"E" AND "EAC" ROSETTES IN ACUTE AND CHRONIC LIVER DISEASE

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Abstract

A significant reduction in the number of 'E' rosettes was observed in patients with liver disease while changes in the percentage of EAC rosettes were insignificant. The increase in the number of null cells with the progress of liver disease is suggestive of increase in the number of defective T cells incapable of forming E rosettes with SRBCS and the depression of cell mediated immunity (JPMA 29:188, 1979).

Introduction

Immunological reactions are known to be implicated in the pathogenesis of acute and chronic hepatic disorders (Eddleston 1974).

Human lymphocytes have been studied in several immunopathological disorders. T and B lymphocytes can be identified by spontaneous non-immune rosette formation with washed sheep erythrocytes and surface immunoglobulin markers respectively. The null lymphocytes are devoid of surface membrane immunoglobulin and the capacity to form rosettes with SRBC.

The object of this study was to determine any possible immunodeficiency or abnormalities in the immune system in patients with liver disease.

Material and Method

Forty-three patients with liver disease and 20 healthy controls were included in this study.

The diagnosis in 15 patients with acute viral hepatitis, 15 patients with cirrhosis and 13 with liver cancer was based on clinical, biochemical and histological findings.

The number of E-rosettes and EAC-rosettes was determined by the modified method described by Jondal et al. (1972). Purification of lymphocytes was done on a Ficoll-Hypaque gradient using heparinised blood. The purified lymphocytes were washed three times by centrifugation at 200G in Hanks balanced salt solution (HBSS) and resuspended in it to a concentration of 4×10 ml and divided into 0.25 ml aliquots.

Preparation of sheep red cells: An aliquot of sheep red cells preserved in Alsever's solution was washed three times in HBSS and 0.5% and 5.0% cell suspensions were made in the Hank's solution.

E-Rosettes: 0.25 ml lymphocytes suspension was mixed with 0.25 ml of 0.5% SRBC and spun at 200G for 5 minutes and incubated in ice for 1 hour.

EAC-Rosettes: 5 ml 5% SRBC were mixed with 5 ml 1:2000 antisheep hemolysin and incubated at 37°C for 30 minutes. The cells were washed once in HBSS and resuspended in 5 ml of the same. 5 ml 1:20 human serum was added and again incubated at 37°C for 30 minutes. The cells were washed three times in HBSS and a 1% suspension was prepared in the same solution.

0.25 ml of EAC were mixed with 0.25 ml lymphocytes, incubated at 37°C for 30 minutes and spun at 200G for 5 minutes.

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Interpretation: For E and EAC rosettes fresh preparation was counted on a glass slide. Lymphocyte surrounded by three or more SRBC was considered as a rosette. Percentage of rosettes was calculated after counting a total of 200 lymphocytes.

Results

In healthy subjects the percentage of 'E' rosettes in peripheral blood varied from 55.5 to 69.5% (mean 64.18%). A significant reduction in T cells occurred in patients with acute viral hepatitis ($t=11.06$, $P < .001$), cirrhosis of the liver ($t=15.7$, $P < .001$) and liver cancer ($t=21.1$, $P < .001$) as shown in the accompanying table.

Table: T and B Lymphocytes

Groups studied	*Number studied*	*T lymphocytes (%)* Mean	Range	*B lymphocytes (%)* Mean	Range	*Null Cells* (%)* Mean
Healthy controls	20	64.18	55.5—69.5	36.05	30.0—45.5	—0.23
Acute viral hepatitis	15	28.40	9.0—49.0	36.33	24.0—46.0	35.27
Cirrhosis of liver	15	22.90	9.0—41.5	33.30	28.0—43.0	43.80
Primary liver cancer	13	22.88	12.0—29.5	35.15	26.0—44.0	41.97

* Null cells = 100 — (T cells + B cells)

EAC rosettes in control subjects ranged from 30.0 to 45.5% (mean 36.05%). The variations in B cells between the healthy and the diseased groups were insignificant.

The percentage of null cells varied from —0.23% in the healthy controls to 43.8% in patients with liver disease.

Discussion

E and EAC rosette technique has been used by several investigators to determine the percentage of T and B cells in the peripheral blood (Denman, 1973; Jondal et al., 1972; Strong et al., 1975).

The number of total circulating lymphocytes and B lymphocytes remains normal in the acute and resolving hepatitis but a significant reduction in the number of T cells has been reported by Edgington and Chisari (1975). Similar findings have been presented in this study.

The number of T lymphocytes as determined by the percentage of E-rosettes was low in most of the patients with liver disease while only minor variations were found in the number of B lymphocytes. These findings suggest that the null cells are generated from T lymphocytes defective in respect to E rosette formation (Edgington and Chisari, 1975).

A significant reduction of cell mediated immunity as determined by the impairment in the response to DNCB (Zuberi et al., 1978) and a significant reduction in the rosette forming T cells (Lodi and Zuberi, 1977) reported by us earlier has also been confirmed in this study.

Reduction in the number of T cells has been found by Wybran and Fudenberg (1973) in patients with malignancy and by Lodi and Zuberi (1977) in patients with primary liver cancer. Similar findings have been observed in this series. The present study and those reported earlier (Zuberi et al., 1978; Lodi and Zuberi, 1977) show progressive diminution of cell mediated immunity with the progress of liver disease from the acute to the chronic stage.

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