Cytokine 61 (2013) 485–490

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Maintenance of CMV-specific CD8$^+$ T cell responses and the relationship of IL-27 to IFN-γ levels with aging

Min Sun Shin [a,1], Jin Soo Lee [a,b,1], Naeun Lee [a], Won-Woo Lee [a,c], Sang Hyun Kim [a,d], Insoo Kang [a,*]

[a] Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520, USA
[b] Department of Internal Medicine, Inha University School of Medicine, Incheon 400-712, Republic of Korea
[c] Department of Microbiology and Immunology, College of Medicine, Seoul National University, 28 Yongon-dong, Chongno-gu, Seoul 110-799, Republic of Korea
[d] Department of Microbiology, College of Medicine, Kangwon National University, 192-1, Hyoja-Dong, Chunchon, Kangwon-Do 200-701, Republic of Korea

ARTICLE INFO

Article history:
Received 1 May 2012
Received in revised form 4 October 2012
Accepted 27 November 2012
Available online 29 December 2012

Keywords:
CD8$^+$ T cells
IL-27
Human
Aging
Cytomegalovirus (CMV)

ABSTRACT

We investigated whether healthy young (age ⩽ 40) and elderly (age ⩾ 65) people infected with cytomegalovirus (CMV) had similar levels of CD8$^+$ T cell cytokine production and proliferation in response to an immunodominant CMV pp65 peptide pool given the role of CD8$^+$ T cells in controlling viral infection and the association of CMV with immunosenescence. Plus, we determined the effects of aging and CMV-infectious status on plasma levels of IL-27, an innate immune cytokine with pro- and anti-inflammatory properties, as well as on its relationship to IFN-γ in that IL-27 can promote the production of IFN-γ. The results of our study show that young and elderly people had similar levels of CD8$^+$ T cell proliferation, and IFN-γ and TNF-α production in response to CMV pp65 peptides. Plasma levels of IL-27 were similar between the two groups although CMV-infected young and elderly people had a trend toward increased levels of IL-27. Regardless of aging and CMV-infectious status, plasma levels of IL-27 correlated highly with plasma levels of IFN-γ. These findings suggest the maintenance of CMV pp65-specific CD8$^+$ T cell proliferation and cytokine production with aging as well as the sustaining of circulatory IL-27 levels and its biological link to IFN-γ in young and elderly people irrespective of CMV infection.

1. Introduction

CD8$^+$ T cells are critically involved in host defense against viral infections [1]. These cells can directly kill virus-infected cells. Also, cytokines such as IFN-γ and TNF-α produced from CD8$^+$ T cells participate in controlling viral infections. In particular, IFN-γ can stimulate monocytes and macrophages to produce cytokines and chemokines as well as promote MHC class I molecule expression on virus-infected target cells [2]. In mice infected with viruses, the deficiency of IFN-γ production from T cells was associated with delayed viral clearance [3]. In addition, inhibition of hepatitis B viral gene expression was abrogated in mice that were neutralized of IFN-γ and TNF-α [4].

Alterations in T cell immunity occur with aging. These changes include involution of the thymus, the organ where T cells mature, with a decreased generation of naïve T cells [5]. In addition, expansion of memory CD8$^+$ T cells with reduced T cell receptor (TCR) repertoire is found in humans with aging [5–7]. Cytomegalovirus (CMV), which establishes persistent infection, has been suggested as a potential driving force for the age-associated expansion of memory CD8$^+$ T cells based on the observations showing the increased frequency of CMV-specific cells in the expanded memory CD8$^+$ T cells [8–10]. CD8$^+$ T cells, a major cellular source of IFN-γ, are critically involved in defending the host against viral infection and reactivation [1]. These findings suggest a possible change in cytokine production and proliferation of CMV-specific CD8$^+$ T cells in humans with aging, leading to the altered proportion of CMV-specific CD8$^+$ T cells. However, it is still controversial whether CMV-specific CD8$^+$ T cells have altered function in the elderly [11–14].

The cytokine IL-27 is composed of the p27 and Epstein–Barr virus-induced gene (EBI3) subunits (reviewed in [15,16]). The p27 and EBI3 subunits are produced primarily by dendritic cells (DCs) and macrophages. The receptor complex for IL-27 comprises WSX-1 (IL-27 receptor alpha) and gp130, which is also shared by other cytokines including IL-6 and IL-11 receptors. IL-27 appears

Abbreviations: CMV, cytomegalovirus; TCR, T cell receptor; EBI3, Epstein–Barr virus-induced gene; Th, T helper; DCs, dendritic cells; EAE, experimental allergic encephalitis; CIA, collagen-induced arthritis; PBMCs, peripheral blood mononuclear cells; CFSE, carboxyfluorescein diacetate; SEM, standard error of mean; NK, natural killer.

* Corresponding author. Address: Department of Internal Medicine, Section of Rheumatology, S525C TAC, 300 Cedar Street, New Haven, CT 06520, USA. Tel.: +1 203 785 6678; fax: +1 203 785 7053.
E-mail address: Insoo.kang@yale.edu (I. Kang).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.cyto.2012.11.024

to have both pro- and anti-inflammatory effects [15,16]. IL-27 is known to promote IFN-γ-producing T helper 1 (Th1) cell response [17]. Similarly, enhanced IFN-γ production from human naïve $CD8^+$ T cells by IL-27 was reported [18]. Recent work showed that IL-27 could increase the production of the anti-inflammatory cytokine IL-10 by a subset of $CD4^+$ T cells in mice and humans [19–21], suggesting an immune regulatory function of this cytokine. Also, IL-27 suppressed the development of experimental allergic encephalitis (EAE) and collagen-induced arthritis (CIA), mouse models of multiple sclerosis and rheumatoid arthritis, respectively [19,22,23]. Viruses including influenza A and hepatitis B viruses induced the production of IL-27 [24,25]. In fact, patients infected with hepatitis B virus had increased levels of IL-27 in blood compared to healthy controls [25]. These data indicate the involvement of viral infection in producing IL-27 with pro- and anti-inflammatory properties. However, little is known about the effect of aging and CMV-infectious status on blood IL-27 levels and the relationship of such cytokine levels to IFN-γ levels in humans.

In this study, we investigated the effects of aging on cytokine production and proliferation of $CD8^+$ T cells in response to the CMV immunodominant protein pp65 in healthy people. We also determined whether CMV-infectious status and aging affected circulatory levels of IL-27 and its relationship to IFN-γ levels. Healthy young (age $\leqslant 40$) and elderly (age $\geqslant 65$) people had similar frequencies of $CD8^+$ T cells that proliferated and produced IFN-γ and TNF-α in response to CMV pp65 protein in peripheral blood. In addition, young and elderly people had similar levels of plasma IL-27 although the ones infected with CMV had a trend towards increased levels of plasma IL-27 compared to the ones uninfected with this virus. Of interest, IL-27 levels correlated with plasma IFN-γ levels in young and elderly people regardless of CMV-infectious status, which supports the biological link of the two cytokines. These findings indicate that CMV-specific $CD8^+$ T cell responses, as measured by cytokine production and cell proliferation, are preserved with aging and that circulatory levels of IL-27 and its relationship to IFN-γ is maintained in the elderly regardless of CMV infectious status.

2. Materials and methods

2.1. Human subjects and cells

Healthy young (age $\leqslant 40$, $n = 85$) and elderly (age $\geqslant 65$, $n = 59$) subjects were recruited for this study (mean age ± SD, 28.8 ± 5.2 and 73.9 ± 5.8). There was no gender difference between the two groups (males to females, 23:62, 16:43 for young and elderly groups, respectively, $P = 0.994$ by Chi-square test). Six of the recruited subjects were smokers. 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 [26–29]. This study was approved by the institutional review committee of Yale University. Peripheral blood was drawn after informed consent. Peripheral blood mononuclear cells (PBMCs) were prepared from blood on FicollPAQUE gradients. The CMV infection status (IgG) was determined by ELISA (BioQuant Diagnostic Kits, San Diego, CA).

2.2. Analyses for cytokine production and cell proliferation

To measure cytokine production by CMV-specific $CD8^+$ T cells, PBMCs from CMV-positive individuals were stimulated for 6 h with or without a CMV pp65 peptide pool (ProMix™ HCMVA (PP65), Proimmune, Sarasota, FL) in the presence of Golgi plug (BD Pharmingen, San Diego, CA) during the last 5 h of stimulation. Stimulated cells were fixed and permeabilized using appropriate buffers (Cytofix/Cytoperm buffers, BD Pharmingen) followed by staining with antibodies to APC-Cy7-CD3, Pacific Blue-CD8, APC-IFN-γ and FITC-TNF-α or isotype controls (BD Pharmingen). Stained cells were analyzed on an LSRII® flow cytometer. For determination of $CD8^+$ T cell proliferation in response to CMV pp65 peptides, PBMCs were stained with carboxyfluorescein diacetate (CFSE, Molecular Probe, Eugene, OR) and stimulated for 7 days with or without the CMV pp65 peptide pool (Proimmune). Cells were then analyzed on an LSRII® flow cytometer. The frequency of CMV pp65-specific $CD8^+$ T cells which proliferated or produced IFN-γ or TNF-α was obtained by subtracting the frequency of proliferating or cytokine-producing $CD8^+$ T cells in unstimulated samples from the frequency of the same cells in samples stimulated with CMV pp65 peptides.

2.3. Measuring plasma IL-27 and IFN-γ

IL-27 and IFN-γ levels in plasmas were measured by commercially available ELISA kits (BioLegend, San Diego, CA and ebioscience, San Diego, CA, respectively) according to the manufacturers' instructions.

2.4. Statistical analysis

The unpaired Student's *t*-test, Pearson and Spearman correlations were done for statistical analyses as appropriate using SPSS 19.0 (IBM, Chicago, IL). *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Young and elderly people have similar frequencies of IFN-γ- and TNF-α-producing $CD8^+$ T cells in peripheral blood in response to CMV pp65 protein

We measured the frequency of IFN-γ- and/or TNF-α-producing $CD8^+$ T cells in young and elderly people using flow cytometry after stimulating PBMCs with an immunodominant CMV pp65 peptide pool (Fig. 1A, representative figure). A large number of $CD8^+$ T cells producing these cytokines could produce both IFN-γ and TNF-α at the single cell level in response to CMV pp65 peptide pool. In both groups, the frequency of $CD8^+$ T cells producing IFN-γ and/or TNF-α was typically less than 2% of total $CD8^+$ T cells and was not different between young and elderly people people (mean frequency (%) ± standard error of mean (SEM), 0.96% ± 0.20 vs. 1.84% ± 0.65) (Fig. 1B). Young and elderly people had similar frequencies of $CD8^+$ T cells producing IFN-γ or TNF-α alone (Fig. 1C and D). Similarly, the frequency of $CD8^+$ T cells producing both cytokines was not different between the two groups (Fig. 1E). These findings suggest that the frequency of CMV pp65-specific $CD8^+$ T cells that produce IFN-γ and TNF-α is sustained in humans with aging.

3.2. Young and elderly people have similar levels of $CD8^+$ T cell proliferation in response to CMV pp65 peptides

We measured the proliferation of $CD8^+$ T cells in response to the CMV pp65 peptides in $CD8^+$ T cells in young and elderly people (Fig. 2A, representative figure). The frequency of proliferating cells was not different between the two groups (mean frequency (%) ± SEM, 13.8% ± 3.02 vs. 14.6% ± 2.47) (Fig. 2B). The frequency of CMV pp65-specific proliferating cells correlated with the frequency of $CD8^+$ T cells producing IFN-γ and/or TNF-α in response to the CMV pp65 peptides in the young ($r = 0.724$, $P = 0.012$) (Fig. 2C). However, such correlation was not found in the elderly (Fig. 2D). These observations suggest that an age-associated alteration may occur in the relationship between the capacities of