incorporate myristate, are no longer localized to membranes and, most importantly, do not transform the cells. The behavior of these constructed mutants is consistent with the interpretation that the mutant p60*src* protein now lacking myristate may be no longer capable of finding the sensitive membrane-bound targets to induce transformation. Similar experiments deleting the myristate acceptor site in the *gag-abl* protein have shown that myristate may have a role in transformation in this system as well (*14*).

Whether there are specific inner membrane receptors for myristylated proteins as suggested here remains to be established. Certain observations suggest that interplay of myristylated proteins at the membrane is important in transformation and growth control. For example, the activity of type I cyclic AMP–dependent kinase has been shown to be depressed fivefold in *src*-transformed cells (*15*), and the cyclic AMP–dependent kinase regulatory subunit can be a substrate for calcineurin (*16*). Myristylation may prove useful for characterizing the cellular sites where these interactions take place, and for studying the physiology and biochemistry of transforming proteins and their proto-oncogene homologues.

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Antibodies to Peptides Detect New Hepatitis B Antigen: Serological Correlation with Hepatocellular Carcinoma

Abstract. *The expression of a previously unidentified gene product, encoded by the hepatitis B virus (HBV) genome, has been achieved with a recombinant SV40 expression vector. Antibodies against synthetic peptides representing defined regions of this protein were used to screen cells infected with recombinant virus as well as tissues naturally infected with HBV. A 24,000-dalton protein (p24) was detected in cells infected with recombinant virus and a 28,000-dalton protein (p28) was detected in tissues infected with HBV. The peptides or recombinant-derived protein were used as antigens to screen sera from individuals infected with HBV. Specific antibodies were detected predominantly in sera from patients with hepatocellular carcinoma. The presence of p28 in tissues infected with HBV and the appearance of specific antibodies in infectious sera establish the existence of an additional marker for HBV infection.*

Analysis of the viral gene products encoded by the hepatitis B virus (HBV) has been hampered by the lack of a permissive tissue culture system and the narrow host range demonstrated by HBV. The cloning of the HBV genome (*1*) has provided a means by which these problems can be circumvented. DNA sequence analysis of these cloned HBV genomes (*2, 3*) identified the genes encoding the surface and core antigens and, in addition, revealed two open reading frames (denoted P and X) on the L strand (*4*). It has been suggested that the endogenous DNA polymerase found associated with core particles is encoded by region P, since the translation product of this sequence is homologous to conserved regions of retrovirus *pol* gene products (*5*). Region X represents an open reading frame for which a protein product has yet to be assigned. Definition of the hepatitis B surface antigen (HBsAg), core antigen (HBcAg) and "e" antigen (HBeAg) on the genome has allowed the production of these classical HBV markers in eukaryotic expression systems. In addition to providing an alternative source from which to obtain HBV gene products, these techniques can be used to generate large quantities of materials for an HBV vaccine (*6*). We now report the expression of the HBV X gene product as well as its detection in tissues infected with HBV with antibodies of predetermined specificity. We also detected circulating antibodies specific for the X protein in human serum samples infected with HBV. Together, these findings establish the existence of a new marker for HBV infection.

Hepatitis B virus DNA was cloned into plasmid vector pBR322 at the Bam HI restriction site, resulting in a full genome interrupted in the pre-S region and retaining both Bam HI sites (Fig. 1A). One Bam HI fragment contained the HBsAg coding sequence [1350 base pairs (bp)] while the other fragment contained 94 percent of the X coding sequence and all of the core gene (1850 bp). Expression of the S gene was accomplished as described (*7*). The DNA sequence containing the X gene (1850-bp Bam HI fragment) was ligated to a pBR322-SV40 cloning vector (pBRSV) at the Bam HI site. The plasmid sequences in pBRSVHBV permitted the replication of this hybrid DNA in *Escherichia coli* prior to transfection into eukaryotic cells. A restriction enzyme digest with Hae II removed all of the plasmid sequences and positioned the HBV fragment in the sense orientation to the SV40 late promoter (SVHBV-3). Construction of the expression vector SVHBV-3 removed the first 66 bp of the putative X gene, including the methionine initiation codon ATG. We predicted that the X sequences present in the construct (Fig. 1B, amino acid residues 23 to 154) would fuse with the SV40 structural protein VP2 sequences present in the vector.

Table 1. Reactivity of human liver samples with peptide 99 and peptide 142. The diagnoses are abbreviated as follows: AH(B), acute hepatitis, type B; ASC, asymptomatic HBV carrier; CH, chronic hepatitis, type B; LC, liver cirrhosis, type B; HCC, hepatocellular carcinoma, type Ba.

Diagnosis	Total number of samples	Samples reactive with one or both peptides	Percentage of total
AH(B)	5*	0	0.0
ASC	68	4	5.8
CH	149	20	13.4
LC	19	3	15.7
HCC+LC	11	8	72.7
Normal	2	0	0.0
Total	254	35	13.7

*Three of the AH(B) samples were $HBsAg^+$ and two were $HBsAb^+$.

The resultant fusion protein would contain 91 amino terminal amino acids of VP2 (initiation codon of VP2 through the Hae II site at position 831 of the SV40 late region) (*8*) and 132 of the 154 amino acids of X. The predicted size of this VP2-X fusion protein is 24,500 daltons (24.5K). SVHBV-3 virions were made according to the method of Hamer *et al.* (*9*). The sequences that provide SV40 late region functions were deleted in the construction of the SV40 expression vector and therefore necessitated complementation by a temperature-sensitive SV40 early region mutant (tsA_{239}) to produce recombinant virus (*9*). SVHBV-3 or wild-type SV40 were used to infect BSC-1 cells and the cell lysates were analyzed for the presence of an X-gene product.

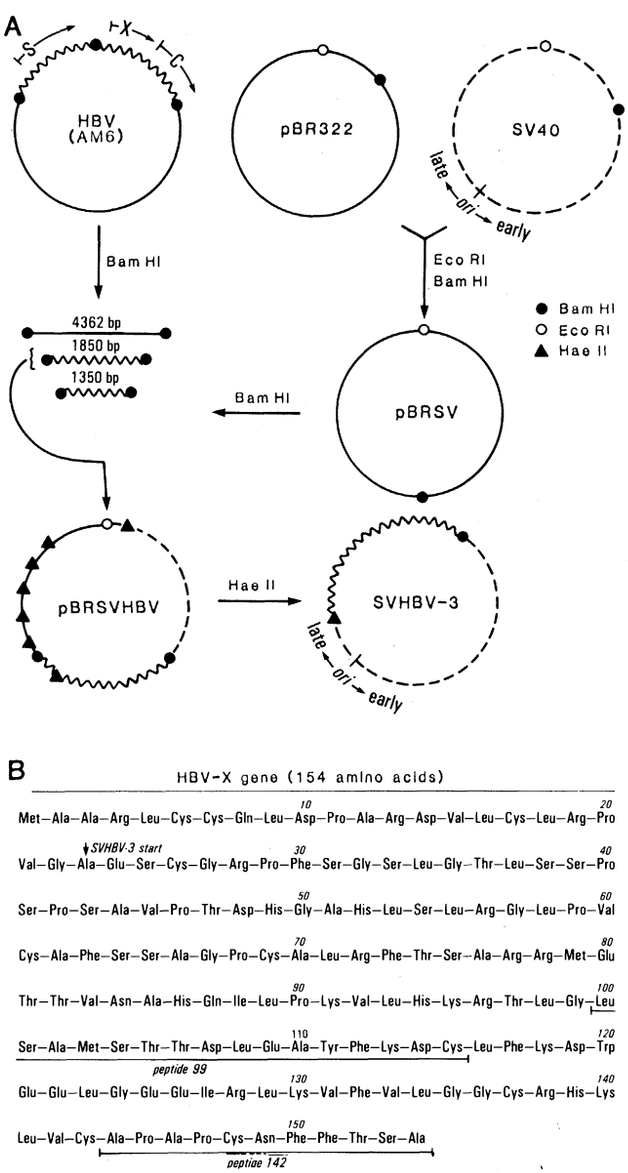


Fig. 1. (A) Construction of an SV40-HBV DNA expression vector containing the HBV X gene. SVHBV-3 DNA was constructed from HBV clone AM6 (*7*) by using the Hae II–Bam HI SV40 expression vector described by Hamer *et al.* (*9*). (B) The predicted amino acid sequence of the X protein, translated from the HBV DNA sequence reported by Galibert *et al.* (*2*). The putative X protein contains 154 amino acids (approximately 17,000 daltons of protein). SVHBV-3 contains amino acid residues 23 through 154 of this sequence, in addition to the 91 amino terminal amino acids of SV40 structural protein VP2. Peptides 99 and 142 are underscored.

Two peptides, representing different regions of the putative X protein, were synthesized. Peptide 99 includes amino acid residues 100 to 115, and peptide 142 includes residues 144 to 154 (Fig. 1B). Antisera to the peptides were generated in rabbits and used as probes for the detection of the putative X protein in the cell lysates (*10*). The antisera to both peptides recognized a 24K protein in lysates prepared from SVHBV-3 infected cells but not in control lysates prepared from cells infected with wild-type SV40. Figure 2, lane 1, shows the reactivity of the antiserum to peptide 99 with the cell lysates. The reactivity was blocked by prior incubation of the antiserum with the competing peptide (Fig. 2, lane 2). Similarly, antiserum to peptide 142 reacted with a 24K protein (lane 3), and this reactivity was blocked by peptide 142 (lane 4). No reactivity was detected at 24K in the control lysates with either antisera (lanes 5 and 6). The antiserum to peptide 99 recognized two additional proteins, one at 45K and the other at 19K (lane 1). Since the reactivity of the 45K protein was not blocked by the competing peptide (lane 2), it was considered nonspecific. The reactivity of the 19K protein was blocked by prior incubation of the antiserum to peptide 99 with the peptide (lane 2). However, the presence of the 19K protein in the control SV40 infected cells (lane 5) suggested that it was not SVHBV-3 related.

The antisera to both X peptides were then used to screen eight samples of human tissue: two hepatoma cell lines and six liver samples. One of the hepatoma cell lines, PLC/PRF/5, is known to contain integrated HBV DNA sequences (*11*). HBsAg has been detected in this line (*12*), while all standard assays for HBcAg and HBeAg have proved negative. The other line, HepG2, has no evidence of integrated HBV DNA sequences (*12*). Cell lysates prepared from these cell lines were tested for reactivity with the antiserum to peptide 99 by immunoprecipitation. An X-specific protein at 28K (p28) was detected in PLC/PRF/5 cells (Fig. 3, lane 1). The reactivity was inhibited by prior incubation of the antiserum with peptide 99 (lane 2) but not by incubation with a nonrelated peptide (lane 3). The control serum was negative (lane 4). No X-specific reactivity was detected when HepG2 lysates were reacted with either the preimmune control serum or antiserum to peptide 99 (lanes 5 and 6, respectively).

The six samples of human liver were screened for the presence of an X-related protein in a Western blot assay. Of the two samples shown in Fig. 4, one sample was taken at autopsy from an individual serologically negative for HBV (Fig. 4, lanes 1, 2, 5, and 6); the other was removed from a patient with chronic persistant hepatitis B whose liver was positive for HBcAg on immunofluorescent staining (Fig. 4, lanes 3, 4, 7, and 8). Cell lysates prepared from these two liver samples were tested with the antisera to peptides 99 and 142. The antiserum to peptide 99 reacted with a 45K protein (p45) in both liver samples (Fig. 4, lanes 1 and 3), and with a 28K protein (p28) in the infected liver only (Fig. 4, lane 3). Both reactivities were blocked when the antiserum to peptide 99 was incubated with peptide 99 (Fig. 4, lanes 2 and 4). When the antiserum to peptide 142 was reacted with these cell lysates, a 40K protein (p40) (lanes 5 and 7) and p28 (lane 7) were detected, but again, only p28 was specific for the infected liver. These reactivities were blocked by prior incubation of the antiserum with peptide 142 (lanes 6 and 8). We also detected p28 in three of four additional liver tissues infected with HBV when these were assayed with antiserum to peptide 99 (data not shown).

Proteins p45 and p40 are probably normal cellular proteins that cross-react with the antisera to the X peptides. They appeared in the uninfected liver sample (Fig. 4, lanes 1 and 5) and could also be detected in cell lysates from liver of uninfected chimpanzee, monkey, rabbit, and chicken (data not shown). These proteins are not liver-specific because they appeared in human kidney, pancreas, placenta, and lung (data not shown). In contrast, p28 appeared only in liver infected with HBV (lanes 3 and 7), was recognized by both antisera, and could be detected in the HBV-containing hepatoma cell line PLC/PRF/5 (Fig. 3). Therefore p28 is regarded as an HBV specific protein.

The detection of p28 in human liver tissues infected with HBV suggested that the X protein may act as an immunogen during HBV infection. Therefore, we screened 254 samples of human serum for the presence of circulating antibodies to the X protein, using an enzyme-linked immunosorbent assay (ELISA). Of these samples, 35 were reactive with either one or both of these peptides (Tables 1 and 2). The results indicate that there is a relation between the diagnosis of hepatocellular (HCC) and the appearance of antibody to the X protein.

Two of the positive serum samples, from individuals with HCC, were reacted with BSC-1 cells infected with SVHBV-3 or wild-type SV40 in a Western blot assay (Fig. 5). Lane 1 shows the reactivity of the antiserum to peptide 99 with the SVHBV-3 cell lysates. Both human serum samples were reactive with p24 (lanes 2 and 3), whereas an uninfected, human serum control was nonreactive (lane 4). To confirm that the reactivity detected with the human serum was directed against the X portion of p24 and not to the VP2 sequences, these serum samples were assayed with SV40 infected BSC-1 cells (lanes 5 to 8). No additional bands other than those observed in the control lanes (1 and 5), were detected in the SV40-infected cells. These findings confirmed the presence of circulating antibodies to the X protein in the human sera and further established the antigenic similarity between p24 and the native X protein.

The size of the HBV-encoded X pro-

Table 2. Data for ten of the serum samples that reacted positively in ELISA.

Diagnosis	ELISA value*		Serology		
	With peptide 99	With peptide 142	HBsAg	HBeAg	HBeAb
ASC	0.219	0.202	+	+	−
LC	0.264	0.641	+	−	−
CH	0.350	0.000	+	−	+
CH	0.311	0.000	+	+	−
CH	0.321	0.529	+	+	−
CH	0.226	0.213	+	−	−
HCC	0.312	0.369	+	+	−
HCC	0.000	0.319	+	−	+
HCC	0.141	0.208	+	−	+
HCC	0.206	0.000	+	−	+

*Antisera were tested against microtiter wells coated with 1 μg of peptide 99 or 142. Bound human antibodies were detected by monoclonal antibodies to human immunoglobulin G conjugated to horseradish peroxidase. Results are the average of two experiments (minus background), and values were recorded at $A_{490\ nm}$.

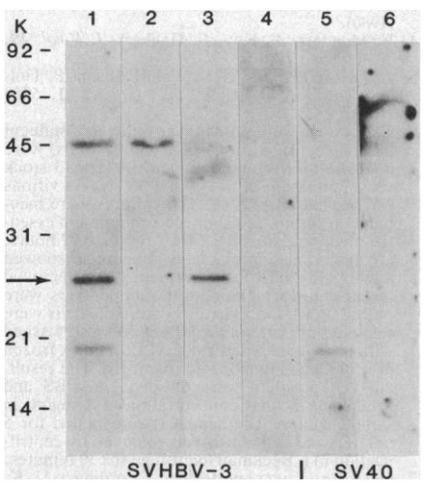


Fig. 2. Reactivity of the antisera to the X peptides with the recombinant SVHBV-3 directed X gene product (*16*). Cell lysate (50 to 100 μg) was loaded onto denaturing polyacrylamide gels (12 percent) and subjected to electrophoresis. The proteins were electrophoretically transferred to nitrocellulose sheets as described (*17*). (Lanes 1 and 3) The strips were reacted with the antisera to peptides 99 and 142, respectively; (lanes 2 and 4) the strips were reacted with antiserum that had been incubated with peptide 99 or 142, respectively; (lanes 5 and 6) the antisera to peptides 99 and 142 were reacted with the control, SV40-infected cell lysates.

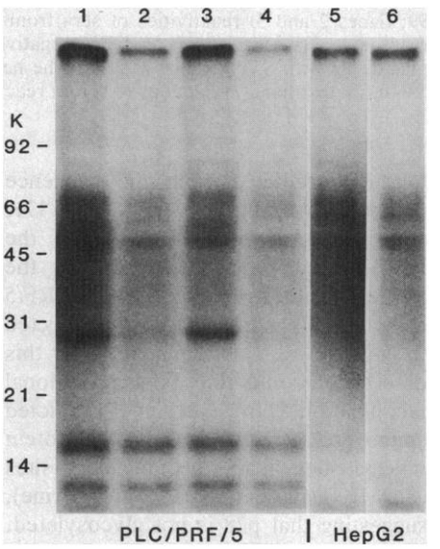
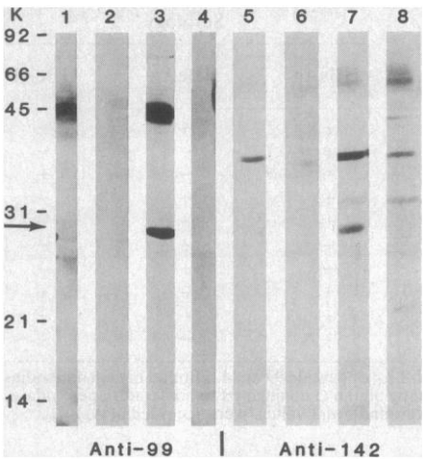


Fig. 3. The reactivity of the antisera to the X peptides with two human hepatomas. PLC/PRF/5 and HepG2 cells were grown in DMEM with 10 percent fetal calf serum to subconfluency (day 6 of a 1:5 dilution from a confluent culture). Cells were collected and lysates prepared as described (*16*). Cell lysate (50 μg) was incubated for 15 minutes in RIPA with 2 percent Trasylol buffer (PBS containing 1 percent noniodet P-40, 0.05 percent sodium deoxycholate and 0.1 percent sodium dodecyl sulfate) and radioactively labeled with 3 mCi of ^{125}I by the chloramine-T reaction. The immunoprecipitations, performed as described (*18*), were as follows: (lane 1) with antiserum to peptide 99; (lane 2) with antibody that had been incubated with peptide 99; (lane 3) with antibody blocked with a nonrelated peptide; (lane 4) with a preimmune serum control. HepG2 cell lysates did not react with the preimmune serum control (lane 5) or with antiserum to peptide 99 (lane 6).



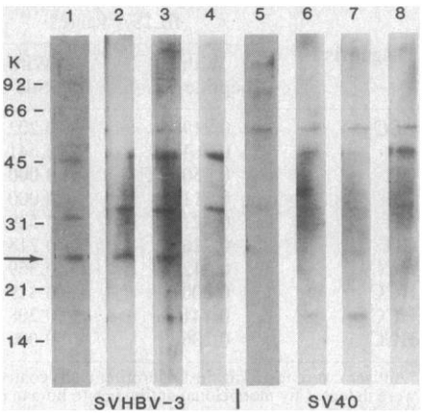


Fig. 4 (left). The reactivity of antiserum to the X peptides with human liver samples. Liver sections from humans were snap frozen in liquid nitrogen and ground into a fine powder with a mortar and pestle. Cell powders were solubilized and boiled in sample buffer, subjected to gel electrophoresis, and blotted as described previously. Nitrocellulose strips were developed as described (*19*). A serologically HBV-negative liver cell lysate is shown in lanes 1, 2, 5, and 6 and a chronic HBV infected liver is shown in lanes 3, 4, 7, and 8. Odd numbered lanes are cell lysates reacted with peptide antibodies and even lanes are reacted with antibodies absorbed with the competing peptide. Lanes 1 to 4 were reacted with antiserum to peptide 99 and lanes 5 to 8 were reacted with antiserum to peptide 142. Fig. 5 (right). The reactivity of human antisera to X protein with recombinant infected cells. Lysates of BSC-1 cells infected with either SVHBV-3 or wild-type SV40 were prepared, separated by electrophoresis, and blotted as described (*17*). Nitrocellulose strips were incubated overnight with a 1:50 dilution of human serum, washed, incubated with a 1:100 dilution of rabbit antiserum to human immunoglobulin G, washed, then developed as described in Fig. 2. (Lane 1) Reactivity of antiserum to peptide 99; (lanes 2 and 3) reactivities of sera from two hepatocellular carcinoma patients; (lane 4) reactivity of a serum from an HBV negative individual; (lane 5) reactivity of antiserum to peptide 99 with SV40; (lanes 6 and 7) the hepatoma serum samples reacted with the control SV40 lysates; (lane 7) the control serum reacted with SV40-infected cells.

tein, as predicted from the sequence reported by Galibert *et al.* (*2*), is 17K (Fig. 1B). However, the size of the HBV-related protein detected by the antisera to the X peptides in PLC/PRF/5 cells, as well as in human HBV-infected tissues is 28K. One explanation for this difference could be posttranslational modification. However, the predicted amino acid sequence of the X protein reveals no available glycosylation sites (that is, asparagine-x-threonine/serine), suggesting that p28 is not glycosylated. Another possibility is that p28 represents the undefined protein that is covalently bound to the 5′ nick in the L strand of the HBV genome (*13*). However, deoxyribonuclease treatment of cell lysates from both PLC/PRF/5 cells and liver samples infected with HBV did not alter the mobility of p28, arguing against its being a DNA-bound protein. The increased size of p28 could be the result of a fusion product between two HBV viral genes or a viral-cellular fusion. Based on genome proximity, an HBs-X or X-HBc fusion is possible [an X-HBc fusion has been found in duck hepatitis B virus (*14*)]. However, p28 did not react with commercial antibody to HBs (Ausria) or HBc (Corab) or rabbit antisera against synthetic peptides representing HBsAg or HBcAg (data not shown). The possibility of an X-cellular fusion remains intriguing. A recent report reveals a conserved HBV-host DNA junction in human hepatomas (*15*). The authors suggest that integration is by way of a specific viral sequence located within the X region. If integration did occur by this process, it would position the X gene next to cellular sequences in all hepatomas.

The detection of circulating antibodies to the X protein in the sera of patients with HBV establishes the X-gene product as an authentic HBV-encoded viral antigen, HBxAg, expressed during an HBV infection. Therefore HBxAg can be used as an additional marker for HBV infection. Antibodies to the X protein were detected predominantly in the sera of patients with HCC. However, that these antibodies are an indicator of HCC remains to be established.

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10. Peptide synthesis was performed as described [J. L. Bittle *et al.*, *Nature (London)* **298**, 30 (1982)]. The peptides were conjugated to keyhole limpet hemocyanin (KLH) and injected into two rabbits each. Both peptides elicited antibodies of significant titers (>1:1280) as measured by ELISA against the peptide antigen (50 pmol per well) [N. Green *et al.*, *Cell* **28**, 477 (1982)].
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16. Cell lysates were prepared as follows. Confluent monolayers of BSC-1 cells (2×10^7 cells) were infected with the recombinant SVHBV-3 stock virus [a mixture of tsA_{239} and SVHBV-3 virions (*7*)] or wild-type SV40. The cultures were incubated in serum-free Dulbecco's minimum essential medium (DMEM) at 40°C for 48 to 72 hours, at which time approximately 50 percent showed a cytopathic effect. Supernatants were removed and the flasks containing the monolayers were placed on ice for 5 minutes before the cells were removed by scraping. Cell pellets were washed with phosphate buffer saline (PBS), quick frozen at −70°C, and lyophilized overnight. The resultant cell powders were dissolved in PBS and brought to a final concentration of 2 mg/ml in sample buffer. The samples were boiled for 5 minutes and cell debris was removed by centrifugation in a Beckman microfuge for 30 minutes.
17. Gel methods were performed according to U. K. Laemmeli [*Nature (London)* **227**, 680 (1970)]. The electroblotting was according to H. Towbin, T. Staehelin, and J. Gordon [*Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979)]. The nitrocellulose blots were incubated at 4°C overnight in Blotto for reduction of nonspecific binding [D. A. Johnson *et al.*, *Gene Analyt. Technol.* **1**, 3 (1984)]. Nitrocellulose strips were incubated for 3 hours at room temperature with a 1:250 dilution of antiserum in a final volume of 10 ml of Blotto. The strips were washed in Blotto prior to a 1-hour incubation with ^{125}I-labeled *Staphylococcus aureus* protein A (10^6 count/min per 1 ml). The strips were washed as above, rinsed with water, dried, and autoradiographed. In cases where blocking with peptide was performed, the antisera were incubated with 100 μg of peptide overnight at 4°C or for 2 hours at 37°C prior to incubation with the nitrocellulose strips. Molecular weights were standardized (Bio-Rad).

18. The radioimmune precipitation assays (RIPA) were done as follows. Radioactively labeled hepatoma lysates 1.5×10^6 count/min of each were reacted with 10 μl of antiserum for 60 minutes. The antigen-antibody complexes were precipitated with Formalin-fixed *S. aureus*. The pellets were washed twice with 500 m*M* LiCl, 100 m*M* tris (*p*H 8.5), and analyzed for radioactivity. Where competition with peptide is indicated, the antiserum was incubated with 100 μg of peptide for 60 minutes prior to the addition of the labeled antigen. All pellets were dissolved in 40 μl of sample buffer, boiled for 3 minutes, centrifuged to remove cell debris, and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis as described in Fig. 2. The gels were dried and autoradiographed.
19. Nitrocellulose strips were developed with a 1:50 dilution of antiserum as described in Fig. 2, with two exceptions: (i) 50 m*M* tris (*p*H 7.6) and 150 m*M* NaCl containing 1 percent bovine serum albumin, 5 percent fetal calf serum, and 0.2 percent Tween 20 was used in place of Blotto in all incubations and washes and (ii) the antigen-bound antibodies were detected with horseradish peroxidase–conjugated goat antibody to rabbit immunoglobulin G, and the complexes were developed with 4-chloro-1-napthol.
20. We are grateful to D. Milich and B. Knowles for providing the PLC/PRF/5 and HepG2 cells. Human liver tissues were generously provided by F. Chisari, A. Yu, and J. Dienstag, and the monoclonal antibodies by P. Kaplan. All HBV-infected serum samples were a gift from K. Mitamura. A special thanks is extended to D. Jones, L. Kaplan, S. Morrell, A. Struthers, and D. Willett for excellent technical assistance and E. Bray for help in preparing the manuscript. This is publication No. 3554-MB from the Research Institute of Scripps Clinic.

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Vaccinia Virus Recombinants: Expression of VSV Genes and Protective Immunization of Mice and Cattle

Abstract. *Vesicular stomatitis virus (VSV) causes a contagious disease of horses, cattle, and pigs. When DNA copies of messenger RNA's for the G or N proteins of VSV were linked to a vaccinia virus promoter and inserted into the vaccinia genome, the recombinants retained infectivity and synthesized VSV polypeptides. After intradermal vaccination with live recombinant virus expressing the G protein, mice produced VSV-neutralizing antibodies and were protected against lethal encephalitis upon intravenous challenge with VSV. In cattle, the degree of protection against intradermalingually injected VSV was correlated with the level of neutralizing antibody produced following vaccination.*

The development of vaccinia virus as an infectious eukaryotic cloning vector (*1–3*) suggests a novel alternative to vaccines derived from whole viruses or viral subunits. Partly because of the role of vaccinia virus as a smallpox vaccine, attention has focused on the construction of recombinant viruses expressing genes of human pathogens (*4–10*). However, the origin of vaccinia virus from cowpox or a related virus and its ability to infect a variety of domesticated animals raise the possibility of veterinary as well as medical uses. Vesicular stomatitis virus (VSV), a member of the rhabdovirus family, causes a contagious disease of horses, cattle, and pigs, characterized by vesicular lesions on the tongue and oral mucosa. Severe outbreaks of the New Jersey serotype of VSV (VSV_{NJ}) have caused substantial economic losses in the United States, Mexico, and parts of Central and South America.

VSV contains a single negative strand of RNA which encodes five messenger RNA's (mRNA's) and five proteins. Complementary DNA copies of mRNA for the G, M, N, and NS proteins of VSV have been cloned and sequenced (*11–13*), and the G and N genes of the Indiana serotype (VSV_I) have been expressed in eukaryotic cells (*14, 15*). To facilitate cloning of the G and N genes into vaccinia virus, a previously used plasmid vector, pGS20 (*3*), was modified (*16*) to contain a single Xho I site downstream of a promoter region that was isolated from a vaccinia virus gene encoding a 7500-dalton (7.5-kD) protein. Into this plasmid, designated pMM34, we placed the G or N gene from VSV_I so that the vaccinia virus transcriptional and VSV translational start sites were juxtaposed. The resulting chimeric gene was flanked by segments of the vaccinia virus thymidine kinase (TK) gene. The G protein gene from VSV_{NJ} was modified (*17*) so that it also could be inserted into the Xho I site of pMM34 as well as into the single Sal I site of another plasmid vector, pLTP1, which contains the promoter region of a vaccinia virus gene encoding a protein of 28 kD (*18*).

Vaccinia virus hybrids containing the chimeric genes were formed by homologous recombination, selected on the basis of their TK^- phenotype and identified by hybridization to a ^{32}P-labeled

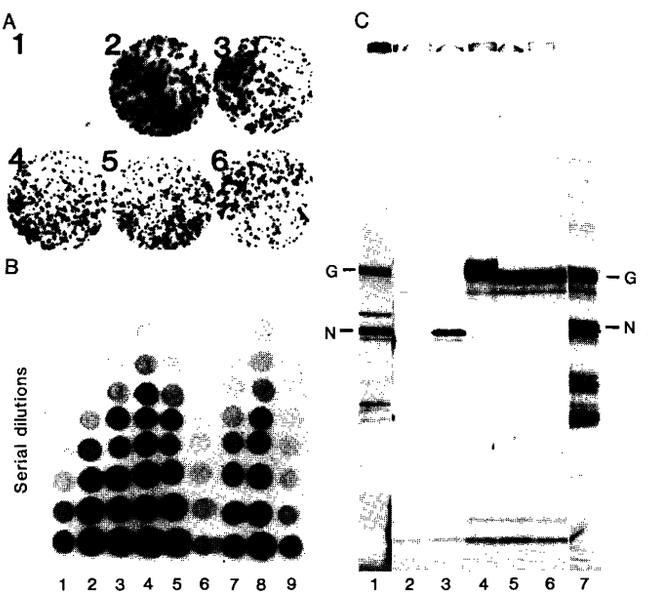

Fig. 1. (A) Antibody binding to recombinant virus plaques. Duplicate monolayers of CV-1 cells contained plaques produced by wild-type vaccinia virus (dishes 1 and 4), recombinant virus v50 (dishes 2 and 5) or v51 (dishes 3 and 6). Binding of antiserum to VSV_{NJ} (dishes 1 to 3) or vaccinia virus (dishes 4 to 6) was followed by incubation with ^{125}I-labeled staphylococcal A protein (*9*). Autoradiographs are shown. (B) Kinetics of expression of chimeric genes. Monolayers of CV-1 cells were infected with 30 PFU per cell of wild-type vaccinia virus or recombinants v50 or v51 in the presence or absence of arabinosylcytosine (Ara-C) at a concentration of 40 μg/ml. Serial twofold dilutions of cytoplasmic extracts at varying times after infection were spotted on nitrocellulose paper and were then incubated with antiserum to VSV_{NJ}, washed, and incubated with ^{125}I-labeled staphylococcal A protein. An autoradiograph is shown. Series 1, wild-type vaccinia virus, 6 hours; series 2, v50, 2 hours; series 3, v50, 6 hours; series 4, v50, 12 hours; series 5, v50, 12 hours, Ara-C; series 6, v51, 2 hours; series 7, v51, 6 hours; series 8, v51, 12 hours; series 9, v51, 12 hours, Ara-C. (C) Characterization of VSV polypeptides made by vaccinia virus recombinants. Monolayers (25 cm^2) of CV-1 cells were infected with 30 PFU per cell of wild-type or recombinant vaccinia virus or with VSV_I or VSV_{NJ} in medium containing 0.01 m*M* methionine. After 2 hours at 37°C, 100 μCi of [^{35}S]methionine (1000 Ci/mmol) was added and the incubation was continued for an additional 10 hours. The preparation of cytoplasmic extracts, immunoprecipitation, and polyacrylamide gel electrophoresis was similar to that described (*4*). Antiserum to VSV_{NJ} was used to precipitate polypeptides from cells infected with VSV_{NJ}, v50 and v51 whereas antiserum to VSV_I was used for polypeptides from cells infected with VSV_I, wild-type vaccinia virus, v37 and v38. Series 1, VSV_I; series 2, wild-type vaccinia virus; series 3, v38; series 4, v37; series 5, v50; series 6, v51; series 7, VSV_{NJ}.