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Immunosenescence comes of age

Symposium on Aging Research in Immunology: The Impact of Genomics

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The Symposium on Aging Research in Immunology: The Impact of Genomics took place on 4 and 5 September 2006 in Paris, France, and was organized by B. Grubeck-Loebenstein.

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Introduction

Ageing of the immune system (immunosenescence) contributes to the increased susceptibility of the elderly to infectious disease and to the poor outcome of vaccination. Defence against pathogens is compromised mainly because of changes in adaptive immunity mediated by T and B lymphocytes; however, all components of the immune system are affected (Fig 1). Dissecting the crucial alterations responsible for dysfunctional immunity in old age will facilitate the development of rational interventions to reconstitute appropriate immune function. Given the increasing proportion of elderly people in most countries and their disproportionate consumption of health-care resources, this issue is rapidly gaining in importance. The meeting, which was dedicated solely to studies of immunosenescence, filled two days with the 'A to Z' of immunity, covering topics ranging from development to senescence, innate immunity to adaptive immunity, and genes to environments, in organisms ranging from mice to monkeys and humans. Understanding and eventually modulating immune dysfunction in the elderly now beckons.

Lymphocyte development and ageing

The cells of the immune system turn over rapidly and therefore need constant replacement from the pool of haematopoietic stem cells (HSCs). If the HSCs themselves aged, it would compromise all downstream events that depend on their integrity, including production of immune cells and subsequent immune responsiveness (Rando, 2006). Evidence for age-associated alterations in the ability of HSCs to reconstitute the haematopoietic system of an animal derives from findings of increased self-renewal with age, resulting in an expansion of the HSC pool size even when transplanted into young animals (D. Rossi, Stanford, CA, USA). However, purified HSCs from old mice showed less activity on a per-cell basis and tended to generate more myeloid cells—for example, macrophages—than lymphocytes. Expression profiling of young and old HSCs revealed that genes mediating lymphoid fate and function were systematically downregulated, whereas myeloid-specification genes were upregulated, with age. The concerted nature of these changes suggests epigenetic involvement as a mechanism that contributes to HSC functional decline with age. There is also a gradual decline in the ability of murine HSCs to progress through the various stages of B-cell-differentiation (K. Dorshkind, Los Angeles, CA, USA). This reflects, in part, the microenvironmental changes involving altered production of interleukin 7 (IL-7) by stromal cells as they age (M. Cancro, Philadelphia, PA). B cells must also compete for the cytokine BLys (or B-cell activating factor (BAFF)), the receptor levels of which determine survival. Declining B-cell production in aged animals results in selective accumulation of marginal zone and memory B cells at the expense of the follicular pool of B cells. The follicular pool is responsible for producing protective immune responses to newly encountered pathogens, such as influenza H5N1. Loss of the declining stem-cell function, and the resultant decline of the follicular B-cell compartment, leads to enhanced infectious disease-related morbidity with ageing (J. Cambier, Denver, CO, USA). Hence, age affects both HSCs and the environment that determines their fate.

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Lymphocyte dynamics and turnover

Similar to B cells, T-cell production and differentiation alter with age, both in the thymus and in the periphery. Turnover of T-cell subsets is difficult to study in humans; however, advances have been made through pioneering work in which deuterated glucose given to young and old volunteers is incorporated into dividing T cells to distinguish the fraction of the pool proliferating during the labelling period (P. Beverley, Compton, UK). In naive, memory and regulatory T-cell subsets, label uptake and loss occurs at the same rate in both young and old people (Vukmanovic-Stejic *et al*, 2006). The only difference was that CD8 memory cells, once labelled, had a much longer half-life than any other subset in the elderly—if they had recently divided, they persisted for extended periods. Also similar to B cells, the number of peripheral T cells is stable with age. Consequently, T-cell diversity in aged individuals can be compromised as T-cell clonal expansions come to predominate in the peripheral pool. Clonal expansions of CD8 cells found in old mice might be either antigen-independent (random T-cell receptor (TCR) usage) or antigen-driven (E. Clambey, Denver, CO, USA), possibly depending on the nature of the pathogens present in their environment. Some expanded clones in mice, as in humans, seem to be highly stable in adoptive-transfer experiments (more than 4 years), with a lifespan far in excess of that of the donor mice. By contrast, other clones disappear rapidly (within 2 months) and are likely to be antigen-driven. Antigen-driven clones characterized by low CD8 expression, high levels of the negative receptor programmed-death 1 (PD-1), high integrin-α4 levels and limited growth capacity are gradually replaced by antigen-independent clones with normal CD8 expression, low integrin-α4 levels and unlimited growth capacity (Clambey). Although antigen-driven clones might be found most frequently in aged humans, this dichotomy suggests that many independent mechanisms can contribute to age-altered CD8 T-cell homeostasis.

Genetics of longevity

The above-mentioned findings indicate that genetic and environmental factors can influence ageing for B and T cells, both in humans and in mice. Genetic differences contribute intrinsic factors (R. Miller, Ann Arbor, MI, USA). Analysis of T-cell subsets in 900 sibling mice showed that one group of allelic differences led to changes detectable in early life, and another group led to changes detectable in middle age. Young mice with T-cell-subset patterns that resembled those of old mice tended to die at comparatively early ages, regardless of whether they were laboratory stocks, wild-derived stocks or hybrids of laboratory and wild mice. Most of the genetic effects were additive such that, taken together, multiple alleles had notable effects on age-sensitive T-cell subsets. Clearly, genetic studies in humans are more of a challenge than in mice. However, C. Franceschi (Bologna, Italy) reported differential levels of expression of several hundred genes with age, many of which seem to be macrophage-specific. This is consistent with the long-held view that ageing in humans is accompanied by increased basal levels of inflammation. Such chronic inflammation is symptomatic of dysregulated immunity, causing tissue damage and changing the microenvironment from one supporting immune responses to one suppressing responses. This has been dubbed 'inflammaging'.

Innate immunity

So, what are the age-associated changes that can be directly measured in macrophages, dendritic cells, neutrophils, natural killer (NK) cells and so on? These might be at least as important, if not more so, than the changes to adaptive immunity discussed above (Solana *et al*, 2006). The number and proliferation of a particular subset of 'natural' T cells with NK-cell and regulatory functions, bearing invariant Vα14Jα18 receptors (iNKT cells), is decreased in the elderly; however, whether these changes have any clinical impact is not yet known (R. Solana, Córdoba, Spain). Neutrophils from old people retain normal chemotaxis and superoxide-generation capacity, but are compromised in phagocytosis in the healthy elderly and more so in the traumatized elderly (J. Lord, Birmingham, UK); these findings have important implications for infection in the elderly. Trauma, in the form of burn injury in mice, resulted in the death of old animals from infections that young animals were able to resist. This susceptibility of old mice correlated with higher levels of pro-inflammatory IL-6 and decreased T-cell function, and could be in part reversed by oestrogen treatment (E. J. Kovacs, Maywood, IL, USA). Dendritic cells—the essential bridge between innate and adaptive immunity—are similar in young and old people in terms of their response to cytokines (although those from the elderly secrete more IL-6 and tumour necrosis factor-α (TNFα)), surface phenotypes and morphology, whereas chemotaxis and, as with neutrophils, phagocytosis are impaired (S. Gupta, Irvine, CA, USA). Gene arrays indicate only a small number of differences between young and old dendritic cells, far fewer than in T cells. Nonetheless, functional impairment in antigen presentation was found, such that dendritic cells from young or old people stimulated naive CD8 cells equally well, but those from the elderly failed to stimulate CD4 cells appropriately.

Clinical implications of immunosenescence

As mentioned above, complications from acute infectious are likely to be more severe in the elderly owing to impaired innate immunity. However, questions remain concerning 'normal, healthy' ageing and the important clinical issue of responses to vaccinations in old age. In a mouse model of the highly relevant human pathogen influenza, the virus is cleared from the lungs more slowly in old animals, correlating with a delayed and decreased peak of cytotoxic T-cell production (D. Murasko, Philadelphia, PA, USA). Therefore, cellular responses are crucial for controlling the virus, but do not function adequately in old animals. Although there is an accumulation of memory cells (the clonal expansion referred to above), they are not solely responsible for this decrease in the virus-specific response. Both memory and naive T cells in old, but not young, mice are resistant to apoptosis, and do not 'make space' for new responses. In the mouse model, cell-transfer experiments showed that both the old environment and the old cells contributed to the problem—young cells did not deplete when transferred to an old environment and old cells did not deplete when transferred to a young environment. The factors inducing apoptosis resistance have not yet been identified; however, it is clearly important to do so and to search for them in humans.

Apoptosis-resistant cells that accumulate in old mice and humans—and fill the 'immunological space'—might be dysfunctional in several ways. In young mice, the number of T cells staining with soluble major histocompatibility complex (MHC)–peptide multimers carrying influenza epitopes was similar to the number of cells producing the antiviral and pro-inflammatory cytokine interferon-γ (IFNγ) on antigen stimulation. However, in old mice, the number of tetramer-positive cells exceeded the number of IFNγ-producers, indicating that some cells bearing antigen-specific receptors failed to respond appropriately to receptor ligation (H. Ertl, Philadelphia, PA, USA). This is similar to the situation in elderly

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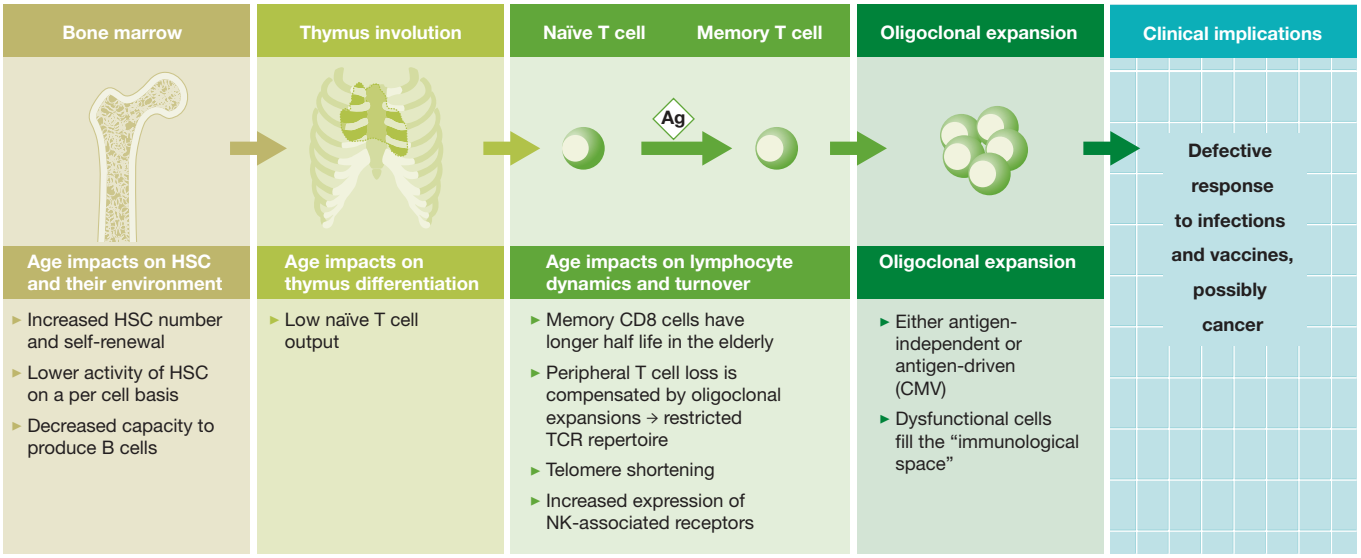

Fig 1 | Human immunoageing trajectory. All compartments are affected by age. Ag, antigen; CMV, cytomegalovirus; HSC, haematopoietic stem cells; NK, natural killer cells; TCR, T-cell receptor.

humans, who have been found to accumulate large clonal expansions, primarily—and for unknown reasons—of cytomegalovirus (CMV)-specific CD8 cells (Pawelec *et al*, 2005). In the mice, this lack of reactivity was not due to poor antigen presentation by dendritic cells (Ertl). The reason for poor reactivity remains unknown; however, responses could be restored, in part, by vaccination using an adenovirus vector AdC68 that naturally infects chimpanzees rather than mice, as a way of improving immunizations by modifying the vaccine product. This might also be possible in humans by using better adjuvants for vaccination (E. Nagy, Vienna, Austria). Deciphering the mechanisms by which adjuvants enhance responses in order to design 'elderly-specific' vaccines will become increasingly important. This applies not only to infectious diseases but also possibly to vaccinating against cancer, as illustrated by differences in responses to anticancer immunizations in young and old mice. In a breast cancer model, preventive vaccination using DNA encoding certain cancer antigens was successful in protecting 90% of the young mice, but only 60% of the old mice, from developing metastases. This correlated with lower levels of IFNγ and IL-2 in old mice (C. Gravekamp, San Francisco, CA, USA). The production of IL-6, which is a potential inhibitor of vaccine-induced T-cell responses, was high in both young and old mice. Increasing IFNγ and IL-2, and depressing IL-6 production in the elderly, would therefore seem to be desirable.

The function of naïve and memory T cells in ageing

Clearly, vaccination can only be effective if cells able to respond are present (Nikolich-Zugich, 2005). These might be either naive cells that require stimulation with vaccine containing novel antigens, or memory cells that require increasing by previously encountered antigens. This raises the question of whether old individuals have naive cells towards the end of their lives and, if so, whether they are fully functional. In mice, naive T cells from old animals do seem to be impaired. CD4 cells show decreased helper activity—and, in this case, transfer into a young environment fails to restore immune competence; this is not because of altered trafficking of lymphocytes through the tissues (L. Haynes, Saranac Lake, NY, USA). Helper function could be restored by a mixture of pro-inflammatory cytokines—IL-1, IL-6 and TNFα; therefore, judicious local use of these as adjuvants might be beneficial. However, elderly humans might have few naive cells that can be targeted in this way. Even CD8 cells with an apparently naive phenotype—CD45RA+ CD28+—expressed fewer additional naive markers—CD62L and CCR7—than those from the young, had shorter telomeres and had restricted TCR repertoires (B. Grubeck-Loebenstein, Innsbruck, Austria). In fact, the presence of cells with an unusual phenotype, more reminiscent of memory cells, seemed to correlate with better responses to influenza vaccination in the elderly. If the elderly mostly rely on their memory cells for pathogen responses in later life, it is crucial to know whether these are retained and function normally, and, if not, what can be done to improve this situation.

A diverse TCR repertoire must be maintained at all times to respond to all types of antigen. Repertoire attrition would be predicted to be a bad prognostic sign in the elderly. Given the high levels of peripheral cell turnover (Beverley), their potential for proliferation could be rapidly exhausted. In humans, telomere shortening with each cell division results in proliferative arrest—replicative senescence—unless cells express telomerase. Extensive proliferation results in CD28 downregulation and, although there are other co-receptors that can upregulate telomerase, for example, CD134, CD137 and inducible T-cell co-stimulator (ICOS), 'end-stage' cells that have lost both CD27 and CD28 cannot do so (A. Akbar, London, UK). Repetitive stimulation therefore results in ever lower levels of telomerase, at which time the telomeres begin to shorten (R. Hodes, Bethesda, MD, USA). In some cases, transfection of the catalytic component of telomerase, hTERT, can result in stabilization of telomere lengths and extended growth of the cells *in vitro*; for example, human immunodeficiency virus (HIV)-specific hTERT-transfected CD8 cells have been cultured for more than three years without acquiring karyotypic abnormalities and retain their ability to control

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HIV infection *in vitro* (R. Effros, Los Angeles, CA, USA). In the absence of continuous telomerase expression, however, memory cells might become impaired as they turn over *in vivo*. Signals regulating this turnover have not been determined; however, peripheral levels are well-maintained in the healthy aged—only the unhealthy might have less peripheral cells per unit blood, that is, be leukopaenic. Antigens do not seem to be required in infections caused by acute agents that do not persist. After the clearance of an acute viral infection in mice, memory cells persist for the lifetime of the animal, and undergo clonal expansions and accumulations of memory cells even in the absence of the original antigen. These persistent memory cells remain fully functional and even become more effective with time (D. Woodland, Saranac Lake, NY, USA). So, what maintains them over extended periods in the absence of antigenic stimulation? The answer is possibly cytokines such as IL-7 and IL-15. CD8 cells carrying low levels of the receptor IL-7Rα already had a limited TCR repertoire in the young, but this was even more restricted in the elderly, with poor responses to TCR-mediated stimulation. These memory cells might be maintained by IL-15 but they are dysfunctional owing to replicative senescence, whereas high IL-7Rα expressers are functional (I. Kang, New Haven, CT, USA). Distinguishing between functional and dysfunctional CD8 cells with the same TCR specificity, on the basis of markers such as IL-7Rα, might be important when considering interventions that would benefit from deletion of the apoptosis-resistant dysfunctional cells, the accumulation of which fills the immunological space and contributes to decreased immunity in the elderly.

Gene arrays of functional and dysfunctional cells might provide clues as to the reasons for their differences. Analysis of gene expression between CD28+ (functional) and CD28- (partly dysfunctional) CD8 T cells revealed that the latter upregulated several stimulatory NK receptors (N.-P. Weng, Bethesda, MD, USA). Many of these differences decreased after culture in the 'maintenance' cytokine IL-15. Both CD28+ and CD28- cells responded in a similar manner to IL-15, and this was the same in both the young and the old. Intriguingly, IL-15 also induced loss of CD28 expression in CD28+ CD8 cells, indicating that maintenance of memory cells by IL-15 could cause the generation of CD28- cells with age.

These and other data suggest the accumulation of clonal expansions of CD8—and, to a lesser extent, CD4—cells in elderly humans and mice. In the absence of foreign antigen, these events still occur in mice; however, in human ageing, there are probably always foreign antigens driving clonal expansions. This might also apply to 'premature' immunosenescence, such as that caused by HIV infection (V. Appay, Paris, France). In humans, longitudinal studies of the elderly (aged more than 85 years) have revealed an overriding importance of persistent CMV and a lesser contribution of Epstein–Barr virus—but not herpes simplex virus—to this phenomenon. CMV-specific CD8 cells begin to accumulate during middle age (G. Pawelec, Tübingen, Germany). The number of different clonal expansions initially increases with age and is a risk factor predicting mortality; however, at the terminal phase, repertoire contraction occurs, which is even more highly predictive of mortality. The CMV-specific CD8 clones that accumulate contain functional cells—producing IFNγ on specific stimulation—and a larger fraction of dysfunctional (anergic) cells. It is the latter that cause inversion of the CD4:8 ratio in severely affected donors, which is a prime risk factor for incipient mortality. Age-associated alterations of TCR diversity might occur with surprising suddenness (J. Goronzy, Atlanta, GA, USA). Diversity seems to be well-maintained in both naive and memory CD4 cells up to 60–65 years of age, despite thymic output mostly ceasing by approximately 50 years of age. However, repertoire diversity in individuals aged 75–80 years was a mere 1% of that in the younger group, suggesting the rapid loss of clonal heterogeneity between late middle age and early old age. These were not longitudinal studies; however, the findings are consistent with the data from the longitudinal studies described above for CD8 cells, in which the same individuals were followed over time and displayed a highly significant negative association between the number of different clonal expansions present and the remaining survival time. Therefore, if these data are confirmed, an enormous future challenge will be to find out exactly what happens at the crucial time point of repertoire crash, and how to prevent it. One possibility could be caloric restriction, which, in monkeys, helps to maintain naive T-cells, and to preserve TCR repertoire diversity and gene-expression patterns at youthful levels (J. Nikolich-Zugich, Beaverton, OR, USA).

Conclusions

All components of the immune system are altered as ageing proceeds (Fig 1); however, the T-cell and B-cell compartments seem to be particularly susceptible. The most severe clinical impact is probably a result of the loss of diversity in the TCR and B-cell-receptor repertoire, owing to the accumulation of dysfunctional cells, and decreased thymic and bone-marrow output. Several interventions discussed at the meeting could conceivably contribute to the restoration of appropriate immune function in the near future.

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