Studies on Lymphocyte Subpopulations and NK Cell Activities in Epileptic Patients

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Summary: The subpopulations of T lymphocytes, B lymphocytes and macrophages of the peripheral blood in 58 cases of epilepsy were studied with McAb by IFA method. The activity of natural killer (NK) cells of peripheral blood monocyte was investigated by $^{51}$Cr-releasing method. 22 healthy donors of about the same age served as controls. The results showed that the percentage of T$_3$ and T$_4$ lymphocytes in epileptic patients was decreased, and the ratio of T$_4$/T$_8$ was markedly reduced as compared to control group. The percentage of T$_8$ lymphocytes was increased. There were no significant changes in B lymphocytes and macrophages. The NK cell activity also showed decrease. The results suggested abnormality in cellular immunity in epilepsy, which may be involved in the pathogenetic mechanism of the disease.

Key words: T lymphocytic subpopulations, B lymphocyte, monocyte, NK cell activity, epilepsy

It has been documented previously that immunological abnormalities were found in epileptic patients$^{[1-3]}$. Auto-antibodies could also be detected in some cases of epilepsy$^{[3]}$, so it was presumed by many authors that immunological disorders might be involved in the pathogenetic mechanism of epilepsy$^{[4-6]}$. But investigations concerning subpopulation of lymphocytes and natural killer (NK) cell activity in epilepsy are still limited. In order to elucidate the cellular immunologic alterations in epilepsy, we studied the subpopulations of lymphocytes and macrophages by use of McAb and NK cell activity with $^{51}$Cr-releasing technique in 58 cases of epilepsy.

MATERIALS AND METHODS

Patients

A total of 58 epileptic patients including 38 males and 20 females with their age ranging from 16-45 years, average age 23 years, were investigated. The diagnosis was established based on clinical symptoms and electroencephalography. The patients had not received any treatment prior to the study, and they were not subjected to any immunologic diseases. 20 healthy donors, 20-45 years of age, served as controls.

Reagents

Monoclonal antibodies (McAb), anti-T lymphocytes (T$_3$, T$_4$, T$_8$), anti-B lymphocytes and anti-monocytes were provided by Prof. SE Liang-ru of the Institute of Biological Products, Wuhan.

Rabbit anti-mouse IgG labeled with fluorescein was obtained from the Institute of Biological Products, Beijing.

$^{51}$Cr was purchased from the Institute of Atomic Energy, Beijing.

Methods

1. Indirect immunofluorescence assay
of subpopulation of peripheral blood monocytes (PBMC).

PBMC were separated from 6 ml heparinized blood by Ficoll-Hypaque density gradient centrifugation. After 3 times washing with RPMI-1640, the cell suspension was adjusted to the concentration of $1 \times 10^7$ cells/ml by 0.01 M PBS (pH 7.2). 50 µL of McAb solution (dilution 1:100) was added to 20 µL cell suspension and it was incubated at 4°C for 30 min. Then, the cells were washed 3 times with 0.01% BSA-PBS by centrifugation. 40 µL of rabbit anti-mouse IgG labeled with fluorescein were added to each well and incubated at 4°C for another 30 min. After 3 times washing by centrifugation, the supernatant was discarded, and smear was made of the sedimented cell suspension, the percentage of positive cells was determined by counting at least 200 cells on each smear under the fluorescent microscope.

2. Assay of NK cell activity

The details of the chromium release technique have been described previously [6,7]. PBMC separated by Ficoll-Hypaque density gradient were suspended in RPMI-1640 (Gibco) containing 10% fetal calf serum at a concentration of $5 \times 10^6$ cells/ml. PBMC and chromium-labeled K562 target cells, 0.1 ml/well, were incubated together on triplicate culture plates for 4 h at 30°C in 5% CO₂, then 100 µl supernatant was removed and released $^{51}$Cr was counted in a Beckman gamma counter. Results were calculated as follows:

$$\frac{\text{experimental cpm} - \text{background cpm}}{\text{maximum cpm} - \text{background cpm}} \times 100$$

**RESULTS**

1. The subpopulations of lymphocytes

The proportion of T₃ cells in the epileptic patients was found to be significantly lower than that in healthy subjects (P<0.01). T₄ lymphocytes were lower than in healthy controls and the ratio of T₄/T₈ lymphocytes was markedly decreased. There was statistically significant difference in these values between epileptic patients and controls (P<0.01; table 1).

The percentage of B lymphocyte in peripheral blood of patients was higher than in healthy controls, the percentage of monocytes was lower than in healthy controls, but there was no statistically significant difference.

2. NK cell activity of PBMC in epileptic patients

In 20 of the 58 patients with epilepsy NK cell activity has been investigated. In healthy control group the average activity of NK cells was $42.20 \pm 14.40\%$ and in patient group it was $35.41 \pm 16.24\%$. The difference was significant (P<0.05; table 2).

According to $\bar{x} \pm SD$, the normal range NK cell activity was 27.80 — 56.60%. If the values higher than 60% and lower than 25% are taken as abnormal, then, according to this standard, in the control group only one case was below and 3 cases were over the range, and in epileptic patient group 8 cases (40%) were below the lower and no case was above the upper limit (fig.1).

The relationship between T cell population, the ratio of T₄/T₈, and NK cell activity has also been studied, and no correlation was seen among them.

**DISCUSSION**

Previous study has shown that rosette formation and blastogenesis of lymphocytes are reduced in patients with epilepsy [12,33]. In our investigation it was found that in patients with epilepsy the level of T₃ lymphocytes was markedly lower than in healthy controls. Since both, rosette forming lymphocytes and T₃ lymphocytes, reflect the level of total T lymphocytes, the result of this study is consistent with previous reports [12,33], but the cause of the reduction is not clear.

The results of our investigation showed that the ratio of T₄/T₈ cells was decreased significantly as compared with