Immunosenescence and Anti-Immunosenescence Therapies: The Case of Probiotics

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ABSTRACT

Aging is a complex process that negatively impacts the development of the immune system and its ability to function. Progressive changes in the T and B cell systems over the life span have a major impact on the capacity to respond to immune challenge. These cumulative age-associated changes in immune competence are termed immunosenescence. This process is mostly characterized by: (1) shrinkage of the T cell repertoire and accumulation of oligoclonal expansions of memory/effector cells directed toward ubiquitous infectious agents; (2) involution of the thymus and the exhaustion of naive T cells; and (3) chronic inflammatory status. Here we discuss possible strategies to counteract these main aspects of immunosenescence, in particular the role of the normalization of intestinal microflora by probiotics. A better understanding of immunosenescence and the development of new strategies to counteract it are essential for improving the quality of life of the elderly population.

IMMUNOSENESCENCE

Aging is a post-maturational process that, due to a diminished homeostatic capacity and increased vulnerability, reduces responsiveness to environmental stimuli. It may be defined as a systemic loss of molecular fidelity that, after reproduction, reaches levels that exceed repair, turnover, or maintenance capacity. Loss of molecular fidelity occurs because energetics determines maintenance of the vital structure and functional integrity of the biomolecules. Hence, molecular fidelity is maintained for time periods. Thus, aging at molecular level results from an increasing entropy that exceeds repair and turnover capacity. This progressive loss of molecular fidelity increases predisposition to illness and death. Infectious and inflammatory diseases are increased in frequency and severity in the elderly, however, suggesting a key role for immunity in the survival of the elderly, because susceptibility to these diseases depends at least in part on immune function. In particular, rate of death from infectious diseases continues to accelerate with age. Thus, a better understanding of the aging of the immune system may provide the most important clues for slowing the inevitable decline associated with the passage of time. In the elderly, many alterations of innate and clonotypic immunity have been described and viewed as deleterious, hence the...
term immunosenescence. Some immunological parameters are commonly notably reduced in the elderly and, reciprocally, good function is tightly correlated to health status. In fact, age-related changes of the immune system are directly or indirectly involved in the peculiar susceptibility to infectious diseases, autoimmunity, and cancer of the elderly and in their decreased responsiveness to vaccination, as well as in the pathogenesis of the more relevant age-related diseases, such as cardiovascular and neurodegenerative diseases, diabetes, and osteoporosis. 3–6

Concerning innate immunity, studies performed on old mice have shown a functional decline of monocytes and macrophages, a reduced expression of Toll-like receptors on activated splenic and peritoneal macrophages, and an impaired production of several cytokines. Furthermore, aged phagocytes such as, macrophages and neutrophils, have impaired respiratory burst and reactive nitrogen intermediate production, which result in a decreased ability to destroy pathogens. Moreover, aged dendritic cells are less efficient in activate both T and B cell populations and aged natural killer (NK) cells show a decreased ability of killing tumor cells.7,8

However, an increased number and percentage of NK cells has been found in the elderly and centenarians; more interestingly, the centenarians with the highest NK function and number of NK cells have preserved endocrine conditions and muscle mass. In addition, an age-related expansion of CD28−CD4+ cells occurs, enriched in cells expressing NK receptors. According to this scenario, it has been proposed that the increase with age of cells with NK features—CD8+/CD4−, CD4+/CD8−, or CD4+/CD8+—could cause prevailing of the innate immunity in the oldest old, although, as previously stated, the increase in NK cell numbers is not accompanied by an increase in functional activity.9–12

On the other hand, the in vivo production of proinflammatory cytokines is upregulated in old subjects. Tumor necrosis factor (TNF)-α, Interleukin (IL)-1, and IL-6 are the classical proinflammatory cytokines. Their ability to activate both local and systemic effect is notorious. Locally, they contribute to the activation of the inflammatory cells and, together with the chemokines, which that induce the expression of adhesion molecules, cause their local recruitment. When the causes of the inflammatory reaction exhibit a higher intensity, the production of these cytokines is increased and they are released in the circulation provoking the systemic inflammation. On the other hand, IL-10 is an “inhibitory” cytokine because it precludes the activation and some effector functions of T lymphocytes and mononuclear phagocytes. IL-10, mainly, inhibits the release of proinflammatory cytokines, so acting in choosing the type of immune response and in turning off the inflammatory processes. Thus, we can observe an abnormal elevation of proinflammatory cytokines during inflammatory responses, and an age-related increase of IL-6 levels has also been reported in plasma, serum, and supernatants.13–20

Senescence of clonotypic immunity is claimed to be principally a result of the declining effectiveness of T cells. Lifelong and chronic antigenic load results in the major driving force of immunosenescence, which impacts on human lifespan by reducing the number of virgin antigen non-experienced T cells (further reduced by thymus involution) and simultaneously filling the immunological space with expanded clones of memory and effector, antigen-experienced T cells. In the meantime, this lifelong and chronic antigen load is responsible for the chronic inflammatory status that characterizes aging (see below). 2,5,6

Gradually, the T cell population shifts to a lower ratio of naïve to memory cells, the thymus pumps out fewer naïve T cells with age, and those T cells remaining, especially the CD8+ subset, also show increased oligoclonality with age. So, the repertoire of cells available to respond to antigenic challenge from previously unencountered pathogens shrinks. In addition, older organisms often are overrun by memory cells that carry a single type of T cell receptor (i.e., clonal expansion). Thus, the memory cells from old individuals might recognize a limited set of antigens despite being plentiful in number. Many of the clonal expansions crowding an elderly person’s immune system result from previous infections by so-called latent viruses. Actually, in the el-
derly, a high number of CD8+ cells are specific for a single epitope of herpetic viruses: in some individuals, more than 10% of peripheral CD8 react against a single CMV epitope. Despite an apparent surplus of memory cells, older animals are not necessarily more able to fight off the pathogens that those cells recognize. Memory T cells usually carry the CD28 surface protein, which helps stimulate the cells to divide when antigen is present. But old memory cells tend to lose CD28 and, as a result, multiply less robustly when exposed to antigen than do younger cells.21–24

Furthermore, one of the main characteristics of immunosenescence is a decrease in thymic epithelial space and thymic cellularity, collectively called thymic involution. In mice, loss of thymic epithelial space is caused by a gross reduction in thymus size, whereas in the human thymus there is an increase in perivascular space, which is progressively replaced with fat in the aging thymus.25,26

In contrast to T cells, no evidence for a loss of B cell function has been found as neither the total number of B cells nor immunoglobulin-secreting cells have been shown to be profoundly decreased with age. However, the B cell repertoire is influenced by aging during an actual immune response, where the spectrum of expressed immunoglobulin genes, as well as the frequency of somatic mutations, affects the quality, though not necessarily the quantity, of the antibody response. What appears as an intrinsic defect in somatic mutations seems to be caused by suppressive influences exerted in vivo by aged CD4+ T cells possibly reflecting both the age-associated shift from type 1 to type 2 cytokine patterns and the age-related impairment of CD40-CD40L system. Finally, one of the most dramatic examples of age-associated repertoire changes is the appearance of oligoclonal expansions of CD5 B cells producing antibodies against self-antigens, albeit with no known pathophysiological consequences. More interestingly, in the elderly and centenarians there is an increase of memory CD27+ B cells with a decrease of virgin CD27− B lymphocytes. The decrement of virgin CD27− B lymphocytes and the concurrent increase of memory CD27+ B lymphocytes can be one of the events that might have an impact on the antibody repertoire of the elderly. In fact, chronic antigenic load may fill immunological space with expanding clones of memory antigen-experienced B cells, which affect the clonotypic immune response to new extracellular pathogens.27–29

As a consequence T and B immunosenescence and, likely, mortality and morbidity will occur earlier in people who have been exposed to an antigenic overload (due to chronic infections). The opposite situation will happen both in people exposed to a lower antigenic load and in people equipped with an immunogenetic background able to efficiently fight infections. This means that the ability of our immune system is progressively worn out by the attack of pathogenic antigens. In developed countries, the improved hygienic conditions and the consequent lower level of bacterial contamination of food and water, could definitively have reduced the antigenic overload, preserving the immune system by rapid exhaustion.2,4,30

Thus, immunosenescence is mostly characterized by: (1) the shrinkage of the T cell repertoire and the accumulation of oligoclonal expansions of memory/effector cells directed toward ubiquitous infectious agents; (2) the involution of the thymus and the exhaustion of naive T cells; and (3) chronic inflammatory status.

This chronic inflammatory status appears to be the prevalent mechanism driving tissue damages associated with different age-related diseases. Aging is accompanied by an age-dependent upregulation of the inflammatory response due to the chronic antigenic stress that impinges throughout life upon innate immunity and has potential implications for the onset of inflammatory diseases. In fact, chronic inflammation is involved in the pathogenesis of all age-related diseases: Alzheimer’s disease, atherosclerosis, diabetes, and even sarcopenia and cancer—to mention but a few—have an important inflammatory component. Increased levels of circulating inflammatory mediators may result from a constant, low-grade activation of cytokine-producing cells or a dysregulated cytokine response following stimulation. Low-grade increases in the levels of circulating proinflammatory cytokines and of C reactive protein are strong predictors of all-cause mor-
tality risk in longitudinal studies of several elderly cohorts. The effects of inflammatory mediators are independent of pre-existing morbidity and of other traditional risk factors for death in survival analyses, suggesting that cytokines trigger/exaggerate pathological processes. So we could conclude that age-related diseases are “the price we pay” for an active immune system that defends us in youth but harms us later.2,4,13–20,30–33

**ANTI-IMMUNOSENESCENCE STRATEGIES**

Nowadays individuals can live until 80–120 years, but for most individuals this increase in life span is not free of disabilities and diseases, which still represent the dark side of aging and longevity. Indeed, human senescence is often affected by a variety of diseases, which share an important immunoinflammatory component and are characterized by the immunological changes typical of immunosenescence. Here we discuss possible strategies to counteract the main aspects of immunosenescence in aging. Some means of such intervention that could be applied in the elderly without ethical problems do already exist to.

Several interventions, including exercise, have been proposed to restore immune function in older populations. The findings from some, but not all studies, support the possibility that exercise may attenuate immunosenescence. In recent years, the role of exercise in modulating immune response has been examined using models that may have clinical relevance, such as the response to vaccines and novel antigens. Taken together, the accumulated data suggest that exercise may be an efficacious therapy for restoring immune function in the elderly. In general, long-term exercise interventions appear to show the most promise.34

Another possible strategy to counteract immunosenescence is to reduce as much as possible the antigenic load represented by pathogens, such as influenza virus and cytomegalovirus (CMV). Strategies of specific vaccination should be applied to prevent, not only morbidity and mortality, but also any additional persistent stimulation of the immune system in the elderly. In the future it can be imagined that a vaccine could be developed against chronic, non-directly lethal viral infections that can cause persisting antigenic stimulation, thereby exhausting the immune repertoire. The possibility of using these vaccines would dramatically increase the immunocompetence of elderly subjects mainly if it takes into account the “infectious nature” of immunosenescence. Because CMV seems to be one of the main driving force of immunosenescence and the number of CMV+ subjects increase with age, immunization strategies against CMV could be potentially highly protective, as they should avoid the accumulation of terminally differentiated T cell clones. At this moment there is no major viable anti-CMV vaccine; however a large clinical trial on the utilization of a vaccine to prevent herpes zoster and postherpetic neuralgia in older adults has been published. This is not a vaccine aimed to eradicate the latent herpes zoster infection, but to significantly decrease the clinical infections of herpes zoster.22–24,35–37

Moreover, the accumulation of replicative senescent T cells with aging is a problem of great importance, so it is tempting to suggest an effective approach to diminish telomere loss. CD8+ T cells show the greatest telomere length erosion, a sign of proliferation due to chronic antigenic stimulation. Vaccination to decrease chronic viral load exhausting the immune system would be also beneficial for telomere length maintenance.38,39

As mentioned above, one of the main characteristics of immunosenescence is thymus involution; thus there have been many approaches for reversing thymic involution in animal models, such as the implantation of pituitary-derived epithelial cell lines or injections of growth factors and hormone agonists. Recently, the role of IL-7 to counteract thymic involution in animal models has been proposed. IL-7 is a cytokine playing a crucial role in the development and maintenance of the peripheral T cell pool. Available data in mice suggest that IL-7 gene therapy in aging restores early thymopoiesis without reversing involution. However, it has been suggested that in humans IL-7 availability in the plasma is not correlated
with thymic involution, and that IL-7 production at the thymic level could represent a more important limiting factor.\textsuperscript{36,40}

As previously stated, aging is accompanied by a low-grade inflammation held responsible for many age-related diseases, so a decrease in the rate of inflammation should prevent the activation of the immune system. The age-associated increase in proinflammatory cytokines and increased autoimmune disease in the elderly raise the possibility that some proinflammatory cytokine-blocking antibodies or soluble receptors could also be beneficial for the elderly. Moreover, there are other less potent, well-known inflammation-modulating drugs, such as statins and non-steroidal anti-inflammatory drugs, that have few side effects and can be used with safety even in very old subjects. On the basis of this, it is reasonable to assume that anti-inflammatory treatments could be useful to counteract and reduce the age-dependent inflammatory status.\textsuperscript{36,41,42}

Finally, another crucial intervention is to provide elderly subjects with a correct dietary intake. It is well accepted that nutrition can influence or even play a leading role in the development of various diseases such as infections, cancer, and cardiovascular diseases. Macronutrients such as anti-oxidants, dietary fiber, omega-3 PUFAs, as well as micronutrients such as Vitamins D, E, zinc, iron, copper, and selenium are of particular interest. Thus nutritional interventions could be beneficial for the prevention, retardation, or even reversal of established immunosenescence.\textsuperscript{43,44}

**PROBIOTICS**

As known, the immune system is characterized by the presence of large repertoires of clonally distributed lymphocytes, each of them capable of recognizing a defined antigenic determinant present on a given microorganism and thus covering the entire universe of possible antigens. The reason why such a new and extremely complex immune system based on lymphocytes emerged is still a mystery, but a reasonable hypothesis suggests that a major driving force was that of taking under control the intestinal microflora characterized by an enormous variety of bacterial species. Indeed, a profound derangement of intestinal microflora is present in the frail elderly and may contribute to the age-related inflammatory status. This derangement of the intestinal microflora likely represents an important source of continuous antigenic stimulation and contributes to immunosenescence.\textsuperscript{45,46}

Thus, knowledge of age-related changes in the gastrointestinal tract flora might be important in the treatment and prophylaxis of diseases, and in maintenance of health among the elderly. Recent studies indicate shifts in the composition of the intestinal microbiota, which may lead to detrimental effects for the elderly host. Increased numbers of facultative anaerobes, in conjunction with a decrease in beneficial organisms such as the anaerobic lactobacilli and bifidobacteria, have been reported. These changes, along with a general reduction in species diversity in most bacterial groups, and changes to diet and digestive physiology such as intestinal transit time, may result in increased putrefaction in the colon and a greater susceptibility to inflammatory disease.\textsuperscript{46,47}

Therapeutic strategies to counteract these changes include dietary supplements containing prebiotics, probiotics, and a combination of these. Prebiotics are non-digestible, by the host, food ingredients that have a beneficial effect through their selective metabolism in the intestinal tract. This concept, reviewed in terms of resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption, of fermentation by intestinal microflora and of selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing, allows us to consider prebiotics the fructo-oligosaccharides, galacto-oligosaccharides, and lactulose. The first significant introduction of the probiotic concept was by Metchnikoff in the early 1900s, who believed that the complex microbial population in colon was having an adverse reaction on the host and reported that Bulgarian peasants who consumed large quantities of fermented milk experienced longevity, attributed to the health promoting effects of the live microorganisms. Currently, probiotics are defined as live cultures of micro-organisms administered orally and acting beneficially on
host health. They favorably influence both development and stability of the microflora, inhibit colonization by pathogens, influence the mucosal barrier by their trophic effect on intestinal epithelium, and stimulate both specific and non-specific components of the immune system; moreover, they may well replace antibiotics whose resistance is steadily increasing. The mechanisms by which probiotics exert their effects are still uncertain, but are thought to be multifactorial, including chemical inhibition of pathogenic bacteria (decreasing luminal pH, producing inhibitory compounds, or reducing substrate availability to other bacterial populations) or stimulation of the immune response (enhancing humoral immune responses, promoting the intestinal immunological barrier, and stimulating non-specific host resistance to microbial pathogens thereby facilitating immune elimination). Moreover it has been shown that probiotics downregulate hypersensitivity reactions, such as food intolerance and atopic eczema, and increase phagocytosis.36,48–52

However, in studying effects of prebiotics on the immune response to vaccination in the elderly, no changes in serum proteins, albumin, immunoglobulins, and secretory IgA have been observed. Antibodies against influenza B and pneumococcus increased significantly from weeks 0 to 8, with no significant differences between groups (a group received prebiotics while the other group received placebo). Antibodies against influenza A did not increase. No effects of prebiotics on cytokine secretion by cultured monocytes were observed.53

On the other hand, increases in the proportions of total helper (CD4+) and activated (CD25+) T lymphocytes and NK cells (CD56+) in elderly subjects following supplementation with *Bifidobacterium lactis* HN019 have been observed. The *ex vivo* phagocytic capacity of mononuclear and polymorphonuclear phagocytes and the tumoricidal activity of NK were also elevated after *B. lactis* HN019. The greatest changes in immunity were found in subjects who had poor pretreatment immune responses. Thus these data demonstrate that dietary consumption of this probiotic in a milk-based diet may offer benefit to elderly consumers to combat some of the deleterious effects of immunosenescence on cellular immunity.54,55

In a further study, the relationship between fecal microbiota composition and frailty in the elderly was studied. Fecal samples from volunteers with high frailty scores showed a significant reduction in the number of lactobacilli. Both the *Bacteroides*/Prevotella and the *Faecalibacterium prausnitzii* groups showed a significant reduction in percentage of total number of hybridizable bacteria in the elderly with high frailty scores. In contrast to this, the number of Enterobacteriaceae was significantly higher in samples from very frail volunteers. Hence, frailty seems to be associated to significant changes in gut microbiota composition. Since frailty depends on the proinflammatory status, it seems that normalization of microflora, responsible for this status, might be relevant in its prevention.19,20,46

To conclude, at present aging must be considered an unavoidable end point of the life history of each individual. Nevertheless our increasing knowledge about the mechanisms regulating aging allows us to envision many different strategies to cope with, and delay, the processes in order to endow everybody with a long and good final time in life. Therefore, a better understanding of immunosenescence and the development of new strategies to counteract it are essential for improving the quality of life of the elderly population.

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**REFERENCES**


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