Intrahepatic lymphoid cells in the acute phase and remission of autoimmune hepatitis in children

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Abstract

In order to characterize intrahepatic lymphoid cells we have examined 53 liver biopsy specimens from children with autoimmune hepatitis: 31 specimens from children in the acute phase and 22 specimens from children in remission. The differences between these two groups consisted in different degree of inflammation, fibrosis and lymphocytes subpopulations. The proportions of lymphocytes CD4+, CD4/CD8 ratio and the lack of expression of CTLA-4 suggest an important factor in the development of autoimmune mechanisms with the leading role of autoreactive CD4+. It has been confirmed that cellular immune responses in autoimmune hepatitis do take place in the liver, even in the remission phase, playing an important role in mediating cellular injury. In summary, the CD4+/CD8+ and CD79a phenotype can be good activity markers in autoimmune hepatitis in children.

Key words: autoimmune hepatitis, histological activity, lymphocyte subsets

Introduction

Autoimmune hepatitis (AIH) is a chronic necroinflammatory disease with an immune mediated liver injury. AIH in children is a rare, progressive disease with an implication of a severe hepatic injury. Although there are no pathognomonic markers, cell mediated immune reactions constitute important factors in hepatocellular damage also affecting the morphological extent of parenchymal injury and inflammatory infiltrates in the portal tracts and lobules. When a diagnosis is performed in the acute phase, liver histology is characterised by necroinflammatory changes accompanied by fibrosis and/or cirrhosis. Since they may not disappear completely during a pharmacologically induced clinical remission the patients require life-long monitoring and therapy [11]. T lymphocytes play a central role in the immunopathogenesis of AIH. The presentation of self antigens or molecular mimicry leads to their activation and expansion in the liver. They also initiate B cell production of autoantibodies, cytokines and hepatocyte damage [5].

The present study is based on a histological and immunophenotypical analysis of the liver inflammation in the state of acute phase and during remission of the disease. The aim of the study was to analyse the morphological extent of parenchymal injury in consecutive biopsies before and after Encorton and/or Immuran treatment. Correlations were also sought between histological activity and fibrosis with various lymphocyte subsets.
Patients, material and methods

We analyzed 31 (from total number of 53 liver biopsies) taken from children with autoimmune hepatitis in the acute phase aged at disease diagnosis 5–17 years (mean 11.7±3.2) and 22 liver biopsies from children in remission. The diagnosis was based on increased ALT activity (699±657 U/l), increased gammaglobulin concentration (36,02±12,66 g/l), increased IgG concentration (3169±1321 mg/dl) and presence of circulating autoantibodies (ASMA, ANA) according to international criteria [2]. The other reasons of hepatitis (viral infections, Wilson’s disease and alfa-1-antitrypsine deficiency) were excluded. Liver biopsy had been performed in all patients before treatment was started. All patients were treated with prednisone and azathioprine. The medication doses were adjusted to efficacy and toxicity.

Informed consent was obtained from all parents of the patients. The research was carried out in accordance with the recommendations of the Bioethic Commission in the hospital.

Histological examination

All liver biopsy specimens were divided into 2 parts. The first one was fixed in 10% neutral buffered formalin and embedded in paraffin. Sections displaying at least 7 portal tracts were routinely stained by Hematoxylin-Eosin, Periodic Acid-Schiff method with Diastase, Gomori silver stain, Azan method. In the course of evaluation histological activity of the disease, each sample was described using Batts-Ludwig classification [3] in order to assess the grade (inflammatory infiltrates) and stage (fibrosis/cirrhosis) of the disease. The following categories of lesions were investigated: piecemeal necrosis, lobular inflammation and focal necrosis, portal inflammation, fibrosis. The second part of the liver biopsy was frozen in liquid nitrogen, fixed in acetone, preserved in –70°C and used for detection of CD25 and CTLA-4 expression.

Immunohistochemical study

The phenotype of the inflammatory reactions was examined on paraffin embedded tissue and frozen tissue using monoclonal antibodies directed against CD3 (T cells), CD4 (T helper cells), CD8 (cytotoxic and suppressor T cells), CD25 (IL-2 receptor), CD79a (B cells, plasma cells, 43 kDa polypeptide), CTLA-4 (CD152, cytotoxic T lymphocyte antigen) by using EnVision system DAKO. Immunophenotype of the cells was described using semiquantitative method for portal tracts: 0: no cells, 1: diffuse single cells <10 cells, 2: between 10 and 40 cells, 3: more than 50 cells and for lobules: 0: no cells, 1: diffuse single cells, 2: foci between 5 and 10 cells or less than 5 foci per lobule, 3: more than 10 cells or more than 5 foci per lobule.

Statistical analysis

Fischer Exact test was used to find a statistically significant association between grade (G) and stage (S) in two groups. Chi-square Independence test was applied for the purpose of comparison the various immunophenotypes of inflammatory infiltrates in the portal tracts and lobules with a grade and stage of the disease.

Results

Morphological changes

Morphological changes were different in the acute phase and in remission. In the acute phase the inflammatory infiltrates were polymorphic composed of granulocytes, plasma cells but with predominance of lymphocytes, with prominent interface hepatitis and lobular focal hepatocyte necrosis. The necroinflammatory activity, described as a grade of the disease, was severe in 72%, moderate in 25% and slight in 3% of the examined material. We found cholestasis with focal gigantocellular transformation in 6,8%, inflammatory reaction in bile ducts in 20% and lymph follicle formations in portal tracts in 13,6%. Liver fibrosis, described as a stage of the disease was characterized by numerous fibrous portal septa and architectural distortion in 68%, while single portal septa occurred in 28% of biopsy specimens and no fibrosis in 4% of them.

In the remission phase the inflammatory infiltrates were less abundant or inconspicuous. No active changes were seen in 42%, while slight inflammation was found in 55% of the material and marked changes only in 3%. Lymphoid cells, without granulocytes as seen in acute phase, were detected inside portal tracts without any infiltration of bile ducts. Steatosis of the hepatocytes was observed in 10,2%. Liver fibrosis was characterized by numerous fibrous portal septa and architectural distortion in 27%, as singular fibrous septa between portal tracts and in 42% and no changes were observed in 31% of biopsy specimens. The demonstration of the different grade and stage in acute phase and remission is shown in Fig. 1 and Fig. 2.

Comparison of the lymphocytic immunophenotype

Portal infiltration in acute phase contained lymphoid cells with predominance of T helper cells CD4+, plasma cells CD79a+ and cytotoxic cells CD8+ (Fig. 3A). In the areas of piecemeal necrosis we found T helper cells CD4+. T cytotoxic cells CD8+ infiltrated focally bile duct epithelium. The lobular inflammation was different and consisted mainly of cytotoxic cells CD8+ and only few helper cells CD4+ and plasma cells CD79a+. Very few lymphocytes expressing the CD25 marker were situated predominantly in portal tracts (Fig. 3B).

Chi-square test of independence performed on liver specimens from patients with acute phase-group 1ab (significance level 0.05) showed dependency between grade of the inflammation and phenotypes of CD3+ cells in the portal tracts and lobules, CD4+ cells in the portal tracts and CD79a+ cells in the portal tracts (Table 1). By means of Chi-square test we rejected the null hypothesis and found dependence in the Cd4+/CD8+ cell ratio in all biopsy specimens according to the grade of the disease in the acute phase.
In the biopsy specimens from children in remission we found the same compartmentalization of lymphoid cells, but inflammatory infiltrates were mild, usually without piecemeal necrosis and we did not found any dependency according grade and stage of the disease. Furthermore we did not find any expression of CTLA-4 in liver biopsy specimens neither in the acute phase nor in remission.

**Table 1**

Chi-square test of independence of liver specimens from patients with acute phase (significance level 0.05) G – grade of the disease; S – stage of the disease; PB – portal tracts in the liver; ZR – lobules in the liver

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Test statistics</th>
<th>Critical value $\chi^2$</th>
<th>p-value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>G vs. CD3PB</td>
<td>T = 37,3943</td>
<td>15,5073</td>
<td>&lt;0.0001</td>
<td>Dependent</td>
</tr>
<tr>
<td>G vs. CD3ZR</td>
<td>T = 44,0794</td>
<td>21,0260</td>
<td>&lt;0.0001</td>
<td>Dependent</td>
</tr>
<tr>
<td>G vs. CD79aPB</td>
<td>T = 38,3415</td>
<td>21,0260</td>
<td>0.000135</td>
<td>Dependent</td>
</tr>
<tr>
<td>G vs. CD4PB</td>
<td>T = 48,8988</td>
<td>21,0260</td>
<td>&lt;0.0001</td>
<td>Dependent</td>
</tr>
<tr>
<td>G vs. CD4PB/CD8PB</td>
<td>T = 49,5364</td>
<td>41,3371</td>
<td>0.007282</td>
<td>Dependent</td>
</tr>
<tr>
<td>G vs. CD25ZR</td>
<td>T = 17,0612</td>
<td>15,5073</td>
<td>0.029477</td>
<td>Dependent</td>
</tr>
<tr>
<td>S vs. CD4PB/CD8PB</td>
<td>T = 44,0073</td>
<td>41,3371</td>
<td>0.027739</td>
<td>Dependent</td>
</tr>
</tbody>
</table>

Null hypothesis = *two samples are independent*. Dependent = *We reject the null hypothesis*. Independent = *There is not enough evidence to reject the null hypothesis*.

In the biopsy specimens from children in remission we found the same compartmentalization of lymphoid cells, but inflammatory infiltrates were mild, usually without piecemeal necrosis and we did not found any dependency according grade and stage of the disease. Furthermore we did not find any expression of CTLA-4 in liver biopsy specimens neither in the acute phase nor in remission.

**Discussion**

It is known that T cells play a central role in the immunopathogenesis of autoimmune hepatitis and lymphocytes CD4+ and CD8+ cells are thought to be critical for the disease activity [7]. Humoral response involves hypergammaglobulinemia and circulating autoantibodies of different specificities.
Cellular immune response concerns circulating T cells, indicating on T-cell dependent autoantibodies production [10].

In the present study, we analysed intrahepatic lymphoid cells situated in the portal tracts and lobules. We found not only T cells, predominantly CD4+, but also B cells CD79a+ in portal tracts correlating with the disease activity. The differences in the immunophenotypes of the inflammatory infiltrates were present in different phases of autoimmune hepatitis: [i] in the acute phase predominance of CD4+ and CD79a+ cells in abundant, severe inflammatory infiltrates, [ii] in remission mild inflammatory infiltrates with CD8+ cells predominance. The distribution of various helper cells and B cells predominated in the portal tracts. Cytotoxic CD8+ lymphocytes predominated in the lobules. The increase of CD4+ cells in portal tracts, observed in the acute phase might reflect their important role in cytokine production, but also their direct cytotoxic effect on hepatocytes in the areas of piecemeal necrosis. The direct cytotoxic effect on hepatocytes of CD4+ cells was also observed in chronic viral hepatitis, since they are often present in piecemeal necrosis areas [9]. In the acute phase, the CD4+/CD8+ ratio of intrahepatic lymphocytes in the portal tracts was correlated with the grade and stage of the disease, suggesting that helper/inducer CD4+ cells can positively regulate cytotoxic T-cell activity. Small amount of CD25+ cells (practically only in the lobules) and their correlation with the grade of the disease could be defective in self-tolerance maintenance. Inability of T-reg (CD4+CD25+) cells to regulate CD8 T-cell function may initiate autoimmune cell damage [7].

The CD4+ T cells clones expanded from blood or liver tissue of AIH patients react with major epitopes of LKM1 antigen in a HLA-class II restricted manner and produce a large amount of IFN-gamma providing evidence of a massive Th1 response in autoimmune hepatitis. Th1 cells exert direct cytotoxic effect on hepatocytes and participate in production of autoantibodies by autoreactive B cells providing effective help for their production [4]. Our observation concerning increased ratio of the CD4/CD8 intrahepatic lymphocytes present in the portal tracts of the liver in AIH patients confirm and extend the observations of others indicating on the leading role of autoreactive CD4+ T cells in pathogenesis of liver injury in AIH [6]. Cytokines produced by these cells possibly induce other cytotoxic effectors present in CD8+ T cell population. Cytotoxic T cells play dominant role in pathogenesis of AIH since other cytotoxic cells such as NK lymphocytes seem to be rather infrequent in the liver of AIH patients. This background may favor the development of a variety of cytotoxic responses involving both CD4+ and CD8+ lymphocytes as well as other cytotoxic reactions more dependent on humoral activities [7]. Characteristic feature of AIH is the tendency to the development of an antigen specific and non-specific immunosuppressive state in the phase of spontaneous remission [8]. The mechanism of this reaction is not known but may involve apoptosis of autoreactive CD4+ T cells. Increased number of CD8+ T cells and decreased amount of CD4+ T cells observed here in the phase of remission, confirm the leading role of CD4+ T cells in pathogenesis of autoimmune hepatitis [11].

The lack of CTLA-4 expression, which is an inhibiting receptor for immune response, could be also an important factor in the development of autoimmune mechanisms, as this receptor is present on the surface of circulating lymphocytes, but absent in situ in the liver [1]. Cellular immune responses in autoimmune hepatitis do take place in the liver, even in the remission phase of autoimmune hepatitis, playing an important role in mediating cellular injury. In summary, the CD4+/CD8+ and CD79a phenotype can be good activity markers in autoimmune hepatitis in children.

Acknowledgment

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References