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Neuroimmune Networks: Physiology and Diseases, pages 39–49

Neuromodulation of Natural Killer Cell Activity

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INTRODUCTION

During the past two decades, it has been shown that the immune system can be modulated not only by "classical" means (Jerne, 1955; 1974; Benacerraf and McDevitt, 1972; Gershon, 1974; McDevitt, 1980) but also by mechanisms controlled by the central nervous system (CNS) (Solomon, 1969 a,b; Besedovsky and Sorkin, 1977). For example, both psychosocial and environmental stressors have been shown to affect the humoral and cellular components of immunity in laboratory animals and man (Ader, 1981; Tecoma and Huey, 1985; Arora et al., 1987). Depressed immune responsiveness in patients with cerebral tumors (Brooks et al., 1972), Huntington's disease (Morrell, 1979), or Guamanian-Parkinsonism-dementia complex (Hoffman et al., 1978) illustrate that diseases of the CNS are associated with changes in immune competence. In addition, individuals with psychosis (Kovaleva et al., 1977), bereavement and depression (Bartrop et al., 1977), and emotional stress also exhibit impaired immune reactivity (Solomon, 1969a,b). Investigations of experimental animals subjected to stress by overcrowding or avoidance conditioning have shown impaired immune responsiveness (Rassmussen et al., 1959; Solomon, 1969a,b), decreased resistance to viral infections (Rassmussen, 1969), and an increased incidence of neoplasia (Riley et al., 1981), suggesting a link between behavior and disease susceptibility.

Epidemiological evidence that individuals with marked depression and anxiety are more prone to certain tumors has been offered on several occasions in recent years (Stavraki et al., 1968; Crisp, 1970; Grissom et al., 1975; Solomon, 1981; Solomon and Amkraut, 1981). Several lines of laboratory evidence suggest an influence of the CNS on tumor growth. For example, pinealectomy in rodents is followed by an increase in weight of transplantable tumors (Kallenbach and Malz, 1957; Barone and Das Gupta, 1970), a rise in mitotic index (Bindoni, 1971), and an increased spread (Rodin, 1963; Das Gupta and Terz, 1967; Stavraki et al., 1968; Baron and Das Gupta, 1970). Chemical sympathectomy renders rats highly receptive to chemically induced experimental tumors (Stein-Werbeowsky, 1974). The influence of a particular region of the CNS on the growth of some transplantable tumors has also been investigated. Bindoni et al (1980a,b) noted increased tumor growth and higher mitotic index in rats or mice following destruction of the tuberoinfundibular region of the hypothalamus. Survival time, too, was shorter in the lesioned animals than in the untreated controls or in animals with lesions in other cerebral areas (Bindoni et al., 1980a,b).

Natural killer (NK) cells, in addition to being important in the defense against viral diseases, are suggested to play a key role in immune surveillance against neoplastic growth (Herberman and Ortaldo, 1981; Kiessling and Wigzell 1979; Welsh, 1987). This population of large granular lymphocytes (LGL) is cy-

totoxic for a variety of cells, including virally transformed (Williams et al., 1977), lymphoma (Nunn et al., 1976), and those derived from solid tumors (Brooks et al., 1980). Unlike other immune components (e.g., cytotoxic T lymphocytes [CTL] [Borberg et al., 1972; Smith et al., 1977], lymphokine-activated killer [LAK] cells [Yron et al., 1980], or tumor-infiltrating lymphocytes [TILs] [Rosenberg et al., 1986] known to be important in tumor control, NK cells lyse tumor and other target cells "nonspecifically," i.e., without any prior stimulation with a specific antigen (Nunn et al., 1977).

There have been several reports implicating the CNS in modulation of NK cell activity. For example, suppression of NK cell activity can be produced by foot-shock and tail-shock, and this effect can be antagonized by naltrexone (Shavit et al., 1984). Moreover, both central and parenteral administration of morphine reduces NK cytotoxicity (Shavit et al., 1986). These results suggest that NK cell activity is modulated in part by the endogenous opioid system. Neuropeptides such as β-endorphin or met-enkephalin enhance NK cell activity in vitro by activating pre-NK cells and increasing their recycling capacity (Mathews et al., 1983a,b). Corticotrophin-releasing factor (CRF) released following stressful stimuli (Britton et al., 1984) has also been shown to modulate NK cell activity. Irwin et al. (1987a,b) reported that the CRF administered intraventricularly produced a dose-dependent suppression of rat splenic NK cell activity. Neither systemic CRF nor CRF in vitro significantly altered NK cell activity. The immunosuppressive effect of central CRF was antagonized by central, but not by systemic, preadministration of a CRF antagonist, α-helical oCRF (residues 9–41). Irwin et al. suggested that CRF released in the brain may have a role in the central modulation of NK cytotoxicity.

There are other lines of evidence suggesting that the CNS can regulate NK cell activity. Renoux et al. (1982) reported that removal of the left cerebral cortex of mice causes a severe depression in NK cell activity. In contrast, removal of the right cerebral cortex had no significant effect. Our laboratory has recently examined the relationship between lateralization of the CNS and immune functions. We have observed that mice of the strongly lateralized line have lower NK cell activity than mice of either the weakly lateralized line or of the heterogeneous line (Fride et al., 1988). Saxena et al. (1982) have shown that hypophysectomy of newborn mice is followed by profound depression of NK cell activity, probably attributable to absence of growth hormone production, since NK cell activity was restored when the growth hormone was administered. Rapid restoration of NK cell activity with lymphokines such as interferon (IFN) or interleukin-2 (IL-2) in vitro or polyinosinic-polycytidylic acid (Poly[I]:Poly[C]) in vivo in mice with a hypothalamic lesion indicates that the hypothalamus exerts its influence not at an early stage of differentiation, but rather when pre-NK cells (agranular LGL) are converted into active NK cells (LGL). This critical step has been reported as being mediated by IFN (Minato et al., 1980; Itoh et al., 1982). These findings immediately suggest that the hypothalamus controls NK cell activity by regulating the release of factors required for LGL maturation (Forni et al., 1984).

MODULATION OF NK CELL ACTIVITY THROUGH THE BENZODIAZEPINE/GABA RECEPTOR CHLORIDE IONOPHORE COMPLEX (SUPRAMOLECULAR COMPLEX) IN THE CNS

In our laboratory there has been a long-standing interest in the neurochemical bases of anxiety (Tallman et al., 1980; Skolnick and Paul, 1983). Pharmacological, biochemical, and behavioral evidence has suggested that the benzodiazepine/GABA receptor chloride ionophore complex ("supramolecular complex") mediates the antianxiety effects of benzodiazepines, barbiturates, and other pharmacologically important agents (Usdon et al., 1982). More recently, several lines of evi-

dence suggested that the supramolecular complex is involved in the *physiological* control of stress and anxiety (Ninan et al., 1982; Havoundjian et al., 1987). Since the role of the "supramolecular complex" in the neural modulation of immunity had not been investigated, we recently initiated such studies. Thus, we recently found that the administration of the benzodiazepine receptor (BzR) "inverse agonists" FG 7142 (N'-methyl-β-carboline-3-carboxamide) and DMCM (3-carbomethoxy-4-ethyl-6,7-dimethoxy-β-carboline) produced a profound suppression of T-cell functions in mice (Arora et al., 1987). Since β-carbolines like FG 7142 have been demonstrated to produce a BzR-mediated behavioral, somatic, and endocrine syndrome reminiscent of stress or anxiety in rodents and primates, including man (Ninan et al., 1982; Dorow et al., 1983; Insel et al., 1984), our findings (Arora et al., 1987) suggested that the benzodiazepine receptors in the CNS and the pathways subserved by these receptors may be important in the neural control of immunity.

In the present study, we investigated whether the supramolecular complex also modulates NK cell activity. Male B10.BR mice (Jackson Laboratories, Bar Harbor, ME) were injected with FG 7142 (5–50 mg/kg) or an equal volume of vehicle. Spleens were removed 2 and 24 hr later and NK cell activity measured using chromium-51 (^{51}Cr) release assay as described (Arora and Shearer, 1981; Petitto et al., 1988). A dose-dependent suppression of NK cell activity was observed both at 2 hr (Fig. 1A) and 24 hr (Fig. 1B) after administration of FG 7142. A similar dose-dependent suppression of NK cell activity was observed, at other effector:target (E:T) cell ratios (100:1 and 25:1; data not shown). The doses of FG 7142 needed to suppress NK cell activity (Petitto et al., 1988) were consistent with those that produce both behavioral and endocrine changes in rodents reminiscent of stress or anxiety (File and Fellow, 1985; Stephens and Kehr, 1985) and those that suppressed T-cell functions (Arora et al., 1987). Pretreatment of mice with the specific, high-affinity BzR antagonist Ro 15-1788 (10 mg/kg) 15 min prior to administration of FG 7142 (25 mg/kg) resulted in a significant reduction of this suppression (Fig. 2). In this series of experiments, FG 7142 suppressed NK cell activity by 35.6% (compared with vehicle-treated animals), which was reduced to 16.6% in mice

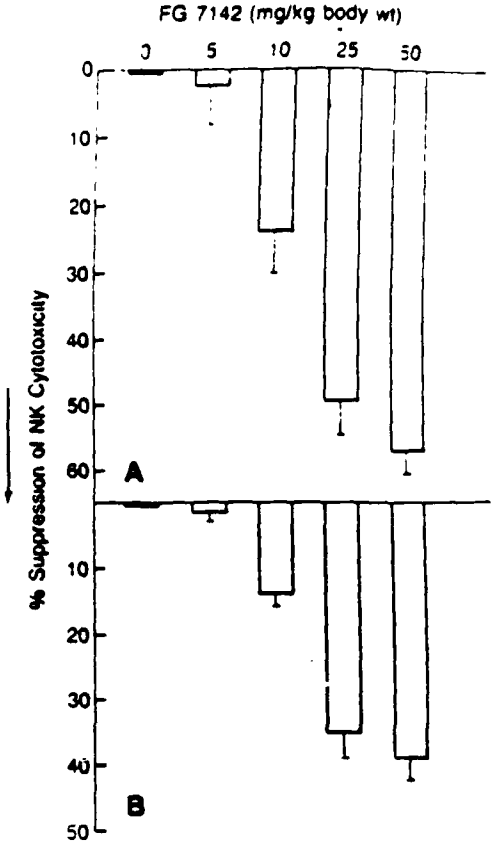

Fig. 1. *Inhibition of NK cell activity by FG 7142: dose-response relationship. Male B10.BR mice were injected with FG 7142 (5–50 mg/kg) or an equal volume of vehicle. Spleens were removed 2 hr (A) or 24 hr (B) later and NK cell activity measured on ^{51}Cr-labeled YAC-1 target cells as previously described (Arora and Shearer 1981; Petitto et al., 1988). Values represent the mean percentage of suppression ± S.E.M. of 10 mice at an E:T ratio of 50:1. Similar dose-response relationships were observed at the other E:T ratios tested (100:1 and 25:1). Analysis of variance followed by Fisher's Protected Least Significant Difference Test indicated that the 25 and 50 mg/kg groups were significantly different from the vehicle treated group (*P < 0.01).*

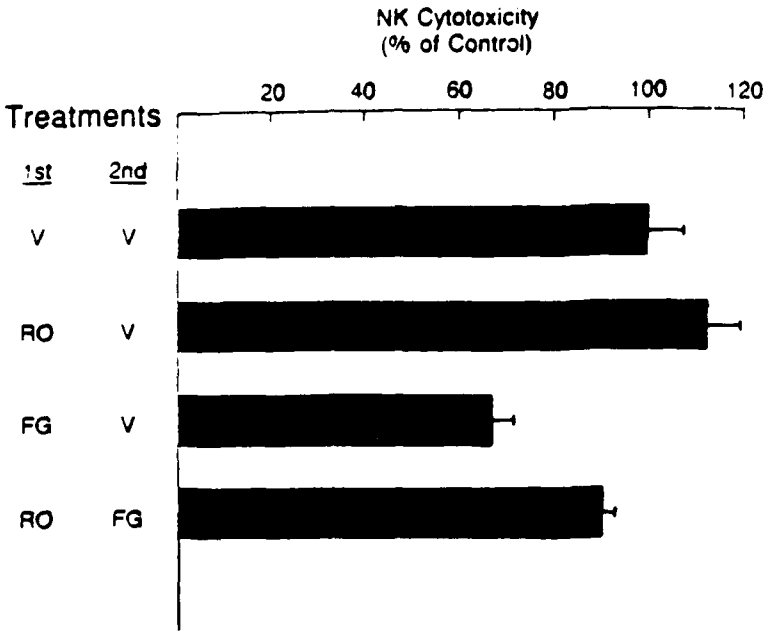

Fig. 2. *Ro 15-1788 blocks FG 7142-induced suppression of NK cell activity. Male B10.BR mice were pretreated with either Ro 15-1788 (10 mg/kg) or an equal volume of vehicle 15 min prior to treatment with either FG 7142 (25 mg/kg) or vehicle. Spleens were removed 2 hr later and NK cell activity determined as previously described (Arora and Shearer, 1981; Petitto et al., 1988). Values are expressed as the percentage of NK cytotoxicity found in vehicle-treated mice. Values represent mean ± S.E.M. of eight mice at an E:T ratio of 50:1. V, vehicle; RO, Ro 15-1788; FG, FG 7142.*

pretreated with Ro 15-1788 (Fig. 2). Ro 15-1788 did not reduce NK cell activity when administered alone (Fig. 2).

Several observations in this study suggest that the suppression of NK cell activity by FG 7142 is mediated via occupation of BzR in the CNS. Direct addition of FG 7142 (1–10 μM) to the ^{51}Cr release assays during a 4-hr incubation period had no effect on NK cell activity (data not shown). Furthermore, neither Ro 15-1788 nor inverse agonist FG 7142 bind with high affinity to peripheral benzodiazepine receptors (pBzR) (Marangos et al., 1982; Schoemaker et al., 1983) that are present on cells of the immune system (Zavala et al., 1985; Ruff et al., 1985; Moingeon et al., 1985; Zavala and Lenfant, 1987). Finally, the antagonism of FG 7142-induced suppression of NK cell activity by Ro 15-1788 is consistent with the ability of this compound to block the effects of both BzR agonists (i.e., substances with benzodiazepine-like qualities) and inverse agonists (Skolnick and Paul, 1983). These findings suggest that the BzR inverse agonists may be useful tools to study neural–immune interactions and support the hypothesis (Arora et al., 1987) that the pathways subserved by the supramolecular complex may play an important role in the neural modulation of immunity.

Recent studies have demonstrated that the Long-Sleep (LS) and Short-Sleep (SS) mouse lines, bidirectionally selected for their hypnotic sensitivities to a single dose of ethanol, are also differentially sensitive to other depressants such as barbiturates (McIntyre and Alpern, 1985, 1986; Marley et al., 1986) and benzodiazepines (McIntyre and Alpern, 1986), as well as convulsants such as 3-carbomethoxy-β-carboline, picrotoxin, and bicu-

culline (Phillips and Dudek, 1983; McIntyre and Alpern, 1986). Thus, LS and SS mouse lines represent a unique *genetic model* that can be utilized to assess the role of the supramolecular complex in the neural modulation of immune functions. Since the well-described difference in drug sensitivities of these lines is mediated through inherent differences in biochemical and biophysical properties of the supramolecular complex (Marley and Wehner, 1986; McIntyre et al., 1988), the assessment of NK cell function could be accomplished without confounding pharmacological intervention. Spleen cells from male LS and SS mice (Institute for Behavioral Genetics, University of Colorado, Boulder, CO), were tested for NK cell activity by using a ^{51}Cr release assay as previously described (Arora and Shearer, 1981; Petitto et al., 1988). NK cytotoxic activity ranged from 6.0 to 16.9% in the LS mice and from 3.6 to 7.0% in SS mice, respectively (Fig. 3). The NK cell activity of the LS line was higher than in the SS line at each E:T ratio tested, with differences ranging from 67 to 142%. (Significant differences in the total numbers of cells per spleen were also observed between these lines. The number of viable cells per spleen was 68% higher in the LS line [$178.8 \pm 15.3 \times 10^6$) than in the SS line [$106.3 \pm 6.5 \times 10^6$] [$P < 0.001$, Student's t test].) Since NK cell activity is assayed with equal numbers of effector spleen cells from each line, the greater number of splenic leukocytes in LS mice thus greatly enhanced the genetic differences in NK cell activity between LS and SS. In toto, these observations, in concert with the findings that benzodiazepine ligands affect immune functions (Arora et al., 1987), provide additional support for the hypothesis that the supramolecular complex (in the CNS) regulates NK cell activity.

INFLUENCE OF SEXUAL BEHAVIOR ON NK CELL ACTIVITY

Sexually active individuals have been reported to have higher risks for contracting diseases such as hepatitis B, gonorrhea, syphilis, and acquired immune deficiency syndrome (AIDS) (Dietzman et al., 1977; Judson et al., 1980; Ma and Armstrong, 1984). We recently observed that sexual behavior, recognized as one of the most efficient routes of disease transmission, itself is immunosuppressive and may trigger events that increase an individual's vulnerability to infection (Ostrowski et al., 1989). The hypothesis that sexual behavior influences NK cell function was examined in male Golden hamsters (*Mesocricetus auratus*). Hamster sexual behavior is well-described, brief, stereotypic, and readily measured (Beach and Rabedeau, 1959). Three groups of male hamsters were tested: mated, sham-mated, and virgin males. Mated animals achieved five or more ejaculations within 45 min in at least four of five weekly (5–7 day interval) mating trials. Intromissive and ejaculatory behaviors were recorded during each mating session as previously described (Beach and Rabedeau, 1959; Ostrowski et al., 1989). Virgin males were treated in the same manner as mated animals except that they were placed in a clean mating chamber without a female. Sham-mated males were exposed to sexually receptive females for 20 min sessions and permitted to investigate and mount the females, but not to intromit or ejaculate (Ostrowski et al., 1989). Splenic NK cell activity was measured as previously described (Arora and Shearer, 1981; Petitto et al., 1989). NK cell activity decreased 2 hr after both a single sexual encounter and after the last of five weekly mating sessions, but not 16 hr after mating (Fig. 4). Thus, NK cytotoxicity was suppressed equally in both sexually active males and in males copulating for the first time. Since many hormones (pituitary, adrenal, and gonadal hormones) that surge with sexual behavior (Kamel et al., 1975) have been shown to influence immune functions (Hochman and Cudkowicz, 1979; Targan, 1981; Kuribayashi et al., 1981; Saxena et al., 1982; Mathews et al., 1983a,b; Johnson et al., 1984), current research efforts in our laboratory are aimed at identifying neural and/or

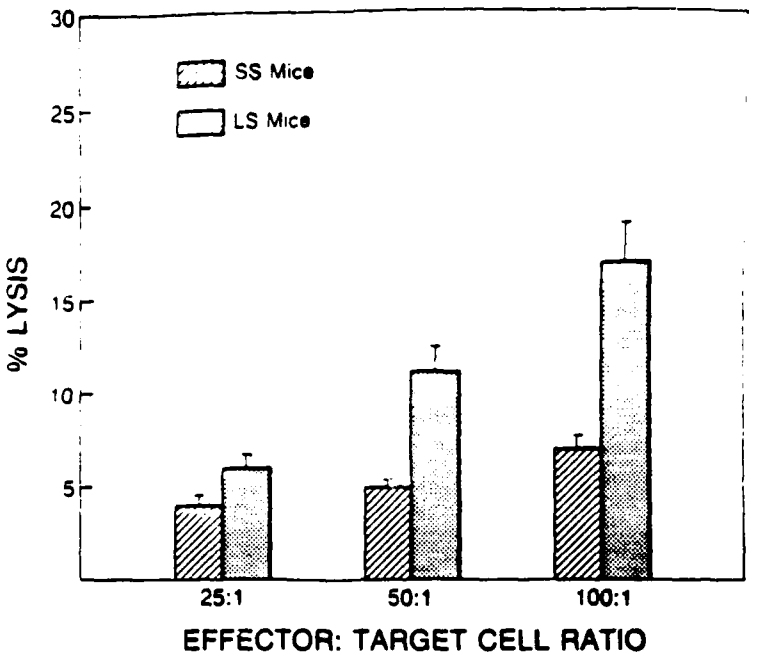

Fig. 3. *Comparison of splen[ic] NK cell activity in the Short-Sleep and Long-Sleep mouse lines. NK effector cell activity was tested as previously described (Arora and Shearer, 1981; Petitto et al., 1988) using YAC-1 tumor target cells at E:T ratios of 100:1, 50:1, and 25:1. Values represent the mean ± S.E.M. of eight animals. The NK cell activity in LS mice was 142, 122, and 67% greater than the SS at E:T ratios of 100:1, 50:1, and 25:1, respectively.*

hormonal factors that may contribute to the copulation-linked NK suppression.

EFFECT OF BEREAVEMENT ON NK CELL ACTIVITY

Recently considerable interest has been focused on changes in immune function that occur in persons undergoing bereavement following the death of a spouse. In addition to other immune functions (Bartrop et al., 1977; Schleifer et al., 1983), NK cell activity during bereavement has also been evaluated (Locke et al., 1983; Irwin et al., 1986). Irwin et al. (1987a) compared NK cell activity in women whose husbands had recently died with that found in age-matched women who had not experienced recent adverse life events. Bereaved women had significantly lower NK cell activity than women whose husbands were healthy. In a second study, depressive symptoms and NK cell activity were measured longitudinally in women before and after the death of their husbands. The results suggested that depressive symptoms, and not merely the death of the spouse, were related to a reduction in NK cell activity during bereavement. Other investigators have also found a relationship between the severity of depressive symptoms and impaired NK cell activity (Locke et al., 1983; Irwin et al., 1986). Longitudinal study of widows utilizing concurrent measures of physical symptoms and immune functions such as NK cell activity will help to ascertain the potential health consequences of stress-related alterations in the immune system.

CONCLUDING REMARKS

Although the studies reported here clearly point to a role of the CNS in modulating the

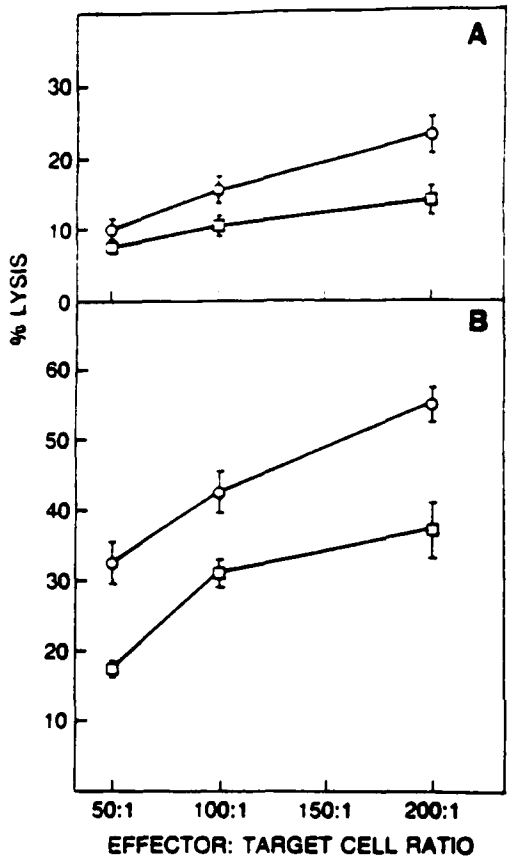

Fig. 4. *Splenic NK cell activity of male hamsters 2 hr after either a single mating session (A) or males mated weekly for 5 weeks (B). NK cell activity was suppressed in mated males (□) when compared with virgins (○) In A, $F = 5.51$, $P < 0.03$; in B, $F = 18.09$, $P < 0.002$. Values represent group means ± S.E.M.*

activity of NK cells, the underlying mechanism(s) is still unclear. There is much evidence to suggest that many endogenous and external factors, as well as regulatory cells, affect the differentiation, maintenance, and function of NK cells (Herberman and Santoni, 1984). Among the activating signals, lymphokines such as IFN and IL-2 have been found to play a major role affecting the lytic function (Trinchieri et al., 1978) as well as the growth and differentation of NK cells. Treatment in vitro with IFN has been shown to increase the kinetics of lysis and the recycling of NK cells (Timonen et al., 1982). In addition, IL-2 has been found to sustain the growth of NK cells (Dennert et al., 1981; Brooks et al., 1982) and to augument NK cell activity synergistically with IFN (Kuribayashi et al., 1981). Cytokines such as β-endorphin or met-enkephalin enhance NK cell activity in vitro by activating pre-NK cells and increasing their recycling capacity (Mathews et al., 1983a,b).

In contrast to the positive regulation of NK cell activity, three kinds of mechanisms may be responsible for its down-regulation: 1) direct inhibition of the development of NK cell lineage or NK cell function, 2) inhibition by suppressor cells, and 3) inhibition of the function of accessory cells. Several substances such as prostaglandins (Brunda et al., 1980), hormones such as hydrocortisone (Hochman and Cudkowicz, 1979), and 17-β-estradiol (Seaman et al., 1978) have also been shown to be directly or indirectly involved in down-regulation of NK cell activity.

While the mechanism by which FG 7142 suppressed NK cell activity is unclear, the neuroendocrine changes produced by BzR "inverse agonists" include increased plasma levels of catecholamines, β-endorphin, and glucocorticoids (Crawley et al., 1985; File and Pellow, 1985). It is possible that one or more of these substances may contribute to the observed immunosuppression. Stress has also been shown to reduce INF levels (Glaser et al., 1986), and both catecholamines and glucocorticoids can decrease IL-2 production (Hirsch et al., 1985). It is possible that the stress-inducing FG 7142 suppressed NK cell activity by affecting the levels of INF and/or IL-2. Additionally, the differential expression of both NK cell activity and cell number observed in the LS and SS mice maybe due to regulation of NK cell activity by cytokines (catecholamines and glucocorticoids) and/or lymphokines (IFN and IL-2). Further, since many hormones (pituitary, adrenal, and gonadal) that surge with sexual behavior (Kamel et al., 1975) have been shown to influence immune functions Hoch-

man and Cudkowicz, 1979; Targan, 1981; Kuribayashi et al., 1981; Saxena et al., 1982; Mathews et al., 1983a,b; Johnson et al., 1984), current research efforts are aimed at identifying neural and/or hormonal factors that may contribute to the copulation-linked NK suppression.

Alternatively, the suppression of NK cytotoxicity observed in our studies could be attributed to immunologic mechanisms involving selective emigration of NK cells from the spleen, less effective target cell recognition, diminished rate of target cell killing, changes in the number of NK cells or their regulation by monocytes, or altered cytotoxicity of other immune cells including T lymphocytes and mononuclear phagocytes. T lymphocytes regulate NK cell activity (Ricardi et al., 1982). A subtle change in T-lymphocyte population, not yet detectable by proliferative tests, might fully account for down-regulation of NK cell activity.

As we have seen, NK cell function is extremely sensitive to many endogenous and external factors. Modifications of NK cell activity may be only a sensitive indicator of any perturbation within the CNS. The establishment of a causal nexus between the central nervous system and natural killer cell activity may thus become substantial in our understanding of the neural modulation of natural killer cell activity.

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