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Inhibition of an Early Step of Poliovirus Replication by Disoxaril (WIN 51711)

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Disoxaril, 5-[7-[4(4,5-dihydro-2-oxazolyl)phenoxy] heptyl]-3-methylisoxazole, inhibits the replication of several picornaviruses (Otto et al., Antimicrob. Ag. Chemother. 27 (1985) 883–886). Measurements of viral RNA synthesis and electronmicroscopy showed that disoxaril inhibits the replication of poliovirus types 1 and 2 in HeLa cells prior to uncoating by stabilizing the virus capsid: The arrival of viral RNA for new RNA synthesis was inhibited completely only when the inocula were preincubated with disoxaril for 15 min at 37°C at 0.3 µg disoxaril/ml for poliovirus type 1 and 0.03 µg disoxaril/ml for poliovirus type 2. Simultaneous addition of the compound and virus resulted in reduced inhibition of viral RNA synthesis. The inhibitory effect of the compound could be partially reversed up to 25 min p.i. if the compound was eluted from the cells. Disoxaril pretreated poliovirus was not inhibited from entering HeLa cells (entry by receptor-mediated endocytosis via coated pits and vesicles into endosomes) (cf. Zeichhardt et al., J. Gen. Virol. 66 (1985) 483–492).

Neutralizing Monoclonal Antibodies Obtained After In Vivo Priming with VP1 of Poliovirus Type 1 Recognize Epitopes Located Between Amino Acid Residues 93–104 of VP1

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Hybridomas obtained after in vivo immunization of mice with virus particles usually produce neutralizing antibodies which do not react with isolated polypeptides. However, after spleen cells from mice immunized in vivo with VP1 of poliovirus type 1, Mahoney,
Neutralizing Antibodies Against Poliovirus Type 1, Mahoney in Rabbits and Mice Using a Synthetic Peptide of Sequence 93–104 of VP 1

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A peptide corresponding to sequence 93–104, immunodominant in poliovirus type 1 (Mahoney) has been synthesized with two additional cysteines at both ends of the peptide. A rigid conformation of the peptide by formation of a closed circle should induce more homogeneous antibodies. The peptide was linked to carrier proteins keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA) by glutardialdehyde and such emulsified in: I Freud's adjuvans, II freshly precipitated aluminiumhydroxide (Aloxide) and III monophosphoryl lipid-A containing trehalose dimicholat (RAS). Mice and rabbits were injected five times subcutaneously every ten days. All animals produced antibodies, binding to peptide in an enzyme-linked immunosorbent assay (ELISA). Most of them also bound to virus with moderate titers compared to antiserum to virus. Three out of five rabbit antisera neutralized virus to serum dilutions of up to 1 to 42 and two mice sera up to 1 to 16 with respect to 100 TCID_{50}.
Gangliosides are Potential Receptors for Influenza C Virus

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9-O-Acetyl-N-acetylneuraminic acid (Neu5, 9Ac2) has been shown to be a high-affinity receptor determinant for attachment of influenza C virus to erythrocytes. We present evidence that Neu5, 9Ac2 is the primary receptor determinant required for influenza C virus in order to attach to tissue culture cells and to initiate an infection. Bovine brain gangliosides which contain this type of sialic acid were found to be potential receptors for influenza C virus. Several cell lines, which are resistant to infection by this virus, become susceptible after incubation with bovine brain gangliosides prior to infection. This result indicates that lack of appropriate receptors on the cell surface is a major reason for the restricted cell tropism of influenza C virus.

Characterization of the Surface Glycoprotein of Influenza C Virus by Monoclonal Antibodies

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We have isolated fifteen hybridomas secreting monoclonal antibodies directed against the surface glycoprotein gp of influenza C virus (Johannesburg/l/66). The antibodies were analyzed for their ability to inhibit the hemagglutination; the acetyltransferase and the hemolytic activity of influenza C virus. The results obtained indicate that the surface glycoprotein of influenza C virus in addition to fusion activity also has hemagglutination and acetyltransferase activity.

Expression of the Influenza Virus Hemagglutinin in Insect Cells by a Baculovirus Vector

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A cDNA sequence of the FVP hemagglutinin gene has been inserted into the BamHI site of the pAc373 polyhedrin vector. Recombinant virus was obtained after cotransfection of this construct, pAc-HA651 and authentic AcNPV DNA. The hemagglutinin gene is located in the polyhedrin gene of the recombinant virus genome. Immunofluorescent labelling, immunoprecipitation and immunoblot analyses revealed that cultures of Spodoptera frugiperda (SF) cells produced immune reactive hemagglutinin after infection with the re-
combinant virus. - Digestion with endoglycosidases H and F showed that the hemagglutinin is glycosylated and that the oligosaccharides are processed. The hemagglutinin is expressed at the cell surface and has hemolytic capacity that is activated by post-translational proteolytic cleavage. When chickens were immunized with SF-cells expressing hemagglutinin, they developed hemagglutination inhibiting and neutralizing antibodies and were protected from infection with FPV. The hemagglutinin gene has also been expressed in larvae of tobacco budworm, and the gene product was found to be biologically active. - These observations demonstrate that the hemagglutinin is synthesized and processed in cultured insect cells or in whole animals in a similar fashion as in FPV-infected vertebrate cells and that it has full biological activity.

**Influence of the Phorbol Ester TPA on the Tryptic Phosphopeptide Pattern of the Nucleoprotein of an Influenza A Virus**

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The nucleoprotein of influenza A viruses is a phosphoprotein, the in vivo phosphorylation of which is influenced by the phorbol ester TPA. Phosphopeptide patterns, obtained by tryptic mapping, show specific differences in the presence and absence of TPA. - TPA has been shown to stimulate the protein kinase C and the enzyme also phosphorylates isolated nucleoprotein in vitro. - These results, combined with the calcium influx and an accumulation of diacylglycerides 3 hours after infection, suggest that that protein kinase C plays a role in the replication cycle of influenza A viruses.

**Significance of Bacterial Proteases in Influenza Virus Infection**

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Several strains of *Staphylococcus aureus* (Staph.) have been found to secrete serine proteases that activate some influenza A viruses (IV) by proteolytic cleavage of the hemagglutinin. When mice were co-infected intranasally with an appropriate strain of IV and Staph. the animals came down with a fatal pneumonia, while infection with IV or Staph. alone did not induce relevant pathological changes. Only low titers of virus could be found in the lungs of mice infected with the virus alone. However, addition of trypsin to lung homogenates increased infectivity of such tissue samples. Co-infection with Staph. produced high virus titers in the lung that could not be increased by in vitro treatment with trypsin. Thus, co-infecting bacteria can play an essential role in the development of influenza pneumonia by providing a protease suitable for cleavage activation of the hemagglutinin.
Serological Influenza-Diagnosis: Comparison Between CFT and Subtype and Immunoglobulin Class Specific IFT

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Low titers (20–40) obtained by CFT, the usual method for influenza serodiagnosis do not allow to differentiate between acute and remote infection. As a rule this differentiation is possible with subtype and immunoglobulin class specific IFT. We show this by results from 127 patients with confirmed influenza-infection. The individual immune response after influenza-infection varied considerably: 19.7% of the patients had produced IgG-, IgA-, and IgM-antibodies, 62.2% IgG- and IgA-antibodies and 7.9% only IgG-antibodies. IFT-positive/CFT-negative reacted 3.1%, CFT-positive/IFT-negative reacted 2.3%. In 4.7% of the patients, neither with IFT nor with CFT antibodies were detected despite of virus isolation. Totally in 27.5% of the patients it was not possible to detect an acute influenza-infection by CFT, in contrast to the detection of immunoglobulin class specific antibodies by IFT. – This work was supported by the Federal Ministry of Research and Technology (01 ZR 051).

Cell-Type Dependent Expression and Secretion of Hepatitis B Virus Large Surface Protein

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A transcriptional active gene fragment of hepatitis B virus was isolated from hepatoma cell line PLC/PRF/5 and transfected to HeLa-cells and mouse L-cells. All three cell lines expressed the small and the middle surface protein, but the L-cell did not express the large protein. Growth of the transfected HeLa cells as nude mouse tumor caused exclusive expression of the large protein. Under these conditions no viral surface antigen was secreted. In vitro the HeLa cell produced and secreted all three surface proteins as filamentous particles. The data suggest i) that secretion of large protein requires an excess of small protein, ii) that a high proportion of large protein favours formation of filaments in addition to 20 nm particles, and iii) that expression of large protein is regulated in a cell-type specific way.

Synthesis of Antibodies to Hepatitis B Surface Antigen by Cultured Lymphocytes from Hepatitis B Vaccine Recipients

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To study immunity to hepatitis B surface antigen (HBsAg) at the cellular level, peripheral blood lymphocytes were isolated 2–20 days after the 1st, 2nd and 3rd vaccination with
hepatitis B vaccine (H-B-Vax®, MSD). The lymphocytes were cultured for 7 days in the absence and the presence of pokeweed mitogen (PWM). Culture supernatants were analysed for specific antibodies against HBsAg (anti-HBs), total IgG and total IgM by enzyme immunoassays. After the 1st vaccination, no anti-HBs was detectable, whereas anti-HBs could be measured 6 days after the 2nd and, in a higher extent, 6–15 days after the 3rd vaccination. PWM inhibited the synthesis of anti-HBs. The influence of PWM upon the synthesis of total IgG and total IgM varied considerably.

Treatment of HBsAg Positive Chronic Active Hepatitis with Recombinant Alpha A Interferon: Results of a Phase II Clinical Trial

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Thirtyone individuals with HBsAg positive chronic active hepatitis received $10 \times 10^6$ U of recombinant leucocyte alpha A interferon / m² body surface area 2 or 3 times a week for 3 months. This resulted in loss of HBeAg in 14/31 which was permanent in 12/31. HBsAg was eliminated in 3/31 and permanently in 2/31. Effect of treatment was dependent on interferon dose, level of viral replication before treatment and superimposed LAV/HTLV III infection. Reversible side effects were frequent well tolerated and included flu like symptoms, slight hairloss, depression, leucopenia and thrombocytopenia. The results promising require, however, still optimization of the treatment schedule and a better understanding of interferon action.

Immunogenicity of Hepatitis B Core Antigen: Monoclonal (Mouse) and Polyclonal (Human) Anti HBC-Antibodies Identify the Same Epitope

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Using hybridoma technology, a panel of monoclonal antibodies (MAB) to Hepatitis B core Antigen (HBcAg) was established. As demonstrated by cross-inhibition studies each MAB recognized the same epitope on the HBcAg. - Binding of the MAB's to HBcAg is inhibited by preincubation with polyclonal human anti-antibodies. - Vice versa each single MAB was able to completely prevent the binding of the polyclonal human anti HBc-antibody. - The results indicate: a) polyclonal human anti HBc-antibodies are monospecific; b) HBcAg exhibits only one immunodominant epitope. - In consequence it was possible to develop a competitive one step enzyme-immunoassay for the detection of anti HBc-positive sera using a single MAB. - In comparison with already established test-systems our assay was found to be superior with regard to sensitivity and practicability.
Vaccination Against Hepatitis B: Persistence of Anti-HBs and Revaccination

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Anti-HBs concentrations were followed in 1070 persons vaccinated against hepatitis B between 1980 and 1982. In 27% of vaccinees initially seroconverted antibody levels declined to or below 10 IU/l within 4 years. As shown earlier in a smaller group the persistence of anti-HBs was dependent of the maximal antibody response measured 4 weeks after the third immunisation. The rate of decrease of anti-HBs seems to be similar in all persons, without being influenced by age, sex or dose of vaccine. Revaccination was done in 70 persons with anti-HBs levels below 10 IU/l. Within 4–6 days after the booster dose anti-HBs increased rapidly leading in 85% of vaccinees to anti-HBs concentrations considerably higher than after the first booster.

The Kinetics of Hepatitis A Virus RNA Replication in Human Diploid Fibroblasts (MRC-5 Cells)

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Under one step growth conditions, first signs of viral metabolic activity are detected 2–4 days after the onset of infection. Therefore, early events, from adsorption to the time viral synthesis is initiated, together or in part, are slow processes. Furthermore, HAV replication is highly protracted, with viral metabolic activity basically occurring during the first two weeks of infection. Thereafter, the cells remain persistently infected. – The synthesis of total viral RNA (i.e. negative strand template RNA, genomic RNA, and viral RNA), as well as synthesis and encapsidation of genomic RNA occur in parallel to the appearance and accumulation of progeny infectious virus. – The appearance of a subgenomic vRNA about 2000 nucleotides long in close association with ribosomes is yet another feature that distinguishes HAV from typical Picornaviruses.

Translation of the Hepatitis A Virus Genome in vitro

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Hepatitis A Virus (HAV) shares many characteristics with the picornaviruses. Its genome consists of a linear, single-stranded RNA of messenger-sense polarity. However, unlike other picornaviruses, HAV fails to shut off host cell metabolism and, hence, analysis of viral protein synthesis meets with difficulties. To circumvent this situation, HAV genomic RNA was translated in a rabbit reticulocyte system, optimized in respect to K⁺, Mg²⁺, tRNA, and viral RNA concentrations. The kinetics of translation of HAV RNA and poliovirus RNA then proved to be similar over a period of 60 min. Translation products of HAV RNA
consisted in at least 12 polypeptides with mwts between 15 and 200 kd. Seven of them with mwts between 15 and 46 kd became evident within the first 10 min of translation. The five larger ones accumulated after 30 to 60 min only. – Processing of the polypeptides larger than 46 kd has so far not been observed.

Role of γ-Interferon in Pathogenesis of Hepatitis A Virus Infection

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Peripheral blood lymphocytes (PBL) were collected from patients, who had suffered acute Hepatitis A Virus (HAV) infection at different times after onset of icterus. This cell population expressed both cytotoxic activity against autologous HAV-infected skin fibroblasts and the capacity to release γ-IFN upon stimulation with HAV-infected fibroblasts. PBL, incubated with uninfected fibroblasts and PBL of anti-HAV negative persons, incubated with autologous HAV-infected fibroblasts produced no γ-IFN and specific lysis also could not be detected. Although the highest activity of HAV-specific lysis, demonstrable 2–3 weeks after the onset of icterus, is not strongly correlated with the height of IFN-production, the coincidence of both parameters indicates, that cytotoxic T-cells and its product γ-IFN might attribute to the elimination of the HAV-infection in man.

Duck Hepatitis B Virus: Subcloning of the Viral Genome and Preparation of Strand-Specific Probes

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The hepatotropic DNA viruses HBV, DHBV, GSHV and WHV are characterized by a small circular DNA genome of about 3 kbp length and a mechanism of replication, the central feature of which is the reverse transcription of an RNA intermediate into minus strand DNA. For the specific detection of replicative intermediates and of viral transcripts, strand-specific DHBV probes were prepared. – DHBV F 1–6 DNA of 3.0 kbp length was ligated with pSP 65 DNA in the Eco RI position. The recombinant DNA molecule was cloned after transformation of E. coli HB 101. From DHBV DNA positive transformants two were selected (21-2 and 24-4) which contain the DHBV DNA insert in different orientation, as determined by restriction enzyme analysis. RNA probes synthesized from these two clones were specific for the detection of viral mRNA (24-4) and for viral minus strand replication DNA species (21-2), respectively. The probes are useful for the study of viral replication and transcription of viral genes and should add to our understanding of the biology of DHBV in hepatocytes as well as in nonhepatocytes.
The Duck Hepatitis B Virus P-Frame: Use of Specific Antisera to Identify P-frame Products

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The P-frame of hepatitis B virus presumably codes for the proteins involved in viral replication. However, neither the nature of these putative products nor their mode of biosynthesis have been elucidated. To approach this problem, 7 segments, in sum 80%, of the duck hepatitis virus (DHBV) P-frame have been expressed as fusion proteins in E. coli and antisera prepared against them and against 4 peptides. These anti-fusion protein sera contain a significant titre towards the respective P-segment and could also immunoprecipitate P-specific polypeptides synthesized in vitro (SP-6). Intact viral cores containing DHBV-DNA and endogenous polymerase activity were immunoprecipitated from infected duck liver using anti-capsid (C) serum. Neither Western blotting of large amounts (20–40 µgC) of this material, nor immunoprecipitation after radioiodination have revealed in vivo synthesized P-products. The reason for this negative finding is still unclear.

Duck Hepatitis B Virus: Cloning of the Viral Genome and Study of the Natural Course of Infection

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The Pekin duck model of hepatitis B is a convenient system for the study of viral replication and antiviral strategies. For the detailed characterization of viral nucleic acid species, the DHBV genome was cloned from serum. DHBV was isolated by ultracentrifugation of serum; the viral genome was extracted, linearized with Eco RI and ligated with pBR 322 DNA. E. coli HB 101 was transformed with the recombinant DNA. From DHBV DNA-positive colonies plasmid-DHBV DNA was prepared. The 3.0 kbp DHBV DNA insert was purified; its physical structure was established by restriction enzyme analyses. The restriction pattern of DHBV DNA F 1–6 is identical to DHBV DNA 16-t (Mandart et al., 1984) but differs significantly from DHBV DNA 3 (Sprengel et al., 1985). Using cloned viral DNA as a probe, viral DNA species from infected serum and liver were analyzed by molecular hybridization techniques. Congenitally infected ducks have high levels of viral DNA in serum with a gradual decline over months. The mechanism of this reduction of viral particles in serum and its correlation with viral replication in the liver are presently being investigated.
Transcription of Woodchuck Hepatitis Virus DNA in the Hepatocellular Carcinoma of Woodchuck

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Transcription of Woodchuck Hepatitis virus (WHV), a member of the Hepadnavirus family, was studied in liver carcinomas of chronically infected woodchucks (Marmota monax). Replication of WHV-DNA was studied in tumors and non-tumor livers by Southern blots. In ten tumors with and without viral replication the transcription of WHV was studied by Northern blots. RNA of these tumors was hybridised with subgenomic fragments of the WHV-DNA cloned in the gemini 2 vector. These clones contain a single reading frame or part of it. In seven tumors the two major RNA's were transcribed (3.7 and 2.1 kb), as it has been shown in chronically infected livers by Moróy et al., 1983. Sometimes larger transcripts were observed. No transcription despite of presence of WHV-DNA could be detected in one tumor. In two tumors with integrated WHV-DNA only one major transcript (3.5 eg. 2.5 kb) could be seen. Hybridisation with the subgenomic clones revealed that in both tumor transcripts in contrast to chronically infected liver the C-gene and a part of the preS1-gene are deleted.

Proteins of Hepatitis Delta Virus in Serum and Liver

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Hepatitis delta virus (HDV) proteins were identified by immunoblot using HD patients sera as source of antibody. Two proteins, P27 and P29, copurified with HDV particles from the serum of an acutely infected chimpanzee. The same proteins were also found in sera from viremic HD patients and from HD infected woodchucks. Detection of HD proteins by immunoblot as marker of HD viremia is superior to detection of HD antigen by ELISA because it is possible even in the presence of excessive HD antibody. P29 was also found in HD infected liver but P27 was missing. However, variable patterns of other HD reactive proteins (P26, P22, P16, P15, P13) were observed. These proteins were predominantly localized in the nucleus. P22 from a woodchuck liver was found to be bound to RNA.

Detection of Hepatitis Delta Virus RNA by Nucleic Acid Hybridisation

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The Hepatitis Delta Virus (HDV) is a defective virus which needs HBV as a helper. HDV is composed of a circular RNA of 1.75 kb and the Delta Antigen. The coat of the HDV is the
HB,Ag. – HDV is a powerful pathogen. In both simultaneous infection with HBV and superinfection of chronic HB,Ag carriers it can cause serious liver-damage. Superinfection of chronic HB,Ag carriers with HDV often results in chronic active hepatitis and cirrhosis. – HDV infection is diagnosed by detection of HD Ag, Anti-HD-IgM and IgG and, since recently, by nucleic acid hybridisation of its RNA with specific probes. – In this study, hybridisation-conditions for HDV-RNA were optimized. Strand-specific probes like M13 primer-extension-probe or SP6-Riboprobe showed a much higher sensitivity than nick-translated probes. However, when riboprobe is used, the problem of background hybridisation arises. Characterization of the HDV-RNA by Northern-blotting showed the typical band at 1.75 kb, which contained both genomic and antigenic RNA, and some minor species of RNA, the predominant one of these banding at 0.8 kb. This species only contained genomic RNA. – HDV-RNA was found in the serum of 68% of patients with chronic active hepatitis or cirrhosis and Anti-HD-IgM. All of these patients had HD Ag in the liver. 50% of the HDV-RNA-negative patients had also HD-Ag in the liver and 67% of these Anti-HD-IgM in the serum. Only 5 of 11 HDV RNA-positive patients had HBV-DNA in the serum.

Characterization of Proteins Associated with the Hepatitis Delta Virus (HDV)

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The number and size of proteins associated with HDV from serum and liver of a chimpanzee at the acute stage of HDV infection were analyzed by immunoblotting. HDV positive serum was sedimented through a sucrose gradient (10-30%) and HDV-associated antigens (HDAg) and HBsAg determined in each fraction by ELISA or RIA. Peak fraction of HDAg were pooled and subjected to SDS PAGE and subsequently blotted on nitrocellulose. HDV-associated proteins were detected by incubation with human anti-HD positive serum and 125I Protein A. Two proteins of 30 and 28 kd were visualized by immunoblotting. Immunoblots of HDAg extracts from liver tissue (chimpanzee) with 7 M guanidinium HCl resulted in two proteins of 28 and 15 kd. Extracts of a human liver from a patient with chronic HDV infections were 13 and 12 kd in size. However, direct homogenation of chimpanzee and human liver in boiling mix subjection to PAGE and subsequent Western blotting resulted in two major protein bands of 30 and 28 kd. – These data indicate that HDV proteins are unstable and rapidly degrade to smaller fragments.

Structural Analysis of p19 and p24 Core Polypeptides of Primate Lymphotropic Retroviruses (PLRV)

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Several monkey species have antibodies crossreacting with human T-cell leukemia virus type I (HTLV-I). The main core polypeptides of these primate lymphotropic retroviruses
(PLRV) have very similar molecular weights. To discriminate individual PLRVs we have compared two dimensional tryptic peptide maps of \(^{125}\)I-labelled core polypeptides p19 and p24 of ten isolates originating from humans and six simian species. Peptide maps showed homologies between all HTLV-I related isolates. According to the relationship of their peptide maps we have classified the PLRVs into three groups, the human and chimpanzee isolates, macaque viruses, and green monkey virus. In contrast p24 and p18 of HIV (Human immunodeficiency virus) and related simian isolates (SIV) were completely different to those of HTLV-I related isolates, therefore they represent a separate group of PLRV. With respect to p24 and p18, the isolate of an African green monkey (SIVagm) is more closely related to HIV than to the viruses of sooty mangabey (SIVsm) and rhesus monkey (SIVmac). However, those of SIVsm and SIVmac are more closely related to each other than to SIVagm and HIV.

OA MuLV Induces Osteoblastic Differentiation of Bone Precursor Cells

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The target cell for OA MuLV and the effects of virus infection on bone cells were studied using primary skeletoblastic cell cultures of neonatally OA MuLV infected NMRI mice, in vitro infected primary bone cell cultures, and a permanent osteoblastic cell line (MC 3T3-E1). Seven days after infection of NMRI mice cells were obtained by fractionated dissociation of calvaria. Cultures from early fractions containing lesser differentiated osteoblastic cells showed a higher rate of infection than those from late fractions (differentiated osteoblastic cells). The osteoblastic differentiation followed by alkaline phosphatase activity (ALP) was higher in cultures from infected mice than in those from controls. In vitro infection of primary cultures from periosteum resulted in a decrease of cell growth associated with an increase of osteoblastic differentiation. The differentiation of calvaria cells was not influenced by virus infection. Infection of MC 3T3-E1 cells was followed by reduction of ALP activity associated with an increased cell growth. However, the cells were still responsive to chemical induction of differentiation. These data indicate that in bone tissue, undifferentiated progenitor cells are the target for OA MuLV and virus infection increases osteoblastic activity. This fact possibly explains the critical role of OA MuLV in bone tumorigenesis. In contrast, infection of the permanent MC 3T3-E1 osteoblastic cells was followed by a reversible block of differentiation.

Osteosarcomagenesis in vitro: The Critical Role of Bipotential Praeskeletoblasts in Mouse Mandibular Condyles

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Mandibular condyles of neonatal mice represent a so-called secondary type of cartilage, containing well-defined cell layers of different states of differentiation. In vitro, bipotential
precursor cells, present in such tissues shift their differentiation program. They express alkaline phosphatase, collagen type I, bone sialoprotein and osteonectin, and finally become bone cells. Twenty-four hours after in vitro infection of mandibular condyles with FBR MSV only cells of the progenitor cell layer were found to be infected. Further cultivation resulted in increasing loss of cellular organization, polymorphism, mineralization of remnant cartilage matrix and invasive growth of fibroblastic perichondreal cells into the condylar tissue and underlaying collagen sponge. Seven days after infection the tissue acquired the appearance of an osteosarcomatous lesion. After implantation of infected mandibular condyles into syngeneic mice, a transplantable fibro-chondrosarcoma tumor line was established. Tumor induction could not be observed after FBR MSV infection of similar tissue such as Meckels cartilage, or Xiphoid. The data suggest that the presence of chondroprogenitor cells in condylar tissue, inhabiting the capacity of bidirectional differentiation, is a prerequisite for FBR MSV-induced osteosarcomagenesis in vitro. This system facilitates a detailed morphological and molecular analysis of early steps in viral osteosarcomagenesis.

Structural Analysis of the Polymerase Gene of Friend Murine Leukemia Virus

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Friend murine leukemia virus (F-MuLV) causes erythroleukemia in mice. In order to study the biology of this virus and the functions of its genes we have sequenced the genome of an highly infectious F-MuLV. The polymerase gene of this virus codes for a protease, the reverse transcriptase and an endonuclease. The sequence obtained showed a high degree of homology to Moloney murine leukemia virus and to AKV in the sequences coding for the protease and the reverse transcriptase. In contrast, the sequence coding for the carboxyterminus of the endonuclease was much less conserved. This might represent the specificity of the viral integration in the host genome. The knowledge of the sequence now allows to study the functions of defined regions of the genome in leukemogenesis.

Construction of an Infectious Friend Murine Leukemia Virus Containing a Selectable Marker

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Induction of disease by the murine leukemia viruses is assumed to involve insertional activation of cellular genes. Localization of newly integrated virus is hampered by the presence of the large number of endogenous viruses in the cellular genome. As a tool to study the localization of possible common integration sites of Friend murine leukemia virus and to determine a possible activation of cellular genes we have constructed a virus containing a selectable marker. A bacterial suppressor tRNA gene (sup-F) was inserted into the LTR without destroying its biological function. Rat-1 cells were transfected with this DNA. We
obtained several cell clones producing infectious virus. The virus collected from these cells has been injected into susceptible mice which, after tumor development, will be used for the preparation of genomic libraries. These will then be used for screening of sup-F harbouring proviruses which should facilitate determination of possible common integration sites.

**Human Sera React to the Major Internal Gag-Protein of Mammalian Retroviruses**

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During the course of our studies of antibodies in human sera, against HIV, we occasionally observed a reactivity against the viral p24 in persons without any risk of having contracted a corresponding virus infection. Reactivity with viral p24 was only detectable in sensitive Western Blots and usually not in ELISAs. – In extension of these initial observations, HTLV I and retroviruses of other mammals (Baboon endogenous virus, BEV; Friend murine leukemia virus, FLV) and chicken (Rous sarcoma virus, RSV) were employed as antigens. Both sucrose density-gradient banded viruses as well as purified major gag-antigens (p27, p30) were used. – It could easily be demonstrated that the gag protein of mammalian retroviruses is recognized by antibodies not only from people suffering from HIV infection, but also from patients with multiple sclerosis and other neurological diseases or with teratocarcinomas and even from healthy blood donors. In contrast, p27 of RSV was not recognized. – We are presently determining what proportion of the human sera react with animal retrovirus gag-antigens and whether a disease-specific pattern can be observed. The origin of these gag-reactive antibodies remains obscure at present. We either observe a biologically trivial true cross-reaction, which may, however, play a practical role in sensitive serodiagnostic procedures, or the reactivity of “interspecies-specific antibodies” formed in response to a yet unknown endogenous or exogenous human retrovirus strain.

**Differential Expression of the c-src Gene During in vitro Differentiation**

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We have investigated the expression of the proto-oncogene c-src, the cellular homology of the Rous sarcoma virus transforming gene v-src. Our previous results on c-src expression during ontogenesis of vertebrates suggest that the physiological function of c-src appears to be more closely related to differentiation processes than to proliferation processes. This statement is supported by data obtained from experiments with the promyelocytic human cell line HL-60. Induction of monocytic and granulocytic differentiation of HL-60 cells by 12-O-tetradecanoyl-phorbol-13-acetate (TPA), retinoic acid (RA) and dimethyl-sulfoxide (DMSO) is associated with an activation of the pp60<sup>c-src</sup> kinase, but not with increased c-src gene expression. Control experiments exclude an interaction of TPA and DMSO themselves with the tyrosine-specific pp60<sup>c-src</sup> kinase. Using embryonal carcinoma cell lines, which also
can be induced to differentiate in vitro, we are currently analyzing whether the differentia-
tion-dependent expression of the c-src gene product is restricted to monomyelocytic cells or
can be generally observed during cellular differentiation processes.

Sequence Studies of a Human Reverse Transcriptase Related DNA Fragment

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S71 is a human endogenous retroviral element related to the simian sarcoma associated
virus (SSAV). - DNA sequencing of a 1kb stretch of S71 showed this region to contain a
40–60% homology to the putative reverse transcriptase coding region of murine leukemia
C-type retroviruses. Sequence comparisons also revealed a weaker homology to the human
enogenous provirus 4-1 (Repaske et al., 1985). – The 3' half of the reverse transcriptase
coding sequence of the mouse C-type retrovirus AKV contains a 40 amino acid region in
which seven proline residues are clustered. All seven are found at corresponding positions in
S71 whereas only two proline residues are conserved in 4-1. – This data indicates that the
reverse transcriptase region of S71 is relatively closely related to that of murine leukemia C-
type retroviruses.

Molecular Cloning of a Chicken cDNA Closely Related to c-src

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To study the expression of c-src and of c-src related genes, a chicken cDNA library was
established and screened with the 0.8 kb PvuII fragment of v-src. 10 positive clones were
obtained. Restriction analysis and hybridization experiments revealed that all clones are
derived from the same genetical locus, which is however different from the locus of the c-src
gene. The largest src-related cDNA clone (3.4 kb) was sequenced and shows up to 72%
homology to v-src. Further comparison to published DNA sequences revealed homology to
all known tyrosine kinases, especially to Isk, a tyrosine kinase isolated from the LSTRA cell
line. – In a Northern Blot study the 3.4 kb clone hybridized to a 3.8 kb mRNA from chicken
embryo fibroblasts. A signal of the same size was obtained by Bishop with a PvuII fragment
of v-src and assumed to be the c-src mRNA. – On the basis of the results presented here, the
3.4 kb cDNA clone represents a novel tyrosine kinase gene. Furthermore the expression of c-
src requires further investigation.
Attenuation in the Control of c-myc Gene Expression?

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DMSO (dimethylsulfoxide), a potent inducer of differentiation in HL60 cells, causes a rapid decrease of cytoplasmic steady state c-myc RNA. This decrease is regulated mainly at the level of transcript elongation. Elongation is blocked within the untranslated c-myc leader. We propose that the c-myc leader is able to undergo two alternative conformations, an one-stem-and-loop structure allowing readthrough of the RNA polymerase into the coding part of the c-myc gene, and a two-stem-and-loop structure causing transcription arrest. We compare the attenuation model for the VP1 gene of the simian virus 40 (SV40) (Hay and Aloni, 1982) with our proposed attenuation model for c-myc gene expression. – Supported by Deutsche Forschungsgemeinschaft (SFB 31).

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Hay and Aloni: Cell 29 (1982) 183–198
Eick and Bornkamm: Nucl. Acids Res. 14 (1986)

Epstein-Barr Virus DNA Contains Insertional Element Downstream of EBNA-2 Exon

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Restriction endonuclease and electron microscopic heteroduplex analyses of cloned DNA sequences of the Jijoye and M-ABA strains of Epstein-Barr virus (EBV) had revealed several regions of partial or non-homology in the EBNA-2 open reading frame located in U2 (1), a feature which allowed the differentiation of 2 types of EBV (2). This report describes an additional region of divergence caused by a 105 base pair (bp) insertion, 100 bp downstream of the Jijoye EBNA-2 exon when compared with the B95-8 EBV DNA sequence. The insertion is flanked by 19 bp direct repeats and constitutes a truncated duplication (18% mismatch) of an adjacent sequence on the 3' side. The structural characteristics, both primary and secondary, of the insert and the surrounding genomic region indicate that it is derived from the Alu-family of human interspersed repeated sequences.

References
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2. Zimber, U., H. K. Adldinger, G. M. Lenoir et al.: Geographical prevalence of two types of Epstein-Barr virus. Virology (in press)
Generation of a Variant t (2; 8) Translocation of Burkitt Lymphoma by Site-Specific Recombination via the \( \kappa \)-Light Chain Joining Signals

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We have analyzed the Burkitt lymphoma line BL64 in which a reciprocal translocation joins the immunoglobulin kappa light chain locus on chromosome 2 to the \( c-myc \) gene on chromosome 8. The breakpoints on the two marker chromosomes 8q+ and 2p− occurred 5' of the J5-segment within the conserved nonamer and heptamer recombination sequences. Both signals were detected directly adjacent to the breakpoints in sequences of chromosome 8 suggesting that the translocation in BL64 was catalyzed by enzymes normally involved in V-J recombinant. The distance between the \( c-myc \) gene and the breakpoint in Y amounts to at least 70 kb on the DNA level. In one allele of the \( c-myc \) gene somatic mutations were found in the promoter-leader region. This allele is transcribed and supposed to be involved in the translocation. Using transient expression assays, no functional differences of the \( c-myc \) promoters isolated from both alleles could be detected in various cell types. These results suggest that in Burkitt lymphoma the translocation occurs during an early stage of B-cell differentiation and that \( c-myc \) is activated by its location in the Ig chromatin structure.

Regulation of a TPA Induced EBV Gene in Raji Cells

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Epstein-Barr-Virus (EBV) infected cells can be induced by the treatment with TPA or other chemical or biological inducers to express early antigens (EA) and to produce virus. The induction of Raji cells, a nonproducer cell line derived from an African Burkitt's lymphoma leads only to the expression of EA. The activation of a strong induced region, which is in part duplicated in the EBV genome “DR”, was studied in detail. It could be shown by 'nuclear run-on' assay, that the treatment of Raji cells with TPA and IUdR activates transcription of the DR-Region. The 2.8 kb RNA encoded by this region is detectable about 5 hours after induction as shown by Northern blot analysis. This induction is protein synthesis dependent indicating, that other gene functions (either EBV or cellular) are required for activation. – DR-Promoter-CAT-Constructs were transiently transfected into EBV positive and negative lymphoid cells and tested for CAT activity after treatment with various EBV inducing agents. The construct could only be activated in EBV positive cells. This indicates, that at least part of the functions required for the activation of the DR-Region are encoded by EBV.
Truncation does not Abrogate Transcriptional Downregulation of the c-myc Gene by Sodium Butyrate in Burkitt's Lymphoma Cells

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In many cases of Burkitt's lymphoma with t (8; 14) translocation and most murine plasmacytomas the c-myc gene is truncated suggesting that separation of the body of the gene from its physiological promoters and upstream regulatory sequences is one of the important mechanisms leading to c-myc deregulation. The fact that c-myc mRNA disappears early after induction of differentiation in various cell systems, suggests a regulatory role of the c-myc gene product for the option of the cell either to proliferate or to undergo differentiation. Burkitt's lymphoma cells show morphological and phenotypical changes characteristic for B-cell differentiation in response to sodium butyrate, a potent inducer of differentiation in various cell systems. We report here, that the treatment of a variety of different Burkitt's lymphoma cell lines with sodium butyrate, irrespective of the type of translocation or of the association with Epstein-Barr Virus, leads to a rapid decrease of c-myc steady state mRNA. This is due to a reduced transcription rate of the c-myc gene, as shown by "nuclear run-on" analysis. The fact that sodium butyrate is capable of downregulating an intact as well as a truncated c-myc gene indicates that an important target site of transcriptional regulation is located downstream of the dual promoters and the first exon.

Expression of an EBNA 2 Variant and Generation of Specific Antisera

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The Jijoye Epstein-Barr virus (EBV) strain is characterized by a substitution of 1.8 kb in the C-terminal part of EBNA 2 gene compared to B95-8 or M-ABA virus. Protein immunoblot analysis using human EBNA 2 positive sera indicated that an immunological variant to the EBNA 2 of B95-8 (type A) is encoded by the Jijoye virus (type B). In order to generate a specific EBNA 2B antiserum the NaeI/NsiI DNA fragment of the Jijoye virus containing 237 bp of the C-terminus from the EBNA 2B gene was cloned in an E. coli expression vector (pME3). The resulting fusion protein contained 79 C-terminal amino acids of the viral protein and a 37000 Dalton part of the bacterial anthranilate synthase. Rabbit antisera generated against this fusion protein reacted specifically with two proteins of 73000 and 77000 Dalton from Jijoye cells and three other cell lines carrying type B virus while no proteins could be identified in the type B cell line BL 29. In addition, using these sera directed against the pME3 fusion protein no reaction could be observed with the EBNA 2A protein from the B95-8 and several other cell lines containing type A virus. Therefore, these rabbit sera seem to be a useful and specific tool for further investigations.
Level of Epstein Barr Virus LMP Expression in Latently Infected Cell Lines Depends on Serum, TPA and n-Butyrate

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The BNLF1c reading frame of the EBV genome is known to code for a part of a membrane protein of 60 000 Daltons expressed in latently infected cell lines (LMP) and an unidentified protein of presumably 28 000 Daltons. Antisera against both proteins were generated in rabbits. These sera reacted with a protein varying in size between 60 000 and 65 000 Daltons found in all EBV harbouring cell lines examined so far. In addition, a second protein of 49 000 Daltons on SDS-PAGE in B95-8, P3HR-1, and M-ABA cells was identified. Furthermore, these sera exhibited a positive cytoplasmic immunofluorescence in 1 to 10% of the cells depending on the cell line examined. Analysing the non-producer cell line Raji, the number of immunofluorescence positive cells and the amount of the 60 000 Dalton protein was rapidly increased by addition of fresh medium with 10% fetal calf serum, by the tumour promoter TPA or to an even higher extent by n-butyrate. No synergistic effect of TPA and butyrate could be observed. The kinetics of induction reached a maximum at 24 h after addition of medium with 10% fresh serum. TPA or/and n-butyrate and decreased after 24 to 48 h. Because of lack of the synergism mentioned above and because of a difference in expression kinetics of LMP and EA-komplex following induction of EBV lytic cycle LMP seems not to be a member of the EA-complex despite of its inducability.

Identification and Characterization of EB Viral Membrane Proteins by Synthetic Oligopeptides

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Using a computer program that predicts the secondary structure of proteins and superimposes values for hydrophilicity, surface probability and flexibility, we identified potential membrane proteins derived from open reading frames encoded in the EB viral genome. Antigenic sites from four of those membrane proteins encoded by the open reading frames BLLF1, BILF1, BILF2 and BALF4 were selected and oligopeptides were synthesized by solid phase peptide synthesis. The peptides were used as antigens in ELISA assays to screen sera from NPC and infectious mononucleosis patients and from healthy individuals. Sera from NPC and infectious mononucleosis patients showed elevated reactions to all peptides; the highest reactions were found to BALF4-derived peptide in NPC patients and to the BLLF1-derived peptide in fresh infections. Only very low antibody titers could be identified against the peptide from reading frame BILF1. For immunization of rabbits, the NH2-terminal amino acids of the peptides were covalently linked with palmitic acid and high-titered antisera against the individual oligopeptides could be obtained. Those rabbit sera were used for the identification of the corresponding EB viral proteins on Western blots, by immunoprecipitation and immunofluorescence. – Supported by SFB 217 TP B3.
Diagnosis of EBV-Correlated Infections with Recombinant Gene-Products of EBV

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For diagnosis of EBV-correlated diseases we chose five relevant antigens of EBV: p150 VCA BcLF1, p138 EA BALF2, p54 EA BMRF1, gp250/350 MA BLLF1, EBNA-1 BKRF1. The coding sequences of these antigens, analysed by computer programs to predict antigenic sites, were cloned in E. coli or – as in the case of gp250/350 – in CHO (Chinese hamster ovary) cells. The products were tested for their antigenicity and stability by immunoblotting. The identified recombinant antigens were purified by gel filtration and ion exchange chromatography (HPLC). The purified EBV proteins were evaluated in Western blots following antibody class specific staining and used in ELISA tests in order to screen sera from EBV-negative individuals, patients with acute EBV infection, convalescents and from patients with nasopharyngeal carcinoma. From the results combined with diagnostic significance suggestions of antigen/antibody class specific test combinations were derived for the various conditions. – Supported in part by BMFT 01ZR 85069 and 01ZR 102/5.

Characterization of the BVRF2 Reading Frame of EBV

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Previously we had mapped by hybrid-selected translation a 49kD protein to the BamHI fragments d and l (Seibl and Wolf, 1985). The open reading frame BVRF2 spans the BamHI fragments, V, d and I in rightward orientation. A late active promoter was mapped in the BamHI d fragment (Baer et al., 1984). Single-stranded DNA of a M13 clone containing several hundred base pairs of BVRF2 selected the mRNA for the 49kD protein proving the transcription of the p49 mRNA in rightward orientation. By Northern blotting we further analyzed the transcription of this gene. To prove that p49 is encoded by BVRF2 and to further characterize the protein we expressed the 3' end of BVRF2 as a fusion protein with β-galactosidase in E. coli. The latter was partially purified and used to immunize a rabbit. The fusion protein was recognized by a pool of sera from patients with nasopharyngeal carcinoma in Western blots. Using the rabbit serum we were able to characterize the 49kD protein as late protein in Western blots and immunoprecipitations. – Supported by DFG Wo227/4.

Secretion of the Two Major Epstein-Barr Viral Membrane Proteins from Recombinant Chinese Hamster Ovary Cells into Culture Medium

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The two major envelope proteins of EBV (gp250/350) are encoded by the same reading frame (BLLF1). The smaller variant gp250 is generated by a partial splice event which
other tumors and in normal individuals no IgG/MA antibodies were detectable. Our data removes an internal part of the transcript encoding gp350 to yield the mRNA encoding gp250. These two proteins are candidates for a EBV-subunit vaccine. - The yield of gp250/350 expression in recombinant CHO cells is not very high and amplification of the MA BaLLFl encoding sequences in these cells is not possible, perhaps due to toxic effects. We now present a eukaryotic expression plasmid construct where the sequences encoding the transmembrane and anchor region are removed. Following transfection of CHO cells, this plasmid results in secretion of the EBV membrane proteins into culture medium. Furthermore, amplification by methotrexate selection was successful, yielding high levels of gp250/350 expression.

Studies on the Expression of the EB Viral Membrane Protein (BNLF1-MA) in Latently Infected Cells

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The protein encoded by the open reading frame BNLF1 (BNLF1-MA) has been connected with the target for cytotoxic T-cell reaction directed against EBV-infected lymphocytes; due to Northern and dot blot experiments, this gene is transcribed in latently infected cells in addition to the nuclear antigens (EBNAs) and is the only one of these polypeptides with characteristics of membrane proteins. Using sera against synthetic oligopeptides derived from the amino acid sequence of BNLF1-MA we could show that this protein is synthesized in Burkitt lymphoma cell lines in a truncated form lacking 138 amino acids at the amino terminal end (Modrow and Wolf, PNAS 83, 1986). Using those antipeptide sera in immunofluorescence tests on latently infected cell lines, a positive reaction in about 20–30% of the cells was obtained. A similar amount of BNLF1-MA producing cells could be identified by in situ hybridization using 3H-cytidine labeled DNA probes. Since latent viral products (EBNA 1–3) should be detectable in more than 90% of the cells, we suggest that BNLF1-MA may be not a typical product of the latent EBV infection but a protein expressed in 20–30% of the cells respectively. In vivo those cells are eliminated by cytotoxic T-cells; in rare cases and in combination with additional factors only the truncated form of BNLF1-MA may be produced and those cells may develop to lymphomas.

Detection of IgA Antibody to EBV Membrane Antigen in Patients with Nasopharyngeal Carcinoma

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The presence of IgA antibody to membrane antigen (MA) of Epstein Barr-virus was tested in sera from nasopharyngeal carcinoma patients, patients from other tumors than NPC and normal individuals. Only 54% of the sera from NPC-patients showed a positive reaction, sera from control groups were negative. After adsorption of the sera with Staphylococcus aureus Protein A, 100% of the NPC-patients had IgA/MA antibody titers, in patients with
indicate, that preadsorption of sera with \textit{Staph. aureus} Protein A renders the diagnostic test significantly more sensitive for the detection of NPC and can be used for trials on the prognosis of patients. Furthermore our data show the importance of antibody tests to EB-viral membrane antigens, especially when those are available in larger amounts by recombinant DNA-techniques (see also \textit{Motz} et al., no 125). – Supported by Stiftung Volkswagenwerk.

**Expression of the Epstein-Barr Virus BamHI M Region**

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In previous experiments we correlated the proteins encoded in the BamHI region to single open reading frames. By Northern blotting we further substantiated the model of the transcription of this DNA area derived from hybrid-selected translations, sequence data (\textit{Baer} et al., 1984) and mapped active promoters. When the protein synthesis was blocked simultaneously with the superinfection, the BamHI M fragment was still transcribed in different cell lines. A 2.6 kb mRNA transcribed from the left part of the fragment was detectable. From this mRNA, the BMRF1 reading frame is translated into a 47 kD protein which is posttranslationally modified into a 54 kD phosphoprotein. A second 1.9 kb mRNAs is transcribed from the right part of the BamHI M fragment where the BMLF1 reading frame is located. To identify the protein, we expressed large parts of the BMLF1 reading frame in \textit{E. coli} as a fusion protein with \beta-galactosidase. The fusion protein was recognized by a pool of sera from NPC patients on Western blots and used to immunize rabbits. The sera can be used to characterize the BMLF1 protein. – Supported by DFG Wo227/4.

**Expression of an Early EBV-Antigen (EA-D) in \textit{E. coli}**

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The coding region of two phosphopolypeptides pp50 and pp58 (Röckel and Müller-Lantzsch, Virology 147 (1985) 253–263) being the major component of EA-complex in non producer cells NC37 following TPA-induction was determined on the EBV-genome. Both proteins are recognized by the monoclonal antibody R3 directed against EA-D (Pearson et al., J. Virol. 47 (1983) 193–201). The 1.34 kb BclI–BglII-fragment being derived from the BamHI-M-region of EBV-M-ABA-genome was inserted in frame into the BamHI-site of tryptophan-regulated \textit{E. coli} expression vector pATH1 (T. J. Koerner, unpublished). This fragment is including the complete BMRF1 open reading frame (\textit{Baer} et al., Nature 310 (1984) 207–211. The resulting fusion protein having a molecular weight of 80 k is recognized not only by EA+ sera but also by the monoclonal antibody R3. – The finding that the monoclonal antibody R3 is reactive with the phosphoproteins pp50 and pp58 and also identifies the product of the BMRF1 open reading frame presents evidence for the BMRF1 open reading frame being the coding region of pp50 and pp58. In diagnosis of nasopharyngeal carcinoma (NPC) the fusion protein is qualified for detecting anti EA-IgA antibodies.
Characterization of an Epstein-Barr Virus Protein Expressed Immediately After the Superinfection of Latently Infected Cells

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The 2.0 kb HindIII K fragment, located in the B95-8 deletion, but not the neighboring fragments hybridized in Northern blots with a 1.8 kb mRNA, isolated from induced P3HR1 and Raji cells and from Raji and X50-7 cells superinfected with P3HR1 virus. When the protein synthesis is blocked simultaneously with the superinfection by cycloheximid or anisomycin, only a few mRNAs characteristic for the switch from latency to the lytic cycle can be expressed. Under these conditions the 1.8 kb mRNA is transcribed. The Hind III K fragment consists mainly of the single open reading frame BJ'LF4. Therefore BJ'LF4 seems to encode a protein which is expressed immediately after the superinfection of latently infected cells. We expressed the 3' half of the reading frame as fusion protein with β-galactosidase in E. coli. The protein was partly purified and used to immunize rabbits. Using the rabbit serum we were able to immunoprecipitate a 68 kD protein from Raji and X50-7 cells superinfected with P3HR1 virus early after infection. – Supported by DFG Wo227/4.

Characterization of Natural Epitopes of the Epstein-Barr Virus Nuclear Antigen 1 (EBNA 1)

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Several groups have already mapped the coding region of EBNA 1 in the Bam HI K-fragment of the EBV-DNA. In order to characterize some natural epitopes of the EBNA 1 protein the Hind III-K-fragment of the EBV strain M-ABA was cut into fragments of about 200 bp by using DNase. These fragments were then cloned into the E. coli expression vector according to all three reading frames in both directions at the 3' end of the lac Z protein. After transformation the bacterial colonies expressing a specific epitope were identified. The method used was colony immunoblotting. – The results were verified by the protein immunoblotting method. Two different epitopes of the EBNA 1 protein could be detected. The sequence analysis localized the epitopes to different regions of repetitive sequences in the region coding for the EBNA 1 protein. After immunization of rabbits with the EBNA 1 epitopes an antiserum was obtained which reacts specifically with the EBNA 1 protein of different EBV strains in the immunoblots.
Expression of MHC Antigens in Burkitt's Lymphoma Cells and Corresponding Lymphoblastoid Cell Lines

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Cell surface density and molecular structure of HLA class I and II antigens of Burkitt's lymphoma (BL) cells and Epstein Barr Virus (EBV) transformed lymphoblastoid cell lines (LCL) established from the same patient were examined. Using a radioimmuno assay with a monoclonal antibody against a common determinant of class I molecules we found a 1.2–25 fold higher density of HLA class I molecules on LCLs than on BLs in all cell pairs studied. In 6 of 8 cell pairs, class II antigens were also expressed in higher amounts on LCLs. In addition, immunoprecipitation of radiolabeled components and subsequent analysis on SDS-polyacrylamide gel electrophoresis revealed qualitative differences between the class I molecules of BL and LCL cells in all 6 cell pairs studied. The observed changes in HLA class I expression may well play a role for the immunological growth control of EBV transformed cells, allowing BL cells to escape from immune surveillance mechanisms.

Effect of Monensin on Cytomegalovirus DNA Replication

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Monensin, at concentrations which depend on the multiplicity of infection, was found to prevent DNA replication of human cytomegalovirus (HCMV) as well as production of viral progeny. The effect of the drug on HCMV DNA-synthesis was fully reversible. Delayed addition was effective only until 24 h postinfection. Induction of viral DNA polymerase was not impeded by the inhibitor. Monensin did not affect, on the other hand, DNA replication of herpes simplex virus. Analysis of protein- and glycoprotein synthesis revealed that monensin interfered with the production of a number of HCMV-specific polypeptides. By the use of a monospecific antiserum, evidence was obtained that the drug interfered with the processing of a 135 kd glycopolyepptide.

DNA-Binding Properties of the Major Immediate-Early Protein of the Murine Cytomegalovirus (MCMV)

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Herpesviruses differ in the number of their ie gene products, but at least one IE protein has transactivating properties. The mode of IE protein-DNA interaction, resulting in promoter activation, is unclear. The following possibilities can be envisaged. 1. IE proteins
bind DNA. 2. IE protein complexes bind DNA. 3. IE proteins bind DNA after interaction with cellular factors. – The ie1 gene products of MCMV have been demonstrated to activate promoters in trans. We examined DNA-binding properties of the major IE protein (pp89) and of its processing product (pp76) using CT-DNA-cellulose-chromatography. pp89 revealed a high affinity for DNA, whereas pp76 showed very little DNA-binding properties. DNA-binding of pp89 however required the presence of a cellular DNA-binding factor(s). Thus, if direct or indirect DNA-binding is a prerequisite for regulation, only pp89 but not pp76 can activate promoters. Regulative activity is either direct after pp89-factor interaction or indirect after factor modification by pp 89.

Characterization of Early Gene Products of the Murine Cytomegalovirus (MCMV) with Monoclonal Antibodies

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To examine how the ie1 gene product, pp89, is involved in the induction of early gene expression, early genes must be identified and isolated. For this purpose monoclonal antibodies (mAb) against early proteins of MCMV were prepared. – One mAb stained antigen located in the cytoplasm and immunoprecipitated a 52 K and 67K early protein. Staining with a second mAb resulted in faint homogenous nuclear fluorescence during the early phase, while at late times the antigen accumulated in large aggregates in the nucleus. This mAb precipitated a 40K and a 47K early protein. The third mAb produced speckled nuclear staining in the early phase, whereas at late times the distribution of antigen resembled that of the second antigen. The immunoblot with the third mAb showed a 36K, 37K, and 39K early protein. Immunoprecipitation with the mAb after in vitro translation of early RNA revealed the 36K and 39K proteins.

Complex Formation of IE-Proteins of the Murine Cytomegalovirus (MCMV)

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Gene expression of MCMV is subdivided into three coordinately regulated phases: immediate-early (IE), early and late. The ie1 proteins transactivate promoters, but it is not clear whether this function is associated with the 89K gene product (pp89) or the processed 76K protein (pp76). Because in regulatory viral proteins complex formation and function is often associated, the sedimentation patterns of pp89 and pp76 were investigated. When early gene expression was allowed for less than three hours, both proteins sedimented as monomers. In the “delayed” early (more than 6 h p.i.) or late phase, only pp76 was detectable as complex. Cellular proteins appeared not to be involved in complex formation, a possible participation of early proteins is presently under study. Events of the “delayed” early phase are responsible for complex formation of pp76. A role in transactivation is unlikely, since only pp89 was found to bind to DNA. The potential recognition of pp76 by cytolytic T lymphocytes requires further analysis.
Human Monoclonal Antibodies Against Cytomegalovirus

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Human monoclonal antibodies were made by fusion of the Spaz 4 cell (a mouse x human myeloma) with either peripheral blood lymphocytes from patients with an acute CMV infection or spleen cells stimulated in vitro with CMV antigen prior to fusion. — With both methods we could obtain several hybridomas secreting anti-CMV antibodies. Four of these hybridomas were established as stable producers, secreting between 20–60 µg/ml of antibodies since more than 2 years now. Three of these antibodies are neutralizing antibodies of the IgG1 subtype; the 4th antibody is an IgG3, recognizing an internal structural protein of the virus. — Clinical trials for prophylaxis of CMV infection in bone marrow transplantation patients have been initiated recently with 2 of the neutralizing antibodies.

Immediate-Early Genes of the Murine Cytomegalovirus: Nucleotide Sequence and Structure of Immediate-Early Gene 2

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The number of immediate-early (IE) genes differs among different herpesviruses. At least one IE gene product is necessary to initiate the early phase of the viral gene expression. The product of the IE gene 1 (ie1) of the murine cytomegalovirus (MCMV) activates promoters in trans. The transcription of ie1 is controlled by a strong enhancer which also influences the expression of another IE gene, ie2. The ie2 codes for a 43K protein which is not immunoprecipitated by murine antisera against MCMV IE proteins. It could only be detected after in vitro translation of hybrid-selected RNA. The function of the ie2 gene product is unknown. Therefore, as a first step, the ie2 gene was analyzed in more detail. — The 43K protein is translated from a 1.75 kb mRNA, a spliced molecule which contains 3 exons of 75 n, 109 n, and 1277 n. The 1277 n exon at the 3' end of the 1.75 kb mRNA has an open reading frame of 1173 n, which could encode a polypeptide of 391 amino acids with a calculated molecular weight of 43.8K.

Immediate-Early Genes of the Murine Cytomegalovirus: Nucleotide Sequence and Structural Organization of the Immediate-Early 1 Gene Coding for a Protein Active in Transcriptional Regulation

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The region of the murine cytomegalovirus (MCMV) genome abundantly transcribed at immediate-early (IE) times is located between map units 0.769 and 0.817. It contains two
independent transcription units (ie1 and -3, ie2), which are transcribed in opposite directions. The ie1 codes for pp89, the major IE protein, which can activate other promoters in trans. The pp89 is translated from a 2.75 kb polyadenylated mRNA, transcribed from map units 0.796 to 0.780. Nuclease protection experiments with end-labelled DNA and labelled cRNA revealed that the mRNA is a spliced molecule and contains three minor exons of 300 n, 107 n, 191 n and a major exon of 1748 n at the 3' end. A 1785 n open reading frame starts at the 5' end of the second exon and encodes a polypeptide of 595 amino acids. The DNA and protein sequence of this MCMV gene shows no region of significant homology to the major IE gene of human CMV, whereas the structural organization and functional properties are similar.

The Structural Phosphoprotein pp150 of the Human Cytomegalovirus and its Genomic Organization

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Human cytomegalovirus (HCMV) particles contain a phospho-protein of about 150 K (pp150) in their matrix; the protein appears particularly reactive in Western blot analyses with human antisera. The gene for pp150 was mapped by screening a bacteriophage Lambda gt11 cDNA expression library with monospecific rabbit antisera. Subsequent hybridization of cDNA with cosmid and plasmid clones containing the entire HCMV strain AD169 genome mapped the gene to HindIII fragments J and N. The genomic segment is transcribed into an abundant late 6.2 kb RNA. The nucleotide sequence of this region was determined, and transcription initiation and polyadenylation sites of the transcript were located by primer extension and nuclease protection experiments. Polypeptide secondary structure analysis revealed multiple β-pleated sheets in hydrophilic clusters, providing a possible explanation for the immunogenic properties of the polypeptide.

Structure and Kinetics of a 5Kb-RNA Encoded within the IE-Region of Human Cytomegalovirus

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An RNA-class of about 5kb is encoded within the immediate-early (IE) region of human cytomegalovirus strain Ad169. This transcript is distinct from all other RNAs of HCMV thus far described. Extensive Northern-blot analyses demonstrated that this RNA is present in RNA preps from all phases of the infectious cycle. Through S1-analyses, RNase-protect-
tion and primer extension using synthetic oligonucleotides, 3'-end and 5'-end were determined. In addition the 3'-coding sequence was established thus providing the whole nucleotide sequence of the coding region. A typical polyadenylation signal was found at the 3'-end. Upstream to the putative initiation site, however, no typical TATA-box was detected. Since no longer open reading frame was found in the sequence, this RNA might have some regulatory rather than a protein-coding function.

Antibody to Envelope-Proteins of Human Cytomegalovirus (HCMV)

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We have compared the immune response to HCMV using both an ELISA and a neutralization test (NT). When antibody titers in human sera and in hyperimmune-gamma globulins were tested in parallel a relatively low correlation coefficient (r_s=0.52) was found.—In addition, we analyzed the immune response to HCMV glycoproteins in human sera using monoclonal antibodies which had been characterized by immunofluorescence, by immunoblot and by NT. These monoclonals were used to obtain enzyme-labelled antigens (ELAs). With these glycoprotein ELAs we again looked for specific antibody activity in human sera. This time a close correlation with the NT-titers was found. Therefore, an enzyme immunoassay can now be used for the selection of blood donations for HCMV hyperimmune-gamma globulins.

Exclusion of False-Positive Results in Dot-Hybridization for the Detection of HCMV-DNA in Clinical Specimens

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Human cytomegalovirus (HCMV) can be detected in clinical specimens by isolation of virus in cell culture or by hybridizing extracted DNA to 32P-labelled viral DNA fragments.—In a reconstruction experiment with urine containing HCMV AD 169, we could show that hybridization unlike cell culture can detect noninfectious virus particles.—Some of the tested urine samples showed false-positive hybridization results, as confirmed by negative serology. From hybridization of DNAs extracted from clinical specimens and different microbial DNAs to CMV-BgII-d and in parallel to a mixture of pBR322 and E. coli DNA, we conclude that false positive results are due to trace contamination of the CMV-DNA probe with residual vector and E. coli DNA sequences.—False-positive results on the basis of cross-hybridization between CMV-BgII-d and DNAs of human cells are unlikely, as proven by controls using DNA from human and animal cell lines.
Analysis of Interferon-Sensitive Subclones Selected from an Interferon-Resistant NIH 3T3 Line

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A NIH 3T3 subclone, clone 1, was found to be deficient with respect to the antiproliferative response as well as to the antiviral effect against lytic RNA-viruses, e.g. encephalomyocarditis virus (EMCV) and vesicular stomatitis virus (VSV). Induction of 2–5 A synthetase and dsRNA dependent protein kinase by IFN as well as inhibition of retrovirus production e.g. murine leukemia virus (MMuLV), indicate a defect other than the lack of a functional IFN receptor. A very low level of 2–5A dependent RNase was discussed to be the reason for the defects described. Development of a selection system, based on IFN treatment and subsequent virus challenge by EMCV allowed the isolation of protected colonies derived from the unprotected cell population. Characterisation of these subclones led to a dose response comparable to the IFN sensitive L 929 cells with respect to EMCV. In contrast, VSV protection as well as inhibition of cell growth was much less extensive resembling the original NIH 3T3 line. This implicates at least partial independent mechanisms leading to each of the effects. No difference in the level of 2–5A dependent nuclease could be demonstrated, implying another defect besides a nonfunctional 2–5A system, responsible for the partial IFN resistance of the original NIH 3T3 line.

Detection of Interferon (IFN) mRNA in Individual Mouse Macrophages by in situ Hybridization

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Infection of cultured mouse bone-marrow macrophages with Newcastle Disease Virus (60 HAU/10⁶ cells) resulted in high titers of IFN – 1,5 to 3 × 10⁴ IU/ml – that reached peak levels 8–10 h following induction. This multiplicity resulted in infection of 100% of the cells as determined by immunofluorescence. Analysis by in situ hybridization revealed the presence of IFN-α and β mRNA 2 h after infection in 50–60% or 25–30% of the cells, respectively. 6 h after infection specific hybridization signals were seen in 60–80% of the cells and a strong increase in the number of grains per cell was observed. A great heterogeneity in grain densities per cell was observed following hybridization with both IFN cDNA probes; however the cells were labeled homogeneously, when hybridization was done with a probe coding for the MHC antigen H-2K. In addition, specific hybridization signals were found in 5–15% of non-induced control cells in the absence of detectable antiviral activity in the corresponding culture supernatants, suggesting that very low amounts of IFN α and β are synthesized constitutively by cultured mouse bone-marrow macrophages.
Role of Interferon During Persistent Infection of Macrophages with Herpes simplex Virus

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Splenic macrophage cultures from HSV resistant C57BL/6 mice survived HSV infection in vitro. In contrast, macrophages from HSV susceptible DBA/2 mice were completely lysed by the virus. Resistance was mediated by the production of interferon early after infection. During prolonged culturing macrophages from C57BL/6 mice continued to produce infectious virions, indicating establishment of a persistent infection. At this time, interferon was undetectable. However, as shown by the addition of an anti-interferon serum, interferon was involved in the maintenance of the persistent infection. During the acute phase of virus infection, induction of viral proteins and DNA replication were identical in macrophages from resistant or susceptible mice. Later on, viral DNA content and the number of cells expressing HSV antigens decreased in macrophages from C57BL/6 mice. However, single cells remained to express viral proteins and to produce infectious particles. The results show that macrophages can be persistently infected with HSV due to their genetically controlled properties.

Interferon-Alpha 2, Interferon-Gamma, and Interferon-Alpha 2 Plus-Gamma in a Combination Therapy of Dendritic Keratitis. A Controlled Clinical Study

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Fourty-five patients with virologically confirmed dendritic keratitis were treated in a randomized, double-blind controlled study with a basic therapy of trifluorothymidine (TFT) eye drops. In addition they received different human recombinant interferon (rHu IFN) eye drops. The following results were obtained for average healing times: TFT plus one drop daily of rHu IFN-alpha 2 arg (30 million iu/ml): 3.3 days, TFT plus rHu IFN-gamma (30 million iu/ml): 3.9 days, TFT plus a mixture of alpha plus gamma (0.3 million iu/ml each): 6.1 days. TFT plus a mixture of alpha plus gamma (1.5 million iu/ml each): 3.3 days. High-titer gamma interferon did not significantly differ from high-titer alpha interferon in the combination therapy of dendritic keratitis. A mixture of alpha plus gamma at a moderate titer (1.5 million iu/ml each) was as effective as a high-titer monopreparation. Adding a low-titer interferon mixture gave no better therapeutic results than antiviral monotherapy. Thus it seems possible to save about 90% of interferon commonly used in the combination therapy of dendritic keratitis by applying a mixture of different suitable interferons instead of interferon monospecies.
**Vaccina-Specific Early RNA is Degraded in Interferon Treated Chick Embryo Fibroblasts**

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The molecular mechanism of interferon action on poxvirus specific immediate early protein synthesis was studied in interferon treated chick cells. Under conditions of over 90% inhibition of poxvirus specific thymidine kinase induction RNA for, this early enzyme is synthesized but does not accumulate. Northern blot analysis reveals strong degradation of residual thymidine kinase RNA. Blot hybridization analysis using total vaccinia DNA and restriction fragment N as probes demonstrate a generally reduced steady state amount of vaccinia specific early RNA's in interferon treated chick embryo fibroblasts. Expression of the chloramphenicol acetyltransferase gene inserted into an infectious vaccinia recombinant is inhibited in the interferon treated cells.

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**Is the Formation of Herpes simplex Virus (HSV) Proteins Interferon Sensitive?**

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HSV replication is sensitive to IFN in cell culture and in vivo. We have claimed (1972–1985) that in normal primate cells, IFN or poly(I):poly(C) may inhibit HSV at stages before the formation of infectious virion particles. Thus, we have described that the formation of immediate early (= alpha) and later occurring viral protein classes, of HSV DNA and (in human fibroblasts) of accumulation of viral normal-length transcripts are impeded by IFN (Lipp and Brandner, in: The Biology of the Interferon System 1984, eds.: H. Kirchner and H. Schellekens, Elsevier (1985)). Similar observations have been made by Kirchner and co-workers on mouse macrophages (1986, Meetings communication). In contrast, Muñoz et al. (J. gen Virol. 65 (1984) 1069) have described that IFN-alpha prevented, in human tumor cells, the formation of infectious viral progeny but not of the viral proteins. Similar observations in untransformed human cells are from Chatterjee et al. (J. Virol 56 (1983) 419). Considering these divergent results we have begun to reinvestigate the IFN effect on HSV protein synthesis. Preliminary results are in accordance with an effect of IFN on HSV translation. – (Supp. by Grant Br281/12.1, DFG).
In vitro Antiviral Activity of Recombinant Human Tumor Necrosis Factor (TNF)

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We observed antiviral activity of hu rTNF on certain cell lines (HEL, WI-38, HEp-2) in a typical in vitro antiviral assay. Pretreatment with TNF led to protection from infection with VSV, EMCV or HSV. We observed inhibition of the cytopathic effect, virus-yield reduction and suppression of formation of viral proteins. (2'-5') (A)n-synthetase, an enzyme induced by IFN, was also induced by TNF in confluent HEp-2-cells. No IFN-mRNA could be detected in Northern blot analysis of RNA from TNF-treated cells. Anti-IFNα-antibodies, however, present during pretreatment with TNF led to a partial reduction of the antiviral TNF-effect, while anti-IFNα or anti-IFNγ did not. The amount of this induced IFNβ-like activity was not sufficient to account for the observed virus-yield reductions suggesting an antiviral activity of TNF itself.

Inhibition of Herpes simplex Virus in Macrophages by Interferon

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Mouse macrophages grown from spleen cells of mice were found to be very sensitive to interferon α/β [IFN] activity against Herpes simplex virus type 1 [HSV-1]. Therefore, we have used these cells to investigate the level at which IFN blocks the replication of HSV-1. – HSV α, β and γ protein syntheses was strongly reduced in IFN α/β acts at a very early step of the viral replication cycle. Isolation of infected cell nuclei prior to the begin of viral replication showed equal levels of HSV-DNA in pretreated and control cells. These results rule out IFN action on virus uptake. Run-on transcription experiments revealed a delay of transcription from the ICP4, ICP0 and the thymidinkinase-gene. In contrast, we found inhibition of total ICP4-RNA steady state levels throughout the replication cycle. These results suggest effects of IFN α/β on both transcription and stability of viral RNA.

Herpes simplex Virus-Specific Protein Synthesis, Glycoproteins and Viral DNA in Chronically and Latently Infected Burkitt Lymphoma Cells

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In chronically HSV type 1 infected BL cells (BJAB, Raji) viral proteins are synthesized continuously in variable amounts during the growth cycle. Reisolated viruses of these chron-
ichly infected cell lines show a changed glycoprotein pattern and syncytia formation in Vero cells after several months of persistence. – After addition of human anti HSV sera to the culture media, the infection becomes latent. During establishing latency viral protein synthesis stops rapidly; the contents of viral DNA decrease in 14 days to less than one copy per cell. After removal of the antibodies the cells show again the characteristics of a chronic infection.

Relevance of the Glycosylation for the Infectivity of Herpes simplex Virus

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Five exoglycosidases and three endoglycosidases were used to determine the glycosylation pattern and its relevance for the infectivity of HSV-1. Digestion with exoglycosidases yielded a reduction of infectivity up to 4.5 log units. By control experiments it could be shown that the proteolytic activity detected in some of the enzyme preparations had no effect on fully glycosylated virions. SDS-PAGE analysis yielded decreases of the rel. mol. wts. of glycoproteins to be expected from glycosidase-treatment. Digestion with endo H did not result in a decrease of infectivity but a decrease of the rel., mol. wt. of gB. This suggests that i) gC and gD are “complex-type”-glycosylated, ii) gB is to 30% glycosylated in a “high-mannose-type”-manner and iii) the removal of these carbohydrates does not decrease the infectivity of HSV-1. Digestion with endo F decreased the infectivity by 90% and resulted in a decrease of the rel. mol. wts. of the viral glycoproteins. Experiments with PEG could not restore the ceased infectivity suggesting that endo F caused a block of infectivity at the level of adsorption. The data presented provide evidence that O-linked carbohydrates might play a role in HSV-1 infectivity.

Analysis of the Humoral Immune Response Against Herpes simplex Virus (HSV-1) Structural and Nonstructural Proteins

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In the present study the protein specificity of antibodies (abs) against HSV-1 structural and nonstructural proteins has been analyzed by WBA and RIPA-PAGE. Sera containing anti-HSV IgM were tested class-specifically in WBA and RIPA-PAGE for the reactivity of IgG and IgM abs. It could be demonstrated that IgG and IgM abs are directed against viral immediate-early (IE), early (E) and late (L) proteins. – Following acute primary HSV-1 infection the earliest IgM ab response was found to be directed against viral glycoproteins, subsequently viral nonglycosylated structural and nonstructural proteins were recognized by IgM abs. Already early in infection IgG abs against viral glycoproteins and other viral structural proteins with an apparent mw of 56, 45 and 39 kD could be detected. Viral IE and E proteins were poorly recognized by IgG abs in acute primary HSV-1 infections. In acute recurrent HSV-1 infections IgM abs exhibited a less complex pattern of reactivity with viral proteins, predominantly viral nonglycosylated structural proteins were recognized by
IgM abs. IgG abs from patients with acute recurrent HSV-1 infections reacted strongly with a variety of viral structural and nonstructural proteins. In latently infected, a long lasting and prominent ab response against gB and gD could be shown, whereas abs against other viral structural and nonstructural proteins seemed to be produced temporarily.

**Electron Microscopy of Chronic and Latent Herpes simplex Virus Type 1 Infections in Burkitt Lymphoma Cells**

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Persistent HSV(AK) and HSV(F) infections of Raji and BJAB cells, under a four-day splitting cycle, are controlled by HSV specific direct immunofluorescence, followed by embedding of samples for EM thin sections. The fate of variable amounts of HSV producing cells is slow cell lysis while non-productive cells maintain a capacity for cell reproduction. Morphological signs of productive infection are swelling and decrease of mitochondria, dissipated intranuclear nucleocapsids and extranuclear enveloped virus particles. Human anti HSV serum (10%) suppresses extra- and intracellular virions and nucleocapsids within 7 days as it is examplified with BJAB-HSV(F), until a state of latency is firmly established.

**Herpes simplex Virus Type 1 (HSV) Genome Variation in Reisolates from Chronically Infected Burkitt Lymphoma Cells**

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Virus was isolated from HSV-1 (F) strain (Roizman) or (AK) strain (Ulm) chronically infected Raji and BJAB cells at 9, 18, 23 and 31 months post infection (p.i.). Progressive variations – deletions and insertions – from 20 to 2300 base pairs (bp) were detectable by restriction enzyme analysis, especially near the termini of UL component and in the middle of US component of the viral genome. Special interest was set on fragments with deletions: Bam HI-B and Bam HI-J of isolate Raji-HSV(F) 23 months p.i. All reisolates (9–31 months p.i.) have the ability to induce syncytia on Vero cells, while parental (F) and (AK)strains are syn⁺ (free of syncytial formation).

**Detection of Herpes simplex Virus (HSV) DNA Sequences in Human Brains**

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From seven adults and two neonates with mortal diseases unrelated to HSV, DNA samples of brain tissue (1 g) were separated in viral (high density) and cellular (low density)
fractions by two cycles of CsCl density gradients. Bam HI restrictograms were hybridized with HSV type 1 fragments: U_{1} (Bam HI-C) and tk gene (-Q), origin of U_{1} (-N) and ICP4 gene (-Y). Neonatal brains were free of HSV DNA. All seven temporal grey and white matter of adult brains and only two brainstems contained HSV DNA sequences, found in both (viral and cellular) fractions. Only one adult brain sample is consistent with a linear organization of its HSV genome; in six adult brains HSV DNA exists in an incomplete form.

**Novel Small RNAs in HSV Infected Cells**

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In eucaryotic cells, small nuclear- and small cytoplasmic RNAs (sn- or scRNAs) are associated with distinct proteins, forming SNRNPs or SNCRNPs. In the present study we analyzed the protein composition as well as the small RNA pattern in noninfected and Herpes simplex virus type 1 (HSV-1) infected vero cells. We found that concomitantly with the shut off of host cell messenger RNA synthesis, synthesis of U-SNRNAs is stopped. Due to their stability, however, U-SNRNAs are still present in HSV infected cells 36 h p.i. Besides these RNAs two novel small RNAs were detected in infected cells, which we termed HVR1 and HVR2. Based on the relative mobilities in urea gels, the apparent chain length of these newly synthesized RNAs were determined to be 255 and 154 nucleotides, respectively. The small RNA binding proteins SM, RNP, RO and LA were found to increase up to 15-fold after HSV-infection. The presented data suggest that new, virus-coded small RNAs are synthesized, which might play a role in maturation and regulation of HSV-coded RNA transcripts.

**Isolation of DNA Clones Encoding Conserved Epitopes of the Glycoproteins gB of Bovine Herpesvirus Type 2 (BHV-2) and Herpes simplex Virus Type 1 (HSV-1)**

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Libraries of small fragments of BHV-2 and HSV-1 DNAs were established with the expression vector lambda gt11 and screened with monoclonal antibodies directed against cross-reacting epitopes present on a 130K glycoprotein of BHV-2 (gB-BHV-2) and glycoprotein gB of HSV-1. Using one of the lambda gt11-gB-BHV-2 clones as a probe it could be shown that the gB-BHV-2 gene maps colinearly to the gB gene of HSV-1 in the U₁ segment of the genome. It could be demonstrated that one group of cross-reacting epitopes is clus-
tered in a region of approximately 100 amino acids. Nucleotide sequencing revealed a highly conserved region in the gB genes of BHV-2 and HSV-1. The determination of the nucleotide sequences coding for the common epitopes is in progress.

Characteristic Changes in Restriction Enzyme Pattern of a Fetal Equine Herpes Virus Type 1 (EHV-1) Isolate During Development to the Vaccine Strain RAC-H

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The attenuated live vaccine strain Rac-H was developed from a fetal isolate by continuous propagation in porcine embryonic kidney cells (256 passages). Strain Rac-H (256th p.) DNA proved to have a restriction enzyme pattern different from other EHV-1 isolates and strains. For comparison of DNA preparations from high and low passaged Rac-H strain we used digestion with the restriction enzyme BamHI. At least up to the 185th passage DNA patterns showed to be similar to those obtained from field isolate DNA. We assume, that mutations resulting in the altered fragment pattern of the vaccine strain do not correspond to the loss of virulence, which was observed during propagation before the 185th passage.

Reactivation of PRV Infection of Swine: A Model of Sudden Deafness of Man?

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In order to study a possible involvement of viruses for disturbances of inner ear functions (sudden deafness, nausea) BALB-C mice were infected intranasally with PRV. These experiments clearly showed that infection migrated via the neural route into the inner ear and led to lesions (J. Virol 57 (1986) 335. – The particular importance of latency and reactivation was investigated by morphologic, immunologic and molecular virological (in situ hybridization) examination of infected swine during acute, latent and reactivated phases. – Electrophysiological examination of the hearing ability revealed significant differences, even single-sided deafness. Analysis of brain sections of hybridization in situ with PRV-DNA gave distinct patterns of distribution for viral sequences and emphasized the involvement of central segments of the hearing and vestibular pathways. – The possible association of sudden hearing loss with viral infections in particular of the Herpes group could have consequences for therapy. – Part of this work was supported by the W. Sander Foundation (84.018.1).
The Neural Persistence of Herpes simplex Virus Requires the Inhibition of the Lymphohaematogenous Spread of Infection

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In mice genitally infected with herpes simplex virus (HSV) no infectious virus can be isolated from the inflamed draining lymph nodes, contrary to the positive results in the lumbosacral ganglia. Attempts to reactivate an abortive infection eventually established in lymph node or spleen cells by stimulation with phythaemagglutinin or lipopolysaccharide rendered no positive results, not even when adult, but immunodeficient mice were used as test animals. Isolation of infectious virus from lymph node and spleen cells was successful in immature 4 to 6-week old mice, particularly when these had undergone pretreatment with cyclophosphamide, silica, antimacrophage serum and/or cortison; 5 days p.i. being the date of optimum virus yield. HSV-1 infected mice were more frequently positive than those with HSV-2, and genetically sensitive animals more so than resistant mice. The data indicate that the lymphohaematogenous spread of the virus is inhibited in favour of the neural dissemination by means of an active defence mechanism, probably by macrophages and/or NK-cells.

Herpes simplex Virus-Mediated DNA Amplification in an SV40-Transformed Hamster Cell Line: SV40 DNA Structure Before and After Infection with HSV

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Infection with HSV-1 induces amplification of SV40 sequences in particular SV40-transformed hamster cell lines. – The cell line Elona (Brandner et al., J. Clin. Microbiol. 5 (1977) 250) in which SV40 has integrated once in the genome as a partial tandem is specially suited for further detailed studies on this amplification process. Elona is not permissive for SV40, but T-antigen and the origin of replication are functional. – Elona cells are permissive for HSV-1. After infection with HSV-1, SV40 sequences get amplified. Restriction and sedimentation analyses show the amplified DNA to be high molecular weight head-to-tail concatemers of SV40 DNA molecules. One cell clone (Elona C13) was isolated having only one incomplete SV40 genome integrated. Southern blot analyses indicate the amplification of flanking cellular sequences after infection with HSV-1. – A genomic library of Elona was constructed in the lambda vector L47 to isolate sequences flanking the integrated SV40 DNA. Hybridization with a deduced probe shows the amplification of cellular sequences after HSV infection. – This study was supported by Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 31).
Genomic Termini of Bovine Herpesvirus Type 1 (BHV-1) Cleavage Site of a Virus-specific Terminase and Interaction with the Cellular Genome

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Bovine herpesvirus type 1 (BHV-1) replicates after circularization of the linear virion DNA and forms concatemers in host cells. The comparison of the nucleotide sequence of clones carrying the left and right genomic termini, junction fragments of the fused genome termini, and fragments spanning a related internal junction revealed that (i) the concatemeric fragment harbors the cleavage site of a terminase, which cuts the concatemers into virion DNA molecules. The recognition sequence of this terminase is conserved in class D herpesviruses. (ii) A 10% proportion of the termini is elongated by varying numbers of nucleotides. The nucleotide sequence of these tails appears in the cellular DNA. An interaction of the virion DNA with the cellular DNA is discussed in respect to persistence.

Herpes simplex Virus Induces Amplification of Recombinant Plasmids that Lack a Herpesviral Origin of Replication

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DNA in defective interfering HSV particles consists of concatemeric repeat units that are composed of subgenomic HSV DNA segments linked to an HSV-specific origin of replication. Recombinant plasmids containing a putative HSV origin are amplified upon introduction into mammalian cells, whenever herpesviral DNA replication functions are provided in trans (Stow, EMBO J. 1 (1982) 863; Weller et al., Molec. Cell. Biol. 5 (1985) 930. – In this context it appears remarkable that in an SV40-transformed cell line, upon infection with HSV, large concatemeric SV40 DNA molecules have been detected. The repeated units of this amplified DNA species do not contain any HSV-specific origin element. Consequently, it could be demonstrated that plasmid constructions containing SV40 DNA sequences instead of an HSV origin of replication behave similar to herpesvirus amplicons (Spaete and Frenkel, Cell 30 (1982) 295) in the presence of HSV-specific DNA replication functions. – This study was supported by Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 31).
Determination of the Nucleotide Sequence Flanking the Deletion (0.762 to 0.789 Map Units) in the Genome of an Intraperitoneally Avirulent HSV-1 Strain HFEM

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Herpes simplex virus type 1 (HSV-1) strain HFEM which harbours a deletion of 4.1 kbp in its genome (0.762 to 0.789 map units, Hpal DNA fragment P of HSV-1), is apathogenic for mice and tree shrews by the intraperitoneal application route. The exact position of this deletion was determined by DNA sequence analysis. This analysis was performed using the recombinant plasmid pU18HSF-Xml-B which harbours the flanking genome regions (0.752 to 0.762 and 0.789 to 0.7895 map units) of the deletion in the genome of HSV-1 HFEM and the recombinant plasmids pU18HSF-Xml-B, pU18HSF-AS, and pHSF-BBsH-D, harbouring particular regions of the genome of the virulent HSV-1 strain F at the coordinates 0.752 to 0.761, 0.786 to 0.790, and 0.762 to 0.771 respectively. The comparison of the DNA sequences of this region with the DNA sequences of the corresponding genome regions of the pathogenic HSV-1 strain F and HSV-1 strain 17 showed that the 5' end of the deletion in the genome of HSV-1 HFEM starts at the nucleotide position 3774 of the BamH1 DNA fragment B from HSV-1/17. This position is 71 bp upstream of the UdR junction of the HSV-1 genome. The 3' terminus of the deletion ends at the nucleotide position 7226 of the BamH1 DNA fragment B from HSV-1/17. The position is within the incomplete ninth repetitive box (ACTCCCACGCACCCCC) and is located 36 bp upstream of the 3' end of the IE 110 mRNA. In addition it was found that the BamH1 DNA fragment B from the genome of HSV-1/17 possesses two deletions of about 150 and 600 bp located in the corresponding BssHII DNA fragment C and D. - This study was supported by the Deutsche Forschungsgemeinschaft, project Da 142/1-3

Cross-Reactions Between the Simplex Viruses of the Alphaherpesvirinae

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Monoclonal antibodies (mAbs) directed against a 130K glycoprotein of the bovine herpesvirus type 2 (BHV-2) cross-react in several serological tests with the human herpes simplex virus types 1 and -2 (HSV-1, -2). The antigen of HSV involved in the cross-reaction was identified as the glycoprotein gB. Indirect immunofluorescence using 0.5 μm cryosections of HSV-1 infected cells and the mAbs revealed either a patchy intranuclear or a cytoplasmatic membrane labelling. Immuno-gold IEM of ultra thin cryosections of infected cells showed that two antibodies detect epitopes on the capsids and in distinct areas in the nuclei, whereas other stain envelopes of viruses in the cytoplasm and after egress. Western blot analysis of capsid preparations isolated from different compartments of infected cells
are in agreement with the hypothesis that gB is transported into the nucleus were it attaches to the capsids. Further investigations will show whether biochemical differences are detectable between gB molecules localized in different cellular compartments.

Parameter of the Cellular Immunity as an Additional to the Estimation of Virus-Specific Antibodies in the Diagnosis of Herpes Virus Infections in Kidney Transplanted Patients

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Patients after organ transplantation have a higher risk to get infected by e.g. cytomegalovirus, herpes simplex virus, varicella zoster. – We studied the course of 30 kidney transplanted patients during the first 3 months after transplantation; immunosuppressive therapy with ciclosporin. Additionally to the virus-specific titers (ELISA) of the IgG and IgM class against CMV, HSV, VZV the ratio of T₄/T₈ lymphocytes in the peripheral blood was estimated. – So far this T₄₈ ratio shifted to lower values – in some cases below 0.5 – if infectious diseases caused by e.g. CMV, HSV occurred days to weeks later. This would mean that the T₄₈ ratio earlier indicates to an outcoming infectious disease apparent or inapparent in clinical course than it could be seen by e.g. seroconversion of virus-specific IgM and IgG. The T₄₈ ratio doesn’t seem to be influenced by ciclosporin medication.

Serologic Evidence for the Occurrence of Human Infections with Marburg- and Ebola-Virus in the Republic of Liberia

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Sera collected 13 years ago from 348 residents of the Republic of Liberia were tested for antibody to Marburg- and Ebola-virus using Elisa and immunoblot techniques. Antibody to Marburg virus (MV) was found in 18.1 % and to Ebola virus (EV) in 10.6 %. Distribution of seropositives was completely independent of sex and of tribal affiliation. The incidence of antibody was only 4.1 % in people who had lived in the Savannah region for more than ten years and amounted to 23.2 % in the rest of the population. In certain divisions of a rubber plantation as many as 52 % of people were seropositive for either virus. The highest prevalence of infection was seen in the age groups between 20 and 39 years. There was no indication of a professional risk. Antibody against both viruses was found in 7.4 % of sera. EV antibody occurred five times as often in MV positive individuals than in the whole population and in EV positive sera antibody to MV was present 10 times as often as in the whole population. These positive correlations indicate that there must exist a common epidemiological risk factor for both infections.
EMC Virus Myocarditis in Mice. Electrocardiographic and Morphologic Findings

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NMRI mice, 5 weeks of age, were inoculated intraperitoneally with 0.1 ml EMC virus suspension containing 10 PFU. Only male mice were used. Every other day 10 mice were examined electrocardiographically and histologically. **Histology:** Day 2: early patchy necroses, no evidence of lymphocytic infiltration. Day 6: beginning of focal lymphocytic infiltration, widespread patchy necroses. Day 10: heavy lymphocytic infiltrates, occasional calcification. Day 14: diminishing infiltration, fibrosis becoming prominent. **ECG:** Day 4: ST-displacements predominant. Day 8: intraventricular conduction defects becoming more common in addition to AV- and SA-blocks; first extra beats observed; QRS voltage starting to decrease. Day 12: virtually all animals show ST-displacement; otherwise no major changes observed in comparison to day 8. – Chronologically, the occurrence of ECG abnormalities correlates well with histopathologic findings in the acute stage of myocarditis. Our findings are in accordance with clinical and morphological findings in acute viral myocarditis of man.

HN-Mutation During Persistence of Sendai Virus in Mice and Comparison to ts-Mutants Isolated After Mutagenesis with 5'-Fluoruracil

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C129-mice were intracerebrally infected with Sendai-virus (D52). 85 days after infection the brain cells were cocultivated with uninfected mice brain cells. From this persistently infected cells infectious and temperature sensitive (ts) mutants were isolated (MG-la). The protein pattern of the purified MG-la particles differs from the wildtyp pattern. The hemagglutination (HN) protein band was more prominent and of lower molecular weight. In contrast to the other viral proteins the HN-band was not recognised by an anti-Sendai serum, but by an anti-HN-monoclonal antibody. That the differences are due to genomic mutations may be concluded from the weak hybridisation signal with HN-mRNA-specific cDNA probes, which doesn’t correlate with the HN-protein abundance: – To study the role of HN in viral persistence we isolated ts-mutants of Sendai wildtyp virus after mutagenesis with 5'-Fluoruracil. The HN-protein of three ts-mutants show the same immunogenic properties as MG-la in immuno-blot. We’ll use these mutants to study the course of infection in mice and different cell culture systems.
A Seroepidemiological Survey of Hantavirus Infections in Rodents Captured in Western Germany

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A total of 282 sera of wild-living mice (20 Mus musculus, 98 Apodemus sylvaticus, 52 Ap. flavicollis, 112 Clethrionomys glareolus) and of 77 sera of wild Brown Rats (Rattus norvegicus) was collected in Western Germany and tested by the indirect immunofluorescence antibody assay using Vero-E-6 cells infected with Hantavirus strains Hantaan 76–118, NE-Puumala, NE-Hällnäs, and Prospect Hill. 3 out of 20 sera from Mus musculus (15%) reacted with Hantavirus antigens, titers ranging between 1 : 32 and 1 : 128. All the other mouse sera were negative. 21 serum samples obtained from Brown Rats were positive by IF assay (27.3%), titers ranging between 1 : 16 and 1 : 256. All the positive rat sera reacted with Hantaan 76–118. Only one showed an additional reaction with the NE-Hällnäs strain. Also 287 sera from laboratory rats collected during the last ten years in Europe from different breeding colonies were tested against 4 Hantavirus strains. 22 sera (7.7%) were positive, titers ranging between 1 : 10 and 1 : 1280. Further work is necessary to locate the natural foci of Hantavirus in Clethrionomys glareolus in Western Germany.

Lack of Infectivity During Sendai Virus Persistence

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Different cell lines persistently infected with Sendai virus were investigated for viral infectivity: at the beginning of persistency an increase of infectivity was detected, which could be explained by adaptation of the virus to the host cells. However, although compared to an acute infection the infectivity is about four magnitudes lower, the virus particles are capable of multiple-cycle replication in non-permissive cells. In a later stage of persistency, a lack of viral infectivity was observed. In contrast to acute Sendai virus infection, no activation was obtained by trypsin treatment. On the other hand a small number of these virus particles are activated by elastase and thermolysin. From these findings it appears that the virus population is heterogeneous and consists of F protein mutants. It could be concluded that infectivity is lost during Sendai virus persistency due to lack of selection leading to a heterogeneous virus population.
An Increase of the Intracellular Calcium Concentration Leads to a Disassembly of Budding Measles Virus at the Plasma Membrane

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We have shown recently that the budding of measles virus is connected with a polar growth of actin filaments. Calcium ions are known to play a key role in the regulation of assembly and disassembly of actin filaments as well as in the regulation of contractility. Therefore calcium ions may also influence the budding process of measles virus. We modulated the calcium concentration in measles virus infected cells by use of the calcium ionophore A23187. In the presence of calcium, but not of magnesium or barium the ionophore induced (1) a disappearance of virus structures and microvilli from the cell surface, (2) a random redistribution of virus hemagglutinin at the cell surface, (3) a dissociation of nucleocapsids from the protoplasmic membrane face, and (4) a significant reduction of the cell bound infectivity. The data indicate, that calcium ions are able to influence the morphology of budding virus particles at the plasma membrane and may play a role in virus morphogenesis. – Supported in part by Gemeinnützige Hertie-Stiftung, Frankfurt/M.

Genome Organisation and Translation Strategy of Flaviviruses

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From the comparison of the complete sequence of genome RNA of west nile virus with partial sequences of the virus specific proteins and with published data of other Flaviviruses the following conclusions can be drawn: 1. All known virus proteins are encoded on the genome consecutively in one long open reading frame in the order 5' - C - preM - E - NS1 - NSX - NS3 - NSY - NSS - 3'. – 2. The structural proteins and NS1 are cotranslationally inserted into the ER membrane and set free from the precursor by cellular signalase. The remaining non-structural proteins are set free by a different protease which is possibly virus specific. – 3. All proteins contain hydrophobic sequences which could function as transmembrane sequences. This finding is in accord with the well known fact that all Flavivirus proteins are membrane associated.

Adenovirus-12 Early Region 1A Protein(s) Indirectly Associates with Viral and Cellular DNA

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The mechanism(s) mediating positive and negative regulation of transcription by Adenovirus early region 1A proteins is at present unknown. Whether E1A gene products
interact with RNA-Polymerase II, specific DNA nucleotide sequences (enhancer elements) or counteract a cellular repressor bound to the viral or cellular genome, all remains formal possibilities. – In order to verify one of the possible interactions of the E1A proteins mentioned above, we have looked for a direct or indirect binding capability of Ad12 E1A proteins to double-stranded (ds) DNA. Nuclear protein extracts, prepared from infected KB cells, have been passed through columns of viral and cellular ds DNA-cellulose. After elution with salt gradients, each fraction was screened for E1A polypeptides by immunoprecipitation using E1A-specific antipeptide antibodies. We have detected an E1A protein with a MW between 38K and 45K, which elutes from the column at 0.2 to 0.3 M KCl. A second column run of the fractions eluted at 0.2-0.3M salt showed no or very little binding of the E1A polypeptide to ds DNA. However, incubation of the E1A protein containing fractions (eluted from the first column) with nuclear extracts of uninfected cells, restores the capability of this E1A polypeptide to interact with viral or cellular DNA, suggesting that the binding of the viral protein is possibly mediated by (a) cellular protein(s). We are currently characterizing the binding conditions, a possible sequence specificity and the cellular component(s) of the DNA-bound viral/cellular protein complex. – (Supported by the Deutsche Forschungsgemeinschaft through SFB 102-A11).

Expression of the Ad12 E1B 58K Protein in E. coli

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The E1B 58K protein of adenovirus (Ad) is involved in oncogenic transformation of cells and exhibits essential viral functions during productive infection e.g. DNA replication, transport of viral mRNA, shut off of host cell protein synthesis. Since only limited amounts of E1B proteins are synthesized in infected cells, we decided to express the E1B 58K gene in E. coli. Such an expression system also offer the possibility to synthesize parts and to raise antibodies against various domains of the proteins and to investigate possible functions in more detail. – The expression vector which has been used (piWiT15) directs transcription from a synthetic promoter controlled by the Lac operator. The clone pB58N-gal codes for the N-terminal first 200 amino acids of the Ad12 E1B 58K protein fused to β-galactosidase. Total protein extracts from pB58N-gal transformed bacteria contain a fusion protein of expected MW (150kD) and in addition fusion proteins of 140kD and 120kD. Expression clone pB58, a derivative of pB58KN-gal, codes for the whole 58K protein. As the described proteins are specifically degraded in E. coli, we are currently testing the T4 pin gene function in respect to stabilization of these proteins. Results about the expression of the corresponding adenoviral proteins in E. coli and the specificity of antibodies, directed against these proteins will be dicussed. – (Supported by the Deutsche Forschungsgemeinschaft through SFB 102-A11).
Temperature-Sensitive Cell Cycle Mutants of Human KB Cells: Adenovirus Functions Required for Induction of Cellular DNA Synthesis in G1 Arrested Cells

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Both infection and transformation of cells by adenoviruses lead to drastic changes in the host cell cycle. Little is known about the viral genes responsible for these changes and about the cell cycle regulating cellular genes affected by early adenovirus functions. — We have isolated temperature-sensitive (ts) cell cycle mutants of human KB cells. At the nonpermissive temperature (39.5°C) the ts defect leads to an arrest of these mutants in the G1 phase of the cell cycle. Infection with Ad2 or Ad12 induces cellular DNA synthesis in the G1 arrested mutants. Infection experiments with the Ad5 deletion mutants d1312 and d1313 indicate that the expression of both early viral regions, 1A and 1B, is required to induce G1 arrested cells to enter the S phase at the non-permissive temperature. — The viral genome of Ad2 and Ad12 replicate in all mutants at 34°C. In contrast to Ad2, however, Ad12 does not replicate at 39.5°C in cell line ts SC 8. — Two-dimensional gel electrophoresis of cell extracts from uninfected and infected cells grown at 34°C and 39.5°C have shown that the expression of most cellular proteins which are shut off or greatly reduced in uninfected cells at the nonpermissive temperature are expressed again at 39.5°C after infection with wt adenovirus and mutant d1313. — (Supported by the Deutsche Forschungsgemeinschaft through SFB 102-A11).

The Adenovirus Glycoprotein E3/19K Inhibits T-Cell Recognition by Blocking Cell Surface Expression of Histocompatibility Class I Antigens

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We have established human cell lines, which express the E3/19K protein of adenovirus 2 by using a transfection system. We found that the E3/19K protein binds to human histocompatibility antigens (HLA) thereby blocking their terminal glycosylation and transport to the cell surface (1). The reduced level of class I antigens on the cell surface impairs T-cell recognition of E3/19K+ cells in vitro (2). The same effects are found in normal human cells infected with adenovirus 2. The observed mechanism might be important for the establishment of persistent infections in vivo. — The construction and expression of hybrid proteins allowed us now to identify the domains of class I antigens necessary for E3/19K association. E3/19K binds to the domains which are crucial for T-cell recognition.

References
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Transcriptional Control of the Ad12 E1a Promoter within the ITR and at the CAP Site

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In order to identify target sequences within the Ad12 E1a promoter which are required for efficient transcription, the activities of different promoter fragments were compared in a transient expression assay. So far two important positions have been detected: 1. Deletion of the leftmost 152 bp of the Ad12 DNA reduces E1a promoter activity 5 to 10-fold, deletion of the leftmost 170 bp at least 10-fold. The deleted DNA includes the Inverted Terminal Repetition (ITR, -164 bp) and is functionally important for transcription from both TATA boxes as well as from the first one alone. The presence of early Ad12 gene products does not overcome this reduction. ITR fragments at the 3' end of the CAT gene in either orientation restrict the control element to DNA sequences between positions 144 and 170 and demonstrate that they serve as a transcriptional enhancer. 2. Transfection of increasing amounts of DNA of the CAT constructs containing a promoter fragment from position 0-525 results in maximal CAT activity at 15 µg of transfected DNA followed by a rapid promoter inactivation. Smaller promoter fragments yield increasing CAT activity according to increasing amounts of transfected DNA. Northern analysis suggests that this control mechanism occurs at the level transcription initiation. Two in limited amounts occurring nuclear proteins of BHK cells binding between positions 400 and 494 can be identified by "Gel Shift" tests. These factors are stimulated in an Ad12 transformed hamster cell line. The target for these factors could be mapped to the E1a CAP site.

Isolation of Cellular Transcriptional Control Sequences by Amplification of a Reconstituted SV40 Promoter

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In order to isolate cellular transcriptional control sequences, we transfected BHK cells with selectable plasmids containing the neo gene under the control of a defective SV40 promoter (p892 neo) or without any eukaryotic promoter sequence (pSV0 neo). G 148 resistant cell clones were isolated and genomic analysis showed multiple insertions of the neo gene into the BHK genome. In order to obtain single copy integration, BHK cells were transfected with DNA fragments of resistant cell clones. Northern analysis of the new clones showed that comparable amounts of neospecific RNA according to wt RNA were synthesized. Therefore we conclude, that cellular sequences have functionally completed the defective promoter of p892 neo. To facilitate the cloning of the single copy insertion sites, we tried an amplification by fusion with COS 1 cells (SV40 transformed monkey cells containing the permissive factor and T-Antigen which are necessary for SV40 replication). In most cases an amplification up to 1000 fold was achieved. The amplified DNA fragments were cloned into pUC 12 and are under investigation.
Oligomerization and Origin DNA Binding Activity of SV40 Large T Antigen

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Simian virus 40 (SV40) large T antigen exists in multiple molecular forms, some of which are separable by zone velocity sedimentation of soluble extracts of infected monkey cells. Three subclasses from infected cells have been separated and characterized: 5S, 7S and 14S. Newly synthesized T antigen occurs in the 5S form, a monomer. The 14S form represents a tetramer and the 7S form, a dimer. The rate of oligomerization of newly synthesized 5S T antigen into tetramers in vivo was greater at early times after infection than at late times. The DNA binding properties of each subclass were investigated after immunopurification. The affinities of the 3 forms for SV40 DNA and for a synthetic 19 bp sequence from binding site I are very similar (K_D 0.4 nM). The specific activity of DNA binding was greatest for the 5S and 7S subclasses and least for the 14S subclass. Moreover, the specific activity of the 5S and 7S forms increased sharply at about 40 h after infection, whereas that of the 14S form remained at a constant low level throughout infection. The binding stoichiometry of T antigen was consistent with the idea that stable tetramers do not bind to viral DNA at all, and that the observed low binding activity derives from contamination with dimers. A model relating oligomerization and DNA binding of T antigen in infected cells will be presented.

Binding of SV40 T Antigen to SV40 DNA Binding Sites I, II and III

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During lytic infection SV40 T antigen binds specifically to three different regions of the SV40 DNA to initiate DNA replication and to regulate early and late transcription. We constructed plasmids containing either 22 basepair synthetic oligonucleotides representing site I or II or combinations of binding site II and III with or without the SV40 specific flanking regions. T antigen bound to site I with very high affinity, whereas isolated site II was not bound specifically. Measurable specific binding could be restored to some extent by combining site II with the 3' SV40 flanking region. Binding to site III remained weak with or without the 3' and 5' flanking sequences. Binding to DNA fragments containing both sites II and III was higher than binding to fragments with either II or III added together. Thus flanking regions not bound directly by T antigen could influence binding affinity probably by changing the DNA structure of the nearby binding site.
Regions on the Polypeptide Chain of Simian Virus 40 Large T Antigen Necessary for Oligomerization and T-p53 Complex Formation

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The SV40 large T antigen is composed of 708 amino acids. It occurs in monomers and various oligomers as well as in complexes with the cellular oncoprotein p53. To detect distinct regions on the polypeptide chain of large T antigen which are essential for the formation of these homo- and heterologous forms we have analyzed T antigen from various SV40 deletion mutants. We found that an area between amino acids 110 and 152 and additionally a C-terminal region between amino acids 591 and 634 are essential for oligomerization. Only this C-terminal region between amino acid 591 and 634 but not the N-terminus up to amino acid 152 seems also to be critical for T-p53 complex formation. Analyzing the potential influence of the phosphorylation of T antigen we found that only phosphorylation of amino acid 124 seems to be important for oligomerization but not for T-p53 complex formation.

Non-Defective and Non-Oncogenic Host Range Mutants of Adenovirus 12

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The non-oncogenic and non-defective host range mutants of Adenovirus 12 having a constant deletion in the left end show enhanced cytolysis and quick cytopathogenic effect in Vero and human cell lines compared to wild type Adenovirus 12 infection in the same cell line. The mutants produce E2 coded single stranded binding protein in larger amounts but failed to produce E1B 19K protein in infected cells. The relationship of the deleted E1A with the other observed functions in bringing about host range, enhanced cytolysis and reduced oncogenicity are being studied. Preliminary studies using rabbit reticulocyte lysate in vitro translation system and hybrid selected m-RNA specific for E1 region indicate presence of functional m-RNA for E1B 19K region. This suggests that E1A has a role in the translation of E1B 19K specific m-RNA.

Further Characterization of E1A Deletion Mutants of Adenovirus 12

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Host range mutants of Ad 12 efficiently replicate in simian Vero cells. They have a 69 bp deletion in E1a (pos. 834–902) and additions of viral sequences at the right terminus of the
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genome. The deletion in E1a eliminates the transforming potential of E1a in vivo and in vitro. The ability to stimulate expression of the other early viral and some cellular genes is not affected. Recombinants have been constructed which carry only the deletion in E1a. Other mutants have been isolated which have only additions to the right terminus of the genome. Plaque assay data obtained with these viruses map the ability to enhanced replication in Vero cells to the E1a region. – Supported in part by grant IV b 5 – FA 9794 from the Minister für Wissenschaft und Forschung des Landes NRW.

Analysis by Monoclonal Antibodies (PAb 1601–1636) of Epitopes of Simian Virus 40 (SV 40) Nuclear and Cell Membrane Tumor (T Antigen) and of Antibody Cross Reactions with Polyoma, BK, JC and Lymphotropic Papovavirus T-Antigens

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We have characterized the series PAb 1601–1636 of monoclonal antibodies (mab) against SV40-transformed cells (EMBO J. 3 (1984) 1485; J. Cancer Res. Clin. Oncol. 109 (1985) 54A). Epitopes recognized by these mabs are located also at the inner region of the T protein and most of them are expressed on the cell membrane of native SV40 tumor cells. The COOH-preterminal sequence .. thr (701) pro pro pro glu pro (706) .. is recognized by PAb 1605. The terminal synthetic undecapeptide containing this epitope has been attached covalently to the membrane of native untransformed cells rendering them with a surface immunofluorescence reaction typical for SV40 tumor cells. – Finally we demonstrate similar peptide studies at the N-terminal and cross reactions of 35 of the mabs with one or more of the T-antigens of BK, polyoma, and JC virus. None of the antibodies reacts with the lymphotropic papovavirus T antigen. – Supported by Deutsche Forschungsgemeinschaft, Br. 281/11.1, 2, Stiftung Müller Fahnenberg and Ciba-Geigy GmbH, Wehr.

Cellular Proteins Mediate Binding of Adenovirus Type 12 E1A Tumor Antigens to Viral Transcriptional Complexes

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Adenovirus type 12 transcriptional complexes were isolated from the infected cells during early phase. Sedimentation analysis identified a fast sedimenting complex type I and a slow sedimenting complex type II. Both complexes made viral specific RNA and contained viral DNA, which in type II but not in type I had nucleosomal configuration. Analysis of the proteins, of the complexes with antiserum against Ad12 E1A-β-galactosidase fusion protein expressed in E. coli (12-1A-FP, demonstrated the following; a) type I complex contained E1A 45k protein which coprecipitated with cellular proteins of mol. wt. 42, 58 and 60k. (b)
type II complex contained E1A 48k protein, which coprecipitated with major cellular proteins of 35, 42–43k and minor proteins of 58, 60, 68, 86 and 120–150k. The association of E1A specific and cellular proteins to transcriptional complexes, was sensitive to both IMNaCl and DNAse I establishing the deoxyribonucleoproteins nature of the complexes. Treatment of transcriptional templates with IMNaCl or DNAse I released E1A proteins which still remained strongly bound to a conglomerate of cellular proteins. These findings indicate that E1A specific antigens do bind to viral DNA but this binding is indirect and mediated by some of the cellular proteins described above.

Vaccinia Virus, Herpes simplex Virus and Carcinogens Induce SV40 DNA Amplification in Transformed Human Cells and Support Replication of Adeno-Associated Virus

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Chemical or physical carcinogens as well as infection with herpes simplex or vaccinia virus induce amplification of SV40 DNA in the human SV40-transformed cell line, NB-E. These findings demonstrate that human cells similar to Chinese hamster cells amplify integrated DNA sequences after treatment with initiators or viruses with initiator-like properties. In addition, it is shown that similar to herpesgroup viruses a virus of the poxvirus group – vaccinia – induces DNA amplification. As shown for other systems, the carcinogen- or virus-induced DNA amplification is inhibited by (co)infection with adeno-associated virus (AAV-5). Concomitantly, this initiator-treatment induces AAV DNA replication and AAV antigen synthesis, revealing vaccinia virus as a helper virus for AAV.

Persistent Infections of Humans with Adenoviruses

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The molecular mechanism of persistence of adenoviruses (Ad) in the organism is still unknown. Therefore, we have tested human tonsillar tissue for infectious virus and for viral DNA-sequences by DNA-DNA hybridization with genomic Ad 2 DNA and cloned Hind III fragments. We hereby observed cases in which infectious virus could neither be isolated directly from ground tissue probes nor in cell cultures established therefrom and propagated for weeks or months. Yet, after application of the “In situ hybridization” we were able to demonstrate adenoviral sequences in cells located in the periphery of cryostat sections of such tonsillar tissue. Only part of the cells harbored viral sequences. Cell cultures established from such tonsillar material exhibited positive hybridization signals, too, but again in only a portion of the cells. – In Southern-Blot hybridizations “off-size” bands were detectable besides bands comigrating with adenoviral marker fragments. In some cases viral DNA fragments were missing. Additionally, different intensities of various bands suggest that parts of the viral genome are overrepresented.
Screening for DNA-Binding Proteins by Phosphorus- and Biotin-Labeled DNA-Probes

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A modern approach to study DNA-binding in a mixture of proteins consists of fractionation of proteins by gel electrophoresis and transfer to nitrocellulose filters by electro- or diffusion-blotting. We describe the use of nick translated biotin-labeled DNA-probes in DNA-binding studies. – Phosphorus- or biotin-labeled DNA-probes were applied to the filter by a specially designed vacuum-filtration apparatus. It hereby became possible to detect DNA-binding activity by even 10 ng of calf thymus core histones dotted on nitrocellulose filters. Furthermore, chromosomal proteins with a lower affinity to DNA (histone H1 and HMG-nonhistone proteins) are detectable among other DNA-nonbinding proteins. – In summary our results demonstrate that the DNA-binding capacity of proteins transferred to nitrocellulose filters can be tested with phosphorus- as well as with biotin-labeled DNA-probes. The sensitivity of both labelling systems appears equal.

Prediction of Antigenic Determinants in Amino Acid Sequences

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During the last years, large amounts of DNA and protein sequences became available due to the rapid progress in sequencing techniques; three-dimensional structures, however, will be available for few proteins. For most purposes it is sufficient to obtain data on the secondary structure, hydrophilicity, flexibility, surface probability and modification of the amino acid sequence. Those predictions are mainly important for immunological questions. Consequent application however, may result in valuable information for three-dimensional arrangements of proteins. In this paper we present a computer program with access to sequence libraries which calculates secondary structures of proteins, superimposes those predictions with additional values and creates a two-dimensional graph. A further algorithm was developed and included which combines structural and other parameters in order to recognize antigenic sites. This algorithm was used to predict epitopes on proteins which had been characterized by X-ray crystallography (lysozyme, myoglobin, poliovirus VP1) and on experimentally well characterized viral polypeptides (EBV, HIV, FMDV); a good coincidence in antigenicity profiles could be obtained. This program can be directly combined with the UWGCG program collection.
Pseudorabies Virus Glycoprotein gII: Its Relevance in Virus Neutralization, Studies on Determination of Immunogenic Epitopes

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The immune response of Pseudorabies virus (PRV; herpes suid 1) -infected swine was found to be directed against the major glycoproteins gI, gII, and gIII. Monoclonal antibodies against gII neutralized PRV in vitro in the presence and absence of complement. Individual MCA were also able to protect mice against lethal challenge with PRV. Thus, the gII-complex is important in virus neutralization in vitro and in vivo. Viral mutants (MAR) resistant to neutralization with individual anti-gII MCA were selected. One mutant was only resistant to neutralization with that MCA used for selection, which also did not recognize the expressed gII. Other MAR mutants were neither neutralized by the MCA used for selection nor by different other MCA against gII, although gII was expressed in all these MAR mutants.

Expression of the Genome of Pseudorabies Virus (Herpes suid 1) During latency

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The activity of the genome of Pseudorabies virus (PRV) was investigated in two latently infected pigs (60 weeks p.i.). PRV-specific antigen(s) could be detected in the brain by immunohistochemical staining of thin sections. For this purpose a polyclonal goat antiserum was used, which recognizes immediate early (IE) protein of PRV. Monoclonal antibodies specific for the major glycoproteins (gI, gII, gIII) and for the major capsid protein of PRV did not react. In situ cytohybridization with strand-specific probes (cRNA synthesized with SP6- or T7-Polymerase) revealed the presence of PRV IE-specific RNA in the brain. In some other organ tissues of both animals, PRV-specific DNA sequences could be also detected by Southern blot hybridization, which, however, did not represent the complete viral genome. Transfection of permissive cells with DNA extracted from these organs lead to the production of PRV.
Variable Copy Number of a Direct Repeat Sequence at the 3'-end of the Gene Coding for gIl of Pseudorabies Virus

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The structural gene of the glycoprotein complex gIl of Pseudorabies virus (PRV; Herpes suid 1) was recently mapped in the unique long part of the genome on BamHI/SalI-fragments 1A and 1G (Mettenleiter et al., Virology 152 (1986) 66–75). The 3'-end of the gIl-mRNA is located in fragment 1G. Southern blot analysis of DNA derived from different PRV strains revealed a heterogeneity in size of fragment 1G ranging between 0.6 to 1.2 kbp. After subcloning in M13 the DNA sequence of different 1G fragments of 3 PRV strains (Phylaxia, Ka, Dessau) was compared. All the fragments analysed exhibited nearly identical DNA sequence, except of single point mutations and a duplication of 6 nucleotides unique for strain Ka. The fragments differed in the presence of variable copy numbers (3 to 30) of a direct repeat sequence of 15 nucleotides. This variability was observed both between the different virus strains and between different 1G fragments from a given virus population. We conclude that the size heterogeneity of fragment 1G depends on the present copy number of the repeat unit. Finally this repeat unit seems not to be located in the coding region of the gIl gene.

Formation of the Disulfide Bridge-Linked Glycoprotein Complex of Pseudorabies Virus

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Glycoprotein complexes were identified in pseudorabies virus. The formation of the gIl-complex will be shown. One precursor protein (pgIl, 110K) of the three disulfide-linked glycoproteins of the complex (gIlα, gIlb, gIlc) could be immunoprecipitated as a monomere under non-reducing and reducing conditions; e.g. no disulfide bridge-linked complex of the precursor could be identified. After processing of pgIl to the mature forms gIlα, gIlb and gIlc these proteins were identified as monomers in infected cells. The gIl-complex was immunoprecipitated not before 6 h p.i. Electronmicroscopical studies of petri dishes of the same experiment show the first envelope particles in the cytoplasm of the infected cells at the same time (6 h p.i.) as the formation of the glycoprotein complex. This indicates that the nuclear membrane fraction plays an important role in the formation of gIl-complex of the virus.
Porcine Bone Marrow Cells were Infected by Pseudorabiesvirus (Herpes suid 1) After in vitro- or Experimental and Genuine in vivo-Infection

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The infectibility of porcine bone marrow cells (BM) was studied after in vitro- and in vivo- infection with Pseudorabiesvirus (PRV). PRV infection was shown by viral multiplication, immunofluorescence (IF), electronmicroscopic examinations, Western blotting and radioimmuno-precipitation of PRV-protein as well as by PRV-specific hybridization of viral DNA and RNA. Age-dependent decreasing frequencies of PRV replicating BM between 1 in 3–35 (a) and 1 in 38–101 (b) were shown by Infectious Center Assays with PRV-infected cultured BM of pigs less than 12 weeks (a) and more than 5 months (b) of age. IF-studies detected a nearly constant ratio of 65–70% BM positive for immediate early, early and late viral proteins and 30% PRV-major capsid protein-positive BM. PRV was isolated from BM of experimentally infected pigs between dpi. 1 and 3 (max. 1/13 000 PRV replicating BM) and viral RNA was detected between dpi. 1 and 6. Thus infection of BM with PRV was shown to be established before infection of CNS. PRV-infection of BM was corroborated by studies on naturally infected pigs.

Pseudorabiesvirus (Herpes suid 1)-Infection Results in Gradual or Total Loss of NK-Activity Mediated by 8/1+, T4−, T8− Bone Marrow Cells

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Pseudorabiesvirus (PRV) infectible mononuclear cells from peripheral blood (PBL), thymus and bone marrow (BM) of pigs were characterized by a panel of monoclonal antibodies (mab) directed against T helper (T4)-or T killer/suppressor (T8)-cells and a mab (8/1) recognizing a T cell differentiation/activation antigen (used as a pan T cell marker). PRV infectible cells were mainly located in thymus and BM and all those thymocytes and nearly 30% of the infectible BM were characterized as 8/1+, T4−, T8-cells by Infectious Center Assays, depletion experiments and double immunofluorescence-studies (FACS/dual laser). Only a few cells of this population were found in spleen, liver and PBL (< 1%). Studies on cell populations showing cytolytic activity revealed, that two different natural-kill (NK) active populations could be found in pigs. (1) 8/1+, T8+, T4− PBL, which were not discernible from CTL/CTLp by mab, were detected in PBL only. (2) 8/1+, T4−, T8− NK cells only located in BM. BM-NK cells showed gradual or total loss of activity after in vitro- or in vivo-infection by PRV.
DNA Sequence of the Control Region of the Murine Polyoma Virus K

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K virus, a polyoma virus causing lytic and persistent systemic infections in mice was isolated from organs of lytically infected newborn mice. The genome was cloned and the DNA region containing the control elements of virus expression was sequenced and compared with the corresponding DNA regions of SV40, LPV, BKV, JCV and mouse polyoma virus strains. In spite of significant homologies in sequence and structure of the “minimal origin” among K virus and the other polyoma virus genomes structural variations allow to classify K virus as an individual member of the polyomavirus family. Long repetitive sequences with enhancer functions, as described for SV40 could not be found within this region, although a 600 bp fragment without open reading frame was detected between the start-point of early transcription and DNA replication indicating that this DNA stretch might provide control functions similar to those of other polyomavirus enhancer structures.

Genomic Heterogeneity within Regulative Sequences of the Human Polyomavirus JC Strain GS

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The human polyomavirus JC persists in systemic organs without cell damage and causes in rare cases progressive multifocal leucoencephalopathy (PML) a disease associated with a lytic JCV infection of the CNS. Since the highly variable lytic JCV genomes are known to contain a celltype specific enhancer/promoter element we asked whether JCV genomes cloned from the kidney and the brain of one PML case are virus isolates identical in sequence and structure. Protein coding sequences of both genomes were identical as shown by sequence analysis and the control region revealed corresponding base sequences and structural elements in the “core origin of replication”. The putative enhancer/promoter element, however, showed a complex pattern of insertions and structural changes suggesting a celltype specific rearrangement of transcriptional control elements.

Molecular Differences of Four Cloned Aleutian Disease Virus (ADV) Strains

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The 3.5 KB BamHI to HindIII genome fragment of the low virulent ADV Pullman and the high virulent Utah strains, the cell culture-adapted lymphotropic ADV-SL3 isolate and the apathogenic ADV-G variant were cloned in pUC18 and pUC19. The ADV Pullman and Utah DNA was derived from virions, isolated from infected mink, whereas the ADV RF
DNA from the latter strains originated from cell culture. After induction of lac operon-dependent protein expression, capsid-specific antigens were only detected in recombinants of pUC19. The expressed proteins of ADV Utah and Pullman had a MW of 57 and 34 KD, the corresponding proteins of ADV-G were each 2 KD smaller and those of ADV-SL3 showed an additional loss of 2 KD. – These results are discussed with emphasis on the pathogenicity and persistence of ADV.

Detection of Aleutian Disease Virus (ADV) DNA Sequences in Tissues of Persistently Infected Mink

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Aleutian disease virus (ADV)-DNA was detected in tissues of persistently infected mink by in situ-hybridization using a^{35}SdCTP-labelled cloned DNA as a probe. – Spleen and mesenteric lymph nodes frequently were found to contain viral DNA. Positive cells could also be detected in bone marrow, thymus, liver, kidney, lung and small intestine. – These studies confirm the concept of a lymphotropism of ADV in vivo. Only a few cells in lymphoid tissues were found to contain viral DNA, suggesting that only certain subpopulation(s) of lymphocytes support the viral replication. The state of viral DNA in positive organs revealed by Southern blot hybridization of whole cell DNA is currently under investigation. So far, some spleen and bone marrow samples were found to harbour viral replicative forms, thus suggesting ADV replication.

Temperature Sensitivity of Aleutian Disease Virus in vitro is Due to Reduced Progeny DNA Synthesis

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The growth of ADV in feline kidney cells is characterized by a reduced production of infectious virus at 37°C. After 3 consecutive passages of ADV at 37°C, the virus titer drops below the detection limit of the fluorescence focus assay (10^4 FFU/ml). The production of viral antigen and viral RNA as well as the replication of viral replicative form DNA was found to be not defective at 37°C. However, the synthesis of viral progeny DNA was diminished. By 50 h p.i., about threefold less progeny DNA was synthesized at 37°C compared to 32°C. The reduction of the synthesis of new viral DNA strands may account for the reduced production of infectious virus.
Correlation of HPV 16/18 DNA Detection and Cytological as well as Histological Findings in Specimens from the Cervix Uteri

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In order to clarify the prognostic significance of the demonstration of HPV sequences in cells taken from cervical swabs for the ultimate development of cervical cancer routinely taken swabs were screened for HPV nuclei acid sequences using phosphorus – as well as biotinlabeled HPV 16/18 DNA-probes. So far, cells from 516 patients have been tested. Out of this group 18.9% (98/516) exhibited positive hybridization results and 67.6% (349/516) were negative. Results from 69 patients (13.5%) were equivocal. Positive hybridization signals were registered in all groups of the Papanicolaou rating system. In the group with nonpathological smears 17.6% (48/273) exhibited positive hybridization results. When taking follow-up swabs we confirmed the first hybridization result in 10 out of 17 cases. Correlating histological and hybridization data we detected positive signals in 2 of 4 squamous cell carcinomas and in 1/2 adenocarcinomas. In cases rated carcinoma in situ 9 out of the 15 specimens analyzed (59.9%) and one out of five dysplasias were also positive. We detected viral sequences in 42.8% (317) specimens without any histological indication of malignancy.

Papillomavirus Infections in Cervical Tumors of Austrian Patients

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Colposcopically directed cervical punch biopsies from 233 Austrian patients were screened by Southern blot hybridization for the presence of human papillomavirus (HPV) DNA. The biopsies represented different stages of cervical intraepithelial neoplasia (CIN), invasive carcinomas, as well as samples from histologically normal cervical epithelium. HPV DNA was detected in 53.4% of the tumors and in 37.5% of the biopsies from healthy epithelium, respectively. The most frequent HPV type in tested biopsies was HPV 16 (34.3%), followed by HPV 6 and/or 11 (15.9%) and HPV 18 (6.9%). HPV 10-related sequences have been identified in 3 invasive carcinomas. HPV 16 prevailed especially in biopsies of invasive carcinomas (48.4%), CIN II and CIN III (39%). Interestingly, HPV 16 DNA was also detected in 22.5% of the histologically normal epithelia. HPV 6 and/or 11 dominated only in samples from mild dysplasias (26.3%). In about 14% of HPV-positive biopsies mixed infections have been detected. The incidence of HPV 6 and/or 11 in CIN I and HPV 18 in cervical cancers as compared with data from other continents indicates the possibility of considerable geographical fluctuations.
Molecular Characterization of the Genome of Molluscum contagiosum Virus

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Virions of the molluscum contagiosum virus (MCV), a member of the poxviridae, were isolated directly from lesions of individual patients of two countries (Germany and Hongkong) and characterized by restriction enzyme analysis. The comparative analysis of the DNA cleavage patterns of 40 independently isolated virus samples revealed that MCV isolates can be classified into two different types. The majority of MCV isolated from clinically typical skin lesions showed similar DNA cleavage patterns and were termed MCV type 1, whereas one isolate derived from a vaginal lesion showed a completely different DNA cleavage pattern and therefore was termed MCV type 2. To confirm these results, DNAs of MCV type 1 and 2 were separately labelled with $^{32}$P and were cross-hybridized by the Southern blot hybridization method to nonradioactive DNAs of MCV type 1 and 2. This study revealed that both viral DNAs hybridized to each other, indicating their close relationship. For further investigation a defined gene library of MCV DNA sequences was established. The BamHI DNA fragments of the viral genome of MCV type 1 prototype isolate 1/80 and MCV type 2 were inserted into the bacterial plasmid vector pAT153. All cloned BamHI DNA fragments were individually identified by digestion of the recombinant plasmid DNA with different restriction enzymes and screened by hybridization of recombinant plasmid DNAs to viral DNA. Using this defined gene library the relatedness of the DNA sequences of MCV genome to the genome of other members of the poxvirus family can now be investigated in more detail. This study is in progress. This study was supported by a grant from the Förderverein zur Bekämpfung der Viruskrankheiten e.V.

Expression of HPV8 Proteins

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Human Papillomaviruses (HPV) induce benign skin tumors in patients with *Epidermodysplasia verruciformis* (E.v.). About 90% of the E. v. associated skin carcinomas contain HPV5 or HPV8 DNA. Although the viral DNA was shown to persist extrachromosomally in high copy number, no viral proteins could be identified. To raise antisera against proteins from HPV8, we cloned DNA-fragments from open reading frames (ORF) E1, E2, E4, E6, E7 and L1 in procaryotic expression vectors. With the resulting HPV8/β-gal fusion proteins we immunized rabbits and guinea pigs. The sera were used to test protein extracts from an HPV8-induced E.v.-wart and from a plantar wart in immunoblot assays. The anti-L1 serum recognized an HPV8-specific protein in the E.v.-lesion. The molecular weight of 60kD correlates with the size of the expected L1-product. No early proteins could be detected so far.
An Enhancer of Human Papillomavirus 8 is Trans-Activated by the Bovine Papillomavirus 1 E2-Function

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Using the chloramphenicol acetyl transferase (CAT) system subgenomic fragments of HPV 8 DNA were tested for enhancer activity. Such activity could be demonstrated neither in mouse fibroblasts (C127 cells) or in human epithelial cells (HeLa). In BPV1 transformed C127 cells, however, the 1406 bp EcoRI-PvuII-fragment which covers the noncoding region led to a clear increase of CAT gene expression. The fragment was active independent of its orientation both upstream and downstream of the CAT gene. Cotransfection experiments using expression vectors for different BPV1 genes as well as transfection experiments of C127 cells transformed with BPV1 deletion mutants suggest that the E2 gene product of BPV1 has a transactivating effect on the HPV 8 enhancer. Qualitative and quantitative differences were noted between HPV 8 specific transcription in C127 and BPV1 transformed C127 cells indicating that BPV1 influences authentic HPV 8 transcription.

Cloning and Sequencing of a Cellular DNA-Fragment Cross-Hybridizing with HSV-2

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Cross-hybridizing experiments revealed hybridization between the cloned Herpes simplex virus type 2 “joint” region and a 15 kb Hind III fragment of Human Embryonal Lung Cell (HEL-) DNA, persisting under stringent conditions. This cellular fragment was rescued out of a λ-library, and was subcloned into pBR322. The region homologous to viral DNA was first localized on a 1920 bp Sst II subfragment which was subcloned into pNO1523, then on a 230 bp subfragment between a Bgl I and Hpa II restriction site. This fragment, together with adjacent regions, was sequenced by the Maxam-Gilbert method. Sequence analysis revealed a 65% GC-content, an open reading frame of 210 bp, and four “stemloop”-structures. A 120 bp region could be determined, representing a 70% homology to the HSV-2 L-S inversion region sequence. – The most intense cross-hybridization with the viral “joint” region was observed within the Bam HI-G fragment on a 520 bp Sst II subfragment. The nucleotide sequence of this fragment was analyzed. The GC-content totaled to 81%; furthermore 11 “stem-loop”-structures and a 16 × directly repeated sequence of 15 nucleotides was detected. A 3’ 120 bp DNA-sequence, corresponding to the HSV-2 L-S inversion region, showed the highest homology with the cellular DNA.
A Mutation in Bovine Papillomavirus 1 (BPV-1) Open Reading Frame E4 Affects the Persistence of Viral DNA

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We have analysed the functions of the overlapping open reading frames E2 and E4 of BPV-1 by mutagenesis with an XbaI linker carrying a TAG termination codon. Mutations in E2 (Ncol) or E2 and the 3' end of E4 (KpnI) have following features in a C127 cells transfection assay: reduced focus forming activity, integration of the viral DNA and stability of the transformed phenotype. Embryonic C57bl mouse fibroblasts immortalised by these mutants could be grown in soft agar. A mutation near the 5' end of E4 (Thul111) had contrary effect: about 50% focus forming activity of wild type, extrachromosomal persistence of the mutant DNA in varying, partially very high copy number and instability of the transformed phenotype correlated with the loss of the viral DNA. No asynchrony of viral and cellular DNA replication could be observed. We assume that E4 plays a role in long term maintenance of extrachromosomally persisting viral DNA molecules.

Molecular Cloning and Characterization of a Novel Papillomavirus Type from a Patient with Hodgkin’s Disease

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A patient with Hodgkin’s disease developed macular skin lesions 12 years after treatment with X-rays and chemotherapy. DNA was extracted from the lesions and a 7.9 kb papillomavirus genome was cloned into the vector pIC 20H after partial Hind III digest. As seen by Southern blot analysis this isolate showed homology with human papillomavirus (HPV) 14 and to lesser extent with several viruses, which occur in skin lesions of patients with epidermodysplasia verruciformis (EV). The pattern of DNA homology between the cloned HPV and other HPV types differed from that of HPV 14 and the extent of cross-hybridization with HPV 14 as measured by reassociation in liquid phase at $T_m-20$ was below 20%. Both viruses differed in their physical maps of restriction enzyme cleavage sites. This data infer, that the cloned DNA represents a new HPV type belonging to the group of EV-viruses.

Characterization of the Regulatory Regions of Human Papillomaviruses 19 and 25

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Human papillomaviruses (HPV) 19 and 25 belong to the group of epidermodysplasia verruciformis (EV) associated HPVs. In contrast to HPV 5 and 8, HPV 19 and 25 were not
detected in skin carcinomas of these patients. We sequenced the RR of HPV 19 and 25. The sequence analysis of HPV 5, 8, 19 and 25 revealed a strong continuous homology between HPV 19 and 25, whereas HPV 5 and 8 showed insertions and rearrangements. Six sequence motifs are shared by the 4 EV specific HPVs. The palindrome ACCGNNNNCGGT, which is found within the RR of all PVs, occurs 3 times within the EV specific RR. It plays a role as binding site for the E2 protein, that acts as a controlling element for transcription (J. Schiller, pers. comm.). Intermingled with each palindrome we found SV40 late promoter-like sequences. A unique feature of HPV 19 and 25 is a block of over 20 alternating AT residues at the 3’ end of the RR.

Simian Foamy Virus (SVF) Strain LK-3: Cloning of Proviral DNA and Detection of DNA Homologies to SFV Prototypes

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Foamy viruses (spumavirinaes subfamily of the retroviridae) cause persistent infections in various mammalian species, probably including man. SFV strain LK-3 was isolated from an African green monkey and reveals T-lymphotropic properties. In various infected cells proviral LK-3 DNA was detected by hybridization to a 32P-labelled viral cDNA probe prepared by “in vitro” reverse transcription (J. Gen. Virol. 67 (1986) 1993–1999). It was characterized as non integrated, linear, double-stranded DNA of about 14 kb, containing “gaps” sensitive to nuclease S1. Probably two whole genomes are linked by single-stranded DNA to form a tandem structure as described for the visna-lentivirus. Proviral DNA from infected Molt-4 cells was cleaved with XhoI and cloned in lambda L.47.1. Positive plaques were identified by hybridization to the 32P-labelled viral cDNA. An LK-3-specific 3.1 kb insert was isolated and subcloned in pUC 8. With viral insert DNA as a probe, strong hybridization occurred to DNA from cells infected with SFV-3, whereas other SFV prototypes and human syncytium forming virus showed weak hybridization signals under less stringent conditions. – Supported by the Deutsche Forschungsgemeinschaft (Ne 213/4-2).

Characterization of H. saimiri Proteins and their Relatedness to EBV Encoded Polypeptides

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Herpesvirus saimiri shows some similarities with the human lymphotropic Epstein-Barr virus concerning biology and tumorigenicity. Lytic infection with H. saimiri results in the successive production of more than 30 virus-induced proteins. Map positions of the corresponding genes on the viral genome were identified by hybrid-selected translation studies. Most of the map positions found in hybrid-selected translation could be confirmed by
immunoprecipitation with various antisera directed against H. saimiri proteins and some additional gene products were detected. Furthermore we could localize the genomic regions coding for DNA-binding proteins and for the virus-specific thymidylate synthase. In translation experiments with EBV mRNAs two EBV-induced proteins were immunoprecipitated by H. saimiri-specific antisera. Additional EBV-specific sera were found to crossreact with various H. saimiri-induced polypeptides. – Supported by Sander Foundation 82.024.1.

Human Immunodeficiency Viruses (HIV): Comparative Morphology, Antigenic Structure and Virus Cell Interaction

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HTLV-IIIb, LAV-2 and SIV were studied by IEM, surface replica, and thin section EM. During morphogenesis virus core components are assembled concomitant with budding at the cell surface; the spherical RNP-core complex 18 nm in thickness is apposed to the virus envelope. During “maturation” this complex rearranges into a tubular core shell with p24 antigenicity and the RNP-nucleoid. At the inner side of the envelope the p17 membrane protein is located. “Immature” HIV are densely studded with probably 72 surface knobs 15 nm in diameter. On the viral envelope gp41 and gp120 determinants are revealed. Sera from HIV infected persons show a close qualitative correlation between labelling density of the envelope and neutralizing capacity. During virus “maturation” knobs are lost progressively. This shedding is discussed as a possible pathomechanism.

Antigenic Properties of Synthetic Peptides Corresponding to Regions on LAV/HTLV-III Envelope Glycoproteins

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Three peptides corresponding to residues 500–511, 579–589, and 611–620 on LAV/HTLV-III envelope glycoprotein precursor gp160 were synthesized. Criteria for the selection of the peptides were conservation of amino acid residues and hydrophatic character of the region. 107 sera from AIDS-patients, LAV/HTLV-III positive persons and normal donors were tested for the presence of antibodies recognizing the peptides. 91% of the positive sera show reactivity against peptide 500–511, 23% against peptide 579–589, and 16% against peptide 611–620. The peptides were not recognized by sera from the control group. The limits of detection for peptide recognition of positive sera were characteristically below a serum dilution of 1:100,000.
HIV Related Retroviruses of African Green Monkeys

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There is as yet no real animal model for AIDS. During the course of our studies of STLV-I in various primate species it was observed that some of the animals, notably among the African Green Monkey and Baboons, also possessed antibodies that reacted with HIV in ELISAs and Western blots. - The African Green Monkeys (Cercopithecus aethiops) raised in a closed colony at the Paul Ehrlich-Institute are more than 50% seropositive for HIV, but obviously healthy. Viruses were isolated from these animals (STLV-III AGM) and were shown to be cytopathogenic for cultured T-lymphocytes of human and macaque origin. Thus far however, the isolates replicate in low titers only in vitro. Efforts to characterize STLV-III AGM proteins and DNA and in vivo inoculation of STLV-III AGM into macaques have been initiated. - The availability of STLV-III AGM allows to address the question why African Green Monkeys remain healthy, although this virus is cytopathogenic in vitro. If STLV-III AGM turns out to be able to induce AIDS-like disease (SAIDS), a model would be at hand for in vivo therapeutic and vaccination studies.

Comparison of the Cytopathic Effects Induced by German HIV in Different Cell Systems

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Human immunodeficiency viruses (HIV) obtained from AIDS-or LAS patients showed differences in cytopathic effects(CPE) which they induced in fresh peripheral lymphocytes (1). - Two of the cytopathic isolates from German patients were used in this study to compare the case of infection, virus-production and cytopathic effects for different lines of T-lymphocytes (Molt 4, CEM, HUT 78), for HL 60, for freshly prepared cord lymphocytes as well as for cord lymphocytes which were immortalized by STLV I. - Some of the uninfected cell lines produced spontaneous syncytia. As a consequence, the production of the cytopathic effects of the viruses was difficult to judge based on the morphology of the cells alone. Cell-vitality, therefore, was determined using a fluorescence technique which allowed to readily distinguish between live and dead cells. - All of the established cell lines proved to be much more resistant to infection by both virus strains than freshly prepared cord lymphocytes, but even between cell lines of similar origin, significant differences in infectability as well as virus production were observed. These experiments demonstrate that the production of HIV is strongly dependent on cellular events. –

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Detection and Serological Classification of Simian Immunodeficiency Viruses

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Simian immunodeficiency viruses (SIV) are relevant to understand the origin of the human immunodeficiency virus (HIV) and to establish animal models for human AIDS. We have examined 856 sera of 34 nonhuman primate species for antibodies to HIV and SIV by a combination of ELISA and radioimmunoprecipitation (RIP). Sera of green monkeys and three closely related species were frequently positive in both assays. SIV isolates from green monkeys, sooty mangabeys, and rhesus monkeys were classified and compared with HIV by crossreactions of respective antisera in the RIP assay. With respect to the peripheral envelope glycoprotein the simian viruses represent a subgroup different from HIV since the simian sera reacted with the homologous and heterologous glycoproteins of the SIV isolates but not with gp120 of HIV. The core polypeptides were bidirectionally crossreactive between each of the four viruses with the exception that sera of green monkeys lacked antibodies to any core polypeptides. However all isolates were distinguishable by tryptic peptide maps of the core polypeptides p18 and p24. (see abstract Jurkiewicz et al.).

Evidence for Strong Biological and Genetic Variation of HIV

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Human immunodeficiency viruses (HIV = LAV, HTLV III, ARV or AAV) were cultured from either peripheral blood lymphocytes (PBL), plasma, whole blood or cerebrospinal fluid (CSF) of AIDS-, ARC- and asymptomatic patients mainly from Frankfurt. The isolates were shown to be LAV/HTLV III-related by reverse transcriptase tests, crossreactivity in immunofluorescence assays, electron microscopy and Southern blot analysis, probed with the HTLV III-specific probe lambda BH10. When the HIV isolates were grown on fresh human PBL striking differences in the morphology of the cytopathic effect induced in the PBL were found among them, which were also reflected in the virus titres and the speed of growth. In immunofluorescence assays the HIV isolates showed different sensitivities for detecting virus-antibodies from patients sera. Restriction analysis of a molecularly cloned isolate revealed differences to the published restriction patterns of cloned HIV, and multiple variants within a given patient. - The observed variations between isolates from a confined area argue for a high mutation rate of HIV. The question is raised whether the very different clinical courses of infection with HIV correlate with particular subtypes. Furthermore the development of effective vaccines against HIV will depend on availability and characterization of a wide spectrum of variants. Further studies will have to show which epitop of the individual mutants are important for neutralizing antibodies.
Structure, Antigenic Epitopes and Variety of the HIV-1 Envelope Protein

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Independent isolates of human immunodeficiency virus (HIV) exhibit a striking genomic diversity, most of which is located in the viral envelope gene. Since this property of the HIV group of viruses may play an important role in the pathobiology of HIV, we analyzed the amino acid sequences of the envelope proteins of eleven different viral strains, three of which represent sequential isolates from a single patient. Using a computer program that predicts the secondary protein structure and superimposes values for hydrophilicity, surface probability and flexibility, we identified several potential antigenic sites in the envelope proteins of HIV. Interestingly, the majority of these predicted epitopes in the exterior envelope protein (gp120) were found in regions of high sequence variability among the independent and also in the sequential viral isolates which are interspersed with highly conserved regions; about 80% of the amino acids were found to be conserved and only two antigenic sites could be identified. These findings give insight into the secondary and possible tertiary structure of variant HIV envelope proteins and should facilitate experimental approaches directed towards identifying and fine mapping of HIV envelope proteins. – Supported in part by BMFT II-005-86 A102.

Purification and Drug Mediated Inhibition of HIV Transcriptase and Cellular DNA Polymerases Alpha, Beta and Gamma

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Reverse transcriptase (RNA dependent DNA polymerase, RDDP) was isolated from density gradient banded Human Immunodeficiency Virus (HIV) by four chromatographic steps. Enzymatic (template primer and ion requirements) and kinetic parameters (Michaelis constant, Hill coefficient for several substrates) were determined. – In order to find inhibitors which are specific for HIV RDDP the effect on HIV RDDP and on the partially purified cellular DNA polymerases of a large number of drugs was tested and compared by the inhibitory dose (ID50), the concentration of the drug at which the enzymatic activity is inhibited by 50%. Most known RDDP inhibitors are equally or even more active on cellular polymerases. In contrast, tetracyclines exhibit a relatively specific effect on HIV RDDP. However, for therapeutic applications a substance has to be found, which shows a very specific inhibitory effect on HIV RDDP and nearly no effect on the cellular polymerases. Under all substances tested, only phosphonoformic acid and its derivatives which are examined in collaboration with ASTRA (Sweden) fulfills this requirement.

11 Zbl. Bakt. Hyg. A 267/1
Tissue Tropism of Borna Disease Virus

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Borna Disease Virus (BDV) has been regarded until recently as a strictly neurotropic agent that propagates along neural pathways. The development of monoclonal antibodies against viral antigens and of highly sensitive immunohistochemical techniques allowed the assessment of the exact distribution of viral antigens in the neural and non-neural tissues. In the nervous system (NS) virus specific antigen could be demonstrated beside neurons in astrocytes, oligodendroglial cells, ependyma, choroid plexus epithelium and in the pigment epithelium of the eye. In the peripheral NS axons were invariably positive, but the Schwann cells proved to be consequently non-permissive to BDV. Among non-neural tissues, the lacrimal, sebaceous and salivary glands, smooth muscle cells, epidermis, pituitary and adrenal glands and the brown fat were permissive to BDV-infection, while the liver, kidneys, lungs and skeletal muscle were not. For the elective vulnerability of these tissues and cell types the specific distribution of surface receptors may offer a plausible explanation.

Spread of Borna Disease Virus in the CNS After Ocular Inoculation

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Borna Disease is a slow virus infection. The distribution of the virus is mostly restricted to the nervous system (neurons, glial cells). – Following ocular inoculation of rats with Borna Disease Virus brain infection ensues by a centripetal spread of the agent mainly along the optic nerve. Borna specific antigen can be determined immunohistochemically first 26 days in the nuclei of neurons of the central nervous system. – Despite the unilateral inoculation, immunoreactive cells appear to be localized symmetrically in the diencephalon and the brain stem; this phase is followed by the rapid spread of Borna Disease Virus over the whole brain. Beside axonal and transsynaptic mechanisms oligodendrocytes are probably also involved in the propagation of infection.

Amyloidosis in Scrapie Infected Hamsters

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Protein deposits which are host encoded and have all the properties of amyloids are found in brains of scrapie infected hamsters. These so-called Scrapie-associated-fibrils (SAF) are absent from scrapie-spleens, when compared with brain materials of similar scrapie titers. –
With a delay of approximately 20 days the formation of SAF-protein in intraperitoneal infected hamsters follows a dramatic increase of scrapie titer up to a plateau in brains. Clinical signs start only after a considerable amount of SAF has accumulated. According to these observations infection with scrapie leads to a lethal, organ specific amyloidosis.

Characterization of the Major Immunogenic Components in Borna Disease Virus Infections

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Borna Disease (BD) virus infections lead either to fatal encephalomyelitides (horse, rabbit, adult rat) or life-long persistencies without disease (1-day old mouse, rat, chicken). Independently from disease or tolerant infection all BD virus infected animals react with a strong humoral immune response. The first and most prominent answer is directed against a complex of virus-induced proteins, the s (soluble)-antigen (s-ag). S-ag enriched supernatants can be prepared from infected brains by sonication and ultracentrifugation at 100,000 x g. Immunogenic s-ag components were characterized by Western-blot-analyses. We found (1) that this antigen complex consists of two major proteins with apparent molecular weights of 60 and 40 KD, (2) that these proteins are recognized by polyclonal serum- as well as oligoclonal CSF-antibodies from all tested species (rabbit, horse, mouse, rat, rhesus monkey), and (3) that monoclonal antibodies equally well react with epitopes on s-ag proteins. Two out of three monoclonal antibodies bind to the 60 KD protein. The reactivity pattern to s-ag components could not be correlated with the presence or absence of the neutralizing capacity of those antibodies.

Antigen Expression in Borna Disease Virus-Infected Rat Astrocytes in vitro

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The fact, that class II MHC-antigen (Ia) is expressed in the brain of Borna disease virus (BDV)-infected rats, stimulated in vitro investigations employing astrocytes. Astrocytes can be induced in vitro to express Ia-antigen by γ-Interferon (IFN-γ) or, as it has recently been reported, after Coronavirus-infection (1). Therefore, we studied the expression of Ia-antigen on the surface of persistently BDV-infected astrocytes in vitro. The cells were obtained from the brain of newborn Lewis rats and were cultured for three weeks before being infected with BDV. They were found to be positive for both glial fibrillary acidic protein (GFAP) and BDV-specific intracellular antigens, using immunofluorescence and peroxidase-antiperoxidase technique. Infected astrocytes did not express Ia-antigen on their surface, but could be induced to express Ia-antigen after treatment with recombinant IFN-γ or Concanavalin-A supernatant. We therefore conclude that induction of Ia-antigen on astrocytes is not a general consequence of viral infections.

References
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PDGF-Like Molecules in Conditioned Medium from SSV-Transformed Cells Lead to Growth-Stimulation and Transformation of Normal Cells

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We have reported an immunological relationship between human PDGF and the SSV transformation-specific glycopeptide (SSV-TrSgp) (Virology 136 (1984) 414–424). SSV-TrSgp represents a proteoglycan-like molecule which is released from SSV-transformed cells. With regard to the putative biological activity of this molecule we found that non-fractionated cells, serum and virus free tissue culture supernatant shows growth-stimulating activity with PDGF-like characteristics on NIH 3T3 cells. Furthermore, the same tissue culture supernatant induces anchorage-independent growth of NRK cells and causes dramatic morphological alterations of various cell lines and non-established fibroblasts. Evidence that the SSV-TrSgp is involved in these processes comes from two additional findings: 1) After fractionation of supernatant from SSV-NP cells by gel filtration a major growth-stimulating and transforming activity is present in the void volume (> 150 kD); 2) Using the same supernatant for preparative SDS-PAGE a high-molecular-weight (> 200 kD) growth-stimulating activity can be electroeluted.

RNA-dependent RNA Polymerase Activity in Infectious Bursal Disease Virus (IBDV)

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IBDV is a member of the family Birnaviridae. Naked icosahedral virus particles are single-shelled; they are composed of several polypeptides and contain two segments of double-stranded (ds) RNA with mol. wts. of 2.2 x 10^6 and 1.9 x 10^6. An RNA dependent RNA polymerase activity is associated with infectious IBDV particles. To induce enzymic activity it is essential to remove Ca^{2+}-ions; however, Mg^{2+}-ions have to be present which are superior to Mn^{2+}. Enzyme activity is enhanced by non-ionic detergent and pH 8.5. At 41°C, the reaction is linear with time for at least one hour, then reaches a plateau 2 to 3 h later, and finally ceases after about 5 h. Reaction products are 14S dsRNA as virion RNA and 24S single-stranded RNA hybridizing to both genome segments. In view of the nature of the products, IBDV polymerase acts as transcriptase as well as replicase.

Genome of Infectious Bursal Disease Virus (IBDV): Electron Microscopic Investigations and Demonstration of a Genome-Linked Protein

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The two double-stranded (ds) RNA segments of IBDV were released from virus particles by treatment with proteinase K in the presence of SDS and were then investigated with the electron microscope. Linear dsRNA molecules were observed, and their lengths were com-
pared with those of the completely sequenced reovirus type 3 segments M3 and S2. Mol.
wts. of $2.2 \times 10^6$ and $1.9 \times 10^6$ were calculated. These results indicate that the genomes of
the other members of the family Birnaviridae are also smaller than those reported. As seen
under the electron microscope, heating of IBDV particles in the presence of SDS without
proteinase K results in dsRNA molecules which are circularized by protein. Electrophoresis
of radioactively labeled particles in different gel systems indicates that this protein is the
90kd structural protein of IBDV which is supposed to represent all or part of the RNA
dependent RNA polymerase.

Identification of the Fusogenic Protein of Semliki Forest Virus (SFV)

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*Aedes albopictus* cells infected with SFV can be induced to fuse from within at pH 6. We
have applied hydrophilic reagents to covalently modify the proteins at the cell surface. Each
reagent was applied at pH 7 to cultures before the pH was lowered and to cultures after the
cells had been exposed to pH 6 at 4°C. Several chemicals reacting with amino-, guanidino-
sulfide- and disulfide groups inhibited the fusion only when applied after low pH exposure.
On the basis of these results, which implicate that low pH induces a conformational change
of a protein, radioactive reagents were utilized and the SFV E1 envelope protein could be
identified as the protein undergoing the conformational change.

Molecular Cloning of the Complete Genome of Insect Iridescent Virus Type 6
and Construction of the Physical Map of the Viral DNA; Further Evidence for
Circular Permutation and Terminal Redundancy of the Viral Genome

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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. Upon denaturation and reannealing of
native linear CIV DNA (238 kbp), duplex DNA circles of a smaller size (211 kbp) with
protruding tails were formed. A defined and complete gene library of the whole CIV DNA
sequences was established using the insertion of 31 EcoRI DNA fragment (A to e') into the
corresponding site of the plasmid vector pACYC184. Furthermore the CIV DNA was
cleaved with restriction endonucleases BamHI, Ncol, Sall, Sphi, and/or double digested with
BamHI/Sall, and the resulting DNA fragments were inserted in the BamHI, BamHI/Sall, and
Sphi sites of the bacterial plasmid vector pAT153 and in Ncol site of the plasmid vector
pKm2. All cloned fragments were individually identified by hybridization experiments. The
physical map of the viral DNA was constructed for restriction endonucleases ApaI, SmaI,
BamHI, Sall, and Ncol. Although the CIV genome is linear, due to circular permutation the
Analysis of the Repetitive DNA Sequences in the Genome of Fish Lymphocystis Disease Virus

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The Fish Lymphocystis Disease Virus (FLDV), a member of the iridovirus family, caused an important fish disease, which is characterized by papilloma-like lesions. The genome structure of FLDV was found to be circularly permuted and terminally redundant. A defined and complete gene library representing 100% of DNA sequences of the viral genome was established. This gene library contains e.g. the complete EcoRI DNA fragments (A to M) of the viral genome. The individual recombinant plasmids were used for further characterization and structural analyses of the viral genome. Detailed Southern blot hybridization experiments revealed the presence of repetitive DNA sequences located within the EcoRI DNA fragment M (1.5 kbp) at 0.718 to 0.736 viral map units and the EcoRI DNA fragment B (12.7 kbp) at 0.034 to 0.165 viral map units. The fine mapping of EcoRI DNA fragments B and M revealed that only a part of the DNA sequence of EcoRI DNA fragment B spanning the map coordinates 0.034 to 0.057 is homologous to the DNA sequences of EcoRI DNA fragment M. Further heteroduplex mapping, DNA hybridization experiments, and molecular subcloning of the EcoRI DNA fragments B and M confirmed that nearly the complete DNA sequences of EcoRI DNA fragment M (0.718 to 0.736 mu) are identical to the DNA sequences of an EcoRI/PstI DNA fragment (0.034 to 0.057 mu) located in the EcoRI DNA fragment B (0.034 to 0.165 mu). The further characterization of these repetitive DNA elements by nucleotide sequencing is necessary for the understanding of the functional activity of these DNA elements. – This study was supported by the Deutsche Forschungsgemeinschaft, project Da 142/2-1.

Diagnostic Tests for the Detection of Infections with the Human Parvovirus B19

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The Human Parvovirus B19 is the causative agent of erythema infectiosum. During the viraemia viral DNA can be detected by DNA hybridisation. A 700 kb DNA fragment of the parvovirus genome, cloned in M13 and pGem was used as radioactive probe. With this technique a parvovirus positive blood probe could be detected which serves as antigen
source for the establishment of an IgM/IgG and antigen capture assay. A flatbottom microtiter plate was coated with anti-\( \gamma \) resp. anti-\( \lambda \), incubated with test sera, antigen and a monoclonal anti-Parvovirus B19 antibody. After a further incubation with a peroxidase-conjugated antimouse IgG orthophenylendiamin and perhydrol were added, the enzymatic reaction stopped with 1N H\(_2\)SO\(_4\) and the optical density measured. For the detection of viral antigen a strongly anti-parvo positive serum was coated, test serum added and the procedure performed as described above. Comparing the antigen ELISA to the DNA-hybridisation, the DNA-hybridisation has a 1000 fold higher sensitivity and 0.06 pg/ml DNA could be detected. The detection of specific IgM7IgG enables a serological diagnosis of an acute and past parvovirus infection. Blood donations can be screened with the antigen capture assay.

Neutralization Antigenic Sites of Foot- and Mouth Disease Virus (FMDV)

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Using FMDV strain O;K, neutralizing monoclonal antibodies (nmab) with four different specificities were isolated. The nmab were first characterized with regard to their neutralization pattern. Two nmab showed a quite narrow but different neutralization pattern. In contrast, the third nmab reacted with all O subtypes tested, but not with the serotypes A and C. Crossneutralization with certain A and C subtypes was observed with the fourth nmab. The latter two nmab reacted with VP1 by Western blotting, and by ELISA with a synthetic peptide whose sequence (AA 139–160 of VP1 from O;K) has been shown to be important for the induction of neutralizing antibodies. The respective experiments with the two narrow reacting nmab were negative. Thus, two nmab may recognize a continuous sequence in a defined region of VP1. This hypothesis can now be tested directly since neutralization resistant variants have been isolated. Preliminary respective nucleic acid sequencing data will be presented.

Analysis of the HSV-2 Induced Suppression of Humoral Antibody Formation by B-Cell Enumeration

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HSV-2 but not HSV-1 induces suppression of humoral antibody formation against HSV-1 and 2 as well as SRBC and HBs-antigen. I.P.-injection of silica partially release this suppression; therefore MØ are the first target (J. gen. Virol. 67 (1986) 1015). In order to analyse more exactly the mechanism of this suppression, we determined the number of HSV-specific IgM- or IgG producing B-cells. Primary or homologous and heterologous secondary infections (high and low dosis) with HSV-1 induce a phase of “nonreactivity” of the spleen at day 8 against a high dose; Secondary infections by HSV-1 at day 8 are manifested by no antibody secreting B-cells in the spleen, humoral antibody response how-
ever is regular. At day 21 a typical B-cell IgG-booster response is manifested in the spleen. The HSV-2 induced suppression wanes more than 50 days p.i. This indicates that antigen presentation and memory cells are stable, only the switch to IgM or IgG production seems to be damaged by HSV-2. This was confirmed by analysis of the HSV-specific very low B-cell response: Some specific B-cells appear very late if compared to HSV-1. Some suppressor T-cells also seem to be produced by HSV-2. Suppression was demonstrated up to 50 days after infection with HSV-2. Generally HSV-2 induces a low total spleen cell response if compared to HSV-1.

The Genome of Mycoplasmavirus L3 is Circularly Permutated and Terminally Redundant

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MVL3 is a polyhedral virus with a diameter of about 60 nm. The viral genome is a linear double-stranded DNA of 26·10^6 mol wt. The infection is non-lytic but cytocidal. Circular permutation and terminal redundancy are shown by three different methods. – (1) Restriction patterns of L3 DNA show a nearly constant decrease in mol wt of some fragments. The difference equals 8% of the total genome size, indicating the shift of the permutation. (2) The determination of Eco RI binding sites on L3 DNA by electron microscopy reveals three populations of molecules. The ends from one class to the other were shifted by 1 μm, i.e. 8% of the genome size. (3) Treatment with Exonuclease III, generating protruding single-stranded ends, followed by renaturation, showed homology in the terminal regions. The homology extends to about 8% of the genome length.

Expression of Protooncogenes in Embryonic, Adult and Neoplastic Tissue of Xiphophorus

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The genome of Xiphophorus contains several protooncogenes which are homologous to the transforming genes of certain retroviruses. Specific crossing between different genotypes of Xiphophorus lead to spontaneous formation of benign and malignant melanoma in the offspring. In order to obtain a better understanding of the functions of protooncogenes and oncogenes in nontransformed and in neoplastically transformed cells respectively, we studied the expression of c-src, c-ras, c-sis, c-erbA, c-erbB and c-myc during embryogenesis, in adult tissue and in melanoma of Xiphophorus. During embryogenesis c-src, c-ras and c-sis are differential expressed. In adults c-ras and c-myc are tissue-specifically expressed and show no overexpression in melanoma. c-src is also expressed with tissue-specificity, the highest level was found in melanoma. High levels of c-erbB and c-sis related mRNA's were detected only in melanoma cells. This data indicate a normal function of protooncogenes more in differentiation than in proliferation and coordinated deregulation of several oncogenes during melanoma formation.
Phage DNA Restriction Enzyme Analysis as a Tool for Epidemiological Typing. C. diphtheriae as an Example

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Epidemiological classification of pathogens allows to monitor person to person spread. An increasingly used method in this context consists in analysis of DNA restriction enzyme patterns. Since toxigenic C. diphtheriae are always lysogenized, we applied this method to analyse phage DNA. – In one approach, DNA of UV-induced and replicated phages was digested with restriction enzymes. The obtained fragments were submitted to agarose gel electrophoresis and visualized by ethidium bromide staining. Another method consisted in digestion of prophage DNA. The various fragments then obtained by agarose gel electrophoresis were transferred to nitrocellulose and hybridized with a $^{32}$P-labeled phage DNA. – Prophage DNA analysis even allows to typify strains that are phage resistant and non lysogenic and therefore cannot be investigated by phage typing. Further studies with isolates from epidemics in Germany and Sweden are to prove that this method is a meaningful extention of conventional assays.

Autoimmunity After Viral Infection: Production of Monoclonal Antibodies Against Golgi-Antigen After Infection of Mice with the Anti-Golgi Inducing Agent (“AGIA”)

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Recently we described Golgi autoantibodies induced by a transmissible agent in the mouse system. Further investigation showed, that Golgi antibodies were present in all infected animals already at day 8 p.i. with a maximum between two and four weeks p.i. Sometimes autoantibodies against cytoskeletal elements and mitochondrial structures could also be detected. In order to characterize the autoantigens, monoclonal antibodies were established. The highest yield of hybridomas producing Golgi autoantibodies was obtained 9–14 days after infection. We also succeeded in producing monoclonal antibodies against other cellular antigens. Using poly- and monoclonal antibodies in immunoelectron microscopy we were able to decorate the Golgi apparatus. Some Golgi antibodies specifically immunoprecipitated antigen of $^{35}$S-labelled culture cells.

A Parapoxvirus Infection in Harbour Seals (Phoca vitulina) in a Rearing Station at the North Sea Coast in Germany

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In a rearing station for Harbour Seals (Phoca vitulina) at the German North Sea coast a pox disease with proliferative skin alterations was observed during the time between July
and November 1985. All 27 seals got infested. In thin sections of biopsy skin specimens poxvirus inclusion bodies of type B were found. By negative staining of triturated suspensions of these biopsy specimens typical parapoxvirus particles were detected. So far all attempts failed to propagate the virus in embryonic cells of sheep skin and kidney. Inquiries in three other rearing stations along the North Sea coast revealed that similar skin alterations had been seen in at least two places, namely at Norden (summer 1985) and at Pieterburen/Netherlands.

Characterization of a Poxvirus Isolated from a Child

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A 6-year-old girl at Lüneburg/Lower Saxony, who had not been vaccinated against smallpox, developed a skin lesion with central necrosis at the left hand in April 1985. An orthopoxvirus strain was isolated by Nasemann et al. (1986). Since this strain produced efflorescences of 1.5 to 2.0 mm in diameter with a haemorrhagic center on the chorioallantoic membrane and cytoplasmic inclusion bodies of type A V+, it was incorporated into the group of cowpoxlike viruses and termed H-CP-LSax. The DNA restriction patterns of this virus strain were compared with those of other orthopoxviruses using the endonucleases BamH I, Hind III, Sma I, Sac I, and Kpn I. The Sma I and Hind III fragmentation patterns of the isolate H-CP-LSax were nearly identical with those of the cowpoxlike virus strain EP 267 which had been isolated from an Asian Elephant (Elephas maximus) at the zoological garden at Frankfurt in November 1977. The girl had no contact with zoo animals. She lived in close contact with some pet animals like cats, rabbits, a dog, and a guinea pig.

Determination of Viral Glycoprotein Antigen with the ELISA as an in-vitro Assay for the Potency of Rabies Vaccines

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The content of rabies virus glycoprotein antigen (glp-ag) is correlated with the immunogenicity of tissue culture vaccines against rabies. An ELISA based on the determination of the glp-ag with monospecific, polyclonal anti-glp-antibodies directed against the rabies virus strains ERA, Flury LEP and PV therefore may replace the invivo protection test in the mouse (NIH-test). – Such an ELISA was standardized, which is based on the fixation of different vaccine dilutions starting at 1:16 at the solid phase of microtitre plates, reaction with an excess of anti glp-antiserum for 1 h at +37°C, a following incubation with a peroxidase coupled anti-IgG for 1 h at +37°C and measuring the reaction at 490 nm 30 minutes after addition of the substrate. – Under these conditions we were able to determine the potency of tissue culture rabies vaccines of different virus strain characteristic and procedure of production, tissue culture supernatants, vaccine concentrates, vaccines after
heat treatment and after adsorption to Al(OH)₃. The glp-ag of unknown samples was tested in parallel with a reference vaccine of known potency and quantitated with the parallel line bioassay according to Finney (in “Statistical Method in Biological Assay”, 3.ed., Ch. Griffin and Comp., London and High Wycombe, 1978). – ELISA results were correlated with the results obtained in the NIH-test in a range of 0.3 to 700 IU. Glp-ag was expressed in BHK-cells at 100 h and increased to a maximum at 200 h after infection. – Thermostability of lyophilized vaccines was demonstrated for the PCEC- and Vero-vaccines at +37°C for 120 days and at +56°C for 24 h. However, if vaccines were stored in liquid form potency decreased rapidly in less than 4 h at +56°C. Stability of the glp-ag at +37°C seemed to be influenced by the stabilizer of the vaccine. First results showed a stable glp-antigenicity of the resuspended PCEC vaccine for 120 days at +37°C, but decreasing glp-antigenicity for the resuspended Vero vaccine after 30 days storage at +37°C. In tropics therefore prefer lyophilized vaccines.

Generation of Human Anti-Rubella Monoclonal Antibodies from Human Hybridomas Constructed with Antigen-Specific Epstein-Barr Virus Transformed Cell Lines

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Human monoclonal antibodies (humab) directed against viral antigens were developed by combining immortalization of human primary B lymphocytes by Epstein-Barr virus (EBV) infection with consecutive fusion of selected immortalized lymphoblasts with an established, human lymphoblastoid cell line. Among the antiviral humab producing hybridomas thus generated, were three which produced anti-rubella humab. Rubella virus consists of three structural proteins, the core protein C and the two envelope proteins E1 and E2. By the Western blot technique we were able to show that two humab reacted with the core protein and the third humab with the envelope protein E1. From the hybridomas grown in stationary cultures, highly purified humab preparations were obtained by subjecting the concentrated culture supernatant to immuno-affinity chromatography.

Coronavirus JHM: Selection of Neurovirulence Variants with Monoclonal Antibodies

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Coronavirus JHM infection of rodents can lead to diseases of the central nervous system accompanied by demyelination. This model is important for studies of pathogenesis. The outcome of infection depends on properties of the virus. Virulent and avirulent JHM-viruses are differentiable by the peplomer protein E2. Functions of E2 are attachment, binding of neutralising antibodies and fusion. – To learn more on relations between structure and virulence, we used monoclonal antibodies (MAB’s) to select JHM-variants changed in de-
finded E2-epitopes. Infection of hybridoma lines, which produce neutralising MAB's, leads to selection of JHM-variants which escape neutralisation. No other antigenic or structural changes are detectable. Four different groups of JHM-variants were selected corresponding to epitopes associated with neutralisation and cell fusion. Mice infected with variants changed in epitope E2-Ba develop predominantly chronic disease. JHM-variants changed in one of the other major epitopes cause acute disease like JHM-wild type virus. These data indicate that virulence is associated with defined domains of the peplomer protein E2.

Specific Initiation of DNA Replication in-vitro by DNA Polymerase α-holoenzyme on the Circular Single-Stranded DNA Genome of Porcine Circovirus

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The main replicase in higher eukaryotic cells DNA polymerase α, contains a tightly associated DNA primase. It was demonstrated that this enzyme is able to initiate DNA replication in-vitro on multiple sites of cloned single-stranded SV40 DNA or on single-stranded parvoviral DNA. In contrast we have used the naturally occuring single-stranded circular porcine circo-virus (PCV) DNA to investigate initiation of DNA replication. Purified DNA polymerase α-holoenzyme from calf thymus primes specifically at one preferred site on this eukaryotic, in cells naturally occurring as single-stranded DNA template.

BFDV, Budgerigar Fledgling Disease Virus- an Avian Polyomavirus

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The structural characteristics of budgerigar fledgling disease (BFDV) were analyzed by biochemical and biophysical methods. The results obtained justify classification of BFDV as a member of the polyomaviruses, a papovavirus subgroup. BFDV is related to all members of this subgroup, but is not identical with anyone of them; therefore, it is a new and the first nonmammalian polyomavirus. The virus was able to transform hamster embryo cells in vitro which is a typical feature of polyomaviruses. However, while most polyomaviruses known until now only cause persistent infections without any clinical symptoms in their natural hosts, BFDV is the etiological agent of an acute disease resulting in high death rates of affected birds.

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An Increase of the Intracellular Calcium Concentration Leads to a Disassembly of Budding Measles Virus at the Plasma Membrane

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We have shown recently that the budding of measles virus is connected with a polar growth of actin filaments. Calcium ions are known to play a key role in the regulation of assembly and disassembly of actin filaments as well as in the regulation of contractility. Therefore calcium ions may also influence the budding process of measles virus. We modulated the calcium concentration in measles virus infected cells by use of the calcium ionophore A23187. In the presence of calcium, but not of magnesium or barium the ionophore induced (1) a disappearance of virus structures and microvilli from the cell surface, (2) a random redistribution of virus hemagglutinin at the cell surface, (3) a dissociation of nucleocapsids from the protoplasmic membrane face, and (4) a significant reduction of the cell bound infectivity. The data indicate, that calcium ions are able to influence the morphology of budding virus particles at the plasma membrane and may play a role in virus morphogenesis. – Supported in part by Gemeinnützige Hertie-Stiftung, Frankfurt/M.