reverse transcriptase activity could not be observed.

Inhibition of HDV RNA Replication in Primary Woodchuck Hepatocytes by Ribavirin and Suramin

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Monolayer cell cultures of primary woodcuck hepatocytes, prepared by liver perfusion in situ with collagenase type I, can be infected with hepatitis delta virus (HDV). Replication of HDV was demonstrated by the appearance of genomic HDV RNA 7 days after inoculation. This tissue culture system was used to study the effect of antiviral substances (Ribavirin, Suramin, Alpha-Amanitin and Acyclovir) on the replication of HDV RNA. — Ribavirin inhibited HDV-replication at concentrations > 10 μg/ml, also when added to culture medium at three days post infection, indicating an interference with HDV RNA replication. — Suramin had an inhibitory effect only when added to culture media simultaneously with the infection of cells, in a concentration of 200μg/ml. This concentration had no toxic effects on primary woodchuck hepatocytes. This restriction of an inhibitory effect to the early stage of infection of hepatocytes indicates an inhibition of virus adsorption or penetration. — Alpha-Amanitin showed a weak inhibitory effect in the highest nontoxic concentration of 0.1 μg/ml. Acyclovir had no inhibitory effect on HDV RNA replication.
Learning Deficiencies in Persistently Borna Disease (BD) Virus-Infected Rats

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BD virus leads either to fatal encephalomyelitis or persistent infection in a variety of animal species. Virus persistence in neurons causes no clinical symptoms, but is, however, associated with the presence of high amounts of virus-specific proteins. We therefore hypothesized that slight neuronal disfunctions could have occurred which might only be detectable under experimental conditions. We monitored the brain functions of intracerebrally infected (titer 10⁵ ffu/g brain) but healthy rats by two basic types of learning tasks. Spatial discrimination learning ('y-maze' and 'hole-board') was significantly less successful in BD virus-infected compared to mock-infected rats, referring to functional hippocampal disturbances. A further complex three-step learning experiment (conditioned reaction suppression, Skinner box) was designed to check emotional information processing. In infected rats, we found a significantly diminished ability to learn aversive reactions compared to control rats, referring to disturbancies of amygdala/cortex functions. In conclusion, BD virus persistence in apparently healthy rats can serve as a potent system to study subtle disfunctions of the brain which are only detectable by methods of experimental psychology.

Regular Appearance of IgM Autoantibodies Directed Against Triosephosphate Isomerase in Acute EBV Infection

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During the acute phase of infections mononucleosis (IM) IgM autoantibodies against two cellular proteins with a molecular weight of 26 kd and 29 kd (p26/p29) appear. The antigens are serologically demonstrable in EBV infected and non-infected B cell lines, as well as in primary human lymphocytes, human erythrocytes, and other cells derived from human organs. Following isolation and purification sequence determination was begun. P29 was recognized as triosephosphate isomerase (TIM), a glycolytic enzyme, after comparison with the Dayhoff chart and consideration of further proteinchemical characteristics. P26 is blocked N-terminally and has not yet been identified for certain. Prior to EBV infection antibodies against TIM and p26 are not detectable. Both regularly occur as IgM antibodies in the course of IM, persisting for about 6 months. A switch to IgG does not occur. During EBV infection various autoantibodies are produced, varying from patient to patient possibly due to the different mechanisms of autoantibody production. Detecting anti-TIM and anti-p26 antibodies, we are the first to describe autoantibodies that occur in all cases of IM. The role for pathogenesis in IM is being investigated.
Role of Delayed Type Hypersensitivity (DTH) Reaction for the Control of Virus Infection

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DTH is usually revealed as local inflammation developing after deposition of antigen. We wanted to know whether DTH is involved in terminating infection of the mouse with lymphocytic choriomeningitis (LCM) virus and have ascertained the cells that mediate both these phenomena. After inoculation into the footpad the LCM virus multiplies locally. Beginning around day 6, the foot exhibits a DTH reaction which is measurable as swelling, and at the same time the virus concentration declines. The swelling consists of two phases in which CD8+ and CD4+ T lymphocytes, respectively, are centrally involved; only the second CD4+ cell-mediated part requires monocytes. For virus elimination CD8+ cells are essential; CD4+ cells alone are not antivirally active, but they improve the performance of the former. Since this effect is also seen by treating the mice with interleukin-2, we assign the helper/inducer cells some accessory function; the same is true with regard to monocytes.

Treatment of Lymphocytic Choriomeningitis Virus-Infected Mice with Interferon-γ Monoclonal Antibody Blocks Generation of Virus-Specific Cytotoxic T Lymphocytes and Virus Elimination

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In actually infected mice the lymphocytic choriomeningitis (LCM) virus multiplies to high titers in essentially all tissues. Around day 6, virus clearance sets in, which is mediated by CD8+ cytotoxic T lymphocytes (CTL), probably by releasing (or inducing other cells to release) antiviral cytokines. To ascertain whether interferon-γ (IFN-γ) plays a role, infected mice were injected once intravenously with monoclonal antibody neutralizing this lymphokine. Administration 1 day after infection blocked virus elimination from spleen and liver and decreased generation of CTL. Limiting dilution analysis revealed absence of activation of CTL precursors. In contrast, when the antibody was given 3 days after or 1 day before the virus, neither clearance nor generation of CTL was measurably affected. We conclude that in the generation of LCM-viral CTL an early event is dependent on constitutively produced IFN-γ; when its activity is blocked, CTL do not mature, resulting in the inability of the animal to terminate the infection.
Morphology of Immune Stimulating Complex (ISCOM)

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Monomeric protein, prepared by the dissection of a microorganism, represents a poor antigen. To arrange a number of identical antigens in multimeric complexes, such as micelles, virosomes or immuno stimulating complexes (ISCOMS), is an efficient way of presenting antigens to the immune system. By integrating a larger number of identical antigens into such a complex, or better into ISCOMS, high titered and long lasting antibodies can be raised. – ISCOMS are assembled from Quil A (a saponin-like substance), lipids and amphipathic proteins. Quil A and lipids form unstable structures. After integration of hydrophobic proteins a stable 3D-structure is built. ISCOM architecture was analysed by negative staining, freeze drying, high resolution shadowing and tilting experiments followed by rotational image analysis. An ISCOM is formed from 20 identical morphological units as a pentagonal dodecahedron with a hole on each of the 12 pentagonal faces. In this open and stable structure the ISCOM is presenting protein determinants to the immune system. ISCOMS can be stored over years, still intact and immunogenic, and induce both antibody- and cell-mediated immunity.

Reference
Morein B.: The ISCOM antigen presenting system. Nature 332 (1988) 287–288

Site-Restricted Persistent Cytomegalovirus Infection After Selective Long-Term Depletion of CD4-Positive T Lymphocytes

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We have established a murine model system for exploring the ability of a CD4-subset-deficient host to cope with cytomegalovirus infection, and report three findings. – First, an antiviral response of the CD8 subset of T lymphocytes could be not only initiated but also maintained for a long period of time despite a continued absence of the CD4 subset, whereas the production of antiviral antibody proved strictly dependent upon help provided by CD4 subset. Second, no function in the defense against infection could be ascribed as yet to CD4-CD8- T lymphocytes, which were seen to accumulate to a new subset as a result of depletion of the CD4 subset. – Third, even though with delay, the CD8+ effector cells raised in the CD4- subset-deficient host were able to clear vital tissues from productive infection and to restrict asymptomatic, persistent infection to acinar glandular epithelial cells in salivary gland tissue.
Functional Characterization of the Envelope Protein Complex of HIV-1 Using Synthetic Oligopeptides

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Following computer assisted analysis of the amino acid sequence of various HIV-1 isolates we synthesized a series of oligopeptides derived from protein regions of gp120/gp41 with differing degrees of conservation. Using sera derived from HIV-1 positive individuals in different stages of the progressing disease, the peptides were tested for reactivity with antibodies in ELISA test systems. For a highly variable antigenic region (V3) we constructed a consensus peptide (p315–326) which showed relatively good reactivity. Patients in the final stage of AIDS had a very limited immune reaction, preferentially with two peptides (p102–112, p315–326); asymptomatic individuals, in comparison, showed a broad spectrum of reactivity despite major differences in individual serum samples, probably due to amino acid variations in the respective virus isolates. Using consecutive sera derived from the same patient decreasing antibody titers to defined epitopes could be demonstrated with progressing disease. Cellular immune response was tested in T-cell proliferation assays by incorporation of \textsuperscript{3}H-thymidine. A peptide derived from a conserved protein region (p435–446) induced T-cell proliferation of about 50% of the peptides tested. In conjunction with the data concerning the reactivity of antipeptide sera, these results were combined into a functional map of the envelope protein complex of HIV-1.

Polymerase Chain Reaction (PCR) and Virus Isolation for Detecting HIV-Infection in HIV-Seropositive Hemophiliacs

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Lymphocytes (PBL) were separated from heparinized blood samples (20 ml) of 25 HIV-seropositive hemophiliacs, who were classified into the different groups of the CDC scheme. For virus isolation 90% of the PBL were cocultivated with PBL from normal healthy individuals. For PCR the DNA of the PBL was prepared from 10% of the patient's PBL and a 142 bp DNA fragment from a highly conserved region of the env-gene (gp 41) (position 7801–7942 for the isolate ARV 2) (Sanchez-Pescuador et al., Science 227 (1985) 484) was amplified using the SK 68/SK 69 primer pair (Ou et al., Science 239 (1988) 295)). Amplified DNA was detected by Southern blot hybridization with a specific DNA probe. – Whereas p24 antigen was detectable only in 4, and decline of p24 antibody only in 3 cases, HIV was isolated in 15 cases. In all of these and in 6 more cases the PCR yielded positive results. In 4 cases all parameters tested were negative. Remarkably, these 4 patients were fully immunocompetent (3 class IIA and 1 class IIIA). – Aided by the Federal Ministry of Youth, Family, Women, and Health.
Heat Inactivation of Human Immunodeficiency Virus Type 2 in Clotting Factor Concentrates

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The transmission of HIV-1 with pharmaceutical plasma protein preparations is reduced by screening plasma donations for anti-HIV-1 and is safeguarded by virus inactivation measures in the manufacturing process. Currently, screening tests for HIV-2 are not available. It is assumed, however, that HIV-2 is equally well inactivated as HIV-2 by virus inactivating production steps. To prove this hypothesis, we performed validation experiments, comparing the inactivation of HIV-2 with that of HIV-1 during the manufacturing process for several products. Heat treatment at 60°C in solution (pasteurization), using cell culture medium or different stabilized plasma protein solutions, inactivated HIV-1 and HIV-2 equally well within 10–70 min. C-type retroviruses turned out to be less heat sensitive. Special purification procedures like rivanol precipitations or treatment with low concentrations of surface-active substances eliminated the infectivity of HIV-1 and HIV-2 to the same extent.

Constitutive Expression of Sendai Viral P-Protein with SV 40-Derived Vectors: Partial Stability of Expressed P-Protein in Transfected LTK-Cells

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The full-length cloned Sendai virus P-gene was inserted in different Simian virus 40-derived expression vectors. After transfection (LTK-cells) geneticin (G 418) resistant cells were cloned. Clones were selected for positive signals in Southern blots with a P-specific probe and in immunofluorescence with a Sendai virus specific antisera. In these selected clones it was possible to detect by immunoblotting a protein which comigrated with the P-protein of viral particles (79 kd). – The appearance of a band at 72 kd which reacts with a P-specific antiseraum seems to depend on degradation of P-protein, which only occurs in transfected cells. This makes it very likely that the P-protein in infected cells is stabilised by association with the nucleocapsid or other viral proteins. – These results allow further experiments to complement defects within the polymerase complex, particular in persistent viruses. Furthermore, it is now possible to study effects of single viral proteins on cellular functions.
Reduced Sendai Virus Release from Persistently Infected CL-E-8 Cells is Correlated with a Small Number of Nucleocapsids Attached to Cellular Structures

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A cell line (C1-E-8) persistently infected with Sendai virus shows a reduced release of virus particles and reduced amounts of viral mRNAs, but identical copy numbers of viral genomic RNA when compared to the acute infection. Immunofluorescence and phase contrast data showed in C1-E-8 cells large inclusion bodies which stained for the viral capsid proteins NP, P, and L. With conventional and immuno-electron microscopy these inclusion bodies could be identified as aggregating viral nucleocapsids. After cell fractionation to separate soluble and cytoskeletal fractions about 95% of the nucleocapsids were found to be not attached to cellular organelles such as the cytoskeleton. These nucleocapsids contain all the three capsid proteins NP, P and L and viral 50s RNA, but no RNA of defective virus particles (DIP). The data presented here suggest a disturbance in the attachment of nucleocapsids to the cytoskeletal framework during the virus maturation in persistently infected cells. The aggregated nucleocapsids correlate with a reduced release of virus particles and may influence the viral gene expression.

Different Palmitoylation of Paramyxovirus Glycoproteins

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Fatty acid acylation has been recognized as a widespread modification of viral and cellular proteins. Currently two types of acylation can be distinguished: myristoylation, whereby myristic acid is attached cotranslationally to a glycine residue at the N-terminus of proteins, and palmitoylation, which involves the posttranslational addition of different fatty acids in a (thio)ester type bond. Here we report about remarkable differences in the palmitoylation of various paramyxovirus glycoproteins. SDS-PAGE and fluorography of ³H-fatty acid labeled virus particles revealed that the fusion protein of Newcastle-Disease virus and the hemagglutinin-neuraminidase of Simian virus 5 are palmitoylated, whereas the glycoproteins of Sendai virus are fatty acid free. The fatty acid attachment is labile to treatment with reducing agents which indicates a reactive thioester bond with cysteine residues.
Structure and Function of the Nucleoproteins of Influenza A Viruses

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The nucleoproteins (NP) of influenza A viruses are multifunctional phosphoproteins. – With respect to the amino acid sequences of the NPs of different species, two subtypes can clearly be defined: all sequenced human strains belong to subtype 1; all other sequenced strains belong to subtype 2, with the exception, that the NPs of swine strains can belong to subtype 1 or 2. There is no significant difference in the secondary structure prediction between both subtypes. On the nucleic acid level there seems to be a separate evolution of the subtype 2 NP genes of isolates from the west coast of the USA, e.g. A/seal/Massachusetts/1/80 (H7N7) and the European, Australian resp. Asian isolates, e.g. A/whale/Pacific Ocean/19/76 (H1N3). – The analysis of two temperature-sensitive (ts) mutants of the NP influenza virus with different phenotypes and defects in the NP revealed that the highly conserved region between residues 147 and 185 might be a functional domain during virus maturation (ts19), while the region around residues 314 to 332 might be a functional domain during viral c/vRNA synthesis (ts81), probably in cooperation with the PB2 protein.

Analysis of the Antigenic Determinants of the Hemagglutinin of Influenza A/ Turkey/Oregon/71 Virus

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Monoclonal, neutralizing antibodies (mAB) to the hemagglutinin (HA) molecule of A/turkey/Oregon/71 (H7N3) have been prepared and used to establish an antigenic map. – In competition experiments with a newly developed competition-ELISA three antigenic domains could be defined, which possess neutralizing properties. Accordingly, the mAB’s obtained could be arranged in three groups. Some mAB’s of one group showed reactivities with mAB’s of another group. This may indicate that parts of the antigenic sites are either structurally overlapping or in close proximity. Alternatively, it is possible that the binding of one antibody to any of these sites allosterically affects the binding of antibodies at topologically distant sites. – Comparable results were obtained with antibody-resistant variants and mutants adapted to a novel host. – Amino acid sequence analyses of the variants, which are under way, will help to localize the antigenic domains in the HA-molecule.
Insertion of a 28 S Ribosomal RNA Sequence into the HA Gene of an Influenza Virus

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During adaptation of the apathogenic avian influenza virus A/Turkey/Oregon/71 (H7N3) of chicken embryo cells (CE-cells), whose haemagglutinin (HA) is not cleavable in this system, infectious variants with proteolytically activated HA were obtained. The cleavability increased in a number of different cell types tested and led to an aggravation in pathogenicity for chickens. Nucleotide sequence analyses revealed that during adaptation 54 nucleotides were inserted into the HA-gene immediately adjacent to the cleavage site. The nucleotide sequence of this insert corresponds to a sequence of a region found in the 28 S ribosomal RNA of mammalian cells. This insertion, which is due to a recombination between the 28 S ribosomal RNA of the CE-cells and the HA gene, is most likely responsible for the alterations of the biological properties of the turkey virus.

Alternation in Biological and Structural Properties of an Influenzavirus (A/Sea/VMass/80) During Adaptation to Embryonic Chicken Cells

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Influenzavirus A/Sea/Mass/80 (H7/N7), which caused considerable loss in harbor seal population of the east coast of the United States in 1979/80, could be adapted to embryonic chicken (CE) cells by serial passages. In contrast to the original virus the hemagglutinin of the adapted virus became proteolytically cleaved in a number of cell-types, previously not permissive. Correspondingly, infectious virus was produced in CE-cells and all the other cell types tested. Furthermore, the variants were found to be highly pathogenic for chickens. Whereas intramuscular infection with the apathogenic original virus did not become clinically manifest, application of the variants led to a systemic, lethal infection. Nucleotide sequence analysis indicate that the alteration in the biological properties are based on an insertion of 9 nucleotides immediately adjacent to the cleavage site of the hemagglutinin, which encodes for 3 additional arginin-residues. By means of this insertion the connecting peptide exhibited structure known for highly pathogenic avian influenzaviruses. These findings not only support the concept that the structure of the hemagglutinin cleavage site determines infectivity and pathogenicity of influenzaviruses (Klenk and Rott, 1988), but also shows for the first time, that a mammalian influenzavirus can acquire pathogenic properties for chickens by selection during adaptation to a novel host.

References

H.-D. Klenk, R. Rott: Adv. Virus Res. 34 (1988) 247–281
The Structure of the F-Protein as a Determinant for the Pathogenicity of Newcastle Disease Virus

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Proteolytic activation of the F protein is an important determinant for the pathogenicity of NDV. To analyze the molecular basis underlying the differential susceptibility to proteolytic enzymes, the complete nucleotide sequences of the F genes of three pathogenic strains, Warwick, Field Pheasant and Texas have been determined as well as the sequences of two apathogenic isolates, Ulster and La Sota. The F gene sequence of a chemically induced mutant of strain Ulster with an increased host range has been determined, too. – The sequences show high homology, positions of cystein residues and glycosylation sites are conserved. – Pathogenic and apathogenic strains differ in their consensus sequences at the cleavage site which is for pathogenic strains Arg-Arg-X-Arg-Lys-Arg and for apathogenic strains X-Arg-Lys-X-X-Arg. The mutant has the consensus sequence X-Lys-X-Arg-Arg and therefore represents an intermediate stage as far as cleavability of the F protein and the number of basic residues at the cleavage site are concerned. These results demonstrate that the cleavability is increasing with the number of basic residues and that for a universal cleavability two pairs of basic residues are necessary.

The O/D Variation of an Influenza A Virus (H1N1) Based on the Loss of an Oligosaccharide from the Haemagglutinin Receptor Binding Site

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An isolate of influenza A virus (H1N1) which was propagated in MDCK cells shows an O/D change in its haemagglutination behaviour. The O (original)-virus only agglutinates guinea pig red blood cells, whereas the D (derived)-virus agglutinates chicken as well as guinea pig red blood cells. The viral nucleotide sequences reveal that the O/D variation is based on an amino acid change at position 133 (H3 numbering) from threonine to isoleucine. This mutation eliminates the glycosylation site at amino acid 131, which is near the receptor binding site. These results indicate that receptor specificity is modulated by oligosaccharides. – The nucleotide sequence of both populations shows a single lysine at the cleavage site. All other influenza virus haemagglutinins which have been examined have an arginine at this position.
Processing of the Influenza Virus Hemagglutinin in Insect Cells

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The hemagglutinin of fowl plague virus has been expressed in insect cells using a recombinant baculovirus as a vector. The hemagglutinin is inserted into membranes and transported to the cell surface. In polarized insect cells it is found only in the apical plasma membrane. The hemagglutinin is acylated and activated by posttranslational proteolytic cleavage. In contrast to these modifications which are similar to those occurring in vertebrate cells, there are significant differences in glycosylation. The carbohydrate was characterized by digestion of the hemagglutinin with endoglycosidases H and D and glucanase F followed by HPLC analysis of the liberated oligosaccharides. In addition to mannose-rich oligosaccharides containing 5–9 mannose residues, that are also found in hemagglutinin derived from vertebrate cells, there was an abundance of shorter oligo-mannosidic side chains. These results suggest that, when grown in insect cells, the complex oligosaccharides of the hemagglutinin are replaced by small, truncated side chains, which may well have modulating effects on its biological activities.

Influenza/Staphylococcus aureus Coinfection in Mice. Treatment with Immunopotentiators

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Combined influenza A H1N1, strain PRB, and Staphylococcus aureus infection of NMRI-mice with a low dose of virus (100 pfu/ml) leads to an illness with high incidence of morbidity and low lethality. Bodyweight changes during this experimental infection were positively influenced by application of Tiprotimod and combined Tiprotimod/A 844 392 treatment (p < 0,05). For A 844 392 given alone, no significant effect on body-weight changes and/or lethality was detectable. An increase of antibody titers was found in all treatment groups. Combined influenza A and Staphylococcus aureus infection of NMRI-mice with a high virus dose (1000 pfu/ml) leads to an illness with high incidence of morbidity and high lethality. In contrast to low dose infection conditions bodyweight changes and antibody titers were not positively influenced by application of both immune response modifiers.
Immunoblot Analysis of the Serological Response in Hantavirus Infections

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The causative agent of Korean hemorrhagic fever with renal syndrome (HFRS) was first detected and isolated from Apodemus agrarius and designated Hantaan virus. Another antigenically related virus which could be isolated from Clethrionomys glareolus is the causative agent of nephropathia epidemica (NE), a mild form of HFRS. All these antigenically related viruses have been grouped in a separate genus (Hantavirus) in the family Bunyaviridae. Sera from patients with NE or HFRS were tested for specific antibody responses to antigens of Hällnäs virus and Hantaan virus strain 76-118. A Vero EG derived cell line persistently infected with Hällnäs virus strain B1, and Vero E6 cells freshly infected with Hantaan virus type strain 76-118 were used as antigens in the immunofluorescence assay (IFA) and the immunoblot. The convalescent-phase sera of NE patients tested in this study regularly revealed a marked reaction with a 52 Kd protein of Hällnäs virus and a 50 Kd protein of Hantaan virus. A convalescent serum from a patient with HFRS and a rat antiserum against Hantaan virus could recognize the 50 Kd band of Hantaan virus, but showed no apparent reactivity with the 52 Kd component of Hällnäs virus in the standard dilutions. Some sera could additionally identify minor bands in the 55 Kd and/or 67 Kd region of the blots. A one-way cross reactivity between Hantaan and Hällnäs viruses was also evident from the results of the IFA in that NE convalescent sera reacted with both viruses whereas KHF convalescent or anti-Hantaan sera gave strongly positive results with Hantaan virus but only faint reaction with Hällnäs virus. – BMFT project 0318973A.

Viral Infections as Aetiological Factors of the Seal Death: Coinfection with Herpes- and Morbilliviruses

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The aetiological factors of the seal mass mortality in the North-Sea are still insufficiently investigated. From more than hundred clinically sick animals five individual isolates were recovered which reacted with canine distemper virus (CDV)-specific antibodies. The majority of the seals collected at the island of Sylt had virus-specific antibodies (including those which neutralized the Rockborn strain of CDV), whereas animals collected at the shores of Jütland were free of antibodies. From both groups of diseased seals serveral herpesvirus isolates were received from the lungs and the brains. These isolates have been characterized with both biological and serological methods. Furthermore, neutralizing antibodies against both viruses were found in half of the seals. It is of considerable interest for the pathogenesis of this disease that a coinfection of herpesviruses and CDV-like viruses occurred in some of the animals, even in the same organs. (lung, brain). This was not only demonstrated by virus isolation but also by the wide spread of herpes- and morbillivirus-specific antigens in samples of the same individuals. The results strongly suggest that under the pressure of ecological
changes in the environment of the seals and due to yet undefined immunosuppressive events a coinfection or cooperation of the viruses from two different groups (morbillivirus and herpesvirus) are mainly responsible for the current epidemic.

**Model of Complete Synthesis and Progressing of the Structural Proteins of the West Nile Flavivirus**

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The genome of WN virus is an infectious ss-RNA 10,960 nt in length. One long ORf of 10,920 nt is present, coding for a polyprotein with the viral structural proteins C (14 kd), preM'(21 Kd) and E (53 Kd) located in the N-terminal region. - The 5'-part of the genome was translated in cDNA, cloned into a SP 6-transcriptional vector and transcribed by SP 6 RNA polymerase. The 2.7 kb mRNA was translated *in vitro* in absence and presence of RER membranes. The synthesized proteins were analyzed by fingerprints, by radiosequencing and by chemical sequencing of the termini. In comparision with the authentic viral structural proteins the data allow to propose the following model of the synthesis of the viral structural proteins:

a) The polyprotein is cleaved cotranslationally by signalase into the mature membrane proteins preM and E and a precursor of the core protein C.

b) This precursor is shortened at the C-terminus in a posttranslational step which generates mature C protein.

c) The membrane protein preM is proteolytically cleaved when virus particles are released from the host cell; in the extracellular virus the C-terminal part remains associated to the virus particles as M protein whereas the N-terminal part of the preM protein is lost from the virus.

**Purification and Characterisation of the Nonstructural Protein NS1 from Flavivirus West Nile Virus (WNV) Infected BHK Cell**

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We determined the complete primary structure of the nonstructural glycoprotein NS1 (50 kD) of WNV. Terminal sequence data were obtained by determination of amino- and carboxytermini of the NS1 protein together with the derived amino acid sequence of the NS1 coding genome region. Comparative studies among nine derived flavivirus NS1 sequences available today, shows that the amino acid number of 352 is exactly conserved. Additionally, the different NS1 proteins exhibit homologous hydrophobicity profiles suggesting that its structural properties are conserved. Furthermore within the NS1 the position of all 12 cysteine residues are also conserved and they will form six intramolecular disulfides. The analysis of the N-glycosylation pattern shows that the NS1 protein contains two high mannose-type carbohydrate site chains. Computer studies indicate that also two N-glycosylation sites are conserved.
Hog Cholera Virus – Identification of HCV Gene Products with Antisera Against Fusion Proteins Synthesized in Bacteria

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The genome of hog cholera virus (HCV) consists of an RNA of 12 kb in length. The RNA is not polyadenylated and contains one large open reading frame. In contrast to the much better described pestivirus bovine viral diarrhoea virus (BVDV) little is known about genomic organization and the HCV-induced proteins. Starting from RNA isolated from purified virions, cDNA was synthesized and cloned in lambda gt 11. HCV-derived cDNA clones could be identified with a goat antiserum prepared against HCV. Two independent cDNA clones – 0.8 and 1.8 kb in length – were expressed as bacterial fusion proteins. After purification the respective polypeptides were used to induce antisera in rabbits. It could be shown that these antisera react in Western blots and radioimmunoprecipitation with the bacterially synthesized proteins. According to metabolic labeling of HCV infected cells, radioimmunoprecipitation assays and SDS-PAGE, one antiserum recognizes the authentic virus encoded protein.

Isolation and Characterization of the Acetylesterase of Two Coronaviruses (BCV and HEV)

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Bovine coronavirus (BCV) as well as a murine coronavirus (JHM-strain of mouse hepatitis virus) have been shown recently to contain an acetylesterase. This enzyme, which releases acetyl residues from position C-9 of sialic acid, inactivates receptors on erythrocytes rendering the red blood cells resistant against hemagglutination by BCV (1). The acetylesterase activity is correlated with gp65 (E3), which is also responsible for the hemagglutinating activity of BCV. – We have found that hemagglutinating encephalomyelitis virus (HEV), a porcine coronavirus also has an acetylesterase similar to the enzyme of BCV and MHV-JHM: (i) the enzyme is inhibited by diisopropylfluorophosphate (DFP), which binds covalently to the serine in the active site of serine esterases; (ii) incubation of purified virions with radiolabeled DFP (1H-DFP) resulted in selective labelling of gp65 indicating that the esterase activity is a function of this protein. The acetylesterase of HEV and BCV was isolated from purified virions by detergent treatment. Following sucrose gradient centrifugation, purified enzyme was obtained.

Reference

1. Vlasak, R., W. Luytjes, W. Spaan, and P. Palese: Proc. Natl. Acad. Sci. 85 (1988) 4526–4529
Membrane Integration and Intracellular Transport of the E1 Protein of Mouse Hepatitis Virus A59 (MHV A59)

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The E1 matrix glycoprotein of MHV A59 spans the viral membrane three times. In infected cells the E1 protein accumulates in intracellular membranes of the RER and the Golgi and thus determines the compartment of particle formation. – In this study we analyzed the process of membrane integration of the E1 protein by coupled in vitro transcription/translation. Membrane spanning domains I and III act as signal sequences and as stop transfer sequences as well. Membrane integration of the three hydrophobic domains can be uncoupled from ongoing protein synthesis, but depends on association of the peptides with the ribosomes. Peptides smaller than 65 amino acids can be translocated post-translationally after dissociation from the ribosomes. – After microinjection of in vitro transcribed 5'-capped and 3'-polyadenylated mRNA, the E1 glycoprotein accumulated in the Golgi, but could not be detected at the plasma membrane. Analyses of the transport properties showed that the E1 protein carries a non-linear Golgi-specific retention signal which is located within the membrane spanning domains.

Kinetics of the Formation of RF and RI in Poliovirus-Infected Cells

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The mechanisms involved in the synthesis of poliovirus RNA have not been fully elucidated. Three forms of viral RNA can be isolated from infected cells – single-stranded RNA of positive orientation which functions as messenger RNA or becomes encapsidated as viral RNA; double-stranded RNA (replicative form – RF); complex RNA consisting of a double-stranded core with protruding single-strands (replicative intermediate – RI). While it has been recognised that the synthesis of plus-strand RNA is structurally associated with RI, little is known about the function of RF and its quantitative relation between RI and RF. It is currently believed that RF and RI coexist throughout the infectious cycle, although there is some doubt whether RF is a precursor of RI or the dead-end product of plus-strand synthesis and as such derived from RI. – We have isolated the total cytoplasmic RNA of poliovirus 1-infected cells at various intervals after infection and analysed aliquots by agarose gel electrophoresis and subsequent hybridisation to (+)- and (−)-strand specific probes. We were able to detect RI from 2 h.p.i. on while RF appeared no earlier than 5 h.p.i. From 3.5 h.p.i. to 5 h.p.i. a number of virus specific RNAs were recorded, the MW of which appeared to be successively reduced from the size of RI to RF, indicating a transition of RI to RF in the later stages of poliovirus infection. Hence, we conclude that RF is the dead-end product of plus-strand synthesis associated with RI. – To obtain further information on the RI/RF transition we have attempted to influence its course by addition of inhibitors of transcription and translation.
Molecular Analysis of the Antigenic Structure of Foot- and Mouth Disease Virus (FMDV)

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The knowledge of the FMDV antigenic structure is necessary for the development of recombinant vaccines against viral infections. For this purpose neutralizing monoclonal antibodies (Mabs) were prepared against FMDV strain 01Kaufbeuren and synthetic peptides. The monoclonal antibodies were characterized by immunological methods and could be divided in six different groups. The antibody binding sites were localized by competition experiments and amino acid sequence comparison of nine different FMDV strains tested in a plaque reduction assay. In addition Mab resistant mutants were selected and the protein sequence of their complete P1-coat protein region were determined by c-DNA sequencing. Regarding these results we have identified at least 6 different epitopes involved in virus neutralisation. Two of them are conformation dependent, the others represent sequential epitopes.

Lactate Dehydrogenase-Elevating Virus (LDV)-Induced Autoimmunity: Antiviral Antibodies Crossreact with Intermediate Filament Antigens

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Preliminary data suggested that “molecular mimicry” might not be the reason of the mechanism which triggers the regularly occurring anti-Golgi autoantibody formation after LDV-infection of mice. However, there was evidence for cross-antigenicity between the virus and intermediate filaments. In order to prove this observation monoclonal antiviral antibodies were established after immunization of mice with inactivated LDV prior to infection with the virus. While no anti-Golgi autoantibodies could be observed, this procedure favoured the selection of hybridomas synthesizing antibodies showing a strong cross-reaction with the viral envelope protein VP3 and intermediate filaments. Therefore we suggest that LDV shares epitopes with intermediate filaments, thereby initiating the autoimmune response against this host cell component. The mechanism stimulating the formation of anti-Golgi autoantibodies after LDV-infection remains to be elucidated.
Lactate Dehydrogenase-Elevating Virus Induces Antibodies Reactive with Surface Antigen of Etiologically Independent Transformants

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Immunocompetent mice infected with lactate dehydrogenase-elevating virus (LDV, nine isolates tested) developed antibodies which were reactive with a cell surface antigen (TSA) expressed by a Moloney sarcoma virus (Mo-MSV) transformed STU mouse cell line. These cells exhibit a glycosylated 46K surface antigen (gp46) probably coded for by cellular gene(s). The LDV-induced anti-TSA antibodies were exclusively restricted to the IgM isotype and were unable to immunoprecipitate a iodinated surface antigen of TSA positive cells. Monoclonal anti-TSA antibodies were found to react with the gp46 positive Mo-MSV transformant PV-TC-77, but in addition with the gp46 negative transformants MSV85 C13 (Mo-MSV transformed; BALB/c), MethA (MCA transformed; BALB/c), and the widely used L cells. Competitive binding studies indicated that anti-gp46 monoclonal antibodies did not block monoclonal LDV-induced anti-TSA antibody reactivity. LDV-infected STU mice were protected against growth of syngeneic TSA positive tumor cells as early as 23 days p.i., to a lower degree at 5 month p.i. when anti-TSA antibodies in the serum of infected animals had decreased. We suppose that LDV induced anti-TSA antibodies are the basis of the long known LDV-induced inhibition of the growth of certain murine tumors.

Simulation of Antibody-Mediated Neutralization of Poliovirus by Chemical Cross-linking

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Among the 3 main mechanisms of neutralization the one is the most important which is believed to stabilize the virus capsid by cross-linking subunits with one or few antibodies. This is supported by the observation that upon cleavage of the antibodies into Fab fragments, the virus infectivity is completely restored, while the Fabs remained on the virus surface. This effect was simulated by cross-linking poliovirus with the bifunctional dimethyl-3,3dithiobispropionimidate by introducing few bridges, which reduced infectivity by more than 99%. It could be shown that VP1-VP3 were cross-linked, probably at the NAg3a-3b, which is the one domain on the virus surface with 2 lysines at a distance suitable for cross-linking. Upon cleavage of the cross-links using mercaptoethanol infectivity was restored as well. It was further demonstrated that cross-linked virus penetrated into HeLa cells like antibody-neutralized virus, and it could be reisolated. This again supports the idea that cross-linking blocks a cooperative conformational change of the virus shell which ultimately leads to liberation of the RNA.
Detection of Enteroviruses by Hybridization with Digoxigenin Labeled c-DNA

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Enteroviruses have high sequence homologies. Therefore it is possible to classify a sample as member of the Enterovirus group by means of nucleic acid hybridization. We established a nonradioactive hybridization method with Digoxigenin (Boehringer Mannheim) and compared it with the radioactive method. dUTP with Digoxigenin bound to its spacers was used for the nonradioactive labeling (random priming). The detection system worked with anti-Digoxigenin-antibodies conjugated to alkaline phosphatase. Two probes were used for hybridization, a 2.5kb fragment of the 5'-end and a 3.6kb fragment of the 3'-end of a Coxsackie B3 c-DNA (Dr. R. Kandolf, MPI Martinsried). RNA of 14 Enterovirus strains (Coxsackie B 1–6, Coxsackie A9, polio 1–3, echovirus 4, 9, 11, 30) was tested. By comparison the radioactive method showed a 10fold higher sensitivity than the Digoxigenin system. The sensitivity limit of Digoxigenin was 5-50 ng total RNA, but both methods were able to detect 10 µg total RNA of cells infected with enteroviruses. The probe with the 5'-end of the CB3 c-DNA was able to detect all of the 14 strains, the 3'-probe did not give signals for the three polioviruses. These differences show that the group specific homologies of the enteroviruses must be higher in the 5'-part of the genome than in the 3'-part and that the polioviruses differ more from the Coxsackieviruses than those from each other and from the echoviruses. This presented non-radioactive labeling method with a CB3' c-DNA probe might be useful for identifying enteroviruses in clinical specimens in future.

Treatment of EMC-Virus Myocarditis in NMRI-Mice with Calcium Antagonists

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The concept of calcium overload implies that the susceptibility of the myocardium to necrotization varies in parallel with the availability of extracellular calcium. The influence of two different calcium antagonists has been investigated on histopathologic changes during EMC-virus-myocarditis in mice. Six weeks old male NMRI-mice were inoculated i.p. with 100 PFU of the myocardiotropic variant of the EMC-virus. Mice were treated with Verapamil (30 mg/kg bw/day) or Fendilin (100 mg/kg bw/day) per os, beginning one day post infection. Control animals were fed with standard diet. On day 90 post infection hearts were fixed for histopathologic examination. – After histopathological examination hearts of both treatment groups show less pronounced inflammatory infiltration, necrosis, fibrosis and calcification compared with controls. In addition an improvement on bodyweight changes was observed (p < 0,05).
Treatment of EMC-Virus Myocarditis in NMRI-Mice with Levamisol and Isoprinosine

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The effect of the immunopotentiators Levamisol and Isoprinosine has been investigated on EMC-virus myocarditis. Six weeks old male NMRI-mice were inoculated i.p. with a virus suspension containing 100 PFU of the myocardiotropic variant of the EMC-virus. Mice were treated with Levamisol (2.5 mg/kg bw/day) i.p. or Isoprinosine (250 mg/kg bw/day) per os at different times. After left ventricular puncture hearts were fixed for histologic examination. – Levamisol administration increases lethality and the number of myocarditic lesions in this animal preparation. – Treatment with Isoprinosine leads to an improvement on body-weight changes and lethality up to day 12 post inf., whereas no therapeutic effect could be documented on day 40 post inf. Hearts of treated animals show marked inflammatory infiltrate (p < 0.05). No significant effect was detectable on lethality, hemodynamic and other histological parameters.

Treatment of EMC-Virus Myocarditis in NMRI-Mice with Mediator-Antagonists

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Mediators such as leukotrienes and 5-hydroxytryptamine may cause cardiac damage in viral myocarditis either directly or via vasoconstrictive properties. – Six weeks old male NMRI-mice were inoculated i.p. with a virus suspension containing 100 PFU of the myocardiotropic variant of the EMC-virus. Mice were treated with the 5-HT₂-Antagonist Ketanserin (30 mg/kg bw/day) per os up to day 72 post inf., or the leukotrien-antagonist FLP 55712 (20 mg/kg bw/day) i.p. up to day 12 post inf.. After left ventricular puncture hearts were fixed for histologic examination. Long-term treatment with Ketanserin increases the number of postmyocarditic lesions in this animal model. Hearts of treated mice show marked lymphocytic infiltration (p < 0.001). Treatment with FLP 55712 leads to similar results. – Therefore it might be concluded that 5-hydroxytryptamine and leukotrien mediated reactions are involved in the local and temporary limitation of the inflammatory process in hearts with EMC-virus myocarditis.
P90, a Multifunctional Enzyme of the Birnavirus IBDV

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Infectious Bursal Disease Virus (IBDV) is a member of the birnavirus family. Viral particles contain three different size classes of proteins and a genome consisting of two double-stranded (ds) RNA-molecules. The larger RNA-segment A codes for most of the viral structural proteins; the smaller segment B codes for a single polypeptide with a molecular weight of 90,000 d (p90) which is covalently linked to both ends of the viral genome. p90 probably represents the RNA dependent RNA-polymerase. In addition to this replicase- and transcriptase-activity, other enzymatic functions are associated with p90: The guanylyltransferase and the methyl-transferase-activities required for cap synthesis at nascent viral mRNA-molecules. In purified IBDV-particles, guanosin-monophosphate binds covalently to serin- or threonin-residues in p90, indicating guanylyl-transferase-activity. The incorporation of $^{3}$H-labelled methyl-groups into nascent mRNA-molecules in the presence of adenosyl-L-methionin is indicative of methyl-transferase-activity associated with IBDV-particles.

Replication of Herpes Simplex Virus Type 1 in Differentiated Human Promyelocytic HL-60 Cells

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The replication of HSV-1 (ANG) in native and in DMSO- and TPA-stimulated HL-60 cells was examined. Results of virus titrations and of infectious center assays revealed a replication of virus in native and TPA-stimulated cells, but an abortive infection in DMSO-treated HL-60 cells. Viral DNA synthesis was detected in non-treated and TPA-treated cells, but not in DMSO-treated cells. Specific viral IE (ICP4) and E (tk, pol) transcripts were detected in all three cell types. Viral L transcripts (gC), in contrast, were almost exclusively detected in native and TPA-treated cells. Viral IE protein (ICP4) was found in all three cell types, whereas the synthesis of E protein (pol), dE protein (gB), and L proteins (VP5, gC) was greatly reduced in DMSO-treated cells. These data suggest a block of HSV replication in DMSO-treated HL-60 cells at the level of early gene translation.
Recombinational Events on SV40-DNA in HSV-Infected Cells

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Infection with HSV of various SV40-transformed cell lines leads to strong amplification of SV40-DNA. In the cell line Elona we observed bidirectional overreplication of integrated SV40 including flanking cellular sequences and also extrachromosomal rolling circle amplification of DNA consisting exclusively of SV40-sequences. Rolling circle amplification seems to be restricted to the situation, where SV40 is integrated in a partial tandem manner, whereas bidirectional overreplication also occurs in cells harboring a truncated SV40-genome. In our study further evidence for recombinational events resulting in excision of free SV40 molecules is presented by analysis of amplified DNAs generated after transfection of SV40-plasmid constructs and superinfection with herpes simplex virus. This study was supported by Deutsche Forschungsgemeinschaft (SFB31: Tumorentstehung und -entwicklung).

Lack of Glycoprotein C Expression in Persistently with Herpes Simplex Virus Infected JOK-1 cells

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Infection of the human B-cell line JOK-1 with herpes simplex virus type 1, resulted in the establishment and maintainance of a persistent infection. Only a small proportion of cells produced infectious virus particles, but considerably more cells of the infected cultures contain viral DNA. Although viral glycoproteins are expressed in infected JOK-1 cultures which were investigated with a polyclonal antiserum against all glycoproteins of HSV, immunofluorescence analyses with a monoclonal antibody raised against glycoprotein C revealed that infected JOK-1 cells failed to express this protein. However, mRNA coding for the protein was found in such cells. In all other tested B-cell lines HSV-1 infection led to extinction of the cell cultures accompanied by expression of gC. The observed phenomenon must be a so far unknown intrinsic property of the JOK-1 cells which we are going to investigate in more detail.
Kinetics of Expression of the Herpes Simplex Virus Type I DNA Polymerase Gene

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Expression of the herpes simplex virus type 1 (HSV-1) DNA polymerase gene in HSV-1 strain ANG-infected African green monkey kidney cells was monitored for 36 h during infection by analyzing transcript initiation, synthesis as well as cellular localization of the protein with the aid of monospecific antibodies, and by determining the enzymatic activity. Using cloned and native DNA probes the transcript analysis showed that initiation of pol transcripts occurred as early as 1 h p.i. both within the sequences encoding the origin of DNA replication (ORI) and at their 5'-and 3'-flanking sequences. The pol RNA species initiated within the ORI, sequences were maximally present at 2 h p.i. and only detected up to 4 hrs p.i. suggesting that these transcripts play an important role in the initiating events of viral DNA replication. Pulse-, pulse-chase- and immunoprecipitation experiments revealed the de novo-synthesis of two major polypeptides of 136 kd and 130 kd that were present in equimolar amounts from 4 to 8 h p.i. correlating with the maximal HSV DNA polymerase activity of these cellular extracts. Pol de novo-synthesis peaked at 2–4 h p.i. and declined by 4-fold at 4–6 h p.i. whereas once synthesized the enzyme was found to be stably present up to 36 h p.i. In alignment with this were the results of the indirect immunofluorescence analysis showing that the protein is detectable up to 4 h p.i. in prereplicative centers and from 6–24 h p.i. in nuclear regions with ongoing viral DNA replication.

Autonomy of the Intrathecal Antibody Response in Herpes Simplex Virus (HSV)-Encephalitis

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The HSV-encephalitis is the most frequently occurring sporadic acute encephalitis in man. An independent intrathecal HSV-specific antibody response is earliest observed as from the second week after onset of disease. A cerebrospinal fluid (CSF)/serum ratio of HSV-specific antibody activity greater than two is indicative for HSV-encephalitis, when paired CSFK/serum samples are diluted to the same IgG-concentration. – Beyond this quantitative autonomy of the intrathecal antibody response a qualitative one can be revealed by comparison of the CSF/serum reaction patterns against individual viral proteins. As demonstrated in this work, the locally synthesized antibody response in HSV-encephalitis may be of an expanded heterogeneity. But even if there are no additional bands in the CSF reaction pattern, differences in clonal heterogeneity or immunoglobulin class response may be demonstrated by competitive liquor-serum-blot-technique (LISBT).
Restriction of Herpes Simplex Virus Infection in Mice by Virus-Neutralizing and Non-Neutralizing Monoclonal Antibody

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Virus-neutralizing and non-neutralizing monoclonal antibodies specific for herpes simplex virus type 1 were compared with a polyclonal antiserum for their ability to influence the course of infection in genitally infected C 57 BL/6 mice. All three antibody types administered 24 and 72 h p.i., enhanced virus elimination from the mucous membranes, particularly the neutralizing monoclonal antibody. Clinical lesions were entirely inhibited by each antibody. Whereas the number of infected spinal ganglia on day 5 p.i. was reduced by all antibodies indicating their influence on viral elimination, induction of premature latency, as demonstrated by the small number of positive ganglia homogenates, was effected by the polyclonal antiserum and the neutralizing monoclonal antibody only, as was the total suppression of virus spread into spleen, kidney and adrenal glands.

These results suggest that virus neutralization has no essential role in the antibody effect on virus elimination from mucous membranes and ganglia. Presumably, a cooperation of antibodies with macrophages and/or NK cells occurs. A complex process within the tissue is discussed for the induction of premature latency by neutralizing antibodies.

Regular Appearance of IgM Autoantibodies in Acute EBV Infection. Isolation and Characterization of the Cellular Autoantigens p26/p29

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Sera taken from patients in the acute phase of infectious mononucleosis (IM) contain IgM antibodies that cause a weak granular cytoplasmatic fluorescence in EBV genome positive and negative cells. Applying the same sera on PVDF membranes two bands were detected when reacting with electrophoretically separated lysates from human and certain animal cells. The molecular weight of these cellular antigens was determined as 26 kd and 29 kd (p26/p29). Whereas p29 was exclusively found in the cytosol fraction, p26 was present in the mitochondrion, "l"-microsome and Golgi fraction. Both proteins were purified from Raji cells by preparative PAGE and electroelution. These preparations were used for immunisation of rabbits and further protein chemical characterization. P29 was identified as triosephosphate isomerase by sequence determination. P26 is blocked N-terminally. Cyanogen bromide cleavage followed by sequence determination of the fragments hints to a mitochondrial ATPase. Epitope homology (molecular mimicry) as well as unspecific B-cell-stimulation by the EBV are possible mechanisms leading to autoantibody production.
Diagnosing Acute EBV Infection by Detecting Autoantibodies Directed Against Triosephosphate Isomerase

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In sera of patients with infectious mononucleosis (IM) antibodies of the IgM class against triosephosphate isomerase (TIM) were detected. TIM was gained from Raji cells by a simple method of lysis. Following separation by PAGE and transfer to a PVDF membrane the protein was used for detection of IgM autoantibodies in 5680 sera deriving from EBV examinations. In no case IgM a-TIM was detected prior to EBV infection. In the acute phase of IM (IgM a-VCA positive) IgM a-TIM was always measurable, including all cases in which the heterophile antibody test failed. IgM a-TIM antibodies are present several months p.i., similar to IgM a-CVA. A switch to IgG does not occur. Sera from patients recovering from IM are negative for a-TIM autoantibodies. In other acute viral infections (HCMV, HSV, VZV, HBV, rubella-, mumps-, measlesvirus) IgM a-TIM autoantibodies were not found, whereas in 7 out of 20 patients with acute hepatitis A virus (HAV) infection IgM a-TIM was demonstrated. All of them were IgG A-VCA/a-EBNA positive. Our investigations show that the appearance of IgM a-TIM is specific for acute EBV infection. Postulating an unspecific stimulation of the corresponding B cell clones in the course of acute HAV infection could be a possible explanation for the appearance of a-TIM antibodies.

Presentation of Cytomegalovirus Immediate-early Antigen to Cytolytic T Lymphocytes is Selectively Prevented by Subsequently Expressed Viral Early Gene Products

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Protective immunity against murine cytomegalovirus infection is mediated by pp89-specific CD8+ T lymphocytes. The regulation of antigen processing and presentation to these lymphocytes (CTL) was studied in infected cells. Recognition by CTL of the nuclear phosphoprotein pp89, the immunodominant viral antigen expressed in the immediate-early phase of infection, was prevented by the subsequent expression of viral early genes. The surface expression of major histocompatibility complex class I proteins and their capacity to present externally added pp89-derived antigenic peptides were not affected. Because CTL recognition of a viral early antigen occurred under the same conditions, a general failure in processing and presentation was excluded. Since neither the rate of synthesis nor the amount of pp89 were modified, absence of recognition by CTL was due to a selective interference with pp89 antigen processing and presentation during the early phase of cytomegalovirus gene expression.
Processing and Transport of Glycoprotein B of HCMV

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The processing and the intracellular transport of the major envelope glycoprotein (gB) of human cytomegalovirus (HCMV) was investigated using antisera against different epitopes of gB, various glycosylation inhibitors and cell fractionation techniques. The epitopes recognized by the antisera were defined by their reactivities with different fragments of gB expressed in E. coli. – The results indicated that gB is synthesized as a 100kd precursor which after cotranslational glycosylation yields a 150kd product. Still in the nuclear/ER fraction this product is modified and transported into the inner nuclear membrane as well as into the Golgi; there it is further modified and cleaved to yield the mature 55kd glycoprotein (gB55). The gB55 represents the C-terminal region of the precursor with the very C-terminal epitope missing or modified. Complete glycosylation is not required for transport or cleavage. The precursor as well as the gB55 are phosphorylated.

Sodium Butyrate Induces Replication of Cytomegalovirus (HCMV) in Human Endothelial Cells

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Nonpermissive cultured human endothelial cells were found to produce infectious HCMV when treated for 12 p.i. with 1–2 mM sodium butyrate. Indirect immunofluorescence 72 h p.i. revealed that the majority of the cells synthesized viral antigen. Drug-treated infected cultures exhibited viral DNA synthesis as determined by isopycnic centrifugation of DNA from pulse labelled cells. Furthermore, butyrate-induced infected cells showed enhanced production of HCMV gB which was identified by immunoblotting and immunoprecipitation with gB-specific monoclonal antibody. Nuclear nucleocapsids as well as enveloped cytoplasmic virions were observed by transmission electronmicroscopy. In view of their polarized growth behaviour butyrate-induced human endothelial cells thus provide a novel system to study aspects of directed cellular transport of HCMV-specific products.
Immune Response to Envelope- and Nucleocapsid Proteins of the Human Cytomegalovirus

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For better identification of the various proteins of HCMV, viral antigens were $^{35}$S-methionine labelled and immunoprecipitated with two monoclonal antibodies. The SDS-PAGE pattern of the immunoprecipitation was compared with Western-Blot analysis of purified nucleocapsids and virion particles. We were able to show that mkAb 3-H6, directed to envelopes and dense bodies, precipitated >200,152,137,104,72,61,55,50,44,32,26 and 20 kD proteins. MkAB B-H8, directed to nucleocapsids, immunoprecipitated 135,78,67,53,38,33 and 26 kD proteins. The same pattern of proteins was identified by Western-Blot analysis of a highly purified nucleocapsid preparation with a pool of 5 HCMV positive sera.

T Cell Recognition of HCMV-Determinants Expressed as β-Galactosidase Fusion Proteins in E. coli

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A set of recombinant DNA clones expressing overlapping fragments as HCMV pp65 β-galactosidase fusion proteins in E. coli was isolated from an open reading frame expression library. pp 65 of human cytomegalovirus (HCMV) has been reported to have stimulating capacity in cellular immune response (1,2). CMV β-galactosidase fusion proteins expressed by clones C35 and C74 induced the proliferative response of polyclonal T cells from healthy CMV seropositive donors as compared to the control. In contrast the clone C47, also expressing a pp65 β-galactosidase fusion protein, showed no significant stimulating activity. The results suggest that a T cell epitope exists within the 65 KD protein which is shared by the overlapping sequences of the two clones C35 and C74 but not by C47. Our data show that the use of CMV β-galactosidase fusion proteins expressed in E. coli offers new possibilities to screen for the localization of T cell epitopes within a protein.

References
1. Forman, S. J. et al.: J. Immunol. 134, (1985) 3391–3395
2. Liu, Y.-N. C. et al.: Int. J. Cell Cloning G (1988) 352–364
The ad 12 Host Range Mutant CS-I Contains an Additional Deletion in the E1B Gene

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The adenovirus 12 host range mutant CS-I replicates in simian Vero cells about 100 fold better than the wild type virus. Previous analysis of the genome revealed two alterations: additional viral sequences at the right end of the genome and a deletion of 69 bp in the first exon of the E1A gene (pos.834–902). We have now sequenced most of the E1B region of these mutant and found a second deletion around the cap site of the E1B RNAs (pos.1510–1541) and an A-T exchange in pos. 1545. However, no differences in the E1B expression are found when wt and CS-I are analyzed by Northern blotting. The deletion affects the ATG codon of the E1B 19K protein but not the reading frame of the 58K protein. Identical deletions in both E1A and E1B were found in two other isolates of Ad 12 mutants adapted to growth in Vero cells.

Expression of the Retroviral Construct pZIP-NeoSV(X) 1-E1A in Different Cell Lines

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As shown previously we have cloned the early region 1A (E1A, bp 443–1706) of the adenovirus type 12 into the retroviral vector pZIP-NeoSV(X)1. This construct was transfected into the packaging cell line psi2. NIH3T3 cells were infected with virus containing supernatants of the psi2 cells. Southern blot analysis of NIH3T3 DNA showed the integration of the intact pZIP-NeoSV(X)1-E1A provirus into the NIH3T3 genome. – The expression of the construct was tested by RNA dot blot analysis in a) psi2 cells, b) NIH3T3 cells and c) HeLa cells (after transfection). In contrast to the transfected HeLa cells, the expression of RNAs containing E1A sequences was surprisingly low in the transfected psi2 cells and not detectable in infected NIH3T3 cells. Nevertheless the infected NIH3T3 cells showed a very high expression of the neomycin phosphotransferase gene. – Supported by the Deutsche Forschungsgemeinschaft through SFB 102/A20.
Detection of Antibodies Directed Against Specific Gene Products of Human Papillomaviruses in Human Sera

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Bacterially derived β-gal fusion proteins of genes from HPV-16, -18 and -6b were used for the detection of HPV specific antibodies in human sera. The ages of the patients ranged from 16 to 104 years. In 75 out of 180 sera antibodies directed against the L2 gene products of HPV-6b, -16 and -18 could be detected. Four of the sera reacted with the early proteins E4 and E7 from HPV-16. A serum from a cervical carcinoma patient reacted with E4, E7 and E2 from HPV-16. In summary 44.4% of the sera appeared to be HPV antibody positive. This value may indicate that the actual HPV infection rate is higher than the prevalence rates determined by DNA hybridization studies.

Reduced Growth Potential of Cells Expressing High Levels of Lymphotropic Papova Virus Large T-Antigen

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During the analysis of Lymphotropic Papova Virus (LPV) transformed embryonal hamster cells we found that cells expressing high levels of large T-Antigen (T-Ag) were genetically unstable and segregated cells expressing very low levels of T-Ag. These cells had a growth advantage and eventually dominated the culture. We asked the question whether the high levels of T-Ag were responsible for the reduced growth rate. SV40- and LPV-T-Ag-expression vectors containing a selectable marker gene were used to study the effect of an active or destroyed T-Ag-gene on the selection process following transfection of the vectors. Results indicate that expression of LPV- but not SV40- T-Ag has a marked inhibitory effect on cell growth. Drug selected cells expressing high levels of LPV-T-Ag show a transformed phenotype.
Identification and Characterization of the Repetitive DNA Sequences in the Genome of Molluscum Contagiosum Virus

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The Molluscum Contagiosum Virus (MCV), a member of the family poxviridae, induces epidermal proliferation in man. The genome of MCV type 1 (MCV-1; 188 kbp) had been characterized by physical mapping using a defined gene library of the viral genome and by DNA-DNA hybridization. The physical maps of the viral genome were constructed for the restriction endonucleases BamHI, ClaI, EcoRI, and HindIII. Detailed hybridization experiments revealed the presence of repetitive DNA sequences located within the terminal regions of the viral genome, e.g. BamHI DNA fragment B (18 kbp; 0 to 0.095 mu) and E (10.9 kbp; 0.944 to 1 mu). The fine mapping of this particular regions indicates that the repetitive DNA sequences are located within the HindIII DNA fragments J1 (4.2 kbp; 0.962 to 0.985 mu), K (4.0 kbp; 0.014 to 0.036 mu), P1 (2.7 kbp; 0 to 0.014 mu), and P2 (2.7 kbp; 0.985 to 0 mu). The further characterization of these repetitive DNA elements by nucleotide sequence analysis is in progress now.

Coding Capacity of the Repetitive DNA Sequences in the Genome of Fish Lymphocystis Disease Virus

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The complete DNA nucleotide sequence of the repetitive DNA elements in the genome of fish lymphocystis disease virus (FLDV) isolated from two different species (flounder and dab) was determined. The size of these repetitive DNA elements was found to be 1413 bp which corresponds to the DNA sequences of the 5' terminus of the EcoRI DNA fragment B (0.034 to 0.052 map units (m.u.) and to the EcoRI DNA fragment M (0.718 to 0.736 m.u.) of the FLDV genome causing lymphocystis disease in flounder and plaice. The degree of DNA nucleotide homology between both regions was found to be 99%. The repetitive DNA element in the genome of FLDV isolated from other fish species (dab) were identified which are located within the EcoRI DNA fragment B and J of the viral genome. The DNA nucleotide sequence of one duplicate of this repetition (EcoRI DNA fragment J) was determined (1410 bp) and compared to the DNA nucleotide sequences of the repetitive DNA elements of the genome of FLDV isolated from flounder. It was found that the repetitive DNA elements of the genome of FLDV derived from two different fish species are highly conserved and possess a degree of DNA sequence homology of 94%. The DNA sequences of each strand of the individual repetitive element possess one open reading frame (150 to 339 amino acid residues) which terminates downstream of the 3' (+60 to +207 bases) and 5' terminus (-7 to -232 bases) of the repetitive DNA sequences. The analysis of the EcoRI FLDV-f DNA fragment M for promoter activity revealed that the DNA sequences of this particular region are able to substitute the function of the chloramphenicol-acetyltransferase gene promoter in procaryotic system. – DFG Projekt Da 142/ 2–4.
Replication of the Woodchuck Hepatitis Virus in Primary Hepatocyte Culture of the Woodchuck

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Primary hepatocyte cultures of woodchucks were used for investigation of the replication of Woodchuck Hepatitis Virus (WHV) and Hepatitis Delta Virus (HDV). In situ liver perfusion with collagenase I was performed from animals without serological markers of WHV infection and from animals which were chronically infected with WHV. Hepatocytes negative for WHY were inoculated with highly viremic sera of the acute phase of a WHY infection on the day after seeding of cells. Sera of chronic carriers with low titers of infectious particles failed to infect cells. At day three an increasing number of relaxed circular WHY DNA was found. At day five replicative forms of WHF DNA could be detected and persisted up to thirty days. - Hepatocytes of a chronic HDV carrier showed HDV RNA and WHY DNA up to 25 days after seeding. Replicative forms of WHY DNA accumulated to complete minus- and double strands after ten days. - The effects of antiviral substances (Acyclovir, Ribavirin) on WHY replication are at present under investigation.

Characterization of a HbeAg Like Protein in the Serum of Woodchucks with Acute or Chronic WHY Infection

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We wanted to examine, whether an e-antigen like protein can be detected in the serum of WHY infected woodchucks. Core proteins present “c” and “e” epitopes. Therefore we used recombinant core protein to generate antiserum in rabbits. This antiserum showed a titer of 1 : 40 in the anti-HBe RIA. Its specificity was confirmed by immunodiffusion. Crossreaction between HBeAg and WHeAg was less than 1%. Maximal titers of WHeAg were $10^{-2}$ in acute and $10^{-3}$ in chronic infection. WHeAg was detected in all four acutely infected animals tested. It was present simultaneously with WHsAg and WHY DNA. 9 out of 12 chronic WHY carriers tested were WHeAg positive. The antibodies will be used for epitope mapping with recombinant fragments of core protein.
Detection of IgG Antibodies to the Hepatitis B Core Antigen by a Direct Enzyme-Immunoassay

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Antibodies (anti-HBc) to the core antigen (HBc-Ag) of the hepatitis B virus are currently being tested in the routine laboratory by inhibiting the binding of a horseradish peroxidase labelled anti-HBc to the HBcAg. In this process, a relatively high number of borderline results are observed whose specificity could not hitherto be further checked. As an alternative method, we have developed a classical ELISA to anti-HBc IgG. Microtiter wells were coated with recombinant HBc-antigen (Uy et al., Virol. 155,89). Serum samples were incubated in it, to the appropriate dilution. The binding of anti-HBc was tested, with a peroxidase labelled, affinity purified anti-IgG was tested. – In the case of 80 hepatitis B virus carriers, 40 amongst them acute cases, and 60 convalescents, very good agreement was found between both tests. Of 995 random samples of serum from blood donors, 41 reacted positively in the competitive test; in the direct test, however, only 18. Of the 23 sera which reacted differently in the tests, 18 were only weakly positive in the competitive test. We presume that these are unspecific reactions.

Cytotoxicity by Borna Disease Virus-Specific CD4 T Cells

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The pathogenesis of Borna disease (BD) is based on a T-cell-mediated immunopathological reaction. Previously, we have established a T-cell line specific for the 38/39 kd BD-virusspecific antigen. This virusspecific T cell line has been characterized as a CD4-positive. BD can be induced in immunosuppressed infected rats upon passive transfer of this cell. Further characterization in vitro revealed cytotoxic activity towards a BDV infected astrocyte cell line. This cytotoxicity was greatly enhanced if the level of MHC class II expression was increased by IFN, and if the specific antigen was added to the test system exogenously. Additionally, the highest specificity of lysis on infected vs. uninfected target cells was found 6–10 days after the last restimulation of effector cells. The possible importance of cytotoxic T cells in the pathogenesis of BD is currently under investigation.
Scrapie in Dura Mater Material of Hamsters: The Development of Titre and the Inactivation of Infectivity in Commercial Transplant Material

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Scrapie in sheep, goat and cattle as well as Creutzfeldt-Jakob disease (CJD) in man are virus-induced amyloidoses representing as spongiform encephalopathies. – Recently, a case of CJD in a 28-year old woman has been suspected to be caused by iatrogenic transmission via a transplant of a cadaveric dura mater, a material used predominantly in neurosurgery, but also used in orthopedic, otologic, urologic, gynecologic, cardiac, and dental surgery. The dura mater used for transplantation is manufactured industrially. – The model system scrapie in hamster has been used to show that low infectivity titres may contaminate dura mater material in the preclinical, symptomless phase. Such contamination increases heavily during the clinical phase of the disease. Conventional industrial manufacture procedures do not eliminate the infectivity except an alkaline treatment is included.

Immune-Mediated Rejection of Fetuses After LCMV Infection

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Intravenous infection of mice with lymphocytic choriomeningitis virus (LCMV) during the early stage of pregnancy (day 0 through 4 post conceptionem [p.c.]) leads to intrauterine death of the offspring. Infection with LCMV at later stages of pregnancy (day 8 through 18 p.c.) does not result in rejection of fetuses. In mice infected at the early stage of pregnancy and treated simultaneously with monoclonal antibodies directed against CD4\(^+\) T lymphocytes, death of the offspring can be prevented and birth takes place as normal. In contrast, monoclonal antibodies against CD8 are not capable of preventing rejection of the fetuses. This is especially remarkable since CD8U2+ T cells represent the most important antiviral-protective and immunopathological T-cell population in the peripheral blood and in the spleen after LCMV infection. LCMV infection of pregnant mice represents a unique model for an immune-mediated rejection of fetuses and at the same time indicates the activity of different antiviral T cells in different organs.
Further characterization of the proviral DNA in foamy virus (LK-3)-infected cells

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Proviral DNA of the T-lymphotropic simian foamy virus strain LK-3 was cloned and characterized. It is present in infected cells as a non-integrated, linear duplex of about 13 kbp, containing a site sensitive to nuclease S1 near the center of the molecule. After completing the double-strand with Klenow-polymerase, the gap fragment was cloned and sequenced. It contains a polypurine tract (PPT) similar to the putative initiation site of plus-strand DNA synthesis of retroviruses, indicating discontinuous DNA-synthesis as proposed for other foamy viruses and the lentiviruses visna and HIV. Nuclease S1 treatment of DNA from LK-3-infected cells generates an additional 1.7 kbp duplex identified, by Southern blot hybridization, as the LTR region of the genome. This fragment seems to represent "strong stop" DNA, an intermediate of retroviral DNA synthesis. So far, no integrated viral sequences could be demonstrated under conditions restrictive for viral cytopathogenicity, as presence of neutralizing antibodies or AZT in the culture medium. – Supported by the Deutsche Forschungsgemeinschaft (Ne 213/4–3).

Molecular Cloning of Two West African HIV-2 Isolates that Replicate Well in Macrophages

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HIV-2-related viruses were isolated from a Gambian dying of exclusively neurological disease (HIV-2<sub>D194</sub>) and from an asymptomatic Ghanian (HIV-2<sub>D203</sub>), and stable infections of cell lines were established. Both strains were found to be markedly different from the HIV-2<sub>ROD</sub> prototype. Like HIV-1 biological subtype c, they grew slowly and induced few or no syncytia but eventually produced high levels of particle-associated reverse transcriptase (RT) in cultures of peripheral blood lymphocytes. Each produced even higher levels of RT when fresh human monocytes/macrophages were used as target cells. The viruses were molecularly cloned after a single passage in culture. Restriction-site analysis showed heterogeneity within each isolate. Nucleotide sequence analysis of HIV-2<sub>D194</sub> revealed that genetically it is a member of the prototypic HIV-2 family, displaying 13% divergence versus HIV-2<sub>ROD</sub> and HIV-2<sub>NHZ</sub>, as compared to 9% divergence between HIV-2<sub>ROD</sub> and HIV-2<sub>NHZ</sub>. In contrast, HIV-2<sub>D203</sub> is the most highly divergent HIV-2 strain yet described: it is equidistant in relation between the known HIV-2 strains and the SIV<sub>MAC</sub> isolates (23–25% divergence) and may belong to a family of its own.
Inhibition of HIV Replication by Xylan-Polyhydrogen-Sulfate: HOE/BAY 946, a New Antiviral Compound in Clinical Trial

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HOE/BAY 946, a xylanpoly-hydrogensulfate, inhibited HIV-replication and virus-induced syncytia formation in lymphocyte cultures at concentrations above 25 µg/ml totally. An inactivating effect of the compound on the virions could not be demonstrated. Pretreatment of lymphocytes with HOE/BAY 946 or a transitory administration did not prevent HIV-infection, demonstrating that the drug must be permanently present for exhibiting fully antiviral activity. Lymphocytes incubated with virus in the presence of HOE/BAY 946 followed by the removal of the drug and unbound virus were infected, suggesting that inhibition of virus adhesion to the cells was not sufficient to avoid their infection. Treatment of a permanently HIV-infected cell line resulted in a drastic reduction of virus shedding and points to an additional mode of action. Two clinical pilot studies with HOE/BAY 946 were started recently in Germany.

Recombinant Fusion Proteins of the Inner Membrane Protein p16 of HIV-2 and P17 of HIV-1: Comparison of the Immunoreactivity with HIV-Antibody-Positive Sera

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In a suitable procaryotic vector system we expressed several fusion proteins which represent p 16 (HIV-2 ROD) and p 17 (HTLV III B) partially or almost completely. The recombinant antigens were tested in the Western blot system with sera of HIV-1 and HIV-2 antibody positive patients. In 68% of the HIV-1 positive sera tested we found a clear reaction with a fusion protein representing the p 17 of HIV-1. However, no reactivity was seen in any of the tested HIV-2 antibody positive sera with recombinant antigens from the p 16 region of HIV-2. There was also no cross-reactivity of these HIV-2 fusion proteins with HIV-1 antibody positive sera. Considering these results it could be supposed that the inner membrane protein p 16 of HIV-2 is less antigenic than the corresponding p 17 of HIV-1.
Hoe 602, a New Drug with Antiviral Activity Against Herpesviruses

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Hoe 602 (2-amino-9-(1,3-bis-(isopropoxy)-propyl-2-oxymethyl)-purine) is highly active against Herpes virus infections in vivo, but not in cell culture in vitro. Mice infected systematically with Herpes simplex virus 1 (HSV 1) were treated successfully by oral or parenteral application of the drug. Hoe 602 showed significantly more antiviral activity against HSV 1, HSV 2 and Cytomegalovirus (CMV) than aciclovir and treatment by topical application was also highly effective. Hoe 602 is converted to ganciclovir in a cascade of three intermediate products. The antiviral activity of the compound is comparable to ganciclovir in mice, dependent on the manner of application. In contrast to ganciclovir enteral resorption of Hoe 602 is as effective in monkeys as in mice. Thus, the prodrug seems to be suited for oral treatment of severe Herpes virus infections in humans.

Expression of Baboon Endogenous Virus-Related Sequences in Human Teratocarcinoma Cell Lines

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Human teratocarcinoma (TC) cell lines produce particles which resemble retroviruses by morphological, biological and biochemical criteria (Löwer, J. et al., J. Gen. Virol. 68, (1987) 2807). These particles have been called HTDV (Human TC Derived Viruses). - The poly-A+ RNA population of TC cells have been screened for sequences related to known endogenous retroviral sequences. The probes used are derived from the pol genes of Baboon Endogenous Virus (BaEV) and ERV-1 and 4-1, two human retroviral sequences. A 5.9 kb band could be detected which hybridizes to all BaEV and ERV-1 probes of regions well conserved between retroviruses. - The LINE-1 family of the mammalian genome displays some distinct homologies to all known reverse transcriptases. The expression of these sequences seems to be very restricted as only in the human TC cell line NTera2D1 a cytoplasmic polyadenylated LINE1-mRNA (≈ 6.5 kb) could be detected (Skowronska, J. and M. Singer PNAS 82 (1985) 6050). - A LINE-1 probe detects the 6.5 kb band in the cytoplasmic RNA population of all TC cell lines investigated. In addition, a band comigrating with the above mentioned 5.9 kb species is stained at low stringency. The intensity of this band in different TC cell lines correlates with the intensity of the band which hybridizes to the BaEV probes as well to the amount of particles observed by electron microscopy.