Effects of Hepatitis B Virus Infection on the Interferon Response in Immunodeficient Human Hepatocyte Chimeric Mice

Masataka Tsuge,1,2,3 Shoichi Takahashi,1,3 Nobuhiro Hiraga,1,3 Yoshifumi Fujimoto,1,4 Yizhou Zhang,1,3 Fukiko Mitsuji,1,3 Hiromi Abe,1,4 Tomokazu Kawaoke,1,3 Michio Imamura,1,3 Hidenori Ochi,1,3,4 C. Nelson Hayes,1,3 and Kazuaki Chayama1,3,4

1Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, and 2Live Science Division, Natural Science Center for Basic Research and Development, and 3Liver Research Project Center, Hiroshima University; 4Laboratory for Liver Diseases, the RIKEN Center for Genomic Medicine, Hiroshima, Japan

Complementary DNA microarray analysis of human livers cannot exclude the influence of the immunological response. In this study, complementary DNA microarray analysis was performed under immunodeficient conditions with human hepatocyte chimeric mice, and gene expression profiles were analyzed by hepatitis B virus (HBV) infection and/or interferon treatment. The expression levels of 183 of 525 genes upregulated by interferon treatment were significantly suppressed in response to HBV infection. Suppressed genes were statistically significantly associated with the interferon signaling pathway and pattern recognition receptors in the bacteria/virus recognition pathway \((P = 1.0 \times 10^{-8} \text{ and } P = 1.2 \times 10^{-8}, \text{ respectively})\). HBV infection attenuated virus recognition and interferon response in hepatocytes, which facilitated HBV escape from innate immunity.

Chronic hepatitis B virus (HBV) infection is associated with the development of virus-related liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Interferon \(\alpha\) (IFN-\(\alpha\)) has been used for the treatment of chronic hepatitis B, and many large clinical trials and meta-analyses have demonstrated the effectiveness of interferon \([1–3]\). However, the effect of IFN-\(\alpha\) therapy is unsatisfactory, and the molecular basis for tolerance to IFN-\(\alpha\) is not clearly defined.

DNA microarray technology has enabled genome-wide analysis of gene transcript levels with the use of clinical tissues and animal models, which has yielded insights into the molecular features of several liver diseases \([4–6]\). However, it has been difficult to determine whether the changes in gene expression were caused by viral interference or by the human immune response, because all of these studies that used clinical and experimental samples were analyzed under the influence of adaptive immune responses. Recently, Mercer and colleagues developed a human hepatocyte chimeric mouse model \([7]\). These mice were derived from severe combined immunodeficiency (SCID) mice, which are severely immunocompromised, and the mouse liver cells were extensively replaced with human hepatocytes \([7, 8]\). With the use of this chimeric mouse model, in which HBV can continuously infect human hepatocytes, the effect of drugs and the response of viral infection can be analyzed in human hepatocytes under immunodeficient conditions \([9]\).

In this study, we performed microarray analysis with human hepatocyte chimeric mouse livers to assess the direct impacts of HBV infection and IFN treatments on gene expression profiles. We successfully demonstrated that HBV infection attenuated the expression of IFN-stimulating genes under immunodeficient conditions, which suggests that HBV proteins might afford escape mechanisms from cellular innate immunity.

METHODS

A serum sample was obtained from a HBV carrier after obtaining written informed consent for the donation and evaluation of the blood sample. The inoculum was positive for Hepatitis B surface and Hepatitis B e antigens, with slightly elevated levels of serum alanine aminotransferase and high-level viremia (HBV DNA load, 7.1 log copies/mL). The studied patient was infected with HBV genotype C. The experimental protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Hiroshima University Hospital ethical committee (approval ID: D08-9).

The \(\text{uPA}^{+/+}/\text{SCID}^{+/+}\) mice were prepared and the human hepatocytes were transplanted as described elsewhere \([8]\). The experiments were performed in accordance with the guidelines of the local committee for animal experiments at Hiroshima University.

Sixteen chimeric mice, in which \(>90\%\) of the liver tissue was replaced with human hepatocytes, were divided into
RESULTS

To analyze the direct effects of IFN in human hepatocytes, we compared the gene expression profiles between groups A (mice without HBV infection) and B (mice with IFN treatment). Of the 1,714 genes that remained after screening with the Welch T test, 685 genes showed a >3.0-fold change between groups. Of these 685 genes, 525 genes were up-regulated and the other 160 genes down-regulated by IFN. The top 20 IFN-regulated genes are listed in Supplementary data table 6. GO analysis revealed that 8 (40%) of the top 20 genes that were upregulated with IFN treatment were related to immune response.

To analyze the effect of HBV infection in human hepatocytes, we compared the gene expression profiles between groups A (mice without HBV infection) and C (mice with HBV infection). Among the 1,714 genes that remained after screening, 373 genes showed a >3.0-fold change between groups. Of these 373 genes, 159 genes were up-regulated and the other 214 genes down-regulated by HBV. The top 20 HBV-regulated genes are listed in Supplementary data table 7. Several oncogenic genes such as growth differentiation factor 15 and glial cell derived neurotrophic factor were included in the top group. Most of the top 20 genes that were downregulated with HBV infection were associated with transcriptional regulation.

To examine whether HBV infection may alter the effect of IFN response in human hepatocytes, we compared gene expression profiles among all groups. As mentioned above, 525 genes were upregulated by >3.0-fold by IFN in the absence of HBV infection. A comparison of groups C (mice with HBV infection but no IFN treatment) and D (mice with both HBV infection and IFN treatment) revealed that 183 (34.9%) of the 525 genes showed statistically significantly reduced IFN response with HBV infection (P < 0.01) (Supplementary data 8A). The top 20 genes in which IFN response was significantly changed by HBV infection are shown in Table 1. The mRNA expression levels of 11 selected genes among the 183 genes with reduced IFN response were also analyzed by real-time PCR, and the reductions in IFN response by HBV infection were verified (Supplementary data 8B). Additionally, we used IPA software to analyze the influence of HBV infection on the IFN response of these 183 genes by means of a pathway-oriented approach. Pathway analysis revealed that several pathways were affected by HBV infection (Table 2). The IFN response was statistically significantly attenuated by HBV infection in the pathways related to IFN signaling and pattern recognition of bacteria and viruses (P = 1.0 × 10⁻⁸ and P = 1.2 × 10⁻⁸, respectively).

DISCUSSION

Elsewhere we have demonstrated a human hepatocyte chimeric mouse model that can be chronically infected with hepatitis B and C viruses [9–11]. This mouse model facilitates analysis of the effect of viral infection and the response to medication under immunodeficient conditions. In this study, we performed complementary DNA microarray analysis using the chimeric mouse model and obtained gene expression profiles to analyze
the direct influence of HBV infection and IFN-α treatment on human hepatocytes.

To avoid contamination with mouse tissue, human hepatocyte chimeric mice, in which liver tissue is largely (>90%) replaced by human hepatocytes, were used in the present study. However, a small amount of mouse-derived cells, such as interstitial cells, bile duct cells, and vascular cells, still remain in the chimeric mouse livers. Because of high homology between the human and mouse genomes, the signals from microarray analyses may be influenced by cross-hybridization with mouse mRNA. It is difficult to produce uPA<sup>+/−</sup>/SCID<sup>+/−</sup> mice >10 weeks old without hepatocyte transplantation, and a previous study demonstrated that it is feasible to use microarray analysis in a functional genomics analysis of chimeric mice [12]. Therefore, to compensate for the contamination, the mice in group A, which were neither infected with HBV nor treated with IFN, were used as negative controls.

To analyze the effect of IFN treatment, we compared gene expression profiles between groups A (mice without IFN treatment) and B (mice with IFN treatment); 525 genes with >3.0-fold upregulation following IFN treatment were observed. Among them, chemokine (C-X-C motif) ligand 9, chemokine (C-X-C motif) ligand 10, and chemokine (C-X-C motif) ligand 11, which promote T cell adhesion, were remarkably highly induced with IFN treatment (Supplementary data table 6) [13]. These results suggest that the antiviral effects of IFN might involve not only direct activation of IFN-stimulated proteins such as myxovirus resistance protein A and double strand RNA-dependent protein kinase but also activation of immunity via chemokines.

Second, we compared the profiles between groups A (mice without HBV infection) and C (mice with HBV infection). As shown in Supplementary data table 7, more than half (12) of the top 20 genes upregulated by HBV infection localized to the cell membrane or the extracellular region, but 14 (70%) of the 20 downregulated genes localized to the nucleus. In addition, GO analysis demonstrated that genes related to cell cycle and DNA modification were affected by HBV infection. We speculate that HBV infection promotes cell growth and DNA damage in the hepatocyte nucleus and activates the immune response in the cytoplasm. From the clinical standpoint, some healthy HBV carriers develop hepatocellular carcinoma without chronic hepatitis or cirrhosis. The present results strongly support this observation, showing that most of the affected genes are known to be associated with carcinogenesis.

Clinically, HBV is known to develop tolerance to IFN treatment in patients with chronic hepatitis B, although the mechanism is not clear. We analyzed the IFN response with and
Table 2. Pathway Analysis of 183 Interferon-Induced Genes With Interferon Responsiveness Downregulated by Hepatitis B Virus Infection

<table>
<thead>
<tr>
<th>Canonical pathways</th>
<th>( P )</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon signaling</td>
<td>1.00 \times 10^{-8}</td>
<td>IFIT3, SOCS1, IFIT1, MX1, IFNAR1, JAK2, STAT1, TAP1, IRF1</td>
</tr>
<tr>
<td>Role of pattern recognition receptors in recognition of bacteria and viruses</td>
<td>1.20 \times 10^{-8}</td>
<td>IL12A, OAS2, OAS3 (includes EG:4940), IFIH1, PIK3R3, TLR4, NOG, TICAM1, DDX58, CASP1, TOD, JAK2, RIPK2</td>
</tr>
<tr>
<td>Type 1 diabetes mellitus signaling</td>
<td>2.00 \times 10^{-4}</td>
<td>SOCS1, IL12A, RIPK1, GAD1, SOCS6, SOCS2, IFNAR1, JAK2, STAT1, IRF1</td>
</tr>
<tr>
<td>Prolactin signaling</td>
<td>2.70 \times 10^{-4}</td>
<td>PIK3R3, SOCS1, SOCS6, SOCS2, NMI, JAK2, STAT1, IRF1</td>
</tr>
<tr>
<td>TREM1 signaling</td>
<td>3.50 \times 10^{-4}</td>
<td>TLR4, NOG, ICAM1, CASP1, JAK2, TLR3, CASP5</td>
</tr>
<tr>
<td>Production of nitric oxide and reactive oxygen species in macrophages</td>
<td>3.90 \times 10^{-4}</td>
<td>PIK3R3, TLR4, RND3, PTPR2A, PM141, RHOU, IFNAR1, MAP3K8, IFN8, JAK2, STAT1, IRF1</td>
</tr>
<tr>
<td>Pathogenesis of multiple sclerosis</td>
<td>1.10 \times 10^{-3}</td>
<td>CXCL10, CXCL9, CXCL11</td>
</tr>
<tr>
<td>Activation of IRF by cytosolic pattern recognition receptors</td>
<td>2.60 \times 10^{-3}</td>
<td>IFIH1, RIPK1, DDX58, STAT1, IFIT2, ISG15</td>
</tr>
<tr>
<td>Dendritic cell maturation</td>
<td>2.60 \times 10^{-3}</td>
<td>B2M, PIK3R3, TLR4, ICAM1, IL12A, IL1RN, IFN8, JAK2, TLR3, STAT1</td>
</tr>
<tr>
<td>Interleukin 12 signaling and production in macrophages</td>
<td>3.60 \times 10^{-3}</td>
<td>PIK3R3, TLR4, IL12A, IFNAR1, MAP3K8, IFN8, JAK2, STAT1, IRF1</td>
</tr>
<tr>
<td>Sphingosine-1-phosphate signaling</td>
<td>3.60 \times 10^{-3}</td>
<td>PIK3R3, S1PR2, RND3, CASP1, RHOU, CASP4, CASP5, CASP7, CASP6</td>
</tr>
<tr>
<td>JAK-STAT signaling</td>
<td>4.00 \times 10^{-3}</td>
<td>PIK3R3, SOCS1, SOCS6, SOCS2, JAK2, STAT1</td>
</tr>
<tr>
<td>Growth hormone signaling</td>
<td>4.70 \times 10^{-3}</td>
<td>PIK3R3, SOCS1, SOCS6, SOCS2, JAK2, STAT1</td>
</tr>
<tr>
<td>Retinoic acid mediated apoptosis signaling</td>
<td>8.50 \times 10^{-3}</td>
<td>TNFRSF10B, PARP8, TNFRSF10, TIPARP, IRF1</td>
</tr>
</tbody>
</table>

**NOTE.** B2M, beta-2-microglobulin; CASP1, caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase); CASP4, caspase 4, apoptosis-related cysteine peptidase; CASP5, caspase 5, apoptosis-related cysteine peptidase; CASP7, caspase 7, apoptosis-related cysteine peptidase; CXCL9, chemokine (C-X-C motif) ligand 9; CXCL10, chemokine (C-X-C motif) ligand 10; CXCL11, chemokine (C-X-C motif) ligand 11; DDX58, DEAD (Asp-Glu-Ala-Asp) box polypeptide 5B; GAD1, glutamate decarboxylase 1 (brain, 67kDa); ICAM1, intercellular adhesion molecule 1; IFIH1, interferon induced with helicase C domain 1; IRF1, interferon-induced protein with tetratricopeptide repeats 1; IRF2, interferon-induced protein with tetratricopeptide repeats 2; IFIT1, interferon-induced protein with tetratricopeptide repeats 3; IFNAR1, interferon gamma receptor 1; IL1RN, interleukin 1 receptor antagonist; IL12A, interleukin 12A (natural killer cell stimulatory factor 1), cytoplasmic lymphocyte maturation factor 1, p35; IRF, interferon regulatory factor; IRF1, interferon regulatory factor 1; IRF6, interferon regulatory factor 8; ISG15, ISG15 ubiquitin-like modifier; JAK2, Janus kinase 2; MAP3K8, mitogen-activated protein kinase kinase kinase 8; MX1, myxovirus (influenza virus) resistance 1, interferon inducible protein p78 (mouse); NDN, N-myc (and STAT) interactor; NOD1, nucleotide-binding oligomerization domain containing 1; NOD2, nucleotide-binding oligomerization domain containing 2; OAS2, 2′,5′-oligoadenylate synthetase 2, 69/71kDa; OAS3, 2′,5′-oligoadenylate synthetase 3, 100kDa; PARP8, poly (ADP-ribose) polymerase family, member 8; PIK3R3, phosphoinositide-3-kinase regulatory subunit 3 (gamma); PIK3R2, phosphoinositide-3-kinase regulatory subunit 2, alpha; RHOU, ras homolog gene family, member U; RIPK1, receptor (TNFRSF)-interacting serine-threonine kinase 1; RIPK2, receptor-interacting serine-threonine kinase 2; RND3, Rho family GTPase 3; S1PR2, sphingosine-1-phosphate receptor 2; SOCS1, suppressor of cytokine signaling 1; SOCS2, suppressor of cytokine signaling 2; SOCS6, suppressor of cytokine signaling 3; STAB1, signal transducer and activator of transcription 1; TRAP1, ATP-binding cassette, sub-family B (MDR/TAP); TICAM1, Toll-like receptor adaptor molecule 1; TIPARP, TCDD-inducible poly(ADP-ribose) polymerase; TLR3, Toll-like receptor 3; TLR4, Toll-like receptor 4; TNFRSF10B, tumor necrosis factor receptor superfamily, member 10b; TNFRSF10, tumor necrosis factor receptor (ligand) superfamily, member 10; TREM1, triggering receptor expressed on myeloid cells 1.

without HBV infection, focusing on the 525 upregulated genes with IFN treatment and using all obtained gene expression profiles. Interestingly, 63.3% of the expressed genes maintained an IFN response, but in 34.9% of those genes, IFN responses were attenuated by HBV infection (Supplementary data 8A). Genes corresponding to interferon signaling, including suppressor of cytokine signaling 1 (SOCS1) and interferon regulatory factor 1, and those corresponding to pattern recognition of bacteria and viruses, including nucleotide-binding oligomerization domain containing 1 (NOD1) and receptor-interacting serine-threonine kinase 2 (RIPK2), were statistically significantly associated with HBV-mediated attenuation to IFN response (\( P = 1.0 \times 10^{-8} \) and \( P = 1.2 \times 10^{-8} \), respectively). According to these results, HBV infection significantly up-regulated SOCS1 expression and reduced the IFN responsiveness of SOCS1. Thus, SOCS1 might support chronic infection of HBV in escaping the effects of innate immunity or IFN therapy. On the other hand, genes involved in recognition of viral infection were also inhibited following HBV infection. Both NOD1 and RIPK2 are related to innate and adaptive immune responses [14, 15]. We speculated that inhibition of NOD1 or RIPK2 expression facilitates HBV survival. Although further study is needed, these results may have important implications for the mechanisms of viral escape from innate immunity.

In conclusion, we performed complementary DNA microarray analysis using human hepatocyte chimeric mice. With this system, we could analyze the direct effects of IFN treatment and HBV infection without the confounding effects of the lymphocyte immunological response and obtained evidence that HBV infection attenuated the virus recognition and IFN response in
hepatocytes, by which means HBV could evade innate immune detection and response.

**Supplementary Data**

Supplementary data are available at *The Journal of Infectious Diseases* online.

**Funding**

This work was supported by the Ministry of Education, Sports, Culture and Technology and the Ministry of Health, Labor and Welfare (Grants-in-Aid for scientific research and development).

**Acknowledgments**

This work was performed at the Research Center for Molecular Medicine, Faculty of Medicine, Hiroshima University. We thank Mari Shiota, Rie Akiyama, and Ruri Mikami for their excellent technical assistance and Aya Furukawa for clerical assistance. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**References**


