HEPADNA VIRUSES

William Robinson, Katsuro Koike and Hans Will, Organizers
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Hepadna Viruses

Virus Replication

O 001 DUCK HEPATITIS B VIRUS DNA SYNTHESIS, William S. Mason, J.-M. Lien, D. J. Petcu, C. Aldrich, J. Goldberg and J. M. Taylor, Fox Chase Cancer Center, Philadelphia, PA 19111.

Structural analysis of virion DNA and of intermediates in hepadnavirus DNA synthesis have led to a model in which the minus strand is synthesized by reverse transcription of viral RNA and the plus strand by copying of the completed minus strand. A protein covalently bound at or near the 5' end of the minus strand has been proposed as the primer of the reverse transcription reaction. The synthesis of the plus strand is apparently primed by a short oligoribonucleotide which remains attached to the DNA even after assembly of mature virus particles.

Recent work from our laboratories and others has addressed a number of problems including the size, origin, and attachment site of the protein bound to the minus strand, the structure and origin of the plus strand primer, and the sites not only of initiation, but also of termination of minus and plus strand synthesis. Evidence derived from studies on duck hepatitis B virus will be presented in support of the following points: (1) The site of protein attachment is the 5' terminal nucleotide of the minus strand (dG, nucleotide 2537), as expected if the protein has a role in priming. (2) The oligoribonucleotide attached to the 5' end of plus strand DNA has both the cap structure and sequence of the 5' terminus of the RNA pregenome, from which it is believed to arise. (3) The minus strand of virion DNA is terminally redundant. (4) The plus strand of virion DNA is incomplete. The 5' ends of the DNA map to nucleotides 2488 and 2489, within and just downstream of the direct repeat element DR2, whereas the 3' ends are located to multiple sites just upstream of DR2. This suggests that the RNA primer of plus strand DNA synthesis blocks elongation of the plus strand through DR2 during the process of virion assembly. (5) A portion of full length minus strands and almost all incomplete minus strands isolated from replicative intermediates fractionated upon hydroxylapatite chromatography as though single stranded. Annealing of these species with a synthetic "strong-stop plus" DNA conferred double strand behavior upon hydroxylapatite chromatography, suggesting that a strong stop plus species does not form prior to completion of the minus strand.

These results will be reviewed in the context of current models of hepadnavirus DNA synthesis.

O 002 REVERSE TRANSCRIPTION OF HEPADNAVIRUSES, Christoph Seeger, Department of Microbiology, N.Y. St. College of Veterinary Medicine, Cornell University, Ithaca, N.Y. 14853.

Hepadnaviruses replicate their relaxed circular DNA genome by reverse transcription of a RNA intermediate, the pregenome RNA (1). Initiation of first strand DNA occurs by a protein of unknown structure and origin (2). Second strand DNA synthesis is primed by a RNA oligomer, which is derived from pregenome RNA (3,4). The 5' ends of first and second strand DNA map to identical direct repeats, 11-12 nucleotides in length, separated by 46-223 nucleotides from each other. They are termed DR1 and DR2. DR1 is part of the terminal redundant portion of pregenome RNA and thus is present at the 5' and 3' ends of the RNA template for reverse transcription. At least three important functions in genome replication can be attributed to DR1 and DR2: i) DR1 serves as the priming site for first strand DNA synthesis; ii) DR1, including five nucleotides at its 5' end, serves as a primer for second strand DNA synthesis; and iii) DR2 serves as the primer binding site for the RNA primer containing DR1 sequences. All three steps require specific enzymatic activities: i) The recognition of the DR1 priming site by the priming protein; ii) a RNaseH like activity for determination of the 3' end of the RNA primer and iii) transport and annealing of the RNA primer to the primer binding site at DR2. The hepadnavirus genome contains a 2.6 kb open reading frame, termed "pol", that is believed to encode the viral reverse transcriptase. To address the question of whether this open reading frame encodes additional enzymatic activities which play a role in production of viral progeny, a biochemical analysis of the pol gene product(s) has been initiated. The pol gene is being produced in a bacterial expression system to assay pol products for enzymatic activities using virus specific RNA and DNA templates.

1) J. Summers and W. S. Mason, Cell 29, 403 (1982).
2) W. H. Gehrlich and W. S. Robinson, Cell 21, 801 (1980).
3) J. M. Lien, C. E. Aldrich and W. S. Mason, J. Virol. 57, 229 (1986).
4) C. Seeger, D. Ganem and H. E. Varmus, Science 232, 477 (1986).

Transcription, Control, Tissue Specificity, Host Range

O 003 MOLECULAR ASPECTS OF REPLICATION AND PATHENOGENESIS OF THE HUMAN LEUKEMIA AND AIDS RETROVIRUS, William A. Haseltine, Joseph Sodroski, Craig Rosen, Wei Chun Goh, Ernest Terwilliger, Andrew Dayton, Roberto Patarca, Laboratory of Biochemical Pharmacology, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115. Retroviruses have been shown to be the etiologic agents of human leukemias and lymphomas and the acquired immune deficiency syndrome and related disorders. These viruses are unusual amongst retroviruses both with respect to virus genomic structure and replication cycle. The human leukemia virus is poorly transmissable and poorly infectious. It has the capacity, upon co-cultivation with infected cells, to transform primary T4+ lymphocytes in vitro. The virus contains a 1,500 region located between the envelope gene and the 3' LTR. This region encodes several proteins, one of which is an avtivator of LTR directed transcription. This gene product also induces - directly or indirectly, genes associated with T cell proliferation. The role of the X-region encoded genes in virus replication and transformation will be discussed. The AIDS virus encodes at least four genes (sor, 3' orf, art and tat) in addition to gag, pol, and env genes common to all retroviruses. Mutation analysis reveals that the 3' orf gene is nor required for virus growth. The viruses lacking the sor gene can relicated slowly. The tat and art genes are absolutely required for viral growth. Both the tat and art genes are appear to be post-transcriptional regulators of epression of virus structural proteins. The mechanism of action of these genes will be discussed. The envelope gene protein of the AIDS virus has been shown to be cytotoxic to T4+ cells. The mechanism of the AIDS virus T4+ cell killing will be discussed.

O 004 HEPADNA VIRUS GENE EXPRESSION IN VIVO, Hans Will, Thomas Weimer, Rolf Sprengel, Marietta Stemmler *, Claus Schröder *, Doris Fernholz, Da F. Wan, and R. Schneider, Max Planck Institut für Biochemie, Am Klopferspitz, 8033 Martinsried, FRG, and * Deutsches Krebsforschungszentrum, Im Neuenheimer Feld, 69 Heidelberg, FRG.

Depending on the type of infection Hepadna virus DNA is propagated in extrachromosomal or chromosomally integrated form. The different consequences for gene expressions have been rarely studied. We therefore initiated a systematic analysis of viral protein expression in acutely infected, chronically infected, and HCC liver tissues derived from human patients. Antibodies have been prepared to viral proteins expressed in E. coli which were used to analyse by immunoblotting core-, surface-, pre-S-, X- and pol-protein expression in vivo. The in vitro synthesized proteins have also been used to search for anti-pol and anti-X antibodies in vivo.

Core antigen (21 kd) expression and synthesis of a 38 kd C-Pol protein was found to correlate with extrachromosomally replicating virus. The structure of the 38 kd C-Pol protein is reminiscent of retroviral reverse transcriptase intermediates, and may therefore represent a cleavage product of the HBV reverse transcriptase. Tissue containing integrated viral DNA did not contain a normal capsid protein but often high levels of pre-S and surface-proteins. This suggests a selective shutoff of core antigen synthesis after DNA integration. However, substantial amounts of C-Pol proteins of different sizes were detected frequently and primarily in human HCC tissues. A similar analysis has been performed for WHV, GSHV, and DHBV infected liver tissue. The possible role of these aberrant C-Pol proteins in tumorigenesis is currently under investigation. Antibodies to pol-protein have been detected in sera of patients. This supports our finding that pol-frame related proteins are expressed in vivo.

To investigate which regions of the pol-frame may encode enzymatic functions we compared all known HBV Pol-protein sequences, and 5 cloned DHBV sequences (one American, two German, and two Chinese isolates). The comparative sequence analysis revealed conserved regions which are good candidates specifying enzymatic domains, and highly variable regions which more likely function as spacer between different domains.

Genome Structure and Gene Products

O 005 INTRACELLULAR ACCUMULATION OF THE HEPATITIS B VIRUS LARGE ENVELOPE POLYPEPTIDE INDUCES HEPATOCELLULAR INJURY IN TRANSGENIC MICE.

F.V. Chisari, D.R. Milich, A. McLachlan, P. Filippi, J. Buras, C.A. Pinkert, R.D. Palmiter and R.L. Brinster. Res. Inst. Scripps Clinic, La Jolla, CA 92037, Univ. of Washington, Sch. of Med., Seattle, WA 98195, Univ. of Pennsylvania Sch. of Vet. Med., Phila., PA 19104.

Recently we reported (J. Virol. 60:1986) that hepatocellular overproduction of the HBV large envelope polypeptide, relative to the major envelope polypeptide, traps all of the envelope polypeptides in a pre-Golgi compartment in transgenic mice containing the entire HBV envelope region under the control of the mouse albumin promoter. We now report the development, in such mice, of a lesion characterized by hepatocellular necrosis, lobular inflammation and significant transaminase elevation. Hepatocellular injury begins at 2-3 months of age, is proportional to intracellular envelope polypeptide concentration, and occurs in the absence of an HBV specific cellular and humoral immune response. Infection by hepatotropic mouse pathogens was not detected. We conclude that overproduction of the large envelope polypeptide causes the intrahepatocellular accumulation of cytotoxic quantities of HBV envelope polypeptides. Since similar overproduction of the large envelope polypeptide has not been observed in HBV infection the relationship between this lesion and the pathogenesis of hepatocellular injury in human viral hepatitis is currently unknown.

O 006 STRUCTURE AND FUNCTION OF HEPATITIS B VIRUS PROTEINS, Wolfram H.Gerlich and Klaus H.Heermann, Department of Medical Microbiology, University of Göttingen, D3400 Göttingen, Germany

The envelope of hepatitis B virus consists of three coterminal proteins which are referred to as large, middle and small surface protein. Complete virions and filaments of excessive surface antigen (HBsAg) from the blood of virus carriers contain up to 20% of large protein, while 20 nm HBsAg particles contain less than 2% of large protein. - Transfection of HeLa cells by a partial HBV genome leads to a regulated expression of large protein. In vitro, the cells secreted HBsAg filaments rich in large and small protein. Grown as a nude mouse tumor the cells produced almost exclusively large protein which was no longer secreted. The findings suggest that the large protein needs an excess of small protein to be secreted, and that a high proportion of large protein favors formation of filaments. - The middle protein is known to bind human serum albumin which has been polymerized by glutaraldehyde. The biological relevance of this phenomenon was questionable, because of the chemical treatment. We have recently found that the middle protein binds in vivo a serum factor which is most likely monomeric human serum albumin. The low titer of the binding factor suggests that the serum albumin needs a limiting co-factor. - The relative immunogenicity of the three envelope domains was studied in guinea pigs and BALB/c mice. Using selective ELISAs, the antibody titer against the pre-s domains of the middle and the large domain was 12% of the titer against the gene s product when 20 nm particles from human serum were used for immunization. This proportion increased to 25% when filaments or virions were used for immunization. Only pre-s2 specific antibodies were detected in the immune blot. This suggests that not only gene s epitopes but also the pre-s1 epitopes of the large protein are conformational. - The viral core protein exists in several forms. Without the pre-core sequence it forms a $P22^{C}$ which assembles even in bacteria to core particles. The core particles carry a dominant conformational epitope which contains the sequences around aminoacid 80. As shown by deletion mutants, formation of the particles requires the protamin-like carboxy-terminal domain. In bacteria, core particles package in the absence of other viral gene products preferably RNA. - Virion core particles from infectious serum, but not core particles from hepatocytes, contain besides the major core protein P22 three larger proteins (P35, P38, P41) which bind antibody against $P22^{C}$. Though the nature of these proteins is not yet clear, it is probable that they are fusion products of the core gene and a another viral gene. - Expression of the core gene with preceeding pre-core sequence does not result in the formation of core particles. As shown by in vitro translation in presence of microsomes, the pre-core sequence acts as a signal sequence for translocation of the product to the lumen of the endoplasmatic reticulum. Proteolytic processing at the amino- and the carboxy-end leads to the secretable form of the viral core protein $P16^{e}$ which is known as e-antigen.

O 007 SYSTEMATIC LOCATION OF IMMUNODOMINANT EPITOPES IN PRE-S-CODED DOMAINS OF THE HBV ENVELOPE PROTEINS, Stephen B.H. Kent, Karen Parker, Div. Biology 147-75, California Institute of Technology, Pasadena, CA 91125; A. Robert Neurath, Nathan Strick, Lindsley F. Kimball Res. Inst., The New York Blood Center, New York, NY 10021.
There is great current interest in the "mapping" of epitopes on the protein constituents of pathogenic microorganisms. Recently, this has been approached by the parallel synthesis of hundreds of small (6-8 residue) peptides, followed by assay of their cross reactivity with, for example, immune sera from post-infection individuals or neutralizing monoclonal antibodies. Here we present an alternative, more generally effective strategy for the systematic location of epitopes that has resulted in a number of interesting observations including the discovery of a novel class of biologically active peptides. Our approach is based on the synthesis of large (approx. 30 amino acid residue) peptide fragments of the protein under study. This has several advantages. A total of only 2xn residues of synthesis completely spans in overlapping fashion a protein of "n" residues; furthermore, initial screening of the relatively few large fragments is more convenient and allows subsequent more detailed studies to be focused on the few immunochemical hotspots of the protein under study, rather than needlessly duplicate these efforts over the whole molecule. In addition, large peptides are more likely to contain complete epitopes, and have a pronounced tendency to adopt conformations similar to those in the native protein. This allows the location of epitopes dependent on secondary or even tertiary structure, as long as significant contributions arise from continuous segments of the peptide chain. Application of these concepts to the pre-S-coded domains of the M- and L-proteins of the HBV envelope has resulted in location of two immunodominant epitopes of the virus and the location of the host-cell receptor binding site on the L-protein. All three epitopes have been successfully mimicked by approx. 30-residue synthetic peptides. The peptides mimicking the immunodominant epitopes are inherently strong immunogens, invoking powerful immune responses in experimental animals without coupling to carrier molecules. The molecular basis of this immunogenicity, i.e. the presence of both T-cell and B-cell epitopes in these peptides, has been further disected. The general implications of these observations for the development of synthetic vaccines will be discussed.

O 008 BIOLOGICAL ROLE OF PRE-S SEQUENCES OF THE HEPATITIS B VIRUS ENVELOPE PROTEIN, A. Robert Neurath, Stephen B.H. Kent, Nathan Strick and Karen Parker, The New York Blood Center, New York NY 10021; California Institute of Technology, Pasadena CA 91125
One of the 4 reading frames (ORF's) on HBV DNA codes for the envelope (env) protein of the virus. This ORF is subdivided into preS1, preS2 and S-regions. Several mRNA's hybridizing with the env gene have been isolated. These mRNA's encode 3 distinct protein products showing variations at their N-termini but having a common C-terminus. These mRNA's code for: S- protein; M-protein (=S+preS2) and L-protein (S+preS2+preS1). HBV-infected hepatocytes secrete 3 types of particles: virions, tubular forms and ~20 nm spherical particles. All these particles contain S-protein. The subviral particles usually also contain M-protein. Although present in low amounts in subviral particles, L-protein is an essential component of the env of HBV. The preS region of the env protein has important biological functions. These are: 1) involvement in virus attachment to hepatocytes; 2) high antigenicity and immunogenicity; 3) elicitation of virus-neutralizing antibodies; 4) regulation of the genetic restriction of the immune response; and 5) regulation of virus particle assembly. Work with synthetic peptide analogs and the corresponding antisera led to the localization of functionally important domains to defined regions of the preS sequence. The corresponding findings related to the first 3 functions listed above, will be described in detail.

O 009 THE ASSEMBLY OF HEPADNA VIRIONS AND SUBVIRAL PARTICLES, D. Persing, B. Eble, K. Simon, G. Enders, C. Chang, V. Lingappa, H. Varmus, and D. Ganem, University of California, San Francisco, CA 94143.
The biosynthesis and assembly of hepadnaviral surface and core antigens has been examined in vitro and in vivo. Both HBV surface and presurface proteins are initially synthesized as transmembrane proteins. PreS2 and S proteins undergo a spontaneous aggregation and budding reaction in the endoplasmic reticulum (ER) to produce secreted 20 nm subviral particles. This reaction is specifically inhibited by overexpression of preS1 proteins, which are themselves incapable of performing it and hence are not exported as subviral particles. PreS1 proteins remain locked in a transmembrane configuration in the ER; additional interactions with other viral components presumably are required for their export. Myristylation of pre S1 proteins may play a role in these membrane interactions. By contrast, the precore region is less important for viral assembly. DHBV genomes bearing a frameshift mutation in preC are infectious, and progeny genomes recovered from infected ducks retain the mutation without reversion. Thus, proper nucleocapsid and virion assembly can proceed in the absence of preC proteins.

Carcinogenesis - I

O 010 STRUCTURE AND FUNCTION OF INTEGRATED HBV DNA, Katsuro Koike, Midori Kobayashi, Katsuyuki Yaginuma, Yumiko Shirakata, Shinako Takada, Chikara Miyazaki, Dept. of Gene Research, Cancer Institute, Toshima-ku, Tokyo 170, Japan.

The integration of hepatitis B virus (HBV) genome is closely related to the development of HBV-associated hepatocellular carcinoma (HCC). Various integrated forms of HBV DNA in HCC cell lines and tissues from adult patients were analysed and showed the subgenomic fragment of HBV DNA was integrated into host chromosome with or without rearrangement of the viral as well as the chromosomal DNA. But these data did not sufficiently clarify (i) the time of HBV integration following viral infection, (ii) the mechanism of HBV DNA integration nor (iii) the causal relation between HBV integration and development of HCC. Most of the integrated HBV DNA, studied so far, were from the adult advanced tumors, where extensively rearranged structure could probably be created during the progression stage of tumor. Almost all the integrated forms were not expressed in the tumor except a few cases of the HBsAg-producing tumor cells.

To clarify some of those points, attention was directed to HBV integration in children chronically infected with HBV, to see early stage of tumor development. We examined integrated HBV DNAs in the HCC of an 11-year-old boy and in the liver of a chronic active hepatitis of his younger brother. Essentially multiple patterns of integration were observed in which integrated HBV DNAs corresponded to most, if not all, of the viral genome and were similar in both HCC and chronic active hepatitis cases. Cell population(s) in the HCC are assumed to be heterogeneous as to HBV integration. One junction of the integrated HBV DNA was restricted to the 5'end of the viral minus-strand, while the other junction was located at various sites. Furthermore, direct evidence for the integration of HBV sequences in chronic active hepatitis prior to tumor development was obtained.

Since one of the viral-chromosomal junctions was restricted to the region of the C-terminus of the X gene and the same region spanning from upstream of preS to the C-terminus of the X gene, containing intrinsic promoter/enhancer sequences, were conserved in all the integrated HBV DNAs, there is a possibility that an X gene-related product may participate in the early step of a tumorigenic process. To study the possible functions of conserved region of integrated HBV DNA in the establishment of malignant transformation, DNA transfection experiments were carried out by HBV DNA fragment inserted into expression vector using mouse NIH3T3 cells as a recipient. After cotransfection with a selective marker pSV2-neo-SV-gpt, HBV DNA was detected in some of the colonies selected in the dish, which were able to grow rapidly. Within those, a few independent colonies produced solid tumor in nude mice. A HBV sequence incorporated into the tumor was a small part within the conserved region. Molecular cloning of the HBV and surrounding mouse chromosomal sequences is now in progress.

Hepadna Viruses

O 011 REPLICATION AND INTEGRATION OF HEPATITIS B VIRUS GENOME, K. Matsubara, T. Tsurimoto, K. Ueda, O. Chisaka, T. Tokino, Institute for Molecular and Cellular Biology, Osaka University, Yamada-oka, Suita, 565, Japan.

We have transfected a human hepatocellular carcinoma cell line with three tandemly arranged hepatitis B virus (HBV) genomes, and obtained a clone that stably produces and releases Dane-like particles. The tansfecting DNA was integrated into cellular DNA and was transcribed into 3.5Kb and 2.1Kb RNA, whose starting sites were in the cohesive end region and in the preS region, respectively. No closed circular vial DNA was detected extrachromosomally in the virus-producing cells, suggesting that DNA in the Dane-like particle is produced by copying the RNA. This system allows to elucidate hitherto unidefied roles of the HBV genes, such as X gene.

Comparison of integrated HBV genome structure in hepatoma cells has revealed that the viral DNA "invades" into cellular DNA by making use of the cohesive end region. In most of the integrants, the viral X gene has been impaired. We are in the process of analysing the properties of target host DNA sequences.

Analyses of the integrant DNA's suggest that frequent rearrangements of cellular DNA take place after the HBV DNA integration: In some cases, the cellular DNA's flanking HBV DNA are from different chromosomes.

These observations will be discussed in conjunction with the later development of hepatocelluar carcinoma.

O 012 **FUNCTIONAL ACTIVITY OF TRANSCIPTIONAL ENHANCERS AND OTHER REGULATORY ELEMENTS IN THE HBV GENOME.** **D.A. Shafritz, R. Tur-Kaspa, R. D. Burk, B. Bloom, D. Moore and Y. Shaul. Albert Einstein Coll. of Med., Bronx, NY, Mass. General Hosp., Boston, MA, and Weizmann Institute of Science, Rehovot, Israel.**

One mechanism by which steroid hormones regulate expression of viral and cellular genes is thru hormone receptor mediated activation of specific gene regulatory sequences. Recently, we identified a glucocorticoid responsive element (GRE) in the HBV genome, which contains a glucocorticoid receptor binding DNA consensus sequence (-CAA--TGTCCT). To determine whether the glucocorticoid receptor protein binds directly to the HBV GRE, we performed nitrocellulose filter binding assays with partially purified receptor and specific fragments of HBV DNA containing either the GRE sequence (mp 351-366) or the HBV enhancer (mp 1080-1234). DNAase I "footprint" analysis was used to identify specific sequences protected by glucocorticoid receptor protein (GRP). Nitrocellulose filter assays showed that a fragment of the HBV genome containing the GRE, but not the HBV enhancer, formed a complex with partially purified rat liver GRP. DNAase I "footprint" analysis showed protection of a 30 nucleotide segment of the HBV genome (mp 340-370) which contained one full GRE consensus sequence (-CAA--TGTCCT) and a second partial repeat of this sequence (TGTCCT). The HBV GRE did not serve as an enhancer alone and thus represents a "conditioning" sequence for the HBV enhancer, located approximately 700 nucleotides downstream from the GRE. This physical separation of the GRE from the enhancer is so far unique to HBV, as is the presence of the GRE directly in the coding sequence of a known gene, the S gene of HBV (mp 155-833).

To further study modulators of HBV gene expression, we have tested the effect of recombinant human α and γ interferons on HBV enhancer activity, using CAT plasmid vectors containing the HBV enhancer and SV 40 promoter, or the HBV enhancer and HBV core gene or S gene promoters. Transient expression of chloramphenicol acetyl transferase (CAT) was determined following introduction of these plasmids into PLC/PRF/5, Hep-3B, SK-Hepl or HeLa cells. 100 U/ml α-interferon reduced CAT activity 2-3 fold in all cell lines, whereas γ-interferon had no effect. Reduction in CAT activity was noted with all plasmid constructs containing the HBV enhancer. No effect of α interferon on CAT activity was noted using $pA_{10}CAT_2$, pSV_2CAT or RSV LTR CAT constructs. A control vector, pGEM CAT plasmid containing 2'-5' oligo A synthetase regulatory sequences, was markedly stimulated by 100 U/ml α-IFN. These studies indicate that HBV enhancer activity is inhibited by recombinant α but not γ interferon, and this may in part explain interferon mediated suppression of virus replication during persistent HBV infection. One possible mechanism for reduction of HBV enhancer activity by α-IFN could be thru a change in activity of a cellular factor following interaction of α-IFN with its receptor on the cell surface. Precisely how this trans-acting factor might interact with the HBV enhancer will require further study.

O 013 **CHROMOSOME ABERRATIONS AND GROWTH FACTOR ACTIVATION IN HEPATOCELLULAR CARCINOMA FROM HV CARRIERS** H.P.Wang, Xi-xian Fu, Y.Lee, O. Hino*, R. Snyder+, and C.E. Rogler. Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, *Dept. of Pathology, Cancer Institute, Tokyo, Japan and +Penrose Research Laboratory, Zoological Society of Philadelphia, Phila, PA.

Persistent infection with HBV or WHV leads to hepatocellular carcinoma (HCC). Most HCCs contain integrated viral DNA in the tumor, however, some HCCs completely lack integrated viral DNA. Studies of HBV and WHV integrations cloned from HCCs have shown that integrated viral DNAs can function as mediators of chromosomal aberrations including the translocation and deletion of genomic DNA. Sequencing of viral-cell DNA junctions has implicated the viral DR1 sequence in both the initial integration mechanism and the generation of chromosomal aberrations. The specific integration and excission of viral DNA may play a key role in a "hit and run" mechanism of viral mediated hepatocarcinogenesis. One cellular DNA deletion associated with an HBV integration was localized to chromosome 11p13, immediately adjacent to the Wilms tumor locus. In addition, RFLPs have been used to detect the loss of alleles from chromosome 11p in six of fourteen cases and chromosome 13q in five of fourteen cases. The possible role of chromosome 11p in HCC is supported by the observation that the gene for Insulin-like growth factor II (on chromosome 11p) is actively transcribed in many human HCC cell lines. A survey of liver tissues from WHV carrier woodchucks revealed high steady state levels of IGF-II mRNA in 93% of the tumors studied and in all the preneoplastic nodules analyzed. These data support a model in which IGF-II activation may stimulate the focal growth of hepatocytes leading to development of preneoplastic nodules and HCC. WHV persistent infection predisposes the liver to development of preneoplastic nodules as is revealed by the development of nodules in WHV carriers given partial hepatectomies.

Carcinogenesis - II

O 014 ONCOGENES IN HUMAN AND DUCK HEPATOCELLULAR CARCINOMA (HCC), Jian-ren Gu, Department of Biochemistry and Molecular Biology, Shanghai Cancer Institute, 2200 Xie-Tu Road, Shanghai, People's Republic of China.

Transfection of DNAs from 10 human HCC was performed in NIH3T3 cells. Six transfectants gave rise to positive foci; three of them were identified as having the activated human N-*ras* gene. Two out of ten samples of HCC DNA demonstrated DNA rearrangements. Significantly, N-*ras* mRNA was overexpressed in 12 of 17 HCC specimens. Using transformed 3T3 cells as an immunogen, a cell-specific monoclonal antibody was prepared. The *ras* product, p21, was strongly positive in transformed cells, while all normal hepatocytes were immunologically negative for this protein. Similarly, c-*myc* messenger was also overexpressed in six out of eight HCC specimens. Therefore, the N-*ras* and c-*myc* are likely candidates for two classes of proto-oncogenes involved in human hepatogenesis or tumor progression.

DNAs from two duck hepatic tumors and one tumor of the surrounding liver tissue were used to transfect NIH3T3 and RAT-1 cells. Duck c-*mht* sequences (*Eco*RI fragments: 5.2, 3.2 kb) were identified in liver DNA transfectants derived from both tumor types. Our results strongly suggest that c-*mht* was activated in duck neoplasia, even during the precancerous stage. No DHBV-DNA was observed in the transformed cells. This system will provide a useful model for the further study of target proto-oncogenes activated by viral or chemical carcinogens.

O 015 HEPATOCELLULAR CARCINOMA ASSOCIATED WITH GROUND SQUIRREL HEPATITIS VIRUS INFECTION, Patricia L. Marion, Hans Popper, Roberto R. Azcarraga, Mary Jo Van Davelaar, Gabriel Garcia and William S. Robinson, Division of Infectious Diseases, Stanford University Medical Center, Stanford CA 94305-5308.

Although persistent infection with hepatitis B virus and woodchuck hepatitis virus has been associated with development of hepatocellular carcinoma (HCC) in the host, little has been known of such an association with ground squirrel hepatitis virus (GSHV), which is closely related to the woodchuck virus. Colonies of GSHV-infected and uninfected Beechey ground squirrels were observed for tumors for a period of 6 years. Tumors developed in 16 squirrels after a minimum of 2.4 years of observation per animal; each of the 16 animals was over 4 years of age when the tumor was detected. The predominant type of tumor was HCC, which appeared in 7 of 33 GSHV-bearing animals studied and in 2 of 27 squirrels with antibody to the virus. Fifty percent (7 of 14) of GSHV carriers over 4 years of age had HCC at necropsy. No HCC appeared in 24 GSHV marker-free squirrels, 13 of which were over 4 years of age. Integrated GSHV DNA was found in the HCC DNA of 3 of 5 carrier animals examined, paralleling the frequent findings of integrated hepatitis B and woodchuck hepatitis viral DNA in human and woodchuck HCC. Our data indicate that long-term infection with GSHV is associated with development of HCC, as is persistent infection with the other mammalian hepadnaviruses.

O 016 TWO DIFFERENT MECHANISMS FOR HEPATITIS B VIRUSES INDUCED HEPATOCELLULAR CARCINOMA, P. Tiollais, A. Dejean, A. Marchio, J. Etiemble, T. Möröy and M.A. Buendia, Recombinaison et Expression Génétique (INSERM U163, CNRS UA271) Institut Pasteur, 75015 Paris.

To understand the mechanism of HBV/WHV induced carcinogenesis at a molecular level, we have chosen two different approaches : first, a systematic screening for activation of protooncogenes in liver tumors of chronically infected woodchucks. Second, a detailed analysis of a single HBV integration site present in a particular human HCC.

Enhanced expression and allelic alterations of the c-myc oncogene were found in three HCC out of nine. Variations in the size of the c-myc transcripts, ranging from 2.0 kilobases (kb) to 5.6 kb, as well as in the level of c-myc gene expression, 5-50-fold higher than in adjacent liver tissues, were observed among the three HCC. Rearrangements of the c-myc locus were either upstream of the gene or within the first intron. Cloning and sequencing of the break-point region from one of the three tumors showed that the c-myc gene was truncated and joined to a unique cellular sequence of unknown function. WHV DNA was not integrated near the c-myc coding exons, excluding a direct role of the virus in c-myc activation.

From a comparison of a HBV integration site present in a particular HCC with the corresponding unoccupied site in the non-tumorous tissue of the same liver, we report that HBV integration places the viral sequence next to a liver cell sequence which bears a striking resemblance to both an oncogene (v-erb-A) and the supposed DNA-binding domain of the human glucocorticoid receptor and human oestrogen receptor genes. We suggest that this gene, usually silent or transcribed at a very low level in normal hepatocytes, becomes inappropriately expressed as a consequence of HBV integration, thus contributing to the cell transformation.

Delta-virus

O 017 MOLECULAR BIOLOGY OF HEPATITIS DELTA VIRUS. John L. Gerin, Div. of Molecular Virology and Immunology, Georgetown University Medical Center, Rockville, MD 20852.

The hepatitis delta virus (HDV) is a defective human RNA virus that requires helper functions of hepatitis B virus (HBV) for its replication. HDV is a significant human pathogen for the world population of HBV carriers and causes both acute and chronic liver disease including cirrhosis. HDV infection occurs worldwide and outbreaks of delta hepatitis have been reported in certain high risk groups (intravenous drug addicts) and general populations. In acute and chronic infection, HDV exists in serum as a 36 nm particle that consists of hepatitis delta antigen (HDAg) and RNA encoated by the envelope surface antigen (HBsAg) of the host HBV infection. Virion HDAg consists of two polypeptides (24 kd and 27 kd) that share a common antigenic determinant. Virion RNA is detected as two forms with apparent sizes of 1.7 kb and 2.0 kb. Neither RNA species is polyadenylated and both have the same (genomic) polarity. Biochemical and morphological data from several laboratories indicate that the two RNA species are different physical forms of the same single-stranded RNA, a linear form (1.7 kb) and a closed circular form (2.0 kb). The approximate equivalence of HDV infectivity in chimpanzees and RNA concentration in an acute phase serum, the presence of the RNA within a virus-like structure and the epidemiological association between serum RNA and delta hepatitis provide strong evidence that the RNA represents the genome of HDV. Virion RNA has been cloned and sequenced. The 1680 base sequence contains a number of open reading frames, one of which, on the anti-genomic strand, codes for a protein that is recognized by standard reagents for HDAg. In vitro translation of HDV RNA from HDV-infected liver and serum virions generated 24 and 27 kd translation products only from liver extracts and not from virion RNA preparation. While HDV contains only genomic RNA, the infected liver contains a number of RNA species of both genomic and anti-genomic polarity some of which are larger than genomic size. Current data indicate that HDV is a negative strand virus with unusual biological properties and has no known counterpart in animal virology; HDV may, in fact, resemble certain unusual agents of higher plants in genome structure and replication strategy.

O 018 PROTEINS OF HEPATITIS DELTA VIRUS, Wolfram H. Gerlich and Feruccio Bonino, Department of Medical Microbiology, University of Göttingen, D3400 Göttingen, Germany, and Gastroenterology Unit of San Giovanni Hospital, Torino, Italy.

Hepatitis delta virus (HDV) forms particles of 36 nm size and 1.25 g/ml density in cesiumchloride. It is enveloped by the surface antigen (HBsAg) of its helper virus hepatitis B virus (HBV). The composition of the HDV envelope is more similar to the 20 nm HBsAg particles than to complete HBV in that it contains very small amounts of large envelope protein. HDV specific proteins were identified by immune blotting with IgG from a chronic HDV/HBV patient. Two proteins, P27 and P29 copurified in size chromatography and density centrifugation with HDV particles from an acutely infected chimpanzee. IgG from an HBV patient did not react with the two proteins. The two proteins were also found in serum pellets from some chronic HDV patients and from HDV infected woodchucks. The signal for P29 was always stronger than that for P27. N-linked glycoside was undetectable in both proteins.
P29 was also present in HDV infected livers from the chimpanzee, from a chronic HDV patient, and from two of three woodchucks. P27 was not found in the livers. However several other protein bands occurred in variable number and intensity (P26, P22, P16, P15, P13). The HDV proteins were enriched in the cell nuclei but a certain amount was also found in the microsomal fraction. In one woodchuck liver P22 was the predominant protein species. P22 was bound to RNase sensitive particles of 15 to 30 nm size. Thus it forms apparently a ribonucleoprotein-complex.

Hepadna Viruses

O 019 REPLICATION OF HEPATITIS DELTA VIRUS GENOME, John Taylor,[1] Pei-Jer Chen,[1] Mark Kuo,[1] Janet Goldberg,[1] Ganjam Kalpana,[1] William Mason,[1] Barbara Werner,[2] and John Gerin,[3] Fox Chase Cancer Center, Philadelphia, PA 19111; [2]State Laboratory Institute, Massachusetts Department of Public Health, Jamaica Plain, MA 02130; and [3]Division of Molecular Virology and Immunology, Georgetown University School of Medicine, Rockville, MD 20852.

The human hepatitis delta virus is a defective virus in that its transmission, both naturally and experimentally, has only been detected in the presence of an hepadna virus. We have used the RNA from the liver of a woodchuck infected with both delta and woodchuck hepatitis virus to create a cDNA library in the vector lambda gt11. Screening this library for delta-related sequences we obtained a recombinant with a 670b insert that was subcloned in M13, both for sequencing and for the generation of strand-specific probes. We have thus examined both quantitatively and qualitatively the delta-related sequences in the livers of experimentally infected chimpanzees and woodchucks. As recently reported (1) our findings include evidence that the 1700b virion RNA is single-stranded, of one polarity (designated genomic), and of a circular conformation. In the liver of infected animals there are species of both genomic and antigenomic RNA, a major fraction of which can exist in a circular conformation. The delta virus in these and other ways shows striking similarities to certain plant pathogens: viroids, virusoids and satellite RNAs. Extrapolation from these similarities has provided insights both into the mechanism by which the delta genome is replicated and into the level of dependence of this replication on the coinfection with an hepadna virus.

(1) Chen, P.-J., et al.; Proc. Natl. Acad. Sci. U.S.A., in press.

Immunological Aspects and Pathogenicity

O 020 DHBV INFECTION OF DUCKS AND ASSOCIATED IMMUNITY, Michael S. Halpern[1], William S. Mason[2], Donald Ewert[1], Anna P. O'Connell[2], and James M. England[3], [1]The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104; [2]Institute for Cancer Research, Philadelphia, Pennsylvania 19111; [3]Department of Pathology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104

A study of immune responsiveness in Pekin ducks infected with the duck hepatitis B virus has been initiated. Immunofluourence assays with fixed tissue sections were used to characterize antibody reactivity in sera obtained from DHBV-infected ducks. Under conditions of experimental infection, antibody to core but not surface antigen was detectable, with a majority of the ducks infected at 8 days posthatch showing a transient anti-core humoral response. Antiviral antibody was not detected in the sera of congenitally DHBV-infected ducks. In addition several of the infected ducks, but none of the uninfected ducks examined, exhibited autoantibody reactivity for α islet cell-associated antigen.

In an attempt to characterize mechanisms that might serve to modulate antiviral immune responsiveness, patterns of viral antigen accumulation were examined in the spleens of DHBV-infected ducks. Viral antigen was detected in Ig^+ cells located to splenic germinal centers of experimentally DHBV-infected ducks. These cells have several properties in common with follicular dendritic cells, and we would tentatively identify them as such. Possible immunological effects of viral antigen accumulation in these cells will be discussed.

O 021 HEPATOCARCINOGENESIS IN DUCK. Masao Omata, Katsutaro Hirota, Susumu Takano, Osamu Yokosuka, Kunio Okuda. 1st Dept. of Medicine, Chiba University School of Medicine, Chiba, Japan

We have previously shown that various histological changes including hepatocellular carcinoma could be observed in the liver of duck hepatitis B virus (DHBV) infected ducks obtained from China. In addition, we demonstrated the integration of DHBV DNA in the duck tumor (Proc Natl Acad Sci USA 1985:82:5180-5184). We also demonstrated that the infection rate of 114 ducks obtained from various parts of China was 63% in "Chinese" ducks, and only 4% in white Pekin. Advanced liver diseases and hepatocellular carcinoma were found in the infected "Chinese" ducks. However, hepatocellular carcinoma has not yet been reported in persistently infected domestic ducks in the United States. These data suggested enviromental and host factor might be important in the development of significant liver diseases in ducks, and experimental transmission study under controlled laboratory condition is required to test the pathogenic role of DHBV.

By inoculating DHBV positive serum derived from Chinese ducks to one day old white Pekin obtained from a Japanese virus free duck farm, we can establish carrier state of the virus in all (Hepatology 1984:4:603-607). We administered three different hepatocarcinogens, namely 3'-methyl-4-dimethyl aminoazobenzene (DAB) in group 1, acetylaminofluorne (AAF) in group 2, and aflatoxin B1 in group 3 carrier ducks. Group 4 carrier ducks did not receive the hepatocarcinogens. None of 44 ducks in the four groups who died at ages of 2 weeks to 1 year developed hepatocellular carcinoma. Hepatocellular carcinoma was observed in 3 of 3 aflatoxin given carrier ducks between 1 to 2 years of age. Episomal viral DNA was detected in the tumors, but high molecular viral DNA, suggestive of viral DNA integration into host genome, was not detected by Southern blot hybridization. In group 4, hepatocellular carcinoma was not seen until the end of 2nd year. However, 2 of 4 duck older than 2 years of age developed huge hepatocellular carcinoma, and viral integration was found in the neoplastic cells. No tumor was observed in group 1. One duck in group 2 developed hepatocellular carcinoma.

Under controlled laboratory condition, we observed the development of hepatocellular carcinoma in experimentally infected white Pekin ducks. In this system, we may be able to test the interaction of chemical carcinogen or its promotor and hepatitis virus infection in hepatocarcinogenesis.

O 022 PATHOLOGY OF HEPADNA AND DELTA VIRUSES INDUCED HEPATITIS AND LIVER CANCERS. Hans Popper, Mount Sinai School of Medicine, New York, N.Y. 10029

The histologic investigation of wild and particularly laboratory infected animals supplements findings in man. Chimpanzees exhibit alterations which assist in the characterization of human lesions and serve explanation of pathogenesis. Chimp hepatitis A (HA) is a periportal necroinflammation leaving the perivenous zone intact, while hepatitis B (HB) is panlobular and characterized by close approximation of lymphocytes to hepatocytes, sometimes even found within them, suggesting a lymphocytotoxic effect similar to the circumscribed HA lesions. In chimp hepatitis non-A, non-B (NANB), significant lymphocytic infiltration restricted to portal tracts is associated with cytoplasmic alterations in the parenchyma, with lymphocytes mainly in sinusoids, suggesting rather a cytotoxic effect. NANB, becoming chronic in about 50% of infected chimps, reveals sustained portal lymphocytic infiltration, with transient recurrences of circumscribed eosinophilic parenchymal lesions. Chimp HB may develop a carrier state characterized by minor histologic changes but many surface antigen loaded ground-glass cells. In carrier chimpanzees, superinfection with HB and NANB viral agents produces more severe lesions characteristic of the specific infective agent and associated with depression of the relatively high HB viral (HBV) replication. The same holds true for infection with the delta agent, which produces in HBV carriers the most severe cytotoxic alterations of any chimp viral hepatitis with the greatest dilution of the infective agent. The lesion is usually short-lived except in the rare chimps with low grade of HB viral replication, characterized by serum anti-e. Thus, similar to man, chronic HBV infections are susceptible to superinfection with other viruses, characterized by depression of HBV replication, in contrast to exacerbations of necroinflammation caused by the HBV in which the replication markers increase. These episodic attacks in man, characterized by circumscribed necroinflammation, are an important factor in transition to cirrhosis and probably also to hepatocellular carcinoma (HCC).

Woodchucks, as best examined animals, Beechey ground squirrels, and Chinese ducks develop upon the respective hepadna virus infection, hepatitis which also histologically resembles human HB. Chronic stages are also associated with hepatocytes loaded with viral antigens. Superinfection with the delta agent of carriers has succeeded in woodchucks and also in ducks. In both species this was followed by development of HCC. In all three species, HCC has been found associated with integration of the respective viral DNA into the host genome. Upon laboratory infection, HCC has developed within two years in 100% of woodchucks, earlier after newborn infection, but so far only in exceptional squirrels.

Hepadna Viruses

O 023 ANTIVIRAL THERAPY OF CHRONIC HEPATITIS B VIRUS INFECTION, William S. Robinson, Stanford University School of Medicine, Stanford CA 94035-5701.

More than 175 million people are chronically infected with hepatitis B virus (HBV) and these infections are associated with chronic hepatitis, cirrhosis, liver cancer and early death in large numbers worldwide. Such chronic human carriers represent the only important reservoir of this virus and transmission of the virus by certain routes leads to new infections. Antiviral therapy of chronic HBV infection has been investigated with the goal of favorably effecting the course of the associated liver disease and eliminating the contagiousness of infected individuals. Treatment of chronic infections with interferons and/or adenine arabinoside (araA) always diminishes and sometimes abolishes viral replication during the period of drug administration. Open treatment trials have suggested that these antivirals used alone or in combination were reasonably tolerated and could result in suppression of virus replication beyond the treatment period in some patients. When treatment was accompanied by the loss of circulating virus, liver disease improved and serum was no longer infectious for susceptible chimpanzees. A recent randomized, double-blind, three-limbed controlled trial of 64 patients comparing interferon and araA in combination, araA alone, and placebo required dosage reduction in all patients because of toxicity in some and failed to show different responses among the three groups. New approaches for antiviral therapy are underway.

Epidemiology and Vaccines (Joint Session)

O 024 ADENOVIRUS AS THE CARRIER OF HEPATITIS B VIRUS OR HIV SURFACE ANTIGEN GENES, Paul P. Hung, Wyeth Laboratories, Inc., Microbiology Division, P.O. Box 8299, Philadelphia, PA 19101.

Early region 1 of the adenovirus type 5 genome was replaced with a DNA sequence containing the gene coding for the hepatitis B surface antigen (HBsAg) flanked by the major late promoter from adenovirus and the tripartite leader sequence and polyadenylation signals from simian virus 40. HBsAg produced upon infection with the hybrid adenoviruses was glycosylated and secreted into the culture medium as particles that were essentially indistinguishable from the 22-nm particles found in human serum.

Recombinant adenoviruses carrying the hepatitis B virus surface antigen coding sequence in the adenovirus E3 region were constructed using DNA from either adenovirus type 5 or an adenovirus type 5 E3 region deletion mutant. Both of these recombinant adenoviruses replicated as efficiently as wild-type adenovirus in all human cells tested, including the human diploid cell strain, WI-38. This indicates that insertion of the hepatitis B virus surface antigen gene into the E3 region does not significantly affect viral replication. Human cells infected with these recombinant adenoviruses secreted immunoreactive hepatitis B virus surface antigen. Since a practical small animal model for human adenoviruses was lacking, a hamster model was developed to evaluate the immunogenic potential of these recombinant adenoviruses. Upon intranasal inoculation, both wild-type adenovirus and the adenovirus E3 region deletion mutant replicated in the lungs of these animals and induced an antibody response against adenovirus. Hamsters similarly immunized with the live recombinant adenoviruses produced antibody against both adenovirus and hepatitis B virus surface antigen.

Evidence to date strongly suggests that the human immunodeficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome (AIDS). Safe vaccine strategies for the prevention of AIDS center around the injection of purified HIV env glycoprotein produced by mammalian cell lines or the immunization of live recombinant vaccinia virus. Successful vaccination will require delivery to the immunocompetent host, those portions of the envelope glycoprotein that induce protective immunity. As one approach, we have investigated the ability of an adenovirus which contains HIV env gene to deliver and produce the HIV protein in infected cells. We show that such live adenovirus 7 recombinants can be isolated, that they retain the env gene sequence, and that human cells infected with these recombinant viruses secrete authentic env glycoprotein, gp 120 in tissue culture.

O 025 HEPATITIS B VACCINE, Robert H. Purcell, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

Plasma-derived vaccines, the prototype for which was prepared by the National Institute of Allergy and Infectious Diseases in 1975, have been safe and effective but very expensive. In part because of unfounded fear that plasma-derived vaccines might contain human immunodeficiency virus (HIV), such vaccines have not been as widely accepted in the United States as they should be. In part to circumvent this problem, the first recombinant technology-derived vaccine, a hepatitis B vaccine prepared in yeast, was developed by Chiron Corporation and prepared by Merck Sharp and Dohme. It was recently licensed for use in the United States and should be available in early 1987. However, because of the expense of purifying the antigen from yeast components, the yeast-derived vaccine will cost approximately as much as the plasma-derived vaccine. Other recombinant technology-derived vaccines, principally expressed in yeast or in mammalian cells, are in the final stages of development or early clinical testing.

Other approaches to the development of hepatitis B vaccine include expression of viral antigens in vaccinia, immunization with synthetic peptides representing important epitopes of the viral envelope and use of anti-idiotype antibodies as immunogens. All the these approaches are in research or preclinical phases of development. Recent interest in the immunology of hepatitis B vaccines has centered around the relative importance of the "minor" components of the surface envelope (preS-1 and preS-2-containing gene products) and the "major" envelope components (S gene products). Epitopes that stimulate immunity to hepatitis B in the chimpanzee model have been identified in both regions, and these studies are being extended to identify other potential neutralizing epitopes of the virus.

O 026 A MEDICAL AND PUBLIC HEALTH APPROACH TO HIV INFECTION: THE MILITARY EXPERIENCE Robert Redfield, Walter Reed Army Institute of Research, Washington, DC.

Currently neither curative therapy or a protective vaccine for HIV is available and no one can predict when these scientific solutions will be accomplished. In the meanwhile, science has provided the necessary tools to attempt to control the spread of this deadly virus. No matter how convincing the alibis for why so little has been done, we know enough now to develop and execute an aggressive national public health policy to effectively limit the morbidity and mortality that HIV will cause in the future. By 1984, science has provided us with an understanding of the etiology and the methods of transmission of HIV, and science and industry had developed meaningful and accurate diagnostics test of this infectious disease. In addition, our forefathers, who faced syphilis under similar conditions, have provided a carefully developed platform for control of a sexual transmitted disease. Despite this, the medical and public health community had been slow to implement the application of the above.

The military has approached HIV more aggressively then the civilian sector. The military has recognized HIV as a medical problem, developed policy based on medical issues and steered by medical leadership, and executed this policy exploiting the scientific advances available. The model which I put forth for the public health control of HIV is based on the experience I gained as a consequence of the opportunity I had to practice medicine in the military during the early HIV years. The components of my proposal include the following:

1) Care for the sick
2) Research for a better tomorrow
3) Education for all
4) Routine screening
5) Case tracing
6) Medical and public health leadership of program.

Most individuals infected today do not know they are infected. The danger of harboring HIV in ignorance could be averted if people were tested. The military program minimizes that danger. Physicians and society should recognize HIV for what it is. HIV is a medical problem; and although there political, civil liberty, or moral concerns, HIV demands a medical solution and medical leadership. Testing for HIV and subsequent case tracing are extremely important components of the military medical HIV control program. Testing and case tracing should become a part of the routine practice of medicine.

Genome Structure and Gene Products

O 100 ANTI-preS ANTIBODIES IN HEPATITIS B VIRUS INFECTION
A. Alberti, P. Pontisso, A. Fraiese
Istituto di Medicina Clinica, Clinica Medica 2°, Universita'di Padova,Padova,Italy

The preS/anti-preS system is a new serologic marker of HBV infection and antibodies to preS proteins may be relevant in the phase of virus neutralization. We have therefore developed methods to investigate the kinetics of the anti-preS response in acute hepatitis B. Total anti-preS antibody activity (anti-preS(1) and anti-preS(2)) was studied by radioimmuno-precipitation of intact Dane particles while anti-preS(2) antibody was measured by RIA, using recombinant CHO/HBsAg particles, rich in preS(2) protein, as solid phase antigen. Antibody to the virus pHSA receptor, borne on the preS(2) protein, was also investigated by hemagglutination inhibition. In acute hepatitis B, antibody precipitating Dane particles was always detected during the incubation period, before appearance of anti-HBc IgM and in the absence of anti-preS(2) and of anti-receptor reactivities. PreS(2) positive particles did not adsorbe the antibody to HBV in this phase, suggesting that anti-preS(1) represents the earliest antiviral response in hepatitis B. Anti-preS(2) antibody became detectable in serum in 26 out of 30 patients during acute phase of hepatitis and remained persistently detectable up to 6 months later. Soon after the appearance of anti-preS(2), anti-receptor antibodies were also detected. The anti-preS(2) and anti-receptor response correlated with virus clearance and with seroconversion to anti-HBe. Antibody to preS(1) and preS(2) and to the virus pHSA receptor were absent in 6 cases of acute hepatitis progressing to chronicity and were rare in sera of chronic hepatitis cases (12%,25% and 12%, respectively). These results confirm that the anti-preS antibody response may be relevant in virus clearance.

O 101 ENHANCER-MEDIATED EXPRESSION OF HBV GENES. Gary Bulla and Aleem Siddiqui. Department of Microbiology/Immunology, University of Colorado Medical School, Denver.

Recent data from our laboratory and others implicate the HBV enhancer in mediating efficient expression of heterologous genes in human hepatoma cells. We have also shown this to be true for the expression of HBc/eAg in tissue culture cells. In this study, we have looked at the global effect of the enhancer or various HBV genes. Native or enhancer-less recircularized HBV DNA was transiently transfected into HepG2 cells and the pattern of overall viral gene expression assessed in terms of both, RNA and proteins. Such a system presents the best in vitro simulation available to study events in the HBV life cycle that take place inside a liver cell following internalization and uncoating of the virus. For RNA assessment, Northern, S1 protection and transcription run-on analyses were employed. The expression of viral gene products were monitored by means of radioimmune assays and SDS-PAGE analyses following specific immunoprecipitations. The subcellular distribution of various HBV gene products were also studied by means of standard fractionation procedures and immunofluorescence. Our results and significance of the enhancer in regulating gene expression during the HBV life cycle will be discussed.

O 102 PRODUCTION OF HEPATITIS B VIRUS IN VITRO BY TRANSCIENT EXPRESSION OF CLONED HBV DNA IN A HEPATOMA CELL LINE. C. Chang[1,2], K.S. Jeng[1], C. Hu[1,2], S.J. Lo[1], T.S. Su[1,2], L.P. Ting[1], C.K. Chou[1,2], S.H. Han[1,2], E. Pfaff[3], J. Salfeld[3], and H. Shaller[3]. 1. Grad. Inst. of Microbiol. & Immunol., Natl. Yang-Ming Med. College, 2.Dept. of Med. Res., Veterans General Hospital, Shih-Pai, Taipei, Taiwan, R.O.C. 3. Inst. for Microbiol. & ZMBH Univ. of Heildelberg, Im Neuenheimer Feld 230 and 282, 6900 Heidelberg, FRG.

Transfection of human hepatoma cell lines with cloned HBV DNA resulted in the secretion of large amonts of hepatitis B surface antigen (HBsAg) and core-related antigens (HBc/HBeAg) if well differentiated cell lines were employed. Synthesis of both viral antigens was the highest in cell line HuH-7 and continued for approximately 25 days. Particles resembling Hepatitis B Virions (Dane particles) by morphology, density and by the presence of the preS1 surface antigen were released fromthe transfected HuH-7 cells into the culture medium. These partiles roduced in vitro were also indinstinguishable from the naturally occuring Hepatitis B Virions in containing the virus associated DNA polymerase and mature HBV genomes. Restriction analysis of these transfecting HBV DNA sequence. Results from radioimmunoprecipitation indicate that all four open reading frames are translated. Antisera specific for X-gene products identified in cell extracts two hitherbo unknown HBV gene products. Partial characterization of these two polypeptides has been performed.

O 103 **MOLECULAR STUDIES OF FIBROBLASTS TRANSFECTED WITH HEPATITIS B VIRUS DNA**, M.A. Gerber, M.L. Chen, A. Hood and S.N. Thung, Dept. of Pathology, Mount Sinai School of Medicine, New York, N.Y. 10029.
Co-transfection of NIH 3T3 mouse fibroblasts with a head-to-tail tandem of cloned hepatitis B virus (HBV) genome and DNA coding for a methotrexate-resistant dihydrofolate reductase resulted in a cell line producing HBsAg and HBeAg. After subcloning of the transfected cells, two subclones (D7 and F8) were selected which secreted significantly different amounts of HBsAg (D7 0.24 pg/cell/day vs F8 0.02 pg/cell/day). DNA extracted from the subclones revealed only integrated and no extrachromosomal HBV DNA sequences as determined by the Southern blot technique with a ^{32}P-labeled full-length HBV DNA probe. HBV DNA in D7 cells showed multiple integration sites in the host genome, whereas F8 cells revealed only two bands of integrated HBV DNA. Similar bands were demonstrated by alkaline phosphatase-conjugated, single-stranded synthetic oligonucleotide probes specific for gene S and gene C. Using a biotin-labeled HBV DNA probe for in situ hybridization, HBV DNA was found in the nuclei of almost all D7 cells with predominant localization to a single chromosome after chromosome spreading, while smaller amounts of HBV DNA were detected in 10 to 20% of F8 cells. These studies suggest that 2 subclones derived from a transfected cell line show different integration patterns of HBV DNA and that the amount of integrated HBV DNA is proportional to the amount of HBsAg produced. (Supported by NIH grant DK 30854).

O 104 THE HBV ENHANCER AND trans-ACTING FACTORS. Shahid Jameel and Aleem Siddiqui. Dept. of Microbiology/Immunology, University of Colorado Medical School, Denver.
We have previously shown by means of gene transfer experiments that the HBV enhancer exhibits cell-type specificity for human hepatoma cells and that this is due to the presence of trans-acting factor(s) present in the liver cells. With the long-term aim of understanding the factor(s) involved in ubiquitous and/or tissue-specific expression, we have studied the interactions between the HBV enhancer and proteins present in nuclear extracts from human hepatoma (HepG2) and non-hepatoma (HeLa,293) cells. Gel retardation and DNaseI protection analyses show the binding of at least one ubiquitous and two (maybe three) liver cell-specific factors to the enhancer DNA in vitro. The ubiquitous factor and one liver cell-specific factor binds to a site between nucleotides 1220 and 1380. The other liver cell-specific factor binds to multiple (at least three) sites spanning the entire enhancer region. Fine mapping of the binding sites as well as mutational studies to test their functional significance in vivo are in progress. In a related study, we have also investigated the ability of other viral trans-acting proteins to activate or repress expression mediated by the HBV enhancer and/or the HBcAg/HBxAg promoters. The results and significance of this study will also be discussed.

O 105 HIGH LEVEL EXPRESSION OF HEPATITIS B VIRUS C-GENE IN HUMAN CELLS,
Olivier Jean-Jean, Massimo Levrero, *Hans Will and Michel Perricaudet, ER 272 - IRSC, CNRS, 94800 Villejuif, France and *Max Planck Institut für Biochemie, 8033 Martinsried, FRG.
HBV C-gene expression in human cells has been studied using a highly efficient adenovirus based expression vector. A plasmid containing the C-gene and the precore sequence under the control of the Ad2 major late promoter with its tripartite leader sequences have been constructed. An adenovirus transformed cell line (293 cells) has been used in transient expression assays. Culture medium and cell fractions were tested by RIA for cAg/eAg as well as cAg reactivity alone. Culture supernatants contained high levels of cAg/eAg but virtually no cAg reactivity. High levels of cAg/eAg as well as cAg reactivity were found in the cytoplasm of transfected cells while lower but still significant levels of cAg/eAg were present in the nuclei. Immunoblot analysis of total cell extracts and cell fractions extracts demonstrated the presence of several core proteins 20 to 28 kd in size. Only two of them were enriched in nuclei. Sedimentation analysis revealed that part of the cAg/eAg reactivity is associated with membrane, and another part is assembled into typical 27 nm particles. In conclusion, we have developed a very efficient expression system that allows to synthesize large amounts of several C-gene products that are amenable to functional analysis.

O 106 HUMAN T CELL CLONES RECOGNIZE SOLUBLE OR ENDOGENOUSLY EXPRESSED HBsAg AS PRESENTED BY HLA MATCHED STIMULATOR CELLS, Yide Jin, Bernard Moss, J. Wai-Kuo Shih and Ira Berkower, FDA, Center for Drugs and Biologics, Bethesda, MD 20892, NIAID, Lab. of Viral Diseases, NIH, Bethesda, MD 20892.
HBsAg specific T cell clones were established from one donor who received plasma-derived vaccine and a second donor who received yeast recombinant vaccine. All clones respond to soluble HBsAg and also respond to stimulator cells infected with vaccinia recombinants containing HBsAg genes. The first donor yielded one clone which responds to cells infected with vaccinia recombinants containing 'pre S + S' but not 'S' alone, while all clones from the second donor recognize the S antigen with or without pre S. The pre S specific clone was restricted to HLA class I and was insensitive to leupeptin inhibition of antigen processing. The S specific clones were all restricted to HLA class II, and 2 were sensitive to leupeptin inhibition, while 3 were insensitive. The leupeptin sensitivity of each clone was identical for exogenous and for endogenously expressed antigen. Mixing experiments ruled out the possibility of antigen secretion by infected cells and reuptake by antigen presenting cells. Thus, HBsAg vaccines can elicit T cells immune to pre S or S antigens, and capable of direct recognition of virally infected stimulator cells in an HLA restricted fashion. Such T cells may be important in preventing infection or in limiting the severity of liver disease following infection with hepatitis B virus.

O 107 ADSORPTION OF HBsAg IN HIGH-TITERED CHRONIC CARRIER SERA TO POLYSTYRENE IS MEDIATED BY SERUM COMPONENTS AND MAY INVOLVE HBsAg-ASSOCIATED POLYALBUMIN RECEPTORS, Ridha Khelifa, Laboratory Centre for Disease Control, Health and Welfare Canada, Ottawa, Ontario K1A 0L2.
The ability of HBsAg in chronic carrier sera to adsorb to polystyrene tubes was assayed by incubating different dilutions of high-titered HBsAg-containing sera in the tubes and using ^{125}I-labeled anti-HBs to detect any adsorbed antigen. The HBsAg in all sera tested was found to adsorb to tubes in significant amounts which increased with antigen concentration. When the tubes were precoated with undiluted normal human serum (NHS) subsequent HBsAg adsorption was not affected; whereas precoating the tubes with fetal calf serum (FCS) drastically inhibited antigen adsorption by more than 95%. When NHS-coated tubes were post-coated with FCS, only a slight (10%) decrease in adsorption was noted. On the other hand, when FCS-coated tubes were post-coated with NHS a small amount of adsorbed HBsAg was observed (10 to 20% of positive control values). HBsAg adsorption was quantitatively inhibited when glutaraldehyde-polymerized human serum albumin (PHSA) was added to tubes simultaneously with HBsAg, while polymerized bovine serum albumin had no effect. The addition of PHSA to adsorbed HBsAg did not cause antigen elution or masking. Taken together, the above observations indicate that some as yet unidentified component(s) present in HBsAg-containing or in normal human serum mediate the observed HBsAg adsorption. the inhibitory effect of PHSA on this adsorption raises the question of whether the HBsAg-binding components present in human serum may constitute a natural analogue of PHSA. Further investigations on this important aspect are in progress.

O 108 TRANS-ACTIVATION OF THE HBV ENHANCER BY THE X PROTEIN OF HBV, C. H. Lee, D. F. Spandau, and C. T. Hu, Department of Pathology, Indiana University School of Medicine, Indianapolis, IN 46223
The X gene of HBV encodes a protein of 154 amino acids. In this study, experiments were performed to elucidate its biological function. The plasmid pHBE7 was constructed so that the *cat* gene would be driven by the HBV enhancer and the promoter of the C gene. The other plasmid, pSVX-X, contains the X gene driven by its own promoter and the SV40 enhancer. Transfection of PLC/PRF/5 cells with pHBE7 resulted in a certain degree of expression of the *cat* gene. However, when pHBE7 and pSVX-X were co-transfected into PLC/PRF/5 cells, a dramatic increase in CAT activity was observed, indicating a *trans*-activating function derived from pSVX-X. If a deletion or a frame shift mutation is created in the X gene of pSVX-X, the *trans*-activating function is lost. These results suggest that the X protein of HBV can *trans*-activate the HBV enhancer.

O 109 HEPATITIS B VIRUS X GENE EXPRESSION IN HUMAN CELLS AND ANTI-X ANTIBODIES DETECTION IN CHRONIC HBV INFECTION, Massimo Levrero, Olivier Jean-Jean, *Hans Will and Michel Perricaudet, ER 272 - IRSC, CNRS, 94800 Villejuif, France and *Max Planck Institut für Biochemie, 8033 Martinsried, FRG.

To characterize the X gene product we developed a very efficient adenovirus based eukaryotic vector. High levels of expression of the HBV X gene in human cells have been obtained by using a plasmid containing the entire X ORF preceded by the adenovirus type 2 major late promoter (MLP) and its tripartite leader sequence (plasmid pMLP-X). An adenovirus transformed human embryo cell line (293-cells) and HepG2 cells transfected with the pMLP-X plasmid produced a protein of about 17 kd. This protein was identified by immunoblot analysisusing an X-MS2 fusion protein. By cell fractionation we were able to show that the X protein is at least in part present in cell nuclei. These results raise the question whether the X gene product has any effect on cellular gene functions. Cellular extracts containing the X protein have been used to screen by immunoblot analysis 21 sera from healthy people or patients with no HBV history, and 24 sera from patients affected by HBV related chronic hepatitis. Of all sera tested 4 reacted with the X product, all were from HBeAg andHBV-DNA+ve patients affected by severe chronic active hepatitis. Studies are in progress to compare X-proteins expressed in eucaryotic and procaryotic cells as a substrate for anti-X antibody detection.

O 110 Molecular studies of human hepatitis delta (δ) virus. Shinji Makino,[1] Sugantha Govindarajan,[2] Toshio Kamahora,[1] Chien-Kou Shieh,[1] Ming-Fu Chang[1] and Michael M.C. Lai[1], Department of Microbiology and Pathology[2], University of Southern California School of Medicine, Los Angeles, California 90033

We have molecularly cloned a human hepatitis delta virus genome from the serum of a patient with active delta (δ) virus infection. This was achieved by isolating the RNA genome of the delta virus particles purified directly from the patient's serum. The RNA was studied by RNase T_1-resistant oligonucleotide fingerprinting. The cDNA primer was prepared according to the sequence of some of the T_1-oligonucleotides, and cDNA clones were then generated. We have obtained a series of overlapping cDNA clones, with the biggest clone having a size of 1.5 kilobases. These clones are arranged in a relationship consistent with the circular structure of the delta RNA genome. Partial cDNA sequences have been obtained, which reveals a high degree of divergence from the published sequence of the delta RNA obtained from the virus serially passaged in chimpanzees (Wang et al, Nature 323, 508 1986). The homology between these two delta RNAs is less than 90%. The biological significance of the sequence divergence is being investigated.

The cDNA clones were used as the probes to study the expression of delta virus RNA in the patients' liver tissue. At least four subgenomic RNA species were detected. Preliminary data suggest that these RNA species are different from those detected in the infected chimpanzee's liver. The origin and structure of these RNA species will be reported.

O 111 EXPRESSION OF HEPATITIS B VIRAL ANTIGENS USING RECOMBINANT AMPHOTROPIC RETROVIRAL VECTORS, A. McLachlan, D.R. Milich, A.K. Raney, J.L. Hughes, J. Sorge, and F.V. Chisari, Dept. of Basic and Clin. Res., Scripps Clinic and Res. Fndtn., La Jolla, CA 92037.

An amphotropic retroviral expression system has been developed which expresses HBV antigens. The retroviral expression system used permits HBV antigen expression from unspliced recombinant retroviral transcripts and confers G418 resistance to cells by expression of a spliced retroviral transcript. The unspliced recombinant retroviral transcript can also be packaged to produce infectious recombinant retrovirus particles.

The properties of HBV surface and core antigens produced in cell lines transfected with the retroviral constructs have been characterized. In addition to the expression of HBsAg, the influence of the expression of pre-S(1) and pre-S(2) on the properties of HBsAg were analyzed. Expression of pre-S(1) containing HBsAg almost completely abolished the secretion of HBsAg. Expression of HBV core antigen was characterized utilizing sub-genomic DNA fragments with and without the pre-core sequence. Inclusion of the pre-core sequence resulted in the expression and secretion of HBeAg. The core sequence alone coded for the expression of nuclear HBcAg and concomitant secretion of HBeAg.

Infectious recombinant retroviruses were recovered using either (i) Moloney murine leukemia virus and the murine amphotropic retrovirus 4070 as helper viruses or (ii) from retroviral packaging cell lines. These recombinant retroviruses were characterized for their ability to transmit antigen expression, via infection and selection, to a variety of cell lines. Recombinant retroviruses which efficiently transmit expression of HBsAg and HBc/eAg have been observed.

O 112 DISTINCTIVE PROPERTIES OF HEPATITIS B VIRUS ENVELOPE PROTEINS AND THEIR ASSEMBLY
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Microbiology Div., Wyeth Laboratories, Inc., P.O. Box 8299, Phila., Pa. 19101

Hepatitis B virus (HBV) large, middle and major Surface (S) antigen genes were inserted into the E1 region of adenovirus 5. Their transcription and translation were regulated by the insertion of a second copy of the adenovirus major late promoter and tripartite leader upstream of the HBV sequences. The recombinant adenovirus containing the HBV Large S gene (HS1.HP) expressed 90% large, 4% middle and 6% major S antigens in the cells. No antigens were detected in the supernatants from HS1.HP infected cells. The recombinant adenovirus containing middle S gene (HS2.HP) expressed predominantly middle S (82%) and some major S antigen (18%), and the recombinant adenovirus which contained major S gene (HS.HP) expressed major S antigen. Both middle and major S antigens were observed in the cell lysates and supernatants as detected by radioimmunoassay (RIA) and by immunoprecipitation and PAGE. Secreted S antigens from HS2.HP and HS.HP-infected cells sedimented to 1.22g/cm^3 in a CsCl isopycnic gradient. RIA-positive fractions of CsCl gradients of HS2.HP and HS.HP supernatants contained 22nm particles. Analysis of cells co-infected with HS1.HP and either HS.HP or HS2.HP recombinant virus showed that expression of similar quantities of large S antigen together with middle or major S antigens inhibited the secretion of the middle S and major S antigens into the supernatants. These results suggest that coordinated regulation exists in the biosynthesis of these different S proteins in the HBV infected cell.

O 113 EVIDENCE FOR HEPATITIS B VIRUS MIDDLE S PROTEIN BINDING TO HUMAN LIVER MEMBRANES.
P. Pontisso, M. Bankowski, M. Peeples. Rush University. Chicago, IL 60612.

The middle S protein of the HBV envelope contains the pre-S2 encoded sequence and has been proposed as the binding protein to human hepatocytes. We have tested recombinant sub-viral particles containing middle S (Prof. Tiollais laboratories, Institut Pasteur, Paris) for binding activity to human liver plasma membranes. The binding assay was first assessed in preliminary experiments using membranes of Vero cells, where we have previously demonstrated high affinity receptors for sub-viral particles containing only the small S protein (Peeples et al, submitted). Human liver tissue was obtained from cadaver kidney donors. Sinusoidal front-enriched membranes were purified (Hubbard et al. J. Cell. Biol. 1983, 96:217) and appeared as vesicles of 80-180nm diameter by electron microscopy. ^{125}I-labeled small S and middle S particles did not bind directly to liver plasma membranes. However, after the addition of polymerized human serum albumin, 43% specific binding was observed only with middle S particles. This binding could not be detected using polymeric bovine or monomeric human albumins. In addition, neither different cellular fractions of human hepatocytes nor purified mouse liver plasma membranes, were able to bind middle S particles, regardless of the presence of polyalbumin. The interaction between middle S and human liver membranes was completely inhibited by sera obtained during the early phase of acute hepatitis B, while 60% inhibition was achieved with their IgG fractions. In conclusion, a specific binding of middle S containing sub-viral particles to human liver plasma membranes has been demonstrated and our results indicate that a human polyalbumin bridge is required as an intermediate receptor.

O 114 HEPATITIS B VIRUS INHIBITS HUMAN BETA-INTERFERON SYNTHESIS BY ALTERING INTERFERON-SPECIFIC RNA LEVELS.
Robert H. Schloemer and Jr-Shin Twu. Indiana University School of Medicine, Indianapolis, Indiana.

Individuals chronically infected with hepatitis B virus (HBV) have a deficiency in the in vivo interferon response. To determine whether hepatitis B virus (HBV) regulates the expression of the human beta-interferon gene, a series of recombinant bovine papilloma virus plasmids containing the cloned human beta-interferon gene and/or fragments of the HBV genome were constructed. Cells were transfected with the recombinant plasmids and permanent cell lines containing the plasmids and the neomycin resistance gene were established by G418 selection. A 1828 bp BamHI HBV DNA fragment containing the gene for core antigen, but not a 2755 bp BglII HBV DNA sequence encoding the surface antigen, suppresses the production of interferon. Paralleling the inhibition in the synthesis of interferon is a decrease in the amount of interferon-specific RNA. Employing a hybrid gene in which the expression of the chloramphenical acetyltransferase (CAT) gene is under the direction of the regulatory region of the beta-interferon gene, it was determined that the expression of the CAT activity was inhibited not only by the entire HBV genome but also by the 1828 bp BamHI HBV DNA fragment. A frameshift mutation within the gene for the core antigen abolishes the inhibitory activity. These results suggest that HBV suppresses the expression of the human beta-interferon gene by interacting with the regulatory region present at the 5' end of the interferon gene.

O 115 Karyotype abnormalities and chromosome instability in hepatocellular carcinoma, Daniela Simon and Barbara B. Knowles, Wistar Institute, Philadelphia, PA 19104.

Karyotype analysis of cell lines derived from hepatocellular carcinoma biopsies reveals multiple and diverse chromosome abnormalities with some chromosomes consistently rearranged in each hepatoma-derived cell line. From analysis of the peripheral blood lymphocytes of one patient with hepatocellular carcinoma, we found multiple signs of chromosome instability including breaks, minutes and chromosome pulverisation; some of the breaks localized to the sites involved in abnormalities in the cell line derived from this patient. To further examine the role of HBV in the manifestation of chromosome instability we have investigated peripheral blood cultures of a series of chronic carriers of HBV. On the basis of these results we hypothesize that chromosome instability, be it genetically determined or HBV-induced, is a factor in the etiology of hepatocellular carcinoma.

O 116 EXPRESSION OF HEPATITIS B VIRUS ANTIGENS IN XENOPUS OOCYTES. David N. Standring, James J.-H. Ou & William J. Rutter. Hormone Research Institute and Department of Biochemistry & Biophysics, University of California, San Francisco, CA 94143.

We have been studying the expression of both the preS/S and preC/C loci in Xenopus oocytes programmed with synthetic HBV mRNAs. Oocytes injected with S mRNA secrete 22nm S antigen particles while preS-1 and preS-2 mRNAs direct the synthesis of the authentic appropriately glycosylated antigens which are not, however, secreted. By co-injecting different ratios of preS and S antigen mRNAs we have shown that high levels of preS expression blocks S antigen secretion. Conversely, high level expression of S antigen drives the cosecretion of small amounts of either preS-1 or preS-2. Thus the oocyte system offers a promising approach to studying the assembly and secretion of HBV particles.

We have also demonstrated that C antigen mRNA gives rise to cytoplasmic core antigen (p22) whereas pC mRNA generates a complex pattern of intracellular products together with a series of heterogeneous secreted proteins ranging in size from 15-23 kd. The major secreted products found after a long incubation are 15-16 kd and have e antigen activity by RIA. By sequence analysis of the secreted product and by the analysis of the secretion of a mutant lacking the carboxyterminus of pC we have demonstrated that the essentially quantitative production of e antigen from pC involves removal of a 19 amino acid signal sequence from the aminoterminus of pC and further processing at the carboxyterminus.

O 117 PRODUCTION OF HBV PARTICLES IN HEPG2 TRANSFECTED CELLS. C.Sureau, J-L. Romet-Lemonne, J. Mullins and M. Essex. Department of Cancer Biology, Harvard University School of Public Health, Boston, Massachusetts. U.S.A.

The introduction by transfection of cloned circular HBV DNA into HepG2 cells followed by culture under selective conditions has resulted in the isolation of 40 transformants. They were expanded as individual cultures before being tested for the presence of intracellular HBV DNA- sequences, production of core-related antigens (HBc/eAg) and surface antigen (HBsAg) in the supernatant. HBV DNA sequences were detected in the total cellular DNA extract of 22 clones. HBV DNA was detected in the supernatant of one clone which was also positive for release of both HBc/eAg and HBsAg in the culture medium. This clone designated HepG2T14 was shown to produce HBV DNA containing particles identical to virions found in infectious human sera by criteria of antigenicity, physical properties, morphology, and DNA polymerase activity. These results indicate the achievement of an <u>in vitro</u> production of HBV-like particles by a clone of HepG2 cells. Processes involved in the production, assembly and secretion of Dane particles and envelope particles seem to be reproduced in this cell-line. In addition we have evidence for production of viral DNA and HBV virions from integrated viral DNA sequences. Three major viral transcripts were detected in the total cellular RNA extract, and further identification of these molecules will be presented. The present system will also enable us to further study the viral proteins by immunoprecipitation and SDS PAGE analysis after metabolic radiolabelling.

O 118 EXPRESSION OF HBV GENES IN WELL- AND POORLY-DIFFERENTIATED HEPATOMA CELL LINES. Ling-Pai Ting, Hsiao-Kuey Chang, Chungming Chang, and Cheng-Kung Chou, Graduate Institute of Microbiology & Immunology, National Yang-Ming Medical College; Department of Medical Research, Veterans General Hospital; Shih-Pai, Taipei, Taiwan, R.O.C.

On the basis of cellular morphology and secreted pattern of plasma proteins, human hepatoma cell lines can be divided into two groups, the well-differentiated hepatoma cell lines and the poorly-differentiated hepatoma cell lines. Several heterologous CAT expression plasmids driven by the HBsAg or HBcAg gene promoter in the presence or absence of HBV enhancer were used to transfect all these cell lines. In well-differentiated cell lines, the HBV enhancer sequence can stimulate the transcriptional activity of HBcAg and HBsAg gene promoter about 2-11 fold and 6-100 fold, respectively. However, in poorly-differentiated cell lines, the transcient CAT expression driven either by the HBsAg or HBcAg gene promoter is very low. But the HBV enhancer still stimulates the HBsAg and HBcAg gene promoters about 2-15 fold and 5-60 fold, respectively. These results suggest that the transcriptional activity of both HBsAg and HBcAg gene promoters in poorly-differentiated cells is very low compared to in well-differentiated cells. The stimulating effect of HBV enhancer on both promoters in poorly-differentiated cells is about the same as in well-differentiated cells. Several protected regions around the HBV enhancer sequence were identified by the nuclear extracts of HuH-7 cells.

O 119 HEPATITIS B VIRUS PARTICLES ARE PRODUCED IN A CELL CULTURE SYSTEM BY TRANSIENT EXPRESSION OF TRANSFECTED HBV DNA, K.Yaginuma, Y.Shirakata, M.Kobayashi and K.Koike, Dept. of Gene Res., Cancer Institute, Tokyo 170, Japan.

An in vitro system for the production of hepatitis B virus (HBV) particles was established by the transient expression of transfected HBV DNA using a human hepatocellular carcinoma cell, HuH-7, as a recipient. The 3.6 kb and 2.2 kb major transcripts were observed, which were similar to those in virus-infected liver cells. Analyses of both transcripts revealed the micro-heterogeneity of 5' ends.

The formation of core particles observed in the cytoplasm and the virus particles secreted in the culture medium contained the replicative intermediates of HBV DNA, and banded at densities of 1.35-1.36 g/cm^3 and 1.22-1.24 g/cm^3, respectively. Furthermore, the in vitro mutagenesis of the template HBV DNA demonstrated that the P gene as well as the C gene products were essential for the production of HBV particles. On the other hand, mutations of the X gene product did not affect the virus production in this system. The cohesive end region was found to play an important role in the regulation of 3.6 kb RNA transcription. Thus, the present in vitro system has the advantage of genetic study over other systems which use transformants with integrated HBV DNA.

Carcinogenesis, Immunology and Pathogenicity

O 200 HEPATITIS DELTA IN HEMOPHILIACS: PRELIMINARY OBSERVATIONS IN HEPATITIS B ANTIGEN POSITIVE AND NEGATIVE PATIENTS. WA Andes, SM Lemon, ML Smiley, CA Johnson, RL Janco, WI Hanna, PC Davis, NL Krejmas. The Southeastern Hemophilia Group, New Orleans, LA.

The transmission of hepatitis viruses to hemophiliacs is well known but the current prevalence of hepatitis delta has not often been studied. We conducted a retrospective serologic review of approximately 600 hemophiliacs. Review of a portion of the HBsAg chronic HBsAg carriers for anti-HD was made and related to their biochemical studies, history of hepatitis, coagulation factor use and current findings. Ages ranged from 7 (2 pts) to 58 years. 15 patients had persistant HBsAg as early as 1978. 8/15 antigen carriers had anti-HD antibody noted in plasma samples taken as early as 1984. 7/15 had no anti-HD. Coagulation factor usage was quite variable and 2/15 patients had used only plasma. 2 additional patients were of interest because no circulating HBsAg had ever been detected yet they evidenced high titer anti-HD. Biochemical values ranged from 21 to 215 AST units and 3/8 with anti-HD antibody had had acute hepatitis but only 1/7 without anti-HD antibody had had hepatitis. Both patients with anti-HD, but not HBsAg, had had hepatitis (1970,1980). Two patients (1 anti-HD (+), 1 anti-HD (-)) developed AIDS and a third, anti-HD (-), is under consideration for liver transplantation.

Estimates of hepatitis delta exposure in hemophiliacs may be more frequent than previously recognized. Even patients not judged to have been carriers of HBsAg may have been exposed. The importance of hepatitis delta exposure in such patients and the relationship of this exposure to AIDS and chronic liver disease in such patients is unknown but warrants close observations in the future.

O 201 HETEROGENEOUS HBV DNA REPLICATION AND INTEGRATION PATTERNS AMONG CIRRHOTIC NODULES AND HEPATOCELLULAR CARCINOMA NODULES WITHIN THE SAME AND DIFFERENT LIVERS, Naoto Aoki and William S. Robinson; Stanford University, Stanford, CA 94305-5701. Individual cirrhotic nodules (CN) and hepatocellular carcinoma nodules (HCNs) of three HBsAg (+) autopsy cases were analysed by Southern blot and slot blot hybridization with an HVB DNA probe. DNA extracted from 20 different CNs and 3 separate HCNs of liver No. 1 were examined with HindIII digestion. Free replicative forms (RF) of HBV DNA wre detected in all CNs (20/20). High molecular weight bands (HMWBs) which suggested clonal integrations of HBV DNA in host DNA were detected in 5 of 20 CNs (5/20). The HMWBs were different in each CN and all were of the 3 HCNs. One HCN (A) showed a distinctively different HMWB pattern. Fifty-seven different CNs and a single main HCN were exmained. Most of the CNs contained less than one copy of the HBV genome/haploid cell genome. RFs (2/57), heterogeneous DNA fragments (HF) (5/57), and HMWBs (1/57) containing viral DNA were detected after HindIII digestion of DNA extracted from CNs. BglII digestion of CN DNAs revealed a subgenomic viral DNA fragment of 2.8 Kbp in 10/16. Fifty-four different CNs and 3 HCNs of liver No. 3 were examined. Most of the CNS contained less than one copy of the HBV genome/haploid cell genome. Fourteen of these CN DNAs were examined by Southern blot after Hind-III digestion. Faint HF were detected in 3 of 14 NCs (3/14) and other (11/14) did not (i.e., contained less than 0.5 pg HBV DNA per 10 g of HindIII digested CN DNA). All 3 HCNs revealed the same clonal integration pattern of HBV DNA. Thus the state of HBV appears to be different in almost every CN, many cells in CNs as well as in HCNs are of clonal origin, and HCNs within the same cirrhotic liver sometimes represent metastatic lesions and sometimes different tumors of multifocal origin.

O 202 IN VITRO AND IN VIVO SYNTHESIS OF THE HEPATITIS B VIRUS SURFACE ANTIGEN AND OF THE RECEPTOR FOR THE POLYMERIZED HUMAN SERUM ALBUMIN FROM RECOMBINANT HUMAN ADENOVIRUSES, Annick Ballay, Massimo Levrero, *P. Tiollais and M. Perricaudet, ER 272 - IRSC CNRS, 94800 Villejuif, France and *Institut Pasteur 75015 Paris, France.
We have constructed recombinant viruses, derived from Ad5, which harbor the genes of hepatitis B virus (HBV) coding for the surface antigen HBsAg and for the receptor for polymerized albumin (pHSA) under the control of adenovirus promoters. Thus, we have successively tested the E1A promoter as well as the major late promoter (in association with its three leader sequences). The recombinant viruses have been constructed by using plasmids, which harbor the very left-end of the Ad5 genome and the genes of HBV under the control of adenovirus promoters. These plasmids have been used to replace the very left-end of the Ad5 by viral sequences from the recombinant plasmid. The recombinant virus is defective for E1A, but can be propagated on the 293 cells which complement this defect. Infection of 293 cells or Vero cells by the recombinant viruses shows that these viruses are able to direct the synthesis of polypeptides carrying the HBsAg and the receptor from pHSA which are correctly assembly and excreted out of cell as typical 22 nm particles. A yield of 10 ug/10^6 cells is obtained with the construction using the major late promoter after 3 days of infection. Moreover, the inoculation of rabbits with the purified recombinant adenovirus elicits the synthesis of antibodies directed against the HBsAg and the receptor for pHSA (Ballay et al., EMBO J., 4, 3861-3865, 1985). These results constitute the basis for the development of adenovirus as a live vaccine for humans.

O 203 HDV-INDUCED CHRONIC LIVER DISEASE:FEATURES OF HBV AND HDV REPLICATION.
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To clarify if high level HDV replication and/or continuing HBV replication are related to more severe illness,we studied 30 patients with HDV-induced chronic hepatitis(HDV-CH).All had been HBsAg+,anti-HD+,IgM anti-HBc - for six months;mean age was 29.4 yrs(range 5-55).Four were HBeAg+;four were anti-HIV+(EIA + blotting);three of these were drug addicts.HBcAg and HDAg were tested on frozen sections by IF.HBV-DNA and HDV-RNA were measured in sera by hybridization with an HBV-DNA probe and a cDNA HDV riboprobe.Liver HDAg and levels of HDV-RNA were closely related. When HDAg+ cells were < 10% (8/30 pts) HDV-RNA levels were always low (+/- or + ;absent in 1),while when they were > 10%(22/30 pts) HDV-RNA was often high (++ to ++++ in 55.5%).Levels of HDV-RNA were unrelated to histological activity,which was present in 61% of 18 pts with low level and in 75% of 12 pts with high level HDV-RNA.Patients with high levels of HDV-RNA had cirrhosis less frequently than those with low levels (42% vs 67%).HBV-DNA coexisted with HDV-RNA in 12/30 pts.Four of these had high levels of HBV-DNA,HBcAg in the liver and HBeAg in serum.Two of them were HIV infected and one was a young child.The remaining 8 pts had low levels of HBV-DNA and did not express HBcAg or HBeAg.Presence of HBV-DNA was linked to a higher prevalence of active disease(83% vs 56% of 18 pts without HBV-DNA)but not of cirrhosis(58% vs 56%).CONCLUSIONS:HBV replication can be found in chronic HDV infection,but synthesis of HBV gene products is usually suppressed.Immunosuppression,(e.g. HIV),may allow their expression. HDV-RNA levels are not related to activity of HDV-CH, but concurrent HBV replication is often present in active disease.

O 204 MOLECULAR MARKERS OF WHV AND HDV INFECTION AT THE LEVEL OF THE SINGLE CELL.
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In situ hybridization is a powerful technique that may be applied to identify cells containing viral nucleic acids in cell populations or tissue samples. The method is particularly suited to the examination of virus-infected tissue, since infected cells are often found in foci. In addition, by careful tissue preparation combined with the judicious use of genomic or anti-genomic or alternatively subgenomic (gene) probes, it may be possible to identify cells containing viral nucleic acid replicative intermediates. These approaches may be combined with viral antigen detection or Southern and Northern blot analysis.

Using a combination of the above methods, we have detected woodchuck hepatitis virus (WHV) DNA in hepatocytes, splenic red pulp and germinal center lymphocytes, pancreatic acinar cells and peripheral blood lymphocytes of experimentally infected animals. We have also detected hepatitis delta virus (HDV) genomic and anti-genomic RNA in infected hepatocytes in a chimpanzee biopsy sample taken at the peak of an acute HDV infection. Genomic RNA and HDAg detected by immunoperoxidase were present in a majority of hepatocytes while anti-genomic RNA was present in only a small minority of hepatocytes. Independent analysis demonstrated that 70% of HBsAg-positive cells contained HDAg but only 8% of HDAg-positive cells contained detectable HBsAg.

O 205 TREATMENT OF CHRONIC TYPE B HEPATITIS WITH RECOMBINANT LEUCOCYTE ALPHA A INTERFERON: RESULTS OF A PHASE II TRIAL.
Georg Hess[1], Wolfram Gerlich[2], Christian Weber[3], Norbert Drees[3], Karl-Hermann Meyer zum Büschenfelde[1], [1] I. Medical Department, 6500 Mainz, FRG, [2] Department of Microbiology 3400 Göttingen, FRG, [3] Hoffmann LaRoche AG, 7889 Grenzach-Wyhlen, FRG.

A defect in alpha interferon production presents the rationale to treat individuals with chronic type B hepatitis with interferon.

Thirtyone individuals with biopsy proven HBsAg, HBeAg and HBV-DNA positive chronic hepatitis were included into the study, they were anti-delta negative. Individuals were randomized to receive 10 x 10^6 units of recombinant alpha A interferon (Hoffmann LaRoche) per m^2 body surface area two or three times a week for 12 weeks intramuscularily. Hepatitis B markers were analysed by commercial tests (Abbott Laboratories, N.-Chicago, Ill.), HBV-DNA according to Scotto et al (Hepatology 1983).

Among 31 individuals treated 3 lost HBsAg and HBeAg, 11 remained HBsAg positive but became HBeAg negative, all other patients remained HBsAg and HBeAg positive. Six individuals had hepatitis B antigens reappearing in the serum, this was permanent in 3 (1 individual HBsAg, 2 individuals HBeAg). Individu-als receiving interferon thrice a week had higher response rates (10/14 vs 4/17) when compared to individuals receiving interferon twice a week. Anti-HTLV III positive individuals did not respond, so did individuals with HBV-DNA concentrations over or equal to 520 pg/10 ul and HBeAg titers equal or higher than 1 : 10.000. The ratio of T4/T8 cells did not correlate to response.

Flue like symptoms, hairloss, increase of depression and leucopenia were reversible side effects of interferon treatment and more pronounced in individuals receiving interferon twice a week.

In summary, interferon appears beneficial in chronic type B hepatitis, the effect to be confirmed in an ongoing controlled phase III trial.

O 206 CELLULAR AND ANTIBODY RESONSES TO HBV PRE-S PROTEINS IN MAN,
Colin R. Howard, Sheila E. Brown, Barbara Sisley, Carolynne Stanley, Shih-Hui Chen and Michael W. Steward, London School of Hygiene and Tropical Medicine, London WC1E 7HT, England.

Human immune responses to pre-S antigens have been measured using synthetic peptides and compared to responses against S proteins. Some recipients of a plasma-derived vaccine antigenically negative for pre-S are primed at the cellular level after the third vaccine dose in the absence of pre-S(2) antibodies. All vaccine recipients had demonstrable lymphocyte responsiveness to a peptide consisting of residues 139 to 147 representing a major a determinant of HBsAg. 71% of acute convalescent sera, 80% of patients with chronic liver disease and hepatitis B immunoglobulin contain high affinity antibody to pre-S2. Our findings indicate (1) priming of cellular responses to pre-S(2) in vaccinees in the absence of antibody, (2) antibody to pre-S(2) is long-lasting after infection, and (3)pre-S2 responses do not correlate with recovery from infection. Further analysis of the pre-S2 region has been undertaken by immunization of BALB/c mice with peptides of varying length. Maximum antibody titres have been obtained with a peptide containing residues LQDPRVRGLY within the pre-S2 region and the affinity of the antibodies induced by the different peptides has been related to their immunogenicity.

O 207 INFECTION AND TISSUE TROPISM OF WHV DURING THE NATURAL COURSE OF EXPERIMENTAL VIRAL INFECTION OF WOODCHUCKS. B. Korba, B. Tennant[1], E. Gowans[2], F. Wells, R. Clarke and J. Gerin. Georgetown Univ., Rockville, MD and [1]College of Veterinary Medicine, Cornell Univ., Ithaca, NY. and [2]Div. of Med. Virology, Inst. of Med. Vet. Science, Adelaide, Australia

Previous studies from our laboratories have demonstrated that lymphoid cells of chronically-infected woodchucks are infected by WHV. These infections are not quiescent as evidenced by the presence of viral RNA transcripts in peripheral blood lymphocytes (PBL) and lymphnodes and the demonstration of WHV replication in lymphoid cells of the spleen. To assist in determining how the infection of extraphepatic tissues participates in the overall course of hepadnavirus infection, we have examined the state of WHV nucleic acids in nine tissues from 44 experimentally-infected woodchucks by Southern and Northern blot and in situ hybridization analyses. Included in this survey were the primary components of the lymphoid system, pancreas, kidney, gonads and liver. Groups of animals were examined at each of several stages in the course of viral infection leading to chronicity and the development of hepatocellular carcinoma. Different kinetics of infection, levels of viral nucleic acids and viral genomic forms were observed in the various tissues throughout the course of infection. These observations are currently being correlated with markers of immune response and liver disease. This study should contribute to our understanding of the natural course of hepadnavirus infections.

O 208 DEVELOPMENT OF HEPATOCELLULAR CARCINOMA IN A CHIMPANZEE WITH NON-A, NON-B HEPATITIS H.K.Linke, R.R.Lesniewski, R.J.Carrick, M.F.Miller, D.A.Peterson & E.Muchmore, Abbott Labs, No.Chicago, IL 60064 & LEMSIP, NY Univ.Med.Ctr., Tuxedo, NY 10987.
The relationship between hepadnavirus infections and hepatocellular carcinoma (HCC) is well documented in many animal species. Examination of HCC tissue from humans has demonstrated prior exposure to HBV through the presence of antigens, antibodies, and/or DNA. In many cases, however, no such markers are demonstrable and other viruses or environmental factors must be considered for HCC. In light of this, there have been a few case reports which implicate Non-A, Non-B hepatitis (NANB) in human HCC. Here we report HCC in a chimpanzee with a history of chronic NANB. The chimpanzee Humperdink received a human NANB inoculum in 1978. Within 5 weeks the animal experienced a slight elevation of liver enzymes (~2X), which quickly returned to baseline. Over the next 6 years the animal was uneventfully inoculated with various preparations to test for hepatitis contaminants. Throughout these experiments, Humperdink remained negative for serologic markers of HBV. Liver biopsy samples taken in late 1984, however, revealed a vacillating hepatitis infection with ultrastructural changes consistent with NANB H-strain. Subsequently he developed a progressive liver carcinoma and died in July, 1985. Tissue and blood samples obtained prior to death were examined extensively for HBV and hepatitis delta markers without success. Sensitive hybridizations of liver and tumor tissue failed to demonstrate copies of HBV DNA. In addition, a cell-free suspension of tumor-like tissue induced a classic NANB-H strain infection in a recipient chimpanzee. The chronology of clinical events and the presence of a NANB infectious agent in tumor tissue suggest a complicity of NANB in the etiology of HCC.

O 209 INTERLEUKIN-2 RECEPTOR EXPRESSION AND GAMMA-INTERFERON PRODUCTION IN HDV-RELATED CHRONIC LIVER DISEASE. S.Magrin,A.Craxì,C.Carini°,M.Messina,G.Antonelli§,F.Dianzani§,C.Ausiello*,L.Pagliaro. Clinica Medica R, Palermo; °Cattedra di Immunologia,Roma; §Cattedra di Virologia,Roma; *Ist.CNR Tipiz. Tissutale,l'Aquila, ITALY.
Hepatitis delta virus(HDV) induces liver cell damage through unknown mechanisms(direct cytophatic effect?).Since the interleukin system(IL-2 and IL-2 receptors) and gamma-interferon(γ-IFN) were shown to be lowered in chronic hepatitis B virus(HBV)-infection,we have comparatively assessed these parameters in HBsAg+ liver disease with and without chronic HDV superinfection.Twenty-six patients with biopsy-proven chronic liver disease(14 HDV-CLD; 12 HBV-CLD) were studied.All were anti-HBe+,and the percentage of HBV-DNA+ subjects was similar in both groups (25% and 21%).None was homosexual or drug addict;all were anti-HIV negative(by ELISA).Twelve healthy individuals,age and sex matched,were used as control.Peripheral blood mononuclear cells were stimulated with PHA for 72 h; IL-2 receptors(IL-2R) expression was assessed by indirect immunofluorescence using monoclonal antibody. IFN activity was evaluated on human Wish cells by Sindbis virus haemagglutination yield reduction after a single growth cycle.Geometric mean titers(gmt) of IL-2R were comparable in HDV-CLD(48.8%),HBV-CLD(41.9%)and normal controls(43.6%).Synthesis of γ-IFN was increased in both HDV-CLD(gmt 1178 IU/ml,range100-30,000)and HBV-CLD(gmt 1467 IU/ml,range 30-30,000)compared to normals(gmt 562 IU/ml,range 30-3,000).In conclusion,no defect in IL-2R expression was seen in patients with HDV-CLD.By contrast,both groups of patients showed an increase in γ-IFN production.The fact that either patients with HIV or HBV with high level of replication were not included in our study could account for the difference with previous reports. Further investigations regarding IL-2 production are in progress.

O 210 COMPARISON OF TWO SOLID PHASE IMMUNOASSAYS FOR ANTI-HD DETECTION AND QUANTITATION
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Two solid phase competitive immunoassays for the detection of the antibody to hepatitis delta antigen (anti-HD) were developed, namely, ABBOTT ANTI-DELTA EIA and ABBOTT ANTI-DELTA RIA. The assays utilize delta antigen (HDAg) as a solid phase "capture" reagent and either ^{125}I-Labeled or horseradish peroxidase conjugated anti-HD IgG as a probe "detector" reagent. The two assays were found to measure the same molecules and to correlate well regarding anti-HD titers. When 1541 HBsAg reactive serum specimens were assayed for anti-HD, 119 (7.72%) were positive by the EIA and 120 (7.79%) by the RIA, giving an agreement of 99.8%. Both assays showed equivalent specificity when a population of 552 serum specimens obtained from individuals with no history of previous exposure to hepatitis B virus were tested. The RIA procedure was utilized to study the prevalence of anti-HD among HBsAg reactive drug addicts and hemophiliacs in the United States and Europe as used as anti-HD prevalence among HBsAg reactive individuals in other continents. Anti-HD prevalence varied among continents with a high incidence among HBsAg reactive drug addicts and hemophiliacs. Similar prevalences were also obtained by EIA procedure.

O 211 Purification and Western Blot Analysis of the Delta Hepatitis Antigen Derived from Infected Liver (Authors Joseph Puig, Howard A. Fields)

The hepatitis delta antigen (HDAg) was purified from the liver of an HBsAg chronic carrier chimpanzee experimentally superinfected with the hepatitis delta virus (HDV). The organ was homogenized in isotonic buffer containing protease inhibitors and centrifuged to remove structural debris. The supernatant was chromatographed through Sepharose CL-4B equilibrated with PBS containing protease inhibitors and 25% glycerol. The HDAg positive fractions were pooled and applied to an immunoaffinity column containing immobilized human anti-delta IgG coupled to Sepharose CL-4B by the meta-periodode oxidation method. The HDAg was eluted with 3M NaSCN containing 25% glycerol. The two-step procedure gave a yield of approximately 15% based upon a quantitative enzyme immunoassay. HDAg purity was ascertained by SDS-PAGE and silver stain analysis. The electropherogram demonstrated three bands corresponding to molecular weight of 43,000, 40,000, and 28,000 daltons. Western blot analysis using horseradish peroxidase conjugated human anti-delta IgG revealed a major polypeptide corresponding to a molecular weight value at 26,000, four minor polypeptides between 20,000 and 25,000, and two polypeptides at 14,000 daltons. None of the polypeptides identified by Western blot analysis were visualized by silver staining indicating that the delta-specific polypeptides are present in relatively low quantities or that the delta antigen(s) is refractory to silver stain. The 26,000 dalton polypeptide, purified by preparative SDS-PAGE and transferred to nitrocellulose, was solubilized with DMSO and used as an immunogen to produce monospecific rabbit anti-delta.

O 212 Simultaneous Detection of HBsAg and Antibody to Human Immunodeficiency (HIV) Virus in Human Plasma. Richard S. Smith, Douglas Richman, and Sharyn Viel. Johnson and Johnson Biotechnology Center, Inc., VA Medical Center, and University of California San Diego

Several studies have indicated that approximately 5-10% of individuals with a history of hepatitis B infection also have antibody to human immunodeficiency virus (HIV). Due to the seriousness of AIDS and hepatitis in donated blood, various enzyme immunoassays (ELISAs) have been developed to detect HBsAg and antibody to HIV separately. Recently, we described an ELISA with synthetic peptides in the env region of HIV to detect antibody in AIDS patients. These peptides were used instead of HIV viral lysates in this assay. This peptide assay for HIV has been combined with a sandwich monoclonal ELISA to detect HBsAg in a one well assay procedure. Antibody to the HIV env peptides are detected by a sandwich type immunoassay with HRPO. HBsAg bound to the plastic is detected with a monoclonal antibody conjugated with the same enzyme. This combination assay accurately detected antibody to HIV and HBsAg antigen in several panels of sera that had been characterized for HIV antibody and HBsAg by licensed procedures. The combination HIV-HBsAg assay can detect 1 nanogram of HBsAg standard. This combination assay for HIV antibody and HBsAg was as sensitive as the combined results of licensed separate procedures for the detection of HBsAg and HIV antibody.

O 213 SURROGATE TESTING FOR NON-A NON-B HEPATITIS: CONFIRMATORY ASSAY FOR ANTI-CORE REACTIVE SPECIMENS J. M. Staller, R. M. Pennington, and L. Valdivia, Hepatitis/AIDS Research and Development, Abbott Laboratories, Abbott Park, IL 60064, USA.

With the advent of Corzyme screening in American blood banks as a surrogate marker for Non-A Non-B Hepatitis, it has become necessary to develop confirmatory assays for the presence of antibodies to Hepatitis B Core Antigen (anti-core). Recent studies have correlated the presence of anti-core in blood donors to transfusion associated NANB. An assay has been developed for confirming samples which are reactive in anti-core assays. This confirmatory assay is composed of small centrifugable particles coated with human dane core (DCP). DCP's are incubated with an anti-core reactive sample. After a short incubation, the DCP's are removed from solution by centrifugation. The supernatant is assayed for anti-core activity. Several groups of samples were tested.

		Corzyme		Confirmatory Assay	
Sample Type (#)		Positive	Negative	Positive	Negative
HBsAg/Anti-HBs Negative	(48)	1	47	1	47
Anti-HBs Positive	(33)	32	1	32	1
Corzyme Borderline	(49)	--	--	45	4

Results show that 92% of borderline samples are true positives.

O 214 A HYBRID HBV-HOST TRANSCRIPT IN A HUMAN HEPATOMA CELL LINE. Tsung-Sheng Su, Veterans General Hospital, Taipei, Taiwan, Republic of China.

To study the role of hepatitis B virus (HBV) involved in hepatocarcinogenesis, a panel of 11 human hepatoma cell lines were screened for HBV expression by Northern blot analysis. Among them, one of the cell line showed to have a major species of RNA about 4.0 kb in size and several minor species of RNA ranged from 3.3 kb to 2.2 kb in size hybridizable to HBV sequence. A S1 nuclease mapping analysis suggested that the major species of RNA, its 5' end of HBV sequence was mapped at pre-S region and its 3' end of viral sequence was mapped at DR region. By genomic cloning and cDNA cloning, we demonstrated that this RNA is a hybrid of HBV and host. There is about 2 kb of sequence at the 3' end of RNA is host origin. By somatic cell hybrids analysis, the virus was found integrated at chromosome 4. The functional significance of this hybrid RNA is being investigated.