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Maintenance of naïve CD8 T cells in nonagenarians by leptin, IGFBP3 and T3

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Abstract

Research into the age-associated decline in the immune system has focused on the factors that contribute to the accumulation of senescent CD8 T cells. Less attention has been paid to the non-immune factors that may maintain the pool of naïve CD8 T cells. Here, we analyzed the status of the naïve CD8 T-cell population in healthy nonagenarians (≥ 90 -year-old), old (60–79-year-old), and young (20–34-year-old) subjects. Naïve CD8 T cells were defined as CD28⁺CD95[−] as this phenotype showed a strong co-expression of the CD45RA⁺, CD45RO[−], and CD127⁺ phenotypes. Although there was an age-associated decline in the percentage of CD28⁺CD95[−] CD8 T cells, the healthy nonagenarians maintained a pool of naïve CD28⁺CD95[−] cells that contained T-cell receptor excision

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Appendix A. Supplementary data Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mad.2009.11.003.

circles (TREC)⁺ cells. The percentages of naïve CD28⁺CD95[−] CD8 T cells in the nonagenarians correlated with the sera levels of insulin-like growth factor binding protein 3 (IGFBP3) and leptin. Higher levels of triiodothyronine (T3) negatively correlated with the accumulation of TREC[−]CD28[−]CD95⁺ CD8 T cells from nonagenarians. These results suggest a model in which IGFBP3, leptin and T3 act as non-immune factors to maintain a larger pool of naïve CD8 T cells in healthy nonagenarians.

Keywords

Nonagenarians; Naïve CD8; TREC; IGFBP3; Leptin; T3

1. Introduction

Immune aging is associated with both a gradual loss of naïve T cells and an accumulation of memory or senescent T cells that can be influenced by two factors: the rate of production of new T cells by the thymus, and the rate of utilization and maturation of T cells that drives the development of the pool of senescent T cells in the periphery (Gruver et al., 2007; Hadrup et al., 2006). The number of available naïve T cells is determined by the rate of thymic involution and the progressive development of memory and senescent CD8 T cells, which can deplete the pool of naïve T cells (Herndon et al., 1997). Although thymic output, as determined by the relative number of T-cell receptor recombination excision circles (TRECs), declines by age 30–40 in humans, the overall output and long-term maintenance of naïve T cells have a significant effect on the maintenance of an immune response in older individuals (Lorenzi et al., 2008; Ye and Kirschner, 2002). In mice, strains that exhibit rapid thymic involution also exhibit accelerated immune senescence in the periphery at old age (Hsu et al., 2005). Increased adiposity of the thymus is associated with decreased thymic output and genetic factors have also been found to have deleterious effects on thymopoiesis and rates of thymic involution (Hsu et al., 2005; Li et al., 2003; Wang et al., 2006).

Survival to the tenth or eleventh decade of life is associated with protection from immune senescence (Franceschi et al., 1996; Ostan et al., 2008; Sansoni et al., 2008). It has been shown that individuals who survive to the age of 100 years exhibit an “inverted” immune risk profile (IRP), *i.e.*, stable maintenance of a high CD4/CD8 ratio with low numbers of CD8⁺CD28[−] cells over time (Strindhall et al., 2007). Research into the immune factors that contribute to the loss of naïve T cells has indicated that chronic viral infections, including chronic Epstein–Barr (EBV) and hepatitis B infections, as well as chronic infection with cytomegalovirus (CMV), are associated with the loss of naïve T cells (Colonna-Romano et al., 2007; Koch et al., 2007; Pawelec et al., 2006; Vasto et al., 2007). Numerous other immune factors have been identified that can promote the age-associated development of a pool of senescent, dysfunctional, oligoclonally expanded CD8 T cells (Ouyang et al., 2003). Notably, it has been established that chronic inflammation is associated with elevated levels of TNF α , IL-6, and/or CRP in the sera and the elevation of these factors has been linked to both enhanced immune activation and systemic functional defects (Glaser et al., 2005; Johansen et al., 2008).

There have been fewer studies of the non-immune factors that enable maintenance of the naïve T-cell pool in the elderly. Hormonal changes, including changes in the levels of insulin-like growth factor-1 (IGF-1), have been proposed to be linked to longevity and, in young animals, IGF-1 levels have been shown to exhibit a positive relationship with thymopoiesis (Fahy, 2003; Yang et al., 2008). The relationship between hormones and immune function in nonagenarians is not well known. In the present study, we analyzed CD8⁺ T cells obtained from subjects who were recruited for the Louisiana Healthy Aging Study with ages ranging from 20 to 101. We examined the population of CD28⁺CD95[−] T cells, as well as

CD28⁺CD95⁺ and CD28⁻CD95⁺ CD8⁺ T cells, in up to 39 young (ages 20–34) subjects, 41 old (ages 60–79) subjects and 55 nonagenarians (ages ≥90). We found that the CD28⁺CD95⁻ population of CD8⁺ T cells exhibited the highest number of TRECs compared to CD28⁺CD95⁺ and CD28⁻CD95⁺ CD8⁺ T cells, and this population declined slowly with age. The size of this population correlated with the percentage of the naïve T CD8⁺ T cells, even in nonagenarians. In nonagenarians, but not in old and young individuals, there were significant correlations between the size of the CD28⁺CD95⁻ naïve CD8⁺ T-cell population and the serum levels of insulin-like growth factor binding protein 3 (IGFBP3), growth hormone (GH), leptin, and T3. We propose a model in which non-immune factors that promote thymocyte development and thymopoiesis, including IGFBP3 and leptin have a positive impact on the naïve CD8⁺ T-cell pool even in nonagenarians. Risk factors for a reduction in the pool of CD28⁺CD95⁻ naïve CD8⁺ T cells or accumulation of CD28⁻CD95⁺ end-stage effector memory CD8⁺ T cells in nonagenarians, included high levels of GH and low levels of T3.

2. Materials and methods

2.1. Subjects

A subset of 135 participants from an ongoing study known as the Louisiana Healthy Aging Study population were studied. The demographic distribution shows that there were 13% African-American and 87% Caucasian in the present Louisiana Healthy Aging Study cohort. All available data are included for the analyses. The health status of the subjects approximated that of the general population (or healthier than the general population and thus the nonagenarians cohort is comprised of individuals who are considered more “functional”). Subjects with thyroid disease or being treated for thyroid disease were excluded and the TSH had to be within a normal range for all subjects to be accepted.

Subjects with diabetes, unstable cardiovascular disease, or mental health problems requiring pharmacologic intervention were excluded from the study. Also excluded were those subjects who had a cardiovascular or cerebrovascular accident in the 3 months prior to evaluation, who had severe high blood pressure, who had evidence of aneurysm, who were taking certain medications used for myasthenia gravis, or who had uncontrolled asthma, an asthma-like condition or emphysema/chronic obstructive pulmonary disease (COPD). All subjects that had been diagnosed with cancer, who were undergoing immunotherapy or receiving immune suppressants, who showed symptoms of allergy or a recent history of infection, were also excluded. The studies were approved by the institutional review boards at University of Alabama at Birmingham, Louisiana State University Health Sciences Center, and Pennington Biomedical Research Center. All participants provided informed consent.

2.2. Measurements

Leptin, IL-6, and TNFα were analyzed on a Luminex 200 IS using a multiplex kit from Millipore (St. Charles, MO). Growth hormone and T3 were analyzed on a Siemen's Immulite 2000 (Deerfield, IL). IGF-1 and IGFBP3 were analyzed using ELISA kits from Diagnostic Systems Laboratories (Webster, TX).

2.3. Purification and preparation of peripheral blood mononuclear cells (PBMCs)

Heparinized peripheral blood, isolated at the Pennington Biomedical Research Center (PBRC), was collected and spun to isolate the buffy coat layer. The buffy coat layer was then centrifuged through a Histopaque gradient (Sigma–Aldrich, St. Louis, MO) for 30 min at 1700 rpm, and PBMCs were collected from the interface.

2.4. TREC assay

PBMCs from healthy donors were labeled with anti-CD8, anti-CD28 and anti-CD95 antibodies and separated with a FACSVantage (BD Biosciences, San Jose, CA). Quantification of TRECs in isolated CD28⁺CD95⁻CD8⁺, CD28⁺CD95⁺CD8⁺ and CD28⁻CD95⁺CD8⁺ T cells was performed by real-time quantitative PCR as previously described (Douek et al., 2000). Briefly, genomic DNA was isolated from at least 50,000 purified cells for each subpopulation of CD8⁺ T cells. Real-time quantitative PCR was done on 5 µL of DNA (equivalent to 50,000 cells) with the primers: CACATCCCTTTCAACCATGCT and GCCAGCTGCA GGGTTTAGG, and probe FAM-5'-ACACCTCTGG TTTTGTAAAGGTGCCCACT-3'-TAMRA (Operon, Huntsville, AL). PCR reactions contained 0.5 U Platinum Taq polymerase (Invitrogen, Carlsbad, CA), 3.5 mmol/L MgCl₂, 0.2 mmol/L dNTPs, 500 nmol/L each primer, 150 nmol/L probe. Conditions were 95 °C for 5 min, then 95 °C for 30 s, 60 °C for 1 min, for 40 cycles. A set of known concentrations of the TREC DNA from human thymus genomic DNA were used to generate a standard curve (Douek et al., 2000), and numbers of TREC in samples were calculated by the Bio-Rad IQ5 (Hercules, CA) software.

2.5. Phenotype of cells

The phenotype of cells was examined by standard flow cytometry procedures as previously described (Chen et al., 2005). This involved six-color immunofluorescence staining of cell samples using a combination of FITC-, PE-, APC-, Pacific-blue, PE-Cy7, and APC-Cy7 conjugated antibodies for anti-CD8, anti-CD28, anti-CD95, anti-CD45RO, anti-CD45RA, and anti-CD127. Each experiment included cells incubated with isotype controls (not shown on the figures). A total of 100,000 events for each sample were recorded and analyzed on an LSR-II (BD Biosciences). The analysis was performed using FlowJo Software (TreeStar, Ashland, CA). Forward angle light scatter was used to exclude dead and aggregated cells.

2.6. Correlation and statistical analysis

The results are expressed as the mean and standard error of the mean (SEM). The two-tailed Student's *t*-test was used for statistical analysis. A one-way analysis of variance (ANOVA) was used when more than two groups of samples were compared. For correlation of the percentage of CD28⁺CD95⁻ CD8 T cells and GH, the Fisher exact correlation test was used.

Correlation between different variables was determined using a linear regression analysis. A linear regression was chosen for most of the analysis to enable the readers to envision the absolute values of the %CD8 T cells and levels of non-immune factors that were measured. A *P* value <0.05 was considered to indicate a statistically significant difference or correlation. Statistical analysis of the data was also carried out after the data were normalized by logarithmic transformation of the CD8 and levels of the non-immune factors.

3. Results

3.1. The pool of CD28⁺CD95⁻ T cells declines with age but persists in healthy nonagenarians

In our previous studies, we found that the strongest single T-cell parameter that age was correlated was the percentage of CD28⁺CD95⁻ T cells (Hsu et al., 2006). The mean percentage of CD28⁺CD95⁻ CD8T cells was approximately 34% in the young individuals and this declined to 18% in the group of old individuals and to 6% in the healthy nonagenarians (Fig. 1a and b, left panel). However, within the group of young individuals the percentages of CD28⁺CD95⁻ T cells varied considerably, and the percentages of CD28⁺CD95⁻ cells in a substantial number of old individuals and some nonagenarians were similar to the lower range of percentages observed in the young individuals, suggesting that a pool of CD28⁺CD95⁻ CD8 cells can persist in the elderly. There were corresponding increases in the mean percentages of

CD28⁻CD95⁺ T cells (Fig. 1a and b, right panel) and there was a significant negative correlation between the percentages of CD28⁺CD95⁻ and CD28⁻CD95⁺ CD8 T cells in all age groups (Fig. 1c).

3.2. TREC⁺ cells are mainly present in the CD28⁺CD95⁻ population in old individuals and nonagenarians

TREC numbers decline with cellular proliferation, with thymic involution, and after thymectomy (Douek et al., 1998). TREC numbers therefore can be an indicator for long-lived naïve or recent thymic emigrant T cells (Hazenberg et al., 2001). We sorted the CD8 T cells by FACS into CD28⁺CD95⁻, CD28⁺CD95⁺ and CD28⁻CD95⁺ cell populations (>200,000 cells per subject), which were then analyzed using the TREC assay. In all age groups, the highest numbers of TREC⁺ cells were seen within the CD28⁺CD95⁻ T-cell populations, suggesting that this phenotype defines the naïve T-cell population. The mean number of TREC⁺ cell within the CD28⁺CD95⁻ CD8 T-cell population was higher in the group of young individuals (~7000 TRECs/100,000 CD28⁺CD95⁻ CD8 T cells) than the group of old individuals and nonagenarians (~2000 TRECs/100,000 CD28⁺CD95⁻ CD8 T cells). However, the mean number (Fig. 2a) and the mean percentage (Fig. 2b) of TREC⁺ cell within the CD28⁺CD95⁻ CD8 T-cell population in the nonagenarians was similar to that observed in the group of old individuals. The mean number of TREC⁺ cells in the CD28⁺CD95⁺ CD8 T-cell population was low (approximately 400 TREC⁺ cells/100,000 CD28⁺CD95⁺ CD8 cells in young subjects and only about 100 TREC⁺ cells/100,000 CD28⁺CD95⁺ CD8 cells in the nonagenarians) and there were no detectable TREC⁺ cells in the CD28⁻CD95⁺ CD8 T-cell population, which indicated that these cells have undergone multiple divisions.

3.3. CD28⁺CD95⁻ T cells express high levels of expression of CD45RA and CD127

Although the CD28⁺CD95⁻ phenotype exhibited the highest number of TRECs, to further verify if these cells contain the most naïve T cells, compared to the CD28⁺CD95⁺ and CD28⁻CD95⁺ CD8 T cells, we analyzed the expression of CD45RA, which is an established marker of resting, naïve T cells, and CD45RO, which is a marker of activation and is commonly found on memory and effector cells (Rutella et al., 1998). We confirmed that the expression of CD45RA was high on the CD28⁺CD95⁻ CD8 T cells and that the majority of these cells expressed only low levels of CD45RO (Fig. 2c, black). The CD28⁺CD95⁺ subpopulation of CD8 T cells expressed high levels of CD45RO as did the CD28⁻CD95⁺ subpopulation of CD8⁺ T cells (Fig. 2c, gray). Moreover, the high expression of CD45RA on the CD28⁺CD95⁻ CD8⁺ T cells did not differ among the three age groups. In contrast, the CD28⁻CD95⁺ exhibited an age-associated increase in the expression of CD45RA, with the CD28⁻CD95⁺ CD8⁺ T cells from the group of healthy nonagenarians expressing levels of CD45RA that were similar to the levels expressed on the CD28⁺CD95⁻ CD8 T cells (Fig. 2d, gray). We also analyzed the expression of CD127 (the IL-7R alpha chain), which is expressed on all mature CD8⁺ T cells after emigration from the thymus. Down-regulation of CD127 is associated primarily with T-cell activation, whereas memory cells express high levels of CD127 (Kaech et al., 2003). We found that both CD28⁺CD95⁻ and CD28⁺CD95⁺ subpopulations of CD8⁺ T cells expressed high levels of CD127 (Fig. 2c) whereas the expression of CD127 was dramatically lower on the CD28⁻CD95⁺ cells (Fig. 2d). We did not observe age-associated differences in the expression of CD127 in any of these subsets of CD8⁺ T cells. Taken together, these data indicate that CD28⁺CD95⁻ CD8⁺ T cells are primarily composed of naïve CD8⁺ T cells, that the CD28⁺CD95⁺ CD8⁺ T cells are primarily composed of memory cells, and that the CD28⁻CD95⁺ CD8⁺ T cells are primarily composed of terminally differentiated effector memory CD8 T cells.

3.4. Elevated levels of leptin correlate with the size of the naïve CD8⁺ T-cell population

To identify the factors that may contribute to the pool of naïve CD8 T cells in the old and healthy nonagenarians, we determined the serum concentration of different hormones and other factors that have been implicated in thymopoiesis, including leptin and the stress-related proinflammatory cytokine IL-6. Leptin has since been shown to have little or no effect on the immune system of normal, unstressed mice, but has been reported to be thymo-stimulatory in settings of thymic stress (Hick et al., 2006; Howard et al., 1999) and has been shown to protect against inflammatory cytokine and stress-induced thymic atrophy (Gruver and Sempowski, 2008). Although the mean concentration of leptin (logarithmically transformed) in the sera of the healthy nonagenarians was significantly lower than the concentration in the sera of old individuals, it did not differ significantly from the concentration in the sera of the young individuals (Fig. 3a). Moreover, in the nonagenarians, there was a significant positive correlation between the percentage of CD28⁺CD95⁻ CD8 T cells and the concentrations of leptin in the serum (Fig. 3b), suggesting that the persistence of naïve CD8⁺ T cells in nonagenarians is associated with higher levels of leptin.

IL-6 has been shown as one of the immune risk factors in aged individuals (Glaser et al., 2005; Johansen et al., 2008). There were, however, no significant differences in the mean serum concentrations of IL-6 (logarithmically transformed) among the three age groups (Fig. 3c). The percentage of CD28⁺CD95⁻ CD8 T cells appears to negatively correlate with the concentration of IL-6 in the serum of the nonagenarians; however, this correlation did not reach the level of statistical significance and a similar correlation was not observed in the group of old individuals (Fig. 3d).

3.5. IGFBP3 correlates with the preservation of naïve CD8⁺ T cells

IGF1 binding protein 3 (IGFBP3), is the best studied and most abundant of the IGFBPs, and carries greater than 80% of serum IGF1 and IGF2 in heterotrimeric complexes (Baxter, 2001). Although the mean concentration of IGFBP3 in the sera was similar between the young and old groups, it was approximately 50% lower in nonagenarians (Fig. 4a). In contrast, the mean concentration of IGF-1 was approximately 50% lower in the sera of the old individuals than in the young individuals and although the levels were significantly lower in the nonagenarians, the magnitude of the decline was small (Supplementary Fig. 1a). The percentages of CD28⁺CD95⁻ CD8 T cells showed a positive and significant correlation with the serum concentrations of IGFBP3 in both the young individuals ($P = 0.02$, Fig. 4b, left) and in the healthy nonagenarians ($P = 0.01$, Fig. 4b, right). Although a similar positive correlation of the percentage of CD28⁺CD95⁻ CD8⁺ T cells with the serum levels of IGFBP3 was observed for the group of old individuals, this correlation did not reach the level of statistical significance (Fig. 4b, middle). IGF-1 levels did not correlate with the percentage of CD28⁺CD95⁻ CD8⁺ T cells in any age group, although the trend of correlation was positive in young individuals but was negative in both old individuals and nonagenarian (Supplementary Fig. 1b-d).

GH also has been implicated in thymopoiesis (Aspinall and Mitchell, 2008; Dorshkind et al., 2009). We found that the range of GH serum concentrations in the young subjects was very wide (0.5–18 $\mu\text{U/ml}$) and that, although less pronounced, the range of GH serum concentrations in the nonagenarians was also wide. There were significant differences in the mean concentration of GH in the serum among all three age groups (Fig. 4c). A higher mean serum concentration of GH was found in nonagenarians than in the older group of individuals. We, however, found a significant correlation between a higher percentage of CD28⁺CD95⁻ CD8⁺ T cells and lower concentrations of GH in nonagenarians (GH less than 1.1 for nonagenarians) (Fig. 4d, right panel) ($P = 0.015$, the Fisher exact test).

3.6. T3 correlates with a low percentage of terminally differentiated effector memory CD8 T cells in nonagenarians

In addition to its effects on the metabolism, T3 has been shown to promote thymopoiesis (Ribeiro-Carvalho et al., 2002). We found a significant decline in the mean serum concentration of T3 with age with a statistically significant difference in T3 levels between each of the three age groups (Fig. 5a). There appeared to be a positive trend of correlation between the percentage of CD28⁺CD95⁻ CD8⁺ T cells and T3 levels in old individuals (Fig. 5b, middle). In contrast, there was a significant correlation between lower percentages of terminally differentiated memory TREC⁻CD28⁻CD95⁺ CD8⁺ T cells and higher serum concentrations of T3 in nonagenarians (Fig. 5c, right).

4. Discussion

In normal, healthy individuals, thymic production is primarily responsible for the maintenance of the pool of naïve CD8 T cells in the periphery. Thymic production is highest in young individuals and declines as thymic involution occurs in early adult life; however, some thymic function can be maintained in middle-age and even in advanced late age (Nasi et al., 2006; Naylor et al., 2005; Sempowski et al., 2002). We have previously shown that the percentage of CD28⁺CD95⁻ CD8⁺ T cells exhibits the best correlation with age in humans (Hsu et al., 2006). Using primates as models, Cicin-Sain et al. (2007) have shown that CD28⁺CD95⁻ CD8⁺ T cells represent a naïve pool of CD8 T cells in aged primates. Messaoudi et al. (Messaoudi et al., 2006) have further shown that calorie restriction is associated with an increase in this population. The current studies confirmed our previous finding (Hsu et al., 2006) but revealed that although there was a highly significant negative correlation between CD28⁺CD95⁻ cells and age, a pool of these cells was maintained in healthy nonagenarians. Notably, we found that the number of TREC⁺ cells in the pool of CD28⁺CD95⁻ CD8⁺ T cells was not significantly lower in the group of healthy nonagenarians than the group of old individuals and were only threefold lower than in the group of young individuals, suggesting that either very long-term naïve T cells are maintained or the atrophied thymus remains capable of generating functional T cells, albeit in small numbers. TREC⁺ CD28⁺CD95⁺ and TREC⁺ CD28⁻CD95⁺ cells represent a very small population of CD8 T cells from young and old groups of subjects. The TREC⁺ CD28⁻CD95⁺ CD8 T cells were completely absent in nonagenarians and this is likely that in nonagenarians, fewer TREC⁺ T cells progress from CD28⁺CD95⁻ to CD28⁺CD95⁺ and CD28⁻CD95⁺.

It has been reported previously that CD45RA expression is not limited to naïve CD8⁺ T cells, but rather, there is a general accumulation of CD45RA^{high} CD8 cells with age (Okumura et al., 1993) and that these CD45RA^{high} CD8 T cells are non-proliferating end-stage differentiated effector cells (Hoflich et al., 1998). The high levels of expression of CD45RA and CD127 on the CD28⁺CD95⁻ T cells are consistent with the concept that this subset of cells represents resting or naïve cells. Our observation of an age-associated increase in CD45RA^{high} cells in the CD28⁻CD95⁺ CD8⁺ T cells is consistent with this previous finding. As the CD28⁻CD95⁺ T cells in nonagenarians are TREC⁻, they may represent terminally differentiated cells in these subjects. Taken together, these data suggest that the recent thymic emigrant CD8 T cells can still be found in nonagenarians and that it is the significantly reduction in the size of this pool of cells that results in the defective immune responses observed in the aged.

Our studies warrant future determination of the mechanistic connection of high levels of leptin, IGFBP3, and T3 with the maintenance of naïve immune system in the elderly. A role for leptin in modulating immunity was first suggested by the occurrence of chronic thymic atrophy, and low numbers of lymphocytes in leptin-deficient obese (*ob/ob*) mutant mice and in leptin receptor function-deficient diabetic (*db/db*) mutant mice (Hick et al., 2006; Howard et al.,

1999; Matarese, 2000; Trotter-Mayo and Roberts, 2008). Leptin has since been shown to have no effect on the immune system of normal, unstressed mice, but has been reported to be thymostimulatory in settings of thymic stress (Hick et al., 2006; Howard et al., 1999). It has been shown to protect against inflammatory cytokine and stress-induced thymic atrophy (Gruver and Sempowski, 2008) and implicated in the prevention of thymic involution (Dixit et al., 2007). These effect may be associated with its ability to modulate stress responses; for example, it reduces the peak corticosterone response to lipopolysaccharide (Gruver and Sempowski, 2008). Bruunsgaard et al. (2000) found no significant differences in leptin levels in old compared to young individuals, but nonagenarians were not specifically analyzed. We found the levels of leptin increase with age but significantly decline beyond age 90. Interestingly, those nonagenarians with higher concentrations of leptin also had higher percentages of naïve CD8 T cells, suggesting that increased levels of leptin with age may be a factor to promote the maintenance of the naïve T-cell pool in nonagenarians. As we did not find a significant correlation between leptin and TNF α or leptin and IL-6 in the nonagenarians (Supplementary Fig. 2), the higher levels of leptin most likely modifies the naïve CD8 T-cell pool by mechanisms other than the blocking of the inhibitory effects of these stress-associated cytokines.

IGFBP3 has autocrine and paracrine actions affecting cell mobility, adhesion, apoptosis, survival, and cell cycle (Butt et al., 1999,2000;Firth and Baxter, 2002;Lee et al., 2005). It can promote the expansion of primitive CD34⁺CD38⁻ hematopoietic stem cell precursors (Chang et al., 2007) and was able to expand hematopoietic human progenitor cells in NOD-SCID recipients (Liu et al., 2003). Lower levels of IGFBP3 and IGF-1 are associated with decreased survival after 100 years of age in humans (Arai et al., 2008) and have been associated with higher hemoglobin levels in older individuals (Landi et al., 2007). Centenarians appear to be characterized by low IGF-1-mediated responses and high levels of anti-inflammatory cytokines such as IL-10 and TGF β (Salvioli et al., 2009). In the healthy nonagenarians in this study, we found a significant correlation between a high percentage of naïve CD8 T cells and high levels of IGFBP3 but not IGF1. We also found that in nonagenarians, individuals with higher levels of GH also exhibited a lower percentage of naïve CD8 T cells. The insulin-IGF signaling pathways have been shown to play an important role in aging in mouse and humans (Franceschi et al., 2005). We therefore propose that IGFBP3 may exert positive effects on the development and maintenance of naïve CD8 T cells that are distinct from the activities produced by IGF-1 or GH.

Despite atrophy of the thyroid gland, thyroid dysfunction is not more prevalent among centenarians than among younger old people (Andersen-Ranberg et al., 1999). T3 has been shown to promote thymopoiesis by modulating the extracellular matrix-mediated interactions between thymocytes and the thymic micro-environmental cells (Ribeiro-Carvalho et al., 2002, 2007). Our results suggest that higher T3 levels may be more closely associated with the prevention of the accumulation or expansion of terminally differentiated CD8 T cells than the maintenance of the pool of naïve T cells. Although Hodgkinson et al. (2009) found that in women age 55–70, T4 was positively correlated with the percentages of total memory T lymphocytes, we found no significant correlation of T3 or T4 (data not shown) with these cells in the present 60–79 year-old group.

In summary, our results suggest that increased leptin, IGFBP3, and T3, are indicators of the healthy immune system development and possibly compensatory mechanisms either for the immune system or other biologic activities that have an overall beneficial impact on immune aging. These non-immune factors may improve metabolism directly within the thymus in addition to other beneficial effects that could promote maintenance of naïve CD8 T cells during later adult life. Identification of effective therapies to prevent immune senescence and preservation of naïve CD8 T cells may be possible. Clinical trials of low levels of leptin,

IGFBP3, and T3 are likely to be safe and may have other anti-aging benefits in addition to prevention of thymic involution or maintenance of naïve CD8 T cells. Such preventative approaches are reasonable considering preventative approaches are being carried out to delay or minimize the effects of other age-related defects such as osteoporosis. A major challenge for the future will be to design longitudinal studies to determine how and when these hormones exert their beneficial effect on naïve CD8 T-cell maintenance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Age-associated decline in CD28⁺CD95⁻ subpopulation and increase in CD28⁻CD95⁺ subpopulation. Single cell suspensions were prepared from the PBMCs of up to 135 individuals ($N = 39$ in age between 20 and 34 years; $N = 41$ in age between 60 and 79 years; $N = 55$ in age ≥ 90 years of age). Cells were stained with fluorescent conjugated antibodies for CD8, CD28, and CD95. The expression of CD28 and CD95 on live CD8⁺ T cells was analyzed. (a) Representative contour plots are shown for CD8 T cells from a representative subject from each indicated age group. The quadrants for the different subpopulations are shown. (b) The percentage of CD28⁺CD95⁻ and CD28⁻CD95⁺ CD8⁺ T-cell population is shown for each individual in the three age groups. The horizontal bar represents the mean of individual analyzed in each age group (*** $P < 0.005$ between the indicated groups). (c) The percentage of CD28⁺CD95⁻ CD8 T cells was plotted against the percentage of CD28⁻CD95⁺ CD8 T cells in the same individual in the indicated age groups.

Fig. 2.

The CD28⁺CD95⁻ population are TREC^{hi} naïve CD8 T cells. (a and b) Single cell suspensions prepared from PBMCs were sorted into CD8⁺CD28⁺CD95⁻, CD8⁺CD28⁺CD95⁺ and CD8⁺CD28⁻CD95⁺ T cells. Quantification of TRECs was performed by real-time quantitative PCR (>200,000 cells per subject). TRECs were not detectable (ND) in the CD28⁻CD95⁺ population of nonagenarians. The result represents the mean $\pm$ SEM of (a) TREC number per 100,000 cells and (b) percentage of TREC⁺ cells in the indicated CD8 T-cell population for 6-8 individuals per group (** $P < 0.01$; *** $P < 0.005$ comparing the indicated cell population with CD28⁺CD95⁻ cells from the same age group). (c and d) Single cell suspensions were prepared from the PBMCs of up to 22 individuals ($N = 6$ in age between 20 and 29 years; $N = 8$ in age between 60 and 79 years; $N = 8$ in age ≥ 90 -year-old). Cells were labeled with fluorescent conjugated antibodies for CD8, CD28, CD95, CD45RA, CD45RO and CD127, and analyzed on a BD LSR-II flow cytometer. Representative histograms of expression of CD45RA, CD45RO, and CD127 on (c) CD28⁺CD95⁺ (gray) and (d) CD28⁻CD95⁺ (gray), compared to CD28⁺CD95⁻ (black) CD8 T cells.

Fig. 3.

Elevated leptin correlates with naïve CD8 T cells. (a) Serum concentrations of leptin were determined for each indicated age group of individuals (** $P < 0.01$ between the indicated groups of comparison). The number (N) of all subjects in each age group that was available for each test was shown. (b) A regression analysis was carried out to determine the correlation of the log value of serum concentrations of leptin and the percentage of CD28⁺CD95⁻ CD8 T cells in the indicated age groups of individuals. A log transformation of the value was used to provide a more normal distribution for leptin. The regression correlation coefficient (R^2), P value, and number (N) of samples used for each analysis were shown. (c) Serum concentrations of IL-6 were determined in each indicated age group of individuals. A log transformation of the value was used to provide a more normal distribution for IL-6. The number (N) of all subjects in each age group that was available for each test was shown. (d) A regression analysis was carried out to determine the correlation of the log value of serum concentrations of IL-6 and the percentage of CD28⁺CD95⁻ CD8 T cells in the indicated age groups of individuals. The regression correlation coefficient (R^2), P value, and number (N) of samples used for each analysis were shown.

Fig. 4.

IGFBP3 correlates with preservation of naïve CD8 T cells. (a) Serum concentrations of IGFBP3 were determined for each indicated age group of individuals ($***P < 0.005$ between the indicated groups of comparison). The number (N) of all subjects in each age group that was available for each test was shown. (b) A regression analysis was carried out to determine the correlation of serum concentrations of IGFBP3 and the percentage of CD28⁺CD95⁻ CD8 T cells in the indicated age groups of individuals. The regression correlation coefficient (R^2), P value, and number (N) of samples used for each analysis were shown. (c) Serum concentrations of GH were determined for each indicated age group of individuals ($*P < 0.05$ between the indicated groups of comparison). The number (N) of all subjects in each age group that was available for each test was shown. (d) The percentage of CD28⁺CD95⁻ CD8 T cells in the indicated age groups of individuals was plotted against serum concentration of GH. The Fisher exact correlation test was used to determine the correlation between the two variables. Dotted lines represent the mean of each variable from the indicated group of individuals.

Fig. 5.

Increased T3 correlates with the decrease in terminally differentiated CD8 T cells. (a) Serum concentrations of T3 were determined for individuals in the indicated age groups (* $P < 0.05$; *** $P < 0.005$ between the indicated groups of comparison). A regression analysis was carried out to determine the correlation of serum concentrations of T3 and the percentage of (b) CD28⁺CD95⁻ and (c) CD28⁻CD95⁺ CD8 T cells in the indicated age groups of individuals. The regression correlation coefficient (R^2), P value, and number (N) of samples used for each analysis were shown.