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The Alignment of the Physical and Genetical Maps of Bacteriophage T4
W. RÜGER, E. NIGGEMANN, I. GREEN, and J.M. GAEHTGENS

While T4 wild-type DNA is not cleaved by restriction endonucleases, the DNA of a
T4 mutant, in which 5'-hydroxymethyl cytosine is replaced by cytosine (T4 dC-DNA), is
digested by these enzymes. We have constructed physical maps of the cleavage sites gen­
erated by the restriction endonucleases Smal/Xmal, Kpnl, Sall, BglII, Xhol and Xbal (1, 2).
These enzymes sub-divide T4 dC-DNA into 73 defined fragments. The alignment of the
physical and the genetical maps then was achieved with three sorts of experiments:
a) the identification of some EcoRl restriction fragments on the T4 physical map which
are known to carry defined T4 genes (3, 4)
b) the localization of the T4 rII region with the aid of two deletion mutants
c) the hybridization of early and late mRNA to the T4 restriction fragments, which allows
the identification of those restriction fragments carrying the early and the late regions.

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Morphological Aspects of Cell Transformation
C.B. BOSCHEK, B.M. JOCKUSCH, R. BACK, R.R. FRIIS, and H. BAUER

Neoplastic transformation of chicken embryo fibroblasts by Rous sarcoma virus is
accompanied by a number of structural alterations to the cell surface and to the cyto­
skeleton. These changes are induced by a single viral gene, termed src, which is known
to code for a phosphoprotein, pp60src. To study the dynamics of the morphological
changes, fibroblasts were infected with the temperature sensitive, transformation defective
mutant viruses, ts NY68 at the restrictive temperature and transformation was then in-
duced by rapidly shifting to the permissive temperature. At various times after temperature shift the cells were fixed and examined by a number of microscopic techniques which yielded the following results: After 15 min, evaginations on the surface of the plasma membrane (ruffles) were observed in the SEM. Concomitantly, immunofluorescence showed accumulations of the contractile proteins, actin and α-actinin on the cell surface. By use of the cathodoluminescence mode in the scanning microscope it was possible to show that the ruffles themselves contain contractile proteins. A number of hours later the actin-containing cytoskeleton stress fibres become disorganised, the ruffles disappear and the cells assume the typical rounded-up, transformed morphology. The early presence of actin and α-actinin within ruffles as well as the dissociation of the stress fibres indicates that one or more of the contractile proteins may serve either directly or indirectly as a target site for pp60src.

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Studies on Retrovirus Glycoprotein Synthesis in Wild-Type and TS Mutant Infected Cells
V. BOSCH¹, B. MASON², and R.R FRIIS¹

We have studied viral glycoprotein synthesis in wild-type retrovirus infected cells by immunoprecipitating labelled cell extracts with antiglycoprotein sera. In pulsed, chased or long-term labelled cells, the major glycoprotein related component electrophoreses slower than gp85 but faster than the disulfide linked VGP (i.e. gp85-S-S-gp35). Its mobility is identical under reducing or non-reducing conditions and, due to its presence in pulsed cells, is identified as the precursor to the glycoproteins, Pr92. Gp85 cannot be detected in infected cells or on the cell surface. These results point to the cleavage of Pr92 occurring after budding as suggested by Klemenz and Diggelmann (1979 J. Virol. 29, 285-292. Concomitant with or after cleavage, there is an increase in molecular weight possibly by glycosylation.

The mutant ts668 produces particles at the non-permissive temperature (42 °C) which are fifty times less infectious than particles produced at 35 °C. Virions produced at 42 °C contain virtually no gp85 but instead a glycoprotein with molecular weight approximately 120,000 dalton which is not immunoprecipitable by anti-gp85 sera. However, the immunoprecipitable components within the cell are identical to those in wild-type – i.e. Pr92 can be detected in normal amounts. It is as yet unclear why these components cannot be incorporated into virions and we hope that studies on this may yield information on the late steps of virus maturation and budding.

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Transformation Defective Temperature Sensitive Mutants of Rous Sarcoma Virus have a Reversibly Defective »SRC« Gene Product
ANDREW ZIEMIECKI and ROBERT R. FRIIS

A phosphoprotein of molecular weight 60,000 d (pp60src) has been identified as the putative product of the “src” gene of Rous sarcoma virus, the gene responsible for the
transformed phenotype. Furthermore, a protein kinase (pp60src K) activity has been demonstrated to be associated with immune precipitates containing pp60src. Using several transformation defective temperature sensitive mutants of Rous sarcoma virus we have demonstrated that at the non-permissive temperature very little pp60src K activity can be detected despite almost normal synthesis of pp60src. When such mutant infected cells are shifted to the permissive temperature there is a rapid increase in detectable pp60src K activity (within 15 min) which is not prevented by cycloheximide at concentrations sufficient to prevent de novo synthesis of pp60src. Furthermore, if mutant infected cells are shifted from the permissive to the non-permissive temperature there is a rapid loss of kinase activity (within 15 min). These rapid changes in pp60src K activity are paralleled by changes in the degree of phosphorylation of the mutant pp60src.

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Protein Kinase Activity in Normal and Avian Sarcoma Virus Transformed Cells

MASATOSHI OWADA, PETER DONNER, and KARIN MOELLING

We have compared the in vitro temperature sensitivity of the kinase activities from cells infected with mutants which are temperature-sensitive for transformation with that of the parental wild-type viruses. A two- or threefold greater inactivation rate was observed with the mutants. Loss of kinase activity is not directly correlated with loss of phosphate content of pp60src.

A kinase activity was found to be associated with the normal cellular pp60src in normal chicken cells which was 100-fold lower than in transformed cells. Kinase activities not associated with a 60k polypeptide will be discussed.

Poly(A)-containing 35S RNA from the Rous sarcoma virus SR-B wild-type and a mutant temperature-sensitive for transformation, M1100, were translated in vitro in a cell-free system. A 60k polypeptide was synthesized in both cases and was found to be associated with a kinase activity for the wild-type whereas that of the mutant was reduced 30-fold.

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Reactivation and Inactivation of Mutant PP60SRC-Associated Kinase Activity by Temperature Shifts

ANDREW ZIEMIECKI and ROBERT R. FRIIS

A phosphoprotein of molecular weight 60,000 d (pp60src) has been identified as the putative product of the "src" gene of avian retroviruses, the gene responsible for the transformed phenotype. Furthermore, a protein kinase activity has been demonstrated to be associated with immune precipitates containing pp60src. Using several Prague ts mutants defective in transformation we have demonstrated that at the non-permissive temperature very little kinase activity can be detected despite almost normal synthesis of pp60src. When
such mutant infected cells are shifted to the permissive temperature there is a rapid increase in detectable kinase activity (within 15 min) which is not prevented if the shift is done in the presence of cycloheximide at concentrations sufficient to prevent de novo synthesis of pp60src. Therefore the rapid increase in kinase activity is partly due to reactivation of the responsible molecule(s) made at the non-permissive temperature. Furthermore, if mutant infected cells are shifted from the permissive to the non-permissive temperature there is a rapid loss of kinase activity (within 15 min). Thus at the non-permissive temperature, the functional kinase molecule(s) made at the permissive temperature are rapidly inactivated.

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Investigations of the src Gene-Product of Rous Sarcoma Virus

H. RÜBSAMEN, R. R. FRIIS, H. BAUER, P. PRESEK, H. GLOSSMANN, E. EIGENBRODT, and W. SCHONER

The product of the transforming gene of Rous Sarcoma virus (RSV) (1) is a phosphoprotein of 60,000 dalton molecular weight, pp60src. In immunoprecipitates of pp60src, a protein-kinase activity can be demonstrated (2). This activity may be an enzymatic function of pp60src, since it is more heat labile in vitro when derived from cells transformed by RSV mutants which are temperature-sensitive for transformation (ts td) (3).

We have determined the amount and the phosphorylation of pp60src in cells infected with several such mutants mapping in src and were unable to find differences between permissive and nonpermissive temperature and between the mutants and wildtype. In contrast, the kinase activity is very much different between the viruses and strongly reduced at nonpermissive temperature. It is, however, not possible at present to draw conclusions concerning a possible role of the kinase in the transformation-process. The kinase has been characterized enzymatically and partially purified. Its analogy to a regulatory cellular kinase will be discussed.

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Processing of Viral Polyprotein Precursors by a Restrictive Protease associated with the Viral Structural Protein p15

K.E.J. DITTMAR, P. DONNER, and K. MOELLING

The viral structural protein p15 carries a restrictive proteolytic activity which cannot be dissociated from the viral protein during ion exchange chromatography and size frac-
tion procedures. It is a thiol protease and can be labeled with $^3\text{H}$-NEM, a specific agent for thiol groups, in all nondefective avian leukosis and sarcoma viruses (ALSV) so far tested.

The ALSV gag precursor Pr76 and the ALSV reverse transcriptase precursor Pr180, either synthesized by metabolic labeling or in an in vitro translation system, can be processed via all known intermediates into the viral structural polypeptides by treatment with p15 in vitro. In contrast, the avian glycoprotein precursor gPr91 is not processed by p15.

If avian viral p15 is applied to immune-precipitated labeled murine gag polyprotein precursor Pr65, cleavage is achieved, but results in cleavage products different from the mature viral structural polypeptides.

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The Conformation of Moloney Murine Leukemia Proviral DNA Sequences in Target and Non-Target Cells of Balb/Mo Mice

MICHAEL BREINDL and RUDOLF JAENISCH

Digestion experiments with micrococcal DNase and DNase I were performed to study the conformation of Moloney murine leukemia virus (M-MuLV) DNA sequences in the chromatin of target and non-target cells of Balb/Mo mice. The following results were obtained: 1. The proviral DNA sequences are in target and nontarget cells organized in nucleosomal structures. 2. The genetically transmitted copy of the viral genome is in lymphatic target cells but not in non-target (liver) cells preferentially digestible with DNase I. This shows that this copy of the viral genome is integrated into the mouse genome at a site where it is specifically activated in lymphatic cells. 3. Leukemogenesis is accompanied by an amplification of proviral M-MuLV sequences in target cells. The amplified copies of the viral genome are not present as free proviral DNA but are also integrated into the mouse genome and organized in nucleosomal structures. 4. The majority of the amplified and reintegrated proviral M-MuLV DNA sequences in the target cells is in a transcriptionally active, i.e., DNase I-sensitive chromatin conformation. In non-target cells the proviral M-MuLV sequences remain DNase I-resistant throughout the lives of the mice.

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Active Immunisation of STU Mice with Purified Envelope Polypeptides from Friend Leukaemia Virus: Evaluation of Humoral Immune Response and Protective Effect

G. HUNSMANN and J. SCHNEIDER

The humoral immune response of STU mice to envelope glycoproteins of Friend leukaemia virus (FLV) was investigated. Furthermore the induced immunity was challenged with an erythroleukaemia inducing dose of FLV. 8-10 weeks old mice were immunised
twice with 32S aggregates of gp85 suspended in PBS or complete Freund adjuvant. Serum antibodies from repeated bleedings of individual mice were examined by cytotoxicity tests and immunoprecipitation. The development of erythroleukaemia after challenge was monitored with white blood counts, spleenweight and titration of infectious virus from spleens. An antibody response was detectable only in those animals which had received 5 to 50 μg of gp85. Adjuvant increased the serum cytotoxicity against AKR virus producing cells and elicited antibodies precipitating envelope polypeptides of BALB xenotropic and feline leukaemia viruses. A significant reduction of virus induced splenomegaly was recorded four weeks after challenge in the group which had received 0.5 μg of FLV gp85. The number of nucleated cells in the peripheral blood correlated well with the extent of splenomegaly. Small spleens of immunised mice contained 10 to 100 times less infectious virus than large spleens of the same group. Thus, the immunological effector mechanism of this protective effect is probably distinct from the antibody response.

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ENV-Gene Polypeptides of Friend Leukaemia Virus: Purification and Association within the Envelope

J. SCHNEIDER, H. FALK, and G. HUNSMANN

On the envelope of mature Friend murine leukaemia virus roughly 10% of surface glycoproteins are coupled to membrane polypeptides by disulfide bridges. The remaining 90% of these glycoproteins are noncovalently associated in the same fashion. This fraction is converted into the covalently linked form by oxidation. The resulting amphiphilic heterodimer polypeptide gp84/86 is recovered almost quantitatively as aggregates, named rosettes, by solubilisation of the viral membrane with Triton X-100 and subsequent velocity sedimentation. gp69/71 and p12E/15E were purified from these protein micelles by molecular sieve chromatography after reduction of disulfide bonds. Electron micrographs of rosettes as well as p12E/15E showed structures differing from native viral knobs. Isolated gp84/86 could be reassociated and then displayed more similarity to these viral surface projections. As shown by peptide mapping, the primary structures of glycoproteins and membrane polypeptides, respectively, appeared to be highly related. Furthermore it was shown by two dimensional polyacrylamide gel electrophoresis and reelectrophoresis of purified gp85/86 that the larger component gp86 comprised gp71 associated with p15E and p12E while the smaller gp84 was formed by gp69 bound only to p12E.

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C-Type Retroviruses in Man. II. Virus Detection in Tissue Culture Cells

R. LÖWER, J. LÖWER, H. FRANK, and R. KURTH

Teratocarcinoma belong to the group of testicular (rarely ovarian) germ cell tumors which, in addition to embryonal carcinoma cells, often contain tissues from all three germ layers.
In tissue cultures of human teratocarcinoma cells, an occasional, albeit extremely low rate of spontaneous C-type virus synthesis can be observed in electron micrographs.

Two of three teratocarcinoma cell lines growing permanently in our laboratory can be induced to differentiate in vitro by addition to the culture medium of 5–20 μg/ml iodo­deoxyuridine (for 24 h), 10−6 M dexamethasone and 1% dimethyl sulfoxide. Cell differentiation can be followed light microscopically and can also be monitored by the vastly enhanced synthesis of β-hcg (human chorionic gonadotropin), which is normally synthesized by differentiated placental trophoblasts.

The two teratocarcinoma cell lines, which can be induced to differentiate can also be induced to synthesize, in a proportion (approx. 10%) of the cell populations, easily detectable amounts of C-type particles. Similar independent observations on the induction of cellular differentiation and C-type virus production in human teratocarcinoma cells have been made by Dr. David Bronson (University of Minnesota, Minneapolis).

Preliminary virus characterization experiments indicate that the viruses possess a manganese-dependent reverse transcriptase and can be banded in sucrose density equilibrium gradients at 1.16 g/ml (intact particles) and 1.23 g/ml (virus cores). Incorporation of 3H-uridine suggests the presence of RNA in the particles.

Detailed biological, immunological and biochemical experiments have been initiated to determine the origin of the teratocarcinoma-derived viruses and to define their possible relationship to other known primate retrovirus strains.

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Human C-Type (Tumor) Viruses. I. Detection of Anti-Viral Immunity in Healthy Individuals and in Patients

R. KURTH1, R. LÖWER1, J. LÖWER1, and F. KATZ2

The majority of healthy children and adults react immunologically with purified envelope antigens of mammalian C-type tumor viruses. This serological reaction may well be due to the so-called "interspecies-specific" antigenic determinants shared by various mammalian and the yet putative human C-type viruses.

In radioimmunoprecipitation assays, anti-viral titers are only low (1:5) to intermediate (1:1280). On the other hand, average antibody affinities are, in general, unexpectedly high (KR values > 109 litres/mole). Both parameters taken together indicate that man has encountered the corresponding antibody-inducing antigens (viruses?) repeatedly, but always in low amounts.

The serological investigations of the anti-viral immune status in groups of patients with various malignant and autoimmune disorders showed an increased immunity in patients with Hodgkin’s disease, melanomas and testicular tumors. Immunological, biochemical and electron microscopical investigations of teratocarcinoma cells led to the demonstration of human C-type virus particles (see also accompanying presentation by R. Löwer et al.).
Biological Aspects of the Latent State of an Episomal Papovavirus Genome in Primate Cells

PETER KRIEG, MARTIN STEFFEN, EBERHARD AMTMANN, and GERHARD SAUER

Circular HD papovavirus DNA persists as an episome over at least two hundred cell generations in phenotypically normal primate cells (Vero cells of Cercopithecus aethiops origin). HD DNA fails to integrate into the genome of the host cell and is perpetuated exclusively as free circular molecule from one cell to another. In the latent state the HD genome is proliferated without production of virions; the latent viral DNA is, however, transcribed into RNA. Vero cells bearing latent viral genomes show certain characteristics of transformation such as growth under low serum and breakdown of actin cables. The viral DNA replication is under stringent host cell control. Under certain conditions of passaging of the cells viral DNA molecules can segregate within originally HD DNA containing cell lines of clonal origin. There may arise cells that have lost the HD DNA owing to segregation. Trypsinisation of HD virus producing cells at short intervals of time (every third day or up to weekly intervals) causes rapid loss of virus producing cells. Sometimes only a few producer-cells remain amongst millions of nonproducer-cells. In contrast, passaging of the virus-producing cell population in intervals of three weeks leads to a steady increase of producing cells within the cell culture (as shown by in situ hybridization and immunofluorescence) such that almost one-half of the cell population is comprised. The relationship between viral DNA replication and the physiological state of the host cell may depend on a factor for replication of DNA which is predominantly used for synthesis of cellular DNA.

Persistence of Bovine Papillomavirus DNA in Fetal Calf Brain Cells

E. AMTMANN\textsuperscript{1} und H. MÜLLER\textsuperscript{2}

Fetal calf brain cells were infected with Bovine Papillomavirus (BPV) in the sixth passage. The virus was isolated from skin warts and purified by two subsequent density equilibrium centrifugations in CsCl followed by sedimentation through a sucrose gradient. - Cells were passaged in weekly intervals at a splitting ratio of 1:2. Three weeks after infection DNA was isolated from the cells and analysed in Southern blot hybridisation. - As a result it was shown that BPV DNA persisted for at least three passages in the cells as an unintegrated superhelical genome. - In additional Southern blot experiments DNA was analysed, which was derived from two spontaneous occurring equine fibroma-like tumors, termed equine sarkoid. Only unintegrated, superhelical papilloma DNA could be revealed.
Role of Fast-Sedimenting SV40 Nucleoprotein Complexes in Virus Assembly

E. FANNING and I. BAUMGARTNER

Fast-sedimenting virion-like SV40 nucleoprotein complexes were extracted from nuclei of productively infected cells. These complexes were compared with CsCl-purified SV40 virus by zone velocity and equilibrium density centrifugation, electron microscopy and plaque assay (1). Although they cosedimented at 240S with purified virus and banded at the same buoyant density after fixation in formaldehyde, they were unstable at high ionic strength, slightly larger in diameter and more infectious. Resedimentation experiments showed that the labile complexes dissociate to empty capsids, DNA-protein complexes and free protein at high ionic strength. Control experiments indicated that these complexes are unlikely to be degradation products of mature virus. In fact, the kinetics of formation of labile fast-sedimenting complexes and stable virus suggest that labile SV40 complexes may represent intermediates in virus assembly. These results are summarized in a new model of SV40 assembly in which viral chromatin is not introduced into empty capsids, as proposed earlier (2), but rather capsid proteins are deposited on the viral chromatin to form a labile encapsidated structure which subsequently matures to a stable particle.

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Association of SV40 T-Antigen with a Host Protein

B. NOWAK, B. BUKAU, and E. FANNING

Simian virus 40 (SV40) codes for two early proteins, the 90K and 17K tumor antigens, which can be immunoprecipitated from extracts of SV40-transformed cells and productively infected cells with serum from tumor-bearing hamsters. Immunoprecipitated proteins from 35S-methionine and 32P-labeled extracts were analyzed by SDS-gel electrophoresis and autoradiography. In addition to the two virus-coded T-antigens, a third protein was observed in extracts of transformed cells. The molecular weight of this protein ranged from 50K to 55K, depending on the species of the cell. One-dimensional partial proteolysis peptide mapping indicated that the 55K and 90K proteins are not closely related. All SV40-transformed cells examined contained the 55K protein, but it could also be detected in uninfected and infected monkey cells with certain tumor sera. These results suggest that the 55K protein is encoded by the host cell rather than the virus.

Cell fractionation experiments demonstrated that the 55K and 90K antigens are found
mainly in the nucleus of the cell. Sedimentation analysis of the extracts from transformed cells showed that the 55K protein co-sediments with a highly phosphorylated form of the 90K antigen at about 25S. This complex was not observed in extracts of productively infected cells.

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Significant Increase of SV40-Induced, Malignant Cell Transformation in vitro by City Smog Extracts

N. SEEMAYER and N. MANOJLOVIC

Interaction of environmental carcinogens and viruses could be of great importance in human carcinogenesis. - We analyzed the effect of city smog extracts and of carcinogenic polycyclic aromatic hydrocarbons on SV40-induced cell transformation in vitro.

Samples of city smog from the heavy industrialized Ruhr-area (West Germany) were extracted by organic solvents and further fractionated. Logarithmically growing Syrian hamster kidney cell cultures were exposed for 18 hours to different concentrations of city smog extracts and their fractions. Thereafter cells were infected with SV40 at a MOI of 500. In parallel experiments hamster cells were exposed to different concentrations of Benzo(a)pyren or Dibenz(a,h)anthracene also followed by SV40 infection. Using various fractions at different concentrations we found a 2-10 fold increase in transformation frequency in comparison to the SV40 control. Pretreatment of cells with the carcinogenic polyaromates led to a 2-3 fold increase of transformation frequency. Inoculation of in vitro transformed cells to Syrian golden hamsters produced malignant tumors. The results demonstrate importance of combined effects of chemical carcinogens from the environment and viruses in cell transformation.

Isolation of Virus-Like Particles from Biopsy Specimen of Hyperplasia multilocularis mucosae oris Heck (Focal Epithelial Hyperplasia, FEH)

R.H. DENNIN and D. PETZOLDT

Hyperplasia multilocularis mucosae oris Heck was discovered 15 years ago in USA. Since several years this disease is also known in Europe. An infectious etiology was assumed early in discovery, but only recent reports claimed the demonstration of virus-like particles in ultra-thin sections by electron microscopy. But so far it failed to isolate virus particles. From a biopsy of one patient suffering from FEH virus-like particles could be isolated. The morphological characteristics indicate similarities with viruses of the papova-virus group. Further characterization was done by immuno-electron microscopy.
Microinjection of Epstein-Barr Virus DNA: Expression of Viral Genes

G. W. BORNKAMP, J. HUDEWENTZ, H. WOLF, G. BAYLISS, and A. GRÄSSMANN

Raji TK- cells, known to express viral genes after superinfection with P3HR-1 virus, were attached to plastic petri dishes coated with either anti-lymphocyte-IgG, concanavalin A or phytohemagglutinin. The cell volume of the small and fragile lymphocytes was increased by fusion of the cells to polykaryons. 24 h after injection of viral DNA 90-100% of the cells expressed the early antigen complex (EA) and 70-80% showed uptake of 3H-labeled thymidine indicating that thymidine-kinase is induced by the injected DNA. - After 48 h 20-30% of the injected Raji cells were also induced to virus capsid antigen (VCA) synthesis. - Human diploid fibroblasts, african green monkey kidney cells and rat fibroblasts, which are naturally not susceptible to Epstein-Barr virus injection, could also be induced to EA synthesis by injection of P3HR-1 virus DNA. - Microinjection of defined DNA fragments provides now a tool to map biological functions of the viral genome.

Protein-Synthesis in EB-(P3HR-1) Virus Superinfected Burkitt-Lymphoma Cells

WALTER W. BODEMER and WILLIAM C. SUMMERS

Burkitt-Lymphoma derived cells such as Raji, Daudi, P3HR-1, BJAB and its converted subclone B1 were superinfected with the P3HR-1 virus. We were able to demonstrate a different response of these various cells upon superinfection in terms of protein synthesis. Raji cells were the most permissive cells; i.e. rapid shut-off of host protein synthesis and synthesis of new proteins (18) which were not detected in non-superinfected cells. In contrast, all other cells used showed a delayed shut-off and only synthesis of some new proteins (2-8). Superinfection of BJAB and BJAB-B1 cells, especially, was highly dependent on the virus preparation.

For detection of EB-virus associated antigens we performed immunoprecipitation experiments. Antisera from IM- as well as BL-tumor patients were incubated with 35S-labeled protein extracts from superinfected Raji cells. The precipitated proteins were analyzed by high resolution gel-electrophoresis. We found 6 proteins with apparent molecular weights of 155K, 130K, 100K, 55K, 30K, and 45K. One protein, the 155K, could not be detected with all IM-sera. Only serum samples from a later stage of disease (about 30 d) contained an antibody specific for this protein. In general, all these 6 proteins were precipitated by IM- and BL-tumor patient sera.

Superinfection of Raji cells has been carried out under conditions allowing synthesis of early proteins (low moi and PAA-treatment). Therefore we conclude that these proteins represent early antigens. They may belong to the EA (early antigen) complex or to the EMA (early membrane antigen).
Analysis of Epstein-Barr Virus (EBV) Induced Surface Polypeptides

B. GEORG, N. MÜLLER-Lantzsch, N. Yamamoto, and H. Zur Hausen

The EBV-producing cell line P3HR-1 induced by the tumor promotor TPA and NC37 cells superinfected with P3HR-1-EBV were surface labeled with $^{125}$I by the lactoperoxidase method. NP40 soluble cell extracts were analyzed by immunoprecipitation with human VCA+MA+ sera and SDS polyacrylamidgel electrophoresis for virus-induced cell surface polypeptides. Two dominant polypeptides with molecular weights of 80000 and 250000 were specifically precipitated. In addition, only traces of polypeptides with 140000 in molecular weight were identified on P3HR-1-EBV producing cells. The surface of superinfected NC37 cells contained two de novo synthesized polypeptides of 80000 and 140000 in molecular weight. Our experiments demonstrated that the 140000 polypeptide is a late polypeptide and Ara-C sensitive, while the 80000 polypeptide is Ara-C insensitive. Both polypeptides were found to be identical in size with $^{35}$S-methionine and $^{125}$I labeled 80000 and 140000 envelope polypeptides from purified virus particles. These results may indicate that the identified polypeptides of 250000, 140000 and 80000 carry antigenic determinants of the EBV-induced membrane antigen complex.

Recombinants Between Different Strains of Herpesvirus saimiri

GÜNThER KEIL, BERNHARD FLECKENSTEIN, and CAREL MULDER

A number of Herpesvirus saimiri (H. saimiri)-strains have been isolated which, in part, are different in oncogenicity for some primate species. Cleavage of unique (L) and repetitive (H) parts of the viral genomes (M-DNA) with various restriction endonucleases yields stable and reproducible markers for strain identification. Some enzymes (group I) find recognition sites in both H- and L-DNA; other endonucleases (group II and III) cleave only in L-DNA or H-DNA, respectively; Sal I (group IV) does not cleave the M-genome of some H. saimiri strains within the entire length.

When permissive cell cultures were infected with long overlapping restriction fragments of H. saimiri M-DNA by the calcium phosphate technique, infectious virus could be rescued within 3 to 12 weeks. Co-transfection of long L-DNA-fragments (Kpn I, Sma I-cleaved) and Bam HI-fragments (group II enzyme) of M-DNA from different H. saimiri strains led to the isolation of eight recombinant viruses. Cleavage maps were constructed for each of these isolates using restriction endonucleases Aos I, Bam HI, Knp I, Sac II, Sal I, Sma I, and Xho I. In all recombinants, the terminal repetitive H-DNA stretches with adjacent external L-segments originated from one H. saimiri strain and the internal part of the L-region from the other parental strain. This method allows to generate recombinants without use of conditionally lethal mutants; the recombinant viruses may be useful in trying to localize coding sequences for viral proteins and to correlate certain genomic loci with oncogenic properties.
Herpesvirus Aotus Type 2, a Virus Related to the Oncogenic Herpesviruses of New World Primates

RÜDIGER RÜGER, MUTHIAH D. DANIEL, and BERNHARD FLECKENSTEIN

The highly oncogenic herpesviruses of New World monkeys Herpesvirus saimiri (H. saimiri) and Herpesvirus ateles (H. ateles) have a linear genome (90–110 megadaltons = md) with an extremely guanine + cytosine heterogeneity and terminal tandem repetitions (H-DNA) of variable length. In owl monkeys (Aotus trivirgatus) we found another herpesvirus of similar genome structure, Herpesvirus aotus (H. aotus) type 2. The DNA of this virus (M-genome) analyzed by cleavages with restriction endonucleases, analytical centrifugation in the CsCl-density gradient, partial denaturation for the electron microscopy, and reassociation kinetics, showed the arrangement of light sequences (L-DNA, 71 ± 1,5 md), inserted between repetitive H-sequences at both ends. The length of an H-DNA-repeat unit corresponds to 1.15 to 1.35 md. The G + C-contents of the various DNA-classes corresponds to as follows.

|          | H. aotus type 2 | H. saimiri  | H. ateles  |
|----------|----------------|-------------|------------|
| H-DNA    | 68.7%          | 70.6%       | 74.6%      |
| L-DNA    | 40.2%          | 35.8%       | 37.5%      |
| M-DNA    | 48.8%          | 45.4%       | 47.1%      |

There are base-sequence homologies between H. saimiri and H. ateles, but we found no cross-reaction between H. aotus type 2 and these viruses. There is also no relation to H. aotus type 1 and 3. Till now an in-vitro transformation of peripheral lymphocytes of primates was not possible with H. aotus 2. In experiments with marmosets it is not likely that this virus is oncogenic. A functional analysis of these virus genomes may contribute to the identification of transformation specific sequences in oncogenic herpesviruses in primates.

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Characterization of an EBV-Like Virus from African Green Monkey Lymphoblasts

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A lymphoblastoid cell line (AGM-2206) has been established from the peripheral blood of an African green monkey. Approximately 1% of these cells showed fluorescence for EBV capsid antigens (VCA), when examined with human EBV-VCA positive sera by indirect immunofluorescence. Titration of monkey and human sera on AGM-2206 and P3HR 1 cells revealed a partial crossreactivity between capsid antigens of the AGM- and the human EB-virus. Neither human nor monkey sera reacted with an EBNA-like antigen in AGM lymphoblasts. Cleavage patterns of AGM-EBV-DNA with the restriction endonucleases ECoRI and Hind III showed differences when compared to human EBV isolates from B 95-8, P3HR 1 and QIMR-WIL cells. Hybridization of labeled EBV DNA from
B 95-8 cells to fragments of AGM-EBV DNA blotted onto nitrocellulose filters resulted in some hybridization to a small number of AGM-EBV DNA bands. In addition, the AGM-EBV transforms AGM B-lymphocytes very effectively. Thus, the isolation of a transforming, B-lymphotropic EBV-like particle from an African green monkey, may permit investigations on primary replication sites, pathogenesis and possibly also on cofactors of EBV-associated oncogenesis.

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Herpesvirus Tupaia: Isolation and Characterisation of a Third Herpesvirus of Tupaia: Oncogenic Properties of Tupaia Herpesviruses

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Previously Tupaia (tree shrew) herpesviruses (THV-1 and 2) have been described. THV-1 had been isolated from degenerating lung tissue culture of Tupaia. In contrast, THV-2 had been isolated by us from a malignant lymphoma centrocytic tumour cell culture of Tupaia. Isolation and characterisation of a third herpesvirus of Tupaia (THV-3), which was isolated from the tumour cell culture of a Hodgkin’s sarcoma (Hodgkin’s disease, Lymphocytic depletion type) of Tupaia, is the subject of this study. THV-3 was efficiently propagated on Tupaia embryonic or baby fibroblasts or kidney cell cultures. DNA isolated from THV-3 was investigated using the restriction endonuclease Eco RI. It was found that the Eco RI cleavage pattern of THV-3 DNA resulted in DNA fragments different from those of THV-1 and THV-2 DNAs. Although the resulting cleavage pattern of these three virus types are different from each other, most of the DNA fragments comigrate on agarose gels.

The response of rabbits to THV-1, 2 and 3 was investigated. Several New Zealand rabbits were inoculated with Tupaia herpesviruses. It was found that the THV-2 induced hyperplasia of thymus if the animals were inoculated at the age of 4-15 days. The infectious virus was recovered from the cultured spleen of infected adult rabbits, when the animals were sacrificed 14 months after inoculation.

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Analysis of Integrated Adenovirus DNA Sequences in Transformed and Infected Cells

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The pattern of integration of adenovirus DNA have been analysed in adenovirus type 12 (Ad12)-transformed hamster cells, in Ad12-induced rat brain tumor cells and in adenovirus type 2 (Ad2)-infected human cells (1, 2, 3). The cellular DNA's were isolated, cleaved with various restriction endonucleases and analysed by the Southern technique using
Abstracts 3. Workshop of the Virology Section of the DGHM

[3H] labeled viral DNA or specific fragments of viral DNA as hybridization probes. Viral DNA sequences in the bulk of cellular DNA were detected by autoradiography. The results of these experiments can be summarized as follows:

1. In Ad12-transformed hamster cells and in Ad12-induced rat brain tumor cells multiple copies of the intact Ad12 genome are colinearly integrated, perhaps predominantly in repetitive sequences of cellular DNA. Free viral DNA is not present in these cells. The sites of integration are different in individual cell lines. We are currently investigating the organization of these sites in detail.

2. In Ad12-transformed hamster cells only early viral genes are expressed, in Ad12-induced rat brain tumor cells the early and some of the late genes are expressed into mRNA.

3. Many, but not all of the 5'-CCGG-3' sequences in the integrated Ad12 DNA in transformed hamster cells are methylated at the internal C residue (4). Virion DNA, however, is not methylated extensively (5). There is evidence that in transformed cells those regions of the genome which are expressed are less methylated than those which are not expressed.

4. In human cells productively infected with Ad2, a large number of viral genome copies are linked to cellular DNA early post-infection. There are only a few sites of recombination which perhaps lie in repetitive cellular DNA sequences.

5. We have isolated and started to characterize 18 morphological revertants of one of the Ad12-transformed hamster cell lines. Viral DNA sequences were lost from the genomes of these lines (6), although in general the patterns of integration are very stable with passage in culture and even in the animal.

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Studies on the Sites of Integration of Viral DNA in Adenovirus Type 12-Transformed Hamster Cells

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We have started to characterize the cellular sites of adenovirus type 12 (Ad12) DNA integration in Ad12-transformed hamster cells. The Ad12-transformed hamster lines T637 and HA12/7 contain multiple copies of the entire viral genome. Most of the viral DNA molecules are integrated intact (Sutter et al., Cell 14, 569, 1978). The total DNA from the transformed cell lines was cleaved with several restriction endonucleases and analyzed by the Southern technique. In particular, restriction enzymes have been used which cleave Ad12 DNA to a large number of fragments (Hinf I, Msp I). After cleavage with the Eco RI
restriction enzyme, rather large DNA fragments (17-33 Kb) are generated which contain the terminal viral DNA fragments (Eco RI-A and C) plus cellular DNA.

The data indicate that the viral DNA is integrated at a limited number of cellular sites. It is currently being investigated whether these cellular sequences constitute repetitive sequences. There is no evidence for the persistence of unit length free Ad12 DNA. In the HA12/7 line the existence of tandemly integrated Ad12 DNA is very unlikely, in T637 cells it cannot yet be rigorously ruled out. It is not known whether the cellular sites in different cell lines have any similarities. Integration patterns are different in different cell lines investigated.

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Selectivity in the Integration of Viral DNA Sequences in Adenovirus-Infected Cells

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Human adenovirus type 2 (Ad2) infects human cells productively and grows to high titers. Viral DNA synthesis starts 6-8 h post-infection. Among the intracellular forms of viral DNA, a high molecular weight (hmw) form sedimenting in alkaline sucrose gradients at 40-90S has been characterized. The hmw form of Ad2 DNA represents viral DNA sequences covalently linked to cellular DNA. The integrated form of Ad2 DNA can be detected early after infection (2-4 h) and is possibly due to parental sequences. The integration event does not require viral DNA replication, but replication of the cells. The integrated viral sequences were also analyzed using restriction endonucleases. After infection of human cells with Ad2, the nuclear DNA was isolated at 4, 6, 8, and 10 h post-infection, and the double stranded hmw DNA was selected by zone sedimentation and by chromatography on BND-cellulose. The DNA was then cleaved with the restriction endonucleases Bam HI or Eco RI, the fragments were separated on 0.6% agarose slab gels and analyzed by the Southern technique. Viral specific sequences were detected by hybridization with 32P nick translated Ad2 DNA or specific viral DNA fragments and autoradiography of the filters. The results demonstrate viral DNA fragments in a limited number of bands in molecular weight regions higher than the Bam HI-A virion DNA fragment (mol. wt. 8.8 \times 10^9). These fragments, which do not correspond in size to any of the virion DNA fragments, are due to viral and cellular sequences linked to each other.

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Characterization of Adenovirus Type 12 After Adaptation to a Human Melanoma Cell Line

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Longtime passages of human adenovirus type 12 in a human melanoma cell line (Nki-4) resulted in the selection of a mutated virus carrying inserted DNA-sequences of different
sizes. This mutant grows faster than the wildtype virus, leading eventually to the disappearance of wildtype genomes not only in Nki-4-cells but also in reinfected KB cells. DNA-analysis by restriction enzyme cleavage revealed, that the adapted virus population carried DNA insertions located at the right end of its genome (E-fragment Hind III), with molecular weights from approximately $2.5 \times 10^5$ to $9 \times 10^5$. Cloning of the mutant led to virus populations, which showed again a clear heterogeneity in their cleavage patterns similar to the uncloned virus. Blotting experiments permitted the detection of DNA homologies to the inserted sequences exclusively in Nki-4 DNA, but not in DNA derived from normal human cell lines or KB-cells. Origin and biological function of the inserted DNA sequences remain to be clarified.

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**On the Structure of Poliovirus**

KLAUS WETZ and KARL-OTTO HABERMEHL

Cross-linking of poliovirus with bifunctional imidoesters reveals VP1 and VP3 to be adjacent proteins. Enzymatic iodination of VP1 by the lactoperoxydase-method shows this protein to have partial contact with the surface of the protein shell. This result is also supported by tryptic proteolysis of the AEC (artificial empty capsid), resulting in a predominant cleavage of VP1. Chemical reaction with a lysine-specific monofunctional reagent mostly modifies VP1 and VP3 whereas VP2 is modified to less extend and VP4 not, although each of the proteins contains a certain number of lysine. This refers VP1 and VP3 to be in contact with the surface of the protein shell, whereas VP2 and especially VP4 should be more inner proteins.

This assumption fits together with experiments concerning UV irradiation of the virus which very efficiently leads to intramolecular cross-links of the RNA and cross-linking of the capsid proteins to the RNA. Analysis of these proteins covalently linked to the RNA shows that VP4 by far has the most contact with the RNA, which means in all probability this protein to be located towards the inside of the capsid. Next to this protein VP2 has the highest amount bound to the RNA. Since VP2 is poorly accessible for chemical modification one might assume that this protein is mostly buried inside the capsid shell. VP1 having contact with the RNA as well as with the surface of the shell might therefore have a more elongated shape. VP3, adjacent to VP1, rather is positioned towards the outside as it has poor contact with the RNA.

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**Cross-linking of Poliovirus by UV Irradiation**

KLAUS WETZ

When poliovirus is submitted to UV irradiation structural and functional changes occur: 1. intramolecular cross-linking of the RNA; 2. intermolecular cross-linking of RNA-protein; 3. cross-linking of protein-protein; finally 4. splitting of the RNA.

29 Zbl. Bakt., 1. Abt. Orig. A 246
These structural modifications are responsible for the loss of infectivity of the virus. After very short periods of irradiation cross-linking of the RNA predominantly occurs, being one of the main reasons for the decrease of infectivity. Cross-linking of RNA-protein also appears after short times and increases after longer times of irradiation. Protein-protein cross-linking and RNA split products, visible after longer periods of irradiation, scarcely are responsible for the loss of infectivity.

Cross-linking of the RNA is very efficient pointing to a close package inside the virus. In contrast to the protein-protein cross-links the RNA-protein cross-links are cleavable and the proteins bound can be detected after RNase digestion. The smallest capsid protein VP4 is predominantly associated with the RNA, followed by VP2 and VP1 while VP3 is bound only in a small amount.

UV irradiation causes conformational changes of the capsid and reveals a new 100S RNP particle of the same ratio of RNA to protein as in the 155S virion. Irradiation renders this RNP RNase sensitive which then causes further degradation to AEC (artificial empty capsid).

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Replication of ME Virus at a Poliovirus-Induced Membrane Complex

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Poliovirus induces a smooth membrane complex in HEp-2 cells in the presence of 2.6 mM guanidine, which inhibits poliovirus replication. Under these conditions when host cell RNA- and protein synthesis are already shut off, the cell still allows a superinfecting picornavirus - the guanidine resistant ME virus - to exploit the polio-induced complex and to modify it successively into an ME virus dependent complex.

The poliovirus-induced membrane complex is characterized by a sedimentation coefficient of 410S. When cells are preinfected for 4 hours with poliovirus in the presence of guanidine and subsequently superinfected with ME virus for another 6 hours, this complex becomes heavier; its sedimentation coefficient is increased to 515S. After superinfection of 10 hours the complex sediments at 700S and is similar to a component which is induced after single infection with ME virus.

The replication of the superinfecting ME virus takes place at the polio-induced complex. It could be shown that the heavy complexes modified by ME virus contain an RNA polymerase activity specific for ME virus. Single stranded RNA, “Replicative Form” and “Replicative Intermediate” of ME virus could be isolated from the heavy complexes as demonstrated by gel electrophoresis.

The utilization of the poliovirus-induced membrane complex results in a shortened latent period of the superinfecting ME virus, which means that a polio preinfected cell fits the replication of ME virus better than a noninfected cell: ME virus is reproduced with a latent period shortened by more than one hour (from about 5 h.p.i. to 4 h.p.i.) when the cell is preinfected with poliovirus for at least three hours in the presence of guanidine.
Multiplication of the thermosensitive mutants 141 and 216 of echovirus 12 is impaired at 40 °C; however, these mutants induce in the infected cell RNA and protein synthesis in near-to-wild type amounts. It could be shown that the observed incorporation of 3H-uridine into acid-precipitable material indeed reflected synthesis of the virus-specific species of RNA sedimenting with 35S and 18S. The proteins synthesized in mutant-infected cells at 36 °C and 40 °C were analyzed by SDS-PAGE: a protein pattern typical for echovirus 12 was observed, except that a band comigrating with VP4 was missing in the 40 °C extracts. This demonstrates that the viral 35S RNA is capable to serve as messenger. To test the hypothesis that ts 141 and ts 216 are defective in maturation extracts from cells infected at 36 °C and 40 °C with these mutants were submitted to sucrose gradient centrifugation: neither intact virions sedimenting with 156S nor 80S and 13-14S particles, the presumptive precursors of assembly, were detected in extracts from cells infected at 40 °C; all three types of particles were present in the 36 °C extracts. Obviously, ts 141 and ts 216 are defect in a step before the formation of 13-14 S particles. Isoelectric focussing of mutant virion particles showed that the mutant particles had an IEP slightly different from that of wild type. We conclude that the mutants ts 141 and ts 216 carry a defective in structural protein (VP4?) which may be the cause for the impairment of maturation.

8 thermosensitive mutants of echovirus 9, strain Barty, were isolated after mutagenesis with 5-fluorouracil. The plating efficiency on GMK cells of these mutants was reduced at 40 °C as compared to 36 °C: the ratio of plaques formed at 40 °C to that at 36 °C was $10^{-2}$ to $10^{-4}$. All 8 mutants were considerably less pathogenic for newborn mice than the parent strain: the PD₅₀ in PFU/mouse was $5 \times 10^3$ for the Barty wild type, and $8 \times 10^4$ to $> 10^6$ for the mutants. A biochemical characterization was initiated to possibly establish a correlation between the nature of the defect and the virulence of the mutants. Heat stability of virus particles in vitro was used as a direct test for a defect in structural protein: 5 of the 8 mutants, ts 35, ts 75, ts 88, ts 115 and ts 117 showed a heat stability at 44 °C which was different from wild type; consequently, they are supposed to carry a mutation in one of the genes coding for capsid protein. In isoelectric focussing one of the mutants, ts 219, was found to behave slightly different from wild type; thus, ts 219, too, probably is a structural protein mutant. The analysis of the mutant capsid proteins by SDS-PAGE yielded for all 8 mutants a band pattern indistinguishable from that of the Barty parent strain. More detailed investigations of the capsid proteins by fingerprint techniques and isoelectric focussing are in progress. A clear correlation between pathogenicity and biochemical properties of the mutants could not be established so far.
Strain Barty, a mouse-pathogenic echovirus 9 and strain Hill, the apathogenic prototype of echovirus 9 were characterized and compared in biological and biochemical terms. Both strains replicate in GMK cells but not in HeLa or KB cells. The Barty virus forms plaques under an agar-overlay, whereas plaque formation of the Hill virus is blocked under these conditions; under an agarose-overlay, however, both strains form plaques. Infectivity of the Hill strain is more thermolabile in vitro than that of Barty. Barty virion particles sediment in a sucrose gradient with 156S — as observed with all other picornaviruses investigated so far —, whereas the particles of the Hill strain form fast sedimenting aggregates under the same experimental conditions. Isoelectric focussing of the virion particles revealed that Barty virus exists essentially in one conformation with a characteristic IEP; purified virions of the Hill strain, however, were found to represent a mixture of particles with various IEP's. The protein patterns of the two strains in SDS-PAGE being mostly similar, showed a characteristic difference: VP1 of Barty is a double band, whereas Hill contains a single band in a position just between the two VP1 bands of Barty. To get an idea about the relevance of the biochemical properties for pathogenicity, we started to characterize an echovirus 9 field strain, isolated in our laboratory, and three variants of the Hill strain which had acquired the capacity to form plaques under agar. The field strain apparently resembles closely the Barty strain; the Hill variants have the protein composition of the parent, but, interestingly, they resemble in other regards, e.g. behaviour in sucrose gradients, the Barty strain.

During an outbreak of infections on a maternity ward in October 1978 a highly pathogenic enterovirus was isolated. By cross-neutralization tests and neutralization kinetics it has been identified as an echovirus 11 prime strain. In search for markers of pathogenicity we compared prime and prototype strain by some biological and biochemical parameters. Both strains are nonpathogenic for newborn mice. Both strains form plaques under an agarose overlay, whereas under agar only Porz virus is plaque-forming. In single cycle growth curves the prototype is slightly delayed as compared to Porz virus and the final virus yield of the prototype is 10 times lower. The adsorption kinetics of both strains to GMK cells is very similar as measured by infectivity. In SDS-PAGE the VP3 of Porz and prototype virus differs markedly. According to first results also differences are found in VP1 as revealed by proteolytic digestion of the isolated bands and subsequent SDS-PAGE. Isoelectric focussing, however, showed no differences between both viruses. Porz virus is stable under saccharose gradient centrifugation in low salt concentration, whereas the prototype no longer forms a band under these conditions (aggregates?).
A Circumscribed Outbreak of Enterovirus Infections on a Maternity Ward

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In October 1978, a 6 day-old child became ill on a maternity ward with clinical signs of a septicemia and died. Subsequently, 6 additional children suffered from aseptic meningitis (birth dates: Sept. 16; Oct. 1; 3; 5; 8; 9), 5 of them within the first 10 days after birth, one child 4 weeks old. From these 6 children all of whom required intensive care, virus was isolated at various times of disease and from various sites (CSF, pharynx, rectum). A total of 25 isolates was recovered. The agent was difficult to type, but in cross-neutralization tests it turned out to be a prime strain of echovirus 11. Though further environmental investigations in 49 people yielded no additional isolate, the path of infection could be reconstructed by clinical and serological data. Determination of neutralizing antibodies of the IgM and IgG class was helpful to identify recent infections, and to obtain information on the type specificity of the IgM reaction. A laboratory infection with the agent permitted to collect detailed virological and serological data.

Is Coxsackievirus B4 Involved in Chronic Cardiopathy?

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Using the determination of Coxsackievirus (C.v.)-specific neutralizing IgM-antibody as a diagnostic method in cases of clinically presumed viral cardiopathy, we could distinguish two types of kinetics of these antibodies. In cases of acute cardiac disease the IgM-titers dropped after about 20 weeks below a titer of 1:8, our threshold of detection. We have found 6 cases of chronic cardiac disease, however, in which C.v. B4-specific IgM-antibody persisted for up to more than two years in the presence of an unusually high titer of total C.v. B4 antibody. We find this result interesting in view of the data, which correlate the presence of IgM-antibodies with the presence of the corresponding antigen in the organism.

As the kinetics of IgM-antibodies in acute non-cardiotropic C.v. infections resembled those in acute cardiac C.v. infections (as diagnosed by the presence of specific IgM-antibody), the pathogenesis of these diseases might be similar. For the pathogenesis of chronic cardiopathy with C.v. B4-IgM-antibody persistence, however, additional factors might be required, the nature of which is unknown up to now.

Concentration of Enteroviruses from Water by Means of Seitzfilters

J. STEINMANN

Virological investigations, using poliovirus type I, were carried out on the detection of enteroviruses from seawater, surface water, and sewage by means of Seitzfilters.
When examining the influence of pH on the adsorption to the positively charged filters the best results were obtained at pH 7.0. Several eluents were compared. It was found that beef extract (3%, pH 10.0) was an effective eluent. Experiments with different types of water (5-10 l) showed that the efficiency after adsorption to Seitzfilters and elution with beef extract was 70-90%. Preliminary results of investigations in a sewage treatment plant indicate that Seitzfilters are suitable for monitoring the efficiency of various treatment processes for removing humanpathogenic enteroviruses.

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**Infectivity and Pathogenicity of Myxoviruses**

R. ROTT

The objective of the studies presented was to define a molecular basis for infectivity and pathogenicity of influenza virus. It is demonstrated that activation of the HA glycoprotein by posttranslational proteolytic cleavage is indispensable for the formation of infectious influenza virus. There are two preconditions for influenza virus to be pathogenic: 1. The presence on the virus particle of a cleaved HA molecule essential for the infectivity, and 2. an optimal genome composition. In naturally occurring avian influenza viruses there is a direct correlation between the cleavability of the hemagglutinin, the potential of the virus to be produced in infectious form in a wide range of host cells, and their pathogenicity for chicken. It is concluded that nature selects an optimal gene constellation for each individual field strain. In these viruses the structure of the haemagglutinin is the determining factor for pathogenicity.

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**Influenza A: Host Range Recombinants and Mouse Virulence**

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Recombination between fowl plague virus FPV/Rostock (Hav1N1) and mouse-lung adapted human strains England/1/61 (H2N2) resp. PR8 (HON1) results in recombinants with high pantropic potential and neurotropism in the suckling mice.

We could show that a combination of the hemagglutinin from FPV (Hav1) with certain polymerase proteins from mouse-lung adapted strains in recombinants produces generalized infections in mice. Infectious virus could be isolated from lungs, brains and blood even after intranasal infection.

A correlation between pantropic potential in vivo and the ability to replicate in mouse-embryo-fibroblasts could be shown.
Comparative Structural Studies on the Hemagglutinin of Pathogenic and Nonpathogenic Influenza Viruses

F.X. BOSCH

Previous studies showed that among naturally occurring avian influenza viruses the structure of the hemagglutinin (HA), i.e. its proteolytic cleavability in a wide spectrum of host cells directly determines the pathogenicity of the virus (Bosch et al., Virology 95, 197-207, 1979).

In the present studies the HA of seven Hav1 influenza strains (four of them being pathogenic for fowl) were biochemically analyzed. Both uncleaved HA as well as HA fractionated by different methods were investigated by one- and twodimensional gel electrophoresis technics including isoelectric focussing.

The results obtained indicate that the structural basis for the biological differences of the viruses is localized on the HA₁ subunit of the hemagglutinin; whereas the HA₂ subunit appears to have a highly conserved structure among both pathogenic and nonpathogenic viruses.

The Hemagglutinin-Gene of Different Hav1-Influenza Virus

VOLKER VON HOYNINGEN-HUENE

Amongst the different avian influenza viruses, those belonging to the Hav1 serotype are excepional in that they include both pathogenic as well as apathogenic strains. Pathogenicity has been shown to be determined by the proteolytic cleavability of the hemagglutinin (HA) molecule. Thus, among these strains, there must be structural variation in the HA-glycoproteins.

We have attempted to study the genetic basis of this phenomenon. ³²P-labelled viral RNA of seven Hav1-strains was prepared and separated by PAGE. Isolated RNA-4-segments were analyzed by molecular hybridisation and RNase-T₁-finger-printing. Whereas the hybridisation data show a clear genetic relationship of declining order, this is not completely supported by the fingerprint patterns. This indicates, that they do not descend one from another, but belong to different lineages which might have developed from a common ancestor.

Serological and Biochemical Studies on Structural Variability of Influenza Virus Neuraminidase

FRIDHELM MAYWALD and F.X. BOSCH

The neuraminidase of influenza viruses can be distinguished serologically into different subtypes. Also within a particular serological subtype antigenic differences can be found.
In our investigations of the neuraminidases of the N1-type we hoped to obtain knowledge on the structural differences underlying the antigenic variability. Serological studies showed that the antigenic alteration of neuraminidases from human and animal pathogenic strains - in a manner similar to the hemagglutinins - progress in a determinable direction. The serologically distinguishable enzymes show a distinct difference in their molecular weight and peptide composition. The results will be discussed in view of the basic mechanism of the neuraminidase variability.

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Characterization of a Temperature-sensitive Mutant of an Influenza Recombinant with a Defect in the NS Gene

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We undertook to clarify the as yet undetermined function of the nonstructural (NS) protein of influenza virus by isolation of ts mutants with a defect in the NS gene. From 30 ts mutants obtained by mutagenizing an influenza virus recombinant with RNA segments from fowl plague virus and virus N, we could identify by recombination and hybridization experiments one mutant with a defect only in RNA segment 8 (NS gene). This mutant was characterized by biochemical and biological tests. The results show that at the nonpermissive temperature RNA and protein synthesis are normal except for hemagglutinin. No intact virus particles are assembled. Although the NS protein is synthesized early in the replication cycle, the findings presented suggest that at least one of its function is a late one.

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Nonstructural Proteins (NS) with Altered Migration on SDS-Urea Polyacrylamide Gel Electrophoresis (PAGE) Synthesized in Cells Infected with Temperature Sensitive (ts) Mutants and a ts-Recombinant Clone of Flowl Plague Virus

SUSAN SPRING1 and C. SCHOLTISSEK

Two temperature-sensitive (ts) mutants and one ts recombinant of fowl plague virus were isolated and each was chosen to have a ts lesion in the gene coding for the NS protein on the basis of complementation and recombination tests with 7 groups of previously characterized ts mutants. These ts clones synthesized an NS protein which had an altered migration on PAGE in 25% SDS-urea gels. Revertant clones from one mutant had an NS protein which differed in migration from the original wild type parent and from the ts mutant. The second mutant also had a P2 protein with altered migration on PAGE. The fact that the parental virus for these ts clones was a recombinant heterozygous for RNA segments 3 and 6 and possessed a recombinant segment 5 may have led to the ready isolation of 3 clones with a ts lesion in the NS segment. With the aid of these mutants it should be possible to get more information concerning the function of the NS protein.

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Intracistronic Complementation with Fowl Plague Virus

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In the case of influenza A viruses one has temperature-sensitive (ts) mutants which possess at least one defective gene out of the 8 single stranded RNA segments of the genome. The ts mutants of KP-virus can be divided into 7 of the possible 8 recombination groups. ts 18 and ts 236 had not yet been definitely assigned to one of these groups. On the basis of hybridization experiments they clearly belonged to recombination group 3 (RNA synthesis negative, transport defect) however, they were distinguished from the other ts mutants by their plaquing behaviour. After recombination with other ts mutants in the same recombination group, plaques were formed, which could not be further passaged. The uncommon plaque behaviour which was observed with ts 18 and ts 236 can be explained by intracistronic complementation. - This was confirmed by further biological tests.

Reconstitution of the Membrane of Influenza Virus

R. T. C. HUANG

Envelope glycoproteins of influenza virus were artificially incorporated into liposomes, using octylglycoside as detergent which could be removed by dialysis. The prepared liposomes contained spike-structures and had specific hemagglutinin and neuraminidase activities. The total amounts of proteins incorporated into liposomes were the same irrespective of the lipid composition. Similarly, the neuraminidase activity of liposomes remained unchanged by altering lipids. However, the hemagglutinating and cell-adsorbing properties of liposomes varied greatly depending on the composition of lipids. Electron microscopical examination revealed that reconstituted viral membranes containing the cleaved hemagglutinin could fuse with cell membranes whereas those containing uncleaved hemagglutinin could not. It was concluded from these results, that membrane fusion is involved in the penetration of influenza virus into host cells and that cleavage of hemagglutinin is necessary for this fusion process.
gp II, with molecular weights of 100,000 and 80,000, respectively. When analysed in the
presence of mercaptoethanol gp I was observed as a single, uncleaved polypeptide chain,
whereas gp II was found to consist of two subunits, gp 65 and gp 30. Evidence was ob-
tained that gp I and gp II share common amino acid sequences. Analysis of intracellular
protein synthesis revealed that gp I is the primary gene product which may be converted
into gp II by proteolytic cleavage. – Trypsin treatment of CEF grown virions, which contain
predominantly gp I, converted this glycoprotein into gp II; the specific infectivity of such
preparations was increased up to 50-fold. These results indicate that cleavage of gp I into
gp II is essential for maximal viral infectivity.

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Studies on the Receptor-Destroying Activity of Influenza C Virus

A. NAGELE, G. HERRLER, and H. MEIER-EWERT

The receptor-destroying activity of influenza C virus (JHB/1/66) was investigated by
adsorption onto and elution from chicken erythrocytes. – It was found, that viruses grown
in CEF-cells showed more or less the same time course of elution, regardless whether gp I
was converted to gp II by trypsin or not. – After elution, the polypeptide pattern of intact
virus was found in the supernatant. – When the virus was pretreated for 30 min at 50 °C
to 60 °C the binding capacity to erythrocytes could be increased. – Pretreatment of the
virus for 30 min at 49 °C reduced the amount of elutable virus to 50% of the controls.

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Inhibition of Influenza C Virus Replication by Actinomycin D, α-Amanitin
and UV-Light

THOMAS PETRI and HERBERT MEIER-EWERT

The effect of specific inhibitors of host cell nuclear RNA synthesis on the virus replica-
tion of influenza C was studied in chick kidney cells and compared with the results found
for influenza A virus. It was shown that similar doses of the inhibitors produced a com-
parable reduction in virus-yields for both types of influenza virus. The inhibition of the
virus protein synthesis in infected cells is dependent on the concentration of the drugs and
on the time of addition. The inhibitor-sensitive phase for influenza C virus is slightly longer
compared to influenza A. – The result of this study shows that this characteristic feature
of other orthomyxoviruses is shared by influenza C virus.
Study on the Immunity Against Influenza C in Humans and Animals

C. PFEIL-PUTZIEN and H. MEIER-EWERT

440 human sera were tested for antibodies against influenza C in haemagglutination inhibition (HI) tests, using virus strain C/JHB/1/66 as antigen. It was found that 43% of the total possessed HI-antibody titers \( \geq 1:10 \). In the age groups 0-5, 6-10, 11-15, 16-20 and \( \geq 25 \) years the highest percentage of positives (62%) was found in the 11-15 year old patients. From 113 sera out of 7 animal species (cow, horse, pig, sheep, roe, dog, rabbit) only 5 sera (2 sheep and 3 roe) showed HI activity after rigorous treatment against nonspecific inhibitors. Further investigations must confirm the presence of true antibodies in these animal species. In addition, we describe the virus isolation from a seven months old child who showed mild upper respiratory tract symptoms. The virus isolate was characterized as a typical influenza C virus and designated C/Bavaria/1/79.

Alteration in the Protease Susceptibility of a Glycoprotein of NDV Induces Change in Pathogenicity

W. GARTEN und H.-D. KLENK

Chemically induced mutants of the apathogenic NDV strains La Sota and Ulster have been isolated that exhibit an altered susceptibility of glycoprotein F to proteolytic cleavage. Cleavage of glycoprotein HN is not altered with the mutants. Fingerprint analysis of the tryptic peptides indicates that the mutation resulted in an altered primary structure of glycoprotein F. With the other viral proteins no differences were found between mutants and wild type viruses. In contrast to the wild types, the precursor \( F_0 \) is activated by proteolytic cleavage, when the mutants are grown in MDBK and BHK21 cells. The mutants are, therefore, able to undergo multiple cycle replication in cells that are permissive for the wild types. This increase in host range at the cellular level is paralleled with one mutant by an increase in pathogenicity for the chick embryo. These observations support the concept that the susceptibility of the glycoproteins to proteolytic cleavage is an important determinant for the pathogenicity of this virus.

ELISA for Detection of IgG- and IgM-Antibodies After Mumps Virus Infection

M.-E. NICOLAI-SCHOLTEN und W. HÖPKEN

An ELISA (Behringwerke Marburg) has been used to titrate specific IgG- and IgM-antibodies in sera from patients with known virological and clinical data.
On the whole 45 acute and convalescent serum samples from patients were tested in parallel for IgG- and IgM-antibodies to mumps virus by the ELISA, in addition 42 acute sera were tested for IgM-antibodies. In 1 patients sera the development of IgG- and IgM-antibodies before, during and up to 14 months after a mumps virus infection was controlled. Also examined for crossreacting antibodies were 23 paired sera from patients of whom, parainfluenza virus Type 1, 2 or 3 was isolated from a throat swab.

Both tests were more sensitive than the complement fixation, antibodies were detected earlier and with higher titers. Low IgG-titers were also partially found in sera from patients with a parainfluenza virus infection. These results restrict the mumps specificity of the IgG-ELISA. The IgM-test in contrast was specific, no crossreacting antibodies between mumps and parainfluenza viruses were detected. The IgM-ELISA is suitable for rapid detection of mumps virus antibodies in single sera taken in the acute phase of illness. But it must be strongly stressed, that for correct interpretation of a positive IgM-result complete clinical data on the patient must be known.

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Antigenic Relationships Between Measles and Canine Distemper Viruses

JOHN R. STEPHENSON and VOLKER TER MEULEN

The morbilliviruses, Measles, Canine Distemper, Rinderpest and Peste des petits ruminants are highly contagious agents which cause diseases of clinical importance. Many studies have been carried out to characterize antigenic relationships among these viruses. Although cross-neutralization has been frequently reported among them, attempts to analyse the degree of the cross-reactivity lead to many conflicting reports. Using immune precipitation with hyperimmune rabbit sera, convalescent measles sera and sera from patients with subacute sclerosing panencephalitis, followed by gel electrophoresis, antigenic relationships between the individual polypeptides of Measles and Canine Distemper viruses have been examined. No antigenic differences were observed between virus isolates from patients with acute measles or subacute sclerosing panencephalitis. Antigenic cross-reactivity was present between the L, N, F0, F1 and M polypeptides of measles and Canine Distemper viruses. However, as no cross-reactivity with rabbit hyperimmune sera was observed between the H polypeptides, these were interpreted as strain-specific antigens. Furthermore, the N polypeptides were interpreted as group-specific antigens since they showed the highest degree of cross-reactivity. Both convalescent measles sera and sera from subacute sclerosing panencephalitis showed high antibody titres to all measles antigens except the L and M polypeptides. However, these sera contained only low activities to the N and F1 polypeptides from Canine Distemper virus.

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Determination of the Concentration and Quality of Rubella Virus Antibody Molecules

G. HOPPE

A method for the determination of the concentration and quality of rubella virus antibody molecules has been developed by appropriate modification of the method of equili-
Sodium-23 filtration. The technique has been used for determining the concentration and quality of rubella antibodies in humans present at different time intervals after rubella infection. It was found that the quality of antibodies changes from low quality to high quality antibodies within 60-80 days after onset of rubella rash. Therefore, determining the quality of rubella antibody molecules can be utilized for discriminating between recent and past rubella infection. Since it was found that the difference of quality of antibodies is not due to the relative concentration of IgM and IgG antibodies, this discrimination is independent from the presence or absence of rubella IgM antibodies.

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Antibody Development after Rubella Vaccination post partum in Previously Rubella-Seropositive Women

H. W. DOERR, M. KAPP, and W. SCHMIDT

56 non-selected women, who displayed rubella-wildvirus induced antibodies (1: \geq 16/HIT), were subcutaneously vaccinated with the rubella strain HPV77/DE5 (Behring, Marburg, FRG) 1-2 days post partum (p.p.), 2-3 months post vaccinationem (p.v.) the development of the specific antibodies was tested in parallel with blood samples drawn off at the date of vaccination. In 29 cases we found titer changes in the rubella HIT (6 \times 1:32/64, 11 \times 1:128/256, 5 \times 1:512/1024, 7 \times 1:2048/4096), in 21 (11) cases we observed a (significant) titer rise (first titers: 13 \times 1:16-64, 4 \times 1:128/256, 4 \times 1:512/1024) and in 6 persons a decrease, among them 2 significant ones (each from 1:512 to 1:128). The rubella HIT subsequent to IgM immunadsorption (SPIT) was found positive in only 1 case (p.v.).

Serologic investigations using other virus antigens presented the following results: a) virus-specific IgM positive: CMV/ELISA (9 women p.p., 7 ones furthermore and 6 ones additionally p.v.), HSV and VZV/ELISA (1 p.v.), EBV/IFT (0); b) significant titer rises in CFT: CMV (6), EBV (2), HSV (5), VZV (2), Mumps (0).

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Significance of Carbohydrate Chains for Conformation and Biological Functions of Semliki Forest Virus (SFV) Glycoproteins

GEORG KALUZA

The envelope glycoprotein E1 and the precursor glycoprotein p62, from which the envelope glycoproteins E4 and E3 are derived, occur in infected cells in two different antigenic forms. One of them is "mature" and has a conformation corresponding to that of the glycoproteins in the SFV envelope. The other one is "immature" and converted into the final form by modification which includes glycosylating processes. The final conformation remains stable during release of virus and is stabilized by intramolecular disulfide
bonds. Reductive cleavage of these bridges alters physical and immunological properties of E₁ and E₂ causing loss of infectivity and of hemagglutinating activity of the virion. A reformation of disulfide bonds at original positions is possible, if the proteins remain in the membrane. It was not observed after dissociation of virus by detergents. The findings indicate that the conformation of the glycoproteins achieved due to a proper composition of the carbohydrate side chains, is very important for their correct transport to a defined target and thus for virus release. The biological functions of the virus particle similarly depend on the tertiary structure of the membrane constituents, while the carbohydrate side chains appeared to be without direct influence.

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Isolation of Infectious RNA and Replicative Intermediates of European Swine Fever Virus

BERND FRENZEL and HINRICH MEYER

Possible members of the genus “Pestivirus”, also designated as “non-arbo togaviruses”, are the viruses of bovine viral diarrhea/mucosal disease (BVD/MD) and European swine fever/hog cholera (ESP/HC). A serological relationship between the viruses of BVD/MD and ESP/HC was described. This antigenic relationship, however, is not definitely confirmed by biochemical data.

To show that the RNA of ESP/HC virus is infectious and that infected cells contain replicative intermediates, and to provide the probes for hybridisation experiments, we isolated both forms from infected cells. Infected cells were used because we could not get sufficient quantities of purified virus from the supernatant of tissue cultures for the isolation of infectious RNA.

For the isolation of infectious RNA PK-15 cells were infected with 10 moi. and incubated for 48 hours. After the incubation period the RNA was extracted three times by the phenol-SDS method, incubated with DNA' se and extracted again with phenol-SDS. The remaining RNA was sedimented in a sucrose gradient (15-30% sucrose, 16 h, 4 °C, 55,000 × g, Spinco SW 27) and fractionated. In transfection experiments infectious ESP RNA was determined in the 42 S region of the sucrose gradient, as shown by the immunofluorescence test. For the isolation of replicative intermediates we proved the incorporation of 3H-uridine into TCA-insoluble material in the presence of different concentrations of actinomycin D. We found that most of the 3H-uridine was TCA-insoluble 9 hours post infection in the presence of 2 µg/ml actinomycin D. At this time we isolated the RNA as described before. Than we precipitated the single-stranded and partial single-stranded RNA by 2 M LiCl. A sample of the remaining soluble material was sedimented in a sucrose gradient (5-20%, 149,000 × g, 12 °C, Spinco SW 50). The radioactive material banding in the region of 20 S was found to be resistant against RNA’se A thus suggesting the presence of double-stranded RNA. In melting experiments in the presence of 0.1 M NaCl in 0.01 M Tris/HCl, pH 7.4, the melting temperature was determined to be above 80 °C.
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Protein Synthesis in Coronavirus JHM Infected Cells

H. WEGE

The murine coronavirus JHM induces a variety of central nervous system disorders in mice and rats and can be used as a model for studying virus induced demyelination. This report deals with the polypeptide composition of the virus and the synthesis of JHM specific proteins in infected cells.

The JHM virus has been labeled with radioactive sugars and aminoacids, purified and analysed by polyacrylamide gel electrophoresis. The virus contains six major proteins, four of which are glycosylated. The molecular weights of the proteins range from 22,000 to 170,000. During infection host cell protein synthesis is shut off. Three major and four or five minor proteins are synthesized at late times of infection. Only four of these proteins are specifically immunoprecipitated by antiserum raised against purified virus, suggesting that the virus specifies non-structural, as well as structural proteins. Combining pulse chase experiments with immunoprecipitation using anti-JHM serum indicates that the four immunoprecipitable proteins are processed by cleavage and glycosylation to yield all six virion proteins. A model describing the synthesis of these proteins will be presented.

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In vitro Translation of the Virus JHM mRNA Encoding the Major-Corona Structural Protein, p60

S.G. SIDDELL

The major protein synthesized in JHM infected cells comigrates with the virion structural protein p60. Polyadenylate-containing RNA extracted from the cytoplasm of infected cells directs the synthesis of a similar, if not identical 60,000 molecular weight polypeptide in messenger-dependent cell free systems derived from both mouse L cells and rabbit reticulocytes. The identity of the in vitro synthesized polypeptide was established by comigration with virion p60, specific immunoprecipitation with antiserum raised against purified p60 and by tryptic peptide fingerprinting. The polyadenylate-containing cytoplasmic RNA which encodes p60 sediments in formamide-containing sucrose gradients at 17S. The in vitro translation of messenger RNA released from infected cell polysomes by puromycin and fractionated on formamide-containing, sucrose gradients indicates that it is a subgenomic RNA that functions as the messenger for p60 in the cell. This is the first evidence that the replication strategy at coronaviruses involves, in common with other positive-stranded RNA animal viruses, subgenomic mRNAs.

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Retinal Alteration in Untreated and Immunosuppressed Borna Virus Infected Rabbits

H. KREY, M. GIEREND, and H. LUDWIG

During the course of Borna virus infection in rabbits the virus reaches the eye along the optic nerve. A characteristic multifocal retinopathy appears at the ocular fundus which
finally results in blindness of the experimental animal. Due to accumulation of antigen in the retinal pigment epithelium focal immunopathological reactions occur which eventually involve the entire retina. Histology reveals focal destruction of the outer retinal layers and perivascular lymphocytic infiltrates in the choroid. Further experiments under immunosuppression showed that only small spotlike lesions appeared at the ocular fundus and that involvement of the entire retina could be stopped. Some animals did not show any retinal focus. Compared to the untreated group only a minimal amount of choroidal inflammatory reaction could be seen under immunosuppression. - The immunopathological reaction and its interruption by immunosuppressive treatment is discussed in detail.

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The Structure of the Ribonucleic Acid of Infectious Bursal Disease Virus (IBDV)

H. MÜLLER and G. STEGER

Infectious bursal disease virus (IBDV) is the causative agent of a highly contagious disease of young chickens (Gumboro disease) with severe necrotic lesions in the bursa of Fabricius. We could show that the genome of IBDV consists of two segments of double-stranded ribonucleic acid (dsRNA) with molecular weights of $2.2 \times 10^6$ daltons. For comparative purposes, infectious pancreatic necrosis (IPNV) a virus of salmonids also containing a bisegmented dsRNA - was included in our studies. The dsRNAs of both virus types are very similar with respect to their molecular weights, GC-contents and melting behaviours. In view of their prominent structural characteristics of the genome it seems justified to place both of these viruses into a separate taxonomic group.

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Characterization of a New Infectious Agent Detected in Various Human Cytomegalo- and Herpeszoster Virus Stocks

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Whilst testing the susceptibility of mink lung cells to several strains of CMV and VZV, it was found in many cases that plaques developed and cell lysis occurred 3–4 days after infection. Detailed analysis showed that this CPE was not due to CMV or VZV but to an infectious agent which has the following properties. This new agent grows lytically on mink lung cells, swine, bovine and lamb kidney, and lamb testicular cells. It grows without
producing morphological changes and with a low titre on human fibroblasts. The double-stranded DNA of the agent sedimented closely to HSV DNA marker in velocity gradients in neutral sucrose and has a buoyant density of 1.684 g/ml in CsCl. The DNA from different isolates when cleaved with 19 different restriction enzymes, for any of them gave very similar patterns. These patterns were not like those of any previously analysed animal virus. Protein analysis showed no similarities between these isolates and other known viruses. An analysis of 1.292 human sera samples showed that 2% had a NT titre of 1:640–1:2560, and the agent has been directly isolated from a patient with acute swelling of the parotid gland. Our results demonstrate that the majority of the CMV and VZV stocks, and fresh isolates contain this unknown agent the presence of which can be easily screened for on mink lung cells.

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**Modifications of the Solid Phase Radioimmunoassay for Detection of Hepatitis A Virus (HAV) and Hepatitis A Antibodies (Anti-HAV)**

G. DÖRING and B. FLEHMIG

From purified human IgG with a high antibody titer against hepatitis A virus F(ab')₂ and Fab fragments were prepared. IgG, F(ab')₂ and Fab each were radiolabelled with ¹²⁵I and each of the preparations was used as a tracer in RIA for the detection of HAV and anti-HAV and anti-HAV-IgM. The influence of time of incubation and temperature on the immunoreactivity is shown. The results are discussed with respect to diagnostic of hepatitis A.

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**Isolation of Hepatitis A Virus Particles with Different Physicochemical Properties**

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Particles of hepatitis A virus (HAV) were purified from a series of stool samples by means of repeated banding in CsCl and sedimentation through sucrose gradients. It could be shown that individual samples differed with respect to buoyant density, sedimentation behaviour and polypeptide composition of the isolated particles. HAV particles banding at 1.34 g/ml in CsCl and sedimenting with 160 S revealed a spectrum of 4 polypeptides comparable to the pattern reported for enteroviruses. Two of these polypeptides, however were either completely absent or present only in trace amounts in particles banding around 1.33 g/ml and sedimenting with less than 160 S. Yet, the latter particles contained at least one additional polypeptide with a molecular weight of between 43000 and 50000. The significance of these findings with respect to antigenicity and infectivity of individual HAV samples is discussed.
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The Significance of Demonstrating Anti-HBc of the IgM Class (IgM-Anti-HBc) for the Diagnosis of Acute and Chronic Hepatitis B Infection

M. ROGGENDORF, F. DEINHARDT, G. G. FRÖSNER, R. SCHEID, and B. BAYERL

A sensitive enzymeimmunoassay was used to determine IgM-anti-HBc (Gerlich et al., J. med. Virol., in press). - Anti-μ coated flat-bottomed microtiter plates were incubated with dilutions of patient serum and later with HBcAg extracted from human liver. After another incubation with peroxidase coupled anti-HBc, orthophenylenediamine was added and the optical density (O.D.) measured. - The specificity of the test was proved by testing IgM- and IgG fractions of sucrose gradients from sera of patients with fresh hepatitis B and by testing rheumatoid factor positive sera. The test proved to be highly sensitive. In sera of patients with fresh hepatitis B IgM-anti-HBc could be demonstrated in dilutions up to 10^{-7}. - Six months after onset of hepatitis, IgM-anti-HBc could be demonstrated in 82% of the tested patient sera. In the sera of 11 of 12 patients with histologically proved chronic active hepatitis and in 96% of 27 HBeAg-positive carriers of HBsAg, IgM-anti-HBc could be demonstrated. The usefulness of the IgM-anti-HBc test as a diagnostic parameter is restricted by the high prevalence of IgM-anti-HBc in chronic hepatitis B infection. The demonstration of IgM anti-HBc in chronic hepatitis over a period of several years may be explained by an immunological inductive persistence of HBcAg.

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Significance of IgM-Anti-HBc for Blood Donor Screening

G. G. FRÖSNER, M. ROGGENDORF, F. DEINHARDT, B. BAYERL, R. SCHEID, and R. ZACHOVAL

According to Hoofnagle and coworkers (New Engl. J. Med. 298, 1579, 1978) HBsAg negative but anti-HBc positive blood may be infectious for hepatitis B. This raises the question whether anti-HBc positive blood should not be used for blood transfusion. 61 of 1966 (3%) Bavarian blood donors investigated here were anti-HBc positive and HBsAg and anti-HBs negative. IgM-anti-HBc is present during the convalescent stage of acute hepatitis B and usually also in patients who have developed HBsAg positive chronic liver disease after acute hepatitis B. Therefore it may be a better indicator for infectivity of sera than determination of total anti-HBc (IgG and IgM). The determination of anti-HBc of the IgM-class diminished considerably the number of potentially infectious blood donors: only 2 of 61 anti-HBc positive blood donors (one was also anti-HBe positive) showed IgM-anti-HBc. Both donors had relatively low anti-HBc titers (50% inhibition titer) (IgG and IgM) of < 1:100 measured by radioimmunoassay (Corab, Abbott). In addition, 7 of 63 anti-HBc and anti-HBs positive donors were also IgM-anti-HBc positive.

If further studies indicate that a considerable number of IgM-anti-HBc positive donors transmit hepatitis B to recipients, this relatively low percentage of positive donors (about 0.5%) can be discarded of without creating a blood supply problem. Only some of the
so-called "healthy" HBeAg negative carriers of HBsAg which are usually not infectious are IgM-anti-HBc positive (41 of 123 = 33%), whereas most patients with chronic aggressive hepatitis show this marker (11 of 12 = 92%). Therefore IgM-anti-HBc may not only be a marker for continuing viral replication, but also for the activity of chronic liver disease.

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A Soluble System for the Replication of Adenovirus Type 2 DNA

THERESIA REITER, CHRISTINE LALLY, and ERNST-L. WINNACKER

Although the general pattern of Adenovirus type 2 (Ad2) DNA replication is well understood (1), the functional organization of this process has remained elusive. In order to approach this problem, a soluble system has been developed which is able to replicate exogenously added Ad2 DNA. The protein extract with the desired specificity is obtained by low-salt extraction of nuclei isolated between 8-12 h. p.i. of Ad2 infected HeLa cells. The most favorable substrate at protein concentrations of 5-10 mg/ml is the virion derived protein/DNA complex. Only background synthesis was observed in the absence of viral DNA, in the presence of viral DNA without the terminal protein and in the absence of ATP. The product synthesized under optimal conditions for DNA synthesis was characterized through sedimentation analysis, electron microscopy (kindly performed by Dr. H. Delius, Heidelberg) and restriction enzyme digestion. The specificity of the system was attested to by the presence of the terminal protein on the 5'-termini of newly initiated DNA strands. Studies have been initiated on the properties of extracts derived from mutant virus infected cells, on the protein composition of the extract, the role of host factors as well as 5'-5' Dinucleoside tetraphosphates in the initiation process. (This work was supported by DFG grant Wi 319/5 and 6).

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Recombination Events in Adenovirus Infected Cells

CHRISTIAN MEINSCHAD, KARIN ULLRICH, and ERNST-L. WINNACKER

This presentation describes an approach towards the analysis of recombination events in human cells. The model system used in this study are the human adenoviruses type 2 and 5 which had been shown previously to recombine efficiently in their permissive host cells (1). Our assay is based on the observation, previously employed in the mapping of adenovirus mutants (2), that the double-stranded genomes of these related serotypes when digested with certain restriction endonucleases, share certain restriction sites while other are lacking in one or the other parent. Upon recombination between these DNA molecules new DNA fragments arise which are not present in digests of either of the parental DNA
molecules and which are easily distinguished and recognized by gel electrophoresis. Using this rapid approach we could indeed demonstrate the presence of recombinant DNA fragments in mixed infections with the wildtypes 2 and 3. Since the recombination frequencies increase from 5% in the first to over 30% in subsequent mixed infections, we have been able to isolate single recombinant virus clones. An analysis of the crossover events on the nucleic acid as well as on the protein level is in progress.

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Tupaia Adenovirus. I. Isolation and Characterization

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An adenovirus was isolated from a kidney of an apparently healthy Tupaia (tree shrew) and termed (TAV). Electron microscopy of purified TAV revealed the presence of typical adenovirus particles with a capsid diameter of about 72 nm. The host range study revealed that Tupaia kidney cells are the cells of choice for the efficient propagation and the plaque assay of TAV. This cell clone was used for the continued propagation of TAV. The buoyant density of TAV DNA was 1.706 g/ml as determined by isopycnic CsCl centrifugation. Analysis of TAV DNA by different restriction endonuclease revealed specific DNA pattern. The molecular weight of the viral DNA was found to be $2.15 \times 10^6$ daltons. TAV hemagglutinated rat and human O-type erythrocytes. One line of identity between human adenovirus and TAV was found by immunodiffusion test. TAV immortalised some mammalian cells. The characterisation of these immortalised cells and an extensive study of the oncogenicity of TAV in vitro and in vivo is required for the final classification of TAV as an oncogenic adenovirus.

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Tupaia Adenovirus. II. Physical Map of the Genome

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A physical map of the Tupaia adenovirus (TAV) was constructed. DNA extracted from virions by standard procedures was cleaved with different restriction endonucleases and the cleavage sites of Bam HI, Eco RI, Kpn I, and Sma I were determined from the sizes of the restriction fragments obtained after complete, incomplete, and double digestion. Fragment sizes were determined by agarose gel electrophoresis and the correlation between the position of the Eco RI restriction fragments and the base distribution in the viral genome was established by partial denaturation mapping.
Analysis of the Structural Proteins of Avian Adenoviruses

INGE MAICHLE-LAUPPE and G. MONREAL

The virion polypeptides from 7 strains of avian adenoviruses out of 5 different serotypes were compared by SDS-PAGE: FAV 1 (OTE); FAV 4 (KR 5); FAV 5 (340); FAV 7 (YR36); FAV 8 (TR 59) (764) (HUNG VI). – Each serotype displays a characteristic protein pattern. Adenovirus strains belonging to the same serotype (764, TR 59, HUNG VI) show almost identical virion polypeptides. However DNA restriction enzyme cleavage of these 3 strains reveals genome differences. – Polypeptide analysis of empty capsids compared to infectious virions allows a preliminary identification of viral core proteins. Their molecular weights of 8000–14000 daltons are considerably smaller than those of human adenoviral core proteins.

Different Expression of the Common Antigenic Determinants of Herpes Simplex Virus Type-1 (HSV-1) and Bovine Herpes Mammillitis (BHM) Virus

G. PAULI, B. NORRILD, and H. LUDWIG

HSV and BHM virus share a common antigen. The cross reacting determinants are carried by a glycoprotein with an apparent mol. weight of 125000. In serologic investigations a clear one-way neutralisation can be observed. The goal of the following studies was to measure the expression of the common antigenic sites at the surface of infected cells. This was done with the binding of IgG and 125I labeled Protein A, as well as with an antibody depending cytotoxicity test. It could be shown that the common antigen is exposed on HSV infected cells, but not on BHM virus infected cells. The different localisation will be discussed.

Histone Synthesis in Human Fibroblasts Infected by Human Cytomegalovirus

K. RADSAK and B. SCHMITZ

Serum-starved human foreskin fibroblasts were infected by human cytomegalovirus (Towne strain) that is thought to induce host cell DNA replication during lytic infection (1). At various times postinfection the cultures were pulse labelled with either ³H-thymidine or ¹⁴C-thymidine and ³H-lysine in order to examine DNA synthesis and histone synthesis, respectively. Isopycnic centrifugation of labelled DNA in CsCl revealed that precursor incorporation into host cell DNA was enhanced over the control around 24 hours postinfection and was decreasing after onset of viral DNA synthesis which reached a peak.
around 72 hours postinfection. For analysis of histones $^3$H-lysine-labelled proteins of lysates of unfractionated cells and of chromatin preparations were subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and subsequent fluorography. Comparison of the fluorograms from the various pulses postinfection suggested that $^3$H-lysine incorporation into histones exhibited no major variations concurrent to the changes of host cell DNA synthesis. In contrast, herpes simplex virus type I was found to progressively extinguish histone synthesis in the course of the cellular infection. Furthermore, histone synthesis in phosphonoacetic acid-treated HCMV-infected cultures was not enhanced over that in mock-infected controls. These observations do not support the view that human cytomegalovirus induces host cell DNA replication under the conditions used.

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Type Specific Differences in the Release of Newly Synthezised Herpes Simplex Viruses from HEP-2 Cells

H. HAMPL, J.R. SCHLEHOFER, and K.-O. HABERMEHL

Depending on the type of herpes simplex virus two different ways of virus release can be observed: release of newly synthesised virions from flat areas of the cellular membrane on the one hand (HSV-1) and from microvilli on the other hand (HSV-2).

Comparative SEM and TEM (stereo surface replica) investigations on the HSV-1-infected (strain THEA) HEP-2 cells show newly synthesised viruses in large amounts on the surface of both rounded up and fused cells whereas cells infected with the strain HFEM of HSV-1 exhibits only a few virus particles on the cellular membrane. Less virus particles can be detected on the surface of HEP-2 cells after infection with HSV-2.

Different mechanisms of the release of virus particles can be observed in cells infected with HSV-2. The virions bud mainly from the microvilli (strain D 316) or filopodia (strain DD) whereas in cells infected with HSV-1 large amounts of virus particles are released from microvilli-free areas of the cellular membrane.

Identification of virus particles on the surface was performed by measurement of the particle-size in high resolution stereo surface replicas and by HSV-specific antibodies. In addition single particles or budding processes were analysed by tilting and rotating the specimen in the goniometer stages of both the scanning and the transmission electron microscope.

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Cell-Line and Virus-Strain Depending Differences in the Morphology and Permeability of Herpes-Virus-Infected Cells

J.R. SCHLEHOFER, H. HAMPL, and K.-O. HABERMEHL

Herpesviruses induce in culture cells different morphological and functional alterations of the cellular membrane depending on the type or strain of the virus as well as on the cell line. These differences in the cellular response concern (aside from the various CPE's):
- the permeability for $^{51}$Cr: enhanced permeability of the membrane by HSV-2 in HEp-2 cells or by HSV-1 in chick embryo fibroblasts (CEF), but reduced permeability by HSV-1 in HEp-2 cells.

- the resistance to detergent: enhancement of the stability of the membrane by HSV-1 in HEp-2 cells in contrast to CEF or human embryo fibroblasts (HEF).

- the structure of the surface of the infected cells: relatively low alterations by HSV-1 or HSV-2 in HEp-2 cells (depending also on the virus-strain) but severe alterations in CEF (more severe alterations by HSV-1 than by HSV-2) and moderate changes in HEF.

- the mechanisms of the release of the virions: differences between HSV-1 and HSV-2-particles.

- the maturation of the virions: typical maturation of virus by budding from the inner nuclear membrane in HEp-2 cells, but atypical maturation steps with many unenveloped nucleocapsids in the cytoplasm in CEF where the particles bud from cytoplasmic membranes.

The surface alterations are demonstrated by comparative scanning- and transmission-electron microscopic studies of identically prepared coverslip cell cultures.

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Supergiant Herpesvirus Genomes

ANGELIKA EBELING¹, BERNHARD FLECKENSTEIN¹, NICOLE BERTHELOT², and PETER SHELDRICK²

Human cytomegalovirus contains linear double-stranded DNA-molecules of about 150 megadaltons (md). Thus the cytomegalovirus-genome has a significantly higher molecular weight than the DNA-molecules of all other known herpesviruses. We found two new herpesviruses, Herpesvirus aotus type 1 and type 3 (H. aotus 1 and 3), possessing “supergiant” DNA-molecules also. Intact H. aotus 1 and 3 DNA shows a density of 1.714 g/ml after analytical centrifugation in CsCl gradients, corresponding to an average G + C-content of 55%. Density centrifugation of fragmented DNA reveals an intramolecular density heterogeneity; about 25% of DNA fragments have about 49% G + C, while the majority of the sequences band at the position of 57% G + C DNA. Sedimentation coefficient (ca. 65S) and contour length (ca. 22 PM 2 units) of H. aotus 1 and 3 DNA indicate about 143-148 md. When single strands of H. aotus 3 were examined in the electron microscope, foldback structures became apparent which allow to conclude on the presence of inverted repetitive sequences. The genomes consist of a long ($UL = 105$ md) and a short ($Us = 19$ md) unique region, flanked at both sides by inverted repetitive sequences of 1.5 md and 2.4 md, respectively. Thus the general plan of those genomes is the same as in human cytomegalovirus. Reassociation kinetics, $T_m$-determination of heteroduplex-molecules and comparative cleavages with a number of restriction-endonucleases (Eco RI, Kpn I, Hind III, Sac II, Sal I, Sma I, Xba I) indicate a high degree of base-sequence-homology between H. aotus 1 and 3. A certain degree of base-sequence-homology was found by cross-hybridisation between DNA of H. aotus 1 and 3 and human cytomegalovirus.
Experimental Infection and the State of Viral Latency in Adult Tupaia with Herpes Simplex Virus Type 1 and 2; Infection of Juvenile Tupaia with Temperature Sensitive Mutants of HSV Type 2

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The susceptibility of adult Tupaia belangeri (tree shrew) to infection with herpes simplex virus (HSV) was investigated. Adult animals were inoculated intraperitoneally with different HSV 1 or 2 (10^5-10^6 plaque forming units per animal). All HSV strains had a killing effect irrespective of the age of the animals with the exception of HSV-2, strain HG-52. The highest pathogenicity of HSV 1 and 2 in these animals was expressed by the predominant clinical picture of a lethal herpetic hepatitis. The reaction of the juvenile Tupaia to infection with temperature-sensitive mutants of HSV was also investigated. Information concerning this reaction is important because the physiological body temperature of the tupaia varies from 36 °C at night to more than 41 °C during daytime. It was found that juvenile tupaia completely survived an infection with temperature-sensitive mutants of HSV-2 HG-52. Although these infections were not lethal, they induced a protection against a second infection of a lethal dose of wild type HSV regardless of type and strain. Infectious HSV was recovered from the spinal cord of those animals which had survived the viral infection at low dose. This indicates that HSV resided in a latent state in Tupaia. The genomes of the recovered viruses were also compared to the viral DNA of the inoculated virus using restriction endonucleases.

Studies in Ectromelia: Epidemiology, Oral Immunization, Transfer to Other Species

G. T. WERNER

Epidemiological studies in colonies of mice showed that ectromelia is transmitted rather through the respiratory tract. The infection through the percutaneous route is less significant. Oral vaccination with vaccinia virus does not guarantee a reliable protection of the single animal against a subsequent challenge infection with ectromelia virus (50 x LD50). The oral vaccination, however, can stop the spread of an epidemic going through a colony of mice. The mousepox virus causes disease in rats, if the animals are immunosuppressed through total body irradiation (150 g weighing rats were given 2 x 350 R). The animals showed clinical symptoms, the mortality was 30%. Ectromelia virus was isolated from spleen, liver and lungs. In immunocompetent animals ectromelia virus caused no symptom. If induced an immunity against poxviruses. In rabbits ectromelia virus produced an immunity against a challenge infection with otherwise lethal doses of vaccinia virus strain Elstree.
Antioncogenic Activity of Two Influenza A strains on Polyoma Virus Induced Tumors in Newborn Wistar Rats

G. NOSS¹ and G. STAUCH²

Simultaneous application of influenza virus and polyoma virus to newborn Wistar rats resulted in significant reduction of tumor rates as compared to controls given polyoma virus, only. This antioncogenic activity (AOA) could be established for two influenza A strains (A/PR 8 and A/Hongkong). The AOA was comparable under the different application schedules used: injection of polyoma and influenza virus on different sites or as a combined vaccine. It was found that the growth of brain tumors was suppressed to a similar degree as that of renal sarcomas. Ether treatment of influenza virus, tested on strain A/PR 8, did not decrease its antioncogenic potential. In one experiment, however, only a significant reduction of brain tumors was seen, while the rate of renal sarcomas did not differ significantly from that of controls.

Experimental and Clinical Studies of the Virostatic Agent Adenin-Arabinoside-Monophosphate

H. STICKL, G. WERNER, and L. EGERER

Adenin-Arabinoside (Ara-A) acts virostatic against several DNA-viruses. The low solubility of the compound poses certain problems. The monophosphate of Ara-A, Ara-AMP, is 50-times more soluble. It is highly effective in experimental encephalitis with herpes simplex virus and vaccinia virus in mice and in rabbits. Special emphasis was laid upon the question, if in an successfully treated experimental encephalitis any morphological damage or late effects can be seen in the brain. The brains of mice were examined six weeks after infection with vaccinia virus strain Ma 1 and subsequent treatment with Ara-AMP (100 mg/kg). The histology revealed minor meningeal irritation (cellular infiltration of the meninges and the meningeal vessels) in 1/3 of the cases. In 2/3 of the animals the histology was normal. In herpetic keratitis the combined treatment of debridement of the epithelial lesion and subsequent application of Ara-AMP ointment were highly successful.

Inhibition of Virus Replication by pppA₂p₅A₂p₅A

H. BUGANY, M. NAIN, and R SIEGERT¹

In Interferon (IF) treated cells the activity of the synthetase of an oligonucleotide, with the structure pppA₂p₅A₂p₅A, is strongly increased (1). This nucleotide activates

¹ With support of DFG.
a mRNA degrading nuclease in vitro and appears to be one of the pathways for antiviral cell activity of IF. We studied the effect of this nucleotide and a derivative carrying no triphosphate on the 5'-end, on the reproduction of virus in the plaque-reduction test or focus-test. We found strong inhibition of plaque formation by Vesicular stomatitis virus in rabbit kidney cells, Vero cells and primary chicken embryo fibroblasts; by Herpes virus in rabbit kidney cells and by Semliki forest virus in primary chicken embryo fibroblasts, when using both nucleotides at a concentration of 260 μM, but not by Semliki forest virus in BHK 21-cells and by Polio virus in HeLa-cells. Inhibition occurred only, by treatment of the cells at the time of virus infection, pretreatment or treatment after infection were without effect. There was also strong inhibition of the reproduction of Rous sarcoma virus in primary chicken embryo fibroblasts as well as in transformed cells. We assume that all these different effects are due to a variable penetration of the nucleotides into the cells. Our findings indicate that the pppA3-dependent antiviral cell activity inhibits the growth of viruses from all classes.

We thank Dr. Friis for cooperation on the Rous sarcoma virus assay.

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Inhibition of Antiviral Activity of Interferon by Ganglioside and Ganglioside-Derived Sialo-Oligosaccharides³

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Gangliosides (G.) specifically bind to interferon (I.) thereby inhibiting its antiviral activity (1). The binding was shown to be partly reversible by free sialic and sialyl-lactose indicating that the ceramide of G. might not be essential for the ligand-I. interaction (2). Presently we compared the ability of G. and G.-derived sialo-oligosaccharide to inhibit the antiviral capacity of interferon.

The results show:
1. The infection of rabbit kidney culture cells by VSV is not influenced by pretreatment of cells or virus with sialo-conjugates.
2. Mono-sialo-gangliotetraitol, the reduced sugar moiety of G. GGtet₁ (II₃ Neu-Ac-GgOse₄-Cer) inhibits interferon antiviral action although to a lesser degree than parent G.

A stronger inhibition is achieved with a dimeric form of the lipid-free sialo-sugar biz (monosialo-gangliotetraientyl) amine, that may possibly provide two interferon binding sites.

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³ Supported by DFG.
Stabilization of Isometric DNA Viruses against Thermoinactivation by Lowered Ionic Strength

R. WIGAND¹, GABRIELE SPRUNCK¹, P. BACHMANN² and G. BRANDNER³

The thermostability of isometric DNA viruses is increased, if the ionic strength is being diminished before heating. If unpurified virus material from cell cultures is heated under conditions (temperature, time), which lead to a diminution of infectivity of 3 to 4 log₁₀, this titer diminution is 1 to 3 log₁₀ less, if the ionic strength is decreased by diluting the material in dist. water (1:100) before heating. A dilution in Eagle's MEM (1:100) does not have the same effect. This property was found in members of 4 DNA virus families: adenovirus 5, herpesvirus 1, SV40 and bovine parvovirus. In contrast, members of pox-(vaccinia) and picornaviruses (polio 2, coxsackie B3) were found to be less thermostable under conditions of low ionic strength. The observed effect may be of practical importance for heat desinfection of viruses and for their persistence in the environment.

Data Processing in Clinical Virology

K.-O. HABERMEHL

Efficient virus diagnosis requires on the one hand an exactly defined course of different procedures covering a broad spectrum of viruses. On the other hand, to achieve useful results for the physician, it is necessary to combine all clinical data of one individual patient with all laboratory results, in order to obtain an interpretation. To accomplish these requirements, we developed a data processing system for clinical virology with the following concept:

1. All personal of the Institute should be able to handle the system without any special knowledge.
2. Using a dialogue-system, one should be able to give the informations into the computer in clear text or in abbreviations of any variable length of the clear text. Code numbers should be avoided as far as possible.
3. All data of patients are stored indefinitely, remaining available for immediate access and integration with the data of a new submission of the same patient.
4. According to the input of the submissions, the system prints detailed working instructions for the different working places of the laboratory, mentioning the old titers or results from the patient, in order to facilitate the course of the examination.
5. After the input of the results, the system prints a diagnostic survey with all results and clinical data including the data, diagnoses and remarks of the old submissions. This enables the virologist to give a preformed interpretation of the results, a virological diagnosis or remark.
6. Upon request, the system gives a detailed letter to the doctor with the old and new data including a virological interpretation in form of a diagnosis with additional remarks.
7. The integrated system allows a detailed statistical analysis of all data with any desired logical connection including alphabetical listing of the patients and of the data files, or establishing of a serum bank.

Using a small decentralized computer-system, one has immediate access to all data, including individual changes in the data files. - The system has been proven since 3 years in our diagnostic work with submissions from 15000 patients per year.

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Comparative Evaluation of Three Preparation Techniques in Diagnostic Electron Microscopy

H. GELDERBLOM, H REUPKE, and I. TISCHER

Electron microscopy may help considerably in rapid viral diagnosis and in cases of noncultivable agents. Thus negative staining of diagnostic specimens is performed routinely in many laboratories. However, little is known about the efficiency of the current preparation techniques. In praxi, the concentration of particles within the specimen often impedes the successful morphological diagnosis.

Using 3H-labeled adenovirus we have therefore reexamined and compared quantitatively, A the conventional 2-step preparation technique, B sedimentation of particles onto the grid using the Airfuge and C agar-agarose filtration.

In addition the influence of methodological parameters, i.e. choice and pH of the contrast stain, surface properties of the grid, and time dependence onto the quality of the E.M. specimen was investigated. Quali- and quantitative aspects of the different preparation techniques will be discussed with special consideration of routine diagnostic procedures.

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Problems of Electron Microscopic Diagnosis of Rotavirus Infection

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The electron microscopic (EM) detection of rotavirus in stool samples is limited by low stability of the virions. Standard methods (preparation: negative staining, PTA 2%, pH 7.2 and EM-investigation: time/grid in EM, 20 minutes) show a significant correlation between storage (time and temperature) and detectable virus concentration (particle per grid per time). Freezing and thawing of virus suspensions (commercially available rotavirus antigen and rotavirus bovine strain) revealed that virus particles were no longer detectable in EM and the infectivity was lost. Storage at 6 °C and 18 °C, respectively, shows a successive decrease of virus concentration and after 4 days no virus particles could be detected in the electron microscope.
By the use of a specific immunosorption to insoluble adsorbentia (controlled-pore glass, polystyrene particles) the separation of IgM and IgG is performed in serum specimens which originate from patients presenting different virus infections, for the virus-specific IgM proof with routine serological methods (CFT/CMV; HIT/rubella, measles, mumps; NT/HSV). The specificity of the IgM antibodies was examined with the ME method, the IgG contamination of the particles with peroxidase-labelled anti-gamma antibodies.

The results of the virus-specific IgM tests seen in the HIT’s are in a good agreement to the conventional methods (Ig separation by density gradient centrifugation) resp. to significant titer rises. The new method, however; was found a little bit less sensitive (especially in the serodiagnosis of measles and mumps). While the new technique was also successfully applied for the detection of neutralizing IgM and IgA antibodies to HSV 1, no sufficient results are available to demonstrate CMV specific IgM antibodies by CFT compared to other methods (IFT, ELISA).

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Comparison of Various Methods of Determining Antibodies Against Herpes Simplex Virus Type 1 Proved ELISA as the Only Assay for Selecting Sero-Negative Patients

J. HILFENHAUS, F. BEHRENS, and H. MOSER

Anti herpes simplex virus type 1 (HSV1) antibody titers of sera from adult donors were determined by the complement fixation (CF), the microneutralization and the ELISA test. The most sensitive method for antibody determination was the ELISA while the CF assay and the microneutralization assay were considerably less sensitive. Although antibody titers obtained with these three methods did not correlate very well, it may be concluded that ELISA antibody titers were up to 40 fold higher than neutralizing antibody titers and up to 100 fold higher than CF antibody titers. Due to the higher sensitivity of ELISA only 3 of 36 blood donors tested proved to be seronegative with this assay while 9 donors of the same group were found seronegative with the microneutralization assay. In vitro stimulation of peripheral lymphocytes with a partially purified HSV1 particle antigen was achieved for all ELISA seropositive donors, including those lacking neutralizing antibody
titers. Thus only ELISA seronegative patients reacted negatively in this stimulation assay. We may therefore conclude that ELISA is an appropriate method of selecting human seronegative patients who are also negative with respect to their specific cellular immune response whereas donors with negative CF or neutralizing antibody titers cannot be considered as in fact seronegative.

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Improved Application of the Indirect Immunofluorescent Tests for Early, Rapid and Differential Serological Diagnosis of Virus Diseases

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The human lymphoid cells (Raji, RPMI 8226), maintained as suspension cultures, synthesize numerous virus antigens (influenza-, adeno-, RS-, mumps, measles-, herpes-, varicella-, cytomegalo-, rubella-, coxsackie-, Echo-virus) after infection. Standardized volumes of infected, acetone-fixed cells on slides are suitable for detection of specific serum IgG, IgM and IgA types of antibodies. The prepared slides can be stored without alteration for at least one year at \(-20^\circ C\), and for at least six months at \(4^\circ C\) in an air tight container. The clinical trials with measles and mumps diseases proved the high specificity and good practicability of these tests. The epidemiological and clinical findings in the case of influenza infection indicate the possibility for subtype specific differential diagnosis.

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Isolation of Leucocytes from Trouts for Virological Studies

P.-J. ENZMANN

Leucocytes from the peripheral blood of rainbow trouts were isolated by two steps of centrifugation in density gradients. The blood cells were centrifuged first into a cushion of "Uromiro" followed by two density gradient centrifugations in "Percoll". Four bands could be identified. About \(10^3\) cells from each band were centrifuged onto microscope slides and then stained with fluorescent antibodies. Isolated leucocytes from uninfected trouts could be infected and assayed for virus after about 40 h. Granulocytes and lymphocytes were visible with a specific fluorescent pattern. Isolated leucocytes from trouts infected 6 weeks ago and showing no signs of disease were analyzed for virus directly after isolation. As above, several cells containing viral antigens could be detected. The described method offers the possibility of diagnosis in the case of persistent VHS-infection.