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Mapping of DNA Restriction Enzyme Fragments of Adenovirus 15/H9 Intermediate Strains

ADRIAN, TH., BASTIAN, B., MÜLLER, CHR., WAGNER, V., and WIGAND, R.

17 adenovirus strains were found to be antigenically related to prototype Ad 15 by neutralization. No relationship to Ad 15, but to Ad 9 could be detected by hemagglutination-inhibition; we therefore named them Ad 15/H9 intermediate strains. – After analysis of the genome by five different restriction enzymes, the fragment patterns obtained deviated widely from the prototype Ad 15, but only slightly from Ad 9. Differences could also be observed among the variants. After digestion by five restriction enzymes, altogether six genome types could be established among the 17 intermediate strains. To map the variations on the genome of the 15/H9 strains, two methods were employed: the double digestion of the DNA and DNA fragments together with the determination of the terminal fragments made it possible to construct a physical map. The second method depends on a particularity of adenoviruses: the DNA is covalently linked with a 55 kD protein at the 5′ terminus. After digestion of the DNA, which does contain this protein, the terminal DNA fragments do not migrate into the agarose gel; after an additional digestion with pronase B, they do migrate into the gel. Thus the terminal fragments were determined by comparing the fragment patterns with and without previous pronase B treatment.

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The DNA of Choristoneura fumiferana Nuclear Polyhedrosis Virus. Molecular Cloning and Restriction Mapping

ARIF, B.1 and DOERFLER, W.

The nuclear polyhedrosis virus of Choristoneura fumiferana is a member of the baculovirus group and contains a double stranded covalently closed circular genome. Analyses by EcoRI, XbaI, HindIII, BamHI and BglII revealed a complex pattern indicative of a large

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Studies of Infected Brain Samples, Using Measles Virus cDNA Clones as Probes

BACZKO, K., BILLETER, M., and TER MEULEN, V.

Using measles virus genomic RNA as template 4 different groups of cDNA clones were synthesized. These clones represent different parts of the genome. Group 1 represents the 3' end, groups 2 and 3 are from the middle of the genome and group 4 contains the 5' end. Using these clones and those derived from measles virus mRNAs (Rozenblatt et al.) it is possible to detect in Northern blots the different measles virus mRNAs. - For further experiments RNA was extracted from rat brains, human control and SSPE brains, subjected to analysis in Northern blots using the available measles cDNA clones as probes. The measles virus specific RNA profile of infected rat brain was similar to that of lytic infected cell cultures. However, the results were different with SSPE brain RNA. It was constantly possible to detect sequences from the 3' and 5' end of the viral RNA, while other RNAs were partly missing. Especially, no “M” mRNA could be detected. This leads to the conclusion that in SSPE the majority of the virus persists as a deletion mutant.

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Immunoprevention of Friend Virus-Induced Murine Leukemia. Sequence Specific Antibodies Against the Viral Envelope Polypeptide

BAYER, H., GRUBER, W., SCHNEIDER, J., and HUNSMANN, G.

Erythroleukemia induced by Friend virus (FLV) in STU mice can effectively be prevented by vaccination with purified FLV envelope glycoprotein (gp70) (1). - We selected for synthesis type specific oligopeptides from the aminocacid sequence (2). These peptides were covalently linked to carrier proteins and injected into rabbits. The specificity of the antisera was examined by enzyme-linked immunosorbent assay. - The peptides were immunogenic in rabbits and four of five elicited antibodies to the native gp70. - We now want to test the biological activity of those sequence specific antisera and use synthetic peptides and their respective antisera for vaccinations and immunotherapy.

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Mapping of the e-Epitop in Core-Protein of Hepatitis B Virus by Limited Proteolysis

BAUMGARTEN, U. and GERLICH, W. H.

Core-Particles were isolated from infected human liver and phosphorylated in vitro with $\gamma^{32}$P-ATP. The core-antigen of the particles was inactivated by traces of SDS. The e-antigen was set free by SDS and it was completely stable at 2% SDS, 100 °C. Core-Particles were digested with V8 protease, Lys C protease or Clostripain and the fragments were detected after SDS gelelectrophoresis by autoradiography or staining with peroxidase labeled anti-HBe antibody. The fragmentation patterns and the distribution of $^{32}$P and e-antigenicity suggested, that the major e-epitopes are located around amino acid 80 of the core-protein sequence. This site is very hydrophilic and well accessible to proteases. The DNA-binding carboxy-terminal region (position 150–185) is protected against proteolysis and probably the site of in vitro phosphorylation.

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Acylation of Semliki-Forest-Virus Glycoproteins and Endogenous Lipids in vivo and in vitro

BERGER, M. and SCHMIDT, M. F. G.

Acylation through the covalent binding of fatty acids is a feature of membrane proteins of diverse origin (Schmidt, 1982). Using BHK-cells infected with SFV we performed short pulse/chase experiments which revealed, that the precursor glycoprotein p62 serves as the primary polypeptide acceptor for fatty acids 2 to 3 min prior to its cleavage to E2. Labeling of p62 with $^3$H-fatty acids could be achieved in pulses as short as 30 seconds. Due to an even more rapid incorporation of $^3$H-palmitic acid into a large number of endogenous lipids only a few lipid species could be excluded with certainty as potential acyl-donors for protein-acylation. A more direct analysis of protein-acylation was attempted through the acylation of exogenous protein in vitro by microsomal preparations derived from BHK-cells. Incubations of such preparations with $^{14}$C-palmitoyl-CoA and deacylated glycoproteins of Semliki Forest virus led to the enzymatic transfer of $^{14}$C-palmitic acid into Semliki Forest virus glycoprotein E2.

Transcription of Herpesvirus saimiri in Productively Infected and Transformed Cells

BODEMER, W., KNUST, E., DIETRICH, W., and FLECKENSTEIN, B.

The transcription of the oncogenic primate herpesvirus H. saimiri was analyzed in lytically infected owl monkey kidney cells and in the H. saimiri transformed T-lymphoid
cell line # 1670. Total cellular, polyadenylated RNA was isolated, used as a template for the synthesis of $^{32}$P-labelled complementary DNA (cDNA) with AMV-reverse transcriptase and the cDNA was hybridized with cloned DNA fragments representing the entire genome. It became apparent that one short genome region between map units 0.89 and 0.91 is predominantly transcribed in the transformed 1670 cells. The direction of transcription could be determined from the intensity of hybridization of the $^{32}$P-labelled cDNA to two adjacent Hind III fragments. Northern blot hybridizations indicated that this transcript is a polyadenylated RNA of about 2.5 Kb. For comparison, we performed similar experiments with RNA from lytically infected cells. cDNA hybridization and Northern blot analyses indicated that the same viral DNA sequence is abundantly transcribed during the immediate-early phase of viral replication. This suggests that this immediate-early gene product of H. saimiri may play a role in maintenance of transformation and/or persistence of viral genomes in transformed cells.

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The sis Oncogene in Primate Tumors

BORN, MARTINA and VON DER HELM, K.

Cells transformed by primate simian sarcoma virus (SSV) express a 115000 dalton protein (p115) that is immunoprecipitated by a goat antiserum (antiSSV) prepared against disrupted SSV (SSAV)-infected and transformed cells. The protein can be phosphorylated in vivo and in vitro at the tyrosine residue.

To determine whether our goat anti-SSV serum recognizes phosphoproteins in human sarcomas similar to the p115 found in SSV-transformed primate cells and whether such proteins show a phosphokinase activity, we chose the immunoprecipitation of tumor cell lysates and labelled the immunoprecipitates with $^{32}$P-gamma-ATP. - We were able to detect phosphopolypeptides of about 120000 to about 130000 dalton in lysates of cultured cells derived from two dermatofibrosarcomas and a reticulosarcoma, respectively, as well as in fresh fibrosarcoma tumor material. - All phosphopolypeptides detected in human sarcomas can be phosphorylated in vitro at serine and threonine residues.

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Replication of Herpes Simplex Virus in Human Lymphocytes: Analysis of the Target Cell Population and its Possible Relevance in Generalized HSV Infections

BRAUN, R., TEUTE, H., REISER, H., KIRCHNER, H., and MUNK, K.

We have previously shown, that Herpes simplex Virus (HSV) replicates in not more than 1% of mitogen stimulated human T lymphocytes and that the replicating T cell subset may be both of the helper or suppressor subtype. Nevertheless, analysis of HSV replication in sorted OKT 4+ and OKT 8+ T cells revealed, that again only a minor part of these T cells supported productive infection. Various experimental approaches showed, that only T cells expressing on their surface Ia antigen, a product of the immune response region, actively produced the virus. Such Ia expressing T cells are not found in the peripheral blood of normal donors, but are present in various amounts in cord blood samples and in the blood of transplantation patients. T lymphocytes containing increased numbers
of Ia expressing cells are susceptible to HSV infection without any prestimulation. Thus it appears, that Ia expressing T lymphocytes may play an important role in generalized HSV infections.

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**EBV-Inducing Factor: A Novel Growth Factor**

**BAUER, G.**

We have recently demonstrated the existence of a humoral factor that induces latent EBV genomes (*Bauer* et al., Virology 121 (1982) 184-194). The factor has been purified from calf serum and has been characterized (*Bauer* et al., J. Biol. Chem. 257 (1982) 11405-11410, 11411-11415). – It is shown here that purified EBV-inducing factor exerts growth promoting activity on NIH 3T3 cells. Nanogram amounts of factor allow normal growth when added to 3T3 cells kept in plasma. Biochemical analysis ensures that the growth promoting activity is identical to the EBV-inducing factor and is different from known growth factors. – The factor described here is localized in platelets but can also be isolated from a variety of malignant and nonmalignant cells. – The conversion from an inactive to an active state in-vivo is demonstrated.

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**T-Antigen Mutants Derived from SV40-Transformed Cells**

**BURGER, C.¹, HUBER, B.², and FANNING, E.²**

SV40 T antigen is a multifunctional regulatory protein which controls the lytic cycle of infection and the transformation of nonpermissive rodent cells by SV40. In order to distinguish the biochemical functions of T antigen in lytically infected cells from those in transformed cells, the ability of T antigens from 8 different transformed cell lines to replicate SV40 DNA was tested. Excision and replication of integrated viral DNA upon fusion with uninfected monkey cells, and DNA transfection experiments, revealed that only 3 lines contained replication-competent T antigen. SV40 DNA was excised and replicated in all lines upon fusion with the Cos 1 line, which provides competent T antigen in *trans*. The 5 mutant T antigens were then investigated biochemically. All of them were phosphoproteins of wild-type size. All formed complexes with the p53 host protein and bound ATP. However, 3 of them displayed abnormal DNA binding properties. The DNA replication defects in the other 2 mutants are unknown. We are presently cloning the mutant genomes to determine the structure of the mutant DNA.

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**Sero logical Surveillance of Renal Transplanted Patients for Cytomegalovirus Infections**

**DENNIN, R. H., SCHULZ, E., SACK, K., and HERHAHN, J.**

Clinical manifestation of cytomegalovirus (CMV) infection is rare among healthy adults. In contrast patients with renal transplantation exhibit relatively high incidence rates from
Acute CMV-infection. Mostly rejection crisis, deterioration of kidney function coincide with CMV-infection after transplantation - hence there is strong requirement for rapid and reliable CMV-diagnostic with respect to differential diagnosis. Therefore frequent serologic assessment of serologic CMV-status with the ELISA-technique were performed to evaluate the usefulness of this method. This was done with parallel measurement of circulating free serum-IgG-concentration: The virus specific titers essentially follow partly the drastic variation of free serum-IgG - as demonstrated also with Elisa-antibody-titers against other viruses, Although the development of CMV-specific IgG- and -IgM-titers and the relation of both allow to assess and to follow up primary and secondary CMV-infections resp. it has to be considered that in cases of immunosuppressed patients titer changes in the post transplantation period could be the result of a general antibody alteration.

Structural Analysis of Herpesvirus saimiri DNA by Molecular Cloning

DIETRICH, W., KNUST, E., SCHIRM, S., BODEMER, W., and FLECKENSTEIN, B.

The major population of Herpesvirus saimiri genomes (M-genomes, 45% GC) consists of a unique L-DNA region (36% GC) flanked by repetitive H-DNA (70.8% GC) of variable length. The terminal H-sequences of M-DNA were identified by 32P-labelling with T4 DNA polymerase and comparison of labelled fragments with several H-DNA cleavage maps. By this means the relative orientation of L- and H-DNA could be determined. - For the cloning of the whole L-DNA region fragments generated by Eco RI or Kpn I were inserted into the respective sites of pACYC 184, λ Charon 4a or pJC81. The terminal fragments of L-DNA were cloned in the new vector pWD7 after cleavage with Kpn I and Sma I. With double digest analysis of cloned fragments or hybridisation of 32p labelled cloned HindIII fragments with partial digests of the fragments Kpn I C or Eco RI C detailed HindIII cleavage maps could be derived for about 40% of the L-DNA region.

Transduction and Expression of Nonselectable Genes Using Retroviral DNA Vector

DOEHMER, J., STRATOWA, C., HUGHES, J., and HOFSCHNEIDER, P. H.

We have used the integrated and the nonintegrated forms of Moloney mouse sarcoma virus (MSV) as eukaryotic vectors for introducing the Hepatitis B surface antigen (HBsAg) (1) or the rat growth hormone (rGH) (2) genes into NIH 3T3 mouse fibroblasts. The ability of the proviral forms of MSV to transform 3T3 cells morphologically was used as a dominant selective marker. Several transformed cellular clones were isolated and analyzed for expression of the inserted genes. - Those cellular clones containing the rGH gene were shown to produce a spliced mRNA and to release growth hormone into the culture medium, which cochromatographed with bona fide rGH. Addition of dexamethasone resulted into enhanced production of rGH. - Those cellular clones containing the HBsAg gene release the HBsAg into the culture medium. HBsAg made by mouse fibroblasts is indistinguishable in its physical and immunological properties from HBsAg obtained from HBV infected
patients. HBsAg as well as rGH appear to be useful expression markers to study eukaryotic signals.

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Retrovirus-related Genes in Normal Uninfected Cells may Play a Part in Cellphysiology

DORNBURG, R., MONDAL, H., and HOFSCHEIDER, P. H.

Retention of retrovirus-related genes in vertebrates during evolution raises the question of possible association of retroviruses with common cell physiology. In the past few years we have shown that the differentiation of organs during embryogenesis is correlated with the expression of retrovirus-like particles (retroviral elements) in Japanese quails and in human beings. Here we describe a similar phenomenon in uninfected, virus-free domestic chicken. These particles possess reverse transcriptase distinct from the enzymes of the AMV/ASV group as reported earlier. Simultaneous detection tests now reveal that they also contain high molecular weight (63–70S) RNA. This RNA can only be hybridized to little extent (15 to 20%) to calf-thymus-DNA primed cDNA of RAV-O-RNA and vice versa. For further characterization molecular cloning of a DNA complementary to that RNA is in progress.

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Characterization of Human Papillomavirus DNA Isolated from a Cervical Carcinoma

DÜRST, M., GISSMANN, L., and ZUR HAUSEN, H.

A 7.2 kb fragment as well as other submolar fragments were identified in the DNA of an invasively growing cervical carcinoma after hybridization with 32P-labelled HPV 11 DNA under non stringent conditions. The 7.2 kb fragment and two other fragments were cloned in lambda L 47 and subcloned in pBR 322 for further analysis. The former fragment was found to be in an episomal state and only to a very small extent in monomeric form in the tumor DNA. Hybridization of the 7.2 kb fragment with other HPV types showed this to be a new type thus designated as HPV 16. The submolar fragments showed cross-hybridization with HPV 3, 6, 10, 11 and 16 only under conditions of low stringency. There is evidence that these molecules contain repetitive as well as unique cellular DNA.
Molecular Cloning and Physical Mapping of Murine Cytomegalovirus DNA

EBELING, A., KEIL, G., and KOSZINOWSKI, U.

The DNA of the murine cytomegalovirus (MCMV), Smith strain is cleaved by the restriction enzyme HindIII into 16 fragments, ranging in size from 0.64 to 22.25 megadalton (md). 15 of the 16 fragments were cloned in the plasmid pACYC177 in the E. coli strain HB101 RecA-. With the help of HindIII/XbaI and HindIII/EcoRI double cleavage experiments, subcloning of the HindIII/XbaI fragments obtained after double cleavage in the plasmid pACYC184 and hybridizations of 32P labeled clones to MCMV-DNA cleaved with different restriction enzymes we could establish physical maps for the enzymes HindIII, XbaI and EcoRI. From these data we determined the molecular weight of the genome to about 235 kilobasepairs or 155 md respectively. All fragments were found in equimolar concentrations and no cross hybridization was found between any of the fragments. Therefore we conclude that the MCMV-DNA consists of a long unique sequence without large terminal and/or internal repeat regions. Thus the structural organisation of the MCMV-DNA is fundamentally different from the genome of human CMV.

Myocarditis in Experimental Poliomyelitis. A Contribution to the Pathogenesis of the Disease

EGGERS, H. J., ROSKOPF, ULRIKE, and ARNOLD, G.

Myocarditis in patients with poliomyelitis has been reported latest since 1912. The pathogenesis of this myocarditis, however, is not understood, in particular, virus multiplication - to our knowledge - could not be unequivocally demonstrated. Newborn, 10 day- or 6 week-old NMRI-mice were inoculated intracerebrally with 5 to 800 PD50 of poliovirus 2, MEF1. Virus titers of $\geq 10^6$ PFU per g organ were obtained within 48 h in the brain and spinal cord, whereas no definite virus multiplication was recorded in liver, kidneys, or lungs. In contrast, significant virus replication was demonstrated in the heart ($10^{5}$-5 PFU per g). Some of the animals had profound electrocardiographic changes. Virus replication could be inhibited in the animals by the polio-virus-specific inhibitor arildone. Histologically, myocardial fibers exhibited various degrees of degeneration and necrosis with adjacent infiltration of lympho- and histiocytes. The lesions were disseminated throughout the myocardium. These studies indicate that myocardial involvement in poliomyelitis is due to virus multiplication, and define an extraneural site of poliovirus replication in the organism.
Bovine Cytomegaloviruses

EHLERS, B.1, STORZ, J.2, GELDERBLOM, H.3, and LUDWIG, H.1

Six herpesvirus strains from the US and Europe, two of them from cases of malignant catarrhal fever, the others from different clinical entities, were characterized with respect to their biology and molecular biology. Morphogenesis and growth properties of these viruses are similar to known cytomegaloviruses. The DNA of one strain has a molecular weight of $96 \times 10^6$ d, as determined by electronmicroscopic length measurement. After restriction enzyme analysis the DNAs of the six viruses show very similar cleavage patterns, which differs completely from those of other bovine herpesviruses like BHV-1, -2, -3 and -6. Because of the above described properties the strains were classified as BHV-4, a new group of bovine herpesviruses.

Effect of Macrophage-Blocking Agents on the Pathogenesis of Scrapie in Mice

EHLERS, B. and DIRINGER, H.

The effect of RES-blockade by Dextran sulfate 500, Trypanblue and Silica on the pathogenesis of scrapie in STU mice was studied by comparison of incubation times. Groups of 6–10 animals were treated with blocking agents either 24 h, 6 h, 2 h before scrapie infection or 72 h afterwards. Only DS 500 (1 mg i. p./animal) prolonged the incubation time from 160 to 200 days and also produced survivors. This effect was correlated to histologic changes in the spleen.

Excision of Amplified Viral DNA from Ad12 Transformed Hamster Cell Line T637 in vivo and in Isolated Nuclei

EICK, D. and DOERFLER, W.

In the adenovirus type 12 (Ad12)-transformed hamster cell line T637, about 20–22 copies of viral DNA persist in an integrated form. Spontaneously arising morphological revertants have lost most or all of the viral DNA. One copy or part of one copy can persist after reversion. The excision event leading to the loss of amplified Ad12 DNA in the revertants can be mimicked in vitro by incubating isolated nuclei of T637 cells. After incubation of T637 nuclei for 1 h at 37 °C, the same viral off size DNA bands disappear that have been lost in morphological revertants. In cell line T637, analysis of the structure of viral DNA fragments, that proved sensitive to endogenous nuclease digestion, revealed that these fragments may contain palindromic sequences. These sequences were lost from the DNA of morphological revertants and from DNA extracted from T637 nuclei after auto-
digestion. Further work will concentrate on the elucidation of the structure of the presumptive palindromes in cell line T637.
(Supported by SFB74-C1 of the Deutsche Forschungsgemeinschaft.)

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The Pathogenesis of Genital Herpes Simplex Virus-Infection in Acyclovir Treated Mice

EIS, A. M. and SCHNEWEIS, K. E.

C 57BL mice, vaginally infected with herpes simplex virus type 1 (HSV-1), were treated with acyclovir (ACV); intraperitoneal doses of 50 mg ACV/kg were applied at 36 h after virus inoculation and repeated every 12 h over a period of 7 days. – Compared to the controls, virus replication in the vagina was only moderately diminished, and virus elimination from the vagina was not accelerated. Nevertheless, no clinical symptoms became manifest and none of the test mice died. Viral latency was also drastically diminished. – This was shown not to be due to an inhibition of virus replication in the acute phase of the ganglionic infection, but to prevention of virus invasion into the nervous system. The data can be well interpreted by the assumption that the virus replication was predominantly inhibited in the inferior layers of the epithelium of the mucous membranes.

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Interaction of Human Oligo-Astrocytoma Cells with SV40

FISCHER, H.I and SCHWECHHEIMER, K.2

Cell cultures from biopsies of human brain tumors allowed differential expressions of the SV40 functions as monitored by the viral antigens and their growth behaviour. A human brain tumor classified by routine method as mixed oligo-astrocytoma grade II was successfully grown in tissue culture. One part of the cells (about 40%) were specifically stained by an antibody to glial fibrillary acidic protein (GFAP) which is regarded to be an astrocyte-specific cell marker. – After infection (transformation) with SV40 most of the cells showed specific T- and V-antigen fluorescence. The supernatants contained free virus. 5 passages after transformation the supernatants became virus free and only the GFAP expressing astrocytes with T-antigen positive nuclei and enhanced growth were found. – The oligodendrocytes were abolished by cell lysis due to virus propagation. The evident correlation to human progressive multifocal leukoencephalopathy will be discussed.

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Transcription of Polymorphic Regions of the Epstein-Barr Virus Genome in Five Different Virus Strains

FREESE, U. K., LAUX, G., POLACK, A., MÜLLER-LANTZSCH, N., and BORNKAMM, G. W.

Epstein-Barr-Virus (EBV) infected lymphoblasts are a model to study viral persistence in non tumorigenic transformation. The close association of EBV to undifferentiated
nasopharyngeal carcinoma (NPC) and to endemic Burkitt's lymphoma (BL) suggest a role for the virus in the development of malignant transformation of human cells. We studied viral gene expression in the prototype strain M-ABA-EBV and compared the transcriptional pattern to the pattern of five additional EBV-virus strains, which differed in their genome structure by restriction fragment polymorphism due to deletions, insertions or varying length of tandem repeat arrays. Some of the deletions correlate with the loss of biological properties of the respective virus strain. We find changes of the transcriptional pattern in all these instances which allow gross assignment of biological functions to distinct transcription units of EBV.

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Host Target Proteins for Onc Gene-Specific Phosphorylation

FRIIS¹, R. R., EIGENBRODT, E., and RÜBSAMEN, HELGA

Temperature sensitive src mutants of Rous sarcoma virus have been used to study host protein phosphorylation during the onset of transformation. The behaviour of several glycolytic enzymes as well as the 36,000 d target protein (Radke and Martin, PNAS 76 (1979) 5212; Erikson and Erikson, Cell 21 (1980) 819) during the onset of transformation has been analyzed. Several stages of column chromatography including two additional steps of affinity chromatography (5'-AMP Sepharose and Blue Sepharose) have made possible the separation of the 36,000 d (38,000 d in our hands) protein from cytosolic malic dehydrogenase (cMDH) (also 38,000 d, pI 7.5) which has been an unavoidable contaminant in previous purification schemes. The purified 38,000 d protein appears to be distinct from cMDH according to V8 protease partial digestion, exhibits rapid phosphorylation after temperature shift, exclusively in tyrosine, and to a higher molar ratio than had previously been reported. A 50,000 d phosphoprotein copurifies with the glycolytic enzyme enolase, becoming slowly phosphorylated in tyrosine after transformation, but to a modest molar ratio.

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Cloning and Characterization of Human Papillomavirus DNA from Flat Warts

FUCHS, P. G. and PFISTER, H.

Human Papillomavirus (HPV) DNA was extracted from flat warts of an individual patient and cloned in pBR322. It was hardly related with HPV3 and 10, which prevail in this kind of lesions. Reassociation kinetics revealed about 55% cross-hybridization with HPV2, which is most frequently associated with common warts. This identified the new isolate as HPV2 subtype, which is only distantly related with the prototype HPV2a (50% cross-hybridization is arbitrarily defined as a borderline between two HPV types). DNA cleavage patterns and physical maps underlined the heterogeneity. The new subtype was also detected in another verruca plana case and in a wart biopsy which was not clearly defined. It is presently tested if the new subtype causes tumors that differ in morphology and histology from HPV2a induced lesions.
Insertion of Adenovirus Type 2 DNA Proceeds without Deletion of Cellular DNA: The Mechanism is Different from that of Bacteriophage λ or of Retroviruses

GAHLMANN, R. and DOERFLER, W.

The adenovirus type 2 (Ad2)-transformed hamster line HE5 contains viral DNA covalently linked to cellular DNA. The viral genomes exhibit an internal deletion. The sites of linkage between viral and cellular DNA, the unoccupied cellular site equivalent to the insertion sequence, and the internal link between the two fragments of viral DNA have been cloned in prokaryotic vectors. The nucleotide sequences at these sites have been determined. The results of this analyses are the following. 1. Viral DNA has been inserted at a cellular sequence that is represented once or a very limited number of times. 2. The cellular DNA sequence at the site of insertion is completely preserved, not a single nucleotide has been deleted. 3. At the right terminus of viral DNA eight nucleotides have been deleted and ten at the left. The sequence data clearly demonstrate that adenovirus DNA inserts into cellular DNA by a mechanism different from that of bacteriophage λ or of retroviruses. Thus, there may be yet another mechanism of inserting foreign DNA into eukaryotic DNA.

(Supported by SFB74-C1 of the Deutsche Forschungsgemeinschaft.)

Structural Basis of the Fusion Capacity of the Influenza Hemagglutinin

GARTEN, W. and KLENK, H.-D.

Two enzymes are involved in the proteolytic activation of the influenza hemagglutinin. A trypsin-like enzyme which is provided by the cell and a second enzyme which is associated with the virion and eliminates arginine from the C-terminus of HA₁ and thus exhibits the specificity of an arginine carboxypeptidase. – Carboxypeptidase activity was found in the virus envelope by a sensitive radio assay which utilizes a matrix bound synthetic peptide terminating with [(3)H]-L-arginine. The carboxypeptidase was separated from viral glycoproteins by isoelectric focusing and affinity chromatography. The enzyme is host-specific as its isoelectric point depends on the host cell. Elimination of arginine from the hemagglutinin cleavage site is blocked by a specific inhibitor (2-mercaptomethyl-3-guanidinoethyl-thiopropanoic acid). The fusion capacity of influenza virus measured by hemolysis is the same whether arginine is present or not at the C-terminus of HA₁. When the amino-terminus of HA₂ is shifted in carboxyterminal direction as occurring after cleavage with chymotrypsin or thermolysin hemolysis is not found. This observation demonstrates that the fusion capacity depends on a specific sequence of HA₂-N-terminus.
Morphometric Studies with Brains of Scrapie-Infected Hamsters

KAATZ, R., BECKMANN, H., SCHIFFER, J., PLEGER, G., GATZKE, H. D., and DIRINGER, H.

Brains of inbred female hamsters (CLAC) were investigated 61, 77, 124, and 148 days after i.p. infection with quantitative histological methods using an IBAS 1 image analysis system. Healthy and pathologically altered glial cells and neurons were counted in the thalamus and in the white matter of the cerebellum and compared with normal controls. The measured data were printed as cell densities and analysed with IBAS 1 statistic and graphic software. At day 61 already there is a distinct increase in the number of pathologically altered glia cells in scrapie brains as compared with the controls. Neuron density remained unchanged in the thalamus and decreased in the white matter of the cerebellum not before day 148. Quantitative histological methods possibly will demonstrate glial cell reactions even earlier than 60 days after an i.p. scrapie infection.

Characterization of a New Type of Defective Parvoviruses

GEORG-FRIES, B., BIEDERLACK, S., BANTEL-SCHAAL, U., and ZUR HAUSEN, H.

An apparently helper-dependent parvovirus was isolated from a penile flat condylomatous lesion by inoculating this material into a culture of human fibroblasts and by coinfecting these cells with adenovirus type 12. The virus shares biological properties with characterized adeno-associated viruses (AAV) in its dependence on adeno- or herpes-viruses for expression of viral structural antigens; but it differs in its DNA and in the size of its proteins from the four AAV types found so far in primates. – Approximately 60% of the adult population reveal antibodies to structural components of the new type of defective parvoviruses, tentatively labelled as AAV 5. The seroepidemiology differs from that reported for other AAV serotypes. The pattern resembles more closely that of herpes simplex viruses than that reported for seroconversion against adenovirus group specific antigens. These results could suggest that herpes simplex (HSV) rather than adenoviruses represent the natural helper of AAV 5.

Presence of a New Type of Human Papillomavirus (HPV 16) in Genital Tumors

GISSMANN, L., DÜRST, M., IKENBERG, H., and ZUR HAUSEN, H.

Human Papillomavirus DNA of a new type (HPV 16) was identified in a cervical carcinoma biopsy and molecularly cloned in lambda L 47 (Dürst et al., these proceedings). – HPV 16 DNA was used as probe to test additional genital tumor biopsies for the presence
of homologous sequences. - 22% of cervical carcinomas in situ, 29% of vulval cancer, 17% of penile cancer biopsies as well as 58% of cervical carcinomas of German origin and 35% from African patients were found to be positive. In contrast only 6% (2 out of 33) of genital wart specimens harboured HPV 16 specific DNA. Both positive biopsies contain also HPV 6 or HPV 11 DNA, respectively, suggesting a helper dependence for HPV 16 replication.

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**The Significance of the Borna Disease Virus Infection for the Non-Mammal, the Chicken**

GOSZTONYI, G., LEISKAU, T., and LUDWIG, H.

Chickens can be infected with Borna Disease (BD) virus. Six weeks p.i. approx. half of the animals die, showing severe symptoms with disturbances in coordination and paralysis. The surviving animals enter varying phases of disease progression, followed by moderate recovery. In the severely diseased chicken, infectivity can be demonstrated in the brain and retina. Immunohistochemically specific antigen is found in astrocytes in the vicinity of the ependyma of the ventricles. In the sera low titres of antibodies against the major BD virus-specific antigen are present, although having no neutralizing activity. In the chronic phase (12 to 24 months p.i.) virus antigen is found abundantly in nerve cells, accompanied by perivascular inflammatory infiltrates. High titres of neutralizing antibodies are present and the brain infectivity is no longer demonstrated. This infection is similar to that of some mammals, the protracted phasic course, however, seems to be characteristic for chickens. The mediation of infection by astrocytes from cerebrospinal fluid spaces to nerve cells has not been observed until now in the mammalian brain.

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**Purification and Characterization of Bovine Interferon-β**

GOTTSCHALK, M. and AHL, R.

Interferon-β was produced in rolling tubes in virusinfected primary bovine kidney cells. In the crude preperations two different interferons-β could be distinguished physicochemically and serologically. The main component comprising 80% bound to Blue Sepharose in 0.5 M NaCl. This interferon-β was purified by affinity chromatography on Blue Sepharose and Phenyl Sepharose up to a specific activity of $6 \times 10^7$ U per mg protein. The recovery was about 70%. In the course of purification bovine interferon-β revealed significantly less hydrophobic properties than human interferon-β. Antisera against bovine interferon-β did not react with interferon-α recovered from IBR/IPV virusinfected cattle and likewise with interferon-β from equine cells. Human and porcine interferon-β was neutralized to a small extent.
Major Immunogenic Components of the Bovid Herpesvirus 1 and 6 and their Cross-Reaction

GREGERSEN, J.-P., PAULI, G., and LUDWIG, H.

IBR- and IPV-viruses, both classified as bovid herpesvirus 1 (BHV-1), are distinguishable by restriction enzyme assay of their DNAs, but do not differ in their immunogenic components. Three major glycoproteins with apparent molecular weights of 93,000, 74,000 and 69,000 can be identified by immunoprecipitation from BHV-1-infected cell extracts. Two of these (GP 93, GP 74) are important for neutralization of the virus, whereas the third (GP 69), not being a structural protein, does not participate in the neutralization reaction. - Goat herpesvirus (BHV-6) which shows a strong cross-reaction with BHV-1, likewise exhibits three major glycoproteins. Their molecular weights are comparable to those of the BHV-1 glycoproteins, and the BHV-6 GP 69, correspondingly, plays no role in neutralization. - All described major glycoproteins are involved in the cross-reaction between BHV-1 and BHV-6.

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Alteringes of the Cytoskeleton After Infection with HSV

HEEG, U., HILLER, G., HÄRLE-GRUPP, V., and FALKE, D.

Previously we have shown that in HSV-induced polycaryocytes (low MOI) microfilaments (MF) polymerize to very long fibers, whereas after high MOI early decay is to be seen (Arch. Virol. 70 (1981) 233). More detailed analysis of the MF-system revealed early during cell-rounding a knob-like appearance of actin on the surface of cells and internal break-down. After high MOI with a fusing strain before fusion an intermediate stage of rounding can be observed with similar knob-like distribution of actin. Another fusing strain of HSV did not show this intermediate stage of rounding. Anti-Keratin sera revealed during rounding no break-down of intermediate filaments, however, the fibers are condensed later as shown by peroxidase-stained ultrathin sections. - Microtubules (MT) and the intermediate fibers are transported into the polycaryocytes with the nuclei without apparent destruction. It can be concluded that MT after fusion first polymerize before they break down. No reconstitution of the MT-system can be observed after a Colcemide-pulse. - Fibronectin (FN) on the surface of cells is lost after infection with rounding and fusing strains of HSV. This loss has to be considered an early process of HSV-replication. This was confirmed in ultrathin sections after labelling of anti-FN antibodies with peroxidase labeled antibodies. By use of a variety of ts mutants no dissociation could be obtained between loss of FN and virus synthesis. Some protease inhibitors did not inhibit loss of FN.

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Posttranslational Modification of Mumps Virus Glycoproteins

HERRLER, G. and COMPANS, R. W.

Like other members of paramyxovirus family, mumps virus contains two glycoproteins. The larger of both proteins, designated HN, is responsible for the hemagglutinating and
neuraminidase activities of mumps virus. The mature form of HN is an oligomer, the monomers of which are held together by disulfide bonds. The second glycoprotein, designated F, has cell fusing activity. Its biological activity requires cleavage into the products F₁ and F₂, which are connected by disulfide bonds. Both formation of HN oligomers and cleavage of F are posttranslational events which are shown by pulse-chase experiments in infected vero cells to occur with the same kinetics. Analysis of the time course of appearance of mumps virus glycoproteins on the cell surface suggested that oligomerization of HN and cleavage of F occur immediately after their exposure on the plasma membrane.

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Cellular Immunopathologic Responses in Borna Disease in Rats

HERZOG, S.¹, NARAYAN, O.¹, and FRESE, K.²

Borna disease carriers were established by intracerebral inoculation of adult Lewis rats followed by a single dose of cyclophosphamide (150/kg) one day later. These non-responsive carriers were adoptively immunized with spleen cells from normal and infected donors. Transfer of immune spleen cells into immunosuppressed carriers resulted in encephalitis and clinical disease. Normal spleen cells failed to produce either inflammatory lesions or clinical symptoms.

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Characterization of a 70 kd Protein in Human Leukemic Sera that is Related to SiSVp30 and/or BaEVp30 Proteins: Presence of Kinase-Activity?

HOFHERR, J.¹, SCHETTERS, H.², ERFLE, V.¹, WEBER, W.³, and HEHLMANN, R.²

The 70 kd protein in human leukemic sera (human protein, HP) that crossreacts serologically with the core proteins p30 of simian sarcoma/simian sarcoma associated virus (SiSV) and/or of baboon endogenous virus (BaEV) and shows peptide similarity with BaEVp30 and SiSVp30 (Hehlmann et al., Cancer Res. 43 (1983) 392) is further characterized. HP does not reveal any detectable crossreactivity with several C-type retroviral reverse transcriptases. HP appears, however, to possess protein kinase activity when assayed in a kinase system that includes purified HP and IgG. Radioactivity is observed in HP as well as in the heavy and light chains of the IgG molecules. There is suggestive evidence that HP preferentially phosphorylates the amino acid serine.
IE-Transcription in the Region of the Transforming DNA Sequence of Human Cytomegalovirus Strain AD 169

JAHN, G., KNUST, E., KRAUS, R., NELSON, J. A., MCDougall, J. K., and FLECKENSTEIN, B.

The transcription of the human cytomegalovirus (HCMV) strain AD 169 was investigated at immediate early (IE) time after infection. Viral total cell RNA's were analyzed from a region of the long unique section of the prototype arrangement of the viral genome (0.06 to 0.16 map units). This region includes the transforming sequence of HCMV strain AD 169, a 2.9 kbp-DNA segment extending between map units 0.123-0.14. Size and localization of four predominant transcripts in cycloheximide-treated cells were determined: a 1.9 kb RNA between 0.06 and 0.085 map units, a 2.3 kb RNA between map position 0.085 and 0.095, a 2.2 kb transcript between 0.115 and 0.130 map units, and a 5 kb RNA, extending between map units 0.130 and 0.15. The 5 kb transcript was characterized in more detail: The direction of transcription was determined by hybridization with $^{32}$P labeled cDNA synthesized from IE-RNA. In contrast to the other three predominant IE-RNA's, the 5 kb transcript is found in the poly (A -) fraction and is detectable in high quantities during the late phase of virus replication. There is no indication that expression of the DNA coding for 5 kb RNA is functionally related to transforming activity of HCMV.

Obesity Syndrome in Borna Disease Virus Infected Rats

KAO, M., GOSZTONYI, G., and LUDWIG, H.

Newborn rats infected with Borna Disease (BD) virus acquire a persistent, tolerant infection (Hirano, Kao and Ludwig, J. gen. Virol. (1983) in press). Of rats infected i.c. at an age of 1-2 months, approx. 40% die with typical symptoms of BD. The survivors enter a stage of adipositas 4-5 months p.i. and gain almost triple the body weight of normal rats. Adipose tissue preferentially covers the abdominal cavity. Brain sections clearly show that the rats acquire a prominent hydrocephalus internus with considerable reduction of the ammonshorn tissue. Virus-specific antigen can immunohistologically be demonstrated in neurons of the hypothalamus and hippocampus. Infectious virus can only be recovered from the brain until 5 months p.i. Some of the obesity rats harbor low titres of neutralizing antibodies in their sera, whereas all of them have antibodies against the major BD virus-specific antigen. Besides the obesity syndrome reported in canine distemper virus-infected mice, this is the first report of a virus-induced adipositas in rats.
The Functional Characteristics of Antigenic Sites on the Hemagglutinin of Influenza A/FM/1/47 (H1N1) and their Distribution on Other Subtype A Influenza Viruses

KAMMER, K.

Antibodies against the hemagglutinin of FM1 have been induced by immunizing BALB/c mice with intact virus. Monoclonality of the antibodies was achieved by agar cloning of hybridoma cultures. The antibodies displayed strict strain specificity in inhibition of hemagglutination. By contrast, in ELISA one of them reacted strongly with heterologous influenza strains A/PR/8/34 (H1N1) and A/FPV/Rostock/34 (H7N1) attached to the solid phase in a continuous buffer system of pH 2-12 according to Teorell and Stenhagen (1939). Particular experimental conditions were required for optimal reactions with PR8 and FPV of another monoclonal antibody: coating with virus had to be carried out at extreme pH values (pH 2 and 12), and antibody binding had to proceed at acidic pH (pH 4). Identity of the latter determinant site on the viral strains was further verified by the finding that antibodies dissociated from immune complexes formed with FM1 could establish binding with PR8 and FPV.

Transcription in Cells Infected with Mouse Adenovirus

KAUSEL, G., ANTOINE, G., SCHILLING, R., and WINNACKER, E.-L.

The aim of the present study was to initiate transcription mapping of mouse adenovirus F1. Since VA RNA can be isolated in large amounts in the related human adenovirus type 2 system, this RNA was selected to begin mapping with. - A very low amount of low-molecular weight RNA is produced after productive infection of 3T3 cells with mouse adenovirus F1. As could be demonstrated by 32P-labelling and silver staining, cytoplasmic RNA isolated from 3T3 cells infected with AdF1 contained an additional RNA species in the molecular weight range of Ad2 VA RNA. The additional RNA species was not observed in non-infected cells.

In vivo Activated Cytolytic T-Lymphocytes Recognize Membrane Determinants Induced in the Immediate Early Phase of the Infection with the Murine Cytomegalovirus

KEIL, G., REDDEHASE, M. J., and KOSZINOWSKI, U.

Infection of the permissive mouse embryo fibroblasts (MEF) with the murine cytomegalovirus (MCMV) leads to a coordinately regulated synthesis of viral proteins which in accordance with the situation described for other herpes-viruses can be arranged in at least
three groups denoted alpha (immediate early), beta (early) and gamma (late). Infected cells in experimentally controlled distinct stages can be used as a probe to define the replication stage specificity of cytolytic T-lymphocytes (CTL). CTL were generated in vitro by interleukin-mediated antigenfree expansion of in vivo activated interleukin-receptive CTL-precursors (IL-CTL-P), obtained from draining lymph nodes after infection of Balb/c mice with MCMV. It was found that different sets of CTL are activated in vivo which detect immunogenic membrane determinants in parallel to the temporal pattern of MCMV gene expression. The existence of CTL capable to recognize virus-induced immediate early determinants prior to synthesis of structural proteins and virus assemply suggests a functional role of these cells in the regulation of persistent/latent infections.

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The Carbohydrate Chains of the Influenza Hemagglutinin

W. KEIL, R. GEYER, H. NIEMANN, and H.-D. KLENK

The distribution of the carbohydrate side chains on the FPV hemagglutinin has been elucidated. HA₁ has 4 complex type side chains attached to asparagine residues 12, 28, 123, and 149. The potential attachment site at asparagine 231 is not glycosylated. HA₂ has a high mannose type chain at asparagine 406 and a complex type chain at asparagine 478. Carbohydrate analyses of the individual side chains demonstrates that there are differences in sugar composition and sulfation of the side chains located in different positions on the polypeptide and that all side chains show considerable microheterogeneity. Comparison with other hemagglutinins demonstrates that the side chains in positions 12 and 478 are conserved. Together these results strengthen the concept that the structure of the polypeptide chain plays an important role in determining the carbohydrate structure.

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Characterization of DNA Sequences from a Human Hepatoma Cell Line Containing Integrated Hepatitis B Virus (HBV) DNA

KOCH, S., KOSHY, R., FREYTAG VON LORINGHOVEN, A., and HOFSCHEIDER, P. H.

HBV DNA is found integrated in the chromosomes of many liver tumors. To study such viral sequences in relation to host cellular DNA, we have cloned three different DNA fragments containing integrated HBV DNA from the human hepatoma cell line PLC/PRF/5. Using restriction mapping, DNA sequencing and hybridization following Southern, we have investigated the structure and origin of viral and human sequences. The biological activity of the cloned DNA showing expression of a viral antigen was seen after transfection into animal cells. The integration sites are found to lie in a fixed single-stranded “gap” of the otherwise double-stranded viral genome suggesting a functional role of this structure. Integration was such that viral promotors could lead to expression of cellular genes. In addition, deletion of host DNA was observed. These results are discussed with respect to mechanisms of transformation.
Expression of the Chloramphenicol Acetyltransferase Gene under the Control of the E1a or E1b Promoter of Adenovirus Type 12 DNA

KRUCZEK, I. and DOERFLER, W.

There is considerable evidence supporting the notion that DNA methylation at highly specific sites plays a role in the regulation of gene expression (for review Doerfler, Ann. Rev. Biochem. 52 (1983) 93-124). We have shown previously that methylation at the 5'-ends of the early regions of viral DNA correlates with the inactive state of these regions (Kruczek and Doerfler, EMBO J. 1 (1982) 409-414). We have initiated a study using the promoters of several Ad12 early gene segments to regulate the expression of the chloramphenicol acetyl-transferase (CAT) gene in mammalian cells. So far, we have succeeded to demonstrate that insertion of the E1a or E1b promoter of Ad12 DNA into the pSVO vector (Gorman et al., Molec. Cell. Biol. 2 (1982) 1044-1051) primes the expression of the CAT gene after transfection of the vector into mouse cells. It will be interesting to investigate what effect, if any, methylation of the promoter will have on gene expression in this experimental system.

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Initiation of the Adenovirus DNA-Replikation

LALLY, C., ANTOINE, G., DÖRPER, T., GRÖGER, W., and WINNACKER, E.-L.

We have studied the initiation of adenovirus replication in vitro with an emphasis on both enzymological and structural aspects. In particular, the significance of host-cell components, viral DNA sequences and the covalently-bound terminal protein for the initiation reaction in vitro using infected nuclear extracts was investigated. - 1) The presence of one or more HeLa cell nuclear factors is essential for the initiation of human Ad2 replication in vitro. This cellular component may be supplied by cells other than the host cell e.g., uninfected mouse 3T3 cells. - 2) The presence of the terminal protein on template DNA is not absolutely required for in vitro initiation since infected 3T3 cell extracts can initiate replication using protein-free cloned termini of the mouse adenovirus genome. - 3) The first 17 bp of the Ad genome have been chemically synthesized and cloned but do not serve efficiently as template for the initiation event. A longer DNA segment, 37 bp, has recently been synthesized and is currently being tested in the in vitro initiation assay. Such methods should allow the determination of the minimal DNA sequence which may be efficiently recognized as template for the replication of Ad in vitro.

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A Subclass of SV40 T-Antigen Binds Stably to Cell Surfaces

LANGE-MUTSCHLER, J., Schnee, J., and HENNING, R.

Small amounts of simian virus 40 (SV40) T-antigen occur on the cell surface of SV40-infected and transformed cells (surface T). We recently observed that T-antigen binds in
vitro to cell surfaces of living cells. We now provide evidence that in vitro cell surface bound T-antigen belongs to a small subclass of T-antigen (5%) with a high cell surface binding affinity. Surface T and in vitro cell surface bound T-antigen revealed compelling similarities: (i) Occurrence in fine microclusters on living suspended cells shown by immunofluorescence microscopy; (ii) the same apparent molecular weight (Mr = 90000 d); (iii) metabolic stability for at least 12 h; (iv) insolubility in presence of EDTA or at high ionic strength and solubility in detergents (0.5% NP40). – These combined results suggest that both antigens possess characteristics like integral membrane proteins. This new experimental approach allows the study of structural and possibly functional features of cell surface associated T-antigen in the absence of intracellular T-antigen.

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The Unintegrated Episomal State of Bovine Papilloma Virus-1 is Dominant Over the Integrated State Induced by SV 40

LEHN, H. and SAUER, G.

SV 40 integrates into the host cell DNA while papilloma-virus genomes accomplish transformation and tumor formation without integration. In order to study the molecular mechanism of integration we have linked defined SV 40 sequences covalently with bovine papilloma virus DNA (BPV-1). These hybrid genomes had been molecularly cloned in the pBR 322 plasmid. After transfection, one of the plasmids, which contained the complete SV 40 tumor-antigen coding region, transformed the cells and induced tumor antigens. Another plasmid with a truncated tumor-antigen coding region transformed, but failed to induce SV 40 tumor-antigen. Both plasmids exist as episomes in transformed cell clones. This suggests that the episomal state of BPV-1 dominates over the process of integration which is known to be induced by SV 40.

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Expression and Functional Mapping of the Autographa californica Nuclear Polyhedrosis Virus (AcNPV) Genome

LÜBBERT, H., ESCHE, H., and DOERFLER, W.

We have started to investigate the functional organization of the genome of the baculovirus Autographa californica Nuclear Polyhedrosis Virus (AcMNPV). Restriction maps for the enzymes EcoRI, BamHI, BglII and Amal have been established. Out of the 24 EcoRI fragments, 21 have been cloned in prokaryotic vectors. We hybrid-selected early (6 h post-infection) and late (24 h post infection) viral RNA on the cloned DNA fragments and translated the purified RNA in an in vitro system. Proteins were analyzed on SDS-polyacrylamide gels. Five early proteins (9-25 and 43-49 map units) and 31 late proteins were mapped on the viral genome. More recently, we started to investigate the possibility of RNA-splicing by hybridizing RNA to viral DNA and by digesting the hybrids with nuclease S1. Subsequently, the resistant hybrids were analyzed on neutral or alkaline gels as well as on two-dimensional gels. So far, we have not obtained evidence for splicing by the experimental procedures employed.

(Supported by a grant from the Federal Ministry of Research and Technology, Bonn, Germany.)
Characterisation of the Glycoproteins of Pseudorabies Virus Using Monoclonal Antibodies

LUKÁCS, N., THIEL, H.-J., METTENLEITER, T., and RZIHA, H.-J.

Purified pseudorabies virus has three major glycoproteins: gA (117K), gB (90K) and gC (126K and see below), characterisable by radioimmunoprecipitation with monoclonal antibodies. The in vitro translation product of gA (non-glycosylated) had molecular weight (MW) 80K. The earliest form of gA identifiable in vivo had MW 82K. Fully glycosylated gA (117K) was seen after 30 min pulse labelling. Thus far precursors of gB have not been found. gC is a complex of disulphide linked glycopeptides. Unreduced gC had MW 146K, splitting up upon reduction into 126K (gC), 66K (gC1) and 55K (gC2) polypeptides. Two monoclonals recognised both the 126K and 66K polypeptides. We suppose that gC1 and gC2 arise through proteolysis of gC. The earliest detectable in vivo gC product had MW 110K.

Studies on the Virus Structures and Antigens After Transfection of Moloney Murine Leukemia Virus (M-MuLV) Mutants. Immunoelectron-microscopic and Ultrastructural Investigations at Mouse- and Hamster Cells with Replica- and Ultrathin Section Preparations

MANNWEILER, K., STUHLMANN, H., HOHENBERG, H., RUTTER, G., and JAENISCH, R.

For studying the expression of M-MuLV structures and gp70 antigen at cell surfaces hamster cell fibroblasts (WOBR) transfected with different cloned proviral DNA were investigated by immunoelectron-microscopic methods. In contrast to biological transfection assay, where some cloned proviral DNA from Mov-substrains were either infectious (pMov-3, pMov-9) or failed to induce infectious virus (pMov-2, pMov-7, pMov-10) (1), all cotransfected WOBR clones produced virus structures (particles) and virus gp-antigen at the plasma membrane; sometimes only detectable with replica-immunocytochemical preparations (2). These structures only differed quantitatively as well in their distribution on individual cells as within the culture and qualitatively at ultrathin section preparations in their ultrastructural composition of the particles visible as incomplete forms or morphological aberrant particles (Mov-7).

References
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2. Mannweiler, K. et al.: J. Microsc. 126 (1982) 145-149
PLC/PRF/5 Cells Produce HBeAg

MARQUARDT, O. and FREYTAG VON LORINGHOVEN, A.

The human hepatoma cell line PLC/PRF/5 is persistently infected with hepatitis B virus (HBV). It produces the HBV surface antigen when cultured in vitro while efforts to detect other virus antigens, the core antigen (HBcAg) or the “e” antigen (HBeAg) were unsuccessful until now. HBeAg should be viewed as converted HBcAg. - The respective genes can be considered inactive in cells cultured in vitro. In contrast, in vivo cultured PLC/PRF/5 cells grown as tumors in male athymic NMRI nu/nu mice produce HBcAg and/or HBeAg with a buoyant density in CsCl of 1.31–1.32 g/ml which is the same as that of HBV core particles. It must be concluded that at least some genes coding for HBcAg in PLC/PRF/5 cells are not damaged but silent when cells are cultured in vitro. The production of both structural HBV antigens and extrachromosomal HBV-specific DNA which has been shown earlier is seen compatible with the persistence of HBV in PLC/PRF/5 cells which is by virtue of stable DNA integration in host chromosomes. Moreover, the formation of virus particles in the cells cannot be excluded suggesting future infectivity studies.

Transformation of Primate Cells by Human Brain Tumor DNA

MELBER, K., KRIEG, P., and SAUER, G.

DNA from human brain tumors was used in transfection experiments with recipient primate (CV-I) cells. Four out of 14 brain tumor samples induced in the recipient cells a transformed phenotype as shown by growth of these cells in medium containing low serum. A preliminary characterization of the transforming principle by sucrose density gradient sedimentation of uncleaved and sonicated DNA consistently revealed a transforming fraction between 5 and 7 kb. Furthermore, tumor DNA was fractionated by a CsCl/ethidium bromide equilibrium density gradient to separate superhelical DNA from linear and relaxed circular DNA. After transfection of recipient CV-I cells, superhelical DNA transformed CV-I cells with a high efficiency while relaxed circular and/or linear DNA showed little transforming activity. These data suggest that the transforming DNA sequences persist as superhelices in an extrachromosomal state.

Analysis of Different Transforming Polyproteins of Acute Avian Leukemia Viruses

MOELLING, KARIN, BUNTE, TH., DONNER, P., and GREISER-WILKE, IRENE

Several avian oncornaviruses express their transforming proteins as polyproteins which are covalently linked to the viral structural protein p19. Using monoclonal antibodies against p19, the polyprotein of MC29, p110gag-myc, was localized in the cellular nucleus and upon purification binds to double-stranded DNA. The transforming proteins
of three transformation-defective deletion mutants were isolated and showed a reduced ability to bind to DNA. Two other members of the MC29 class, MH2 and CMI1, were analyzed analogously. While the polyprotein of CMI1 behaved similarly to the gag-myc protein of MC29, the MH2 polyprotein exhibited several novel properties. It was located in the cytoplasm and interacted with single-stranded RNA in contrast to the MC29 and CMI1 polyproteins. It does not appear to be of the gag-myc type. Another cytoplasmic polyprotein, the gag-erbA protein of AEV, did not bind either to DNA or RNA. - The polyprotein from FSV was purified 4000-fold as an active tyrosine-specific protein kinase which phosphorylates vinculin in the absence of exogenous phospholipids. Other substrates were also phosphorylated in vitro.

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Monoclonal Antibodies Against Structural Proteins of Human Cytomegalovirus

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Monoclonal antibodies against human cytomegalovirus (HCMV) were prepared by injecting purified virus particles into Balb/c mice. At least four groups of stable hybridoma cell lines have been obtained. Most hybridoma cell lines produce antibodies that detect a major outer membrane virion glycoprotein of about 58000 dalton and also a 130000 dalton glycoprotein. Another class of monoclonal antibodies is directed against a 155000 dalton phosphorylated nucleocapsid protein, and the third class detects a 29000 dalton phosphorylated membrane protein (pp 71). By hybrid-selection of CMV mRNA against CMV DNA cosmid clones, followed by immunoprecipitation of the in vitro translated products with the antibody 355, the pp 71 was shown to be a structural protein encoded by CMV DNA between map positions 0.21 and 0.36. Similar methodology may be generally useful for physical mapping of DNA sequences coding for other virion proteins.

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The Symmetry of the Capsid of PCV

ÖZEL, M., TISCHER, I., and GELDERBLOM, H.

The structure of the isometric porcine circo virus (PCV) was determined after high resolution negative staining, and after different rotational, uni- and bidirectional shadowing techniques were performed. - The analysis of the 17 nm capsid of PCV by the Markham rotation technique revealed an icosahedral symmetry of \( T = 4 \) and a total number of 42 capsomeres for this smallest autonomously-growing vertebrate virus.
Borna Disease Virus: Properties of Cell-Bound and Cell-Free Virus

PAULI, G. and LUDWIG, H.

Characterization of Borna Disease virus has proved difficult, because the virus appears to be cell-bound and yields only low titers in tissue culture. Successful attempts to increase virus titers and to release cell-associated virus from persistently infected cells are described. N-butyrate could be shown to increase the amount of infectious virus. Concomitant results obtained by treatment of infected cells with increasing NaCl concentrations indicated that a fraction of the virions is bound to the plasma membrane and can be released from the cells. This released virus has a higher buoyant density in sucrose gradients (1.22 g/cm³) than virus obtained following ultrasonication of infected cells (1.18 g/cm³). Similarly, cell-free virus is more resistant to temperature inactivation than cellbound virus. Additional results suggest that cellular proteins liberated by ultrasonication may be involved in this inactivation process. No difference in the growth properties of released and cell-bound virus could be observed.

Cloning of Bovine Papillomavirus 3 DNA and Comparison of the Genomes of BPV3 and 4

PFISTER, H.t, CAMPO, M. S.2, COGGINS, L. W.2, and HETTICH, I.3

The DNAs of bovine papillomaviruses (BPV) 3 and 4 are smaller than those of other papillomaviruses (7.2 kb vs about 7.9 kb). BPV3 and 4 cause epithelial tumors on the skin and the esophageal mucosa, respectively. The latter are closely associated with esophageal carcinomas. BPV3 was molecularly cloned in Lambda L47 using the single Eco RI cleavage site and was compared with BPV4. Both viruses cross-hybridized under stringent conditions but shared at most 8% of their DNA sequences as deduced from reassociation kinetics. According to heteroduplex analysis and Southern blot hybridization with subgenomic DNA fragments, the homologous sequences were distributed over the whole genome. Both molecules were in register when aligned at the BPV3/Eco RI site and the BPV4/1, 7 kb Eco RI site (Bam HI = 0 kb). Relaxed hybridization conditions revealed a relationship between BPV3, BPV1 and HPV13 DNAs.

Transcriptional Control Signals Contained in the Long Terminal Repeat Sequences of Mouse Mammary Tumor Virus

PONTA, H., HYNES, N. E., KENNEDY, N., CARROZZA, M. L., VAN OOYEN, A.t, BEATO, M.2, HERRLICH, P., GÜNZBURG, W., SALMONS, B., KNEDLITSCHEK, G., and GRONER, B.

Chimeric genes containing the MMTV LTR and the Herpes TK gene have been transfected into cultured cells and used to study regulation of RNA synthesis. Three observa-
tions have been made. First, the transcription of MMTV RNA is controlled by glucocorticoids. By constructing a series of 5′ and 3′ deletion mutants in the LTR we have shown that the hormone sensitive region is located in the 202 bp 5′ to the LTR cap site. Second, we have seen that the hormone responsive LTR sequence confers hormone sensitivity to a downstream RNA initiation site. Third, two LTRs cooperate to cause hormone independent transcription. In constructions containing only one LTR we detected no LTR initiated transcripts in the absence of hormone. This has been observed with endogenous and exogenous, 3′ and 5′ LTRs transfected into two different cell types.

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Distinct Maturation Stages of Cytolytic T-Lymphocytes Constitute the Cellular Immune Response During Acute Infection of Mice with the Murine Cytomegalovirus

REDDEHASE, M. J., KEIL, G., and KOSZINOWSKI, U.

Infection of Balb/c mice with the murine cytomegalovirus (MCMV) served as a natural virus-host system to study the in vivo maturation of cytolytic T-lymphocyte precursors (CTL-P). Two principal types of Limiting Dilution (LD) analysis, the expansion and the restimulation LD assay, led to the detection of two distinct maturation stages within the lineage of virus-specific self restricted CTL. A low frequent set representing on an average 15% of the specifically activated CTL-P in an immune lymph node generates cytolytic activity in vitro in the absence of further antigen under conditions provided by growth and differentiation interleukins. This set of cells was denoted interleukin-receptive CTL-P (IL-CTL-P). IL-CTL-P belong predominantly to the lymphoblast fraction and the majority express the Thy 1+, Lyt 1+ , 2+ phenotype distinct from their in vitro grown progeny which are Thy 1+, Lyt 1-, 2+. Since IL-CTL-P are not influenced by selective effects of antigen in vitro we consider them to be the best available representatives of the genuine in vivo activated immune repertoire. Analysis of the fine specificity of IL-CTL-P will allow to define the identity of those immunogenic determinants which are of importance in the antiviral immune response.

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Herpes Simplex Virus DNA Caused Generalized and Lethal Herpesvirus Infection in Tree Shrew

RÖSEN, A.1, SCHOLZ, J.1, GELDERBLOM, H.2, and DARAI, G.1

Infectivity of isolated viral genome has been demonstrated in vitro for a number of RNA and DNA viruses. But no unequivocal evidence has been reported for the induction of acute clinical symptoms as a manifestation of viral infection using viral DNA in the animals so far investigated, with the exception of a few DNA tumour viruses and cloned hepatitis B DNA. The proof of the infectivity of isolated viral DNA in vivo would be an extremely useful tool for the determination of gene and gene function responsible for the pathogenicity of herpes simplex virus (HSV) and therefore its prevention in man. Knowledge concerning the dynamics of development of HSV infection, the mechanisms of virus spread
and the manifestation of disease is of special interest. The generalized herpesvirus infection and herpetic hepatitis as a manifestation of HSV infection is common feature of neonatal infections, pregnant women, and immunosuppressive patients. – The infectivity of the genome of HSV in tissue culture is well documented. The infectivity of the HSV genome was investigated in vivo using the animal model system tree shrew, which is highly susceptible to HSV. Individual tree shrews were inoculated intrahepatically with 0.2 ml transfection mixture containing 25 μg HSV DNA, 50 μg salmon sperm DNA, and 250 μg DEAE-Dextran. Seven out of nine tree shrews transfected with HSV DNA showed generalized herpesvirus infection. The new progeny virus was found in different organs of transfected animals.

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Characterization of Polypeptides of HBcAg After Limited Proteolysis by the Western Blotting Technique

ROGGENDORF, M., VON DER HELM, K., and DEINHARDT, F.

The physicochemical and immunological properties of polypeptides obtained from HBcAg (of human and bacterial origin) by treatment with SDS and digestion with pronase E were investigated. Transferring HBcAg from human liver with and without digestion from SDS-PAGE to nitrocellulose papers and incubating with serum containing only anti-HBc resulted in one band with a mol. wt. of 21000, whereas incubating with serum containing anti-HBc/anti-HBc resulted in a band with the mol. wt. of 21000 and additional smaller bands. After digestion with pronase E four additional bands could be detected. Using HBcAg from E. coli, incubation with anti-HBc resulted in one band of about 16000 with digested HBcAg and one band with native HBcAg at the mol. wt. of 21000. Additional bands were detected after incubation with serum containing anti-HBc/anti-HBc ranging from 14000 to 21000. HBeAg purified from human serum resulted in one band with the mol. wt. of 18000 after incubation with anti-HBc/anti-HBc. HBeAg proved to be highly resistant to proteases. Immunologically active proteins with the mol. wt. of 14000 could be detected 20 h after treatment with pronase E.

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Cloning and Characterization of Human T Cell Leukemia Virus (HTLV)-Proviral Information

RÜBSAMEN, HELGA1, WONG-STAAL, FLOSSIE2, GALLO, R.C.2, and MULLINS, J.I.3

From the DNA of leukemic cells obtained from an Israeli case of adult T cell leukemia (UK), two different clones were derived using the vector λJJ1. Clone UK 1 contained 18.2 Kb of eukaryotic DNA, while clone UK 4 contained only 10.3 Kb. Restriction analysis and hybridization to the probes pCI (HTLV LTR), pCRCH (envelope information from the
US case CR) and HL 60 DNA revealed that both clones contained defective proviruses. The viral information in UK 1 (7 Kb) consisted of 1 LTR and sequences from the central and 3' end of the provirus and was found to be monoclonally integrated into the tumor cells. In contrast, UK 4 contained 2 LTR sequences and a 5 Kb deletion in the center for the viral genome. This information was present in only 5 to 10% of the cells. Interestingly, the LTR sequences of UK had a SstI site, which had also been found in a Japanese isolate of ATLV, but which was not present in CR. - The cellular flanking sequences in both clones showed no evidence for hybridization with a set of 12 probes for viral oncogenes, including ras and myc. So far, we have no firm evidence that these loci are occupied in other HTLV - associated tumors by virus.

The support of this study by an ICRETT - grant from the International Union Against Cancer (H.R.) is gratefully acknowledged.

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Sequence Homologies of the Genome of Cytomegalovirus with Cellular DNA

RÜGER, R. and FLECKENSTEIN, B.

DNA from a cosmid-cloned gene library of the human cytomegalovirus (CMV) strain AD 169 was used for the detection of viral DNA sequences in human tissues of various origins. Surprisingly some of the cosmid clones hybridized in Southern blot studies with intermediate repetitive DNA sequences of uninfected human cells. Homology was shown with the Eco RI fragments U, O, R and b and with the Hind III fragments D and S. All hybridization reactions were carried out under stringent reannealing conditions. Similar or identical hybridization patterns were found with DNA from placenta tissues, peripheral white blood cells, lymphoblasts in vitro immortalized with Epstein Barr virus, several hematopoietic tumor cell lines, colon carcinoma biopsies and adjacent non-tumorous tissues, and with DNA from patients with serological evidence for preceding CMV infection and patients sero-negative in ELISA. Homologous sequences were also found with DNA from owl monkey kidney cells and Chinese hamster ovary cells. The intermediate repetitive DNA in the CMV-genome is not related to human Alu-sequences. The occurrence of repetitive cellular DNA sequences in the viral genome indicates that purified virion DNA should not be used in the search for virus-specific nucleic acids in human tissues.

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Sequence Analysis of Mouse Hepatitis Virus JHM mRNA7: Evidence for an Unusual RNA “Splicing” Event

SKINNER, M. A. and SIDDELL, S. G.

cDNA was copied from polyadenylated, JHM infected cell RNA and inserted into pAT153. Virus-specific clones were identified by hybridisation, with DNA copied from genome RNA, and by HART. Two overlapping clones were subcloned into M13 vectors for sequencing by the dideoxynucleotide method. The sequence of mRNA7 is 1767 nucleotides long including a 1355 base open-reading frame encoding a basic (nucleocapsid) protein of 40700 mol. wt. Close inspection of the 5'-noncoding sequence and corresponding regions of the genome shows that a leader, derived from the 5'-end of the genome, is spliced onto the 5'-end of each subgenomic message. The mechanism for this splicing event is
unknown but may involve polymerase “jumping”. It is not an example of conventional mRNA splicing, MHV replication being independent of nuclear functions. Also the sub-genomic messages are not produced by processing of larger precursors.

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Characterization of Bornavirus-Specific Antigens in Infected Cells

SCHEEFERS, H.

Borna disease is a chronic encephalomyelitis of horses and sheep. The causative virus has not been characterized so far, but a virus specific antigen can always be demonstrated by immunofluorescence microscopy in the animal or in permanent infected tissue culture cells. Two Bornavirus specific antigens were isolated by immunoaffinity chromatography from infected cell homogenates (total rat brain, rabbit brain or MDCK cells). These two antigens were detected by immunautoradiography using sera from infected animals (rats, rabbits). The two proteins which cause the typical nuclei fluorescence in infected cells shown with immunofluorescence microscopy are not glycosylated and have an apparent molecular weight of 36 kD and 38 kD. The antigens are neither host proteins nor modified constituents of the infected tissues. One to two micrograms of these proteins can be found in one infected rat brain.

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Phosphorylation State and Interrelationship of Subclasses of SV40 Large T Antigen

SCHEIDTMANN, K. H., ECHLE, B., and WALTER, G.

SV40 large T antigen regulates viral DNA replication and transcription and plays an essential role in SV40 induced transformation. Such different functions might be carried out by subclasses of large T which might be interconvertible by reversible phosphorylation. We have isolated subclasses of large T differing in their DNA-binding behaviour or their degree of oligomerization and investigated their interrelationship and phosphorylation state. Two principally different subclasses can be distinguished: Newly synthesized large T is phosphorylated to a limited extent, it exists predominantly in mono- and dimeric form and has a high DNA-binding activity. Older molecules are highly phosphorylated, they are converted to higher aggregates and have a reduced DNA-binding activity. We suggest that in vivo large T binds to DNA while in a low phosphorylation state and that sequential phosphorylation modulates this interaction.

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Subcellular Distribution and Oligomerization State of SV40 Large T Antigen

SCHICKEDANZ, J., SCHEIDTMANN, K. H., and WALTER, G.

SV40 large T-antigen is a nuclear protein, which exists in different oligomerization and phosphorylation states. We investigated the subcellular distribution and the kinetics of
oligomerization of T-antigen after its synthesis. SV40-infected cells were pulse-labeled and chased for various times and after stepwise detergent extraction "cytoplasmic" and "nuclear" protein fractions were obtained. The amount of T-antigen in either fraction was determined by immuno-precipitation and SDS-PAGE. The oligomerization state and the association with viral nucleoprotein complexes (NPC) was analysed by sedimentation in sucrose gradients. - Our data can be summarized and interpreted as follows: After its synthesis, T-antigen is rapidly transported to the nucleus, presumably as mono- or dimer; there it is temporarily tightly associated with nuclear structures (NPC). After 2-4 h T-Ag is gradually converted to tetramers and higher complexed forms and appears in the soluble nucleoplasmic fraction. The association with NPC's may reflect a participation of T-antigen during replication and transcription.

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Initiator-Like Selective Gene Amplification by Herpes Simplex Virus and its Inhibition by Co-Infection with Defective Paroviruses

SCHLEHOFER, J. R., HEILBRONN, R., GEORG-FRIES, B., GISSMANN, L., and ZUR HAUSEN, H.

Herpes simplex viruses type 1 and type 2 induce amplification of (integrated) SV40 sequences in SV40 transformed chinese hamster embryo cells as efficiently as chemical or physical carcinogens. This can be shown by in situ hybridization of cellular DNA to 32p-labelled SV40 DNA after HSV infection or carcinogen treatment compared to controls. - The amplification is inhibited by coinfection with the defective parovirus AAV-5 recently isolated from a penile condylomatous lesion. Under these conditions (AAV-5 infected cells plus HSV infection or carcinogen treatment, respectively) AAV-5 DNA is synthesized effectively and AAV-5 specific antigens are expressed as revealed by hybridization with cloned AAV-5 DNA or by immunofluorescence with monoclonal antibodies. In addition, AAV-5 infection appears to induce a selective killing of "initiated" cells as demonstrated by comparing the number of surviving cells under the conditions of this assay.

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"In vivo" and "in vitro" Effects of Retroviruses Isolated from Bones During the Early Latent Period of Radiation-Induced Osteosarcomas

J. SCHMIDT, LUZ, A., and ERFLE, V.

We have previously shown that during the early latent period of radiation-induced osteosarcomas in mice infectious endogenous retroviruses can be isolated from spleen, bone marrow and bone tissue. Ecotropic and xenotropic viruses from bone tissues of BALB/c, C57BL/6 and NMRI mice were now investigated for host range properties, MCF inducing – and cell transforming potential "in vitro". Oncogenicity of the viral isolates was tested in mice. - One ecotropic, XC-positive retrovirus isolated from irradiated NMRI mouse – bone tissues induced both foci of altered cell growth in NIH 3T3 cells and malignant lymphomas (70%, 7/10) and osteopetrosis (30%, 3/10) within 12 months after infection. Similar data were obtained with retrovirus isolated from radiation-induced osteosarcomas of the NMRI mouse.
Abstracts of Papers at the 8th Workshop of the Virology Section of the DGHM

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Structural Polypeptides of Adult T-Cell Leukemia Virus. Detection and Isolation

SCHNEIDER, J.¹, YAMAMOTO, N.², HINUMA, Y.², and HUNSMANN, G.¹

Sera from patients with adult T-cell leukemia (ATL) regularly react in an immunofluorescence test with cells producing ATL-virus (ATLV). To identify the immunoreactive polypeptides ATLV-producing cells were radio-labeled and immunoprecipitates analysed by electrophoresis (SDS-PAGE). A glycoprotein, gp68, was the major intracellular antigen. Pelleted virus contained immunoreactive p28, p24, p19, and p15 in addition to small amounts of gp46. gp46 was also found on the surface of virus-producing cells and in the virus-free culture fluid like envelope glycoproteptides of other retroviruses. Several findings indicate that gp68 is the precursor of gp46: 1. Localization of the glycoproteins; 2. gp68 is synthesized earlier than gp46; 3. Partial proteolysis produces similar patterns of fragments from both glycoproteins. Furthermore gp68 and p24 were isolated from producer cells and virus. The isolated polypeptides allowed us to quantitate respective antibodies in human and primate sera and to produce component-specific heteroantisera for future typing of viral isolates.

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Neuropathogenicity of HSV in Mice: Protection Against Lethal Encephalitis by Coinfection with a Nonencephalitogenic Strain

SCHRÖDER, C. H., KÜMEL, G., GLORIOSO, J., KIRCHNER, H., and KAERNER, H. C.

Intraperitoneal infection of susceptible mice with a nonpathogenic strain prevents the lethal outcome of a challenge infection with a pathogenic strain, even if the challenge precedes the protective infection. This could be explained by the finding that the protective infection blocks the initial replication of the challenge virus. In addition to this effect, intraperitoneal infection with the protective HSV-1 strains leads to the induction in the CNS of a status refractory to direct intracranial infection with HSV-1. This process is also inducible locally by intracerebral inoculation of a nonreplicative mutant. The results may indicate that the difference between HSV-strains with respect to their neurovirulence can be explained by the differential induction of or the sensitivity to this protective effect. Detergent soluble extracts of infected cells or infected and u.v. irradiated cells that did not contain live virus protected the animals against coinfection with encephalitogenic challenge virus. The experiments define this protective effect as an antigen induced immediate host defense mechanism unrelated to interferon or NK cells yet active within a very short time post infection.
Ultrastructural Studies on Cytotoxic T Lymphocyte-Target Cell Interaction in Brains of Adult Mice Experimentally Infected with Lymphocytic Choriomeningitis (LCM) Virus

SCHWENDEMANN, G., LÖHLER, J., and LEHMANN-GRUBE, F.

Convulsive illness and death of adult mice infected by intracerebral inoculation with LCM virus is generally assumed to result from the activity of LCM virus-specific cytolytic T lymphocytes (CTL), although the evidence is scant. The virus replicates to high concentrations in the central nervous system, and the immunofluorescence method reveals infection of leptomeninges, ependyma and choroid plexuses. Moribund mice exhibit a choriomeningitis characterized by inflammatory accumulations of activated monocytes and T lymphocytes. By use of the electron microscope we could demonstrate cytolysis of leptomeningeal and epithelial cells of the plexus that were invaded with processes or in toto by transformed T lymphocytes. Similar observations had been reported on allogeneic CTL-target cell interactions in vitro. We interpret our findings to mean that the pathogeneic mechanism of murine lymphocytic choriomeningitis is indeed destruction of virus-infected target cells by CTL.

Modulation of Hepatitis B Infection by Intravenous Application of Anti-HBe-Hyperimmunoglobulin

STEPHAN, W.¹ and PRINCE, A. M.²

Repeated administration of an intravenous immunoglobulin containing anti-HBe and anti-HBc, free of anti-HBs, before and after inoculation of 10⁴.⁹ CID50 hepatitis B virus markedly prolonged the incubation period of hepatitis B in experimentally infected chimpanzees. Similar administration of an immunoglobulin preparation containing anti-HBc but free of anti-HBe and anti-HBs, or administration of a single dose of anti-HBe and anti-HBc containing immunoglobulin intramuscularly three days before or after inoculation of HBV, did not appear to modulate HBV infection. These observations suggested that anti-HBe may have biological activity in the modulation of HBV replication.

Phosphorylation and DNA-Binding Properties of Free and Host Cell Protein p53-Complexed Simian Virus 40 T-Antigen

STÜRZBECHER, H., MONTENARH, M., and HENNING, R.

Simian Virus 40 (SV40) T-antigen occurs in SV40-infected and -transformed cells in free forms and in high-molecular-weight complexes with the host cell protein p53. To attempt
correlating the phosphorylation and DNA-binding properties we started to compare the phosphorylation of free T-antigen and of T-antigen complexed with p53. After sequential immunoprecipitation using monoclonal anti-p53-antibodies followed by anti-T-sera the phosphorylation of T-antigen was determined by either densitometric scanning of Coomassie Blue stained, $^{32}$P-labeled T-antigen bands followed by counting for $^{32}$P-c.p.m. or by double labeling with $^3$H-leucine and $^{32}$P-phosphate. In contrast to previous studies of other laboratories we found that T-antigen bound to p53 is significantly underphosphorylated. - Under typical strong binding conditions (pH 6.0) free T-antigen bound expectedly well to dsDNA whereas T-antigen-p53-complexes remained almost completely unbound. This observation and the above described phosphorylation properties explain in part our previous observations describing the apparent positive correlation between the phosphorylation of total T-antigen and its dsDNA-binding affinity.

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Peptide Specific Antibody as a Tool for the Investigation of the Transforming Protein PP60$^{SRC}$

TAMURA, T.1, BAUER, H.1, PIPKORN, R.2, and BIRR, CHR.2

Previous investigations from this laboratory have suggested that the transforming protein pp60$^{src}$ of Rous sarcoma virus, is pleiotropic and/or multifunctional. So far, phosphokinase activity of pp60$^{src}$ has been defined, but other functions or specific targets for pp60$^{src}$ have not been identified with certainty. In order to study the various functions of pp60$^{src}$ we have undertaken the production of antibodies against different antigenic sites (epitopes) of pp60$^{src}$ which may allow detection of specific configurations and functional stages of that protein. For this purpose we have synthesized oligopeptides which correspond to certain regions of the primary structure of pp60$^{src}$ and which, due to a high hydrophilicity value, were hoped to correspond to epitopes of pp60$^{src}$. The specificity of the peptides was tested by reaction with antibody from tumor bearing rabbit (TBR sera), and 5 out of 8 peptides were found to correspond to pp60$^{src}$ epitopes. The respective peptide-specific IgG's were isolated and found to precipitate pp60$^{src}$ more or less quantitatively, but all removed pp60$^{src}$ kinase activity totally in a competition test. Phosphorylation of antibodies was observed only with those which are directed against peptides located between amino acid no. 315 and 506.

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Monoclonal Antibodies Specific for Rous Sarcoma Virus Coded Structural and Transforming Proteins

TANAKA, T., LÖWER, J., and KURTH, R.

Monoclonal antibodies specific for pp60$^{src}$, reverse transcriptase and structural polypeptides of RSV have been prepared. Antibodies were characterized in solid phase RIAs, immunoprecipitation and protein kinase assays and by immunofluorescence. Initial experiments employing anti-pp60$^{src}$ antibodies indicate that pp60$^{src}$ may be differentially distributed intracellularly depending on whether transformed avian or mammalian cells are studied.

13 Zbl. Bakt. Hyg., I. Abt. Orig. A 255
Characterization of a Transformation Specific Glycopeptide in SSV-NP Cells

THIEL, H.-J., HAFENRICHTER, R., and GREGER, B.

An autologous goat antiserum against simian sarcoma virus (SSV) infected non producer cells (SSV-NP cells) recognizes an SSV transformation specific glycopeptide (SSV-TrSgp) (Thiel et al., Virology 112 (1981) 642-650). Gel filtration of this component on a Sephacryl S-200 column indicates a molecular weight of about 200,000 dalton. - The antigenicity of the SSV-TrSgp is completely destroyed after treatment with proteases. On the other hand, incubation with chondroitinase AC or ABC degrades the molecule to some extent, but does not affect its antigenicity as measured by immunoprecipitation. - The biochemical properties (molecular weight of glycopeptides, degradation by chondroitinases) are indicative of a proteoglycan-like molecule. The SSV-TrSgp appears to be cell membrane associated as evidenced by EDTA and trypsin treatment of pulse labeled cells as well as by surface iodination. Its potential role in cell transformation by SSV will be discussed.

Physicochemical and Biological Properties of a Very Small Porcine Virus (Porcine Circo Virus)

TISCHER, I., GELDERBLOM, H., VETTERMANN, W., and KOCH, M. A.

The Porcine Circo Virus (PCV) is presented as a member of a group of isometric animal viruses so far not encountered. The genome of this virus consists of single stranded circular DNA. - The virus was isolated from permanent cultures of pig kidney cells where it multiplies without recognizable CPE. PCV in pig kidney cells shows phenomena of latent or persistent infection. The responsible mechanisms are discussed. - Preliminary experiments in animals together with Drs. Weinhold and Mields showed that PCV multiplies in pigs and that infected animals develop antibodies. About 80% of unselected pig sera from different parts of Germany contain PCV specific antibodies. So far no clinical disease can be associated to PCV.

Human Monoclonal Antibodies Neutralizing Polio Virus Type 3 and Autoantibodies against Cytoskeletal Structures

UHLIG, H., RUTTER, G., and DERNICK, R.

Our aim was to obtain human monoclonal antibodies against polio and measles viruses. Palatine tonsils of three randomly selected children were chosen as a source of human B-lymphocytes: unfractionated cells were infected with Epstein-Barr virus (EBV) B 95-8 and transferred to microculture wells. Supernatants of cultures showing transformed cells were screened for neutralizing antibodies against polio viruses (type 1 Mahoney and Sabin, type 2 MEF-1, type 3 Saukett) and for antibodies against measles virus by indirect immuno-
fluorescence staining with chronically infected HeLa cells as antigen source. About 2–5% of culture supernatants contained neutralizing antibodies against polio virus type 3. Antibodies against measles virus or polio virus types 1 and 2 were never detected. However, 10–14% of microcultures produced autoantibodies, the majority of which were directed against cytoskeletal structures. – Some characteristics of selected antibodies are presented.

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Production of Hepatitis Be-Antigen in E. coli

UY, A., GERLICH, W. H., HEERMANN, K. H., KÖCHEL, H., and THOMSSEN, R.

The gene for the core protein of hepatitis B virus was inserted next to the lac promoter of plasmid pGL 101 and transfered to E. coli. The viral core protein produced in E. coli had a mol. weight of 25000 in SDS gelelectrophoresis. The core protein had predominantly e-antigenicity during early growth phases of bacterial cultures. In later phases the e-antigen titer remained constant whereas the core antigen titer increased till the stationary phase was reached. Core antigenicity was found only with particles larger than 20 nm. The e-antigenicity was found with structures of approximately 50000 mol. weight. The data suggest, that e-antigen is not only a decay product of core particles, as reported previously, but it may be also a precursor.

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Coronavirus Infection of Rats: Virus Induced Immune Reactions against Brain Antigens

WATANABE, R., WEGE, H., and TER MEULEN, V.

Intracerebral infection of Lewis rats by the murine coronavirus JHM is followed by a demyelinating encephalomyelitis (SDE). The histological changes consist of marked primary demyelination and perivascular infiltrations of mononuclear cells. Similar infiltrations are typical for experimental allergic encephalomyelitis (EAE), an autoimmune disease induced by sensitisation with a mixture of myelin basic protein (MBP) and Freund's adjuvant. Based on this observation we started to investigate the sensitisation of lymphocytes against neuroantigens and viral proteins by ³H-thymidine incorporation in vitro. Infected rats develop disease within 8–25 days. Lymphocytes of diseased rats are sensitized against MBP and virus. Adoptive transfer of MBP-stimulated lymphocytes from diseased rats was followed by the development of EAE-like lesions in the recipients. By contrast, lymphocytes of infected BN-rats, an inbred strain which does not develop neurological symptoms, are not stimulatable by MBP. Furthermore, Lewis-rats which survived SDE, are no longer capable to develop EAE. These results suggest, that during this virus infection an immune reaction against neuroantigens develops, which may contribute to the pathogenesis of SDE.
Coronavirus Infection of Rats: Persistency and Demyelination in the Central Nervous System

WEGE, H., WATANABE, R., and TER MEULEN, V.

Several diseases of the central nervous system (CNS) are associated with demyelination. An autoimmune reaction, a virus infection or a combination of both could contribute to the pathogenesis of such diseases. Infection of rats with coronavirus JHM can lead to acute or subacute demyelinating encephalomyelitis (AE or SDE). The type of disease depends on properties of the virus, the age and immune status of the rat and the host genetic background. A high rate of SDE can be induced by infection with temperature sensitive mutants. Clinical symptoms (paresis, incoordination and paralysis) develop 14–160 days post infection. Many rats survive this phase, but weeks to months later a relapse of disease can occur. Neuropathological changes of such animals are old remyelinated plaques and fresh demyelinating lesions with infiltrations of monocytes. Infectious virus can be isolated. As a basis for genetic studies we compared the susceptibility of 13 rat inbred strains. Some strains are highly resistant, do not develop symptoms, but pronounced inflammatory changes are found. This animal model allows to study pathogenetic mechanisms which could be of relevance for virus induced CNS diseases of man.

Reaction of Monospecific Antisera with Antigenic Sites of the Infectious Poliovirus and its Precursor Particles

WIEGERS, K.-J. and DERNICK, R.

Monospecific antisera raised against isolated structural polypeptides VP1, VP2 and VP3 show different reactions with intact infectious poliovirus and its precursor particles from infected cells in the immunoprecipitation reaction. During assembly of the 5S protomer, via 14S pentamer and 80S procapsid to 160S infectious virus (1), antigenic determinants recognized by the VP1 and VP2 antisera disappear from the surface of the precursor particles. However, antigenic sites recognized by the VP3 antisera are expressed maximally on the surface of the mature infectious virus. The same monospecific antisera show a strong intertypic cross reaction with type 2, strain MEF-1, and type 3, strain Saukett, polypeptides. The expression of these intertypic determinants is also lost during the assembly of the virus from the precursor particles.

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Production of Infectious Hepatitis B Virus by Inoculation of Cloned HBV DNA in Chimpanzees

WILL, H.¹, CATTANEO, R.¹, DARAI, G.¹, DEINHARDT, F.², SCHELLEKENS, H.³, and SCHALLER, H.¹

HBV DNA cloned in E. coli has been tested for its ability to induce hepatitis B in chimpanzees. Double stranded circular HBV DNA or tandemly linked HBV DNA integrated into plasmids induced all serological and biochemical markers of hepatitis B infection when transferred into the liver of chimpanzees by injection or transfection. To demonstrate that infectious hepatitis B virus were present in the serum, a second chimpanzee was inoculated with acute phase serum of a transfected animal. Two weeks after intravenous injection, the second chimpanzee developed hepatitis B this proving the presence of infectious hepatitis B virions, in the serum of a transfected animal. This approach allows to obtain HBV virus stocks originating from a defined nucleotide sequence and opens new perspectives in the molecular biology of HBV.

Characterization of Plasmids Containing HBV DNA After Replication in Chimpanzee Liver

WILL, H.¹, PFAFF, E.¹, DARAI, G.¹, DEINHARDT, F.², and SCHALLER, H.¹

In an attempt to demonstrate that cloned HBV DNA is infectious, a mixture of HBV DNA containing plasmids was inoculated into chimpanzee liver cells. Six weeks later HBV and procaryotic DNA was detected in liver cells of the animal (Will et al., Nature 299 (1982) 740-742). To analyse the structure of these molecules in detail, a plasmid rescue experiment was performed by transforming E. coli cells with DNA isolated from chimpanzee liver. E. coli colonies containing the ampicillin resistance marker of the recombinant DNA molecules used for inoculation of the animal were selected. Plasmid DNA was isolated from 60 colonies, analysed by restriction enzyme cleavage and tested for the presence of HBV DNA and for expression of HBV antigens in tissue culture cells. Five different plasmid species were identified, all containing different amounts of HBV DNA but none being identical with any of the plasmids used for inoculation. Two of the plasmids produced the HBV specific HBe antigen in tissue culture cells although none of the plasmids used for inoculation expressed this antigen. These data indicate that the plasmids underwent structural changes during replication in the liver cells of the chimpanzee. It is unclear, whether some of the plasmids were autonomously replicating molecules in the liver or whether they needed complementation by helper virus molecules. Further analysis of the structure of the plasmids may help to answer this question and also allow to locate the origin(s) of replication of HBV within a subgenomic HBV DNA fragment.
New Family of Middle Repetitive DNA Sequences in the Mouse Genome with Structural Features of Solitary Retroviral Long Terminal Repeats

WIRTH, T., GLOGGLER, K., BAUMRUKER, T., SCHMIDT, M., and HORAK, I.

Screening of a 129/J mouse genomic library under non-stringent hybridization conditions with a xenotropic virus-like LTR probe revealed a family of insertion element-like sequences with structural features of solitary retroviral long terminal repeats, called LTR-IS. They are interspersed among variable flanking regions of mouse DNA and lack any viral structural genes. LTR-IS elements start and end with 11 bp inverted repeats and contain signals implicated in RNA polymerase II transcriptional regulation, CCAAT, TATAAA and AATAAA. The members of the family are homologous but non-identical, about 500 bp long elements, with 4 bp target site duplications on both sites of the element. The number of LTR-IS is about 500 per mouse haploid genome.

Sensitive Detection of Hepatitis B Core Antibody Using a Direct Radioimmunoassay

WOLFF, W. and GERLICH, W. H.

A novel assay for antibody (antiHBc) against hepatitis B core antigen (HBcAg) was developed. Flexible microtiter plates were coated with HBcAg, incubated with test samples and thereafter with 32P-labelled HBcAg. Labelling was achieved in vitro by the viral protein kinase. This assay was tenfold more sensitive than conventional inhibition assays, using labelled antiHBc as reagent. It detected a large proportion of positive persons (166/392) in a population with an increased risk of blood transmitted hepatitis infection, which were not detected by the inhibition assay. Such results were rare (7/232) in healthy blood donors. The weak antibody activity, detected only by the new assay, may be due to a very limited hepatitis B infection or it may be caused by cross-reacting antibody against other antigens.

Detection of Serum Antibodies to Adult T-Cell Leukemia Virus (ATLV) in Primates

YAMAMOTO, N.1, SCHNEIDER, J.2, SCHMITT, J.3, THOMSSEN, R.4, HINUMA, Y.1, and HUNS MANN, G.2

Out of 471 sera from individual non-human primates (27 species, 3 families of prosimians, 6 families of simians) 35 reacted positive in three types of immuno assays for antibodies to ATLV structural polypeptides. While 60% (32/53) of African green monkeys...
were positive, chimpanzees and crab-eating monkeys reacted only sporadically. The virus persists in African green monkeys since ATL polypeptides were detected in peripheral blood lymphocytes after in vitro cultivation. The major intracellular polypeptide recognized by positive sera was a glycoprotein, gp68, the putative precursor to viral envelope poly­peptides. The precipitation of isolated 125I-labeled gp68 was used to detect and quantitate ATLV serum antibodies. Results of all three assays agreed well. It is unknown at present whether these new ATLV isolates are oncogenic in primates. Our results may indicate spreading of ATLV from Africa to susceptible primates including man in other parts of the world.

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Intracellular Transport of Picornaviruses as Investigated by Ionophores and Weak Lysosomatropic Bases

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To investigate the intracellular transport of poliovirus type 1 and Maus Elberfeld (ME) virus during their early and late reproduction Ca++-ionophore A23187, chloroquine, NH₄Cl, and monensin were used for differential inhibition of the viral reproduction:
- Virus uptake is not regulated by Ca++. The Ca++-ionophore impairing the Ca++-influx into the cells does not interfere with the reproduction of both viruses (up to 30 µM).
- Lysosomes mediate the early reproduction, most probably the uncoating. The lysosomotropic weak bases chloroquine (1 mM) and NH₄Cl (60 mM) inhibit the reproduction of both viruses before their RNA synthesis sets on. Low concentrations of chloroquine (0.1 mM) and NH₄Cl (10 mM), respectively, result in an intermediate stimulation of viral reproduction.
- Release of both viruses proceeds without control of the golgi apparatus. Up to 20 µM monensin which blocks the flow of certain export proteins from the golgi apparatus to the cell surface, does not inhibit the viral reproduction.