The Gracefully Aging Immune System

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Prolonged life expectancy in the 20th century has been one of humankind’s greatest triumphs. However, the substantial increase in the human life span has ushered in a new concern: healthy aging. Because infectious diseases prominently contribute to morbidity in the particularly vulnerable elderly population, strategies for preventing these diseases would have a clear impact on improving healthy aging. Thus, vaccines and immunization strategies tailored for the elderly population are needed, and vaccines should be developed to take into consideration the peculiar age-induced variations of immune responsiveness. The conference “Ageing and Immunity” recently held in Siena, Italy, has reviewed and discussed several possible causes of immune senescence, as well as strategies for counteracting this waning of immune responsiveness and for restoring immunocompetence. In addition, examples of diseases that should be targeted by vaccination in the senior population were considered.

ENSURING HEALTHY AGING

Improved sanitary conditions and progress in medical care have greatly prolonged human life expectancy, but these changes have led to a rapid aging of the world population, both in developed and developing countries. By the year 2030, the percentage of the population that will be elderly (≥60 years of age) is predicted to be over 25% of the total population, of which 75% will be living in less developed countries. Maintaining this population in healthy conditions therefore represents a major public health challenge.

Aging enhances susceptibility to severe infections, which represent a major cause of morbidity and mortality in the elderly population. Therefore, preventing infectious diseases is one of the necessary steps toward healthy aging. The preventive strategies (vaccines) that are currently used and are effective in controlling many infectious diseases may not be suited for treating the elderly population because the aged immune system does not react with the same rules as that of a younger adult. To this end, a thorough investigation of the features of aging immune reactivity will be necessary, both in healthy and pathological or frailty conditions, so as to custom design vaccination and therapeutic strategies that can be maximally effective without increasing side effects. Thus, inasmuch as aging represents one of the main health problems of the 21st century, vaccines specifically directed to the elderly become a public health priority both in developed and developing countries.

AN AGING IMMUNE SYSTEM

The second conference on “Ageing and Immunity,” held in Siena, Italy, 22 to 24 April 2012, was organized by Fondation Mérites to review the progress made in the last 2.5 years after the previous 2009 workshop (1). A series of critical issues has been identified and addressed in order to set a framework for a more thorough understanding of immune senescence and for a targeted approach to ensure healthy aging to the human population.

A new baseline. The metabolic changes of the aging body, including the increased presence of apoptotic cells and of oxidative stress, even in healthy conditions, induce the immune system to change its “quieter” state to a different, often higher level of basal activation. Consequently, the immune reactivity of healthy elderly people is qualitatively and quantitatively different from that of healthy adults. Thus, different “normal” thresholds should be considered in the healthy aging population. By identifying the features of the normal response by the aging immune system, it would then be possible to better identify pathological responses. Progress in understanding the cellular and molecular basis of immune senescence and immune responsiveness in the elderly will allow us to develop a better targeted and effective immunization strategy.

Combating immunological aging. Differences in immune responses in aged individuals often lead to a state of immunological frailty or immunosenescence. Immunological frailty can be addressed through diverse strategies, including preventing infectious diseases (vaccination), ensuring adequate nutrition and physical exercise, and maintaining a high level of intellectual challenge. However, implementation of such measures is a problem of resources and of public health strategies, in particular in less developed countries.

Vaccination in the elderly. There is evidence that vaccination in the elderly delays the transition from so-called “young-old” to “old-old.” However, it remains difficult to identify which age groups within the elderly population should be particularly targeted by vaccination. Reliable and robust biomarkers of vaccine-induced protection in the elderly (correlates of protective immunity) are needed. In addition, immune reactivity of the elderly to vaccination should be improved without triggering potentially adverse inflammatory reactions. By integrating the progress made in understanding the molecular basis of immune senescence with vaccinology and gerontology, current scientific knowledge could be translated into the development of vaccination strategies for the poorest countries.

THE BURDEN OF INFECTIOUS DISEASES IN THE ELDERLY

Infectious diseases are a major cause of morbidity and mortality in the elderly population. There are many reasons for this increased susceptibility. Inadequate response to infectious agents is not only caused by immune senescence but can be caused by malnutrition, which has a powerful immunosuppressive effect, by less effective barriers (such as more permeable skin, less mucous and saliva, and less active muscles), by concomitant chronic medical conditions, and by the increased use of medications. Malnutrition is a critical concern in the elderly population in developing countries, such as in Africa, where between 10 and 50% of the aged population has a body mass index (BMI) of <20, as opposed to 6% in Switzerland. The risk of death from infectious disease in people over 70 years of age has been calculated to be minimal with a BMI of 25 to 30; however, this risk increases greatly in people over 70 with a BMI of <20 (2). Within developed countries, infections variably affect the elderly depending on the
environment (for example, nursing homes versus hospitals versus communities, season, or sex). In these populations, infections mainly consist of urinary and respiratory tract infections and pneumonia, pertussis, Helicobacter pylori-dependent gastritis, and reactivation of varicella zoster virus (VZV). Also important are periodontal infections; incidence is increased by the age-impaired salivation and oral functions. Periodontal infections can contribute to systemic cardiovascular and respiratory diseases (3) and, by causing tooth loss, contribute to inducing malnutrition. These interrelated causes contribute to defects at every level of the immune response and thus to an increase in infectious disease burden in the elderly.

**DIFFERENCE IN THE IMMUNE RESPONSE IN THE ELDERLY**

**Innate immunity and inflammation.** The relatively nonspecific innate immune response, and its effector cells such as macrophages, polymorphonuclear leukocytes, and natural killer cells, is generally considered less prone to immune senescence than are mechanisms and effectors of adaptive immunity. However, most of the data supporting this perception have been generated in mice. By evaluating the available data on innate responses in aging humans, it is evident that several innate activities are also affected by old age; these differences result in a decreased capacity in immediate defense against pathogens and in reduced ability to initiate adaptive immunity, together with enhanced inflammatory reactions (4, 5). For example, polymorphonuclear leukocytes show age-related defects (5–7). In particular, response to chemotactic stimuli and infiltration of inflamed tissues is impaired, as well as production of reactive oxygen species and intracellular killing capacity, with slower and less efficient resolution of the infectious event. The changes in innate responses appear to be in part due to intrinsic “senescence” that compromises the activation pathways, but they are in large part due to the changes occurring in the aging body and to the concurring infectious diseases.

**Antigen-presenting cells.** Responsiveness to vaccines depends among other factors on the capacity of antigen-presenting cells, such as dendritic cells (DCs), to take up and adequately present the vaccine to T cells and to trigger a protective response. Recent evidence shows that both myeloid (mDC) and plasmacytoid (pDC) DC populations are constitutively activated (in terms of intracellular cytokine production) in older (≥65 years of age), compared with young (age 21 to 30 years) adults (8). The constitutively inflammatory microenvironment (inflammaging) causes an increased “basal” level of innate/inflammatory activation and may lead to enhanced inflammation-dependent tissue damage during infections (9). However, it may also cause an increased activation of down-regulatory mechanisms that may result in inadequate response to infectious challenges. The role of DCs in inducing immunological tolerance also appears to be affected in old age, with consequent increase of the incidence of autoimmune diseases [reviewed in (10)].

Toll-like receptor (TLR) stimulation, which strongly activates DCs from younger individuals, does not substantially augment cytokine production in old DCs. The decreased in TLR signaling in old age has been proposed to be caused by decreased TLR expression (somewhat controversially) or to unresponsiveness of TLR to challenge [reviewed in (6)]. The latter may be a direct effect of aging (decreased functionality of the signaling pathway) or it could be due to an anti-inflammatory rebound—increased expression of regulatory molecules, such as TIR8/SIGIRR and interleukin 37 (IL-37)—caused by the abundance of endogenous inflammatory stimuli in the aging organism. Recent data suggest that aging can affect the expression of microRNA (miRNA) regulating the activation of the nuclear factor-kB inflammatory pathway in mDCs, contributing to the decreased TLR-dependent DC activation (11). Besides impaired TLR-dependent cytokines, old DCs and old monocytes also show decreased TLR-dependent expression of the costimulatory molecule CD80, two markers associated with protective response to influenza vaccination (8, 12). These data suggest that improving vaccine efficacy in the elderly may need to include strategies for increasing TLR responsiveness in DCs (Fig. 1).

**T cell responses.** The most evident changes in immune mechanisms during aging are those in adaptive immune mechanisms (Fig. 1). Defense against intracellular infections is chiefly mediated by T cells. By examining the different T cell subsets, several defects can be identified that eventually affect adaptive immunity, including the ability of naïve old T cells to activate, proliferate, and differentiate into robust effector cells; the ability of homeostatic mechanisms to maintain and preserve T cell subset balance; and the ability to mount an immune response while dealing with a latent persistent infection [for example, herpesviruses such as cytomegalovirus (CMV)] (13, 14).

Alterations in signaling pathways in aging CD4 T cells are linked to decreased responsiveness to infections and impaired generation of protective immunity to vaccines. For example, T cell receptor (TCR) signaling is altered in old CD4 T cells. Although TCR expression is apparently unaffected, TCR-dependent extracellular signaling–regulated kinase (ERK) phosphorylation is reduced in parallel to a posttranscriptional increase in the phosphatase DUSP6 (which inhibits ERK). The increase of DUSP6 is due to the age-dependent decline in mir181a, which controls DUSP6 expression (15). Reconstitution of mir181a levels, or transcriptional silencing/pharmacological inhibition of DUSP6, markedly improves responsiveness of elderly CD4 T cells, including T helper cell (Th1) differentiation.

In another example, upon activation aged CD4 memory T cells exhibit increased levels of DUSP4, another phosphatase that curtails the nuclear activity of phosphorylated ERK and c-Jun N-terminal kinase (JNK) (16). The increased transcription of DUSP4 is due to lower energy production and increased 5′ adenosine monophosphate–activated protein kinase (AMPK) activity. Silencing of DUSP4 expression in elderly CD4 T cells restores their ability to provide helper activity for B cell differentiation and antibody production (16). The identification of activated negative-feedback loops involving several members of the DUSP family in aged CD4 T cells opens the opportunity that signaling pathways in T cells can be directly targeted so as to optimize vaccine responses in the elderly.

Antiviral protection is mainly mediated by CD8 T cells, another T cell subset that is greatly affected in the elderly. Aging leads to a shift in the CD8 T cell repertoire from naive to highly differentiated CD28–negative effector memory T cells. This shift is accelerated by latent infection with cytomegalovirus. An important issue that needs to be clarified is the mechanism by which the accumulation of end-differentiated effectors occurs, whether by continuous regeneration or impaired elimination (resistance to apoptosis). The latter does not appear to be the case because indeed highly differentiated CD8 effector memory T cells in the old are particularly susceptible to induction of apoptosis but can be rescued from apoptosis by IL-15 (17). Thus, organs such as the bone marrow that produce increased levels of IL-
15 in old age are niches rich in differentiated CD8 effector memory T cells that can compensate for the senescence-dependent impairment of regenerative capacity (18).

T cell immunity is highly dependent on the rapid expansion of effector cells and subsequent cell survival to form long-lived memory T cells. Proliferation is associated with considerable genomic stress and the activation of DNA damage response pathways, which are increasingly compromised with immune aging (19). Best known is telomeric erosion, in part due to a cumulative replicative history, in part due to a decline in repair mechanisms, in particular the expression of telomerase (20). Age-associated telomeric erosion is seen in naïve T cells and more so in CD8 effector cells, in which telomerase expression appears to limit clonal expansion (21). Reduced telomerase expression in naïve CD4 T cells contributes to the accelerated immune aging in rheumatoid arthritis (22). Nontelomeric DNA damage responses also decline with age. End-differentiated CD8 CD28 T cells overexpress miR-24, which down-regulates the expression of histone variant H2AX (17). As a consequence, DNA damage responses to genotoxic stress are impaired in these cells with reduced serine phosphorylation of ataxia telangiectasia mutated (ATM) and p53. Also, naïve CD4 T cells from patients with rheumatoid arthritis have reduced expression of the ATM-MRE complex, which causes increased frequencies of DNA double-strand breaks and impaired p53 activation in T cells from these patients (23). Functional consequences are increased apoptosis susceptibility and a decreased replicative capacity, which dampens T cell responses.

When looking at local T cell responses, such as in mucosal or skin barriers, age-related impaired responsiveness is also evident...
(24). A prototypical T-dependent skin reaction, cutaneous delayed type hypersensitivity (DTH) in response to recall antigens, is strongly decreased in older individuals. However, this is not due to defective T cell functions but rather to inadequate activation of dermal blood vessel endothelial cells that do not allow extravasation of memory T cells and their entry into the skin. This is due to inability of tissue macrophages to release the endothelial cell activator tumor necrosis factor-α (TNF-α) upon challenge. However, isolated cutaneous CD163+ macrophages from old subjects can secrete TNF-α after stimulation with TLR ligands in vitro, indicating that there is no intrinsic macrophage defect. The increased presence in old skin of CD4+Foxp3+ regulatory T cell (Treg cells) (25) is possibly responsible for shifting macrophage functional programming toward M2 (deactivation, anti-inflammation), reducing their capacity to produce TNF-α (26).

**B cell responses.** Impaired humoral responses in older people may be caused in part by a reduced B cell repertoire (Fig. 1) (27). A comparison of the antibody responses to winter vaccination (flu+pneumococcus) of two age groups (<50 and >65 years) has demonstrated that older individuals have reduced serum immunoglobulin M (IgM) and IgA pneumococcal responses, although they have no difference in IgG levels. Repertoire analysis (performed with CDR3 spectratyping) showed a clear change in the repertoire at day 7 after vaccination with a return to the baseline levels at day 28 in younger individuals, whereas these changes were much less evident in older people. In particular, changes in the IgA repertoire were quite substantial, with little amplification at day 7 and no complete return to baseline at day 28. In particular, IgA1 levels were flat in old individuals, although they increased and then decreased in younger people. Overall, the antibody response to the vaccine of the older group comprises antibody subclasses characteristic of T-independent types of responses, as opposed to the younger group (28).

**IMPROVING IMMUNE RESPONSE IN THE ELDERLY**

What can we do for improving the immune reactivity of the elderly, decreasing the infection burden? Physical exercise is strongly recommended. It has been demonstrated that moderate physical exercise greatly improves the immune reactions of elderly people—for instance, by inducing much better protective antibodies after influenza vaccination (29). Moreover, vaccines are one of the most cost-effective health interventions and have proven to be crucial in preventing mortality and morbidity linked to a large number of pathogens. Sustained protection against infectious diseases, not only in childhood but also in adulthood and in older ages, should become a goal of a comprehensive global health program.

Systematic information on infections in the aged populations and data on aging immune responses are needed to guide program development. Although most available data are mostly collected in the high-income countries of the world, the middle- and low-income settings remain largely undocumented. The World Health Organization (WHO), through a joined effort of their aging and life-course (ALC) and immunization departments (IVB/IVR), is developing a research agenda on the use of vaccines and immunization during the life course of an individual, which could eventually result in increased quality of life and substantial savings to the health system. Developing such a research agenda requires combining findings on populations and gerontology with immunology and molecular biology, to generate knowledge that eventually will lead to policy for the vaccination of groups beyond infancy. Data will be collected in populations of different ages and of different areas of the world, so the information generated can lead to global policy. As a first step, WHO has prioritized epidemiology and disease burden, immunological status/immune senescence and related methodology, implementation/programmatic research with selected vaccines, and socioeconomic analysis to assess and compare benefits of different interventions. A global systematic review of disease burden (such as respiratory infections), with emphasis on morbidity throughout the life of an individual has been initiated, and methodology to assess the immunological status (such as T cell status) of a major collection of dried blood samples (several thousand samples) from individuals from all regions of the world and of different ages is being validated. The immediate next step will be to apply the measurement to a representative part of the collection in order to assess the effective resting naïve T cell pool (a marker of aging).

**IMPROVING VACCINE EFFICACY IN THE ELDERLY**

One key strategy to improve immune response in the elderly is vaccination. Strategies to better protect the elderly may include vaccination at younger ages, when the immune system is fully competent. An additional interesting approach might be vaccination against pathogens, such as CMV, which have been implied in the induction of immunosenescence, and/or at improving the durability of immunological memory. The possibility to protect against CMV and to induce long-lasting memory thanks to vaccine by using strong adjuvants may open the way to such approaches (30, 31). A very interesting example of immune senescence due to infection is that of chronically infected HIV-1 patients (32). These precociously suffer of diseases typical of old age (such as cancer or cardiovascular diseases) and show signs of immune senescence (such as inflammation, low tissue CD4 cells, low levels of naïve CD8+ T cells, high levels of CD8’CD28- memory T cells, or a huge increase in CMV-specific cells). Thus, preventive strategies that will be designed for elderly people may apply to HIV patients and vice versa.

**TAILORED VACCINES FOR THE ELDERLY**

One approach to vaccinating elderly populations is to develop new vaccines that target diseases affecting elderly subjects. Several diseases preferentially affect the elderly population. For instance, infection with *Clostridium difficile* (the most frequent cause of nosocomial diarrhea) causes considerable problems in most industrialized countries because available treatments are suboptimal, with the elderly population being at an increased risk of infection–related morbidity and mortality. Any vaccine would need to be tailored to the responsiveness of the aged population that, at variance with younger adults, responds only to toxin A and not to toxin B or their combination (33).

Another approach would be to adapt existing vaccines to the elderly. The suboptimal efficacy of some vaccines in an elderly population may result from the age-related impairment of immune functions, as discussed above, but also to the increase in comorbidities. It is therefore important to redesign existing vaccines in order to improve their efficacy in the aged, immunological frail population. Changes in the vaccination schedule, vaccine dose, route of immunization, as well as combination with an adjuvant or administration of a biological response modifier have been attempted without impressive results (34). In the case of anti-influenza vaccination, the intradermal inoculum appeared to yield a small im-

The mechanisms by which adjuvants rescue immune responsiveness in the elderly are being actively investigated and involve the controlled triggering of local innate/inflammatory events that then inform a potent adaptive immune response. The inflammation-related side effects that are attributed to vaccine adjuvants are the focus of major efforts in safety investigation. The recent cases of narcolepsy in children after flu vaccination with AS03-adjuvanted pandemic influenza vaccine in countries such as Sweden (41), Finland (42), and the United Kingdom (43) never occurred in elderly people or at any age when using other adjuvants, such as MF59. Although the precise causes of the narcolepsy events are not yet fully clarified, it should be noted that a similar incidence of narcolepsy cases have occurred also in China after administration of nonadjuvanted vaccine, and it correlated with the presence of concurring bacterial lung infection (44). A deeper understanding of the mechanisms of adjuvant-induced amplifying effects is required to better tune the development of stronger vaccine responses in immunologically frail elderly individuals.

**NEW TECHNOLOGIES FOR IDENTIFYING SUBTLE IMMUNOLOGICAL CHANGES**

A major issue in further defining and responding to immune senescence is that changes that distinguish normal immune reaction from inadequate responsiveness can be so subtle that their identification may be elusive. New technologies are being developed and becoming available to researchers that provide multiparametric, high-throughput evaluation of dynamic immune cell behavior and allow identification of the slightest anomalies (Fig. 2).

**Proteomic analysis.** An example of a new technology for assessing the cellular protein profiles is mass cytometry (cytometry by time-of-flight mass spectrometry (Cy-TOF)), a recently developed technology in which fluorochromes are replaced with heavy metal isotopes. The quantity and mass of the stable isotopes are detected by means of an inductively coupled plasma mass spectrometer (ICP-MS), which currently allows for the analysis of spectra encompassing 100 isotope channels at an acquisition rate of 500 cells per second. The high resolution of the time-of-flight (TOF) mass analyzer used in the mass cytometer, combined with the intrinsically discrete nature of isotopic masses, allows the perfect discrimination of isotopes.
separated by only one atomic mass unit, with negligible spectral overlap. This very high resolution dramatically increases the number of parameters that can be measured simultaneously per single cell, especially when compared with fluorochrome-tagged reagents. The application of CyTOF to immunophenotyping bone marrow and peripheral blood mononuclear cells of healthy individuals has allowed the simultaneous evaluation of at least 34 distinct parameters at the single-cell level (45, 46). The single-cell data from such studies may represent the reference against which immunological variations in the elderly can be identified. Data analysis with the software SPADE allows visualization of data clustering in a two-dimensional (2D) tree structure (47).

As an example of possible applications of CyTOF-based high-content screening, a 36-parameter study of CD8+ cytotoxic T cells has allowed the quantitative identification of different subsets specific for Epstein-Barr virus, CMV, and influenza, and the simultaneous characterization of their subcellular complexity in terms of plasma membrane markers, intracellular cytokines, and granule components (48). It is reasonable to expect that the possibility of measuring a greatly enhanced number of parameters at the single-cell level, because it is made possible by mass cytometry, will afford new opportunities for improving immunological profiling, diagnosis, and preventive/therapeutic intervention and will allow to evaluate how age and concomitant conditions affect the immunological parameters normally triggered by infections and/or by vaccination.

*Systems biological approaches.* Another new approach for assessing the immunological changes induced by vaccination and their relationship to effective protection relies on systems biology methods. The aim is that of exploiting retrospective information on the mechanisms underlying the efficacy of successful vaccines (that up to now is evaluated retrospectively upon infection) to understand how vaccines trigger protective immunity and, based on efficacy-predicting profiling, implement a new generation of effective-by-design vaccines (49).

The identification of molecular signatures induced rapidly after vaccination, which correlate with and predict the later development of protective immune responses, represents the initial strategy to prospectively determine vaccine efficacy, as for instance has been done in the case of the yellow fever vaccine (50). Such a strategy would be particularly useful when evaluating the efficacy or immunogenicity of untested vaccines, as well as in identifying individuals with suboptimal responses amongst high-risk populations, such as infants or the elderly (51).

The study of a biological system, such as the development of protective immunity, requires a detailed understanding of its constitutive elements but also a holistic understanding of how those elements interact to produce the complex molecular and cellular chain of events that give rise to immune responses. Although available large-scale profiling platforms can capture the state of entire systems, exploiting this information to gain in-depth understanding of the organization of those systems remains a challenge. To simplify the analysis and interpretation of large-scale data sets, sophisticated computational approaches are being developed. The resulting modular analysis frameworks can be used to develop novel tools for the assessment of the immune system in large-scale human immunology studies. As an example, transcriptomic analysis of blood samples from healthy individuals versus patients with a bacterial infection has allowed the identification of signatures not only specific for the disease but also the disease stage/activity and the effect of therapy (52). New high-throughput screening platforms can reduce analysis to minimal blood volumes (100 μL) and, together with the advanced computational approaches of modular analysis, simplify complexity so as to bring about data interpretation and classification rapidly and in a straightforward fashion.

This approach has been used to assess the effects of aging on the B cell repertoire. Changes in the immunoglobulin repertoire and B cell functional responses in elderly people were examined using high-throughput DNA sequencing to sequence the immunoglobulin heavy chain V(D)J rearrangements. Methodologically, the most complete and reliable results were obtained with the combined use of both genomic DNA and cDNA libraries (run in duplicate) as template, gene-specific primers, and 454 sequencing (more expensive but providing 500 base pair) that can capture the entire V(D)J in one read (53). Clonal expansion (as induced by influenza vaccination in adult subjects) does not influence the repertoire diversity. A study with 70 healthy subjects 1 to 88 years old was performed to assess clonal expansion upon influenza vaccination. Some participants were sampled twice 1 year apart. Sequence analysis shows little age-related change in V, D, J segment usage, V-D and D-J junction properties, or complementary determining region 3 (CDR3) features during adult life. Hypermutation frequency of the V genes in the total repertoire was also comparable between adult subjects of various ages. Clonal expansion analysis, however, revealed that limited numbers of large expanded clones are more frequently observed in elderly people than in younger adults. In addition, the large clones in elderly people distinguish themselves from those in younger people in that they appear to persist over time. How this behavior of elderly B cells in response to challenge correlates with chronic viral infection status remains to be elucidated.

T cell responses also depend on the available repertoire of naïve and memory T cells. T cell generation in the thymus dramatically decreases with age, giving rise to the notion that the repertoire contracts with age. Although recent studies have questioned the role of the thymus to maintain T cell homeostasis (54, 55), increasing oligoclonality is found for CD8 T cells (56–58), and an abrupt repertoire contraction has been described for CD4 T cells with older age (59). More detailed studies using massive parallel sequencing of T cell receptor genes are currently underway. These studies will have to be supplemented by functional studies quantifying the precursor pool in the human naïve and memory T cell repertoires that is specific for a given antigen. One of the newly developed high-throughput cellular screening (HTCS) approaches is based on the polyclonal stimulation and expansion of limiting the number of naïve T cells in multiple replicate cultures with antigen and autologous antigen-presenting cells in order to generate libraries that can be repeatedly screened for the presence of antigen-specific T cells. As proof of principle, two antigens are used, represented by two different types of microbes, *Candida albicans* and *Staphylococcus aureus*. Validation is provided by parallel assessment of T cell repertoire ex vivo from infected patients. Multiparametric flow cytometry analysis allows detecting the phenotype of T cells and their capacity to produce cytokines during time from priming (in vitro or in vivo). The HTCS approach allowed the clear distinction of T<sub>17</sub> memory subsets induced by the two microbes, based on different capacity to produce IL-10 (primed by *S. aureus*)

versus interferon-γ (primed by C. albicans) (60). Memory T cell libraries can be used for analyzing the antigen-specific repertoire, and their cross-reactivity with other antigens (such as C. albicans–specific memory T cells can recognize other Candida strains and also loosely related Saccharomyces cerevisiae). Methodologically, the 14-parameter flow cytometric analysis of T cell phenotypes is efficiently evaluated with the SPADE software that allows the clustering of data into a 2D tree representation of qualitative and quantitative subpopulations (47). This HTCS approach can be used to interrogate the T cell repertoires of elderly people in order to assess their memory status and the reactivity to vaccinations.

SPECIFIC PROBLEMS
Influenza. One specific concern is the limited efficacy of influenza vaccines in the elderly. In this population, low efficacy has been linked to reduced serum antibody responses. To fully understand the mechanisms of decreased antibody production with age, valid and reliable models are needed: Mouse models cannot be used because they do not accurately reproduce the human situation. In humans, investigating the mechanisms underlying the age-dependent fading of immunity should be studied with model antigens to which individuals have never been previously exposed in their life because the previous repeated exposure to the virus can substantially and variably affect the response levels of different individuals. Thus, influenza antigens do not represent the most ideal antigens to study immune responses requisitely triggered by vaccination. Despite these limitations, considerable information can be collected by examining the functions of plasmablasts (B cells in the process of becoming antibody-producing plasma cells) in cohorts of adults versus elderly people upon vaccination with the influenza vaccines (such as the inactivated 2009 seasonal TIV influenza vaccine or the 2009 monovalent pandemic H1N1 vaccine). By measuring different parameters (the numbers of vaccine-specific plasmablasts, the quantity of polyclonal antibodies secreted by plasmablasts, and the specificity and affinity/avidity of the secreted antibodies) (61), it turns out that elderly people develop a lower number of flu-specific plasmablasts, which produce the same amount of antibodies as young cells, with normal affinity/avidity against variant influenza strains. It is noticeable that the antibodies induced by seasonal flu vaccination in elderly people do strongly recognize the pandemic H1N1 virus, whereas those of young people do not (62). Thus, elderly subjects appear to have a reduced capacity of B cell amplification, and their memory B cell repertoire is shaped by the age-associated influenza exposure history, generating a different response pattern.

Pneumococcal disease. The pneumococcal disease nowadays is restricted to elderly people and risk groups (for example, smokers), with an overall case fatality rate of ~12%. Pneumococcal polysaccharide vaccines have been used for a long time to prevent pneumococcal diseases in the elderly and in risk groups. Their efficacy and effectiveness has always been controversial. There is substantial agreement that the presently used 23-valent polysaccharide vaccine is 50 to 80% effective for the prevention of invasive pneumococcal diseases in healthy adult subjects, but it is not effective in the elderly because it does not prevent the disease nor the increased mortality due to the disease. Recently, the 13-valent conjugate pneumococcal vaccine has been licensed by the European Medicines Agency for use in subjects aged ≥50 years, with indication against invasive pneumococcal diseases, based on comparative immunogenicity data with the polysaccharide vaccine. Immunogenicity was shown to be always noninferior, and in most instances superior to that of the polysaccharide vaccine for the common serotypes. Effectiveness data for the conjugate vaccine are not yet available but are probably awaited by 2013 from a big study of effectiveness on community-acquired pneumonia presently underway in the Netherlands. Public health decisions will have to be made in the near future about the best strategy of use for the two vaccines, that will have to take into account the current open questions on immunological, effectiveness, ethical, organizational, and communication issues (63).

Varicella zoster infections. Herpes zoster is caused by reactivation of latent VZV. T cell memory and consequent cell-mediated immunity to VZV (VZV-CMI) prevents latent VZV in sensory neurons from reactivating. VZV-CMI declines greatly with aging, and reactivation occurs more frequently and with more painful outcomes. Specifically, there is a substantial loss of VZV-specific CD4+ early effectors and CD4+ effector memory cells and lower CD8+ early effectors. A vaccine against VZV is available and strongly recommended for elderly people. The vaccine is able to restore VZV-CMI, although its efficiency is a function of the age of the vaccinee, and wanes with time (64, 65). To improve efficacy, several attempts are underway. Increasing the virus dose in the vaccine and repeated administrations do not yield any appreciable improvement. Studies with adjuvants and a trial with an adjuvanted recombinant glycoprotein vaccine are underway.

CONCLUDING THOUGHTS
The aging of the population worldwide imposes new agendas in the priorities for research priorities in general and in the field of vaccination in particular. Vaccines have been considered up to recently as particular to the pediatric age. However, although young children remain an ideal target for vaccination, diseases that will hit at older ages are gaining increasing importance as the composition of the population changes. (66). In this respect, prevention of diseases that particularly affect older age groups acquires a higher and higher profile and needs dedicated answers. Despite the impressive progress experienced in the past decade in the field of immunology, molecular biology, and systems biology, a great body of work still remains to be done to apply this progress in scientific knowledge to the field of aging and of immunosenescence in particular (67).

REFERENCES AND NOTES


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