ULTRASTRUCTURE OF HEPATOCYTES OF A Rhesus Monkey INFECTED WITH HEPATITIS C VIRUS.

ABSTRACT

Ultrastructural alterations of hepatocytes of an experimentally infected rhesus monkey with hepatitis C virus were presented. During the follow-up period (70 days) maximal structural hepatocyte changes were seen at the 7th week post-infection, when the ALT level reached a peak. These results were compared with HCV-infected chimpanzee hepatocytes. Hepatocytes response to HCV infection is animal dependent and different cytoplasmic structures were observed in rhesus and chimpanzees monkeys. Both animal species can be considered for HCV experimental studies.

INTRODUCTION

Flaviviruses comprise several strong etiologic agents in human diseases, the most common being yellow and dengue fever. Hepatitis C virus (HCV), another member of the Flaviviridae family, may induce an infection that can develop to a chronic stage, liver cirrhosis and hepatic cellular carcinoma (Houghton 1996).
It was shown in several experiments (Jackson et al. 1979, Negro et al. 1992, Shimizu 1992) that HCV obtained from human sera can infect chimpanzees successfully. However, nowadays chimpanzees are virtually unobtainable for research purposes and finding an alternative animal model constitutes a priority in this field. We have investigated the susceptibility of rhesus monkeys to HCV infection (Vitral et al. 1997). Animals were followed for a period of 10 weeks and showed serological and histological evidences that infection had been apparently succeeded.

Formerly we presented shortly some ultrastructural alterations of rhesus monkey hepatocytes caused by HCV infection (Barth et al. 1997). In the present paper we intend to show, using electron microscopy, the morphological evolution of HCV infected hepatocytes to liver tissue regeneration.

MATERIAL AND METHODS

A rhesus monkey (RH 23) was inoculated intravenously with 15ml of pooled HCV/RNA positive plasma samples as formerly described (Vitral et al. 1997). Liver wedge biopsies were obtained by surgery weekly up to 70 days post-infection. For ultrastructural studies, small pieces of the liver tissue were fixed in 2.0% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded aceton and embedded in Araldit. Ultrathin sections were standard stained with uranyl acetate and lead citrate.

RESULTS

During the first two weeks post-infection no significant events in hepatocytes morphology could be observed using electron microscopy (Figures 1, 2). At the end of the third week, some dispersed foci in liver tissue appeared and some hepatocytes (Figures 3, 4) presented vacuolization and a sparsely structured cytoplasm. In sequence, the infection culminated from the 5th to the 7th week post-infection presenting numerous hepatocytes destroyed.

Patches of microtubuloreticular aggregates, like to membrane webs (Egger et al. 2002) were detected in hepatocytes 6 weeks post-infection (Figure 5) as well as bundles of fibres. Decorated microtubules appeared in sample of 5 weeks post-infection (Figure 6). At 7 weeks of infection, the maximal effect of hepatocytes destruction could be observed (Figures 7-9). Numerous cells died and the cell membranes could not more be recognized. Vacuoles of several sizesfullfill the cytoplasm. Mitochondria showed a condensed aspect and the nuclei, nevertheless still rounded, showed a disorganized chromatin pattern. These damaged hepatocytes were substituted by adjacent cells that started cell division at the 8th week post-infection (Figure 10). They showed a facetted contour in the sections and smooth intercellular membranes like recently differentiated cells.

DISCUSSION

Ultrastructural features of hepatocytes of HCV infected monkeys are different if comparing rhesus and chimpanzees. Rhesus monkey hepatocytes do not show type I-IV alterations, that are respectively reticular inclusion bodies, convoluted membranes, tubular structures and microtubular aggregates, considered a cell response to virus infection (Shimizu 1992, Pfeifer et al. 1980). In rhesus monkeys, a strong fragmentation of the cisterns of the smooth endoplasmic reticulum (ER) and of the endoplasm (rough endoplasmic reticulum, rER), was observed, resulting in numerous small and fewer great unit membrane covered vesicles. This aspect was not so evident in chimpanzees (Jacob et al. 1990, Shimizu 1992, Schimizu et al. 1979, Schimizu et al. 1990). It was obser-
ved in addition a general disorganization of cytoplasmic organelles and the appearance of more or less spherical aggregates of microtubuloreticular structures, probably originated from the ER (Ghadially 1997) in rhesus monkey hepatocytes. They are different from the type IV alterations and were not detected in chimpanzees by Shimizu et al. (opus cit.).

Recently, Egger et al. (2002) using tetracycline-regulated cell lines by stable transfection of U-2 OS human osteosarcoma cells with different HCV cDNA constructs, monoclonal antibodies to HCV and immunogold electron microscopy, found that the viral structural core and E1 proteins and the non-structural NS3, NS4A, NS4B and NS5A proteins occurred on this membranous web. They demonstrated also that the membranous web was induced by the NS4B viral protein and is related to the rER. The core and E1 proteins were found also inside the lumen of the rER. Considering both, the viral structural and non-structural proteins accumulated on the membranous web in HCV infected cells, Egger et al. (2002) proposed that this structure forms the viral replication complex. A web-like inclusion body was also detected in a HCV infected liver biopsy of a chimpanzee by the same authors and formerly by Pfeiffer et al. (1980). The morphological aspects of the microtubuloreticular structures that occurred inside rhesus and chimpanzee hepatocytes and the membranous webs inside the cells of the transfected cell lines, by analogy, are similar rER-derived structures expressing the viral proteins.

Changes in hepatocyte nuclei and mitochondria morphology were less pronounced than the cytoplasmic changes in rhesus monkey HCV infected hepatocytes. The nuclei volume remained more or less constant, while the chromatin was progressively fragmented and the nucleoplasm appeared "empty". Mitochondria suffered shrinkage during cell degradation. Cell membranes between hepatocytes developed to be rounded and partially disapeared, so that no cell individualization was observed at the 7th week post-infection (maximum of cytopathic effect and ALT elevation) in focal virus infected hepatocytes of rhesus monkeys.

Multivesiculate cytoplasm of hepatocytes of chronic HCV-Infected human patients was shown by Bosman et al. (1998), but it seems that there the virus-like particles (VLPs) do not lay on or inside these ER-derived vesicles.

In all experiments using HCV-infected monkeys there was an agreement (Negro et al. 1992, Shimizu et al. 1990, Vitral et al. 1997) that the most intensive effect of virus infection and presence of viral RNA coincide with the maximum of alanine aminotransferase (ALT) elevation and around the 7th week of infection.

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Fig. 1 - Non-infected rhesus monkey (control) showing a sinusoid and surrounding hepatocytes. Figs. 2 - 7 days post-infection, showing no alteration of cell organelles; note the well preserved ergastoplasm next to the nucleus. Figs. 3 - 14 days post-infection; cell vacuolization is starting. Figs. 4 - 21 days post-infection; several hepatocytes arranged around a sinusoid containing an inflammatory polymorphonuclear cell and an erythrocyte, present disorganization of cell structures. Figs. 5 - 42 days post-infection; dense mitochondria, a strongly vesiculate endoplasmic reticulum and microtubulo-reticular aggregates are present. Fig. 6 - 35 days post-infection; decorated microtubules occur inside some hepatocytes. (Bar = 1.4 μm for Figs. 1 and 2, 3.3 μm for Figs. 3 and 4, 0.5 μm for Figs. 5 and 6)
Figs. 7 - 42 days post-infection; complete disorganization of hepatocytes and strong vacuolization are characteristics of this stage. Figs. 8 - 42 days post-infection; hepatocyte membranes thickness is increased. Figs. 9 - 42 days post-infection; lipid droplets are present; no individualization of hepatocytes and sinusoids is detected in virus infected areas of the liver. Figs. 10 - 63 days post-infection; a densely filled cytoplasm, numerous well structured mitochondria and faceted cell contours are indicators of tissue regeneration. (Bar = 3.3 μm for Fig. 7, 1.4 μm for Fig. 8 and 2.3 μm for Figs. 9 and 10)