CHAPTER II

LITERATURE REVIEW

CLINICAL FEATURES OF RABIES

In human,rabies presents in one of two forms;encephalitic or paralytic which are analogous to furious and dumb rabies in dogs respectively. A wound or abrasion of the skin,usually inflicted by a rabid animal, is the major portal of entry of virus which is excreted in saliva. A few cases have been reported after non-bite exposures. These included contact with aerosolized rabies virus in caves inhabited by rabid bats (17) and in laboratory accidents with infected aerosolized tissues (18,19). Exposure of the conjunctiva , oral mucous membranes , genitalia and abrasions to the saliva of rabid animals can also cause rabies (20). A lick by a rabid animal is dangerous only to the damaged skin (21). Rabies has occured in 4 persons who received corneal transplants from donors who died of undiagnosed rabies (22,23,24). Not every rabid animal bite necessarily results in clinical rabies. Rabies mortality varies from 35 to 57% in unvaccinated bite victims (20,25,26). The risk of developing clinical rabies depends on the virus content of the saliva, severity of the bite and location of the wound.

Once infection occurs, the clinical course can be divided into 5 stages (20) : the incubation period, the prodrome, the acute neurologic phase, coma and death or recovery.

1. Incubation Period

The interval between exposure and the appearance of illness attributable to rabies is the incubation period. This period is usually 1-2 months but ranges from 1 day to 6 years (1,5,20,21,27). It depends upon the size of the viral inoculum, the severity of the wound and length of the neural path from the wound to the CNS. Severe bites on the head have shorter incubation periods than bites on the extremities (28).

2. Prodrome

The initial symptoms of clinical rabies occur when the virus invades the CNS. These symptoms are usually either variable or nonspecific, and may be described as tingling , itching , burning , coldness , numbness or as simply as pain at the site of bite. The symptoms, starting at the site of the bite, gradually spread to involve the whole limb and occasionally involve the whole body. About one-third to two-thirds of all patients with rabies can be expected to notice some sort of abnormal sensation in the region of the bite at the start of illness. Other symptoms include fever , easy fatigability , gastrointestinal symptoms , musculoskeletal pain which may

resemble influenza or an upper respiratory tract infection. These features are non-diagnostic and also occur in some patients who have been bitten but never develop the disease.

3. Acute Neurologic Phase

Encephalitic rabies : This form of rabies occurs in more than two-thirds of human cases. The earliest neurologic feature in encephalitic rabies is hyperactivity, which may resemble intense anxiety reaction or nervousness but can be aggravated by internal stimuli such as thirst or fear, or external stimuli such as bright light or a loud noise. Within hours to days , the patients exhibits alternating intervals of confusion and calm. During confusional state, signs of autonomic disturbances are occasionally observed. As the disease progresses, confusion becomes extreme, and may evolve to wild agitation and aggressiveness. This period of irritability is gradually replaced by depression of consciousness and coma. Most encephalitic rabies patients have aero- and hydrophobia but may not persist throughout the whole course. The clinical pattern of encephalitic rabies is shown in Figure 1.

Paralytic Rabies : One-third of human cases may present as paralytic rabies. It is less readily diagnosed because of its atypical presentation resembling Guillain-Barre' syndrome (GBS). Certain animal vectors, different virus strains, a bite on the lower limbs, and unsuccessful postexposure immunization may predispose to this clinical presentation. The major signs in

encephalitic rabies appear late in paralytic cases. The most striking clinical difference between encephalitic and paralytic rabies is the relative sparing of consciousness in the latter group. Weakness usually starts in the bitten extremity and progressively involves all limbs. During the early phase, the obvious signs are myoedema at percussion sites (29) and piloerection. This myoedema is noted since the start of neurological phase, even during the prodrome, and persists until the preterminal stage. Only half of the paralytic patients experience aero- and hydrophobia. The course of the disease is less fulminant than in the encephalitic type and survival is longer, particularly when ventilatory support is available. The clinical pattern of paralytic rabies is shown in Figure 2.

4. Coma

Both groups are indistinguishable in this stage. Meticulous history taking and diagnostic laboratory tests are required to exclude other CNS infections especially in endemic areas.

5. Recovery

Despite intensive care of rabies patients combined with treatment using interferon (30) and adenine-arabinoside, the fate of victims has not changed. Since 1972, 3 well-documented cases of recovery have been reported (19,31,32). Diagnosis in these patients rested on a high concentration of rabies

antibodies in blood and CSF in serial observations, the concentrations being too high to have resulted from vaccine.

PATHOGENESIS

Rabies infection almost always follows the bite of a rabid animal harboring the virus in saliva, although it can be acquired via nasal or oromucosal routes and from aerosol exposures. In order to complete its cycle with production of clinical signs and further transmission of the infection, the virus has to be transported centripitally from the site of the bite to the CNS, where multiplication takes place, and then carried centrifugally to neural and extraneural sites such as the salivary glands. This cycle is modified or even interrupted by the influence of genetic susceptibility factors in the host, immunologic pressure and finally by the strain and virulence of the virus. These complex mechanisms undoubtedly play a role in several puzzling aspects of rabies infection : the long incubation period, the two uniquely different clinical manifestrations, abortive infections as well as recovery, and the rapidly progressive fatal outcome once clinical signs appear.

At electron microscopy, virus particles were found budding from the plasma membranes of muscle cells. The virus or viral genome was taken up into the unmyelinated nerve endings at the muscle spindles and at neuromuscular junctions. Rabies virus binding to acetylcholine receptor is inhibited by alpha-bungaro-

toxin and d-tubocurarine (33). Monoclonal antibodies to the alpha subunit of the acetylcholine receptor also specifically block viral binding in vitro (34). Sequence homology of amino acids between rabies viral glycoprotein and snake venom curaremimetic neurotoxins has also been demonstrated (35). However, other receptors must be involved since many cells such as HeLa cells which can be infected do not contain acetylcholine receptors.

Rabies virus may remain at the inoculation site for a consideration period, since amputating the feet of the mice weeks after footpad inoculation markedly reduced mortality rates (36). Immunosuppression increases mortality and some immune mechanisms may be involved in eventual release of virus from myocytes. Rabies virus reaches the CNS by retrograde axoplasmic flow (37,38). Neurectomy (39) and inhibition of axoplasmic flow with colchicine and vinblastine prevents rabies in experimentally infected mice. Sequential studies demonstrate viral accumulation in the dorsal root ganglia and the soma of the motor neurons. There is uncertainty about the relative involvement of sensory and motor pathways in the spinal cord

Once rabies virus reached the CNS, rapid dissemination occurs. Budding from the plasma membrane of neurons and glial cells, and evidence of direct cell-to-cell transmission or direct transsynaptic spread have been documented (40,41). Recently,

Gillet et al (1986) have demonstrated that rabies virus travels by retrograde fast axonal transport (200-400 mm/day) following stereotaxic inoculation into the striatum. The studies by R.T.Johnson (1965,1982) show that there is selective vulnerability of neuronal cells in the limbic system with relative sparing of the neocortex in early stage of invasion. These finding readily explain the behavioral abnormalities of the disease. Eventually, widespread infection of neurons leads to terminal coma and death (42). Neurons are the CNS cells selectively involved, although infection of astrocytes and glial cells has been reported in animals and humans (40,43,44,45). Glial cells are significantly less susceptible than neurons to rabies virus infection in vitro (46). The presence of highly sialylated gangliosides of GT_{1b} and GQ_{1b}, plays a role in the binding of rabies virus to the membrane of chick embryo-related cells, and larger amounts of these gangliosides are found in neurons than in glial cells (47).

The clinical manifestrations of rabies have been considered to be due to direct viral invasion of the nervous system. The relatively scanty inflammation in the brain tissue with absence of cell destruction points to functional derangement as the main cause of lethality. Preliminary studies in mouse neuroblastoma-rat glioma hybrid cells (48,49) and in rabies-infected rat brain (50,51) show that there are modifications in opiate and muscarinic acetylcholine receptor affinity. Neuronal function has been well-demonstrated experimentally (52), with 3

distinct phases of impairment of brain activities and sleep pattern changes.

However, the presence of virus alone may not be the only factor in determining the clinical disease, for access of the virus to the CNS does not necessarily lead to rapid development of symptoms and death. High titers of virus in the brain and spinal cord can be found in animals long before clinical signs of the disease appear (53). The prevalence of abortive canine rabies in the northern part of Thailand was found to be 17% (54). Abortive rabies and recovery from clinical rabies with or without residua has been repeatedly reported in many species (31,32,55,56,57,58,59,60). The mechanisms mediating this resistance are complex and probably involve virologic, immunologic and genetic aspects.

Mice of various strains vary in susceptibility to street rabies (61). In SJL/J mice, resistance is associated with restriction of viral replication within the CNS which is correlated with the early appearance of neutralizing antibody (62). Rabies virulence can also be determined by viral strain. Fixed rabies virus injures neurons more extensively than street virus strains (63). The biological properties of rabies virus correlate with changes in the antigenic phenotype (64). Rabies virus contains 5 proteins. The single glycoprotein is responsible for the induction and binding of neutralizing antibodies, and for conferring immunity to lethal challenge with rabies virus

(65,66,67,68,69). Analysis of variants selected in vitro with antiglycoprotein monoclonal antibodies has revealed that only one amino acid substitution at position 333 of the glycoprotein affects virulence in adult mice (68,70). Rate of cell-to-cell spread, number of infected neurons and the degree of cellular necrosis were much lower in the case of this apathogenic virus,although the distribution of infected neurons in the brain was similar for both viruses (71). Defective interfering particles may be another factor in resistance and possible survival (6,72). Natural infection with small doses of less pathogenic virus strains might allow the host time to impart an adequate immune response. A very high level of interferon was detected in rabies-infected rat brain without beneficial effect because its production may have been too late (73).

IMMUNE RESPONSE TO RABIES INFECTION

Both humoral and cellular mechanisms are important in successful clearance of rabies virus. Depletion of B cells by anti - mu - serum treatment, and depletion of T cells with antithymocyte serum or of T and B cells with cyclophosphamide potentiate infection (74) . Adoptive transfer in these cyclophosphamide-treated mice with combined T and B cells soon after infection, reduced the mortality in more than either cell type alone (75). The efficacy of post-exposure protection by inactivated tissue culture vaccines correlates with cell-mediated cytotoxic response (76). Lethal infection with street rabies

virus strain, on the other hand, is always associated with lack of such a response (7). Return of a cytolytic T cell response in immunosuppressed mice correlates with recovery (8,9).

In contrast, some authors have suggested that an immune response to rabies virus may contribute to the disease process (77). Immunosuppressed mice take longer to succumb to rabies infection than immunocompetent mice, and the onset of paralysis after experimental immunosuppression is temporally related to the return of immune responsiveness (2,3,78). Passive transfer of rabies immune serum or sensitized cells to infected immunosuppressed animals accelerates the appearance of paralysis and death. Histologic examination then reveals marked inflammation and degeneration of CNS parenchymatous tissue (3,79). This "early death phenomenon" was first observed in monkeys and mice , and later in humans with unsuccessful immunization attempts (5,36,80,81,82). Therefore, immune responses may have dual effects in rabies infections.

Host immune response may play a role in determining the clinical presentation of either encephalitic or paralytic rabies in mice (2). Rabies infected immunocompetent mice usually exhibited an ascending paralysis of the limbs, while immunosuppressed mice developed encephalitis with only minor paralysis.

In human rabies, the immune response may also influence

clinical manifestrations. Patients with a cellular response to rabies virus, as determined by lymphocyte proliferation assay in vitro, manifest clinically as encephalitis rather than paralysis and tend to die faster (1), though this finding does not exclude differences in the strain and distribution of virus as determining factors. The less fulminant and slower clinical course in paralytic rabies may be due to low levels of viral glycoprotein on the neuronal surface membrane. This has been shown in street virus strain infection of neural cell lines and laboratory rodents compared to fixed virus strain (83). In addition, the autoimmune phenomenon of cellular reactivity to myelin basic protein is observed in encephalitic and paralytic human rabies. Patients with this reactivity may have more rapidly progressive disease (1). The role of myelin basic protein has been noted in patients with postinfectious and postvaccinal encephalomyelitis where the degree of cellular or humoral response was correlated with the disease severity (84,85,86,87). In human rabies , however, MBP reactivity associated with accelerated death may merely reflect an epiphenomenon associated with widespread damage.

In contrast to post-vaccinal encephalomyelitis, antimyelin basic protein antibody has not been detected in the serum or CSF of rabies patients. This is also true for antirabies antibody; only 20% of rabies patients had seroconversion during the early stage. Our attempt to detect antibody to P_2, a neuritogenic antigen in experimental allergic neuritis, in 3

patients with paralytic rabies also failed (unpublished data). An autoimmune phenomenon similar to those in the acute GBS was proposed by Chopra et al (82), in view of neuropathologic changes in these patients. There is need for further detailed studies of the pathogenetic mechanisms involved.

It is intriguing that only 20% of rabies patients develop neutralizing antibody to rabies virus despite a long incubation period of weeks to months. Recent study showed that these patients with rabies may have defect in immune recognition to N protein. Rabies vaccinees usually have antibodies to N and G components by day 10 after the first vaccine injection. Antibodies to individual antigen were comparable in levels at all timepoints. In rabies patients, neutralizing antibodies may appear as early as 3 days after onset of the first symptom of the disease. Levels of anti-N antibody are lower than those directed against G protein in all neutralizing antibody positive patients. These results suggest that the process of immune recognition and of antibody development in human rabies is more likely to occur early in the pre-clinical phase, and that reactivity to N protein may be crucial for elicitation of neutralizing antibody (88). Furthermore, circulating mononuclear cells with Leu-7 (CD 57) phenotype were all markedly diminished in peripheral blood of all 7 rabies patients (16). Defects in immunorecognition (ie. poor neutralizing antibody development) and defects in killing mechanism may allow virus to spread throughout the nervous system

in the very early phase. Acceleration of the disease course then may be potentiated by immune destructive processes in the latter phase.

NATURAL KILLER (NK) CELLS

1. Characteristics and Distribution

NK cells have been identified in many vertebrate species and have been operationally defined as cells capable of mediating spontaneous in vitro cytotoxicity against a variety of target cell populations without apparent prior sensitization(89-92). NK cells have been considered to be distinct from specific cytotoxic T lymphocytes (CTL) because they have not been shown to have clonally distributeed specificity, restriction for products of the major histocompatibility complex (MHC) at the target cell level, or immunologic memory.

Morphologic analysis of NK cells has demonstrated that these cells have a homogeneous appearance of large granular lymphocytes (LGL) and distinctive physical characteristics that allow their enrichment on low Percoll density gradients(93,94). Taken together, these morphologic characteristics and unique functional capacities clearly distinguish NK cells from T cells,B cells, monocytes, and other hematopoietic and lymphoid elements. Despite their homogeneous morphology, it has become apparent that NK cells are extremely heterogeneous and the precise relationships between NK

cells, T cells, and myelomonocytic cells have not been clearly defined. The heterogeneity of NK cells has become apparent through characterization of the cell surface antigens expressed by these cells. Using various monoclonal antibodies, studies have shown that the majority of NK cells in human peripheral blood express antigens such as NKH1 (CD 56 or Leu 19), (95,96), IgG-Fc receptor (CD 16)(97-100), T10 (CD38)(101,102), Mo1 (C3bi receptor CD 11b)(103-105), and T11 E rosette receptor (CD2) (96,100,102). Approximately 60% of NK cells express HNK-1 antigen (CD 57) (106,107) and 20-30% express CD3 (102,108,109) or CD 8 antigens (110). Relatively few NK cells express T4 or Ia antigen, and no NK cells are thought to express B cell-restricted markers such as B1 and B2 nor monocyte restricted markers such as MY4 and MO2. Thus, the majority of NK cells are $NKH1^{+}$ and express markers characteristic of both T cells (CD 2) and myeloid cells (CD 11b,CD16). Other antigens such as HNK-1, CD 3, and CD 8 are expressed only on subsets of NK cells.

A substantial proportion of NK cells shares with T lymphocytes the expression of the CD 2 and CD 7 T cell markers but, in contrast to mature T cells, NK lymphocytes are $CD\ 3^{-}$ $CD\ 5^{-}$, do not express either TCR α/β or γ/δ, and do not productively rearrange either type of TCR genes(111-113). NK cells have no phagocytic and adherence properties(90). T lymphocytes that are either $\alpha\beta^{+}$ or $\gamma\delta^{+}$ may express, particularly upon activation, a cytolytic activity that resembles

that of NK cells. These T lymphocytes should not be termed NK cells. Either T lymphocytes displaying "NK-like" activity or "non-MHC-requiring" cytolysis may be a more appropriate term. Purified $NKH1^{+}$ $T3^{+}$ cells were found to represent 2.5% of PBMC and 24 % of the total number of $NKH1^{+}$ cells. These $NKH1^{+}$ $T3^{+}$ have the typical morphology of large granular lymphocytes and exhibited spontaneous cytotoxicity against K562 target cells and this lytic activity could be inhibited by anti-T3 monoclonal antibody(108).

The distribution of NK cells inside and outside the lymphoid system has been a subject of considerable interest. Studies in mouse (114), rat (115,116) and man(117,118) have indicated that NK cell activity is readily detectable in spleen and peripheral blood but can't be detected in bone marrow and thymus. Whether NK cell activity exists in the lymph node is still the subject of controversy. However, a characteristic pattern of augmentation of NK cells by appropriate stimuli can still be demonstrated (119,120). This pattern of distribution suggests that at least the majority of functionally active NK cells lack the ability to recirculate.

2. Mechanism of NK cell mediated lysis

The study of the mechanisms by which natural killer cells kill their targets has recently undergone intensive research activity. The membrane target structure of NK cells have

not been identified, and it is not known whether different NK cells react with the same or with different target antigens. At the effector cell level, it is not known which membrane molecules play important roles in effector/target cell binding or if all NK cells utilize the same membrane structures to interact with various target cells. Although NKH1(CD56), CD 16, and HNK-1(CD57) antigens have been useful in defining NK cells, these antigens do not appear to play a significant role in the cellular interaction between NK cells and their targets. More specifically, anti-NKH1 antibodies do not affect proliferation of NK cells, effector cell conjugation with targets, or triggering of NK cell lysis(108). Anti-CD 16 antibodies inhibit ADCC and have been shown to activate NK cells(121,122) but these reagents do not affect their function (99,100). Similarly, anti-HNK-1 has not been found to affect NK cell function or proliferation.

Recent findings suggest that NK killing is complex involving possibly multiple mechanisms as well as multiple mediators. While some investigators thought five years ago that pore-forming protein (PFP, also termed perforin or cytolysin) was a central component in NK-mediated killing, it is becoming clear that its role may be more restricted than originally speculated(121,122). NK cells degranulate upon binding to their target cells, releasing PFP (perforin or cytolysin) monomers into the intercellular space. In the presence of extracellular calcium, perforin undergoes a conformational change that allows it to bind to the target membrane and to assemble

into complement-like pores, resulting in irreversible damage to the target cell(121). When the membrane leaks, ions and water tend to flow down their electrochemical gradients toward equilibration, and so there is a drop in membrane potential. If the holes in the membrane are of limited size, there is an additional effect, known as the Donnan effect. Since large molecules inside the cell can not pass out, they then will attract water and salts from the extracellular space through these membrane pores which will lead to cell swelling and lysis(122).

Other mechanisms of killing have also been demonstrated recently. It has been shown that DNA of some, but not all, target cells undergoes fragmentation into repeat units of 150-180 base pairs during attack by NK cells(123). Whether the endonuclease activity is directly introduced from outside or is endogenously activated from inside the target cell during the lethal event has remained speculative. Further studies have shown that target cells may possess an endogenous suicidal pathway which is turned on by the killer cell(124). These findings have recently been used as a strong argument against the pore-formation model(125).

Recent experiments have identified slow-killing molecules in human NK cells (126-129) that are antigenically related to tumor necrosis factor (TNF) and lymphotoxin (LT). An other candidate mediator of NK cytotoxicity is proteoglycans

(130,131). Its role, initially detected in NK cell granules, also requires further studies.

3. Regulation of NK cell function

It has been established that various lymphokines including interferon (IFN)(132,133) and interleukin-2(IL-2) (134-136) are capable of activating NK cells and enhance their cytolytic function. These two lymphokines have been suggested to be the major and basic substances regulating the development of NK cell activity (137,138). The mechanisms by which IFN and IL-2 influence NK function are complicated and probably involve both the induction of new effector cells from inactive precursors (pre-NK cells) and an augmentation of the cytotoxic potential of mature NK cells(139). In addition, NK cloned cell lines were responsive to IL-3 as well as IL-4. IL-3 acts as a "maintenance factor", being unable to induce cell cycle progression but preventing death of the cloned cells in the absence of IL-2 or IL-4. IL-4 appears to act as a "progression factor" with direct effect on cell proliferation as well as having additive effects with IL-2. Although less effective than IL-2, cell treatment with IL-4 also enhanced cytotoxicity. Considerable synergies can be obtained in mouse peritoneal NK cell activity using local injections of suboptimal doses of both IL-2 and gamma interferon (140). However, as it has been demonstrated that exposure of lymphocytes to IL-2 induces IFN production (141,142), it is not clear whether IL-2 itself is sufficient to

augment the cytotoxicity or acts solely through the induction of interferon (143). In addition to inducing division of antigen- or mitogen- activated T cells, IL-2 can affect the proliferation of at least some NK cells in vitro (144,145).

The other important regulatory factors include those that emanate from the central nervous system (146,147). Electrolytic lesions of the hypothalamus,hippocampus and some adjacent structures of rodents result in alterations in the number and functions of natural killer (NK) cells and T-lymphocytes in the spleen, thymus, and blood (148-153). Experimental animals stressed by overcrowding or avoidance conditioning, display impaired immune responsiveness (154-155) and decreased resistance to viral infection (156). Placement of bilateral electrolytic lesions in the preoptic-anterior hypothalamic area(AHT) of rats resulted in decreased splenic NK activity(146). Additionally, decreased NK activity in AHT rats was abrogated by hypophysectomy, suggesting that neural modulation of NK cells was affected by pituitary hormones or peptides(146). A physiological explanation of the mechanisms involved in this regulation suggests that a variety of stimuli, including hypoglycemic stress, psychological(157) and other stress situations (158) affect the pituitary gland directly or through complex interactions with other hypothalamic areas. These in turn influence the basal production of neuroendocrine or immune system hormones, such as β -endorphin, IFN and IL-2 released into the peripheral circulation (146). These

neuropeptides, particularly β -endorphin, influence NK activity via highly specific binding with calmodulin, a ubiquitous calcium binding protein (159,160). The activated calmodulin serves as the major intracellular receptor for calcium, regulates several calcium dependent enzymes (161,162) and controls the cyclic nucleotide metabolism (163,164), cellular secretion (165), microtubule polymerization (166), contractile processes (167) and the action of certain immune system hormones, particularly interferon (168). These conditions are required for the cytotoxicity of NK cells.

In contrast, the factors reported to inhibit NK cell mediated cytotoxicity in vitro are prostaglandins E_1, E_2 and D_2 (169-173). The mechanism of suppression has not been established, but appears to operate through an elevation of intracellular cAMP (171,174). In addition, there are endogenous suppressor cells which influence NK cell activity. Amongst these are macrophages (175,176), thymocytes (177), granulocytes (178) and certain subpopulations of peripheral blood lymphocytes (179,180). However, the mechanism of action of such suppressors is not clear.

4. Role of NK cells in vivo

4.1 NK cells can lyse target cells that have undergone malignant transformation. Normal cells appear to be generally resistant to lysis by NK cells. In animal models, NK

cells have been shown to play an important part in immmune surveillance against the establishment of primary tumors, as well as in controlling the spread of distant metastases. The cytolytic activity of NK cells can be enhanced by IL-2 *in vivo* and *in vitro* (135), enabling them to lyse tumor cell targets that are resistant to unstimulated NK cells. The effectiveness of these lymphokine-activated killer cells has been evaluated in a series of clinical trials in which patients received recombinant IL-2 alone or in conjunction with lymphokine-activated killer cells generated *in vitro*. The metastatic tumors regressed in some of the patients who were treated with lymphokine-activated killer cells or IL-2 (181). Such a response was seen most often in patients with metastatic renal-cell carcinoma or melanoma.

4.2 A number of studies have identified a potentially important role for these cells in the immune response to certain viral infections (11). In these experiments, NK cells responded rapidly to viral challenge and mounted both a proliferative and a cytolytic response several days before a more specific T cell response could be mobilized. Deficiencies of NK cells have been documented in a variety of clinical circumstances, including Che'diak-Higashi syndrome, leukocyte-adhesion-molecule (CD 11/CD 18) deficiency and X-linked lymphoproliferative syndrome.

Increased NK cell activities and elevated IFN levels have also been shown in a wide variety of bacterial and

parasitic infections. However, it is still not clear the exact role of these responses (127-134).

4.3 NK cells may exert a regulatory influence on an immune response. Cells expressing HNK-1 phenotype are found in the follicular area of human lymphoid tissues (182,183), thus which may play a role in B cell differentiation (182). Moreover, at least some NK cells are capable of secreting IFN and IL-2, as well as other lymphokines , but the mechanism whereby NK cells can be triggered to secrete these lymphokines is not known.