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ISOLATION AND CHARACTERIZATION OF THE AVIAN SARCOMA VIRUS TRANSFORMATION-SPECIFIC PROTEIN pp60^{src}

The sarcoma virus-specific protein pp60^{src} has been isolated from Schmidt-Ruppin D (SR-D) virus particles and was found to be indistinguishable from pp60^{src} of transformed cells by V8 partial proteolytic cleavage. pp60^{src} and its associated protein kinase activity were purified by DEAE-phosphocellulose and Sephadex G-150 column chromatography using casein and immunoglobulin (IgG) of src-specific sera as substrates. In a glycerol density gradient analysis pp60^{src} sedimented as a monomer. A tendency to form large aggregates was observed particularly in the absence of non-ionic detergent. The protein kinase phosphorylated tyrosine in vitro in various acceptor proteins like immune IgG, casein, actin and chicken histones. pp60^{src} was located in the viral membrane. During incubation isolated membranes with [γ-^{32}P]ATP in vitro about 10 polypeptides were phosphorylated in tyrosine.

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DEMONSTRATION OF RSV CODED pp60^{src} IN VIVO AND THE IMMUNE RESPONSE AGAINST pp60^{src} IN THE NATURAL HOST THE CHICKEN

The virus protein pp60^{src} is a 60K protein kinase responsible for fibroblast transformation after ASV infection. It was found and extensively characterized beginning 1977. However, up to this date, it was not investigated, whether pp60^{src} can indeed be found in vivo in tumors and whether chickens, bearing ASV induced tumors, are able to initiate an immune response to pp60^{src}.

We demonstrated the presence of pp60^{src} in tumor tissue by the protein kinase assay. In SRV-D infected chickens an immune response against pp60^{src} was also found. This was shown by precipitation of ^{35}S-Methionine labelled lysates of SRV-D transformed chicken embryo fibroblasts. This result means that in spite of the intracellular presence of the so-called endogenous pp60^{src} in normal chicken cells chickens are not tolerant to pp60^{src}.
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STUDIES ON THE EXTRACELLULAR PP60
AND ITS ASSOCIATED KINASE ACTIVITY

Indirect immunofluorescence with purified pp60 spec-
nic antisera, immunoprecipitation of 32P-labeled pp60
and its associated protein kinase showed considerable
amounts of pp60 and its kinase activity being present
on the outer cell surface of chick embryo fibroblasts and
mouse cells transformed by the Schmidt-Ruppin strain (SR-A
of Rous sarcoma viruses. Within 5 min after medium change,
the pp60-associated kinase activity could be detected
in the culture medium. After 60 min the linear release of
kinase activity reaches a plateau. To prove that the
occurrence of pp60 kinase in the incubation medium is
not due to cell lysis, the time dependent release of sev-
eral glycolytic enzymes, known to be viability markers, was
measured. Hexokinase and pyruvate kinase could not be de-
tected. The very small amounts of lactate dehydrogenase
and pyrophosphatase in the medium did not increase during
the incubation time. Whether or not the extracellular
pp60 and its kinase activity are involved in the trans-
formation mechanisms is unknown. Transfer experiments show
that released pp60 kinase activity is rapidly taken up
by normal chick embryo cells.

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PHOSPHORYLATION OF PP60 AT TYROSINE AND ITS ROLE IN
TRANSFORMATION

A 60,000 molecular weight phosphoprotein, pp60,
is thought to be the product of the "src" gene of Rous
sarcoma virus responsible for transformation. Using
transformation defective temperature sensitive mutants
of Rous sarcoma virus we have investigated the relation-
ship between phosphorylation of pp60 and transformation.
We found that phosphorylation of mutant pp60 at tyro-
sine residues correlates well with the ability of the
protein to induce transformation.
Monoclonal Antibodies against the Avian RNA Tumor Virus Protein p19

Balb c /3T3(A31) cells transformed with SR-D avian sarcoma virus were tumorigenic in newborn syngeneic mice. The serum of the tumor-bearing mice was shown to precipitate the sarcoma-specific protein pp60src and the gag precursor of the avian virus structural protein p19, Pr75gag, from 35S-methionine labeled SR-D transformed chicken embryo fibroblasts in an immunoprecipitation.

The spleen cells of two of these tumor-bearing mice were used for the production of hybridomas by fusing them with the plasmacytoma cell line P3-NS1-1-Ag4-1 (NS1). The hybrid cells were selected in HAT-containing medium. From 97 surviving cultures, only four were positive in a radio-active antibody binding assay against proteins of transformed and normal cells and of transforming and nontransforming viruses. Although the serum from the tumor-bearing mice precipitated the pp60src protein, the positive hybridoma supernatants only precipitated the precursor Pr75gag and the structural protein p19.

The anti p19-producing hybridomas were cloned two times in soft agar and remained stable antibody producers in tissue culture for at least 2 months. For production of high-titered ascites fluids, hybridoma cells were inoculated into pristane pretreated (Balb c x C57BI)FI mice. The anti p19 monoclonal antibodies precipitated the putative transformation specific gag fusion protein with a MW of 75,000 from avian erythroblastosis virus (AEV) transformed bone marrow cells, as shown in an immunoprecipitation with 35S-methionine labeled cells.

On the Biosynthesis of Rous Sarcoma Virus Glycoproteins

The mature glycoproteins of Rous sarcoma virus, gp85 and gp35, are synthesized from a glycosylated precursor, Pr92env. We have examined the nature of the processing event(s) leading from Pr92env to gp85 and gp35 as well as the site of processing. We could show that sialation is an event in the processing and, in addition to cleavage, it may be the only other processing event. Processing of Pr92env occurs as a late step in the biosynthesis of viral glycoprotein and may occur at the cell surface since unprocessed Pr92env can be detected there. We have no evidence for processing occurring extracellularly in released virions as has been proposed by others. We have also examined three ts mutants of RSV and have shown them to be defective in the processing of Pr92env. Pr92env is synthesized in normal amounts at the non-permissive temperature. Virions released at the non-permissive temperature contain approximately normal amounts of the internal viral proteins but lack viral glycoprotein and are approximately 50 x less infectious than virions released at the permissive temperature.
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ON THE MECHANISM OF THE PROCESSING OF THE GAG-PROTEIN
PRECURSOR FROM ONCORNAVIRUSES

The gag-protein p15 of the avian oncornaviruses processes its own precursor pr76. We have evidence now that precursor pr76, when purified from infected cells in a native protein state, can autocatalytically cleave itself. As the first cleavage event p15 is cleaved off, which then will process the remaining precursor. Precursor pr76, which contains canavanine instead of arginine is not processed autocatalytically, but can be cleaved by "normal" protein p15, containing arginine. Two types of cleavage sites at the precursor are discussed.

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INTERMEDIATE TRANSFORMING PHENOTYPES INDUCED BY POTENTIAL TUMOR PROMOTERS IN NORMAL AND tsRSV-INFECTED CHICKEN EMBRYO CELLS

12-O-tetradecanoylphorbol-13-acetate (TPA) which is a potent tumor promoter in mouse skin, induced morphological alterations in tsRSV-infected chicken embryo cells (CEC) at the nonpermissive temperature (41°C), as well as in normal CEC. Biological and biochemical effects of TPA on tsRSV-CEC have been investigated: 1) Stimulation of cell proliferation accompanied by criss-cross growth. 2) Inhibition of colony formation in soft-agar of tsRSV-CEC at 36°C at the concentration of 1-100 ng/ml TPA. 3) Enhancement of hexose-uptake in tsRSV-CEC at 41°C, which reaches 50% of the level seen at 36°C. 4) TPA is known to stimulate transiently mouse epidermal ornithine decarboxylase (ODC) activity. It stimulated ODC-activity in normal (20fold) and in tsRSV-CEC (2fold). The activity was found to be 50-100fold higher in transformed CEC than in normal CEC. Rapid recovery of the activity in tsRSV-CEC was observed to precede recovery of morphological transformation after down-shift (36°C). 5) The protein kinase activity associated with pp60SRC, the protein encoded by the RSV-sarcoma gene, and the amount of the pp60SRC polypeptide were not affected by TPA. The kinase activity of the normal cell homologue of pp60SRC, pp60Ssrc, remained constant. 6) Protein kinase activity of partially purified pp60SRC was again not influenced by TPA when actin was used as acceptor protein.
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ON THE STRUCTURE OF OLIGOSACCHARIDES IN GP85-ROSETTES
FROM FRIEND LEUKEMIA VIRUS (FLV)

At least four (asialo) oligosaccharide fractions of the N-acetyllactosaminic (AL), two of the mixed (M), and four of the oligomannosidic (OM) type were obtained from FLV gp85 by a sequence of degradation (with pronase, endo H, neuraminidase and by hydrazinolysis) and separation procedures.

The AL oligosaccharide fractions contained mainly mannose (Man), galactose (Gal), N-acetylglucosamine (GlcNAc) and, in some cases, also fucose (Fuc) and N-acetylgalactosamine (GalNAc).

The M oligosaccharides contained Gal besides Man and GlcNAc. Methylation analyses revealed the presence of 4-, or 3-substituted Man - in addition to the common 2-substituted Man, 3,6-disubstituted Man and internal 4-substituted GlcNAc; they also comprised terminal Gal, 3-substituted Gal, and external 4-substituted GlcNAc.

Methylation and exoglycosidase digestion analyses of the OM oligosaccharides showed that the smallest species is composed of three terminal and one 3,6-disubstituted α-Man, as well as one (internal) 3,6-disubstituted β-Man. The next larger OM oligosaccharides contain in addition one or two 2-substituted Man, whereas the largest species further comprises 4-substituted Man residues.
"gp85-Rosettes" were isolated from FLV, and from those gp69/71 employing gel chromatography in the presence of SDS and 2-mercaptoethanol. A complete component analysis of gp69/71 was carried out, correlating the amino acid and sugar analyses via the common hexosamine values. A minimum molecular weight based on methionine was calculated to be 13700 D. Comparison with the molecular weight determined previously by ultracentrifugation (Moennig et al., 1974) led to a value of 54800 D (four times the minimum). The carbohydrate content was found to be approximately 20 weight percent. FLV gp69/71 is shown to be closely related to other retroviral envelope glycoproteins through statistical comparison of amino acid composition (S Q-values).

Moennig, V., Frank, H., Hunsmann, G., Schneider, I., Schäfer, W. (1974) Virology 61, 100-111

Prevention of Friend Virus Induced Murine Erythroleukemia by Active Immunization with Isolated Viral Envelope Glycoproteins.

Adult STU mice were repeatedly immunized with purified Friend virus (FLV) envelope polypeptides gp85, gp71 and pl5E as well as the major core polypeptide p30. The antigens were emulsified in complete Freund's adjuvant. After the last boost animals were challenged with a dose of FLV inducing fatal leukemia in 100% of controls. 20/20 mice immunized with a dose of 100 μg of gp85 were completely protected while 3/7 animals receiving 10 μg of gp85 died with leukemia. Vaccination with gp71 or pl5E was less effective than gp85 and p30 was ineffective. 100 μg of gp71 protected 4/6 mice while the same dose of pl5E prolonged the medium survival time for 35 days. Only animals that had developed a strong antibody response survived the FLV challenge. The serum antibodies of these animals displayed type-, species- and interspecies specific reactivity. Only a marginal cellular reactivity to gp85 was detected.

To elucidate the mechanism of this immunity we measured viremia by the radioimmuno assay for the internal viral polypeptide p30. Animals totally protected were not viremic later than 7 days post FLV challenge. Mice of the immunized groups not surviving showed a sharp decrease of serum antibodies to gp85 and a simultaneous appearance of virus in blood about 2-4 weeks before death from leukemia.

Thus, the suppression of virus spread by antibody seems to be important for immunoprevention against this virus-induced leukemia. Between 6-12 months of age AKR mice succumb to a thymic leukemia induced by an endogenous virus. However, 40% of these mice immunized as juveniles with AKR gp85 survive 12 months. Active immunization with purified viral envelope polypeptides may be effective against certain type-c virus-induced malignancies of other species as well.
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CHARACTERIZATION OF mRNAs OF FRIEND MURINE LEUKEMIA VIRUS

Friend Murine Leukemia Virus (F-MuLV) envelope protein can be used for vaccination of mice against F-MuLV induced leukemia. Our plan is to localize the immunological determinants responsible for this protection and the in vitro synthesis of a vaccine containing only a single determinant. The sequences on the F-MuLV envelope specific mRNA coding for the immunological determinants of the viral coat protein are being determined.

Viral mRNAs are isolated and characterized as follows: Cells infected with F-MuLV are lysed under RNase inhibiting conditions and RNAs are extracted. Hybridization of the RNA to F-MuLV proviral DNA (Oliff et al. 1980, J. Virol. 33, 475-486) is used to separate viral mRNAs from poly(A) containing cellular RNA. Envelope specific 21S mRNA is isolated by velocity centrifugation. In vitro translation of 21S mRNA allows comparison of in vivo and in vitro products of the envelope gene. Hybrid arrested translation of viral envelope specific mRNA yields peptides which can be screened for the presence of immunological determinants.

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DIVERSE EFFECTS OF THE PHORBOL ESTER, TPA, ON RETROVIRUS AND SV40 GENOME EXPRESSION IN VIVO AND IN VITRO.

1. Mice were treated with TPA i.p. every 2 days for one week and then infected with Friend Spleen Focus Forming Virus. Nine days after infection, the number of spleen foci was counted. In TPA-treated mice, a 5-fold increase was noted.

2. In virus-producing cells (mouse/F-MuLV; rat/SSV1), TPA exerted an increase of virus release. 3. No virus induction by TPA was found in Moloney MuSV-transformed non-producer mouse and hamster cells. 4. Cell transformation of 3T3 cells by Moloney MuSV was not enhanced in the presence of TPA. 5. In B cell mitogen-stimulated mouse spleen cell cultures, TPA inhibited the induction of endogenous virus formation. 6. In SV40-transformed tumorigenic mouse, hamster and tupaia cells, TPA enhanced the amount of non-integrated virus DNA. 7. In fusion & rescue experiments, the number of SV40 capsid antigen positive cells was not influenced by TPA. 8. Infection of semipermissive cells with SV40 was not enhanced in the presence of TPA.

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HORMONE RESPONSIVE EXPRESSION OF A CLONED MOUSE MAMMARY TUMOR VIRUS GENE IN TRANSFECTED L CELLS

The GR strain of mice contains five copies of the MMTV proviral gene per haploid genome. The breeding of congenic mice in combination with RIA of viral proteins in the milk of lactating mice and Southern blotting analysis of MMTV specific Eco RI fragments allowed the correlation of a single unique proviral gene with virus production and mammary tumor formation in vivo. In order to probe structural differences among proviral genes all five copies of MMTV have been cloned into λ recombinant molecules. The proviral genes were found to be similar but not identical in their primary DNA structure. Cloned MMTV DNA was cotransfected with the HSV thymidine kinase gene into Ltk-cells. L cells were found to faithfully replicate transfected MMTV DNA and express it into viral mRNA and protein indistinguishable from those found in mammary tumors. Transfected proviral copies were shown to be subjected to hormonal induction in L cells since dexamethasone stimulated viral mRNA production about ten fold.

C-TYPE RETROVIRUSES IN MAN: PARTICLE PRODUCTION IN FIVE HUMAN TERATOCARCINOMA (TC) CELL LINES.

Teratocarcinomas are defined as germ cell tumors which consist of embryonal carcinoma cells and derivatives of all three germ layers.

In five human teratocarcinoma cell lines (Tera 1, Tera 2, GH, HC, ER), selected for cells which are able to form domes and vesicles in tissue culture, spontaneous production of C-type like particles has been observed by electron microscopy.

In preliminary studies, the particle-producing cells exhibit some of the characteristics of trophoblasts. Particle morphology differs from known C-type virus morphology in two aspects (see presentation by Boller, K. et al.): i) there is no electron-lucent space between envelope and core, ii) particles with collapsed cores, a sign for virus maturation, have never been observed.

In our hands, the particles are so far not infectious as tested in extensive cocultivation experiments with a broad spectrum of indicator cells. Furthermore, a reverse transcriptase activity could not be demonstrated in the supernatant of teratocarcinoma cultures. We therefore assume that all human teratocarcinoma derived particles - designed "HTD viruses No. 1-5" - are defective and thus not infectious.
C-TYPE RETROVIRUSES IN MAN: COMPARISON OF C-TYPE PARTICLES DERIVED FROM HUMAN TERATOCARCINOMA (TC) CELLS WITH RETROVIRUSES OF MONKEYS

In order to compare human teratocarcinoma-derived (HTD)-particles with known exogenous and endogenous retroviruses of primates, we have chosen the simian sarcoma associated virus (SSAV) and the baboon endogenous virus (BEV) as prototypes. In immunological radioimmuno competition assays employing purified primate virus structural proteins, cell lysates of human TC cells were unable to compete for antibodies specific for a wide variety of primate and other retrovirus proteins. In contrast and as positive control, TC cells superinfected with BEV were able to compete in appropriate RIAs and also exhibited a reverse transcriptase activity in the culture supernatant.

In superinfected TC cells, both HTD-particles and the superinfecting virus can be distinguished morphologically, even when budding from the same cell.

The sum of presently available morphological, immunological and biochemical data suggests that HTD-particles represent a new group of viruses, basically with retrovirus morphology.

C TYPE PARTICLES IN HUMAN TERATOCARCINOMA CELLS: AN ELECTRON-MICROSCOPIC SURVEY OF THE VIRUSES AND THE CELLS.

Virus-like particles, found in cell cultures derived from human teratocarcinomas (see the presentation of R. Löwer et al.), share structural features with known C-type particles of baboon, mice and chicken as judged by electron-microscopy, but also show distinct differences.

In common are the presence of an envelope, the budding at the cytoplasmic membrane and the mode of budding, during which the virus core is assembled. Because of these characteristics human teratocarcinoma derived (HTD)-particles are classified as C-type viruses.

The electron-lucent space between core and envelope, which exists in all known C-type particles, has never been observed in HTD-particles. In addition, collapsed cores, a sign of mature C-type particles, have not been found in HTD-particles. In ultrathin sections, HTD-particles have a diameter of about 125 nm, in contrast to the 100 to 110 nm exhibited by C-type particles of animals.
SPECIFICITY OF THE HUMAN IMMUNE RESPONSE AGAINST PURIFIED PROTEINS OF THE BABOON ENDOGENOUS VIRUS BaEV.

It is still an unresolved issue whether human RNA tumor viruses (oncoviruses) exist and, if so, what the nature of their involvement in human disease might be. One approach to investigate human exposure to oncoviruses has been the search for antibodies against primate oncovirus proteins in human sera by means of radiolabeled precipitation assays (RIAs). Human sera were previously shown to possess antibodies capable of recognizing envelope glycoproteins of certain type C viruses such as SSV/SSAV and FLV. We have investigated the reaction of human sera from blood donors and patients against purified p30 and gp70 proteins of the baboon endogenous virus BaEV. The human sera tested did not show any positive reaction with purified BaEV p30 and gp70, when it was purified from BaEV grown in the human cell line. Positive immune reactions were obtained with gp70 from BaEV grown in the dog cells. The most interesting antibody reaction was seen with the sera from patients with teratocarcinomas, where 70% of the samples tested were positive.

Competition of the immune reaction could be obtained with a variety of oligosaccharides and glycoproteins. Deglycosylation of the glycoprotein gp70 by digestion with neuraminidase and endoglycosidase H led to a complete loss of antibody reactivity.

We conclude, that the positive immune reaction against BaEV glycoprotein is not the consequence of prior exposure of man to BaEV or related oncoviruses but is mediated by heterophil human antibodies that react with the carbohydrate moieties of oncovirus envelope antigens.

EXPRES5ION OF RETROVIRAL RELATED GENES DURING EMBRYOGENESIS OF JAPANESE QUAIL.

In a wide variety of animal species retroviral related genes have been retained during evolution and some of this genes are expressed during embryogenesis. This findings suggest that retroviral genes may be involved in embryogenesis. We have earlier shown (H. Mondal et. al. Cold Spring Harbor Symp. on Cell Proliferation; 1980, Vol. 7, 1239 - 1250) that retrovirus-like particles containing reverse transcriptase have been released in the media cultivated with the cells of various differentiating organs of Japanese quail which was not known to contain endogenous retroviruses. Here we characterize the particles thoroughly and attempt to show that the expression of retroviral related genes is not an effect of cell culture but a regular physiological phenomenon during cytodifferentiation. Embryonic tissues at successive stages of development of quails instead of dying in cell culture, have been directly tested for the presence of viral information. Reverse transcriptase bound mostly to the retrovirus-like particles (400 - 600 S) and sometimes to the retrovirus-like cores (80 - 100 S) appears in tissues of 3 day-old embryos of quails. The activity increases and ultimately disappears with the advancement of age. In quail embryonal tissues reverse transcriptase is bound to structures which are biophysically and biochemically similar to retroviruses. Therefore, it may be assumed that particles isolated from quail embryos are of retroviral nature. These and other data also indicate that retroviral related genes are universally present and are expressed at certain stages of embryonic differentiation. Whether they are directly involved in embryogenesis or whether they can only be used as a signal to monitor the gene expression during embryogenesis that has to be shown by further experiment.
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COMPARISON OF T-ANTIGEN-ASSOCIATED HOST PHOSPHOPROTEINS FROM SV40-INFECTED AND -TRANSFORMED CELLS OF DIFFERENT SPECIES.

Simian Virus 40 (SV40)-infected and -transformed cells contain, in addition to the virus-coded tumor antigens, one or more 48-56K host proteins which can be specifically immunoprecipitated with anti-SV40 tumor serum. At least part of this class of proteins exists as a complex with the SV40 large T antigen. The 55K proteins associated with the large T antigen in SV40-transformed monkey, mouse and human cells and SV40-infected monkey cells were compared by two dimensional gel electrophoresis and V8 partial proteolysis peptide mapping. Although these proteins differed slightly in apparent molecular weight and peptide pattern, they migrated identically in isoelectric focussing gels. These results suggest that the 55K proteins associated with large T antigen are very closely related to each other. Despite their similarities, the 55K proteins from different host cells form complexes of different stabilities with large T antigen, as judged by spontaneous dissociation of the complexes with time, and differential immunoprecipitation with tumor serum and a monoclonal antibody directed against the 55K proteins. In productively infected monkey cells, the amount of 55K phosphoprotein-T antigen complex increased with time after infection.

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PURIFICATION AND PROPERTIES OF SV40-TUMOR ANTIGEN.

Two forms of SV40-tumor antigen have been concentrated from lytically infected monkey kidney cells. The two forms sediment at 16 and 5 S, respectively. They show different binding capacity towards DNA and phosphocellulose. Both forms, however, bind preferentially to the DNA fragment carrying the origin of SV40 DNA replication.
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MULTIPLE FORMS OF SV40 TUMOR ANTIGEN.

Subclasses of SV40 large T antigen in SV40-transformed and infected cells separated by zone velocity sedimentation in sucrose density gradients have been characterized. Three forms of large T antigen were distinguished: a 5-6S form, a 14-16S form and a 23-25S form. These forms appeared to differ biochemically and biologically. Differential labeling experiments suggested that the 5-6S form was less highly phosphorylated than the faster sedimenting forms. The 23-25S form which was complexed with one or more host phosphoproteins, as reported recently (1,2), was prominent in extracts of transformed cells, but was also detected in productively infected cells.

Pulse-chase experiments suggested that the 5-6S large T antigen is a precursor of the more stable faster sedimenting forms of T antigen. Monkey cells infected with a tsA mutant of SV40 at 41°C contained only 5-6S large T antigen, implying that this form is not active in initiation of SV40 DNA replication. In pulse-chase shift-down experiments, DNA replication resumed and the 5-6S large T antigen which had accumulated at 41°C was partially converted at 33°C to a fast-sedimenting form. However, shift-up experiments demonstrated that the fast-sedimenting large T antigen, once formed, remained stable at 41°C though it was unable to function in initiation. These experiments suggest that different biological functions of large T antigen may be carried out by different subclasses of this protein.

1. Lane and Crawford. 1979. Nature 278, 261.
2. McCormick and Harlow. 1980. J. Virol. 34, 213.

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ANALYSIS OF TRANSCRIPTIONALLY ACTIVE CHROMATIN

Selectively isolated SV40-chromatin was fractionated by sucrose density gradient centrifugation, and various fractions were assayed for their ability to carry out RNA synthesis in vitro. The fast sedimenting fraction (faster than previrions and mature chromatin) proved to be particularly active as shown by pulse-labelling both in vitro and in vivo. Poly-A-containing messenger species (19 and 16 S) and larger poly-A-free precursor molecules were associated with the fast-sedimenting chromatin. Mature and replicating viral chromatin contain comparatively fewer mature and nascent RNA sequences.
Human brain tumors with episomal SV40 genomes.

Three out of nine human brain tumors were shown to contain unintegrated SV40 genomes with wild-type restriction enzyme cleavage patterns. As revealed by the Southern blot analysis only every tenth to twentieth tumor cell harbored one circular SV40 genome of wild-type size. There was no expression of viral antigens, as both immunofluorescence and immunoprecipitation assays failed to demonstrate either T- or capsid antigens. SV40 virus was induced in one case by fusion with normal human brain cells. The virus is fully infectious and is indistinguishable from wild-type SV40. This is the first direct demonstration of wild-type SV40 DNA sequences in human brain tumors.

Cloning of human papillomavirus DNA

To date it has not been possible to grow human papillomaviruses in tissue culture. Neither by viral purification from clinical material nor by purifying DNA from total cellular DNA usable amounts of viral DNA could be obtained. Therefore the following viral DNA's were cloned in E.coli K12 using pBR 322 as vector:

- HPV 1 isolated from plantar warts
- HBV 6 isolated from condyloma acuminatum
- HPV 8 isolated from a patient with Epidermodysplasia Verruciformis

The genomes of the different viruses were analyzed by restriction enzyme cleavage. By hybridizing single fragments with each other, a slight sequence homology was indicated.
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THE TRANSCRIPTION OF EPISOMAL BOVINE PAPILLOMA VIRUS IN TUMORS.

Bovine papilloma virus (BPV-1) induces sarcoid connective tissue tumors in horses and solid tumors in hamsters. The pattern of transcription both in a hamster tumor and in productively infected cells (within a bovine papilloma) were compared with each other. In both cases there was one prevailing RNA species (1300 bases) which mapped between 0.31 and 0.60 relative units on the physical map. In addition, within the papilloma at least 5 further RNA species were present, which are either missing or are underrepresented within the tumor. One of the frequently present species within the productively infected cells mapped between position 0.00 to 0.20. This region is not required for transformation.

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BOVINE PAPILLOMAVIRUS DNA IN EXPERIMENTALLY INDUCED HAMSTER TUMORS.

The syrian hamster is susceptible to bovine papillomavirus (BPV) infection, which leads to fibromas, fibrosarcomas, chondromas or meningeomas, depending on the route of inoculation. Two fibromas, which had been induced by subcutaneous injection of BPV 1, were analyzed by the Southern blot technique to look for the state of viral DNA. A blot of uncleaved tumor DNA revealed three bands, co-migrating with components I, II, and III of viral DNA. Furthermore, a faint band could be detected, which migrated slower than component II DNA. The same pattern was observed after cleavage of tumor DNA with the restriction endonucleases Sal I and Sac I, which do not cleave viral DNA, indicating that BPV 1 DNA persists in a free form. In agreement with this assumption plasmid DNA could be purified by CsCl-ethidium bromide gradient centrifugation and banded at a density of 1,607 g/ccm. Part of component I DNA was trapped, however, by cellular DNA at a density of 1,565 g/ccm. It could be released by DNA fragmentation with Sal I prior to gradient centrifugation. From this it is concluded that BPV 1 DNA persists extrachromosomally as a plasmid but is associated with cellular DNA.
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THE CHARACTERIZATION OF THE DNA OF HUMAN PAPILLOMA (HPV) 6

The viral DNA obtained from condylomata acuminata (HPV 6) was purified by centrifugation of DNA from genital warts in CsCl-Ethidiumbromide gradients and subsequently cloned in E.coli. The restriction enzyme map of the cloned DNA is in agreement with that of the non-cloned viral DNA.

By hybridization studies using the cloned viral DNA as a probe, HPV 6 sequences were found in seven other condylomata acuminata, and a HPV 6 subtype was identified. Furthermore, preliminary results indicate a partial hybridization between cloned HPV 6 DNA and the DNA obtained from flat condylomas of the cervix.

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CHARAKTERISIERUNG EINER DNA-NEGATIVEN MUTANTE VON ADENOVI-RUS TYP 2

In dieser Arbeit werden Eigenschaften einer temperaturesensitiven, DNA-negativen Mutante von Adenovirus Typ 2 beschrieben. Diese Mutante H2 ts206, die bereits vor einigen Jahren isoliert wurde (P. Kathmann et al., J. Virol. 19, 43-53 (1976)), synthetisiert virale DNA nur bei 32 C, nicht aber bei 40 C. Temperatur-shift Experimente haben jedoch gezeigt, daß der Defekt nur mittelbar die DNA Synthese beeinflußt und stattdessen sehr früh nach der Infektion (2 Stunden) exprimiert wird. Untersuchungen der "frühen" viralen RNA Synthese durch RNA/DNA Hybridisierung zeigten dann, daß in mit ts206 infizierten Zellen zwar normale Mengen virusspezifischer RNA im Zellkern aber nicht im Zytoplasma gefunden werden.

Die Mutante wurde durch Marker-Rescue mit ts-DNA und Wildtyp (wt) Fragmenten in einem sehr engen Bereich bei der Koordinate 30 % des viralen Genoms kartiert. An bekannten RNA Spezies kartieren in diesem Bereich die niedermolekularen VA-RNA Moleküle, deren Funktion bisher unbekannt ist. Unser Befund erhär tet Spekulationen, wonach niedermolekulare RNA Spezies am "Processing" von nuklearer RNA bzw. an der Reifung zytoplastischer RNA beteiligt sein sollen.
INTEGRATION, EXPRESSION AND METHYLATION OF VIRAL-DNA IN ADENOVIRUS TYPE 2-TRANSFORMED HAMSTER CELLS

We have investigated the patterns of integration of adenovirus type 2 (Ad2) DNA in 5 transformed hamster cell lines (HE1-6), by using the Southern blotting technique. Blots were hybridized to the different EcoRI and BamHI restriction fragments of Ad2 DNA labeled with [32p] by nick translation.

In three of the cell lines (HE1-3) the EcoRI-B fragment is present in its entire length. The EcoRI-B fragment encodes the 72K DNA binding protein (DBP). The mRNA for the 72K protein as well as the protein itself was found to be present in only one cell line - HE1.

Investigations of the methylation patterns of the HpaII sites (5'-CCGG-3') along the viral genome show that the EcoRI-B fragment is not methylated in the cell line in which this fragment is expressed (HE1), but totally methylated in the two cell lines lacking expression of DBP.

A detailed analysis of the leader region of the 72K protein shows that the early leader is missing in the HE2 and HE3 cell lines but at least part of the main late leader is present. By cloning the region of the late leader (70.7-73.6) and using it as a probe for hybridization to RNA blots, we could show that the late leader is used in transformed cells, which express the 72K protein, and also early after infection in the productive system. (Supported by SFB74 and the State of Northrhine-Westfalia, IIB5 - FA8381).

EXPRESSION OF INTEGRATED ADENOVIRUS TYPE 12 DNA IN TRANSFORMED HAMSTER CELLS AND IN REVERTANTS

We have analyzed the size distribution and map positions on the adenovirus type 12 (Ad 12) genome of cytoplasmic RNA isolated from five lines of Ad12-transformed hamster cells and from morphological revertants of one of the lines, T637. These revertants are characterized by the loss of all or most of the Ad12 genome equivalents integrated in the DNA of line T637. The Ad12-specific RNAs in human cells productively infected with Ad12 have also been investigated. Size classes of Ad12-specific RNA were determined by electrophoresis and subsequent transfer of RNA to nitrocellulose filters. Ad12-specific RNA sequences were detected by hybridization with [32p]-labelled Ad12 DNA or specific restriction endonuclease fragments of Ad12 DNA. The results are summarized in Table below. (Supported by SFB74).

Table:
The degrees of homologies to various parts of the Ad12 genome are indicated. The numbers in brackets refer to the number of size classes of Ad12-specific RNA found.

| Cell Lines | Prot. | Eco 3 | Eco B | Bam C | Bam B | Eco A |
|------------|-------|-------|-------|-------|-------|-------|
| ClAC 1     | (2)   | (4)   | (3)   | (2)   | (2)   | (2)   |
| Ad52-1     | (2)   | (2)   | (2)   | (2)   | (2)   | (2)   |
| M4/7       | (2)   | (2)   | weak  | (2)   | (2)   | (2)   |
| Ty17       | (2)   | (2)   | homologies | (2) | (2) | (2) |
| Ty2        | (1)   | (1)   |       |       |       |       |
| T637       | (2)   | (2)   | (2)   | (2)   | (2)   | (2)   |
| RE1/12     | (2)   | (4)   | (3)   | (2)   | (2)   | (2)   |
| RE4/12     | (2)   | (4)   | (3)   | (2)   | (2)   | (2)   |
| RE3/12     | (2)   | (4)   | (3)   | (2)   | (2)   | (2)   |

*Ad 12 L and RE-Ad 12 refer to RNA isolated late (45-60 h p.i.) and early (8-10 h p.i.), respectively from productively infected cells.

b Eco A' refers to the first terminus of Ad12 DNA comprising approximately 44.5 kbp.
Sequencing the Adenovirus 2 Region Coding for the 3' Terminus of the DNA Binding Protein Messenger RNA

The coding region for the single-stranded DNA binding protein (DBP) of adenovirus 2 (Ad2) is located between map positions (m.p.) 61.6 to 68.6 on the Ad2 genome. The leftwardly transcribed messenger RNAs for the DBP (Mr 73,000) have common 3' termini, but differ in their splicing pattern and in the 5' leader sequences used early (m.p. 75.1-75.2) and late (m.p. 71.9-72.0) after infection. To localize the 3'-terminal regions of the DBP mRNAs we first identified and mapped a small restriction fragment BglII J/AluI-2 (m.p. 61.51-62.34) hybridizing to poly(A) containing fragments of the DBP mRNA. Both strands of BglII J/AluI-2 (296 nucleotides) were then sequenced using the Maxam and Gilbert technique. Inspection of the DNA sequence revealed a possible poly(A) addition signal AATAAA for the DBP mRNA at a distance of 135 nucleotides from the AluI site at m.p. 61.51. On the opposite DNA strand at a distance of 114 nucleotides from the same AluI site the probable poly(A) addition signal of the hexon mRNA can be seen.

Sequence Analysis of Adenovirus Type 12 Mutants Adapted to African Green Monkey Kidney (VERO) Cells

Ad12 has been adapted to growth in Vero cells (1). Plaque-purified mutants have a higher infectivity than the wild-type virus in Vero cells and a lower infectivity in human Hela cells.

One of the mutants (SL) has an insertion of 284 base pairs at the right hand end of the terminal Hind III E fragment, another one (LL) an insertion of 176 base pairs at the same locus. In the left terminal Hind III fragment both mutants have a deletion of 70 base pairs.

The insertions consist of wild-type sequences. In the mutant SL the genome at the right hand end starts with wild-type sequences 1 to 294 followed by number 11, 12 and so on of the wild-type genome.

In the mutant LL the genome starts from right to left with the wild-type sequences 1 to 180 of the left end of the wild-type genome followed by number 4 to 7 (number 8 is deleted) 9 and following ones of the right hand end of the wild-type genome.

The deletion in the Hind III G fragment of the mutant DNA consists of about 70 base pairs and is localized between number 739 and 906 of the wild-type.

(1) Werner, G., zur Hausen, H.
Virology 86, 66 - 77 (1978)
Undifferentiated sarcomas were induced in hamsters by the injection of adenovirus type 12 (Ad12) into newborn animals. Tumor incidence was 63% among the survivors, 47% in male animals, 75% in female animals. The mean latency period was 52 days. Histologically it could be shown that the tumors infiltrate local muscular tissue, and tumor cells can be found inside lymphatic vessels.

The DNA was extracted directly from tumor tissue and analyzed by restriction enzyme analysis and Southern blotting for 11 different tumors. In principle, the patterns of viral DNA integration were very similar to those described previously for Ad12-transformed hamster lines maintained in culture for many years. The intact Ad12 genome appeared to be integrated colinearly in multiple copies. Among different lines the genome copy number varied between 1 to 2 and about 25 to 30. Using the isoschizomer restriction endonuclease pair HpaII and MspI, it was found that the total cell DNA as well as the integrated Ad12 DNA extracted from tumors directly was very poorly methylated at the 5'-CpCGG-3' sites. Surprisingly, the patterns of methylation shifted to higher levels upon explantation and repeated passages (4-24) of the tumor cells in culture. Although mechanism and significance of changes in the extent of DNA methylation are not understood, it can be clearly demonstrated that the extent of methylation at 5'-CpCGG-3' sites can be influenced by culture conditions of the cells. (Supported by SFB74 and the Friedrich-Ebert-Stiftung).

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MECHANISMUS DER REPLIKATION VON MAUS-ADENOVIRUS FL DNA

Maus-Adenovirus FL hat ein liniäres, doppelsträngiges DNA Genom vom Molgewicht 19,6x10^6 Daltons. Es ist damit etwa 10% kleiner als die Chromosomen der menschlichen Adenoviren. Der Replikationsmechanismus ist jedoch ähnlich dem der menschlichen Adenoviren mit den molekularen Enden als Ursprungs- und Endpunkte der DNA Replikation. Die Gegenwart einer umgekehrten terminalen Repetition haben wir auf 2 Arten identifiziert. Im Elektronenmikroskop (H. Delius, EMBL, Heidelberg) erkennt man nach De- und Renaturierung einsträngige Ringe. Durch Sequenzierung der viralen DNA im terminalen Bereich konnte die Länge der Repetition auf 93 Basenpaare festgelegt werden. Beim Vergleich dieser Sequenz mit denjenigen anderer Adenoviren stellt man fest, daß allen Adenoviren eine Sequenz von 9 Basenpaaren zwischen den Positionen 9-17 der molekularen Enden gemeinsam ist. Diese Sequenz lautet: 5'-ATAATAAAC-3'. Diese Sequenz ähnelt in ihrer Basenzusammensetzung einem eukaryontischen Promotorelement. Der Sequenzhomologien wegen ist es vielleicht nicht verwunderlich, daß Maus-Adenovirus FL DNA auch in vitro in Kernextrakten aus mit menschlichen Adenoviren infizierten Zellen repliziert. Unsere Versuche ermöglichen es uns nun, das identifizierte Replikon zu minimalisieren und als Vektorsystem für den Gentransfer in Mäusezellen auszuarbeiten.
The presence of a DNA terminal protein complex, as first demonstrated for avian adenovirus by Robinson et al. (1973) was investigated by Tupaia adenovirus (TAV). The TAV DNA protein complex was isolated from purified virions according to the procedure described by Sharpe et al. (1976) for adenovirus 5 DNA-protein complexes. DNA-protein complexes were released from purified virions with 4M guanidinium hydrochloride and isolated by sedimentation in sucrose gradients containing 4M guanidinium chloride. After dialysis against 100mM NaCl, 10mM Tris-HCl, 1mM EDTA, pH 8.0 the isolated DNA-protein complexes were examined by electron microscopy and agarose gel electrophoresis after restriction endonuclease cleavage. TAV DNA was prepared under conditions where terminal proteins remain attached to the termini of adenovirus DNA. Subsequent electrophoresis showed that the terminal restriction fragments and the uncleaved TAV DNA-protein complex migrated into native agarose gels only after protease treatment. This indicates that TAV DNA when isolated from virions contains proteins attached to its termini. The infectivity of TAV DNA and the DNA-protein complex was determined using calcium phosphate technique. Purified TAV DNA which had been treated by pronase and freed of proteins by phenol extraction caused a CPE on TEK cells similar to that caused by intact virus. RNase treatment or heating to 60°C for 60 min did not abolish the infectivity of TAV DNA (4-8 plaques/2 µg DNA). DNase completely destroyed the infectivity. The purified TAV DNA-protein complex at a 10-fold lower DNA concentration (0.2 µg) showed an enhanced infectivity even after exposure to RNase or heat (87-152 plaques/0.2 µg DNA). This indicates that the infectivity of the TAV DNA-protein complex was increased by about 200-fold when compared to TAV DNA.

ADENOVIRUS 37, A MEDICALLY IMPORTANT, HITHERTO UNRECOGNIZED VIRUS

During the last years numerous adenovirus strains have been isolated in various parts of Europe from ocular and a few from genital sites, belonging to a new type 37 of subgroup D of human adenoviruses. The virus is closely related in hemagglutination-inhibition to Ad19 and 10, and more distantly related in neutralization to Ad19, 10, 13, and 30. The virus has escaped correct identification in the past, and has been found to be untypable, or registered as Ad19, 10, or 10/19 by different investigators. De Jong et al. (Abstracts of the 4th International Congress for Virology, 141, 1978) have described it as an intermediate adenovirus strain 13-30/10-19. The relationship in neutralization is unilateral, and the degree of cross-reaction depends on the individual antisera used. The virus has a unique genome type, as shown by restriction endonuclease analysis of its DNA, and by serological and biochemical criteria the virus is to be considered as a new species (type). In addition, sera from patients show a monotypic rise of neutralizing antibodies against Ad37. Identification is performed by neutralization with homologous antiserum.
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CLASSIFICATION OF FOWL ADENOVIRUSES (FAV) ACCORDING TO STRUCTURAL PROTEINS AND DNA RESTRICTION PATTERNS

Fourteen FAV strains out of the eleven serotypes (McFerran 1977) were purified:

- FAV 1 (OTE, CELO);
- FAV 2 (SR-48);
- FAV 3 (SR-49);
- FAV 4 (KR-5);
- FAV 5 (340);
- FAV 6 (CR-119);
- FAV 7 (YR-36);
- FAV 8 (TR-59, 764, Hung VI);
- FAV 9 (A-2);
- FAV 10 (C-2B);
- FAV 11 (UF-71).

Patterns of structural proteins and DNA restriction patterns of all strains were analyzed. Serotypes showing no cross reactivity in the serum neutralization assay differ in the molecular weights of all structural polypeptides and have no common DNA restriction bands. However, serotypes showing only slight cross neutralization proved to be rather related regarding their DNA cleavage and polypeptide patterns, which of course was true for members of the same serotype. The identity of OTE and CELO could be demonstrated and the eleven FAV serotypes reduced to five basic types (type 1: OTE, CELO; type 2: SR-48, UF-71, A-2, SR-49; type 3: KR-5, C-2B; type 4: 340; type 5: CR-119, YR-36, TR-59, 764, Hung VI).

The likewise studied hemagglutinating duck virus strain 127, the causative agent of the egg drop syndrome 1976, has a markedly shorter DNA than the FAV serotypes ($16 \times 10^6$ D vs. $28 \times 10^6$ D of FAV). Its proteins look similar to human adenoviral proteins, but its genome does not hybridize either to fowl adenovirus nor to human adenovirus DNA.

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THE FIBERS OF FOWL ADENOVIRUSES

Laver et al. (1971) demonstrated that pentons dissociated by dialysis from particles of the fowl adenovirus (FAV) serotype 1 strain CELO carried two fibers, a short and a long one.

Purified virions out of the eleven FAV serotypes were examined by electron microscopy: FAV 1 (OTE, serologically identical with CELO); FAV 2 (SR-48); FAV 3 (SR-49); FAV 4 (KR-5); FAV 5 (340); FAV 6 (CR-119); FAV 7 (YR-36); FAV 8 (TR-59, Hung VI); FAV 9 (A-2); FAV 10 (C-2B); FAV 11 (UF-71).

All virus strains showed two fibers protruding from one penton base. According to the serotype the length difference between the two fibers was more or less pronounced. The double fibers could not only be demonstrated on isolated pentons, but on the virions itself.

Pentons with two fibers seem to be a common feature of the group of fowl adenoviruses, separating them from all other adenovirus groups known.

Laver, W.G., Younghusband, H.B. and Wrigley, N.G. (1971) Purification and properties of chick embryo lethal orphan virus (An avian adenovirus). Virology, 45, 598

* Supported by DFG Grant Ma 834
EPSTEIN-BARR VIRUS STRAIN AND GROUP SPECIFIC ANTIGENIC DETERMINANTS DETECTED BY MONOCLONAL ANTIBODIES

In order to differentiate Epstein-Barr virus (EBV) strains by serological markers we prepared hybridomas producing monoclonal antibodies against polypeptides of the QIMR-WIL EBV strain. These monoclonal antibodies were screened by indirect immunofluorescence techniques. Immunoprecipitation, by using EBV positive monoclonal antibodies and the protein A method, of 125I-labeled polypeptides from purified QIMR-WIL EBV particles, revealed that a series of monoclonal antibodies against the four main surface and envelope polypeptides precipitating p340, p340/p240, p140, and p80 were obtained. When 125I-labeled polypeptides from purified P3HR-1 and B95-8 EBV particles were immunoprecipitated, it could be demonstrated that several anti p340 (QIMR-WIL) antibodies recognized strain specific antigenic determinants, while anti p340/p240 (QIMR-WIL) as well as anti p140(QIMR-WIL) antibody clones reacted with antigenic sites which are in common either among B95-8 or in addition to P3HR-1 polypeptides.

COOPERATIVE EFFECT OF AN ACTIVATED SERUM FACTOR AND INDUCERS OF EARLY ANTIGEN SYNTHESIS OF EPSTEIN-BARR-VIRUS.

Alkali- or acid-treatment of serum, followed by neutralization, activates a factor that potentiates the induction of early antigens of Epstein-Barr-virus by inducers such as I UdR,m-butyric acid, anti-IgM and the tumor promoter TPA. In the presence of activated factor the induction is more efficient when compared to untreated serum in the following parameters: 1) the rate of induction is faster, 2) the percentage of cells that finally express early antigens is increased, 3) the dose response of induction is much more sensitive. The activated factor is a weak inducer by itself. The factor is present in the sera from various species such as man, horse, calf and chicken.

The cooperative effect between the factor and chemically different inducers indicates the existence of a common step in the induction of EBV antigens by those agents. The factor may play a role in the regulation of EBV in vivo.
EVIDENCE FOR THE PERSISTENCE OF EPSTEIN-BARR VIRUS IN THE PAROTID GLAND

Primary infection with Epstein-Barr virus is followed by a lifelong carrier state. Virus can be isolated from the saliva for many months or years. It was not clear, however, whether circulating lymphocytes which are enriched in the oropharyngeal tissue may occasionally enter a lytic cycle or if EBV is produced in a specific site of the body resembling the behaviour of Marek's disease Herpesvirus.

In situ hybridization with frozen sections from tonsils, parotid glands and other tissues revealed that EBV genomes are present in the tissue of the parotid gland of healthy persons. The data from in situ hybridizations could be confirmed using stringent reassociation kinetics.

Isolation of EBV from the ducts of the parotid gland has been reported during the course of our work by Morgan et al. (The Lancet, Dec. 1979). This finding is in perfect agreement with our data.

EPSTEIN-BARR VIRUS INDUCES CELL FUSION

Nasopharyngeal Carcinoma of Man (NPC) is thought to be induced by Epstein-Barr virus (EBV), however, the virus genome positive cells of the tumor are epithelial in origin and lack receptors for EBV. Microinjection studies have shown that EBV can be expressed in receptor negative cells once the barrier to penetration has been overcome. It has been suggested that EBV might enter receptor negative cells after fusion of such cells with EBV carrying lymphocytes. Previously it was thought that the fusion might be brought about by one of the many syncytial forming viruses which inhabit the respiratory tract. Using a recently developed technique for the preparation of monolayers of human lymphoblastoid cells we have been able to observe EBV induced cell fusion. Superinfection of immobilized Raji cells with EBV derived from P3HR1 cells leads to the formation of multinucleate cells (Nature 287, 164-165, 1980). Further studies have shown that superinfected Raji cells can fuse not only to uninfected Raji cells but also to cells devoid of receptors for EBV such as human embryofibroblasts and human T-lymphoblasts. In a series of experiments using metabolic inhibitors and amino-acid analogues it was shown that partial expression of the EBV genome was sufficient to induce cell fusion. The possibility that the fusion was caused by virion proteins integrated into cell membrane upon infection was excluded by a series of experiments which showed that induction of EBV early antigens (using IUDR, phorbolesters, butyric acid) in immobilized cells was sufficient to cause cell fusion. The efficiency with which EBV induces cell fusion and the fact that multinucleate and up to tetraploid cells can be observed in NPC biopsies lends support to the hypothesis that EBV induced cell fusion plays an important role in the etiology of EBV induced NPC.
USE OF ENZYMIMMUNOASSAYS FOR THE DEMONSTRATION OF ANTIBODIES TO DIFFERENT ANTIGENS OF EPSTEIN-BARR VIRUS

The application of enzymimmunoassays with automatic reading for the detection of antibodies against different antigens of EBV requires the use of purified and characterized antigen preparations because nonspecific reactions which are readily detectable by immunofluorescence could otherwise lead to indistinguishable falsely positive results.

For the demonstration of antibodies of the IgG class directed against EBNA, VCA and EA, the different antigens were coated directly to the solid phase. The plates were incubated consecutively with patient serum and peroxidase coupled antihuman IgG. Finally the substrate was added.

For EBNA preparation nuclei of B95/81 cells were isolated and extracted. The preparation of VCA was carried out by two times pelleting the clarified supernatant of 12 liters of P3HR1 cell suspension culture. EA was prepared by isoelectric focusing of the extracts obtained from induced B95/81 cells.

The demonstration of antibodies to EBNA (anti-EBNA) in sera of patients with acute and past infectious mononucleosis (IM) and patients with nasopharyngeal carcinoma (NPC) proved to be 5 to 10 times more sensitive than anti-EBNA determined by anticomplementary immunofluorescence (ACIF). A similar sensitivity could be found in the demonstration of antibodies to VCA (anti-VCA).

The demonstration of anti-VCA of the IgM class (anti-VCA IgM) is of major importance for the diagnosis of acute IM. The demonstration of anti-VCA IgM was carried out by a reverse ELISA in which anti-μ is coated to the solid phase. After consecutive incubation with patient serum VCA and peroxidase coupled anti-VCA the substrate is finally added.

All tested sera of patients with acute IM proved to be anti-VCA IgM positive.

TUMOR PROMOTORS AS METHYLATION INHIBITORS

THE INFLUENCE OF 12-O TETRADECANONYL-PHORBOL 13-ACETATE (TPA) ON THE METHYLATION OF HERPESVIRUS SAIMIRI AND HERPESVIRUS ATELES GENOMES IN PERSISTENTLY INFECTED TRANSFORMED CELL LINES.

The DNA of oncogenic primate herpesviruses herpesvirus saimiri and herpesvirus ateles persists in transformed cells in an episomal form, in which the circular DNA molecules contain deletions and duplications of viral DNA-sequences. In contrast to viral DNA sequences formed during lytic infection episomal DNA from transformed cells is further modified by methylation of the 5-position in the 5' CpG dinucleotide, as indicated by restriction enzyme analyses and its density in CsCl.

Treatment of transformed cells with the methylation inhibitors 5-adenosylhomocysteine (SAH), 5'-deoxy-5'-S-isobutyl-thio-adenosine (SIBA), and sinefungin leads to a reduction of the density of episomal viral DNA.

Similar results were obtained with the tumor promoter TPA or butyrate in two different cell lines transformed by H. saimiri and three cell lines transformed by H. ateles. In addition tumor promoters as well as methylation inhibitors seem to reduce the number of persisting viral gene copies in the infected cell. Since phorbol esters are known to induce virus specific protein synthesis in herpesvirus transformed lymphoid cells, our findings support the hypothesis that methylation of persisting viral DNA in eucariotic cells has a function in the long term regulation of gene expression.
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GENE EXPRESSION OF ONCOGENIC PRIMATE HERPES VIRUSES IN TUMOR CELL LINES AND LYTICALLY INFECTED CELLS

Highly oncogenic primate herpesviruses such as herpesvirus saimiri and herpesvirus aetæs give rise to lytic infection in owl monkey kidney cells (OMK cells) and (as well as) persistent infections in T lymphocytes.

We studied the expression of viral genes in persistently infected cells, which may contain up to 340 viral gene copies per single cell. In lytically infected cells about 50% of the viral DNA sequences are transcribed into nuclear RNA, whereas cytoplasmic RNA contains only 30% of virus-coded RNA. The amount of viral coded RNA in lytically infected cells depends on the time after infection and varies in the range of 0.5 to 10%.

Hybridization with cosmid-cloned L-DNA from H. saimiri indicates that the right region of genome is transcribed to a greater extent. However, in persistently infected cells, despite the presence of large amounts of viral DNA, virus-specific RNA is barely detectable. In vitro translation of RNA from lytically infected cells results in the synthesis of about 15 proteins, which partly comigrate with in vivo labeled proteins from virus infected cells or viral structural proteins. Immunoprecipitation with various monkey sera indicates that some of the in vivo labeled proteins are virus-specific. Except for pATNA, a nuclear antigen detectable in cells transformed by H. aetæs, no virus specific protein could be detected in transformed lymphoid cells. Further experiments are planned to find the reason for the discrepancies between the great amount of viral DNA and the lack of gene expression.

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CHARACTERIZATION OF HERPESVIRUS SAIMIRI AND HERPESVIRUS AETELES PROTEINS

H. saimiri and H. aetæs are highly oncogenic in various New World monkeys. It has been shown that one strain of H. saimiri (H. saimiri 11 A) is unable to induce tumors and protects the infected animals against challenge inoculation with wild-type virus. In order to characterize the different virus strains, we purified virions and analysed the proteins of the following strains on SDS-polyacrylamide gels: H. saimiri 11, H. saimiri 11 A, H. saimiri 4322, H. aetæs 73 and H. aetæs 810. 21 polypeptides were identified in all three H. saimiri isolates; the molecular weight revealed no differences. 19 virion proteins were identified for the H. aetæs strains; these proteins were different for H. aetæs 73 and H. aetæs 810 and distinguishable from H. saimiri proteins. Surface iodination of H. saimiri virions and capsids detected three proteins as part of the virus envelope. In order to further characterize viral proteins of the oncogenic wild-type H. saimiri 11 and the attenuated strain H. saimiri 11 A derived from it, we analysed immunoprecipitated proteins synthesized early and late during lytic infection on SDS-polyacrylamide gels. By this technique one polypeptide could be identified, which was present in cells infected with H. saimiri 11 wild-type but not in those infected with H. saimiri 11 A.
THE AMP- AND ADP-DEPENDENT PHOSPHORYLATING CAPACITY OF THE HSV-THYMIDINE-KINASE

The HSV-induced TK phosphorylated dThd, dCyd and acyclo-guanosin. This enzyme also shows thymidilate-kinase-activity. ATP and dCTP serve as good phosphate donors, whereas dGTP and GTP are not very active.

We have shown that a TK-activity appears in HSV-infected TK- cells able to use besides ATP as well ADP as AMP as phosphate donors. These TK-activities can be partially separated by glycerol-gradient-centrifugation and polyacrylamide gel elektrophoresis. The AMP-dependent activity has a somewhat higher M.W. and the ADP-activity a somewhat lower M.W. than the ATP-dependent TK (80000 D). Km- and Vmax-values show that the ADP-TK has the highest affinity to dThd and that the ATP-TK exhibits the highest specific activity. The AMP-TK is selectively inhibited by α-Phenanthrolin, Jodocetamide, Jodosobenzoic acid and N-Ethylmaleimid. P-Chlormercurlbenzolcarbd deppresses all activities. In experiments performed with different divalent cations in the assay we observed different abilities of the three activities concerning the use of these effectors. They also differ with regard to temperature stability, pH-profile and salt-inhibition. The 32P-labeled phosphate group of AMP and ADP was transferred to dThd. The AMP-activity was purified 1300-fold by AMP-Sepharose-columns (J. gen. Virol., in press).

The HSV-type I-TK-activities differ from the type II-activities in isoelectric focusing experiments. Besides this the cellular TK can be separated from the virus-coded enzyme.

From these results it is concluded that the HSV-induced TK is a multifunctional enzyme-complex with different catalytic sites.

DNA-BINDING GLYCOPROTEINS IN HSV 1-INFECTED BHK 21 CELLS

The extracts of HSV 1-infected and not infected BHK cells, labeled with (3H)-glucosamine (2 h p.i., 10 hours) were passed through a column with ds-DNA-cellulose. The bound proteins were eluted by 1,7 M NaCl and separated by SDS-PAGE. In the autoradiograph of the gel three virus-specific bands of Mr 145 000, 128 000 and 54 000 could be seen.

These bands were shown to be proteins by proteolytic digestion with Staph. aureus protease.

In order to ensure that the (3H)-glucosamine had been incorporated specifically into glycoproteins, the DNA-binding proteins were passed through a Lens culinaris hemagglutinin (LcH)-sepharose column. The retained glycoproteins were eluted by a buffer containing methyl-α-D-mannopyranoside and separated by SDS-PAGE. In this gel two of the three virus-specific bands could be identified by the PAS staining method. The third virus-specific band could not be detected probably because it did not contain sugar residues specific for LcH or because it did not contain sialic acid which is the sugar residue preferentially stained by the PAS method.

The biological function of the three virus-specific DNA-binding glycoproteins is not known. Whether they play a role in the attachment of the replicating Herpes-DNA to the nuclear membrane or to the nuclear matrix remains to be established.
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HSV-INDUCED DISORGANIZATION OF THE CYTOSKELETON

Virus infections of eucaryotic cells result in cellular modifications which are rec-
ognized as cytopathic effects - cell rounding or cell fusion. Using different
strains of HSV-1 we induced these effects and examined the arrangement of the
cytoskeleton of the infected cells. During cell fusion the "stress fibers" are re-
leased from the plasma membrane, can rearrange to very long fibers and finally
are destructed, while the intermediate filament-system appears to be unaffected.
Examination of the cell rounding process revealed that the stress fibers are not
decomposed but arrange as a network close to the cytoplasmic surface of the
plasma membrane, and the intermediate filaments collapse to the surface of the
nucleus. Preliminary experiments indicate that the microtubules remain unaf-
fected during cell fusion but are destructed during cell rounding. From these
results we conclude distinct mechanisms which lead to either cytopathic effect.
Since plasma membrane and cytoskeleton are responsible for many important
features of eucaryotic cells the experimental system we use should allow some
hints upon the modified interaction of these structural elements of the cells
during virus-induced cell pathology.

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VIRAL INTERFERENCE IN HSV-1: FORMATION OF NONINFECTIOUS
PROGENY DNA SIMILAR IN STRUCTURE TO STANDARD VIRAL GENOMS

Intracellular progeny DNA was isolated and characterized from cells infected
with standard herpes simplex virus or from cells coinfected with standard virus
and with a virus stock obtained by serial passages at high multiplicity of
infection. This virus stock was shown to contain an excess of variant virus
particles interfering with the replication of infectious progeny virus. In both
infections similar amounts of unit length viral DNA were synthesized.
Restriction endonuclease digestion of the DNA from the coinfection experiment
yielded fragments typical for viral standard DNA notably the end fragments
which suggests a correct maturation of the intracellular DNA. Despite this
obvious similarity of the two DNA species the specific infectivity of progeny
DNA formed in cells infected with an interfering virus stock was lower by two
log than progeny DNA from standard virus infections as determined by
transfection assays. With the aim of excluding various forms of DNA
modification as the cause of interference, DNA-methylation and the eventual
presence of ribonucleotides in the DNA was studied. In both (6-3H)-uridine
labelled DNA preparations methylation was below the level of detection of ten
5-methyl cytosine residues per unit length viral DNA molecule. Low values were
also obtained for the uridine content of the DNA. Almost 100% of the
radioactivity incorporated could be recovered as deoxyribonucleotides and less
than 0.07% as uridine.
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NEUTRALIZING HERPES-SIMPLEX-VIRUS-ANTIBODIES IN A MOUSE MODEL: INFLUENCE OF GENETIC FACTORS AND DRUGS

Investigations concerning the pathogenesis of Herpes-simplex-infection in mice indicate the importance of cellular defense mechanisms (Nature 265, 630-632 (1977)) against challenge with this virus, whereas little is known about the influence of humoral antibodies.

Comparison of some mouse strains with different H-2-haplotypes on B 10-background did not show significant differences between HSV-antibody-levels indicating no influence of MHC-genes on HSV-antibody formation.

Determination of neutralizing HSV-antibodies in some other strains revealed titers always to be much higher in female mice than in males suggesting an involvement of sex-linked mechanisms of regulation.

Levels of HSV-antibodies can be increased by use of some nonspecifically acting drugs, as silica, dextran sulfate 500,000 and Indomethacin.

Bestatin, a peptide known in vivo to stimulate DNA-metabolism exclusively of T-Lymphocytes (Biochemical Pharmacology 28, 3131-3137 (1979) ) effects an increase of HSV-antibody-titers when given between the third and eighth day after i.p.-infection; the maximum effect is achieved the fifth day post infection. The extent of antibody response is dose dependent showing a 4-fold increase already at doses of 0.1 mg/kg and 6-fold at 62.5 mg/kg.

Bestatin does not effect an acceleration of virus elimination from liver, spleen and thymus when given at the day of infection.

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COMPARISON OF THE DNA OF VARIOUS BOVID HERPESVIRUS TYPE 1 (BHV - 1) ISOLATES (IBR/IPV) BY RESTRICTION ENZYME ANALYSIS

The DNA cleavage pattern of 25 BHV - 1 isolates, obtained from IBR/IPV - outbreaks in Southern Germany during the years 1977 - 1980, and of IBR-SP and IPV-SCH (G. C. Straub 1965) were compared after digestion with the endonucleases Bgl II, Hind III, Bam HI, and Eco RI. The results indicated (i) great similarities among the genomes of all isolates tested, without a temporal or regional cluster of different virus strains, (ii) no clear cut differentiation between viruses of the IBR - and the IPV - form of the disease, and (iii) most of the isolates of the years 1977 - 80 showed identical DNA fragment pattern.
In order to differentiate serologically identical virus strains deriving from clinical cases of IBR and IPV, respectively, we compared the genomes of 4 IBR and 4 IPV isolates by means of restriction enzyme analysis. Furthermore we compared the genomes of a wildtype IPV virus strain (passage 10) and its attenuated derivative (passage 450) which is used as vaccine strain. The biological properties of the latter ones additionally have been examined by neutralization and immunoprecipitation experiments, and no difference could be observed. The digestion patterns with various restriction endonucleases showed a close similarity between IBR and IPV isolates as well as between the wildtype and the attenuated IPV virus strain, but a few consistent differences were found. Conclusions drawn from these results, including observations on preliminary mapping data are discussed.

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STRAIN DIFFERENCES IN PSEUDORABIES VIRUSES

Pseudorabies virus isolates were collected from dogs and cats which died from the disease after feeding of raw pig meat. Although the different isolates could be differentiated by various biological markers like growth properties, fusion activity and plaque morphology no significant serological differences existed. The DNA of these isolates and of Pseudorabies virus strains obtained from pigs and cows was analyzed by cleavage with various bacterial restriction enzymes. The strains could be grouped to at least 3 categories. They were different from vaccination strains used for immunization of pigs. Analysis of the DNA appears to be a powerful tool for tracing epizootologies in nature. It will be discussed how far results obtained by molecular "fingerprinting" correlate with the pathogenesis of the disease in different animal species.
IBR - IPV VIRUSES: GENOMESTRUCTURE AND DISEASE

Bovid Herpes Virus 1 infections which include the clinical entities infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) are of major economical interest. Isolates from the respiratory tract, the central nervous system, the conjunctiva, aborted fetuses and tissue culture cells prepared from healthy fetuses as well as from severe infections of the genital tract could not be confidently differentiated by serological tests. By restriction endonuclease analysis of the DNA from such isolates the viruses could be grouped into "IBR-like" and "IPV-like" strains. It was of interest that virus isolates from healthy fetuses which had an "IBR-like" genomestructure gave rise to abortion in pregnant heifers, whereas nothing is known about abortogenic activity of "IPV-like" viruses included vaccination strains.

GENOME STRUCTURE OF TUPAIA HERPESVIRUSES.

Previously Tupaia (tree shrew) herpesviruses (THV-1, 2, 3, 4) have been described. THV-1 to 4 were efficiently propagated, plaque-purified and cloned on tupaia embryonic fibroblasts. The analysis of THV-1 to 4 DNAs by the restriction endonucleases Kpn I, Hpa I, Cla I, Hind III, Bgl II, and Eco RI shows characteristic fragment patterns of DNA bands. Minor bands were not detectable. There are many similarities and dissimilarities in size and in the restriction enzyme cleavage patterns between the four Tupaia herpesviruses. Molecular weights of the DNA fragments of THV-1 to 4, produced by digestion with restriction enzymes Hind III and Eco RI was determined. The molecular weight of THV DNA was also determined by electron microscopic measurement of the contour length of the DNA. The measurement of 38 molecules of THV DNA resulted in a molecular weight of 129 to 133 x 10^6 Dalton. Single-stranded DNAs of THV-2 were prepared for electron microscopy by heating the viral DNA in formamide and isolating the single-strands from agarose gels as described previously. It was found that single-stranded THV DNA does not display any extended stem-loop structures. This conclusion was based on examination of single-stranded THV DNA under conditions favoring intra-strand hybridization. Self-annealed single strands of THV DNA did not form stem-loop structures. With respect to the structure of their genomes, the tree shrew herpesviruses are unique when compared to the DNAs of other known herpesviruses from different species. This result rules out the existence of isomeric forms of THV DNAs, but we cannot exclude very short repeated nucleotide sequences on THV genome at this stage of investigation.
The pathogenicity of THV-1 to 3 in Tupaia as its indigenous host was studied. Several juvenile Tupaias were inoculated intravenously and intraperitoneally with THV-1 to 3. Intravenous inoculation of these viruses led to death of the infected animals. In contrast, the majority of intraperitoneally inoculated animals survived the infection. The animals were dissected after death or after sacrifice when moribund. The major observation was inflammatory hemorrhagic necrosis of lungs. The tissues and whole blood of these animals were titrated for determination of virus titre in different organs. The titrations were performed using Tupaia embryonic fibroblasts as described previously for plaque assay of THV-2. High titres of infectious virus were recovered from lung, spleen, and liver. Two of those animals which survived the acute infection were splenectomized 24 months after administration of THV-1 and 2 for demonstration of the latent state of viruses in lymphoblastoid tissues of spleen. The spleen of these Tupaias was transferred to tissue culture and the infectious viruses were recovered from spontaneously degenerated culture at the first passaging in vitro. The DNA of these recovered viruses was extracted and cleaved by different restriction enzymes and the resulting DNA fragments were subsequently separated by agarose gel electrophoresis and compared to the corresponding DNA fragment patterns obtained from the viral DNAs of the inoculated virus.

References: (1) Scholtissek, C.: Curr. Top. Microbiol. Immunol. 70, 101-119 (1975). (2) Radsak, K., et al.: Arch. of Virol. 65, 45-54 (1980)
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A COMPARISON OF INTRA- AND EXTRACELLULAR VACCINIA IHD VIRUS

Most of the biological and biochemical experiments on vaccinia have been done using virus preparations prepared from cell-associated or intracellular virus pools. When infected with vaccinia strain IHD rabbit kidney cells (RK 13) produce as much as 25% of the total virus progeny in an extracellular form, released into the medium (1). This offers the possibility to compare both intra- and extracellular virus isolated from the same culture.

Paralleled by a lower density (1.236 versus 1.271 g/cm³) the extracellular virus shows approximately twice the phospholipid content of the intracellular variant. The percentage of phosphatidylethanolamine and phosphatidylserine is significantly increased whereas the content of a previously unidentified lipid component is decreased. This component has now been identified as acyl bis (monoacylglycerol) phosphate. In addition the two virus forms show differences in the pattern of their proteins accessible to lactoperoxidase catalyzed iodination. Specific for the extracellular vaccinia type is an unlabelled 37K-protein representing 5-7% of the total structural proteins. When the production of the extracellular vaccinia variant is inhibited by isonicotinoyl-methyl-chlorbenzoylhydrazin treatment (2) no particle-associated 37K can be found.

(1) Payne, L.G., J. Virol. 31, 147-155 (1979)
(2) Kato, N., Eggers, H.J., and Rolly, H., J. Exp. Med. 129, 795-808 (1969)

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CONDITIONS FOR IHD VIRUS SPECIFIC MICROVILLI FORMATION DURING SYNCHRONIZED VIRUS ASSEMBLY

Formation of pox virus specific microvilli late in the infectious cycle is inhibited by rifampicin or hydroxyurea treatment. Under conditions of synchronized maturation following removal of rifampicin or hydroxyurea microvilli appear on the cell surface concomitantly with the formation of mature virus particles in the cell. The inhibition of microvilli formation in the rifampicin treated cell is due to the antivaccinia effect of the drug since a rifampicin resistant vaccinia mutant is able to induce microvilli formation even in the presence of the inhibitor. If, however mature virus particle formation is prevented by addition of sodium fluoride, cycloheximid, actinomycin D, or isatin-9-thiosemicarbazone after reversal of the rifampicin block no microvilli can be detected. Two temperature sensitive pox virus mutants with different defects in virus maturation are able to induce microvilli formation even at the permissive but not at the restrictive temperature. It is concluded that induction of microvilli on the surface of the infected cell can only occur if mature virus particles are formed. Alterations of the cell surface after infection like early antigen, hemagglutinin and cell fusing activity are not required for microvilli induction.
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RELATIONSHIP BETWEEN MINK ENTERITIS VIRUS (MEV) AND CANINE PARVOVIRUS (CPV): COMPARISON OF THE RESTRICTION SITE MAPS OF THE REPLICATIVE FORM DNA.

Since 1978 outbreaks of an apparently new contagious enteric disease in dogs were observed almost simultaneously throughout the world. The causative agent was found to be a parvovirus (referred to as canine parvovirus, CPV) which biologically proved closely related to feline panleucopenia virus (FPV). Whether or not CPV can be distinguished from FPV and MEV (mink enteritis virus, probably a strain of FPV) by serological means is still in dispute.

Therefore we decided to compare the genomes of CPV and MEV by restriction enzyme analysis of their replicative form (RF) DNAs. Out of 79 mapped sites 68, or 86%, were found to be common for both types of DNAs indicating that CPV and MEV are closely related viruses. Whether they evolved from a common precursor or whether CPV is derived from MEV, however, can not be deduced from our present data.

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TRANSFEKTION MIT PARVOVIRUS LU III DNA

The possibility of infection of NBK-cell-cultures with the DNA of parvovirus Lu III was investigated. The well introduced calcium-phosphate-DNA-co-complexing method of GRAHAM et al. (1973) was used for this system. With this method the DNA of parvovirus Lu III turned out to be infectious. The infectivity of the DNA preparations was destroyed by DNase I but was resistant against RNase. By direct electron microscopy and immunoelectron-microscopy it could be shown that the particles emerging after transfection were identical with Lu III virus particles used for DNA-preparation.

Ref.: Graham, F.L., van der Eb, A.J.: A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 52, 456-467 (1973)
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PROPAGATION OF HEPATITIS A-VIRUS (HAV) IN FRHK-4/R CELLS

Recently we reported the propagation of HAV strains GBG, GBM and GJA in a fetal rhesus kidney cell line (Frhk-4) (Flehmig, B., Med. Microbiol. Immunol., 168, 239-248, 1980). The HAV is demonstrated in cell culture medium by immune electron microscopy (IEM) and radio immunoassay (RIA). In the course of seven serial passages in Frhk-4 cells the HAV was adapted to the cell culture. In the 1st passage HAV was first detectable in the supernatant of infected cell culture 8-10 weeks after infection by RIA and in the 7th passage the first supernatant positive for HAV was detected two weeks after infection. With indirect immuno fluorescence 3 days after infection a specific granular fluorescence in the cytoplasma in cells infected with HAV from the 7th passage is shown. A persistent infection of Frhk-4 cells with HAV is shown over a period of 6 months. HAV produced in Frhk-4 cells appeared at a density of 1.29-1.34 g/cm$^3$. In the 1.32g/cm$^3$ fraction typical HAV-particles are shown by electron microscopy.

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KINETICS OF THE REPLICATION OF HEPATITIS A VIRUS IN CULTURES OF HUMAN HEPATOMA CELLS.

Hepatitis A virus (HAV) was propagated serially in monolayer cultures of an HBsAg producing hepatoma cell line. Already after four successive passages synthesis of hepatitis A antigen (HAAg) could be detected by immunofluorescent staining in the cytoplasma of individual infected cells as early as 24 hours after infection. The virus remained strictly cell associated even after several weeks of incubation and no cytopathologic change were ever observed. Accumulation of HAAg in the infected cultures as revealed by a quantitative radioimmunoassay therefore could be ascribed 1) to the continuous synthesis of antigen in infected cells and 2) to the continuous spread of infection within the monolayer by direct cell to cell contact. The latter phenomenon leads to the formation of foci of HAAg-positive cells which readily can be detected by immunofluorescent staining after five to ten days after infection. We have used this phenomenon to establish a rapid and sensitive system for the quantification of HAV infectivity.
FURTHER CHARACTERIZATION OF HEPATITIS A VIRUS

Hepatitis A virus (HAV) from stools of hepatitis A patients was shown previously to grow in the human hepatocellular carcinoma cell line PLC/PRF/5; subsequently HAV has been grown also in another human hepatocellular carcinoma cell line (Hep 3 B 2.17) and in human embryo fibroblasts.

The temperature stability of HAV was studied in the PLC/PRF/5 line: at 85°C HAV is inactivated within 1 min., whereas at 56°C the titre is reduced only by 2 log 10 in 6 hours. At 32°C and 37°C the titre decreased slowly, and infectivity is still detectable after 2 and 1 week respectively. At room temperature the titre remains stable for one week and afterwards is reduced by 2 log 10 until the 4th week, whereas no virus infectivity is detectable after 8, 12 or 15 weeks of storage.

When irradiated with 60Co (doses of 150, 300, 600 and 1200 krad) a titre reduction of only 2,25 log 10 is observed with the highest exposure.

No interference with poliovirus type 3, ECHO virus type 30, coxsackie A 9 and B 3 viruses was detected, nor was haemadsorption or haemagglutination observed with erythrocytes from guinea pig, rat, mouse, rabbit, chicken, pigeon, marmoset and man (blood group O) at 4°C, 23°C or 37°C.

CLONING OF THE HEPATITIS A VIRUS GENOME

Hepatitis A virus (HAV) particles have been isolated from stool of a patient. RNA was purified from the particles and cDNA prepared by the AMV reverse transcriptase. The cDNA has been cloned into the Pst 1 site of plasmid pBR 322 and transformed into E. coli X 1776.

Insertions in the plasmids have a size of 300-800 nucleotides, most of them hybridize to HAV specific RNA from a hepatoma cell line. Water lysates of two of the clones were found to be positive in a RIA for HAV antigens.
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HEPATITIS B VIRUS-SPECIFIC SEQUENCES IN CHROMOSOMAL DNA OF HUMAN LIVER CARCINOMA AND CIRRHOSIS

There is considerable evidence correlating the elevated incidence of primary liver carcinomas in humans and the high prevalence of hepatitis B virus in countries where the virus is hyperendemic. The serum of those patients contains viral antigenic markers at a much higher frequency than the serum of normal controls.

We have examined DNA from liver tissue obtained from several cases of liver disease, by means of hybridization (Southern, E.M. 1975). Hepatitis B virus specific sequences have been detected integrated in the genomic DNA of 3 out of 7 carcinomas and in 2 out of 5 cases of cirrhosis. Sequences were also found in the uninvolved areas of a liver containing an angiosarcoma. These observations further strengthen the suggestion that hepatitis B virus may have a role in some carcinomas of the liver. The detection of sequences in the cirrhotic livers is of interest when it is viewed in the context of the fact that cirrhosis often progresses to carcinoma of the liver.

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Heterogeneity of HBV-DNA Restriction Enzyme Pattern as a Problem of Cloning

Restriction enzyme patterns of HBV-DNA prepared from the plasma of 14 healthy carriers were examined. Nine of them were of subtype ad and 5 of subtype ay. The HBV-DNA was labelled with 125I-dCTP by the endogeneous polymerase. DNA was digested with the restriction endonucleases Eco R1, Bam H1 and Xba I. Restriction enzyme patterns differed between and within serological subtypes.

Three of 5 specimen of subtype ay had no Eco R1 site and more than one Bam H1 site. Therefore two of them had to be cloned with Bam H1 as two fragments of 1.7KB and 1.5KB. The lack of an Eco R1 site was surprising as all published experiments used this enzyme for cloning the entire HBV-genome.

Of 9 specimen of subtype ad 9 had a single cut with Eco R1 8 with Xba I and 1 with Bam H1. One was cloned with Eco R1 and another with Bam H1 in pBR 322. Restriction maps were prepared and the beginning of the S-gene from one clone was sequenced.
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COVALENT DNA-PROTEIN-COMPLEX OF HEPATITIS B VIRUS

Hepatitis B Virus DNA contains after extraction from virions with SDS a tightly bound protein which is removed only by protease digestion. Using restriction analysis and partial denaturation studies, the protein binding site was located at the 5' end of the complete DNA-strand. The binding is presumably covalent, because it was resistant against 0.1 N NaOH or heating to 90°C. The 5' ends of bulk DNA-Strands do not contain free hydroxyl groups after treatment with phosphatase. This finding is consistent with the linkage of proteins via a phosphodiester group to the 5' ends. However, the incomplete strand of the HBV-DNA did not contain detectable amounts of tightly bound protein. The function and the origin of the 5' bound protein is unknown. It may serve as a co-factor in the synthesis of the complete strand and/or as a sequence- and strand-specific endonuclease in the generation of genome-length DNA from replicative intermediate.

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IMMUNE ELECTRON MICROSCOPY OF PARTICLES ANTIGENICALLY RELATED TO HUMAN NANB-HEPATITIS IN THE SERUM OF A CHIMPANZEE INFECTED WITH A HUMAN NANB-AGENT

Four chimpanzees were inoculated with plasma taken at the acute stage from patients who were infected during an outbreak of hepatitis NANB. The agent was transmissible from the infected chimpanzees to two further chimpanzees. All infected animals developed acute hepatitis and typical ultrastructural alterations in the cytoplasm of hepatocytes (Pfeifer et al. Virchows Arch. B.Cell.Path. 33, 233-243, 1980). Immunoglobulin from HNANB convalescents, who were involved in the outbreak, agglutinated particles which were isolated from one chimpanzee serum only at the acute stage, but not before infection. This spherical particles were mostly pleomorphic with an average diameter of 33 nm, but some larger particles with a 28 nm core and an envelope of 20 nm thickness were also found in the immune complexes. Another chimpanzee did not develop particles, but antibodies half a year after the acute stage. Agglutinating antibodies were also found in ten of twenty sporadic cases of HNANB. They appeared three to twelve months after the acute phase, but not at the onset of the disease.
DIFFERENT STRATEGIES IN THE REPLICATION OF TOGAVIRUSES

Both, alphaviruses and flaviviruses contain a single molecule of single-stranded 42S infectious RNA of a length of about 12,000 nucleotides as genetic material. The genome RNA of the flavivirus West Nile (WN) virus has been isolated and labeled at its 3'-terminus in vitro using RNA ligase and 5'-[^32]pCp. Sequence analyses of this end-labeled RNA have shown that it contains the 3'-terminal sequence Ap9p9pApUpCpUoH. The replicative form, a double-stranded RNA molecule containing 42S RNA of both positive and negative polarity, has been isolated from WN virus-infected BHK cells. The termini of this RNA species also have been labeled in vitro and have been subjected to sequence analysis. The results of these analyses show that the two 42S RNA molecules present in this complex are strictly complementary to each other and that the positive-stranded molecule does not contain a 5'-terminal cap structure which is present in the mature 42S plus-strand genome RNA. These results are compared to the corresponding data concerning alphavirus-specific nucleic acids.

STRUCTURE OF MYXOVIRUS-SPECIFIC CORE PROTEINS

The core protein of alphaviruses is thought to interact with both the viral RNA and the glycoproteins embedded in the virus envelope.

The amino acid sequence of the core proteins of Sindbis and Semliki Forest virus was determined. Comparison of the sequences obtained showed that the core protein of alphaviruses has a polar structure. In the N terminal part most of the basic amino acids are located and clustered in histone-like sequences. In the C-terminal half of the protein the sequences are highly conserved with about 70% identity of primary structure between the two viruses investigated. The potential significance of the polar structure of the core protein for the architecture of the virus particle is discussed.
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THE VIRAL CORE INFLUENCES PROPERTIES OF THE SEMLIKI FOREST VIRAL MEMBRANE.

The membrane of Semliki Forest virus contains three glycoproteins. At least one of them, E2 spans the lipid bilayer making contact to the viral core. This interaction is thought to be the driving force of virus budding. Evidence is presented indicating that some properties of the virus surface can be influenced by the viral core.

The virus particle is devoid of SH-groups essential for infectivity, since a number of SH-reagents employed under the usual conditions, were without effect on infectivity. However, 0.1 M-iodoacetamide and vinylpyridin destroyed infectivity very effectively. It could be shown that iodoacetamide is bound specifically only to the core protein. As a result of this carboxy methylation, alterations in the viral core can be postulated, which are followed by changes in membrane properties such as adsorption of virus to susceptible cells and pH for optimal hemagglutination.

A similar inactivation of viral infectivity is caused by weak acid solutions; under these conditions, the viral core has been reported to shrink; and pH 5.8 - 6.0 is necessary for optimal hemagglutinating activity.

Our working hypothesis assumes a common reason for the observed effects: due to the existence of a protein bridge across the lipid bilayer of the viral membrane, a structural alteration of the viral core causes a structural change of the envelope glycoprotein(s), which in turn modify the properties of the membrane.

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SEROLOGICAL DIAGNOSIS OF ACUTE TICK BORNE ENCEPHALITIS BY DEMONSTRATION OF ANTIBOIES OF THE IgM CLASS

A sensitive enzymimmunoassay is described for the demonstration of specific antibodies to Tick Borne Encephalitis Virus of the IgM class (anti-TBEV IgM).

Anti-μ coated flat bottomed microtiter plates are incubated with dilution of patient serum (2 hours at 37°C) and later with purified TBEV. In the next step peroxidase coupled anti-TBEV immunoglobulin is incubated for another 2 hours. After washing of the plates, orthophenyldiamine is added and the optical density measured at 510 nm. At early stage after onset of illness anti-TBEV IgM could be demonstrated up to dilutions of 10⁻². The specificity of the test system was proven by preincubation of the patient sera with anti-μ which inhibited totally the positive reaction in the assay.

By the described method Tick Borne Encephalitis (TBE) could be serologically diagnosed in 80 sera of 54 patients. Anti-TBEV IgM could be demonstrated in sera drawn up to nine months after onset of illness.

The evaluation of epidemiological data revealed a peak of the number of infections in the age group of the 31-40 year olds. The seasonal distribution revealed that most of the infections occur from June to October with a small peak in July.
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IMMUNOLOGIC AND BIOCHEMICAL COMPARISON OF TICK-BORNE ENCEPHALITIS (TBE) VIRUS ISOLATED IN DIFFERENT EUROPEAN COUNTRIES.

TBE virus strains isolated either from ticks or human material in Finland, Germany, Czechoslovakia, Switzerland and Austria were compared by competitive radioimmunoassays and peptide mapping of the isolated structural glycoprotein by limited proteolysis followed by SDS-polyacrylamide gel electrophoresis. Closely related viruses as louping ill virus and the far eastern subtype of TBE virus as well as more distantly related flaviviruses - West Nile virus, Murray Valley encephalitis virus and Locco virus - were included into these studies. Competitive immunoassays employing whole purified virions did not reveal any immunological differences between all the European TBE virus isolates tested, whereas the far eastern subtype and louping ill virus could be differentiated showing a slightly weaker competition. The peptide maps obtained for the isolated glycoprotein by means of limited digestion with proteases of different cleavage specificities revealed a remarkably homogeneous overall peptide pattern for the European isolates tested, the maps of the eastern subtypes and louping ill virus being similar but no identical. Completely unrelated patterns were obtained for West Nile, Murray Valley encephalitis and Rocio virus.

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DETECTION OF RUBELLA SPECIFIC IgM ANTIBODIES: COMPARISON OF DIFFERENT METHODS.

The specific IgM antibody titre in sera of patients with clinical symptoms of rubella infection was determined. Therefore the IgM fraction was isolated by the following different methods and titres were compared to each other.

1) Isolation of serum IgM by immune adsorption to heavy chain specific anti-human IgM antibodies covalently coupled to polyacrylamide microimmuno-beads.
2) Exchange chromatography by DEAE-cellulose columns.
3) Isolation of IgM by ACA-34 gelchromatography
4) Rubella-HIT by use of the SPT-technique
   a) according to Krech and Wilhelm (1979)
   b) according to the Latex-adsorption method (Doerr, 1979)
5) Alternatively we eliminated IgG and IgA by use of protein A, anti-IgG and anti-IgM, covalently bound to CPG (Geisen et al., 1979)
6) Determination of antibody titres in the 19 S fraction (control).

The titres derived by the different methods showed qualitatively good correlations. The quantitative measurement of IgM titres, however, revealed a lower sensitivity of the column chromatographic methods.
The murine coronavirus JHM contains six major proteins, four of which are glycosylated. These are gp180, gp98, gp65, pp60 (a phosphoprotein), gp25 and p23. In infected cells host cell protein synthesis is shut off and four viral polypeptides are synthesized. These polypeptides, are ip150, ip65, ip60 and ip23. Pulse chase experiments indicate that ip150 and ip23 give rise to the glycoproteins gp180/98 and gp25 respectively. The mRNAs encoding the intracellular viral polypeptides have been isolated, translated in vitro, and the products identified by electrophoresis, immunoprecipitation and tryptic peptide mapping. The mRNAs have also been sized in sucrose formamide gradients and agarose gels after glyoxal treatment. A 17S (0.6 x 10^6) mRNA encodes ip60, a 19S (0.93 x 10^6) mRNA encodes ip23 and a 27S (2.76 x 10^6) mRNA encodes a 120K polypeptide, the non-glycosylated form of ip150. These results and the arrangement of the subgenomic mRNAs showed that coronaviruses display a novel strategy of replication in which the synthesis of different virion proteins is directed by separate subgenomic mRNAs. This strategy allows for not only the control of viral protein synthesis but also accommodates, if necessary, different pathways of post-translational modification for each viral protein.

Murine coronavirus A59 and bovine coronavirus L9 were metabolically labeled with different radioactive markers and their glycoproteins E1 and E2 were isolated.

E1 could be labeled with 2H-mannose, 1H-fucose, 1H-galactose, 6H-glucosamine, and H-palmitic acid. Its oligosaccharides resemble in their sugar composition the commonly known N-glycosidically linked complex type side chains. E2, on the other hand, could be labelled only with galactose and glucosamine. It does not contain fatty acids, and sugar constituent analyses confirmed the absence of mannose and fucose. In contrast to E2, glycosylation of E1 is not inhibited by tunicamycin and 2-deoxy-arabino-D-xosose and 95% of the carbohydrate label could be removed from E1 under reductive alkaline conditions.

These findings support the concept of O-glycosidic carbohydrate protein linkages in E1 which have not previously been described for viral glycoproteins.
CHARACTERIZATION OF INTRACELLULAR VIRAL RNA IN JHM-VIRUS INFECTED CELLS.

The murine coronavirus JHM is interesting as an experimental animal model for virus persistency and demyelinating central nervous system diseases. As a basis for studies on the replication strategy of coronaviruses we characterized the JHM-virus specific intracellular RNA.

After infection of Sac(-) cells with the murine coronavirus JHM the synthesis of seven major and two minor RNA species is induced. These RNAs are polyadenylated and single stranded. Their mol. wts. were estimated by electrophoresis in agarose gels containing methylmercury hydroxide. The values for the major species are $6.67 \times 10^6$ for RNA of genome size, $3.42 \times 10^6$ for RNA 2, $2.76 \times 10^6$ for RNA 3, $1.35 \times 10^6$ for RNA 4, $1.19 \times 10^6$ for RNA 5, $0.93 \times 10^6$ for RNA 6 and $0.62 \times 10^6$ for RNA 7. The minor species have a size of $4.7 \times 10^6$ (RNA a) and $1.5 \times 10^6$ (RNA b). No gross difference in number and amount of each RNA species was found between total cytoplasmic RNA, polyadenylated cytoplasmic RNA and RNA extracted from pelletted polysomes. The pattern of RNA extracted early or late in infection remains unchanged.

Several of these RNAs were identified by translation in vitro as physiological mRNA coding for viral proteins.

These data support the concept that the coronavirus proteins are derived from a set of independent subgenomic mRNAs.

CORONAVIRUS JHM-INFECTION IN RATS - A MODEL OF SUBACUTE DEMYELINATING DISEASES OF THE CENTRAL NERVOUS SYSTEM.

Chronic central nervous system diseases of man can be induced by a virus infection. A typical neuropathological finding in such disorders are demyelinated lesions in selected areas of the CNS. Experimental studies in this field are greatly faciliated by laboratory animal infections leading to similar disease pictures.

Infection of rats by the intracerebral route with JHM virus can lead to different CNS diseases. Besides an acute encephalomyelitis we find a subacute demyelinating encephalomyelitis occurring after prolonged incubation times of several weeks to months. Infectious virus can be recovered from diseased rats. Plaques of primary demyelination are also found in clinically silent animals. Uncloned and cloned virus including selected temperature sensitive mutants have very different degrees of neurovirulence and capacity to induce demyelination. Besides the virus clones used for inoculation, host factors like age, genetic background and the immune status influence the development of diseases. Conspicuously high rates of demyelination are obtained by injection of mutants in rats preimmunized by maternal antibodies against JHM virus. This model offers many experimental possibilities to study the interaction of virus and host in the pathogenesis of subacute demyelinating diseases.
Rapid Localization of $^{3}$H-Labeled VSV-Proteins in SDS Polyacrylamide Gels

The distribution pattern of $^{3}$H-labeled VSV-proteins in slab gels has been recorded with three instruments: a thin-layer scanner, a Betacamera and a Linear Analyzer, all of which have previously been used with success in monitoring paper chromatograms and thin-layer chromatograms. Individual radioactive protein bands may be localized with all three instruments although they differ markedly in sensitivity. The scanner is relatively insensitive when compared to the Betacamera and the Linear Analyzer. Due to the large size of the spark chamber (20 x 20 cm) the whole area of a slab gel can be monitored simultaneously with the Betacamera. The Linear Analyzer has two main advantages:

1) A single gel lane can be monitored in full length,
2) the disintegrations are collected and integrated, thus giving a quantitative distribution pattern of the different protein bands in that lane.

The "betagram" technique has a very low detection limit and is thus less time-consuming than fluorography. However, the resolution of the "betagram" does not reach the quality attained with fluorography.

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Does VSV Induce Two Glycoproteins in HeLa Cells?

Particles of VSV contain 5 proteins which are called L, G, N, NS and M. These polypeptides are coded by the viral genome which becomes expressed in HeLa cells upon infection. Close inspection of polyacrylamide gels, which were loaded with $^{35}$S-met labeled extracts of VSV infected cells, reveal a 6th band, migrating slightly faster than the G-protein. The 6th polypeptide, $G_{M}$, is associated with membrane vesicles in a manner that it becomes protected from proteolytic cleavage. $G_{M}$ incorporates $^{3}$H mannose, $^{3}$H glucosamine and $^{3}$H galactose like the G-protein.

Tryptic glycopeptides of both proteins show an almost identical elution pattern when chromatographed on DEAE-Sephadex A25 columns. Limited digestion of $^{35}$S-met labeled $G_{M}$- and G-protein with an arginine specific protease produces an oligopeptide pattern with minor differences indicating a sequence fragmentation of $G_{M}$. This interpretation was corroborated by tryptic fingerprints of $^{35}$S-met labeled G- and $G_{M}$-protein.

Preliminary data suggest that $G_{M}$ is lacking a part of the C-terminal region which seems to be essential for the incorporation of the glycoprotein into the budding virus particle.

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**ISOLATION AND CHARACTERIZATION OF THE RNP’S OF TWO FISH-PATHOGENIC RHABDOVIRUSES (VHS-V and RVC)**

VHS-virus (viral haemorrhagic septicaemia of rainbow trout) and RVC (Rhabdovirus carpio; spring viremia of carp) were propagated in RTG-cells and in FHM-cells, respectively. The viruses were treated with 2% Triton X-100. RNP and membrane fragments were separated by addition of 2M ammonium sulphate. In the RNP-fraction of VHS-V we found 3 polypeptides: L, N, M1 with molecular weights of 110 kd, 44 kd and 22-25 kd. The membrane-fraction contained two proteins: G and M2 with molecular weights of 63 kd and 17-19 kd. The RNP of both viruses banded at a density of 1.22 - 1.26 g/cm³ in sucrose gradients and at a density of 1.29 - 1.32 g/cm³ in CsCl gradients. The relative low density of the viral RNP may be explained by detergent molecules bound to hydrophobic regions of the RNP.

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**STRUCTURAL BASIS AND MECHANISM OF PROTEOLYTIC CLEAVAGE OF INFLUENZA VIRUS HEMAGGLUTININ.**

Infectivity and pathogenicity of avian influenza viruses have been shown to be directly correlated to the proteolytic cleavability of the hemagglutinin (HA) glycoprotein in the infected cell (Bosch et al., Virology 95, 197-207 (1979). Of 9 antigenically identical HA’s of H avi virus strains only 5 are be cleaved in all cell types tested, the others, like most influenza virus hemagglutinins, have a very limited host range with regard to proteolytic cleavability. We have analyzed the structural differences of these hemagglutinins.

The results obtained allow us to draw the following conclusions: 1. The amino acid sequence at the cleavage site determines the proteolytic cleavability of influenza virus hemagglutinin. 2. The activation process itself consists of two reactions: First a trypsin-like enzyme of cellular origin opens the peptide bond between arginin-glycin. This cleavage reaction activates a second proteolytic activity which removes basic amino acid(s) from the C-terminus of HA. This second activity appears to be virus-associated since it is also activated after in vitro cleavage of HA with trypsin both on the surface of infected cells as well as on purified virus particles.

When the carbohydrate side chains of HA are modified by inhibitors of glycosylation (deoxyglucose, tunicamycin), the cellular enzyme still cleaves HA but the second activity can be blocked indicating the possible glycoprotein nature of the virus associated activity.
When the hemagglutinin of influenza A virus is activated by proteolytic cleavage a connecting piece located between cleavage fragments HA₁ and HA₂ is eliminated. With fowl plague virus the connecting piece is a basic peptide containing several arginine and lysine residues, whereas with all other strains analyzed it is a single arginine. Structure and Variation in Influenza Virus. W. G. Laver and G. Air, Eds., Academic Press, 1980). The available evidence indicates that elimination occurs by the sequential action of a trypsin-like protease that cleaves an arginine-glycine linkage, followed by a carboxypeptidase B, that removes the basic amino acid(s) from the newly created C-terminus. The basic linker is removed not only by in vivo cleavage in infected cells, but also after in vitro cleavage with trypsin as the only enzyme added to purified virus. Thus, it appears that the virus contains carboxypeptidase A activity. Removal of the basic linker results in a charge shift of the hemagglutinin towards a more acidic isoelectric point. A similar shift is also observed when glycoprotein F of Newcastle disease virus is cleaved. Thus, there is evidence that the influenza hemagglutinin and the paramyxovirus F protein are activated by the same mechanism involving removal of a basic linker by the sequential action of two proteolytic enzymes.

The oligosaccharides linked N-glycosidically to proteins arise from an oligosaccharide (GlcMan₉GlcNAc₂) pre-assembled on a carrier-lipid, dolichol diphosphate (Do1-PP). The sugar analog fluorogluucose (FGlc) inhibits multiplication of enveloped viruses because it strongly inhibits glycosylation of the envelope glycoproteins (1). FGlc affects the assembly of the dolichol-linked oligosaccharide because an essential intermediate, Dol-P-Man, becomes limiting (2). However, the glycosylation of influenza virus-hemagglutinin is not completely inhibited. Residual glycosylation occurs via a dolichol-linked intermediate with only 5 Man residues (3). In cells deprived of ATP, the pool of Dol-P-Man is also decreased, and glycosylation occurs, in part, via the alternate pathway. For as yet unknown reasons, processing of the hemagglutinin in the presence of FGlc, or in cells deprived of ATP, is impaired.

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3. R. Datema, R. T. Schwarz, and J. Winkler, (1980) Eur. J. Biochem. 110, 355-361.
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THE GLYCOSYLATION SITES OF THE INFLUENZA A HEMAGGLUTININ

Glycosylation of the influenza hemagglutinin is initiated at the rough endoplasmic reticulum by the en bloc transfer of oligosaccharide residues containing mannose and N-acetylglucosamine from a polyisoprenol derivative to the nascent polypeptide chain. After a trimming process, fucose and galactose are attached presumably in the Golgi apparatus. The mature hemagglutinin contains 2 major types of N-glycosidically linked oligosaccharide side chains, the complex type I and the mannose-rich type II. 6 of the 7 potential glycosylation sites of the fowl plague virus hemagglutinin have carbohydrate side chains. On HA1 -Asn(12)-Gly-Thr-Asn(28)-Ala-Thr; -Asn(123)-Gly-Thr and -Asn(149)-Ala-Ser have type I side chains. -Asn(231)-Asp-Thr- is not glycosylated. Fragment HA2 contains a carbohydrate chain of type I at position: -Asn(42)-Trp-Thr- and a type II chain at -Asn(496)-Asn-Thr. The carbohydrate chain of -Asn(123)- is sulfated. Comparison of different influenza A hemagglutinins indicates that the glycosylation patterns are strain dependent supporting the notion that the primary structure of the polypeptide is an important determinant for the carbohydrate moiety of the glycoprotein.

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SPECIFIC COVALENT MODIFICATION OF VIRAL MEMBRANE PROTEINS THROUGH FATTY ACIDS. DISTRIBUTION AND STRUCTURE

Selective covalent binding of fatty acids to the glycoproteins of several enveloped viruses has been detected. All enveloped virus species tested so far afford this property: Sindbis virus, Semliki Forest virus, Influenza A viruses, Newcastle disease virus, Sendai virus, Vesicular stomatitis virus, Friend leukemia virus, murine coronavirus, and West Nile virus. Not all viral glycoprotein-species of the various enveloped viruses contain fatty acids. In influenza virus only the MA2-subunit of the hemagglutinin shows fatty acid binding; but no acyl chains could be detected in the HA1-subunit or in the neuraminidase. Likewise fatty acid was found to be bound to the F-protein of Newcastle disease virus but only trace amounts (if any) could be detected in the HA-glycoprotein.

Using partial digestion of 3H-palmitate labeled virus particles with subsequent analysis of "naked" particles and the released distal spike fragments the linkage-region for the fatty acids could be located to the carboxy-terminal region of the respective glycoprotein.

To establish the structure of the linkage between fatty acid and the polypeptide-backbone of the spike glycoprotein 3H-palmitate labeled HA2 or E1 plus E2 (from Semliki Forest virus) were digested proteolytically. Labeled hydrophobic peptides were then separated and their amino acid composition determined. Regardless of their origin all 3H-palmitate containing peptides contained serine whereas other amino acids varied considerably. This supports our hypothesis that fatty acids of viral glycoproteins are linked to the hydroxyl group of serine.
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REGULATION OF INFLUENZA VIRUS PROTEIN SYNTHESIS

The genome of the influenza viruses consists of 8 segments of RNA which contain the genetic information for 9 viral polypeptides. The synthesis of these polypeptides is controlled with respect to the amount synthesized and the time of synthesis. Studies on RNA extracted from infected cells at various times post-infection have shown that the mRNA for each protein is present throughout infection at a relative abundance which reflects the abundance of the polypeptide for which it codes. Thus control is chiefly at the level of transcription although translational control may also occur.

An approach to the study of these control processes is the use of conditional-lethal mutants. We have investigated protein synthesis in cells infected with ts 236, a mutant of fowl plague virus with a defect in the P3 polypeptide, a constituent of the polymerase complex. Synthesis of the HA polypeptide could not be detected in cells infected at the restrictive temperature. However, using RNA extracted from cells infected at the restrictive temperature, the unglycosylated precursor to HA was synthesized in vitro. Down-shift experiments in which mRNA synthesis at the permissive temperature was blocked with actinomycin D confirmed that the HA mRNA was produced at the restrictive temperature. These results suggest that at the restrictive temperature the mRNA for HA is produced but that its translation is restricted. Since ts 236 contains a lesion in the gene coding for the P3 polypeptide, P3 may have a role in controlling the translation of the HA polypeptide.

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ON THE HEMOLYTIC AND CELL FUSION ACTIVITIES OF INFLUENZA- AND PARAMYXOVIRUSES

Influenza viruses have been found to cause extensive hemolysis and fusion of cells. It is shown that an optimal pH exists for each virus strain at which hemolysis and cell fusion occur most strongly and that for these activities proteolytic activation of hemagglutinin is essential. Furthermore, it is demonstrated that the hemolytic activity differs among virus strains and that under optimal conditions influenza viruses hemolyse more efficiently than paramyxoviruses. These results support the view that influenza viruses can fuse membranes. In addition, a practical application of influenza viruses as effective fusogens of cells has been suggested.
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PHYSICO-CHEMICAL AND BIOLOGICAL DIFFERENCES BETWEEN PATHOGENIC AND NONPATHOGENIC INFLUENZA VIRUS RECOMBINANTS

Comparative studies on pathogenic and nonpathogenic influenza virus recombinants obtained in vitro with cleaved hemagglutinin have been carried out in order to find differences in their physico-chemical or biological properties.

It was shown that there is no correlation between the pH- and temperature-sensitivity of the recombinants and their pathogenicity. Furthermore, there is no increase in the formation of defective particles under von Magnus conditions of nonpathogenic recombinants compared to pathogenic ones. But there is an essential difference concerning the replication at 41°C. At this temperature nonpathogenic recombinants produce virus-specific proteins, but very little infectious virus, and the spread of the virus is inhibited when cells are infected with a low MOI. One may assume that the same effect occurs in chickens having a body-temperature of 41°C. The reason for this reduced virus production at 41°C is not yet known, but it does not depend on a higher temperature-lability of the nonpathogenic recombinants.

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INTERSPECIES-TRANSMISSION OF HUMAN AND ANIMAL INFLUENZAVIRUS-STRAINS AND THEIR SIGNIFICANCE IN RELATION TO THE ECOLOGY OF INFLUENZAVIRUSES

Wild and domestic animals play an important role in the ecology of influenza viruses. Particularly birds and swine are thought to represent reservoirs for human influenza viruses. Our own investigations during the last three years demonstrate interspecies-transmissions from birds to man and from swine to man and vice versa. We isolated one H2N2 and one H2Nav2 virus from feral ducks; the hemagglutinins of these react with homologous titer with the human prototype strain A/Singapore/1/57 and shows no cross-reactions with the similar, but drifted strain A/dk/Germany/73. Furthermore, we isolated 6 swine influenza viruses (HswIN1) from feral ducks and demonstrated the infectivity and contagiosity of one of these viruses for swine. From Italian pigs we isolated an H3N2-virus, which is identical with the human Hong Kong/1/68-strain, and high antibody titers were found against this strain and the Victoria/3/75-strain in swine. Six swine influenza viruses were isolated from swine in Belgium, which are identical with the avian swine influenza viruses (HswIN1) isolated from birds. This is the first reported evidence for the crossing of a species-barrier from birds to mammals in nature.
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REACTOGENICITY AND ANTIBODY RESPONSE TO POLYVALENT
AQUEOUS AND Al(OH)₃ ADSORBED TWEEN-ETHER SPLIT PRODUCT
INFLUENZA VACCINE IN PRIMED AND NON-PRIMED ADULTS

A comparative clinical trial with an Al(OH)₃ adsorbed
polyvalent tween-ether split influenza vaccine and a tween
ether split fluid vaccine of equal antigenic content was
performed in young adults. Two vaccinations were given
28 days apart. Local reactions were more frequent in the
Al(OH)₃ group. There was no significant difference in
hemagglutinin inhibition and anti-neuraminidase response
to the two different vaccine types, regardless as to whether
the volunteers were primed or not primed.

Antibody response to A/Brazil/11/78 (H1N1) revealed that
about 50% of the volunteers born after 1955 were primed
but titers were strikingly low.

From these and other studies it can be concluded that
Al(OH)₃ has no substantial adjuvant effect in commercial
influenza split-product vaccine for humans.

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IN VITRO TRANSCRIPTION OF INFLUENZA C VIRUS

An RNA-dependent RNA-polymerase activity of Influenza C
Virus (JHB/I/66) is described in an in vitro assay, con-
taining Triton-solubized virus, the four ribonucleoside-
triphosphates ATP, CTP, GTP and 3H-UTP, the dinucleotide
ApG, Mg²⁺ and Na⁺.

The activity was measured as the quantity of acid-preci-
pitable radioactivity synthesized per time unit. The activity
is proportional to the amount of virus, and depends
on the presence of ApG as a primer. Optima for pH, tempera-
ture and Mg²⁺ concentration were determined to be: 8.3 -
8.5 10 mM and 32 ° C.

Mn²⁺-ions decrease the activity in the presence of Mg²⁺
and ApG.

The rate of synthesis is constant for about one hour and
decreases to zero rapidly thereafter. This effect is
probably due to nonspecific hydrolysis of the substrate
molecules, since subsequent addition of new substrate
reconstitutes the activity.
SEPARATION OF GLYCOPEPTIDES AND OLIGOSACCHARIDES OF THE
GLYCOPROTEINS HN₀ AND F₀ FROM NEWCASTLE DISEASE VIRUS (NDV)
NDV strain 'Ulster' was propagated on MDBK cells in the
presence of [6-3H] GlcN. The labelled glycoproteins were
isolated from purified virus by preparative SDS-PAGE and
digested with pronase.
Fractionation of the glycopeptides thus obtained on ConA-
Sepharose followed by subfractionation on Biogel P-4
resulted only in a poor separation. The total glycopep-
tides were therefore treated with endo H, and the cleavage
products (oligosaccharides and GlcNAc-peptides) were
separated from the endo H-resistant glycopeptides over
Biogel. 20% (48%) of the 3H-GlcN label were thus released
in the case of HN₀ (F₀). The oligosaccharides from endo H-
resistant glycopeptides were set free by hydrazinolysis.
The oligosaccharides released by endo H were fractionated
(and the GlcNAc-peptides separated off) by successive
ConA and Biogel P-4 chromatography. Large glycans with
moderate affinity to ConA (one from HN₀, at least three
from F₀), as well as large (one from HN₀ only) and small
oligosaccharides (at least three from HN₀ and four from
F₀) with high affinity to this lectin were obtained.
Biogel P-4 chromatography of the oligosaccharides from
endo H-resistant glycopeptides revealed at least two
(four) very large oligosaccharides of this type in F₀(HN₀).

DISTRIBUTION OF VIRAL ANTIGENS AND VIRAL STRUCTURES
ON HELA CELLS LYTICALLY AND PERSISTENTLY INFECTED WITH MEASLES
VIRUS (EDMONSTON).
HeLa cells persistently infected with the Edmonston strain of
measles virus show a polar accumulation of virus-specific
surface antigens. In contrast, HeLa cells lytically in-
fected with the same virus strain reveal a homogeneous
distribution of virus-specific surface antigens. These dif-
fferences are highly significant and can be observed inde-
pendent of events of mitosis. Immunofluorescence staining
on persistently infected cells with rabbit anti-measles
serum against the viral polypeptides P and NP and with mo-
noclonal antibodies against HA indicates that the polar
distribution of surface antigens corresponds to a local
accumulation of antigens within the cells. In addition, SEM
studies on persistently infected cells show a polar arrange-
ment of budding virions and nucleocapsid structures, whereas
lytically infected cells reveal a homogeneous distribution
of these structures. These results indicate that budding
processes of virions on persistently infected cells are re-
stricted to distinct areas of the cell surface and that
this property represents a morphological marker of this
type of infection.
BORNA DISEASE: PREFERRED SITES IN THE CNS

Borna Disease virus containing brain suspension was inoculated into the thalamus area of the left brain hemisphere. The rabbits were investigated at different stages of the disease and infectious virus as well as antigen were quantified from defined areas of the CNS. Virus and antigen appeared first in the limbic system concentrating in the pyriform cortex and hippocampus ventralis, followed by a centrifugal spread. This finding will be discussed with respect to known clinical symptoms which suggest that the limbic system might primarily be involved in the manifestation of the disease.

LIGHT MICROSCOPICAL AND IMMUNOHISTOLOGICAL INVESTIGATIONS OF THE CNS OF BORNA DISEASE VIRUS INFECTED RABBITS

Borna Disease (BD) which naturally occurs in horses and sheep can experimentally be transmitted to a variety of animal species including rabbits. This disease affects the CNS and can be grouped to the slow virus infections. Our report concentrates on the characterization of cells in perivascular infiltrates located intraparenchymal and meningeal. Animals were intracerebrally infected as usual. Approx. 2 to 3 weeks post inoculation neurological symptoms could be observed. Animals sacrificed in a prefinal stage show a panencephalomyelitis together with severe meningitis. Besides histologic examinations selected regions of the brain were monitored for the presence of immunoglobulin in B-cells using the PAP- and the direct immunofluorescence technique. Histologically a meningeal reaction is evident. The characteristic periveneous infiltrates were preferentially seen in the limbic system. Furthermore glial nodules, neuronophagia as well as occasional intranuclear inclusion bodies were observed. The immunohistological tests only revealed a positive reaction in a few mononuclear cells. These results show - as assumed from preliminary immunological data - that the perivascular cuffs are mainly composed from T-cells including a few B-cells.
THE CLINICAL EXPRESSION OF BORNAVIRUS INDUCED RETINO-CHOROIDITIS DEPENDS ON THE VASCULAR SUPPLY OF THE RETINA

The vascular supply of the retina differs considerably in various animal species. In rabbits the retina is almost completely avascular and supplied by the choroidal blood flow. In Rhesus monkeys a retinal vascular system supplies the inner layers whereas the outer layers depend on the choroidal flow. In this study Rhesus monkeys and rabbits were experimentally infected with Borna disease virus. In both species retino-choroidal inflammatory lesions were observed but the clinical expression and histo-pathological findings were different. In rabbits lesions appeared in the posterior polar region of the retina. Histopathology revealed destruction of the outer retinal layers and lympho-plasmacellular infiltrates in the choroid but not in the retina. In Rhesus monkeys lesions occurred in the peripheral zones of the retina in early stages of the disease and progressed towards the posterior polar region. Histologically destruction of outer retinal layers was observed and inflammatory infiltrates occurred in the iris, the ciliary body and the choroid as well. In the retina perivascular lympho-plasmacellular infiltrates were observed. In both species infectious virus and specific antigen were found in the outer retinal layers but not in the choroid. The clinical course of the disease obviously depends on the vascularity of the target organ which implies that immuno-pathological events are of pathogenetic importance. In species with avascular retinas immunocompetent cells accumulate in the choroid, no retinal infiltration with lymphocytes is observed here. When the retina is vascularized immunocompetent cells reach their target - the outer retinal layers - via the retinal and the choroidal vasculature.

INFLUENCE OF IMMUNOSUPPRESSIVA ON BORNA DISEASE IN RABBITS

Borna Disease virus infected rabbits were treated with different concentrations of cyclophosphamide, glucocorticoids or both in combination. The comparison with untreated, infected rabbits showed a drastic alteration in the clinical picture, a considerable prolongation of the survival time, and differences in body weights and body temperatures during the course of the disease. The immunosuppressed animals had no or low amounts of antibodies in the serum and cerebrospinal fluid, but they harbored infectious virus and high amounts of specific antigen in the brain. Immunohistologically, differences in location and distribution of antigen as compared to positive untreated animals could not be detected. In the immunosuppressed animals perivascular infiltrates were not observed in the different regions of the brain.
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POLYCLONAL MITOGEN STIMULATION OF SPLEEN LYMPHOCYTES FROM SCRAPIE INFECTED MICE

In mice infected with the Scrapie agent either i.p. or i.c. increasing titers of infectivity are first observed in the lymphoreticular system (1). Possibly this replication alters some properties of the lymphocytes either directly or indirectly. This has been tested for mitogen stimulated 3H-Thymidine incorporation into the DNA of lymphocytes 4-60 days after infection.

In comparison to control mice we observe a 2 to 3-fold increase of 3H-thymidine incorporation into lymphocyte DNA of Scrapie infected mice between 10 and 20 days post infection. Thereafter a 50% reduction is observed. These results are obtained with ConA as well as with LPS or PHA and they are independent of either cell number or mitogen concentration.

Therefore Scrapie infection seems to have induced an alteration of both B- and T-lymphocytes. It is noteworthy that the period of increased incorporation of 3H-thymidine ends when the titer of Scrapie infectivity in the lymphoreticular system has reached its final plateau.

1) Kimberlin, R.H., Sci. Progr. Oxf. 63, 461-481, 1976

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HIERARCHY OF VIRUS-SPECIFIC PROTEINS IN ECHOVIRUS 12, TRAVIS- AND ECHOVIRUS 9, STRAINS BARTY- AND HILL-INFECTED CELLS

Like all picornaviruses ECHO 12 and ECHO 9 possess a single-stranded RNA serving as mRNA with a coding capacity for a single protein with a molecular weight of 270,000 Dalton. This large protein is being cleaved via several intermediates into the much smaller capsid proteins. For ECHO 12 and ECHO 9 we detected 17, 14 and 13 virus-specific proteins, respectively. These proteins were separated by electrophoresis and subsequently subjected to partial enzymatic digestion. With the aid of the digestion patterns we characterized the precursor-product relationships of these virus-specific proteins.

By block of the initiation of the translation of these proteins with hypertonic medium we constructed a genetic map of the viruses. The principle of this method is analogous to the pactamycin mapping and allows an assignment of the proteins to the coding RNA sequences. We obtained a pattern of polypeptide cleavages analogous to polio and other picornaviruses: the capsid region is close to the 5-terminus, the replicase region probably close to the 3-terminus, and in the center of the genome a protein corresponding to polio-NCP X. The general similarity to the polio- and EMC-virus system is obvious.
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ANTIGENIC VARIATION IN PATHOGENIC AND APATHOGENIC STRAINS OF ECHO 11

Having noticed differences according to several criteria between a pathogenic variant of ECHO 11, Porz virus, and the Gregory prototype strain (Mertens et al., 1979), we compared a total of five ECHO 11 strains with each other in search of possible markers of pathogenicity. Two of the three additional strains (Aus I and Aus II), isolated during one epidemic near Melbourne in 1979, were obtained from Dr. Ann Donaldson of Fairfield Hospital, Fairfield, Australia. The third strain was U-virus, isolated in 1958 by Dr. Lennart Philipson. All strains except Gregory are apathogenic for mice; all except Gregory form plaques under agarose and agar overlay, whereas Gregory is plaque-forming only under agarose.

SDS-PAGE of the capsid proteins revealed differences between all five strains. This result was confirmed and extended when isolated capsid proteins were digested before PAGE. Gregory and U-virus resemble each other in VP 0, VP 2, and VP 3, but are clearly different in VP 1. The two Australian strains were identical in VP 1 and VP 3, but differ in VP 0 and VP 2. Porz virus was different from all other strains, tending more towards Gregory and U-virus. Isoelectric focusing of virus particles revealed no differences between the strains, whereas in isotonic saccharose gradients, Porz virus and Aus II did not aggregate and the other strains did. Cross neutralization tests showed either a prime situation (e.g. Aus II in relation to Aus I), or antigenic variation.

The comparison of five ECHO 11 strains showed that there is no unique feature which could serve as a marker of pathogenicity. Considerable genetic instability has to be reckoned with, giving rise to different strains even within one epidemic.

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ALTERATION OF THE CELLULAR SURFACE MEMBRANE AFTER MIXED-INFECTION WITH POLIO- AND ME VIRUS

Mixed-infection of HEp-2 cells with polio- and ME virus revealed that virus-induced membrane changes do also occur when viral reproduction is inhibited (cf. Zeichhardt, Habermehl & DIEFENTHAL, J.gen. Virol. 1981, in press). ME virus - when inhibited by interfering poliovirus - determines the alterations of the cellular surface, whereas the unaffected poliovirus dominates the changes in the cytoplasm. Comparative scanning (SEM) and transmission electron microscopy (TEM) (for methods cf. Schlehofer, Hampl & Habermehl, J.gen. Virol. 44, 433, 1979) revealed for infections with:

(i) ME virus: Compared to mock-infected cells ME virus induces large membraneous structures deriving from pycnotic cells and stretching over several cells like "cloths" (8 h.p.l.). These cloths originating from giant blebs represent huge vacuoles. The cellular surface membrane is smoothened and the number of microvilli is reduced. Cellular organelles and cytoplasm are disintegrated as represented by nuclei with margination of condensed chromatin, swollen mitochondria and large vacuoles. (ii) Poliovirus: Compared to ME virus infections poliovirus-infected cells lack the cloths; however, the cells are even more pycnotic exhibiting long filipodla and rough surfaces with condensed microvilli (8 h.p.l.). The cytoplasm contains typical vesicles and the lobed nucleus comprises less condensed chromatin than after ME virus infection. (iii) ME and poliovirus, simultaneously: The cytoplasmic and nuclear changes are specific for poliovirus infections, whereas the alterations of the cellular surface are characterized by the ME virus induced cloths and blebs. The ME virus specific alteration predominates also in cytolysis membrane damages as expressed by 51-Cr-release from infected cells: The ME virus typical 51-Cr-release - more enhanced than after poliovirus infection - even dominates, when ME viral reproduction is suppressed by interfering poliovirus.
LOCALIZATION AND CHARACTERIZATION OF TWO IMMUNOGENIC REGIONS ON THE COAT PROTEIN VP$_{Thr}$ OF FOOT-AND-MOUTH DISEASE VIRUS (FMDV), SUBTYPE O$_{1K}$, INDUCING NEUTRALIZING ANTIBODIES

The structural coat protein VP$_{Thr}$ was chemically and enzymatically cleaved and the products were separated by gel and ionexchange chromatography. Cyanogen bromide cleavage yielded four cleavage peptides; enzymatic digestion of the whole 146 S virus particle with trypsin and an arginin specific protease (mouse submaxillary gland protease) generated two peptides derived from VP$_{Thr}$ as detected by electrophoresis. The order of the cleavage peptides was determined by their elution profiles, molecular weights and N- and C-terminal amino acids. In two of four cases, virus-neutralizing antibodies were induced after immunization of STU-mice with purified cyanogen bromide peptides of VP$_{Thr}$. Only one of two peptides obtained by arginin specific digestion induced neutralizing antibodies, and none of the tryptic fragments. We conclude that these immunocompetent protein sequences lost by tryptic digestion are located at the beginning and at the C-terminal end of the smaller fragment of arginin specific digestion corresponding to the middle and the C-terminal end of VP$_{Thr}$.

MAJOR ANTIGEN OF FOOT AND MOUTH DISEASE VIRUS: CLONING OF cDNA AND EXPRESSION IN E. COLI

Double stranded DNA copies of the single stranded genomic RNA of Foot and Mouth Disease Virus have been cloned into the E. coli plasmid pBR322. The coding sequence for structural protein VP1 was identified and inserted into a plasmid vector where the expression of this sequence is under control of the phage lambda P$_{1}$ promoter. In an appropriate bacterial host the synthesis of antigenic polypeptide can be demonstrated by radioimmunoassays.
INVESTIGATIONS ON THE POLYPEPTIDES OF INFECTIOUS BURSAL DISEASE VIRUS (IBDV)

Infectious bursal disease virus (IBDV) is the causative agent of a highly contagious disease of young chickens resulting in severe necrotic lesions in the bursa of Fabricius (Gumboro disease). IBDV is a non-enveloped icosahedral particle with a diameter of about 60 nm. We could demonstrate that its genome consists of two segments of double-stranded RNA with molecular weights of $2.2 \times 10^6$ and $2.5 \times 10^6$ daltons.

Our further investigations on structure and biosynthesis of IBDV have shown that in polyacrylamide-gel electrophoresis five structural polypeptides with molecular weights of 90 kd, 48 kd, 40 kd, 32 kd and 28 kd can be resolved. Two of them (40 kd and 32 kd) presumably have carbohydrate side-chains since they bind Concanavalin A. Tryptic peptide analysis of virus-specific proteins revealed that the 40 kd polypeptide is derived from the 48 kd polypeptide by posttranslational modification. When isolated lymphoid cells of the bursa of Fabricius, the thymus or the spleen, or chicken embryo fibroblasts are infected with IBDV at a high multiplicity of infection, virus-specific polypeptides can be demonstrated exclusively in cells of the bursa of Fabricius, which represents the target organ of IBDV. Fluorescein-conjugated antibodies against IBDV show that even in bursal lymphocytes only 20 to 25% of the cells are infected. This may be one of the reasons why virus-specific bands are difficult to resolve from host bands by polyacrylamide-gel electrophoresis of infected cells.

TOPOGRAPHIC AND ANTIGENIC DEMONSTRATION OF CHARACTERISTIC ULTRASTRUCTURES AT THE SURFACES OF VIRUS-INFECTED CELLS BY COMPARATIVE, HIGH-RESOLUTION EM STUDIES OF SURFACE REPLICAS WITH LARGE AREAS.

The development of a semi-automatic washing device for surface replicas (1) has made it possible to produce routinely replicas with large areas for high-resolution EM studies. Thus, surface replicas of freeze-fractured and etched coverslip cell cultures (2) and of C.P.-dried preparations of the same culture (produced under identical physical parameters) permit comparative EM studies on the morphogenesis (budding processes) of virus-specific structures at the plasma membrane such as incorporation and penetration in or through the individual membrane facets (ES, EF, PF). By using immunological markers (Ferritin) in replica immuno-cytochemistry (RIC), studies can be carried out in the TEM on the topographic distribution in overviews as well as on the antigenic characterization of the specific structures (100-150 nm) in the high-resolution range.

Alterations occurring at the plasma membranes of HeLa and BHK cells during the course of infection with measles and towl plague virus as well as their morphogenesis are demonstrated, observed and discussed.

(1) Hohenberg, H., and Mannweiler, K.: Mikroskopie (Wien) 35 (1980), in press.
(2) Hohenberg, H., and Mannweiler, K., in preparation.
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COMPARISON OF SOME METHODS FOR THE PHYSICAL SEPARATION OF IgM AND IgG ANTIBODES IN DIAGNOSTIC SERUM SPECIMENS

Using rubella-IgM-positive sera and serum pools of low (borderline) specific IgM titers, we compared with each other the ultracentrifugation on sucrose gradients in a swing out rotor (SW 41) and a vertical rotor (VTi 65, Beckman), and two column chromatographic methods (Protein-A-Sepharose, DEAE-Sephacel, Pharmacia). Both native serum and serum pretreated in order to increase IgM in relation to IgG, were tested. Pretreatment consisted of dialysis of serum specimens against distilled water, which precipitates the serum euglobulins including IgM, and only little IgG. Fractions from sucrose gradients were assayed for IgM and IgG (commercial immunodiffusion plates) as well as specific rubella antibodies (HI); in the IgM peak fractions of chromatographic eluates, IgA was assayed additionally.

With native serum, ultracentrifugation in the swing out rotor was the only method to produce reliable results according to sensitivity, HI-profile, and lack of IgG contamination in IgM fractions. With euglobulins, however, similar results were obtained with the vertical rotor. Column chromatographic methods were insufficient both with native serum and euglobulins. The method of euglobulin precipitation appears promising in that it may allow to use the vertical rotor, and thus shorten the diagnostic process by 18 - 20 hours.

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THE REACTIVITY IN ELISA-ASSAY OF MONOCLONAL AND POLYCLONAL ANTIBOIES AGAINST AN INFLUENZA A VIRUS (A/FM/1/47)

The requirements for optimal binding to an antigen are different for monoclonal and polyclonal antibodies. Optimal binding to the hemagglutinin of an influenza virus occurred with monoclonal antibodies at a narrow pH range, while the same antigenic sites displayed an unaltered binding capacity over a much broader pH range if the antigen is integrated into the virus particle. Such differences are not seen with polyclonal antibodies.

In a number of ELISA-techniques short fixation with methanol, acetone or formaldehyde of coated antigens is routinely applied. However, these antigens showed a drastic change, up to complete loss of their antigenicity to a variety of monoclonal antibodies. Binding of polyclonal antibodies was far less restricted by the same treatment.
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ENZYME-IMMUNOASSAY FOR THE DETECTION OF VIRAL ANTIBODIES WITH VIRUS-INFECTED CELLS

A modified enzyme-immunoassay with simplified antigen-preparation for the detection of viral antibodies in patients' sera is described. Virus-infected and non-infected cells as controls are used for "CELL"-ELISA performed in microtiterplates instead of purified virusantigen prepared by complex procedures. Fixation with cold aceton is necessary for reliable adherence of the cells to the plastic. This requires use of aceton-resistant microtiterplates. Alkaline phosphatase is enzyme system of choice for this test.

Present investigations for the detection of antibodies (IgG) in patients' sera with "CELL"-ELISA were mainly performed using herpes simplex virus I-, but also mumps virus- and measles virus-infected vero-cells. The results indicate a satisfactory correlation to those obtained from conventional serological tests (NT-test and IF-test). In contrast to this, the attempts to detect antibodies to enteroviruses with the "CELL"-ELISA-technique were not successful up to now.

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DEVELOPMENT OF AN ELISA TEST FOR THE DETECTION OF ANTIBODY AGAINST MARBURG AND EBOLA VIRUS

An indirect Elisa technique for the detection of antibody against Marburg and Ebola viruses was developed and tested. Antigen for the coating of polystyrol plates was cultivated in Vero cells and was inactivated with B-propiolactone before use. Reaction of human anti-sera with the antigen could be blocked by pretreatment of the plates with sera from immunized guinea pigs or hamsters. Such a blocking test would permit species independent detection of antibody.

Persons who have experienced Marburg virus infection 13 years before have still now high titers of antibody against this virus. In addition these sera showed elevated titers against Ebola virus as compared to control sera.

We have also tested a group of 64 human sera from Mobai, Sierra Leone, for antibody against these viruses. About 30% exhibited highly positive reactions with Ebola virus and some less with Marburg virus, too.

Further investigation will be necessary to decide whether Ebola virus infection is prevalent in this region or not.
IgM-RHEUMATOID FACTORS (IgM-RF): THEIR INFLUENCE IN THE INDIRECT IMMUNOASSAY (ELISA) FOR (VIRUS) SPECIFIC IgM ANTIBODIES AND THEIR ELIMINATION WITH HUMAN IgG COATED LATEX PARTICLES

All indirect immunoassays - antigen adsorbed to a solid phase - for specific IgM antibodies can indicate a false positive result if the sample contains specific IgG and IgM-RF at the same time.

The relative dependence on the content of both parameters is demonstrated with the sample of virus specific (herpes simplex, measles) IgM-ELISA's.

An absorption procedure for the serum to be tested with human IgG-coated latex particles (RF-latex reagent, Behringwerke) for the elimination of IgM RF is described.

The efficiency of this absorption procedure is proven with a sensitive IgM RF ELISA (detection limit approx. 0.15 I.U. per ml) as well as with ELISA's for virus specific IgM antibodies in extreme combinations: serum mixtures with a high IgM-RF content in presence of falling specific IgG titers and vice versa.

Results show that
1) IgM-RF concentrations even below the detection level of conventional RF-tests (RF latex, Waaler Rose) can lead to a false positive result in the test for specific IgM antibodies, the degree depending on the concentration of specific IgG.
2) IgM-RF are effectively removed by the proposed absorption procedure to an amount of about 130 I.U. per ml:
   A serum containing virus specific IgG five times above the average titer (1:16240) without specific IgM but simultaneously an unusual high IgM RF content (288 I.U. per ml) gets negative (1:20) in a specific IgM ELISA after the absorption.
3) Virus specific IgM antibodies are not reduced by this procedure.

INDUCTION OF INTERFERON-NEUTRALIZING ANTIBODIES IN SERUM OF PATIENT

Recently, Treuner et al., reported the successful treatment of a nasopharyngeal carcinoma with HuIFN-β (Lancet 1, 817-818, 1980). In the course of IFN-therapy a HuIFN-β neutralizing activity appeared in the serum of this patient. We demonstrated that such activity is due to IgG class antibodies.

This is the first report demonstrating evidence for antigenicity of interferon in a homologous host system.
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PURIFICATION AND MOLECULAR CHARACTERIZATION OF HUMAN FIBROBLAST INTERFERON

Human fibroblast interferon was purified to homogeneity from serum containing culture medium and obtained in a completely volatile buffer for the first time. The 20 to 50,000-fold purification was achieved by a combination of affinity chromatography (Con A or Blue Dextran Sepharose) with high performance liquid chromatography. Material with an average specific activity of $4 \times 10^5$ U/mg was characterized by SDS PAGE with and without fluorescence labeling, amino acid and amino sugar analysis, partial amino and C-terminal sequence analysis and tryptic peptide patterns, all performed at the picomole level. In some preparations, low activity levels were found at 10,000, 17-18,000, 35,000 and 40,000 besides the usual 20-21,000 dalton positions on SDS PAGE.

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ENDOGENOUS INTERFERON IN THE CEREBROSPINAL FLUID OF HERPES ENCEPHALITIS PATIENTS

Low titers of antiviral activity were found in the cerebrospinal fluids (CSF) of herpes encephalitis patients when using a sensitive interferon assay. With this dye-uptake assay on human fibroblasts, trisomic for chromosome 21, one international unit of human fibroblast or leukocyte interferon per milliliter can unequivocally be determined. Eight CSF samples taken during the first ten days of illness were antivirally active whereas only one of 11 taken later proved positive. With the exception of one serum no interferon was found in the serum samples taken simultaneously with the CSF samples. By antibody neutralization using specific antisera against human fibroblast and leukocyte interferon it was found that the antivirally active substance is identical with or closely related to human leukocyte interferon.
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SAFETY TEST FOR THE CONTROL OF VIRUS REPLICATION OF NUCLEAR POLYHEDROSIS VIRUS FROM NAMESTRA BRASSICAe IN VERTEBRATES

The purpose of this safety study was to determine whether the Nuclear Polyhedrosis Virus (NPV) of *Mamestra brassicae* can replicate in vertebrates. As a possible indication of a virus replication, the detection of antibodies against virions and/or polyhedrin (matrix protein) was assumed. After feeding of NPV (purified NPV, biological active virions and UV-inactivated virions) to NMRI-mice no antibodies against virions as well as against polyhedrin could be detected within sixty days p.i. After application of NPV by aerosol, no antibody induction could be observed, too.

In our experiments NPV was not able to induce antibodies after application over respiratory or alimentary tract. Therefore, we conclude that no virus replication had taken place in vertebrates. Based upon these results, there is no hygienic risk from this point of view for the application of NPV in biological pest control, especially NPV from *Mamestra brassicae*.

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PCMB-TREATMENT BLOCKS REVERSIBLY THE ECLIPSE OF THE BACTERIOPHAGE φX 174

The p-Chloromercuribenzoic acid (PCMB), a sulfhydryl residue reagent, causes a reversible inactivation of the bacteriophage φX 174 wt and its hostrange mutant φX 174 HaHb with an approximate equal efficiency.

The PCMB inactivation kinetics resemble a sigmoid curve. By a subsequent treatment with an equal high concentration of Mercaptoethanol (ME) it is possible to restore the biological activity completely.

The PCMB-treated phages are able to become reversibly attached to the bacteria, but the eclipse step is blocked. Apparently, sulfhydryl residues and/or changes in the distribution of the charge participates in this step, because PCMB-treated phages show a changed electrophoretic mobility.

Untreated, eluated eclipsed phages are biologically inactive and cannot be reactivated by ME.
CONTINUOUS REPLICATION OF POTATO SPINDLE TUBER VIROID (PSTV) IN PERMANENT CELL CULTURES OF POTATO AND TOMATO

Potato spindle tuber viroid (PSTV) replicates continuously in callus cultures of PSTV-infected wild type potato (Solanum demissum) and tomato (Lycopersicon peruvianum L. Mill) plants and in cell suspensions derived from in vitro-inoculated potato protoplasts (Solanum tuberosum HH 258). The persistence of PSTV replication in these cell lines through at least fourteen subculture passages, which corresponds to a continuous replication over a period of more than one year, was demonstrated by infectivity assay and by polyacrylamide gel electrophoresis of isolated nucleic acids. This continuous de novo synthesis of PSTV was substantiated by the incorporation of $^{32}$P-orthophosphate into viroid RNA. These cell lines with continuous and effective replication of a viroid should provide an excellent material for the investigation of viroid replication at the cellular level.

EFFECTIVE SCREENING METHOD FOR DETECTION OF MYCOPLASMA HYORHINIS CONTAMINATION IN VIRUS STOCKS.

Mycoplasmas are ubiquitous prokaryotes that frequently contaminate cell cultures and stocks of viruses. Growth of mycoplasmas in a cell-free medium may be slow and difficult, particularly in the case of M. hyorhinis, whose strains often are growing apparently cell-dependent and therefore were termed "auxotrophs". M. hyorhinis belongs to this group and is one of the major contaminants in virus stocks. It was found that mink lung cells (MvILu, ATCC-CCL64) is the cell of choice for the rapid propagation of M. hyorhinis. In all cases MvILu were infected with M. hyorhinis strains (17981, 23839, 25021, 27717, and 29052), plaques developed and the cell lysis occurred 3 to 5 days after infection. These properties of MvILu allowed the establishment of a test for the detection of M. hyorhinis contaminations in cell cultures and virus stocks using MvILu cells as an indicator cell line. The DNA from different M. hyorhinis strains was cleaved with 27 different restriction enzymes. The resulting DNA fragments were electrophoretically separated on 0.5% agarose slab gels and stained with ethidium bromide. It was found that restriction enzymes Bst EII, Xho I and Hind III are the enzymes of choice for analysis of DNA of this mycoplasma. The resulting DNA cleavage patterns for M. hyorhinis could easily be distinguished from that of contaminating DNA which originated from known viral DNA of stocks. The most unequivocal procedure for detection and recognition of mycoplasma contaminating tissue cultures is to isolate the organisms in suitable cell-free media and identify the isolates by specific serological methods. However, this procedure suffers the joint disadvantages that it can be slow, involving days or weeks, and that cell-free cultivation may be difficult to achieve with some species, notably M. hyorhinis. Our method of analysis of DNA of mycoplasma gives a rapid and exact result and it could provide a useful alternative to difficult microbiological tests.
DEOXYTHYMIDINE-5'-TRIPHOSPHATASE: PURIFICATION AND CHARACTERIZATION OF A NEW ENZYME IN HUMAN SERUM.

An enzyme activity in human serum has been found to catalyze the conversion of dTTP to dTDP. No measurable hydrolysis of nucleoside triphosphate to the diphosphate was observed with any of the other standard ribo- and deoxyribonucleoside triphosphates except dUTP up to an amount of about 50% compared with that of dTTP. The characteristic of this enzyme activity is completely different from any comparable enzyme described to date (1). The enzyme has been purified to near-homogeneity from human serum with an apparent $K_m$ of 25 $\mu$M. The enzyme is inhibited non-competitively by dGTP ($K_i = 68 \mu$M). The speculation of the biological function of the dTTPase is to be involved in the ribonucleotide reductase system stimulating the production of the other three deoxyribonucleoside triphosphates and being regulated itself by dGTP in a non-competitive way.

1) Dahlmann, N., Müller, D.: Hoppe Seyler, Dez. 1980

DETECTION OF HERPESVIRUS (PSEUDORABIESVIRUS) DNA IN ORGAN TISSUES OF ACUTELY AND LATENTLY INFECTED PIGS.

Pseudorabiesvirus (PsRV), the causative agent of Aujeszky disease in pigs, establishes latent infections as other herpesviruses, too. In our studies various organ tissues of experimentally infected animals were investigated for the presence of PsRV and PsRV DNA. By cocultivation methods infectious virus was only detectable up to 5 to 6 weeks p.i. After immunosuppressive treatment of latently infected pigs virus reactivation occurred in nearly all cases. The presence of viral DNA in various organs and neural tissues of these animals could be demonstrated by in situ cytohybridization. The amount of viral genomes per cell of some of these tissues (5 - 20 genome equivalents) was determined by reassociation kinetics.