Detecting Plasma Cell Precursors in Autoimmune Hepatitis

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Abstract. Background: Autoimmune hepatitis (AIH) is a non-resolving inflammatory liver disease. Inflammation showing plasma cells under hematoxylin-eosin (H&E) stain is typical of AIH. In many cases, however, only lymphocytes and occasional granulocytes are found. It was recently noticed that the antibody to multiple myeloma oncogene 1/IRF4 (MUM1), stained plasma cells and their precursors. Patients and Methods: Liver biopsies from 11 patients were stained with H&E and with anti-MUM1. Clinically, four patients were suspicious of AIH, four had viral hepatitis C, two nonalcoholic steatohepatitis (NASH) and one, fatty liver. Counting was performed in three high-power fields. Results: In patients having clinical suspicion of AIH, H&E revealed plasma cells in only one of the four cases. On the other hand, MUM1 immunostain revealed such cells in all four cases, MUM1-expressing cells were found in the portal triads, in the zone corresponding to interface inflammation and in the parenchyma surrounding the interface hepatitis. MUM 1-expressing cells ranged from 26 to 44 in AIH, from 2 to 15 in viral hepatitis C, from 1 to 3 in NASH and from 0 to 2 in the case with fatty liver (AIH vs. viral hepatitis C/NASH/fatty liver, p<0.05). Conclusion: These preliminary results suggest that MUM1 immunostain may be of help in endorsing a presumptive clinical diagnosis of AIH and may add valuable information in the differential diagnosis between AIH and the other liver diseases examined here. This appears to be the first report in which MUM1 immunostain has been applied to assess the presence of plasma cell precursors in AIH.

Autoimmune hepatitis (AIH) is a non-resolving inflammatory disease of unidentified etiology that causes progressive destruction of the hepatic parenchyma (1). If left untreated it can lead to liver cirrhosis and hepatic failure. The diagnosis of AIH requires characteristic clinical features, laboratory data and the exclusion of other diseases associated with chronic hepatitis and cirrhosis (1).

The histological picture in sections stained with hematoxylin and eosin (H&E) is typified by interface hepatitis showing necro-inflammatory disruption of the limiting septa, lobular disarray, central necrosis, focal parenchymal necrosis, rosettes, and by bridging fibrosis or cirrhosis (2). The inflammatory infiltration contains both lymphocytes and plasma cells. Plasma cell infiltration is typical of AIH, but it is not specific as plasma cells can also be encountered in viral hepatitis (3). In contrast, the absence of plasma cells from the infiltrate does not preclude the histological diagnosis of AIH. In fact, in many cases, H&E stain shows only lymphocytes, occasional eosinophils and/or neutrophils (4). In the absence of plasma cells in H&E sections, pathologists may raise doubts in confirming a presumptive clinical diagnosis of AIH. It should be emphasized that The International Autoimmune Hepatitis Group (IAIHG) (4) postulated that a diagnosis of definitive AIH should not be made without liver histology.

Due to difficulties in demonstrating plasma cells in H&E-stained liver sections displaying inflammatory changes, sections were stained with antibodies to CD138 and the multiple myeloma oncogene 1/IRF4, MUM1 (DakoCytomation) to detect plasma cells, as a complement to routine stains for liver biopsies at this laboratory.

Patients and Methods

Liver biopsies from 11 patients were investigated. Sections were stained with routine stains for liver biopsies, namely H&E, periodic acid Schiff (PAS), PAS-diastase, Perl’s reaction for detecting iron storage, Sirius stain (without contrast) to highlight collagen, Gordon-Sweet to stain reticulin, and antibodies to cytokeratin 7 (CK7 Leica Microsystems, Wetzlar, Germany) to evidence bile ducts, CD138 and MUM1 (DakoCytomation, Glostrup, Denmark) to detect plasma cells.

Sections were scrutinized at ×10 magnification to assess the presence of CD138- and MUM1-stained cells. Staining with CD138 was soon discontinued as it labeled other structures, including part of the background. On the other hand, MUM1 stained only plasma cells, as demonstrated by staining colonic biopsies with chronic

Key Words: Liver, plasma cell precursors, MUM1, differential diagnosis, autoimmune hepatitis.

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inflammation, carrying a lymphatic follicle: while MUM1-stained plasma cells, the lymphocytes in the follicles remained unstained. In the absence of plasma cells in H&E-stained sections, MUM1-expressing cells were regarded as plasma cell precursors. MUM1 is a 50 kDa protein encoded by MUM1 gene, and a member of the interferon regulatory factor family of transcription factors. MUM1/IRF4 is expressed in the nuclei and cytoplasm of plasma cells and a small percentage of germinal center (GC) B-cells located in the “light zone.”

The numbers of MUM1-labeled cells found in three high-power fields (×40 objective) showing a priori the highest number of MUM1 positive cells, was calculated in the portal triads, the septal domain and the surrounding liver parenchyma.

Data was analyzed by the Mann-Whitney U non-parametric test. Statistical significance was defined as p<0.05.

Results

Clinical data showed that four patients had suspicion of AIH, four patients viral hepatitis C, two nonalcoholic steatohepatitis (NASH) and one fatty liver.

H&E sections from the four patients having clinical presumptive diagnosis of AIH displayed severe interphase hepatitis. The inflammatory infiltrates contained lymphocytes and few eosinophils. Plasma cells could not be discerned at high-power examination (×40) in three (Figure 1) out of the four cases. On the other hand, MUM1-immunostain in all four cases revealed distinct MUM1-expressing cells within the confines of the portal triads, in the zone corresponding to interface inflammation and in the parenchyma surrounding the interface hepatitis (Figure 2).

Table I. The numbers of MUM-1 expressing cells (range) in liver biopsies from 11 patients having either autoimmune hepatitis (AIH), chronic hepatitis C, non-alcoholic steatohepatitis (NASH) or fatty liver.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>MUM1-expressing cells (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune hepatitis</td>
<td>4</td>
<td>26-44</td>
</tr>
<tr>
<td>Chronic hepatitis C</td>
<td>4</td>
<td>2-15</td>
</tr>
<tr>
<td>NASH</td>
<td>2</td>
<td>1-3</td>
</tr>
<tr>
<td>Fatty liver</td>
<td>1</td>
<td>0-2</td>
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Table I shows that the numbers of MUM-expressing cells in three HPF ranged from 26 to 44 in the four cases with AIH. MUM-expressing cells were more often found in the zone corresponding to interface hepatitis. MUM1 labeled cells were not found in the bile duct epithelium. The difference in the number of MUM1-expressing cells between AIH and the other liver ailments shown in Table I was significant (p<0.05).

Discussion

AIH is not an uncommon disease. In Sweden, the incidence of AIH is 1/100,000 individuals/year and the prevalence 11/100,000 individuals/year (5).

In this preliminary work, three out of four cases with a presumptive clinical diagnosis of AIH showed no plasma
cells in H&E-stained liver sections, neither among the round cells found in the portal triads, nor at the interface, or the surrounding parenchyma. On the other hand, MUM1 immunostain detected plasma cell precursors in all four cases, more frequently at the interface and less frequently in the portal triads and the surrounding parenchyma. Plasma cell precursors were significantly more common in AIH than in chronic hepatitis C and in NASH.

MUM1 is a lymphocyte-specific transcriptional factor, member of the interferon regulatory factor (IRF) family, known to contribute to regulation of immunoglobulin gene expression in B-cell differentiation within germinal centre light zones (3). MUM1 is the missing link for identification of the transition from BCL6 positivity to CD138 expression. Defects in IRF4 may cause multiple myeloma, a malignant tumour of plasma cells.

These preliminary results suggest that MUM1 immunostain may be of help in endorsing a presumptive clinical diagnosis of AIH in liver biopsies and add valuable information in the differential diagnosis between AIH and the other liver diseases studied here.

Further studies will be necessary before MUM1 can be incorporated as a complement to routine stains in liver biopsies showing lymphocytic inflammation of portal triads with interface hepatitis in H&E stain.

This appears to be the first report in which MUM1 immunostain has been applied to assess the occurrence of plasma cell precursors in AIH.

References