AN INVERSE RELATIONSHIP BETWEEN AUTOIMMUNE LIVER DISEASES AND STRONGYLOIDES STERCORALIS INFECTION

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Abstract. A case-control study was undertaken to describe the prevalence of Strongyloides stercoralis infection among patients with autoimmune liver diseases, such as primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH), and primary sclerosing cholangitis (PSC). This study covered 4,117 patients who were admitted to hospitals in Okinawa, Japan, between 1988 and 2006. During this period, 538 patients had the following chronic liver diseases: PBC, AIH, PSC, chronic viral hepatitis group, and alcoholic liver disease. The other 3,579 patients who were hospitalized and underwent parasitologic tests served as controls. The frequency of S. stercoralis infection in the autoimmune liver diseases group (1.0%) was lower than that found in the control group (7.0%; \( P = 0.0063 \)). None of the female patients with PBC born before 1955 had S. stercoralis infection, which was also statistically significant (\( P = 0.045 \)). We hypothesized that immunomodulation induced by S. stercoralis infection may lower the incidence of autoimmune liver disease.

INTRODUCTION

Primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH), and primary sclerosing cholangitis (PSC) are chronic liver diseases that likely have an autoimmune basis to their pathogenesis. Although their etiology remains obscure, environmental and genetic factors may play an important role in the development of these diseases. The majority of surveys concerning environmental factors and autoimmune liver disease have focused on PBC.1 Some epidemiologic studies have revealed that environmental factors may be associated with the onset of PBC in genetically susceptible hosts.2 Helminth infections are highly prevalent in many developing countries, where autoimmune diseases are uncommon. An inverse epidemiologic relation between helminth infection and autoimmune diseases has been described in some reports.3,4 Experimental data also showed that parasitic helminths can block autoimmune diseases in mice.5,6 Moreover, clinical trials have been reported involving the application of Trichuris suis ova in the treatment of inflammatory bowel diseases.7 Immunomodulation induced by chronic helminth infection could be the reason that autoimmune disease was less prevalent among patients with helminthiasis.8,9 Strongyloides stercoralis is the most common human parasitic helminth. S. stercoralis lives in warm and humid soil, and its filariform larvae usually infect through the skin of bare feet. After infection, larvae migrate to the duodenum and grow into mature females. Rhabditiform larvae hatched from eggs are ejected from the host; however, some develop into filariform larvae and infect through the large intestine and the skin around the anus (autoinfection). Therefore, once the host is infected, this worm is able to complete its life cycle and proliferate within the host, and the infection can persist for decades.10 Although hyperinfection occasionally occurs in patients with human T cell lymphotropic virus type 1 or immunosuppression, many patients chronically infected with S. stercoralis have an asymptomatic infection or mild disease.11 Few epidemiologic studies of parasite infection in patients with liver diseases have been described.12–14 Hegab and others12 reported a high incidence of opportunistic intestinal protozoa in pediatric patients with chronic liver diseases, but no S. stercoralis or other helminths were detected. Gaburri and others13 described a higher prevalence of intestinal parasites in patients with alcoholic cirrhosis; however, no patients with autoimmune liver disease were implicated in their study. Until now, no study has focused on the relationship between chronic helminth infection and autoimmune liver diseases, such as PBC, AIH, or PSC. We report herein the results of a case-control study to describe the prevalence of S. stercoralis infection (also known as strongyloidiasis) among patients with autoimmune liver diseases, particularly PBC. We subsequently discuss the effect of chronic helminth infections on autoimmune liver diseases.

MATERIALS AND METHODS

Patients. This study involved 4,117 patients who were admitted to the following hospitals between 1988 and 2006: the First Department of Internal Medicine, Ryukyu University Hospital, Nakagami Hospital, and Yonabaru Chuo Hospital. All three hospitals are located in Okinawa, Japan. All patients were Japanese and residents of Okinawa. Informed consent was obtained from all individuals. In all patients, a detailed history was taken, and a clinical examination was carried out. All of these patients underwent biochemical evaluation including liver function tests using standard automated techniques.

Diagnosis of S. stercoralis infection. One fecal sample from each subject was routinely collected on their admission. Infection of S. stercoralis was diagnosed using the agar plate culture method.15

Diagnosis of PBC. The diagnosis of PBC was based on the following criteria: a detectable anti-mitochondrial antibody (AMA), a persistent increase in biliary enzymes for at least 6 months, and/or compatible or diagnostic liver histology. In cases of PBC without detectable AMA, criteria were modi-
The diagnosis of AIH was based on the criteria of the International Autoimmune Hepatitis Group.\textsuperscript{16} Serologic tests for anti-nuclear antibodies, anti-smooth muscle antibodies, and anti-liver/kidney microsomal antibodies were performed using standard immunofluorescence techniques.

**Diagnosis of PSC.** The diagnosis of PSC was based on biochemical evaluation, endoscopic retrograde cholangiopancreatography findings, and histology that were compatible with PSC.

**Statistical analysis.** Two-tailed Fisher exact test was used to compare the prevalence of *S. stercoralis* infection. \( P < 0.05 \) was considered significant. The Mann-Whitney \( U \) test was used to compare peripheral eosinophil counts.

## RESULTS

**Strongyloides stercoralis infection in chronic liver diseases.**

The study population was composed of 2,438 men and 1,679 women, with a mean age of 54.8 ± 17.5 (SD) years. During the study period, 538 patients were found to have the following chronic liver diseases: PBC (74 cases), AIH (30 cases), PSC (1 case), hepatitis B virus (HBV; 108 cases), hepatitis C virus (HCV; 255 cases), HBV and HCV (4 cases), and alcoholic liver disease (66 cases). The other 3,579 patients who were hospitalized and underwent tests for chronic liver diseases: PBC (74 cases), AIH (30 cases), PSC (1 case), hepatitis B virus (HBV; 108 cases), hepatitis C virus (HCV; 255 cases), HBV and HCV (4 cases), and alcoholic liver disease (66 cases). The other 3,579 patients who were hospitalized and underwent tests for chronic liver diseases: PBC (74 cases), AIH (30 cases), PSC (1 case), hepatitis B virus (HBV; 108 cases), hepatitis C virus (HCV; 255 cases), HBV and HCV (4 cases), and alcoholic liver disease (66 cases). The other 3,579 patients who were hospitalized and underwent tests for chronic liver diseases: PBC (74 cases), AIH (30 cases), PSC (1 case), hepatitis B virus (HBV; 108 cases), hepatitis C virus (HCV; 255 cases), HBV and HCV (4 cases), and alcoholic liver disease (66 cases). The other 3,579 patients who were hospitalized and underwent tests for chronic liver diseases: PBC (74 cases), AIH (30 cases), PSC (1 case), hepatitis B virus (HBV; 108 cases), hepatitis C virus (HCV; 255 cases), HBV and HCV (4 cases), and alcoholic liver disease (66 cases). The other 3,579 patients who were hospitalized and underwent tests for chronic liver diseases: PBC (74 cases), AIH (30 cases), PSC (1 case), hepatitis B virus (HBV; 108 cases), hepatitis C virus (HCV; 255 cases), HBV and HCV (4 cases), and alcoholic liver disease (66 cases). The other 3,579 patients who were hospitalized and underwent tests for chronic liver diseases: PBC (74 cases), AIH (30 cases), PSC (1 case), hepatitis B virus (HBV; 108 cases), hepatitis C virus (HCV; 255 cases), HBV and HCV (4 cases), and alcoholic liver disease (66 cases). The other 3,579 patients who were hospitalized and underwent tests for chronic liver diseases: PBC (74 cases), AIH (30 cases), PSC (1 case), hepatitis B virus (HBV; 108 cases), hepatitis C virus (HCV; 255 cases), HBV and HCV (4 cases), and alcoholic liver disease (66 cases). The other 3,579 patients who were hospitalized and underwent tests for chronic liver diseases: PBC (74 cases), AIH (30 cases), PSC (1 case), hepatitis B virus (HBV; 108 cases), hepatitis C virus (HCV; 255 cases), HBV and HCV (4 cases), and alcoholic liver disease (66 cases).

### Table 1

**Prevalence of *S. stercoralis* infection**

<table>
<thead>
<tr>
<th>Birth year</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1910s and before</td>
<td>32/189 (16.9)</td>
<td>15/123 (12.2)</td>
<td>47/312 (15.1)</td>
</tr>
<tr>
<td>1920s</td>
<td>62/489 (12.7)</td>
<td>26/311 (8.4)</td>
<td>88/800 (11.0)</td>
</tr>
<tr>
<td>1930s</td>
<td>72/585 (12.3)</td>
<td>22/397 (5.5)</td>
<td>94/982 (9.6)</td>
</tr>
<tr>
<td>1940s</td>
<td>28/373 (7.5)</td>
<td>8/289 (2.8)</td>
<td>36/662 (5.4)</td>
</tr>
<tr>
<td>1950s</td>
<td>6/340 (1.8)</td>
<td>1/230 (0.4)</td>
<td>7/570 (1.2)</td>
</tr>
<tr>
<td>1960s</td>
<td>1/247 (0.4)</td>
<td>0/168 (0.0)</td>
<td>1/415 (0.2)</td>
</tr>
<tr>
<td>1970s and after</td>
<td>0/215 (0.0)</td>
<td>0/161 (0.0)</td>
<td>0/376 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>201/2,438 (8.2)*</td>
<td>72/1,679 (4.3)</td>
<td>273/4,117 (6.6)</td>
</tr>
</tbody>
</table>

* \( P < 0.001 \) versus female.

The total prevalence rate of *S. stercoralis* infection was 6.6% (273 of 4,117; Table 1). The prevalence rate of *S. stercoralis* infection in men and women was 8.2% (201 of 2,438) and 4.3% (72 of 1,679), respectively. The prevalence rate in men was significantly higher than in women (\( P < 0.001 \)). The prevalence rate of *S. stercoralis* infection was significantly decreased in younger generations, and none of the female patients born after 1955 were infected.

The frequency of *S. stercoralis* infection in chronic liver diseases is shown in Table 2. The rate of *S. stercoralis* infection in the autoimmune liver diseases group (1.0%) was lower than that found in the control group (7.0%; \( P = 0.0046 \); odds ratio, 0.127; 95% confidence interval = 0.018–0.914). Only one patient with PBC had strongyloidiasis: a 63-year-old man. None of the patients with AIH or PSC had strongyloidiasis, although these subgroups were too small for statistical comparison.

The frequency of *S. stercoralis* infection in the chronic viral hepatitis group (3.8%) was slightly lower than that in the control group and that in the alcoholic liver disease group (9.1%) was slightly higher, although these differences were not statistically significant.

To evaluate the immunologic response, we analyzed peripheral eosinophil counts in patients with autoimmune liver diseases and in patients with *S. stercoralis* infection. Peripheral eosinophil counts were measured in 95 of 105 cases of autoimmune liver diseases and 246 of 273 cases of strongyloidiasis. In patients with *S. stercoralis* infection, peripheral eosinophil counts were significantly higher (506.2 ± 504.9 \(/ \text{mm}^3\); reference range, 70–440/\text{mm}^3) than those in patients with autoimmune liver diseases (158.4 ± 211.4 \(/ \text{mm}^3\); \( P < 0.0001 \); Table 3).

### Table 2

**Prevalence of *S. stercoralis* infection in chronic liver diseases**

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Number of <em>S. stercoralis</em>-positive patients/number of tested patients (%)</th>
<th>Male</th>
<th>Female</th>
<th>Born before 1955</th>
<th>Born after 1955</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune liver diseases</td>
<td>1/105 (1.0)*</td>
<td>7</td>
<td>98</td>
<td>85</td>
<td>20</td>
</tr>
<tr>
<td>PBC</td>
<td>1/74 (1.4)</td>
<td>3</td>
<td>71</td>
<td>62</td>
<td>12</td>
</tr>
<tr>
<td>AIH</td>
<td>0/30 (0.0)</td>
<td>3</td>
<td>27</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>PSC</td>
<td>0/1 (0.0)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chronic viral hepatitis</td>
<td>14/367 (3.8)</td>
<td>238</td>
<td>129</td>
<td>249</td>
<td>118</td>
</tr>
<tr>
<td>HBV infection</td>
<td>4/108 (3.7)</td>
<td>88</td>
<td>19</td>
<td>61</td>
<td>47</td>
</tr>
<tr>
<td>HCV infection</td>
<td>102/55 (3.9)</td>
<td>147</td>
<td>108</td>
<td>185</td>
<td>70</td>
</tr>
<tr>
<td>HBV and HCV infection</td>
<td>0/4 (0.0)</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Alcoholic liver disease</td>
<td>6/66 (9.1)</td>
<td>60</td>
<td>6</td>
<td>53</td>
<td>13</td>
</tr>
<tr>
<td>Control</td>
<td>252/3,579 (7.0)</td>
<td>2,133</td>
<td>1,446</td>
<td>2,758</td>
<td>821</td>
</tr>
<tr>
<td>Total</td>
<td>273/4,117 (6.6)</td>
<td>2,438</td>
<td>1,679</td>
<td>3,145</td>
<td>972</td>
</tr>
</tbody>
</table>

* \( P = 0.0046 \) versus controls.

DISCUSSION

Our results clearly showed that the frequency of *S. stercoralis* infection among patients with autoimmune liver dis-
ease, especially PBC, was lower than that in the control group. To our knowledge, this is the first report to describe an inverse relationship between autoimmune liver diseases and chronic helmint infection.

Okinawa is located in the southwestern part of Japan, belonging to the subtropical zone. Okinawa has been recognized as an area of endemic S. stercoralis infection. In this study, the prevalence of S. stercoralis among women was found to be 4.3%, and most of the patients positive for S. stercoralis were older than 40 years of age. In a community-based mass survey in Okinawa, Asato and Hayashi gave respective reports for the prevalence of S. stercoralis infection in women of 7.7% and 14.9%, respectively. This argues against the possibility of study bias in this study, suggesting that these results may properly reflect the geopidemiology of S. stercoralis infection even though this was a hospital-based case-control study.

Correlative studies should be viewed with caution as the data cannot separate the issues of cause and effect. At least two hypotheses can be proposed to explain the low prevalence of S. stercoralis infection among patients with PBC and other autoimmune liver diseases: 1) S. stercoralis infection decreases the risk of the onset of autoimmune liver disease or 2) autoimmune liver diseases reduce the susceptibility to S. stercoralis infection. The latter hypothesis would not seem plausible, because S. stercoralis infection is percutaneous; thus, the presence of PBC should not affect the infection by S. stercoralis. In contrast, serious liver dysfunction increases the risk of strongyloidiasis. In this study, most patients with PBC and other autoimmune liver diseases were in an early phase in development of the disease. Therefore, their daily activities, lifestyle, and sanitary environment, which have most effect on the susceptibility to helmint infection, should be the same as those in the control group. Thus, we propose the hypothesis that S. stercoralis infection may reduce the onset of autoimmune liver disease.

Epidemiologic studies have revealed that regions of the world with high rates of helmint infections consistently have a reduced incidence of autoimmune diseases, such as Crohn disease, insulin-dependent diabetes mellitus, and multiple sclerosis. The inverse relationship between helmint infection and autoimmune diseases could be a reflection of the imbalance of two different types of T-helper cells, namely Th1 and Th2. Th1 and Th2 cells are reciprocally cross-inhibitory; helmint infections, including strongyloidiasis, are associated with responses stimulated by Th2-type cytokines such as interleukin (IL)-4, and this impedes the development of Th1 cells that seem to be involved principally in organ-specific autoaggressive disorders. In this study, peripheral blood eosinophilia, which suggest Th2 involvement in the immune response, were observed in patients with strongyloidiasis but not in patients with autoimmune liver diseases. As well as the Th1-Th2 cross-inhibitory process, the anti-inflammatory network seems to play a crucial role in downregulation of autoimmune diseases. Helminths induce CD4+ regulatory T cells and promote the production of powerful immunomodulatory molecules such as IL-10 and transforming growth factor-β. The presence of this strong anti-inflammatory regulatory network could help to prevent the development of both Th1-type and Th2-type immune responses.

PBC is an organ-specific autoimmune disease that is characterized by chronic progressive destruction of small intrahepatic bile ducts, with portal inflammation and fibrosis. Autoreactive T lymphocytes that infiltrate the liver may play a major role in the bile duct damage that accompanies the disease. Studies analyzing the cytokine mRNA expression have shown that predominantly Th1 cells are activated in association with damaged bile ducts. Although the studies of cytokine profiles in patients with PBC revealed slightly different patterns according to the tissue examined and stage of the disease, a number of investigators reported that the expression of interferon-γ (Th1 cytokine) mRNA in liver tissue was increased and that interferon-γ mRNA levels correlated positively with the severity of inflammation and the level of serum γ-glutamyltransferase. These results support the idea that Th1 cells are activated in PBC and play an important role in its pathogenesis.

However, in view of the eosinophilic reaction observed, especially in patients with early-stage PBC, the Th1-Th2 dichotomy may not always exist. Therefore, it is hypothesized that concurrent helmint infection induces a Th2-type immune response, which may prevent the Th1-type hepatic inflammation in PBC. Alternatively, because regulatory T cells suppress the differentiation of Th0 into either Th1 or Th2 cells, these regulatory cells may also be involved in the im-
munomodulatory effect of concurrent infection on the course of PBC.

T cells seem to also play a central role in the immunopathogenesis of AIH. Recent experimental evidence suggests that immunoregulatory dysfunction characterized by decreased numbers of CD4+ regulatory T cells may occur in AIH.32 Such observations suggest that a decrease in the number of regulatory T cells and their ability to expand may lead to autoimmune liver disease, and CD4+ regulatory T cells induced by chronic helminth infection may therefore impede the development of AIH.

In conclusion, our results clearly showed an inverse relation between autoimmune liver disease and S. stercoralis infection. Immunomodulation induced by chronic S. stercoralis infection may account for this negative relationship. Further study of the immunomodulation and anti-inflammatory network induced by helminth infection may be highly informative toward the goal of inhibition of the onset and development of autoimmune liver diseases.

References


