T-cell immunity in the aging human

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Age and immunity: immunosenescence

Immunosenescence is an ill-defined term commonly used to describe the differences observed between younger and older individuals in a broad range of immune parameters. Humans and mice are the best-studied, but indications of immunosenescence have also been reported in primates, dogs, cats and horses.[1] Although often referred to as age-associated changes to immunity, and almost always assumed to be detrimental to immune responses, in most cases, the differences between old and young are neither known to be detrimental nor demonstrated to have changed with age in that individual. This would require longitudinal studies of the same individuals over time, which are clearly challenging in long-lived species like humans, but are also quite rare even in mice, mostly due to cost issues. Although there are, in fact, numerous longitudinal studies being conducted in different human populations, in the past these have mainly focused on psychosocial or socioeconomic parameters, or some aspects of health, and have rarely included much in the way of biogerontological investigation, with an even smaller number focusing on hematology and immunology. Thus, most of the available data derive from cross-sectional cohort studies, which are of course very valuable for mapping overall differences between young people born and raised at the end of the 20th century compared with those from the beginning of it. But it is obvious that these cohorts will differ markedly in multiple potentially confounding factors that cannot or can only be imperfectly controlled, i.e. differences in genetics, pathogen exposure, medical care, nutrition, and many others. Hence, delineation of risk factors including immunological parameters associated with diseases of aging that we identify as differences in the current elderly compared to the young may not necessarily be relevant to the latter as they themselves age. Clearly, correlating certain measured parameters in the elderly with disease states in those populations is not without value and it might well be possible to extrapolate this to those currently younger. However, establishing individual risk factors that will be clearly relevant to disease development in the young would ideally require longitudinal studies of individuals. But in addition to taking a very long time, these would also only be relevant to future populations born and raised under the same conditions. This is extremely unlikely of course. So, as usual in human medicine, we are left with imperfect compromises, whereby we can examine factors in populations already advanced in years which are relevant to health outcomes, frailty, morbidity and mortality in those populations within a reasonably short follow-up time. Identified factors relevant in this context can then be selectively tested on younger populations to determine whether they also represent risk factors at a younger age. This approach is feasible but, unfortunately, we already know that risk factors in people highly selected for survival in very old populations are different from those relevant in middle age, simply because of earlier mortality in younger people with those risk factors.

What can one do? Animal models may be informative for certain immune parameters, but translation from mouse to human in the clinical context is usually problematic, and investigating monkeys is hardly chronologically advantageous compared to humans. One could consider examining people with genetic phenotypic premature aging syndromes, but it is unclear to what degree these various genetic lesions have anything to do with 'normal' aging. Hence, one is forced to come to the conclusion that this problem is *per se* intractable and that compromises will always be necessary. An approach that we are taking is to combine cross-sectional with longitudinal studies in both young and old groups at the same time. This study, designated BASE II,[2] is collating data from 2200 people sharing the same general environment in Berlin: one-third of them young middle-aged and two-thirds older (60-80 years). The database will contain individual subject information on genetics, medical parameters, psychological state, cognitive function, socio-economic factors, nutrition and immunology. In this way, we will attempt to dissect out interacting risk factors in older people and compare these in a broad manner with the same measurements in the younger control population, while following both longitudinally. Immunology data are not yet available from this study, but a small number of other longitudinal studies have provided limited data on immune parameters, mostly independent of other risk factors. Predominant amongst these were the OCTO and NONA immune studies of the very elderly (>85) in the small Swedish town of Jönköping, data from which gave rise to the concept of the "immune risk profile" associated with 2-, 4- and 6-year all-cause mortality in this population.[3] Other studies with considerable immunology data currently being worked up include the excellent Leiden 85+, Newcastle 85+, Belfrail and Baltimore longitudinal studies. Published results are eagerly awaited.

T-cell immunosenescence

Numerous cross-sectional studies in young-*versus*-old humans have identified phenotypic differences in innate and adaptive immune parameters, the most striking of them being in the distribution of T-cell differentiation phenotypes. Reports with less data, but equally convincing, have shown that similar differences are also seen for B cells.[4] Because most studies agree that the elderly possess fewer naïve T and B cells, and reciprocally more memory cells, the common-sense consensus interpretation of the data is that these differences are caused by antigen exposures over the life course, presumably mostly to pathogens. The innate immune system is molded much less markedly or not at all by such exposures, fitting with the majority of findings that age-associated differences are nowhere near as obvious in innate as in adaptive immune signatures.[5] Thus, these major differences in T- and B-cell distribution are reflections of the adaptive changes to adaptive immunity, as its name suggests, and are by no means reflections of chronological or biological aging.[6] That they are really changes and not only differences can be discerned from innumerable murine experiments exposing naïve mice to antigen,

and to some extent from *in vitro* experiments with human cells. Alterations in the distribution of T-cell phenotypes are also observed in humans, strikingly so, for example when people become infected with a certain beta herpesvirus, HHV5, cytomegalovirus (CMV). Even in infants, the redistribution of T-cell phenotypes from naïve-predominant to memory-enriched occurs rapidly,[7] as it does in CMV-uninfected transplant patients receiving a CMV-positive graft.[8] Other persistent herpesviruses may have similar effects, especially Epstein-Barr virus (EBV), but do not leave quantitatively the same imprint on T-cell distribution as CMV does.[9] Why CMV should be unique in this respect is not known, but it clearly has an enormous impact on immune signatures throughout life. In at least 2 studies, the usual marked accumulations of late-stage differentiated $CD8^+$ memory T cells is absent in that small fraction of older people not infected with CMV,[10,11] whereas the attrition of naïve cells is also seen in CMV-seronegative subjects. Hence, one of the hallmarks of "immunosenescence" is entirely due to the requirement for the host to maintain control of latent CMV infection throughout life. Indeed, in a Kaplan-Meier analysis of survival of very old people from Leiden, The Netherlands, those with the highest *in vitro* $CD8^+$ T cell-mediated pro-inflammatory responses to CMV antigens (including high TNF production) enjoyed a significant survival advantage, whereas higher levels of naïve T cells were not beneficial.[12] This illustrates the theme of adaptation very well, in that naïve cells are required for defense against new exposures that were probably rare in this old population, whereas the continued control of latent CMV infection was of paramount importance.

All that is not to say that there are no other problems in T-cell immunity in the elderly. The output of immune cells from the bone marrow (BM) is skewed towards myeloid cells and away from lymphoid progenitors in old mice and probably humans too. This is a result of differences in the hematopoietic stem cell (HSC) microenvironment of the BM stroma, macroenvironment of the aged organism and intrinsic effects in the HSC themselves.[13] The B cells that are produced may also be functionally compromised, and the production of naïve T cells is certainly greatly reduced or absent after puberty due to the physiological process of thymic involution. This again cannot be considered an aging process, as it is an evolutionarily conserved developmental event observed in many different species, starting at puberty, or even before. However, the manifestations of thymic involution may contribute to effects on T-cell immunity first seen many years thereafter (i.e. after the reproductive period when there is no longer selective evolutionary pressure for maintenance of effective immunity). It is also clear that in addition to the adaptive changes described above, there is an effect of aging at the individual cell level, as may be best illustrated by the poorer function of naïve cells from old-*versus*-young mice specific for experimental antigens not encountered before (in models where all T cells carry the same transgenic antigen receptor). The assumption here is that T cells with a naïve phenotype have been circulating since birth and that rather than being newly-produced cells from thymic remnants in the old mice, they have been exposed to the aged mouse environment over its lifetime.[14] The conclusion from experiments of this type is that such old cells are functionally compromised. This results from dysfunctional signal transduction for T-cell activation, as also is believed to apply in humans.[15]

This brief perspective has allowed only a very superficial view of the complexities of immune aging, but I hope will serve to stimulate more critical thinking on the planning of experiments to investigate immunosenescence and interpretation of the data in human studies in future.

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Current pre-clinical and clinical advances in the *BCR-ABL1*-positive and -negative chronic myeloproliferative neoplasms

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Though it has been remarkable to have witnessed the major advances in the understanding of the molecular pathogenesis of the chronic myeloproliferative neoplasms (MPN) over the past three decades, many challenges remain. The advances began with the identification of the *BCR-ABL1* gene in chronic myeloid leukemia (CML) in 1985, leading to the introduction of ABL1 tyrosine kinase inhibitors (TKIs), and the *JAK2*^{V617F} mutation in polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) in 2005, leading to the JAK inhibitors.[1-3] CML is now arguably the most successfully treated human malignancy. Despite these remarkable achievements, the quest for cure, functionally defined as treatment-free remission after discontinuation of TKI therapy, remains difficult.[4] In the *BCR-ABL1* negative MPNs, a similar degree of success has not been achieved, perhaps because of the rather surprising clonal complexity of these disorders and the increasing molecular evidence of the need for *JAK2*^{V617F} mutation to cooperate with other genetic aberrations in the initiation and progression of the disease. This clonal complexity needs to be elucidated further in order to recognize clinically relevant candidate therapeutic targets. Herein, we review some of the topical challenges and successes in the biology and therapy of the MPNs that were discussed at the 7th post-American Society of Hematology CML-MPN workshop, which took place in Atlanta on December 11-12, 2012, and up-dated prior to this publication.

1. Unraveling the impact of epigenetics in the classical MPNs

It has been speculated that some of the heterogeneity in CML might be attributable to differences in patients at the epigenetic level. Few studies have addressed the DNA methylome in CML. Dunwell *et al.* identified two genes (*TFAP2* and *EBF2*) that showed increased methylation at the time of blast crisis, while hypermethylation of the autophagy-related gene *ATG16L2* was associated with poorer response to TKI therapy.[5] Jelinek *et al.* studied the incidence of abnormally methylated promoters in 10 selected genes, the frequency of which was shown to increase during advanced phase disease and following resistance to imatinib. They showed that abnormal methylation of the SRC suppressor gene *PDLIM4* was associated with shorter overall survival independently of disease phase or imatinib responsiveness.[6] A recent study showed aberrant hypermethylation of *CEBPA* gene promoter and a significant association of this hypermethylation with the CML stage.[7]

Bazeos *et al.* collected CD34$^+$ cells from newly diagnosed patients with CML in the chronic phase of disease and compared their DNA methylation profiles with analogous cells collected from the same individuals at the time of achieving at least complete cytogenetic response (CCyR) as well as from healthy controls (A Bazeos *et al.*, personal communication, 2012). Unsupervised hierarchical clustering revealed a series of differentially methylated probes, which were associated with untreated CML and represented a CML signature. Profiles obtained from patients who had achieved CCyR following treatment with tyrosine kinase inhibitors showed similar DNA methylation patterns to those of healthy persons with some residual CML signature marks. The investigators correlated their finding with clinical parameters but were unable to demonstrate major differences in methylation patterns between newly diagnosed individuals and concluded that the CML methylation signature is remarkably homogenous. It is possible that the CML phenotype requires acquisition of a *BCR-ABL1* fusion gene in association with a series of specific epigenetic changes.

There is also interest in assessing the relationship between microRNA (miR) expression and CML, particularly in patients who are resistant to TKI treatment. Patients responding to imatinib have demonstrated an increased expression of some miR (-150 and -146a) and decreased expression of other miR (-142, -199b, -145).[8] Earlier studies also demonstrated an association between a downregulation of miR-203, possibly due to CpG methylation (or simply genomic instability), and the *ABL1* upregulation in newly diagnosed patients.

Studies on the genetic architecture of MPN have discovered an unexpected high level of complexity. Most mutant genes in MPN fall in three functional classes: JAK/STAT signaling (mutations in *JAK2*, *MPL*, *CBL*, *LNK*, and rare mutations in SOCS), epigenetic gene regulation (*TET2*, *EZH2*, *ASXL1*, *DNMT3A*, *IDH1*) and spliceosome factors (*SRSF2*).[9] Patients with MPN who transform to acute myeloid