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Abstracts of Papers Presented at the 42nd Meeting of the Deutsche Gesellschaft für Hygiene und Mikrobiologie Hannover, October 4–6, 1989

Section Virology

Serologic Variants of Adenoviruses

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Many adenovirus field strains exhibit serological relationship to two different prototypes. Differences were found by SN and HI. Examples are intermediate strains (15/H9, 17/H29; H = Hemagglutinin), doubly neutralizable strains (3+16/H16, 11+35/H35), or strains cross-reacting in HI with two different types (21/H21+35). The variants were further characterized by DNA restriction analysis using at least seven endonucleases. While the DNA of serologically identical strains showed slight variations, only, the comparison of the variants with the serologically related prototypes resulted in only moderate DNA relationship (50 to 70% comigrating fragments, with few exceptions). It is possible that the serologic variants have originated by recombination of two prototype-like strains. Respective data will be presented for AV15/H9 intermediate strains.

Human Retroposon S71 Contains a Representative of a New Class of Repetitive Sequences Expressed in Human Tissue

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S71 contains a human endogenous retroviral element related to simian sarcomavirus (SSV) and its associated virus SSAV. By sequence analysis and comparison with the corresponding SSV/SSAV sequences the genomic organization of S71 element was determined to be 5' gag–S-NRS-pol–LTR 3'. S-NRS represents a region of 1130 bp in S71 that consists of nonretroviral sequences. A probe containing the complete S71 element was used to screen two human cDNA libraries under low-stringency conditions. 21 Clones were isolated which had yielded strong hybridisation signals with the S71 probe. Hybridisation of these clones with different fragments of the S71 genome revealed that 12 of these clones contain S-NRS related sequences. Five of the isolated clones were sequenced and compared with S71. One
of them contains a homologous to the U3-region of the S71 LTR. The outer four clones sequences with 65–82% identity to S71 S-NRS on nucleotide level. The deduced amino acid sequences of the four S-NRS clones are only 30–70% homologous to S71 S-NRS. None of the sequences represent a contiguous open reading frame. These results indicate that S-NRSs probably do not have protein encoding function. Hybridisation of human genomic DNA shows that S-NRS belongs to a multicopy family of related sequences.

A Molecular Clone for the Human Spumaretrovirus with Biological Activity

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Human immunodeficiency viruses, human T lymphotropic viruses, and the human spumaretrovirus (HSRV) constitute the natural occurring human retroviruses. Molecular cloning and sequencing of HSRV revealed four open reading frames (ORFs) additional to gag, pol, and env. Three additional ORFs, bel 1–3, are located between the env gene and the 3'LTR, one additional ORF, s1, is located in the central region of the genome. Functional analysis of the bel and s genes requires an infectious molecular clone of HSRV, which was not available so far. We have constructed such a clone (pHSRV). The biological activity of the clone was confirmed by a variety of criteria: induction of characteristic CPE in susceptible cells infected with cell-free supernatant from cultures transfected with pHSRV; indirect immunofluorescence; radioimmunoprecipitation of viral proteins and electron microscopy. Furthermore, it is shown that pHSRV is able to transactivate the HSRV-LTR.

A Short Repetitive Nucleotide Sequence Causes Size Heterogeneity of Bovine Herpesvirus Glycoprotein IV

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Glycoprotein IV of bovine herpesvirus 1 (BHV-1) is one of the major immunogenic glycoproteins of the viral envelope and is involved in absorption and/or penetration of the virus. It is essential for the production of infectious virus and cannot be deleted from the viral genome. In contrast to the analogous proteins of herpes simplex virus (gD) and pseudorabies virus (gp50) the gIV of BHV-1 shows a size heterogeneity among different strains. This indicates that in at least one part of the polypeptide backbone the aminoacid composition is variable. Comparison of the nucleotide sequences of four BHV-1 strains revealed that the size heterogeneity in these strains is due to different repetitions of a 90 bp sequence, which is located upstream the sequence coding the membrane spanning domaine of gIV. We are currently testing the possibility to introduce heterologous sequences into this part of gIV to analyse whether the gIV can contain antigenic epitopes of different viruses without affecting the functional activity of the gIV.
Investigation on the Prevalence and Clinical Course of Delta Virus Infection in South-West-Germany

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Aim of this investigation was to answer three questions: 1. Prevalence of Delta hepatitis in South-West-Germany? 2. Prevalence of inapparent HDV infection? 3. Is the clinical course of Delta hepatitis different from that of hepatitis B?

Material and methods: Six hundred and fifty-three HBsAg pos. and one hundred and fifty-two HBsAg neg./anti-HBc/HIV pos. sera were reinvestigated for anti-Delta IgG and IgM (DELTAK, DELTA-IGMK, SORIN). Data on the clinical course were taken from patients history.

Results and discussion: The prevalence of Delta hepatitis is 4.3% (28/653) in HBsAg pos. patients. The prevalence of inapparent HDV infection in HBsAg neg./anti-HBc pos. drug addicts is 4.6% (7/152). From this identical prevalence of HDV infections with apparent and unapparent HBV infections, we conclude, that about every other HDV infection is correlated with clinical hepatitis. Anti-Delta IgM was detected in 22 of the 28 HBsAg/anti-Delta pos. sera as well as in 2 of 7 anti-Delta/anti-HBc pos. sera of HBsAg neg. drug addicts. Autoantibodies were detected in 82% of the patients (23/28). All cases, in which liver biopsies were done, demonstrated anti-Delta IgM and autoantibodies. Thus, the detection of anti-Delta IgM and autoantibodies indicates chronic a. H.

Identification and Characterization of the Repetitive DNA Sequences within the Genome of Molluscum Contagiosum Virus Type 1

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The Molluscum Contagiosum Virus (MCV), a member of the family poxviridae, induces epidermal proliferation in man. The genome of MCV type 1 (MCV-1; 188 kbp) had been characterized by physical mapping using a defined gene library of the viral genome and by DNA-DNA hybridization. The physical maps of the viral genome were constructed for the restriction endonucleases BamHI, ClaI, EcoRI, and HindIII. Detailed hybridization experiments revealed the presence of repetitive DNA sequences located within the terminal regions of the viral genome, e. g. BamHI DNA fragment B (18 kbp; 0 to 0.095 mu) and E (10.9 kbp; 0.944 to 1 mu). The fine mapping of these particular regions indicates that the repetitive DNA sequences are located within the HindIII DNA fragments J1 (4.2 kbp; 0.962 to 0.985 mu), K (4.0 kbp; 0.014 to 0.036 mu), P1 (2.7 kbp; 0 to 0.014 mu), and P2 (2.7 kbp; 0.985 to 1 mu). Nucleotide sequence analysis was carried out. It was found that the HindIII fragments J1 and K each contained an inverted repeat of 1682 bp and 1675 bp, respectively. The homology between the both repetitive DNA elements was found to be 99%. The analysis of the coding capacity of the determined DNA sequences revealed the presence of 9 and 5 open reading frames in the HindIII DNA fragment K and the corresponding region in the HindIII DNA fragment J1, respectively.
Expression of Human Immunodeficiency Virus Type 1 gag Gene Encoded by a Recombinant Herpes Simplex Virus Type 1

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The genome of DNA viruses is a valuable vector for expression of foreign genetic elements. In the present study the genome of an apathogenic herpes simplex virus type 1 (HSV-1) strain HFEM was used for manipulation and development of a novel eukaryotic vector. A recombinant virus (HSV-1 X HIV-1 gag) was constructed by inserting the human immunodeficiency virus type 1 (HIV-1) gag gene within the DNA sequences of the BamHI DNA fragment B of the viral genome. Previous to the insertion of the HIV cDNA sequence the immediate early promoter (IE4) of HSV-1 was inserted upstream from the gag gene. The recombinant virus was characterized in detail for determining the newly acquired phenotypic and genotypic properties. These analyses revealed that the recombinant virus is stable, replicates efficiently in the cell cultures, and expresses the specific gag gene product of HIV-1 at a high level. – BMFT Project U-047-88.

Investigation on the Degree of Destruction of HIV-1-Infected Cells from Blood of HIV-1-Positive Patients in Different Stages of the HIV-1-Infection

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Methods: From EDTA-blood samples of HIV-1-positive patients (ELISA, confirmed by Immunoblot) during different clinical stages (CDC-classification) the lymphocyte fraction was isolated by the ficoll-gradient technique. This cell suspension was depleted for CD8-positive cells by the magneto-immunobead method (Dynabeads®); from the remaining cells those expressing HIV-1-gp120 antigens were isolated by anti-gp120 coated Dynabeads. Evaluation was done by scanning electron microscopy.

Results: Almost no intact cell structures could be found in samples from patients during the AIDS stage: the most observed structures resembled fragments of cells, especially cell membranes; in contrast, samples prepared by the same procedure from HIV-1-positive patients of the earlier stages the moiety of intact cell structures was relatively high.
Prevalence of Antibodies to Human Herpesviruses (CMV, EBV, HSV, VZV) and Hepatitis B Virus (HBV) in HIV Seropositive Persons. Association to Progression of HIV Infection

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The initial immunological disturbance in HIV infected patients is aggravated by persistent (reactivated) infections e.g. by viruses of the herpes group. Such opportunistic viral infections are considered to play an important part as cofactors in the pathogenesis of AIDS. In the present study the results of HIV antibody testing as well as of tests for serological markers of CMV, EBV, VZV, HSV, and HBV in HIV positive persons are analysed. Since July 1985 2140 persons were found HIV positive for the first time in the Dep. of Virology of Frankfurt. The monthly incidence of HIV carriers is 30–50 new cases. Between 1.10.88 and 20.7.89 HIV antibody tests were performed in 10358 patients. 337 persons were HIV positive (3.25%). Compared to a control group of HIV negative persons (n = 9660), HIV positive in-patients (n = 307) and outpatients (n = 30) showed an increasing prevalence of antibodies to Herpes viruses. The difference was particularly evident for CMV-IgG (100% prevalence in hospitalized, 91.8% in out-patients and 63.5% in HIV negative patients). Anti-HBc, with a low prevalence in controls (16.5%) was markedly associated with progression of HIV infection (69.5% prevalence in out-patients, 84.6% in in-patients). The antibody pattern to opportunistic viral infections may be regarded as a marker of pathogenicity in HIV infected patients.

Comparison of the Immunoreactivity of Recombinant Proteins and Synthetic Peptides with HIV Positive Sera

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Restriction fragments of pBH10-RIII and synthetic oligonucleotides were cloned in expression vectors in E.coli. Fusion proteins with β-galactosidase and aa 260–547, aa 549–736, aa 737–863, aa 597–611 of gp160 (env), aa 118–512 of p55 (gag) of HIV-1 and aa 589–603 of gp140 (env) of HIV-2 were tested with 60 positive sera by immunoblotting. Fusion protein aa 589–603 was tested with 12 HIV-2 positive sera. Fusion protein aa 597–611 was recognized by 100% of sera, 98.3% of the sera reacted with fusion protein aa 549–736. The fusion protein aa 260–547 was detected by 81.6% and fusion protein aa 737–863 by 63.3%. Fusion protein aa 118–512 (gag) was recognized by 43.3% of sera corresponding to different CDC groups. The fusion protein aa 589–603 was recognized by 100% of HIV-2 positive sera. – Synthetic peptides were prepared via the Fmoc-strategy and purified by reversed phase chromatography. Peptides were selected corresponding to HIV-1 gag aa 105–115, aa 129–135, aa 234–243 and HIV-1 env aa 504–518, aa 507–518, aa 512–518 and HIV-2 env aa 589–603 and aa 597–603. The same sera were applied to an ELISA-test with the synthetic peptides. The peptide gag aa 105–115 was detected by 23.3% aa 129–135 by 41.7%, and aa 234–243 by 3.3% of sera. Peptide env aa 504–518 was
recognized by 63.3%, aa 507–518 by 73.3%, aa 512–518 by 61.7%, and aa 597–611 by 100% of sera. Peptides env aa 589–603, and aa 597–603 from HIV-2 were both recognized by 91.7% of sera. – Antigenicity of recombinant proteins and synthetic peptides was demonstrated to be comparable.

Prevalence of HSV-2-Typespecific Antibodies in Different Groups of Patients. A Comparison Between Enzyme-Linked Immunosor­bent Assay (ELISA) and Western-Blot

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Because of the strong crossreactivity of the two serotypes of Herpes simplex Virus (HSV) it is very difficult to distinguish between HSV-1 and HSV-2-typespecific antibodies in patients’ sera. Although genital herpes can be caused by HSV-1 in 14% of the cases, HSV-2 is the cause of most recurrent genital herpes (86%). The serological differentiation of past HSV-2 from past HSV-1 infections is necessary for identifying pregnancies likely to be complicated by recurrent maternal HSV-2 infection. By improving the enzyme-linked immunosorbent assay (ELISA) for HSV-2-antibodies and additional testing of sera by Western blot, we were able to specifically identify HSV-1- and HSV-2-antibodies in serum samples. – For the ELISA, HSV-2-antigen was immobilized on 96-well microtiter plates (Nunc), and patients’ sera were added. Antibodies were detected by biotin/streptavidin peroxidase. – For the Western blot the electrophoretically separated HSV-2-antigen was used. The antigen was blotted to a polyvinylidenedifluoride (PVDF) membrane (Immobilon™, Pharmacia) and incubated with the test sera. Antibodies against typespecific glycoproteins of HSV type 2 (gG-92), forming a sharp band, were visualized by the application of biotin/streptavidin peroxidase. Our results showed that the ELISA and the Western blot correlated in 91.6%. Serum samples with high antibody titers against HSV-1 showed false positive reaction in the HSV-2-ELISA in 8.4% of the cases. The optical density of these sera ranged ±0,2 around the cut-off value of the ELISA. These samples could easily be identified by Western blot. – Serological studies showed, that 30% of the prostitutes (n = 20) have antibodies against HSV-1 and 25% against HSV-2. In 40% both types of antibodies could be found. Furthermore 70% of female patients in gynecological treatment (n = 20) had antibodies to HSV type 1 and in 10% of the cases against both serotypes. 20% showed no antibody reaction. Roughly the same prevalence of HSV-1 and HSV-2-typespecific antibodies was found in 20 patients from the dermatological department.

Protection Against Herpes Simplex Virus Infection by Nonneutralizing Monoclonal Antibodies

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A nonneutralizing monoclonal antibody (mab), directed against glycoprotein gC of herpes simplex virus type 1 (HSV-1) and neutralizing mab to glycoprotein gB were evaluated for their ability to protect mice from genital HSV-infection. The nonneutralizing mab had
only little effect on virus replication in the mucous membranes, but completely protected the mice from peripheral skin lesions, neurological illness and death. Progression of the virus to the central nervous system was obviously inhibited by a modified course of the ganglionic infection: the number of ganglia presenting infectious virus and final latency were reduced, although early latency was not induced. In addition to the effects of the nonneutralizing mab, the neutralizing mab effectively shortened viral shedding from the vagina and converted proliferative ganglionic infection into latency. The different modes of action of the monoclonal antibodies are discussed in context to the possible defense mechanisms of the humoral immunity against virus proliferation in mucous membranes and in ganglia.

Bovine Herpesvirus 1 Thymidine Kinase and Glycoprotein H Genes are Expressed from A 5.1 Kb mRNA

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The thymidine kinase (TK) gene of bovine herpesvirus 1 (BHV-1) can serve as an integration locus for foreign DNA into the viral genome, because expression of a functional active TK is not essential for viral replication. The TK-gene of BHV-1 was located by conversion of TK- negative cells to the TK+ -phenotype and marker rescue experiments. It contains an open reading frame of 1068 bp. Sequence comparison with other herpesviral TK-proteinsequences revealed, that only certain parts of the amino acid sequence as the nucleotide binding loop are highly conserved. Hybridization of RNA from infected cells with TK-DNA showed, that TK-mRNA has a size of 5.1 kb, whereas the size of TK-mRNAs of other herpesviruses measures about 2 kb. 5'- and 3'-end of the TK transcription unit were characterized using nuclease S1-analysis. An open reading frame, when translated into protein, is homologous to the glycoprotein H (gH) of Herpes simplex virus type 1 and lies downstream the open reading frame of the tk-gene. We conclude, that the expression of BHV-1-gH correlates with the expression of thymidine kinase. Preliminary results suggest, that transcription of both genes is initiated from one common promoter.

Presumed Transcription Regulating Domain of Histone H3 is Cleaved off Early During Infection by Foot-and-Mouth Disease Virus Protease 3C

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In foot-and-mouth disease virus (FMDV) infected cells disappearance of the nuclear protein histone H3 and the simultaneous appearance of a new chromatin associated protein termed Pi can be observed. We have sequenced the amino terminus of Pi and clearly showed that protein Pi derives from histone H3 by proteolytic cleavage. The 20 N-terminal amino acid residues are specifically cleaved off early during infection. In addition using an in vitro transcription/translation assay with different FMDV clones we showed that the histone H3 - Pi transition is FMDV 3C protease dependent. This protease until now has only been found
to be responsible for the processing of the viral polyprotein. The 3C protease (i) is the only FMDV protein required to induce this histone H3–Pi transition, (ii) no other viral protein can perform this specific cleavage, and (iii) no viral precursor fusion protein is necessary for this specific cleavage, as it is reported for the processing of the poliovirus P1 precursor-polyprotein. The 3C mediated histone H3 cleavage is not restricted to chromatin derived from natural host cells. As the deleted part of the histone H3 corresponds to the domain presumed to be involved in the regulation of transcriptional active chromatin in eucaryotes, it is postulated that this specific cleavage of H3 is a mechanism which FMDV utilizes to switch off host cell RNA synthesis, as is reported for picornaviruses. In combination with the reported mechanism of host cell translation shut off by cleavage of the cap-binding protein complex, this specific histone H3 cleavage could contribute to the almost complete breakdown of host cell functions during infection.

**Stable Expression of Bovine Herpesvirus 1 Glycoprotein Iv in Murine and Bovine Cells**

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The gene encoding glycoprotein IV (gIV) of bovine herpesvirus 1 (BHV-1) was cloned into eucaryotic expression vectors. The strong enhancer/promoter sequences of the murine cytomegalovirus (MCMV) and the metallothionine promotor were used to control expression of the gIV gene. Both constructs expressed the gIV in transient transfection assays. Transformed cell-lines could only be isolated after transfection with the gene under control of the inducible metallothionine promotor whereas constitutive expression of gIV seemed to be toxic. The recombinant gIV was glycosylated and transported to the cell surface as shown by radioimmunoprecipitation after iodination of living cells immunoelectronmicroscopy and FACS-analysis. Transfectants show reduced susceptibility to BHV-1 infection. FACS-analysis revealed that the recombinant gIV is recognized by monoclonal antibodies against gIV and rabbit sera obtained after immunization with purified gIV and after infection with gIV expressing recombinant vaccinia virus. Sera from cattle infected with field virus also reacted with the transfectants. Expression of mutated gIV genes should lead to the functional analysis of the gIV, especially to define regions which are important for the binding of neutralizing antibodies and for adsorption of the virus on the cell surface.

**Computer Aided Protein Design of Herpes Simplex Virus 1 (HSV-1) Thymidine Kinase (TK)**

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The TK of HSV-1 contains three regions of homology to highly conserved sequences of other nucleotide binding enzymes. These are the residues 49 to 66 (nucleotide binding loop), residues 161 to 168 and residues 317 to 319. Comparison of the viral and cellular TK
protein sequences and 3-D structure analysis led to the hypothesis that the Asp 161 might be involved in binding of acyclovir which is used as a therapeutic for HSV infection. Site specific mutagenesis which replaced Asp 161 by Asn led to a polypeptide with no TK activity. The same was found when residues 184 to 306, which are not present in cellular TKs, were deleted. Both mutant genes were integrated into vaccinia virus to study the biochemical properties of the resulting polypeptides and to analyze the importance of the mutated sequences for the functional activity of the enzymes.

Characterization of Polyomavirus JC Replication in Different Cell Lines

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The human polyomavirus JC replicates cytolically in oligodendroglia cells of the CNS and in an attenuated manner in kidney cells. Examination of the control region of JCV organspecific variants JCV-GS/B and JCV-GS/K supported the assumption of transcriptionally regulated control in persistent JCV infections. To further analyse the control functions involved in organspecific expression we asked for the ability of JCV variants to replicate in CNS and kidney cell lines. JCV DNA was cloned into the vectors pUC and pBR322. The vectors carried the complete JCV control domain and an intact early gene region. DNA replication was tested by Southern blot analysis and restriction cleavage with Dpnl. In the hamster cell line BHK and the JCV transformed hamster cell line of glial origin (HJC) replication was not detected up to 96 hours post transfection (p.t.). In contrast the human glioma cell line U138 and the Adenovirus transformed kidney cell line 293 revealed replication of both organspecific variants as early as 48 hours p.t. Nevertheless the amount of replicated DNA of the kidney variant was clearly reduced in glioma cells and replicated DNA was detected later than with the CNS variant. From these data we conclude, that JCV replication is regulated not only host type specific, but also in a cell type specific manner.

Immune Response after Vaccination with a Highly Purified Killed Hepatitis A Vaccine

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Young human volunteers were vaccinated with a killed hepatitis A vaccine produced in human embryo fibroblast cells. Different amounts of vaccine were administered by the intramuscular route. 4 weeks after the first vaccination with a 0.3µl vaccine 36.6% of the volunteers seroconverted. 4 weeks after the second injection the seroconversion rate was 95.1%. Anti-HAV was determined quantitatively and results showed anti-HAV titers 20 to 100 times higher than those after gamma globulin administration. One year after the vaccination 19/19 volunteers still had anti-HAV titers in their sera 20 to 50 times higher than those after gamma globulin administration.
Antibody to Hepatitis -C-Virus (HCV) in Patients with Chronic Non-A, Non-B Hepatitis

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Recently, an assay for circulating antibodies to a blood-borne Non-A, Non-B hepatitis virus, the HCV, has become available by cloning the genome of HCV and using a polypeptide synthesized in recombinant yeast clones of the HCV. – We have investigated a total of 100 sequential serum samples from a well characterized group of 29 patients with chronic Non-A, Non-B hepatitis and 4 control patients with liver diseases other than Non-A, Non-B hepatitis. – 65 sera from 22 patients with chronic Non-A, Non-B hepatitis were found to be positive for anti-HCV. – Sequential serum samples revealed stable anti-HCV findings in all but two patients. The antibody was not detected in 7 sera of patients with liver diseases unrelated to Non-A, non-B hepatitis. – Our data suggest that HCV is a major cause of Non-A, Non-B hepatitis.

Bone Tumor-Inducing Murine Leukemia Viruses Induce Osteogenic Differentiation of Osteoblast-Like Cells in vitro

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RFB and Akv, two murine leukemia viruses known to induce osteopetrosis and osteomas in newborn mice were used to study the effects of virus infection on osteogenic differentiation and proliferation of osteoblast-like cells in vitro. Following infection of primary periosteal cells ALP activity increased significantly, indicating induction of osteogenic differentiation. Cell proliferation was reduced particularly in RFB-infected cells. In primary osteoblast-like cells this effect was less pronounced. – Permanent osteoblast-like MC3T3-E1 cells grown in a threedimensional (3D) cell culture form mineralized osteoid within 6 weeks. After infection with RFB and Akv increased amounts of bone matrix were observed. Immunohistochemical analysis showed higher amounts of collagen type I, fibronectin and tenascin in infected cultures than in controls. Northern Blot analysis of infected cells grown in 3-D cultures revealed increased expression of genes, which are characteristically expressed in differentiated osteoblasts. – The data suggest that in vitro infection of skeletoblast precursor cells and immortalized osteoblast-like cells with bone tumor-inducing murine leukemia viruses is followed by enhanced osteogenic differentiation and increased bone matrix production.
Expression and Characterization of the Terminal Protein 1 (TP1) of the Epstein-Barr Virus in Insect Cells with a Baculovirus Expression System

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The terminal protein 1 is the putative product of an EBV gene expressed in latency. The very special feature of this gene is that the coding sequence is only created by circularization of the linear viral genome at the terminal repeats. It codes for a protein of about 54 kDa, which is likely to be a membrane protein. - A BamHI genomic fragment, containing the first exon of TP1 and a BamHI/Smal fragment of a TP1 cDNA derived from a M-ABA/Cbl cDNA library were expressed in the tryptophan regulated E.coli expression vector pATH. Rabbit antiserum were raised against the resulting fusion proteins. A PstI/Smal fragment of the above cDNA clone which contains the whole reading frame of TP1 was cloned in a pAC 409 Baculo transfer vector and after recombination with a wild type Baculovirus expressed in insect cells. The rabbit antiserum raised against the procaryotic fusion proteins recognized a protein of about 55 kDa and a dimer of the same protein in extracts of infected insect cells in the Western blot. Immunofluorescence studies of living cells showed that the protein is located in the plasma membrane. A number of human EBV-positive antiseria – exclusively sera from NPC patients – could be identified which show an antibody response to TP1. This result indicates that the protein is expressed during the life cycle of EBV. Additionally, a protein of about 54 kDa which specifically reacts with TP-positive antiseria could be detected in the membrane fraction of the EBV-positive cell line M-ABA.

Topography of DNA-Protein Interactions in the Regulatory Regions of Epidermodysplasia verruciformis-Associated Human Papillomavirus Genomes

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Human papillomaviruses (HPV) associated with the epidermodysplasia verruciformis syndrome (ev) represent a group of closely related viruses, clearly distinct from other HPVs. The most interesting features of ev-viruses are: (i) high oncogenic potential of some specific virus types, (ii) extremely narrow host range. To get some insight into the molecular basis of processes controlling the viral expression we studied the sequence-specific DNA-protein interactions within the genomic regulatory regions. Using the nuclear extracts of HeLa cells and a combination of exonuclease III- and DNase I-footprinting techniques the protein binding maps have been constructed for HPV8 and HPV19, the prototypes of viruses with high versus low oncogenic potential. The sequences in question showed a complex array of protein binding domains, covering almost the entire length of the regulatory regions. A cluster of prominent binding sites, which overlapped with the sequences of the motif 44 (M44), a highly conserved element in most of the ev-viruses, was investigated in more detail. In transient CAT assays the M44 motif of HPV8 as dimer or trimer was found to act as a strong expression activator. The analysis of M44 sequences in band-shift tests revealed
partially different sets of DNA binding proteins, interacting with the elements from HPV 8 and 19. One of these proteins, at least in case of HPV8, was shown to be the regulatory factor AP1. A part of M44 sequences in the vicinity of the AP1 binding site display a homology to enhancer elements in other small DNA viruses.

Non-Radioactive Detection of Hepatitis B Virus DNA

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Semiquantitative detection of Hepatitis B Virus (HBV) DNA in sera of infected individuals has become an important means of modern serological hepatitis diagnostics. It enables investigators to draw conclusions on infectivity, prognosis and therapeutical success. Molecular hybridization using radioactively (i.e. $^{32}$P phosphorus) labeled DNA probes with subsequent autoradiography is characterized by both high sensitivity and specificity. Alternatively Digoxigenin-labeled probes which are detected by enzyme immunoassay have been employed. Using this method 1–2 pg of HBV DNA in aqueous solution have been detected with high specificity on membranes made of cellulose nitrate. Testing sera of hepatitis patients 1 pg of HBV DNA has been detected on nylon membranes of 1.2 μm pore size ($^{32}$P: 0.1 pg). Unfortunately specificity remained disappointing even after centrifugation, digestion by proteinase K and treatment of the sera with phenole/chloroform.

Prevalence of Unrecognized HIV Infection in a University Hospital

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Objective. To assess the risk for laboratory personnel resulting from patients with unknown HIV infection, sera sent in to the Institute for Medical Virology and Immunology (IMVI Essen) for immunologic and/or virologic testing were anonymously screened for HIV antibodies.

Methods. Between July and December, 1988, a total of 6252 sera from different departments of the University clinics of Essen were selected for screening under code on the basis that no HIV antibody test was requested for diagnostic purposes at the same time, and sera from patients found to be anti-HIV positive during the previous 4 years were disregarded (n = 61). Sera reactive in ELISA (Enzygnost-Anti-HIV, Behring) were further tested in a second (Dupont) and third ELISA (Pasteur) and in a confirmatory test (Western blot -WB-, Dupont).

Results. In 11 of 6252 sera from patients not suspected for HIV infection, HIV antibodies were confirmed by WB (0.176%) (table).
Number of anti-HIV positive sera with unknown HIV status in anonymous screening

| month  | Jul | Aug | Sep | Oct | Nov | Dec | total |
|--------|-----|-----|-----|-----|-----|-----|-------|
| sera tested: | 485 | 1207 | 975 | 1270 | 1150 | 1165 | 6252 |
| confirmed positive: | 2 | 0 | 1 | 1 | 1 | 2 | 11 |

An additional 4 sera showed less than 2 specific WB bands and were considered indeterminate. Sera from unknowingly infected patients were sent in most often from the internal (n = 5) and dermatology departments (n = 3), whereas sera with indeterminate HIV antibody results mostly originated from patients who received tumor treatment (n = 2).

Conclusions. The prevalence of 0.176% unknown HIV infections reflects a higher rate than generally assumed but possibly results from a selection in a university of patients with immunological disorders and underlying HIV infection. An overall prevalence of 1% anti-HIV positive sera suggests the strict adherence to infection control measures in medical laboratories.

Partial Characterization of the Nonstructural Protein 125/80 kD of the Bovine Viral Diarrhea Virus and its Potential Role in Cytopathogenesis

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Bovine viral diarrhea virus (BVDV) is a member of the pestivirus group. In culture of bovine cells, a non cytopathic (ncp) and a cytopathic (cp) biotype can be distinguished. Using radioimmunoprecipitation a monoclonal antibody (MAb) detected a 125 kD non-structural (ns) polypeptide in cells infected with ncpBVDV. In cells infected with cpBVDV an additional 80 kD protein was precipitated. A second set of MAbs was directed against the 48 kD minor glycoprotein. The distribution of both proteins in cells infected with the two biotypes was analyzed by immunofluorescence analysis (IFA). None of the two proteins was detected on the surface of live, infected cells. The ns protein was homogenously distributed in the cytoplasm of cells infected with both biotypes. The second set of MAbs displayed different staining patterns in cells infected with each of the biotypes. In cells infected with cpBVDV, a homogenous cytoplasmic fluorescence was visible. In cells infected with ncpBVDV, the stain was largely restricted to the perinuclear zone, giving a distinct staining with each of the MAbs. The possible significance of these results for cytopathogenicity are discussed.
Molecular Mimicry: An Epitope of Identical Sequence on Influenza B Virus und UlsnRNP is Recognized by Human Autoantibodies

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In sera of patients with a variety of inflammatory rheumatic diseases autoantibodies to selfantigens (autoantigens) are found. One of the hypotheses on the molecular basis of autoimmune diseases which is discussed intensively since decades, assumes that structure- or sequence-related epitopes of virus and host proteins might be involved in initiation of autoimmune processes (molecular mimicry). – To search for crossreactive or sequence-identical epitopes of cell and virus proteins, we started to map in detail antigenic regions and individual epitopes on autoantigens. A most detailed study has been performed for the UlsnRNP specific p68 protein which is the major target of autoantibodies in systemic rheumatic diseases such as mixed connective tissue disease and systemic lupus erythematosus. – By immunoblotting and ELISA assays performed with recombinant p68 fusion proteins and peptides four antigenic regions and many patient specific epitopes could be mapped. In one of the antigenic regions an epitope 5 amino acids long could be identified which is also present on the matrix protein M1 of influenza B viruses. With affinity purified p68 specific autoantibodies the reaction with the M1 influenza B virus protein and vice versa could be experimentally verified. – These results demonstrate for the first time that autoantibodies from patients with rheumatic diseases recognize an epitope of identical sequence on a highly prevalent and pathogenic virus and a major autoantigenic target. Whether the existence of this common epitope is by chance or causally related is currently investigated.

Detection of Antibodies Against Cytomegalovirus (HCMV) Induced “Early”-Antigens by Immunoblotting

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Contradictory informations about the diagnostic assessment of antibodies against the “early”-antigens of human cytomegalovirus (HCMV) have been numerous published. We investigated the correlation between the incidence of antibodies against HCMV “early”-proteins and the state of infection. Ninety-six HCMV-IgG-positive sera (ELISA) were tested for specific IgG-antibodies against HCMV “early”-antigens by immunoblotting. Three groups were examined: (a) 29 renal transplantation patients, (b) 33 AIDS-patients and (c) 34 randomly selected healthy individuals. Each group yielded approximately the same percentage of positive immunoblots (59%, 58%, 59%). Sera belonging to group (a) or (b) reacted stronger and recognized a greater number of polypeptides (6±4; 5±3) when compared to healthy persons (4±3). All immunoblot-positive sera reacted at least with the 66kD protein (major “immediate early”-protein). Fourteen out of 19 HCMV-IgM-positive sera (ELISA) belonging to group (a) or (b) recognized “early”-antigens (74%). Twenty-two out of 43 HCMV-IgM-negative sera (51%) reacted as well. We conclude that an acute HCMV infection does not cause the formation of antibodies against “early”-antigens in all individuals and furthermore these antibodies can persist during a subclinical/latent infection.
Association of Bacteria for Susceptibility Testing as an In-vitro Model of Aerob and Anaerob Mixed Infections

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Polymicrobial etiology involving anaerobes and aerobes is predominant in a variety of clinically important infections. Amongst the pathogens most frequently isolated from such infections are β-lactamase producing strains. Some in vitro and in vivo findings are indicating that in mixed infections certain interactions between components of the polybacterial associations may occur. Enzymatic destruction of the antibiotic by one species, may protect other concomitant fundamentally sensitive organisms from the activity of the drug. We therefore investigated the effect of associations of aerobic and anaerobic pathogens (E. coli, H. influenzae, S. pyogenes, E. faecalis, S. aureus, S. epidermidis, B. fragilis, B. melaninogenicus) on their respective MBCs of ampicillin (AMP), AMP plus sulbactam (SUL), amoxicillin (AMX), AMX plus clavulanate (CLA), and metronidazole (MET). The experiments were done by means of a broth dilution method (10 ml) with serial twofold dilutions of AMP, AMX, MET, and constant concentrations of SUL (1mg/l and 5mg/l), and CLA (2.5mg/l and 5mg/l). After incubation at 35°C, cfu/ml of the species present in each tube were determined. Our results are indicating that (i) susceptibility testing of single strains of polymicrobial infections not necessarily reflects their behaviour in polymicrobial infections, (ii) β-lactamase production by one of the strains associated may lead to an increase of MBC\textsubscript{AMP} or MBC\textsubscript{AMX} up to 64-fold for non-β-lactamase producing strains, (iii) SUL and CLA are able to inhibit β-lactamases of associated pathogens, (iv) destruction of MET by E. faecalis lends additional support to the use of the combination AMP+SUL or AMX+CLA in aerobidanaerobic infections including E. faecalis.

Definition of the Family Toroviridae

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Berne virus (BEV), isolated from a horse, is the prototype of the Toroviridae, a proposed new family of positive-stranded RNA viruses. BEV virions consist of a peplomer-bearing membrane which envelops a tubular nucleocapsid of helical symmetry; this structure can be straight (resulting in a tubular particle) or bent into an open torus (conferring the shape of a kidney or biconcave disc to the virion). The nucleocapsid contains a single polyadenylated RNA molecule of > 25 kb and the most abundant polypeptide, an 18.3kD basic phosphoprotein with nucleic acid binding properties. Upon infection, a set of 5'-coterminal subgenomic mRNAs is synthesized by leader-independent transcription; only the unique 3' sequences of each mRNA are translated. – The combination of virion structure, nucleocapsid protein size and leader-independent transcription is unique in virology and justifies a family status for BEV and related viruses. However, coronaviruses have a similar genome organization, a 5'-coterminal nested set of mRNAs and sequence similarities in the second ORF of the polymerase gene of BEV. Since the latter suggest common ancestry, toro- and coronavirus together may be considered as a third evolutionary cluster of positive-stranded RNA viruses, in addition to the alpha- and picornavirus superfamilies.
Cytotoxic T-Lymphocytes can Discriminate Between Cells Infected with Different Strains of Epstein-Barr-Virus (EBV)

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In order to characterize viral and nonviral structures responsible for the recognition of EBV-infected lymphocytes by the immune system, we studied the reactivity of EBV-specific cytotoxic T cells towards autologous EBV-positive lymphoblastoid cell lines (LCLs). LCLs were established from different EBV-positive donors by either spontaneous outgrowth of cells transformed by the donors own virus or by infection with a laboratory strain of EBV (B 95–8). These cell lines were used for the generation of EBV-specific cytotoxic T cells by weekly stimulation and addition of interleukin 2; cells were cloned by limited dilution. In chromium release assays these CD8 positive T cell lines and clones were able to discriminate between two autologous lymphoblastoid cell lines infected by either the “own” virus strain or the B 95–8 strain, respectively. Further experiments using various concentrations of effector cells showed that structures recognized by different T cell clones were expressed only in a certain percentage of cells (20–40%) of each LCL, and that nonviral components differently expressed on different LCLs also seem to play a role CTL/LCL interactions.

Bovine Herpesvirus 4: Vector for Life-Virus Vaccines?

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Bovine Herpesvirus 4 (BHV-4) has biological properties similar to the cytomegaloviruses of other species. It is thought to be apathogenic in cattle. The genome consists of about 150 000 bp. A unique region is flanked by repetitive units of 2.2 kbp. The repetitive units were used for the integration of the immediate early region of the murine cytomegalovirus because sequence- and Northern blot-analysis gave no indication for transcription within the repeated sequences. The heterologous sequences, however, were rapidly lost during the propagation of the recombinant virus. Therefore an EcoRI-fragment was isolated which contains both unique and repetitive DNA. This 4.5 kbp fragment was sequenced and used for the construction of a recombination vector. The possibility to use this system for the construction of a lifevirus vaccine is discussed.
Specific Binding of Vaccinia Recombinant-Derived HIV-1 GP 160 to CD 4+ Cell Lines

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The worldwide spread of AIDS requires the rapid development of a vaccine against its etiological agent HIV-1. The HIV-1 envelope glycoprotein gp 160 has been chosen as the most likely candidate for such a vaccine. We have developed a method for large-scale production and purification of gp 160 from recombinant vaccinia virus-infected Vero cells. This protein has been shown to be able to elicit T-cell proliferative responses and cross-reactive antibodies in animals. - In this study we have analysed the ability of the protein to bind to the CD 4 receptor. – We have demonstrated that

I) recombinant gp 160 binds to different CD 4+ cells like CEM-, MT4- and H9 cells,
II) recombinant gp 160 does not bind to CD 4- cells like Vero cells,
III) denatured recombinant gp 160 does not bind to CD 4+ cells.

The large-scale production and purification of recombinant gp 160 leads to a native and biologically active protein, which binds specifically to the CD 4 receptor.

Direct Detection of HIV-1 in HIV-Seropositive Hemophiliacs: Polymerase Chain Reaction with pol- and env-Specific Primer Pairs

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In addition to virus isolation from the peripheral blood lymphocytes (PBL) the polymerase chain reaction (PCR) provides another tool for direct detection of HIV. We use the PCR to ascertain HIV infection in HIV seropositive hemophiliacs. – We investigated blood samples from a group of patients with two different primer pairs from the pol- and env-region of the HIV-1 genome (env: SK68/9, On et al., 1988; pol.: P5/6, Laure et al., 1988). – HIV-1 specific amplified DNA was detected by electrophoresis of the PCR products through NuSieve agarose gels followed by Southern blotting and hybridization with specific radiolabeled oligonucleotide probes. – Samples from randomly selected HIV- seronegative blood donors served as controls for the entire experimental procedure. – The results are discussed with regard to the total amount of positive cases compared with the use of only one primer pair and to the number of virus isolations.
Studies on the HBs Specific Immune Response in vitro

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Anti-HBs response of HBs vaccine recipients within an ongoing immunization course was studied in vitro. Antigen specific antibody production and proliferative response as well as the expression of CD23 and CD25 and the secretion of sCD23 were detected. It was the aim of the study to analyze the effect of various cell stimuli (mitogens, lymphokines and antibodies against cell surface antigens) on the antigen-specific immune response in vitro. PWM driven enhancement in HBs specific antibody production was shown to be time dependent. In contrast to IL2 and IL6, incubation with IL4 led to a significant increase in anti-HBs antibody synthesis. Low doses of IL4 led to a significant increase in HBs induced CD23 expression. Moreover, IL4 induced sCD23 secretion is enhanced by HBs antigen. IL2 receptor expression of HBs vaccine non-responder PBMC is reduced as compared to the responder group. CD25 receptor expression of responder PBMC is influenced by antigen as well as IL2 whereas no modulation can be seen with non-responder PBMC. The capacity of non-responder cells to respond to HBs antigen is reduced whereas the capacity of these cells to respond to IL2 is markedly enhanced. In summary, our data show that the HBs specific immune response in vitro is influenced by lymphokines.

Detection of HPV-6b, -16, and -18 Specific Antibodies in Human Sera

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E. coli expressed β-Gal fusion proteins of all HPV-16 ORFs, L1 and L2 of HPV-6b and -18 were used for the detection of HPV antibodies in human sera by Western-blot analysis. 38.9% of sera from women (n = 560) and 32.2% of sera from men (n = 96) were found to contain antibodies directed against the L2 gene products of the HPV types mentioned above. 9.6% of female and 12.5% of male sera exhibited antibodies directed against the HPV-16 E4 and/or E7 gene products. On the other hand in an individual female serum antibodies directed against HPV-16 E1 and E2 could be found, and another one harboured antibodies directed against HPV-16 E6. All of the antibodies were of IgG type. In order to specify whether antibodies against papillomaviruses are associated with sexual activity, we used sera from 100 female individuals of age 14 years old. We tested also the sera from the same individuals which were subsequently taken at time intervals of 3 and 10 years. It could be shown that L2 antibodies were present in the sera over the whole period of time. However, also an increase in the frequency of antibodies directed against HPV-16 L2 with growing age of the women was observed. These data indicate that beside the distribution of human papillomaviruses by sexual intercourse other routes of papillomavirus infection may exist, for instance perinatal infection.
Identification of Herpes Simplex Virus Type-1 Glycoproteins Interacting with the Cellular Surface

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In an attempt to identify structural proteins of HSV-1 binding to the cell surface we analyzed complexes formed between \(^{14}\text{C}\) labelled HSV-1 (ANG) proteins and cell surface structures of RC 37 cells biotinylated in vivo. Complexes specifically formed between viral proteins and biotinylated cell components were affinity purified on streptavidin agarose, eluted, and subsequently analyzed by SDS-PAGE and autoradiography. Viral proteins with binding activity in this assay were identified as gC, gB, and gD of HSV-1 ANG. Identical results were obtained with the HSV-1 strain Kos 321 and with biotinylated Wi-38 and HEp-2 cells. The specificity of the results was controlled in competition experiments using unlabelled virions. Most likely the interaction between viral glycoproteins and the cell surface occurs in a non-cooperative manner as shown by experiments with gC-negative, gC/ gE-negative and gB-temperature sensitive viral mutants. Independent binding of gC, gB and gD could also be demonstrated by inhibition experiments with monospecific rabbit sera. Further experiments revealed differences in salt stability and pH dependence between gD, gB, and gC with respect to their binding ability. Thus, binding of gD could be disrupted at 500 mM NaCl and had a pH optimum between pH6 and pH7, whereas binding of gC and gB showed a pH optimum near pH7 and was stable even at salt concentrations above 1000mM NaCl. The results given above provide direct evidence for a functional role of gC, gB, and gD in binding of HSV-1 to the cell surface.

Molecular Mechanisms of the Antiviral Activity of Zinc Sulfate

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Already 22 years have passed since Falke et al. have described inhibitory effects of zinc ions on HSV replication. Later studies were restricted to the inhibition of HSV DNA synthesis, and hence viral replication, by the selective block of the HSV DNA polymerase. – We present a study on antiviral effects of zinc at all stages of HSV replication. – Zinc ions of a concentration of 15 mM completely inactivate free HSV virions of either serotype within 2 to 4 h. As demonstrated by electron microscopy, the mechanism of this effect is due to irreversible binding of the metal ions to the viral membrane, presumably to mercaptan groups of the viral glycoproteins. Adsorption of zinc inactivated virus is reduced to some extent only whereas a heavy impact has to be presumed on virus penetration. Only in a small concentration range (100 to 200 \(\mu\text{M}\) Zn in culture medium) \(\text{ZnSO}_4\) is inhibitory to the virus replication in infected cells without serious toxicity to the cells themselves. – Therefore the mechanism of the in vivo efficacy of zinc preparations against HSV lesions of the mucosa is mainly based upon inactivation of free virus and only partly due to virustasis or inhibition of HSV adsorption.
Expression of Recombinant Human C3D/EBV Receptor and Subsequent Infection with Epstein-Barr Virus of Epithelial and Fibroblast Cell Lines

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Lymphotropism has long been considered the central feature in the biology of EBV. Results demonstrating EBV genomes and expression in epithelial cells have not easily been explainable. Recently CR2, the receptor for complement factor C3d, has been proven to serve as EBV receptor. The expression of the receptor is limited to B-lymphocytes and certain epithelial cells in undifferentiated state. A model has been proposed: (1) EBV enters the easily infectable but inefficiently producing B-lymphocytes via the receptor* (2) epithelial cells are poorly infectable but able to produce virus as shown in the parotid gland and in oral hairy leukoplakia. — In the present study, the tissue tropism of EBV has been expanded by transfecting pNSV.CR2.neo, an eukaryotic expression vector promoting the transcription of a cDNA insert encoding human CR2 derived from Raji cells in murine L cells, HeLa cells and CNE-2 cells, the latter representing a human EBV negative cell line from nasopharyngeal carcinoma. The receptor could be demonstrated in immunofluorescence tests using a monoclonal antibody. After infection of the transfected cells by P3HR1 virus signals resulting from incubation with sera from NPC patients in immunofluorescence tests could be obtained. The latent protein complex EBNA was expressed for at least 51 days p.i. as shown in anticomplement-indirect immunofluorescence tests. The results indicate that the receptor is a primary determinant of the tissue tropism. Evidence for a dominant lytic infection was not visible, even after treatment with TPA/TPA and butyric acid/retinoic acid.

Cap-Independent Translation Initiation of Foot- and Mouth Disease Virus

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The genom of foot- and mouth disease virus (family of Picornaviridae) is a single-stranded molecule of mRNA polarity containing approximately 8,500 nucleotides. The 5’-end of the RNA is not capped, but a small peptide (VPg) is covalently linked to this end. — In contrast to the normal eucaryotic translation initiation (scanning-model by M. Kozak), the translation of picornaviruses starts cap-independent at an internal AUG codon. The translation of foot- and mouth disease virus initiates approximately 1,300 nucleotides downstream from the 5’-end of the RNA at the 11. and 12. AUG. — By direct mutagenesis of nucleotide sequences in the 5’ non-coding part of foot- and mouth disease virus we can show that a region of 400 nucleotides is essential for in vitro translation initiation. RNA-protein binding assays indicate that components of a protein extract enriched for initiation factors (ribosomal salt extract) of rabbit reticulocytes or rat liver protects specific positions of the 5’ non-coding region against RNase digestion. Several other experiments also indicate direct RNA-protein interactions. Currently experiments are performed to further characterize these (host-) factors.
Identification of an Epstein-Barr Virus (EBV)-Encoded Target Antigen for Recognition by Cytotoxic T Cells

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Experiments done either with synthetic peptides or with cells transfected with the gene encoding the EBV latent membrane protein BNLF1 suggested that this protein is a target for EBV specific cytotoxic T-cells (CTLS). As such experiments do not closely resemble the natural situation we wanted to expand on this topic and established a system which should better reflect in vivo conditions. We constructed two recombinant vaccinia viruses, one carrying the complete BNLF 1-MA reading frame, the other encodes a truncated form of BNLF 1-MA containing the third exon only which was found on EBV infected Burkitt Lymphoma in addition to the downregulated full length protein. The continuous coding sequence of the complete gene which is necessary for expressing foreign proteins in recombinant vaccinia was cloned from genomic fragments and synthetic oligonucleotides. After infection of several cell types including freshly isolated PBLs the expression of both forms of LMP was clearly demonstrated in immunoblots and by immunofluorescence. - EBV-specific cytotoxic T-lymphocytes (CTL) generated by repeated stimulation with autologous EBV infected lymphoblastoid cells in the presence of IL-2 were reacted with autologous PHA stimulated blasts infected with the two vaccinia viruses and the wild type virus as a control. CTLs of two persons recognized the full length BNLF1 protein but not the shortened protein whereas CTLs of two other persons did not recognize any of the two BNLF1 proteins. From these experiments we conclude that 1., the N-terminus of the BNLF1 protein contains a determinant which is recognized by some EBV-specific CTLs and 2., that there are additional proteins which are targets for other EBV-specific CTLs.

Positive and Negative Regulation of the EBV BMLF1 Promoter

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The expression of the Epstein-Barr virus regulation gene BMLF1 is regulated by a promoter/enhancer complex located upstream from the short ORF BSLF1 (transcribed as a spliced unit). The region 5'-proximal to the TATA box contains consensus sequences for binding transcription factors (e.g. NF-1, AP-1). We analyzed the activity of this control region in different cell systems using appropriate reporter assays (chloramphenicol acetyltransferase and Hepatitis B virus surface antigen as reporter genes). - A 127 bp AluI/BstEII fragment including the TATA box was shown to be sufficient to promote the transcription in Raji cells induced to lytic EBV expression by different procedures. Responsiveness to phorbol ester was shown in EBV negative cells (3T3). The upstream region of the BMLF1 promoter (1333 bp BstEII/SstI fragment) was identified as regulatory segment in transfection assays showing both positive and negative effects depending on the presence and state of EBV. - In EBV negative cells (HeLa), the upstream element clearly responded to the EBV trans-activator BRLF1. This specific trans-activation of the distal control region by BRLF1 (expression vector pKSVR kindly provided by A. Sergeant, Lyon) was down-regulated in latently infected cells (Raji) while in EBV producer cells (P3HR-1) trans-activity by BRLF1 was...
detected. Following insertion into a heterologous expression system the BMLF1 upstream control region enhanced transcription of the SV40 early promoter independent of its orientation.

Functional Characterization of the Regulatory Region of the Murine Polyomavirus K (KV)

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The murine K Virus causes a fatal disease in newborn mice and a clinically inapparent infection in immunocompetent animals. The virus replicates in vivo only in selected cell types and no cell culture system is available. Comparison of the K Virus sequence with the other polyomavirus strains demonstrates a high similarity in coding regions, whereas putative elements of transcriptional control (bp 4625 and 340) share almost no homologous DNA domains. To characterize the function of this region the complete 486 bp control element was cloned into the vector 3MCAT. This vector carries the chloramphenicolacetyltransferase gene and inserted control elements direct the CAT gene expression. In the positive control vector pSV2CAT the expression of the CAT gene was regulated by the SV40 promoter/enhancer element. The transfection rate was determined by cotransfection of β-galactosidase encoding vector and protein expression was measured by a fluorometric assay. The analyses showed, that the murine cell lines 3T3 NIH, 3T3 Balb/c, 3T3 Swiss, Meth A and L-cells do not allow expression of the CAT gene under control of the KV regulatory domain, whereas the transfected murine cell line 3T6 expresses CAT at a basal level. The expression is comparable to the rate in the non-murine cells BHK and CV1. These data indicate, that the expression of KV in vitro is restricted to specific cell types. Current experiments address the question for murine cells allowing KV expression.

Glycoprotein gIII Mediates Binding of Pseudorabies Virus (PrV) to a Heparin-Like Cellular Receptor

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We have previously shown that mutants of PrV that do not express the nonessential glycoprotein gIII adsorb less readily to cells than does wildtype virus. We show here that the first interactions of PrV with target cells occur via binding of the virus to a heparin-like component on the cell surface and that the viral protein mediating this binding is glycoprotein gIII. This conclusion is based on the following findings: 1) Heparin inhibits adsorption of wildtype PrV effectively as measured by plaque formation as well as by attachment of radioactively labelled virus to cells. However, it affects adsorption of gIII mutants only slightly. 2) While wild-type PrV binds well to matrix-bound heparin binding of a gIII mutant is dramatically reduced. 3) Pre-treatment of cells with heparinase reduces plaque
formation and adsorption of wildtype PrV by 90% but does not affect gIII mutants. 4) Of the PrV membrane proteins glycoprotein gIII binds most abundantly and specifically to heparin sepharose beads. Our results indicate that binding of PrV via glycoprotein gIII to a heparin-like cellular component promotes efficient adsorption. In the absence of gIII or of the heparin-like cellular receptor the virus adsorbs by an alternative less efficient mode.

The Polypeptide Profile of European Isolates of Bovine Herpesvirus 1 (BHV1) Recovered Between 1960 and 1985

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Bovine herpesvirus 1 (BHV1) causes two well established entities, namely infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV). Encephalitis, a third entity, is less well understood. Whereas IBR usually follows horizontal virus spread by aerosols, IPV is acquired by venereal contact. It remains controversial if the conditions were associated with distinct viruses. Restriction endonuclease analysis of viral DNA or evaluation of viral proteins following separation in SDS-polyacrylamide gels may be used to distinguish between BHV1.1, BHV1.2 and BHV1.3. The proteins of European field isolates, recovered from distinct disease episodes within the time period 1960 to 1985, were compared with the proteins of established laboratory strains. Of 74 field isolates 43 were classified as BHV1.1, 31 as BHV1.2 and none as BHV1.3. BHV1.2 was most regularly recovered from cattle with genital afflictions. However, some BHV1.2 strains originated from animals with IBR and others from aborted fetuses as well. The first recognition of BHV1.1 among the field isolates in 1972 coincided with sequential waves of severe IBR outbreaks throughout Europe. Results obtained with laboratory strains indicated that BHV1.1, obviously of limited pathogenic potential, must have existed at earlier times. Together with published data the results suggest that recognition of a strain as BHV1.1 or BHV1.2 does not necessarily reflect a specific pathogenic potential nor a distinct organ tropism. Nevertheless, the severe IBR incidences were clearly associated with a virulent BHV1.1 variant.

Mapping of the DNA-Binding Site of the L1 Gene Product of Human Papillomavirus Type-16

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It has been demonstrated that the late gene products L1 and L2 of certain papillomavirus types exhibit DNA-binding activity. Hepatitis B virus core proteins are also an example for the interaction of viral capsid proteins with nucleic acids. In this case DNA binding is mediated by carboxyterminally located clusters of basic aa residues. Similarly, the L1 gene product of HPV-16 contains also groups of basic amino acids at its carboxyterminus. To examine whether this region is in fact the DNA binding site of L1, we expressed different
parts of this gene in *E. coli* as β-gal fusion proteins. The vector system which we used allows the cleavage of the viral protein from β-gal by collagenase digestion. Out of the different expression products only the whole L1 protein and its carboxyterminal part bound DNA. To specify the DNA binding site, the coding sequence for the last 30 amino acids of L1 was fused to β-gal. By this measure DNA binding activity could be transferred to β-gal, which did not formerly exhibit such property. Collagenase digestion and use of L1 specific polyclonal antibodies ensured that DNA binding was a genuine attribute HPV-16 L1 gene products.

**Sialic Acid is the Cellular Receptor Determinant for the Avian Polyomavirus BFDV**

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Budgerigar fledgling disease virus (BFDV) represents a new and the first non-mammalian member of the polyoma virus genus (1). BFDV possesses uncommon biological (2) and structural properties when compared with mammalian polyomaviruses (3). The apparently broad host range of BFDV prompted us to study early events in virus-host cell interactions. We found that erythrocytes obtained from a large number of avian and mammalian species are agglutinated by BFDV. Red blood cells were rendered resistant to agglutination by treatment with neuraminidase (NA) from either *Vibrio cholerae* or Newcastle disease virus. The latter enzyme differentiates between α 2,3- and α 2,6-linkages, cleaving preferentially sialic acid attached to galactose via an α 2,3-linkage. NA-treated cells were resialylated using specific sialyl-transferases and CMP-sialic acid. Only a transferase attaching sialic acid in an α 2,3-linkage to Gal was able to restore receptors for BFDV. Accordingly, cultured chicken embryo cells were resistant to BFDV infection following destruction of this receptor by NA treatment.

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**Improvement of Diagnosis of Cytomegalovirus (HCMV) Infections in Immunosuppressed Patients by Detection of HCMV Using a Monoclonal Antibody Directed Against HCMV-Early-Antigen**

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Primary or recurrent HCMV infections can cause severe clinical problems in immunocompromised patients. As far as active HCMV infections in these patients are concerned, the diagnostic significance of serological data can be restricted. Therefore, direct
Detection of cytomegalovirus (antigen) in body fluids has become increasingly important. For that purpose, in our study blood and urine specimens from 60 immunosuppressed patients (52 renal transplant recipients, 2 heart and 2 liver transplant recipients, 4 patients with AIDS) were tested for HCMV. Indicator cell cultures (foreskin fibroblasts) were inoculated with urine and leucocytes, respectively, and 2 resp. 7, 11, and 18 days after inoculation, subjected to an immunoperoxidase staining (IPS) using a monoclonal antibody (Dupont, F.R.G.) directed against HCMV early antigen. As controls, cell cultures inoculated with leucocytes were examined for HCMV-specific cytopathic effects (CPE) for 60 days. Additionally, leucocytes from 30 patients were subjected to in-situ-hybridization with biotin-labeled HCMV AD 169-DNA (Eco RI J-fragment) probes. Serum samples were tested for the presence of HCMV-specific antibodies of Ig classes G, M., and A by ELISA (Behring, F.R.G.). – HCMV was detected in 6 blood and 8 urine samples from 11 patients. 2 blood samples were positive by both IPS and CPE, 3 by IPS only, and 1 by CPE only. Hybridization assays were all negative. In virologically positive cases, titres of HCMV-specific antibodies amounted to (reciprocal titres): IgG ≥ 640 (10 cases, with a significant rise of titre in 1 case), IgM ≥ 40 (4 cases), and IgA ≥ 320 (3 cases). – Our results indicate that, for laboratory diagnosis of active HCMV infections, the detection of HCMV early antigen in urine is, with regard to practicability and rapidity, superior to the test for viremia and is to be considered a helpful extension of serological testing. Moreover, with urine samples being easily obtainable, the detection of HCMV early antigen in urine is especially appropriate, too, for controlling the course of active HCMV infections.

Identification of the Major Immunogenic Antigens of the Human Spumaretrovirus

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The human spumaretrovirus (HSRV) is a distinct member of the foamy virus subfamily of Retroviridae. Its genome of about 11 kilobases has been sequenced. It comprises the typical retroviral genes gag, pol, and env, and at least three more open reading frames possibly coding for regulatory proteins. Thus far, not much is known about the gene products of HSRV. By means of radioimmunoprecipitation, we have identified and partly characterized the major immunogenic antigens of HSRV. Radiolabeled viral proteins precipitated by HSRV-positive sera (but not by HSRV-negative control sera) were in the range of 32 to 170 kilodalton (kDa) apparent molecular weight. Labelling with 14C-glucosamine, or with 35S-methionine in the presence of tunicamycin led to the identification of three viral glycoproteins of 170, 130 and 48 kDa apparent molecular weight, respectively. These glycoproteins most likely represent env gene products. A phosphorylated protein of 60 kDa may be related to the gag gene of HSRV. The results of this study show that radio-immunoprecipitation provides a powerful diagnostic and research tool to identify HSRV-positive sera.
Analysis of the Nonstructural Proteins of the Flavivirus West Nile Virus (WNV)

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The primary structure of the nonstructural proteins NS1, NS2a, NS2b, NS3, NS4b and NS5 of the WNV has been determined. The nonstructural proteins were isolated from nuclear membrane fraction of WNV infected BHK cells. Aminoterminal sequence data of these purified proteins were determined. Together with the published amino acid sequence of the nonstructural coding genom region (Castle et al., 1986, Virology 149, 10–26) we obtained the sequences of the nonstructural proteins NS1 (50 kD), NS2a (19 kD), NS2b (14 kD), NS3 (70 kD), NS4b (27 kD) and NS5 (97 kD). The gene order, the sizes of the virus coded proteins and the processing of the nonstructural proteins appears to be identical between the flaviviruses.

“Autotoxicity” in the Pathogenesis of Infections Caused by Paramyxoviruses and Influenzaviruses

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It is well accepted that the functional impairment and killing of infected cells, hypersensitivity and autoimmunity contribute to the symptoms and pathology of viral diseases. We will discuss evidence for an additional mechanism can be defined as the uncontrolled activation of host effector functions not focused on viral antigens. “Autotoxicity” is evident in the following situations: (i), Certain paramyxoviruses and influenza viruses are capable of activating the generation of reactive oxygen species in phagocytes in the absence of antiviral antibody. This reaction is triggered by the binding of viral surface glycoproteins to the plasma membrane of the phagocytes. When injected into the blood stream, these viruses exert toxic effects independent of viral replication. – (ii), Liver damage is observed in a model of murine influenza despite the apparent lack of viral replication in this organ. This effect may be mediated by cytokines. – (iii), In Canine Distemper, demyelination occurs despite the apparent lack of viral replication in oligodendrocytes, the cells which form myelin. Degeneration of uninfected oligodendrocytes is also observed in dissociated brain cell cultures. As measured by luminol-dependent chemiluminescence, antiviral antibody stimulates the generation of reactive oxygen in microglial cells by linking Fc receptors with viral antigen expressed on the surface of infected cells, e.g. astrocytes. In these cell cultures, oligodendrocytes can readily be destroyed by reactive oxygen species generated by xanthine/xanthine oxidase.
Recombinant Antigens for Cytomegalovirus-Diagnostics

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Only limited information is available about the course of antibody development in cytomegalovirus (CMV) infection. A prerequisite for the improvement of antibody testing are well characterized viral proteins. Our investigations focused on the expression of portions of single, immunogenic structural proteins in bacterial systems. The recombinant antigens were tested in immunoblot assays for their reactivity with human sera. All sera, judged to be positive for IgG against CMV in the ELISA reacted with parts of the phosphorylated tegument protein pp150. However, only a limited number of such sera reacted with the recombinants from another tegument protein called pp65. First results indicate that recombinant CMV-proteins can serve as reliable antigens for serodiagnosis. Moreover, the antibodies made against these recombinant proteins proved to be useful for the detection of viral antigen in patient material.

The Pathogenesis of the Breda-Virus Infection and the Combined Infection with Cryptosporidium sp.

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Bredavirus is grouped into the family of Toroviridae. The virus was first described in 1982 as causative agent for diarrhoea in newborn calves. Strain I and II occur in newborns, strain III which was detected in Ohio causes severe diarrhoea in 6–12 weeks old calves. – 26 h after oral inoculation virus is present and multiplying in epithelial cells from mid-small-intestine caudally. From small intestinal villi epithelial cells are sloughed which results in villous atrophy (v. a.). Additionally focal necrosis of cryptocytes and multifocal lesion in surface cells of the large intestine are present. 48 h p. i. villi fuse, which results in further reduction of absorptive capacity. Viral material is present in M-cells and macrophages below the lamina muscularis mucosae. – In experimental combined infections with Bredavirus II and Cryptosporidium sp. gnotobiotic calves are more severely affected. Alteration occur in these animals all over the small intestine with villous atrophy and epithelial alterations in the large intestine.
Acute Infections with Hepatitis A Virus and Hepatitis B Virus:
Age Distribution and Seasonal Peaks in German and Foreign Populations (1984–1989)

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About 112,000 sera (January, 1984 to August, 1989) were analyzed retrospectively for hepatitis A or hepatitis B markers; 83,000 of these were evaluable. Age distribution and seasonal distribution of acute hepatitis A virus infections in groups of patients with German or foreign names were determined. Most cases of acute hepatitis A in the foreign population occurred between September and December, and most patients were below 15 years of age. In the German population no seasonal peaks could be found. However, there was one peak between 5 and 9, and another between 20 and 49 years of age. – We did not see seasonal peaks of acute infections with hepatitis B virus. No age was preferentially affected, but infections occurred earlier in foreign population than in German people. – In both groups cases of acute infections with both hepatitis A and hepatitis B viruses were only rarely found. More foreign people than German had markers for hepatitis A or hepatitis B (81% vs. 59%).

Examinations of the Prevalence of the Hepatitis C Virus
Among Different Collectives

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We determined the prevalence of antibodies against the hepatitis C virus among different collectives. This agent, presumably a togavirus, at least causes one part of hepatitis Non-A, Non-B infections. – One group consisted of 200 patients with suspected hepatitis Non-A, Non-B during the years 1982–1989. Another group comprised 40 men infected with HIV 1. 37 drug addicts who died from drug abuse in 1988 also were investigated. Finally, we examined 20 dialysis patients with persistent infections with the hepatitis B virus. They were compared with 20 dialysis patients without markers for hepatitis B. All these groups were compared with 500 healthy blood donors. The results are discussed.
Pseudorabies Virus (PrV) Glycoprotein gII: Isolation and Characterization of a gII-Expressing Cell Line

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The glycoprotein complex gII belongs to the essential membrane proteins of Pseudorabies virus (PrV). Therefore mutational analyses of viral gII require growth of the respective mutant in a complementing, gII-expressing cell line. A cell line capable of supplying gII in trans was isolated after co-transfection of a genomic PrV-DNA fragment encompassing the complete viral gII-expression unit and the plasmid pSV2neo conferring resistance against the antibiotic G418. The viral expression unit is usually silent but can be transactivated after superinfection by Herpes simplex virus (HSV-1). Transient expression of the PrV “immediate early” protein is not sufficient to induce transactivation. By immunofluorescence and radioimmunoprecipitation using gII-specific monoclonal antibodies expression of authentic gII could be demonstrated. Attempts to isolate a constitutively gII-expressing cell line failed, presumably due to the toxicity of the expressed glycoprotein. Results regarding processing of gII without the context of a PrV-infection will be presented. Furthermore, availability of this cell line enables us to specifically mutate the gII-gene in the PrV genome and characterize the resulting mutants biologically.

Comparison of Measles Virus (MV)-Specific T Cell Lines from Lewis Rats Primed with Purified Structural Proteins or Bacterial Fusion Proteins

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Cellular and humoral immune reactions are important for the pathogenesis of viral infections. In our animal model of MV encephalitis, Lewis rats develop a subacute CNS disease process (SAME) in the presence of only low levels of neutralizing antiviral antibodies. The contribution of the MV-specific cellular immunity to this disease is yet unknown. Therefore, the in vitro reactivity of polyclonal lymphocyte cultures to MV structural components was determined. Additionally, the possibility to replace purified viral antigens by bacterially expressed fusion proteins was studied. T cells were primed by immunization with antigens in emulsified in Freund’s complete adjuvant. T cells prepared from regional lymphnodes were taken into culture 9-12 days later and lymphoproliferation was measured by the incorporation of tritiated thymidine in the presence of specific and irrelevant antigens with a panel of antigen-specific T cell lines. With all polyclonal T cell populations except those primed with recombinant haemagglutinin (pBD-H) a specific proliferation was obtained when either whole inactivated MV or the immunizing antigen was used for restimulation. While the nucleocapsid (N) or matrix (M)-specific cell lines recognize equally well pBD-N and virion purified N (Nv), or pBD-M and Mv respectively, such substitution for pBD-H and Hv was not found. Our observations indicate the induction of a differential T cell dependent immune response against the procaryotically expressed H compared to the H glycoprotein purified from virion.
Can Autoantibodies Against Triosephosphate Isomerase Contribute to Hemolysis in Viral Infections?

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In the course of acute Epstein-Barr virus (EBV) infection IgM antibodies always occur against two cellular antigens that were characterized as proteins with a molecular weight of 26 kD (p26) and 29 kD (p29), respectively. Purified p29 was identified as a monomer of human triosephosphate isomerase (hTPI). P26 is a so far unknown protein that possesses a high homology with triosephosphate isomerase of rabbits (rTPI). – The two autoantibodies are produced only as IgM class antibodies, there is no switch to IgG. Presumably these antibodies are monoclonal in 40 percent of the patients. – In 25 percent of the cases with acute hepatitis A virus (HAV) infection anti-hTPI/rTPI antibodies were found, too. Acute EBV infection as well as acute HAV infection may be complicated by hemolysis of different extent. IgM anti-hTPI/rTPI antibodies purified by affinity chromatography caused an increased $^{51}$Cr release from human erythrocytes (Rh negative, group 0). The contribution of these autoantibodies to hemolysis in acute viral infections is likely and will be further investigated.

Hog Cholera Virus – Expression of Structural Proteins by Vaccinia Virus Recombinants

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Hog cholera virus causes one of the most important epidemics of swine. Recently the genome was completely cloned and sequenced¹² and the genome localization of some proteins was determined. – The virus probably consists of four structural proteins, three of which are glycosylated (gp44/48, gp33, gp55). For further studies on structural features and biological functions of these proteins or their precursors a HCV cDNA clone covering the whole structural protein coding region was cloned in vaccinia recombination vectors. – By radioimmunoprecipitation and Western blot the molecular weights of the proteins expressed by Vaccinia virus recombinants are identical to those in HCV infected cells. – This system provides also a promising tool for characterization of neutralization epitopes by in vitro mutagenesis. The use of such mutants for protection studies in the natural host will be discussed.

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Induction of Polyclonal and Monoclonal Antibodies Against HIV1 and HIV2 by Immunization with Immune Complexes

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By a new immunization scheme monospecific antibodies against core proteins of HIV1 and HIV 2 and the reverse transcriptase were raised in mice. HIV antigens were separated by SDS-PAGE, transferred onto nitrocellulose and incubated with human serum of HIV infected individuals. The resulting immune complexes were visualized by goat anti-human antibodies conjugated with alkaline phosphatase. For immunization the respective immune complexes coupled to the nitrocellulose were implanted subcutaneously in mice without any other adjuvant. High antibody titers of high specificity were induced by this immunization procedure. With regard to the number of positive clones producing monoclonal antibodies against HIV proteins the immunization using immune complexes as described here was more efficient than immunization with the complete inactivated virus injected with adjuvants.

Recombinant Hepatitis B Virus (HBV) Core Particles Carrying Immunodominant B-Cell Epitopes of the HBV Pre-S(2) Region

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A fusion peptide comprising HBV core antigen (HBc) T-cell recognition sites and an immunodominant HBV envelope pre-S(2) antibody binding site has recently been shown to induce MHC restricted T-cell help for the fused B-cell recognition site (PNAS, 85 : 1610, 1988). In an outbred population the presence of additional T-cell epitopes would be desirable. An ideal HBV vaccine would comprise the high immunogenicity of core particles with the presence of conserved B-cell epitopes from the surface antigens, inducing virus neutralizing antibodies. We have previously constructed a plasmid expressing authentic HBc (subtype ayw) under tac promoter control (F. Schödel, unpublished and Infect. Immun. 57 : 1347, 1989) in E.coli. Core particles purified from this expression system provide protection even across species barriers from acute woodchuck hepatitis virus infection in woodchucks (F. Schödel, K. Manneck, K. Fuchs, H. Will and M. Roggendorf, unpublished). In first attempts we tried to express HBV pre-S(2) epitopes as aminoterminal fusions with HBc, all attempts resulted in no detectable expression due to instability or toxicity. We then inserted oligonucleotides coding for two overlapping pre-S(2) epitopes (DPRVRGLYFPA) (O. Immunol. 137 : 2703, 1986) at the site where in Duck Hepatitis Virus core antigens an insertion of 39 amino acid is found compared to the mammalian Hepatitis Viruses and predicted to be at the surface of particles. The insertion results in the expression of stable fusion proteins with pre-S2 antigenicity in Western-blots. The chromatographic behaviour of HBc/pre-S(2) chimaera on Sepharose 4B is similar to that of authentic recombinant core particles, suggesting that they assemble to particles. A rabbit anti-preS(2) 120–145 antiserum (kindly provided by R. Neurath) recognizes the HBc/pre-S(2) particles in a non-
denaturing ELISA type assay, a monoclonal antibody to the second epitope does not, which shows that a part of the inserted sequence is accessible on the particles. Since we can now express HBc particles carrying pre-S2 epitopes in E.coli the immunologic properties (induction of T-cell and B-cell responses) of purified particles can be investigated.

Adenovirus Infections – Clinical Importance and Diagnosis

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In a clinical survey adenoviruses (AV) were isolated in 45 out of 418 stool specimens of infants (AV 40/1 in 13 cases). 85% of the infants were ill in the first year of life. Gastroenteritis was above all caused by AV 40/1, respiratory infects occurred to the same degree with all types. Enteritis – when combined with a respiratory infection – was considered to be only an accompanying enteritis. 74% of the infants required hospitalization. – Concerning the laboratory diagnosis virus isolation is predominant. The cultivation of viruses in cell cultures takes about a week and allows to differentiate the types. The demonstration of antigens by immunological means (latex agglutination/ELISA) is more rapid (20 min/2h) and detects AV 40/1 very well despite less sensitivity. As confirmatory tests they reduce the time for virus isolation in cell cultures by 2–5 days. – For the diagnosis of AV 40/1 one should give priority to immunological tests, for the other AV-types, virus isolation confirmed by immunological methods has to be recommended. –

Some Aspects of the Pathogenesis of CAA in Embryonated Chicken Eggs and One-Day-Old Chickens

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Infections with chicken anaemia agent (CAA), a small unclassified DNA virus, has become an emerging threat for the poultry industry. For diagnostic purposes and for control of SPF breeding flocks and poultry products sensitive detection procedures are required. To establish the sensitivity of different host systems for CAA, a titration of the infectivity was performed in cultured cells of the MDCC-MSB1 lymphoblastoid cell line in chicken embryos and in one-day-old chickens. – When CAA is inoculated into the yolk sac of 5 day-old embryonated chicken eggs, the embryos can develop normally into chickens. However, at 10–14 days post-hatching the mortality is higher and the anaemia is more severe than in chickens inoculated at one-day-old with the same virus dose. – In both groups CAA antibodies could be detected several weeks post-hatching as determined by virus neutralization test and immunofluorescence test. – MDCC-MSB1 cells were found as sensitive for the detection of CAA as the two assay methods in vivo.
A new ELISA Developed for Identification and Typing of Herpes Simplex Virus (HSV) – Enzygnost®-HSV (Ag)

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The characterization of an enzyme-linked immunosorbent assay, Enzygnost-HSV (Ag), for the identification and typing of HSV is discussed. – Differentiation of HSV was evaluated against 26 laboratory HSV strains. It was shown that the typing indices were valid over a broad dilution range, which is essential for samples of both low and high antigen content. – In a preliminary clinical study (n = 93 specimens) direct testing revealed an identification sensitivity of 89.5%, a typing sensitivity of 90.9% and overall specificity of 93.9%. In a confirmatory test, a 100% agreement for both identification and typing was obtained. – On the basis of these results, it was concluded that Enzygnost-HSV (Ag) is a suitable alternative to the cell culture method, since it overcomes the failure of virus isolation due to possible inactivation resulting from improper transport and/or storage of the specimens. – In comparison with conventional HSV detection and typing test systems, Enzygnost-HSV (Ag) offers the following advantages:
– ease of performance (within 4h),
– less expensive,
– subjective reading of test results using ELISA photometers,
– computer-controlled automation using microtitre plates is possible.

Enzygnost®Anti-EBV IgG: Development of an ELISA for the Detection of Human Epstein-Barr-Virus

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A new Enzygnost®Anti-EBV IgG test has been developed for the determination of EBV-specific IgG antibodies. It has been shown that this test recognizes the EBV-VCA, EBV-EBNA and EBV-EA-D specificities of the IgG antibodies. – As a result of the careful selection of antigens coupled with an excellent choice of conjugates, the test exhibits very high sensitivity and specificity. In a comparison with a commercially available test, Enzygnost®Anti-EBV IgG gave a sensitivity of 99.9% and a specificity of 96.9%. – Cross-reaction with other antibodies has not been detected by indirect techniques. Anticoagulants have been shown to have no influence on test results. The test can be completed in 2.5 h and can be automated by means of the BEP II system. On the basis of these preliminary results, Enzygnost®Anti-EBV IgG is a suitable alternative to the commonly used fluorescence tests.
Detection of Rotavirus RNA/Particles by a Combined Silver-Stained Polyacrylamide Gel/Electron Microscopy Method and Comparison with Four Commercial Enzyme Immunoassays with Respect to Reading Procedures

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Four different commercial immunoassays, two enzyme-linked immunosorbent assays (ELISA) (Rotazyme II, Abbott and Enzygnost Rotavirus-Ag, Behring) and two latex agglutination assays (LX) (Rotascreen, Viramed and Rotalex, Orion Diagnostica) were compared with a combination of silver-stained polyacrylamide gel RNA-analysis and electron microscopy (RNA/EM) for detection of rotavirus in fecal specimens. During 4 1/2 years we registrated by infants < 3 years during epidemic phases in children hospitals a prevalence of rotavirus infection (PRI) of 46.7% (43 of 92). After pretesting 842 specimens, because of this PRI only 389 probes (192 positive and 197 negative in RNA/EM as reference test) were analysed for true calculation of diagnostic sensitivity (dSe), of diagnostic specificity (dSp) and of positive and negative predictive values (pPV, nPV). The dSe was for Enzygnost Rotavirus-Ag and Rotazyme II: 90.6%, for Rotascreen: 78.7% and for Rotalex: 74%. The dSp ranged from Rotalex: 98% over Rotazyme II: 95.9% and Enzygnost Rotavirus-Ag: 91.4% to Rotascreen: 89.8%. The pPV was calculated: Rotalex 97.3%; Rotazyme II 95.6%; Enzygnost Rotavirus-Ag 91.1% and Rotascreen 88.3%. The nPV was 91.3% (Rotazyme II), 90.9% (Enzygnost Rotavirus-Ag), 81.2% (Rotascreen) and 79.4% (Rotalex). With respect to reading procedures of all tests only the coloured or dirty supernatants of 48 (12.3% stool specimens) could be regarded as a strong factor for false positive and false negative results of both LX.

Antibodies to Human Adenovirus Group: Detection by Enzyme-Linked Immunosorbent Assay, Western Blot and Complement-Fixation, and Prevalence in Different Age-Groups in Frankfurt/Main, West Germany

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Sera of 48 persons in the age between 20- to 30-years were tested parallel with the biotin/avidin Western blot (b/aWB), with the biotin/avidin enzyme-linked immunosorbent assay (b/aELISA) and with the complement fixation technique (CFT). Antigens used in all tests of the study were preparations of the human adenovirus type 2. Specific IgG-antibodies directed against two immunoblotted group-specific major adenoviral polypeptides, the hexon epitope α-antigen and the penton base β-antigen, were found in more cases (85%) than antibodies found with the b/aELISA (81%) and with the CFT (48%). The prevalences of IgG- and IgA-antibodies to human adenovirus were studied by b/aELISA in 579 serum samples obtained from 12 different age-groups of Frankfurt/Main, West Germany. The lowest IgG-antibody prevalence (52%) was measured in the six- to 12-month age-group, increasing to 95% in the six- to seven-years age-group. A lot of adenovirus positive sera with specific IgA-antibodies were measured in the one- to two- (30%) and two- to three- (16%) years age-groups. A second peak of IgA-antibody prevalence (12%) appeared in the six- to seven-years and seven- to eight-years age-groups, where the highest prevalence of anti-adenovirus IgG was seen.
High Level Expression of an Active HIV Reverse Transcriptase in E. coli

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The HIV pol encoded reverse transcriptase plays an important role in the viral life cycle. Reverse transcriptase is necessary for viral replication, therefore it is an attractive candidate for antiviral therapy. Detailed functional and structural analysis like e. g. crystallographic studies or neutron solution scattering are predisposition for the development of new inhibitors affecting reverse transcriptase activity. – For this purpose we have constructed a plasmid which allows high level expression of the active reverse transcriptase after introduction into E. coli. By partially adopting the E. coli codon usage and adding the original amino- and carboxy terminal sequence by synthetic oligonucleotides we were able to produce the authentic enzyme in E. coli in considerable amounts (up to 10% of the total E. coli protein) and in a very stable form, as shown by Coomassie staining and by Western blot analysis. Mutants with additional amino acids fused at the aminoterminal sequence show a lower expression and an increased accessibility to the bacterial proteases. – The activity of the authentic and of the amino terminally altered enzyme was compared by standard methods and by an activity gel procedure. Enzyme activity could be detected only at 66 kD and 130 kD, whereas no activity could be detected at 51 kD.

The recombinant produced enzyme can be used for purification and crystallographic studies.

Glycoproteins of Hog Cholera Virus – Characterization and Genome Localization

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The genome of hog cholera virus (HCV) consists of an RNA of 12 kb in length. The RNA contains one large open reading frame(1) which is probably translated into a polyprotein and then processed proteolytically. – Metabolic labeling and radioimmunoprecipitation with a polyspecific antiserum led to the identification of four different glycoproteins – gp55, gp48, gp44 and gp33 – in HCV infected cells(2). Inhibition of N-linked glycosylation through treatment of infected cells with tunicamycin resulted in distinct changes in migration behavior of these proteins in SDS-PAGE. – Various cDNA fragments located in the region coding for HCV encoded glycoproteins were expressed as fusion proteins in bacteria. The purified fusion proteins were used to prepare antibodies specific for the respective HCV glycoproteins. These serological reagents were used in radioimmunoprecipitation assays and Western blots. The following conclusions can be drawn: 1.) gp48 and gp44 represent differently processed forms of a single protein. 2.) The order of the glycoproteins on the HCV genome is NH₂-gp44/48-gp33-gp55-COOH.

References
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Seroepidemiology of Human Papillomavirus 8 (HPV8)

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In patients with epidermodysplasia verruciformis (ev) HPV8 induces lesions with a higher risk of malignant conversion. There is evidence that the virus occurs also in the normal population. As HPV8 can not be propagated in tissue culture capsid antigens are not readily available for seroepidemiologic studies. We therefore expressed the major structural protein L1 of HPV8 and fragments thereof as β-gal fusion proteins in bacteria. The expression vector pROS encodes a FXa-cleavage site, which allows the separation of viral and bacterial moiety by proteolytic digest. Purified viral antigens were used to test 360 sera by Western blot analysis. 12% of the sera revealed antibodies against HPV8 L1 at a dilution of 1 : 20. In some cases the titers exceeded 1 : 150. The antibody prevalence was similar in all age groups. Western blots with L1 fragments showed that the humoral immunoresponse of different persons is not always directed against the same epitopes.

Identification of HBV Variants with a Stop Codon in the Precore-Region in HBeAg-/HBV DNA + Patient's Sera

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The molecular basis for the lack of HBeAg in viremic sera of some acute and chronic infected patients is not clear. In this study it was investigated whether this group of patients is infected with HBV variants which cannot synthesize HBeAg. – Viral DNA isolated from sera of 5 HBeAg negative chronic carriers was amplified (PCR) and sequenced directly or after cloning. In all 5 patient’s sera HBV variants were found which had in common a stop codon in the precore region. – Since the precore protein is the precursor for synthesis of the classical HBeAg, one can conclude that the precore mutation of the HBV variants are responsible for the lack of HBeAg in the serum of these patients. These results also implicate that the expression of HBeAg is not essential for the viability of HBV. At least under immunosuppressive conditions the precore mutation seems not to drastically effect viral replication since a high HBV-DNA titer was observed in the serum of one patient after liver transplantation. – Whether the lack of HBeAg expression is also responsible for the frequent severe and progredient course of infection and the lacking response in interferon therapy is currently investigated with a test specific for precore variant’s infection and with animal models.
Significance of the Nucleoprotein-Specific Immune Response for the Pathogenesis of Influenza Virus Infections

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A highly purified nucleoprotein (NP) preparation from influenza virus infected cells yielded in addition to the commonly known 56 kd protein a 42 kd component which could not be detected in virus particles. Among a series of NP-specific monoclonal antibodies some reacted with both proteins and others were only bound by the 56 kd protein. Among both types of NP-specific monoclonal antibodies only a limited number were bound at the surface of murine cells infected with any type A virus. Another category of antibodies bound to cells infected with a given subtype, but failed to react with the surface of cells infected with a different subtype. The results indicate that only restricted antigenic domains of the native NP and perhaps NP fragments are exposed at the surface of infected murine cells. Additionally, the protective capacity of cell-associated NP was determined by immunization of mice with the purified NP preparation. In parallel, and in order to determine the immunogenic potency of newly synthesized NP, mice were immunized with a vaccinia virus recombinant containing the gene for NP prior to challenge with infectious virus. Although immunized mice produced monospecific antibodies and a cytotoxic T cell response to the employed forms of NP, they were not protected from influenza virus infection.

Molecular Characterization of the RNA S Segment of Nephropathia Epidemica Virus Strain Hällnäs B1

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The S segment RNA of nephropathia epidemica virus (NEV) strain Hällnäs B1 was isolated by molecular cloning of the corresponding cDNA. The RNA is 1785 nucleotides long with the 3’ and 5’ termini being complementary for 23 bases. The viral messenger-sense RNA contains one major open reading frame (ORF) with a coding capacity of 433 amino acids encoding a 49 kDa polypeptide. Compared to the Hantaan S segment cDNA sequencing there is a nucleotide homology of 60% and 61% homology at the amino acid level. Many of the amino acid differences are conservative exchanges. The C-termini of the NEV and Hantaan nucleocapsid proteins are nearly identical. The hydrophilicity profiles are very similar and most of the potential kinase dependent phosphorylation sites have been conserved. In contrast, the following differences are significant: The calculated isoelectric points of the NEV and Hantaan nucleocapsid proteins are 5.6 and 6.7, respectively. The most prominent antigenic determinants predicted by the hydrophilicity profiles are located close to the C-terminus of NEV and close to the N-terminus of Hantaan virus nucleocapsid polypeptides. – BMFT project 0318973A.
Inhibition of Transcription and Cleavage of Histone H3 Following Transient Expression of the FMDV Protease 3C Gene

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Foot-and-mouth disease virus-infected cells suffer from cytopathic effects. One causative agent is the virus protease 3C, as shown by transient expression of respective cDNA in baby hamster kidney cells. In contrast to other cDNA-encoded virus proteins 3C is not detectable by indirect immunofluorescence. It is, however, detectable as de novo synthesized protein 16 hours after transfection by radioimmunoprecipitation. The enzyme is then indistinguishable in size from that found in virus-infected cells, indicating similar autocatalytical release from fused protein. Transcription of protease 3C-encoding cDNA fragments is inhibited, as well as that of co-transfected fragments which do not encode protease 3C, as analysed by Northern blot hybridizations. The shut off of transcription which is one of the cytopathic effects observed in infected cells correlates thus to the production of active protease 3C. The inhibitory molecular mechanisms may involve truncation of the nuclear protein histone H3 at its N-terminus as found by Western blotting. This protein is found similarly truncated in virus-infected cells.

Virusprotein-Specific Antigenicity and Immunogenicity of Tissue Culture Rabies Vaccines

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Pre- and postexposure treatment with inactivated rabies vaccines prevent rabies virus infection without complications in contrast to vaccines of nervous tissue origin. A considerable diversity concerning virus strains (PM, Flury LEP, ERA), cell strains (Wi-38, MRC-5, chick fibroblast, BHK) and concentration procedures (ultrafiltration, ultracentrifugation) exists between vaccines of different producers. The influence of the various criteria on the antigenicity and immunogenicity were tested in in vitro experiments using mono- and polyclonal antibodies against RV proteins with antigen-ELISA, Western blot and immune-electronmicroscopy in unfractionated and fractionated vaccines after rate zonal ultracentrifugation. The immunogenicity was tested in vivo by protection induction (PI) in mice, the results were correlated with the virusspecific antigenicity (VPS-Ag) in tissue culture supernatants. VPS-, immunoglobulin-specific and functional antibody- (AB) and interferon- (IFN) induction was tested in human vaccinees. – RV glycoprotein (GP) and nucleocapsid (NP) concentration in vaccines were correlated with PI in mice. The GP:NP ratio was > 1 in tissue culture vaccines in contrast to brain vaccines. The harvest of GP and NP in TC depends mainly on the cell strain. The virion-associated : soluble GP ratio is production-specific. The concentration virion TC vaccines contain GP, NP, M, L, which induce in vaccinees an early, high and long lasting VPS-, IgG specific AB-response; anti-GP and -NP is induced as early as 3–7 days. IFN-induction is correlated with the applied dosis. No nonresponder was observed and all vaccinees had protecting levels of neutralizing AB from 7–14 days onwards and still after 1 year.
Antibodies against Hepatitis B core Antigen (anti-HBc) and Hepatitis C Virus (anti-HCV) in Blood Donors

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An unsatisfactory reproducibility and comparibility was observed when six available enzyme immune test kits for anti-HBc were evaluated by four German Red Cross Bloodtransfusion Centers. Only 62 of 1838 blood donors were consistently positive, but 228 samples produced discrepant results, although five of the assays used the same inhibition procedure of labeled anti-HBc (Caspari et al., J. Clin. Microbiol., in press). We reexamined the clearly and discrepantly positive samples of this study (besides some negative controls) by an assay which measures the binding of IgG to HBc Antigen by peroxidase labeled anti-IgG. Practically all consistently positive samples were confirmed by the different test principle while practically all discrepant samples were negative. This shows that consistent results by different inhibition assays are indeed reliable while divergent results can be neglected. Anti-HBc is used in some countries as surrogate test for HCV carriers. Using an experimental test kit from Ortho Diagnostics, 2 of 64 confirmed anti-HBc positive and 2 of 389 confirmed anti-HBc negative donors reacted repeatedly as anti-HCV positive. These data do not provide evidence that anti-HBc is useful for elimination of HCV carriers.

Detection of HBV DNA with 32-P-Labeled or Sulfonated Probes

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In this study we compared directly the detection level, sensitivity, and specificity of the most sensitive radioactive and the most sensitive non-radioactive method for detecting hepatitis B virus (HBV) DNA in patient serum by dot blot hybridization, based on our previous experience with 6 different assay systems. The former employed the 32P-labeled HBV RNA probe included in the HepProbe Kit (GIBCO-BRL), detected autoradiographically. An advantage of this kit is the low level of radioactivity of the pre-labeled probe (< 10 μC), thereby permitting its use even in those laboratories lacking a license for radioisotopes. The non-radioactive method involved the use of an HBV DNA probe sulfonated with sodium bisulfite (Chemiprobe Kit, Orgenics), followed by immundetection and an enzymatic color reaction. The detection level of the 32P probe was found to be 0.3 pg HBV DNA, corresponding to 3 x 10^4 genomes in 50 μl serum, compared with only 2 pg with the sulfonated probe. Subsequently, sera from 159 patients with various constellations of HBsAg, and anti-HBe were tested with both methods. The concordance rate was 71% (r = 0.42). Compared with 32P results, sulfonation showed a sensitivity of 80% and a specificity of 67%. Radiolabeling, therefore, still allows the most sensitive and reliable detection of HBV DNA in serum. Sulfonation could eventually provide a feasible alternative to radioactivity if the viral DNA in serum could be sufficiently amplified in vitro, for example with the polymerase chain reaction. Such studies are being planned in our institute.
Influence of Immunosuppression on Diaplacental Tumor Induction by Polyoma Virus in Wistar Rats

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Intravenous inoculation of pregnant Wistar rats with polyoma virus induced tumors, hydronephroses and postnatal antibody formation in their offspring. - Simultaneous peroral application of cyclosporin A during pregnancy resulted in a complete prevention of hydronephroses, a reduction of the rate of tumors and of animals with antibody response.

Immunogenicity of Hepatitis B Virus P-Gene Products

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The frequency and specificity of antibodies to P-gene encoded proteins of human hepatitis B virus was tested in sera of acute and chronically infected patients with and without hepatocellular carcinoma (HCC). For antibody detection an immunoprecipitation gel assay was performed with radioactively labeled polypeptides produced by in vitro translation of RNA of different P-gene regions. Thus, five antigenic regions were identified. All anti-P antibody positive sera reacted with carboxyterminal P-polypeptides, a subset with polypeptides of the aminoterminal and middle region, and none reacted with P-protein derived from the most sequence variable region. Anti-P antibodies were detected at very high frequency in sera of acute (73%) and chronically infected patients without HCC (87%), but less often in HCC patients (27%). Anti-P antibodies appear early in infection and decline prior to HBsAg/anti-HBs seroconversion. - These data indirectly demonstrate the expression of most HBV P-gene sequences and the high immunogenicity of P-proteins in vivo. Moreover, they establish anti-P antibodies as a frequent serological marker of infection and identify the carboxyterminal region of the P-proteins (s) as immunodominant.

Analysis of T-Antigen Expression in a Transformed Hamster Cell Line of Glial Origin (HJC)

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The human polyomavirus JC produces brain tumors in hamsters. In these tumors JCV T-antigen, the major product of the early genes is generally expressed. The protein plays a central role in the regulation of polyomavirus replication. Since tissue culture as a major source for T-antigen is limited to human primary fetal glial cells we characterized JCV T-antigen in the hamster cell line HJC derived from a JCV induced medulloblastoma. - Southern Blot analysis revealed the presence of JCV DNA in HJC cells. The physical state
was predominantly integrated. The transcription of early genes was demonstrated by presence of JCV specific mRNA in Northern Blots. The nuclear localisation of T-antigen could be established by immunohistochemical staining and by immunoprecipitation with a cross-reacting monoclonal SV40 antibody (Pab 416) a molecular weight of 85 to 90 kD was determined. Furthermore from Western Blot analysis it could be assumed that high molecular forms of JCV T-antigen are present in the hamster cell line. Taken together these data demonstrate that the molecular weight and nuclear localization were as expected from SV40 transformed cell lines. Therefore JCV T-antigen in HJC cells shall be used to characterize its DNA binding capacity to the origin region of JC virus DNA.

Serum Antibodies to Coxsackie-B-Viruses Analysed by a Modified Western Blot Technique

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Current laboratory diagnosis of a Coxsackie-B-virus (CBV) infection is mainly based on virus isolation, supported by the detection of rising or high virus-specific neutralizing antibody titers. Since such high titres have also been found in apparently healthy people – probably originated from a subclinical infection and persisting for a year or more – CBV-IgM detection may be a more reliable criteria for serological diagnosis. Attempts to identify these antibodies in routine context using conventional techniques (NT, immunodiffusion) or solid phase immunassays (EIA, RIA, reverse ELISA, immunoblot) have failed, despite high sensitivity and specificity, due to high costs or technical disadvantages. In the present study we developed a modified Western Blot Technique (Modi-Western Blot), which enables a rapid and reliable identification of CBV-IgM antibodies. In this assay the antigen was electrophoretically separated using an integrated electrophoresis system (PhastSystemUnit) and transferred to a solid matrix by diffusion blotting. Subsequent immunodetection was performed using a biotin-avidin amplification and monoclonal antibodies. To appraise the efficiency of the method 51 human serum samples were analysed for virus-specific antibodies (IgM, IgA and IgG subclasses) to Coxsackie-B-viruses. – Group specific IgM responses could be found in 22 of 31 (70.9%) of IgM seropositive cases. Type-predominant IgM antibodies (9/31) were detected in 3 cases against CBV1, in 5 cases against CBV2 and in 1 case against CBV4. Moreover IgG/IgA assay in 8 patients seropositive for IgG and IgM revealed in all cases IgG3 and IgA antibodies, which supports the evidence of an acute CBV-infection.