

# Understanding immunosenescence to improve responses to vaccines

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In the older adult, the benefits of vaccination to prevent infectious disease are limited, mainly because of the adaptive immune system's inability to generate protective immunity. The age-dependent decrease in immunological competence, often referred to as 'immunosenescence', results from the progressive deterioration of innate and adaptive immune responses. Most insights into mechanisms of immunological aging have been derived from studies of mouse models. In this Review, we explore how well such models are applicable to understanding the aging process throughout the 80–100 years of human life and discuss recent advances in identifying and characterizing the mechanisms that underlie age-associated defective adaptive immunity in humans.

The immune system undergoes profound transformations with age, and response patterns to immunological challenges are therefore highly age dependent. Changes that occur in humans after the age of 50 years have received particular attention because of their clinical impact. Such changes have been globally called 'immunosenescence', which is a categorical label and does not imply a functional designation and certainly does not imply a mechanistic designation. The most widely appreciated consequence of advanced age is diminished effectiveness of the immune system<sup>1</sup>. However, immunosenescence is multifaceted and also includes an enhanced susceptibility to autoimmunity that is conceptually difficult to reconcile with the impaired responsiveness of the adaptive immune system<sup>2,3</sup>, as well as constitutive low-grade inflammation that may contribute to a plethora of degenerative diseases, including cardiovascular disease, neurodegenerative syndromes and age-specific ailments such as frailty<sup>4–6</sup> (**Box 1**). Mechanistically, immunosenescence is explained only partially by organismal and cellular senescence. Equally important are physiological differentiation programs, such as the acquisition of memory-like features by naive T cells and the cumulative history of antigen exposure, as well as the infectious load and the dealings with chronic or latent pathogens and adaptations to environmental stressors of the aging host.

Prevention of or compensation for age-related immunological defects is at the core of healthy aging, as the immune system is involved not only in controlling infections and malignancies but also in tissue homeostasis and repair<sup>7–9</sup>. Given the accelerating trends in population aging, healthy aging is not only a question of individual well-being but also an essential objective for maintaining the prosperity and political stability of the world community.

The elderly population increases at different speeds in different countries, but the trend is global and involves economically advanced countries as well as developing countries. Demographic shifts are now most pronounced in Eastern Asia and some European countries in which elderly people already do or will soon outnumber children and the number of people who are actively working will shrink. In the future, 75% of elderly people will be living in less-developed countries that lack efficient social networks and support systems. Preventing or ameliorating infectious diseases in the aging population has therefore been identified as a public health priority by the World Health Organization through a joint effort of their programs on aging and life-course and on immunization<sup>10</sup>.

## Vaccination of the elderly: a work in progress

Vaccination to prevent childhood diseases is one of the most successful and cost-effective interventions in medicine and has completely reshaped the landscape of infectious disease in children and young adults. As infections are a major cause of morbidity and mortality in the elderly, vaccination seems to be the optimal tool for promoting healthy aging<sup>11</sup>. Four vaccines are now recommended for people over 60 years of age to protect them from influenza, pneumococcal, tetanus and pertussis infections and to prevent reactivation of herpes zoster. All four target antigens for which immunological memory already exists. Three induce, at least in part, recall responses, whereas the pneumococcal vaccine now in use is a polysaccharide vaccine that induces a mainly T cell-independent B cell response. Of the four vaccines, only the Tdap vaccine ('tetanus toxoid, reduced diphtheria toxoid and acellular pertussis') gives a satisfactory but diminished protective antibody response in the elderly compared with that in young adults<sup>12</sup>. In contrast, vaccines against influenza or pneumococcal disease do not induce protective immunity in a large proportion of the elderly population but seem to be able to mitigate disease to some degree<sup>13,14</sup>. Similarly, vaccination with the live vaccine against varicella (herpes) zoster virus (VZV) prevents reactivation of herpes zoster only partially or attenuates the severity of post-herpetic neuralgia<sup>15,16</sup>. Primary immune responses are compromised as much or maybe even

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### Box 1 Cardinal features of immunosenescence

- Impaired ability to respond to new antigens
- Unsustained memory responses
- Greater propensity for autoimmune responses
- Lingering, low-grade inflammation

more in the elderly than in the young adult, as illustrated by the poor response to the vaccine against hepatitis B<sup>17</sup>, the side effects of vaccination against yellow fever or the disease severity after infection with West Nile virus or of severe acute respiratory syndrome. More than five decades of experience and the 2009 H1N1 influenza virus pandemic have provided an unparalleled understanding of vaccination against influenza virus<sup>18</sup>. Although most observational studies have reported a small increase in titers of hemagglutination-inhibiting antibodies and mitigation of disease severity, some epidemiological studies have questioned the benefit of the annual vaccination against influenza virus<sup>19,20</sup>. Attempts at improving vaccine outcomes have included the use of adjuvant, higher vaccine doses or booster vaccination, but such strategies have been of limited benefit<sup>21–23</sup>. The live vaccine against influenza virus was not efficacious and is therefore not approved for use in people over 50 years of age<sup>24</sup>. Better understanding of the mechanisms of immunological aging is indispensable for optimal targeting of age-related defects and restoration of responses to vaccines such that that vaccination is becoming an effective tool for promoting healthy aging. Although effective responses to vaccination depend on the cooperative action of the innate and adaptive immune systems, both of which are subject to aging, this Review will focus mainly on the adaptive arm. We will first discuss the effect of aging on the biology of T cells and B cells in mouse models and then discuss similarities and differences in the aging of the immune system in mice and humans.

#### Loss of regenerative capacity in aging mice

Basic principles of immunological aging have been widely studied with mouse models, as mice are the preferred system for immunological studies and a vast array of tools are available<sup>25</sup>. Early studies described age-acquired defects in antibody responses, in particular germinal center function and somatic hypermutation, and provided evidence that the adaptive immune system deteriorates with age<sup>26,27</sup>. One obvious culprit is the substantial loss of naive T cells during aging that, together with the recognition of thymic involution, has led to the paradigm that decreasing regenerative hematopoietic potential is at the core of immunological aging<sup>28</sup>. Indeed, the maintenance of a naive T cell system in the adult mouse is entirely dependent on its ongoing but vastly diminished thymic activity<sup>29</sup>. Loss in regenerative capacity is predicted to account for the loss of naive T cells and also for the contraction in T cell antigen receptor (TCR) diversity<sup>30</sup>. As a result, considerable effort has been focused on understanding the mechanisms of thymic involution and on identifying means of thymic rejuvenation, the subject of several excellent reviews<sup>31–33</sup>. Although diminishing regenerative capacity is greatest for the T cell system, other hematopoietic lineages are affected as well. Age is associated with cell-intrinsic changes in the hematopoietic stem cell (HSC) pool, foremost in their differentiation potential. HSCs committed to the myeloid lineage increasingly outnumber lymphoid-prone HSCs during aging<sup>34,35</sup>. The 'preferential' survival and population expansion of HSCs, DNA damage and defective repair or epigenetic reprogramming, as well as extrinsic factors such

as the cytokine environment, contribute to this increasing imbalance<sup>36</sup>. The frequency of lymphoid-committed HSCs is rate-limiting for the generation of B cells, as diminishing the HSC pool in the young mouse reproduces the subset distribution of B cells characteristic of old mice<sup>37,38</sup>. In addition, age-associated changes in the microenvironment and the regulation of developmental checkpoints result in quantitative and qualitative changes in the generation of B cells<sup>39</sup>. As a consequence, regenerative B cell capacity in old mice is considerably diminished, to as little as 10% that of young mice, and the naive B cell compartment is smaller and less diverse in old mice. In contrast to T cells and B cells, the population of plasmacytoid and myeloid dendritic cells (DCs) essential for adaptive immune responses does not seem to decrease, consistent with the finding that the generation of myeloid cells is favored in the aging host. Available studies have the caveat that the number of DCs is tissue dependent, and most studies of the mouse have focused on DC function rather than DC numbers<sup>40</sup>.

#### T cell- and B cell-intrinsic defects in mouse immunological aging

The availability of mice with transgenic expression of TCRs has made it possible to study age-related defects in the antigen-specific responses of naive and memory T cells. The general consensus is that naive T cells are increasingly dysfunctional with aging, whereas the function of memory cell populations is preserved<sup>25,41,42</sup>. *In vitro* studies have mapped defects to the formation of T cell synapses and early TCR signaling events<sup>43</sup>. The defects have been attributed to changes in the glycosylation of cell-surface molecules or in physicochemical membrane properties; however, the exact mechanisms and in particular the relationship to aging have remained elusive. Diminished production of interleukin 2 after T cell activation has been identified as the single most important consequence, and indeed the addition of exogenous interleukin 2 'rescues' many of the age-related defects in T cell activation<sup>44</sup>. T cell defects can also be improved by the addition of proinflammatory cytokines or ligands of Toll-like receptors, which provides support for the present approach in vaccinology for overcoming poor responses to vaccination through the use of an optimal adjuvant<sup>45</sup>.

Several mouse models of infectious disease have supported the idea that immunological aging is physiologically relevant to the generation of insufficient primary T cell responses. Old mice succumb more readily to infection with West Nile virus or *Listeria monocytogenes* than do young mice<sup>46</sup>. Factors that probably contribute to the greater mortality are the lower abundance and TCR diversity of CD8<sup>+</sup> T cells and their diminished ability to clonally expand, as well as a defect in generating effector CD8<sup>+</sup> T cells that are polyfunctional and produce large amounts of cytokines on a per-cell basis<sup>46</sup>. In contrast, T cell memory responses, once established in the young mouse, are less affected by age, and recall responses are generally intact<sup>42</sup>.

Although the environment of the aged host cannot be ignored, many such age-related deficiencies have been shown to be T cell intrinsic<sup>47</sup>. The response to immunization has been studied in adoptive-transfer experiments in which young and old mice deficient in the coreceptor CD4 were reconstituted with young or old naive CD4<sup>+</sup> T cells with transgenic TCR expression<sup>48</sup>. The resultant immunological defects correlated with the age of the cell, not with the age of the host, and closely resembled findings obtained *in vitro*. Similarly, adoptive-transfer studies of young and old CD8<sup>+</sup> T cells have linked the age of the CD8<sup>+</sup> T cell population rather than the age of the host to such defects<sup>47</sup>.

The mechanisms underlying the 'preferential' dysfunction of naive cells are difficult to pinpoint. Although decreasing regenerative potential

affects the naive compartment more than it affects the memory compartment, decreasing absolute numbers of cells are obviously not the only cause. Peripheral selection that influences the homeostatic proliferation and/or survival of CD8<sup>+</sup> T cells can skew and possibly contract the TCR repertoire<sup>49</sup>. The ‘preferential’ occurrence of intrinsic defects in naive cells, such as defective signaling and loss of replication and differentiation potential, are difficult to explain, as naive T cells have lower homeostatic turnover rates *in vivo* than do memory cells and are therefore less susceptible to replicative senescence. Studies reporting an increase in the longevity of naive CD4<sup>+</sup> T cells with age due to a decrease in expression of the proapoptotic molecule Bim have led to the hypothesis that this longer lifespan predisposes the cells to accumulate defects<sup>25</sup>. Naive T cells can also acquire a ‘semi-memory’ phenotype with age, which indicates that they have entered differentiation pathways. Expression of molecules usually associated with chronic stimulation and exhaustion (such as PD-1 and LAG-3) has even been observed in aged naive T cells<sup>47</sup>.

Cell-intrinsic defects have also been described for mouse B cells, including a diminished ability to undergo class-switch recombination and lower expression of the cytidine deaminase AID and the transcription factor E47 (ref. 39). Some of the T cell defects can be overcome by lineage-specific ablation of p16<sup>INK4a</sup>, an oncoprotein that has been linked to cellular senescence<sup>50</sup>. In contrast, B cell-specific ablation of p16<sup>INK4a</sup> is tumorigenic. Overall, the molecular mechanisms of the cell-intrinsic defects remain poorly understood for mouse T cells and B cells; in particular, it is unclear how they relate to pathways that have been linked to cellular senescence or aberrant cell differentiation.

### Immunological aging: from mice to men

Although the mouse model has been invaluable in identifying immunological principles and experimentally delineating mechanisms, the evolutionary distance between mice and men has introduced differences that do not always allow simple translation of findings obtained with mice to humans<sup>51,52</sup>. Differences exist in innate and adaptive immunity, most obviously in the species-specific regulation and expression of a large number of molecules involved in immunological

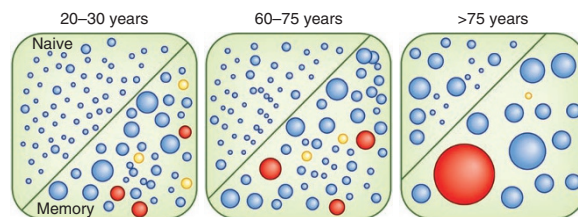
processes<sup>53</sup>. Studies of immunosenescence have added more levels of complexity (Table 1). It cannot be taken for granted that time can be ‘telescoped’; 24 months in a mouse may not be equivalent to 80 years in a human. This point is of particular relevance, as the consequences of cumulative replication constitute an important mechanism of cellular aging. Not only is the replicative history of mouse and human HSCs and lymphocytes over a lifetime very different, the two species also differ considerably in the telomeric length, with telomeres nearly ten times shorter in human cells than in mouse cells. Telomeric erosion and the associated induction of cellular senescence mediated by the tumor suppressor p53 are therefore relevant for human lymphocyte aging but not for that of mice.

Another major difference is that immunological aging is shaped by infections and comorbid conditions that are not truly reflected by most studies of mouse aging<sup>54</sup>. By shaping the repertoire of the immune system, previous infections influence later responses to partially related or completely unrelated antigens<sup>55</sup>. In fact, most of the influenza virus-specific B cell responses in the elderly are based on mutational modifications of the existing immune B cell repertoire rather than the recruitment of new naive B cells<sup>56,57</sup>. Latent infections have a considerable effect on the immunological aging process. It has been estimated that clinically healthy humans are inhabited by about eight to twelve latent viruses, including herpes viruses, polyoma virus, anelloviruses and adeno-associated viruses<sup>58</sup>. Having co-evolved with the host, many of these viruses are highly species specific and are therefore difficult to simulate with animal models. Although they do not cause overt disease (except in immunocompromised people), they shape the immune repertoire and the host environment. Infection with cytomegalovirus (CMV) has received particular attention because CMV-specific responses encompass a large proportion of the entire repertoire in some people and then seem to accelerate immunological aging and depress responses to unrelated vaccines<sup>59–61</sup>. Equally important, many comorbid conditions are in a reciprocal relationship with the immunological aging process in that each accelerates or modifies the other. Telomere shortening, a hallmark of cellular aging, has been observed in several autoimmune diseases<sup>62</sup>. Accelerated immunological aging is possibly best studied

**Table 1 Species-specific characteristics of immunological aging**

	Mouse	Human
Lifespan	2–3 years	75–100 years
Setting of aging studies	Mostly pathogen-free environments	Normal environments
Latent viral infection	A few studies of murine CMV; species comparisons difficult because latent viruses have coevolved with host	Some but not all latent infections seem to accelerate immunological aging
Telomeres	Very long telomeres and constitutive telomerase; telomeres do not signal replicative aging	Telomeres shorter and telomerase suppressed; telomeric erosion in all hematopoietic lineages, which leads to DNA-damage responses and replicative aging
Cellular senescence	Overexpression of p16 <sup>INK4a</sup> in various lineages	Overexpression of p16 <sup>INK4a</sup> in various lineages
Hematopoietic stem cells	Decrease in lymphoid-lineage commitment	Decrease in lymphoid-lineage commitment
B cells	Much less generation of B cells Repertoire contraction Defects in class-switch recombination and somatic hypermutation, possibly due to a lower abundance of AID	Less generation of B cells, extent unknown Repertoire contraction; monoclonal B cell expansion Conflicting findings on B cell-intrinsic defects in class-switch recombination and somatic hypermutation
T cells	Thymic involution Loss in naive T cells  Oligoclonal T cell expansion, mainly of central memory CD8 <sup>+</sup> T cells Impaired calcium influx in naive CD4 <sup>+</sup> T cells after stimulation; defective interleukin 2 production Responses of memory T cells largely intact	Thymic involution T cell generation via peripheral proliferation maintains a diverse naive CD4 <sup>+</sup> T cell compartment up to the eighth decade of life Expansion of terminally differentiated effector CD8 <sup>+</sup> T cell populations, in part due to latent viral infection Impaired TCR sensitivity due to loss of miR-181a and more DUSP6 in naive CD4 <sup>+</sup> T cells Defective responses of memory CD4 <sup>+</sup> or CD8 <sup>+</sup> T cells, inability to provide help to B cells in part due to higher DUSP4 expression Loss of CD27 and CD28, gain in negative regulatory receptors (ILT2, KIRs, KLRG), mostly on effector CD8 <sup>+</sup> T cells
	No equivalent	

**Figure 1** Age and the human CD4<sup>+</sup> T cell repertoire. The TCR repertoire of the naive and memory CD4<sup>+</sup> T cell compartments changes with age (circle size indicates clonal size). By the end of the growth period, a diverse naive repertoire has been established in the young adult (left). Regardless of thymic activity, the naive compartment decreases in size only moderately during the next decades of life while mostly maintaining the overall diversity and distribution of clonal sizes (middle). An abrupt contraction is seen in later life (right). Memory responses to latent infection with CMV and VZV are established in the memory compartment but act very differently during aging. Although the clonal frequency and size of VZV-specific clones diminish with age (yellow circles), T cell clones specific for CMV (red circles) dominate the repertoire in the elderly and contribute to the contraction of diversity in the memory compartment.



in rheumatoid arthritis, in which more DNA damage and the initiation of deranged DNA-repair responses shape T cell homeostasis and function<sup>63</sup>. Studies in the future may therefore take the reverse approach: rather than investigating natural immunological aging in the mouse, researchers will define age-related immunological aberrations in humans and then develop models with which to study those in the mouse. As one example, observations of the effect of infection with CMV have led to several studies of mice demonstrating that chronic infections established early in life influence all-cause mortality and immune responses in later life<sup>64–66</sup>. Such models may now allow longitudinal studies to delineate the mechanisms by which infections accelerate immunological aging.

### Maintenance of the immune repertoire in humans

At first glance, the development of T cells and B cells and the decrease in regenerative capacity seem strikingly similar in mice and men, which might therefore imply easy extrapolation of data from mice to humans. HSCs in humans also undergo a shift to myeloid-lineage commitment at the expense of lymphoid precursors<sup>34</sup>. Data indicate that this shift is accentuated under telomeric stress<sup>36</sup>. In the setting of telomerase deficiency, the transcription factor BATF limits the self-renewal of lymphoid-biased HSCs. The combination of compulsory lymphoid differentiation and diminished self-renewal of lymphoid precursor cells is postulated to lead to loss of pluripotency and dominance of myeloid-lineage HSCs<sup>67</sup>. This mechanism should be particularly operational in humans, in whom telomeric erosion is relevant and is found in HSCs during aging<sup>68</sup>. Indeed, accumulation of DNA damage and higher expression of BATF are features of the aging of human HSCs. As a consequence of HSC failure, the frequency of early or committed B lymphoid progenitor cells (defined by expression of CD45RA, CD38, CD10 and CD19) decreases with age<sup>69</sup>. The diminution of naive B cells, contraction of the immunoglobulin repertoire and emergence of greater frequencies of autoantibodies seem to be at least in part a consequence of decreasing regenerative potential and associated leakage in central selection mechanisms<sup>38,70</sup>. Although this limited capacity is already evident in healthy people and is more evident in frail elderly people<sup>71</sup>, the defect is of increasing concern for those adults who undergo antibody-mediated depletion of B cells, a treatment modality no longer reserved for hematological malignancies but increasingly used as an immunosuppressive treatment for various autoimmune diseases.

As in mice, the human thymus involutes, with the replacement of thymic epithelial cells by fat<sup>72</sup>. Bone marrow-transplantation studies have suggested that thymic function does not sufficiently recover after the age of 40–50 or 50–60 years, depending on the study and the conditioning regimen used to rebuild the repertoire of naive T cells<sup>73,74</sup>. Given the fact that thymic involution is a very controlled process and starts in children as early as 1 year of age, some investigators consider it an evolutionarily selected process of an organ that is

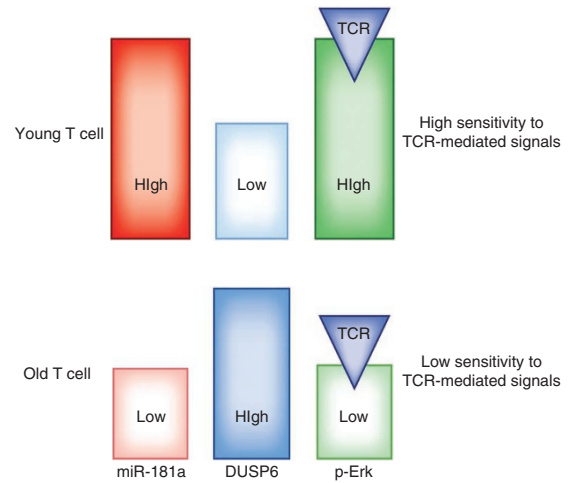
needed during the development of the organism but not during adult life and therefore not a typical example of tissue aging<sup>75</sup>. However, by analogy to mice, thymic involution in humans has also been considered detrimental to immunological function and a central driver of T cell aging<sup>76</sup>. How much thymic T cell generation contributes to T cell homeostasis in the healthy adult has been a matter of debate. The frequency of T cells expressing TCR excision circles is often considered a means of quantifying thymic output<sup>77</sup>, and indeed TCR excision circles in T cells in the peripheral blood decrease exponentially throughout adulthood<sup>78</sup>. However, the age-related loss in TCR excision circles can be explained as a consequence of T cell loss or dilution rather than falling thymic output<sup>79,80</sup>. A study has challenged the paradigm that the thymus is critical in the adult and has provided evidence that the maintenance of naive T cells fundamentally differs in mice and humans<sup>29</sup>. Although even with old age the pool of naive T cells in mice is highly dependent on thymic output, T cell generation in humans throughout adult life is derived mainly from peripheral division. Thymic output seems to be important only throughout the growth stages of human life: early life thymectomy induces accelerated immunological aging; however, it does so only in concert with latent CMV infection<sup>81,82</sup>. That interpretation is consistent with several observations. The turnover of naive T cells (as calculated by the frequency of cells expressing the proliferation marker Ki67) does not change much between the age of 20 and 65 years, which suggests that thymic output is already low during young adulthood with no need for increases in peripheral proliferation with age to compensate for decreasing output<sup>78</sup>. The absolute size of the naive CD4<sup>+</sup> T cell compartment in humans is relatively well maintained into the seventh decade of life<sup>83</sup>. In contrast, the naive CD8<sup>+</sup> T cell compartment shrinks<sup>46</sup>. This difference in attrition is probably not due to a decrease in input, which would affect both lineages (CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells) equally. CD4<sup>+</sup> T cells, although still naive, lose expression of the adhesion molecule CD31 after proliferating<sup>84</sup>. Such CD31<sup>-</sup> naive T cells have a skewed TCR repertoire relative to that of CD31<sup>+</sup> cells, even in young adults, consistent with peripheral selection<sup>85</sup>. However, the global TCR repertoire of naive CD4<sup>+</sup> T cells remains highly diverse (Fig. 1). Only in the eighth decade of life does cell turnover increase, possibly because lymphocyte numbers fall below a critical threshold and the TCR repertoire abruptly contracts<sup>78,86</sup>. Similar results have been obtained with a non-human primate model<sup>87</sup>. According to agent-driven stochastic *in silico* modeling of the human naive CD4<sup>+</sup> T cell repertoire during aging, even complete thymic demise at 20 years of age does not have any influence on repertoire diversity<sup>88</sup>. This model produces repertoire contraction only when multiple inheritable changes in growth behavior are introduced. Restoration of thymic activity in this case neither prevents nor ‘rescues’ the repertoire collapse. In summary, for responses to vaccination in the healthy elderly, thymic activity after the age of 20 years seems to be irrelevant, and defects in T cell homeostasis are due to peripheral selection and growth



and survival patterns. Nevertheless, thymic activity during adulthood or thymic rejuvenation continues to be important in patients who have undergone depletion of their peripheral repertoire during adult life. Obviously, this includes patients infected with human immunodeficiency virus who have begun highly active antiretroviral therapy in late or middle adulthood. An increasing segment of the elderly population has undergone medical intervention, such as chemotherapy or bone marrow transplantation for cancer treatment. Several studies have indicated that low concentrations of TCR excision circles after bone marrow transplantation are predictive of more complications of infections and poor survival<sup>74,89</sup>.

### Aberrant differentiation of naive CD4<sup>+</sup> T cells

Human naive CD4<sup>+</sup> T cells do not develop the severe age-dependent dysfunctionality that is characteristic of mouse T cells. Similar amounts of interleukin 2 are produced when the naive response of T cells from young and elderly people is probed with the following two new antigens: inactivated rabies virus, and recombinant Etr protein of tick-borne encephalitis virus presented by autologous DCs<sup>90</sup>. A high correlation has been found for gene expression by purified naive CD4<sup>+</sup> T cells from young and elderly adults after stimulation of the cells with DCs and superantigen<sup>91</sup>. Under suboptimal stimulation conditions, however, diminished responsiveness becomes apparent. Delineation of the TCR signaling cascade has shown that, in particular, phosphorylation of the kinase Erk is blunted with age<sup>92</sup>. The diminished Erk response is due to an increase in the cytoplasmic concentration of the dual-specificity phosphatase DUSP6 as people age. DUSP6 is constitutively expressed in T cells and has been linked to the attenuation of the threshold at which TCR stimulation is translated into a productive signal<sup>93</sup>. It is one of four phosphatases regulated by the microRNA miR-181a, which has high expression in CD4<sup>+</sup>CD8<sup>+</sup> double-positive thymocytes. The concentration of miR-181a decreases sharply during the transition to CD4<sup>+</sup> or CD8<sup>+</sup> single-positive thymocyte or naive T cell, concurrent with decreasing sensitivity of the TCR in responding to the recognition of autoantigen. The microRNA miR-181a seems to be similarly important in calibrating TCR responses with advancing age (Fig. 2). The concentration of miR-181a in naive CD4<sup>+</sup> T cells decreases with age, and reconstitution of elderly CD4<sup>+</sup> T cells with miR-181a restores responsiveness via repression of DUSP6 translation. The decrease in miR-181a may therefore be an example of antagonistic pleiotropy, as is often discussed in the context of aging, being beneficial in T cell development in the young to prevent autoimmune disease but detrimental in the elderly by setting the TCR threshold too high to respond to exogenous antigen. It is unclear at present whether the mechanisms that regulate miR-181a expression during thymic development and during peripheral T cell aging are shared. However, the finding that miR-181a expression is lower in memory CD4<sup>+</sup> T cells than in naive CD4<sup>+</sup> T cells is consistent with the idea that downregulation is a programmed differentiation process and that aged naive T cells are semi-differentiated, possibly because of recognition of self antigen during homeostatic proliferation<sup>94</sup>. Of note, human memory CD4<sup>+</sup> T cells are less dependent on the Erk pathway than are naive cells and phosphorylate Erk less than naive cells do, as they channel the signal more through the scaffolding molecule hDLg to activate the kinase p38 pathway<sup>95</sup>. But even for memory CD4<sup>+</sup> T cells, reconstitution of miR-181a can improve responses in the elderly. DUSP6 is certainly not the only target of miR-181a that is important for T cell biology. In particular, miR-181a has been found to repress negative regulators of the Notch pathway, and the decrease in miR-181a abundance may therefore dampen Notch signaling<sup>96</sup>.

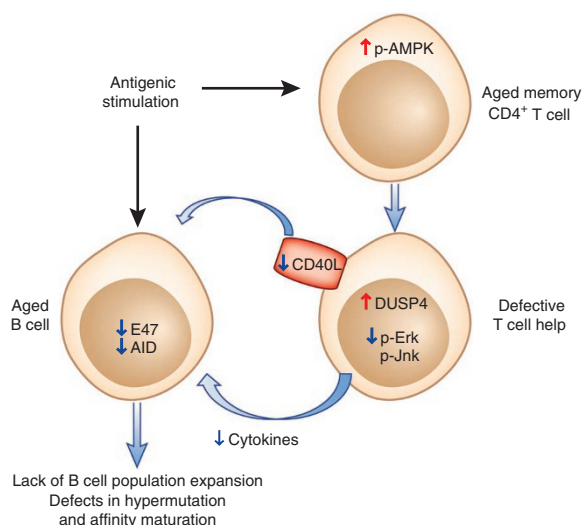


**Figure 2** TCR desensitization in the elderly. One important regulator of TCR activation thresholds is DUSP6, which controls the initial response of Erk and associated positive feedback loops after stimulation of the T cell. DUSP6 expression is regulated by miR-181a. Because of a lower abundance of miR-181a, expression of DUSP6 increases with age, which results in desensitization of the TCR signaling cascade. p-Erk, phosphorylated Erk.

### Intrinsic T cell defects: a consequence of resource allocation

In contrast to results obtained in the mouse studies cited above, studies of human cells have shown that T cell memory established in early age deteriorates during the second half of life. The most obvious example of this is reactivation of latent infection with VZV, which manifests as herpes zoster. Also, the diminished response to the vaccine against influenza virus is due at least in part to a defective T cell memory response. Memory T cells specific to conserved hemagglutinin and neuraminidase epitopes of influenza virus that are not subject to antigenic drift exist in essentially all elderly people. Mechanistic defects seem to be highly dependent on the pathogen. For infection with VZV, a steady decrease in VZV-specific CD4<sup>+</sup> T cells over time has been documented that is boosted only very transiently by vaccination against VZV or reactivation of herpes zoster<sup>16</sup>. In contrast, high frequencies of antigen-specific T cells reactive to CMV persist throughout life (Fig. 1). However, contraction in the diversity of the CMV-specific TCR repertoire has been associated with diminished viral containment, which results in higher antibody titers and increases antigen-specific clonal expansion<sup>97</sup>. The polyfunctionality of antigen-specific T cells seems to be one additional factor for effective T cell responses that diminishes with age with the emergence of more and more monofunctional T cells<sup>46</sup>.

In addition to the quantitative aspects of T cell numbers and repertoire, defects intrinsic to memory T cells develop with age. Interestingly, many of these defects are related to the allocation of resources to proper cellular maintenance, which can be at odds with the ability to respond to external antigenic stimuli with T cell activation and effector function<sup>94</sup>. The past decade has seen great progress in the understanding of how the metabolic pathways in T cells are connected to function<sup>98</sup>. In particular, the activation of T cells and their acquisition of effector function are strictly linked to metabolic activity, which must provide not only sufficient energy in the form of ATP but also precursor metabolites for cellular synthesis. One metabolic master regulator is the AMP-activated protein kinase AMPK, which is activated by a low ratio of ATP to AMP, a greater abundance of reactive oxygen species and, in the context of TCR stimulation,



**Figure 3** Defective T cell–dependent B cell responses in the elderly. Elderly memory CD4<sup>+</sup> T cells respond to stimulation by increasing their expression of DUSP4 because of increased activity of AMPK. DUSP4 curtails sustained activity of phosphorylated Erk (p-Erk) and phosphorylated Jnk (p-Jnk) and impairs the ability of the T cell to provide help to B cells. As a consequence of defective signaling via CD40L, B cell activation and E47 expression are impaired. Defective T cell help coincides with an age-associated intrinsic B cell defect in E47 expression and thus transcription of the gene encoding AID. As a consequence, B cell clonal expansion, immunoglobulin class-switch recombination and hypermutation are impaired.

fluxes in cytoplasmic calcium concentrations. It has been proposed that functional defects in elderly memory CD4<sup>+</sup> T cells are controlled by AMPK<sup>94</sup> (Fig. 3). Studies of the effect of age on the memory function of CD4<sup>+</sup> T cells have identified a negative feedback loop mediated by the dual-specificity phosphatase DUSP4 (ref. 99). This nuclear phosphatase controls nuclear activity of the kinases Erk and Jnk. Its activity peaks 2–4 days after T cell activation, when it is substantially overexpressed in elderly T cells. DUSP4 curtails the expression of the ligand for the costimulatory receptor CD40 (CD40L) and several other functional molecules on activated memory CD4<sup>+</sup> T cells. As a consequence of the greater inducibility of DUSP4 in elderly T cells, their ability to provide help for T cell–dependent B cell responses is severely impaired but can be restored by silencing of *DUSP4*. The upstream mechanism of higher DUSP4 expression is enhanced AMPK activity in aged memory CD4<sup>+</sup> T cells that leads to transcription of the gene encoding the transcription factor Egr-1 and, eventually, DUSP4. This finding raises the interesting possibility that the function of memory CD4<sup>+</sup> T cells in the elderly can be improved by targeting of their metabolic activity.

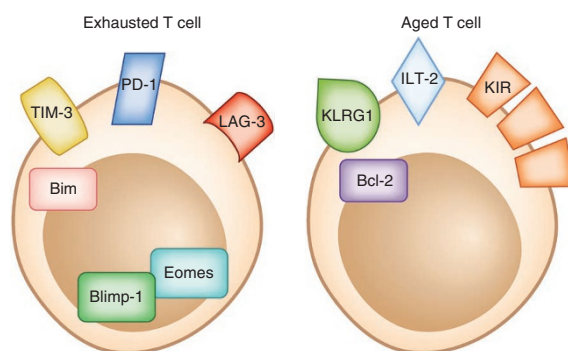
Even in young cells, a considerable amount of cellular resources is committed to maintaining the integrity of the genome; this is increasingly the case with increasing age. Chronic DNA-damage responses are apparent in all hematopoietic lineages in older people, including T cells. In part, these are explained by telomeric dysfunction; an increase in the frequency of double-strand breaks is also found in peripheral T cells during aging<sup>100</sup>. Of note, DNA damage in memory T cells far exceeds that in naive T cells at any age. DNA-damage responses may in part be responsible for the higher expression of p16<sup>INK4a</sup> that is found in human T cells as well as other tissues in the elderly<sup>101</sup>. However, p53-mediated cell-cycle arrest, typical of cellular senescence, is not a consistent feature of elderly T cells. Diminished proliferative activity is generally not permanent and can be over-

come<sup>102</sup>. The proliferative ability of terminally differentiated CD8<sup>+</sup> T cells can be restored by overexpression of the human telomerase reverse transcriptase TERT to increase telomerase activity<sup>103</sup>. The proliferation of naive and memory CD4<sup>+</sup> T cells from elderly people can be improved substantially by the provision of Zn<sup>2+</sup> to upregulate metallothionein expression<sup>91</sup>.

Chronic DNA-damage responses may result from greater genotoxic stress but may also signal inefficient DNA repair. Such a defect certainly is one of the major driving forces in the accelerated immunological aging seen in patients with rheumatoid arthritis. In addition to the diminished telomerase activity similar to that of healthy immunological aging<sup>104</sup>, the expression and function of molecules in the ATM-dependent DNA-repair pathway are diminished, which leads to more nontelomeric DNA damage, a failure of p53 activation and the activation of alternative DNA-repair pathways<sup>100,105</sup>. It will be interesting to determine whether similar mechanisms can also be identified with normal aging.

### Expression patterns of T cell–regulatory receptors

During aging, the expression patterns of T cell–regulatory receptors change considerably (Fig. 4). On balance, the expression of various inhibitory receptors is enhanced, accompanied by a decrease in the expression of members of the costimulatory CD28 family and the tumor-necrosis factor receptor superfamily<sup>106,107</sup>. These changes mostly involve memory CD8<sup>+</sup> T cells. Often most CD8<sup>+</sup> T cells are affected, whereas only very small subsets of memory CD4<sup>+</sup> T cells show such changes in expression. The expression of cell-surface inhibitory receptors is reminiscent of clonal exhaustion, in which chronic stimulation with a high dose of antigen induces high expression of the inhibitory receptors PD-1, LAG-3 and TIM-3 (refs. 108,109). In a mouse model of chronic infection with lymphocytic choriomeningitis virus, *in vivo* blockade of signaling via PD-1 and its ligand restores the production of interferon- $\gamma$ , proliferation and cytotoxic activity of virus-specific CD8<sup>+</sup> T cells and diminishes the viral load. Those findings are paralleled by infection with several human chronic viruses, such as human immunodeficiency virus, hepatitis B virus and hepatitis C virus. Different classes of molecules are expressed in exhausted and aging CD8<sup>+</sup> T cells, and they generally do not segregate together in the same T cell subsets with PD-1, LAG-3 and TIM-3. Such proteins include members of the KIR (killer immunoglobulin-like receptor) and KLR (killer cell



**Figure 4** Age-associated population expansion of terminally differentiated CD28<sup>-</sup> T cells. With increasing age, oligoclonal populations of terminally differentiated effector T cells accumulate; these are frequently specific for latent viruses, in particular CMV. These T cells differ in phenotype, function and survival from the exhausted T cells that develop in response to highly replicating viruses. Blimp-1 and Eomes, transcription factors; Bcl-2, antiapoptotic molecule.

## Box 2 Therapeutic strategies to improve responses to vaccination

- Prevent repertoire contraction
- Broaden memory B cell repertoire during adult life
- ? Treat latent viral infections to prevent repertoire skewing
- Boost miR-181a to increase TCR sensitivity
- Inhibit DUSP6 to increase TCR sensitivity
- Diminish expression of DUSP4 to improve T cell helper function
- Induce zinc-dependent metalloproteins to improve clonal expansion
- Optimize DNA and telomeric repair to sustain proliferative capacity

lectin-like receptor) families, in particular KLRG1 and CD85j of the ILT (immunoglobulin-like transcript) family<sup>107</sup>. Although T cell exhaustion and age-associated expression of negative regulatory receptors seem to be different programs<sup>110</sup>, their functional consequences may be similar and blockade of these receptors may be beneficial in improving antiviral CD8<sup>+</sup> T cell responses. Contrary to that model, preliminary evidence has shown that negative regulatory receptors expressed during aging selectively inhibit proliferation while keeping effector function intact (unpublished observation). They may therefore serve a beneficial function in maintaining a diverse T cell repertoire by limiting the clonal expansion of selected virus-specific clones.

### B cell responses in the elderly

Similar to T cell responses, B cell responses in the elderly are determined by the number and diversity of B cells and potential intrinsic defects<sup>38,111</sup>. The dependence of many B cell responses on T cell help adds another level of complexity. The regenerative capacity of B cells is compromised during aging because of mechanisms very similar to those described for the mouse. As a consequence, older humans have fewer naive B cells. Depending on whether expression of CD19 or CD20 is used to define B cells, total number of B cells is either diminished slightly or not at all because of the population expansion of cells with a classic memory phenotype or less-defined phenotypes<sup>69,70</sup>. Repertoire diversity is compressed, in particular in those people who are frail and not in good health<sup>71</sup>. Several lines of evidence indicate that limitations in the available B cell repertoire impair the antibody response. Through the use of high-throughput parallel sequencing, it has been shown that the lineage structure of the antibody repertoire before vaccination in elderly people is clearly contracted and the mutation load is higher<sup>56</sup>. Responses to the vaccine against influenza in the adult rely strongly on somatic mutation of the memory repertoire rather than recruitment of new antibody sequences. Even the B cell response to the 2009 pandemic H1N1 influenza virus was dominated by antibodies broadly cross-reactive to the hemagglutinin epitopes of multiple influenza strains<sup>57,112</sup>. Enzyme-linked immunospot assays have shown a nearly 90% lower frequency of circulating antibody-secreting cells after vaccination against influenza virus, whereas the functionality of plasmablasts is not impaired<sup>113</sup>. In summary, the data are consistent with the model that the pre-existing repertoire restricts the quantitative response in the elderly. Differences in the quality of the response are less consistent and, where found, may be due to pre-existing hypermutations in prevaccination B cells, which limits the ability to optimize the fit of antibodies to newly arising variants. Alternatively, lower

induction of AID expression in the elderly has been correlated with diminished affinity maturation after vaccination against H1N1 influenza virus and the production of antibodies with lower affinity<sup>114</sup>. Lower AID induction may be an intrinsic defect of elderly B cells, may be caused by defective expression of E47 and may be associated with defective immunoglobulin class-switch recombination in naive B cells or immunoglobulin M-producing memory B cells<sup>115</sup>. The induction of E47 is also regulated by CD40L-mediated signals, which raises the possibility that the lower E47 expression is caused by a T cell defect. Indeed, restoring the helper function of T cells by silencing *DUSP4* in T cells improves E47 induction in B cells<sup>99</sup>. Obviously, both mechanisms are not mutually exclusive but can be synergistic (Fig. 3).

### Vaccination of the elderly: what is next?

With changing demographics around the globe, which has been described as ‘the gray tsunami’, improving responses to vaccination in the elderly is not an option, it is a necessity. Progress over the past decade in optimizing vaccine success has been modest; however, the chances for success are not as dim as sometimes thought. Strategies for creating better vaccines have been modeled mostly on responses to vaccination in the young or have relied on the study of immunological aging in the mouse. Understanding how the immune system ages in humans and identifying molecular pathways that can be targeted to specifically improve the responses to vaccination in the elderly has only just begun (Box 2). Responses to vaccines in the older adult will inevitably depend on available resources in terms of T cells and B cells, yet repertoire maintenance in the human adult seems to be relatively robust and not heavily reliant on the generation of new B cells and T cells. Restoring the generation of precursors to B cells and T cells in the bone marrow and rejuvenating thymic function are challenging objectives and certainly not easily applicable, but these may not be a primary necessity for healthy immunological aging. Exceptions to this are people whose lymphocyte repertoire has been wiped out by medical interventions or by infection with human immunodeficiency virus. Available data from studies of repertoire contraction during infection with CMV and responses to H1N1 influenza virus suggest that a diverse memory T cell and B cell repertoire is as important as maintaining the repertoire of naive cells. In the very old, severe contraction of the repertoire variably occurs and seems to be a consequence of abnormal growth and survival of peripheral cells. As a ‘multi-hit’ model affords the best prediction of repertoire collapse in the very old, identifying and preventing single factors may be sufficient to avert substantial loss in diversity. Higher concentrations of inflammatory cytokines (also called ‘inflammaging’) and tissue aging, which have been reviewed elsewhere<sup>40,116</sup>, certainly contribute to defective responses to vaccination. Cell-intrinsic changes that impair the activation and differentiation of T cells and B cells are rather subtle and may be overcome by temporally limited interventions at the time of vaccination. Negative-feedback loops in signaling and cellular metabolism are emerging as important pathways to understand and potentially modulate the activity of aging T cells.

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1. Weng, N.P. Aging of the immune system: how much can the adaptive immune system adapt? *Immunity* **24**, 495–499 (2006).
2. Goronzy, J.J. & Weyand, C.M. Immune aging and autoimmunity. *Cell Mol. Life Sci.* **69**, 1615–1623 (2012).
3. Goronzy, J.J. & Weyand, C.M. Aging, autoimmunity and arthritis: T-cell senescence and contraction of T-cell repertoire diversity - catalysts of autoimmunity and chronic inflammation. *Arthritis Res. Ther.* **5**, 225–234 (2003).
4. Shaw, A.C., Joshi, S., Greenwood, H., Panda, A. & Lord, J.M. Aging of the innate immune system. *Curr. Opin. Immunol.* **22**, 507–513 (2010).
5. Kanapuru, B. & Ershler, W.B. Inflammation, coagulation, and the pathway to frailty. *Am. J. Med.* **122**, 605–613 (2009).
6. Cavanagh, M.M., Weyand, C.M. & Goronzy, J.J. Chronic inflammation and aging: DNA damage tips the balance. *Curr. Opin. Immunol.* **24**, 488–493 (2012).
7. Boraschi, D. *et al.* Ageing and immunity: addressing immune senescence to ensure healthy ageing. *Vaccine* **28**, 3627–3631 (2010).
8. Boraschi, D. *et al.* Ageing and Immunity. *Sci. Transl. Med.* (in the press).
9. McElhaney, J.E. & Effros, R.B. Immunosenescence: what does it mean to health outcomes in older adults? *Curr. Opin. Immunol.* **21**, 418–424 (2009).
10. Thomas-Crusells, J., McElhaney, J.E. & Aguado, M.T. Report of the ad-hoc consultation on aging and immunization for a future WHO research agenda on life-course immunization. *Vaccine* **30**, 6007–6012 (2012).
11. Chen, W.H. *et al.* Vaccination in the elderly: an immunological perspective. *Trends Immunol.* **30**, 351–359 (2009).
12. Weston, W.M., Friedland, L.R., Wu, X. & Howe, B. Vaccination of adults 65 years of age and older with tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Boostrix®): results of two randomized trials. *Vaccine* **30**, 1721–1728 (2012).
13. Jefferson, T. *et al.* Efficacy and effectiveness of influenza vaccines in elderly people: a systematic review. *Lancet* **366**, 1165–1174 (2005).
14. Nichol, K.L., Nordin, J.D., Nelson, D.B., Moolooly, J.P. & Hak, E. Effectiveness of influenza vaccine in the community-dwelling elderly. *N. Engl. J. Med.* **357**, 1373–1381 (2007).
15. Gagliardi, A.M., Gomes Silva, B.N., Taroni, M.R. & Soares, B.G. Vaccines for preventing herpes zoster in older adults. *Cochrane Database Syst. Rev.* **10**, CD008858 (2012).
16. Levin, M.J. Immune senescence and vaccines to prevent herpes zoster in older persons. *Curr. Opin. Immunol.* **24**, 494–500 (2012).
17. Denis, F. *et al.* Hepatitis-B vaccination in the elderly. *J. Infect. Dis.* **199**, 1019 (1984).
18. Dormitzer, P.R. *et al.* Influenza vaccine immunology. *Immunol. Rev.* **239**, 167–177 (2011).
19. Jackson, M.L. *et al.* Influenza vaccination and risk of community-acquired pneumonia in immunocompetent elderly people: a population-based, nested case-control study. *Lancet* **372**, 398–405 (2008).
20. Wong, K., Campitelli, M.A., Stukel, T.A. & Kwong, J.C. Estimating influenza vaccine effectiveness in community-dwelling elderly patients using the instrumental variable analysis method. *Arch. Intern. Med.* **172**, 484–491 (2012).
21. Couch, R.B. *et al.* Safety and immunogenicity of a high dosage trivalent influenza vaccine among elderly subjects. *Vaccine* **25**, 7656–7663 (2007).
22. Khurana, S. *et al.* MF59 adjuvant enhances diversity and affinity of antibody-mediated immune response to pandemic influenza vaccines. *Sci. Transl. Med.* **3**, 85ra48 (2011).
23. Brown, L.E. The role of adjuvants in vaccines for seasonal and pandemic influenza. *Vaccine* **28**, 8043–8045 (2010).
24. Ambrose, C.S., Luke, C. & Coelingh, K. Current status of live attenuated influenza vaccine in the United States for seasonal and pandemic influenza. *Influenza Other Respi. Viruses* **2**, 193–202 (2008).
25. Haynes, L. & Lefebvre, J.S. Age-related deficiencies in antigen-specific CD4 T cell responses: lessons from mouse models. *Aging Dis.* **2**, 374–381 (2011).
26. Miller, C. & Kelsoe, G. Ig VH hypermutation is absent in the germinal centers of aged mice. *J. Immunol.* **155**, 3377–3384 (1995).
27. Yang, X., Stedra, J. & Cerny, J. Relative contribution of T and B cells to hypermutation and selection of the antibody repertoire in germinal centers of aged mice. *J. Exp. Med.* **183**, 959–970 (1996).
28. Linton, P.J. & Dorshkind, K. Age-related changes in lymphocyte development and function. *Nat. Immunol.* **5**, 133–139 (2004).
29. den Braber, I. *et al.* Maintenance of peripheral naive T cells is sustained by thymus output in mice but not humans. *Immunity* **36**, 288–297 (2012).
- Study providing evidence that thymic function has different importance for adult T cell homeostasis in men and mice.**
30. Blackman, M.A. & Woodland, D.L. The narrowing of the CD8 T cell repertoire in old age. *Curr. Opin. Immunol.* **23**, 537–542 (2011).
31. Berent-Maoz, B., Montecino-Rodriguez, E. & Dorshkind, K. Genetic regulation of thymocyte progenitor aging. *Semin. Immunol.* **24**, 303–308 (2012).
32. Chinn, I.K., Blackburn, C.C., Manley, N.R. & Sempowski, G.D. Changes in primary lymphoid organs with aging. *Semin. Immunol.* **24**, 309–320 (2012).
33. Dixit, V.D. Impact of immune-metabolic interactions on age-related thymic demise and T cell senescence. *Semin. Immunol.* **24**, 321–330 (2012).
34. Pang, W.W. *et al.* Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. *Proc. Natl. Acad. Sci. USA* **108**, 20012–20017 (2011).
35. Beerman, I. *et al.* Functionally distinct hematopoietic stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion. *Proc. Natl. Acad. Sci. USA* **107**, 5465–5470 (2010).
36. Wang, J. *et al.* A differentiation checkpoint limits hematopoietic stem cell self-renewal in response to DNA damage. *Cell* **148**, 1001–1014 (2012).
37. Guerretaz, L.M., Johnson, S.A. & Cambier, J.C. Acquired hematopoietic stem cell defects determine B-cell repertoire changes associated with aging. *Proc. Natl. Acad. Sci. USA* **105**, 11898–11902 (2008).
38. Kogut, I., Scholz, J.L., Cancro, M.P. & Cambier, J.C. B cell maintenance and function in aging. *Semin. Immunol.* **24**, 342–349 (2012).
- Comprehensive review of the influence of age on B cell generation, homeostasis and function.**
39. Cancro, M.P. *et al.* B cells and aging: molecules and mechanisms. *Trends Immunol.* **30**, 313–318 (2009).
40. Solana, R. *et al.* Innate immunosenescence: effect of aging on cells and receptors of the innate immune system in humans. *Semin. Immunol.* **24**, 331–341 (2012).
41. Maue, A.C. *et al.* T-cell immunosenescence: lessons learned from mouse models of aging. *Trends Immunol.* **30**, 301–305 (2009).
42. Lang, A. & Nikolich-Zugich, J. Functional CD8 T cell memory responding to persistent latent infection is maintained for life. *J. Immunol.* **187**, 3759–3768 (2011).
43. Sadighi Akha, A.A. & Miller, R.A. Signal transduction in the aging immune system. *Curr. Opin. Immunol.* **17**, 486–491 (2005).
44. Haynes, L., Linton, P.J., Eaton, S.M., Tonkonogy, S.L. & Swain, S.L. Interleukin 2, but not other common  $\gamma$  chain-binding cytokines, can reverse the defect in generation of CD4 effector T cells from naive T cells of aged mice. *J. Exp. Med.* **190**, 1013–1024 (1999).
45. Coffman, R.L., Sher, A. & Seder, R.A. Vaccine adjuvants: putting innate immunity to work. *Immunity* **33**, 492–503 (2010).
46. Nikolich-Zugich, J., Li, G., Uhrhlaub, J.L., Renkema, K.R. & Smithey, M.J. Age-related changes in CD8 T cell homeostasis and immunity to infection. *Semin. Immunol.* **24**, 356–364 (2012).
- Comprehensive review of how cellular aging and chronic infections shape the CD8+ T cell response with advancing age.**
47. Decman, V. *et al.* Defective CD8 T cell responses in aged mice are due to quantitative and qualitative changes in virus-specific precursors. *J. Immunol.* **188**, 1933–1941 (2012).
48. Haynes, L. & Eaton, S.M. The effect of age on the cognate function of CD4+ T cells. *Immunol. Rev.* **205**, 220–228 (2005).
49. Rudd, B.D. *et al.* Nonrandom attrition of the naive CD8+ T-cell pool with aging governed by T-cell receptor:pMHC interactions. *Proc. Natl. Acad. Sci. USA* **108**, 13694–13699 (2011).
50. Liu, Y. *et al.* Expression of p16<sup>INK4a</sup> prevents cancer and promotes aging in lymphocytes. *Blood* **117**, 3257–3267 (2011).
51. High, K.P., Akbar, A.N. & Nikolich-Zugich, J. Translational research in immune senescence: assessing the relevance of current models. *Semin. Immunol.* **24**, 373–382 (2012).
52. Davis, M.M. Immunology taught by humans. *Sci. Transl. Med.* **4**, 117fs112 (2012).
53. Mestas, J. & Hughes, C.C. Of mice and not men: differences between mouse and human immunology. *J. Immunol.* **172**, 2731–2738 (2004).
54. Nikolich-Zugich, J. Ageing and life-long maintenance of T-cell subsets in the face of latent persistent infections. *Nat. Rev. Immunol.* **8**, 512–522 (2008).
55. Su, L.F., Kidd, B.A., Han, A., Kotzin, J.J. & Davis, M.M. Virus-specific CD4+ memory-phenotype T cells are abundant in unexposed adults. *Immunity* **38**, 373–383 (2013).
56. Jiang, N. *et al.* Lineage structure of the human antibody repertoire in response to influenza vaccination. *Sci. Transl. Med.* **5**, 171ra119 (2013).
- First global analysis of the clonal structure and mutational distribution of the immunoglobulin repertoire in the elderly in the context of vaccination against influenza virus.**
57. Li, G.M. *et al.* Pandemic H1N1 influenza vaccine induces a recall response in humans that favors broadly cross-reactive memory B cells. *Proc. Natl. Acad. Sci. USA* **109**, 9047–9052 (2012).
- Study showing that the response to vaccination against the pandemic H1N1 influenza virus builds on the preexisting B cell memory repertoire.**
58. Virgin, H.W., Wherry, E.J. & Ahmed, R. Redefining chronic viral infection. *Cell* **138**, 30–50 (2009).
59. Pawelec, G., McElhaney, J.E., Aiello, A.E. & Derhovanessian, E. The impact of CMV infection on survival in older humans. *Curr. Opin. Immunol.* **24**, 507–511 (2012).
60. Goronzy, J.J. *et al.* Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals. *J. Virol.* **75**, 12182–12187 (2001).
61. Saurwein-Teissl, M. *et al.* Lack of antibody production following immunization in old age: association with CD8+CD28- T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. *J. Immunol.* **168**, 5893–5899 (2002).
62. Andrews, N.P., Fujii, H., Goronzy, J.J. & Weyand, C.M. Telomeres and immunological diseases of aging. *Gerontology* **56**, 390–403 (2010).
63. Goronzy, J.J., Shao, L. & Weyand, C.M. Immune aging and rheumatoid arthritis. *Rheum. Dis. Clin. North Am.* **36**, 297–310 (2010).



64. Smithey, M.J., Li, G., Venturi, V., Davenport, M.P. & Nikolich-Zugich, J. Lifelong persistent viral infection alters the naive T cell pool, impairing CD8 T cell immunity in late life. *J. Immunol.* **189**, 5356–5366 (2012).
65. Cicin-Sain, L. *et al.* Cytomegalovirus infection impairs immune responses and accentuates T-cell pool changes observed in mice with aging. *PLoS Pathog.* **8**, e1002849 (2012).  
**First study to examine the effect of infection with murine CMV on immunological aging in a mouse model.**
66. Mekker, A. *et al.* Immune senescence: relative contributions of age and cytomegalovirus infection. *PLoS Pathog.* **8**, e1002850 (2012).
67. Mandal, P.K. & Rossi, D.J. DNA-damage-induced differentiation in hematopoietic stem cells. *Cell* **148**, 847–848 (2012).
68. Colmegna, I. *et al.* Defective proliferative capacity and accelerated telomeric loss of hematopoietic progenitor cells in rheumatoid arthritis. *Arthritis Rheum.* **58**, 990–1000 (2008).
69. Ademokun, A., Wu, Y.C. & Dunn-Walters, D. The ageing B cell population: composition and function. *Biogerontology* **11**, 125–137 (2010).
70. Dunn-Walters, D.K. & Ademokun, A.A. B cell repertoire and ageing. *Curr. Opin. Immunol.* **22**, 514–520 (2010).
71. Gibson, K.L. *et al.* B-cell diversity decreases in old age and is correlated with poor health status. *Aging Cell* **8**, 18–25 (2009).  
**First study showing contraction of the B cell repertoire with aging.**
72. Steinmann, G.G., Klaus, B. & Muller-Hermelink, H.K. The involution of the ageing human thymic epithelium is independent of puberty. A morphometric study. *Scand. J. Immunol.* **22**, 563–575 (1985).
73. Hakim, F.T. *et al.* Age-dependent incidence, time course, and consequences of thymic renewal in adults. *J. Clin. Invest.* **115**, 930–939 (2005).
74. Castermans, E. *et al.* Thymic recovery after allogeneic hematopoietic cell transplantation with non-myeloablative conditioning is limited to patients younger than 60 years of age. *Haematologica* **96**, 298–306 (2011).
75. Dowling, M.R. & Hodgkin, P.D. Why does the thymus involute? A selection-based hypothesis. *Trends Immunol.* **30**, 295–300 (2009).
76. Aspinall, R., Pitts, D., Lapenna, A. & Mitchell, W. Immunity in the elderly: the role of the thymus. *J. Comp. Pathol.* **142** (suppl. 1), S111–S115 (2010).
77. Douek, D.C. *et al.* Changes in thymic function with age and during the treatment of HIV infection. *Nature* **396**, 690–695 (1998).
78. Naylor, K. *et al.* The influence of age on T cell generation and TCR diversity. *J. Immunol.* **174**, 7446–7452 (2005).
79. Dutilh, B.E. & de Boer, R.J. Decline in excision circles requires homeostatic renewal or homeostatic death of naive T cells. *J. Theor. Biol.* **224**, 351–358 (2003).
80. Hazenberg, M.D., Borghans, J.A., de Boer, R.J. & Miedema, F. Thymic output: a bad TREC record. *Nat. Immunol.* **4**, 97–99 (2003).
81. Saucedo, D. *et al.* Evidence of premature immune aging in patients thymectomized during early childhood. *J. Clin. Invest.* **119**, 3070–3078 (2009).
82. Saucedo, D. & Appay, V. Altered thymic activity in early life: how does it affect the immune system in young adults? *Curr. Opin. Immunol.* **23**, 543–548 (2011).
83. Czesnikiewicz-Guzik, M. *et al.* T cell subset-specific susceptibility to aging. *Clin. Immunol.* **127**, 107–118 (2008).
84. Kimmig, S. *et al.* Two subsets of naive T helper cells with distinct T cell receptor excision circle content in human adult peripheral blood. *J. Exp. Med.* **195**, 789–794 (2002).
85. Kohler, S. *et al.* Post-thymic *in vivo* proliferation of naive CD4<sup>+</sup> T cells constrains the TCR repertoire in healthy human adults. *Eur. J. Immunol.* **35**, 1987–1994 (2005).
86. Goronzy, J.J. & Weyand, C.M. T cell development and receptor diversity during aging. *Curr. Opin. Immunol.* **17**, 468–475 (2005).
87. Cicin-Sain, L. *et al.* Dramatic increase in naive T cell turnover is linked to loss of naive T cells from old primates. *Proc. Natl. Acad. Sci. USA* **104**, 19960–19965 (2007).  
**Study showing that in nonhuman primates, age-related changes in T cell homeostasis are not linear.**
88. Johnson, P.L., Yates, A.J., Goronzy, J.J. & Antia, R. Peripheral selection rather than thymic involution explains sudden contraction in naive CD4 T-cell diversity with age. *Proc. Natl. Acad. Sci. USA* **109**, 21432–21437 (2012).  
**By *in silico* simulation, this study puts forward the provocative hypothesis that contraction in T cell diversity with age is best explained by a 'multi-hit' model in which adult thymic activity is irrelevant.**
89. Wils, E.J. *et al.* Insufficient recovery of thymopoiesis predicts for opportunistic infections in allogeneic hematopoietic stem cell transplant recipients. *Haematologica* **96**, 1846–1854 (2011).
90. Gomez, I., Marx, F., Gould, E.A. & Grubeck-Loebenstein, B. T cells from elderly persons respond to neoantigenic stimulation with an unimpaired IL-2 production and an enhanced differentiation into effector cells. *Exp. Gerontol.* **39**, 597–605 (2004).
91. Lee, W.W. *et al.* Age-dependent signature of metallothionein expression in primary CD4 T cell responses is due to sustained zinc signaling. *Rejuvenation Res.* **11**, 1001–1011 (2008).
92. Li, G. *et al.* Decline in miR-181a expression with age impairs T cell receptor sensitivity by increasing DUSP6 activity. *Nat. Med.* **18**, 1518–1524 (2012).  
**Study identifying a negative feedback loop in TCR signaling that accounts for diminished T cell responsiveness in the elderly and is controlled by miR-181a.**
93. Li, Q.J. *et al.* miR-181a is an intrinsic modulator of T cell sensitivity and selection. *Cell* **129**, 147–161 (2007).
94. Goronzy, J.J., Li, G., Yu, M. & Weyand, C.M. Signaling pathways in aged T cells—a reflection of T cell differentiation, cell senescence and host environment. *Semin. Immunol.* **24**, 365–372 (2012).
95. Adachi, K. & Davis, M.M. T-cell receptor ligation induces distinct signaling pathways in naive vs. antigen-experienced T cells. *Proc. Natl. Acad. Sci. USA* **108**, 1549–1554 (2011).
96. Fragoso, R. *et al.* Modulating the strength and threshold of NOTCH oncogenic signals by mir-181a-1/b-1. *PLoS Genet.* **8**, e1002855 (2012).
97. Wang, G.C., Dash, P., McCullers, J.A., Doherty, P.C. & Thomas, P.G. T cell receptor  $\alpha\beta$  diversity inversely correlates with pathogen-specific antibody levels in human cytomegalovirus infection. *Sci. Transl. Med.* **4**, 128ra142 (2012).  
**Study showing that the diversity, not the size, of the CMV-specific T cell repertoire controls the latency of CMV infection.**
98. Maciver, N.J., Michalek, R.D. & Rathmell, J.C. Metabolic Regulation of T Lymphocytes. *Annu. Rev. Immunol.* **31**, 259–283 (2013).
99. Yu, M. *et al.* Signal inhibition by the dual-specific phosphatase 4 impairs T cell-dependent B-cell responses with age. *Proc. Natl. Acad. Sci. USA* **109**, E879–E888 (2012).
100. Shao, L. *et al.* Deficiency of the DNA repair enzyme ATM in rheumatoid arthritis. *J. Exp. Med.* **206**, 1435–1449 (2009).
101. Liu, Y. & Sharpless, N.E. Tumor suppressor mechanisms in immune aging. *Curr. Opin. Immunol.* **21**, 431–439 (2009).
102. Di Mitri, D. *et al.* Reversible senescence in human CD4<sup>+</sup>CD45RA<sup>+</sup>CD27<sup>-</sup> memory T cells. *J. Immunol.* **187**, 2093–2100 (2011).
103. Effros, R.B. Telomerase induction in T cells: a cure for aging and disease? *Exp. Gerontol.* **42**, 416–420 (2007).
104. Fujii, H., Shao, L., Colmegna, I., Goronzy, J.J. & Weyand, C.M. Telomerase insufficiency in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA* **106**, 4360–4365 (2009).
105. Shao, L., Goronzy, J.J. & Weyand, C.M. DNA-dependent protein kinase catalytic subunit mediates T-cell loss in rheumatoid arthritis. *EMBO Mol. Med.* **2**, 415–427 (2010).
106. Weng, N.P., Akbar, A.N. & Goronzy, J. CD28<sup>-</sup> T cells: their role in the age-associated decline of immune function. *Trends Immunol.* **30**, 306–312 (2009).
107. Cavanagh, M.M., Qi, Q., Weyand, C.M. & Goronzy, J.J. Finding balance: T cell regulatory receptor expression during aging. *Aging Dis.* **2**, 398–413 (2011).
108. Francisco, L.M., Sage, P.T. & Sharpe, A.H. The PD-1 pathway in tolerance and autoimmunity. *Immunol. Rev.* **236**, 219–242 (2010).
109. Wherry, E.J. T cell exhaustion. *Nat. Immunol.* **12**, 492–499 (2011).
110. Akbar, A.N. & Henson, S.M. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? *Nat. Rev. Immunol.* **11**, 289–295 (2011).  
**Opinion article comparing the similarities and differences of T cell exhaustion and senescence.**
111. Frasca, D. & Blomberg, B.B. Aging impairs murine B cell differentiation and function in primary and secondary lymphoid tissues. *Aging Dis.* **2**, 361–373 (2011).
112. Wrammert, J. *et al.* Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J. Exp. Med.* **208**, 181–193 (2011).  
**Study providing evidence (along with ref. 57) that adaptation of the existing B cell memory repertoire, rather than recruitment of novel specificities, dominates the B cell response in the adult.**
113. Sasaki, S. *et al.* Limited efficacy of inactivated influenza vaccine in elderly individuals is associated with decreased production of vaccine-specific antibodies. *J. Clin. Invest.* **121**, 3109–3119 (2011).  
**Study providing evidence that a decreasing number of antigen-specific antibody secreting cells explains the impaired response to influenza vaccination in the elderly, whereas the quality of the B cell response is maintained.**
114. Khurana, S., Frasca, D., Blomberg, B. & Golding, H. AID activity in B cells strongly correlates with polyclonal antibody affinity maturation *in-vivo* following pandemic 2009–H1N1 vaccination in humans. *PLoS Pathog.* **8**, e1002920 (2012).  
**Study providing evidence (in partial contrast to ref. 113) of B cell-intrinsic defects that impair the quality of the B cell response to vaccination against H1N1 influenza virus in the elderly.**
115. Frasca, D. *et al.* Aging down-regulates the transcription factor E2A, activation-induced cytidine deaminase, and Ig class switch in human B cells. *J. Immunol.* **180**, 5283–5290 (2008).
116. Vukmanovic-Stejic, M., Rustin, M.H., Nikolich-Zugich, J. & Akbar, A.N. Immune responses in the skin in old age. *Curr. Opin. Immunol.* **23**, 525–531 (2011).