CHAPTER I

INTRODUCTION

Rabies is a common cause of viral encephalitides in developing countries including Thailand where canine rabies has not been adequately controlled. It has been estimated that at least 300 people die each year from rabies in Thailand and approximately 50,000 worldwide. Rabies is a universally fatal disease in man, once signs and symptoms develop. Human rabies may present as encephalitis or paralysis. Encephalitic patients usually have a very rapid and progressive course. Most of them die within 7 days after onset. In contrast, paralytic rabies patients may survive up to 3 weeks (1).

Defects in immune responsiveness play a role in pathogenesis of rabies. Virus localization and strain may also contribute to the diversity of clinical manifestations. Immune injury may play a role in disease severity.

Iwasaki, Smith and Tignor (2,3,4) have studied immunopathogenesis in experimental rabies models. Rabies infected immunosuppressed mice live longer than immunocompetent mice. The onset of paralysis following transient immunosuppression is related temporally to a return of immune

responsiveness. Histologic examination also showed a marked inflammatory responses in the CNS of paralyzed immunocompetent animals. This "early death phenomenon" has also been observed in rabies patients (5). Patients who have cellular reactivity to rabies virus antigen, as determined by the in vitro lymphocyte proliferation test, tend to die faster and usually manifest as encephalitis. Patients who have Myelin Basic Protein (MBP) reactivity also have a very rapid and progressive course terminating usually within 17 to 24 hours. These data support the presence of immunopathogenetic as well as autoimmune mechanisms. Results of studies of rabies viral antigen distribution in the CNS of patients with encephalitic and paralytic rabies did not show any correlation with varying clinical manifestations. Antigen was found predominantly in the brainstem, cerebellum and spinal cord regardless of the clinical forms when the survival period was 7 days or less. Studies using a panel of monoclonal antibodies against glycoprotein (G) and nucleocapsidprotein (N) as well as restriction map analysis of isolates from rabid dogs and patients with both presentations did not show any particular differences. Immune mechanisms seem to play role in both enhancing the severity and in determining the clinical presentation of rabies.

The concept of "immune paralysis" is based on the facts that rabies is a strictly neuronotropic virus infection. Absence of viremia and infection at a immunoprivileged site may have been responsible for the failure of host defense mechanisms to

eradicate an established CNS infection. Chances are low for immune cells to recognize the virus thus delaying protective responses and allowing virus to disseminate . Moreover, previous studies found defects in immune responsiveness in experimental models and in man (1,6,7). Recent studies, however, did not support a delay of immune responsiveness due to absence of viremia and to infection that is restricted to the nervous system. Antibody development did not correlate with the period of survival. Furthermore, rabies neutralizing antibodies were found as early as days 3 and 4 after the onset of disease. This suggests that the process of immune recognition develops during the pre-clinical phase or incubation period. This may occur at the inoculation site or at the dorsal root ganglion where initial viral replication occurs and where the blood nerve barrier is relatively scant or deficient. However, no more than 20% of these patients develop neutralizing antibody in the blood. Analysis of antibody to G and N proteins suggests a deficit in immune recognition of antigens, particularly to N protein which is important for elicitation of a rabies neutralizing antibody response.

Examination of cytokines in serum showed that levels of soluble interleukin-2 receptor (sIL-2r) were comparable in groups of fatal rabies and nonfatal encephalitis due to other viruses. However, fewer paralytic rabies patients had elevated sIL-2r. Soluble CD8 levels were elevated in only a few rabies and

non-rabies patients. Interleukin-6 was detectable only in sera of encephalitic rabies patients and in none of the patients with paralysis. This suggest that an inadequate response to N protein rather than defects in immune activation (except in the subgroup of paralysis) may be responsible for the virulence.

Wiktor et al(7) noted in the course of experiments on the development of the cytotoxic T- lymphocyte (CTL) response following rabies immunization, that infection of mice with attenuated strains of virus such as HEP or ERA induced a rabies specific CTL response. Infection with virulent CVS or street virus,however, did not induce such a response , and lethal infection with a street rabies virus strain is always associated with lack of such a response. Moreover,acute infection of mice with virulent rabies virus does not only cause CTL suppression against rabies virus, but also against other concomitant immunizing agents such as influenza virus . Return of a cytolytic T cell response in immunosuppressed mice correlated with recovery(8,9). Therefore, CTL response may be important for protection from rabies. However, inorder for CTL to become active,specific recognition must be intact. This process is relatively slow and only a small number of T cells may usually be involved. After the initial antigen activation, at least one to two weeks is required to amplify the specific response to a functionally effective level. In contrast, natural killer cells appear to be lymphocytic "Minute-men", which can be mobilized quickly.These cells are constantly circulating in relatively

large numbers and require neither antigen processing nor the presentation of antigen for activation (10).

Survival periods in human rabies after first clinical symptoms usually ranges from days to one week except in individuals who have the paralytic presentation where survival periods of more than 2 weeks may be observed (1). Under such circumstance of a rapidly progressive course, NK cells can be expected to be of prime importance in preventing dissemination of virus. Recent evidence in animals have identified a potentially important role for NK cells in the host defence against viral infections other than rabies(11,12). Griffin et al (12) demonstrated that after intracerebral inoculation of sindbis virus, the level of NK activity increased dramatically in spleen and peripheral blood. They found that both normal mice and athymic mice can clear sindbis virus from brain, and have high NK activity in CSF. NK cells are sensitive to cyclophosphamide (13,14). This is consistent with the observations that inflammatory cells in the CSF, both specific and nonspecific, are eliminated by a single dose of cyclophosphamide given after infection. Biron et al(15) reported a female patient who had a marked susceptibility to herpesviruses. Immune function including specific T-cell and antibody responses to viral antigens were normal or near normal except for deficiency of natural killer cells.Cells mediating natural killer function with NK phenotypic markers could not be

demonstrated by any of the methods used in this patient. Her unusual susceptibility to viral infection was also observed during primary infections with cytomegalovirus and herpes simplex virus. This spectrum of sensitivities suggests that natural killer cells have a major defensive role against some but not all viral infections.

In human rabies, Sriwanthana et al (16) showed that number of cells with Leu 7 phenotype (CD 57) in peripheral blood mononuclear cells of 7 rabies patients were diminished. In order to determine whether cells with Leu 7 phenotype are truly diminished or their lower number are due to sequestration , lymphocyte subsets in the brain of a rabies patient were analyzed. Lymphocytes at perivascular cuffs as well as in brain parenchyma from various regions were mostly T cells and none of them were CD 57 cell by avidin-biotin immunohistochemical technique. However, NK function has not been determined in this study. Further, cells with CD 57 represent only 20-60% of NK cells.

To examine the importance of NK cells in human rabies, their number, analyzed by monoclonal antibody to CD 56 phenotype, and function were assessed. Normal healthy controls and patients with meningoencephalitis due to other origin were also studied.