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Age-associated alteration in naive and memory Th17 cell response in humans

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Abstract

Th17 cells produce IL-17 that plays an important role in host defense. However, little is known about whether aging affects human Th17 cells. Here we demonstrated that healthy elderly people (age $\geq$ 65) had a decreased frequency of IL-17-producing cells in memory CD4⁺ T cells compared to healthy young people (age $\leq$ 40) while both groups had similar frequencies of IFN- γ -producing cells in the same memory cell subset as measured by flow cytometry. In contrast, the healthy elderly had increased differentiation of IL-17-producing effector cells but not IFN- γ -producing cells from naïve CD4⁺ T cells compared to the healthy young. The results of ELISA also showed similar findings with increased IL-17 production from naïve CD4⁺ T cells and decreased IL-17 production from memory CD4⁺ T cells in the elderly compared to the young. These findings indicate that aging differentially affects naïve and memory Th17 cell responses in humans.

Keywords

Human; aging; IL-17; T helper 17 (Th17) cells; CD4⁺ T cells

1. Introduction

T helper 17 (Th17) cells, a recently identified subset of CD4⁺ T cells, produce the pro-inflammatory cytokine IL-17 (IL-17A) that plays an important role in defending host against

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microorganisms such as fungus [1, 2]. IL-17 potently induces production of IL-8 and G-CSF from epithelial cells and fibroblasts, leading to activation and recruitment of neutrophils. Indeed, mice deficient of IL-17 receptor A had impaired neutrophil-mediated immune response with increased susceptibility to microorganisms like *Candida albicans* and *Klebsiella pneumoniae* [3–5]. IL-17 also enhances production of IL-6, TNF- α , the acute phase reactants C-reactive protein and serum amyloid A [1]. Other target genes up-regulated by IL-17 are anti-microbial molecule defensin and chemokines including CCL20 that recruits dendritic cells (DC) and T cells.

Previous studies reported the roles for cytokines produced by innate and adaptive immune cells in controlling the development and expansion of Th17 cells [6–8]. These cytokines include IL-1 β , IL-6, TGF- β , IL-21 and IL-23 [6–8]. Indeed, human CD4⁺ T cells expressing high levels of IL-1 receptor I (IL-1RI) or IL-23 receptor (IL-23R) had increased production of IL-17 compared to CD4⁺ T cells not expressing these receptors [9, 10]. Similar to Th1 and Th2 cell development driven by transcriptional factors T-bet and GATA3, respectively, Th17 cell development is regulated by transcriptional factors ROR γ t (human ortholog is RORC) and ROR α [11, 12]. It is also well known that Th1 and Th2 cells have distinct expression patterns of chemokine receptors. CXCR3 and CCR5 are highly expressed on Th1 cells whereas Th2 cells have high levels of CCR3, CCR4 and CCR8 expression [13]. Similarly, Th17 cells have increased expression of CCR4 and CCR6 [14]. Of interest, a recent study reported that CD161, a c-type lectin receptor associated with natural killer (NK) cells, is expressed largely on memory CD4⁺ T cells producing IL-17 in human peripheral blood, although CD161⁺ cells were not found in the naïve CD4⁺ T cell subset [15].

Aging is associated with alterations in T cell immunity that include thymic atrophy and changes in T cell subsets and function [16]. These alterations likely contribute to increased risk of infection with high morbidity and mortality in the elderly [17, 18]. In fact, the incidence of community acquired pneumonia (CAP) increases with aging, and pneumonia is the third most common cause for hospitalization in people age greater than 65 [19, 20]. In the United States, extracellular bacteria including *Streptococcus pneumoniae* and *Klebsiella pneumoniae* account for a large number of cases of pneumonia in the elderly [21, 22]. Also, mortality from candidemia is higher in the elderly than in the young [23]. Thus, it is conceivable that an alteration in the Th17 cell response occurs with aging, leading to increased risk for infection. Here we showed that healthy elderly people (age ≥ 65) had a decreased frequency of Th17 cells in peripheral blood compared to healthy young people (age ≤ 40). Of interest, the differentiation of Th17 cells from naïve CD4⁺ T cells was increased in the elderly compared to the young whereas the frequency of Th17 cells in memory CD4⁺ T cells was lower in the elderly than in the young. Consistent with the latter finding, the frequency of memory CD4⁺ T cells expressing the Th17 cell-associated markers CD161 or CCR4 and CCR6 also was lower in the elderly than in the young. Taken together, these findings suggest that the maintenance of memory Th17 cells but not the differentiation of Th17 cells is impaired in humans with aging, and that such an alteration may contribute to increased susceptibility to infection in the elderly.

2. Materials and methods

2.1. Human subjects

Healthy elderly (age ≥ 65 , n = 38) and young subjects (age ≤ 40 , n = 41) were recruited for this study (mean age $\pm$ SD, 74.2 $\pm$ 10.5 and 27.4 $\pm$ 10.6). There was no gender difference between the two groups (male:female for elderly and young 7:31 and 10:31, respectively, $P = 0.519$ by Chi-square test). Individuals who were taking immunosuppressive drugs or who had any disease potentially affecting the immune system including autoimmune diseases, infectious diseases, malignancy, diabetes and asthma were excluded [24–27]. Informed

consent was obtained from all subjects. This work was approved by the institutional review committee of Yale University.

2.2. Cells and flow Cytometry

Peripheral blood mononuclear cells (PBMCs) were prepared from peripheral blood on FicollPAQUE gradients. Cells were stained with antibodies to Pacific Blue-CD4, PE-CCR6, PE-Cy5-CD45RA, PE-Cy7-CCR4, FITC or APC-CD161 (all from BD Bioscience, San Jose, CA), FITC-IL-1 receptor 1 (IL-1RI, R&D Systems, Minneapolis, MN), APC-CD27 (eBioscience, San Diego, CA) or isotype antibodies. Cells were analyzed using an LSRII® flow cytometer (BD Bioscience) and FlowJo software (Tree Star, Ashland, OR).

2.3. Cell sorting and culture

PBMCs were stained with antibodies to PE-Cy5-CD45RA, PE-Cy7-CCR7 and Pacific Blue-CD4. Stained cells were sorted into naïve ($CD45RA^{+}CCR7^{+}$) and memory ($CD45RA^{+}CCR7^{-}$) $CD4^{+}$ T cells using a FACS Aria® (BD Bioscience). In some experiments, untouched memory $CD4^{+}$ T cells were purified using a magnetic cell purification kit (Miltenyi Biotec, Auburn, CA). Sorted naïve and memory $CD4^{+}$ T cells were cultured in serum free X-VIVO-15 medium (Lonza, Basel, Switzerland) and RPMI 1640 medium supplemented with 10% FCS, respectively. Cells were seeded at 2.5×10^4 in U-bottomed 96-well plates and stimulated for 7 days with beads coated with anti-CD3 and anti-CD28 antibodies (one bead per 10 cells; Invitrogen) or PBS (control). In addition to the antibody-coated beads, cytokines including IL-1 β (25 ng/ml), IL-6 (25 ng/ml), IL-23 (25 ng/ml), TGF- β (5 ng/ml; all from R&D Systems) and IL-21 (25 ng/ml, eBioscience) were added to naïve $CD4^{+}$ T cells. Similarly, IL-1 β (25 ng/ml) and IL-23 (25 ng/ml, eBioscience) were added to some memory $CD4^{+}$ T cells as indicated.

2.4. Intracellular cytokine staining and ELISA

For intracellular cytokine staining, PBMCs or 7 day-cultured cells were stimulated for 4 hours with a combination of PMA (50 ng/ml; Sigma-Aldrich, St. Louis, MO) and ionomycin (1 μ g/ml; Sigma-Aldrich) or PBS (control) in the presence of Golgiplug (BD Bioscience). Stimulated cells were stained with antibodies to Pacific blue-CD4, PE-Cy5-CD45RA or FITC or APC-CD161 as indicated. Cells were fixed, permeabilized and stained with antibodies to Pacific blue, PE or FITC-IL-17A (eBioscience), PE-RORC or PE-Cy7-IFN- γ (BD Bioscience) followed by analysis on an LSRII® flow cytometer. IL-17 and IFN- γ in culture supernatant were analyzed using commercially available ELISA kits (eBioscience) in triplicate.

2.5. Statistical Analysis

The quantitative data were expressed as the mean $\pm$ standard error of mean (SEM). Mann-Whitney test and Pearson correlation were used for statistical analyses as appropriate using SPSS 17.0 (SPSS Inc.). *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. The frequency of $CD4^{+}$ T cells producing IL-17 in peripheral blood is decreased in the elderly compared to the young

We measured the frequency of $CD4^{+}$ T cells producing IL-17 (Th17) and/or IFN- γ (Th1) in peripheral blood of healthy young and elderly people after 4 hours of PMA (phorbol myristate acetate)/ionomycin stimulation using flow cytometry. We identified $CD4^{+}$ T cells producing IL-17, IFN- γ , or both (Figure 1A; representative example). In line with previous studies, the frequency of IL-17-producing $CD4^{+}$ T cells was much smaller than that of IFN-

γ -producing cells [10, 28]. The frequency of IL-17⁺IFN- γ ⁻ CD4⁺ T cells was lower in the elderly than in the young (mean $\pm$ SEM, $0.68 \pm 0.08\%$ vs. $1.20 \pm 0.17\%$, $P = 0.011$) (Figure 1B). Similarly, the former group had a decreased frequency of IL-17⁺IFN- γ ⁺ CD4⁺ T cells compared to the latter one (mean $\pm$ SEM, $0.16 \pm 0.02\%$ vs. $0.48 \pm 0.16\%$, $P = 0.037$) (Figure 1B). In contrast, the frequency of IL-17⁻IFN- γ ⁺ CD4⁺ T cells was not different between the two groups (mean $\pm$ SEM, $20.83 \pm 2.01\%$ vs. $18.56 \pm 2.36\%$, $P = 0.308$). We assessed the ratio of CD4⁺ T cells producing IL-17 to the same cells producing IFN- γ since the balance of cells producing these cytokines could be unchanged despite the decreased frequency of IL-17-producing CD4⁺ T cells in the elderly. The ratio of Th17 to Th1 cells was lower in the elderly than in the young (Figure 1C). Of interest, the frequency of IL-17⁺IFN- γ ⁻ CD4⁺ T cells directly correlated with the frequency of IL-17⁻IFN- γ ⁺ CD4⁺ T cells in young people ($r = 0.634$, $P = 0.002$) (Figure 1D). However, a similar correlation was not found in elderly people ($r = 0.349$, $P = 0.121$). A possible mechanism for the decreased frequency of Th17 cells in the elderly is reduced expression of the transcription factor RORC that is essentially involved in promoting IL-17 production [11, 12]. However, expression levels of this molecule in IL-17⁺IFN- γ ⁻, IL-17⁻IFN- γ ⁺ and IL-17⁻IFN- γ ⁺ CD4⁺ T cells were not different between the two groups (Figure 1E).

It is well known that the development of IL-17-producing CD4⁺ T cells from naïve CD4⁺ T cells requires long-term T-cell receptor (TCR) triggering in the presence of Th17-polarizing cytokines [6–8]. Indeed, IL-17-producing CD4⁺ T cells in PBMCs stimulated for 4 hours with PMA/ionomycin were cells with the memory phenotype (CD45RA⁻) (Figure 1F, representative data). Thus, it is possible that the decreased frequency of IL-17-producing CD4⁺ T cells in the elderly could be secondary to a decreased frequency of memory CD4⁺ T cells. However, the elderly had a trend toward an increased frequency of memory CD4⁺ T cells compared to the young (mean $\pm$ S.E.M., $66.9 \pm 3.1\%$ vs. $59.4 \pm 2.9\%$, $P = 0.094$). Furthermore, the frequencies of IL-17⁺IFN- γ ⁻ cells and IL-17⁺IFN- γ ⁺ cells in the memory CD4⁺ T cell subset without the expression of CD45RA were lower in the elderly than in the young (mean $\pm$ S.E.M., $1.01 \pm 0.1\%$ vs. $1.81 \pm 0.25\%$, $P = 0.001$; 0.23 ± 0.14 vs. 0.67 ± 0.20 , $P = 0.004$). The frequency of IL-17⁻IFN- γ ⁺ cells in the same memory CD4⁺ T cell subset was not different between the two groups (mean $\pm$ S.E.M., $29.7 \pm 2.1\%$ vs. $28.9 \pm 2.9\%$, $P = 0.753$). Taken together, our observations indicate that the balance of Th17 and Th1 cells in human peripheral blood changes with aging, resulting in a propensity toward a decreased frequency of Th17 cells.

3.2. The elderly have a decreased frequency of memory CD4⁺ T cells producing IL-17 that is not restored by Th17-promoting cytokines

Our assay analyzed the frequency of IL-17-producing CD4⁺ T cells in the peripheral blood of young and elderly people after 4 hours of PMA/ionomycin stimulation. Such cells producing IL-17 were cells with the memory phenotype. Thus, we next determined whether the elderly had a decreased frequency of Th17 cells in the memory CD4⁺ T cell subset. We purified memory CD4⁺ T cells from peripheral blood of young and elderly people and stimulated the cells for 7 days with TCR triggering (Figure 2A; representative example). The frequency of IL-17⁺IFN- γ ⁻ cells was lower in the elderly than in the young (mean $\pm$ SEM, $1.39 \pm 0.21\%$ vs. $2.50 \pm 0.26\%$, $P = 0.004$) (Figure 2B). Also, the frequency of IL-17⁺IFN- γ ⁺ cells was lower in the former than in the latter (mean $\pm$ SEM, $0.43 \pm 0.09\%$ vs. $1.09 \pm 0.18\%$, $P = 0.002$) (Figure 2B). In contrast, the frequency of IL-17⁻IFN- γ ⁺ cells was not different between the two groups. Similar findings were observed in measuring IL-17 and IFN- γ in tissue culture supernatant by ELISA (Figure 2C). Cytokines such as IL-1 β and IL-23 are known to promote Th17 cell response in humans [9, 10, 29, 30]. Thus, we investigated whether adding these cytokines during TCR triggering could lead to similar frequencies of IL-17-producing cells in young and elderly people. Addition of these

cytokines during TCR triggering did increase the frequency of IL-17-producing cells (Figure 2A; representative example). However, the frequency of these cells was still lower in the elderly than in the young while both groups had similar frequencies of IL-17⁻ IFN- γ ⁺ cells (Figure 2D). Also, the results of ELISA measuring IL-17 and IFN- γ in culture supernatant showed similar results (Figure 2E). These findings suggest that the decreased frequency of Th17 cells in the peripheral blood of the elderly stems in part from the reduced Th17 cell response in the memory CD4⁺ T cell population.

3.3. The elderly have a decreased frequency of memory CD4⁺ T cells expressing CD161 and a combination of CCR4 and CCR6

In humans, memory CD4⁺ T cells that produce IL-17 are known to express CD161, CCR4, CCR6, and IL-1 receptor I [10, 14, 15]. Thus, we measured the frequency of memory (CD45RA⁻) CD4⁺ T cells expressing these molecules in peripheral blood of young and elderly people (Figure 3A and D; representative examples). The elderly had a decreased frequency of CD161⁺CD4⁺ T cells in the memory CD4⁺ T cell subset compared to the young (Figure 3B). We next sorted memory CD4⁺ T cells from both groups and stimulated them for 7 days with TCR triggering. IL-17-producing cells were more frequently found in the CD161⁺ T cell population than in the CD161⁻CD4⁺ T cell population in both young and elderly people (Figure 3C). Similar to CD161, the elderly had a decreased frequency of CCR4⁺CCR6⁺ CD4⁺ T cells compared to the young (Figure 3D and E). However, the frequency of IL-1RI⁺ memory CD4⁺ T cells was not different between the two groups (data not shown).

3.4. The differentiation of Th17 cells from naïve CD4⁺ T cells is enhanced in the elderly

The decreased frequency of memory CD4⁺ T cells producing IL-17 in the elderly could be secondary to impaired differentiation of Th17 cells from naïve CD4⁺ T cells with aging. Thus, we assessed the development of IL-17-producing cells from naïve CD4⁺ T cells in young and elderly people. Purified naïve CD4⁺ T cells were stimulated with T cell receptor (TCR) triggering in the presence of IL-17-polarizing cytokines including IL-1 β , IL-6, IL-21, IL-23 and TGF- β . Cells producing IL-17 or IFN- γ were noticed in both young and elderly people although the frequency of cells producing both cytokines was fairly low (Figure 4A; representative example). The elderly had an increased frequency of IL-17-producing cells compared to the young (mean $\pm$ SEM, 1.10 $\pm$ 0.27% vs. 0.35 $\pm$ 0.09%, P = 0.041) (Figure 4B). Although the frequency of IFN- γ -producing cells appeared to be higher in the elderly than that in the young, the difference was not statistically significant (mean $\pm$ SEM, 1.32 $\pm$ 0.33% vs. 0.61 $\pm$ 0.11%, P = 0.059) (Figure 4B). Similar findings were observed in measuring IL-17 and IFN- γ in tissue culture supernatant by ELISA (Figure 4C). The role for IL-1RI in differentiating Th17 cells from human naïve CD4⁺ T cells was recently reported [10]. The development of Th17 cells was found mostly in naïve CD4⁺ T cells with the expression of IL-1RI but not in the same subset without the expression of this molecule [10]. Thus, we measured the frequency of naïve CD4⁺ T cells with or without expressing IL-1RI. The elderly had an increased frequency of IL-1RI⁺ naïve CD4⁺ T cells compared to the young (Figure 4D and E), potentially contributing to the increased differentiation of Th17 cells from naïve CD4⁺ T cells with aging. Our findings indicate that the differentiation of Th17 cells from naïve CD4⁺ T cells increased with aging and that the decreased frequency of memory CD4⁺ T cells in peripheral blood of the elderly could not be explained by impaired Th17 cell differentiation.

4. Discussion

Here we have demonstrated that healthy elderly people have a decreased frequency of Th17 cells in peripheral blood compared to healthy young people while both groups had similar

frequencies of Th1 cells. Indeed, the correlation of the frequencies of Th17 and Th1 cells alters with aging, leading to the impaired balance of Th17 and Th1 cell responses. Of interest, the development of Th17 cells from naïve CD4⁺ T cells in response to TCR triggering and Th17-polarizing cytokines was increased in the elderly compared to the young while the frequency of Th17 cells in the memory CD4⁺ T cell subset was lower in the elderly than in the young. These findings suggest that the decreased frequency of Th17 cells in the peripheral blood of the elderly could be secondary to a change in memory Th17 cell response but not in differentiating Th17 cells from naïve CD4⁺ T cells. In line with the results of analyzing memory Th17 cells, the frequency of memory CD4⁺ T cells expressing CD161 or a combination of CCR4 and CCR6, markers associated with human Th17 cells, also was lower in the elderly than in the young. However, the frequency of memory CD4⁺ T cells expressing these molecules was higher than that of memory CD4⁺ T cells producing IL-17. This finding which is consistent with the results of previous studies suggests that none of these molecules could identify a pure subset of Th17 cells [14, 15]. Our study demonstrates that alterations in Th17 cell responses occur in humans with aging, providing a possible explanation for an increased risk of infection in the elderly.

In our study, the frequency of IL-17-producing (Th17) cells in total CD4⁺ T cells was measured in young and elderly individuals after stimulating PBMCs for 4 hours with PMA/ionomycin. Of interest, the frequency of this cell population was lower in the elderly than that in the young although both groups had similar frequencies of IFN- γ -producing (Th1) cells. In this short-term stimulation assay, IL-17-producing CD4⁺ T cells were cells with the memory cell phenotype, suggesting a decreased frequency of Th17 cells among memory CD4⁺ T cells in the elderly. Indeed, memory CD4⁺ T cells from the elderly had a decreased frequency of IL-17-producing cells and reduced IL-17 production in response to TCR triggering compared to the same cell subset from the young. This finding could be explained by several possibilities. For instance, the elderly could have impaired differentiation of effector Th17 cells from naïve CD4⁺ T cells, leading to the decreased development of memory Th17 cells. However, this does not appear to be the case since the results of our study showed that the elderly had increased differentiation of IL-17-producing effector cells from naïve CD4⁺ T cells in response to a combination of TCR triggering and Th17-polarizing cytokines compared to the young. We recognize that the difference in the frequency of IL-17-producing cells in peripheral blood between the young and the elderly could be considered small. However, it is well known that the frequency of Th17 cells in the immune system is substantially lower than that of Th1 cells [10, 28]. Of interest, the frequency of IL-17-producing CD4⁺ T cells correlated with that of IFN- γ -producing CD4⁺ T cells in healthy young people. This suggests that individuals who produce higher amounts of IL-17 from memory CD4⁺ T cells likely have a stronger capacity to produce IFN- γ from the same cell type compared to individuals who produce lower amounts of IL-17. However, this correlation was not found in the elderly. Of note, the elderly have an increased risk for bacterial and fungal infections. IL-17 is a critical component for controlling extracellular pathogens such as *Klebsiella* and *candida* [3–5]. Thus, it is conceivable that the decreased frequency of memory Th17 cells could contribute to increased risk of infections with extracellular pathogens in the elderly [21–23]. A recent study reported decreased IL-17 production from total T cells in response to TCR triggering in males with aging as measured by ELISA [31]. This study did not measure IL-17 which was produced specifically from CD4⁺ T cells as we have done here although this cytokine can be produced from naïve and memory CD4⁺ T cells as well as from non-CD4⁺ T cells such as CD8⁺ T cells and CD3⁺CD4[−]CD8[−] (double negative) T cells [32, 33].

We observed an increased differentiation of Th17 cells from naïve CD4⁺ T cells in response to TCR triggering and Th17-polarizing cytokines with aging. This intriguing finding suggests that the elderly could develop an expansion of Th17 cells from naïve CD4⁺ T cells

in response to microorganism(s) to which they were previously unexposed although the frequency of total naïve CD4⁺ T cells could decline with aging. A recent mouse study reported increased IL-17 production from natural killer T (NKT) cells with enhanced neutrophil activation in aged mice infected with herpes virus, leading to increased mortality due to hepatic necrosis [34]. This suggests that an increased differentiation of Th17 cells from naïve CD4⁺ T cells in the elderly may not necessarily be beneficial to the host given the potent pro-inflammatory property of IL-17. A recent study reported a correlation of the differentiation of IL-17-producing CD4⁺ T cells from naïve CD4⁺ T cells with the expression of IL-1RI in humans [10]. In fact, we noticed increased levels of IL-1RI expression on naïve CD4⁺ T cells in the elderly compared to the young, suggesting a possible mechanism for our observation. Altered expression of IL-1RI on naïve CD4⁺ T cells could be a contributing factor to the increased Th17 cell differentiation in the elderly.

In conclusion, our study demonstrates that the elderly have a decreased frequency of memory Th17 cells and an increased differentiation of effector Th17 cells from naïve CD4⁺ T cells compared to the young. These findings suggest that aging differentially affects naïve and memory Th17 cell responses in humans.

Abbreviations

Th17 cells	T helper 17 cells
Th1 cells	T helper 1 cells
IL-1RI	IL-1 receptor I
IL-23R	IL-23 receptor
PBMCs	Peripheral blood mononuclear cells

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Figure 1.

The elderly have a decreased frequency of Th17 cells but not Th1 cells in peripheral blood compared to the young. PBMCs from healthy elderly (age ≥ 65) and young (age ≤ 40) individuals were stimulated for 4 hours *ex vivo* with PMA and ionomycin or PBS (control) in the presence of Golgiplug. The frequency of CD4⁺ T cells producing IL-17 and/or IFN- γ as well as their expression of CD45RA and RORC were measured using flow cytometry. (A) Representative dot plots showing CD4⁺ T cells producing IL-17 and/or IFN- γ . (B) The frequency (%) of IL-17⁺IFN- γ ⁻, IL-17⁺IFN- γ ⁺ and IL-17⁻IFN- γ ⁺ CD4⁺ T cells in healthy young (n=21) and elderly (n = 21) subjects. (C) Elderly subjects had a lower Th17/Th1 ratio (frequency of IL-17⁺IFN- γ ⁻ CD4⁺T cells/frequency of IL-17⁻IFN- γ ⁺ CD4⁺T cells) compared to young subjects. (D) The frequency (%) of IL-17⁺IFN- γ ⁻ CD4⁺ T cells correlates with the frequency of IL-17⁻IFN- γ ⁺ CD4⁺ T cells in the young but not in the elderly. (E) Expression of RORC (MFI) in CD4⁺ T cells producing IL-17 and/or IFN- γ in the young and the elderly (n = 8 for each group) (F) Expression of CD45RA by CD4⁺ T cells producing and not producing IL-17. Numbers in dot plots indicate the frequency of cells for each quadrant. Bar graphs are shown as mean $\pm$ SEM.

Figure 2.

The elderly have decreased Th17 cell response in memory CD4⁺ T cells that are stimulated with T cell receptor (TCR) triggering alone or a combination of TCR triggering with IL-1β and IL-23 compared to the young. Sorted memory (CD45RA⁻) CD4⁺ T cells from young and elderly individuals were stimulated for 7 days with anti-CD3/CD28 antibodies or a combination of anti-CD3/CD28 antibodies, IL-1β and IL-23. (A) Representative dot plots showing CD4⁺ T cells producing IL-17 and/or IFN-γ. (B, D) The frequency (%) of IL-17⁺IFN-γ⁻, IL-17⁻IFN-γ⁺ and IL-17⁺IFN-γ⁺ CD4⁺ T cells in memory CD4⁺ T cells stimulated as described above in young (n = 9) and elderly (n = 10) people. (C, E) IL-17 and IFN-γ levels in culture supernatant from memory CD4⁺ T cells that were stimulated as above were measured by ELISA in young and elderly people (n = 7 for each group). Numbers in dot plots indicate the frequency of cells for each quadrant. Bar graphs are shown as mean ± SEM.

Figure 3.

The elderly have a decreased frequency of memory CD4⁺ T cells expressing CD161 and a combination of CCR4 and CCR6 compared to the young. (A, B, D, E) PBMCs from young (n = 9) and elderly (n = 11) individuals were stained with antibodies to CD4, CD45RA, CCR4, CCR6, CD161 or isotype control followed by analysis on a flow cytometer. (A) Representative dot plots showing memory (CD45RA⁻) CD4⁺ T cells expressing CD161. (B) The frequency (%) of CD45RA⁻CD161⁺ memory CD4⁺ T cells in young and elderly subjects. (C) The frequency (%) of IL-17-producing cells in CD161⁺ and CD161⁻ cells in memory CD4⁺ T cells. Sorted memory (CD45RA⁻) CD4⁺ T cells were stimulated for 7 days with anti-CD3/CD28 antibodies and stained with antibodies to CD161 and IL-17. Stained cells were analyzed using flow cytometry. (D) Representative dot plots showing memory (CD45RA⁻) CD4⁺ T cells expressing CCR4 and CCR6. (E) The frequency (%) of CD45RA⁻CCR4⁺CCR6⁺ CD4⁺ T cells in young and elderly subjects. Numbers in dot plots indicate the frequency of cells for each gated box or quadrant. Bar graphs are shown as mean $\pm$ SEM.

Figure 4.

The differentiation of Th17 cells from naïve CD4⁺ T cells is increased in the elderly. (A–C) Sorted naïve (CD45RA⁺) CD4⁺ T cells from young (n = 9) and elderly (n = 10) individuals were stimulated for 7 days with anti-CD3/-CD28 antibodies in the presence of IL-1 β , IL-6, TGF- β , IL-21, and IL-23. (A) Representative dot plots showing CD4⁺ T cells producing IL-17 and/or IFN- γ . (B) The frequency (%) of IL-17⁺IFN- γ ⁻, IL-17⁺IFN- γ ⁺ and IL-17⁻IFN- γ ⁺ CD4⁺ T cells. (C) IL-17 and IFN- γ in tissue culture supernatant were measured by ELISA. (D–E) IL-1RI expression on naïve (CD45RA⁺) CD4⁺ T cells was measured in young (n = 9) and elderly (n = 11) individuals using flow cytometry. (D) Representative dot plots showing naïve CD4⁺ T cells that express IL-1RI. (E) The frequency (%) of CD45RA⁺IL-1RI⁺ CD4⁺ T cells in young and elderly subjects.