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**Demonstration of Viral DNA, Viral Antigens, Hepatocellular Ultrastructure, Proliferation Kinetics and Enzyme Activity during Hepadnaviral Hepatocarcinogenesis in Woodchucks**

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Sequential hepatocellular changes were studied during carcinogenesis in woodchucks inoculated as newborns with woodchuck hepatitis virus (WHV), which is closely related to the human hepatitis B virus. When the woodchucks reached 12 months of age, aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) was administered to one group of WHV carriers in the diet at dose levels of 40 µg/kg body weight/day for 4 months and subsequently 20 µg/kg body weight/day (5 days/week) for lifetime. The histo- and cytomorphology of the liver were investigated by light and electron microscopy. The WHV DNA and the viral antigens (WHsAg and WHcAg) were demonstrated in the liver tissue by *in situ* hybridization and immunohistochemical approaches. In addition, the cell proliferation kinetics were investigated using a monoclonal antibody against Ki67. The activities of several enzymes involved in carbohydrate metabolism and in mitochondrial function were analysed by enzyme histochemistry. The cytomorphological and cytochemical analysis permitted the identification of five different types of focal lesions in chronic WHV carriers, namely APF/GCH, APF/GGH, APF, GSF, and GSF/BCF which seem to be integrated into two separate cell lineages leading to hepatocellular neoplasms; the amphophilic and glycogenotic/basophilic cell lineage apparently derived from MDA. The cellular and

subcellular alterations were similar in WHV-infected animals with and without AFB<sub>1</sub>-treatment.

MDA constitute a mosaic of glycogen-rich cells, amphophilic cells and ground glass cells. Well demarcated APF/GCH comprised glycogen-containing cells and glycogen-poor amphophilic cells, but no ground glass cells. The striking difference between APF/GGH and APF/GCH was the presence of a few scattered ground glass cells in APF/GGH. Cells forming APF were poor in glycogen and were characterized by a homogeneous, granular acidophilic cytoplasm due to pronounced mitochondrial proliferation, associated with a distinct diffuse or randomly scattered basophilia. GSF consisted of considerably enlarged hepatocytes excessively storing glycogen and were well demarcated from the surrounding parenchyma. GSF/BCF were made up of glycogenotic cells, glycogen-poor basophilic cells, and various types of intermediate cells.

High levels of WHV DNA replication and WHsAg expression were observed in most MDA, but the expression of WHcAg was limited to a lower number of hepatocytes in MDA under the experimental conditions chosen. Cell proliferation was significantly elevated in MDA in comparison with the liver parenchyma of WHV-free woodchucks. Three different types of proliferative foci of altered hepatocytes (FAH) representing early morphological and metabolic alterations emerged from MDA, namely 1) APF/GGH initially maintaining the viral DNA replication and the expression of WHsAg and WHcAg in many cells, 2) APF/GCH resembling APF/GGH but being free of ground glass cells, WHsAg and WHcAg and 3) GSF largely losing the WHV DNA and the expression of both antigens early during the development. The enzyme patterns of these two different cell lineages are in many respects opposite and mimic different hormonal effects. The amphophilic cell lineage shows a reduced activity of glycogen synthase but increased activities of gluconeogenic and mitochondrial enzymes, reflecting a thyromimetic effect. In contrast, the glycogenesis is associated with a reduction in the activity of enzymes involved in glycogen breakdown and gluconeogenesis, resembling a response of hepatocytes to insulin. Despite the phenotypic diversity in these two hepatocellular lineages, early changes in energy metabolism due to a disturbance in signal transduction pathways appear to be a common denominator. FAH and hepatocellular neoplasms are apparently elicited by a direct interaction of WHV with the hepatocytes. Although progression of FAH to hepatocellular neoplasms may become

independent from viral replication and antigen expression at an early time point, chronic active WHV infection is a prerequisite for the development of a high incidence and number of preneoplastic and neoplastic hepatocellular lesions.

