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A Study on the Role of the CD28/CTLA 4 -B 7 Costimulatory Signal in the Pathogenesis of Diseases in MRL/*lpr* Mice

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Mechanism of Free Radical-Induced Oxidative Injury and Activation of Signal Transduction in Rat Liver Membrane with Acute Hepatitis -Spectroscopic Observation-

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The mechanism of free radical-induced oxidative injury and activation of signal transduction were studied in the rat liver membrane with acute hepatitis using Fourier transform-infrared (FT-IR) and electron spin resonance (ESR) techniques with conventional biochemical ones.

The carbon tetrachloride (CCl_4) -induced oxidative injury and activation of signal transduction were investigated in the liver of female Sprague-Dawley (SD) rats. In the reaction of CCl_4 with NADPH in microsomal membrane, the $CCl_3\cdot$ radical was detected by ESR with a spin trapping reagent of PBN. The FT-IR spectroscopy revealed that absorption band of -C-H in -C=C-H decreased in intensity at 3012 cm-1, but the absorption bands of the phosphate head and choline in the phsopholipids did not significantly change between 1300 and 900 cm-1, indicating the removal of H·from -C=C-H by radicals and the absence of dephosphorylation of phospholipids in the microsomal membrane. In the lipid membrane fraction of the liver homogenate, on the other hand, the accumulation of diacylglycerol (DAG) was observed in addition to phosphatidylcholine, phosphatidylethanolamine and triglyceride (TG) by FT-IR and HPLC. It was estimated to be ca. 10-15% of the membrane phospholipids by weight. DAG is an intracellular activator of protein kinase C (PKC) which regulates cell proliferation and differentiation. The activation of PKC was also observed in liver homogenate in the CCl_4 injected rats, as been expected. It was thus concluded that the CCl_4 -derived radicals

stimulate PKC through the accumulation of DAG in the liver membrane of the rats. . Neither DAG nor TG was found in the lipid membrane of the rats pretreated with PBN of radical trapping reagent.

The lipids extracted from the Long-Evans Cinnamon (LEC) rat liver homogenate were also studied at the stages of pre-hepatitis and hepatitis, i. e., at 10 and 16 weeks of age, respectively. The LEC rat is known to accumulate copper in their liver and produce active oxygen radicals there at the latter stage. The 16-week old rats were observed to develope respective new absorption bands of TG and DG at 1161 and 1070 cm^{-1}. DAG is known to activate the transcriptional factor of SRF to express the proto-oncogene c-fos after binding to PKC in the membrane. Previously, it has been reported that the activation of SRF depends on the concentration of copper in the nuclear protein of the LEC rat liver. The LEC rat develops liver cancer after recovery from hepatitis. Thus, DAG may play an important role in liver cancer development in LEC rats that survive fulminant hepatitis, via activation of PKC and SRF. PKC activities were confirmed to be significantly high during the acute hepatitis stage.

The mechanism of free radical-induced oxidative injury and activation of signal transduction were successfully studied by using spectroscopic techniques combined with biochemical ones.