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Dendritic Cells in Human Aging

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Abstract

Dendritic cells (DCs) play a critical role in linking innate and adaptive immunity. A role of DCs in immunosenescence and chronic inflammation associated with aging has not been investigated in detail. In this article we will briefly review DCs biology and changes associated with human aging.

INTRODUCTION

Dendritic cells (DCs) are heterogeneous, professional antigen presenting cells that are uniquely equipped with molecules and strategically placed between internal and external environment, which enable them to link innate and adaptive immunity (Schuurhuis et al, 2006;Hugues et al, 2006;Reis e Sousa, 2006;Dubsky et al, 2005;Hackstein and Thomson, 2004;Kapsenberg, 2003). Hematopoietic precursor stem cells differentiate into immature dendritic cells (iDCs) which are recruited to the periphery where they continuously sample antigens and process them in an endosomal-MHC II complex-dependent pathway. These iDCs then migrate to the regional lymph nodes where they phenotypically mature and express a number of cell surface molecules and secrete a number of cytokines, which are important in their migration and interactions with T cells, B cells, and natural killer cells. These phenotypically mature DCs interact with T cells, B cells, and NK cells to elicit their specific responses and differentiate into functionally mature DCs.

Aging is associated with progressive decline in immune function (immunosenescence) resulting in an increased susceptibility to infections and cancer and reduced response to vaccination (Powlec et al, 2002;Saltzman and Peterson, 1987). The decline in specific T and B cell functions in aging is characterized by decreased response of T cells to specific antigens, altered cytokine secretion patterns, changes in naïve and memory T cells, decreased cytotoxic T cell responses, failure to produce high affinity antibodies, generation of long lasting memory response, and defects in signal transduction (Powlec et al, 2002;Saltzman and Peterson, 1987;Song et al, 1993;Gupta, 1989;Powers and Belshe, 1993;McElhaney, 1992). Paradoxically, aging is associated with low grade chronic inflammation and elevated circulating levels of pro-inflammatory mediators, including IL-6, TNF- α , IL-1 β and prostaglandin E2 (Ershler, 1993;Fagiola et al, 1993;Brunnsgaard et al, 2003;Trzonkowski et al, 2004;Penninx et al, 2004). However, a role of dendritic cells in immunosenescence and in chronic inflammatory state in aging is poorly understood.

In our study, young subjects were students, residents and staff of the University of California, Irvine between the ages of 21 years to 35 years. Aged subjects were drawn from middle class independently living healthy volunteers between the ages of 65 years and 88 years. A detailed

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questionnaire was used to exclude any significant illness, i.e. metabolic syndrome (diabetes/ cardiovascular disorders), infections, malignancy etc. Furthermore, subjects were required that they would not be taking any antioxidants for at least one week prior to donating blood samples. The study was approved by the Institutional review Board of the University of California, Irvine.

DC NUMBERS, PHENOTYPE, AND DIFFERENTIATION

Dendritic cells in humans are heterogeneous and are subdivided into two major categories: [a] those that are present in peripheral blood. They include myeloid DCs (MDCs) and plasmacytoid DCs (PDCs), and [b] those DCs that are present in the tissues/organs. They include Langerhans cells (LC) in the skin, which contain langerin and birbeck granules; interstitial DCs, which are present in tissues, and interdigitating DCs present in the thymus and other lymphoid organs. *In vitro* monocyte-derived DCs (MODCs), which are generated from monocytes in the presence of GM-CSF and IL-4, are closely related to interstitial or MDCs. Schodell and Siegal (2002) reported decreased number of circulating PDCs and decreased IFN- α secretion in aged subjects. Using multiple cell surface marker analysis to define circulating MDCs and PDCs we did not observe any significant difference in the number of circulating MDCs or PDCs between aged and young humans (unpublished observation). The basis for these conflicting data is not clear. Furthermore, our unpublished study of *in vitro*-derived MODCs show no significant differences either in the generation of MODCs, morphology, or cell surface phenotype in aged subjects as compared to young subjects. The MODCs population obtained from aging monocytes displays a typical DC morphology and expresses DC surface markers, such as HLA class II, CD1a, CD11c, CD54, CD80 and CD86, but not CD14. Our observations are in agreement to data of MODCs reported by Lung et al (2000) and Steger et al (1996). Linton et al (2005), using a T cell receptor transgenic mice model, also observed normal immunophenotype of DCs *in vivo* in aged mice. Pietschmann et al (2000) also observed no difference in the expression of multiple cell surface markers in myeloid-enriched lymphocyte depleted peripheral blood cells, which they termed as DCs, from aged subject except the expression of HLA-DR, which was significantly reduced. These cells appear to be more of monocyte/macrophage type rather than of DCs type. Fujihashi and McGhee (2004) reported aging-associated decreased frequency of *in situ* DCs in Peyer's patches, which may in part be responsible for age-associated mucosal dysregulation, including lack of essential cytokine synthesis for the induction of either sIgA immunity or oral tolerance. Zavala and Cavicchia (2006) observed changes in both morphology and expression (density) of CD1a on LCs in gingival epithelium from elderly subjects. Varas et al (2003) observed that thymic stromal DCs were only slightly affected by age. Increased inflammatory activity accompanies normal aging brain. Stichel and Luebbert (2006) observed that a dense network of DCs pervaded brain areas where substantial histopathological changes are observed in aging mice. All CD11c+ DCs displayed typical dendritic shape and expressed the myeloid specific CD11b. The numbers of LCs are decreased in the skin in aging (Bhushan et al, 2002). Therefore, it appears that during aging changes in DCs may vary with the subsets of DCs and tissue of their residence.

CAPTURE OF ANTIGEN

Immature DCs are remarkably efficient in the capturing of antigens and induction of tolerance (Schuurhuis et al, 2006; Hugues et al, 2006; Dubsky et al, 2005; Hackstein and Thomson, 2004; Kapsenberg, 2003). In contrast, mature DCs are efficient in antigen presentation and induction of immunity. DCs capture antigens by several mechanisms including [a] micropinocytosis; [b] receptor-mediated endocytosis via C-type lectins or Fc γ receptors (uptake of immune complexes or opsonized particles); [c] phagocytosis of apoptotic and necrotic cells, viruses, bacteria including mycobacteria, and [d] internalization of heat shock proteins, hsp70 or gp96-peptide complexes through multiple receptors including TLR2/TLR4

(Dubsky et al, 2005). Therefore, an efficient uptake of antigens is essential for generation of specific immune response.

Since we did not observed any significant difference in the differentiation and maturation of MODCs in aging we examined micropinocytosis of Lucifer Yellow dye and uptake of FITC-dextran by MODCs from young and aged subjects using flow cytometry. A decreased uptake of both Lucifer dye and FITC-dextran by MODCs from aged was observed as compared to MODCs from young subjects (unpublished data). The reduced uptake of antigen may also affect antigen processing and presentation and thus, effective T cell response in aging.

Phagocytosis of self antigens by DCs in the form of apoptotic cells is important for the maintenance of peripheral self tolerance. Under physiological conditions, immature dendritic cells continuously sample self-antigens from apoptotic cells resulting in the peripheral tolerance. In contrast, uptake of necrotic cells promotes the maturation of MODCs resulting in an upregulation of costimulatory molecules and subsequently, generation of immunity against endogenous antigens (Sauter et al, 2000). Therefore, efficient phagocytosis and removal of apoptotic cells is critical for the maintenance of peripheral self tolerance. We examined an uptake of apoptotic cells by MODCs from aged and young subjects. Jurkat T cells were labeled with CFSE and induced to undergo apoptosis by actinomycin D. Apoptotic cells were then co-cultured with MODCs and examined for phagocytosis by flow cytometry. We observed that MODCs from aged subjects display impaired capacity to phagocytose apoptotic cells as compared to young MODCs. Since phagocytosis of apoptotic cells results in an anti-inflammatory response by inhibiting production of pro-inflammatory cytokines and induction of anti-inflammatory cytokines (Fadok et al, 1998), an impaired uptake and therefore inefficient removal of apoptotic cells by DCs from aged human may result in both inflammation and autoimmunity commonly associated with aging.

We also observed that LPS-activated MODCs from aged subjects produce increased levels of pro-inflammatory cytokines IL-6 and TNF- α ; however, no increase in IL-10 production was observed (unpublished data). Furthermore, we did not observe any difference in TLR4 (receptor for LPS) expression in MODCs from aged and young subjects, suggesting that the mechanism of defect is downstream of TLR4.

PATTERN RECOGNITION RECEPTORS

DCs sense pathogens through a variety of pattern recognition receptors (PRRs) such as toll-like receptors (TLRs), C-type lectin receptors and NODs (Kapsenberg, 2003; Akira and Takeda, 2004; Tada et al, 2005; Cambi et al, 2005). Therefore, expression and functioning of these PRRs determine the generation of effective immune responses against pathogens. There are few reports which described a reduction in the expression various TLRs in aged mice macrophages (Renshaw et al, 2002; Boehmer at, 2004; Chelvarajan et al, 2005). However, there are no published reports describing the expression and functioning of TLRs in DCs in aging humans or mice. Our gene array (Affymetric) data did not show any significant difference in the expression of any of the TLRs in MODCs between aged and young subjects (unpublished personal data). Furthermore, the expression of TLR4 at the protein levels, as analyzed by flow cytometry, was also comparable between young and aged individuals. These contrasting observations of decreased TLR4 expression in macrophages from aged mice may due to differences in species and/or cell type (macrophages versus DCs).

MIGRATION OF DCs

DCs present at the entry point of pathogens such as skin and mucosal surfaces and following capturing of antigen migrate to regional lymph nodes to elicit effective adaptive T and B cell responses. During this process, the expression of a chemokine receptor CCR-7, which allows

DCs to migrate to the T cell areas in the lymphoid organs in response to chemokine CCL19 (MIP3- β) is upregulated (Caux et al, 2002; Ardeshtna et al, 2002). Since MDCs from CCR7 knockout mice cannot migrate to lymph nodes, it appears that the interaction between CCR7 and its ligands is essential for normal migration of MDCs from periphery to the regional lymph nodes (Forster et al, 1999). Therefore, we examined migration of MODCs in a transwell chamber in response to MIP3- β . MODCs from aged displayed impaired migration. This defective migration of MODCs may be in part responsible for impaired adaptive immune response in aging. In order to understand the mechanisms of impaired migration we also examined the expression of CCR7, which was comparable between MODCs from aged and young subjects. This would suggest that signaling downstream of surface receptors may play a role in impaired migration of MODCs in aging. Linton et al (2005) in a TCR transgenic mice model reported *in vivo* impaired migration of DCs from aged mice to the draining lymph nodes, which appears to be due to both intrinsic defect of DCs and aged microenvironment. Cumberbatch et al (2002) observed decreased LCs mobilization and the subsequent accumulation of DCs in the regional lymph nodes in aged mice in response to topical challenge with a chemical agent; however, contact hypersensitivity responses were not compromised. In addition, LC migration induced by intradermal injection of TNF- α was also reduced in aged animals. In contrast, using a different model system Pietschmann et al (2000) observed normal transendothelial migration of peripheral blood myeloid-enriched lymphocyte-depleted cells in elderly subjects. These data suggest that the impairment of DCs migration in aging may vary with the type of DCs and the migratory regions/tissue. Since DC migration is intimately linked with their maturation, and consequently with their impact on T cell immunity, it is likely that impaired migration of DCs may play a role in immunosenescence.

Since we observed impairment of both phagocytosis and migration of DCs in MODCs in aged subjects and the cell surface receptors are comparable between young and aged, the defect appears to be in downstream signaling pathways. PI3 kinase has been demonstrated to play a critical role in both phagocytosis and migration of DCs (Del Prete et al, 2004; Stephens et al, 2002; Bhattacharya et al, 2004). Our preliminary data show a decreased activation of PI3K, as evidenced by impaired phosphorylation of AKT, in MODCs in aged subjects. Whether, the decreased PI3K activity is a primary defect or is due to abnormality of its regulatory proteins (e.g. Pten) remains to be determined.

MODULATION OF ADAPTIVE IMMUNE RESPONSES BY DCs

DCs differ from other antigen-presenting cells in that DCs initiate *de novo* immune responses, whereas other APCs (B cells, macrophages) participate in amplifying those immune responses. DCs play an important role in adaptive immunity; induction of tolerance and immunity (Kapsenberg, 2003; Hugues et al, 2006; Reis e Sousa, 2006; Schuurhuis et al, 2006). Immature DCs induce tolerance by an activation/induction of T regulatory cells and deletion of self-reactive clones by apoptosis. In contrast, mature DCs induce T and B cell immune responses. DCs maturation may be induced through pathogen receptors such as TLRs, T cell-derived signals, such as CD40L or antigen-antibody complexes or through exposure to inflammatory cytokines. DCs maturation is associated with several coordinated events, including loss of endocytic/ phagocytic receptors, upregulation of MHC molecules, and co-stimulatory molecules (e.g. CD40, CD58, CD80, CD40L, CD70, 4-1BB-L), changes in adhesion molecules, and secretion of a broad array of cytokines and other inflammatory mediators which allow them to communicate between themselves and other cells of the immune system (Schuurhuis et al, 2006). Therefore, DCs can modulate adaptive immune responses in two ways; [a] through modulation of expression of co-stimulatory and inhibitory molecules on DCs, which regulate the priming of T cells and B cells, and [b] through cytokines produced in response to a pathogen that is largely responsible in determining the type of TH1/TH2/TH3 responses (Kapsenberg, 2003).

Alteration in the expression of these molecules with age would result in impaired T cell priming. As discussed above, MODCs from aged subjects are not defective in the expression of these co-stimulatory molecules at the basal level and following LPS activation. Furthermore, we did not observe any significant difference in HLA-DR expression on DCs at the basal level of following activation with LPS.

Besides the co-stimulatory molecules, DCs express a number of inhibitory molecules such as PD-L1, PD-L2, ICOS L receptors of IGSF (immunoglobulin super family) such as Immunoglobulin-like transcript 3 (ILT3) and , PIR-B and DlgR2 (Ujike et al, 2002; Cella et al, 1997; Shi et al, 2006). Increased expression of these inhibitory receptors on DCs would impair T cell priming. There are no published data regarding expression of any of these molecules on DCs in aging.

Activation of DCs through TLR triggering or CD40 or proinflammatory cytokines results in the secretion of both pro-inflammatory (TNF- α , IL-6, IL-12p70, IL-23) and anti-inflammatory cytokines (IL-10). The secretion of IL-12 by DCs drives IFN- γ production (TH1 response), whereas the anti-inflammatory cytokine, IL-10 inhibits IL-12 secretion by DCs and thereby drives TH2 responses. Recent studies have shown that activation of DCs through a combination of TLR2 and C-type lectin receptors on DCs results in the secretion of IL-10 which leads to a T regulatory (TH3) type of response. Thus, any change in the pattern of cytokine secretion by DCs in aging may also influence the nature of T cell responses. Lung et al (2000) reported normal TNF- α and IL-12 production by MODCs from aged subjects. However, we observed increased production of LPS-induced pro-inflammatory cytokines TNF- α and IL-6 by LPS-stimulated MODCs from aged subjects, whereas secretion of IL-10 was comparable to young controls (unpublished data). By contrast, decreased secretion of TNF- α and IL-6 and increased secretion of IL-10 by LPS-stimulated macrophages from aged mice has been reported (Boehmer et al, 2004; Chelvarajan et al, 2005), which may be due to species difference and/or difference between alterations in macrophages and DCs functions in aging.

There is some controversy regarding capacity of DCs in old age to stimulate T cells and B cells. In animal models of aged mice, DCs of lymph nodes display decreased antigen trapping capacity implying impaired functional activity; however, MODCs from elderly subjects were not impaired in their capacity to induce T cell responses (Grewe, 2001). Steger et al (1997) reported that DCs from young and aged subjects have similar stimulatory capacity to induce proliferation of T cell lines developed in long-term cultures. Our study with freshly isolated naïve T cells from young subjects stimulated with MODCs from aged humans demonstrated that aged MODCs were comparable to young MODCs in inducing proliferation in CD8+ T cells; however, proliferation of CD4+ T cells was impaired, suggesting that aged DCs are defective in stimulating preferentially CD4+ T cells. Sharma et al (2006) reported that the efficacy of autologous DCs vaccines from aged mice in inducing anti-tumor immune response *in vivo* was impaired. This impairment of DC vaccination induced anti-tumor response in aging was observed even when IL-2 was also added. However, *in vivo* co-administration of anti-OX-40 or anti-4-1-BB monoclonal antibodies resulted in normalization of anti-tumor response by aged DC vaccination. These data suggest that with appropriate co-stimulating signals DC-induced anti-tumor responses can be restored in aging mice. Szakal et al (2002) reported that *in vivo* age-related defects in B cell stimulation via the B cell receptor and co-stimulation via CD21/CD21L are related to immune complex-trapping by follicular DCs. Whereas other studies have described no change or even an enhancement (Steger et al, 1996). The conflicting results may be due to different subsets of DCs used (LCs versus myeloid DC versus FDC) and species differences (man versus mouse). Furthermore, it is also likely that functional study of *in vitro* generated DCs may not reflect true *in vivo* changes in adaptive immune responses generated by DCs during aging.

SUMMARY

Peripheral blood PCDCs and MODCs numbers are similar in young and aged subjects and generation of MODCs from peripheral blood monocytes is comparable between young and aged subjects. MODCs from young and aged subjects are phenotypically and morphologically similar. MODCs migration, micropinocytosis and receptor-dependent and -independent phagocytosis are impaired in aging. Impaired phagocytosis is not due to changes in receptors and molecules involved in receptor-mediated phagocytosis CD14, Elmo, and DOC180. LPS-stimulated MODCs from aged secrete increased levels of IL-6 and TNF- α without any change in TLR-4 expression.

A number of investigators have reported that PI3K plays a role as a negative feedback regulator of inflammation (Williams et al, 2004; Fakao and Koyashu (2003); Guha and Mackman, 2002). In DCs, PI3K appears to block MAPK p38 activation. AKT has been shown to block kinase activity of MEKK-3, which is upstream of MAPK p38 (Gratton et al, 2001). Since activation of AKT is positively regulated by PI3K, an inhibition of PI3K or lack of PI3K upregulates p38 activity in DCs (Fukao et al, 2002). Guha et al (2002) have shown that PI3K-AKT pathway in monocytes also suppresses both MAPKs and NF- κ B cascade in response to LPS resulting in decreased production of TNF- α . Therefore, decreased activation of AKT in aging associated with an increased activation of p38 and NF- κ B and increased TNF- α and IL-6 production by aged MODCs (our unpublished observations) would be consistent with negative regulatory role of PI3K-AKT pathway in innate responses in DCs. Since PI3K positively regulate phagocytosis and migration, a decreased activation of PI3K-AKT pathway in MODCs in aging may play an important role in both impaired phagocytosis and migration of MODCs and increased secretion of TNF- α and IL-6. DCs from aged subjects induce decreased proliferative response in naïve CD4+ T cells from young subjects.

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