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Aging is associated with a numerical and functional decline in plasmacytoid dendritic cells, whereas myeloid dendritic cells are relatively unaltered in human peripheral blood

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Abstract

Dendritic cells (DC) are potent antigen-presenting cells that initiate and regulate T-cell responses. In this study, the numbers and functional cytokine secretions of plasmacytoid and myeloid DC (pDC and mDC, respectively) in peripheral blood from young and elderly subjects were compared. Overall, pDC numbers in peripheral blood were lower in healthy elderly compared with healthy young subjects ($p = 0.016$). In response to influenza virus stimulation, isolated pDC from healthy elderly subjects secreted less interferon (IFN)- α compared with those from healthy young subjects. The decline in IFN- α secretion was associated with a reduced proportion of pDC that expressed Toll-like receptor-7 or Toll-like receptor-9. In contrast, there was little difference in the numbers and cytokine secretion function between healthy young and healthy elderly subjects ($p = 0.82$). However, in peripheral blood from frail elderly subjects, the numbers of mDC were severely depleted as compared with either healthy young or elderly subjects ($p = 0.014$ and 0.007 , respectively). Thus, aging was associated with the numerical and functional decline in pDC, but not mDC, in healthy young versus elderly subject group comparisons, while declining health in the elderly can profoundly impact mDC negatively. Because of the importance of pDC for antiviral responses, the age-related changes in pDC likely contribute to the impaired immune response to viral infections in elderly persons, especially when combined with the mDC dysfunction occurring in those with compromised health.

Keywords

Plasmacytoid dendritic cell; Myeloid dendritic cell; Cytokine; Toll-like receptor; Immune senescence

1. Introduction

Dendritic cells (DC) are considered “professional” antigen presenting cells (APC) because of their abilities to capture, process, and present antigens to T cells[1]. DC can express high

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levels of major histocompatibility complex (MHC) and co-stimulatory molecules that activate T cells, including naïve T cells[2]. In addition, DC can secrete chemokines and cytokines that attract T cells and stimulate T cell growth[3–6]. Based on their lineage origins, DC in human peripheral blood can be categorized into two major subsets, myeloid DC (mDC) and plasmacytoid DC (pDC). Expression of myeloid lineage markers such as CD11c are characteristic of mDC, whereas characteristic lymphoid marker expression of pDC are pre-T-cell receptor (pT α) and Spi-B[7–9]. Different subsets of the Toll-like receptor (TLR) family are expressed in mDC and pDC[10], with mDC selectively expressing TLR 1–6, 10 and pDC primarily expressing TLR 7–9[11]. In addition to their phenotypic differences, mDC and pDC have distinct functions. For example, influenza virus or herpes simplex virus infection causes stimulation of mDC to secrete interleukin (IL)–6, tumor necrosis factor (TNF)– α , and IL-12, while their stimulation of pDC leads to secretion of interferon (IFN)– α [12–15]. The secretion of IFN- α stimulates natural killer (NK) cells and augments the IFN- γ secretion from type 1 CD4+ T helper cells (TH1) and CD8+ T cells, a hallmark of a type 1 T cell response (Th1 response)[16,17]. Consequently, the numbers present, ratio, and functional status of mDC and pDC subsets can influence the innate immune response and the subsequent downstream adaptive immune response.

Elderly persons are particularly susceptible to infection and death from infectious pathogens. For example, more than 90% of the annual influenza virus–related deaths occur among persons more than over 64 years old[18]. The decline in cell-mediated immune responses, particularly the cytotoxic T-cell immune response, is largely believed to be responsible for the increased morbidity and mortality from infectious diseases in elderly individuals[19,20]. Therefore, because DC play a pivotal role in T-cell activation and regulation, it is important to understand age-related changes in numbers and functional status of DC. Previous studies examining the role of DC in human aging have resulted in conflicting results[21]. In one study for mDC, it was reported that elderly subjects had lower numbers of mDC in their peripheral blood compared with young subjects, and mDC from elderly subjects secreted less IL-12 than young subjects after stimulation by lipopolysaccharide (LPS)[22]. In contrast, this age-related decline in mDC in peripheral blood was not observed in the report by Agrawal *et al.*[23]. In addition, monocyte-derived DC (MDDC) generated *in vitro*, which resemble mDC in peripheral blood, were reported to have no age-related changes in phenotype or function between young and elderly donor subjects[24]. However, the study by Agrawal *et al.* demonstrated that MDDC from elderly persons were impaired in pro-inflammatory cytokine secretion and phagocytosis[23]. With regard to pDC, it was reported that aging was associated with a decline in frequency and absolute cell counts in pDC found in peripheral blood[25,26]. However, a recent paper by Della Bella *et al.* showed that the number of pDC in peripheral blood was not affected by aging[22]. To date, there is no consensus as to how DC subsets are affected by the aging process.

In this study, we obtained mDC and pDC in peripheral blood from subjects of different age and health status. For comparison, the numbers of each subset present and their functional ability to secrete IL-12, IFN- α , and other inflammatory cytokines upon stimulation were determined. We observed that healthy aging was associated with a decline in numbers and functions of pDC, whereas the numbers and function of mDC in the same groups were relatively unaffected. In contrast to aging with sustained health, aging with declining health

was associated with a significant decline in the numbers of peripheral blood mDC. In concordance with the age-related changes in function of mDC and pDC, we also found that the proportion of pDC positive for TLR-7 or TLR-9 pDC were reduced, whereas the proportion of TLR-2 and TLR-4 positive mDC were unaltered with aging.

2. Subjects and methods

2.1. Recruitment and blood samples

The studies were conducted in three subject populations, healthy elderly, healthy young and elderly with underlying disease. The elderly groups were classified using the Canadian Study of Health and Aging (CSHA) categories 1 and 2 for the healthy elderly (fit and well respectively), and categories 5 and 6 for those with underlying disease, which CSHA qualifies as mildly or moderately frail[28]. The healthy populations were independently living volunteers. The exclusion criteria for healthy subjects have been described elsewhere[27]. Briefly, healthy subjects were excluded from the study if they had underlying chronic disease such as diabetes, autoimmune disease (such as systemic lupus erythematosus or rheumatoid arthritis), or congestive heart failure, were receiving ongoing treatment with immunosuppressive drugs, or were pregnant. Healthy subjects on medication to control hypertension and hypothyroidism were eligible for the study. Frail elderly subjects had chronic illness and impairment sufficiently severe for these subjects to need to live in an assisted living facility ($n = 20$). Each of these frail elderly subjects had at least one disabling disease (Alzheimer, disease, dementia, Parkinson, disease, or chronic obstructive pulmonary disease (COPD)), or a minimum of two significant chronic disease conditions including diabetes, arthritis, and cognitive impairment. All frail subjects were medically stable. None of the frail subjects were receiving chronic immunosuppressive therapy. The age range for young subjects was between 20 and 40 years, and healthy and frail elderly subjects were 60 and older. The subject numbers, ages, and medical information for each group are summarized in Table 1. The studies were approved by the Institutional Review Board at Eastern Virginia Medical School, and all participants provided informed consent.

2.2. Purification of peripheral blood mononuclear cells

Heparinized peripheral blood was obtained by venipuncture (20–30 ml), and peripheral blood mononuclear cells (PBMC) were isolated using the described protocol[27].

2.3. Quantification of mDC and pDC numbers and their expression of TLRs using flow cytometry

Live PBMC (2×10^6) were stained with the following antibodies in a 96-well U-bottom plate: lineage marker (FITC, including CD3, CD14, CD16, CD19, CD20, and CD56), CD123 (PE), HLA-DR (PerCP), and CD11c (APC) (BD Biosciences, San Diego, CA). Cells were incubated with the antibodies for 20 minutes in the dark at room temperature. Cells were washed twice before being acquired using a dual-laser FACSCaliber flow cytometer, and data were analyzed using CellQuest software (Becton Dickinson, San Diego, CA). Lineage marker–negative and HLA-DR–positive cells were designated as DC and gated for further analysis. DC with high CD11c and low CD123 expression were defined as mDC, whereas high expression of CD123 and low CD11c expression were defined as pDC[29].

For TLR expression, PBMC were stained with the similar antibody cocktail mentioned above with the exception that the antibody for CD123 (PE) was substituted with that of a PE-conjugated TLR of interest (eBioscience, San Diego, CA). For TLR-7 and TLR-9, PBMC were fixed and permeabilized for intracellular staining (a procedure similar to intracellular cytokine staining described below), whereas for TLR-2 and TLR-4, staining was performed on live PMBC for cell surface staining (a procedure similar to staining for mDC and pDC described above). The expressions of CD11c and TLR by DC were analyzed in a dot plot. The CD11c-high and CD11c-low DC were designated as mDC and pDC, respectively, and the percentage of them positive for a given TLR expression was recorded.

2.4. Influenza virus

Influenza A/Sydney/05/97 (H3N2) viruses were generated from inoculated chicken eggs from seed viruses obtained from the Centers for Disease Control and Prevention (CDC) using procedures previously described[30].

2.5. Intracellular cytokine staining of DC

PBMC (1.25×10^6) were co-incubated with poly I:C (50 $\mu\text{g}/\text{ml}$, Sigma, St. Louis, MO), CpG ODN2006 (6 $\mu\text{g}/\text{ml}$, MWG Biotech, High Point, NC), live influenza virus (10 HA unit/ml) or control antigen (either RPMI complete medium or diluted allantoic fluid) for 3 hours in a U-bottom 96-well plate. Brefeldin A (BFA, 5 $\mu\text{g}/\text{ml}$, Sigma) was added to the cells, and the incubation was continued for another 15 hours. PBMC were fixed (1% paraformaldehyde, Sigma), permeabilized (permeabilization buffer, Becton Dickinson, CA), and stained with the following conjugated antibodies: lineage marker (FITC), IL-12 (p40/70) or IFN- α (PE), HLA-DR (PerCP), and CD11c (APC) (BD Pharmingen, San Jose, CA). Stained PBMC were subjected to flow cytometry analysis. DC gated from lineage marker-negative and HLA-DR-positive cells were analyzed for their IL-12 or IFN- α cytokine expression versus their expression of CD11c. DC expressing CD11c (high) and IL-12 were defined as IL-12⁺-mDC, whereas DC positive for IFN- α and negative for CD11c were defined as IFN- α ⁺-pDC.

2.6. DC isolation and stimulation

PBMC from five to 10 young or elderly donors were pooled separately, and then used for mDC and pDC isolation with the CD1c (BDCA-1) Dendritic Cell Isolation Kit and BDCA-4 Cell Isolation Kit (Miltenyi Biotech, Auburn, CA). Isolated mDC and pDC (1×10^5) were co-incubated with CpG, poly I:C or influenza virus as mentioned above. The cells were incubated at 37°C overnight, after which the supernatant was collected.

2.7. Cytometric bead array assays

Supernatants from DC cultures were diluted 1:10 before analysis with a human inflammatory cytometry bead array (CBA) assay kit that analyzes the levels of IL-12 (p70), TNF- α , IL-10, IL-6, IL-1 β , and IL-8 simultaneously (BD Biosciences, San Diego, CA).

2.8. IFN- α ELISA

The concentration of IFN- α in DC supernatants was determined using a human IFN- α ELISA kit (BioSource, Camarillo, CA).

2.9. In vitro generation of DC

DC were generated using a previously described procedure[31]. Briefly, PBMC were placed in a T75 flask for 2 hours. Nonadherent PBMC were removed, and adherent PBMC were cultured for 7 days in the presence of IL-4 (500 units/ml) and GM-CSF (800 units/ml) (Genzyme, Cambridge, MA).

2.10. Statistical analysis

Testing of significance in the difference of means of the two age groups was done using the two-tailed Student's *t* test when data were normally distributed. Normal distribution of data was tested using an SPSS program (SPSS Inc., Chicago, IL). Where data were not normally distributed, a modified *t* test, Mann-Whitney rank sum test, or Wilcoxon signed rank test determined the significance of differences between the two groups. Statistical significance was set at $p < 0.05$. The correlation coefficients between two groups were analyzed using the Pearson's correlation coefficient test.

3. Results

3.1. Healthy aging is associated with a selective decline in pDC frequency, whereas the mDC frequency remains constant

The numbers of mDC and pDC in total PBMC from healthy young ($n = 52$; mean age, 28 years) and elderly subjects ($n = 75$; mean age, 74 years) were quantified using four-color flow cytometry. Figure 1A illustrates a representative dot plot showing the mDC and pDC population in PBMC using CD123 and CD11c as markers. The frequency of pDC in healthy elderly subjects was 28.6% less than that of the healthy young counterparts (median, 0.14% and 0.10% in young and elderly respectively, $p = 0.016$, Fig. 1B, left panel). This observation in age-related decline in pDC numbers was confirmed when pDC were quantified using BDCA4 and CD123 as markers (data not shown). Unlike pDC, the numbers of mDC did not differ between healthy young and healthy elderly subjects (median, 0.28% and 0.26% for young and elderly subjects respectively, $p = 0.82$, Fig. 1B, right panel). The ratio of pDC/mDC was 31.2% less in the healthy elderly group than that of the healthy young group (0.80 and 0.55 in healthy young and elderly respectively, $p = 0.024$). Because there is no age-related decline in the numbers of mDC in the healthy subjects, the decline in pDC/mDC ratio is primarily caused by the reduction in pDC with age. These data demonstrate an age-related decline in the numbers of pDC, whereas the numbers of mDC remain relatively unaffected during healthy aging.

3.2. Frail elderly subjects had a significant decline in mDC frequency and a disparity between the pDC and mDC compared with healthy elderly subjects

The numbers of mDC and pDC in peripheral blood from a group of frail elderly subjects ($n = 20$; mean age, 82 years) were compared with those in the healthy young and healthy

elderly group mentioned above. The frail elderly subjects had a 42.9% and 20.0% lower number of pDC than the healthy young and elderly subjects respectively (median, 0.08% for frail subjects, compared with 0.14% and 0.10% in healthy young and elderly subjects respectively, $p = 0.002$ and 0.04 , Fig. 1B left panel). The numbers of mDC in the frail elderly subjects was 41.3% below that of the healthy young or elderly subjects (median, 0.17%, compared with 0.28% and 0.26% in healthy young and healthy elderly subjects, $p = 0.014$ and 0.007 , Fig. 1B, left panel). The lower numbers of mDC in frail elderly subjects differs dramatically from that of healthy subjects, for whom the numbers of mDC did not change with age (Fig. 1B), suggesting that health status in the elderly subjects affects the numbers of mDC in peripheral blood.

Furthermore, the numbers of mDC and pDC among the three subject groups were correlated. The frequencies of mDC and pDC from healthy young and healthy elderly donors correlated positively ($r = 0.68$ and 0.63 respectively, Pearson's test), suggesting a proportional relationship between the two DC populations. However, the mDC and pDC did not correlate well among the frail elderly subjects (Pearson's $r = 0.13$), indicating that aging with diseases is accompanied with disparities in the numbers of peripheral blood mDC and pDC.

3.3. Phenotypic comparison of mDC and pDC from young versus elderly subjects

The expression of MHC II (antigen-presenting molecule), CD11c (adhesion molecule), and CD123 (IL-3 receptor) on mDC and pDC from healthy elderly and healthy young subjects were compared. No significant differences were observed, except that there was a slight trend of lower MHC II expression on mDC and pDC from elderly subjects compared with young subjects ($p = 0.33$ and 0.11 for mDC and pDC respectively, data not shown).

The expression of TLRs on mDC and pDC were compared between healthy young and healthy elderly subjects. We chose the extracellular TLR-2 and TLR-4, and the intracellular TLR-7 and TLR-9, as the representative TLRs for mDC and pDC respectively[11]. Significantly and disproportionately fewer pDC expressed TLR-7 or TLR-9 with advancing age, whereas the proportion of mDC expressing TLR-2 or TLR-4 remained similar between healthy young and healthy elderly subjects (Fig. 2). These data suggest that in healthy aging, the decline in pDC frequency is also accompanied by a reduction in the expression of TLR-7 and TLR-9. In contrast, the mDC were unaltered in numbers and in TLR-2 and TLR-4 expression.

3.4. Frequency of IFN- α -secreting pDC is reduced in healthy elderly subjects

Consistent with reports by other groups[11,32,33], mDC and pDC populations were functionally distinct with respect to their patterns of cytokine secretion in response to stimulation. Stimulation with poly I:C resulted in high amounts of IL-12 secretion in mDC but not pDC. Stimulation by influenza virus infection failed to stimulate IL-12 secretion from either mDC or pDC (Fig. 3A). pDC were the main source of IFN- α when stimulated by influenza virus or CpG, at levels up to 10 times higher than that of mDC (Fig. 3A). Accordingly, we chose the IL-12 secretion by mDC and IFN- α secretion by pDC as indicators for their functional status, allowing comparisons of age-related differences. With respect to pDC, aging was associated with a 63.6% decline in the numbers of pDC positive

for IFN- α (IFN- α^+ -pDC) upon influenza virus stimulation ($p = 0.005$, Fig. 3B). A similar decline in the frequency of IFN- α^+ -pDC was also observed when stimulated by CpG 2006 (a 41.7% decline from the healthy young to healthy elderly subjects, $p = 0.03$, Fig. 3C). No age-related difference was observed in the amount of IFN- α secreted by pDC on a per-cell basis (Fig. 3B and 3C, right panels).

For mDC upon poly I:C stimulation, the numbers of IL-12-secreting mDC (IL-12 $^+$ -mDC) and the amount of IL-12 secreted per cell were comparable between healthy young and healthy elderly subjects (Fig. 3D). Because the magnitude of decline in the numbers of pDC determined by phenotype was less severe (a 28.6% reduction, Fig. 1B) compared with the reduction in the numbers of IFN- α^+ -pDC (a 41.7% to 63.4% reduction, Fig. 3B and C), it is likely that there is a functional decline in addition to the numerical decline within the pDC population with age.

3.5. Isolated pDC, but not mDC, secreted less inflammatory cytokines from healthy elderly subjects compared with healthy young subjects

To examine the functional changes directly, mDC and pDC were isolated from PBMC and their secretion of inflammatory cytokines were compared between healthy young and healthy elderly subjects. Equal numbers of mDC or pDC isolated from healthy young or healthy elderly subjects were stimulated with poly I:C and influenza virus respectively. Upon stimulation by influenza virus, pDC secreted significant amounts of IFN- α , IL-6, IL-8, and TNF- α (Table 2), but very little (if any) IL-12 (p70), IL-1 β , or IL-10 (data not shown). More importantly, pDC isolated from healthy elderly subjects secreted less IFN- α , IL-6 and TNF- α compared with those in healthy young subjects. With respect to IL-8, elderly subjects had a higher baseline level, but the increase over the baseline level was less in response to influenza stimulation. For mDC, secretions of IL-12 (p70), IL-6, IL-8, IL-10, IL-1 β , and TNF- α were comparable between healthy elderly and healthy young subjects (Table 3). IFN- α stood out as the only cytokine not secreted in response to poly I:C stimulation among the seven cytokines tested here (data not shown). These data demonstrate that aging impairs the function of pDC with respect to cytokine secretion, whereas the function of mDC remains relatively unaltered. This observation is consistent with the results obtained using intracellular cytokine staining (Fig. 3).

3.6. Comparable amounts of monocyte-derived DC with similar phenotype can be generated *in vitro* from adherent PBMC from healthy young and healthy elderly subjects

To examine the effect of aging on the DC generated *in vitro*, 10 healthy young (mean = 31 years) and 18 healthy elderly (mean, 75 years) were recruited. Monocyte-derived DC (MDDC) were generated in the presence of GM-CSF and IL-4. As shown in Table 4, similar numbers of MDDC were obtained from the elderly donors compared with their young counterparts (5.6% of PBMC from the elderly vs. 3.6% from the young subjects, $p = 0.08$). In addition, these MDDC were not phenotypically different with respect to their expression of CD86 (co-stimulatory molecule B7.2), MHC I, or MHC II (Table 4). These data shown that aging does not affect the cell yield or the phenotype of DC generated *in vitro*. Because MDDC resembling mDC phenotypically and functionally, these data are consistent with the finding that mDC, or the precursors of mDC, are relatively preserved with aging.

4. Discussion

We observed a numerical and functional decline in pDC with age. The decline in the numbers of pDC that we demonstrated was not associated with a decrease in the numbers of mDC, suggesting a selective impact on pDC during the healthy aging process. Interestingly, a similar phenomenon has been reported in children during the first decade of life, whose pDC numbers decline rapidly (close to a 2.5-fold decline) whereas the numbers of mDC remain relatively stable[34,35]. Conflicting findings have been reported with regard to the age-related changes in pDC from elderly persons. At least two studies demonstrated an age-related decline[25,26], whereas two other studies showed no changes in the numbers of pDC with aging[22,23]. Some have suggested that the decline in pDC numbers results from the selective cell death of pDC during the process of isolating PBMC from whole blood[22]. However, a more recent study argued against this notion and proposed that insufficient numbers of cells acquired during the flow cytometry analysis produced inconsistent findings with age. Thus those data support our observation of an aging-related decline in pDC numbers[26]. We propose that the differences in sample population and sample size also contribute to the discrepancy. In this study, a relatively large sample population was recruited based on our sample-size calculation. This relatively large sample size is dictated by the small difference between the two age groups and the large variation in the numbers of pDC frequency between individuals.

In addition to the numerical decline, Shodell and Siegal also reported a functional decline in pDC with age by measuring the cell numbers of pDC and the IFN- α secretion in supernatants from PBMC stimulated by HSV[25]. Our data add to this observation by demonstrating a reduced frequency of IFN- α^+ -pDC in PBMC and an impaired cytokine secretion from isolated pDC from healthy elderly compared with young subjects. Because the IFN- α secretion is known to be mediated via TLR-7 and TLR-9 when stimulated by influenza viruses and CpG, respectively[36,37], these results suggest that the TLR-7 and TLR-9 expression/signaling pathway on pDC is impaired by aging. Consistent with this notion, the proportion of pDC expressing TLR-7 or TLR-9 was reduced in healthy elderly compared with young subjects (Fig. 2A). In contrast, the expression of TLR-2 and TLR-4 did not change as a function of age (Fig. 2B), consistent with the observations that cytokine secretion from isolated mDC was not impaired with aging (Table 3). To focus on the aging process *per se*, our study on the functional aspects of DC (cytokine secretion and TLR expression) were carried out specifically in healthy elderly subjects. Although it is important to extend this functional study to the frail elderly population, one has to realize the complexity of the study, as chronic diseases and medications will, in addition to aging, likely also affect DC function.

Age-related changes in TLR-1 expression have been reported in human monocytes[38,39]. Murine macrophages also demonstrate a decreased expression in most TLRs[40]. However, we are just beginning to understand how aging affects TLRs and the relationship between TLR expression and cytokine secretion. A study by Agrawal *et al.* shows that the TLR-4-mediated signaling pathway is impaired in aging, although the expression of TLR-4 on DC is comparable between young and elderly subjects[23]. In our laboratory, we observed that the baseline level of TLR-7 or TLR-9+pDC was less in elderly subjects in unstimulated

PBMC, but that overnight stimulation of PBMC with influenza virus eliminated this age-related difference (unpublished observation). Furthermore, when PBMC were stimulated with CpG 2216, a much stronger TLR-9 stimulus than CpG 2006, the age-related difference in IFN- α +pDC no longer reached statistical significance (data not shown). It remains unknown whether this is because the two activation pathways specific for CpG 2006 or CpG 2216 are affected differently by the aging process[41]. Therefore, additional studies are needed to better delineate the relationship between the age-related decline in pDC function and the alterations in TLR-7– and TLR-9–mediated pathways involved in the secretion of IFN- α in pDC[32,33,42].

Both mDC and pDC are rare in peripheral blood but are abundant in lymphoid tissues, where they exert their immunologic activity. The importance of pDC has been implicated in HIV infection, opportunistic infections in AIDS patients with acquired immune deficiency syndrome (AIDS), and in transplantation[29,43–45]. pDC are also considered as effector cells that can be recruited to the infected sites[46]. We believe that the numerical and functional decline in pDC may have implications for the age-related decrease in the Th1 response to influenza[27]. A greater amount of IFN- α is secreted by pDC compared with mDC or monocytes (Fig. 3A and data not shown). Because influenza virus does not stimulate IL-12 secretion from mDC or pDC but can stimulate IFN- α secretion from pDC, a vigorous Th1 response may largely depend on the secretion of IFN- α by pDC after influenza virus infection.

The differential effects of aging on mDC and pDC also support the notion that these DC subsets may have distinct origins and/or developmental pathways[7,47,48]. In human beings, the development of pDC is believed to be lymphoid dependent because of their expression of lymphoid markers such as CD2 and CD5[49,50]. Because aging is known to have detrimental effects on lymphoid tissues, including the thymus, the age-related decline in pDC is consistent with the theory that pDC development is lymphoid dependent.

Our results show that among frail elderly subjects, the numbers of mDC were significantly lower than those detected in healthy young and healthy elderly subjects. This reduction in mDC contrasts with the preserved mDC frequency with age among healthy subjects (Fig. 1B). In addition, the positive correlation between mDC and pDC frequency observed in healthy young and healthy elderly subjects was not evident in this frail elderly group. We cannot determine how much of this difference is from disease, medication, or the combination of older age with these factors (the average age of our frail elderly cohort is 8 years more than that of the healthy elderly group [Table 1]). However, we believe that the increased age in the frail elderly group is not the major contributor to our observation of decreased numbers of mDC in the frail elderly group. We performed a subanalysis comparing the numbers of mDC in the frail elderly subjects to those of age-matched healthy elderly subjects ($n = 37$, selected out from the healthy elderly group by age), and a trend of decline in mDC numbers was also observed (0.19% vs. 0.28% in the frail and age-matched healthy elderly group, respectively), although this difference did not reach statistical significant ($p = 0.174$).

Our results show that MDDC, at least numerically and phenotypically, are not affected by age. Similar results have been reported by Steger *et al.* and Lung *et al.* [51,52]. In their studies, the function of MDDC were also compared between young and elderly subjects in terms of the ability of MDDC to stimulate antigen-specific T-cell response and cytokine secretion, and no age-related changes were found. Because mDC are similar to MDDC, these results are consistent with our notion that aging *per se* does not affect the number and cytokine secretion function of the mDC population.

In summary, we demonstrated that aging is associated with a decline in the function and numbers of pDC, whereas mDC numbers and function are relatively unchanged with healthy aging. In addition, aging with mildly disabling disease is associated with a decline in mDC population. These findings may help to explain some of the age-related increases in morbidity and mortality from infectious diseases, especially with respect to underlying conditions, and support the strategy of targeting pDC for immune modulation to enhance vaccine efficacy in healthy elderly populations.

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Fig. 1.

Quantifications of the numbers of mDC and pDC in healthy young, healthy elderly, and frail elderly subjects (for subject numbers and ages, see data presented Table 1). The numbers of mDC and pDC were quantified using flow cytometry in PBMC stained with lineage markers (FITC), CD123 (PE), HLA-DR (PerCP), and CD11c (APC). (A) Representative dot plot showing the mDC (upper left) and pDC (lower right) populations. (B) Numbers of pDC and mDC in total PBMC from different groups. Horizontal bars represent geometric mean values. * $p < 0.05$. Median, mean, and standard error (SE) are listed under each group.

Fig. 2.

Comparison of TLR expression by pDC and mDC from healthy young and healthy elderly subjects. Fourteen healthy young (mean age, 34.3 years) and 17 healthy elderly subjects (mean age, 72.6 years) were recruited for testing the TLR-2 and TLR-4 expression on cells surface of mDC. The intracellular expression of TLR-7 and TLR-9 in pDC were compared between 13 young (mean age, 26.1 years) and 13 elderly (mean age, 69.4 years) subjects. PMBC isolated from these subjects were stained with lineage markers (FITC), HLA-DR (PerCP), CD11c (APC), and TLR (PE) of interest. Data shown are mean values from each age group with standard errors shown in error bars. * $p < 0.05$.

Fig. 3. IFN- α and IL-12 secretion from pDC or mDC, respectively, from healthy young and healthy elderly subjects. Eighteen healthy young (mean age, 36.1) and 19 healthy elderly subjects (mean age, 74.4) were recruited. PBMC isolated from these subjects were stimulated with influenza virus infection or CpG (for pDC to secrete IFN- α), or poly I:C (for mDC to secrete IL-12). Stimulated PBMC were subjected to intracellular cytokine staining for IL-12 or IFN- α . Only DC (lineage marker-negative and HLA-DR-positive cells) were gated for analysis. (A) Representative dot plot illustrating the distinct pattern of cytokine secretion by

mDC and pDC upon stimulation. (B–D, left panels) Frequencies of IFN- α ⁺-pDC (B and C) or IL-12⁺-mDC (D) in PBMC upon stimulation in young and elderly group. (B–D, right panels) are the mean fluorescent levels (MFL) of corresponding cytokine. In unstimulated PBMC, the numbers of IFN- α or IL-12 positive pDC or mDC was below detectable levels (0.01%). Horizontal bars denote medians. * $p < 0.05$.

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Table 1

Ages and medication information for study subjects

	Healthy young	Healthy elderly	Frail elderly
Number of subjects	52	75	20
Age range (y)	21–40	64–92	61–95
Mean age (y)	28	74	82
Standard deviation of age	4.8	6.1	8.8
Number of medications used			
Range	0–3	0–9	4–15
Mean	0.5	2.8	10.4
Diseases (<i>n</i> and % in the group)			
Depression	1 (2%)	2 (3%)	12 (60%)
Hypertension	1 (2%)	26 (35%)	11 (55%)
Osteoporosis	0 (0%)	5 (7%)	9 (45%)
Sleep disorder	0 (0%)	2 (3%)	7 (35%)
Allergy	2 (4%)	5 (7%)	7 (35%)
Type 2 diabetes	0 (0%)	0 (0%)	6 (30%)
GERD	0 (0%)	2 (3%)	5 (25%)
Arthritis/osteoarthritis	0 (0%)	14 (27%)	5 (25%)
CHF	0 (0%)	1 (1%)	3 (15%)
COPD	0 (0%)	0 (0%)	3 (15%)
Asthma	4 (8%)	3 (4%)	0 (0%)
High cholesterol	0 (0%)	14 (19%)	0 (0%)

GERD, gastroesophageal reflux disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease.

Table 2

Impaired secretion of inflammatory cytokines from pDC isolated from healthy elderly subjects compared with healthy young subjects

	Control		Influenza+	
	Young	Elderly	Young	Elderly
IFN- α^a	23 $\pm$ 12	34 $\pm$ 3	35,564 $\pm$ 11,418	23,117 $\pm$ 6419 [#]
IL-6 ^b	3 $\pm$ 2	21 $\pm$ 12	287 $\pm$ 69	189 $\pm$ 29 [#]
IL-8 ^b	385 $\pm$ 71	691 $\pm$ 92	814 $\pm$ 93	901 $\pm$ 99
TNF- α^b	5 $\pm$ 2	11 $\pm$ 2	451 $\pm$ 6	359 $\pm$ 8 [#]

Cytokine levels (pg/ml), in supernatant of isolated pDC stimulated with influenza virus (10 HAU/ml, A/Sydney/H3N2) overnight. Cytokine levels were measured either by conventional ELISA (a) or CBA inflammatory cytokine kit (b). Values shown are means $\pm$ standard errors in three separate experiments. In each experiment, pDC were isolated from pooled PBMC from five young and 10 elderly donors.

[#] Values are consistently lower in elderly compared with young subjects in all three experiments.

⁺ Values are consistently higher in elderly compared with young subjects in all three experiments.

Table 3

Comparable secretion of inflammatory cytokines from mDC isolated from healthy young and healthy elderly subjects

	Control		Poly I:C+	
	Young	Elderly	Young	Elderly
IL-12p70	22 ± 13	19 ± 11	409 ± 120	428 ± 52
IL-6	0	0	12,607 ± 3204	12,725 ± 2158
IL-8	674 ± 116	818 ± 128	30,079 ± 7027	33,089 ± 5399
IL-10	29 ± 9	29 ± 9	837 ± 211	926 ± 297
IL-1b	0	0	787 ± 208	818 ± 128
TNF- α	14 ± 8	12 ± 7	1421 ± 332	1556 ± 184

Cytokine levels (pg/ml), in supernatant of isolated mDC stimulated with poly I:C (50 μ g/ml) overnight. Cytokine levels were measured with a CBA inflammatory cytokine kit. Values shown are means $\pm$ standard errors in three separate experiments. In each experiment, mDC were isolated from pooled PBMC from five young and 10 elderly donors. Equal numbers of mDC (1×10^5) from young or elderly subjects were seeded for each treatment conditions.

Table 4

Comparable amounts of DC *in vitro* with similar phenotypes can be generated from adherent PBMC in healthy young and elderly subjects

Group	DC yield (% of PBMC)	MFL		
		MHC I	MHC II	CD86
Young (<i>n</i> = 10)	3.6 ± 2.2	1193 ± 332	817 ± 151	2428 ± 267
Elderly (<i>n</i> = 18)	5.6 ± 1.6	1076 ± 224	757 ± 251	2406 ± 454
<i>p</i> Value	0.08	0.28	0.49	0.89

DC were generated *in vitro* from adherent PBMC in the presence of IL-4 and GM-CSF. DC cultures were harvested after 7 days and subjected to immunofluorescent staining. DC were gated on based on their scattering patterns, and their phenotypes were analyzed by flow cytometry. Data presented are the mean levels ± standard deviation from each group.