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Immune signatures associated with mortality differ in elderly populations from different birth cohorts and countries even within northern Europe

Graham Pawelec[a,b,*]

[a] Second Department of Internal Medicine, University of Tübingen, Tübingen, Germany
[b] Health Sciences North Research Institute of Canada, Sudbury, ON, Canada

ABSTRACT

Since associations between lifespan and certain simple immune parameters such as T cell proliferative responses to mitogens were reported in the 1970´s, efforts to dissect out immune parameters correlating with morbidity and mortality have sought to define factors predicting individual longevity. Such "immune signatures" associating with defined clinical outcomes would represent biomarkers of "immunosenescence" that might also provide mechanistic insights into the ageing process. Because appropriate immune function is necessary for a healthy old age, a better understanding of immunosenescence contributing to frailty and death might allow interventions to improve personal and public health. Here, we discuss data from our studies in several different European countries and document significant differences between overtly similar populations. These findings draw attention to the marked variation even between presumably quite homogeneous populations, which may be due to the different birth cohorts studied in addition to numerous other variables. Thus, immunological parameters, and presumably many other factors, are sensitive to context-dependent variation, making it currently difficult to extrapolate biomarkers of longevity from any one human population to another.

1. Introduction

Early studies on mitogen-induced in vitro T cell proliferation had already reported correlations with mortality in the 1970´s (Roberts-Thomson et al., 1974). A decade later, studies from Sweden extended these findings to include some simple parameters such as inversion of the peripheral blood CD4:8 ratio (caused by an accumulation of apoptosis-resistant weakly or non-proliferative CD8 + T cells) and relatively low numbers of peripheral B cells in 85-year-old subjects at baseline. These studies were continued in the 1990´s (the Swedish OCTO/NONA-Immune longitudinal studies) to establish a constellation of immune parameters informative for mortality 2, 4 and 6-years from baseline. We termed this immune signature the "Immune Risk Profile" (IRP) and showed that it was characterised by accumulations of late-stage differentiated CD8 + CD27-CD28- T-cells and excess seropositivity for Cytomegalovirus (CMV). These studies were relatively small and performed in a single center. The question arose as to how representative these results from (mostly female) 85-year-olds born around 1900 in the small town of Jönköping in southern Sweden might be for people from different European countries born at different times. This review briefly presents results from our own further studies in oldest old populations from the Netherlands (Leiden 85-Plus), and Belgium (BELFRAIL). The results reveal some common features of human "immunosenescence" but also many differences between studies.

2. Manifestations of "immunosenescence"

The immune system protects the individual against pathogens but is different in composition and function in younger and older people. Its altered properties with ageing, mostly reflecting remodelling and dysregulation (but dubbed "immunosenescence" as a catch-all designation (Pawelec, 2017)) are therefore believed to contribute to the greater susceptibility to infectious disease in older people and to their increased mortality relative to younger adults. In addition to compromised pathogen resistance, immunosenescence most likely contributes to the pathology of many non-communicable diseases including neurodegeneration, cardiovascular and autoimmune diseases, and cancer (Muller and Pawelec, 2015). Hence, it would not be unexpected to discover that immune signatures hold predictive power for all-cause mortality in human populations. However, this question has seldom been approached even in longitudinal studies seeking correlations between biomarkers and ageing such as the fascinating Dunedin study which identified a constellation of parameters informative already at early

* Correspondence to: Second Department of Internal Medicine, University of Tübingen, Tübingen, Germany.
E-mail addresses: graham.pawelec@uni-tuebingen.de, gpawelec@hsnri.ca.

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middle age (although it did at least look at leukocyte telomere length and C-reactive protein) (Belsky et al., 2015). Hence, many efforts have been initiated to seek biomarkers or constellations thereof ("immune signatures") for predicting morbidity and mortality and which might be potentially informative for understanding mechanisms of immune ageing. Unfortunately, the majority of such studies in humans is cross-sectional, not comparing the same individuals over time, and informative only for differences between currently older and younger populations. Even longitudinal ageing studies in animals are quite rare and suffer from obvious disadvantages of translation to humans. Most of the relatively few longitudinal studies taking mortality as an unequivocal end-point in humans have focussed on the very elderly where mortality can be documented in a sufficiently short period of time. The development of frailty or decreased responsiveness to vaccination may offer alternative endpoints in younger elderly if rigorously controlled, eg. by adopting objective measures of frailty (Fulop et al., 2010). Currently, some large-scale studies in humans are ongoing, and will hopefully also contribute some data on immunological variables in addition to the multiple other variables commonly assessed in these types of studies. One such study for which we already have baseline data is a very extensive study of 25–35 year-olds and 65-85 year olds in Berlin (BASE II) which is currently cross-sectional but will provide 5-year mortality data within the year (Bertram et al., 2014). However, these data are not yet available and the present review will focus on studies on elderly people from Sweden (Jönköping), Holland (Leiden) and Belgium (Flanders), considering cohorts of people > 85 years of age who might *a priori* considered to be from very similar populations.

3. OCTO/NONA-Immune longitudinal studies from the Swedish city of Jönköping

The OCTO/NONA Longitudinal Studies addressed several biobehavioural and medical questions but also included some simple immunological parameters in the 1980´s (Wikby et al., 1994). The OCTO study focussed on individuals born around 1900 who were selected for good health and therefore excluded the majority of 85-year-olds, but the later NONA study examined a representative population of (free-living) individuals, only one-third of whom would have been included in OCTO. The results of these studies led to the concept of an "immune risk profile" (Pawelec et al., 2001) which was in fact quite similar in both OCTO and NONA (Nilsson et al., 2003). However, this IRP was quite weakly associated with 2-, 4- and 6-year mortality; a pro-inflammatory serostatus and cognitive impairment was more predictive of mortality, but the presence of both risk factor clusters in the same person was much more strongly associated with incipient mortality (Wikby et al., 2005). The analysis of simple immune parameters at OCTO baseline showed a cluster of variables associating with all-cause mortality; these included increased absolute numbers of CD8 + T cells (resulting in an inverted CD4:8 ratio) and lack of or very low level responses to T cell mitogens, and also included reduced numbers of B cells (Ferguson et al., 1995). Interestingly, some individuals who had had a normal CD4:8 ratio at baseline exhibited an inverted ratio 2 years later and became less likely to survive until the next follow-up two years after that (Wikby et al., 1998). These dynamic changes were informative for survival of the study subjects that had either been in the IRP group at baseline (16%) or transitioned to it (15%) over the follow-up period of 8 years (Olsson et al., 2000). Some years later, in the NONA-immune study, somewhat more advanced immunological phenotyping showed that the CD8 + T cells that accumulated and were diagnostic for the IRP lacked CD27 and CD28 costimulatory receptor expression (Olsson et al., 2000). Because 100% of subjects in the IRP typed seropositive for antibodies against the human beta-herpesvirus Cytomegalovirus (CMV), whereas 80% of people not in the IRP group were infected with CMV, we hypothesized that the requirement to maintain immunosurveillance against the virus itself might have been contributing to many of the changes noted in the elderly. This idea had in fact first been put forward many years earlier in a paper suggesting that several of the phenotypic differences between young and old people were actually due to the increasing fraction of the population infected with CMV with age, and not an effect of age itself (Looney et al., 1999). Thus, many of the accumulated CD8 + T cells were found to be at a late stage of differentiation, characterised by expression of CD45RA in the absence of CCR7, CD27 and CD28, as well as the expression of CD57 and KLRG-1. These cells have short telomeres and proliferate poorly or not at all. They thus conform to several of the features of "senescence" but as is the case with replicatively senescent fibroblasts, they are not inactive. In fact, limited data from clonality assays indicated that retention of these cells by the individual may be crucial in that the loss of these clones from the periphery was associated with a reduced survival time (Hadrup et al., 2006). This would be consistent with sparse reports that CMV reactivation is more frequent in the elderly (Stowe et al., 2007). More frequent CMV reactivation might also be related to the slightly increased levels of some inflammatory mediators commonly observed in the blood of older people and referred to as "inflammaging" (Franceschi et al., 2000). As already mentioned, the IRP and inflammaging together with cognitive impairment were independent risk factors for NONA subjects´ survival (Wikby et al., 2006). Impressively, these risk factors remained robust in the face of several other states that would have been predicted to strongly influence survival, including Alzheimer´s Disease, cardiovascular diseases and type-2 diabetes. Clearly, the IRP relates to adaptive immune parameters, whereas inflammaging most likely reflects innate immunity. Thus, it would seem more likely than not that these variables will be different in different populations and will impact on mortality predictors in those specific populations. This expectation has been borne out by emerging results from a number of studies in other populations.

Because the early OCTO and NONA studies indicated that a simple IRP predominantly reflecting different frequencies of T cells and B cells, a very simple and broadly-applicable surrogate marker has been examined in a number of longitudinal studies in different countries. Thus, a British study including B-cell data concluded that the IRP did correlate with survival (Huppert et al., 2003). On the other hand, a Spanish study reported no association with 3-year survival (Formiga et al., 2014). Even some studies not focussing on the very elderly have reported that the IRP may be informative for survival (Plonquet et al., 2011), possibly even in HIV-infected patients (not just due to loss of CD4 + T cells (Ndumbi et al., 2015)). Obviously, stratifying populations strictly according to whether their CD4:8 ratio is below or above unity is a very blunt tool and might also need adjusting to the particular population studied. A case in point may be the British Newcastle 85-Plus study, where a CD4:8 ratio < 1.7 associated with survival, rather than an inverted CD4:8 ratio of < 1.0 (Spyridopoulos et al., 2016). Interestingly, a CD4:8 ratio > 4 was associated with mortality in the Newcastle 85-Plus study, which is reminiscent of the results in the Belgian BELFRAIL study (see below).

4. The Dutch Leiden 85-Plus study

As its name suggests, the Leiden 85-Plus study has been longitudinally examining many parameters in people from the age of 85 in Leiden (Lagaay et al., 1992). Underlining the importance of biobanking cryopreserved materials, we were privileged to obtain samples of peripheral blood mononuclear cells (PMBC) from a small number of the Leiden 85-Plus study subjects at 89 years of age for whom 8-year follow-up mortality data were already available. Thus, associations between parameters of interest and survival could be immediately established with no extended intervening waiting period. These individuals were of course already at a very advanced age of and none of them fell into the IRP category. This was not surprising, given that also in the Swedish OCTO/NONA study itself, none of the participants reaching 89 years of age exhibited an inverted CD4:8 ratio, probably

because of selection against those with an IRP (Strindhall et al., 2007). However, several other parameters were identified which did correlate with survival in these Leiden 85-Plus subjects. As in almost all published studies of human immune ageing, even from very early on in the field of immunosenescence research (Pawelec et al., 1997), the frequencies of naïve CD8 + T cells were very low in these subjects. Nonetheless, stratifying the cohort according to whether CD8 + naïve T cell frequencies of fell above or below the median did not reveal an advantage to possessing higher frequencies of naïve cells; in fact, survival was better in the group with below-median frequencies of naive CD8 + T cells (Derhovanessian et al., 2013). Thus, potential limitations to the range of new antigens that these individuals might be able to respond to (Goronzy and Weyand, 2005) was clearly not important for their survival and even seemed to be a hindrance in this particular population. What was associated with survival was an above-median frequency of the very cell type that accumulated in the OCTO/NONA subjects and put them in the IRP group. Thus, some of the very elderly in the Leiden 85-Plus study who had higher frequencies of late-stage memory cells were the ones with a significant survival advantage. This was only the case for individuals whose CD8 + T cells responded in vitro to dominant CMV antigens by producing pro-inflammatory factors (eg. TNF, IFN-γ) but not anti-inflammatory factors at the same time (eg. IL 4) (Derhovanessian et al., 2013). As in OCTO/NONA subjects close to the end of life (Hadrup et al., 2006), this implies that control of CMV is most important in these individuals and did impact on their survival. Another possibly counter-intuitive finding from the Leiden 85-Plus study was that the overall frequency of regulatory T cells (Tregs), which are said to be increased in older people, so inhibiting immune responses and leading to immunodeficiencies of ageing (Jagger et al., 2014) and thus contributing to increased morbidity and mortality, were also not associated with survival. Intriguingly, individuals with above-median frequencies of a subpopulation of Tregs bearing the chemokine receptor CCR4 (believed to be expressed by the most actively inhibitory cells) had better 8-year survival (Derhovanessian et al., 2015). It has to be noted that these Tregs were identified only by their phenotype, and so it remains possible that their function might have been compromised. Nonetheless, a positive association with the frequency of Tregs may imply that better functionality in the feedback mechanisms dampening down immune responses at old age is most important. It is also fascinating to note that Treg frequencies are rare amongst immune parameters by virtue of their not being affected by CMV. Hence one could speculate that the crucial accumulated pro-inflammatory CMV-specific CD8 + T cells required for continued effective CMV immunosurveillance must be counterbalanced by larger amounts of more active Tregs. This emphasizes a repeating theme in studies in ageing: namely, that it is the balance of opposing forces that determines outcome, rather than the absolute values of any single parameter taken in isolation.

5. The BELFRAIL study

BELFRAIL was set up to shed light on some of the factors influencing frailty in later life (Vaes et al., 2010). As often the case thus far, BELFRAIL was not designed predominantly as an immunological study, but after the first wave of recruitment, a substudy of > 200 subjects was included. Their PBMC were cryopreserved and then phenotyped for frequencies of immune cells. At first follow-up, immune parameters were correlated with the development of frailty, followed by an analysis of factors associating with 3-year mortality. Some quite disparate findings have begun to emerge, indicating that this population differs markedly from both the Swedish OCTO/NONA subjects and the Leiden 85-Plus subjects (Adriaensen et al., 2015). Here, a more direct comparison with the former was possible because many BELFRAIL subjects (who were < 89 years of age) did have a CD4:8 ratio of < 1. In OCTO/NONA, the proportion of subjects with a CD4:8 ratio < 1 was around 15% whereas it was only 7% in BELFRAIL. Again different to OCTO/NONA, many BELFRAIL subjects had a CD4:8 ratio > 5, amounting to nearly one-third of individuals; in contrast, this was seen in only a handful of Swedish elderly. The reason for the CD4:8 ratio being > 5 in BELFRAIL was an accumulation of naïve CD4 + T cells, not a decrease of CD8 + T cells. Surprisingly, subjects with a CD4:8 ratio > 5 were more physically frail, and had worse survival in the 3 year follow-up (Adriaensen et al., 2017). This is reminiscent of the findings from the Leiden 85-Plus study, where above-median levels of (in this case CD8 +) naïve T cells correlated with worse survival. The association of the higher CD4:8 ratio with poorer survival in BELFRAIL was seen solely in women; quite strikingly, the survival curves for men were completely unaffected by any CD4:8 ratio. Moreover, in BELFRAIL, it was the CMV-seropositive women with a CD4:8 ratio < 1 (that is, exactly the IRP subjects whose survival in OCTO/NONA was the worst) who had the best 3-year survival, in marked contrast to CMV-seronegatives who did worst (Adriaensen et al., 2017).

Table 1
Summary of cellular immune parameters associated with mortality in the three longitudinal studies OCTO/NONA, Leiden-85-Plus and Belfrail.

OCTO/NONA factors predicting 2-, 4- and 6-year mortality from baseline at 85 yr:
CD4:8 ratio < 1,
poor proliferative response to mitogens,
accumulations of CD27-CD28-CD8 + T cells
attrition of clonally expanded CMV-specific CD + T cells
fewer B cells
CMV-seropositivity
Excess neutrophils
Leiden 85-Plus factors positively associating with 8-year survival from baseline at 89 yr:
Accumulations of CD28- CD8 + T cells responding to CMV antigens
Accumulations of CD4 T cells with a regulatory T cell phenotype
Belfrail factors positively associating with 3-year survival from 85 yr (in women only)
CD4:8 ratio < 1
CMV-seropositivity
Belfrail factors negatively associating with 3-year survival from 85 yr (in women only)
CD4:8 ratio > 5
Accumulations of CD4 + naïve T cells
CMV-seronegativity

6. Conclusions

Despite the originally unexpected differences in immune parameters associated with survival of very elderly northern Europeans (Table 1), certain conclusions can be drawn. First and foremost is that some fairly simple immune characteristics do in fact correlate with incipient frailty and mortality, but that the correlations established for a particular population do not necessarily translate to a different population. This is the case even for the 3 overtly quite similar populations discussed in this paper. Many variables essentially impossible to control for may have been contributing to these differences, underlining the context-dependency of biomarkers that would be desired for predicting clinical trajectories like frailty and mortality. From a practical viewpoint, these findings do raise obvious questions concerning interventions aimed at restoring appropriate immunity. Merely noting that essentially all studies in humans have reported vanishingly small amounts of naïve CD8 + T cells in the elderly has spawned many efforts to consider how to revert these to “younger” status (Ventevogel and Sempowski, 2013); however, as seen in the Leiden 85-Plus data, this may provide no benefit or even a disadvantage, even more strikingly illustrated by BELFRAIL. Reciprocally, interventions directed at deleting accumulations of late-stage differentiated CD8 + T cells or depleting regulatory T cells (Jagger et al., 2014) might also be disadvantageous in some individuals. Hence, any attempts to “rejuvenate” immunity in the elderly would require an assessment of the specific requirements of the individual at a level which we are thus far not able to achieve. The

ultimate tailor-made intervention would require predicting which of the differences assessed in a particular individual, if any, would need to be reverted to the state seen in young people. This would also require extensive knowledge of the individual´s background characteristics. Thus, we can recognize that the BELFRAIL, Leiden 85-Plus, OCTO and NONA populations, despite all being northern European Caucasians of fairly similar age when studied, had quite different birth dates and their life expectancies at birth were quite different. This might well be expected to influence the risk factors for their survival in late life. At birth, the BELFRAIL subjects had a life expectancy of 51 - 57 years but this was only 46–49 years for OCTO born earlier in the past century or even in the 19th century in some cases (Adriaensen et al., 2017). Mortality rates (and hence selection pressures) were not the same in the different cohorts – namely, 30% in 3.3 years for BELFRAIL, 16% in 2 years for the Leiden 85-Plus and 26.5% in 2 years for OCTO. There could be many reasons for these differences - genetics, diet and microbiota, lifestyle, access to health care and exposure to epidemics, wars, pollution and any number of other environmental factors in these different birth cohorts and countries. Thus, the lesson here has to be that even seemingly very similar populations are actually quite different. Thus, accurately identifying IRPs in a prospective manner for individuals from different birth cohorts in different populations will be challenging or even intractable and therefore designing interventions to maintain or restore appropriate immunity in the elderly will be extremely difficult and potentially hazardous.

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