Effects of Alpha-Interferon on Gamma-Interferon Production of Peripheral Blood Mononuclear Cells in Hepatitis B Virus Carriers

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We studied gamma-interferon production of phytohemagglutinin-stimulated peripheral blood mononuclear cells in response to alpha-interferon in hepatitis B virus carriers and healthy individuals. The magnitude of gamma-interferon production was significantly higher in patients with anti-HBe antibody than in patients with HBe antigen and healthy individuals. Furthermore, alpha-interferon augmented the production of gamma-interferon of peripheral blood mononuclear cells from patients with active liver injury (serum alanine aminotransferase (ALT), >40 U/L), but not that from patients with inactive liver injury (serum ALT, <40 U/L) or healthy individuals. These results suggested that alpha-interferon could enhance the cellular immune response against hepatitis B virus by augmenting the endogenous production of gamma-interferon in patients with active liver injury, implying that the responsiveness to alpha-interferon might be responsible for liver cell injury.

KEY WORDS: Cellular immune response; gamma-interferon; alpha-interferon; liver cell injury; hepatitis B virus.

INTRODUCTION

Type B chronic hepatitis is a serious liver disorder and a worldwide public health problem. The mechanisms of its chronic infection must be clarified. Some immunological defects have been found in hepatitis B virus (HBV) carriers (1-4), suggesting that persistent HBV infection is due to a defective immunologic response to the virus. The host defense mechanism against viral infection includes interferons (IFNs) which have antiviral, antiproliferative, and immunoregulatory properties (5). Gamma-IFN is produced during an immune response by activated T cells and can stimulate the recognition process of the immune system by enhancing the HLA display on HBV-infected hepatocytes (6, 7). Based on these findings, some investigators have reported that clearance of HBV-infected hepatocytes may be closely associated with gamma-IFN production (7, 8). Alpha-IFN has both direct antiviral and immunomodulatory activities and is considered to be an important regulator of the local immune response in the liver in patients with type B hepatitis (9-11). Administration of alpha-IFN is also effective in promoting clearance of hepatitis B e antigen (HBeAg) and HBV-DNA in chronic HBV carriers (12-16), but it is not always effective, and the importance of immunological factors determining the outcome has been suggested (16). However, the mechanism of action of alpha-IFN in patients who respond to this therapy is currently unknown.

The aim of this study was to determine whether alpha-IFN could modulate the gamma-IFN production of peripheral blood mononuclear cells (PBMCs) in patients with type B hepatitis. We found that, in HBV carriers, the responsiveness of PBMCs to alpha-IFN was closely related to the activity of liver disease.

MATERIALS AND METHODS

Subjects

This study examined 12 healthy individuals and 38 HBV carriers. HBV carriers were divided into three groups according to their laboratory data.
Table I. Patients' Profiles

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Sex (M:F)</th>
<th>ALT (U/L)</th>
<th>DNA-p (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy individuals (n = 12)</td>
<td>31.8 ± 3.1</td>
<td>12:0</td>
<td>9.3 ± 3.0</td>
<td>ND</td>
</tr>
<tr>
<td>HBV carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A (n = 9)</td>
<td>33.7 ± 8.3</td>
<td>7:2</td>
<td>25.2 ± 9.1^{a,b,a}</td>
<td>2198 ± 4070</td>
</tr>
<tr>
<td>Group B (n = 19)</td>
<td>32.1 ± 9.8</td>
<td>16:3</td>
<td>226.8 ± 212.3^{a}</td>
<td>1435 ± 2662</td>
</tr>
<tr>
<td>Group C (n = 10)</td>
<td>30.5 ± 6.0</td>
<td>6:2</td>
<td>22.4 ± 19.5^{b}</td>
<td>4.8 ± 5.1^{c}</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SD. DNP-p, HBV-DNA polymerase activity; ND, not determined.
*Superscript a, P < 0.01 vs healthy individuals (Student’s t test); superscript b, P < 0.01 vs Group B (Student’s t test); superscript c, P < 0.05 vs Group B (Student’s t test).

Group A patients were positive for HBeAg and had normal levels of serum alanine aminotransferase (ALT) which were persistently less than 40 U/L for at least three months. Group B patients were positive for HBeAg and had levels of serum ALT which were persistently more than 40 U/L for at least three months. Group C patients were negative for HBeAg. All HBV carriers were negative for antidelta antibody or anti-human immunodeficiency virus antibody. The patients’ profiles are given in Table I. Informed consent was obtained from all subjects.

Lymphocyte Preparations

Peripheral mononuclear blood cells (PBMCs) were isolated from heparinized venous blood by Ficoll–Hypaque gradient sedimentation. The interphase PBMCs were suspended at 1.2 × 10^6 cells/ml in RPMI 1640 (GIBCO, Grand Island, NY) culture medium supplemented with 10% heat-inactivated fetal calf serum, glutamine, and antibiotics.

Cultures

An aliquot (1 ml) of PBMCs containing 1.2 × 10^6 cells was cultured for 48 hr with 5 μg phytohemagglutinin (PHA) and simultaneously with or without 10 to 10^4 units/ml alpha-IFN at 37°C in a 5% CO₂ incubator. Recombinant alpha 2a-IFN was kindly supplied by Takeda Chemical Industries, Ltd., Japan. After culture, the tubes were centrifuged to collect the supernatants, which were stored at −20°C until use. Cell viability, evaluated by trypan blue exclusion, was more than 90%.

Gamma-IFN Assays

Gamma-IFN was assayed using a commercial immunoradiometric assay kit (SUCROSEP IRMA, Boots-Celltech Diagnostics Limited, Berkshire, UK), which is a two-site immunoradiometric assay. The concentration of gamma-IFN was measured directly by incubation with an ^{125}I-labeled monoclonal antibody which was specific for human gamma-IFN. The analyte-^{125}I-labeled antibody complex was then immobilized by incubation with sheep anti-gamma-IFN antibody coupled to the solid phase. The assay standard was a preparation of purified natural human gamma-IFN obtained from the Finnish Red Cross Blood Transfusion Service, Helsinki. This was calibrated against the National Institutes of Health (NIH) human gamma interferon reference preparation obtained from the NIH, Bethesda, Maryland. Some results of gamma-IFN induction by alpha-IFN were expressed as a stimulation index (SI gamma = gamma-IFN production in the presence of alpha-IFN/gamma-IFN production in the absence of alpha-IFN).

Hepatitis B Virus Markers

Hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface antigen (anti-HBs), hepatitis B e antigen (HBeAg), antibody to hepatitis B e antigen (anti-HBe), and antibody to hepatitis core antigen were measured with a commercial radioimmunoassay kit (Ausria II, Ausab, HBe RIA kit, CORAB, Abbott Laboratories, North Chicago, IL). Hepatitis B virus DNA polymerase activity was measured according to Kaplan et al. (17).

Statistics

Student’s t test was used for continuous variables and the chi-square method was used for dichotomous variables to determine statistical significance.

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