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Original Article

Responses of Dendritic Cells to TLR-4 Stimulation Are Maintained in the Elderly and Resist the Effects of CMV Infection Seen in the Young

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Abstract

Toll-like receptor 4 (TLR-4) plays a crucial role in the pathophysiology of several age-related diseases. Although poorer function of circulating myeloid dendritic cells (mDCs) has been reported in the elderly, data on TLR-4 function in these cells in older people are lacking. Here, we investigated TLR-4 functionality in the elderly by ex vivo analysis of cytokine production of mDCs in response to LPS in 39 younger (23–34 years) and 61 older (62–77 years) healthy people using flow cytometry. We matched these subjects for Cytomegalovirus (CMV)-serostatus because a latent infection with this ubiquitous herpesvirus is known to affect numerous immune parameters. We found that TLR-4-dependent production of IL-6 and TNF was strongly stimulated in circulating mDCs from the elderly. However, mDCs of more than half of the young donors failed to respond in the same way. This was related to their already highly activated ex vivo state, predominantly observed in CMV-seropositive young donors and associated with lower CMV-specific IgG titres. This may reflect an increasingly important requirement for control of CMV infection throughout life. These data suggest that TLR-4 agonists may be the adjuvants of choice for elderly people, most of whom are CMV-positive, and whose responses to immunization are frequently impaired.

Keywords: Circulating myeloid dendritic cells—TLR-4—Cytokine production—Aging—Cytomegalovirus—Human

Both susceptibility to, and severity of, infectious diseases increase with age. This is predominantly due to age-related changes in both the innate and adaptive arms of the immune system (1). As part of the innate immune system, dendritic cells (DCs) are the most potent antigen-presenting cells and are specialized for the uptake, processing, transport and presentation of antigens to T cells (2). After their activation in the periphery, DCs migrate to lymphoid tissues where they interact with T and B cells to initiate and shape adaptive immune responses. Two main DC subsets with different functions are present in human peripheral blood. Plasmacytoid DCs (pDCs) derive from lymphoid progenitor cells, mainly produce IFN-α and are characterized as having the cell surface phenotype HLA-DR$^+$ CD11c$^-$ CD123$^+$ and being negative for the lineage (Lin) markers CD3, CD14, CD16, CD19, CD20, and CD56. Myeloid DCs on the other hand originate from the myeloid or multi-lymphoid lineages and are defined as Lin$^-$ HLA-DR$^+$ CD11c$^+$ CD123$^-$ (2). Upon stimulation, myeloid dendritic cells (mDCs) mainly secrete IL-6, IL-12, and TNF. DCs express several membrane-bound pattern recognition receptors, which allow them to recognize nonpolymorphic components of different pathogens such as bacteria and viruses. The toll-like receptor (TLR) family is one of the main pattern recognition receptors with 13 members identified so far. Each TLR can recognize distinct pathogen-associated molecular patterns; their expression varies with species, DC subtype and maturation stage, making it important to study the appropriate species. While human pDCs express only TLR-7, TLR-9, and TLR-10, mDCs express TLR-1, -2,

-3, -4, -5, -6, -7, and -8 (3). In contrast to the extensive literature on T and B cells in aging, data on DCs and their function in the elderly are scarce and often contradictory. Several studies have investigated the impact of age on the number of human mDCs and pDCs, showing equal (4–9) or lower (10) numbers of mDCs and equal (4,9,10) or lower (5–7,11,12) numbers of pDCs in the elderly compared with young controls. The reasons for these differences are not known. To the best of our knowledge, only one published study has investigated functions of circulating mDCs in the elderly (5). That study found decreased function in response to TLR-1/2, TLR-2/6, TLR-3, TLR-5, TLR-7/8, and TLR-9 stimulation. As mDCs are the main drivers of Th1-type responses important in triggering adaptive immunity, if confirmed, this lowered response in the elderly could contribute to immune impairment. However, TLR-4 function was not investigated in that study (5) but is crucial to immune responses against multiple pathogens. Single nucleotide polymorphisms in the TLR-4 gene have been associated with susceptibility to several age-related diseases (13), but there are no data on DC stimulation through TLR-4 in the elderly, although in old mice it does seem to be compromised (14). Therefore, we sought to determine if the functionality of this TLR on mDCs is also impaired with human aging. It is now accepted that many age-associated changes in different immune parameters are due to or are exacerbated by a latent infection with human Cytomegalovirus (CMV). CMV is double-stranded DNA virus and a member of the *Betaherpesviridae* sub-family of *Herpesviridae*. The incidence of CMV infection increases with age (15) and can reach more than 90% or even 100% of the elderly in some countries. After primary infection the virus establishes latency in pluripotent CD34+ mononuclear cells. Cells of the myeloid lineage such as monocytes and their progenitors; macrophages and DCs are also sites of latency from which CMV reactivation occurs after pro-inflammatory stimulation and differentiation (16,17). Although several studies have demonstrated an immune-suppressive role for CMV after infection of monocyte-derived DCs *in vitro*, no data are available on whether the presence of a latent infection with CMV is associated with any functional alterations of DCs in vivo. Furthermore, most studies on age-related differences in myeloid dendritic cells have not stratified subjects for CMV-serostatus. Hence, they could be comparing the effects of CMV, not necessarily age, as the majority of elderly donors would have been CMV-seropositive, while the young would be mainly CMV-seronegative. The present study was designed to dissect the impact of age on the functional capacity of circulating mDCs in response to TLR-4 stimulation in the context of a latent infection with CMV, the normal situation in the elderly and thus the most relevant for determining immune status in older people. These findings have implications for selecting the adjuvant of choice for vaccinating elderly people.

Materials and Methods

Subjects

In this study, peripheral blood mononuclear cells (PBMC) from 100 participants of the Berlin Aging Study II (BASE-II) were analyzed (see Bertram and colleagues (18) for detailed cohort description) and were approved by the appropriate Ethics Commissions. Briefly, BASE-II is a multidisciplinary project, collecting a large number of aging-related variables including factors linked to geriatrics and internal medicine, immunology, genetics, psychology, nutrition, sociology, and economics in 2,200 inhabitants of the greater metropolitan area of Berlin, Germany. The study includes older individuals (60–80 years) and young participants (20–35 years). The subcohort for the present analysis consisted of 39 younger donors (18 male and 26 female) with a mean age of 28, and 61 older donors (29 male and 32 female) with a mean age of 68.

Plasma C-Reactive Protein

Plasma C-reactive protein (CRP) levels were determined using an immunological turbidity assay (cobas/Roche, Rotkreuz, Switzerland).

CMV Serology

Anti-CMV IgG titres were measured in plasma using a CMV IgG Kit (Omega Diagnostics Group, Scotland, UK). IgG levels were determined using a semi-quantitative approach, according to the manufacturer's instructions.

Ex Vivo Analysis of DC Function

Using a freeze/thaw protocol modified to maximize cell viability, peripheral blood mononuclear cells were thawed in a 37°C waterbath, the contents of the vial transferred to a centrifuge tube, and an equal volume of culture medium added at room temperature. This dilutes the DMSO cryoprotectant from 10% to 5%, avoiding osmotic shock. After 5 minutes equilibration at room temperature, another equal volume of medium was added and the cells centrifuged at 200*g*. This sediment viable cells but leaves cell debris in the supernatant. The cells were then washed twice in the same way to remove the DMSO cyroprotectant while minimizing contamination with dead cell fragments and soluble materials. Finally, cells were resuspended in X-vivo15 medium (Cambrex, Verviers, Belgium). Viable cells were enumerated using phase-contrast microscopy and plated at 1×10^7 viable cells/mL in U-bottom 96 well plates (1×10^6 cells/well). Peripheral blood mononuclear cells were either left untreated (negative control) or stimulated with 0.5 µg/mL Lipopolysaccharide (Sigma, St. Louis, MO). About 1 µL/mL Golgi Plug (BD Bioscience, Franklin Lakes, NJ) was added to all cultures and the cells were incubated for 6 hours, after which they were harvested, washed and treated with human immunoglobulin, GAMUNEX (Bayer, Leverkusen, Germany) and ethidium monoazide (EMA, MoBiTec GmbH, Göttingen, Germany) to block Fc receptors and label nonviable cells. Cells were then stained with directly conjugated monoclonal antibodies, Lin1-FITC (lineage cocktail containing anti-CD14, -CD16, -CD19, -CD20, -CD56) and HLA-DR-PerCpCy5.5 (BD Bioscience), CD11c-PECy7 and CD123-APC (Bio Legend, San Diego, CA). After 20 min of incubation on ice, cells were washed, then fixed and permeabilized with CytoFix/CytoPerm (BD Bioscience) and stained with IL-6-v450 (BD Bioscience), IL-12-PE and TNF-Alexa700 (eBioscience, San Diego, CA). Samples were measured immediately using an LSR II and the FACSDiva software (BD Bioscience). The spectral overlap between the channels was calculated automatically by BD FACSDiva software after measuring negative and single-colour controls.

Flow cytometry data were analyzed using FlowJo version 7.6.5 (Tree Star, Portland, OR). For this, duplicates were removed using the forward-scatter (FSC) height versus forward-scatter area plot followed by exclusion of EMA+ dead cells (Supplementary Figure 1). Because peripheral blood dendritic cells are characterized by forward-scatter similar to monocytes and SSC similar to lymphocytes, a gate was set based on forward-scatter and side-scatter (SSC) that included both the lymphocyte and monocyte population. Within this population, $Lin1^-$ HLA-DR+ cells were used for further analysis. Myeloid dendritic cells were characterized as $CD11c^+$ $CD123^-$ and

plasmacytoid dendritic cells as CD11c$^-$ and CD123+. Here, we have focused on myeloid DCs assayed for intra-cytoplasmic production of IL-6, IL-12, and TNF. The amount of cytokine production on a per-cell basis was determined using the mean fluorescence intensity (MFI) of cytokine-producing cells, which was standardized according to the MFI of the cytokine-negative population for each donor.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5. Two independent groups were compared using the Mann–Whitney *U* test. The paired *t*-test was used to analyze samples pre- and post-stimulation. Correlations between CMV-titre and the percentage of cytokine-producing cells were assessed using Spearman's correlation analysis. Tests were two-tailed and $p < .05$ considered to indicate statistical significance.

Results

TLR-4-Dependent Functional Capacity of DCs Is Well-Maintained in the Elderly

We first compared functional capacities in response to TLR-4 stimulation in DCs from young and old donors. For this, individuals were grouped as young (23–34 years) or old (62–77 years) and the percentage of cytokine-producing cells after stimulation in vitro with lipopolysaccharide (LPS) was compared. The net frequency of myeloid DCs producing TNF or IL-6 upon stimulation (after subtracting the frequency of DCs spontaneously producing cytokines) was slightly but statistically significantly *higher* in the elderly than the young (Figure 1A), whereas no significant difference was observed in the net frequency of DCs producing IL-12 (data not shown). The total percentage of cytokine-producing cells (without subtracting background) did not differ between the two groups (data not shown). We then sought to determine if there was a difference between young and old in the ability of their DCs to produce both TNF and IL-6 at the same time in the same cell. This assay revealed that the majority of DCs in both young and old donors produced only TNF (IL-6$^-$TNF$^+$, Figure 1B), with a smaller fraction producing either IL-6 alone (IL-6$^+$TNF$^-$) or both cytokines (IL-6$^+$TNF$^+$, Figure 1B). The frequency of DCs producing IL-6 and TNF simultaneously was not significantly different between the young and old groups, although the latter included many individuals with far fewer polyfunctional DC than any of the young (Figure 1B). Due to the very low frequency of mDCs producing IL-12 in both young and old donors, this cytokine could not be included in this analysis of polyfunctionality. Finally, we also explored the level of cytokine production on a per-cell basis, reflected in the MFI of cytokine-producing cells, and found that this was also similar in young and old donors. The DCs from the elderly even responded significantly better than the young in terms of the level of TNF production on a per cell basis after LPS stimulation (Figure 1C). Thus, DCs from the elderly maintain or even increase TLR-4-stimulated functionality as assessed by both the fraction of responding cells and amount of cytokine production per cell, at least as far as IL-6 and TNF production is concerned. This suggests that the quality of the cytokine response by TLR-4-stimulated circulating DCs is well-maintained in older people and might even contribute to their generally higher levels of plasma IL-6 and TNF compared with the young.

Circulating DCs Have a More Activated Phenotype in the Young Compared With the Old

During this study, we observed that in some donors a large fraction of DCs spontaneously produced TNF and/or IL-6 in short-term ex vivo cultures without stimulation by LPS (Figure 2A, lower row). We designated this as an "activated" phenotype. We believe that this heterogeneous distribution of such an activated phenotype was not due to the presence of dead or dying cells in the cultures because although this cannot be completely excluded, we observed

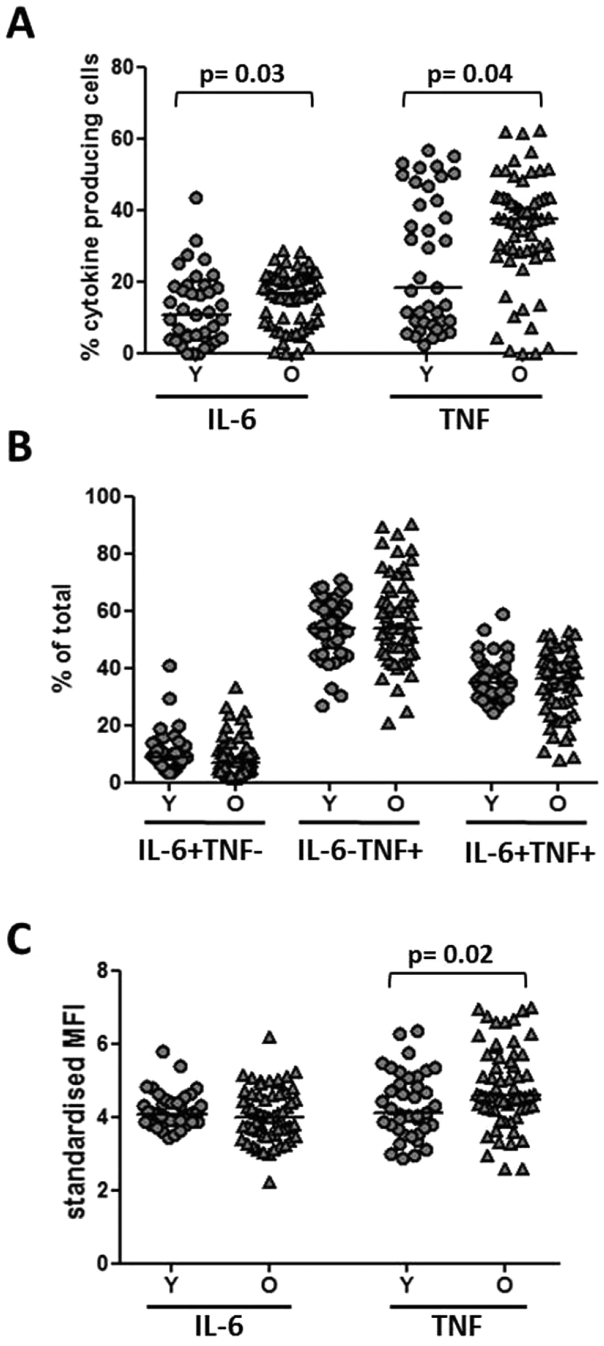

Figure 1. Effect of age on the functional capacity of circulating myeloid dendritic cells. Peripheral blood mononuclear cells from 39 young and 61 old donors were stimulated for 6 h with LPS or left untreated. Production of TNF and IL-6 was determined using flow cytometry to detect cytoplasmic cytokines as described in the Methods section. The frequency of dendritic cells (DCs) spontaneously producing cytokines was subtracted from the frequency of DCs producing cytokines in response to stimulation to give net cytokine production (A). Polyfunctionality analysis comparing the proportion of cells producing only TNF or IL-6 or both cytokines in young and old donors (B). The amount of cytokine production on a per-cell basis determined using mean fluorescence intensity (MFI) of cytokine-producing cells, standardized according to the MFI of the negative population for each donor (C). Horizontal bars represent the median of each group. *p* Values were calculated using Mann–Whitney *U* test.

no obvious correlations between the percentage of EMA$^+$ cells and the background level of TNF and IL 6 production (data not shown). Moreover, although there was a great degree of inter-individual variation in both young and old subjects, there was a clear age stratification in this phenomenon. As shown in Figure 2B, the percentage of donors with mDC spontaneously producing either IL-6 or TNF was significantly greater in the young, especially for TNF. This was not observed for IL-12 (data not shown). Both young and old groups included individuals whose DCs did not produce IL-6 or TNF spontaneously, as well as those which did, but significantly more so of the former in the young. The frequency of DCs producing cytokines from the 16 of 19 young donors (84.2%) with this activated phenotype did not increase significantly after stimulation with LPS, whereas all of the remaining 20 young donors responded to stimulation by increasing the percentage of cytokine-producing cells at least 8-fold, up to-240 fold. In the old group, 5/8 (62.5%)

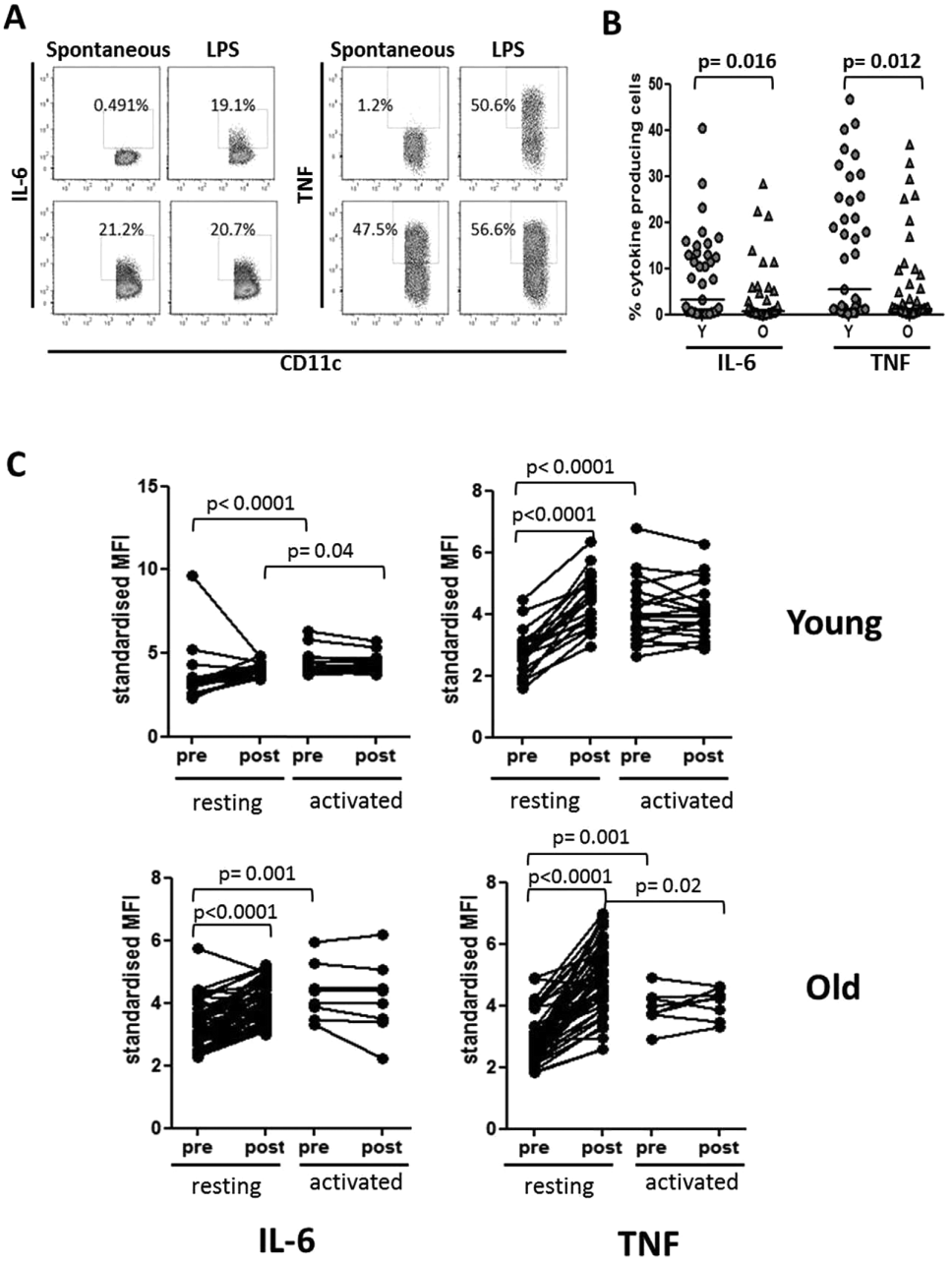

Figure 2. Spontaneous activation status of dendritic cells (DCs) and responsiveness to LPS stimulation. (**A**) FACS plots from two representative donors with low (upper row) or high frequency (lower row) myeloid DCs spontaneously producing IL-6 (left-hand side) and TNF (right-hand side) or upon stimulation with LPS. (**B**). Frequencies of DCs spontaneously producing IL-6 and TNF in young and old subjects. (**C**) Donors were stratified into two groups according to the percentage of DCs spontaneously producing TNF: those with lower than 10%, designated as "resting" (20 young and 52 old subjects) and those with more than 10% of DCs spontaneously producing TNF, designated as "activated" (19 young and 8 old individuals). mean fluorescence intensity values (standardized as described in the Methods section) of IL-6 (left-hand side) or TNF (right-hand side) were compared pairwise between pre-stimulation (pre) and post-stimulation (post) samples in young (upper row) and old individuals (lower row). Horizontal bars represent the median of each group. *p* Values were calculated using Mann–Whitney *U* test. Paired *t*-test was used to analyze pre- and post-stimulation samples.

donors with >10% DCs spontaneously producing TNF also failed to respond, compared with 51/53 (96.2%) responders in the rest. Thus, a higher activated status of the circulating DCs in both young and old donors was associated with nonresponsiveness to further stimulation with LPS (Figure 2A, lower panel). This difference is therefore not associated with age per se. We then asked whether, despite the lack of increase in the frequency of cells producing cytokines, the amount of cytokine produced per cell did increase after stimulation. For this, the MFI of cytokine-producing cells pre- and post-stimulation with LPS was compared for each cytokine in young and old donors. As shown in Figure 2C the amount of cytokine produced by DCs from both young and old donors with an activated DC phenotype (ie, with a high proportion of spontaneously activated DCs) did not increase after stimulation with LPS. This was in contrast to the group with a resting DC phenotype (ie, with low frequency of DCs spontaneously producing cytokines). In the latter group, the MFI for both IL-6 and TNF increased significantly after stimulation with LPS (Figure 2C). Moreover, the MFI of cytokines produced spontaneously was significantly higher in the group with activated DCs compared with those without (Figure 2C). Again, this phenomenon was seen for both young and old donors and therefore not an effect of age per se.

Pro-inflammatory Status of DCs Is Not Associated With Increased Systemic Pro-inflammatory State

In order to determine if this *ex vivo* spontaneously activated DC phenotype was associated with an increased systemic inflammatory status in vivo, we compared the level of CRP, a marker for systemic inflammation, between donors with an activated or resting DC phenotype. Median CRP levels of 0.15 mg/dL (IQR = .22) were measured in individuals with a resting DC phenotype, compared with 0.11 mg/dL (IQR = .21) in donors with a high proportion of activated DCs (p = .22). Accordingly, the explanation that the activated DC phenotype was the result of an acute infection at the time of blood draw was ruled out. Nonetheless, comparing the whole group of elderly (median: 0.170 mg/dL) with the young (median: 0.085 mg/dL) revealed that the former did have significantly higher levels of CRP as repeatedly reported in the elderly (p = .03).

A Latent Infection With CMV Is Responsible for a Higher Activation Status of Circulating DCs

Because of the strong immune modulatory effect of CMV on other immune parameters, we sought to determine how age had an impact on the functional status of DCs in the presence of a latent infection with this virus. For this, individuals were grouped according to CMV-serostatus and age and the percentage of cells spontaneously producing cytokines, or upon stimulation with LPS, was compared. This revealed that the higher activated status of DCs of the young donors described earlier was predominantly seen in the CMV-seropositive group. Thus, in 15/25 (60%) CMV+ young donors more than around 20% of DCs spontaneously produced IL-6 and/or TNF, and these seemed to form a discrete group of subjects relative to those not spontaneously producing these cytokines (Figure 3A). In contrast, such a high proportion of activated DCs was observed in only 2/14 (14.3%) CMV-seronegative young donors (p = .006). At the population level, the difference between CMV-seropositive and –seronegative young donors for spontaneous TNF production just reached statistical significance (p = .048). In the elderly, despite CMV-seropositivity, only 6/52 (11.5%) had DCs with this activated phenotype (Figure 3A). This is the reason for the significantly lower net percentage of cytokine-producing cells after stimulation with LPS in the CMV+ young relative to the CMV+ old (Figure 3B). There was no difference between young and old donors in the CMV-seronegative group (Figure 3). Interestingly, DCs from the majority of CMV-seropositive old donors had a similar phenotype to those from young and old individuals without a CMV infection. Also unexpectedly, some CMV-seronegative young donors nonetheless failed to respond to LPS by increasing the proportion of TNF-producing DCs (Figure 3B, lower right graph). Thus, there are complex interactions between age and CMV infection state that influence the TLR-4-stimulated production of pro-inflammatory cytokines by mDCs.

Pro-inflammatory Status of DCs Is Associated With Lower Anti-CMV IgG Levels in Serum

Having observed this highly activated status of the DCs in more than half of the young CMV-seropositive donors tested, we sought to determine what the reason might be for differences within the infected group. Because we found that in our cohort, old individuals have a higher titre of anti-CMV IgG in serum (median 8.86 IU/mL) compared with young donors (median 4.42; p < .0001), we asked if a higher anti-CMV IgG titre was associated with a lower activation status of the DCs and thus their better responsiveness to stimulation observed in the majority of the elderly. Indeed, we found a significantly lower percentage of cells spontaneously producing IL-6 or TNF with increasing anti-CMV IgG titre in young individuals (Figure 4, circles). In the elderly, we found a similar negative correlation between anti-CMV IgG titre and the percentage of cells spontaneously producing TNF, whereas for IL-6 only a trend was observed (Figure 4, triangles). These data suggest that higher titres of CMV antibody may be ameliorating the stimulatory effect of the virus on circulating mDCs.

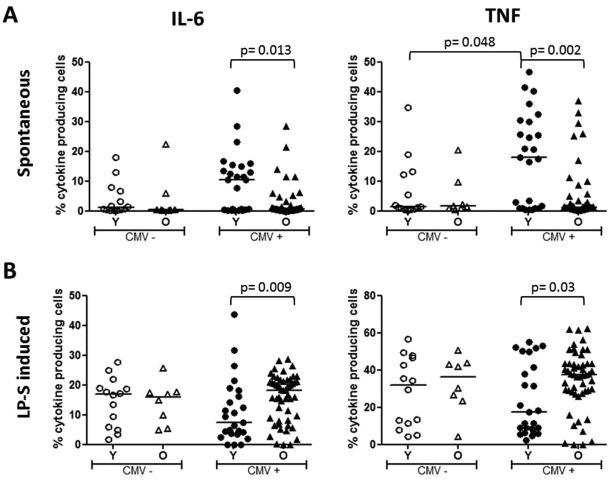

Figure 3. Impact of CMV-serostatus on the activation state and responsiveness of circulating dendritic cell (DC). The frequency of DCs spontaneously producing IL-6 (left-hand side) and TNF (right-hand side) (A) and the net frequency of DCs producing these cytokines following LPS stimulation (B) were compared between CMV-seronegative (15 young and 9 old subjects) and CMV-seropositive (24 young and 52 old individuals) subjects. Horizontal bars represent the median of each group. p Values were calculated using Mann–Whitney U test.

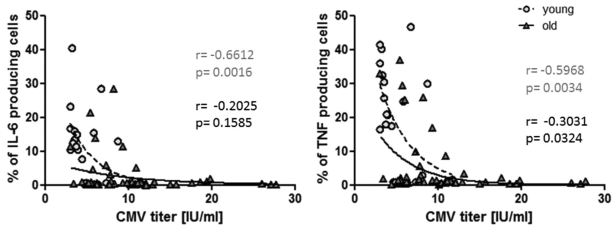

Figure 4. Correlation between anti-CMV IgG titre and the spontaneous production of TNF and IL-6 by myeloid DCs. The percentage of DCs spontaneously producing IL-6 (left-hand side) as well as TNF (right-hand side) was correlated with anti-CMV IgG titre in serum of 23 CMV-seropositive young and 50 CMV-seropositive old individuals. Three donors were excluded from the study due to a titre higher than the threshold of the test. *p* Values were calculated using Spearman correlation test.

Discussion

In this study, we demonstrate that dendritic cells from old individuals maintain their functionality in terms of IL-6 and TNF production when stimulated through TLR-4 ligation. Moreover, possibly due to their tighter control of latent CMV infection as reflected in generally higher anti-CMV IgG titres, the background activation of their mDCs (which is associated with CMV-seropositivity in the young) is greatly reduced. To the best of our knowledge there are no published studies investigating the impact of age on TLR-4-dependent cytokine secretion in circulating mDCs, and the effects of CMV infection thereon. One study reporting reduced TLR function in human mDCs ex vivo (5) did not include TLR-4 stimulation, and did not take CMV infection into account. However, several studies using in vitro cultured monocyte-derived DCs have shown that such DCs from aged subjects respond as well as DC from young individuals (19) or even better upon stimulation with LPS (8,9), which is in line with our findings presented here. Additionally, we report a significantly higher basal activation status of mDCs in many of the young donors. This is in contrast to some previous reports (5,8) showing that DCs from aged subjects have a higher basal level of activation compared with young individuals (5,8,20). The main reason for this may be that these previous studies also failed to take CMV-serostatus into account, as this activated phenotype was mainly present in CMV-seropositive young donors in our study. It is well known that the percentage of people infected with CMV increases with age in most populations and that the majority of young donors is CMV-seronegative, in Western countries at least. In our study we deliberately chose to include a large proportion of CMV-seropositive young donors, whereas if this age group is recruited randomly into a study, the majority will be CMV-seronegative. Our data demonstrate a clear association, but not an absolute correlation, between CMV-seropositivity and the higher activation status of DCs in this age group. Thus, disparate findings in previous reports are possibly due to the inclusion of too few CMV-seropositive donors in the young group. Moreover, we also know that the impact that CMV infection has on immune parameters depends on the genetic background of the individual, at least as far as CD8+ T cells are concerned (21). This was not controlled for in the present study. Discrepancies might also result from technical issues in these nonstandardized assays, such as culture conditions and different kinetics. Whether this activated mDC phenotype identified in about half of the CMV-seropositive young and some of the elderly donors has any clinical implications in our study remains to be investigated. Our data demonstrate that such a higher activated status of circulating mDCs in both young and old subjects is associated with non-responsiveness to further stimulation with LPS, which is in line with previously published data, albeit in response to stimulation through other TLRs (5). On the other hand, we demonstrate that resting DCs from the elderly are stimulated through TLR-4 to produce multiple cytokines at a level comparable to or greater than in young individuals. These findings could be of particular relevance in development of adjuvanted vaccines using TLR-4 agonists for elderly subjects, whose response to primary and secondary immunization is frequently impaired (22). For instance influenza vaccine efficacy is estimated to be only 17%–53% in older adults (23). Currently most licensed Influenza vaccines in the United States are non-adjuvanted, whereas many studies have demonstrated higher effectiveness of vaccines containing adjuvants (24,25). Given reports that DC responses to several other TLR ligands are dampened in the elderly, the data presented here suggest that unimpaired TLR-4 responsiveness would make this the target of choice to activate DCs in vaccines tailor-made for the elderly.

In general, slightly higher serum levels of inflammatory mediators are found in elderly people relative to most younger subjects. This has given rise to the concept of "inflammaging" (26) implicated in many diseases of aging (27). In the present study, the elderly donors did have slightly but significantly higher levels of CRP compared to the young. This suggests that they had higher levels of plasma IL-6 as well, also as commonly reported in the elderly, although we have not yet measured this directly. However, there were no correlations between the activated DC phenotype, CMV infection and CRP levels in our study, suggesting that the CMV-associated high pro-inflammatory status of DCs was not reflected in an increased systemic pro-inflammatory state. These data are consistent with an earlier report that the higher CRP levels in the elderly are not associated with CMV infection (28). The mechanistic link (if any) between a latent infection with CMV and high ex vivo activation status of DCs in some donors requires further investigation. One reason for this might be an active infection of DCs by CMV in these young donors. Kvale and colleagues (29) have shown induction of an activated phenotype, reflected in increased surface expression of CD83, MHC class I and II, in *in vitro* monocyte-derived $CD11c^+$ DCs in response to CMV exposure. Our finding of higher levels of expression of HLA-DR on DCs of young CMV-seropositive donors (data not shown) confirms this activated phenotype seen by Kvale and colleagues and our finding on basal cytokine expression. Another explanation might be CMV reactivation-induced activation of DCs ongoing at the time of blood draw. We do have data on the IgM status of the patients and thus we can probably exclude a recent primary infection or reactivation, although the presence of IgM is a controversial criterion in this regard. Analysis of the activation state of IgM-negative and -positive individuals revealed no significant difference, neither in young nor in old (data not shown). This suggests that the higher activation state of DC is not due to reactivation of CMV but CMV latency in general in the young. On the other hand, the presence of CMV DNA in monocytes has been associated with larger expansion of CD8 T-cells specific for an immunodominant antigen of CMV (30). We do not yet have data on CMV-specific T-cell responses in these donors; however we expect that highly activated DCs will be able to better stimulate T-cells, as has already also been shown in CMV-infected mDCs (29). These data add to our understanding of the complex interactions between CMV and the host immune system as an individual ages, demonstrating a possible selection, maturation and adaptation of the crucially important anti-CMV immune response during the persistent presence of this virus.

It also emphasizes the importance of analyzing the impact of CMV in younger donors and identification of possible mechanisms that might later lead to CMV-associated morbidity and mortality, not only in the very elderly, but at late adulthood. Practical implications for adjuvanted vaccines in the elderly should not be overlooked.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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