KILLER CELL (K-CELL) POPULATION IN CHRONIC LIVER DISEASE

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Summary

Peripheral blood killer cell (K-cell) population of patients with chronic hepatitis was investigated by means of a plaque-assay method using sheep red blood cells. The mean K-cell population of 14 control subjects was 5.1 ± 2.0% (mean±SD), and that of 28 patients with chronic hepatitis was 4.4 ± 3.1%. These 28 patients were divided into three groups: CPH, CAH 2A and CAH 2B. The mean K-cell population of each group was decreased in order of the severity of the disease. Especially, that of patients with CAH 2B was a statistically significant decrease (p<0.01) from control subjects.

In the course most patients with CAH, K-cell population did not change for three months after admission. K-cell population was observed to decrease in the patients with active stage of liver cirrhosis, but not in patients with the terminal stage of liver cirrhosis, even in hepatoma patients. It is suggested that the K-cell population may play an important role of pathogenesis of chronic hepatitis.

Key Words: K-cell, ADCC, chronic hepatitis.

Introduction

Several investigations in the last few years have indicated that the peripheral blood lymphocytes from patients with liver diseases, especially those with chronic aggressive hepatitis, were directly cytotoxic against hepatocytes in vitro. Recent reports supported the hypothesis that antibody-dependent cell-mediated cytotoxicity (ADCC) might be involved in the pathogenesis of liver cell injury, and they also suggested that the effector cells responsible for ADCC had Fc receptors, but lacked surface markers showing nature T- or B-lymphocytes. They are called killer cells (K-cells). In this study, peripheral blood K-cell populations of patients with chronic liver diseases, and correlation between K-cell population and disease activity were investigated.

Patients and Methods

The twenty-eight patients with chronic hepatitis diagnosed by liver biopsy were classified according to the classification of chronic hepatitis proposed by the European group in 1968. Eight patients were diagnosed as chronic persistent hepatitis (CPH), ten as chronic aggressive hepatitis activity moderate (CAH 2A) and ten as chronic aggressive hepatitis activity severe (CAH 2B). In addition, eleven patients with liver cirrhosis and thirteen patients with hepatoma were studied. Fourteen...
healthy volunteers served as control subjects.

All sera were tested for HBsAg by radioimmunoassay or immune adherence hemagglutination.

Statistical significance of the results was assessed by the Student’s t-test.

K-cells were measured by using the modification of the plaque technique described by Biberfeld and Perlmann. Target cells were fresh sheep red blood cells (SRBC). Ten μl of 10⁹/ml SRBC were added to poly-L-lysine treated wells of a microtest plate. After centrifuging, dense SRBC monolayers in microtest plate were made. Lymphocytes were obtained from peripheral blood by centrifugation on a Ficoll-Isopaque gradient after removal of macrophages by silica incubation, and were adjusted to a concentration of 2 × 10⁶/ml in RPMI 1640. Cell-mediated erythrolysis was induced with IgG fraction of a rabbit anti-SRBC serum. One μl of lymphocytes and one μl of appropriately diluted rabbit antibodies were added to the SRBC monolayer, and incubated for three hours at 37°C in a humid atmosphere of air with 5% CO₂. After incubation, ten μl of the mixture of 0.0003% brilliant cresyl blue (Merck), 0.25% glutalaldehyde (Wako) and 1% seaplaque agarose (Biomedical) were added to each well for fixing and staining of the monolayers. The number of plaque-forming cells was counted under a microscope and calculated from the following formula.

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\% \text{ PFC} = \frac{\text{plaque forming cells (PFC)}}{\text{whole lymphocytes}} \times 100
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Results

Plaque-forming cells are shown in Fig. 1. Erythrolysis is reflected by the formation of clear zones of irregular shapes and different sizes. No plaque-forming cell was found when lymphocytes were added to the SRBC mono-

layers in the absence of antibody. Fig. 2 shows the mean K-cell population when lymphocytes from control subjects and from patients with chronic hepatitis were tested in the presence of anti-SRBC antibody. The mean K-cell population of fourteen control subjects was 5.1 ± 2.0% (mean ± SD), and that of twenty-eight patients with chronic hepatitis was 4.4 ± 3.1%.