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The immunoproteasome in steatohepatitis: Its role in Mallory–Denk body formation

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ABSTRACT

Recently it has been shown that the expression of the immunoproteasome increased in proportion to the degree of chronic inflammation in both the liver cell cytoplasm and nuclei in liver biopsies from patients who had chronic active hepatitis or cirrhosis. In the present study, biopsies from patients with steatohepatitis, with or without Mallory–Denk body (MDB) formation, were studied by immunofluorescent staining. Normal liver showed colocalization of FAT10, LMP2, LMP7, and MECL-1 at the mitochondria. Only LMP2 and LMP7 were found in the cell nuclei. Liver biopsies from patients with steatohepatitis and MDB formation, and a case of hepatocellular carcinoma forming MDBs in the tumor cells, showed colocalization of FAT10 and ubiquitin with LMP2, LMP7 and MECL-1 within the MDB. This indicates involvement of the immunoproteasome in MDB formation in steatohepatitis cases and in a case of HCC forming MDBs. Prior studies have shown that the immunoproteasome was involved in drug-induced MDB formation using the same immunofluorescent colocalization approach as was used on these human liver biopsies. The increase in the immunoproteasome subunit proteins was made at the expense of the 26S proteasome. This indicates that the shift from the 26S to the immunoproteasome had occurred in the MDB positive hepatocytes.

Introduction

Steatohepatitis frequently includes Mallory–Denk body (MDB) formation. In the case of alcoholic steatohepatitis, MDB formation occurs in 80% of biopsies (French, 1981a,b). Recently, using a drug-induced mouse model where MDB formation occurred, it was shown that the MDB formation was associated with an up regulation of the immunoproteasome catalytic subunits and a down regulation of the β5 26S proteasome subunit (Bardag-Gorce et al., 2010a). There was a loss of activity in the 26S proteasome causing accumulation of undigested proteins by the liver cells (Bardag-Gorce et al., 2010a). The undigested proteins aggregated and MDBs formed. The switch from the 26S proteasome to the immunoproteasome was associated with the up regulation of the TLR 2/4 pathway, the increase of the catalytic subunits of the immunoproteasome and the expression of TNFa and IFNg receptors (Bardag-Gorce et al., 2010a,b). The increase in the expression of the TLR 2/4 pathway was associated with the up regulation of TNFα and IFNγ receptors and TNFα expression (Bardag-Gorce et al., 2010b). The process of MDB formation is driven by a synergistic activation of genes localized in the MHC1 locus (Oliva et al., 2010). An interferon sequence responsive element (ISRE) on the Fat10 promoter is activated synergistically by the combination of IFNγ and TNFα as shown *in vitro* (Oliva et al., 2010). The genes activated i.e. TNFα, IFNγ, LMP2, LMP7 and MECL-1 cause the formation of the immunoproteasome, and consequently decrease the 26S proteasome activity (Bardag-Gorce et al., 2010a). The whole sequence of events involved in experimental MDB formation was prevented by feeding S-adenosylmethionine (SAMe) due to a gene silencing mechanism. This occurred through epigenetic events, which resulted from SAMe-initiated methylation of histones, including H3K27 (Bardag-Gorce et al., 2007, 2008, 2010a,b; Oliva et al., 2008; Li et al., 2008). This phenomenon has recently been reviewed (French et al., 2010a), as well as the relationship of MDB formation to the development of hepatocellular carcinoma through the proinflammatory mechanism (French et al., 2010b).

Recently, the expression of the immunoproteasome subunits in hepatocytes of liver biopsies from controls and patients with chronic liver disease and cirrhosis has been described (Vasuri et al., 2010). The expression of LMP2, LMP7, MECL-1 and PA28α/β has been shown to increase in proportion to the degree of chronic inflammation, in both controls and chronic liver disease. Using immunoperoxidase localization, it was shown that the immunoproteasome subunits were found in the cytoplasm and the nuclei of hepatocytes. The increase of LMP2 and PA28α in the total liver was also shown by Western blot. There was also an increase in the subunits LMP2 and 7 and PA28α in the nuclear extracts. Their immunoperoxidase results were confirmed in the present study. Here, we focused on changes observed in MDB forming hepatocytes.

Methods

Human tissues

Archived liver biopsies were studied from patients with alcoholic liver disease where Mallory–Denk bodies (MDBs) were

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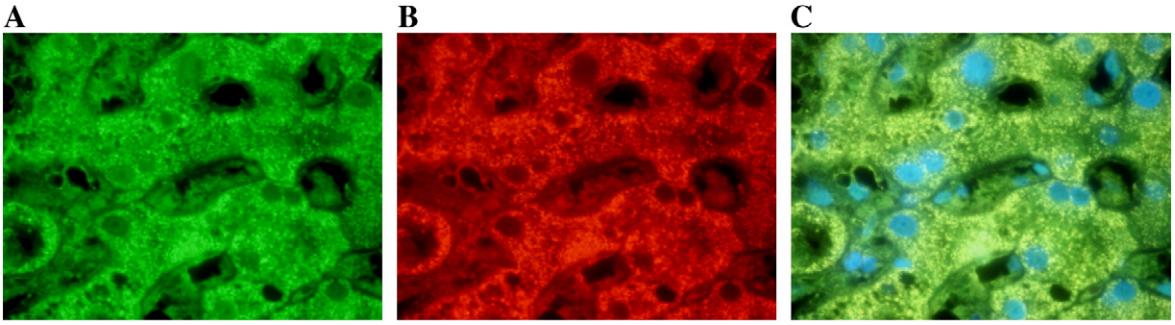

Fig. 1. Control liver double stained with FAT10 and LMP7 antibodies: A — FITC forLMP7; B — Texas Red for Fat10; C — Tricolor blue for DAP1. Note that FAT10 and LMP7 antibodies co-localize around the mitochondria. X654.

found. Different stages from fatty liver, to alcoholic hepatitis, to cirrhosis to hepatocellular carcinoma (HCC) were stained with either FAT10 or ubiquitin antibodies and double stained for LMP2, LMP7, MECL-1 and PA28α and β. A total of 14 biopsies from ALD patients, one with NASH and one autopsy liver from advanced alcoholic cirrhosis were stained, together with one normal liver biopsy obtained in a failed attempt to find a liver metastasis.

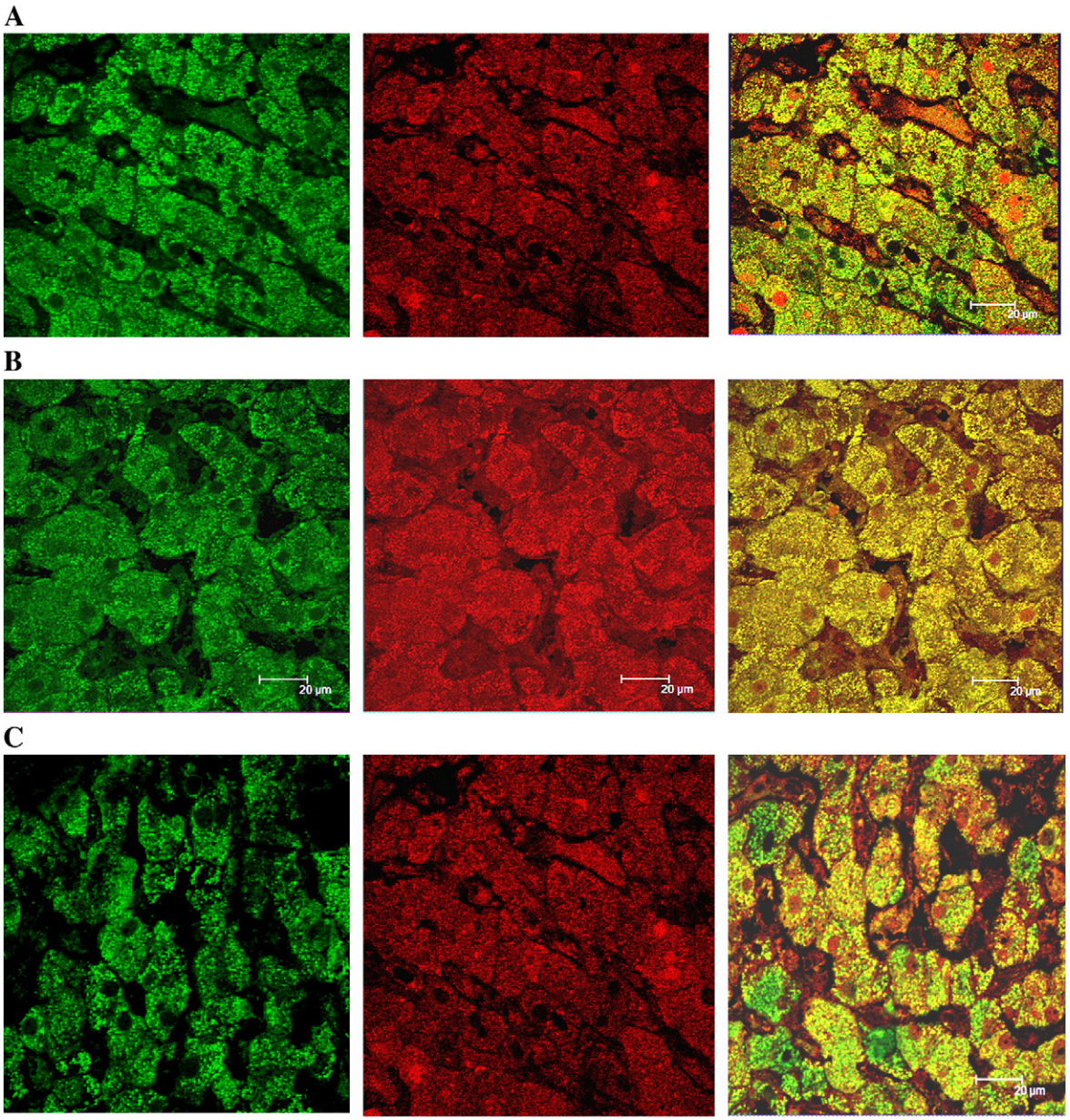

Fig. 2. Normal area of a liver biopsy from a patient with NASH showing co-localization of A — LMP2, B — MECL-1, and C — LMP7 around the mitochondria (green) and FAT10 (Texas red) merged (yellow). Magnification bar 20 µm.