Chapter 16
Immune Parameters Associated with Mortality in Longitudinal Studies of Very Old People Can Be Markedly Dissimilar Even in Apparently Similar Populations

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Abstract While challenging, only longitudinal studies can pinpoint biomarkers dynamically associated with a selected outcome. In human studies of ageing, this challenge is particularly onerous, both in terms of the time required and the outcome selected. While recognizing the limitations of our approach, taking the most robust and unequivocal outcome (all-cause mortality), and taking a very old population as the starting point, we have sought to define peripheral blood "immune signatures" predicting incipient mortality. Studies in overtly similar populations in Sweden, Belgium and The Netherlands reveal certain constellations of immune biomarkers associated with all-cause mortality in people >80 years of age. Unexpectedly, however, these "immune risk profiles" are different in the different populations. Thus, it is unlikely that it will be possible to identify "one-size-fits-all" biomarkers of ageing in different populations, at least when solely focusing on parameters of immunity.

Keywords Immunosenescence · Longitudinal studies · Immune signatures · Immune risk profile · Cytomegalovirus · Inflammaging · Biomarker of aging · Mortality

16.1 Introduction

Untold studies have unequivocally demonstrated by numerous different analytical approaches that the composition and functionality of essentially all aspects of immunity in humans is different in young and old people (see Nikolich-Zugich 2018) for a recent extensive review). Understandably, most studies have been cross-sectional,

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comparing younger with older individuals and assuming that the differences observed were due to changes over time as the person aged. This is obviously challenging to prove, given the long human lifespan, although some ongoing studies are attempting to enable such a long-term aim to be achieved by planning to follow younger populations over their lifespan [for example, the Berlin BASE II study (Bertram et al. 2014)]. Even such long-term commitments to the goal of demonstrating changes over time may generate data that are only relevant to the circumstances of the particular population studied, as we will seek to show from the material reviewed here.

16.2 Immunosenescence, Inflammaging and Disease

The theoretical framework for hypothesizing that changes to the human immune system contribute to ageing, disease and mortality is simple: the vertebrate immune system is believed to have evolved to protect the host against invasive pathogens, so any degradation of this capacity with ageing (loosely termed "immunosenescence") would be expected to cause more susceptibility to infectious disease and death in old people. Additionally, it is hypothesized that age-associated changes to the immune system also contribute to tissue damage via autoimmunity or exacerbated chronic inflammatory states. Thus, unbalanced and dysregulated immune function in older individuals reflects a greater degree of the usual immunopathological side effects that always accompany immune activation, but are better regulated in younger individuals, who also have more resilience for dealing with the "collateral damage" caused by immune "friendly fire". Hence, immune-mediated damage is more widespread; so-called "inflammaging" together with immunosenescence, is believed to contribute to many non-infectious diseases of the elderly, such as neurodegeneration, cardiovascular and autoimmune diseases and cancer (Fulop et al. 2014). However, in most cases these assumptions have remained untested hypotheses and direct evidence supporting such contentions is sparse. Even in experimental animals, usually mice, longitudinalFCD8 studies allowing age-associated changes in immunity to be correlated with clinical outcome are rare- and even when such studies have been performed, data from these short-lived animals would not be expected to be directly translatable to humans. Not only are most studies carried out on inbred, specific pathogen-free strains, but if done on wild animals, mice have evolved very different survival and reproductive strategies which affect longevity. So how can we approach this problem in people? Only species-specific data would be meaningful, preferably from longitudinal studies. The few such studies in humans thus far published are mostly limited to following people very old at baseline for a few years and taking the unequivocal clinical endpoint of mortality within a time frame which is logistically possible to study. Other important and informative clinical outcomes such as frailty development or vaccine responsiveness would be very desirable but are much more challenging to define, standardize and investigate. There is no arguing with mortality. It is to be hoped that at least some of the large-scale human surveys currently acquiring data will indeed be continued long-term and that their coordinators will recognize the importance of

measuring immune parameters in addition to the more commonly-collected data, as we are attempting to do in the Berlin BASE II study (Bertram et al. 2014). It is also to be hoped that more representative data from countries other than Western Educated Industrialized Rich Democratic (Weird) countries (e.g. Alam et al. 2013) will add to our knowledge base on the contribution of immune ageing to health and longevity in older adults. Given our experience summarized below on superficially quite similar populations nonetheless demonstrating markedly different immune risk profiles, the lesson will be that multiple (so-far undetermined) heterogeneous variables will most likely need to be integrated into a holistic picture of individual ageing. Again, this is an aim of the Berlin BASE II study, collating data on diverse fields of genetics, medicine, psychology, socio-economics, cognition—and immunity (Bertram et al. 2014).

16.3 The Swedish OCTO/NONA-Immune Longitudinal Studies

To the best of our knowledge, the first attempt to establish an immune signature associated with for all-cause mortality emerged from biobehavioural studies in Sweden. We were privileged to take part in some of the immunological analyses performed in these pioneering OCTO-Immune longitudinal studies set up by Anders Wikby and colleagues in Jönköping, southern Sweden many years ago (Wikby et al. 1994) in the context of the EU project "T-CIA" (2003–2005, see https://cordis.europa.eu/project/rcn/67245/factsheet/en). This established some simple immune parameters associated with 2-, 4-, and 6-year mortality in non-institutionalized subjects 85 years old at baseline. This was the origin of the concept of the "Immune Risk Profile" (IRP), which initially comprised a cluster of functional parameters (poor proliferative responses of peripheral blood mononuclear cells to T-cell mitogens), accumulations of CD8+ T-cells which resulted in CD4:8 ratio <1, lower B-cell counts, and infection with the common human ß-herpesvirus HHV5 (Cytomegalovirus, CMV) (Wikby et al. 2005; Pawelec et al. 2009). Unusually for this type of study, participants were re-examined and re-sampled every 2 years, which enabled dynamic changes in parameters to be associated with outcome, and not simply baseline parameters. This study showed that 16% of the subjects were in the IRP groups at baseline but a further 15% moved into over the 8-year follow-up (Olsson et al. 2000). Other interesting findings from this seminal pilot study were that a minority of individuals in the IRP group at baseline survived by reverting to a non-IRP phenotype, and that independent risk factors for mortality reflecting "inflammaging" (IL 6 in particular) as well as cognitive impairment were actually more closely correlated with mortality than the IRP—participants with both clusters of risk factors survived least well (Wikby et al. 2005). For more details on the OCTO studies (free-living older adults selected for exceptionally good health) and the NONA studies (representative free-living populations NOT selected

for very good health, but nonetheless with a similar IRP), please see a recent review (Pawelec 2018).

Our concept of the IRP has not been exactly validated by others' studies (or our own, see below), although several publications have appeared using an inverted CD4:8 ratio and IL 6 levels (together) as an IRP under several different circumstances and without knowledge of its relevance to survival. One study coming close to the original IRP definition (i.e. also including B-cells) showed that the IRP did correlate with survival in a UK cohort (Huppert et al. 2003), whereas a Spanish study reported that a CD4:8 ratio <1 at baseline was not associated with 3-year survival (Formiga et al. 2014). In other contexts, some associations with survival have been found under very different circumstances, presumably reflecting the importance of immune control and dysregulation not limited to ageing, for example surviving nosocomial lung infections (Plonquet et al. 2011). The IRP was also reported to be informative in HIV-infected patients (not due to CD4+ T cell loss) (Ndumbi et al. 2015). When adjusting the arbitrary cut-off value of > or < unity for the CD4:8 ratio, closer correlations may indeed be seen, as for example, in another UK study where a CD4:8 ratio <1.7 versus >4 informative for survival (Spyridopoulos et al. 2016). These finding to some extent reflect our own, where we attempted to establish immune signatures associated with survival in Dutch and Belgian populations, as well as the ongoing work with the BASE II study (the latter ongoing and to be reviewed elsewhere).

16.4 The Dutch Leiden 85-Plus Study and the Leiden Longevity Study

The Leiden 85-Plus study surveyed the majority of individuals reaching the age of 85 years in the small Dutch city of Leiden (Lagaay et al. 1992). Illustrating the importance of biobanking precious materials, thanks to participation in the EU project "Lifespan" (see http://www.lifespannetwork.nl/) we were able to access cryopreserved peripheral blood mononuclear cells from individuals 89 years of age at sampling and for whom 8-year follow-up mortality data were already available. At this advanced age, there had been no survivors in IRP+ subjects from the OCTO/NONA cohort, presumably due to selection (Strindhall et al. 2007), so it came as no surprise that we could not replicate the Swedish findings as there were no Leiden 85-Plus subjects in the IRP group at 89 years of age (i.e. none with an inverted CD4:8 ratio). However, immune parameters strongly correlating with remaining 9-year survival emerged from these studies, namely the accumulation of late-stage differentiated CD8+ T-cells (essential component of the Swedish IRP) was associated with better survival in this cohort, provided that these cells responded in a pro-inflammatory manner to CMV antigens, with no anti-inflammatory component (Derhovanessian et al. 2013). This emphasizes the over-riding necessity to maintain adequate surveillance against latent CMV, which was also seen in the Swedish studies (Hadrup et al.

2006). This may seem to go against the grain of inflammaging, but another interesting finding from the Leiden 85-Plus study was that higher levels of suppressive CD4+ T cells (known as regulatory T cells, or Tregs) were also correlated with better survival—perhaps by limiting the side effects of the essential immunosurveillance against CMV (Jagger et al. 2014). The ever-resurgent theme of CMV control was also prevalent in our studies of the remarkable cohort in the Leiden Longevity Study (LLS). Individuals in the F2 generation with long-lived parents and grandparents were less likely to become infected with CMV and less likely to over-react to its presence when then did become infected (Derhovanessian et al. 2010). Hence, amongst all the other putative factors influencing immunoageing and its contribution to mortality, the role of genetics and CMV would always need to be taken into account, as further illustrated by studies on heritability in twins (Goldeck et al. 2016).

16.5 The BELFRAIL Study

Finally, the BELFRAIL study in northern Belgium was set up to examine factors predicting frailty in later life, and to correlate development of frailty with mortality (Vaes et al. 2010). As with many such studies, it was not established primarily to look for immunological parameters, but in collaboration with Prof. Mathei, a substudy to phenotype immune cells in the blood was established. First results were indeed able to correlate immune parameters with the development of frailty (Adriaensen et al. 2015); thereafter, it became possible also to show determine correlations with 3-year mortality (Adriaensen et al. 2017). As with the Swedish studies, in this Belgian cohort, many subjects did have a CD4:8 ratio of <1 at baseline, but far fewer than in OCTO/NONA (7% rather than 16%). Another difference was the preponderance of individuals (over 30%) with a CD4:8 ratio >5, which was only seen in a few Swedish subjects. This was caused by a dominance of naïve CD4+ T cells, without any less CD8+ T cells. Correlations with survival in BELFRAIL were completely different from OCTO/NONA: in BELFRAIL, those with a CD4:8 ratio >5 were more frail and 3-year survival was lower—but only in women (Adriaensen et al. 2017)! There was absolutely no effect in men. To us, even more surprisingly, CMV-seropositive women with a CD4:8 ratio <1 (i.e. those who would have been in the Swedish IRP group with worse 2, 4 and 6-year survival) were the ones who survived best in BELFRAIL. This was in stark contrast to CMV-seronegative Belgian women with a CD4:8 ratio >5 who did worst (Adriaensen et al. 2017). These results are clearly at odds with those from OCTO/NONA. The resolution of this conundrum awaits further studies in the Belgian and other populations, but clearly indicates the heterogeneity and context-dependency of immune system-ageing-longevity trajectories in free-living people in the real world. We hope that our BASE-II study may shed further light on this matter, albeit with the proviso that such data may also only be relevant to people in Berlin now, and not necessarily for those elsewhere, or even in the same place in future.

16.6 Southern Italian Cohorts

To further contribute to knowledge about the impact of immune signatures on healthy ageing we studied a group of elderly from southern Italy which showed the common signs of immune ageing when compared to young individuals with 30% having a CD4:CD8 ratio ≤1 in contrast to 0% among the young. However, when we selected a group of age-matched centenarian offspring we found a higher CD4:CD8 ratio compared to the controls (due to higher frequencies of CD4+ T-cells and lower CD8+ T-cells) (Pellicano et al. 2014) Further, the frequencies of naïve T-cells were higher in the offspring compared to the controls and thereby closer to those of young individuals. Together with a fitter compartment of naïve B-cells (Colonna-Romano et al. 2010) this may reduce susceptibility to new infections in the offspring population and as a consequence an increased life span can be expected. This hypothesis awaits confirmation in follow-up studies. Age-related non-communicable diseases are also correlated with distinct immune signatures, such as increased frequencies of late-differentiated T-cells and a CD4:CD8 ratio <1, as observed in Alzheimer's disease patients compared to age matched controls (Pellicano et al. 2012).

16.7 CMV and the IRP in Other Ageing Studies

A recent study reported that serum IL-6 levels, but not CMV serology, were predictive of pre-frailty among the very old (Cao Dinh et al. 2018). This is consistent with earlier findings from the Newcastle 85+ study, where no correlation of frailty with the IRP (CD4:CD8 ratio <1) but only with inflammatory markers, such as IL-6 was found in a cohort of octogenarians. In contrast, reminiscent of the findings in BELFRAIL, memory/naïve T-cell and B-cell ratios showed the opposite to the results of the Swedish studies (Collerton et al. 2012). However, these studies had not taken mortality as the end point, but in later work on this cohort, Spyridopoulos et al. found that cardiovascular mortality was linked to CMV seropositivity and T-cell senescence (Spyridopoulos et al. 2016). These results are consistent with the earlier large National Health and Nutrition Examination Survey (NHANES) III which also determined that CRP and CMV seropositivity were risk factors for both all-cause and cardiovascular mortality (Simanek et al. 2011). An example that taking frailty or mortality as end-points can lead to opposite findings is also provided by the BELFRAIL study described above, where CMV seropositivity was negatively associated with frailty (Mathei et al. 2011). On the other hand, high anti-CMV titres, but not CMV serostatus per se, were associated with an increased risk for all-cause mortality (Mathei et al. 2015). The striking differences between the sexes in the BELFRAIL study also make clear that women and men need to be studies separately in this type of analysis. The Women's Health and Aging Studies (WHAS) I and II showed that IL-6 levels independently associated with prevalent frailty (Leng et al. 2007) and mortality at follow-up, currently under investigation.

16.8 Conclusions

Long-lived, free-living human populations are so heterogeneous in so many different ways that identifying robust immune biomarkers of ageing may well prove impossible. The same is likely to be true for any other biomarkers of ageing. Despite numerous attempts, little progress has been made in this respect ("While there are several candidates for biomarkers of aging, none have so far proven a true measure of the underlying aging process. A true biomarker of aging must meet certain criteria in order to be both accurate and useful …." see https://www.afar.org/infoaging/biology-of-aging/biomarkers-of-aging/), but the belief remains that collecting sufficient "big data" to cover large numbers of variables will eventually allow sufficient accuracy to be attained (Xia et al. 2017). As illustrated in this chapter, however, thus far, marked immune biomarker differences in several overtly similar European populations, suggest that establishing such biomarkers will remain a challenge for the foreseeable future. How and whether to intervene to modulate aged immune function in the elderly is therefore a crucial question, given this variability. A "one-size-fits-all" approach across different populations in different countries and of different ages is unlikely to be appropriate. Nonetheless, certain parameters do seem to be emerging as lowest common denominators in our and others' studies (Pawelec 2017) is that older people everywhere have low amounts of naïve CD8+ T cells and thus could have holes in their antigen recognition repertoires which might contribute to susceptibility to new pathogens. Developing technologies could theoretically identify such holes in the repertoire in advance and use receptor gene transfer to specifically compensate.

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