A Method to Assess the Distribution and Frequency of Plasma Cells and Plasma Cell Precursors in Autoimmune Hepatitis

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Abstract. *Chronic inflammation exhibiting interface hepatitis and plasma cells in hematoxylin-eosin (H&E)-stained sections is typical of autoimmune hepatitis (AIH), a non-resolving inflammatory liver disease of unidentified cause. Some biopsies may only reveal lymphocytes and occasional granulocytes but no plasma cells. Recent studies on liver biopsies showed that the antibody against multiple myeloma oncogene-1 (MUM1) stained plasma cells (PC), and plasma cell precursors (PCP). Here, liver biopsies from 86 patients were stained with H&E, as well as for MUM1. The portal triad with the highest degree of chronic inflammation (hot-spot PTCI) was chosen for assessing both the topographic distribution and the frequency of MUM1-positive cells. In the 12 untreated AIH cases, MUM1-positive cells were found organized in an irregular ring-like fashion at the peripheral domain of the PTCI, but in none of the three medically-treated AIH cases. Only one out of the remaining 71 liver biopsies exhibited a similar ring-like arrangement, but the PTCI outline was sharp and the number of MUM1-positive cells was low. The highest mean number of MUM1-positive cells at the peripheral domain of the PTCI (59.2 cells) was found in AIH cases (AIH vs. other liver ailments, $p<0.05$). The highest mean number of MUM1-labelled cells in the core of the PTCI (83.3 cells) was found in PBC cases (PBC vs. other liver ailments $p<0.05$). Anti-MUM1 permits assessment of qualitative and quantitative PC/PCP changes evolving in autoimmune liver diseases. It is suggested that MUM1 may be of help in the histological differential diagnosis between autoimmune liver diseases and other liver ailments.*

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Autoimmune hepatitis (AIH) is a non-resolving inflammatory disease of unidentified cause that affects the portal triads, leading to progressive inflammatory expansion beyond the limiting septa with the gradual destruction of the adjacent parenchyma, cirrhosis and eventually to hepatic failure (1). At present, the diagnostic criteria of AIH include the presence of elevated serum IgG or γ-globulins and serum antinuclear autoantibodies (ANA), anti-smooth muscle antigen (ASMA), anti-liver kidney microsomal antigen (LKM), soluble liver antigen (SLA), defined autoantibodies or histocompatibility leucocyte antigen (HLA), and antineutrophil cytoplasmic antibodies (atypical p-ANCA). The diagnosis of AIH is substantiated following a positive treatment response to corticosteroids (1-8).

The International Autoimmune Hepatitis Group (1, 3) has emphasized that a definitive diagnosis of AIH should include characteristic liver histology. In sections stained with hematoxylin and eosin (H&E) the histological features of AIH include moderate or severe inflammatory activity in the outermost confines of the portal tracts bordering the lobules, disruption of the limiting septa (interface hepatitis), lobular disarray, focal parenchymal necrosis, rosette formations and central-portal bridging necrosis or cirrhosis (2). The presence of numerous plasma cells (PCs) is characteristic of AIH but non-specific, as PCs may also be encountered in viral hepatitis (3). Other cases exhibit only lymphocytes, occasional eosinophils and/or neutrophils (4). In the absence of PCs in H&E staining and bearing in mind that the other histological parameters might not be absolutely pathognomonic of AIH (5), pathologists are often reluctant to pass a definitive diagnosis. Because of these limitations, the possibility that the presumptive clinical diagnosis and the laboratory data given by clinicians might influence the histopathological appreciation cannot be totally rejected. In this context, Czaja classified the histological parameters of AIH into compatible, typical, and incompatible (6). This classification was apparently intended to confirm or to reject a presumptive clinical diagnosis of AIH.

Due to the difficulties in the histological diagnosis of AIH in many cases, it appears desirable to develop a reliable

Figure 1. *Autoimmune hepatitis. Irregular ring-shaped arrangement of (MUM1)-expressing plasma cells/plasma cell precursors at the periphery of a portal triad with chronic inflammation (liver biopsy; MUM1 immunostain, ×10).*

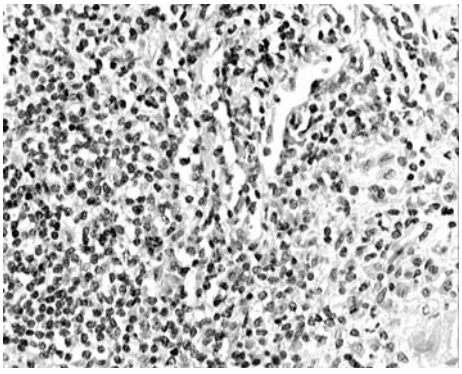

Figure 2. *Autoimmune hepatitis. High-power view of the same liver biopsy as shown in Figure 1, stained with hematoxylin and eosin, showing no plasma cells with this stain (liver biopsy; H&E, ×20).*

method that would permit to diagnose this ailment at the microscopic level, unbiased by the clinical data.

In a preliminary study, we investigated the number of PCs by the aid of immunostaining for multiple myeloma oncogene-1 (MUM1) in 11 liver biopsies (9). MUM1 is a 50kDa protein member of the interferon regulatory family of transcription factors (interferon regulatory factor-4 gene, *IRF4*). MUM1 contributes to the regulation of immunoglobulin gene expression in the final step (late centrocyte) of B-cell differentiation within germinal centre light zones, initiated by centrocyte-follicular dendritic cell contact (10).

When colonic biopsies with chronic inflammation carrying a lymphatic follicle were stained with H&E, it was found that discrimination between plasma cells and lymphocytes in the *lamina propria mucosa* (*lpm*) and in the lymphocytes in the lymphatic follicle, was fairly easy (10, 11). However, when parallel sections were stained with MUM1, not only PCs in the *lpm* were MUM1-positive but also were many

Figure 3. *Hepatitis C. Regular ring-shaped arrangement of (MUM1)-expressing plasma cells/plasma cell precursors, at the periphery of a portal triad with chronic inflammation (liver biopsy; MUM1 immunostain, ×10).*

cells regarded as lymphocytes in H&E (10, 11). Obviously, these MUM1-expressing lymphocytes were PC precursors (PCP) (12). In the lymphatic follicle all lymphocytes in H&E were MUM1-negative.

Since PCPs are apparently not recognized in H&E-stained sections, we investigated a relatively large cohort of liver biopsies stained with MUM1, aiming to visualize both PCs and PCPs in various liver diseases, including AIH.

Materials and Methods

Needle liver biopsies from 86 consecutive patients were investigated. Sections were stained with H&E, periodic acid Schiff (PAS), PAS-diastase, Pearl's reaction, Sirius stain (without contrast), Gordon-Sweet, cytokeratin-7 (CK7; Leica Microsystems, Wetzlar, Germany) and MUM1 immunostain (Dako Cytomation, Glostrup, Denmark). Initially, sections were also stained with CD138, but this was soon discontinued as this immunostain also labeled other structures, as well as part of the background.

Review of filed MUM1-stained sections was carried out blind to the clinical data. Using low-power examination (×4 objective), all portal triads with chronic inflammation (PTCI) were inspected. PTCI showing *a priori* the highest degree of inflammation with the highest number of MUM1-positive cells (hot spot PTCI) was chosen for assessing both the topographical distribution and the number of MUM1-positive cells.

Liver biopsies were considered adequate when containing ≥12 portal spaces.

The topographical distribution of MUM1-positive cells was assessed using a 10× objective. Two cell domains were recorded: a) The outermost layer of inflammatory cells in the PTCI, bordering the parenchyma, and b) the remaining PTCI core.

Counting of MUM1-positive cells was performed in the same two PTCI-cell domains, using ×40 magnification.

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

Figure 4. *Chronic hepatitis. Note occasional (MUM1)-expressing plasma cells/plasma cell precursors in a portal triad with chronic inflammation (liver biopsy; MUM1 immunostain, ×20).*

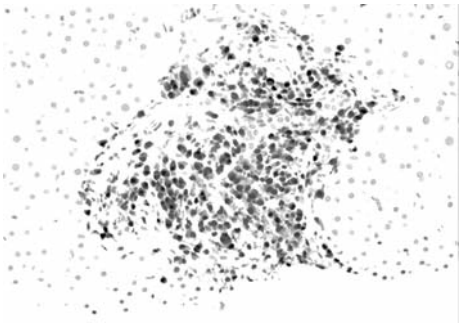

Figure 5. *Primary biliary cirrhosis. Note central agglutination of (MUM1)-expressing plasma cells/plasma cell precursors, about bile ducts in a portal triad with chronic inflammation (liver biopsy; MUM1 immunostain, ×20).*

Results

Distribution of MUM1-labelled cells in the outermost cell layer of the PTCI. The results show that MUM1-positive cells were found organized in an irregular ring-like fashion in the peripheral domain of the PTCI (Figures 1 and 2) in all 12 cases with AIH (100%).

In one of the hepatitis C virus (HCV) cases, MUM1-positive cells were also organized in a ring-like fashion in the peripheral domain of the PTCI but this border was sharp, thus lacking the irregularity of contours recorded in AIH (Figure 3). In the remaining 73 cases listed in Table I, including the three cases of AIH that had received medical treatment, MUM-positive cells arranged in a ring-like pattern were not found (Figure 4). The difference between the occurrence of an of irregular ring pattern of MUM1-positive cells in AIH and the other liver diseases listed in Table I was significant ($p<0.05$).

Figure 6. *Overlap syndrome (autoimmune hepatitis and primary biliary cirrhosis). Note irregular ring-shaped arrangement of (MUM1)-expressing cells at the periphery, as well as in the central core (around bile ducts) of a portal triad with chronic inflammation (liver biopsy; MUM1 immunostain, ×20).*

Table I. *The mean number of (MUM1)-positive cells (range in brackets) at the periphery and within the core of the portal triad with chronic inflammation (PTCI) in 86 liver biopsies.*

		Mean number of MUM1-positive cells	
	No. Cases	Periphery of PTCI domain	Core of PTCI domain
AIH	12	59.2 (22-104)	34.7 (10-92)
AIH (treated)	3	9.3 (2-19)	5.0 (2-7)
PBC	4	22.5 (5-47)	83.3 (48-116)
Chronic hepatitis	17	1.1 (0-9)	5.5 (1-20)
HCV/HBV	23	7.3 (0-30)	20.5 (1-97)
NASH	9	2.8 (0-13)	9.6 (1-31)
Toxic hepatitis	2	2.5 (1-4)	9.5 (5-14)
Cryptogenic cirrhosis	10	0.4 (0-2)	10.2 (1-29)
Liver adjacent to metastasis	6	0.3 (0-2)	3.0 (0-15)

AIH: Autoimmune hepatitis, PBC: primary biliary cirrhosis, HCV/HBV: hepatitis C/B virus, NASH: non-alcoholic steatohepatitis.

Number of MUM1-labelled cells in the outermost cell-domain of the PTCI. Table I shows that the highest number of MUM1-positive cells in the outermost cell layer of the PTCI was recorded in AIH cases (mean=59.2 labelled cells), followed by cases with primary biliary cirrhosis (PBC) (mean=22.5 labelled cells), and by AIH cases that had received medical treatment (mean=9.3 labelled cells). On the other hand, cases with chronic hepatitis, HCV/HBV, fatty liver and non-alcoholic steatohepatitis (NASH), cryptogenic cirrhosis, metastasis or toxic hepatitis had a mean of 7.3 MUM1-labelled cells. The difference between the number of MUM1-expressing cells in the outermost cell layer of the PTCI in AIH and in the other liver diseases listed in Table I was significant ($p<0.05$).

In the remnant PTCI core: Table I also shows that the highest number of MUM1-labelled cells in the remnant PTCI core was recorded in cases with PBC (mean=83.3 MUM1-positive cells) (Figure 5), followed by cases with AIH (mean=34.7 MUM1-positive cells). On the other hand, a mean number of MUM1-expressing cells of 20.5 was found in cases with medically treated AIH, with chronic hepatitis, HCV/HBV, fatty/NASH, cryptogenic cirrhosis, metastasis or toxic hepatitis. The difference between the number of MUM1-expressing cells in the remnant PTCI core in PBC, and the other liver diseases shown in Table I was significant ($p<0.05$).

Clinical data showed that all patients with AIH had type-1 AIH.

Discussion

The present study of histological sections from liver biopsies confirmed the preliminary results (9), namely that MUM1 labels not only PCs but also PCPs.

The qualitative scrutiny of cases with AIH showed that the MUM1-positive cells were arranged in an irregular, ring-like fashion at the periphery of the PTCI domain bordering the liver parenchyma. Liver diseases other than AIH lacked this irregular ring-like arrangement of MUM1-positive cells. Although the significance of this unexpected finding remains unclear, one plausible explanation seems to be that MUM1-positive cells in AIH had been summoned to the parenchyma bordering the PTCI, where the putative autoantigen eliciting the autoimmune reaction should be located. Although the detection of liver-related autoantibodies using immuno-serological approaches has been widely used for diagnosis and prognosis in AIH, the detection of the disease requires the identification and characterization of disease-specific autoantigens (13). Recently, Qifeng *et al*. identified three new autoantigens in AIH, namely (RPS20), Alba-like and dUTPase, as highly AIH-specific biomarkers (13). Consequently, the findings presented in this communication appear to testify to the struggle at the battlefront between autoantibodies (carried by PCs/PCPs at the periphery of the PTCI) and autoantigens (present in the hepatocytes). Rationally, the PTCI might be expanding outwards in AIH, *pari passu* with the relentless progression of the autoimmune inflammatory process in untreated patients. Medical treatment seemed to have abrogated the active autoimmune process, as deduced by the disappearance of the irregular ring-like MUM1 pattern and the dramatic decrease in the number of MUM1-positive cells at the PTCI periphery in the three treated AIH cases.

One of the cases with HCV had MUM1-positive cells arranged at the periphery of PTCI, thus resembling AIH cases. However, the PTCI in this case was incomplete and sharp (Figure 3). In this context it may be of interest to mention that Badiani *et al*. (14) found that out of 1759 patients chronically infected with HCV, 92 had intense interface hepatitis, but only 2 (2%) had typical histological findings of AIH. After applying the scoring system for diagnosis of AIH, only one patient was classified as having definitive AIH. Thus, chronic HCV, intense interface hepatitis and AIH is a rare combination (14). Whether our case of HCV showing incomplete and sharp MUM1-positive cells at the periphery of PTCI had an autoimmune component could not be confirmed by the laboratory data.

Quantitative calculations at the periphery of the PTCI cell domain demonstrated that the number of MUM1-positive cells was highest in AIH than in all other liver diseases investigated here. Interestingly, the second highest number of MUM1-positive at the periphery of the PTCI was recorded in another autoimmune disease, namely in PBC. However, the number of liver biopsies with PBC was too small to permit a definitive conclusion regarding its significance in diagnostic work. Analysis of more liver biopsies in PBC cases by immunostaining with MUM1 is necessary before these preliminary observations can be substantiated.

Notably, when AIH cases were excluded, the mean number of MUM1-positive cells at the periphery of the PTCI cell domain was significantly higher in PBC than in other liver diseases. Moreover, quantitative calculations within the PTCI core domain demonstrated that the number of MUM1-positive cells was highest in PBC than in all other liver diseases investigated, including AIH. Hence, MUM1 immunostaining emerges as an important tool to disclose the high frequency of PCs/PCPs within the PTCI core in PBC. Of interest, Boberg *et al*. (3) found that out of 84 cases considered as classical AIH, as many as 20 (24%) had biliary changes.

We found that the second highest number of MUM1-positive cells in the PTCI core was recorded in AIH, suggesting that bile ducts in the PTCI in cases with AIH might carry autoantigens, although to a lesser degree than in PBC. In this context, it should be understood that PBC and AIH are distinct autoimmune chronic liver diseases that may co-exist in the same patient (overlap syndrome) (15). The wide variation in the prevalence of the overlap syndrome in PBC may in part be due to the difficulty in diagnosing these two distinct autoimmune liver diseases in the same patient as well as in the lack of a uniform definition of this autoimmune setting (15). Interestingly, in one case with overlap syndrome we found that MUM-1-positive cells were found both at the periphery of the PTCI cell domain and agglutinated about bile ducts within the core of the PTCI (Figure 6).

It may be of interest to mention that only one out of the 86 cases lacked MUM1-positive cells in the PTCI. Hence, it would appear that PCs/PCPs are present in almost all liver

diseases showing chronic inflammation, the difference between autoimmune and other liver diseases being a matter of degree, as regards to numbers and distribution.

Both the characteristic immunohistochemical distribution, as well as the increased frequency of PCs/PCPs in AIH, is at variance with reports on AIH in recent literature, where it is stressed that PCs may be lacking from the infiltrate and that none of the histopathological findings in AIH are specific (1-8, 13-15). In contrast, Hoeroldt *et al*. (16), by studying 245 patients recently reported that "histology was consistent with AIH in all cases and did not suggest other diagnosis". Unfortunately, the histological parameters recorded in H&E sections in the 245 patients were not presented.

In conclusion, the results of this investigation strongly suggest that MUM1 immunostaining is a robust biomarker that discloses profound qualitative and quantitative PC/PCP changes occurring in autoimmune liver diseases. MUM1 adds valuable information to the differential diagnosis between AIH, PBC and the other liver diseases presented here. Based on the present findings, MUM1 has been incorporated at this Department into routine staining of liver biopsies, with the aim to diagnose autoimmune liver diseases at the microscopic level, unbiased by clinical data.

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