LIPID PEROXIDE FORMATION IN ISOLATED HEPATOCYTES BY CYTOTOXIC FACTORS PRODUCED FROM LYMPHOKINE-ACTIVATED MACROPHAGES

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Summary
When peripheral blood lymphocytes from patients with chronic active hepatitis were stimulated with liver specific lipoprotein (LSP), considerably higher frequencies of lymphocyte transformation and MIF production were induced. Peritoneal macrophages from guinea pigs were activated by lymphokine-containing lymphocyte culture supernatant and produced a cytotoxic (or cytostatic) factor acting on isolated hepatocytes in culture. The cytotoxic (or cytostatic) factor, which was fractionated by Sephadex G-75 column gel filtration followed by DEAE-cellulose column chromatography, had cytotoxic effect on isolated liver cells and produced a significant amount of lipid peroxide. These results suggested the possibility that the cytotoxic effects may be caused at least partially by the lipid peroxidation formation.

Key Words: macrophage-mediated cytotoxicity, cytotoxic factor, lipid peroxide, liver specific lipoprotein, monokine.

Introduction
It is becoming an accepted idea that autoimmune reactions induced by liver specific lipoprotein (LSP) may play an important role in the pathogenesis of chronic active hepatitis. Although diverse possibilities such as antibody-dependent cell-mediated cytotoxicity, killer T cell-mediated cytotoxicity, antigen-antibody complexes and participation of lymphokines have been proposed in attempts to interpret the immunopathogenesis of liver injury in chronic active hepatitis, none of them permits a clear interpretation of how such immunological mechanisms result in the liver injuries. We have previously reported that activated macrophages may have cytotoxic or cytostatic effects on isolated hepatocytes via effector cell-to-target cell contact or through the production of monokines acting on the hepatocytes. Since lipid peroxidation as a mechanism for liver injury has been reported, particularly in the case of carbon tetrachloride poisoning, we investigated whether lipid peroxide is formed in isolated hepatocytes due to cytotoxic factor produced by lymphokine-activated macrophages.
Materials and Methods

1. Lymphocytes. Lymphocytes were obtained from patients with chronic active hepatitis. All of them showed positive lymphocyte transformation by stimulation with LSP, providing evidence of sensitization to LSP.

2. Preparation of LSP. Subcellular components of rat liver cells were fractionated according to the method of Meyer zum Büschenfelde. The purification of LSP consisted essentially of a series of gel chromatography columns through which the supernatant of rat liver homogenate was passed. The membrane lipoprotein appearing in the void volume on chromatography over Sepharose 6B was shown to contain LSP.

3. Preparation of peritoneal exudate cell suspension. To obtain peritoneal exudate cells (PEC) which mainly consist of macrophages, 20 ml of sterilized mineral oil (Marcol 52, Esso Oil Co.) was injected into the peritoneal cavity of normal guinea pigs. PEC was collected 4 days after oil injection by perfusing the peritoneal cavity with 200 ml of Hanks solution. After the oil phase was decanted, the aqueous phase was centrifuged at 800g for 10 min at 4°C. The pellets were then washed three times with fresh Hanks solution and suspended in Eagle MEM solution containing 10% fetal calf serum, 100 units/ml of penicillin and 100 μg/ml of streptomycin to make a cell suspension of 5 × 10⁷ cells/ml. The cells mostly consisted of macrophages with a few contaminating lymphocytes and granulocytes.

4. Assay of macrophage activation. The peripheral blood lymphocytes were cultured in the presence of LSP at 37°C for 48 hours and the culture supernatant containing the macrophage activating factor (MAF) was added to the PEC prepared from normal guinea pigs as described above. The macrophage activating factor contained in the culture supernatant of activated lymphocytes was estimated by measuring the uptake of [³H]-glucosamine into PEC according to the method of Hammond et al.; the culture supernatant of activated macrophages was added to the PEC and [³H]-glucosamine (1 μCi, specific activity: 20 Ci/mol) was added to the culture after incubation at 37°C for 72 hours. After incubation at 37°C for another 6 hours, 3 ml of phosphate buffered saline was added to each culture and cells were collected on a millipore filter membrane by suction after gentle shaking. The membrane was then washed by 10 ml of ice-cold 5% trichloroacetic acid solution and radioactivity retained on the filter membrane was counted by liquid scintillation spectrometry as described previously. As a control the same experiments were performed without adding LSP to determine the stimulation index. Based on analyses of many normal subjects, we judged that positive activation of macrophages occurred when the stimulation index was greater than 180%.

5. Assay of macrophage-mediated cytotoxicity. To demonstrate that MAF-activating macrophages are capable of exerting cytotoxic or cytostatic action on liver cells, we chose guinea pig hepatocytes as the target cells because these were readily available. Isolated liver cells were prepared from a normal guinea pig according to the method of Bellemann et al. Culture supernatants containing MAF were prepared by stimulating lymphocytes from patients with chronic active hepatitis as described above. These were added to PEC taken from a normal guinea pig, followed by incubation at 37°C for 6 hours. After incubation, cells were separated, washed with fresh Eagle MEM solution and resuspended in the same medium to make a cell suspension of 5 × 10⁶ cells/ml. After PEC were incubated for another 24 hours, the culture supernatant was separated and 0.5 ml of the culture superna-