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The Immune System and the Aging Process in Man (40958)[1]

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Abstract. Life span appears to be under genetic control. Genes linked to the major histocompatibility complex are associated with life span. These genes also play a major role in regulating immune reactivity and suggest that immunological factors may be determinants in the length of life. Many of the immune functions which change with age can be related to the involution of the thymus gland and the decline in thymic hormone levels. Impaired proliferation of T cells from old individuals may be the cellular basis of certain changes in immunological reactivity seen with age. Lymphocytes like arterial smooth muscle cells and fibroblasts from elderly donors appear to have an impaired capacity to proliferate in culture.

The maximal life span, like all inherited characteristics, is determined by genes. Although the genetic processes that regulate life span are not clear, the longevity of mice has been linked to genes of the major histocompatibility complex (1). As genes within this complex regulate many immune functions, it is reasonable to suggest that immunological functions may be important determinants of life span. Immune function is known to change with age. This review will (a) describe changes in immune reactivity that accompany human aging, (b) examine the cellular basis of immune senescence in man, and (c) discuss the clinical consequences of immunological senescence.

Change in the structure and function of the immune system has been appreciated for some time. Pathologists in the last century noted that the weight of organs changed with age. The weight of the lungs tended to increase and the weight of the brain and liver tended to decrease with age. These changes were modest. The most dramatic changes in organ weight were the 50% loss in the mass of the spleen and the 90% loss in mass of the thymus gland between the ages of 20 and 80. These facts were recognized, although the immunological function of these organs was not appreciated. The immunological function of the thymus was not recognized until the studies of J. F. A. P. Miller and of R. A. Good in the early 1960s. Careful anatomical studies carried out in the early 1930s had shown that the cellular mass of the thymus was maintained only to sexual maturity (2). After puberty, rapid involution of the thymus occurred and by the age of 45, the thymus retained only 10 to 20% of its maximal cellular mass.

The thymus gland not only serves as a site of T-cell differentiation but functions as an endocrine organ. The endocrine function of the thymus gland has been recognized for 15 years although precise knowledge concerning the number of thymic hormones and their structure is not complete. The concentration of thymic hormone in serum can be measured by its capacity to cause the differentiation of precusor T cells. Lewis *et al.* (3) showed that the serum level of thymic hormone was constant between birth and 30 years of age. Thereafter, the serum concentration of thymic hormone falls progressively. After the age of 60 thymic hormone can no longer be detected in the serum. The age-associated changes in thymic structure and endocrine function precede the progressive impairment of T-cell-dependent immune function detected after 45 years of age, which in turn is followed by the rise in the chronic diseases of aging (Fig. 1). These sequential events may be related. The capacity of a young thymus gland or thymic hormone administration to reverse age-associated defects in immune function of lymphocytes from old mice

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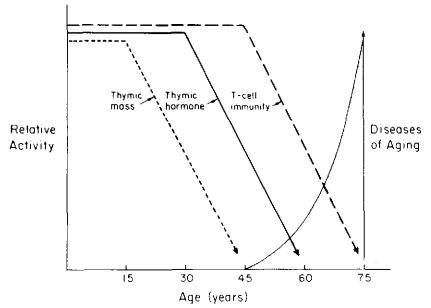

FIG. 1. Temporal relationship between changes in the form and function of the immune system and the diseases of aging.

supports this thesis (4). In addition, studies in man (discussed below) suggest that survival of elderly humans is directly correlated with the degree of impairment in T-lymphocyte function.

As thymic function declines with age, a number of immune functions would be expected to be altered. Fewer immunocompetent T lymphocytes would be differentiated as the thymus gland involutes. Permissive effects of the thymus gland and its hormonal product on postthymic T cells would be lost. T-Lymphocyte function measured *in vitro* or *in vivo* would decline.

The proliferative responses of T lymphocytes incubated with plant lectins or antigens *in vitro* would decrease and delayed-type hypersensitivity reactions, and resistance to viral, fungal, or mycobacterial infection *in vivo* would become impaired with age. A loss of helper and suppressor T-cell functions which regulate B-cell function would also result. Antibody production dependent upon helper T cells would decline. Self-tolerance maintained by suppressor T cells would be compromised and an increased incidence of autoantibodies would occur. Finally, T-cell regulation of B-cell clonal expansion would be reduced leading to the appearance of monoclonal immunoglobulins in the serum.

Clinical data confirm these predicted consequences of immune senescence. The antibody response to xenogeneic erythrocytes, which depends on helper T cells, decreases with age (5). Anti-sheep erythrocyte titer was maximal in serum from individuals between the ages of 15 and 20 years of age. After 20 years of age the titer declined progressively. MacKay and co-workers (6) contrasted the decline in the human antibody response to a foreign antigen (*Salmonella flagellin*) with the increase in antibody response to an autologous antigenic determinant (antinuclear antibody) that occured with age. Hallgren *et al.* (7) reported that other autoantibodies, antithyroglobulin antibody and rheumatoid factor as well as antinuclear antibody, increase with age. Additional evidence of an age-associated autoimmune diathesis is the finding by Day (8) of high levels of circulating immune complexes in the serum of old subjects. Whereas less than 5% of young healthy subjects under 40 years of age had more than 100 μg/ml of immune complexes, 50% of healthy elderly subjects over 60 years of age had more than 100 μg/ml of immune complexes. These findings support the suggestion that helper and suppressor T-lymphocyte function declines with age.

Cell-mediated immunity also declines with age. Delayed hypersensitivity reaction to common skin test antigens was compared in young subjects less than 25 years of age and in subjects over the age of 60 (9). All the young subjects reacted to two or more of these antigens while less than half of the older subjects reacted to this number of antigens. Nearly 25% of the older subjects reacted to none of the antigens. The loss of delayed hypersensitivity to recall antigens in older subjects did not distinguish between a loss of memory for these antigens with time and impaired capacity to manifest a delayed cutaneous hypersensitivity response. When persons of various ages were sensitized with dinitrochlorobenzene and challenged a short time later, only 5% of subjects less than 70 years of age did not manifest a positive reaction (10). In contrast, more than 30% of persons over the age of 70 years failed to develop a positive reaction. This suggested that the impaired delayed cutaneous reactivity of elderly persons reflected an impaired capacity of older subjects to manifest cell immunity and not only a loss of immunologic memory.

It was, of course, possible that impaired cutaneous reactivity was due to changes in the function of skin cells and not T lymphocytes. The response of T lymphocyte to

PPD *in vitro* was measured in tubercular patients of different ages (11). The proliferative response of T lymphocytes incubated with PPD was inversely correlated with age of the lymphocyte donor. Thus, defects in delayed-type hypersensitivity reactions in old subjects was shown to be associated with defects in T-cell function. It had previously been observed that the proliferative response of lymphocytes cultured with the mitogen, phytohemagglutinin (PHA), was also inversely correlated with age (12). Both of these observations suggested that lymphocytes from older subjects were impaired in their capacity to divide in culture.

The finite capacity of humans cells to divide in culture observed by Hayflick suggested an *in vitro* correlate of aging (13). This hypothesis received support when it was found that the number of population doublings of human fibroblasts (14) and arterial smooth muscle cells (15) was inversely correlated with the age of the cell donor. These studies did not distinguish between there being fewer cells with proliferative capacity in the cell explants from old donors or a failure of proliferating cells from old donors to divide as many times. An impairment in the reproductive capacity of proliferating cells was suggested by measuring the size of fibroblast colonies from young or old donors. Colonies from old donors contained fewer cells than did colonies established from young donors (16).

The studies of my laboratory have addressed the cellular basis of immune senescence in man. These investigations have examined the proliferative capacity of lymphocytes from young and old humans. The most obvious explanation for the impaired response of T cells from old humans is a relative or absolute decrease in the number of T lymphocytes with age. We found no evidence that the relative or absolute number of T lymphocytes changed with age despite the involution of the thymus gland, the decline in serum thymic hormone concentration, and the decreased proliferative capacity of lymphocytes that occur with age.

An *increase* in the T γ subpopulation of T lymphocytes has been recognized (17). The long life of T lymphocytes probably explains the maintenance of a constant number of T cells in the peripheral blood of man. Of course, the finding that the number of T cells in one lymphoid compartment, the blood, does not change with age should not be taken as evidence that the total number of T lymphocytes in the body does not change with age. Nonetheless, the fact that the number of T lymphocytes is unchanged with age and the fact that the proliferative defect observed with unfractionated lymphocytes is also manifested in purified T lymphocyte preparations suggest that fewer T cells respond to a proliferative stimulus and/or that the proliferative potential of responsive T lymphocytes from old humans is impaired. We have considered both these possible explanations (18).

The number of T lymphocytes which can be activated by PHA was measured by three independent techniques. The first, limiting dilution analysis, is an all-or-none assay which correlates the percentage of positive responses in sets of replicate cultures with the number of lymphocytes in each set of cultures. The number of initially responsive cells was also assessed by the vesicular stomatitis virus (VSV) plaque assay. The assay is based on the fact that mitogen-activated lymphocytes are susceptible to infection with VSV. The number of infected lymphocytes was determined by spreading the lymphocyte preparation over an indicator L-cell monolayer. Each infected lymphocyte released virus which killed cells in the indicator cell monolayer. The number of plaques in the monolayer reflects the number of virus-infected lymphocytes which in turn reflects the number of activated lymphocytes. The last method to estimate the number of responsive lymphocytes used thymidine incorporation by mitogen-activated lymphocytes in the presence of colchicine. Colchicine, which inhibits cell division, prevents the generation of progeny cells which normally contribute to the total thymidine incorporated by lymphocyte cultures. All three assays indicated that lymphocyte preparations from old subjects had only one-quarter to one-half the number of mitogen-responsive T lymphocytes present in preparations from young subjects. Thus, impaired cell-medi-

ated immunity of old humans was, at least in part, attributable to a reduced number of T lymphocytes which could be activated to proliferate.

When thymidine incorporation by lymphocytes in the presence or absence of colchicine was compared, another defect in T lymphocytes from old donors was suggested. Lymphocytes from young donors incorporated three times as much thymidine in the absence as compared to in the presence of colchicine. In contrast, lymphocytes from old donors incorporated only twice as much thymidine in the absence as compared to the presence of colchicine. As the difference in thymidine incorporated by lymphocytes in the presence or absence of colchicine reflects the proliferation of progeny cells of initially responsive lymphocytes, the finding suggested that the proliferative capacity of initially responsive lymphocytes from old humans was impaired.

The proliferative capacity of mitogen-responsive T lymphocytes was directly assessed by incubating lymphocytes with PHA in the presence of bromodeoxyuridine (BrdU), a thymidine analog (19). DNA strands containing BrdU do not stain brightly at metaphase and allow the identification of cells dividing for the first, second, or third time in culture. The number of lymphocytes dividing for the first, second, or third time was measured after incubation with PHA for 72 hr. Lymphocytes from old donors incubated with PHA for 72 hr contained the same number of lymphocytes dividing for the first time as did cultures from young donors, but only one-half the number of lymphocytes dividing for a second time and only one-quarter the number dividing for a third time. This difference was not due to delayed entry into cell division, or an increased cell-cycle time of lymphocytes from old donors. Thus, these findings support the concept that the proliferative capacity of mitogen-responsive lymphocytes from old donors was less than that of mitogen-responsive lymphocytes from young donors. Thus, lymphocytes from old donors, like fibroblasts and arterial smooth muscle cells, are impaired in their proliferative potential.

The results of our studies suggest a model to explain the proliferative defect of lymphocytes from old persons in culture (Fig. 2). Although the number of T lymphocytes in the blood from old and young donors is the same, there are fewer mitogen-responsive T lymphocytes in lymphocyte preparations from old donors. In addition, the proliferative capacity of responsive T lymphocytes from old donors is reduced. Thus the impaired proliferation of T lymphocytes from old donors is due both to a reduced number of responsive lymphocytes and the impaired proliferative capacity of these cells.

We have considered possible means of enhancing the proliferative capacity of lymphocytes from old donors, and thereby augmenting cell-mediated immune competence. There are no obvious methods of "resetting" the biological clock that defines the proliferative potential of diploid cells. For this reason, we have centered our attention on the nature of the nonresponsive T cells from old donors. On the one hand, such cells may be postmature cells, which have exhausted their proliferative potential. Alternatively, nonresponsive T lymphocytes might include T-lymphocyte precursors which have failed to differentiate and thereby gain responsiveness as a conse-

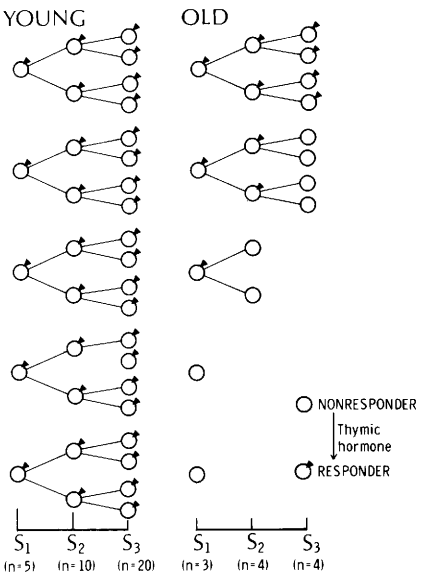

FIG. 2. Model of the age-associated defect in thymidine incorporation by lymphocytes in culture.

quence of inadequate thymus gland function. Experiments in mice suggest that such precursor cells do exist in increased numbers in old animals and that their differentiation can be brought about by thymic hormone (4). Experiments are now being carried out to test the effect of thymic hormone administration in man.

The clinical significance of immunological senescence should be considered. While immune competence has traditionally been related to resistance to viral, bacterial, and fungal disease, evidence also suggests that immunological factors may also contribute to the expression of neoplastic and cardiovascular disease. Vascular damage leading to atherosclerosis has been related to the circulation of soluble immune complexes in experimental animals and man. Serum sickness induced in rabbits and spontaneous autoimmune disease in NZB mice result in high levels of circulating immune complexes. In these models, immune complex nephritis has been recognized for some time. Recently, the premature occurrence of coronary artery disease has also been documented (20). These observations add credence to the association of circulating immune complexes with vascular disease. Human diseases such as systemic lupus erythematosus have also been associated with premature coronary artery disease (21). Although coronary artery disease was related to the treatment of these patients with corticosteroids, it appears more likely that the persistent exposure of the vasculature to circulating immune complexes is the important factor leading to vascular pathology. It remains to be determined whether the increased level of circulating immune complexes in some healthy older humans is a risk factor with respect to cardiovascular disease.

The evolutionary advantage of cell-mediated immunity has been related to the defense of the organism against neoplastic disease. The increased incidence of cancer in immunologically compromised experimental animals and humans supports this hypothesis. Criticism of this concept relates to the fact that immunosuppression is not always associated with an increased incidence of spontaneous tumors and the tumors that frequently occur are usually those of the lymphoreticular system. While it is clear that immune surveillance is not the only factor modulating the expression of neoplastic disease, the hypothesis has heuristic value. Certainly the increasing incidence of cancer and the decline in cell-mediated immunity with age presents an interesting association which may reflect a causal relationship. Critical to such an interpretation would be prospective studies that would relate the future risk of neoplastic disease to the level of T-cell immune competence during middle or late middle age. If viral infection is proved to play an important role in human cancer, the importance of the decline in cell-mediated immunity with age to the occurrence of cancer would seem stronger.

There is good evidence that the susceptibility of humans to viral disease is related to cell-mediated immunity. One of the clearest clinical consequences of lowered T-cell immunity with age is the increasing incidence of herpes zoster with age (22). This viral disease occurs with increasing frequency not only in older subjects but also in patients with neoplastic disease and patients receiving immunosuppressive drugs who have impaired T-cell competence.

Cell-mediated immunity was initially discovered in a search for the factors responsible for resistance to tuberculosis. The increasing incidence of reactivation tuberculosis in the elderly population has become not only an epidemiological fact but also a challenge to the medical profession. The loss of delayed cutaneous hypersensitivity to PPD may not only contribute to the exacerbation of tuberculosis but also contributes to the delayed recognition of tuberculosis in the elderly. One-half of the deaths from tuberculosis today occur in the elderly and in one-half of these patients the disease is not discovered until postmortem examination. Elderly patients, who frequently do not manifest a vigorous inflammatory response may not develop fever, cough, or abnormal chest X ray and fail to manifest a positive skin reaction to PPD. In such patients, therapy may be delayed, often with fatal consequences. Bone marrow or liver biopsy may be essential if the

diagnosis is to be established before the results of bacterial cultures are available.

If the loss of T-cell immunity increased the risk of a variety of diseases, one would predict that the impairment of T-cell immunity would be directly correlated with shortened survival. Studies have offered evidence that the survival of elderly persons is directly related to the severity of impaired T-lymphocyte function (9). While such studies must be regarded as preliminary, the thesis developed in this review that impaired T-cell immunity has significant clinical consequences is supported.

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