Psychoimmunologic and Endorphin Function in the Aged[a]

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IMMUNE FUNCTION IN THE ELDERLY

In humans and all mammalian species which have been studied so far, the immune system undergoes significant changes with advancing age.[1-4] Immunosenescence may be defined as those alterations in immune function which occur to some degree in all older individuals, and which are distinguishable from immunodeficiency secondary to underlying disease, malnutrition, toxic exposure, or genetic disorder. The increased incidence of malignancy, infectious disease, autoimmune disorders, monoclonal gammopathies, and amyloidosis with age is felt to be linked with this decline of immunocompetence.[5-9] In addition, the immunologic theory of aging[10] proposes genetically programmed changes in immune cells as the determinant of maximum lifespan. Support for this theory may be found in the fact that intervention which increases the lifespan of rodents (dietary restriction, hypothermia) also causes profound changes in immune function.[11-13] Conversely, in humans, derangements of immunity such as high autoantibody titers,[14] low suppressor cell activity,[15] and impaired cutaneous hypersensitivity[16] have been correlated with increased mortality.

Immunosenecence is characterized by its high prevalence, interindividual variability, and complexity. The immune system is not uniformly affected by the aging process. For example, total numbers of white blood cells, lymphocytes, and granulocytes, as well as phagocytic function of neutrophils and the complement system do not change appreciably with age.[17-19] The most significant decrements occur in cellular immunity, in such functions as delayed type hypersensitivity,[20] resistance to tumor cells,[21] viruses and protozoans,[6,8] primary allograft rejection, and graft versus host

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disease.[22] The frequency of individuals with autoantibodies,[23] circulating immune complexes, and monoclonal gammopathies[9] increases with age. This dysregulation of immune homeostasis, leading to *vulnerability and failure under stress,* may be attributed to changes in the immune cells themselves and their secretory products, as well as in the cellular milieu in which they function. The most important of these processes appears to be reduced cellular efficiency, since total numbers of immune cells are little altered with age.[24] A brief review of the most important age-related changes is presented below.

Stem cells in mice have a reduced ability to repair injury,[25] capacity for clonal expansion,[26] and ability to migrate to the thymus with age.[27] Macrophages from old mice secrete inadequate amounts of interleukin-1 (IL-1),[28] leading to decreased stimulation of T helper cells and thereby contributing to diminished interleukin-2 (IL-2) production.[29] Abnormal augmentation by splenic T cells in mice appears to contribute to this decline in IL-1 production by macrophages.[30] Increased production of prostaglandin E by macrophages from old animals may also inhibit the proliferative responses of T lymphocytes.[31,32]

B cells appear to function relatively well in old age in both human and animal models. They proliferate normally in response to antigenic challenge; however, their differentiation into mature plasma cells capable of secreting appropriate levels of high affinity antibody is slightly diminished.[33] This decline may be related to changes in the B cells themselves (such as decreased density of surface immunoglobulin[34]), or secondary to T cell dysfunction. In man, decreased primary antibody response to immunization is commonly seen; whereas secondary responses to antigens produce normal titers of antibody.[35–38] A decline in autotolerance, signaled by increased autoantibody production by B cells, is another common finding in older individuals and is also felt to be related to altered B and T cell interactions.[11,22]

T cells are indisputably the component of the immune system most sensitive to the aging process. The onset of decline in T cell function can be demonstrated as early as puberty, at which time thymic involution begins.[39] Thymic hormones, which are required for both T and B cell maturation, begin to decline in the third decade of life; and by age 50, the thymus retains less than 15% of its original mass attained in early adolescence.[40] Although total T cell numbers are not dramatically altered, functionally mature T cells are decreased in number with age.[41] Shifts in T cell subpopulations,[42] changes in surface receptors (*e.g.,* IL-2 and glucocorticoid receptors[43]), impaired adenylate cyclase activity,[44] increased sensitivity to prostaglandin inhibition,[45] prolonged cell cycle duration,[46] and decreased calcium uptake required for proliferation[47] also occur with advancing age. Although helper T cell numbers may be normal or reduced with age, function is usually found to be diminished secondary to both impaired IL-2 production and responsiveness.[48,49] The defect limiting IL-2 production is a diminished number of lyphokine-producing T cells, but there may also be an alteration in IL-2 receptor expression in aged humans and experimental animals.[50] In combination, these changes in the T cell population lead to decreased mitogenesis in response to plant lectins, such as phytohemagglutinin (PHA) and concanavalin A (ConA), mixed lymphocyte reactions, or cytotoxic T cell assays *in vitro.*[51,52] Suppressor T cells have been reported to increase,[53] decrease,[54] or not change with age.[55] The emergence of autoimmune disorders with age points to either decreased suppressor cell function or resistance of other lymphoid cells to suppressive influences.[56] (The onset and course of autoimmune disorders are also related to psychosocial factors, particularly failure of psychological defenses[57]).

Natural killer cells are a heterogeneous subpopulation of lymphocytes contained within the null cell population and comprise approximately 5-15% of peripheral blood

lymphocytes.[58] They are capable of target cell lysis without prior sensitization or major histocompatability complex restriction. In mice, they have been found to play a major role in suppression of local and metastatic tumor growth, lysis of viral-infected cells, and to contribute to the longevity of long-lived strains.[59] The data in humans are controversial, as natural killer (NK) cell activity has been reported to be maintained, increased, or decreased in the elderly.[60–75] A summary of the major reports in the literature is found in TABLE 1. These studies differ greatly in population size and characteristics; but it appears that in healthy populations where an adequate sample size is obtained, NK activity is preserved or increased with age in the absence of disease. Because NK cells are felt to represent a primary line of defense against viral infection and malignant clones of cells, modulation of NK activity is being actively investigated as a potentially valuable approach to the prevention and/or therapy for cancer.

OPIOID PEPTIDES AND THE IMMUNE SYSTEM

Endogenous opioids (endorphins) have been implicated as mediators of a number of responses to stressful stimuli.[76] In view of the close connections between the central nervous system and the immune system and the marked responses of the immune system to stressful stimuli, it is not surprising that the endogenous opioids should play a role in the modulation of the immune system.[77]

In-vivo studies in animals have shown that stressors that produce prolonged endogenous opioid release lead to suppression of splenic natural killer (NK) cell activity.[78] This suppression appears to be secondary to tolerance developing to the acute effects of endogenous opioids on NK cell activity.

Endogenous opioids have also been shown to be involved in modulation of tumor growth.[79–81] The effects of endogenous opioids on tumor growth are not only secondary to the effects they have on the immune system, but also are due to direct effects on opioid receptors present on some tumors and indirectly secondary to the ability of opioids to release growth hormone and prolactin.

In 1979, Wybran *et al.*[82] showed that there are methionine-enkephalin receptors on T cell lymphocytes. Opioids have been shown to increase NK cell activity, alpha-interferon and interleukin-2 production, enhance chemotaxis, release histamine from mast cells, modulate PHA-stimulated lymphocyte proliferation, increase superoxide production and bind to terminal complexes of complement (see REFERENCES 81,83,84 for a review).

The effects of endogenous opioids on NK cells have been particularly well studied. Peripheral blood lumphocyte natural killer (NK) cell activity is a spontaneous cytotoxic function that is involved in mediating host defense to viral and other infections and in natural resistance to malignant cells. Results from three laboratories have shown that beta-endorphin and methionine enkephalin enhance NK activity.[85–87] The dose-response curve has an inverted-U shape. Kay *et al.*[88] have demonstrated that the des-tyrosine endorphins are more potent than beta-endorphin in enhancing NK activity. The ability of endorphins to stimulate NK activity appears to reside in the (6-9) amino acid fragment, *i.e.*, the alpha-helical nonopioid portion of beta-endorphin. The nonopioid endorphin fragment effects are reversed by naloxone, suggesting that the opioid receptor site is of the double-lock variety with the (6-9) portion acting as

TABLE 1. Natural Killer Cell Activity with Aging

Authors	Population	Methods	Results
Increased activity with age			
Onsrud, 1981[60]	15 residents of Salvation Army Home (76-93); 15 SAH workers (20-39)	20 hr Cr^{51} release assay against K562 cells	Increased NK activity in peripheral blood and T lymphocytes. No change in NK activity per unit of blood in old subjects. Increased lymphocytes with + Fc receptors in old.
Batory *et al.*, 1981[61]	44 old (70-98) 69 young (20-45)	4 hr Cr^{51} release assay	Increased NK activity; increased granular lymphocytes; increased lymphocytes with + Fc receptors in old.
Fernandes and Gupta, 1981[62]	50 independently living without significant illness or recent hospitalization (mean 71.2); 43 (mean 32.3)	4 hr Cr^{51} release assay	Increased non-T cell NK activity in males only; no change in T cell NK activity in old subjects.
Abo *et al.*, 1982[63]	112 healthy subjects from 0-88 years; 54 males, 58 females	6 hr Cr^{51} release assay; HNK-1 immunofluorescence	Increased NK activity; increased HNK-1 expression with age. Increased HNK-1 expression in males.
Thompson *et al.*, 1984[64]	17 old (100-103) 25 young (less than 57 years)	Leu 11b and Leu 7 immunofluorescence	Increased percentage of Leu 11b and Leu 7 cells in old.
Tilden *et al.*, 1986[65]	105 healthy subjects (0-79, 12 between 60-79)	4 hr Cr^{51} release assay	Increased NK activity, Leu 7, Leu 11, large granular lymphocytes with age. NK activity correlated with granular lymphocytes and Leu 11 cells.

Lighart *et al.*, 1986[66]	33 aged 75-84 and 35 aged 25-34; all fulfilling strict SENIEUR protocol for immunological studies.	CD-16 monoclonal antibody staining	Increase in CD-16 positive cells in the old subjects. (NK activity not measured.)
Decreased activity with age			
Rabinowich *et al.*, 1985[67]	9 old (patients hospitalized for elective surgery 66-89). 10 young (22-51). None with organic disease.	4 hr Cr^{51} release assay; IL-2 stimulation of NK activity.	Decreased NK activity; decreased IL-2 stimulation of NK activity in old. Decreased PHA responsiveness and IL-2 production in old. Correlation between NK activity and IL-2 production and PHA responsiveness.
Mysliwska *et al.*, 1985[68]	20 old (mean 76); 20 young (mean 25). Old in nursing home without acute or chronic illness; no medications.	4 hr Cr^{51} release assay with murine L 1210 target cells (DBA/2 mice)	Decreased NK activity in old, not accounted for by female predominance in sample.
Rytel *et al.*, 1986[69]	16 old (mean 79) 21 young (mean 28)	K562 target cells	Decreased NK activity in old. Interferon stimulation of NK activity in old less than in young subjects.
Mariani *et al.*, 1986[70]	Not specified	K562 and P815-IgG cell lines. Immunofluorescent antibody staining for CD-4, CD-5, CD-8, CD-16.	CD-5/CD-8 and CD-5/CD-16 clones were less cytotoxic in old subjects than in young subjects.
No change in activity with age			
Sato *et al.*, 1979[71]	7 old (65-75) 8 young (25-35) 11 newborn	16 hr Cr^{51} release assay against RSb and Rsa (human transformed cell lines). Interferon stimulated cytotoxicity.	Cytotoxic activity nonsignificantly lower in old compared to young, increased over levels in neonates. Interferon stimulated cytotoxicity of all groups.

TABLE 1. *Continued*

Authors	Population	Methods	Results
Nagel *et al.*, 1981[72]	200 adults aged 20-95 from BLSA. Healthy.	4 hr Cr^{51} release assay	No change in NK activity with age; no change after 20-month follow-up period in subset. Some increase in levels in 80 to 95-year-old group.
Marcano *et al.*, 1982[73]	11 old (80-114) in nursing home	4 hr Cr^{51} release assay. Antibody dependent cell cytotoxicity; cell-mediated lympholysis; mixed lymphocyte culture.	No change in NK activity in old; decreased T lymphocyte function: ADDC, CML, MLC, in old.
Murasko *et al.*, 1986[74]	260 old (70-106) living in geriatric community; 39 young (23-35)	4 hr Cr^{51} release assay	No difference young vs. old. Those over 90 compared to those 70-84 showed decline.
Tsukayama *et al.*, 1986[75]	13 old (mean 79.5), malnourished, functionally impaired nursing home residents; 9 young (mean 33.7), healthy.	4 hr Cr^{51} release assay	No change in NK activity with age.

the key to allow access to the opioid receptor.[89] A similar double-lock opioid receptor conformation has been proposed for the dynorphin molecule.[90,91]

Of particular interest is the finding that both interferon-[87] and interleukin-2-[89] stimulated NK activity are reversed by naloxone. Radioactively-labeled interleukin-2 is displaced from PHA-stimulated lymphocytes by beta-endorphin and naloxone (Kay, Allen and Morley: submitted for publication). Others have also shown that naloxone decreases the expression of the IL-2 receptor as measured by the monoclonal antibody, anti-TAC. These findings demonstrate that the TAC protein is only one component of the high-affinity receptor for interleukin-2.[92] They suggest, therefore, that the opioids may modulate immune function through their effects on the TAC protein component of the high-affinity IL-2 receptor.

OPIOID PEPTIDES AND AGING

Several studies have provided evidence that the endogenous opioid (endorphin) systems are altered with aging.[93] Concentrations of beta-endorphin and other endogenous opioids have been reported to be lower in the hypothalamus and other central nervous system areas of older rats compared to younger rats.[94–96] In addition, a decrease in opioid receptor binding has been reported in the central nervous system of older animals.[97] The physiological correlate to these reduced biochemical parameters with aging is that older animals have a marked decrease in sensitivity to morphine analgesia.[98,99] Opioid feeding systems also appear to be markedly attenuated with advancing age.[100] In addition, tolerance to opiates occurs more commonly in younger compared to older animals.[101] Taken together, these studies suggest that there is a reduction in the activity of the endorphin system with advancing age. It was for this reason that we felt it would be useful to study the effects of beta-endorphin on natural killer cell activity from young and old individuals.

NATURAL KILLER CELLS, BETA-ENDORPHIN AND AGING

We examined age-related differences in basal natural killer (NK) cell activity and *in-vitro* stimulation with beta-endorphin (BE) and interleukin-2 (IL-2) in 45 free-living elderly volunteers (mean age 73, range 65-89) and 29 young volunteers (mean age 29, range 22-39). Most subjects were studied twice, four months apart. There were significantly higher NK values in the elderly subjects (16.4 $\pm$ 1.7 lytic units/10^6 cells vs. 10.8 $\pm$ 1.0, $p < 0.05$). The percentage of Leu-11a and Leu-19 lymphocytes, which have been correlated with NK activity, was quantified. Leu-11a cells were significantly higher in the elderly (17 $\pm$ 2% vs. 13 $\pm$ 1%, $p < 0.05$), as were Leu-19 cells (23 $\pm$ 2% vs. 16 $\pm$ 2%, $p < 0.05$). Stimulation with BE (10^{-7} or 10^{-8} M) produced a statistically significant increase in NK activity (outside the 95% confidence interval) in 21% of the young and 43% of the old on at least one of the two test days. The concordance rate for stimulation on these two days was 71% in the young and 58% in the elderly, suggesting an increased variability in lymphocyte responsiveness in the older group. Stimulation with IL-2 (10 units/ml) occurred in 57% of both the young and the old groups.

In summary, healthy elderly subjects demonstrate enhanced NK activity both at baseline and after stimulation with beta-endorphin, as compared to a younger population. Analysis of the Leu-11a and Leu-19 lymphocytes subgroups indicates that this enhanced activity may reflect an increase in the proportion of cells with NK activity in the healthy elderly. We suggest that this difference between healthy young and old subjects may represent a cohort effect of increased immune surveillance in those surviving to old age.

NATURAL KILLER CELLS, BETA-ENDORPHIN AND EXERCISE

We also have studied an *in-vivo* model of acute physical stress-exercise, which is known to cause elevations of beta-endorphin in humans.[102-104] Two previous studies have found NK activity to be enhanced after exercise in young subjects.[105-106] Our preliminary results in nine women aged 24-75 indicate that NK activity is significantly stimulated by an acute bout of maximal exercise (6.3 ± .8 lytic units/10^6 cells before exercise vs. 14.1 ± 1.8 lytic units post exercise, $p < 0.01$) in both young and old subjects (FIG. 1). In addition, the ability to stimulate NK cells with beta-endorphin

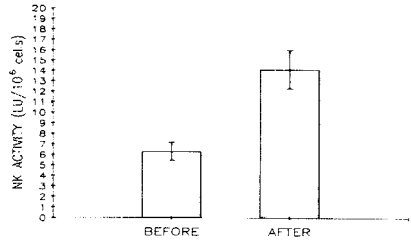

FIGURE 1. The effect of exercise on NK cell activity.

in vitro is markedly suppressed post-exercise (15 ± 4% vs. 1 ± 4%, $p < 0.05$), suggesting that an exercise-induced rise in beta-endorphin may have maximally stimulated these cells and prevented further *in-vitro* augmentation of their activity. These data are compatible with a role for beta-endorphin in the stimulation of NK cells that occurs during exercise.

PSYCHOLOGICAL FACTORS AND NATURAL KILLER CELL STIMULATION IN HEALTHY YOUNG AND OLD SUBJECTS

Clinical depression of a significant degree[107] and bereavement[108] have been associated with immunosuppression. Bereavement, dysphoria and depression are more common among the elderly.[109] Healthy elderly persons with good social support systems tend to show stronger indices of immune function, particularly mitogen

response of lymphocytes.[110] Natural killer cell activity can be enhanced in an elderly population by a relaxation inducing intervention.[111] Dilman relates the process of aging to a complex variety of centrally "programmed," hypothalamically mediated metabolic and immunologic changes in which psychological depression may be an involved variable, in both cause and effect roles.[112]

Physically healthy relatives of patients with rheumatoid arthritis who showed the rheumatoid arthritis-predisposing autoantibody, rheumatoid factor (an IgM anti-IgG), in their sera were more emotionally healthy than their physically healthy relatives without rheumatoid factor.[113] Thus, emotional health may protect against autoimmune disease. The above data support an hypothesis that physically healthy elderly persons are likely to be emotionally healthy as well.

We hypothesize that a cohort of healthy elderly persons will have low levels of dysphoric affect (*e.g.*, depression, helplessness, anxiety) and adaptive coping and personality trait patterns. The same trends might be expected in a younger population of healthy persons but would be less critical to health because of the greater vulnerability of the aging immune system to failure under stress. We correlated state and trait psychological variables with measures of immune function, particularly numbers of natural killer (NK) cells and their function under baseline conditions and under stimulation by beta-endorphin and IL-2, in cohorts of elderly and young persons.

Methods Forty-eight persons ages 65-89 (mean 73), who currently were and for the past five years had been in good physical health and who currently showed no significant cognitive impairments, were compared with 28 healthy persons ages 21-40 (mean 29) on psychological and immunological variables. All subjects were recruited from the clientele or staffs of two senior centers and staffs of two Veterans Administration hospitals.

At baseline (the only data herein reported) the following psychological variables were measured:

1. Anxiety as assessed by the Taylor Manifest Anxiety Scale (short form).[114]
2. Hopelessness as assessed by the Beck Hopelessness Scale.[115]
3. Social desirability (an index of socially acceptable attitudes and beliefs) as assessed by the Marlow Browne Scale.[116]
4. Overall "hardiness" as assessed by the Kobasa Hardiness Scale.[117] (Hardiness, which can be divided into components of commitment, control, and challenge, appears to serve as a buffer against the effects of stressful life events in producing illness[111].)

At baseline, the following immunologic studies were performed:

1. Baseline (NK) cell activity.
2. Maximum observed stimulation of NK cells by beta-endorphin.
3. IL-2 stimulation of NK cells (above the 95% confidence level).
4. Percentage of lymphocytes bearing Leu-11a and Leu-19 markers, both of which are correlated with NK cell activity. (Those psychological or immunologic tests performed on fewer than 20 subjects are not included in this initial report.)

RESULTS

Data were analyzed using the SAS program. There was a trend toward significance between IL-2 stimulation of NK cell activity and "hardiness" ($p < 0.10$, $n = 32$) when all subjects (young and old) were combined. Looking at the young subjects,

maximum beta-endorphin stimulation of NK cells was significantly *negatively* correlated with the Taylor Manifest Anxiety Scale ($p < 0.003$, $n = 26$). Maximum beta-endorphin stimulation was significantly correlated ($p < 0.01$) with the Hardiness Scale in the elderly group ($n = 42$). There was a definite trend toward correlation of hardiness with IL-2 stimulation in the elderly ($n = 19$, $p < 0.10$). (Unfortunately, a considerable number of "hardy" elderly subjects were evaluated prior to inclusion of IL-2 stimulation studies in the research protocol.)

The elderly group showed significantly higher mean hopelessness (6.0 vs 4.3, $p < 0.02$) and social desirability scores (12.3 vs 9.9, $p < 0.03$) than the young group.

INTERPRETATION

A trend toward psychoimmunologic correlation between "hardiness"—a measure of commitment, control, and challenge that is known to correlate with health—and stimulability of NK cells was found especially in old subjects. That stimulation by beta-endorphin and IL-2 were correlated with these adaptive traits may be related to the contribution these peptides make to immune failure in the aged. Healthy old persons are similar to healthy young persons in regard to hardiness, a stress "buffer", but have more dysphoria and are more conventional. However, hardiness appears more strongly related to immune function in the elderly.

Continued psychoimmunologic studies of large numbers of old and young subjects with longitudinal follow-ups every four months will likely reveal more relationships between psychological and immunological status and health.

CONCLUSION

Our preliminary data support the concept that NK activity is enhanced in the elderly. The elderly also demonstrate an enhanced stimulation of NK activity by beta-endorphin. It is suggested that beta-endorphin, which is released from the pituitary in concert with ACTH in response to stress, provides a potential link among stress, altered immunity, and diseases related to dysregulation of the immune system. Support for this hypothesis comes from our finding that beta-endorphin plays a role in the stimulation of NK activity associated with exercise. "Hardiness," a constellation of personality traits and coping patterns reflecting commitment, control, and challenge that has previously been found to be related to health, correlates with beta-endorphin stimulation and tends also to correlate with IL-2 stimulation of NK cell activity. Since IL-2 may be important for augmentation of NK activity *in vivo,* augmented activity would mediate stronger surveillance against malignant and infectious diseases. The correlation found between emotional "hardiness", beta-endorphin and IL-2 stimulation of NK cells suggest a mechanism by which emotional hardiness influences maintenance of physical health, especially in the elderly.

Future studies will be needed to determine whether both superior psychological and immunological functions are necessary to maintain health in old age.

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REFERENCES

1. BILDER, G. E. 1975. Studies on immune competence in the rat: changes with age, sex, and strain. J. Gerontol. **30:** 641-646.
2. JAROSLOW, N., K. M. SUHRBIER & T. E. FRITZ. 1974. Decline and restoration of antibody forming capacity in aging beagle dogs. J. Immunol. **112:** 1467-1476.
3. MATHIES, M., L. LIPPS, G. S. SMITH & R. L. WALFORD. 1973. Age-related decline in response to phytohemagglutinin and pokeweed mitogen by spleen cells from hamster and a long-lived mouse strain. J. Gerontol. **28:** 425-430.
4. NOMAGUCCI, T. A., Y. OKUMA-SAKURAI & I. KIMURA. 1976. Changes in immunological potential between juvenile and presenile rabbits. Mech. Ageing Dev. **5:** 409-417.
5. MAKINODAN, T., S. J. JAMES, T. INAMIZU & M-P. CHANG. 1984. Immunologic basis for susceptibility to infection in the aged. Gerontology **30:** 279-289.
6. GARDNER, I. D. & J. S. REMINGTON. 1978. Aging and immune response. I. Antibody formation and chronic infection in toxoplasma gondii-infected mice. J. Immunol. **120:** 939-943.
7. GRUYS, E. 1979. A comparative approach to secondary amyloidosis: minireview. Dev. Comp. Immunol. **3:** 23-36.
8. PAZMINO, N. H. & J. M. YUHAS. 1973. Senescent loss of resistance to murine sarcoma virus (Moloney) in the mouse. Cancer Res. **33:** 2668-2672.
9. AXELSSON, U., R. BACHMAN & J. HALLEN. 1966. Frequency of pathological proteins (M components) on 6,995 sera from an adult population. Acta Med. Scand. **179:** 235-247.
10. WALFORD, R. L. 1969. The Immunologic Theory of Aging. Williams and Wilkins. Baltimore, MD.
11. FERNANDES, G., E. J. YUNIS, D. G. JOSE & R. A. GOOD. 1973. Dietary influence on antinuclear antibodies and cell-mediated immunity in NZB mice. Int. Arch. Allergy Appl. Immunol. **44:** 770-782.
12. WEINDRUCH, R., S. R. S. GOTTESMAN & R. L. WALFORD. 1982. Modification of age-related immune decline in mice dietarily restricted from or after midadulthood. Proc. Natl. Acad. Sci. USA **79:** 898-902.
13. LIU, R. K. & R. L. WALFORD. 1975. Mid-life temperature transfer effects on life span of annual fish. J. Gerontol. **30:** 129-131.
14. MACKAY, I. R., S. F. WHITTINGHAM & J. D. MATHEWS. 1977. The immunoepidemiology of aging. *In* Immunology and Aging. T. Makinodan & E. Yunis, Eds. 35-49. Plenum Medical Book Co. New York, NY.
15. WECKSLER, M. E. & P. B. HAUSMAN. 1982. Effects of aging in the immune response. *In* Basic and Clinical Immunology. D. P. Stites, J. B. Stobo & H. H. Fedenberg, Eds. 306-313. Lange Medical Publications. Los Altos, CA.
16. ROBERTS-THOMSON, I. C., U. YOUNGCHAIZUD, S. WITTINGHAM & I. R. MAKAY. 1974. Aging, immune response, and mortality. Lancet **2:** 368-370.
17. CORBERAND, J. X., P. F. LAHARRAGUE & G. FILLOLA. 1986. Neutrophils of healthy aged humans are normal. Mech. Ageing Dev. **36:** 57-63.
18. SPARROW, D., J. E. SILBERT & J. W. ROWE. 1980. The influence of age on peripheral lymphocyte count in men: a cross-sectional and longitudinal study. J. Gerontol. **35:** 163-166.

19. NAGAKI, K., S. HIRAMATSU, S. INAI & A. SASAKI. 1980. The effect of aging on complement activity (CH50) and complement protein levels. J. Clin. Lab. Immunol. **3:** 45-50.
20. DWORSKY, R., A. PAGANINI-HILL, M. ARTHUR & J. PARKER. 1983. Immune responses of healthy humans 83-104 years of age. J. Natl. Cancer Inst. **71:** 265-268.
21. LIPSCHITZ, D. A., S. GOLKDSTEIN, R. REIS, M. E. WEKSLER, R. BRESSLER & B. A. WEILAN. 1985. Cancer in the elderly: basic science and clinical aspects. Ann. Intern. Med. **102:** 218-228.
22. BLOOM, E. T., W. J. PETERSON, M. TAKASUGI & T. MAKINODAN. 1985. Immunity and ageing. *In* Principles and Practice of Geriatric Medicine. M. S. J. Pathy, Ed., 57-65. John Wiley and Sons, Ltd. Chichester, England.
23. CAMMARATA, R. J., G. P. RODNAN & R. H. FENNEL. 1967. Serum antigamma globulin and antinuclear factors in the aged. JAMA **199:** 456-458.
24. DYBKAER, R., M. LAURITZEN & R. KRAKAUER. 1981. Relative reference values for clinical, chemical and haematological quantities for healthy elderly people. Acta Med. Scand. **209:** 1-9.
25. CHEN, M. G. 1971. Age-related changes in hematopoietic stem cell populations of a long-lived hybrid mouse. J. Cell Physiol. **78:** 225-232.
26. ALBRIGHT, J. & T. MAKINODAN. 1976. Decline in the growth potential of spleen colonizing bone marrow stem cells of long-lived aging mice. J. Exp. Med. **144:** 1204-1213.
27. TYAN, M. L. 1977. Age-related decrease in mouse T cell progenitors. J. Immunol. **118:** 846-851.
28. INAMIZU, T., M-P. CHANG & T. MAKINODAN. 1983. Decline in interleukin (IL)-1 production with age. Gerontologist **23:** 249.
29. CHANG, M-P., T. MAKINODAN, W. J. PETERSON & B. L. STREHLER. 1982. Role of T cells and adherent cells in age-related decline in murine interleukin-2 production. J. Imunol. **129:** 2426-2430.
30. INAMIZU, T., M-P. CHANG & T. MAKINODAN. 1985. Influence of age on the production and regulation of interleukin-1 in mice. Immunology **55:** 447-455.
31. ROSENSTEIN, M. M. & H. R. STRAUSSER. 1980. Macrophage-induced T cell mitogen suppression with age. J. Reticuloendoth. Soc. **27:** 159-166.
32. LICASTRO, F. & R. L. WALFORD. 1986. Effects exerted by prostaglandins and indomethacin on the immune response during aging. Gerontology **32:** 1-9.
33. GOIDL, E. A., J. B. INNES & M. E. WEKSLER. 1976. Immunological studies on aging. II. Loss of IgG and high avidity plaque-forming cells and increased suppressor cell activity in aging mice. J. Exp. Med. **144:** 1037-1048.
34. WODA, B. A. & J. D. FELDMAN. 1979. Density of surface immunoglobulins and capping on rat B lymphocytes. I. Changes with aging. J. Exp. Med. **149:** 416-423.
35. HOWELLS, C. H. L., C. T. VESSELINOVA-JENKINS, A. D. EVANS & J. JAMES. 1975. Influenza vaccination and mortality from bronchopneumonia in the elderly. Lancet **1:** 381-383.
36. RUBEN, F. L., J. NAGEL & P. FIREMAN. 1973. Antitoxin responses in the elderly to tetanus-diptheria (Td) immunization. Am. J. Epidemiol. **103:** 145-149.
37. LANDESMAN, S. H. & G. SCHIFFMAN. 1981. Assessment of the antibody response to pneumococcal vaccine in high-risk populations. Rev. Infect. Dis. **3(Suppl):** 184-197.
38. MAKINODAN, T. & W. J. PETERSON. 1962. Relative antibody-forming capacity of spleen cells as a function of age. Proc. Natl. Acad. Sci. USA **48:** 234-238.
39. BOYD, E. 1932. The weight of the thymus gland in health and disease. Am. J. Clin. Dis. Child. **43:** 1162-1214.
40. WEKSLER, M. E. 1981. The senescence of the immune system. Hosp. Pract. **16:** 53-64.
41. O'LEARY, J. J., D. P. JACKOLA, H. M. HALLGREN, M. ABBASNEZHAD & W. G. YASMINEH. 1983. Evidence for a less differentiated subpopulation of lymphocytes in people of advanced age. Mech. Ageing Dev. **21:** 109-120.
42. NAGEL, J. E., F. J. CHREST & W. H. ADLER. 1981. Enumeration of T lymphocyte subsets by monoclonal antibodies in young and aged humans. J. Immunol. **127:** 2086-2088.
43. GILLIS, S., R. KOZAK, M. DURANTE & M. E. WEKSLER. 1981. Immunological studies

of aging. Decreased production of and response to T cell growth factor by lymphocytes from aged humans. J. Clin. Invest. **67:** 937-942.

44. ABRASS, I. B. & P. J. SCARPACE. 1982. Catalytic unit of adenylate cyclase: reduced activity in aged human lymphocytes. J. Clin. Endocrinol. Metab. **55:** 1026-1028.
45. DORIA, G., C. MANCINI & L. ADORINI. 1982. Immunoregulation in senescence: increased inducibility of antigen-specific suppressor T cells and loss of cell sensitivity to immunosuppression in aging mice. Proc. Natl. Acad. Sci. USA **79:** 3803-3807.
46. TICE, R. R., E. L. SCHNEIDER, D. KRAM & P. THORNE. 1979. Cytokinetic analysis of the impaired proliferative response of peripheral lymphocytes from aged humans to phytohemagglutinin. J. Exp. Med. **150:** 1029-1041.
47. KENNES, B., C. HUBERT, D. BROHEE & P. NEVE. 1981. Early biochemical events associated with lymphocyte activation in aging. Immunology **42:** 119-126.
48. THOMAN, M. L. & W. O. WEIGLE. 1981. Lymphokines and aging: interleukin-2 production and activity in aged animals. J. Immunol. **127:** 2102-2106.
49. ERSHLER, W. B., A. L. MOORE, K. ROESSNER & G. E. RANGES. 1985. Interleukin-2 and aging: decrease interleukin-2 production in healthy older people does not correlate with reduced helper cell numbers of antibody response to influenza vaccine and is not corrected *in vitro* by thymosin alpha-1. Immunopharmacology **10:** 11-17.
50. THOMAN, M. L. 1985. The role of Interleukin-2 in the aged-related impairment of immune function. J. Am. Geriatr. Soc. **33:** 781-787.
51. MOODY, C. E., J. B. INNES, L. STAIANO-COICO, G. S. INCEFY, H. T. THALER & M. E. WEKSLER. 1981. Lymphocyte transformation induced by autologous cells. XI. The effect of age on the autologous mixed lymphocyte reaction. Immunology **44:** 431-438.
52. TOLLEFSBOL, T. O. & H. J. COHEN. 1986. Expression of intracellular biochemical defects of lymphocytes in aging: proposal of a general aging mechanism which is not cell-specific. Exp. Gerontol. **21:** 129-148.
53. GUPTA, S. & R. A. GOOD. 1979. Subpopulations of human T lymphocytes. X. Alterations in T, B, third population cells, and T cells with receptors for immunoglubulin M (T mu) or G (T gamma) in aging humans. J. Immunol. **122:** 1214-1219.
54. HALLGREN, H. M. & E. YUNIS. 1977. Suppressor lymphocytes in young and aged humans. J. Immunol. **118:** 2004-2008.
55. BARRETT, D. J., S. STENMARK, D. W. WARA & A. J. AMMANN. 1980. Immunoregulation in aged humans. Clin. Immunol. Immunopathol. **17:** 203-211.
56. ANTEL, J. P. & B. G. W. ARNASON. 1979. Suppressor cell function in man: evidence for altered sensitivity of responder cells with age. Clin. Immunol. Immunopathol. **13:** 119-124.
57. SOLOMON, G. F. 1981. Emotional and personality factors in the onset and couse of autoimmune disease, particularly rheumatoid arthritis. *In* Psychoneuroimmunology. R. A. Ader, Ed. 259-278. Academic Press. New York, NY.
58. HERBERMAN, R. B., J. Y. DJEU, H. D. KAY, J. R. ORTALDO, C. RICCARDI, G. D. BONNARD, H. T. HOLDEN, R. FAGNANI, A. SANTONI & P. PUCCETTI. 1979. Natural killer cells: characteristics and regulation of activity. Immunol. Rev. **44:** 43-70.
59. LOTZOVA, E. & K. B. MCCREDIE. 1973. Natural killer cells in mice and man and their possible biological significance. Cancer Immunol. Immunother. **4:** 215-221.
60. ONSRUD, M. 1981. Age-dependent changes in some human lymphocyte subpopulations. Changes in natural killer cell activity. Acta Pathol. Microbiol. Scand., Sect. C **89:** 55-62.
61. BATORY, G., M. BENCZUR, M. VARGA, T. GARAM, C. ONODY & G. G. PETIANYI. 1981. Increased killer cell activity in aged humans. Immunobiology **158:** 393-402.
62. FERNANDES, G. & S. GUPTA. 1981. Natural killing and antibody-dependent cytotoxicity by lymphocyte subpopulations in young and aging humans. J. Clin. Immunol. **1:** 141-148.
63. ABO, T., M. D. COOPER & C. M. BALCH. 1982. Postnatal expansion of the natural killer and killer cell population in humans identified by the monoclonal HNK-1 antibody. J. Exp. Med. **155:** 321-326.
64. THOMPSON, J. S., D. R. WEKSTEIN, J. L. RHOADES, C. KIRKPATRIC, S. A. BROWN, T. ROSZMAN, R. STRAUS & N. TIETZ. 1984. The immune status of healthy centenarians. J. Am. Geriatr. Soc. **32:** 274-281.

65. TILDEN, A. B., C. E. GROSSI, K. ITOH, G. A. CLOUD, P. A. DOUGHERTY & C. M. BALCH. 1986. Subpopulation analysis of human granular lymphocytes: associations with age, gender and cytotoxic activity. Natl. Immunol. Cell Growth Regul. **5:** 90-99.
66. LIGHART, G. J., P. C. VAN VLOKHOVEN, H. R. E. SCHUIT & W. HIGMANS. 1986. The expanded null cell compartment in ageing: increase in the number of natural killer cells and changes in T-cell and NK-cell subsets in human blood. Immunology **59:** 353-357.
67. RABINOWICH, H., Y. GOSES, T. RESHEF & A. KLAJMAN. 1985. Interleukin-2 production and activity in aged humans. Mech. Ageing Dev. **32:** 213-226.
68. MYSLIWSKA, J., A. MYSLIWSKI & J. WITKOWSKI. 1985. Age dependent decline of natural killer and antibody-dependent cell-mediated cytotoxicity activity of human lymphocytes is connected with decrease of their acid phosphatase activity. Mech. Ageing Dev. **31:** 1-11.
69. RYTEL, M. W., N. KERMANI, K. S. LARRATT & P. A. TURNER. 1986. Immune interferon response and natural killer cell activity in the elderly and young adults. (Abstract) J. Am. Geriatr. Soc. **34:** 685-686.
70. MARIANI, E., M. VITALE, P. RODA, A. DEGRASSI, A. R. MARIANI & A. FACCHINI. 1987. T and NK clones in old individuals. (Abstract) Fed. Proc. **46:** 1090.
71. SATO, T., A. FUSE & T. KUWATA. 1979. Enhancement by interferon of natural cytotoxic activities of lymphocytes from human cord blood and peripheral blood of aged persons. Cell. Immunol. **45:** 458-463.
72. NAGEL, J. E., G. D. COLLINS & W. H. ADLER. 1981. Spontaneous or natural killer cytotoxicity of K562 erythroleukemic cells in normal patients. Cancer Res. **41:** 2284-2288.
73. MARCANO, N. B., A. RIVAS, E. F. FIGARELLA, I. BLANCA, G. K. PENCHASZADEH, M. PEREZ-ROJAS & N. E. BLANCO. 1982. Cell-mediated effector mechanisms in aging humans. Arch. Allergy Appl. Immunol. **69:** 7-11.
74. MURASKO, D. M., B. J. NELSON, R. SILVER, D. MATOUR & D. KAYE. 1986. Immunologic response in an elderly population with a mean age of 85. Am. J. Med. **81:** 612-618.
75. TSUKAYAMA, D., R. BREITENBUCHER, S. STEINBERG, T. ALLEN, R. NELSON, G. GEKKER, W. KEANE & P. PETERSON. 1986. Polymorphonuclear leukocyte, T-lymphocyte, and natural killer cell activities in elderly nursing home residents. Eur. J. Clin. Microbiol. **5:** 468-471.
76. MORLEY, J. E. 1983. Neuroendocrine effects of endogenous opioid peptides in human subjects: a review. Psychoneuroendocrinology **8:** 361-379.
77. SOLOMON, G. F. 1987. Psychoneuroimmunology: interactions between central nervous system and immune system. J. Neurosci. Res. In press.
78. SHAVIT, Y., J. W. LEWIS, G. W. TERMAN, R. P. GALE & J. C. LEIBESKIND. 1984. Opioid peptides mediate the suppressive effect of stress on natural killer cell cytotoxicity. Science **223:** 188-190.
79. AYLESWORTH, C. F., C. A. HODSON & J. MEITES. 1979. Opiate antagonists can inhibit mammary tumor growth in rats. Proc. Soc. Exp. Biol. Med. **161:** 18-20.
80. ZAGON, I. S. & P. J. MCLAUGHLIN. 1983. Opioid antagonists inhibit the growth of metastatic murine neuroblastoma. Cancer Lett. **21:** 89-94.
81. MORLEY, J. E., N. KAY, J. ALLEN, T. MOON & C. J. BILLINGTON. 1985. Endorphins, immune function and cancer. Psychopharmacol. Bull. **21:** 485-488.
82. WYBRAN, J., T. APPELBOOM, J. P. FARALY & A. GOVAERTS. 1979. Suggestive evidence for morphine and methionine-enkephalin-like receptors on normal blood T lymphocytes. J. Immunol. **123:** 1068-1070.
83. SOLOMON, G. F., N. KAY & J. E. MORLEY. 1986. Endorphins: a link between personality, stress, emotions, immunity and disease? *In* Enkephalins and Endorphins: Stress and the Immune System. N. P. Plotnikoff, R. E. Faith, A. J. Mungo & R. A. Good, Eds. 129-144. Plenum Press. New York, NY.
84. TESCHEMACHER, H. & L. SCHWEIGERER. 1985. Opioid peptides: do they have immunological significance? Trends. Pharmacol. Sci. **6:** 368-370.
85. FAITH, R. E., H. J. LIANG, A. J. MURGO & N. P. PLOTNIKOFF. 1984. Neuroimmunomodulation with enkephalins: enhancement of natural killer (NK) cell activity *in vitro.* Clin. Immunol. Immunopathol. **31:** 412-418.

86. MATTHEWS, P. M., C. J. FOELICH, W. L. SIBBITT & A. D. BANKHURST. 1983. Enhancement of natural cytotoxicity by beta-endorphins. J. Immunol. **130:** 1658-1662.
87. KAY, N., J. ALLEN & J. E. MORLEY. 1984. Endorphins stimulate normal human peripheral blood lymphocyte natural killer cell activity. Life Sci. **35:** 53-59.
88. KAY, N., J. E. MORLEY & J. M. VANREE. 1987. Enhancement of human lymphocyte natural killing function by non-opioid fragments of beta-endorphin. Life Sci. **40:** 1083-1087.
89. MORLEY, J. E. & N. KAY. 1986. Neuropeptides as modulators of immune function. Psychopharmacol. Bull. **22:** 1089-1092.
90. CHAVKIN, C. & A. GOLDSTEIN. 1981. Dynorphin-specific receptor for the opioid peptide, dynorphin: structure-activity relationships. Proc. Natl. Acad. Sci. USA **78:** 6543-6547.
91. MORLEY, J. E. & A. S. LEVINE. 1983. Involvement of dynorphin and the kappa opioid receptor in feeding. Peptides **4:** 797-800.
92. TSUDO, M., R. W. KOZAK, C. K. GOLDMAN & T. A. WALDMANN. 1986. Demonstration of a non-TAC peptide that binds interleukin-2: a potential participant in a multichain interleukin-2 receptor complex. Proc. Natl. Acad. Sci. USA **83:** 9694-9698.
93. MORLEY, J. E. 1986. Neuropeptides, behavior and aging. J. Am. Geriatr. Soc. **34:** 52-62.
94. DUPONT, A., P. SAVARD & Y. MESAND. 1981. Age-related changes in central nervous system enkephalins and substance P. Life Sci. **29:** 2317-2323.
95. GAMBERT, S. R. 1981. Interaction of age and thyroid hormone status on beta-endorphin content in rat corpus striatum and hypothalamus. Neuroendocrinology **32:** 114-119.
96. BARDEN, N., A. DUPONT & F. LABRIE. 1981. Age-dependent changes in the beta-endorphin content of discrete rat brain nuclei. Brain Res. **9:** 209-215.
97. MESSING, R. B., B. J. VASQUEZ & B. SAMANIEGO. 1981. Alterations in dihydromorphine binding in cerebral hemispheres of aged male rats. J. Neurochem. **36:** 784.
98. SPRATTO, G. R. & R. E. DONO. 1978. Effect of age on acute morphine response in the rat. Res. Commun. Chem. Pathol. Pharamacol. **19:** 23-28.
99. WEBSTER, G. W., L. SHUSTER & B. E. ELEFTHERIUS. 1976. Morphine analgesia in mice of different ages. Exp. Aging Res. **2:** 221-223.
100. GOSNELL, B. A., A. S. LEVINE & J. E. MORLEY. 1983. The effects of aging on opioid modulation of feeding in rats. Life Sci. **32:** 2793-2799.
101. NICAK, A. & A. KOHUT. 1978. Development of tolerance to morphine and pethidine in rats is dependant on age. Act. Nerv. Super. (Praha) **20:** 231-235.
102. FRAIOLI, F., C. MORETTI, D. PAOLUCCI, E. ALICICCO, F. CRESCENZI & G. FORTUNIO. 1980. Physical exercise stimulates marked concomitant release of beta-endorphin and adrenocorticotropic hormone (ACTH) in peripheral blood in man. Experientia **36:** 987-989.
103. CARR, D. B., B. A. BULLEN, G. S. SKRINER, M. A. ARNOLD, M. ROSENBLATT, I. Z. BETTINS, J. B. MARTIN & J. N. MCARTHUR. 1981. Physical conditioning facilitates the exercise induced secretion of beta-endorphin and beta-lipotropin in women. N. Engl. J. Med. **305:** 560-563.
104. COLT, E. W. D., S. L. WARDLAW & A. G. FRANTZ. 1981. The effect of running on plasma beta-endorphin. Life Sci. **28:** 1637-1640.
105. TARGAN, S., L. BRITVAN & F. DOREY. 1981. Activation of human NKCC by moderate exercise: increased frequency of NK cells with enhanced capability of effector-target lytic interactions. Clin. Exp. Immunol. **45:** 352-360.
106. BRAHMI, Z., J. E. THOMAS, M. PARK & I. R. G. DOWDESWELL. 1985. The effect of acute exercise on natural killer cell activity of trained and sedentary human subjects. J. Clin. Immunol. **5:** 321-328.
107. SCHLEIFER, S. J., S. E. KELLER, S. G. SAMUEL, L. D. KENNETH & M. STEIN. 1985. Depression and immunity lymphocyte function in ambulatory depressed patients, hospitalized schizophrenic patients, and patients hospitalized for heriorrhaphy. Arch. Gen. Psychiatry **42:** 129-133.
108. BARTROP, R. W., L. LAZARUS, E. OUCKHURST, L. G. KILOH & R. PENNY. 1977. Depressed lymphocyte function after bereavement. Lancet **1:** 834-836.
109. BLAZER, D. & C. D. WILLIAMS. 1980. Epidemiology of dysphoria and depression in an elderly popultion. Am. J. Psychiatry **137:** 439-444.

110. THOMAS, P. D., J. M. GOODWIN & J. S. GOODWIN. 1985. Effect of social support on stress-related changes in cholesterol level, uric acid level, and immune function in an elderly sample. Am. J. Psychiatry **142:** 735-737.
111. KIECOLT-GLASER, J. K., R. GLASER, D. WILLIGER, J. STOUT, G. MESSICK, S. SHEPPARD, D. RICKER, S. C. ROMISHER, W. BRINER, G. BONNELL & R. DONNERBERG. 1985. Psychosocial enhancement of immunocompetence in a geriatric population. Health Psychol. **4:** 25-41.
112. DILMAN, V. M. 1981. The Law of Deviation of Homeostasis and Diseases of Aging. John Wright PSG. Boston, MA.
113. SOLOMON, G. F. & R. H. MOOS. 1964. The relationship of personality to the presence of rheumatoid factor in asymptomatic relatives of patients with rheumatoid arthritis. Psychosom. Med. **27:** 350-360.
114. TAYLOR, J., 1955. A personality scale of manifest anxiety. J. Abnorm. Soc. Psychol. **48:** 285-290.
115. BECK, A. T., C. H. WARD, M. MENDELSON, J. E. MOCK & J. ERBAUGH. 1961. An inventory for measuring depression. Arch. Gen. Psychiatry **4:** 561-571.
116. CROWNE, D. P., & D. MARLOW. 1960. A new scale of social desirability independent of psychopathology. J. Cons. Psychol. **24:** 349.
117. KOBASA, S. C. 1979. Stressful life events, personality and health: an inquiry into hardiness. J. Pers. Soc. Psychol. **37:** 1-11.