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Article *in* Advances in Cell Aging and Gerontology · December 2002

DOI: 10.1016/S1566-3124(02)13001-X

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The OCTO and NONA immune longitudinal studies: a review of 11 years studies of Swedish very old humans

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Contents

Advances in Cell Aging and Gerontology, vol. 13, 1–16

1. Introduction

From a societal and population perspective, the very old constitute the fastest growing age segment with compromised health and significant requirements for service and health care. Immune studies of elderly populations have mainly been conducted on individuals in their 60s and 70s. Few have focused on samples over 80 years of age and employed longitudinal designs. In the Swedish OCTO (Wikby et al., 1994; Ferguson et al., 1995; Olsson et al., 2000) and NONA (Wikby et al., 2002) immune studies, however, octo- and nonagenarians were deliberately focused upon using the longitudinal design. The main reason for the inclusion of very old individuals in these studies of the immune system is that oldest-old samples provide the potential for a model useful for detecting intra-individual change in a period in life with high probability for compromised health and morbidity. Changes in the immune system may provide presumptive predictors for subsequent mortality and clinical parameters related to the substantial morbidity/co-morbidity seen in late life. From a practical perspective, detection of predictive changes may enable clinical interventions that could assist in improvement of the quality of life for the individuals in this expanding population segment.

The OCTO immune longitudinal study is a population-based study of ageing and the immune system in a sample of Swedish octogenarians. It began in 1989 and ended in 1997. In 1999, the subsequent NONA immune longitudinal study of nonagenarians was initiated. This review summarises results from the OCTO study and the initial wave of the NONA. First, however, we address the significant design and sampling considerations that directed this research.

2. Methodological design and sampling considerations in ageing studies

2.1. Design considerations

The two methods used in studies of ageing in a population are the cross-sectional and longitudinal designs. The most common design is the cross-sectional, a method by which two or more age groups are compared at a single time of examination. Age changes cannot be measured directly but are inferred from the difference in mean values, observed in the different age groups. Caution is necessary in this interpretation, however, since age differences also reflect the fact that birth-cohorts may have been exposed to various environmental exposures and socio-cultural influences. Another confounding effect in cross-sectional studies is that of selective mortality. As a study population ages it becomes more and more selected, since deaths do not occur randomly in a population. If, for example, a low value in a variable is deleterious, death might occur first in individuals with low values and last in individuals with high values. In a cross-sectional study an observed difference in mean values between age groups may falsely be interpreted as a real age change rather than as an effect of selective mortality. Many studies have characterised changes in the immune system with age, but a number of these have yielded conflicting results. This may partly relate to the fact that a majority

of these studies are cross-sectional, limited by single measurements on different individuals.

In a longitudinal design, individuals are followed across time, usually with a number of years in between measurement occasions. This allows the detection of intra-individual change that minimises or overcomes many of the confounding artefacts likely to emerge from a cross-sectional design. Although the longitudinal design represents the superior alternative for conducting ageing research, the use of this design has been very limited, particularly in studies of the immune system. The main reason for objection to the longitudinal design is that such studies are expensive and require sustained effort, financial support, and commitment of personnel. In addition, longitudinal studies require careful coordination, standardised procedures, and control of studied panels to avoid dropouts. Also, there is a risk of confounding between age and time of measurement effects. Time of measurement confounding involves numerous factors, such as the motivation and interest of the subjects as well as experimenter effects including changes in personnel, in their motivation, and in the methods and techniques used across time. Many of these problems can be compensated for by including a younger group for comparisons across measurement occasions, since immune system changes across the limited time periods between these occasions will be negligible in healthy young people compared to the very old. Also, restricted time periods between the measurements and the use of identical methods will prevent time of measurement effects.

2.2. Sampling considerations

Advancing age brings increased disease problems. This is one of the primary problems in the selection and definition of a sample in population studies of ageing. To overcome this, most studies have used various selection schemes to exclude individuals with underlying diseases from participation in studies of the immune system. The stringent *SENIEUR* Protocol (Ligthart et al., 1984) represents an excellent example of a widespread application of a set of exclusion criteria used to select individuals in perfect health, to be able to distinguish between age changes caused by *primary* and *secondary ageing*, i.e. by diseases. The exclusion of *non-SENIEUR* individuals, however, will result in the study of less than 10% of all individuals aged over 80 years in a representative population (Pawelec et al., 2001). Another way to diminish confusion between ageing and disease has been to employ exclusion criteria tailored to the experimental situation (Hallgren et al., 1988; Wikby et al., 1994), i.e. in immune studies to exclude individuals that have immune-related diseases or who use drugs that affect the immune system. Such a strategy, however, will also generate a highly selected sample, since it will exclude about 50% of the individuals in a population aged over 80 years (Wikby et al., 1994).

A way to overcome these selection problems is to examine a population-based sample, combined with careful continuous evaluation of individual health parameters. The clinical variables needed for the evaluation of individual health and morbidity are then of considerable value in the comparison of findings from the application of various protocols and in the categorisation of individuals into

subgroups according to their health status (Nilsson et al., 2003). Thus, the significance of change in health status, an important consideration in these ageing studies, is included rather than excluded.

The establishment of specific markers for ageing, that are independent of morbidity and valid for the population, would be of great assistance in ageing studies. Such markers of ageing, being predictive of longevity, would represent the current status of the immune system independent of disease and, thereby, replace the need for the elaborate exclusion procedures using *SENIEUR* Protocol assessments (Pawelec et al., 2001).

3. The OCTO and NONA immune longitudinal studies

3.1. The OCTO immune study

The OCTO immune longitudinal study was an integrated part of the OCTO longitudinal study on bio-behavioural ageing, in Jönköping, Sweden. The municipality of Jönköping has 110,000 inhabitants and is situated in the south-central part of Sweden. The aim of the OCTO immune study was to explore age changes in the immune system in Swedish octogenarians relative to an array of medical, bio-behavioural, and social variables measured in the OCTO (Wikby et al., 1994).

3.1.1. Sample and design

Census data was used to identify octogenarians living in Jönköping and born in 1897, 1899, 1901, and 1903. A non-proportional sample composed of 100 persons in each of the birth-cohorts was recruited. From these 400 individuals, 324 were examined in the first wave in 1987/1988 of the OCTO study. The persons were then at the ages of 84, 86, 88, and 90 years. At the second wave of the study, the OCTO immune longitudinal study was initiated. Of the 324 examined at baseline of the OCTO, 96 were deceased before the start of the second wave of this study. Another 15 declined to participate, giving a total number of potential participants of 213 for the OCTO immune.

Exclusion criteria were set to diminish confound between ageing, disease, and medications and to secure reliable psychosocial self-reports. Potential candidates were included if they:

- were non-institutionalised;
- had normal cognition according to neuropsychological tests (Johansson et al., 1992);
- were not on a drug regimen that may influence the immune system.

These exclusion criteria were similar to those of Hallgren et al. (1988). Of the potential 213 individuals, 110 met the inclusion criteria. Of these, 102 individuals participated in the first wave. Sixty-nine individuals were available throughout the three waves in the longitudinal analysis and 23 participated in the longitudinal analysis over all four time-points, T1 (1989), T2 (1990), T3 (1991), and T4 (1997) (Table 1). Non-participation at the various measurement occasions was mainly due to mortality in the sample.

Table 1
Characteristics of the subjects included in the OCTO Immune Longitudinal Study

Occasion (time)	Year	Number of subjects investigated	Age (years)	
			Mean	Range
1	1989	102	88	86–92
2	1990	83	89	87–93
3	1991	69	90	88–94
4	1997	23	95	94–100

Fourteen healthy middle-aged volunteers (39 years SD ± 5.8) of men and women working in the laboratories at Ryhov Hospital in Jönköping were included across the measurement occasions for comparative reasons.

3.1.2. Immune components

The very old individuals were examined in their place of residence. Blood samples were drawn in the morning between 08:00 and 10:00 h. The following immune system parameters were investigated:

- Complete blood cell count.
- Differential WBC count.
- Antibody defined T and B cell surface molecules using three colour flow cytometry.
- Proliferative response of PBMC using a mitogen stimulation assay with ConA in cell culture.
- Interleukin 2 production.
- Cytomegalovirus (CMV) and Herpes simplex serology.

3.2. The NONA immune study

Findings in the longitudinal OCTO immune study constituted the background for the subsequent ongoing NONA immune study of nonagenarian individuals living in the municipality of Jönköping. The NONA immune is an integrated part of the NONA longitudinal study initiated to examine the disablement process in late life. The overall aim in the NONA immune is to examine predictive factors for longevity in the very old and to further investigate in greater depth the immune risk profile identified in the OCTO immune. The aim is also to consider immune data in the context of functional and disability parameters examined in the overall NONA. The overall study includes measurements of the following functional and disability domains:

- physical and mental health,
- cognitive functioning,
- personal control/coping,
- social networks,
- provision of service,
- care and everyday functioning capacity.

The NONA immune is also part of the EU-supported program *Immunology and Ageing in Europe*, with collaborations between the NONA immune project and several laboratories participating in this Thematic Network (Pawelec, 2000).

3.2.1. Sample and design

NONA immune is a population-based random sample with no exclusion criteria. Individuals were drawn from the population (census) register of Jönköping. A non-proportional random sampling procedure was employed, including all individuals permanently residing in the municipality, with the goal to have individuals aged 86, 90, and 94 years old. The sampling frame was defined from the census information available in September 1999. As the number of available subjects in the oldest birth cohort was limited, a few subjects were also included from the birth cohorts of 1904 and 1905. Blood samples for the immune system analysis were drawn in 138 individuals, of whom 42 belonged to the oldest birth cohort, 47 were 90-years old, and 49 86-years old.

The mean age of the sample was 90.3 years with a total proportion of women of 70%. While about 60% of them lived in ordinary housing, 40% resided in sheltered housing or in an institution. A comparison between individuals who participated in the in-person testing part of the NONA study ($n=157$), and those who agreed to have blood was drawn ($n=138$), indicated no significant differences for demographics or overall ratings of physical and mental health.

3.2.2. Immune components

Subjects were examined in their place of residence. The blood samples were drawn in the morning between 09:00 and 10:00 h. The following immune and clinical components are studied in the first wave of the NONA immune longitudinal study:

- Complete blood cell count.
- Differential WBC count.
- Proteins, albumin, transthyretin, C-reactive protein, orosomucoid, haptoglobulin, IgG, IgM, IgA, urea, cystatinC, creatinine as indicators of malnutrition, inflammation or kidney disease.
- Antibody defined T cell surface molecules of T, NKT, NK cell populations, using three colour flow cytometry.
- Secretion of cytokines.
- TCR clonotype mapping with Denaturing Gradient Gel Electrophoresis (DGGE).
- CMV and Herpes simplex serology.
- DNA damage and defence in PBMC, comet assay to detect damage, Ferric Reducing Ability of Plasma (FRAP) to measure antioxidant capacity.
- Mt-DNA damage, PCR methodology to detect and quantify levels of mt-DNA^{4977} and heteroduplex Reference Strand Conformational Analysis (RSCA) to analyse the accumulation of point mutations.
- MHC/peptide tetramers to analyse the number of CMV- and EBV-specific CD8+ cells.

4. Results and discussion

4.1. Immune parameters and mortality

Deterioration of the immune system with ageing is believed to contribute to morbidity in humans due to a greater incidence of infections, autoimmune disease and cancer (Pawelec et al., 1999). The relationship between immune parameters and mortality in the OCTO immune study has been reported in two studies (Ferguson et al., 1995; Wikby et al., 1998), indicating that no single immune parameter could predict 2-year survival. Multivariate cluster analysis, on the other hand, allowed the identification of a cluster of immune parameters constituting an "immune risk" phenotype (IRP). This was composed of a combination of a low number of CD4 cells, a high number of CD8 cells, poor T cell proliferation and low IL2 production. The IRP predicted subsequent 2-year mortality using immune data both at baseline in 1989 and two years later (Table 2). Whether these findings can be generalised also to other populations will be tested in the broader-defined NONA immune sample, although the IRP was shown to be independent of the individual's health condition (Nilsson et al., 2003).

The relationship between a reduced immune functional response and mortality has also been described in several previous studies. In humans, Murasko et al. (1987) reported poor responses to three T cell mitogens, Con A, phytohemagglutinin and pokeweed, to be associated with increased mortality in old individuals. In a study of octogenarians, Roberts-Thomson et al. (1974) found that anergy was predictive of 2-year mortality. Old subjects that were anergic had a 2-year mortality rate of 80% as compared with 35% in subjects that were not anergic. An association between anergy and morbidity might have confounded these results, however, which was not taken into account. Wayne et al. (1990) reported, on the other hand, that anergy is associated with higher mortality rates even in healthy people older than 60 years.

4.2. Immune parameters and morbidity

The prevalence and incidence of diseases were examined in the NONA immune by a detailed evaluation of health and morbidity information (Nilsson et al., 2003).

Table 2
Chi square analysis of 2-year survival/non-survival in IRP/non-IRP octogenarians

Survival	IRP	Non-IRP	$P <$
1989–1991			
Survivors	5	59	0.001
Non-survivors	9	16	
1991–1993			
Survivors	9	34	0.05
Non-survivors	9	11	

The examination allowed a comparison of findings from the application of a modified SENIEUR protocol (Ligthart et al., 1984) with results using the exclusion criteria of the OCTO immune study (Wikby et al., 1994; Hallgren et al., 1988). The protocols were applied by the use of medical records, self-reports, laboratory data and information about medication usage. The modified SENIEUR protocol excluded more than 90% of the NONA immune sample. The use of the original protocol, suggesting additional laboratory analysis for exclusion, would probably have excluded even more individuals, demonstrating the need for use of broader criteria in studies of the immune system. The OCTO immune criteria excluded almost 65% of the initial sample compared with 48% in the previous OCTO immune study by use of these criteria (Nilsson et al., 2003).

A step-by-step exclusion procedure was applied beginning with the criterion that reduced the sample most, continuing with the next and so on in decreasing order (Nilsson et al., 2003). Applying the five most common exclusion criteria, cardiac insufficiency, medication, laboratory data, urea and malignancy, the modified SENIEUR protocol excluded 120 of the original sample (87%). When the OCTO immune protocol was applied, medication was found to be the most common criterion, excluding 59 (43%), institutionalisation the second, excluding 54 (39%), and cognitive dysfunction the third, excluding 20 (14%).

The application of the protocols allowed us to define three independent subgroups, very healthy (SENIEUR, $n=13$), moderately healthy (non-SENIEUR/OCTO immune, $n=38$), and frail (non-SENIEUR/non-OCTO immune, $n=87$). Noteworthy, a comparison of the number of T cells across these subgroups indicated no group differences for the IRP (Nilsson et al., 2003), previously identified in octogenarians (Ferguson et al., 1995; Wikby et al., 1998). The IRP might thus serve as a marker of ageing, independent of the individuals' state of health. This is compatible with results in non-inbred mouse populations, showing that clusters of immune markers can predict longevity in old as well as middle-aged individuals independently of the health condition of the animals (Miller, 2001).

4.3. Changes in T-lymphocyte sub-populations

A primary advantage of the longitudinal design is that it enables us to estimate age changes over a specific time period for any specific variable. In the OCTO immune, octogenarians (102 at baseline) were followed for 8 years from T1 in 1989, though T2 1990, T3 1992 and T4 1997. At baseline 14 individuals (14%) with an IRP pattern were identified using cluster analysis. Two years later another 12 individuals (12%) with this immune profile were identified by cluster analysis (Wikby et al., 1998). The new individuals were recruited into the subgroup at-risk due to a significant increase in the CD8+ T lymphocyte levels and simultaneous decreases in the levels of CD4+ cells. Only minor changes in the total number of T cells (CD3+) were found across the 2-year period of time indicating that there were progressive homeostatic changes occurring in the T cell compartment of these octogenarians.

At T4 in the OCTO immune an inverted CD4/CD8 ratio was used to identify individuals at risk (Olsson et al., 2000). The inverted ratio is closely associated

with the IRP. The number of individuals in the sample ($n = 23$) was too small at this time to allow the use of multivariate cluster analysis. The CD4/CD8 ratio can also be used in an evaluation of the immune system status in a number of clinical situations.

An increasing fraction of individuals with a CD4/CD8 ratio less than 1 was continuously recruited over time. At T1 16%, at T2 17%, at T3 23% and at T4 27% of the individuals had an inverted CD4/CD8 ratio. Of the octogenarians followed longitudinally from T1 in 1989 to T4 in 1997 32% originally had (14% prevalent cases at baseline) or developed (18% incidental cases) an inverted CD4/CD8 ratio (Olsson et al., 2000). All the individuals that once moved into the category with an inverted CD4/CD8, never moved back to normal values. They remained in the IRP category until their death.

At T1 of the NONA immune the fraction of individuals with an inverted CD4/CD8 ratio was about 20% (Fig. 1), which is compatible with the proportions found on different occasions in the OCTO immune (Wikby et al., 2002).

Studies of variations in the CD4/CD8 ratio among healthy adults have indicated that about 5% of subjects have an inverted ratio (Amadori et al., 1995). As much as 57% of the variation was under genetic control. Prevalence of 14–27% at different measurement occasions in the OCTO and NONA studies are therefore too large to be explained only by means of normal genetic distributions of these Swedish populations. The findings of 18% incidental cases across the 8 years in the OCTO support this view. Rather, the results suggest other factors affecting very old individuals and influencing the balance between the CD4 and CD8 T cell subsets. While this change in balance involved significant increases in the CD8+ level with CD4+ level being concurrently deceased, the total T cell level (CD3+) remained unchanged. This suggests the T cell homeostasis model is operating in very old individuals.

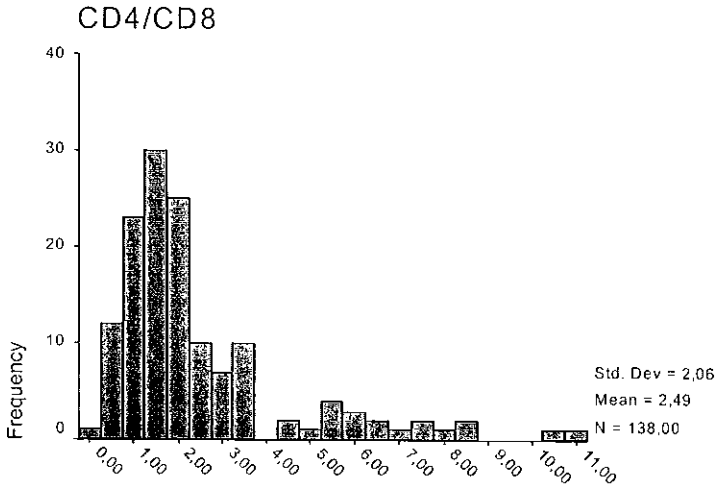

Fig. 1. The frequency of nonagenarians (at baseline) versus the CD4/CD8 ratio. (Reprinted from Wikby et al. (2002), with permission from Elsevier Science.)

According to this model an increase in the number of CD8+ cells is accompanied by a decrease in the number of CD4+ cells keeping the total number of T lymphocytes at a relatively constant level (Mittler et al., 1996). Such a loss in the T cell homeostasis may be associated with age and thymic involution.

4.4. *T lymphocyte sub-populations and chronic viral infection*

More detailed analysis of presumptive factors affecting the CD4/CD8 balance in octo- and nonagenarians indicated that nutritional disorders, medications, inflammatory diseases or cancer were not associated with IRP (Pawelec, 2000). At T4, 8 years after the initiation of the OCTO immune study, however, an association was found between CD4/CD8 ratio, lymphocyte activation and the level of CMV-IgG antibodies in plasma, indicating evidence of a persistent CMV infection (Olsson et al., 2000). No association with persistent Herpes simplex virus infection was found, similar to the findings of Looney et al. (1999). The results were confirmed and even more evident at baseline in the larger NONA sample (Wikby et al., 2002). The prevalence of CMV-IgG antibodies among octo- and nonagenarians was about 90%, significantly greater than for middle-aged (about 60%). The results also indicated that the very old individuals with an inverted CD4/CD8 ratio are unique, having a CMV prevalence of 100%, while those in the range of 1–4 had a prevalence of 90% and those with ratios greater than 4 only 55% (Wikby et al., 2002). Very old individuals with an inverted CD4/CD8 ratio were also characterised by profound T cell changes apparent in a number of T cell subsets, particularly by increases in the number of CD8+CD57+CD28− and CD8+CD45RA+CD27− cells, indicating T cell activation (Table 3). These changes were comparable with findings by Merino et al. (1998), reporting that the expansion of CD8+ CD57+CD28− T cells in the elderly is dependent both on age and CMV status. Similar results have been found by others (Fagnoni et al., 1996; Nociari et al., 1999),

Table 3
CD3+CD8+CD4−, CD3+CD8−CD4+ and CD8+ lymphocyte subsets (numbers of cells/mm^3) in nonagenarians at baseline categorised by their CD4/CD8 ratios and compared with middle-aged[a]

Subset	Very old with a CD4/CD8		Middle-aged ($n=18$)
	Less than 1 ($n=24$)	Greater than 1 ($n=114$)	
CD3+CD8−CD4+	551 ± 41[b,d]	729 ± 32	863 ± 78
CD3+CD8+CD4−	850 ± 68[c]	338 ± 19	387 ± 56
CD8+CD57+CD28−	494 ± 50[c]	212 ± 14	138 ± 37
CD8+CD45RA+CD27−	462 ± 66[c]	197 ± 12	140 ± 43
CD8+CD45RA+CDRO+	330 ± 41[c]	145 ± 9	132 ± 24
CD8+CD57+CD56+	221 ± 45[c]	105 ± 9	53 ± 14

[a] Reprinted from Wikby et al. (2002), with permission from Elsevier Science.
[b] Mean ± SE.
[c] $P < 0.001$ compared to other subgroups.
[d] $P < 0.01$ compared to middle-aged.

demonstrating at a single cell level that these cells proliferate poorly and produce mainly IL4, IL10 and IFN-gamma associated with ageing.

A significant increase in the CD8+ cell compartment in very old individuals with an inverted CD4/CD8 ratio may reflect an attempt to counteract disease problems, such as the CMV infectious process, by generation of clones of protective effector cytotoxic cells. Exposed to a recurrent or persistent viral antigen, like CMV, CD8+ cells lose their CD28 expression with up-regulation of the CD57 cell activation marker. Several authors have reported that clonal expansions in the CD8+CD57+ and CD8+CD28− subsets occur and persist in old individuals (Wang et al., 1995; Schwab et al., 1997; Mugniani et al., 1999; Posnett et al., 1999; Weekes et al., 1999a,b).

Preliminary T cell clonotype mapping, using the DGGE procedure was done in 11 NONA immune individuals by thor Straten et al. (unpublished results) using specific primers covering the TCRBV 1–24 variable regions. The map covers the vast majority of T cells (thor Straten et al., 1998). Significantly greater numbers of distinct bands on the gel, indicating clonal expansion, were obtained in individuals with an inverted CD4/CD8 ratio compared to those with a normal ratio, for whom distinct bands were found to be rare. Interestingly, the number of bands correlated with the number of CD8+CD57+CD28− cells (Wikby et al., 2001).

In vitro studies of the role of the CD28 marker on CD8+ cells have convincingly demonstrated that repeated antigen-induced T cell division leads to a state of T cell senescence with irreversible cell cycle arrest, shortened telomeres, undetectable telomerase, and down-regulation of CD28 expression. These results indicate immune exhaustion (Effros, 1997, 2000). These "senescent" CD8+CD28− T cells, however, show an increased resistance to apoptosis and to retain good functional antigen-specific cytotoxicity (Spaulding et al., 1999).

4.5. The CD3+CD8+ phenotype associated with IRP

In the NONA immune study an extended panel of surface antigen markers was used which enabled simultaneous analysis of several T cell phenotypes. It was found that the predominant phenotypes of the CD3+CD8+ cells, associated with the inverted CD4/CD8 ratio as well as with persistent CMV infection, were CD27−, CD28−, CD56+, CD57+, CD45RA+ and partly double-labelled CD45RA+RO+ cells, representing late differentiation stages of highly overlapping populations (Wikby et al., 2002). The high frequency of the CD45RA+ population is not surprising since the use of the CD45RA/RO marker system seems inadequate to differentiate naive and memory cells. It has been shown that in the differentiation of the CD45RO+ memory marker, cells revert to CD45RA+ (Okumura et al., 1993; Roederer, 1995). The presence of a double-labelled population suggests a transitional double-positive state that may relate to the CD8+ population changes and cell activation.

Recent data suggest that human effector CD8+ express a CD27−CD28− CD45RA+CD57+ phenotype (Pittet et al., 2000). The data also indicated that effector function correlates even better with CD56+ surface expression. Dramatic clonal expansion was confined to this CD56+ subset and found to be associated

with CTL effector function. This conclusion was based on findings of high amounts of intracellular perforin and granzyme B (Pittet et al., 2000). It has also been proposed that the majority of these "effector/senescent" cells are the results of chronic activation (Tarazona et al., 2000). In addition, Pittet et al. (2000) reported a reduced lytic capacity of these cells in vivo, which prevents damage to tissue cells by the presence of specific NK receptors capable of inducing inhibitory signals. Baars et al. (2000) showed that these NKT cells express both Ig-super family and C-type lectin classes of NKR. They suggest that binding may cause inhibition of the activation in the cytolytic machinery. Future studies will examine the possibility whether this suppression is important in individuals with a reduced CD4/CD8 ratio.

4.6. MtDNA damage, DNA damage, antioxidant capacity

DNA, mt-DNA damage and antioxidant capacity of T cells have the potential to be significant contributors to the age-related homeostatic CD4/CD8 changes in the T cell subsets and functions found in the OCTO and NONA studies. To address this question, two studies were performed in the NONA immune sample, comparing nonagenarian subjects with the middle-aged control samples (Hyland et al., 2002; Ross et al., 2002).

Hyland et al. (2002) investigated the antioxidant capacity of plasma using the FRAP assay as well as the levels and types of DNA damage using the alkaline comet assay in peripheral blood mononuclear cells (PBMCs). An increase in the levels of oxidative DNA damage was previously demonstrated when T cells grow older in cell culture (Barnett et al., 1999; Hyland et al., 2000). An increased DNA damage accumulation in the T cells will result in T cell cycle arrest and the prevention of T cell replication, contributing to the progression of immunosenescence. Results from NONA immune indicated significantly higher plasma antioxidant capacity in NONA subjects and similar levels of DNA damage of PBMC, as compared with the middle-aged controls (Hyland et al., 2002). This suggests a relationship between longevity and an intact immune function of NONA T cells, underpinned by an elevated antioxidant defence. There was no association, however, between these results and the homeostatic T cell changes previously identified in the OCTO and NONA immune studies. This supports the view that the T cells from NONA subjects, including those with substantial increases in the CD8+, CD27−, CD28−, CD56+, CD57+, CD45RA+ NKT cell phenotype, represent "non-senescent" cells that maintain proper function.

Studies of mitochondrial DNA damage of lymphocytes of the NONA immune study were performed by Ross et al. (2002). The mitochondria play significant roles in apoptosis and energy production processes and any changes in their functions are, therefore, believed to be of importance for the T cell function and, thereby, immunosenescence. A competitive polymerase chain reaction (PCR) methodology was used to evaluate the level of $mtDNA^{4977}$ in addition to a novel heteroduplex RSCA technique to study the accumulation of point mutations with age. The $mtDNA^{4977}$ was detected at very low concentrations in all NONA samples, independently of the individual's age. No accumulation of point mutation was detected.

The low level of mt-DNA damage and absence of age association, support the idea that a vast majority of T cells are still able to replicate rather than being senescent (Ross et al., 2002).

5. Conclusions and future direction

Large clones of CD8 T cells are frequently found with age in both humans and mice. In apparently healthy adults these clonal expansions increase linearly with age (Ricalton et al., 1998). In mice most cells in the clones are in continuous slow division independent of antigenic stimulation (Ku et al., 2001). It has been suggested that the clonally expanded T cells compete with normal CD8 cells, using IL-15 more effectively or resist the inhibitory effects of IL-2, thereby affecting the immune response. Along with these increased clonal expansions, immune senescence has been characterised by thymic involution and a decrease in naïve T cell production resulting in a progression that may compromise the immune capabilities of the elderly. As suggested by Lemaoult et al. (2000), repeated cycles of clonal selection and expansion in a non-renewing T cell population expectedly would be predicted to alter the peripheral lymphocyte population, including its structural integrity and functional responsiveness. Ultimately the consequences are a loss of diversity and immune protective capabilities in the aged. The results of the OCTO and NONA immune studies suggest that the latter, in fact, is occurring selectively and dramatically in the expanded CD8 T cell population(s) described in the individuals with the IRP.

The ongoing NONA longitudinal immune study is examining factors underlying the dysregulation of homeostasis in the peripheral T cell compartments. Establishment of the clonality of the CD8 cells is expected to better characterise the T cell changes and to provide pathways to examine the factors driving this expansion in the IRP individuals. This along with future cytokine profiling will provide important information on the nature of the expanded cells and their functional capabilities. The analyses of relative relationship to pathogens, particularly of CD8+ T cell responses to latent pathogens such as CMV using tetramer staining (Ouyang et al., 2003), should provide supporting evidence that previously controlled factors could drive the observed T cell changes in the increasingly compromised immune system of the elderly.

In addition to cutting edge immunology studies, future research, also, must be multidisciplinary and include provision of more detailed psychosocial and medical clinical evaluation of the affected individuals in comparison to those elderly individuals with lower risk profiles. The immune alterations may only be a reflection of other significant stress-related factors that may be different, but ultimately result in similar immune system changes. Only carefully controlled longitudinal studies which are not selectively exclusive will provide the information necessary to elucidate the mechanism or mechanisms underlying these important age-associated changes, as well as their ultimate health related effects. Omitting this important additional information will result in ineffective practical approaches to not only understanding, but also improving the health and well-being of ageing individuals. The components

of the ongoing NONA study have been designed to consider, on a broader basis, potential factors impacting upon the latter.

Acknowledgements

The authors acknowledge the support from the Research Board in the County Council of Jönköping and the Research Council in the Southeast of Sweden (FORSS) for funding these projects. We also acknowledge Länsjukhuset Ryhov for provision of laboratory resources for the completion of these studies. The authors are also indebted to our co-workers Sture Löfgren, Bengt-Olof Nilsson, Jan Ernerudh, Jadwiga Olsson, Jan Strindhall and Per-Eric Evrin for their important contributions to these studies. We particularly would like to thank the nursing staff including Annica Andersson, Inga Boström, Gerd Martinsson, Agneta Carholt, Lene Ahlbäck, Lena Blom, Monica Janeblad and Lena Svensson for their efforts in obtaining the blood samples used. We are also indebted to Roberta Valeski, Florence Confer, Margaret Kensinger, Penn State University, United States, and Andrea Tompa, Gunilla Isaksson, Inger Johansson, Cecilia Ottosson, Helen Olsson, Lisa Stark Jönköping, Sweden, for secretarial and technical assistance. We finally acknowledge our ImAginE collaborators, Graham Pawelec and Qin Ouyang, University of Tubingen, Germany, Yvonne Barnett, Paul Hyland, Owen Ross and colleagues, University of Ulster, Northern Ireland, Rosalyn Forsey, Jonathan Powell and Julie Thompson, Unilever, UK, and Per thor Straten, Danish Cancer Society, Copenhagen, Denmark, for successful co-operation.

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