CHAPTER IV

RESULTS

1. Immunoperoxidase staining for detection of NK cells in PBMC of rabies patients

The immunoperoxidase staining by using avidin-biotin-peroxidase complex was modified from methods previously described by Hsu and co-worker (185) and Johnson et al (186).

To determine factors that might affect the outcome of immunoperoxidase staining, different conditions were performed. Various concentrations of mouse anti-human CD 56 and CD 57 (1° Ab)(1:25, 1:50, 1:100 and 1:200 dilutions) were titrated against 2 different concentrations of biotinylated horse anti-mouse IgG (2° Ab)(1:100 and 1:200 dilutions) as shown in table 2 and 3 respectively, dilution of 1:100 of CD 56 and 1:50 of CD 57 monoclonal 1° Ab and 1:100 of 2° Ab resulted in a greater intensity of cytoplasmic staining of CD 56 and CD 57 positive peripheral blood mononuclear cells on a clearer background. There was no difference in intensity of staining using varied 1° Ab incubation times (45 minutes, 1 hr and 1.5 hrs). The chosen incubation time was 45 minutes based on convenience.

2. Specificity of reaction in immunoperoxidase staining, ABC. technique

The control studies were done using normal mouse serum instead of 1° Ab on PBMC from normal controls, rabies and non-rabies encephalitic patients. No reaction was obtained, confirming the specificity of the staining system.

3. Quantitation of natural killer (NK) cells in peripheral blood of rabies patients and normal healthy controls

The numbers of cells with CD 56 phenotype of the rabies patients were not significantly different from those of the normal controls, i.e., 14.3 ± 2.4 % ($\bar{X}$±SD) vs. 14.5 ± 1.8 % ($p>0.05$)(Table 4, Figure 5). Rabies patients had significant reduction of cells with CD 57 phenotype (7.9 ± 2.3 %) as compared to normal controls (17.0 ± 1.9 %, $p<0.001$)(Table 4, Figure 6).

CD 56 cells represent human NK cells much better because > 95 % NK cells are CD 56 positive but only 20-60 % will be CD 57 positive (96,100). Consequently, our results suggest that the number of NK cells was not diminished in rabies patients.

4. The natural killing activities of peripheral blood mononuclear cells in rabies patients and normal healthy controls

The natural killing activity in PBMC of 13 human rabies patients compared to 31 normal healthy controls are expressed in

LU/10^7 PBMC and shown in Table 5 and Figure 7. Natural killing activity of human rabies PBMC was lower than that of normal controls , however, it did not reach a statistical significance (45.3 ± 18.8 vs. 55.4 ± 22.3, $p>0.05$).

5. NK number and function of peripheral blood mononuclear cells in non-rabies encephalitic patients

The number of cells with CD 57 phenotype in non- rabies patients were significantly different from those of the normal controls (11.5 ± 2.1 % vs. 17.0 ± 1.9 % , $p<0.001$)(Figure 6).

As shown in Figure 8, there was no correlation between number and function in these patients. Two patients with tuberculous meningoencephalitis who had a normal CD 56 number appeared to have significantly diminished NK activity. On the other hand, 2 patients with herpes simplex and viral encephalitis of unidentified cause, whose NK activities were within normal range, had significantly diminished CD 56 numbers.

6. In vitro activation

To determine whether the NK cells from rabies and non-rabies encephalitic patients could be further enhanced in vitro , PBMC from 4 rabies, 4 non-rabies encephalitic patients and 10 normal healthy controls were studied. In vitro culture of PBMC with exogenous recombinant α -IFN or IL-2 were performed. When compared with unstimulated culture (C), lytic activity was

increased after culture of PBMC overnight with α-IFN or IL-2 in rabies patients ($p<0.01$, $p<0.05$ respectively) and controls ($p<0.01$, $p<0.01$) but not in non-rabies encephalitic patients ($p>0.05$, $p>0.05$) as shown in Figure 9.