NATURAL KILLER CELL AND LYMPHOKİNE - ACTIVATED KILLER CELL ACTIVITIES IN PATIENTS WITH LIVER CIRRHOSIS RELATİVE TO SEVERITY OF LIVER DAMAGE

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SUMMARY : Although defects on cellular immunity of liver cirhosis (LC) patients have been noted, the results of studies on the natural killer (NK) cell and lymphokine - activated killer (LAK) cell activities are still controversial.

To evaluate the role of severe liver damage on NK and LAK cell activities, 36 patients with LC, 10 chronic active hepatitis (CAH) and 15 healthy controls were examined. The NK cell activity was measured with a 4-hr chromium release assay, and the K562 cell line was employed as target cell. LAK cell activity was measured with chromium release assay and Daudi cell line was employed as target cells. The NK cell activity was significantly decreased (mean ±SD, 40.0313 ± 12.5, $p < 0.05$) in cirrhotic patients compared with controls and patients with CAH. Cirrhotic patients with Pugh's C grade of severity of Liver disease had lower NK activity (29.777 ± 8.68 $p<0.001$). The LAK cell activity was significantly decreased (53.25 ± 5, $P<0.05$) in cirrhotic patients with Pugh's C grade of severity of liver disease compared with controls and had lower LAK activity. The depression of NK cell activity in cirrhotic patients was inversly correlated with prothrombin time, serum bilirubin levels and the NK cell activity in cirrhotic patients with ascites was lower than in patients without ascites. NK cell activity showed significant correlation with serum albumin levels ($r=0.465$, $p<0.01$). These results show that the deminished NK and LAK activity in cirrhotic patients might be related to the severity of liver damage.

Key Words : *Cirrhosis, Natural Killer Cell, Lymphokine, Activated Killer Cell.*

INTRODUCTION

Liver cirrhosis (LC) is thought to be a premalignant condition of hepatocellular carcinoma (HCC) (10).

Natural Killer (NK) cells and lymphokine activated killer (LAK) cells induced by interleukin 2 are normal unprimed mononuclear cells which have cytotoxic activity against a variety of tumor cells (6). They are believed to play an important role in the defense mechanism against malignant tumor cells (12). Although NK and LAK activities are markedly depressed in patients with hepatocellular carcinoma (HCC) (8, 9, 11) the results of studies on the NK cell and LAK cell activity of LC patients are still controversial (2, 8, 9).

This study investigated NK cell activity, LAK cell activity in LC patients and the influence of clinical and biochemical parameters on the NK cell activity of LC patients also evaluated.

MATERIALS AND METHODS

Patients : Thirty - six patients with LC (including 23 with HBsAg positive, 4 with HCV antibodies positive and 9 with non A non B) were studied. There were 28 men and 8 women with ages ranged from 23 to 65 years (mean 48.8 ± 10.67 years). LC was biopsy proven or diagnosed with clinical symptoms and signs accompanied with positive findings on imaging studies (abdominal CT, abdominal sonography and liver scintigraphy). Hepatitis B markers (Abbott) and Hepatitis C virus antibodies (by ELİSA II generation) were detected. Biochemical laboratory tests (aminotransferase, alcaline phosphatase, serum bilirubin, albumin concentrations, prothrombin time) were determind in each patients for correlation with NK activity. Grading of LC was based on the criteria of pugh et al (7). At the same time 10 patients with (CAH) (mean age 33.71 ± 8.69 including 6 with type B CAH, 4 with type C CAH and 15 healthy volunteers), were also examined. All of the CAH patients were biopsy proven and none of them were receiving any form of therapy at the time of the study.

NK cytotoxic activity assay was performed in patients with LC, patients with CAH and healthy volunteers. LAK cytotoxic activity was evaluated in 4 patients with pugh child C, 7 with pugh child B and 4 with pugh child A LC.

Isolation of effector cells : Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats obtained from patients and healthy donors by Ficoll-Hypaque gradient centrifugation. After washing twice in RPMI-1640 medium (Sigma Chemical Co.), adherent cells were removed by incubating PBMCs for 90 min at 37°C, in a plastic petri dish in RPMI-1640 supplemented with 10 % FCS (SEBAK, GmbH Aidenbach), 2mM L-glutamin (referred to as complete medium). Nonadherent cells collected and washed once more, then prepared the effector cell suspension at desired density in complete medium.

Assays for NK and LAK cytotoxic activity : NK and LAK assays were performed as described elsewhere (14). Briefly, cytotoxic activity was performed in a 96-well V-bottomed microtiter plates by incubating various numbers of effector cells with $4x10^4$ ^{51}Cr-labeled K562 and Daudi target cells respectively. LAK assays were performed with the effector cells ($2x10^6$/ml) preincubated with IL-2 (Sigma, St. Louis Mo., cat no. T-3267) at the final concentration of 100 U/ml for 18-h in RPMI-1640 supplemented with 10 % pooled and heat inactivated human AB serum and then washed twice in RPMI-1640 before the assay. Effector cells for NK and LAK activity were prepared at various numbers in 100 µl complete medium and added into appropriate wells containing $4x10^4$ target cells in 100 µl complete medium. After 4-h incubation at 37°C in a humidified 5 % CO2 atmosphere, 100 µl of supernatant was removed from each well and countered in a gamma counter (LKB, 1275 Minigamma gamma counter). The spontaneous release was determined by incubating labeled targets in complete medium alone. The maximal release was determined by adding 100 µl of 2 % SDS. Percentage specific lysis was calculated by the formula :

$$\% \text{ Specific cytolysis} = \frac{(\text{Experimental } ^{51}\text{Cr release} - \text{Spontaneous } ^{51}\text{Cr release})}{(\text{Maximum } ^{51}\text{Cr release} - \text{Spontaneous } ^{51}\text{Cr release})}$$

All determinations were made in triplicate, and data are reported as the means of these determinations.

NK cytotoxicity assay. In order to assess NK cytotoxic activity, chromium-51 release assay was used with a modification of that was described previously (14). The K562 target cells were labelled by incubating $3x10^6$ cells with 150 µCi of the sodium chromate (CJS1, Amersham, UK) for 1 hr at 37°C, pelleted, washed twice with 10 ml of RPMI and resuspended in RPMI plus 10 % FCS at $2x10^5$ cells/ml. Effector : target ratios (E:T) were 50:1, 25:1 and 12:1 in wells of V-bottomed 96-well microtiter plate, in triplicate, with 10^4 target cells/well and incubated for 4 hr at 37°C. After incubation, plate was centrifuged at 400xg for 5 min and 100 µl of supernatant was harvested from each well without disturbing the cell pellet and counted by a gamma counter (LKB, 1275 Minigamma gamma counter).

To determine the spontaneous release, target cells were incubated with 100 µl of medium only. Maximum release was obtained by incubating the

target cells with 100 µl of 2% sodium dodecyl sulfate (SDS) in distilled water.

Cytotoxicity was calculated from the average counts per minute (cpm) released into the supernatants of triplicate samples by the following formula :

$$\% \text{ Specific } {}^{51}\text{Cr release} = \frac{\text{Sample cpm - Spontaneous cpm}}{\text{Maximum cpm - Spontaneous cpm}} \times 100$$

Statistical analysis : The repeated - measures analysis of variance, linear regression and correlation and Kruskall-Wallis variance analysis were used for statistical analysis.

RESULTS :

The NK cell activity was significantly decreased in LC patients as compared to normal controls and CAH patients but there was no statistical difference between the NK cell activity of the CAH group and normal controls. The LC patients with pugh's C grade of severity of liver disease had lower NK cell (Table 1) and LAK cell activities (53.25 ± 5, $p<0.05$) than patients with grades A and B (Table 2).

Although it wasn't statistically significant the depression of NK cell activity in LC patients was inversely correlated with prothrombin time ($r=-0.21$, $p>0.05$), and serum levels of bilirubin ($r= -0.25$, $p>0.05$). The depression of NK cell activity in LC patients was significantly correlated with the serum levels of albumin ($r= 0.465$, $p<0.01$) and the presence of ascites ($p<0.01$).

The LC patients with hepatic encephalopathy did not influecnce the NK cell activity ($p>0.05$) of LC patients.

DISCUSSION

Although defects on cellular immunity of LC patients have been noted (3, 4, 15) the NK cell and LAK cell activity in patients with LC were controversial (2, 8, 9). The NK cell and LAK cell activities have been observed to be normal in some reports (9). Kupffer cells can modulate the activity of NK cells (5) and it is impaired in patients with LC (13) though the immunity of LC patients might be impaired. In our study we found that the patients with

	Natural killer cell activity E/T ratios* (1/50)	P
Pugh's A (N = 10)	48.000 ± 10	**p > 0.05
Pugh's B (N = 14)	41.5 ± 12.0878	
Pugh's C (N = 11)	29.77 ± 8.6	*** p < 0.001

* E / T effector target cell
** Compared with pugh's B
*** Compared with Pugh's A and B

Table 1 : Natural killer cell Activity in liver cirrhosis patients with different grades of pugh's classification.

	Lymphokine-activated killer cell activity E/T ratios* (1/50)	P
Pugh's A (N = 4)	71.10 ± 3.64	
Pugh's B (N = 7)	83.75 ± 3.86	
Pugh's C (N = 4)	53.25 ± 5	** p < 0.05

* E / T effector target cell
** Compared with Pugh's A and B

Table 2 : Lymphokine - Activated killer Cell Activity in liver cirrhosis patients with different grades of pugh's classification

pugh's C grade of severity of liver disease had lower NK and LAK cell activity. The results suggested that the severity of liver damage correlate with NK and LAK cell activities of LC patients.

Hepatic synthesis of proteins was decreased in patients with liver disease (10) and the correlation between malnutrition and impaired immunity in previous studies was explained by liver damage. In our study, the depression of NK cell activity in LC patients was correlated with the serum levels of albumin. It implied that serum level of albumin represent the severity of liver damage.

Prothrombin time shows quantitative total functioning paranchymal mass in patients with LC (1). Although it wasn't statistically significant the depression of NK cell activity was inversely correlated with prothrombin time.

The serum levels of bilirubin which may represent the severity of liver damage was inversely correlated with depression of liver damage but it wasn't statistically significant. Chaung et al didn't find any correlation between ascites and NK cell activity (2). Our results showed a significant correlation between the depression of NK cell activity and the presence of ascites.

We concluded that the deminished NK and LAK cell activity in LC patients might be related to the severity of liver damage.

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