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Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study

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Abstract

Results from the previous times (Times 1–3) of the Swedish longitudinal OCTO immune study indicated that a combination of high CD8 and low CD4 percentages and poor T-cell proliferation in PBL was associated with a higher 2-year mortality in a sample of very old Swedish individuals. The combination of immune parameters was closely related to an inverted CD4/CD8 ratio. In the present study at Time 4 (T4) results are reported from the final follow-up of this longitudinal study, 8 years after it was initiated in 1989. An additional goal at this time point was to examine the immune system alterations in the very old in relation to evidence of lymphocyte activation and cytomegalovirus antibody status. In the present study immune system changes were identified that suggest a loss of T-cell

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homeostasis, as reflected by a decrease in the number of CD4 cells and a very significant increase in the number of CD8 cells in individuals with an inverted CD4/CD8 ratio. When considered over the duration of the OCTO study the inversion occurred in a high percentage (32%) of the individuals included in the original sample and was associated with non-survival. At T4 the changes were apparent in a number of the T-cell subsets, but particularly in the $CD8^+CD28^-$ and $CD57^+$ subsets. T-cell activation was significantly associated with the inversion of the CD4/CD8 ratio. In this very old sample the subset alterations were associated with evidence of cytomegalovirus (CMV) infection.

Keywords: Aging; T-cell; CD4; CD8; Cytomegalovirus; Activation

1. Introduction

In aged humans decreases in CD4 peripheral blood T-lymphocytes have been reported by a number of studies (Smith et al., 1993; Calderon et al., 1995; Belmin et al., 1996; Ogata et al., 1996; Rea et al., 1996). In a sample of very old Swedish individuals, as a part of a longitudinal investigation which began in 1989, our laboratory first reported that low CD4 and high CD8 peripheral blood lymphocytes (PBL) were important components of a group of immune parameters associated with decreased immune function and 2-year mortality (Ferguson, 1995). At Time 1 (T1) in 1989 none of the individual immune parameters were significantly associated with non-survival in a relatively small sample of 86–92-year olds ($n = 102$). Using multivariate cluster analysis a combination of low CD4, high CD8 and poor T-cell proliferation was related to 2-year mortality. In 1993, examining the same sample longitudinally, we confirmed our results, again associating non-survival with changes in the combination of immune parameters, which were also found to be associated with an inverted CD4/CD8 ratio (Wikby et al., 1998). At Time 4 (T4) in 1997, the inverted CD4/CD8 ratio was used to identify individuals being at risk for non-survival, since the number of individuals in the sample ($n = 23$) was too small for the use of multivariate cluster analysis. At T4 additional individuals moved into the subgroup characterized by an inversion of the CD4/CD8 ratio.

The assessment of the PBL CD4/CD8 ratio enables an evaluation of the immune system status in a number of clinical situations. The inversion of the normal PBL 1.5–2.5 ratio is suggestive of genetically determined differences or immune dysfunction. In particular, HIV infection has given prominence to the importance of this determination (Christensen et al., 1996). Besides HIV, the ratio is also useful in other clinical evaluations of the immune system, such as during acute viral diseases, including cytomegalovirus (CMV) infection (Labalette et al., 1994), as well as for allograft rejection (Gorochov et al., 1994), hemophilia (Hassett et al., 1993), malnutrition (Chandra, 1983; Mazari and Lesourd, 1998) and chronic immune system activation (Kern et al., 1999). Thus this ratio has become a fundamental parameter that is increasingly considered in clinical evaluations.

The pattern of PBL changes reported in the OCTO immune study was similar to those seen in a number of these clinical conditions. In certain viral diseases, such as

CMV infection, the progression of the disease can be followed by lymphocyte subpopulation alterations and their relative activation status of the cells, as determined by phenotypic surface markers (Wang et al., 1993). In particular, these changes have been associated with alterations in the CD3CD8 lymphocyte subpopulations. The changes characteristically include increases in the $CD57^+$ and the $CD28^-$ subpopulations in asymptomatic individuals (Wang et al., 1993; Kern et al., 1996; Mugniani et al., 1999).

In addition, there is an increasing number of reports that describe T-cell activation as a component of the immune system changes in aging humans (Xu et al., 1992; Sansoni et al., 1993; Pawelec et al., 1997; Rea et al., 1999). Studies have associated the changes in the number of lymphocytes expressing activation markers both with age and with CMV antibody titers (Weymouth et al., 1990; Born, 1995; Ruiz et al., 1995; Merino et al., 1998; Looney et al., 1999). The results of several studies suggest the balance in the immune system of the elderly is disturbed when viral activation occurs during the carrier state of CMV infection (Musiani et al., 1988; Looney et al., 1999).

Because of the unusual pattern of progression of PBL cell numbers and T-lymphocyte changes associated with non-survival of the very old in our studies, with our fourth (T4) longitudinal evaluation of this elderly population we examined additional lymphocyte phenotypic markers, using three-color flow cytometry analysis. The additional markers included those determining RA and RO, the CD3 costimulatory marker, CD28, and the activation markers CD57 and HLADR. The relationship of these lymphocyte phenotypic markers to evidence of an antibody response, indicating prior CMV infection, was evaluated at T4 in the longitudinal Swedish OCTO immune sample of the very old.

2. Materials and methods

2.1. Subjects

The sample of elderly was drawn from a panel of individuals in the longitudinal OCTO study on biobehavioral aging at the Institute of Gerontology in Jönköping, Sweden. Individuals born in 1987, 1899, 1901, and 1903 were selected for the immune substudy when they were aged 86–92 and included if they: (1) were non-institutionalized; (2) had normal or only mild cognitive dysfunction, according to neuropsychological tests (Johansson et al., 1992) and (3) were not on a drug regimen known to affect the immune system (Hallgren et al., 1988). One hundred and ten met the inclusion criteria at the time the original sample was identified (Ferguson, 1995). Seven individuals declined to provide a blood sample and in one case there was a technical problem with sample collection, providing 102 individuals, 86–92 years old, at the initiation of this study. Of these, 23 subjects participated in the longitudinal analyses through the four time points at T1 (1989), T2 (1990), T3 (1991) and T4 (1997). Non-participation at the follow-up measurement occasions was mainly due to mortality in the sample. At T4 a thorough clinical

examination of the individuals in the sample was done. Results, including measures of serum pre-albumin and albumin levels, body mass index, the triceps skin fold test, and measurement of the upper arm circumference, indicated no differences between the individuals categorized into two groups based on their CD4/CD8 ratios. No evidence of malnutrition was found in either group of the very old in the sample. A healthy middle-aged sample ($n = 15$), 40–60 years old, of men and women working in the laboratories at the Ryhov Hospital in Jönköping was included as controls.

The very old subjects were examined in their place of residence either in ordinary housing or an institutional setting. The blood samples were drawn in the morning between 08:00 and 10:00 h (Wikby et al., 1994).

2.2. Preparation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells were isolated from heparinized venous blood by density gradient centrifugation on Ficol-Isopaque (Lymphoprep®, Nycomed Diagnostika, Oslo, Norway). The PBMC were washed three times in Dulbecco's PBS (D-PBS) and resuspended in RPMI 1640 (Flow Laboratories, Sweden), supplemented with 10% inactivated (56°C, 30 min) fetal calf serum (Flow Laboratories, Sweden), 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin (Gibco, Sweden). Isolated PBMC counts, differential counts, white blood cell counts, complete blood cell counts and examination of the whole blood were performed as previously described (Ferguson, 1995).

2.3. Flow cytometric analysis

Monoclonal antibodies (Mabs), including appropriate isotypic controls, were purchased from Becton Dickinson (Stockholm, Sweden). The data was acquired using FACScan (Becton Dickinson Immunocytometry System (BDIS), Jönköping, Sweden) and analysed using CELLQuest software. The staining protocol for three-colour staining is presented in Table 1. In tube 1 (BDIS) TriTEST Control IgG1FITC/IgG1PE/CD45PerCP was used to create an analysis gate to include CD45-positive lymphocytes in FL3 (CD45PerCP) versus SSC and to set the fluorescence quadrant markers on the FL1 versus FL2 to detect the presence of any non-antigen-specific antibody binding (non-specific staining). In tube 2 (BDI) TriTEST CD4 FITC/CD8PE/CD3PerCP was used to create a gate set on the CD3 positive lymphocyte fraction and to adjust compensation on FL1 versus FL2. As recommended by BDIS, quality of control was performed using daily CaliBRITE beads and FACSComp software for setting the photomultiplier tube (PMT) voltages, the fluorescence compensation and checking instrument sensitivity prior to use. Internal quality control was performed to check consistency for CD markers included in more than one tube and resulted in a variation coefficient of 5–7%.

2.4. Cytomegalovirus (CMV) and Herpes simplex serology

An indirect enzyme-linked immunosorbent assay, the IMx Microparticle Enzyme Immunoassay (MEIA, ABBOT Scandinavia AB, Sweden) was used to detect anti-CMV immunoglobulin G antibodies. The procedure followed the manufacturer's instruction. Antibody titers of >40 were considered to be positive (Gratama et al., 1987). The indirect enzyme-linked immunoabsorbant assay procedure used in the Clinical Microbiology Laboratory at Ryhov, Jönköping was applied to detect Herpes simplex antibodies.

2.5. Data analysis

Statistical analyses were conducted using SPSSPC + ® (Norusis, 1986). Subgroups were compared using One-way Analysis Of Variance (ANOVA) and Chi-square analyses (Fischer test). Correlational analysis was performed using Pearson r correlation coefficients.

3. Results

3.1. Changes in CD4 and CD8 lymphocyte subsets for very old and middle-aged

As reported previously in our studies, the CD4/CD8 ratio formed two distinct subgroups in the very old, one group with a CD4/CD8 ratio less then one and

Table 1
Protocol used for three-color staining

Tube[a]	FITC	PE	Per-CP
1	IgGl	IgG1	CD45
2	CD4	CD8	CD3
3	CD45RA	CD45RO	CD3
4	CD57	CD28	CD3
5	CD8	CD28	CD3
6	CD4	CD28	CD3
7	CD57	CD8	CD3
8	CD57	CD4	CD3
9	CD8	HLA-DR	CD3
10	CD4	HLA-DR	CD3
11	CD8	CD45RO	CD3
12	CD45RA	CD8	CD3
13	CD4	CD45RO	CD3
14	CD45RA	CD4	CD3

[a] TriTEST™.

Table 2
The numbers (cells/mm^3) of CD4 and CD8 and the CD4/CD8 ratio for very old with a CD4/CD8 ratio of less than 1, greater than 1 and for the middle-aged[a]

Cell subset	Very old with a $CD4^+/CD8^+$		Middle-aged ($n = 14$)	$P <$
	Less than 1 ($n = 6$)	Greater than 1 ($n = 16$)		
CD4	890 ± 64[d]	1131 ± 70	1352 ± 124[b]	0.05
CD8	1295 ± 110[c,d]	462 ± 56[b]	567 ± 71[b]	0.0001
CD4/CD8	0.7 ± 0.07[c,d]	3.0 ± 0.4[b]	2.5 ± 0.2[b]	0.01

[a] Values are given as mean $\pm$ SE.
[b] $P < 0.05$ between the group and the group 'less than 1' by Duncan's test for comparison of individual groups:
[c] $P < 0.05$ between the group and the group 'greater than 1' by Duncan's test for comparison of individual groups:
[d] $P < 0.05$ between the group and the group '50-year old' by Duncan's test for comparison of individual groups:

another with a ratio greater than 1 (Ferguson, 1995; Wikby et al., 1998). In this study, using One way Analysis of Variance (ANOVA), significant between group differences occurred in the CD4 ($P < 0.05$) and CD8 numbers ($P < 0.0001$) and in the CD4/CD8 ratios ($P < 0.01$) (Table 2). A post-hoc test for comparison of individual groups showed that the group of very old with CD4/CD8 ratio less than 1 was unique, since it indicated a significant decrease in the number of CD4 cells, compared with middle-aged individuals, as well as a significant increase in the number of CD8 cells and a significant decrease in the CD4/CD8 ratio, compared with the two other subgroups. There were no significant differences between the very old with a CD4/CD8 ratio greater than 1 and middle-aged in any of the lymphocyte subsets.

3.2. Longitudinal changes in the relative number of very old with CD4/CD8 ratios less than 1

An increasing percentage of individuals with a ratio less than 1 were continuously recruited with time in this aging sample. By the last time point (T4) of this longitudinal study 32 of 98 (32%) of the individuals from the original sample had developed a CD4/CD8 ratio of less than 1 (Fig. 1). Longitudinally, of the 98 individuals followed from 1989 to 1997, 16 (16%) had a CD4/CD8 ratio less than 1 at Time 1 (T1) in 1989. One year later at Time 2, 13 of 75 individuals (17%) and 2 years later at T3, 16 of 69 individuals (23%) with CD4/CD8 ratio less than 1 were identified. At T4, 6 of 22 individuals (27%) with a ratio less than 1 were found. Only three of the 16 individuals in the category with CD4/CD8 ratio less than 1 at T1 survived for the duration of the study.

3.3. Numbers and relative numbers of CD57, HLADR and CD28 markers on CD4 and CD8 cells in very old and middle-aged at Time 4

The numbers of cells with the activation markers CD57 and HLADR, with the
markers and with CD28 on CD4 and CD8 were examined for very old individuals with CD4/CD8 ratio less than 1, those greater than 1, and for middle-aged individuals. The results indicate cell number differences between the groups and across the groups occurring in the CD3CD4 and CD3CD8 positive cells. For the CD3CD4 positive cells, the numbers of the $CD28^+$ and $CD28^-$ cells and the $CD57^+$ and $CD57^-$ cells for the old individuals with a CD4/CD8 ratio less than 1 were all significantly different from those of the old individuals with CD4/CD8 ratio greater than 1 and the middle-aged controls (Table 3). For the CD3CD8 positive cells significant differences occurred between the CD 28^-, the $HLADR^-$ and $HLADR^+$, the $CD57^-$ and $CD57^+$ cells, in the individuals with a CD4/CD8 ratio less than 1 compared with the individuals with a CD4/CD8 ratio greater than 1 and the middle-aged controls (Table 4). These results indicate the most profound changes occur in the $CD8^+$ T-cell subpopulation numbers in the individuals with a CD4/CD8 ratio less than 1. In particular, this includes CD8 cell activation, evidenced by increases in the numbers of cells positive for CD57 and HLADR, as well as loss of the co-stimulatory surface marker, CD28. Moreover, correlational analysis indicated significant relationships ($P < 0.001$) between these $CD8^+$ T-cells subsets. The Pearson correlation coefficients 0.79 ($CD28^-$ vs $HLADR^+$), 0.81 ($CD28^-$ vs $CD57^+$) and 0.79 ($HLADR^+$ vs $CD57^+$) were obtained.

The T-cell subgroups were further compared by computing relative numbers of the cells with various markers relative to the numbers of CD4 and CD8 cells.

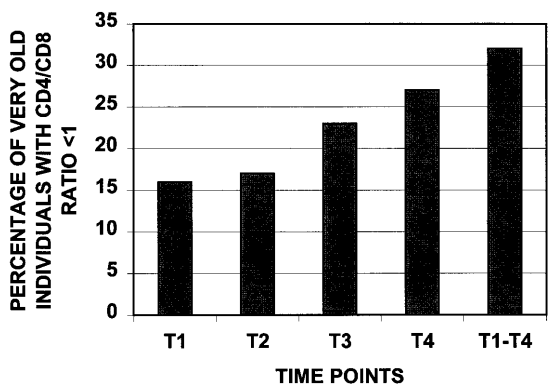

Fig. 1. Longitudinal changes in the percentage of very old individuals with CD4/CD8 ratios less than 1 at times 1–4 of the Swedish OCTO immune study.

Table 3
Differences in the $CD3^+CD4^+$ lymphocytes subpopulations (number of cells/mm^3) in the very old OCTO immune sample categorized by their CD4/CD8 ratios and compared with middle-aged controls at Time 4[a]

Cell subset	Very old with a $CD4^+/CD8^+$		Middle-aged ($n = 14$)	$P <$
	Less than 1 ($n = 6$)	Greater than 1 ($n = 16$)		
$CD3^+CD4^+CD28^+$	$703 \pm 75^{c,d}$	1045 ± 64^{b}	1268 ± 112^{b}	0.01
$CD3^+CD4^+CD28^-$	$310 \pm 81^{c,d}$	130 ± 23^{b}	106 ± 18^{b}	0.01
$CD3^+CD4^+HLADR^+$	311 ± 63	313 ± 62	153 ± 20	NS
$CD3^+CD4^+HLADR^-$	710 ± 98^{d}	853 ± 59^{d}	$1210 \pm 115^{b,c}$	0.01
$CD3^+CD4^+CD57^+$	$351 \pm 126^{c,d}$	163 ± 46^{b}	64 ± 14^{b}	0.01
$CD3^+CD4^+CD57^-$	$643 \pm 77^{c,d}$	$1033 \pm 59^{b,d}$	$1310 \pm 126^{b,c}$	0.01

[a] Values are given as mean ± SE.
[b] $P < 0.05$ between the group and the group 'less than 1'.
[c] $P < 0.05$ between the group and the group 'greater than 1'.
[d] $P < 0.05$ between the group and the group '50-year old'.

One-way ANOVA was used to examine the relative numbers of the activation markers $CD57^+$ and $HLADR^+$ of the CD4 and CD8 cells. The results indicated significant differences between the groups for all of the subsets (Table 5). A post-hoc comparison of groups indicated significant differences between the middle-aged and both groups of very old for all markers. In addition, the comparison indicated for the $CD3^+CD4^+CD57^+$ subset that the very old individuals with a CD4/CD8 ratio less than 1 had significantly higher relative numbers compared with the old individuals with a ratio greater than 1 and the middle-aged.

One-way ANOVA of relative numbers of $CD28^-$ subset of $CD4^+$ and $CD8^+$ cells for very old individuals with CD4/CD8 ratio less than 1, those greater than 1 and for middle-aged individuals indicated differences between groups (Table 5). Post-hoc comparisons of the groups showed significant differences between the middle-aged and one or both groups of very old for these subsets. Moreover, the comparison showed that very old individuals with a CD4/CD8 ratio less than 1 had significantly higher relative numbers of the $CD28^-$ subsets for both $CD4^+$ and $CD8^+$ cells than the individuals with a ratio greater than 1 and the middle-aged individuals.

3.4. Longitudinal examination of the relationship of cytomegalovirus (CMV) IgG antibodies and immune system parameters in very old and middle-aged individuals

The prevalence of individuals with CMV-lgG antibodies in the very old individuals was 90% (89 individuals out of 98) at Time 1 (1989), 88% (59 individuals out of

67) at Time 3 (11991) and 95% (19 individuals out of 20) at Time 4 (1997). The prevalence of individuals with CMV-lgG antibodies in the middle-aged at these times were relatively consistent at 67% (10 out of 15), 67% (10 out of 15) and 65% (10 out of 14).

A one-way ANOVA comparison, between the groups of CMV-lgG positive and negative individuals, of the relative numbers of $CD57^+$, $HLADR^+$ and $CD28^-$ of CD4 and CD8 cells was completed. The results indicated significantly higher relative numbers of the $CD57^+$ and $CD28^-$ cell markers both for the CD4 and the CD8 cells from CMV-lgG positive individuals (Table 6). These results suggest that CMV is associated with cell activation and a decrease in the expression of the CD28 surface marker.

3.5. Cytomegalovirus IgG antibodies and CD4/CD8 ratios

Thirty-one individuals from the 98 that we followed at T1, T2, T3 and T4 either had a CD4/CD8 ratio less than 1 initially at T1 or did change from a CD4/CD8 ratio greater than 1 to a value less than 1 during the study. No individuals changed in the other direction from a CD4/CD8 ratio less than 1 to a ratio greater than 1. All of the 31 individuals (100%) with a CD4/CD8 ratio less than 1 were CMV-IgG positive, while 60 out of 69 individuals (87%) with a CD4/CD8 ratio greater than 1 were CMV-IgG positive ($P = 0.03$, Fischer's test). No relationship to Herpes

Table 4
Differences in the $CD3^+CD8^+$ lymphocyte subpopulations (number of cells/mm^3) in the very old OCTO Immune sample categorized by their CD4/CD8 ratios and compared with middle-aged controls at Time 4[a]

Cell subset	Very old with a $CD4^+/CD8^+$		Middle-aged ($n = 14$)	$P <$
	Less than 1 ($n = 6$)	Greater than 1 ($n = 16$)		
$CD3^+CD8^+CD28^+$	429 ± 35	289 ± 58	426 ± 38	NS
$CD3^+CD8^+CD28^-$	$1009 \pm 121^{c,d}$	280 ± 41^{b}	202 ± 56^{b}	0.0001
$CD3^+CD8^+HLADR^+$	$634 \pm 117^{c,d}$	$291 \pm 55^{b,d}$	$135 \pm 24^{b,c}$	0.0001
$CD3^+CD8^+HLADR^-$	$841 \pm 130^{c,d}$	$301 \pm 33^{b,d}$	$502 \pm 71^{b,c}$	0.001
$CD3^+CD8^+CD57^+$	$758 \pm 135^{c,d}$	259 ± 58^{b}	150 ± 47^{b}	0.0001
$CD3^+CD8^+CD57^-$	$612 \pm 55^{c,d}$	$275 \pm 44^{b,d}$	$401 \pm 42^{b,c}$	0.001

[a] Values are mean $\pm$ SE.
[b] $P < 0.05$ between the group and the group 'less than 1'.
[c] $P < 0.05$ between the group and the group 'greater than 1'.
[d] $P < 0.05$ between the group and the group '50-year old'.

Table 5
The percentage of CD57, HLADR and CD28 cells in the CD3+CD4+ and CD3+CD8+ cells for the very old individuals with a CD4/CD8 ratio less than 1, and greater than 1 and for the middle-aged[a]

Ratio	Very old with a $CD4^+/CD8^+$		Middle-aged ($n=14$)	$P<$
	Less than 1 ($n=6$)	Greater than 1 ($n=16$)		
$CD28^-/CD3^+CD4^+$	$29.4 \pm 9.4^{c,d}$	10.4 ± 1.5^{b}	7.3 ± 0.9^{b}	0.0001
$CD57^+/CD3^+CD4^+$	$32.7 \pm 7.7^{c,d}$	$12.0 \pm 2.3^{b,d}$	$4.3 \pm 0.8^{b,c}$	0.0001
$HLADR^+/CD3^+CD4^+$	30.5 ± 4.7^{d}	25.2 ± 4.0^{d}	$11.2 \pm 1.0^{b,c}$	0.01
$CD28^-/CD3^+CD8^+$	$69.4 \pm 2.5^{c,d}$	$50.7 \pm 5.2^{b,d}$	$27.5 \pm 3.7^{b,c}$	0.0001
$CD57^+/CD3^+CD8^+$	53.7 ± 4.2^{d}	45.6 ± 4.3^{d}	$22.6 \pm 3.3^{b,d}$	0.001
$HLADR^+/CD3^+CD8^+$	42.9 ± 7.0^{d}	45.3 ± 3.6^{d}	$20.8 \pm 2.0^{b,c}$	0.0001

[a] Values are given as mean ± SE.
[b] $P<0.05$ between the group and the group 'less than 1' by Duncan's test for comparison of individual groups:
[c] $P<0.05$ between the group and the group 'greater than 1' by Duncan's test for comparison of individual groups:
[d] $P<0.05$ between the group and the group '50-year old' by Duncan's test for comparison of individual groups:

simplex virus was found to be associated with the changes in this study. The data indicates a significant relationship between prevalence of a persistent CIVIV infection and a CD4/CD8 ratio less than 1 in this sample of the very old individuals (Table 7).

Table 6
The relative percentage of $CD28^-$, $CD57^+$ and $HLADR^+$ subsets of $CD4^+$ and $CD8^+$ cells in CMV-IgG positive and negative individuals for very old and middle-aged at Time 4[a]

Ratio	Very old		Middle-aged		$P<$
	Positive	Negative	Positive	Negative	
$CD28^-/CD3^+CD4^+$	16.9 ± 2.5 (19)[b]	1.7 (1)	8.2 ± 1.3 (9)	5.5 ± 0.5 (5)	0.02
$CD57^+/CD3^+CD4^+$	18.8 ± 3.7 (19)	2.5 (1)	5.1 ± 1.0 (9)	2.8 ± 1.0 (5)	0.02
$HLADR^+/CD3^+CD4^+$	25.1 ± 2.6 (19)	16.7 (l)	11.6 ± 11.3 (9)	10.5 ± 1.9 (5)	0.002
$CD28^-/CD3^+CD8^+$	58.8 ± 3.8 (19)	24.3 (l)	33.4 ± 4.3 (9)	16.9 ± 4.3 (5)	0.0001
$CD57^+/CD3^+CD8^+$	48.1 ± 3.4 (19)	17.7 (l)	26.8 ± 4.5 (9)	14.6 ± 2.2 (5)	0.0001
$HLADR^+/CD3^+CD8^+$	43.9 ± 3.3 (19)	41.4 (l)	22.1 ± 2.9 (9)	18.3 ± 2.1 (5)	0.0001

[a] Values are mean ± SE (n).
[b] Number of individuals.

Table 7
An analysis of the relationship of the CD4/CD8 ratio and CMV antibody status in the very old examined at Time 1, Time 2, Time 3 and Time 4

CD4/CD8	CMV-lgG positive	CMV-lgG negative	P^a
<1	31[b]	0	0.03
>1	60	9	

[a] Fischer's exact test.
[b] Number of individuals in each category.

4. Discussion

To maintain homeostasis the peripheral blood T-lymphocyte balance is closely regulated in complex ways that have not yet been fully determined (Goldrath and Bevan, 1999). In general clonal expansions of specific subpopulations are balanced by programmed cell death in other subpopulations and maintenance of a relatively constant total peripheral blood T-lymphocyte count. When a condition initiating the clonal expansion is corrected, programmed cell death occurs in the expanded subpopulation and homeostasis is restored. In elderly humans an increasing number of studies are reporting age-related declines in peripheral blood T-cells and alterations in some of the peripheral blood lymphocyte subpopulations (Nagel et al., 1983; Sansoni et al., 1993; Franceshi et al., 1995; Ferguson, 1995; Rea et al., 1996; Wikby et al., 1998). Thus very old age may define a unique sample of individuals whose peripheral blood lymphocyte populations are exceptions to the homeostatic mechanisms.

No other study has made an attempt to separate very old individuals by differences in relative CD4/CD8 ratios, as has been done in the longitudinal Swedish OCTO immune study. The considerable value of a longitudinal study is it enables individuals to be followed and changes to be considered with time. As the results demonstrate, this study has defined an increasing number of individuals in the sample with CD4/CD8 ratios less than 1 with age, as well as a progressive increase in the number of individuals that move into that category. This change is driven by the decrease in the CD4 lymphocyte subset and/or the expansion of the CD8 subset. At T4 of the OCTO study the profound increase in the CD8 number of individuals with an inverted CD4/CD8 ratio and its relationship with cytomegalovirus (CMV) infection suggest that CMV infection might be a contributor to this significant expansion of CD8 cells. At the same time the CD4 subset decreases in a compensatory fashion to maintain a constant number of T-lymphocytes in the peripheral blood pool. Perhaps most important is the fact that the individuals who moved into the low CD4/CD8 ratio group subsequently did not move out. The latter suggests there is a persistent underlying factor, or factors, that combined with CMV activation is causing a dissociation of the homeostatic mechanisms for the PBL CD4 and CD8 T-cell counts and their relative numbers in the very old.

An important question about the CD4 and CD8 T-cell subpopulations is their subset composition and relative activation state. With increasing frequency, reports of similar observations of activation markers are appearing in the literature on the aging immune system (Fahey et al., 2000). The risks associated with infectious disease progression and mortality also increase with age (Izaks et al., in preparation). Effros (2000) and her colleagues have convincingly demonstrated that antigen-induced T-cell division leads to irreversible cell cycle arrest, shortened telomeres and absence of the CD28 surface marker. This suggests that these changes may be a significant determinant in the increased incidence of diseases, such as infectious disease and cancer, in the elderly.

Interestingly HIV-1 infection at an older age is related to a shorter time to development of AIDS and to death (Darby et al., 1996; Pezzotti et al., 1996). The PBL changes in HIV disease progression have been associated with changes in lymphocyte activation status. Clinically the relative activation of the PBL has been one of the most important parameters followed to determine the progression of this infection (Smith et al., 1993). Lymphocyte activation changes correlate significantly with the decrease in the CD4 population and an increase in the CD8 cells that possibly is in an attempt to counteract the progression of the HIV infectious process by generation of clones of protective effector cytotoxic T-cells that are $CD8^+CD28^-$ (Posnett et al., 1999). These cells can represent over 50% of all CD8 cells in the elderly and in chronic viral infections such as HIV-1. Recent studies have indicated that much of the CD8 T-cell expansion during viral infection is antigen specific rather than representing bystander activation or cross reactive stimulation of non-specific cells (Murali-Krishna et al., 1998; Tough and Sprent, 1998). Therefore, the combination of chronic lymphocyte activation in response to specific antigens and CD8 clonal effector cell responses are important in the elderly.

At T4 in the OCTO immune study we report a significant association of the cell marker changes characteristic of the individuals at risk for non-survival with cytomegalovirus IgG status. Interestingly this study also examined Herpes simplex antibody levels and, similar to the study of Looney et al. (1999), indicated no evidence of a relationship of the T-cell changes in the elderly with another Herpes virus. As demonstrated in the studies of Wang et al. (1993), there was a significant correlation between the increase in $CD57^+$ and $CD28^-$ T-cells and CMV IgG antibody status. Lenkei and Andersson (1995) also reported a high correlation of CMV antibody titers with lymphocyte activation status and $CD57^+$ expression. The studies of Looney et al. (1999) indicated an increase in the $CD28^-$ CD4 and CD8 T-cells associated with CMV IgG antibody status in the elderly. Merino et al. (1998) reported that expansion of $CD8^+CD57^+CD28^-$ cells in the elderly was dependent mainly on age, but also on CMV carrier status possibly related to repeated antigenic stimulation. Other recent studies have suggested that chronic antigenic stimulation is associated with $CD8^+CD57^+$ and $CD8^+CD28^-$ T-cell clonal expansion and response to CMV peptide specific induction (Mugniani et al., 1999; Weekes et al., 1999a).

The recent results from a number of laboratories are in accordance with findings in this study that indicate immune system changes are associated with CMV

activation. In addition, this study indicated the changes are most profound in the aged individuals with an inverted CD4/CD8 ratio. In particular, these individuals have a very significant CD8 lymphocytosis associated with a major expansion of a $CD8^+CD57^+CD28^-$ T-cell subset. An increasing number of reports indicate CMV can be an important determinant in the immune status of seemingly asymptomatic elderly. It will be critical to confirm this and to examine this relationship in terms of the temporal occurrence of an active infection, the loss of T-cell homeostasis evidenced by persistent changes in the CD4/CD8 ratio, as well as the clonality and functional status of these cells (Weekes et al., 1999b; Effros, 2000). Based on the results of the OCTO study, it seems reasonable to suggest that the combination of old age, lymphocyte activation due to chronic infection, such as CMV, and a related inbalance in unknown factors that regulate homeostatic mechanisms in the immune system may have contributed to increased risk for non-survival observed in elderly in the OCTO study.

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