Immune Response and Aging: Constitutive and Environmental Aspects

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Advancing age is accompanied by a decline of most cell-mediated and humoral immune responses, although the changes are sometimes selective and may appear to represent modifications in immune regulation (1). From all compartments and cells involved in the immune response, the thymus and T-lymphocytes appear to be the most directly affected, whereas monocyte, stem cells, and B-lymphocytes are less involved. Among the various immune phenomena detectable _in vitro_ or _in vivo_, lymphocyte proliferation to mitogens, lectins, and alloantigens, the generation of cytolytic effector cells, delayed-type hypersensitivity, and primary and secondary antibody responses appear to be particularly diminished in the aged.

These changes do have an impact on disease and may be responsible, at least in part, for the increasing susceptibility of elderly individuals to infectious diseases, in particular tuberculosis and apparently acquired immunodeficiency syndrome (AIDS), for a lesser resistance to tumors, for changes in the manifestations of allergic hypersensitivity, and for the development of autoimmune diseases. It cannot be within the scope of this chapter to review all manifestations in which advancing age and changes in the immune responses have been implicated. I shall focus rather on the major cellular and molecular aspects of immunological senescence and on the external factors, such as nutrition, that may influence them.

Theories of aging can be classified broadly into two types. One type of theory states that aging is an orderly genetically programmed event that is the consequence of differentiation during growth and maturation (2). According to this type of theory, immune response cells have a limited proliferative and reproductive capacity that is genetically determined. This is also consistent with the observation of polymorphic immune aging patterns, as observed in various mice strains kept in a similar environment (3).

The other type of theory attributes aging to a progressive accumulation of faulty molecules resulting in cell dysfunction and death. This may be a stochastic event resulting from random synthetic errors, or from progressive damage due to environmental influences. In this case, adverse and environmental factors such as nutritional deficiency could well contribute to the manifestations of immunological aging.
HEMATOPOIETIC STEM CELLS AND DIFFERENTIATION OF IMMUNE COMPETENT PRECURSORS

The maturation of functional T- and B-cells from hematopoietic precursors is a prerequisite for a functioning immune system. Accordingly, the quality and quantity of the stem cells present in the bone marrow and the effectiveness of the aged environment to support stem maturation and differentiation should be evaluated. The total numbers of bone marrow pluripotent stem cells, characterized by their ability to form colonies in the spleen when transferred to lethally irradiated recipients, is unaffected by aging in most strains of mice (1). However, the proliferative capacity of these stem cells may be reduced in the aged.

Aging appears to affect more severely the committed stem cells of the lymphoid compartment and the maturational microenvironment of the bone marrow and thymus. The ability of the aged bone marrow to reconstitute immune responsiveness in irradiated young hosts has been the most common technique used for studying bone marrow aging, but recently new in vitro techniques have also been used. Early experiments using bone marrow reconstitution of irradiated hosts have suggested that age does not affect the production of B-cells and B-cell precursors. More recent work in vitro, however, has led to the conclusion that the aged bone marrow is not as effective as the young bone marrow in supporting B-cell differentiation. Mature B-cells, on the other hand, do not appear to be affected by aging in a major functional way.

Some studies indicate that although the total number of colony-forming cells in the aged bone marrow is not markedly diminished, the number of bone marrow T-cells and their ability to repopulate the thymus in irradiated animals decrease with age (1). As discussed in more detail below, the T-cell compartment appears to be markedly more affected by the aging process than the B-lymphocyte compartment (4). The ability of the aged thymus to serve as a site of T-cell maturation is possibly an important factor and thymic involution has been suggested to be the primary cause of immunosenescence. Although thymic function greatly diminishes with age, the number of peripheral T-cells does not change proportionately. This is in contrast to the situation arising after adult thymectomy, in which animals undergo a gradual decline in the number of T-cells, suggesting thereby that the aged thymus must retain considerable capacity for supporting peripheral T-cells. In the mouse, the capacity of the thymus to support the complete spectrum of T-cell differentiation is lost shortly after birth. However, even in old age, the thymic reticulum is capable of supporting some degree of T-cell differentiation. The role of thymic hormones in maintaining or generating functional peripheral T-cells is still not fully understood. However, several studies have shown that peripheral T-cell functions can be improved in aged animals by in vitro or in vivo exposure to thymic hormones.

In conclusion, the differentiation toward mature lymphocytes is defective in the aged animal, due largely to deficiencies in the maturation environment, and in part to loss of pre-B- and pre-T-cells in the bone marrow. This may lead in the aged
individual to an accumulation of larger numbers of immature cells and/or of memory
cells (1).

B- AND T-LYMPHOCYTE ACTIVATION

The generation of an immune response, be it cellular or humoral, requires the
activation of antigen responsive T- and B-lymphocytes, and their entry into the cell
cycle with subsequent expression of differentiated functions. The entry and transit
of the cell cycle in T-cells require delivery to the cell surface of several signals
triggering the sequential expression of several new proteins, such as the receptors
for interleukin-2 (IL-2) and transferrin (5). Interleukin-2 is synthesized and secreted
by specific subpopulations of T-cells. Interactions between IL-2 and its high affinity
receptor are necessary to drive T-cells from the G1 to the S-phase. The rate of
cell cycle traverse appears similar in aged and young populations, but in older in-
dividuals a lower number of aged lymphocytes enters the cell cycle and in particular
fewer undergo repeat cycles after stimulation.

Both T- and B-lymphocytes use similar intracellular mechanisms for transducing
a membrane event into intracellular activation. Perturbation of some membrane an-
tigen receptors, such as membrane IgM for B-lymphocytes or T-cell receptors for
T-lymphocytes, leads to rapid activation of phospholipase C, which initiates the
hydrolysis of phosphatidyl inositol, 4,5-phosphate. The products of this reaction,
diacyl glycerol and inositol triphosphate, in turn activate protein kinase C and trigger
the release of intracellular stores of calcium. A second major signaling mechanism
involves the cyclic nucleotides cyclic adenosine monophosphate (cAMP) and cyclic
guanosine monophosphate (cGMP), a system that appears to be a down-regulator
of the first mechanism. Mitogen activation of aged T-lymphocytes is defective al-
ready at the earliest steps and problems can be identified at the levels of calcium
mobilization, phosphatidyl inositol phosphate hydrolysis, and protein kinase C ac-
tivation. Alterations in membrane composition and changes in the viscosity of the
plasma membrane may also impair cell activation.

At a later stage, namely during transitions of cells from G1 to G2, which includes
the synthesis of several activation proteins, various defects have been clearly shown
(5). Alterations in the generation of the proto-oncogene c-myc, in the synthesis of
IL-2, and in the expression of IL-2 receptors have been reported (1). The synthesis
of IL-2, a necessary second signal for driving T-cell entry into DNA synthesis, is
low in both aged humans and experimental animals (6). The most significant factor
controlling the quantity of IL-2 produced by aged lymphocytes appears to be the
number of precursors. Whereas the amount of IL-2 produced per precursor cell is
the same whether derived from an aged or young animal, the number of IL-2-produ-
cing cells is greatly reduced in aged individuals. Expression of the IL-2 receptor,
which is synthesized and expressed during the late G1 to G2 portion of the cell
cycle, is also deficient in aged animals. A decrease in the number of activated re-
ceptors per cell and a decrease in the number of cells expressing the receptors may
TABLE 1. Cell cycle events affected by the aging process

<table>
<thead>
<tr>
<th>Cell cycle stage</th>
<th>Event</th>
<th>Changes with age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Go</td>
<td>Membrane composition</td>
<td>Lipid changes affect viscosity</td>
</tr>
<tr>
<td></td>
<td>Membrane potential, ion</td>
<td>Na,K-ATPase activity decreases</td>
</tr>
<tr>
<td></td>
<td>Cytoskeleton</td>
<td>Action polymerization changes</td>
</tr>
<tr>
<td>Go-G1a</td>
<td>Phosphatidylinositol hydrolysis</td>
<td>Decrease</td>
</tr>
<tr>
<td></td>
<td>Protein kinase C activation</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>cAMP/cGMP</td>
<td>Controversial</td>
</tr>
<tr>
<td>G1a-G1b</td>
<td>Expression of new proteins</td>
<td>Low mRNA for IL2, IL2 receptor, GM-CSF, IL1, IL6, IFN-γ</td>
</tr>
<tr>
<td></td>
<td>Expression of activation antigens</td>
<td>Low RL388, transferrin receptor</td>
</tr>
<tr>
<td>G1b-S</td>
<td>Lymphokine signals</td>
<td>Low DNA synthesis initiated in IL2R+ cells</td>
</tr>
<tr>
<td>G2M-G1</td>
<td>Cycle reentry</td>
<td>Impaired, accumulated chromatin damage, low DNA repair</td>
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both be responsible for the decrease in functional proliferation manifested by T-lymphocytes in aging. In addition, there seem to be some other defects limiting aged cell responsiveness to IL-2 at some site distal to receptor expression.

As far as B-cell activation is concerned, following activation with antigens or anti-μ reagents interactions with lymphokines also appear to be required for cell cycle transit and further differentiation (1). At least five T-cell-derived lymphokines have been characterized that influence B-cell growth and differentiation, such as interferon-γ, IL-2, IL-4, IL-5, and IL-6. Up to now, the effect of age on the synthesis and activity of these lymphokines has not been much studied. However, it has recently been shown by our group that messenger RNA levels expressed for a variety of lymphokines such as IL-1, GM-CSF, IL-6, and interferon-γ are also decreased in the elderly (6).

Several studies have indicated that subsequent cell cycles may be more significantly impaired in aged cells than the initial cell cycle, possibly in relation to alteration in chromatin structure and DNA repair mechanisms (1).

In summary, aged lymphocytes display a number of defects that prevent normal cell cycle entry and transit (Table 1). The inability of aged T-cells to enter and progress throughout the cell cycle is not due to a single defect but to a number of deficiencies. In addition to revealing multiple sites at which aging affects the activation sequence, the data available also indicate that only a portion of the lymphoid population is affected. Aged lymphocyte populations are a mosaic of normal active cells and of those that are defective.

AGE-ASSOCIATED ALTERATIONS IN CELL SUBSET DISTRIBUTION

The total number of B- and T-lymphocytes appears to be unchanged by aging, but changes in subset distribution may occur. Several studies in mice and man looking
at the absolute numbers and proportions of T-lymphocyte subsets in aged peripheral blood lymphocyte populations have yielded conflicting results (1). A majority of the reports record a decrease in the proportion of T-lymphocytes, with a decrease in both CD4 and CD8 cells. However, the magnitude of the differences between aged and young populations is less than 20%, which is much less than the degree of functional decline that accompanies aging. A reduced number of functional receptors may also contribute to the ineffective triggering of aged lymphocytes. It must also be kept in mind that changes in T-lymphocyte subset distribution may not be a biomarker of aging but rather a reflection of some underlying disease processes. Indeed, selection of aged blood donors for good health, according to the so-called SENIEUR Protocol (7), indicates that a sizable proportion of the immunological decline phenomena usually attributed to age may in fact be due to additional non-age factors, such as environment (possibly nutrition) and disease.

**IMMUNOLOGICAL REGULATORY CHANGES OCCURRING AS A FUNCTION OF AGE**

Anti-idiotypic antibody production increases with age and is apparently responsible for the reduction in the amount of high-affinity antibody and the decrease in the avidity profile of antibodies produced by the aged. Downregulation by anti-idiotypic antibody seems to become much more important with age. The idiotype repertoire also apparently changes with age and the serum from aged animals is more efficient in suppressing antibody responses. Isotypic regulation, such as IgE regulation, may also be, at least in part, due to auto–anti-isotypic antibodies. We have accordingly started to investigate whether an increase in auto–anti-IgE antibodies may be responsible for the decrease in IgE-mediated allergic manifestations observed in the majority of elderly allergic patients (8).

Regulatory mechanisms mediated by suppressor cells are certainly also present in young and aged individuals. However, controversy remains regarding the role of suppressor cells in the diminished immune responsiveness of the aged. In the aged, several suppressor cell systems may operate. Regulatory T-cells control auto–anti-idiotypic antibody production and directly downregulate some responses but non-T-suppressor cells have also been reported (1).

One of the most intriguing aspects of immune senescence is the emerging data that indicate that aging does not equally affect all tissues and immune organs. In man, most of the data available for study of the immune functions are drawn from peripheral blood lymphocytes. However, several recent studies suggest that mucosal immunity, in particular the response in the gut-associated lymphoid tissue, is not markedly affected by age.

**AGE, IMMUNE FUNCTION, AND DISEASE**

Changes in immune regulation are apparently responsible for the increase in autoimmune phenomena and auto-antibodies that may lead, albeit not obligatorily, to
specific organ or tissue injuries. Such changes in regulation are probably also involved in the age-associated decrease in manifestations of atopic allergy.

The age-related decrease in stem cell kinetics, differentiation, and functions may be critical to an effective response to stress, such as infection. Elderly patients with sepsis often fail to mount leukocytosis and to express fever, which are the result of lymphokine production.

The functional attributes of thymus-derived T-cells include delayed hypersensitivity reactions, production of lymphokines, killing of tumor cells, lysis of virus-infected cells, and transplantation rejection. In individuals above the age of 65 years, delayed cutaneous hypersensitivity reactions to ubiquitous recall antigens are reduced (9). Lymphopenia and anergy appear to have important prognostic significance in old age.

Of particular interest and beyond the genetic determinism of immune senescence is the recently formulated hypothesis that nutrition is a critical determinant of immune competence and risk of illness in old age (9). The fact that malnutrition in infants and children is directly associated with immune deficiencies is well documented. The impact of nutritional deficiency on the immune response is also evident from the phenomenon of acute anergy in patients undergoing severe trauma or operative shock, since this state of anergy can largely be overcome and abnormal immune response restored by nutritional supplementation.

In the elderly, nutritional deficiency may be more selective; in most instances it is probably not sufficiently pronounced to affect the immune response markedly. Very few studies have attempted up to now to correlate immune senescence with the state of nutrition or have attempted correction of nutritional deficiencies and their effects on immune responses in the elderly (9). In a group of apparently healthy individuals, those with clinical hematological and biochemical evidence of nutritional deficiency showed significant reduction in delayed cutaneous hypersensitivity reactions, in the number of T-cells, and in the lymphocyte response to phytohemagglutinin. Nutritional supplementation during a period of 8 weeks resulted in improved skin test responses, an increase in T-lymphocyte numbers, including the CD4 subset, and better lymphocyte proliferative response to phytohemagglutinin (10). In another study, prealbumin levels have been reported to correlate with impaired immune responses (11). Furthermore, nutritional supplementation appeared in a recent study to improve natural killer cell activity and mitogen-induced lymphocyte stimulation responses, to enhance delayed cutaneous hypersensitivity, and to increase IL-2 production (9).

Among the various nutritional elements, zinc appears to be particularly important since, on the one hand, the immune deficiency associated with low zinc diet is well documented, whereas on the other hand moderate zinc supplementation appears to improve delayed cutaneous hypersensitivity. However, megadoses of zinc, as well as of some other elements such as selenium, vitamin A or vitamin E, may also have immunosuppressive effects (9). Appropriate nutritional support may improve the response to immunization in the elderly, for example with influenza virus vaccine. It may also prevent postoperative complications, which occur more frequently and
are more severe in elderly subjects who are malnourished. However, many more studies are required in order to assess more precisely the potentialities of nutritional supplementation in the elderly for improvement of their immune response and prevention of disease associated with immune deficiencies or dysregulation.

CONCLUSION

The combined effects of immunization, improved sanitation, better housing, and good nutrition have resulted in a dramatic decrease in childhood mortality and increase in life span in most industrialized countries. It remains to be seen whether improvement of nutrition in old age, possibly associated with a concurrent and increased stability of the immune responses at or near their optimal level, could be achieved in the elderly by the two pillars of prevention: optimum dietary intake and regular physical exercise.

REFERENCES


DISCUSSION

Dr. Chandra: I should like to make a couple of points. The first is that changes in immune responsiveness with age are not inevitable. There are individuals of 80 or 90 years of age who retain vigorous immune responses, as vigorous as those seen in young people. The
second point is that in those elderly people who have nutritional deficiencies, defined by reduced dietary intake or by estimation of blood nutritional indices or some functional index of nutritional status, an improvement in nutrition is generally accompanied by an improvement in the immune response. In the population we have studied 25% to 30% of individuals, although apparently healthy and without evidence of significant systemic disease, showed evidence of reduced intakes and blood levels of various nutrients, particularly zinc, iron, vitamin C and \( \beta \)-carotene (1). We then provided a small supplement of these micronutrients that could be given to all persons in the group without the need to identify those who showed individual deficiencies. This supplementation improved \textit{in vitro} lymphocyte reactivity, natural killer cell activity, and was associated with a significant reduction in common respiratory illness.

\textit{Dr. de Week:} It is important to stress the heterogeneity of the aging population. Many things can influence immune function and in any population of aged individuals there will be a wide variety of possible reasons for impaired immune responsiveness. Thus, without very large numbers of subjects followed up longitudinally, it will always be difficult to ascribe this or that change to this or that factor.

I should like to ask Dr. Chandra whether his supplementation study resulted in the reversal of anergy in old people as well as in improvement of lymphocyte reactivity.

\textit{Dr. Chandra:} An improvement in skin hypersensitivity response was present but not statistically significant. However, we published a study in 1982 (2) in which we showed that anergy was reversed by diet treatment involving macro- as well as micronutrients after a period of 12 weeks in 7 of 14 individuals who were anergic at the start of treatment.

\textit{Dr. de Week:} I think that the anergy effect, or some measurable \textit{in vivo} effect, will in the future be more important to study than \textit{in vitro} assays, especially in patients submitted to surgical shock. It has been shown recently that patients who do not respond to intradermal injections of particular lymphokines have a much worse prognosis than those who are anergic but still respond to these lymphokines.

\textit{Dr. Schiffman:} In one of our studies we found that there was an improvement in immune status simply as a result of improved food flavor. There was no difference in the amount of food or nutrients consumed, but the addition of odors and flavors seemed to improve the immune status. Could you comment on factors other than nutrition that may improve immunity?

\textit{Dr. de Week:} I have no data of this kind from our own studies.

\textit{Dr. Chandra:} Stress is a very important factor that can affect the immune response to a variety of biochemical and other mediators. Perhaps by adding these pleasant odors to the diet there was a change in the overall satisfaction these individuals derived from their food and the happier state of mind produced may have affected their immune responsiveness.

\textit{Dr. Schiffman:} Although we applied a “life satisfaction” scale, I do not think it was sensitive enough to pick up any differences resulting from these effects on the diet.

\textit{Dr. Guesry:} It seems that nutrition plays a critical role in immunological function and the importance of trace minerals has been stressed. I think protein and long-chain polyunsaturated fatty acids also play a role that may be of considerable importance. I should like to ask Dr. Chandra whether he investigated such factors in those of his patients who had an incomplete response to trace nutrients.

\textit{Dr. Chandra:} We did not do this in our study, but there is a large body of evidence to show the important immunomodulatory effect of lipids. There appear to be two considerations. The first is the amount of fat and the second, the type of fat. If in an animal model you feed more than 16% of the dietary energy as fat, you start to see an immunosuppressive
effect that is more marked with polyunsaturated fatty acids. If you feed more than 40% as fat, the effect is much more marked and the distinction between polyunsaturated and saturated fats disappears.

Dr. Steen: Longitudinal studies of normal aging seem to show that the aging process becomes much more pronounced after the age of 70 to 75 years. Is this reflected in deterioration in immune function as well?

Dr. de Weck: It may be, and some data suggest that after 80 years there may be a very pronounced decline in immune responsiveness. But much of the available data do not include many subjects over the age of 80 and we need more information in the age range of 80 to 100 years.

REFERENCES