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The Relationship of Cytomegalovirus (CMV) Infection with circulatory IFN- α levels and IL-7 receptor α expression on CD8⁺ T cells in Human Aging

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Abstract

The IL-7 receptor alpha (IL-7R α) is the high affinity receptor for IL-7 which is essential for T cell homeostasis. We recently reported an age-associated expansion of human effector memory (EM) CD8⁺ T cells expressing IL-7R α low (IL-7R α^{low}), which could be detrimental to hosts by occupying “immunological space”. We investigated the potential mechanisms for this phenomenon, focusing on cytomegalovirus (CMV) infection and INF- α . In the elderly (age 65), CMV infection was associated with a decreased frequency of naïve CD8⁺ T cells as well as with an increased frequency of total EM and IL-7R α^{low} EM CD8⁺ T cells. However, in the young (age 40), this viral infection was associated only with an increased frequency of IL-7R α^{low} EM CD8⁺ T cells. There was no association found between CMV immune status and plasma levels of IFN- α . In CMV-infected young and elderly people, INF- α levels had no correlation with the frequency of IL-7R α^{low} EM CD8⁺ T cells although this cytokine levels correlated with the frequency of IL-7R α^{low} CD45RA⁺ EM CD8⁺ T cells in CMV-uninfected elderly people. Our findings suggest that the effect of CMV infection on the frequency of CD8⁺ T cell subsets may begin with IL-7R α^{low} EM CD8⁺ T cells and spread to other subsets with aging. Also, IFN- α could be associated with the expansion of IL-7R α^{low} CD45RA⁺ EM CD8⁺ T cells in the CMV-uninfected elderly.

Keywords

Human; aging; cytomegalovirus (CMV); IL-7; CD8⁺ T cells

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Competing Interests.

The authors have no competing interests.

1. Introduction

IL-7, a member of the common cytokine-receptor γ -chain (γ c) family cytokines, is essential for CD8⁺ T cell homeostasis [1]. The IL-7 receptor complex comprises two chains: the high affinity IL-7R α chain and γ c. IL-7 can promote the survival of memory CD8⁺ T cells even in the absence of antigen through up-regulating the anti-apoptotic molecule Bcl-2. In fact, in mice infected with lymphocytic choriomeningitis virus, effector CD8⁺ T cells with high levels of IL-7R α expression survived better to become memory CD8⁺ T cells compared to effector CD8⁺ T cells with low levels of IL-7R α expression [2]. Of interest, a recent study also suggested a potential role of IL-7R α in the development of autoimmunity in that blocking this receptor reduced autoimmunity and T cell activation in lupus-prone mice [3].

Memory T cells can be divided into central and effector memory (EM) T cells based on the capacity to migrate secondary lymphoid tissues (e.g. spleen and lymph nodes). While central memory T cells have such a capacity by expressing the lymphoid tissue homing chemokine receptor CCR7, EM T cells can move to infected and/or inflamed peripheral sites [4]. We recently reported the presence of two unique subsets of EM CD8⁺ T cells with high and low levels of IL-7R α expression (IL-7R α^{high} and α^{low}) in human peripheral blood [5]. Compared to IL-7R α^{high} EM CD8⁺ T cells, IL-7R α^{low} EM CD8⁺ T cells had impaired proliferation and survival with reduced T cell receptor (TCR) repertoire [5]. Also, IL-7R α^{low} EM CD8⁺ T cells had decreased expression of the co-stimulatory molecules CD27 and CD28 that are down regulated with T cell stimulation. This suggests that IL-7R α^{low} EM CD8⁺ T cells are functionally exhausted cells with skewed TCR specificities towards previously encountered antigens.

Alterations in T cell immunity occur with aging, likely contributing to an increased risk of infection and malignancy. One of the most prominent age-associated changes in T cell immunity is probably the expansion of memory CD8⁺ T cells with decreased naïve CD8⁺ T cells [6, 7]. Of interest, IL-7R α^{low} EM CD8⁺ T cells expand with aging in humans [5]. Such cell expansion could be harmful to hosts since it may impair the ability of CD8⁺ T cells to properly develop immune responses to newly encountered microorganisms, like emerging strains of influenza virus, by occupying “immunological space” [8, 9].

Cytomegalovirus (CMV) that establishes latent infection in humans has been suggested as a factor related to memory CD8⁺ T cell expansion with aging in that the elderly infected with this virus had increased numbers of memory CD8⁺ T cells compared to the elderly uninfected with CMV [10, 11]. CMV infection could directly induce the expansion of CMV-specific memory CD8⁺ T cells by immune stimulation. Alternatively, but not mutually exclusively, CMV-associated expansion of memory CD8⁺ T cells could be driven by other factors such as the cytokine IFN- α which can be produced in viral infection. Indeed, IFN- α is known to affect the differentiation of CD8⁺ T cells [12, 13]. However, the relationship among CMV infection, circulatory levels of IFN- α and expansion of memory CD8⁺ T cells, in particular IL-7R α^{low} EM CD8⁺ T cells, is unknown. Here we investigated this question by measuring these parameters in young and elderly people.

2. Materials and Methods

2.1. Human subjects

Healthy elderly (age 65, n = 43) and young subjects (age 40, n = 62) were recruited for this study (mean age $\pm$ SD, 72.9 $\pm$ 8.6 and 28.3 $\pm$ 5.4, respectively). 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 as previously done [5, 7, 14, 15]. The CMV infection status (IgG)

was determined by ELISA (Bio-Quant Diagnostic Kits, San Diego, CA). Twenty eight subjects were CMV positive in the young group whereas 21 subjects were CMV positive in the elderly group. Mean antibody indexes were calculated according to the manufacturer's instruction. Plasma IgG levels between CMV-positive and -negative subjects in both young and elderly groups were different (mean antibody index $\pm$ standard error of mean, young CMV-negative subjects vs. young CMV-positive subjects, 0.378 ± 0.054 vs. 1.832 ± 0.072 , $P < 0.001$ by unpaired t -test; elderly CMV-negative subjects vs. elderly CMV-positive subjects, 0.250 ± 0.053 vs. 2.12 ± 0.146 , $P < 0.001$ by unpaired t -test). There was no gender difference between the two groups (males to females, 16:46, 12:31 for young and elderly groups, respectively, $P > 0.05$ by Chi-square test). Informed consent was obtained from all subjects. This work was approved by the institutional review committee of Yale University.

2.2. Flow Cytometry and ELISA

Peripheral blood mononuclear cells (PBMCs) were prepared from peripheral blood on FicollPAQUE gradients. Cells were stained with antibodies to APC-Cy7- or Amcyan-CD3, Pacific Blue-CD8, PE-Cy7-CCR7, PE-Cy5-CD45RA (all from BD Pharmingen, San Jose, CA) and FITC-IL-7R α (R&D Systems, Minneapolis, MN) or isotype antibodies. Cells were analyzed using an LSRII® flow cytometer (BD Bioscience) and FlowJo software (Tree Star, Ashland, OR). Plasma IFN- α levels were determined using a commercially available ELISA kit (panspecific) according to the manufacturer's instruction (Mabtech Inc., Mariemont, OH).

2.3. Statistical Analysis

Two-way ANOVAs were performed to compare the effects of CMV infection on the primary outcomes for each age group using PROC ANOVA in SAS version 9.2. Some outcomes were log-transformed and the normal property of residuals was checked using Kolmogorov-Smirnov test. The association between IFN- α and the primary outcomes were assessed using Pearson correlation coefficient by CMV infection status in each age group. The statistical tests were performed at a significance level of 0.05.

3. Results and Discussion

3.1. The association of CMV infection with the frequency of CD8⁺ T cell subsets is different between young and elderly people

We analyzed the frequency of CD8⁺ T cell subsets including IL-7R α ^{low} EM CD8⁺ T cells in healthy young (age 40) and elderly (age 65) people who were infected or uninfected with CMV (see details in Supplementary Methods). As previously reported [7], we identified naïve (CD45RA⁺CCR7⁺), central memory (CM, CD45RA⁻CCR7⁺) and EM (CD45RA^{+/}-CCR7⁻) CD8⁺ T cells based on the expression of the lymphoid tissue homing receptor CCR7 and the T cell receptor co-receptor CD45RA (Fig. 1A). EM cells could be further divided into CD45RA⁻ and CD45RA⁺ EM CD8⁺ T cells. IL-7R α ^{high} and ^{low} cells were identified in CD45RA⁻ and CD45RA⁺ EM CD8⁺ T cells (Fig. 1A). The association of CMV infection with the frequency of the CD8⁺ T cell subsets was different between young and elderly people (Fig. 1 and Supplementary Fig. 1). In the elderly, CMV-infected individuals had a decreased frequency of naïve CD8⁺ T cells and an increased frequency of EM (EM cells include both CD45RA⁻ and CD45RA⁺ cells) CD8⁺ T cells compared to CMV-uninfected individuals (Supplementary Fig. 1). The frequency of CM CD8⁺ T cells was not different between CMV-infected and -uninfected elderly people. In the young, both CMV-infected and -uninfected individuals had similar frequencies of naïve, CM and EM CD8⁺ T cells (Supplementary Fig. 1A–B).

In analyzing IL-7R α ^{low} EM CD8⁺ T cells, CMV-infected individuals had an increased frequency of IL-7R α ^{low} EM CD8⁺ T cells compared to CMV-uninfected individuals regardless of age (Fig. 1B). Young and elderly people had different patterns of the association of CMV infection with IL-7R α ^{low} CD45RA⁻ and CD45RA⁺ EM CD8⁺ T cells. The association of CMV infection with IL-7R α ^{low} cells was primarily found in CD45RA⁻ EM CD8⁺ T cells in the elderly while this association was noticed in both CD45RA⁻ and CD45RA⁺ EM CD8⁺ T cells in the young (Fig. 1D–E). Our findings indicate that CMV infection is selectively associated with the expansion of IL-7R α ^{low} EM CD8⁺ T cells in the young while this infection is associated with the altered frequencies of naïve, EM and IL-7R α ^{low} EM CD8⁺ T cells in the elderly. Of interest, IL-7R α expression can be down regulated by TCR stimulation and cytokines such as IL-7 and IL-15 [1]. Thus, repetitive CMV reactivation with stimulation of immune cells including EM CD8⁺ T cells could be a factor accountable for CMV-associated expansion of IL-7R α ^{low} EM CD8⁺ T cells in young and elderly people.

3.2. Young and elderly people have similar plasma levels of IFN- α regardless of CMV immune status

IFN- α is involved in regulating the differentiation of CD8⁺ T cells [12]. Mice deficient of IFN- α receptor on CD8⁺ T cells had decreased CD8⁺ T cell expansion [16]. However, it is unknown about the relationship of circulatory IFN- α levels with the CMV immune status and expansion of memory CD8⁺ T cells in young and elderly people. Thus, we measured plasma IFN- α levels in young and elderly people using an ELISA kit that can detect 11 subtypes of IFN- α (pan-specific) and correlated the results with CMV immune status. Plasma levels of this cytokine were not different between the young and the elderly (mean $\pm$ standard deviation, 9.5 pg/ml $\pm$ 14.7 vs. 10.8 pg/ml $\pm$ 12.3). In addition, in each group, CMV-infected and uninfected individuals had similar levels of plasma IFN- α (Fig. 2A). These findings indicate that circulatory IFN- α levels are not affected by aging or CMV immune status in humans. The absence of the effect of the CMV immune status on plasma IFN- α levels could be secondary to the presence of intact immunity against CMV in healthy people.

3.2. Plasma levels of IFN- α correlate with the frequency of IL-7R α ^{low} CD45RA⁺ EM CD8⁺ T cells in elderly people uninfected with CMV

We next explored whether plasma levels of IFN- α correlated with the frequency of CD8⁺ T cell subsets. In young people, there was no correlation noticed between the frequency of CD8⁺ T cell subsets and plasma levels of IFN- α , regardless of CMV immune status (Supplementary Table 1). Similarly, no correlation was found between these parameters in CMV-infected elderly people. However, in CMV-uninfected elderly people, plasma levels of IFN- α correlated with the frequency of CD45RA⁺ EM and IL-7R α ^{low} CD45RA⁺ EM CD8⁺ T cells (Figure 2B–C). Although the significance of this finding is yet to be determined, it suggests a potential role of IFN- α in expanding some subsets of EM CD8⁺ T cells in CMV-uninfected elderly people. In fact, IFN- α could promote the expansion of short-lived effector CD8⁺ T cells with IL-7R α ^{low} in mice which had the phenotype similar to that of human IL-7R α ^{low} EM CD8⁺ T cells [12].

In this study, we found that the young and the elderly had different patterns of the association of CMV infection with the altered frequency of CD8⁺ T cell subsets. In the elderly, CMV infection was associated with the altered frequencies of naïve, total EM and IL-7R α ^{low} EM CD8⁺ T cells. However, in the young, this viral infection was associated only with the altered frequency of IL-7R α ^{low} EM CD8⁺ T cells. Despite the known relationships among viral infection, IFN- α and CD8⁺ T cell differentiation, there was no association of CMV immune status and plasma IFN- α levels. In CMV-infected young and

elderly people, INF- α levels had no correlation with the frequency of IL-7R α^{low} EM CD8 $^{+}$ T cells although this cytokine levels correlated with the frequency of IL-7R α^{low} CD45RA $^{+}$ EM CD8 $^{+}$ T cells in CMV-uninfected elderly people. Taken together, our findings suggest that the effect of CMV infection on the frequency of CD8 $^{+}$ T cell subsets may begin with IL-7R α^{low} EM CD8 $^{+}$ T cells and spread to other subsets with aging in humans. Also, IFN- α could be associated with the expansion of IL-7R α^{low} CD45RA $^{+}$ EM CD8 $^{+}$ T cells in the CMV-uninfected elderly.

Highlights

- The expansion of human IL-7R α^{low} effector memory (EM) CD8 T cells occurs with age
- Such cell expansion could be detrimental to hosts by occupying “immunological space”
- We studied mechanisms for this phenomenon, focusing on CMV infection and INF- α
- CMV effect on the frequency of CD8 T cells may begin with IL-7R α^{low} EM CD8 T cells
- IFN- α may affect IL-7R α^{low} CD45RA $^{+}$ EM CD8 T cell increase in the CMV-negative elderly

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. The association of cytomegalovirus (CMV) infection with the frequency of IL-7Rα^{low} effector memory (EM) CD8⁺ T cells in young and elderly people

Peripheral blood mononuclear cells (PBMCs) were purified from the blood of young (age 40) and elderly (age 65) donors and stained with antibodies to CD3, CD8, CD45RA, CCR7, IL-7Rα or isotype. Stained cells were analyzed on an LSRII® flow cytometer. (A) Representative data showing the expression of IL-7Rα on naïve (CD45RA⁺CCR7⁺), central memory (CM, CD45RA⁺CCR7⁺), CD45RA⁻ effector memory (CD45RA⁻ EM, CD45RA⁻CCR7⁺) and CD45RA⁺ EM (CD45RA⁺CCR7⁺) CD8⁺ T cells. (B) The frequency of IL-7Rα^{low} cells in CD45RA^{-/+} EM CD8⁺ T cells among CMV-infected and -uninfected young and elderly people. (C–D) The frequency of IL-7Rα^{low} cells in (C) CD45RA⁻ EM CD8⁺ T cells and (D) CD45RA⁺ EM CD8⁺ T cells among CMV-infected and -uninfected young and elderly people. Bars indicate mean. *P* values were calculated by the two-way ANOVAs.

Figure 2. The association of plasma IFN- α levels with cytomegalovirus (CMV) infection in young and elderly people as well as with the frequency of CD45RA⁺ effector memory (EM) CD8⁺ T cells in CMV-uninfected elderly people

(A) IFN- α levels in plasmas of young (age 40) and elderly (age 65) donors were measured by ELISA. (B–C) Correlations of plasma IFN- α levels with the frequency of CD45RA⁺ EM CD8⁺ T cells (B) and IL-7R α^{low} CD45RA⁺ EM CD8⁺ T cells in CMV-uninfected elderly people. Bars indicate mean. *P* values were obtained by the two-way ANOVAs (A) or Pearson correlation analysis (B–C).