Virchows Arch (1997) 431:189–194 © Springer-Verlag 1997

ORIGINAL ARTICLE

Zhong Jiang · Yu Liu · Lou Savas · Lynda Smith
Herbert Bonkovsky · Stephen Baker
Barbara Banner

Frequency and distribution of DNA fragmentation as a marker of cell death in chronic liver diseases

Received: 17 February 1997 / Accepted: 18 March 1997

Abstract To study the early stages of cell death in various types of chronic liver injury, liver biopsies from a total of 26 patients, including 7 with chronic hepatitis C(CHC), 4 with chronic hepatitis B(CHB), 7 with alcoholic liver disease (ALD), 4 with autoimmune or drug hepatitis(AI/DH), and 4 with primary biliary cirrhosis(PBC), were examined by an in situ nucleotidyl transferase assay (ISNTA), which detects DNA fragmentation. Positive nuclei in hepatocytes and sinusoidal lining cells were counted in all parenchymal areas, excluding triads and areas of fibrosis, using a computer with Sigmascan software. The number of positive hepatocytes/mm^2 was similar in the biopsies of patients with CHC, CHB, ALD and AI/DH, but significantly lower in PBC. The number of positive sinusoidal lining cells/mm^2 was significantly greater in biopsies with CHC compared to CHB, ALD, AI/DH and PBC. Double staining revealed that the ISNTA-positive sinusoidal lining cells were also CD68 positive, indicating that they were Kupffer cells. The frequency of ISNTA positivity did not correlate with serum AST or ALT levels, steatosis, cell swelling or cirrhosis. ISNTA-positive hepatocytes were more frequent than acidophilic bodies in every disease category. We conclude that apoptosis may be a common pathway of cell death in different liver diseases, that the high frequency of DNA fragmentation in Kupffer cells in CHC suggests that during chronic hepatitis C infection activated Kupffer cells may be subject to regulatory control by apoptosis and that ISNTA is more sensitive than acidophilic bodies in assessing the degree of cell injury in the liver.

Key words Apoptosis · Liver disease · Hepatitis · Hepatocytes · Kupffer cells

Z. Jiang · Y. Liu · L. Savas · B. Banner (✉)
Department of Pathology,
University of Massachusetts Medical Center, Worcester,
MA 01655, USA,
Tel.: (01) 508-856-6436, Fax: (01) 508-856-2968

L. Smith · H. Bonkovsky
Department of Medicine,
University of Massachusetts Medical Center, Worcester,
MA, USA

S. Baker
Department of Academic Computing,
University of Massachusetts Medical Center, Worcester,
MA, USA

Introduction

Apoptosis is a process by which cell death occurs by a highly specific series of enzyme-mediated reactions, resulting in fragmentation of the DNA [2, 4, 5, 7, 11, 16, 21, 26, 32–34]. Apoptosis may occur as a genetically programmed process for tissue remodeling or cell turnover, or it may be triggered by multiple factors such as inflammatory cytokines, viruses, and chemical or physical agents [4, 5, 7, 9, 11, 21, 23, 33, 34].

The extent to which apoptosis contributes to the acidophilic bodies found in liver diseases is not known, as the acidophilic bodies represent the end stage of cell death, and the mechanisms of their formation are difficult to study by routine histological techniques [17]. In situ nucleotidyl transferase assay (ISNTA) provides a sensitive technique for staining fixed tissue sections for DNA fragmentation, which occurrs early during apoptosis [1, 2, 8, 10, 23, 25, 27, 28]. DNA fragmentation, as detected by ISNTA, has been reported also in necrosis and autolysis [1, 6, 13] suggesting that this assay may not be specific for apoptosis. However, in the absence of other evidence for necrosis, i.e. pyknotic nuclei, disintegration of the plasma membrane and zonal necrosis, it is highly likely that ISNTA-positive nuclei do represent cells undergoing apoptosis. The aims of this study were to use the ISNTA assay, which detects the products of DNA fragmentation in the nucleus prior to cell death, to detect evidence of cell injury at an early stage and to compare chronic liver diseases of different aetiologies with respect to the frequency of ISNTA-positive cells. We also wished to determine whether the acidophilic bodies seen in H&E sections adequately represent the degree of cell injury. The assay revealed DNA fragmen-

tation in otherwise intact cells, suggesting that apoptosis is a common pathway of cell death in different liver diseases. Also, in chronic hepatitis C (CHC) there was a high frequency of ISNTA-positive sinosoidal lining cells, which may be Kupffer cells, suggesting that during hepatitis C virus infection, Kupffer cells are subject to injury and may also undergo apoptosis.

Materials and methods

Cases of chronic liver diseases of diverse etiologies were identified in the surgical pathology files of University of Massachusetts Medical Center (UMMC) between January 1992 and April 1995. A total of 26 liver needle-biopsies were randomly selected from patients with chronic hepatitis C (CHC, $n = 7$), chronic hepatitis B (CHB, $n = 4$), alcoholic liver disease (ALD, $n = 7$), autoimmune or drug hepatitis(AI/DH, $n = 4$) and primary biliary cirrhosis(PBC, $n = 4$). A wedge biopsy from a normal liver resected for trauma was also included to serve as a normal control.

The 7 patients with CHC included 5 men and 2 women, ranging in age from 33 to 59 years. All were seropositive for hepatitis C by second generation enzyme immunoassay and recombinant immunoblot assay tests, and had been referred to UMMC for evaluation for interferon-α (IFN-α) therapy. The patients had no other known causes of their liver disease; ceruloplasmin and alpha-1-antitrypsin levels were normal and titers for anti-smooth muscle antibodies were (< 1/80) low or nondetectable. Hemochromatosis was excluded by examination of sections of liver biopsies stained by Gomori's method for iron (based on the Prussian blue reaction). Two patients had no prior therapy for hepatitis; 5 patients had received standard dose IFN-α and IFN-α plus levamisole, and were being re-biopsied because of relapse. All 7 patients were seronegative for markers of hepatitis B infection.

The 4 patients with CHB were men, ranging in age from 27 to 47 years. All were seropositive for hepatitis B and seronegative for markers of hepatitis C. All were referred for IFN-α therapy. The 7 patients with ALD included 5 men and 2 women, ranging in age from 32 to 73 years. The 4 patients with AI/DH included 2 men and 2 women, ranging in age from 42 to 65. The 4 patients with PBC included 1 man and 3 women, ranging in age from 22 to 64 years. Five patients with ALD and all with autoimmune hepatitis and PBC were seronegative for markers of hepatitis. The markers of viral hepatitis in the remaining 4 cases of ALD or drug hepatitis were not available, but clinical and pathological diagnoses of all these 4 cases were either ALD or drug hepatitis.

All biopsies were performed using a Klatskin- modified Menghini needle. The needle biopsies were fixed in Carnoy's solution and the wedge biopsy was fixed in formalin. All were processed routinely for light microscopy and stained with H + E, Trichrome and Gomori's iron stain.

The principle of the ISNTA is that DNA fragmentation, an early event in the apoptotic process, yields regular arrays of free DNA ends with 3'-OH groups that can be enzymatically elongated by terminal deoxynucleotidyl transferase and labeled with nucleotides that can be visualized subsequently, using the ApopTag Kit (Oncor, Gaithersburg, Md.). Since DNA fragments occurring during necrosis or autolysis may also react in this assay, interpretation of positive staining must include an assessment as to whether other criteria for necrosis are present and whether the cell in question appears otherwise viable.

For staining, paraffin-embedded sections were cut at 5 microns. After deparaffinization the sections were incubated with hydrogen peroxide to quench endogenous peroxidase. Sections were subjected to enzymatic homopolymeric tailing with TdT and digoxigenin-labeled nucleotides for 1 h at 37°C in a humidified atmosphere. The incorporated nucleotides were revealed by incubation with anti-digoxigenin antibody conjugated to peroxidase for 30 min at room temperature. The peroxidase label was then visualized by the diaminobenzidine reaction. All sections were counterstained with hematoxylin.

There were 7 cases available to achieve satisfactory results by double staining for apoptosis and for markers to identify Kupffer cells. Anti-CD68 monoclonal antibody (DAKO, Carpinteria, Calif.) detecting Kupffer cells [20] was used for immunocytochemical staining followed by ISNTA to identify DNA fragmentation. Briefly, the slides were microwaved in phosphate-buffered saline (pH 7.4) for 5 min in an 800 watt microwave oven. Following replenishment of this solution, the slides were microwaved again for an additional 5 min and allowed to cool for 20 min. Following a hydrogen peroxide block of endogenous peroxide and a serum blocking step, the slides were incubated with the primary antibody at a dilution of 1:500 for 45 min followed by brief buffer washes. They were incubated in a cocktail of biotinylated anti-mouse IgG/IgM and anti-rabbit IgG (BioTek, Santa Barbara, CA) for 30 min. The sections were then washed, incubated in avidin/biotin complex (BioTek) for 30 min, washed, then reacted with diaminobenzidine and hydrogen peroxide to visualize the brown product. The slides were further processed using ApopTag Kit as previously described except that anti-digoxigenin antibody conjugated to alkaline phosphatase replaced antibody conjugated to peroxidase in order to visualize apoptotic nuclei as red.

The morphological evaluation was carried out on the H + E stained slides. The following features were noted to be present or absent in each biopsy: acidophilic bodies, steatosis, cell swelling, lobular inflammation, piecemeal necrosis, portal inflammation, cirrhosis and activated sinusoidal lining cells, identified as enlarged or prominent cells along the sinusoids. Lobular inflammation was graded as follows: Grade 1 = patchy (only a few triads or lobules involved) and inflammatory cell infiltrate is sparse; Grade 2 = majority of lobules involved and inflammatory infiltrate is prominent.

Apoptotic cells were counted in ISNTA-stained slides. All parenchymal areas excluding triads and fibrosis were identified using a computer with Sigmascan software (Jandel Scientific, Cort Medera, Calif.). Positive nuclei stained strongly and were easily identified. ISNTA-positive hepatocytes were identified by their location in hepatic cords, and sinusoidal lining cells were identified by their position along sinusoids. Cells in sinusoidal lumens were considered to be inflammatory cells and were not counted. The numbers of apoptotic hepatocytes and sinusoidal lining cells mm^2 were determined. Acidophilic bodies were counted in a similar manner in the H + E stained slides. Serum alanine aminotransferase and aspartate aminotransferase (ALT and AST) values were recorded if available within 1 month of the date of the biopsy.

The diagnostic groups were compared statistically with respect to the frequencies of apoptotic cells/mm^2 in hepatocytes and sinusoidal lining cells, using the SPSS statistic package (Chicago, IL). The assumption of normality of the errors of the principal outcomes was evaluated graphically by plotting histograms of standardized residuals. These measures were transformed using natural logarithms, then normality was assessed graphically again. The Kolmogorov Smirnov goodness-of-fit test for normality was used to determine if significant deviations from normality existed, even though the transformed variables appeared normal. These procedures indicated that the results were normally distributed. One way analysis-of-variance (for completely randomized designs) was then used to test omnibus hypotheses of no difference in population means. In the presence of significant main effects, pairwise comparisons were made using the Tukey's multiple comparisons procedure.

Results

The histologic features of all biopsy specimens are shown in Table 1. Acidophilic bodies were seen in all cases of CHC and CHB, often surrounded by lymphocytes. No specific zonal distribution was noted. All biopsy specimens demonstrated some degree of steatosis, but steatosis was more severe in ALD than in the other

Table 1 Frequencies (%) of Histologic Features in Each Biopsy Group
Percent of biopsies positive for each feature

Histological feature number of cases	CHC *n*=7	CHB *n*=4	ALD *n*=7	AI/DH *n*=4	PBC *n*=4
Acidophilic bodies	100	100	83	75	50
Steatosis	100	100	100	100	100
Cell swelling	15	75	66	75	50
Piecemeal necrosis	77	77	77	50	0
Active sinusoidal cells	100	100	66	50	25
Cirrhosis	15	25	50	50	0
Portal inflammation	100	100	100	100	100
Lobular inflammation					
Grade 1	50	50	29	25	0
Grade 2	15	50	71	50	0

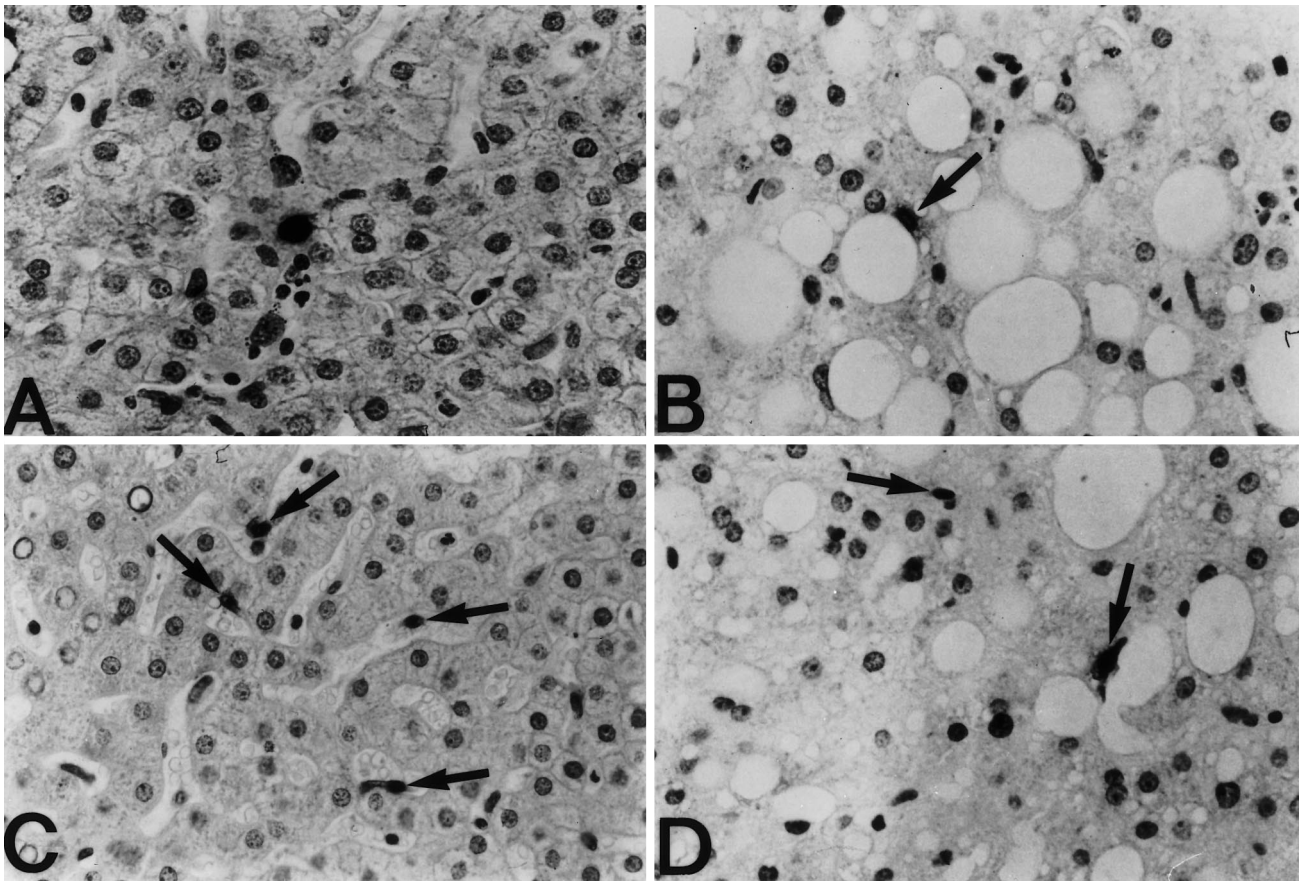

Fig. 1A–D Composite photomicrographs showing ISNTA-positive hepatocytes and sinusoidal lining cells in liver biopsies from patients with CHC and ALD: (A) ISNTA-positive hepatocyte in CHC; (B) ISNTA-positive hepatocyte with steatosis in ALD; (C) ISNTA-positive sinusoidal lining cells in CHC; (D) ISNTA-positive sinusoidal lining cell in ALD. (ISNTA with haematoxylin counterstain, ×200)

groups. Activation of sinusoidal lining cells was found in all cases but was more frequent in CHC and CHB, than in the other diseases. Cirrhosis was seen more frequently in biopsies with ALD and AI/DH than in biopsies with CHB and CHC. No cirrhosis was found in the biopsies with PBC, all of which were Stage 1. Lobular inflammation was present in all groups except PBC. Grade 2 inflammation was most common in alcohol injury and was characterized by polymorphonuclear infiltrates. Grade 2 lobular infiltrates with lymphocytes predominating was present in half the cases of CHB and AI/DH.

In the normal liver only rare ISNTA positive cells were found. Positive cells were single and widely scattered. Positive staining in hepatocytes and sinusoidal lining cells in biopsies with hepatitis C and alcohol liver disease is illustrated in Fig. 1. Single positive cells without nuclear shrinkage or other evidence for necrosis were found in CHC (Fig. 1A). Some steatotic hepatocytes in ALD were also positive (Fig. 1B). The mean numbers (±SEM) of positive hepatocytes/mm^2 for the five disease groups are shown in Fig. 2. Statistical analysis showed that the mean numbers of positive hepatocytes/mm^2 were similar in CHC, CHB and ALD, and significantly differ-

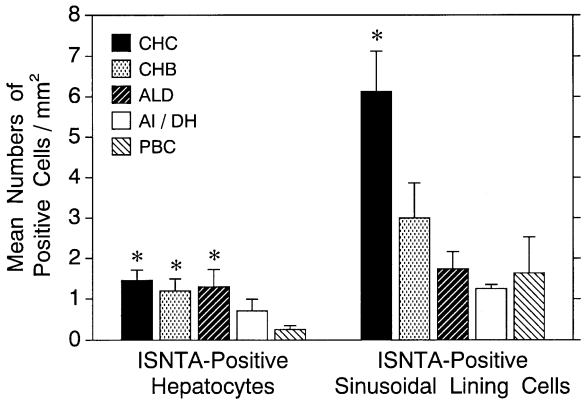

Fig. 2 Composite bar graph showing the mean numbers (± SEM) of ISNTA-positive hepatocytes and sinusoidal lining cells for each diagnostic group. The frequency of occurrence of ISNTA-positive hepatocytes was similar in CHC (n = 7), CHB (n = 4), and ALD (n = 7). The difference between the mean frequencies in these groups and those in AI/DH (n = 4) and PBC (n = 4) was significant (* = Tukey-HSD test, significant at level 0.05). The frequency of ISNTA-positive sinusodial lining cells was significantly greater in CHC compared to CHB, ALD, AI/DH and PBC (* = Tukey-HSD test, significant at level 0.05)

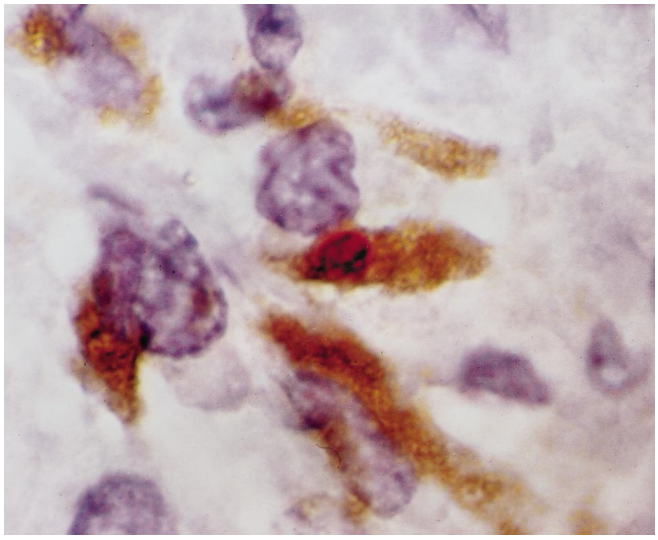

Fig. 3 Liver biopsy from a patient with CHC double stained with ISNTA (red) and CD68 antibody (brown) to identify Kupffer cells. Note the ISNTA-positive Kupffer cells in the center. (Haematoxylin counterstained, ×1000)

ent from the mean numbers in AI/DH and PBC (Tukey-HSD test with significance level 0.05). Serum AST and ALT levels within one month of biopsy were available in 16 patients. There was no correlation between the frequency of ISNTA-positive cells and the levels of AST and ALT, the degree of steatosis, cell swelling, inflammation or cirrhosis (results not shown).

In normal liver ISNTA-positive sinusoidal lining cells were not found. The numbers of ISNTA-positive sinusoidal lining cells for the five disease groups are shown in Fig. 2. In every disease category, positive staining was more frequent in the sinusoidal lining cells than in the

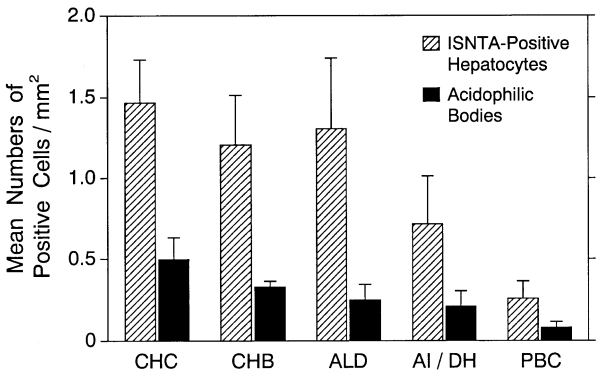

Fig. 4 Bar graph showing the mean numbers (± SEM) of ISNTA-positive hepatocytes detected by ISNTA, and acidophilic bodies counted in H + E-stained sections. In each disease category (CHC, n = 7; CHB, n = 4; ALD, n = 7; AI/DH, n = 4; PCB, n = 4) the frequency of ISNTA-positive hepatocytes/mm^2 was significantly greater than that of acidophilic bodies (* = Tukey-HSD test, significant at level 0.05)

hepatocytes. The numbers of apoptotic sinusoidal lining cells/mm^2 were significantly greater in CHC compared to CHB, ALD, AI/DH and PBC (Tukey-HSD test with significance level 0.05). In 7 cases, including 5 HCV and 2 ALD, double staining revealed that sinusoidal lining cells were also CD68 positive. Only one CD68-negative ISNTA-positive sinosoidal lining cell was found in one HCV biopsy. An example of a sinusoidal lining cell staining positive for both ISNTA and CD68 is shown in Fig. 3.

The mean numbers of acidophilic bodies are shown in Fig. 4. Comparison of the frequencies of ISNTA-positive hepatocytes and the acidophilic bodies counted in H + E-stained sections showed that the frequency of ISNTA-positive hepatocytes/mm^2 was greater than that of acidophilic bodies/mm^2 in every disease category.

Discussion

Apoptosis is a process by which single cells die by a step-wise, enzymatically mediated serial fragmentation of nuclear DNA without cell membrane injury, as opposed to necrosis, in which the DNA is denatured randomly along with other cellular proteins [7, 9, 19, 24]. Apoptotic cells are immediately phagocytosed by neighboring cells. Theoretically there is no inflammatory response to this process, in contrast to necrosis, in which inflammation may be considerable [3]. Apoptosis is thought to occur under many different conditions [4, 5, 11, 18, 22, 33, 34]. It is the mechanism for deleting cells and reshaping tissue during embryogenesis and maturation of tissue, and during the physiologic atrophy in response to aging or hormonal withdrawal [9]. More germaine to this study is that viruses and cytokines, such as TNF and TGFβ, may trigger the endonucleases which mediate apoptosis [14, 15, 19, 26, 35].

In the liver, acidophilic bodies have always been assumed to be necrotic hepatocytes, but they are also

called “apoptotic bodies”. They clearly represent the end stage of cell degeneration, but the mechanisms of their formation are not known. There is mounting evidence that apoptosis may be the mechanism for hepatocellular death in viral infections, such as adenovirus, EBV, and HIV [8, 28, 31]. It is assumed that continuing inflammation in response to pathogens, such as viruses, or continuing assualt on hepatocytes by viruses or mutated viruses is the basis for ongoing cell death in chronic, as opposed to acute liver disease. The acidophilic bodies seen in chronic viral hepatitis may indeed be apoptotic bodies [23]. Our hypotheses were that ISNTA-positive cells would be more frequent in CHC, CHB and autoimmune hepatitis than in the other diseases, and that ISNTA assay would be more sensitive than counts of acidophilic bodies in assessing the amount of cell damage in the biopsies.

There is growing controversy over the specificity of the ISNTA assay used here. Positive staining by this technique has been reported in otherwise indisputable autolysis and necrosis [1, 7, 13]. In our study none of the biopsies showed zonal or bridging necrosis. In apoptosis, the DNA changes precede disintegration of the cell membrane, while in necrosis these changes follow injury of the cell membrane. Our results showed that the ISNTA-positive hepatocytes were single, widely scattered and otherwise viable, with well defined cytoplasm and cell membranes. In addition, our double staining study showed that the ISNTA-positive lining cells expressed CD68. Therefore, we interpreted positive ISNTA staining as apoptosis, since the cells with ISNTA-positive nuclei had morphologically intact cytoplasm and expressed cytoplasmic antigen (CD68). All had been fixed immediately at the biopsy procedure, ruling out autolysis.

In all disease categories, the number of apoptotic cells detected by the ISNTA assay was much higher than the number of acidophilic bodies counted in the biopsies. This was to be expected, since acidophilic bodies, whether they are the end stage of apoptosis or necrosis, should be rapidly removed from the liver. We also expected that, the mean number of ISNTA-positive cells/mm^2 would be high for CHC and CHB. Surprising findings were the lower frequency of ISNTA-positive hepatocytes in autoimmune and drug hepatitis, and the high frequency in ALD. In the latter biopsies, ISNTA-positive hepatocytes were located in areas of steatosis. Although alcohol has been shown to damage hepatocyte membranes directly, and indirectly through metabolites, there is evidence that some metabolic products (acetaldehydes) may induce apoptosis [36]. Apoptosis has not been studied in autoimmune, drug hepatitis, or PBC. Of interest in this study was the lack of correlation between the frequency of ISNTA-positive hepatocytes and the serum levels of ALT and AST. This finding supports the view that apoptosis is not associated with leakage of cellular contents into the extracellular apace [4].

Another unexpected finding was the high rate of ISNTA-positive sinusoidal lining cells. It is possible that some of the cells counted in the sinusoids were circulating haematopoietic cells. However, we tried specifically to avoid counting any cells in the lumen, or cells that resembled WBCs, and counted only cells which were part of the lining of the sinusoid. All of the biopsies were counted in the same manner, and all contained either portal or lobular inflammation. However, even with these unifying features, the number of ISNTA-positive sinusoidal lining cells was significantly greater in biopsies of patients with CHC than in the biopsies from the other disease groups. Using a double staining technique, we found that the ISNTA-positive lining cells were Kupffer cells. One ISNTA-positive, CD68-negative cell was found, but all of the other ISNTA-positive lining cells were also CD68 positive. This strongly suggests that Kupffer cells may be subject to regulatory control [17] and that the hepatitis C virus, cytokines, or possibly alcohol may induce apoptosis in these cells.

In summary, we used an ISNTA assay to detect DNA fragmentation, which occurs in the early stages of apoptosis in liver diseases of diverse etiologies. As expected, injured cells detected by this technique were more frequent than acidophilic bodies. Significant differences were detected in the frequency of apoptotic hepatocytes between CHC, CHB and ALD and the other diseases. An unexpected finding was the high frequency of ISNTA-positive Kupffer cells in all biopsies, but particularly in chronic hepatitis C. This suggests that both Kupffer cells and hepatocytes are subject to regulation by apoptosis in hepatitis C infection. The pathways leading to such effects are uncertain, but likely depend upon cytokines elaborated by several cell types in response to viral infection.

Acknowledgements This work was supported by the Departments of Pathology and Medicine at UMMC, the former for preparation and staining of the tissues and the latter for accumulation of clincial data on the patients. We are grateful for the assistance of Mrs. Karen Balcius in typing the manuscript.

References

1. Ansari B, Coates J, Greenstein D, Hall PA (1993) In situ end-labelling detects DNA strand breaks in apoptosis and other physiological and pathological states. J Pathol (Lond) 170:1–8
2. Arends MJ, Morris RG, Willie AH (1990) Apoptosis: the role of the endonuclease. Am J Pathol 136:593–608
3. Bursch W, Paffe S, Putz B (1990) Determination of the length of the histological stages of apoptosis in normal liver and altered hepatic foci of rats. Carcinogenesis 11:847–853
4. Carson DA, Ribeiro JM (1993) Apoptosis and disease. Lancet 341:1251–1254
5. Cohen JJ, Duke RC, Fadolk VA, Selling KS (1992) Apoptosis and programmed cell death in immunity. Annu Rev Immunol 10:267–293
6. Collins RJ, Harmon B, Grobe GC, Kerr JR (1992) Internucleosemal DNA cleavage should not be the sole criterion for identifying apoptosis. Int J Radiat Biol 61:451–453
7. Cummings CM, Winterford CM, Walker NI (1997) Apoptosis. Am J Surg Pathol 21:88–101
8. Debbas M, White E (1993) Wild-type p53 mediates apoptosis be E1A, which is inhibited by E1B. Genes Dev 7:546–554
9. Farber E (1994) Programmed cell death: necrosis versus apoptosis. Mod Pathol 7:605–609

10. Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119:493–501
11. Gerschenson LE, Rotello RJ (1992) Apoptosis: a different type of cell death. FASEB J 6:2450–2455
12. Gorzyca W (1992) DNA strand breaks occurring during apoptosis – their early in situ detection by terminal deoxynucleotidyl transferase and nick translation assays and prevention by serine protease inhibitors. Int J Oncol 1:639–648
13. Grasl-Kraupp B, Ruttkay-Nedecky B, Kondelka H, Bukowska K, Bursch W, Schulte-Hermann R (1995) In situ detection of fragmented DNA (TUNEL assay) fails to discriminate among apoptosis, necrosis and autolysic cell death: a cautionary note. Hepatology 21:1465–1468
14. Gressner AM, Polzar B, Lahme B, Mannherz H-G (1996) Induction of rat liver parenchymal cell apoptosis by hepatic myofibroblasts via transforming growth factor β. Hepatology 23:517–581
15. Leist M, Gantner F, Bohlinger I, Germann PG, Tiegs G, Wendel A (1994) Murine hepatocyte apoptosis induced in vitro and in vivo by TNF-α requires transcriptional arrest. J Immunol 153:1778–1788
16. Lennon S, Martin S, Cotter T (1991) Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. Cell Prolif 24:203–214
17. Munn DH, Beall AC, Song D, Wrenn RW, Throckmorton DC (1995) Activation-induced apoptosis in human macrophages: developmental regulation of a novel cell death pathway by macrophage colony-stimulating factor and interferon γ. J Exp Med 181:127–136
18. Ogasawara J, Watanabe-Fukunaga R, Adachi M, Matsuzawa A, Kasugai T, Kitamura Y, Itoh N, Suda T, Nagata S (1993) Lethal effect of the anti-Fas antibody in mice. Nature 364:806–809
19. Patel T, Gores GJ (1995) Apoptosis and hepatobiliary disease. Hepatology 21:1725–1741
20. Pulford KA, Rigney EM, Micklem KJ, Jones M, Stross WP, Gatter KC, Mason DJ (1989) KP1 – a new monoclonal antibody that detects a monocyte/macrophage associated antigen in routinely processed tissue sections. J Clin Pathol 42:414–421
21. Que FG, Gores GJ (1996) Cell death by apoptosis: basic concepts and disease relevance for the gastroenterologist. Gastroenterology 110:1238–1243
22. Reed J (1994) Bcl-2 and the regulation of programmed cell death. J Cell Biol 124:1–6
23. Roberts J, Searle J, Cooksley W (1993) Histological patterns of prolonged hepatitis C infection. Gastroenterol Jpn 28 [Suppl 5]:37–41
24. Rosser BG, Gores GJ (1995) Liver cell necrosis: cellular mechanisms and clinical implications. Gastroenterology 108:252–275
25. Schmitz G (1991) Nonradioactive labelling of oligonucleotides in vitro with the hapten digoxigenin by tailing with terminal transferase. Anal Biochem 192:222–231
26. Sun DY, Jiang Z, Zheng LM, Ojcious DM, Young JD (1994) Separate metabolic pathways leading to DNA fragmentation and apoptotic chromatin condensation. J Exp Med 179:559–568
27. Thiry M (1992) Highly sensitive immunodetection of DNA on sections with exogenous terminal deoxynucleotidyl transferase and non-isotopic nucleotide analogs. J Histochem Cytochem 40:411–419
28. Vaux D (1993) Toward an understanding of the molecular mechanisms of physiological cell death. Proc Natl Acad Sci USA 90:786–789
29. Walker PR, Kokileva L, LeBlance J, Sikorska M (1993) Detection of the initial stages of DNA fragmentation in apoptosis. Biotechniques 15:1032–1047
30. Wijsman JH, Jonker RR, Keijzer R, Vande Velde CJ, Cornelisse CJ, van Dierendonck JH van (1993) A new method to detect apoptosis in paraffin sections: in situ end-labeling of fragmented DNA. J Histochem Cytochem 41:7–12
31. Williams GT, Smith CA (1993) Molecular regulation of apoptosis: genetic controls on cell death. Cell 74:777–779
32. Wyllie AH (1980) Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284:555–556
33. Wyllie AH (1992) Apoptosis and the regulation of cell numbers in normal and neoplastic tissues: an overview. Cancer Metastasis Rev 11:95–103
34. Wyllie AH, Kerr JFR, Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68:251–306
35. Yamamoto M, Ogawa K, Morita M, Fukuda K, Komatsu Y (1996) The herbal medicine inchin-ko-to inhibits liver cell apoptosis induced by transforming growth factor β1. Hepatology 23:552–559
36. Zimmerman B, Mapoles J, Simon F (1993) Mechanisms of acetaldehyde mediated growth inhibition: delayed cell cycle progression and induction of apoptosis (abstract). Hepatology 18:152