Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Abstracts of Papers Presented at the Session of the Virology Section of the Deutsche Gesellschaft für Hygiene und Mikrobiologie, Martinsried, March 25–28, 1987

Received July 1, 1987

Herpes Simplex Virus Type 1 (HSV 1) and SV40: Inhibition of Early Gene Expression by Human Tumor Necrosis Factor (TNFα) and Interferon (IFN) α and γ

ALTINKILIC, BORA, M. FRITSCHE, M. LIPP and G. BRANDNER

Abt. Virologie, Inst. f. Med. Mikrobiologie u. Hygiene, Univ., D-7800 Freiburg; Inst. f. Biochemie, Univ. D-8000 München

IFN-α arrests in human fibroblasts HSV1 (McIntyre) gene expression before translation of viral immediate early (IE) proteins, possibly by suppression of normal-length viral transcripts (Lipp, Brandner, 1981, 1984). In contrast, Muñoz and Carrasco (1984) maintained that in Hela cells the HSV1 (KOS) protein and virion formation are complete IFN-α resistant. However, we demonstrate that the viral IE protein translation of both virus strains, KOS and McIntyre, is IFN-α sensitive in both, human fibroblasts and HeLa cells, though significantly less in the latter. In addition, we found that hu. rec. TNF-α, alone or in a synergistic combination with hu. rec. IFN-γ, impedes HSV1 IE protein formation likewise, whereas SV40 early gene expression is more sensitively arrested by IFN-α than by TNF-α plus IFN-γ and is almost resistant against TNF-α alone. Finally we observed that TNF-α alone or in combination with IFN-γ was unable either to increase or to accelerate the cytopathic effect of cells previously infected with HSV1 (human fibroblasts), SV40 (TC7) or rubella virus (vero cells).

Supported by DFG grant Br 281.12.1.

HBsAg can Induce Interferon Gamma in Peripheral Blood Lymphocytes

VOTH, R., S. ROSSOL, G. HESS, H. KIRCHNER and K. H. MEYER

ZUM BÜSCHENFELDE

1. Med. Klinik und Poliklinik, Univ., D-6500 Mainz

Deutsches Krebsforschungszentrum, Abt. Tumorvirologie, D-6900 Heidelberg

Treatment of peripheral blood lymphocytes (PBL) from healthy donors with purified HBsAg resulted in interferon gamma production in a dose dependent manner. PBL were purified
by Ficoll-Paque and adjusted at 5 × 6" cells/ml. Interferon was measured by a highly sensitive enzym-immunoassay, proven to be free of any crossreactivities. 10 ng/ml of HBsAg peaked on day 3 of incubation and resulted in 40 to 60 units of interferon gamma. Interferon alpha could not be detected in the supernatant at any time of incubation. Interferon gamma production by HBsAg was regulated by PGE1, since coincubation of PGE2 together with HBsAg yielded in decreased interferon gamma titers, whereas indomethacin treatment together with HBsAg resulted in 200 units of interferon gamma. TNF, which is known to have antiviral properties similar to those of interferons two fold elevated interferon gamma production, when coincubated with HBsAg.

Despite Low LCM Virus-Specific Cytotoxic T Cell Activity the LCM Virus is Efficiently Eliminated from the Spleens of Acutely Infected Mice

LEHMANN-GRUBE, F. and D. MOSKOPHIDIS

Heinrich-Pette-Inst. f. Exp. Virologie u. Immunologie, Univ., D-2000 Hamburg

The mechanism with which higher organisms control virus infections is uncertain. According to one widely accepted hypothesis cytotoxic T lymphocytes (CTL) destroy infected cells in vivo, thereby blocking further virus replication. In the BALB/c mouse LCM virus-specific CTL are restricted solely by the class I antigen "L". In the BALB/c-derived mutant C-H-2^d" L is altered and cannot act as a restriction element. Although few if any LCM virus-specific CTL are generated, these mice are perfectly capable of eliminating the virus. Also, transfer of immune cells from mutants into infected BALB/c mice or vice versa efficiently accelerates virus elimination from the recipients. We interpret these observations to mean that CTL play a central role in the control of the LCM virus in the mouse, however not by lysis virus-infected cells but rather by releasing (or inducing release of) factors that inhibit virus replication in nearby cells.

Interaction between the PreS2 Domain of Hepatitis B Virus and Native Human Serum Protein

WADECK, F., K.-H. HEERMANN, A. LENZ, and W. H. GERLICH

Dept. Med. Microbiology, Univ., D-3400 Göttingen

We generated a monoclonal antibody (Q19/10) which binds to the glycosylated preS2 domain of hepatitis B viral surface (HBsAg). Binding of this antibody is inhibited by chemically cross-linked human serum albumin and by normal human serum. The natural inhibitor is not present in animal sera except chimpanzee serum, it has a low titer, and it elutes in gel chromatography with monomeric albumin. The preS2 epitope is masked in vivo either partially or completely, depending on the HBsAg titer. Purified HBsAg contains closely bound albumin. In vitro, preS2 does not bind normal human serum albumin, but it binds glutaraldehyde modified monomeric albumin. We suggest that a small subtraction of albumin (approx. 10 pmol/ml) is modified in vivo in a way binds to the surface of hepatitis B virus.
Transactivation as a Possible Mechanism of Hepatitis B Virus (HBV) Associated Oncogenesis

HOFSCHEIDER, P. H., MADLEN WOLLERSHEIM, and P. ZAHM
Max-Planck-Inst. f. Biochemie, D-8033 Martinsried

Transactivation has recently been demonstrated for certain pathogenic and transforming viruses. This finding raised the question whether a transactivating mechanism is implicated in HBV associated malignant transformation. In the experiments reported here we focused on the HBx gene whose function is still unknown. The HBx gene was cloned and expressed in an SV 40 expression vector as well as under the control of the HBs gene promoter. After cotransfection with plasmids containing the bacterial CAT gene being expressed either by viral (SV 40, MMTV, HTLV I, RSV) or cellular promoters (thymidine kinase) a transactivation was demonstrated. Interestingly, a single copy of HBV DNA was found to be sufficient for transactivation in cells of appropriate specificity. As we could show for the hepatoma Ul, an integrated HBV DNA sequence (Ul.4) connected to human DNA sequences also caused transactivation, showing a considerably stronger transactivating effect than the HBx gene alone. It is therefore concluded that a positive feedback effect on the x promoter is exerted by the HBx gene product. Further investigations will have to demonstrate whether the mechanism described is indeed involved in the process of malignant transformation.

Serological Evidence for the Expression of the Reverse Transcriptase of Human Hepatitis B Virus

STEMLER, MARIETTA¹,², JULIA HESS², R. BRAUN², H. WILL³, and C. H. SCHRODER¹

¹ Inst. f. Virusforschung, Deutsches Krebsforschungszentrum, D-6900 Heidelberg;
² Inst. f. Med. Virologie, Univ., D-6900 Heidelberg;
³ Max-Planck-Inst. f. Biochemie, D-8033 Martinsried

Human sera were examined by immuno-blotting for antibodies to the reverse transcriptase of human hepatitis B virus (HBV) believed to be encoded in the P open reading frame (P-ORF). Sera from patients with self-limited (n = 58), acute (n = 23), and chronic (n = 8) hepatitis were analysed. One of the sera with markers of a prior infection and two sera of chronically infected patients reacted with a protein synthesized in E. coli that contains the middle part of the predicted polymerase. Other E. coli fusion proteins containing overlapping or different regions of P-ORF were used to localize antigenic domains. Antibody binding to two different domains representing the middle and the C-terminal region of P-ORF was obtained in the sera of chronically infected patients. Our findings are indirect evidence for the in vivo expression of the complete HBV polymerase reading frame.
Isolation and Characterisation of DHBV Related Hepatitis B Viruses

SPRENGEL, R., E. F. KALETAl, and HANS WILL

Max Planck Inst. f. Biochemie, D-8033 Martinsried; 1 Inst. f. Geflügelkrankheiten, D-6300 Giessen

So far, avian hepatitis B viruses have been detected only in Pekin ducks. Two isolates (DHBV 3 and 16) have been cloned and sequenced. Aim of this study was (i) to characterize supposedly hepatocarcinogenic DHBV isolates of brown spotted ducks of China, and (ii) to investigate whether DHBV related viruses exist in other avian species. A large number of sera from different avian species was tested by DNA spot hybridisation for the presence of DHBV related viral DNA. Positive signals were obtained with sera from Chinese ducks, German geese and herons. Several isolates were cloned, sequenced, and the host range was determined by infection of German ducks and geese. The analysis revealed a close relatedness of all avian hepadna viruses derived from ducks or geese, and no indication for a major genetic difference of Chinese DHBV isolates which could explain its putative hepatocarcinogenic property. DHBV from geese and Pekin ducks did not show species specific sequence differences and were infectious in both avian species. In contrast, the heron hepatitis B virus sequence is highly divergent from DHBV. Preliminary results suggest that this virus is not infectious in ducks or geese.

Studies on the Characterization of the Duck Hepatitis B Virus (DHBV) Associated Polymerase

RADZIWILL, G. and VALERIE BOSCH

ZMBH, Univ., D-6900 Heidelberg

The Hepadna virus P-open reading frame probably codes for the virus associated polymerase. Until now, however, P-frame sequence specific antisera, which react with fulllength P-frame products synthesized in vitro, were unable to detect authentic in vivo P-proteins in virions or in intracellular viral cores. Alternatively, the enzymatic activity can be used to monitor and characterize the viral polymerase. Conditions could be found to open viral cores such that they are permeable to macromolecules but fully retain polymerase activity. After restriction enzyme digestion, only fragments carrying the protein covalently bound to the viral minus strand remain associated with the opened cores and can be labelled by the endogenous reaction. The viral polymerase did not accept any of a number of exogenous templates. This tight association with endogenous template and/or core is an unusual property of the DHBV polymerase.
Procaryotic Expression of gag- and env-Genes of HIV and the Application in Immunoblots

HARZENETTER, S., O. ERLWEIN, A. LENZ, J. v. HINTZENSTERN, G. JAHN, M. BRÖKER, and B. FLECKENSTEIN

Inst. f. Klin. u. Molekulare Virologie, Univ., D-8520 Erlangen

To obtain diagnostic reagents for the detection of acquired immunodeficiency virus (HIV) exposure, we have expressed viral antigens representing gag and env proteins. The vectors preferentially used had been constructed for expression of large quantities of fused proteins in E. coli (Gene Anal. Techn. 3 (1986) 53–57). The plasmid carries the E. coli lac promotor and portions of the E. coli lac Z gene. Stable fusion proteins were electrophoretically transferred and incubated with HIV-antibody positive human sera. Reactive bands were compared with those from immunoblots done with proteins of gradient purified virions; the results of Western blotting with the fusion proteins containing epitopes of gag p15, p17, p24, and env gp41 were at least as sensitive as with conventional ELISA, immunofluorescence, immunoblot, and commercially available ELISA-tests based on recombinant proteins. Several examples with critical sera emphasized the reliability of Western blots with bacterially expressed proteins.

Absence of Anti HIV-2 in Drug Addicts and Haemophiliacs in Munich

GÜRTLER, L. G., W. SCHRAMM, J. EBERLE and F. DEINHARDT

Max v. Pettenkofer Inst., Med. Klinik Innenstadt, Univ., D-8000 München

Anti HIV-1 was found in drug addicts in 1986 in 30–40% (71 of 236 males and 58 of 130 females) and in 85 out of 166 haemophiliacs (51%). Using LAV-2 from L. Montagnier we established an anti HIV-2 ELISA assay and for confirmation an indirect immunofluorescence assay and Western blot test. – Until now we have screened more than 120 drug addicts, only one showed anti HIV-1 and a reaction with gp36 of HIV-2. Of the 70 haemophiliacs screened for anti HIV-2, two anti HIV-1 containing sera showed also staining of gp36. Presently nobody can determine if this kind of reaction with the gp36 is due to a double infection of these patients with HIV-1 and 2, or if this is caused by immunological cross-reaction of anti gp41 with the glycoprotein of HIV-2.

Detection of Antibodies to HIV II (LAV II) in German- and West-African Patients by Immunofluorescence and Immunoblotting

SCHMITZ, H. and A. MEYER

Virol. Abt., Bernhard-Nocht-Inst., D-2000 Hamburg 4

The HIV II obtained from Dr. L. Montagnier was cultivated in different T-cell-lines (CEM, Molt-4). Using the indirect immunofluorescence-technique cross-reactivity of the sera of 227 patients with HIV I infections with HIV II infected CEM-cells was found in
about 10%. In contrast all sera with HIV II infection showed a strong reaction to both HIV I and HIV II antigens. The specificity of the immune reaction was controlled by cross-absorption techniques, using either HIV I or HIV II infected T cell-lines. In the immunoblot anti-HIV II positive sera reacted with all gag-proteins of HIV I. Our preliminary results with sera from Africa and from Germany show that predominantly African people are involved in this infection.

Establishment and Characterization of Kaposi’s Sarcoma Derived Cell Lines

ROTH, W. K., S. WERNER, K. REMBERGER, and P. H. HOFSCHNEIDER

Max-Planck-Inst. f. Biochemie, D-8033 Martinsried; Path. Inst., LMU, D-8000 München

We have cultivated Kaposi’s sarcoma biopsies from six different patients, all suffering from the acquired immune deficiency syndrome (AIDS). The cells growing out from the explant cultures were analysed cytochemically in different passages. A varying expression pattern of endothelial cell markers was found in most of the cell lines, depending on passage number and culture conditions. — Transfection of NIH mouse 3T3 and FISHER rat I cells with the DNA of the cell lines from two different patients revealed a transforming activity of the Kaposi’s sarcoma cellular DNA. The transformed recipient cells developed tumours in nude mice, which histologically resemble Kaposi’s sarcomas or angiosarcomas. The same recipient cells transformed by Polyoma virus DNA or haras oncogene DNA developed tumours with the histological features of fibrosarcomas.

Direct Use of Carrier-Bound Synthetic Peptides as Antigens in HIV and EBV-Specific ELISA Tests and for the Elicitation of Specific Antibodies

MODROW, SUSANNE, BRIGITTE HÖFLACHER, R. MERTZ, and H. WOLF

1 Max v. Pettenkofer Inst., Univ., D-8000 München; 2 Gesellschaft f. Strahlen- u. Umweltsforschung, D-8042 Neuherberg; 3 Genzentrum, D-8033 Martinsried

Since solid phase peptide synthesis was introduced by Merrifield, this method has been established in many laboratories and synthetic peptides have become widespread in their use in natural and medical science. In particular, synthetic peptides were used as specific antigens in diagnostic test systems for the detection of antibodies in patient sera. Monospecific or monoclonal antisera against peptide sequences can be raised in animal systems and are used for the characterization and identification of the native polypeptides in cellular and molecular systems and have proven to be of great value in immunological test systems. Chemical methods for synthesis were considerably improved and adapted to automated systems which allow high yields of the respective products. The consecutive purification steps however are still very expensive and time consuming. For the use in ELISA or RIA tests and for antibody production peptides need to be coupled again to plastic or protein supports.
which may lead to the destruction of secondary structure and a loss of antigenicity. To avoid these problems we have developed a system where side-chain protection groups can be removed without simultaneous cleavage of the synthesized peptide from polystyrene supports. Peptides from EBV and HIV-specific proteins were synthesized and used in very sensitive ELISA tests to screen patients' sera for specific antibodies. In addition, the carrier-bound peptides were shown to be potent antigens for raising antibodies in immunized animals.

Cellular Immune Responses Against Synthetic Peptides from the Latent Membrane Antigen (BNLF1-MA) of Epstein Barr Virus

JILG, W., S. MODROW, H. MAIRHOFER, and H. WOLF
Max v. Pettenkofer Inst., Univ., D-8000 München

We have studied cellular immune responses against two synthetic peptides from the latent membrane antigen (BNLF1-MA) of the Epstein Barr virus, a possible candidate for LYD-MA, (“lymphocyte detected membrane antigen”). The two peptides represent the amino acids 42–53, expressed on the cell surface, and 69–81 situated in the interior of the cell. Five of 15 EBV-positive persons reacted well in a lymphocyte proliferation assay with BNLF1/42–53; however, 2 of 3 EBV-negative individuals also showed borderline reactions. Surprisingly the peptide BNLF1/69–81 showed a strongly antiproliferative effect. Thus, the sequence 42–53 seems to play a role for the recognition of EBV infected lymphocytes by specific T-cells, although an unspecific stimulatory effect of this sequence cannot be ruled out. The biological significance, if any, of the strong inhibitory effect of the BNLF1/69–81 peptide is at present not clear.

Analysis of Class I RNA in Burkitt Lymphoma and Lymphoblastoid Cell Lines of the Same Patients

MARKERT, C., W. JILG, and H. WOLF
Max v. Pettenkofer Inst., Univ., D-8000 München

Recently we were able to show that surface density of class I HLA antigens on Burkitt lymphoma (BL) cells is reduced in comparison to EBV-transformed peripheral lymphoblastoid cell lines of the same patients. Additionally, qualitative differences between BL and LCL cells were detected. In this paper we present data on class I RNA in the same cell lines. – Northern blots of total and poly A+ RNA with a HLA class I cDNA probe revealed one HLA class I transcript of about 2kb in BL as well as in LCL cells. Differences in size of transcripts in BL and LCL cells not detected. – Using slot-blot analysis of total and poly A+ RNA with the same HLA class I cDNA probe and scanning of autoradiograms we could show that RNA data confirmed the previous protein data: expression of HLA class I antigens in BL cells is lower than in LCL cells and down regulation occurs on the transcriptional level. However, this effect is not a direct viral effect as it is also detected with EBV-negative BL cells. The relevance of this phenomenon for the failure of immune surveillance in patients developing Burkitt’s lymphoma has to be proven by further experiments.
Induction of Interferon Alpha by Interferon Gamma in Peripheral Blood Cells of Patients with Active Chronic Hepatitis B and Healthy Donors

ROSSOL, S., R. VOTH, G. HESS, H. KIRCHNER,¹ and K. H. MEYER ZUM BÜSCHENFELDE

¹ Med. Klinik and Poliklinik, Univ., D-6500 Mainz
¹ Deutsches Krebsforschungszentrum, Abt. Tumorvirologie, D-6900 Heidelberg

Treatment of peripheral blood lymphocytes (PBL) of patients with chronic active hepatitis B and healthy donors resulted in increased interferon gamma production. PBL were purified by Ficoll-Paque and adjusted at 5 × 10⁶ cells/ml. Interferon was measured by a highly sensitive enzym-immunoassay. Addition of interferon alpha resulted in interferon gamma production in a dose dependent manner. Maximal interferon gamma activities were obtained on day 3 by incubation with 1000 U/ml interferon alpha. However, only 60% of interferon levels exhibited in healthy donors was observed in patients. Coincubation with antibodies to interferon alpha or gamma led to undetectable amounts of interferon in the supernatants. Taken together these observations remain to be noted when considering the use of interferon alpha in therapy of chronic active hepatitis B.

NK Cell Activation by Cloned Human Tumor Necrosis Factor (TNF)

VOTH, R., S. ROSSOL, G. HESS, and K. H. MEYER ZUM BÜSCHENFELDE

¹ Med. Klinik and Poliklinik, Univ., D-6500 Mainz

Cloned human Tumor Necrosis Factor was shown to activate Natural Killer cells (NK) in a dose dependent manner in various systems. In vivo the nature of NK cells was defined by pretreatment of mice with the NK specific antibody anti-Asialo GM-1. Whereas peritoneal exsudate cells showed less or no NK activity human peripheral blood cells resulted in significantly increased cytotoxic capacity due to NK cells. However, in all systems we failed to detect any interferon in turn to TNF treatment. Thus the addition or injection of both compounds yielded in additive amounts of NK activity. It is therefore concluded that TNF is able to exhibit immunmodulatory capacities similar to those of interferons, hence there is no molecular defined homology of both compounds.

Virus-Induced Autoimmunity: Identification of the Anti-Golgi Inducing Agent (AGIA) as Lactate Dehydrogenase Elevating Virus

WEILAND, E., F. WEILAND, and A. GROSSMANN

Bundesforschungsanstalt f. Viruskrankheiten d. Tiere, D-7400 Tübingen

Anti-Golgi autoantibodies were recently described in both human patients suffering from autoimmune diseases (1; 2) and in mice bearing a certain tumor (3). During our studies of the mouse system we demonstrated that it was not the tumor itself which induced anti-Golgi
Abstracts of Papers at the Session of the Virology Section of the DGHM

autoantibodies but a transmissible agent found to be associated with progressor-Mo-MSV transformants that had been serially passaged in vivo (4). Here we report further characterization of the causative agent and show, that it possesses properties characteristic of lactate dehydrogenase elevating virus (LDV), such as morphology of non-arbo togaviruses, capacity to elevate plasma LDH levels, restriction of replication to a subpopulation of murine macrophages (probably the la subset), extremely high serum-titres (10^11 ID_{50}/ml) during the acute phase of infection and lifelong viraemia. – The nature of the autoantigens awaits further characterization as well as the regulation of the induction phase of the autoimmune response. (Ref. 1–3 cited in 4: E. Weiland and F. Weiland, Clin. exp. Imm. 62 (1985) 167).

Demonstration of Antibodies Against HBx-Protein in Patients with Acute or Anamnestic HBV-Infection

BRAUN, R., J. HESS, M. STEMMLE, H. WILL, J. KÜHN, and C. SCHRODER

Inst. Med. Virology, Univ., D-6900 Heidelberg;
1 Inst. Virus Research, German Cancer Research Center;
2 Max-Planck-lnst. of Biochemistry, D-8033 Martinsried

MS-2 and β-GAL fusion proteins with insertions of parts of the HBc, HBx and HBc reading frame were used to screen human serum samples for the presence of antibodies directed against the HBx-protein. – The results of the study show that antibodies against HBx-protein can be demonstrated by Western-blot in 25% of patients with anamnestic HBV infection (15/60). Furthermore, in 2 of 5 serum samples of patients with chronic HBV infection and in 9 of 20 serum samples of patients with acute HBV infection HBx-antibodies could be demonstrated. In 67 control sera an antibody reactivity with the viral X-protein was found only in one case. To further examine the specificity of the antibody reactivity against HBx-protein immunoprecipitation and competition experiments were performed. The antibody reactivity against MS-2-X could be competed by β-GAL-X, which demonstrates the specificity of HBx-antibodies. The summary of data indicates that antibodies against HBx-protein are present in approx. 25% of Hepatitis B patients. Titer rises are found already in the early course of infection. A prognosis to the outcome of infection however can not be concluded from the presence of HBx-antibodies.

Expression and Replication of Hepadna Viruses in an Established Hepatoblastoma (HepG2) Cell Line

ROTH, K., D. F. WAN, R. RASSHOFER, and H. WILL

Max Planck Inst. f. Biochemie, D-8033 Martinsried;
1 Max v. Pettenkofer Inst., D-8000 München

By cotransfection of cloned, plasmid-integrated HBV- or DHBV-DNA (dimer genomes) and a neomycine (neo) resistance conferring plasmid more than 100 neo-resistant HepG2 clones containing chromosomally integrated viral DNA were obtained. Cell culture media and cell extracts were tested for viral proteins by radioimmunoassay and immunoblot
analyses; secretion of virus particles, replicative intermediates, integrated und extra-
chromosomal viral DNA were analysed by Southern-blot analyses. Five cell lines with
different expression patterns of viral antigens were obtained. Virus productin could be
demonstrated for two of these clones. Extrachromosomal supercoiled HBV DNA, which
serves as template for synthesis of the RNA pregenome in vivo, could not be identified. This
result suggests that integrated viral DNA can serve as template in vitro. For none of the
clones transfected with DHBV DNA virus production could be demonstrated. It is therefore
conceivable that intracellular factors contribute to the host and cell specificity of hepadna
viruses. Using the HepG2 cell system it should be possible to identify such factors, and to
elucidate mechanisms of gene expression and replication in more detail.

Antibodies Against the X-Protein of Hepatitis B Virus in Sera of Patients with
Hepatocellular Carcinoma

LIANG, X.-H., Z.-Y. TANG, M. STEMLER, I. LONCAREVIC, H. WILI, and C. H. SCHRÖDER

1 Inst. f. Virusforschung, Deutsches Krebsforschungszentrum, D-6900 Heidelberg;
2 Shanghai Med. Univ., Shanghai, VR China;
3 Max-Planck-Inst. f. Biochemie, D-8033 Martinsried

The diagnostic relevance of antibodies against the X protein of hepatitis B virus (HBV) is
subject to controversy. We analysed HBsAg positive serum samples (n = 55) of patients
with hepatocellular carcinoma (HCC) for the presence of anti-X antibodies. In an im-
munoblot two X-fusion proteins (β-gal-X and MS2-X) served as substrate. Positive reac-
tions with both fusion proteins were obtained with four of the 55 sera examined. Specificity
of reactions was confirmed by competition experiments. The low fraction of anti-X positive
sera excludes anti-X antibodies as a general marker for HCC. In all four anti-X positive sera
the antibody titer against the X-protein was higher as the one against the nucleocapsid
antigen and in one case anti-X activity was detectable even at a 1:50,000 dilution. It is
conceivable that high anti-X antibody titers in some HCC patients are related to X gene
expression in tumor tissue.

X-Sequence Related Proteins and x-Specific Antibodies

PFAFF, E., J. SALFELD, and L. THEILMANN

Microbiology, ZMBH, Univ., D-6900 Heidelberg

Little is known about proteins encoded by the X-open reading frame of the human
Hepatitis B virus. To identify x-related proteins and antibodies recognizing x-protein, sever-
al expression plasmids were constructed which allow expression of different parts of the x-
frame in E. coli as fusion proteins and the elucidation of antibodies against various x-related
protein sequences. In addition, 35S-labelled x-protein was synthesized from the first AUG in
the x-gene sequence by in vitro transcription and translation using the SP6-Polymerase. The
resulting x-protein of 154 aa was used to assay for x-specific antibodies. Using both ap-
proaches, x-specific antibodies were detected in particular in sera from patients with prim-
ary liver carcinomas, and also in a few sera of patients with HBV-induced liver cirrhosis. Therefore we expect that sequences of the x-open reading are being expressed during the HBV life cycle. – When liver specimens from HBV-infected patients were screened in Western blots for the presence of x-encoded protein(s), one of our x-specific antibodies was found to recognize a protein with a molecular weight of about 22 kd. This protein differs in its size from the one expected for a conventionally expressed x-frame (17 kd) and also in size and occurrence from a 28 kd protein with x-antigenicity detected in several primary liver carcinomas by Moriarty et al.

Expression and Replication of Woodchuck Hepatitis Virus in Chronically Infected Liver and Primary Livercarcinoma

WEIMER, T. and H. WILL

Max Planck Inst. f. Biochemie, D-8033 Martinsried

In this study we examined whether there is a counterselection for viral replication and core gene expression in PLC tissue of woodchucks similar as known for human PLC tissue. The expression of WHcAg and Pre-S proteins was tested with antibodies against the corresponding proteins expressed in E. coli by immunoblot analyses. The molecular forms of the viral DNA (integrated and extrachromosomal, methylated and unmethylated) was analysed by Southern blot- and restriction enzyme digestion. In most of the WHV PLC tissues no or very little WHcAg expression was found. This indicates that also in WHV infected PLC tissue WHcAg expression is often abolished although less frequently as in human PLC tissues. The integrated but not the extrachromosomal WHV DNA was often found methylated which may contribute to core gene inactivation. In contrast to WHcAg, Pre-S1 and Pre-S2 expression was always observed in chronically infected and often in PLC tissues. In some of the PLC tissues there was predominant expression of Pre-S1. The altered expression of the Pre-S proteins in PLC tissue may contribute to the reduced viremia in some of these animals. The mechanism which lead to the altered Pre-S expression in these tissues remains to be determined.

Transactivation by Integrated HBV DNA Sequences

WOLLERSHEIM, MADLEN and P. H. HOFSCHEINER

Max-Planck-Inst. f. Biochemie, D-8033 Martinsried

The BHx open reading frame (ORF) was cloned into the SV40 based expression vector pKSV10 and cotransfected with a plasmid expressing the CAT gene under control of the SV40 early promoter. The results indicate transactivation by a product of the BHx gene. We also tested the integrated HBV DNA sequences U1.4 and U1.5 cloned from the human hepatoma tissue U1. In cotransfection experiments with plasmids expressing the CAT gene under control of the SV40 promoter and the LTRs of HTLV I and MMTV, respectively, transactivation can be demonstrated. By cloning sequences 5 of the HBx ORF in front of the CAT ORF we could identify a promoter signal which might control HBx gene expression in infected liver cells. A transactivating effect on the HBx promoter was observed by the HBV
DNA integrate U1.4, in which the HBx gene is cotranscribed with flanking cellular sequences. Further investigations will be necessary to clarify whether transactivation is a mechanism of HBV induced oncogenesis.

Transactivation by Hepatitis B Virus DNA Possibly Involved in Hepatocellular Carcinogenesis

ZAHM, P. and P. H. HOF SCHNEIDER
Max-Planck-Inst. f. Biochemie, D-8033 Martinsried

From epidemiological studies infection of hepatocytes with hepatitis B virus (HBV) is known to enhance the frequency of developing a hepatocellular carcinoma. To date no evidence demonstrates a particular intramolecular cis-influence of integrated HBV DNA on adjacent cellular sequences. We therefore consider a trans-regulation by HBV DNA as alternative mechanism of HBV related tumorigenesis. – Here we describe a transactivating function of HBV DNA. It was found by the stimulation of chloramphenicol-acetyl-transferase (CAT) activity after cotransfection with cloned HBV DNA. This transactivation depends on the expression of sequences of the HBV X region. It is performed even by a single copy of HBV DNA. In surveying different isolated promoters for effects by HBV DNA we observed transactivation of the promoters of the HSV TK gene and the LTRs of RSV, HTLVI and MMTV. – In the light of these results it is very likely that transactivation by HBV DNA is also performed in HBV infected hepatocytes. It might well be that also cellular genes important in transformation are deregulated by this mechanism.

Development of Vaccines for the Control of Virus-Associated Chronic Disease and Neoplasia

MOTZ, M., S.-Y. GU, Y. ZENG, G. DEBY, W. JILG, H. MAIRHOFER, and H. WOLF
Max v. Pettenkofer Inst., Univ., D-8000 München

Hepatitis B and Epstein-Barr virus (EBV) cause chronic active disease and are linked with neoplasia of man. The question needs to be discussed whether prevention of primary infection is a practical approach to controlling severe disease. Strategies for possible vaccination will depend on the epidemiological situation. In the case of controlled immunosuppression prior vaccination of virusfree individuals would require extremely safe products for vaccination. For Hepatitis B such a product, produced in yeast, is already commercially available and in use. For EBV this approach is not successful as yeast-specific glycosylation patterns destroy viral antigenicity. We have expressed selected parts of the EBV genome in mammalian cells and have obtained a product with good yield and authentic antigenicity. The possible use of this product will also require appropriate adjuvants. For areas with high natural risk for tumor development such as several areas of Africa, Southeast Asia and Southern China in particular (accumulated risk for hepatocellular carcinoma and nasopharyngeal carcinoma of about 1% each), vaccines causing effective long-term immuni-
ty and inexpensive, simple and safe application would be required. Starting from the long proven, very safe Chinese vaccinia strain Tien-Tan we have constructed a recombinant vaccinia strain which includes both EBV membrane and Hepatitis B surface antigens. Both antigens are expressed at high levels.

Serologic Diagnosis and Epidemiology of HIV Infections in Frankfurt, an AIDS Endemic Area of Western Germany

DOERR, H. W., K. MERGENER, TH. KLENK, D. HEINEMANN, and H. RABENAU

Univ. Clinics, Centre of Hygiene, Dept. of Med. Virology, D-6000 Frankfurt/Main 70

Antigen and antibody tests are now available for the serological diagnosis of HIV infections. The ELISA (Abbott Co.) on HIV specific serum antigen revealed positive results in 60% of AIDS patients (n = 26), 40% of LAS (n = 28), 10% of patients with risk (n = 20) and 0% of persons without risk (n = 13). – Antibody analysis of Ig(sub)classes showed a restriction to IgG 1 (found in 100% of HIV-seropositives) and IgG 3 (about 50%) in blood serum and to IgG 1 in cerebrospinal fluid. IgG 2 and IgG 4 antibodies could be detected in 2 respectively 1 case. HIV specific IgA antibodies could not be detected and IgM antibodies only in one case, in which a seroconversion was observed. HIV specific IgM could also be found in cerebrospinal fluids of two patients with incipient cerebral symptoms. These results were confirmed by the Western blot technique. – Basing on routine laboratory diagnosis of serum antibodies to HIV-1 in the years 1985 and 1986, the monthly detection rate of infection amounts to 50 persons on average. The relation of female to male persons changed from 1:7 to 1:3 during a year. This might be influenced by a screening programme performed with female prostitutes, who are found seropositive in about 2.5%. The clinical examination of HIV infected patients admitted to the City Hospital of Offenbach/FRG revealed striking signs in about 35–40%, of AIDS in 6%. The infected persons belong to risk groups. In December 1986 the first case of HIV-2 infection was recorded.

Disease-Correlated Serological Tests for HIV-Infected Individuals Using Recombinant Viral Gene Products

SOUTSCHEK-BAUER, E., M. MOTZ, and H. WOLF

Max v. Pettenkofer Inst., Univ., D-8000 München

On the basis of Western blot analysis, antibodies against various HIV-encoding antigens, e.g. p24 and p41, permitted conclusions on the status and prognosis of HIV-infected individuals. The production of defined recombinant viral antigens in E. coli offers a reliable method for the availability in large quantities of pure, diagnostically relevant antigens. – Based on computer-assisted predictions for the recognition of antigenic epitopes, conserved partial sequences from the env-glycoprotein, the gag protein and the pol protein in E. coli were expressed alone, as autologous and as β-galactosidase fusion proteins. – Test series with HIV-positive and control sera indicate the sensitivity and specificity of the established immunoassays. Prognostic predictions on the course of the infection are possible through
Abstracts of Papers at the Session of the Virology Section of the DGHM

the comparative analysis of various antigens as can be judged from the series of Western blots using recombinant antigens. ELISA tests using recombinant antigens are in preparation.

S71 Contains Sequences Related to the Putative RNase H Domain of Retroviral Reverse Transcriptases

BRACK, R., T. WERNER, C. LEIB-MÖSCH, V. ERFLE, and R. HEHLMANN

1 Abt. f. Molekulare Zellpathologie;
2 Inst. f. Säugetiergenetik, Gesellschaft f. Strahlen- u. Umweltforschung, D-8042 Neuherberg;
3 Med. Poliklinik, Univ., D-8000 München

Molecular clone S71 (Virology 155 (1986) 666–667) contains human endogenous retroviral sequences related to Simian Sarcoma Associated Virus (SSAV). Nucleotide sequencing of the S71 pol region shows the presence of a 1 kb reading frame distinctly related to the 3 carboxyterminal half of the reverse transcriptase of the murine leukemia virus AKV. This region also includes the putative RNase H domain. An amino acid identity of 54% as well as conservation of 60% of the proline positions points to a high degree of homology between the AKV and the S71 pol sequence. Moreover, protein secondary structures calculated for both sequences agree up to 66%, the highest degree of structural conservation being in the RNase H domain. Structural elements found in this region also seem to be conserved in a number of retroviral RNase H domains. We are currently isolating S71 cDNA clones indicating that this sequence may also be expressed in human tissue.

Transcription of Proto-Oncogenes in Rous Sarcoma Virus Infected and Transformed Chick Embryo Cells

BULLACHER, C. and A. BARNEKOWW

Inst. f. Med. Virologie, D-6300 Gießen

To evaluate an effect of cellular infection by Rous sarcoma virus (RSV) and its transforming oncogene, v-src, on the transcriptional activity of proto-oncogenes we tested 18 cellular oncogenes and β-actin performing "nuclear run on transcription assays" using nuclei isolated from RSV infected or transformed chick embryo cells (CEC). A drastic reduction in the transcriptional activity of the c-fgr proto-oncogene could be observed in RSV infected CEC compared to uninfected CEC. The transcription rates of c-fos and c-myc were also reduced but to a lesser extent. From the experiments with a temperature-sensitive, transformation-defective mutant of RSV, we conclude that the alterations observed were caused by the viral infection and are not due to the process of transformation.
FBR Murine Osteosarcoma Virus (FBR MSV)-Induced Osteosarcomagenesis in vitro: Biochemical Parameters

CLOSS, E., J. SCHMIDT, K.-H. ENGELMEIER and V. ERFLE

Gesellschaft f. Strahlen- u. Umweltforschung, Abt. f. Molekulare Zellpathologie und 
1 MEDIS, D-8042 Neuherberg

Mandibular condyles of neonatal mice represent skeletal tissue of so-called secondary cartilage. Skeletal precursor cells present in this tissue are capable of differentiating into cartilage cells in vivo and into bone cells in vitro, thus representing an ideal system for the study of osteosarcoma virus-induced alterations of the differentiation pattern of skeletal tissue. - Infection of mandibular condyles with FBR MSV in vitro results in the transformation of osteogenic precursor cells and in the development of an osteosarcomatous lesion within 7 days. - Control and infected condyles were labelled with $^{45}$Ca and $^{47}$Ca and the uptake and deposition of radiolabelled Ca and the activity of alkaline phosphatase (ALP) were determined. - Transformation of the condyles was not followed by alterations of ALP activity in comparison to uninfected controls. Further, uptake and deposition of radiolabelled Ca was similar in both, transformed and control condyles. In contrast, autoradiographic analysis revealed an irregular pattern of bone matrix mineralization in the virus-transformed tissue, similar to that observed histologically. - The data indicate that morphological alterations observed in virus-induced osteosarcomagenesis in vitro are not associated with alterations in Ca uptake or ALP activity, however, bone matrix mineralization follows the irregular pattern of the transformed tissue.

Studies on the Molecular Genetics of Melanoma Formation and on the Expression of Proto-Oncogenes During Normal Development and Tumorigenesis in Xiphophorus

SCHARTL, M., F. RAULF, W. MÄUELER, and S. M. ROBERTSON

Genzentrum/Max-Planck-Inst. f. Biochemie, D-8033 Martinsried

In Xiphophorus, melanoma formation was assigned by classical genetic findings to the expression of a cellular oncogene, named Tu. Tu was proposed to be negatively controlled by cellular anti-oncogenes or regulatory genes (R). The molecular nature of Tu and R is unknown so far. In order to contribute to an understanding of the molecular biological basis of melanoma formation, we are attempting to isolate and characterize the oncogene Tu. We have identified a restriction fragment length polymorphism associated with Tu. In addition we are analyzing the structure and expression of known proto-oncogenes in various mutants of Xiphophorus affecting melanoma formation. During embryogenesis c-src and c-ras 3 expression peaks during organogenesis, while c-sis expression elevates later, suggesting a function more related to cellular differentiation than to proliferation. In adults, c-ras 1 and 2 are expressed tissue specifically and show no over-expression in tumorous tissue, while c-ras 3, c-src and c-myc demonstrate enhanced expression in melanoma. c-erb B 1, 2 and c-sis are expressed only in tumor cells. These data suggest a coordinated deregulation of several oncogenes during the process of melanoma formation and/or progression. Serum-deprivation of the PSM melanoma cells leads to up to 10 fold elevated levels of c-erb B 1, c-src and c-ras 2 mRNAs. Normal levels are restored within 48 h after stimulation. Serum-deprivation
of the A2 embryonic cells results in a total loss of c-onc gene expression without reconstitution after 48 h. This demonstrates that the expression of these c-onc genes in melanoma cells is not related to proliferation in general but may instead have tumor specific features.

Bone Tumor Induction by Murine Leukemia Viruses (MuLV)

SCHMIDT, J., W. BEHNISCH, A. LUZ, C. LEIB-MÖSCH, and V. ERFLE

Abt. Molek. Zellpathologie, Gesellschaft f. Strahlen- u. Umweltforschung mbH, D-8042 Neuherberg

Murine leukemia viruses (MuLV) isolated from normal and irradiated bone tissue, from spontaneous benign and malignant bone tumors, and from radiation-induced osteosarcomas, as well as the endogenous ecotrophic leukemia virus of the AKR mouse (Akv), previously reported to be non-oncogenic, are capable of inducing osteomas and osteopetrosis together with malignant lymphomas in NMRI mice. The latent periods vary from 6–18 months. – Histological examination of the virus-induced bone tumors showed marked differences in the sizes of the cell layers of the periosteum, in the degree of crypt formation into the mineralized osseous tissue, and in the number of metabolically active osteoblasts. – Restriction mapping of the different virus genomes showed great similarities to that of Akv, but differences in the structure of the glucocorticoid regulatory elements (GRE) and enhancer sequences of the LTR regions. The latter exhibited distinct effects on Dexamethasone-induced CAT activity in NIH3T3 cells. In osteoblast cell lines, Dexamethasone-induced Cat activity correlated also with the Dexamethasone-induced increase of osteoblastic differentiation. However, the different LTR structures could not yet be associated with either bone tumor incidences or with the different latent periods observed with the various virus types.

Evolution of src-Related Proto-Oncogenes in Metazoans

ROBERTSON, S. M., S. OTTILIE, E. STERNECK, G. HANNIG, and M. SCHRATL

Max-Planck-Inst. f. Biochemie, Genzentrum, D-8033 Martinsried

We have previously shown that the sponge is the first organism in phylogenesis to contain the ancestral c-src gene. A genomic library of Spongilla lacustris has been prepared, screened for v-src related sequences, and preliminary sequencing results from one clone indicates homology at the nucleotide and amino-acid level to the v-src gene. Interestingly, hybridization data suggest that the sponge c-src gene may shorter than that of higher eukaryotes by virtue of an internal region of the gene being missing. This observation is consistent with data on the sponge src protein (Angelika Barnekow, pers. comm.) showing a smaller c-src protein product, possibly due to deletion of a region of the protein corresponding to the missing DNA sequence. – As Hydra is the first organism in phylogenesis with differentiated nerve cells, we are using src-related clones isolated from a Hydra genomic bank to study the neuron-specific expression of c-src. – On the basis of DNA hybridization, the proto-oncogene yes is first detected in lower chordates. From a genomic bank of Xiphophorus maculatus we have isolated src- and yes-related clones that may represent an early stage in the src/yes gene duplication/divergence process.
Structure of the Chicken Proto-Oncogene c-mil and its Relationship to Protein Kinase Genes

BISTER, K.,¹ M. KOENEN², A. SIPPEL², and C. TRACHMANN¹

¹ Otto-Warburg-Lab., Max-Planck-Inst. f. molek. Genetik, D-1000 Berlin 33
² Zentrum f. molek. Biologie, Univ., D-6900 Heidelberg

We have previously described the genomic organization and the nucleotide sequence of the chicken c-mil gene within the 3' region that is homologous to v-mil, one of two oncogenes of the highly oncogenic avian retrovirus MH2. We have now isolated c-mil cDNA clones by screening a chicken cDNA library with a v-mil specific probe. Nucleotide sequence analyses of overlapping c-mil cDNA clones revealed the presence of a single large open reading frame encoding a 647-amino acid protein with a calculated molecular weight of 73,192, which is in good agreement with the apparent molecular weight of 71,000–73,000 reported for the c-mil protein detected in cellular extracts. The carboxyl-terminal region of 379 amino acid residues corresponds to the v-mil encoded portion of the MH2 gag-mil hybrid protein with homology to the catalytic domains of protein kinases. The amino-terminal region of the c-mil protein contains sequence motifs, like cysteine rich domains, found in the regulatory domains of protein kinases or in certain receptor molecules.

High-Molecular-Weight PDGF-Like Molecules in Conditioned Medium from SSV-Transformed Cells Lead to Growth-Stimulation and Transformation

KLEIN, R. and H.-J. THIEL

Federal Research Centre for Virus Diseases of Animals, D-7400 Tübingen

Serum-free tissue culture supernatant of SSV-transformed nonproducer cells shows growth-stimulating activity with PDGF-like characteristics on NIH 3T3 and nonestablished mouse fibroblasts. Furthermore, the same tissue culture supernatant shows transforming activity as shown by inducing anchorage-independent growth of NRK and nonestablished goat fibroblasts. – After fractionation of supernatant by gel filtration, both activities are present in the high-molecular-weight fractions (150–300 kD). Growth-stimulating and transforming activity of such fractions can largely be inhibited by antibodies against PDGF or SSV-NP cells. – Using tissue culture supernatant for preparative SDS-PAGE followed by electroelution we also find a high-molecular-weight (>200 kD) growth-stimulating and colony-inducing activity that can be inhibited by our specific antibodies. – We suggest that both activities are due to a highly glycosylated PDGF-like molecule which is released by SSV-transformed cells and was formerly described as SSV transformation-specific glycopeptide (SSV-TrSgp) (Virology 136 (1984) 414–424).
Specific Factors Binding to the E2A Late Promoter Region of Adenovirus Type 2 DNA: No Apparent Effect of 5'-CCGG-3' Methylation

HÖVELER, A. and W. DOERFLER

In the regulation of eukaryotic gene expression, the interactions of proteins with specific signals in the promoter sequence play a decisive role. The methylation of specific promoter sequences causes the inactivation of eukaryotic promoters. We have studied the inactivation of the E2A late promoter of adenovirus type 2 (Ad2) DNA by the methylation of three 5'-CCGG-3' sequences at positions −215, +5 and +23, relative to one of the cap sites (Langner et al., PNAS 81 (1984) 2950–2954). The mechanisms by which DNA methylation interferes with regulatory functions in the late E2A promoter of Ad2 are not known. By DNase I protection analyses, at least six different promoter sequences have been identified which bind specifically to host proteins. Binding of these proteins to unmethylated or 5'-CCGG-3' methylated late E2A promoter sequences has been compared by using the gel migration delay assay or DNase I protection (footprinting) analyses. Apparently, protein binding is not detectably affected by late E2A promoter methylations. It is still conceivable that the functionality of promoter-bound host factors is altered when three 5'-CCGG-3' sequences are methylated.

Supported by the Deutsche Forschungsgemeinschaft through SFB74-C1).

Insertion of Adenovirus Type 12 DNA in the Vicinity of an Intracisternal A Particle (IAP) Genome in Syrian Hamster Tumor Cells

LICHTENBERG, URSULA, CHRISTINE ZOCK, and W. DOERFLER

Inst. of Genetics, Univ., D-5000 Köln

In the Ad12-induced hamster tumor T1111(2) about 10 Ad12 genome equivalents were integrated. One of the integrated copies proved unstable and was lost or rearranged upon of cell line H1111(2). This unstable site of junction between Ad12 DNA and hamster DNA was cloned, sequenced and analyzed. Similarly, the preinsertion site from BHK21 hamster cells was analyzed. At the left terminus of Ad12 DNA, the first 64 nucleotides were deleted. At a distance of 127 nucleotides to the left from this junction site, an internal dispersed fragment of Ad12 DNA (bp 1290 to 1361) was inserted into cellular DNA. To the left of Ad12 nucleotide 1361, about 620 cellular nucleotides followed. Abutting this sequence to the left, the 3' terminal sequence of an endogenous, intracisternal A particle (IAP) genome in hamster cells was detected. The possible significance of the close vicinity of an IAP sequence to an inserted Ad12 genome for the transformation event, the instability of this site, or the transcriptional activity of this region was not known. The preinsertion sequence and its environment were also investigated for transcriptional activities which were found at distances of several 100 nucleotides from the insertion site.

Aided by BMFT BCT 03652 and Fonds der Chemischen Industrie.
SV40-Induced Transformation: The Cellular Protein p53 Appears to be a Substrate for an SV40-Induced Protein Kinase

SCHEIDTMANN, K. H.
Inst. f. Immunbiologie, Univ., D-7800 Freiburg

Stabilization and overexpression of the cellular protein p53 might play a key role in SV40-induced transformation. Stabilization of p53 might be facilitated by complex formation with T antigen, modulation of a regulatory protease and/or posttranslational modification. The phosphorylation state of p53 in normal, SV40-infected or -transformed rat fibroblasts was investigated. In normal cells, p53 appeared as a single species with high turnover, phosphorylation was hardly detectable. In transformed cells p53 was stable and a second form appeared which was derived from the first form by additional phosphorylation as revealed by pulse chase experiments and phosphopeptide analysis. In cells abortively infected with SV40, the highly phosphorylated form of p53 appeared two days postinfection suggesting that SV40 large T antigen induces or activates a protein kinase one substrate of which is p53. This might be an early event in SV40-induced transformation.

Supported by SFB 31 and Landesschwerpunkt 09 Baden-Württemberg.

Specific Interaction of Simian Virus 40 Large T-Antigen with the Cellular Chromatin and the Nuclear Matrix During Course of Infection

SCHIRMBECK, R. and W. DEPPERT
Univ. Dept. Biochemistry, D-7900 Ulm

We have analyzed the subnuclear distribution of SV40 large T during course of lytic infection. At early times post infection, large T was present mainly in the nucleoplasm and at the cellular chromatin: Concomitant with the onset of viral DNA replication, the amount of nuclear matrix associated large T increased drastically. During further course of infection the amounts of both chromatin and nuclear matrix associated large T increased steadily, paralleling the increase in viral DNA synthesis. The biological significance of this correlation was corroborated by analysis of cells infected with the SV40 tsA-mutant tsA58. At the restrictive temperature, tsA-58-mutant large T failed to initiate viral DNA replication in infected cells, and also failed to associate with the cellular chromatin and the nuclear matrix. By blocking viral DNA synthesis with aphidicolin, we were able to show that the accumulation of large T at these nuclear structures does not result from binding of large T to viral chromatin, but reflects an association with cellular components of the chromatin and the nuclear matrix.
Human papilloma virus (HPV) 8 propagates almost exclusively in differentiating keratinocytes of patients with epidermodysplasia verruciformis. Enhancer activity was demonstrated within the 1.4 kb EcoRI-PvuII-fragment, that has to be trans-activated by the product of open reading frame E2 of either HPV 8 or bovine papillomavirus (BPV). The analysis of smaller DNA segments revealed two independent regions, which stimulate CAT gene expression in cis. The major activity is encoded within the 5' part of the upper regulatory region of HPV 8. The positive control sequences appear to be separated by cis-acting negative control sequences. In contrast to these findings in C127 mouse fibroblasts HPV 8 CAT hybrids showed only marginal activity in primary human foreskin fibroblasts or keratinocytes, or in SV40 transformed human foreskin keratinocytes, which could not be stimulated by cotransfection of expression vectors for HPV 8 or BPV 1 E2.

Identification of the Fish Lymphocystis Disease Virus Thymidine Kinase Gene

Fish Lymphocystis Disease Virus (FLDV), a member of the iridovirus family, causes a widely distributed disease in pleuronectes, which is characterized by papilloma-like lesions. A thymidine kinase gene (tk) activity has been reported for two members of the family iridoviridae namely Frog Virus 3 and FLDV. The identification of the tk gene loci in FLDV genome was performed by transformation of 3T3 TK negative to 3T3 TK positive cells using specific viral DNA sequences and transfection assay (calcium phosphate technique). DNA fragments of the viral genome used in this study were obtained from a defined gene library of FLDV genome containing the complete viral DNA sequences. The selection of the converted cells was carried out under the condition of HAT selection procedure. The results of these experiments revealed that the EcoRI FLDV DNA fragment C (10.9 kbp; 0.611 to 0.718 mu) is able to transform 3T3 TK negative to 3T3 TK positive cells. Additional experiments which were performed using the subclone of EcoRI DNA fragment C revealed that the DNA sequences (4.7 kbp) mapped at the coordinates 0.663 to 0.718 of FLDV genome possess the transformation ability, indicating that the tk gene loci is located in this particular region. - The determination of the nucleotide sequences of this interesting gene is in progress now.

DFG project Da 142/2-2.
Characterization of Neutralization Escape Mutants Selected by a Monoclonal Antibody Against VP1 of Poliovirus Type 1, Mahoney

WIEGERS, K. J., H. UHLIG, and R. DERNICK
Heinrich-Pette-Inst. f. Exp. Virologie u. Immunologie, Univ., D-2000 Hamburg 20

Neutralizing monoclonal antibodies obtained after immunization with infectious poliovirus recognize only conformation dependent epitopes and do not react with isolated polypeptides. Recently, we have obtained three neutralizing monoclonal antibodies using an appropriate in vitro immunization system of spleen cells primed in vivo with purified VP1. These antibodies recognized also isolated VP1. The antibody binding sites were located between amino acid residues 93–104 of VP1. We have used one of these antibodies for the selection of neutralization escape mutants. All mutants isolated had an altered VP1 as revealed by isoelectric focusing in urea. From 16 neutralizing monoclonal antibodies recognizing conformation dependent epitopes 5 could neither neutralize nor bind these mutants. We conclude that the region of amino acid residues 93–104 of VP1 is also involved in the formation of the epitopes of this group of antibodies, probably as part of a discontinuous epitope.

Molecular Immunological Aspects of Foot- and Mouth Disease Virus

PFAFF, E. 1, H.-J. THIEL 2, and H. SCHALLER 1
1 Microbiology and Center for Molecular Biology, Univ., D-6900 Heidelberg;
2 Federal Res. Institute for Animal Diseases, D-7400 Tübingen

The major antigenic determinant of foot-and mouth disease virus has been located within the region from amino acid 144 to 159 on coat protein VP1. A synthetic peptide representing this region can protect guinea pigs against viral infection. Immune affinity chromatography using this synthetic peptide eliminates > 90% of the neutralising activity of a hyperimmune serum. To investigate the antigenic determinant in more detail, neutralising monoclonal antibodies (McAb) were raised against complete virus and against the synthetic peptide. These McAbs could be divided into 4 neutralisation groups. The binding sites of the McAbs were localized by different methods e.g. Western blot, ELISA and competition experiments. 2 of the groups of McAbs' bound to the synthetic peptide, isolated VP1 and viral particles and binding could be inhibited completely by the peptide. The McAbs of the two other groups bound only to complete viral particles and weakly to the peptide. To correlate the different neutralisation pattern with the primary structure of the coat protein VP1, the nucleotide sequences coding for the antibody binding sites were determined for all FMDV strains tested. In conclusion this analysis demonstrated that McAbs from the first two groups recognize overlapping sequential epitopes, whereas the McAbs from the two other groups recognize conformational epitopes. – To support these findings, escaped mutants were selected from all McAb groups and sequenced. The results obtained showed amino acid exchanges in the antibody binding site in VP1 in the case of the McAbs which recognize the sequential epitopes. In contrast, in the other cases, amino acid exchanges were only found in the two other coat proteins VP2 and VP3, which by themselves do not induce neutralising antibodies.
Regulation of translation initiation: Foot and Mouth Disease Virus (FMDV) as a model system

KÜHN, R. and E. BECK

Zentrum f. Molek. Biologie (ZMBH), D-6900 Heidelberg

FMDV serves as a model system for structural and functional analyses during the translation process. Translation initiates atypically for eucaryotes at an internal start site approx. 1300 nucleotides downstream from the 5' end of the RNA. We have shown that deletion or insertion mutations in large parts of this so called 5' non coding region affect strongly the efficiency of translation initiation. A hot spot region for mutagenesis, also for point mutations is a pyrimidine-rich stretch 15 bp in front of the 10th AUG, which functions as the start codon. There is evidence that ribosomes bind directly to this initiation site. — The whole non coding region is marked by a high degree of secondary structure and by the ability to bind several cytoplasmic factors. With the help of protein RNA binding assays we are trying to analyse factors which are involved in this process.

Poliovirus-Uncoating in Acidic Organelles is Inhibited by Disoxaril (WIN 51711)

ZEICHHARDT, H. 1, M. J. OTTO 2, M. A. MCKINLAY 2, P. WILLING-MANN 1, and K.-O. HABERMEHL 1

1 Inst. f. Klin. u. Exp. Virologie, Freie Univ., D-1000 Berlin; 2 Dept. Microbiology, Sterling Winthrop Res. Inst., Rensselaer, New York/USA

Stabilization of the capsid of polioviruses type 1 (strain Mahoney) and type 2 (strain MEF) by disoxaril (WIN 51711; 5-[7-[4-(4,5-dihydro-2-oxazolyl)phenoxy]heptyl]-3-methylisoxazole) results in inhibition of poliovirus uncoating in the acidic endosomes and/or lysosomes. Ultrastructural investigations and measurements of the delivery of parental viral RNA into the cytoplasm of HeLa or HEp-2-cells for virus-specific RNA new synthesis revealed that only pretreatment of poliovirus with disoxaril results in maximal inhibition of uncoating (type 1: 0.3 µg disoxaril/ml; type 2: 0.03 µg/ml). Compound-pretreated as well as control virus enter the cell by receptor-mediated endocytosis via coated pits and coated vesicles into endosomes. As soon as the virus interacts with the cell the compound-induced inhibition remains only partial. The compound can be eluted from the infected cells up to 25 min p. i. which results in a partial reversal of the uncoating block. The virus is located in the acidic endosomes at this time after infection.
Diagnosis of Enteroviral Heart Disease Through in situ Nucleic Acid Hybridization

KANDOLF, R. 1,2, P. KIRSCHNER1, D. AMEIS1, E. ERDMANN2, B. KEMKES3, and P. H. HOF SCHNEIDER1

1 Max Planck Inst. f. Biochemistry, D-8033 Martinsried; 2 Dept. Internal Med. I, Univ.; 3 Dept. Cardiac Surgery, Univ., D-8000 München 70

Using cloned coxsackievirus B3 cDNA as an enteroviral diagnostic reagent, an in situ hybridization assay has been developed, which detects the main etiologic agents of viral heart disease in a single hybridization assay including coxsackie B and A viruses as well as echoviruses. Endomyocardial biopsies from 46 patients with clinically suspected myocarditis or dilated cardiomyopathy have been examined together with myocardial tissue from recipient hearts of 9 patients who underwent heart transplantation. Enteroviral infected myocardial cells have been detected in 6 of 21 patients with suspected myocarditis as well as in 4 of 17 patients with dilated cardiomyopathy, including 2 recipient hearts with positive hybridization. 17 patients with myocardial diseases not consistent with suspected viral heart disease – e.g., coronary heart disease, were used as an internal control group and showed negative hybridization. An important observation is that the number of infected myocardial cells is correlated with the severity of clinical symptoms. Interestingly, positive hybridization for enteroviral RNA was also obtained in late biopsies – e.g., 6 months after onset of the disease, indicating persistence of the virus in human myocardium.

Virus Isolations from Children after Sudden Infant Death (SIDS)

MERTENS, TH., ANNA-MARIA EIS, URSULA PIKA, M. OEHMICHEN1, and H. J. EGGERS

Inst. f. Virologie, Univ., D-5000 Köln 41; 1 Inst. f. Rechtsmedizin, Univ., D-5000 Köln 30

From 21 cases (14 m, 7 f) with sudden infant death (SIDS) and 2 cases with near-SIDS (both male, one died) we tried to isolate virus. The mean age was 4 months (0.7–10). Six infants had been immunized with oral polio vaccine, six had not been immunized, and the vaccination history of 11 was not known. The following specimens were examined: lung: 21; intestine: 20; brain: 12; heart: 4; trachea: 1; CSF: 1; spleen: 1. Isolations were attempted in primary monkey kidney cells, in human embryonic lung fibroblasts, and in HeLa cells. (Specimens of 11 cases were examined by ELISA for influenza A/B-, parainfluenza-, adeno- and RS- antigen with negative results.) We isolated poliovirus from 6 cases (4 × type 2, 1 × type 3, 1 × types 1 + 3) and one untyped enterovirus. The agents were detected in the intestine, once with poliovirus 2 isolated in the absence of a vaccination history. In one case, however, poliovirus 2 was isolated from the lung, when oral polio vaccinations had been done 6 and 4 months prior to SIDS.
Genetic Polymorphism of Human Adenoviruses

ADRIAN, TH., U. WOLF, and R. WIGAND

Dept. Virology, Fac. Med., Univ. of the Saarland, D-6650 Homburg

About 1200 patient isolates were collected over a period of 30 years mainly in Europe and the USA and belonged to the adenovirus subgenera A to F. The DNA of these strains was analysed by seven restriction endonucleases. Strains, which were indistinguishable by immunological tests, but showed different DNA fragment patterns were named genome types. Genome types of strains belonging to subgenus C were found to be very variable, as also could be seen for AV3 and AV16 of subgenus B. Moderate differences showed genome types of subgenus A (AV31), subgenus B (AV7 and AV21), subgenus D (AV8 and AV37), and subgenus E (AV4). The variations were located on the genome and found to be distributed randomly. Great differences were seen between the genome types and the corresponding prototypes, but small ones among some of the genome types, which points to a common ancestor for them.

Complex Formation of SV40 Large T Antigen with the Cellular Oncoprotein p53

KRAISS, S., ANDREA QUAISER, and M. MONTENARH

Dept. Biochemistry, Univ., D-7900 Ulm

We analyzed T-p53 complex formation in two SV40-transformed cell lines one of which overexpresses p53. Only in the latter case, we did find elevated levels of T-p53 complexes and additionally free p53. We therefore conclude that the amount of T-p53 complexes is determined by the amount of p53. Sucrose gradient analysis showed that in cells with limiting amounts of p53, free T-antigen and T-p53 complexes exist in predominantly high oligomeric forms. When high levels of p53 are present, lower molecular weight T-p53 complexes were also found. Free T-antigen was predominantly found in low oligomeric forms whereas free p53 rapidly formed high oligomers. We assume that p53 binds preferentially to an already oligomeric T-antigen, but when present in high concentrations it also binds to lower molecular weight forms of T-antigen.

Diagnosis of Epstein-Barr Virus with Recombinant Antigens in Comparison with Conventional Methods

KÜHBECK, R., H. MORGENSTERN¹, P. KOLDOVSKI¹, M. MOTZ, and H. WOLF

Max v. Pettenkofer Inst., Univ., D-8000 München
¹ HNO Clinic, Univ., D-4000 Düsseldorf

Antigenic sites of BALF2 (p138 VCA), BMRF1 (p54 EA) and BcLF1 (p150 EA) were cloned in pUC and pINDU plasmids and expressed in E. coli as fusion and non-fusion
proteins. The proteins were purified by partial precipitation and ion-exchange chromatography. These purified antigens were tested in ELISA with 100 human sera and the results were compared to those of immunofluorescence tests (IF). We were able to show a good correlation between anti-VCA IgG and IgM in IF and anti-p150 IgG and IgM in ELISA as well as anti-EA IgG in IF and anti-p54 IgG in ELISA. Anti-EA IgM in IF were not tested. Low values of specific IgA against p150 and p54 were found mostly by sera which showed borderline titers in the IF against anti-VCA and anti-EA.

Fixation of Unmethylated or 5'-CCGG-3' Methylated Foreign DNA in the Genome of Hamster Cells: Gene Expression and Stability of Methylation Patterns

MÜLLER, U. and W. DOERFLER
Inst. of Genetics, Univ., D-5000 Köln

Most of the studies on unmethylated or in vitro methylated viral promoters and their expression were based on transient expression assays. It was, therefore, mandatory to fix in an integrated form methylated or unmethylated viral promoters in conjunction with indicator genes in the genome of mammalian cells and to test levels of expression. BHK21 hamster cells were cotransfected with the pSV2-neo construct and the plasmid pAd2E2A-CAT which contained the E2A promoter of adenovirus type 2 DNA. G418-resistant cell lines were selected that carried the intact E2A-CAT promoter construct in which these sequences were not rearranged. Of the 18 lines generated by transfecting unmethylated DNA (um), 17 lines expressed the CAT gene. Of the 12 lines generated by transfecting methylated pAd2E2A-CAT DNA (m), 5 lines did not express the CAT gene, 5 lines expressed it weakly, 2 lines expressed the CAT gene strongly. In the m cell lines which expressed the CAT gene to varying extents, the late E2A promoter had become partly or completely demethylated. In contrast, in cell lines that did not express the CAT gene, the late E2A promoter had remained almost completely methylated. What factors determined the degree of "demethylation" of the transfected, methylated construct?

Supported by the Deutsche Forschungsgemeinschaft through SFB74-C1.

A Subgroup-Specific Antiserum Induced by the Central Part of the Human Papillomavirus 8 Capsid Protein

STEGER, G.¹, M. JARZABEK-CHORZELSKA², S. JABLONSKA², and H. PFISTER

¹ Inst. f. Klin. u. Molek. Virologie, Univ., D-8520 Erlangen;
² Dept. Dermatology, School of Med., Warsaw/Poland

The open reading frame (ORF) L1 of papillomaviruses is highly conserved and codes for the major capsid protein. This protein carries group-specific and type-specific antigenic determinants. We have cloned a 600 bp HaeIII-fragment from the central, less conserved part of ORF L1 of human papillomavirus 8 into a procaryotic expression vector, which gives rise to a β-gal-fusion protein. A guinea-pig antiserum against this protein was tested
with antigens from different wart biopsies using indirect immunofluorescence tests and Western blotting. It detected capsid proteins of HPV8 and of HPV5, 9, and 17, which are all closely related with HPV8. The antiserum did not react with structural antigens of HPV1 or HPV3. This indicates that the central part of HPV8 L1 reveals antigens, which are common to viruses associated with epidermodysplasia verruciformis. In contrast, group-specific antigens appear to be encoded by the 5′ and 3′-parts of ORF L1.

Anti-Idiotypic Antibodies for the Induction of Neutralizing Antibodies Against Poliovirus

GRAVENHORST-MÜNTER, ULRlKE and K. WETZ

Heinrich-Pette-Inst. f. Exp. Virologie u. Immunologie, Univ., D-2000 Hamburg 20

Rabbit IgG antibodies specific for poliovirus type 1 (Mahoney) were used to raise anti-idiotypes in guinea-pigs. These antibodies reacted specifically with anti-poliovirus IgG in an ELISA. Furthermore, binding of the anti-idiotypes to anti-poliovirus rabbit IgG was completely inhibited by poliovirus. This suggests that anti-idiotypic antibodies recognized determinants on anti-poliovirus antibodies which are related to the site of poliovirus binding. Reduced competition was observed using heterologous anti-poliovirus antibodies from rats or mice. Anti-idiotypes induced an immune response in rabbits or mice which was specific for poliovirus. All antisera recognized poliovirus in an ELISA to different degrees, however, they did not neutralize the virus. It is therefore concluded that anti-idiotypes hardly become a suitable vaccine against poliovirus.

Two 5′Terminal UMP Residues Missing in Infectious Recombinant Coxsackievirus B3 cDNA are Present in the Genome of cDNA Derived Virus

KLUMP, W., I. BERGMANN, D. AMEIS, R. KANDOLF, and P. H. HOFSCHEIDER

Max Planck Inst. f. Biochemistry, D-8033 Martinsried

The 5′terminus of picornaviral genomes is highly conserved indicating an important function for viral replication. Interestingly, the infectious recombinant cDNA plasmid pCB3-M1 obtained from molecular cloning of full-length reverse-transcribed coxsackievirus B3 cDNA contains the 5′sequence CCAAAA deviating from the 5′ terminal sequence UUAAAA of coxsackievirus B3 wild type RNA. Remarkably, the 5′ sequence of the RNA of cDNA generated virus preparations obtained from independent transfection experiments is identical with the authentic coxsackievirus B3 RNA 5′ sequence UUAAAA. Therefore, the two 5′ UMP residues missing in the infectious recombinant coxsackievirus B3 cDNA must have been recaptured in the course of virus replication. We assume that the two 5′ UMP residues are contributed by the uridylylated virus protein VPg-pUU acting as a primer of RNA synthesis.
Evidence for the Cleavage of Staphylococcus aureus Protein A by the Foot-and-Mouth-Disease Virus Proteinase 3c

MARQUARDT, O.
Bundesforschungsanstalt f. Viruskrankheiten d. Tiere, D-7400 Tübingen

The foot-and-mouth-disease virus (FMDV) proteinase 3c is involved in the posttranslational maturation of virus proteins and possibly further in the pathogenesis of the infected cell. The analysis of such proteolysis may explain the process on a molecular level. Prerequisite is a simple proteinase-specific assay. Its basics have been established by use of recombinant DNA techniques and are communicated here. Earlier work has resulted in the expression of cloned FMDV cDNA including the 3c proteinase gene by E. coli. There, an active enzyme has been produced but the protein could not be identified. In a new approach, the 3c proteinase gene was fused to the Staph. aureus protein A gene present in pRIT2T. Gene expression should result in a fusion protein, the C-terminal part of which is specific for the 3c proteinase and the N-terminal part for protein A. The latter enables the detection of expression products by affinity to antibodies. The experiment showed that in fact protein A-specific polypeptides were produced, however reduced in molecular weight as compared to the protein expressed from pRIT2T alone or from a construction with a truncated 3c proteinase gene. It is concluded that protein A is substrate for this enzyme and converted into products. The detection of a substrate enables one to follow the purification of active enzyme from E. coli.

Demonstration of Neutralizing IgM Antibodies vs Coxsackieviruses Type B

MERTENS, TH., ANNA-MARIA EIS, ANNETTE BRÄUTIGAM, and H. J. EGGER
Inst. f. Virologie, Univ., D-5000 Köln 41

The serologic diagnosis of recent enterovirus infections presents a serious problem in clinical virology. A reliable serologic method would be of great clinical significance (e.g., myocarditis, type 1 diabetes). Since ELISA tests lack type specificity we looked for neutralizing IgM antibodies vs the six coxsackie (cox) B types in sera of patients. IgM was separated by two-step-column-chromatography and tested in a microneutralization test. Out of 239 patients 37% were IgM negative. 40% showed IgM antibodies vs one, 17% against two, 5% against three, and 0.4% against 5 serotypes. There is no good correlation between the serum antibody titer and IgM positivity, and the incidence of antibodies vs 1 or more cox B types does not increase with age of the patients. Heterotypic rises of IgM antibody titers could be demonstrated in a patient from whom cox B 2 had been isolated. We conclude that neutralizing antibodies against cox B viruses do not appear to persist lifelong, that heterotypic IgM boosters do occur and that IgM may persist for extended periods of time.
Production and Characterization of a Monoclonal Antibody vs Coxsackievirus B4

RITZKOWSKY, A., TH. MERTENS, URSULA PIKA, and H. J. EGGERS

Inst. f. Virologie, Univ., D-5000 Köln 41

Coxsackieviruses (cox) types B and other picornaviruses share common antigens. Even human neutralizing antibodies exhibit some cross reactions. When trying to produce monoclonal antibodies (mab) against various cox B virus types we found them to differ substantially with regard to immunogenicity. A mab against cox B4 lacked neutralizing capacity (microneutralization and plaque reduction test). Binding of this mab in a dot blot on nitrocellulose depends on the antigen preparation. It obviously binds to a type-specific internal antigen of VP1 as also shown by Western blot. Immunoblots using digested VP1s of cox B4 show several bands (V8 protease: 2; N-chlorosuccinimide: 3). A comparison with polyclonal antisera proved not possible due to antibodies against V8 protease. Competitive inhibition of binding yielded weak positive results after preincubation with mab followed by incubation with polyclonal type-specific rabbit antiserum.

Different Specificities of Murine and Human Monoclonal Antibodies Against Poliovirus Type 1

UHLIG, H. and R. DERNICK

Heinrich-Pette-Inst., D-2000 Hamburg 20

Murine hybridomas producing poliovirus-specific monoclonal antibodies (mAbs) were obtained from adult Balb/c mice immunized with complete infectious poliovirus type 1, Mahoney. Human B cell lines producing mAbs reactive with poliovirus type 1 were generated by transformation with Epstein-Barr virus (EBV) B 95–8 of tonsillar lymphocytes from several immune donors. Both murine hybridomas and EBV-transformed cells were cloned in semisolid agarose. All neutralizing (Nt-) murine mAbs recognized and neutralized only poliovirus type 1, whereas some human Nt-mAbs neutralized either poliovirus type 1 and 2 or all three serotypes. MAbs reactive with poliovirus type 1 and 3 but not with type 2 were not detected. Immunoprecipitation of radiolabeled poliovirus type 1 with crossreactive human Nt-mAbs was inhibited competitively by preincubation of mAbs with cold poliovirus type 3 and/or 2.

Processing of the FMDV Polyprotein

ZIBERT, A. and E. BECK

Zentrum f. Molekul. Biologie (ZMBH), D-6900 Heidelberg

The RNA genome of Foot-and-Mouth Disease Virus (FMDV) is expressed as a polyprotein which is rapidly processed into the mature proteins. Two viral proteases and at least one host cell protease are involved in this cleavage reactions. Characterizing the polyprotein
cleavage sites of the two viral proteases L/L' and P3c we constructed plasmids with mutations in each of these proteins. RNA derived from these plasmids by use of SP6 RNA polymerase was expressed in the rabbit reticulocyte lysate and proteins were identified by different specific antisera corresponding to nearly all parts of the polyprotein. As a result the only processing site found for the L/L' protease was the junction between L and P1. The other viral protease P3c seems to be responsible for all other cleavage sites except from the P1/P2 junction and the autocatalytic cleavage of the P1AB fusion in the viral capsid.

A Reovirus from the Snake Python regius

AHNE, W., I. THOMSEN, and J. R. WINTON

1 Inst. of Zoology and Hydrobiology, Univ., D-8000 München; 2 Dept. Microbiology, Oregon State Univ., Corvallis, Oregon/USA

A reovirus has been isolated from the snake *Python regius*. The agent replicated in IgH2 cells at 28–30 °C producing syncytia. Negatively stained virions revealed icosahedral particles of 70–75 nm with a double capsid structure. Polyacrylamide gel electrophoresis of nucleic acid showed ten segments of ds RNA with migration patterns different to the RNA of mammalian reoviruses. The agent was not neutralized by antiserum against mammalian reovirus serotypes 1, 2 and 3 and it did not agglutinate human O-erythrocytes.

The Relatedness of the Nucleoprotein(NP)-Genes of the Human H1N1 Influenza A Virus from the Years 1950, 1977 and 1978

ALTMÜLLER, A. and C. SCHOLTISSEK

Inst. f. Virologie (FB 18), D-6300 Gießen

The nucleoprotein(NP)-gene-sequences of four H1N1 Influenza A-virus strains were compared with each other and additional known virus strains. According to our sequence data the strain A/USSR/90/77 does not show remarkably antigenic drift in its NP-gene compared to its progenitor A/FW/1/50. The latter one had disappeared for 27 years and stayed in a yet unknown place. In the strain A/Brazil/11/78 the change of amino acid no. 291 coming from aspartate to glutamate to alanine probably results in the loss of the ability to “rescue” NP-temperature-sensitive mutants of fowl plaque virus in doubly infected chick embryo fibroblast cells. The NP-gene of the last strain A/Cal/10/78 shows intermediate homology between the sequences of human H1N1 and H3N2 strains.
Influenza Epidemic in a Pediatric Practice During January and March 1985

DÖLLER, G., K. Y. TÍHEN, P. C. DÖLLER, and H.-J. GERTH

Abt. Med. Virologie u. Epidemiologie d. Viruskrankheiten, Hyg.-Institut, D-7400 Tübingen
1 Niedergelassener Kinderarzt, Königstraße 10, D-7407 Rottenburg a.N.

We report on influenza infections as established by virus isolation of patients in a general pediatric practice during the influenza season. Because it is difficult to differentiate influenza from other acute respiratory infections, influenza has to be diagnosed by virological methods. From 202 children nasopharyngeal secretions were taken for virus isolation on MDCK-cells. Identification of influenza virus was carried out with FITC labelled specific antibodies (Döller et al., Med. Microbiol. Immunol. 176 (1987) 27–35). From 44 children influenza A subtype H3N2 was isolated, from 61 children influenza A subtype H1N1 and from 13 children influenza B. In 11 cases of influenza A/H1N1 O-D-phase variation was found. Three typical manifestations were observed: 1.) monophasic fever $\geq 38.5^\circ\text{C}$ (81.25%), 2.) biphasic fever (14.4%) and 3.) croup (4.24%). No relationship between influenza type resp. subtype and symptoms could be established. Influenza A/H3N2-activity reached its peak during the 5th and 6th week of 1985, influenza A/H1N1-activity during the 8th and 9th week, followed by an influenza B peak during 10th and 11th week.

The work was supported by the Federal Ministry for Research and Technology (01 ZR 051).

Identification of Coronavirus MHV-JHM mRNA4 Gene Product

EBNER, D. and S. G. SIDDELL

Inst. f. Virologie u. Immunobiologie, Univ., D-8700 Würzburg

Coronaviruses are positive-stranded, RNA viruses which replicate in the cytoplasm of infected cells. The expression of the murine coronavirus, (MHV), genome is mediated by 7 subgenomic mRNAs. The gene products of the mRNAs 7, 6 and 3 are the nucleocapsid, membrane and spike proteins of the virion. The coding sequences of several mRNAs encoding non-structural proteins have been cloned and sequenced, however, the corresponding gene products have not been rigorously identified in vivo. – A cDNA copy of MHV-JHM mRNA 4 has been cloned into the bacterial vector pUR 292 and expressed as a $\beta$-galactosidase fusion protein. The virally encoded polypeptide, synthesized in bacteria, was used to raise antibodies in rabbits. The antiserum directed to the C-terminus of the viral mRNA 4 gene product recognized the authentic polypeptide in vivo and in vitro. The major mRNA 4 translation product, as predicted from sequence analysis, is a 15,000 molecular weight polypeptide.
Cloning and Sequencing of the Nucleoprotein Genes of H3N2 – Influenza Virus Strains from South China

GAMMELIN, M., B. SCHRODER, and CH. SCHOLTISSEK

Inst. f. Virology, Univ., D-6300 Giessen

The genome of Influenza viruses consists of eight single-stranded RNA segments, which are exchangeable during double infection. Since avian Influenza viruses are probably not transferred to human beings under natural circumstances, and on the other hand, human Influenza viruses cannot be transferred directly to birds, the exchange of the single segments takes place in pigs, after double infection. Pigs are probably tolerant of growth of avian and human Influenza virus strains. The nucleoprotein (NP), whose Phosphopeptid-Fingerprint is strain-specific and host cell dependent, is very likely to be responsible for the species specificity. – The following questions are being examined by sequencing and comparison of sequences of the NP-genes of one human (Hk5), one avian (D7/4) and two porcine (Sw6, Sw127) Influenza virus strains: Are there species specific differences in the sequences of the NP-genes? – Is there an adaptation of the NPs to pigs, which would be the new host? – The present results allow the following conclusions: The sequence of the NP-gene of Hk5 possesses high homology to the NP-genes of other human Influenza virus strains and is clearly different to the sequence of the NP-gene of D7/4. D7/4 is very homologeous to the NP-genes of other avian Influenza virus strains. The NP-gene of Sw6 is very similar to the NP-gene of Hk5. On the other hand, the NP-gene of Sw127 is derived from an avian Influenza virus strain, but it shows many base exchanges, which cannot be found in D7/4 and other avian strains. – This means that, firstly, there are species specific differences in the sequences of the NP-genes, and, secondly, the NP-gene of Sw127 is derived from avian Influenza virus strains and is on its way to adaptation to pigs.

Variation in the Receptor Specificity and in the Acetyesterase Activity of Different Strains of Influenza C Virus

HERRLER, G. and H.-D. KLENK

Inst. f. Virologie, Univ., D-3550 Marburg

Previous studies with the strain Johannesburg/1/66 of influenza C virus have revealed that the receptor-destroying enzyme of this virus is a neuraminic O-acetylermesterase and that N-acetyl-9-0-acetylenuraminic acid (Neu5,9Ac2) is a receptor-determinant for attachment of influenza C virus to cells. We have extended our investigation to six other strains of influenza C virus. All strains were found to contain acetylermesterase. Using bovine submandibulary mucin (BSM) as substrate, the maximum variation in the enzyme activity of the different strains was tenfold. BSM and rat serum were found to prevent all strains of influenza C virus tested from agglutinating chicken red blood cells. The hemagglutination-inhibition titers determined for the different strains varied up to 100-fold. The inhibitory activity was abolished after incubation of the inhibitors with acetylermesterase. From these results we conclude (i) Neu5,9Ac2 serves as a receptor determinant for all influenza C viruses; (ii) factors other than the presence of Neu5,9Ac2 also affect the binding of influenza C viruses to cell surface receptors.
Regulation of Viral Gene Expression in Persistent Sendai-Virus Infections

HOMANN, H., C. ECKERSKORN, W. WILLENBRINK, and W. J. NEUBERT

MPI f. Biochemie, Abt. Virusforschung, D-8033 Martinsried

Sendai virus 6/94 causes acute and persistent infections in cell cultures. It is temperature sensitive. cDNA clones covering 75% of the viral RNA-genome were produced. The sequence of the cloned nucleocapsid protein (NP)-gene of Sendai virus 6/94 was determined. It differs from wild-type-virus Sendai-Z by a frame shift mutation at position 1598, possibly representing the ts-mutation. The altered C-terminus is 7 aminoacids longer, which was verified by sequencing the C-terminal tryptic peptide. The persistently infected cell lines P3 and CL-E-8 release 100 times less virus than acutely infected cells. Northern blot experiments showed that the mRNAs for all viral proteins are also strongly reduced. The tryptic peptide pattern of NP-protein from persistently infected E8-cells is slightly different from that of Sendai virus 6/94 NP-protein, as shown by HPLC-analysis. Different tryptic peptides are being sequenced. The NP-protein plays a central role in the regulation of viral transcription and replication. Therefore we assume that the low efficiency of virus replication is due to the alterations found in this protein.

Insects Expressing the Hemagglutinin of Influenza Virus

KURODA, K., A. GRÖNER, F. FRESE, R. ROTT, W. DOERFLER, and H.-D. KLENK

Inst. f. Virologie, Univ., D-3550 Marburg

The cloned hemagglutinin gene of fowl plague virus was introduced into the baculovirus AcNPV using homologous recombination techniques. Previously, biologically active hemagglutinin was detected in cultured insect cells infected with recombinant AcNPV (Kuroda et al., EMBO J. 5 (1986) 1359–1365). To express the hemagglutinin in whole insects, we have now infected larvae of tobacco budworm (Heliothis virescens). The hemagglutinin was synthesized in fat body, epidermis, and trachea, but not in the gut, indicating that the recombinant virus had the same tropism as authentic AcNPV. The hemagglutinin was correctly processed in these bodies into the fragments HA1 and HA2. Homogenates of infected larvae exhibited hemagglutinating activity and could be used for effective immunization of chickens against fowl plague.

Cloning and Sequencing of TBE Virus cDNA

MANDL, CH., HEIDI HOLZMANN, F. X. HEINZ, and CH. KUNZ

Inst. of Virology, Univ., A-1095 Vienna

The genome of Tick-borne encephalitis (TBE) virus consists of a positive-stranded RNA molecule of approximately 10,500 nucleotides, but lacks a polyA structure at its 3'-end. TBE-specific clones from the 5' -terminal part of the genome containing the entire sequence
information for all the structural proteins (C, prM, M and E) were sequenced. – The
deduced amino acid sequence contains characteristic stretches, such as hydrophobic anchor
and positively charged stop transfer sequences, signalase and protease cleavage sites that
function in the processing of the structural proteins. Homologies among flavivirus structural
protein sequences were found to vary between 20 and 90% depending on the proteins and
viruses compared. Glycosylation sites show some degree of homology but are not generally
conserved among flaviviruses. Cystein residues, however, are highly conserved stressing
their importance for the integrity and function of the protein structure.

Treatment of Lymphocytic Choriomeningitis Virus-Infected Mice with
Monoclonal Antibodies Reveals that Lyt-2+ T Lymphocytes Mediate
Clearance of Virus

MOSKOPHIDIS, D. and F. LEHMANN-GRUBE

For studying the mechanism with which higher organisms control a virus infection, the
adult mouse acutely infected with the LCM virus is valuable, because the virus replicates in
all major organs, yet causes few pathologic alterations; hence, termination of the infection is
not confounded by illness. Monoclonal antibodies (MAb) directed against T lymphocytes
and subsets were inoculated once after the animals’ infection and the effects on virus
elimination and antiviral immune responses were determined. Anti-Thy-1 and anti-Lyt-2
MAb diminished virus clearance and activity of cytotoxic T lymphocytes (CTL) but reduced
only partly (anti-Thy-1) or not at all (anti-Lyt-2) the antibody response. Treatment with
anti-UT4 MAb had no effect on virus elimination or CTL activity but abolished the
humoral response. We conclude that for the control of the virus CTL are essential, whereas
antibodies play no relevant role.

Selective Inhibition of Translation of the mRNA Coding for Measles Virus
Matrixprotein at Elevated Temperatures

OGURA, H., K. BACZKO, B. RIMA, and V. TER MEULEN

Measles virus present in SSPE is characterized by defects of one or several envelope
proteins, whereby the matrixprotein is mostly affected. It is not known by which mechanism
these defects are produced. Elevation of temperature may play a role, because rising the
temperature of measles virus infected cells from 35°C to 39°C resulted in immediate
inhibition of M protein synthesis. At 39°C the corresponding mRNA was still present and
the stability of the M protein was not significantly altered. Messenger RNAs extracted
before and after shift up produced nucleocapsid, phospho and matrix-protein in vitro at
32°C and 39°C. The data indicate that MV matrix-protein production is selectively sup­
pressed in vivo at elevated temperatures because of the inability of the translation system to
interact correctly with the matrix mRNA.
The Exposition of Antigenic Determinants of the Semliki Forest Virus Envelope Glycoprotein E\(_2\) can be Altered by Carbohydrate Chains

REPGES, S. and G. KALUZA
Inst. f. Virologie, FB Humanmedizin, D-6300 Giessen

On E\(_2\) of the viral envelope, 6 non competing epitopes a–f can be discerned by means of monoclonal antibodies. Blocking tests revealed that carbohydrate side chains of the viral glycoproteins compete with at least one of them (f). Epitopes b and e cannot be detected, if intramolecular disulfide bonds in the viral envelope are cleaved, or in E\(_2\) of virus particles released in the presence of M\(_6\)N (such particles are devoid of complex carbohydrate chains), or in infected cells after a 15 min – pulse labelling. These two epitopes seem to be located on a critical structural point specific for the final conformation in the spike complex. Antibodies directed to epitope b have neutralizing properties. The data confirm earlier findings, that the final structure of the envelope glycoprotein E\(_2\) is acquired upon particle release.

Molecular Biological Aspects of Virus-Induced Subacute Encephalomyelitis in Lewis Rats

SCHNEIDER-SCHAULIES, S., U. G. LIEBERT, K. BACZKO, and V. TER MEULEN
Inst. f. Virologie u. Immunbiologie, Univ., D-8700 Würzburg

In order to define parameters leading to persistent measles virus (MV) infection of the CNS, the expression of MV specific RNA's and the related proteins were investigated in our model of measles virus induced subacute encephalomyelitis (SAME) in Lewis rats. In vivo results suggest that synthesis of measles virus H, F and M protein is reduced or absent in the brain of SAME animals already 2–3 weeks following infection. The virus specific mRNAs, however, have been shown to be present for all six structural proteins in the subacute type of infection by Northern blot analysis and in situ hybridization with strand specific probes. In contrast, in \textit{in vitro} experiments the matrix protein and probably the H proteins cannot be translated from the corresponding mRNA. – These results indicate specific restriction of the expression of viral M protein in CNS infections supporting measles virus persistence.

Selection of Mutations in the Primary Immunogenic Proteins of Sendai-Virus in Persistently Infected Mice

SCHREIBER, G. A., E. M. KOCH, and P. H. HOF SCHNEIDER
Max-Planck-Inst. f. Biochemie, Abt. Virusforschung, D-8033 Martinsried

C129 mice were inoculated with Sendai-Wildtype-Virus (Strain DS2) or ts-mutants ts1–5 transcription-defective at 39°C. Infectious virus could be shown in the sera of all infected mice on PID 22. Brain cells of the ts4 infected mice were cocultivated with uninfected mice
brain cells (MGB) on PID 26 at 31 °C. However virus released from this culture was ts in the HN-gen product only. This finding can be explained by reversion of the transcription-mutation and establishment of a new mutation in the HN-gen in the organism. Furthermore, there is a mutation in the second surface-antigen F. This mutation which allowed the cleavage of the inactive fusion-protein F0 to the infectious form F1–F2 by a cellular protease was not ts. After 30 days in culture a reversion of the HN-ts-mutation could be seen. These results might implicate the pressure of the immunsystem in the selection of mutants of the surface-antigens. In absence of this selection-pressure in the culture system the revertants become dominant.

Characterization of the Repetitive DNA Sequences in the Genome of Insect Iridescent Virus Type 6 by Molecular Cloning and Physical Mapping

FISCHER, M., J. B. SOLTAU, P. SCHNITZLER, H. DELIUS, and G. DARAI

Inst. f. Med. Virologie, Univ.; Europ. Molec. Biology Laboratory, D-6900 Heidelberg

The genome of Insect Iridescent Virus Type 6 – Chilo Iridescent Virus (CIV) – was shown to be circularly permuted and terminally redundant. A defined and complete gene library of the whole CIV DNA sequences (209 kbp) was established, e.g. this gene library comprises the complete 32 EcoRI DNA fragments of the viral genome. The physical map of the viral genome was constructed for the restriction endonucleases Apal, Asp718, BamHI, EcoRI, Ncol, PvuII, Sall, Smal, and SphI. Although the CIV genome is linear, due to the circular permutation, the restriction maps are circular. The defined gene library of CIV DNA was used for identification of further structural and functional properties of the viral genome. Hybridization experiments and heteroduplex mapping were performed. The repetitive DNA elements were detected in the EcoRI DNA fragments H (9.8 kbp; 0.535–0.582 μm) and C (13.5 kbp; 0.909–0.974 μm). The fine mapping of these regions and hybridization experiments revealed that the DNA sequences from one flank of the EcoRI DNA fragment H (0.535–0.548 μm; 2.65 kbp) are partially homologous to a part of DNA sequences of EcoRI DNA fragment C (0.920–0.944 μm; 5.1 kbp). The further characterization of these repetitive DNA elements by nucleotide sequencing is necessary for the understanding of the functional activity and regulatory role of these elements.

DFG Project Da 142/2–2.

Herpes simplex Virus Induces Overreplication of Cellular DNA-Sequences Linked to Integrated SV40-DNA in a Transformed Hamster Cell Line

GERSPACH, R., G. LIPS, and B. MATZ

Inst. f. Virologie, Univ., D-7800 Freiburg

HSV induces amplification of SV40-DNA-sequences in certain SV40-transformed cells. The hamster cell line Elona contains SV40-sequences integrated once in the genome as a partial tandem. The amplified DNA isolated after HSV-infection has the structure of high
molecular weight head to tail-concatemers of SV40-DNA-molecules. – In this study a genomic library of Elona DNA was constructed in the lambda vector L47, and the sequences flanking the integrated SV40-DNA were isolated. Deduced plasmid clones used as hybridization probes against genomic Elona-DNA show the amplification of the cellular DNA-sequences flanking the SV40-integration site.

This study was supported by Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 31).

Demonstration of Herpes Simplex Virus (HSV-1) Structural Components Exhibiting Cell Receptor Activity in a Cell Blot

KÜHN, J. E., B. R. EING, K. MUNK, and R. W. BRAUN

Institut of Med. Virology, Univ., D-6900 Heidelberg

Specific binding activity of intact RC-37, Hep-2 and HL-60 cells against HSV-1-Ang proteins could be demonstrated in dot blot experiments and Western blots. Different denaturing and “non-denaturing” gel systems were compared. Under denaturing gel conditions cells bound to viral proteins with app. mw’s of 140 kD, 85 kD, 80 kD, 71 kD and 41 kD. Pretreatment of virions with 1% NP40 and subsequent ultracentrifugation on a sucrose cushion showed that binding activity was exclusively located in the pellet containing insoluble material, e.g. predominantly viral capsids. No cell binding activity against the major viral glycoproteins could be demonstrated. The observed cell binding activity was sensitive to trypsin digestion of virions prior to gel electrophoresis. A rabbit anti-HSV-1 serum completely inhibited cell binding in dot blots, in Western blots cell binding against the 41 kD protein was inhibited.

Activation of a Simian Virus 40 Origin of DNA Replication by Herpes Virus Type 1

MATZ, B., G. LIPS, and R. GERSPACH

Institut f. Virologie, Univ., D-7800 Freiburg

Earlier studies have shown that an early gene function of herpes simplex virus (HSV) is involved in the aberrant overreplication of chromosomally integrated SV40 DNA sequences in transformed cells. Subsequently, it could be demonstrated that circular plasmid vector DNA bearing the SV40 genome was amplified when transfected into non-transformed hamster cells upon infection with HSV. – In the present study a series of recombinant plasmid constructions with SV40 and pSPT18 were assayed for activation by HSV infection. Amplification was abolished (i) when the 5'-regulatory region of early transcription was deleted, (ii) when the T-antigen gene was dissected, (iii) when the hairpin structure of the SV40 origin was altered. Deletion of the late region had no effect. – The results indicate a concerted action of SV40 origin, T-antigen and herpesviral DNA replication functions.

This study was supported by Deutsche Forschungsgemeinschaft (SFB 31).
Monoclonal Antibodies Against HSV-1 Specific Proteins in Nuclei of Infected Cells

SCHENK, P.¹, S. PIETSCHMANN², G. PAULI¹, H. GELDERBLOM², and H. LUDWIG

¹ Inst. f. Virologie, Univ., D-1000 Berlin;
² Bundesgesundheitsamt, Robert Koch-Inst., D-1000 Berlin 65

Thirteen different monoclonal antibodies directed against antigens in HSV-1 infected cell nuclei were selected. Immunoblotting and immunoprecipitation revealed that these antibodies belong to four different recognition groups. They react with: 1. the major DNA binding protein (ICP8, 130kd); 2. the ICP35 family; 3. the 65k DNA binding protein, these antibodies were characterized in collaboration with H. S. Marsden (Glasgow); 4. a 60kd protein, present in virions and cells. None of the antibodies neutralized HSV-1. The localization of the antigens in the nuclei were specified by immune electronmicroscopy. The ICP35 family is associated with nucleocapsids, whereas the DNA binding proteins show a patchy distribution. There is evidence that some antigens represent infected cell proteins and are not a virus constituent.

Identification and Nucleotide Sequence Analysis of the Repetitive DNA Elements in the Genome of Fish Lymphocystis Disease Virus

SCHNITZLER, P., H. DELIUS¹, J. SCHOLZ, M. TOURAY, E. ORTH, and G. DARAI

¹ Europ. Molec. Biology Laboratory, D-6900 Heidelberg

The genome structure of the Fish Lymphocystis Disease Virus (FLDV) was found to be circularly permuted and terminally redundant. A defined and complete gene library corresponding to 100% of the DNA sequences of the viral genome (98 kbp) was established. Using this defined gene library, DNA-DNA-hybridization experiments and heteroduplex mapping, two repetitive DNA elements were detected which mapped at the coordinates 0.034 to 0.057 mu (a part of the EcoRI DNA fragment B; 12.7 kbp, 0.034 to 0.165 mu) and at the coordinates 0.718 to 0.736 mu (EcoRI DNA fragment M; 1.4 kbp). The nucleotide DNA sequences of the repetitive DNA element was carried out. This analysis revealed the presence of many small direct and inverted repetitions, e.g. two direct repetition boxes (TTTAAAATTTAAATTAA) started at the nucleotide position 812 and 942 and two inverted repetitions (TTAAATTTAAATTAA) at the nucleotide position 820 and 959. For the evaluation of the regulatory role of these signals the corresponding DNA sequences of the repetitive DNA elements of FLDV are now compared to the known repetitive DNA sequences of other viruses, e.g. pox- and herpesviruses. Furthermore DNA-DNA hybridization experiments revealed that these repetitive DNA elements are conserved in the genome of different strains and types of FLDV, indicating the importance of these DNA elements for virus progeny, e.g. virus replication and encapsidation.

DFG Project Da 142/2–2.
Replacement of Glycoprotein B Gene Sequences in Herpes simplex Virus Type 1 Strain ANG by Corresponding Sequences of the Strain KOS Causes Changes of Plaque Morphology and Neuropathogenicity

WEISE, KERSTIN, H. CH. KAERNER, and C. H. SCHRODER
Inst. f. Virusforschung, Deutsches Krebsforschungszentrum, D-6900 Heidelberg

Glycoprotein B (gB) sequences derived from the peripherally apathogenic HSV-1 strain KOS 321 were transferred to pathogenic HSV-1 ANG strains. In co-transfection experiments the cloned HSV-1 KOS Bam HI G fragment served as donor and genomic DNA of two ANG variants as recipients. One of these variants termed HSV-1 ANG path expresses glycoprotein C (gC), the other termed C18 was a spontaneous gC negative mutant. Both ANG strains are of the syn phenotype whereas HSV-1 KOS 321 is syn+. In this report we show that the syn phenotype of HSV-1 strain ANG path is governed by the syn 3 locus. We present evidence that the absence of gC expression under certain genetically defined conditions is not compatible with the syn+ phenotype. We further provide evidence that the Bam HI G fragment derived from the peripherally apathogenic HSV-1 KOS strain may confer apathogenicity to a pathogenic strain. In converse experiments the Bam HI G fragment of HSV-1 ANG path restored pathogenicity of apathogenic subclones. From our data it could be conceived that gB is one determinant of peripheral neurovirulence of HSV-1.

Microtitration Plate Hybridization and its Application for the Detection of HCMV

FISCHER, K.1, C. METZ1, H. G. BESCHLE1, and R. FRANZE2
1 Behringwerke AG, D-3550 Marburg;
2 Boehringer Mannheim GmbH, D-8122 Penzberg, present address

Using hybridization for the diagnosis of infectious diseases two major problems are radioactive probes and the detection of hybrid formation by immobilizing nucleic acids on nitrocellulose filters. Alternatively biotinylated probes were used in combination with microtitration plate hybridization: 10 pg purified HCMV-DNA could be detected in comparison to filter hybridization where a 32P-labeled probe detected 100 pg and a biotinylated probe about 500 pg DNA. Selfhybridization of the biotinylated HCMV-specific probe gave a sensitivity of detection of 1 pg homologous DNA. Using sandwich hybridization on microtitration plates HCMV was detected in the supernatant of infected fibroblasts and in urine reconstituted with the virus. Microtitration plate hybridization, which has the potential of instrument based analysis, together with non-isotopic probes may lead to a better acceptance of hybridization in the clinical laboratory.
Core Histones Mediate DNA Binding of the Murine Cytomegalovirus Regulatory Immediate-Early Protein pp 89

MÜNCH, K., G. M. KEIL, M. MESSELE, and U. H. KOSZINOWSKI
Bundesforschungsanstalt f. Viruskrankheiten d. Tiere, D-7400 Tübingen

pp 89 is the major product of the transcription unit iel of the murine cytomegalovirus (MCMV). We have reported previously that pp 89 activates viral promoters in trans. In order to analyze the molecular mechanisms of transcriptional activation, we have studied the DNA-binding capacity of pp 89. Chromatography of extracts from infected cells on calf thymus DNA cellulose showed that pp 89 interacts with DNA. This interaction was dissociated at salt concentrations between 0.3-0.6 M NaCl. pp 76, a cleavage product of pp 89, and additional minor iel proteins eluted already at low ionic strength. pp 89 lacks intrinsic affinity for DNA, since cellular factors were required for DNA binding. These factors were identified as core histones. A role for an amino acid sequence homology in the N-terminal region of pp 89 with histone H2B in the pp 89-histone-DNA interaction is discussed.

Semliki Forest Virus (SFV) Induced Cell-Cell Fusion is an ATP-Dependent Event

KEMPF, C., M. R. MICHEL, U. KOHLER, and H. KOBLET
Inst. Hygiene Med. Microbiol., Univ., CH-3010 Bern

SFV infected Aedes albopictus cells which fuse at mildly acidic pH (6.2) were used to investigate cell-cell fusion. It was shown that this polykaryon formation correlated with a decrease in cellular ATP and could be blocked by inhibitors of oxidative phosphorylation. However, if infected cells – in presence of the inhibitors – were exposed for only a short time to pH 6 (e.g. 1 min) the intracellular ATP transiently recovered and polykaryon formation occurred. Using microinjection of the highly fluorescent, non permeable dye Lucifer yellow it was demonstrated that under normal conditions 90% of infected cells had fused with their neighbours. In presence of KCN no such cell-cell fusions could be observed. Thus, it can be concluded that SFV-induced polykaryon formation requires a specific expenditure of energy and that ATP is used early in the fusion process.

Epitope Mapping of TBE Virus Glycoproteins

GUIRAKHOO, F., F. X. HEINZ, and CH. KUNZ
Inst. of Virology, Univ., A-1095 Vienna

The antigenic structure of the E glycoprotein of Tickborne encephalitis (TBE) virus was studied using a panel of monoclonal antibodies (Mabs). 17 epitopes were identified which have different properties with respect to serological reactivity, functional activity or topological localisation. – They were found to cluster in three antigenic domains, A, B, and C –
Most epitopes of domain A are denaturation sensitive. Low pH-induced conformational changes were localized to certain epitopes within domain A. These may play an important role for membrane fusion events in the course of virus infection. The B domain epitopes are also highly conformation-dependent, but their structure is stabilized by disulfide bridges as revealed by reduction and carboxymethylation experiments. These disulfide bridges are probably also responsible for the protease resistant tertiary structure of domain B.

**Measles Virus Encephalomyelitis in Lewis Rats: A Model for Virus Induced Autoimmune Reactions**

LIEBERT, U. G., SIBYLLLE SCHNEIDER-SCHAULIES, K. BACZKO, and V. TER MEULEN

Inst. f. Virologie u. Immunbiologie, Univ., D-8700 Würzburg

Pathogenesis of the measles virus induced central nervous system (CNS) complications in man, subacute sclerosing panencephalitis and post infectious measles encephalomyelitis, includes the development of a defective replication cycle and possibly the generation of autoimmune reactions. In our model of subacute measles encephalomyelitis (SAME) both these aspects have been analyzed. The histopathological abnormalities of SAME are characterized by a non-demyelinating encephalomyelitis with prominent mononuclear cell infiltrations. Molecular biological studies demonstrate a persistent measles virus infection of brain cells with all viral mRNAs present, but restriction of viral gene expression. In animals with SAME a cell-mediated immune response specific for myelin basic protein (MBP) was found. T-cell lines with specificity for MBP isolated from these rats induce encephalitis in naïve recipients. These observations indicate that autoimmune reactions occur in association with a persistent measles virus in CNS tissue which may be involved in the pathogenesis of SAME in rats.

**In Vitro Expression of the Coronavirus MHV-JHM Surface Protein**

PFLEIDERER, M. and S. G. SIDDELL

Inst. f. Virologie u. Immunbiologie, Univ., D-8700 Würzburg

The MHV-JHM virion is comprised of a large infectious RNA together with the nucleocapsid, membrane and surface proteins. The surface protein plays a major role in the pathogenesis of the viral infection. It is the major target of both the humoral and cell-mediated immune response, carries fusogenic properties and determines cell and tissue tropism. To initiate a functional analysis of the MHV-JHM S protein we have cloned and sequenced the S protein gene. The cDNA copy was then inserted into the pGEM vector and full-length, capped RNA transcripts were synthesized using T7 polymerase. The *in vitro* transcripts were translated in a reticulocyte lysate and the products analysed by immunoprecipitation with an S protein specific rabbit antiserum. The antiserum immunoprecipitated a major 136,000 molecular weight product, the expected size of the S protein. With this system we shall map monoclonal antibodies recognizing sequential epitopes by truncating or deleting regions of the S gene. Moreover, the S gene construct can now be confidently inserted into the vaccinia virus expression system and analysis of the functions of S protein domains undertaken in vivo.
Abstracts of Papers at the Session of the Virology Section of the DGHM

Isolation and Characterization of the Influenza C Glycoprotein

FORMANOWSKI, F. and H. MEIER-EWERT

Deprt. Med. Virology, Fac. Medicin, Techn. Univ., D-8000 München 40

The surface glycoprotein of influenza C Johannesburg/1/66 was removed from the virion by treatment with the protease bromelain. The isolated glycoprotein has an estimated molecular weight of 75 000 dalton. The calculated molecular weight of the remaining membrane bound portion of the molecule is 3 000 Dalton. The isolated glycoprotein was purified by sucrose density gradient centrifugation. The position of the protein trimer band in the gradient indicated a sedimentation coefficient of approximately 10 S. – After isolation of the uncleaved glycoprotein (gp I), from MDCK-cell grown virus, with bromelain, the protein is cleaved into two subunits held together by a disulphide bond. The estimated molecular weights of the subunits are 59 000 dalton and 28 000 dalton. – Comparison of the cleaved glycoprotein from cell grown virus to gp 65 from egg grown virus, by SDS-polyacrylamide gelelectrophoresis, showed no difference in the migration of the two proteins. This indicates that cleavage of cell grown glycoprotein by bromelain occurs near the natural cleavage site. This bromelain cleavage site was further analysed.

Structural and Pathogenic Properties of a Reassortant Derived from the Two Serotypes of the Infectious Bursal Disease Virus (IBDV)

MÜLLER, H. K., H. MÜLLER, and H. BECHT

Inst. f. Virologie, Univ., D-6300 Gießen

Infectious Bursal Disease Virus is the causative agent of a severe disease of chickens. The main feature of this infection is the destruction of the Bursa of Fabricius (B.F.). Turkey isolates are not pathogenic for chickens. There is hardly any crosreaction in neutralization tests between chicken isolates (ST 1) and turkey isolates (ST 2). Besides differences in the size of their two RNA-segments, there are differences in the protein pattern. A reassortant was produced by double infection with Cu-1 (ST 1) and 28/82 (ST 2). The larger segment of the reassortant was derived from the chicken strain Cu-1 and the smaller one coding for the virion-associated polymerase was from the turkey strain. The reassortant causes no clinical signs altough many bursal cells are infected. The bursal follicles are not totally destroyed. Therefore the pathogenic property of IBDV depends partly on the smaller segment, which codes for the RNA-polymerase.
Further Results of Seroepidemiological Studies of Haemorrhagic Fever with Renal Syndrome (HFRS) in Germany

PILASKI, J.¹, R. PECENY¹, O. GORSCHEWSKY¹, G. KRATZSCH², M. ZEIERT³, and H. W. LEE⁴

¹ Med. Institute of Environm. Hygiene, D-4000 Dusseldorf
² Praxis Innere Medizin u. Rheumatologie, D-7900 Ulm
³ Sekt. Nephrologie, Klinikum, Univ., D-6900 Heidelberg
⁴ Inst. for Viral Diseases, Seoul/Korea

The indirect immunofluorescent antibody (IFA) technique was applied using Vero-E6 cells infected with different hantavirus strains (76–118, NE-Hällnäs, NE-Puumala, Prospect Hill, SR-11). A serological survey in 3 hospitals at Duesseldorf revealed that 10 out of 820 (1.2%) indoor-patients have hantavirus antibodies. Between July 1985 and December 1986 a total of 5 clinical cases with renal failure has been observed in humans living in the southwestern part of Germany, all being confirmed by serological examination. Hantavirus antibodies were also found in 3 free-living rodent species, i.e. \textit{Rattus norvegicus}, \textit{Clethrionomys glareolus} and \textit{Microtus arvalis}. In August 1986 a seropositive \textit{Clethrionomys glareolus} had been trapped near the living place of one clinical patient.

Differentiation Between Primary and Recurrent Cytomegalovirus Infections

SCHMITZ, H., M. P. LANDINI, and A. BRÜHMANN

Virol. Abt., Bernhard-Nocht-Inst., D-2000 Hamburg 4

As could be shown by Western-Blot analysis an increasing number of epitopes on different protein bands can be detected during the first weeks after onset of a primary cytomegalovirus (HCMV) infection. After several weeks antibodies to numerous protein bands of complete virus particles could be demonstrated. In transplant recipients after reactivation additional bands and broadening of the protein bands was observed. As a result of these observations an inhibition test was developed (Arch. Virol. 90 (1986) 41) which can be used for the differentiation between primary infections and reactivations. This test-principal might be further improved if the polyclonal human antibody tracer could be substituted by monoclonal antibodies directed to "late epitopes". In order to detect such "late epitopes" the protein-patterns obtained with sera of primary cytomegalovirus infection and of reactivations were compared by immunoblotting. From these studies it could be concluded that weak epitopes could be found preferentially on the 150 000 protein. These epitopes were only detected in sera from persons after repeated immunisation during reactivation.
The Variability of the Abundant Structural Phosphoprotein pp65 of the Human Cytomegalovirus and its Antigenic Properties

JAHN, G., B.-C. SCHOLL, B. TRAUPE, B. RÜGER, and S. KLAGES

Inst. f. Klin. u. Molek. Virologie, Univ., D-8520 Erlangen

Human cytomegalovirus (HCMV) particles contain a phosphoprotein of about 65 000 (pp 65) molecular weight in the matrix. The protein is the predominant structural constituent of HCMV particles, mainly found in dense bodies of lytically infected cells. Recent clinical isolates of HCMV do not necessarily have pp 65 as an abundant component; neither do these low passage wild-type viruses have appreciable amounts of dense bodies. The humoral immune response of infected individuals against pp 65 is variable and not always detectable by Western blot analyses. The reactivity did not correlate with the clinical status; however, there was a correlation with the titre in the complement fixation test. We raised antibodies against HCMV phosphoproteins in rabbits for gene mapping and corroborated the relative immunogenic properties of HCMV proteins. Immunization with dense bodies or with gel-purified pp65 resulted in rapid seroconversion. Taken together these experiments show that pp 65 is immunogenic and the weak antibody response in the course of natural infection is probably due to low amounts of pp 65 in wild-type virions. The genomic organization of the gene coding for pp 65 has been recently described in detail (B. Rüger et al., J. Virol. 61 (1987) No. 2).

CD8 Positive T Lymphocytes Specific for Murine Cytomegalovirus Immediate-Early Antigen Mediate Protective Immunity

REDDEHASE, M. J. 1, W. MUTTER 1, K. MÜNCH 1, H.-J. BÜHRING 2, and U. H. KOSZINOWSKI 1

1 Bundesforschungsanstalt f. Viruskrankheiten d. Tiere, D-7400 Tübingen;
2 Med. Universitätsklinik, D-7400 Tübingen

Cytomegalovirus (CMV) disease is one of the leading reasons for mortality in immunocompromised patients. Opportunistic primary or recurrent CMV infection causes interstitial pneumonia in bone marrow transplant recipients, and is also involved in the acquired immunodeficiency syndrome (AIDS). We have established a murine model system for studying CMV disease with particular respect to the virus-specified antigens implicated in protective immunity. Control of virus multiplication in host tissue, protection from virus-caused tissue destruction, and survival were mediated by specifically sensitized T lymphocytes of the CD8 subset. The antiviral effector cells did not recognize structural antigens of the virion, but were specific for the nonstructural immediate-early (IE) antigen(s) of murine CMV. Thus, murine CMV disease provides an example of a role for nonstructural viral antigens in protective immunity.

* M. J. Reddehase et al.: J. Virol. 55 (1985) 264.
M. J. Reddehase, W. Mutter, and U. H. Koszinowski: J. Exp. Med. 165 (1987) 650.
Expression of the Major Immediate-Early Protein of Murine Cytomegalovirus in Vaccinia and Recognition by Cytolytic T Lymphocytes (CTL)

VOLKMER, H.¹, R. WITTEK², S. JONJIĆ¹, and U. H. KOSZINOWSKI¹

¹ Bundesforschungsanstalt f. Viruskrankheiten d. Tiere, D-7400 Tübingen
² Inst. de Biologie Animale, CH-1000 Lausanne

Several differently spliced mRNAs are transcribed from transcription unit Iel of MCMV. An abundant 2.75-kb mRNA codes for the major Iel protein pp 89. This phosphoprotein is transported to the nucleus, where it is able to transactivate genes. An unknown Iel product is recognized by CTL in association with MHC class I gene products. After transfection of Iel in L cells, it still remained unresolved whether the major Iel protein pp 89 or the product of one of the minor Iel RNA species is responsible for target formation. In order to integrate the pp89 open reading frame (orf) into vaccinia, the introns interrupting the pp89 orf were removed by site-directed mutagenesis. By cloning into the expression vector pGS 62, the pp89 orf was brought under control of a vaccinia promoter. By homologous recombination, the pp89 orf was subsequently integrated into vaccinia. Immunoprecipitation revealed the expression of pp89 and its 76kD processing product in cells infected with the vaccinia-pp89 orf recombinant. The nuclear localization was confirmed by indirect immunofluorescence. A cloned line of Ie-specific CTL recognized vaccinia-pp89 orf infected target cells. Therefore, conclusive evidence could be obtained that a regulatory nonstructural herpesviral protein gives rise to a membrane antigen detected by CTL.

Evidence for Human Polyomavirus JC Infection in the Central Nervous System of Non-PML-Patients

DÖRRIES, KRISTINA

Inst. f. Virologie u. Immunbiologie, Univ., D-8700 Würzburg

Primary JCV infection leads to lifelong persistency in men. In rare cases a lytic infection of glia cells results in PML probably as a consequence of late reactivation of a persistent CNS infection. This would require the presence of JCV in non-PML brain. – In search for the evidence of JCV DNA we examined brain material of individuals with several systemic and neurological disorders others than PML by molecular hybridization techniques. Although the concentration of virus sequences was low, JCV supercoiled DNA could be detected corresponding in length with JCV genomes isolated from PML patients. Electro-eluted cellular DNA hybridized with the complete BamHI/PvuII restriction pattern of JCV strain GS thus showing the presence of JCV genomes in the CNS of individuals without PML. – These data support the idea that reactivation of persistent JCV infection may contribute significantly to the pathogenesis of PML.
Replication of HSV-2 Variants in the Adrenal Glands of Mice

PODLECH, J., D. POTRATZ, J. PREUHS, S. MÜLLER, and D. FALKE

Div. exp. Virology, Inst. Med. Microbiol., Univ., D-6500 Mainz

Recently, we described the replication of strains of HSV-1 in the adrenals of mice, independently of the degree of their neuropathogenicity (Arch. Virol. 90 (1986) 207). Therefore, we postulated two types of tropism. Moreover, corticoid hormone levels were increased in the serum. Strains of HSV-2 however replicated less well in the adrenals, some strains lacked this replication completely. We now succeeded in isolating 2 variants from the strain ER, one replicates very well, the other only to a low degree in the adrenals. Therefore, a third type of tropism emerged. Both variants were isolated after several passages along the adrenals and plaque purified. Both lines are stable. We tested other properties of replication in macrophages, Vero-cells, corticoidal cells, type of CPE, replication in the ovaries and the uterus, determined the i. p. and i. c. LD50's. The proteins were labelled with 35S-methionine and 3H-mannose. DNA was cut with RE and analyzed in agarose gels. The strong replication in the ovaries and the uterus seems to correlate to the higher susceptibility of female mice to HSV.

Analysis of the Coding Capacity of the Genetic Information Located Between 0.762 to 0.789 Map Units of the HSV-1 Genome and its Influence on the Viral Pathogenicity

RÖSEN-WOLFF, A., W. LAMADÉ, Y. BECKER1, and G. DARAI

Inst. f. Med. Virologie, Univ., D-6900 Heidelberg;
1 Dept. Molec. Virology, The Hebrew Univ., Jerusalem/Israel

The HSV-1 strain HFEM is apathogenic for the tree shrew and mice by the intraperitoneal (IP) route due to a deletion in the genome coordinates 0.762–0.789. Insertion of the BamHI DNA fragment B (0.738–0.809 mu) or of the MluI DNA fragment (coordinates 0.7615–0.789) molecularly cloned from the virulent HSV-1 strain F restored the IP pathogenicity to strain HFEM. Stable intratypic recombinant virus was established, virulent for both animals, indicating that the viral DNA sequences involved in the IP pathogenicity of HSV-1 is located within the genome coordinates 0.761–0.796. The determination of the nucleotide sequence flanking the deletion in the genome of the avirulent HSV-1 HFEM and the corresponding region of the genome of the virulent recombinant virus RMIC1 was carried out and compared to the DNA sequences of the virulent wild type HSV-1. This analysis revealed the presence of open reading frames (ORF) in this region and their functional activity seem to be involved in the viral pathogenicity. The corresponding DNA sequences should be recognized differently by the cellular elements which control the development of the HSV-1 infection in the tree shrew and the mouse.

DFG project Da 142/1–3.
Combinations of exoglycosidases and endoglycosidases were used to specifically cleave carbohydrate chains and residues from glycoproteins of purified intact HSV-1 virions. The additional proteolytic activity of enzymes was controlled in different assays and was considered in the interpretation of results. Glycosylation of the four major glycoproteins of HSV-1 (gC, gB, gE and gD) was separately examined using monospecific antibodies. Besides some detailed information concerning the glycosylation pattern of gC and gE the results of these studies revealed a strong high mannose glycosylation pattern of gB not being essential for virion infectivity. It could be demonstrated that digestion of virions by protease-free endoglycosidases reduced virion infectivity. This reveals an essential role of N-glycosylation in the infectious process. Further experiments show evidence that the removal of N-linked glycosylation interferes with virus adsorption.

Comparison of the Nucleotide and Aminoacid Sequence of the Glycoproteins gB of Bovine Herpesvirus 2 and Herpes Simplex Virus

Bovine herpesvirus 2 (BHV-2) specifies a glycosylated polypeptide of 130,000 mol. wt. (BHV-2 gB), which cross-reacts with glycoprotein gB of herpes simplex virus type 1 (gB-1). Monoclonal antibodies to both glycoproteins have been used to screen subgenic libraries of BHV-2 and HSV-1 DNA cloned in the expression vector lambda gt11. Inserts of positive recombinant phages were used as probes to identify large DNA-fragments cloned in the lambda EMBL3 vector. Nucleotide sequencing lead to the determination of three open reading frames in the BHV-2 genome. They show extensive homology to the genes encoding ICP 8, ICP 18.5 and gB of HSV-1. Chou and Fasman analyses of the gB molecules revealed similar secondary structures of both glycoproteins. The putative glycosylation sites and 9 cys residues of BHV-2gB could be aligned with identical positions of gB-1 (strain F) indicating further homologies in the tertiary structures.
Ein neues Epstein-Barr-Virus (EBV) – spezifisches Kernantigen wird durch die großen internen Repetitionen kodiert

SAUTER, M. und N. MÜLLER-LANTZSCH

Inst. f. Virologie, Univ., D-7800 Freiburg

Transkriptionsanalysen und Charakterisierung von cDNA-Klonen zeigen, daß die mRNA für das EBNA1-Protein über einen Bereich von 70 kb der EBV-DNA gespaltet wird. Die EBNA1-spezifischen cDNA-Klone enthalten neben dem Leserahmen für das EBNA1 noch zwei weitere Leserahmen für bisher unbekannte Proteine. Der erste offene Leserahmen besteht aus verschiedenen Exons aus den viralen Genomfragmenten Bam HI W und Y (W1, W2, Y1 und Y2; Speck und Strominger, Proc. Natl. Acad. Sci. 82, 8205–8309, 1985). Das Exon W1 wurde in dem Tryptophan-regulierten Expressionsvektor pATH11 exprimiert. Kaninchenserum gegen das erhaltene Fusionsprotein erkennen, von einigen Ausnahmen abgesehen, im Burkitt-Lymphom wie auch in EBV-immortalisierten lymphoblastoiden Zelllinien ein Protein, das je nach Zelllinie ein Molekulargewicht von 50 000–65 000 aufweist. In Zellen, die den nicht immortalisierenden EBV-Stamm P3HR-1 exprimieren, konnte nur ein verkürztes Kernprotein mit einem Molekulargewicht von 30 000 erkannt werden. Dieses neue Kernprotein wird teilweise durch virale DNA-Regionen kodiert, die in dem nichtimmortalisierenden EBV-Stamm P3HR-1 deletiert sind. Daher könnte dieses Protein für die Immortalisierung von B-Lymphozyten durch EBV von großer Bedeutung sein. Versuche zur subzellulären Verteilung zeigen, daß dieses neue EBV-Protein hauptsächlich in der Kernmatrix einer Zelle lokalisiert ist.

Epitopic Mapping of Aleutian Disease Virus (ADV) Proteins

STOLZE, BIRGIT, DANUTA KIEREK-JASZCZUK, and O.-R. KAADEN

Inst. Virology, Vet. School, D-3000 Hannover

ADV, an autonomous parvovirus, consists of two structural proteins of 75 and 85 KD. Additionally, in virus preparations a polypeptid of 25 KD can be detected. Furthermore, a nonstructural protein of 71 KD is encoded by the viral DNA. We analysed the proteins of the ADV-SL3 isolate with Western blot technique. All monoclonal antibodies specific for the structural proteins recognized p85, p75 and p25 but not the p71, which could be detected by two different antibodies. To analyze the epitopes and antigenic relationship between viral proteins, the polypeptides were subjected to chemical or enzymatic cleavage, and subjected to epitopic mapping with monoclonal antibodies. A close antigenic relationship between the structural proteins p75, p85 and p25 was demonstrated. However, the structural and nonstructural proteins revealed no common epitopes.
Overlapping Sets of Viral RNAs Reflect Array of Polypeptides in the Ecori-J and -N Fragments (Map Positions 81.2 to 85.0) of the Autographa californica Nuclear Polyhedrosis Virus Genome

OELLIG, CORNELIA, BRIGITTE HAP, T. MÜLLER, and W. DOERFLER

Inst. of Genetics, Univ., D-5000 Köln

In several parts of the Autographa californica nuclear polyhedrosis virus (AcNPV) genome, nested sets of overlapping RNAs with common 3' or 5' termini have been discovered (Lübbert and Doerfler, J. Virol. 52 (1984) 255–265). The pattern of viral transcription and the arrangement of viral gene products in the region of 81.2 to 85.0 map units were investigated. In this segment of the AcNPV genome, nine size classes of viral RNA with common 3' termini have been identified which range in size from 1.3 to 4.6 kb. The nucleotide sequence of this part of the AcNPV genome was determined. Computer analyses revealed several open reading frames (ORFs) on the rightward transcribed strand. The presumptive polypeptides encoded by the larger ORFs ranged in size from 11.3 to 55.6 kilo Daltons (kD). The amino acid sequence of the 55.6 kD polypeptide showed a clustered sixteen fold repeat of the dipeptide arginine-serine. The results of in vitro translation experiments using hybrid-selected RNAs with homologies to subfragments of the 81.2 to 85.0 map unit region yielded polypeptides of approximately 28, 34-36, and 48-50 kD. The localizations of individual size classes of RNAs in the 81.2 to 85.0 map unit region of the viral genome were determined precisely at the 3' and 5' termini by S1 protection analyses.

Supported by the Deutsche Forschungsgemeinschaft through SFB 74-C1.

Purification and Partial Characterization of a Poliovirus 2 Encoded Protease

KÖNIG, H., and BRIGITTE ROSENWIRTH

Sandoz Forschungsinst., A-1000 Vienna

All poliovirus-specific proteins are generated by proteolytic cleavage of a large precursor protein. These cleavages are mediated by three virus-encoded proteases. Using an artificial peptide substrate we detected an enzymatic activity appearing 3 h post poliovirus infection which cleaves the C-terminal methyl ester bond of this peptide. – This enzyme could only be solubilized in active form by SDS or one of its derivatives. However, it stayed in solution after separation from the membraneous cell fraction in the absence of excess detergent. – The enzyme was purified to > 90% homogeneity by a rapid and simple two step procedure. It was identified as the poliovirus-coded peptide 2A, which is the protease responsible for the first cleavage of the nascent polypeptide chain. – Kinetic analysis as well as heat denaturation experiments showed the enzyme to be heat-labile. It was irreversibly inactivated by exposure to pH below 5. 2A belongs to the class of sulfhydryl proteases, as was demonstrated by inhibition studies.