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The effect of sex on immune cells in healthy aging: Elderly women have more robust natural killer lymphocytes than do elderly men

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ABSTRACT

Immune gender differences have been reported, but are little studied in elderly humans. We compared monocyte and lymphocyte subsets, along with soluble immune mediators in healthy men and women over the age of 70. We also measured natural killer (NK) lymphocyte cytotoxic granule exocytosis, chemokine synthesis, and cytokine synthesis in response to a variety of stimuli. Elderly women had significantly more circulating B cells than men, whereas men had more CD4 central memory T cells and higher monocyte levels. Plasma adiponectin levels were higher in women, plasma retinol-binding protein 4 levels were higher in men, but there were no significant gender differences in C-reactive protein, IL-15, or sphingosine-1-phosphate. Women had a higher ratio of immature $CD56^{bright}$ NK cells to mature $CD56^{dim}$ NK cells, indicating a gender difference in NK cell maturation in the elderly. Comparing sexes, female mature NK cells had more vigorous cytotoxic granule responses to K562 leukemia cells and IFN-γ responses to NKp46 crosslinking. Moreover, female NK cells were more likely to produce MIP-1β in response to a variety of stimuli. These data show that gender influences NK cell activity in elderly humans.

1. Introduction

Men and women differ in several aspects of physiology, including immune responses (Oertelt-Prigione, 2012). Gender differences in immune cells and immune responses can be linked to estrogen, progesterone, and testosterone levels, X-linked genes, socioeconomic factors, and other differences (Oertelt-Prigione, 2012). Few studies have compared men and women in old age, when hormone differences are less marked. This is important because elderly men suffer more infectious disease complications, cancers, and death than do elderly women (Gabriel and Arck, 2014; Kaplan et al., 2002; Siegel et al., 2012).

Lymphocytes comprise innate natural killer (NK) cells and adaptive T cells and B cells. NK lymphocytes immediately respond to infectious disease agents and direct appropriate T cell responses (Caligiuri, 2008). Among NK cells, the less mature $CD56^{bright}$ cells are poorly cytotoxic, but vigorously respond to cytokines by making a variety of chemokines and cytokines, including MIP-1β and IFN-γ (Caligiuri, 2008). The more mature $CD56^{dim}$ cells are potent cytotoxic and cytokine-secreting cells when responding to cellular stimuli, such as K562 leukemia cells (Caligiuri, 2008; Fauriat et al., 2010). $CD56^{dim}$ NK cells themselves are heterogeneous, maturing from an early stage with high CD94/NKG2A heterodimer expression to later stages with less CD94/NKG2A expression and more killer cell immunoglobulin-like receptor (KIR) and CD57 expression (Björkström et al., 2010; Yu et al., 2010).

NK cell numbers are maintained in healthy elderly people, but NK-mediated cytotoxicity and NK cell secretion of immunoregulatory cytokines decline with aging. Aging-related NK defects are due, at least in part, to ineffective support from stromal cells (Hazeldine and Lord, 2013; Nair et al., 2014). Decreased NK cell activity in elderly people correlates with an increased incidence and severity of viral infection, pneumonia, and infectious disease deaths (Hazeldine and Lord, 2013; Ogata et al., 2001). Moreover, low NK function was associated with a 2-fold increase in cancer 2–11 years later (Imai et al., 2000). Removal of senescent cells is important for healthy aging (Baker et al., 2011). Therefore NK cells, which clear

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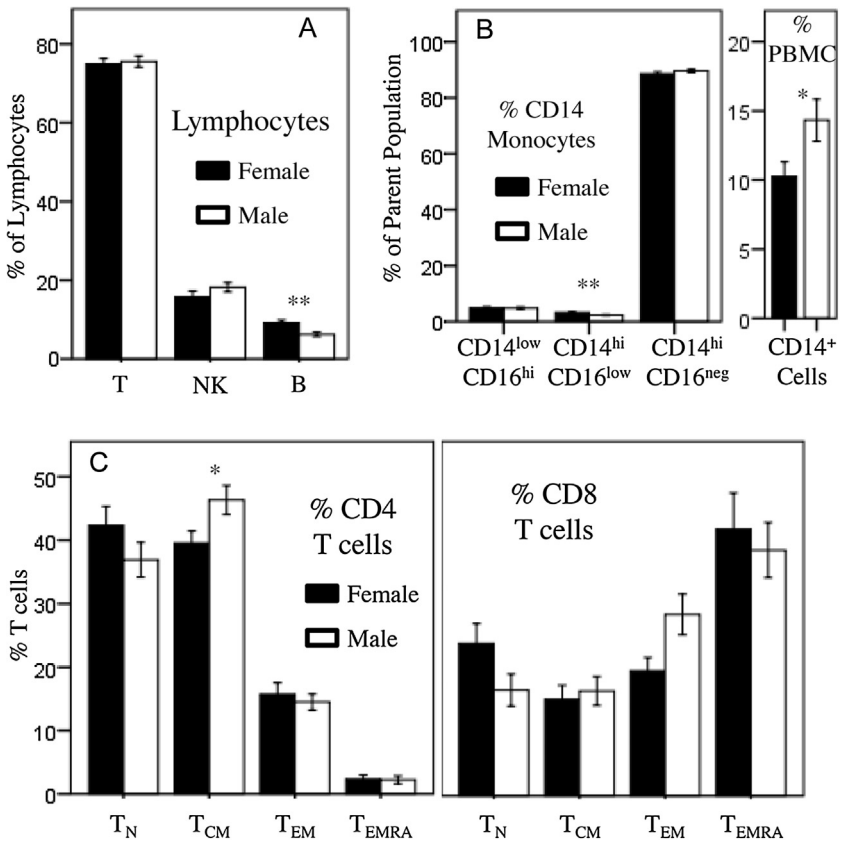

Fig. 1. Gender differences in blood mononuclear cells. T cells, NK cells, and B cells are shown as a percentage of lymphocytes (A). Monocytes are expressed as a percentage of PBMC (B, right panel). Shown are the percentages of $CD14^{+}$ monocytes in the $CD14^{low}CD16^{hi}$, $CD14^{hi}CD16^{low}$, and $CD14^{hi}CD16^{-}$ subsets (B, left panel). The percentage of CD4 and CD8 T cells classified as T_N, T_{CM}, T_{EM}, and T_{EMRA} (C). Plotted are the means and SEM for females (filled histograms) and males (open histograms). Asterisks denote significant mean differences as determined by student's T test (*, $p < 0.05$; **, $p < 0.01$).

senescent cells (Krizhanovsky et al., 2008), are likely important for increasing healthspan.

Many studies have addressed gender-related differences in NK cell numbers or cytotoxic activity toward K562 leukemia cells, but few have investigated gender differences in the elderly and none have examined chemokine and cytokine production. Because of the importance of NK cells in the elderly, our primary goal was to assess how sex affects NK cell maturation, cytotoxicity, chemokine, and cytokine responses to a variety of stimuli. We also measured the levels of serum factors known to affect NK cells. For the first time in any age group, we studied sex-linked differences in NK cell function other than cytotoxicity.

T cells are the major blood lymphocyte and both orchestrate and carry out adaptive immune responses. Both CD4 and CD8 T cells contain naïve (T_N), central memory (T_{CM}), effector memory (T_{EM}), and terminally differentiated effector memory (T_{EMRA}) subsets (Sallusto et al., 2004). As thymus function diminishes with age, T_N and T_{CM} decline and are replaced by T_{EMRA}, especially in the CD8 compartment (Czesnikiewicz-Guzik et al., 2008). T_{CM} are thought to be most important for maintaining functional T cell memory to infectious agents. During aging, T cells acquire HLA-DR and CD57 expression and lose CD28 expression (Czesnikiewicz-Guzik et al., 2008), and develop a senescent phenotype (McElhaney and Effros, 2009). To our knowledge, gender differences in these T cell subsets have not been investigated, especially in the context of the elderly. In the current study, we investigate for the first time gender differences in T cell naïve and memory subsets.

Blood monocytes comprise an important branch of the innate immune system. They immediately respond to infectious disease agents and stimulate T cells. The majority of blood monocytes express CD14 without co-expressing the low-affinity receptor for IgG, CD16 (Ziegler-Heitbrock, 2007). Monocytes that express CD16 with either normal or reduced CD14 levels respond to TLR ligands with relatively high levels of TNF-α and low levels of the anti-inflammatory cytokine, IL-10. $CD16^{+}$ monocytes express high levels of HLA-DR, are excellent antigen presenting cells, and can differentiate into dendritic cells (Ziegler-Heitbrock, 2007). $CD16^{+}$ monocytes also have been associated with glycemia, atherosclerosis and adiposity (Poitou et al., 2011). Because CD16 expression defines monocyte populations with distinct functions, we studied monocyte subsets in elderly men and women.

2. Methods

2.1. Subjects

Male and female subjects >70 years were recruited from volunteer donor pools at the University of Kentucky Sanders-Brown Center on Aging and by advertisements. Venous blood from 26 males (age mean ± standard error of the mean (SEM), 77.8 ± 0.31, range 70–90 years) and 24 females (age mean ± SEM, 77.0 ± 0.91, range 70–85 years) were analyzed between October 2012 and April 2014. Prospective donors were screened by telephone interview to exclude those with conditions previously demonstrated to affect NK cells. Exclusion criteria included hospitalization or serious illness in the prior year, history of immunologic illness (rheumatoid arthritis, systemic lupus, scleroderma, polymyositis, Sjögren's syndrome, transplantation, etc), current use of immunomodulatory medications (e.g., corticosteroids), inability to walk one city block, regular consumption of two or more alcoholic beverages per day