Activation of Class I Major Histocompatibility Complex Gene Expression by Hepatitis B Virus

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Normal hepatocytes express very few class I major histocompatibility complex (MHC I) molecules, but MHC I expression is elevated in hepatitis B virus (HBV) infection. We report here that hepatoblastoma cells with replicating HBV genomes express three- to fourfold-higher levels of MHC I protein and mRNA than do parent cells without HBV DNA. Transient transfection assays demonstrated that the HBV X protein *trans* activated transcription from an MHC I promoter and allowed identification of *cis* elements important for *trans* activation.

Hepatitis B virus (HBV) is a major cause of acute and chronic hepatitis worldwide (7, 11). The hepatocyte damage in HBV infection is not a direct viral cytopathic effect but is thought, rather, to result from the host T-lymphocyte response to viral antigens in conjunction with host class I major histocompatibility complex (MHC I) molecules (5, 7, 9, 11, 20). The MHC I molecules of mammals are cell surface glycoproteins composed of a polymorphic heavy chain and an invariant light chain (15). These molecules play a key role in the recognition and destruction of virus-infected cells by cytotoxic T lymphocytes of the host immune system, since the T cells recognize viral antigens only in conjunction with a histocompatible MHC I molecule. Normal hepatocytes express few MHC I molecules (<10% of spleen cells) (14, 22), but there is elevated expression during HBV-associated hepatitis (7, 11, 21, 23). This increased expression is potentially important in initiating or augmenting the killing of infected hepatocytes by cytotoxic T cells, since the level of expression of MHC I molecules on virus-infected cell surfaces correlates well with the degree of effectiveness with which they are destroyed by T lymphocytes (37).

The basis for increased MHC I expression during HBV infection is unclear. Presumably, some of the effect can be attributed to infiltrating T cells that secrete interferons, which are known to elevate MHC I expression (1, 10, 25, 35). However, it is also possible that HBV can directly increase MHC I expression. To examine this possibility, we utilized HepG2 cells, which are well-differentiated hepatoblastoma cells free of HBV DNA (17), and UP7-4 cells, which are HepG2 cells stably transfected with a dimer of HBV genome and which secrete viral and subviral particles (C. H. Shih, personal communication). These cells were stained with a monoclonal antibody against human MHC I heavy-chain molecules (Dako) and with fluoresceinated rabbit anti-mouse antibodies (13) and were analyzed on a Becton Dickinson FACScan IV flow cytometer. The amount of cell surface MHC I expression on UP7-4 was approximately four times the amount on the HepG2 cells (Fig. 1). Similar results were obtained with clone 2.2.15 (27), which is an independently derived clone of HepG2 with replicating HBV DNA (data not shown). A Northern (RNA) blot of total RNA from HepG2 and UP7-4 cells, probed with an MHC I

heavy-chain cDNA (HLA-B7, courtesy of P. Parham) (31), revealed a threefold increase in the levels of mRNA for the MHC I molecules (Fig. 2A), showing that the increased cell surface expression was correlated with increased steady-state mRNA levels. These results demonstrated that the presence of the HBV genome was associated with increased expression of MHC I genes in the absence of T lymphocytes.

Since interferons are known to increase the transcription of MHC I genes (1, 10, 18, 25, 35), it was possible that HBV was indirectly increasing MHC I expression by an autocrine mechanism, i.e., by increasing expression of beta interferon or interferon receptors or both by the infected host cell. However, two independent lines of evidence militate against this possibility. First, no beta interferon mRNA was detectable in UP7-4 cells by Northern blotting, even after prolonged exposure (data not shown). Second, the mRNA levels of 2'-5'-oligoadenylate synthetase are similarly low in HepG2 and UP7-4 cells (Fig. 2B), as revealed by Northern

FIG. 1. Flow cytometric analysis of cell surface MHC I expression by hepatoblastoma cells with (UP7-4) or without (HepG2) HBV. HepG2 cells with or without replicating HBV were stained for cell surface MHC I heavy-chain molecules (13) and analyzed on a Becton Dickinson FACScan IV.

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FIG. 2. Northern blotting of MHC I heavy-chain (A) and 2'-5'-oligoadenylate synthetase (B) RNA from cells with (UP7-4) or without (HepG2) replicating HBV. Total cellular RNA (20 μ g) was purified, electrophoresed on denaturing gels, and subjected to Northern blotting with the appropriate cDNA probes (3). (A) Band intensities were quantitated with densitometer scanning; (B) the autoradiogram was deliberately overexposed to show the bands in untreated HepG2 and UP7-4 cells. Lanes + IFN, Cells were treated with 200 U of recombinant human gamma interferon (Collaborative Research, Inc.) per ml 20 h before being harvested.

blotting with a cDNA probe (courtesy of M. Revel) (2). Since this is a gene that is highly inducible by all interferons (2) (Fig. 2B), HBV must increase the mRNA levels of MHC I genes independently of any interferon effect.

It has been shown that the HBV X protein (pX) *trans* activates transcription from many disparate promoters (8, 28, 30, 32, 33, 36) and that the X gene is both transcribed and translated in HepG2 cells with replicating HBV genomes (6;

TABLE 1. Effect of HBV gene products on MHC I promoter activity^a

HBV gene expressed	Relative promoter activity
None.....	1.0
X.....	3.9 $\pm$ 0.3
Surface.....	1.1 $\pm$ 0.3
Precore.....	0.7 $\pm$ 0.2
Polymerase	1.5 $\pm$ 0.2

^a HepG2 cells were cotransfected with a plasmid expressing each of the indicated HBV genes (28) and with pP2CAT, a CAT reporter plasmid under the control of the promoter region of the murine MHC I gene H2K^b (34). On day 3, the cells were harvested and the CAT activity in cell lysates was measured (28) and normalized to the activity in the presence of only the expression vector, which was designated as 1.0. The results are the average of three to five independent transfections.

W. Guo, J. Wang, G. Tan, T. S. B. Yen, and J.-H. Ou, unpublished data). Therefore, pX may also *trans* activate MHC I genes in these cells. To examine this possibility, we performed CaCl₂-mediated transient transfections into HepG2 cells with 5 μ g each of a plasmid expressing one of the known genes of HBV (28) and of pP2CAT (originally named pK^bP2NruCAT, courtesy of D. Faller), a reporter plasmid with the chloramphenicol acetyltransferase (CAT) gene under the control of a murine MHC I promoter with ~1 kilobase pair of upstream sequences (34) (Fig. 3). Cotransfection of the X gene, but no other HBV genes, resulted in an approximately fourfold increase in CAT expression from the MHC I promoter (Table 1). The *trans*-activating factor must be the X protein, rather than X RNA or DNA, since *trans* activation was lost when a frameshift mutation was introduced into the X gene (data not shown). Because this level of *trans* activation fully accounts for the increased MHC I mRNA levels in the cell lines with replicating HBV, pX most likely represents the sole factor in these cells which is activating MHC I transcription.

To map the *cis* elements within the MHC I promoter responsive to pX, we deleted the regions upstream of either the *Hae*III or *Hinc*II sites in the MHC I promoter by restriction endonuclease digestion (Fig. 3) and performed

FIG. 3. *cis*-Element requirements for *trans* activation of MHC I by pX. The thin line shows selected restriction sites of the upstream region of the murine MHC I gene H2K^b (34). The thick lines indicate the regions present in the CAT reporter plasmids used. The sequence in the H2TF1/NF- κ B and interferon-responsive element (IRE) regions (16, 18) is shown at the bottom, as are the two sets of mutations introduced into the plasmid pH3CAT. HepG2 cells were cotransfected with the indicated reporter plasmid as well as a plasmid expressing pX (28). The CAT activity was measured (28) and normalized to the activity in the presence of the parent expression vector without pX. Numbers are means $\pm$ standard deviations of three to six independent transfections.

cotransfection studies with pX. A promoter with only ~300 base pairs of upstream sequences (pH3CAT) was *trans* activated to an even higher level than the parent plasmid was, possibly because of loss of a negative element (Fig. 3B). On the other hand, a promoter with ~100 base pairs (pHc2CAT) was not *trans* activated. Therefore, the region between 100 and 300 base pairs upstream of the mRNA start site must be critical for *trans* activation by pX. This region contains two elements conserved in human and murine MHC I genes (1, 12, 16, 18, 29): the interferon-responsive element and a sequence motif recognized by both H2TF1 and NF- κ B, two cellular transcription factors (Fig. 3A). It seemed likely that pX was acting through the H2TF1/NF- κ B motif, since the *trans* activation of human immunodeficiency virus in HepG2 cells has been shown to be dependent on NF- κ B motifs (30, 33). Therefore, we introduced multiple point mutations into this motif in the plasmid pH3CAT by oligonucleotide-directed mutagenesis (Bio-Rad Laboratories) (19) and examined the effect on *trans* activation by pX. These mutations (pH3/EAM/CAT) destroyed the ability of the promoter to be *trans* activated (Fig. 3). However, clustered mutations in the interferon-responsive element (pH3/IREM/CAT) also rendered the promoter unresponsive to pX. This is reminiscent of the *cis* element requirement for induction by interferons: both the interferon-responsive element and the H2TF1/NF- κ B motif are needed for transcriptional activation (16). Therefore, there must be complex interactions between the transcription factors that bind to these two sites.

In summary, our data show that HBV pX can *trans* activate transcription of MHC I genes approximately fourfold in cultured cells. Many different viruses are known to increase expression of MHC I genes; they include adenoviruses, papovaviruses, retroviruses, and reoviruses (4, 24, 26, 34). To this list can now be added HBV. In the case of the other viruses, it is not clear what effect, if any, this action has on disease pathogenesis, since most host cells already express MHC I molecules. However, normal hepatocytes express relatively few MHC I molecules (14, 22). Thus, HBV *trans* activation of MHC I genes in infected hepatocytes can potentially facilitate cytotoxic T-cell recognition of these cells and thus initiate or contribute to the inflammation and necrosis seen in hepatitis B. Therefore, blocking the action of pX in infected cells may provide a novel means of treating HBV-associated hepatitis.

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LITERATURE CITED

- Baldwin, A. S., and P. A. Sharp. 1988. Two transcription factors, NF- κ B and H2TF1, interact with a single regulatory sequence in the class I major histocompatibility complex promoter. *Proc. Natl. Acad. Sci. USA* 85:723-727.
- Benech, P., Y. Mory, M. Revel, and J. Chebath. 1985. Structure of two forms of the interferon-induced (2'-5')oligo A synthetase of human cells based on cDNAs and gene sequences. *EMBO J.* 4:2249-2256.
- Berger, S. L., and A. R. Kimmel (ed.). 1987. Guide to molecular cloning techniques. Academic Press, Orlando, Fla.
- Campbell, I. L., L. C. Harrison, R. Ashcroft, and I. Jack. 1988. Reovirus infection enhances expression of class I MHC proteins on human β -cell and rat RINm5F cell. *Diabetes* 37:362-365.
- Casini, A., A. Alberti, E. Banchetti, E. Schiavon, V. Patussi, P. Pontisso, C. Surrenti, and G. Realdi. 1987. Cell mediated immunity to HBcAg in chronic HBV infection. *Am. J. Clin. Pathol.* 88:494-498.
- Chisaka, O., K. Araki, T. Ochiya, T. Tsurimoto, W. Hiranyawasitte-Attatippaholkun, N. Yanaiharu, and K. Matsubara. 1987. Purification of hepatitis B virus gene X product synthesized in *Escherichia coli* and its detection in a human hepatoblastoma cell line producing hepatitis B virus. *Gene* 60:183-189.
- Chisari, F. V., C. Ferrari, and M. V. Mondelli. 1989. Hepatitis B virus structure and biology. *Microb. Pathog.* 6:311-325.
- Colgrove, R., G. Simon, and D. Ganem. 1989. Transcriptional activation of homologous and heterologous genes by the hepatitis B virus X gene product in cells permissive for viral replication. *J. Virol.* 63:4019-4026.
- Ferrari, C., A. Penna, T. Guiberti, M. J. Tong, E. Ribera, F. Fiaccadori, and F. V. Chisari. 1987. Intrahepatic, nucleocapsid antigen-specific T cells in chronic active hepatitis B. *J. Immunol.* 139:2050-2058.
- Friedman, R. L., S. P. Manley, M. McMahon, I. M. Kerr, and G. R. Stark. 1984. Transcriptional and posttranscriptional regulation of interferon-induced gene expression in human cells. *Cell* 38:745-755.
- Gerber, A. M., and S. N. Thung. 1985. Molecular and cellular pathology of hepatitis B. *Lab. Invest.* 52:572-590.
- Hakem, R., P. LeBouteiller, M. Barad, M. Trujillo, P. Mercier, J. Wietzerbin, and F. A. Lemonnier. 1989. IFN-mediated differential regulation of the expression of HLA-B7 and HLA-A3 class I genes. *J. Immunol.* 142:297-305.
- Harlow, E., and D. Lane. 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Harris, H., and T. Gill. 1986. Expression of class I transplantation antigens. *Transplantation* 42:109-117.
- Hood, L., M. Steinmetz, and B. Malissen. 1983. Genes of the major histocompatibility complex of the mouse. *Annu. Rev. Immunol.* 1:529-568.
- Israel, A., A. Kimura, A. Fournier, M. Fellous, and P. Kourilsky. 1986. Interferon response sequence potentiates activity of an enhancer in the promoter region of a mouse H-2 gene. *Nature (London)* 322:743-746.
- Knowles, B. B., C. C. Howe, and D. P. Aden. 1980. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science* 209:497-499.
- Korber, B., N. Mermod, L. Hood, and I. Stroynowski. 1988. Regulation of gene expression by interferons: control of H-2 promoter responses. *Science* 239:1302-1306.
- Kunkel, T. K. 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci. USA* 82:488-492.
- Mondelli, M., G. M. Vergani, A. Alberti, D. Vergani, B. Portmann, A. L. W. F. Eddleston, and R. Williams. 1982. Specificity of T lymphocyte cytotoxicity to autologous hepatocytes in chronic hepatitis B virus infection: evidence that T cells are directed against HBV core antigen expressed on hepatocytes. *J. Immunol.* 129:2773-2778.
- Montaño, L., G. C. Miescher, A. H. Goodall, K. H. Wiedmann, G. Janossy, and H. C. Thomas. 1982. Hepatitis B virus and HLA antigen display in the liver during chronic hepatitis B virus infection. *Hepatology* 2:557-561.
- Natali, P. G., A. Bigotti, M. R. Nicotra, M. Viora, D. Manfredi, and S. Ferrone. 1984. Distribution of human class I (HLA-A, B, C) histocompatibility antigens in normal and malignant tissues of nonlymphoid origin. *Cancer Res.* 44:4679-4687.
- Paz, M. O. A., F. Brenes, P. Karayiannis, T. P. Jowett, P. J. Scheuer, and H. C. Thomas. 1986. Chronic hepatitis B virus infection. *J. Hepatol.* 3:371-377.
- Rigby, P. W. J., N. B. LaThangue, D. Murphy, and B. I. Skene. 1985. The regulation of cellular transcription by simian virus 40 large T-antigen. *Proc. R. Soc. London B* 226:15-23.
- Rosa, F., and M. Fellous. 1984. The effect of gamma-interferon on MHC antigens. *Immunol. Today* 5:261-263.

26. Rosenthal, A., S. Wright, K. Quade, P. Gallimore, H. Cedar, and F. Grosveld. 1985. Increased MHC H-2K gene transcription in cultured mouse embryo cells after adenovirus infection. *Nature (London)* **315**:579-581.
27. Sells, M. A., M. L. Chen, and G. Acs. 1987. Production of hepatitis B virus particles in HepG2 cells transfected with cloned hepatitis B virus DNA. *Proc. Natl. Acad. Sci. USA* **84**:1005-1009.
28. Seto, E., T. S. B. Yen, B. M. Peterlin, and J. H. Ou. 1988. Trans-activation of human immunodeficiency virus long-terminal repeat by hepatitis B virus X protein. *Proc. Natl. Acad. Sci. USA* **85**:8286-8290.
29. Shirayoshi, Y., P. A. Burke, E. Appella, and K. Ozato. 1988. Interferon-induced transcription of a major histocompatibility class I gene accompanies binding of inducible nuclear factors to the interferon consensus sequence. *Proc. Natl. Acad. Sci. USA* **85**:5884-5888.
30. Siddiqui, A., R. Gaynor, A. Srinivasan, J. Mapoles, and R. W. Farr. 1989. Trans-activation of viral enhancers including long terminal repeat of the human immunodeficiency virus by the hepatitis B virus X protein. *Virology* **169**:479-484.
31. Sood, A. K., J. Pan, P. A. Biro, D. Pereira, R. Smetava, V. B. Reddy, B. W. Duceaman, and S. M. Weissman. 1985. Structure and polymorphism of class I MHC antigen mRNA. *Immunogenetics* **22**:101-121.
32. Spandau, D. F., and C.-H. Lee. 1988. *trans*-Activation of viral enhancers by the hepatitis B virus X protein. *J. Virol.* **62**: 427-434.
33. Twu, J.-S., C. A. Rosen, W. A. Haseltine, and W. S. Robinson. 1989. Identification of a region within the human immunodeficiency virus type 1 long terminal repeat that is essential for transactivation by the hepatitis B virus gene X. *J. Virol.* **63**:2857-2860.
34. Wilson, L. D., D. C. Flyer, and D. V. Faller. 1987. Murine retroviruses control class I major histocompatibility antigen gene expression via a *trans* effect at the transcriptional level. *Mol. Cell. Biol.* **7**:2406-2415.
35. Yoshie, O., H. Schmidt, E. S. P. Reddy, S. Weissman, and P. Lengyel. 1982. Mouse interferons enhance the accumulation of a human HLA RNA and protein in transfected mouse and hamster cells. *J. Biol. Chem.* **257**:13169-13172.
36. Zahm, P., P. H. Hofschneider, and R. Koshy. 1988. The HBV X-ORF encodes a transactivator: a potential factor in viral hepatocarcinogenesis. *Oncogene* **3**:169-177.
37. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. *Adv. Immunol.* **27**: 51-177.