Clinical Immunology (2013) 147, 79–88

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

An altered relationship of influenza vaccine-specific IgG responses with T cell immunity occurs with aging in humans

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Received 17 December 2012; accepted with revision 26 February 2013
Available online 15 March 2013

KEYWORDS
Influenza vaccine;
T cells;
Humoral response;
Human;
Aging

Abstract Alterations in T cell immunity occur with aging. Influenza causes significant morbidity and mortality in the elderly. We investigated the relationship of serum IgG responses with hemagglutinin inhibition (HI) antibody titers and the frequency of distinct T cell subsets in young and elderly people who received the inactivated influenza vaccine. Influenza vaccine-specific IgG responses correlated with the increase of HI antibody titers and the frequency of $CD4^+$ T cells producing IFN-γ and IL-17 in young, but not elderly, people. Also, only in young people, such IgG responses correlated with the frequency of memory T cells, especially central memory cells, $CD45RA^-$ effector memory $CD8^+$ T cells and IL-7 receptor alpha high effector memory $CD8^+$ T cells with potent survival and proliferative capacity. These findings suggest that aging alters the association of influenza-vaccine specific IgG responses with HI antibody titers, cytokine-producing capacity and proportions of memory T cells in humans.

1. Introduction

Age-associated alterations occur in the immune system that comprises innate and adaptive immunities [1–6]. These changes account in part for the development of pathologic conditions like infections, malignancies and inflammatory diseases that are associated with aging. T cells, a component

Abbreviations: TCR, T cell receptor; HI, hemagglutinin inhibition; CM, central memory (CM); IL-7Rα^{high}, IL-7 receptor alpha high; EM, effector memory.

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http://dx.doi.org/10.1016/j.clim.2013.02.022

of adaptive immunity, play a critical role in the immune system. T cells are involved in host defense against microorganisms and malignancy as well as in regulating other immune responses such as antibody production from B cells [2,3]. Alterations in the number and function of T cells are found with aging [2,3]. Aged mice have decreased generation of naïve T cells with atrophy of the thymus as well as expansion of memory T cells with reduced T cell receptor (TCR) repertoire diversity [2,7–9]. In addition, changes in the effector function of T cells, including cytokine production, cell proliferation and cytotoxicity, have been found with aging [2,3,5,10].

Memory T cells can be divided into several subsets. Central memory (CM) T cells with the expression of lymphoid tissue homing chemokine receptor 7 (CCR7) can migrate to secondary lymphoid tissues like the lymph nodes and spleen, while effector memory (EM) T cells can move to inflamed or infected peripheral tissues [11]. The cytokine IL-7 is essentially involved in the maintenance of memory T cells by promoting cell survival [12]. Indeed, two different subsets of EM $CD8^+$ T cells with high and low levels of IL-7 receptor alpha chain expression (IL-$7R\alpha^{high}$ and ^{low}) exist in human peripheral blood. IL-$7R\alpha^{high}$ EM $CD8^+$ T cells have potent cell proliferative and survival capacity compared to IL-$7R\alpha^{low}$ EM $CD8^+$ T cells [13]. Of interest, the proportions of naïve, CM and EM T cells as well as IL-$7R\alpha^{high}$ and ^{low} EM $CD8^+$ T cells change with aging [13,14].

$CD4^+$ T helper (Th) cells can be divided into Th1, Th2 and Th17 cells based on the cytokines predominantly produced by individual Th cell subsets [15]. Th cells can promote humoral immune responses by producing cytokines as well as providing co-stimulation to B cells [16]. Recent studies reported increased antibody production and immunoglobulin isotype class switching by the cytokine IL-17 which is produced primarily by Th17 cells [17]. IFN-γ, the stereotypic cytokine produced by Th1 cells, could increase the generation of IgG2a while IL-4 derived from Th2 cells enhances the production of IgG1 in mice [18]. The findings from previous studies investigating the effect of aging on Th cell cytokines, in particular IFN-γ and IL-4, were largely inconsistent (reviewed in [2]). Of interest, we recently reported a decreased frequency of IL-17-producing memory $CD4^+$ T cells in the peripheral blood of the elderly, compared to young people [10].

Influenza is common but can be a serious medical illness which causes significant morbidity and mortality in the elderly [19]. In fact, most deaths associated with influenza are seen in people age 65 or older [19]. Although the influenza vaccine can provide protection against an influenza virus infection, the vaccine response appears to decline with aging [4,20–23]. The assay for hemagglutination inhibition (HI) antibody titers has been used to determine the response to influenza vaccine. In fact, the levels of the increase in serum HI antibody titers after influenza vaccination were lower in the elderly than in the young [20,24]. Also, the elderly had decreased levels of influenza virus-specific $CD4^+$ and $CD8^+$ T cell responses compared to young adults after vaccination [22,23,25,26]. Such impaired humoral and cellular immune responses to influenza vaccine could stem in part from alterations in the immune system with aging [21,22]. The accumulation of memory $CD8^+$ T cells expressing CD45RA or lacking CD28 expression, which may represent a functionally senescent cell population with reduced telomerase activity, was associated with a blunted increase in serum HI antibody titers in the elderly after influenza vaccination [27–29]. This finding suggests that the age-associated expansion of memory T cells could have a detrimental effect on the development of vaccine responses.

Here we studied the relationship of serum IgG responses with HI antibody titers as well as the frequency of T cell subsets with distinct cellular characteristics in young and elderly people who received influenza vaccine. Although serum IgG responses to influenza vaccine correlated with the increase of HI antibody titers in young people, a similar correlation was not found in elderly people. The frequency of $CD4^+$ T cells producing IFN-γ and IL-17 correlated with IgG responses to influenza vaccine in the young but not in the elderly. In addition, such IgG responses correlated with the frequency of memory $CD4^+$ and $CD8^+$ T cells subsets, especially CM, IL-$7R\alpha^{high}$ EM and $CD45RA^-$ EM $CD8^+$ T cells, only in the young. These findings suggest that aging affects the association of IgG and HI antibody responses specific for the influenza vaccine as well as the relationship of the cytokine-producing capacity and the proportions of memory T cells with the development of influenza vaccine-specific IgG responses in humans.

2. Materials and methods

2.1. Human subjects

Healthy elderly subjects 65 years of age (n = 26) or older and healthy young subjects 40 years of age or younger (n = 29) were recruited for this study. The mean age ± SD for young and elderly subjects was 24.9 years ± 2.2 and 75.3 years ± 6.8, respectively. The gender distribution was not different between the two groups (F:M, 16:13 and 15:11, respectively for young and elderly groups, P = 0.851 by Chi-square test). Twenty six of 29 young subjects and all 26 elderly subjects had received influenza vaccine in the previous year (P = 0.238 by Fisher's exact test). Individuals who were taking immunosuppressive drugs or had a disease potentially affecting the immune system, including cancer and autoimmunity were excluded [10,13,24,26,30,31]. All subjects were vaccinated in October 2011 with a commercially available inactivated subvirion trivalent 2011-2012 influenza vaccine containing the following strains: A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008. Peripheral blood was collected before vaccination and at a mean of 32 days (range, 29–36 days) after vaccination. Informed consent was obtained from all subjects. This work was approved by the institutional review committee of Yale University.

2.2. Flow cytometric analysis

Peripheral blood mononuclear cells (PBMCs) were prepared from blood on FicollPAQUE gradients. Cells were stained with antibodies to APC-Cy7-CD3, Pacific Blue-CD8, PE-Cy7-CCR7, PE-Cy5-CD45RA (all from BD Biosciences, San Jose, CA) and FITC-IL-7Rα (R&D Systems, Minneapolis, MN) or isotype antibodies. For intracellular cytokine staining, PBMCs were stimulated for 4 hours with a combination of phorbol myristate acetate (PMA, 50 ng/ml; Sigma-Aldrich, St. Louis, MO) and ionomycin (1 μg/ml; Sigma-Aldrich) or PBS (control)