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## Host Resistance and Immune Responses in Advanced Age

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Extensive studies suggest that although changes in immunity with healthy aging (termed immunosenescence) occur at the most rapid rate of any physiologic system, it is the compounding effects of age-related diseases and external conditions that result in an overall state of a dysfunctional immunity responsible for the increased risk for and severity of common infections in older adults. Hence, immunosenescence is a predisposing condition, but its contribution to infection risk likely is small until immunity is impaired further as a result of accumulating chronic illness, external conditions, or repeated or chronic infections. This is different from the changes related to immunosuppression that result from certain conditions, such as HIV infection, or immunosuppressive medications that result in unusual, opportunistic infections. Unfortunately, the challenge of studying a diverse population with multiple confounders and the focus of gerontologists on studying normal aging have limited research and understanding of why frail, older adults are so susceptible to common infections and outbreaks of infectious syndromes, including influenza, West Nile virus, and severe acute respiratory syndrome (SARS) and experience frequent vaccine nonresponse.

The high risk for infection in those of advanced age and who have multiple comorbidities is underscored by the high rates of health care–acquired infections in older adults residing in long-term care facilities (LTCF). In

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1999, surveillance of LTCF-acquired infections by the National Nosocomial Infections Surveillance system reported an incidence of 3.82 infections per 1000 resident days of care but with significant variability depending on the type of facility, nature of the residents, definitions used for infections, and type of data analysis [1]. The prevalence of infection rates ranged from 1.6% to 32.7%, and overall incidence rates ranged from 1.8 to 13.5 infections per 1000 resident days of care, with equal variability for specific infections, such as urinary tract or pneumonia. Thus, in establishing infection control policies and vaccination protocols, addressing clear abnormalities in immunity in this varied population must be undertaken. Grouping individuals by disease severity or by level of impairment of specific components of immunity may assist in advancing the ability to identify predictors of impaired host resistance to infections and develop strategies to boost immune response in an at-risk population [2].

Conversely, there is much interest in how recurrent or chronic infections may result in progression of age-related “inflammatory” diseases, especially atherosclerosis. There is some evidence that the interaction of pathogenic burden with host genotype (eg, a mutation of Toll-like receptor (TLR) 4, a surface pathogen receptor on immune cells [discussed later]) may determine the character and enhanced and prolonged inflammatory responses known as “inflamm-aging” (discussed later) that may contribute to cardiovascular disease, autoimmunity, poor host resistance, tumor surveillance, and diminished longevity in the aged [3]. Additionally, factors often not considered immune mechanisms may compromise host resistance and increase infection risk, such as swallowing difficulty or inability to take oral medications [4]. In a vulnerable but less frail population, it is less clear why there remains increased risk for infection and poor vaccine response. The interaction of genetic predisposition for diseases, including susceptibility to certain infections, exposure to chronic pathogens, and other environmental/lifestyle factors, all interact to establish an increasingly impaired foundation of host resistance.

The role of chronic or recurrent infection has not been well studied but also is likely to impair immunity significantly. One study looked at the intra-individual variability on immune senescence markers and found that acute illness, both infectious and noninfectious illness, affected neutrophil (CD16) and T-cell (CD8+ CD28-) markers for up to 30 days but did not look at how this might have compromised host resistance further [5].

## **Overview of immunosenescence**

### *Components of immune response as it relates to host resistance and aging*

Innate immunity is the first line of host resistance against common microorganisms, with a cellular component, made up of neutrophils,

macrophages, epithelial cells, eosinophils, basophils, natural killer cells, keratinocytes, and dendritic cells (DC), and a soluble component made up primarily of the complement cascade. This review focuses on neutrophils and macrophages that can kill pathogens and tumor cells either directly or through the release of proteins, and antigen-presenting cells (APCs) that are critical in regulating subsequent acquired immunity. All these innate immune cells are capable of responding directly and without prior exposure to pathogens via recognition of microbial products (lipids, DNA/RNA, and protein) that bind to surface receptors, including TLR. Toll, a German slang word for jazzy or cool, is the name of a receptor found in fruit fly that codes for dorsal-ventral patterning but also was found to have a role in host defense against fungal infections and has much homology with human immune cell receptors that are important in communication between innate and acquired immunity [6]. In addition to responding directly to pathogens, innate immune cells also provide a link to activation of acquired immunity. DC are the predominant innate immune cells to activate acquired immunity. In response to a pathogen, DC produce bursts of cytokines and other substances that not only kill the pathogen but also recruit and promote the differentiation of other immune cells, including additional DC. Acquired immunity is distinct from innate immunity because of its antigen specificity and the generation of memory responses. This requires activation of lymphocytes (T or B cells) that have specific cell-surface receptors to foreign antigens generated by random recombination of gene segments in the T-cell receptor. These recombinations of molecules and pairings of different variable chains produce a wide repertoire of lymphocytes with specific unique receptors that can recognize virtually any infectious organism or pathogen. Therefore, the acquired immune system has the unique characteristic of specificity of response to a given antigen, whereas the TLR of APC involves pattern recognition of families of proteins common to many pathogens. Because the specificity of the acquired immune response is generated in immune tissues at a distance from the site of infection, however, such as the thymus and lymph nodes, it is necessary to clonally expand antigen-specific lymphocytes to the novel pathogen to have efficacy at a distant site of infection. Acquired immunity requires 4 to 7 days to generate appropriate numbers of cells to counter the infection whereas innate immunity can act in minutes or even seconds.

Another unique feature of acquired immunity is establishment of memory cells, which enables a rapid response on subsequent rechallenge with the same antigen. Cells of the innate immune system interact to play a pivotal role in the initiation and subsequent direction of acquired immune responses. The specificity and regulation of the acquired immune response is dependent on the interaction of fully matured DC with T cells, cytokines and other signaling molecules, and, of course, T cells with the capacity to respond to such signals. Aging, illness, and chronic conditions clearly alter cytokine production and response, altering the integrity of the immune

response, and may not only reduce host resistance but also potentiate inflammatory, age-related diseases, often referred to as “inflamm-aging” [3].

If the interaction of innate and acquired immune cells is ineffective and fails to result in clonal expansion of T cells, the acute immune response is shut off, which could result in impaired host resistance or could be a normal shutting off of the immune response that no longer is needed. Persistent activation of immune cells may result in autoimmune or inflammatory diseases. With aging, it seems the acquired immune cells once “fired” are not removed. This process normally is accomplished by a process called programmed cell death or apoptosis. Aging is associated with ineffective apoptosis of some cells that may populate immune tissues and either prevent fresh cells from being produced or impair activity of those cells (so called suppressor cells). Hence, both premature apoptosis (failing to sufficiently activate an immune response) or inability of immune cells to undergo apoptosis (populate immune tissues with ineffective cells) can result in impaired host resistance [7]. The interface between innate and acquired immunity, that regulation of turning on or off of an immune response occurs, may be particularly vulnerable to the effects of aging and the interaction of age- or comorbidity-related inflammation. It seems this critical interface is where the major age-associated impairment in host resistance occurs and results in increased risk for common infections and poor vaccine responses [7–12].

In summary, studies to date have shown that (1) age-related studies of immunity largely have been limited to the T cells of acquired immunity, with a presumption of essentially intact APC function in healthy older adults; (2) healthy aging changes in immunity, termed immunosenescence, may not have significant clinical relevance in host defense; but (3) rates of common infections and susceptibility to epidemics are increased in chronically ill older adults, and the contribution on immunosenescence in that population may be greater as a result of loss of other redundant host defense mechanisms. Innate immunity is critical to the number of immunocompetent units and the magnitude of the immunologic burst on activation of the adaptive immune system [2].

Cytokines orchestrate the immune response, and specific cytokines are classified as pro- or anti-inflammatory cytokines, depending on their effects on immune function, but the distinction depends on the setting [8,10,11]. Interaction between the different immune constituent cell types of host defense is performed by the strength and balance of cytokine signals. The ultimate signal received and the response and differentiation of effector cells to specific signals likely are affected by aging and chronic illness. In general, activation of acquired immunity that involves cell-mediated immune response is described as a T helper 1 (Th1 or type 1) response and is associated with production of high levels of the cytokines interleukin (IL) 2 and interferon- $\gamma$  (IFN- $\gamma$ ). In contrast, a T helper 2 (Th2 or type 2) response, which is associated with allergic or parasitic infections but not associated with clearance of most bacterial or viral infections, is associated with production

of high levels of IL-10, IL-4, and IL-5 [7]. The relative concentrations of proinflammatory cytokines, defined as those that up-regulate a Th1 response, or anti-inflammatory cytokines that are important in turning off a Th1 response, are influenced by gene activation of effector immune cells and allow further specificity of the eventual outcome of an inflammatory response. These distinctions of pro- and anti-inflammatory are from immunologists' perspective and often are confused with the discussion of inflammatory mediators that increase with aging and age-related diseases, which is particularly true of IL-6, which has pro- and anti-inflammatory characteristics (discussed later). With aging, therefore, it is the interaction of genetic predisposition and environmental exposure that dictates proinflammatory status, cardiovascular disease, autoimmunity, host resistance, tumor surveillance, and longevity versus increased morbidity and mortality resulting from susceptibility to common infections [3].

#### *Changes in innate immunity with healthy aging*

Neutrophils first, then macrophages, use chemotaxis, phagocytosis, and killing via secreted proteins to control invading pathogens and to alert other lines of immune defense. This requires cells to follow a gradient of chemokines by receptor binding to cell adhesion molecules and cellular activation primarily through TLRs by pattern recognition of common microbial peptides. With healthy aging, evidence suggests innate immunity remains intact or is up-regulated in very healthy aging. The frequently reported increase in activated monocytes [8] and nonspecific increase of proinflammatory substances, primarily IL-1 and IL-6, produced by the innate immune system and down-regulation of acquired immunity may reflect a compensatory event by either component, but their causality is unclear [2,10,13].

#### *Changes in phagocytic cells with aging*

For many years, no age-associated decline in neutrophil function was demonstrated, but this may have been because of the challenge of studying neutrophil functions. Studies have been focused on possible alterations in their ability to seek out and migrate toward pathogens, phagocytize, and kill the invading organisms in older adults. Cytoskeletal and membrane changes have an impact on key neutrophil functions, including adherence and membrane fluidity, and are vulnerable to changes in the microenvironment that occur with aging and especially in age-related diseases [14]. More recent studies also demonstrate a decline in the phagocytic ability of neutrophils in older adults [15]. Other studies report that tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), in particular, causes a higher suppression of CD18-mediated, fibronectin-primed, superoxide release in neutrophils from older adults. In addition, neutrophils from healthy older adults are more susceptible to oxidative stress and apoptosis [14]. In looking for a central theme for these age-related changes in neutrophils, impaired signaling elicited by TLR

(although number of TLR seems unaltered) and changes in fluidity and the presence of lipid rafts important in forming an immune synapse to facilitate cell-to-cell communication are found in healthy older adults. In advanced age, activation of TLR2, which recognizes primarily gram-positive bacteria, or TLR4, the predominant recognition protein for gram-negative bacteria, results in altered second messenger integrity leading to altered nuclear transcription factor function, such as nuclear factor  $\kappa$ B (NF- $\kappa$ B) when compared with younger adults [16]. Altered aspects with aging of second messenger pathways include shifts in activation of p42/p44 mitogen-activated protein kinase (MAPK), resulting in a shift in cytokine patterns (discussed later) [14].

Neutrophils undergo spontaneous programmed cell death (apoptosis) without the support of proinflammatory stimulation *in vitro*. Neutrophils from older adults cannot be rescued from apoptosis with proinflammatory cytokines, as can be demonstrated with neutrophils from younger adults, as multiple apoptotic pathways are favored in the aged neutrophils [17,18]. This suggests that although older adults may have adequate numbers of neutrophils, they likely have functional impairments and an inability to sustain activity at the site of infection.

#### *Changes in antigen presentation with aging*

The transition from innate to acquired immunity requires APCs to take up, process, and present specific antigen to T cells expressed in conjunction with the immunohistocompatibility complex II (major histocompatibility complex) molecule that recognizes self-T-cell receptor. DC are the most significant APC, but monocytes, macrophages, B cells, and possibly neutrophils all can function in this capacity. Increased numbers of DC generated *in vitro* from circulating monocytes of very healthy older adults in comparison to younger adults are reported [19]. Processing of the antigen so that it eventually can be presented on the surface of the APC is a complex process, and the proteosomes that are required seem to be altered with aging. There has been study of proteosome activity in the induction of NF- $\kappa$ B in T cells with regard to age that identified impaired degradation of I $\kappa$ B- $\alpha$ , shifting the cytokine response [20]. Proteosome activity also is known to be crucial in antigen processing, and because altered proteosome activity is found in T cells, aging quite likely also could have an impact on antigen processing. In a small study on DC generated *in vitro* from healthy older adults, processing and presentation of antigen seemed intact, and the DC from healthy older adults actually were capable of restoring the proliferative capacity of T cells from older adults and prevented the development of apoptosis in T cells grown to senescence (no longer able to proliferate) in culture [21,22]. In mixing experiments using superantigen, it has been shown that the antigen-presenting capacity of circulating APCs (including DC) actually is greater in APC from healthy

older adults in comparison with younger adult controls and is associated with increased production of IL-12 and IL-10 [23].

*Effect of aging and the environment on dendritic cells regulation of the immune response*

DC are the most potent APC and have a direct role in the priming of T-cell immune responses. There are distinct DC subsets, more pronounced in murine models, with myeloid (DC1) that supports T-cell Th1 response and lymphoid (DC2) that supports T cells differentiating toward a Th2 response. DC1 from old mice (21–24 months) were 4 times less effective than DC1 from young mice (3–6 months) in stimulating syngenic CD4+ T-cell proliferation, stimulating less tumor regression and less DC-specific intercellular adhesion molecule (ICAM)-grabbing nonintegrin expression (important in differentiation to macrophage) but producing more IL-10, which shuts off a Th1 response [24]. The small studies (discussed previously) on monocyte-derived DC (monocytes from peripheral blood, stimulated with granulocyte-macrophage colony-stimulating factor and IL-4) from healthy older adults have not identified differences in number, phenotype, or functional ability to support T-cell proliferation, and DC from healthy older adults are shown to reinduce proliferation of aged T cells [16,21]. This could represent either an in vitro artifact (only responsive cells are selected in culture conditions) or be related to the study of very healthy older adults.

DC regulate immunity as DC sense pathogens through a variety of pattern recognition receptors, such as TLRs, including by lipopolysaccharide, which stimulates TLR4. Different TLRs differentially induce expression of costimulatory or inhibitory surface receptors and the production of proinflammatory (primarily IL-12 and TNF- $\alpha$ ) versus anti-inflammatory (especially IL-10) cytokines [25]. TLR stimulation on DC also results in a direct antimicrobial response, including generation of nitric oxide and vitamin D-dependent antimicrobial peptides, such as cathelicidin [26]. Although there has been little study of potential changes in TLR activation in aging, there is evidence that genetic and environmental factors may alter this signaling between innate and acquired immunity at the level of DC that may alter the host resistance. For example, DC activation by double-stranded DNA, a major constituent of many viruses, to support a Th1 response is enhanced in the presence of keratinocytes and dependent on keratinocyte-derived IFN- $\alpha/\beta$  and IL-18 [27]. Likewise, stimulation of DC by *Mycobacterium tuberculosis* was associated with up-regulation of vitamin D-receptor and the vitamin D-1-hydroxylase genes that leads to induction of the antimicrobial peptide cathelicidin and killing of intracellular *M tuberculosis*, whereas sera from African American individuals, known to have increased susceptibility to *M tuberculosis*, had low levels of 25-hydroxyvitamin D and less induction of cathelicidin messenger RNA, suggesting a link between TLRs, vitamin D-mediated innate immunity, and host resistance that could have relevance to older adults, especially frail older adults who



likely have lower levels of vitamin D [26]. TLRs also seem to play a key role at the subcellular phagosome level that allows DC to discriminate self-antigens from phagocytized apoptotic cells from nonself-antigens from phagocytized microbial cells, and dictate DC maturation, costimulatory molecule expression, and antigen presentation; what effect aging or chronic inflammatory states has on this TLR signaling pathway is unknown [28]. Further, impaired TLR1/2 signaling has been demonstrated in DC isolated from older humans [29].

These data support the hypothesis that genetic predisposition, microenvironment, and aging impair host resistance to infection and response to vaccination by influencing the effect of innate immunity on the development of adaptive immune responses.

*Summary of changes in acquired immunity from aging  
(immunosenescence)*

*Changes in T-cell function in healthy older adults*

The overall impact of normal aging on host immunity is believed to occur primarily along two linked mechanisms. The first is replicative senescence that limits T-cell clonal expansion (resulting from a Hayflick's phenomenon, loss of telomerase activity/telomere length, or a decrease in CD28 costimulatory molecule expression that could be related more to repeated exposure to antigen than age). The second is the developmental changes associated with involution of the thymus that precedes dysfunction of the T-cell component of acquired immunity, resulting in shifts in peripheral T-cell subsets, with expansion of memory T cells with the impaired proliferative capacity (discussed previously) [30]. Studies show a decrease in telomere length with age in T cells and B cells; however, it is demonstrated that there is no significant change in telomerase activity [31]. The loss of CD28 that is seen in age-related shifts in T-cell subpopulations, however, occurs predominately in the CD8 subset, suggesting repeated exposure to antigen characteristic of chronic illness, and these CD8 memory cells with shortened telomeres accumulate as a result of ineffective apoptosis [30]. The age-related decline in T-cell function is preceded by involution of the thymus gland, with dramatic decline in thymic hormone levels. In addition, changes in bone marrow stem cells are described that are distinct from thymic changes. These changes are believed to result in a shift in the phenotype of circulating T cells, with a decrease in the number of naïve T cells and a relative accumulation of memory T cells. As a result of the combination of thymic involution, repeated antigenic exposure, and alteration in susceptibility to apoptosis (increased for CD4+, decreased for CD8+), thymic and lymphoid tissues become populated by anergic (nonresponsive) memory CD8+ CD28- T cells [7]. Likewise, there are significant age-related changes in the CD4+ subset. CD4+ T cells have decreased, but not absent, levels of CD28, which correlate with delay in reaction time to in vitro stimulation and accumulation of suppressive T regulatory cells

that have low CD4<sup>+</sup> expression with CD25<sup>+</sup> expression [32]. The delay in activation is related to impaired costimulatory pathways and significant postreceptor changes that can be elicited after cell-to-cell interaction of T cells with APC, likely the result of physical changes in the cell membrane lipid rafts and an impaired ability to form efficient immune synapses during cell-to-cell interaction. Surface receptors seem to be organized in structures called lipid rafts, and alteration in cholesterol and sphingolipid composition associated with aging may be associated with impaired polarization of lipid rafts on the T-cell surface, resulting in an impaired ability to interact with APC, with the effect more pronounced on CD4<sup>+</sup> T cells [7]. These membrane changes could be a result of changes in the microenvironment, including the hormonal milieu, exposure to free radicals, and other inflammatory mediators (discussed later). As a result of, and in addition to, abnormal surface signaling, there are altered second messenger functions, including autophosphorylation of Lck and activation of ZAP-70 and linker of activated T cells [33–35], resulting in decreased activation of transcription factors, especially NF- $\kappa$ B that is pivotal in regulation of cytokine production, with the most important finding being a decline in IL-2 and impaired proliferation [20,36,37]. These altered second messenger functions may be related to age-related changes in phosphatases' activity related to shifts in the lipid content of the cells that can be mimicked by cholesterol repletion in young T cells [18,38]. So, with aging, there is an accumulation of dysfunctional, clonally expanded, memory T cells that should be removed from immune tissue but also have impaired apoptosis that prevents the efficient removal [14,17,39]. There is a fine balance between anti- and proapoptotic pathways in T cells, including the Bcl-2 family, and caspases that act either as initiators or executioners (beyond the scope of this review) [40,41]. With aging, altered regulation of apoptosis may be tied to decline in CD28; hence, there is more susceptibility of apoptosis in CD4<sup>+</sup> and less in CD8<sup>+</sup> T cells [30,42–44].

The CD28<sup>–</sup> T cells usually are anergic—they do not respond despite exposure to the antigen that would be expected to trigger responses in the T cell. Chronic disease and external factors, such as malnutrition, increased propensity for autoimmune disorders, or repeated, chronic infection (eg, lifelong cycles of reactivation or suppression and reactivation of cytomegalovirus [CMV] and other herpes viruses), are associated with a shift in cytokine propensity toward a Th2 anti-inflammatory response, as evidenced by an increase in IL-10 production and expansion of the CD28<sup>–</sup> cells [7,8,10,21,23]. The presence of CD28<sup>–</sup> T cells either has direct effects on other parts of immunity or is a marker of impaired immunity. One study of 153 residents of assisted living facilities in Rochester, Minnesota, demonstrated that nearly half of the residents failed to generate an antibody response to any of the trivalent components of influenza vaccine, and this correlated with age and the expansion of CD8<sup>+</sup> CD28<sup>–</sup> T cells [45].

This combination of increase in CD8<sup>+</sup> but decrease in CD4<sup>+</sup> T cells, with impaired proliferative response, is called the immune risk phenotype

(IRP). IRP is described as an increase in CD8+ CD28–CD57+ cells, with a CD4:CD8 ratio of less than 1, decreased mitogen-stimulated proliferative response of T cells, and CMV seropositivity, suggesting chronic CMV antigenic stimulation is causative to the aging changes [46]. IRP was found to be associated with increased IL-6 and, together with the presence of cognitive impairment, predicted 58% of deaths in adults over age 85 in a 4-year longitudinal study in Sweden and is linked to frailty [46,47]. Measurement of C-reactive protein (CRP) may be useful particularly in identifying chronic infections, because CRP synthesis is dependent on IL-6 [48]. Despite the universal changes in T-cell response with age, the relevance is unclear, as impaired proliferative response to specific antigen or a mitogen, even after adjusting for relative sensitivity to mitogen, failed to correlate with impaired antibody response to influenza immunization [22].

#### *Changes in B cells in healthy older adults*

Primary and secondary antibody responses to vaccination are found to be impaired, and whereas much is due to altered T-cell support, age-related changes in B cells seem to have similarities to T-cell changes. B cells from older individuals show impaired activation and proliferation that also could be related to changes in costimulatory molecule expression [10]. In addition, the specificity and affinity of antibodies produced in older adults is lower than in younger populations, and there also is alteration in isotype [10,31,49]. A longitudinal study of young and older adults demonstrated that the proliferative responses to influenza antigen on yearly immunization were lower in older adults, as was the percentage of older adults who had protective antibody titers [22]. In parallel with changes in the T-cell compartment of immune tissue, there seems to be an accumulation of antigen-experienced B cells in the immunologic space that have a shrunken immunoglobulin repertoire that makes less quantity and decreased specificity of antibody to new antigen [50]. These accumulated memory B cells are CD27+ CD19+, a marker associated with differentiation of B cells to immunoglobulin-producing cells, and as in the T-cell compartment, also may have impaired apoptosis [51–53]. At the same time, as a result of alteration in self-antigens due to oxidative damage and glycation, there is an increase in autoantibody production.

#### **The impact of chronic illness on adaptive immune function in the aged**

Despite nearly 90% involution of the T-cell generating thymus by age 40, true opportunistic infections are not seen among older adult patients, even those who have significant chronic disease. This suggests that there likely is compensation for lost immunologic tissue of the thymus gland. Typical bacterial infections (ie, pneumonia, urinary tract, and skin and soft tissue infections), however, are a common problem in older adults. Other age-

related infections include viral infections (ie, reactivation of *Herpes zoster*) and significantly increased morbidity and mortality associated with influenza virus and infections that are related to microbial colonization of *Clostridium difficile* or methicillin-resistant *Staphylococcus aureus* in severely ill individuals treated with antibiotics [31]. In addition, changes in immunity create difficulty in detecting active (primary and reactivation) and inactive tuberculosis. Response to vaccination, which requires intact cell-mediated immunity to drive the humoral response, clearly is diminished in many different older adult populations and in aged laboratory animals. Underlying chronic illness increases the risk for influenza infection dramatically and impairs the response to vaccination. The presence of one or two chronic illnesses (such as emphysema, diabetes, or chronic renal insufficiency) is associated with a 40- to 150-fold increase in the incidence rate for influenza pneumonia [10]. Whether or not chronic illness, medication, or other related external conditions compromise immune competence further is not elucidated definitively but is likely. One study on vaccine response in nursing home residents demonstrated that only 50% of residents had an adequate response (ie, a fourfold increase in antibody titers), but the response to vaccination failed to correlate with nutritional status or dehydroepiandrosterone levels [10]. Another study in a nursing home setting reported that although only 36% of 137 vaccinated residents demonstrated a rise in antibody titer, there was no correlation with age, body mass index, or functional status, as measured by the Barthel index [54]. Another study of 154 individuals found that nonresponders to influenza vaccine were characterized by higher levels of anti-CMV IgG, a higher percentage of CD57+ CD28- T cells (exposure to CMV), and increased serum levels of TNF- $\alpha$  and IL-6 [55]. Understanding how age-related inflammatory diseases and altered immunity resulting from chronic infections will be extremely difficult to unravel as it represents a chicken-versus-the-egg story.

Another example of how chronic illness impairs immunity further is that aging and impaired renal function reduce the response to hepatitis B vaccine in renal failure patients. Findings showed that 86% of patients who had creatinine at or below 4 mg/dL had a protective antibody titer after hepatitis B immunization, in comparison to only 37% of individuals who had a serum creatinine above 4 mg/dL. Likewise, age independently was inversely associated with antibody response. Immunization of patients who have chronic renal insufficiency before serum creatinine exceeds 4 mg/dL, therefore, is recommended [56].

It is known that T cell-dependent immune response declines gradually with age. In a review of more than 200 scientific articles that evaluated healthy older adults who were selected by the SENIEUR protocol [57], the magnitude of decline in T-cell-dependent immune response with age is modest (ie, ~25% decline versus healthy older adults) [23] relative to that of the aging mouse model [8,9]. In contrast, the T-cell-dependent immune response of frail older adults is impaired 2 to 3 times more than that of

healthy older adults of ages comparable to those of frail older adults, [23]. Moreover, the greater impairment in immunity in vulnerable older adults is associated with a decline in induction of proinflammatory IL-12 response and increased anti-inflammatory IL-10 [2,23]. These results suggest that changes in the immune tissue microenvironment may play an important role in age-related decline in T-cell-dependent immune response in humans. This is not unexpected, for the aging mouse model had shown previously that the age-related decline in T-cell-dependent immune response is caused by changes occurring in the immune cells (intrinsic changes) and in immune tissue microenvironment (extrinsic changes) [58]. Also consistent with this is a review that analyzed changes in various physiologic functions with age from 469 studies involving more than 54,000 older adults. The comprehensive review included 43 immunologic studies of 372 individuals. They found that the mean annual rate of decline with age in immune functions is greater than the average rate of decline of all other physiologic functions that were assessed. The investigators concluded that the deterioration in immune function in older adults is the result not only of biologic aging but also of the presence of chronic disease [59]. To address this hypothesis directly, a study analyzed the influence of chronic disease burden on T-cell immunity using the Cumulative Illness Rating Scale (CIRS). CIRS is an instrument that measures disease burden in individuals who have various chronic diseases but no evidence of acute deterioration or infection. T-cell immunity was related inversely to disease burden (increasing CIRS), with impaired proliferation to phytohemagglutinin, increased production of immunoinhibitory IL-10, and trends toward decreased immunoenhancing IL-12 but no correlation with chronologic age between 51 and 95 years [2].

### **Inflammatory mediators and immunity**

The finding that impaired immunity is correlated more with comorbidity than age in older adults suggests that changes in the composition of inflammatory mediators that occur in the immune tissue microenvironment of older adults could play an important role in accelerating the gradual age-related decline in type 1 immune response caused by changes in T cells. The fact that excessive production of inflammation actually could be immunosuppressive is counterintuitive, and the effects of this increase in inflammatory mediators as a part of inflamm-aging on the acute immune response largely has not been studied. Part of this seeming contradiction is that many of the large epidemiologic studies measure serum cytokine levels and fail to address what effect IL-6 (perhaps the most recent studied of the inflammatory mediators) in particular has on acute immunity to infection or vaccination.

Many studies have shown an increase in plasma or serum levels of IL-6, IL-8, IL-10, and TNF- $\alpha$  and a decrease in IL-1 [8,10]. An earlier study defined markers of "inflammation" as albumin less than 3.8 g/dL,

cholesterol less than 170 mg/dL (bottom decile), IL-6 greater than 3.8 pg/mL (top tertile), and CRP greater than 2.65 mg/L (top tertile). The study found a strong association with mortality in subjects who had three or four markers of inflammation, with the adjusted odds ratios for 3- and 7-year mortality 6.6 and 3.2, respectively, compared with those who had no abnormal markers. Subjects who had one or two markers were at more moderate and statistically insignificant increased risk for 3- and 7-year mortality with adjusted odd ratios of 1.5 and 1.3, respectively [60]. Clearly, these markers are nonspecific and have many causes in addition to inflammation. Longitudinal studies suggest that higher circulating levels of IL-6 and other inflammatory mediators are associated with and are predictive of functional disability and increased mortality in older adults who had no functional impairment at entry into these longitudinal studies [61]. An association also exists between physical activity and lower levels of serum IL-6. Moreover, higher serum IL-6 levels are reported in many chronic diseases, with slight (27% to 72%) increase in relative risk for mortality but significant increase in coronary heart disease, stroke, and congestive heart failure in subjects 70 to 79 years of age who do not have evidence of cardiovascular disease at baseline [60,62].

It remains unclear, however, what increased serum IL-6 levels represent. The association with disease more likely is from a hormonal effect of IL-6, secreted by adipose tissue, and mediated by catabolic changes in somatic muscle, rather than on an immunologic basis. Few studies have been done comparing how circulating levels of IL-6 or other markers of inflammation correlate with traditional measures of cell-mediated immunity. One mouse study using a cecal ligation infection model demonstrated that high inflammation, including plasma IL-6 levels, were associated with high mortality during acute infection, but mortality in the chronic phase of the infection was correlated with immunosuppression and very low IL-6 levels [63]. In one study, only one out of 32 patients who had Alzheimer's disease demonstrated a decline in production of IL-6 and TNF- $\alpha$  associated with severe dementia in comparison to IL-6 and TNF- $\alpha$  levels among patients who had mild to moderate dementia [62]. A recent review describes 14 studies that report increases in IL-6 with aging but IL-6 itself is shown inhibitory to mycobacteriostatic activities in macrophages, suggesting a causative role for the increased susceptibility to tuberculosis in advanced age [62]. Chronic illness likely contributes further to dysregulation of immune response. A study comparing IL-2 and IL-6 levels in young adults, healthy older adults, and "almost-healthy" older adults (individuals who do not meet the SENIEUR protocol because of no history of regular exercise or the use of medications for conditions, such as hypertension or osteoarthritis) reported lower levels of IL-2 and higher levels of IL-6 in the "almost-healthy" older adult population [57,64]. Another study assessed the association between prior CMV infection, proinflammatory status, and effectiveness of the anti-influenza vaccination in 154 individuals during the influenza season

and analyzed associations age and/or inclusion by the SENIEUR protocol, with response to the vaccine as determined by antihemagglutinins (HI), TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, corticotropin/cortisol axis, anti-CMV antibodies, and CD28+CD57- lymphocytes. Nonresponders of younger and older ages (not specified) were characterized by having higher levels of anti-CMV IgG and higher percentages of CD57+ CD28- lymphocytes (known to be associated with CMV carrier status) together with increased concentrations of TNF- $\alpha$  and IL-6 and decreased levels of cortisol. Influenza vaccine induced increases in TNF- $\alpha$  and IL-10 in all nonresponders, whereas cortisol increased only in the young. It was concluded that CMV carrier status was eliciting elevated proinflammatory potential that could contribute to unresponsiveness to influenza vaccine [55].

Preliminary studies of acute infections in humans have suggested an association of increases in inflammatory mediators, especially increases in IL-6 and IL-10, with poor outcome, including severe community-acquired pneumonia and Q fever [65,66]. In addition, association of IL-10 polymorphism is found associated with severity of illness in community-acquired pneumonia [67] and persistent increased levels of IL-10 and soluble TNF receptor I 1 week after development of pneumococcal pneumonia correlated with older age [62]. Clearance of inflammatory mediators in the sputum (IFN- $\gamma$ , TNF- $\alpha$ , IL-6, and IL-8) is reported an early marker of clearance of pulmonary tuberculosis [68]. Finally, there is evidence that proinflammatory cytokines can be modulated with medication, as IL-6 levels were attenuated in severe pneumonia requiring mechanical ventilation by the addition of glucocorticoids, and IL-6 and CRP were reduced by the addition of aspirin in subjects who had chronic stable angina [69,70].

The relationship between aging, inflammatory mediators, response to infection, and the progression of chronic inflammatory diseases is important but complicated. There likely is a final common pathway interaction of these factors that alters the microenvironment of an acute response to infection that, together with accumulation of anergic/nonresponse T and B cells, results in crossing the threshold of host resistance, resulting in the marked increase in common infections, susceptibility to epidemics, and the poor vaccine response in chronically ill, older adults. Despite these challenges, there are many opportunities to intervene.

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