Impaired Dendritic Cell Function in Aging Leads to Defective Antitumor Immunity

Annabelle Grolleau-Julius,1 Erin K. Harning,1 Lisa M. Abernathy,1 and Raymond L. Yung1,2

1Divisions of Geriatric Medicine and Rheumatology, Department of Internal Medicine, University of Michigan and 2Geriatric Research Education and Clinical Centers, Ann Arbor Veterans Affairs Health System, Ann Arbor, Michigan

Abstract

We recently reported that bone marrow–derived dendritic cells (DC) from aged mice are less effective than their young counterparts in inducing the regression of B16-ovalbumin (OVA) melanomas. To examine the underlying mechanisms, we investigated the effect of aging on DC tumor antigen presentation and migration. Although aging does not affect the ability of DCs to present OVA peptide6257–264, DCs from aged mice are less efficient than those from young mice in stimulating OVA-specific T cells in vitro. Phenotypic analysis revealed a selective decrease in DC-specific/intracellular adhesion molecule type-3–grabbing nonintegrin (DC-SIGN) level in aged DCs. Adoptive transfer experiments showed defective in vivo DC trafficking in aging. This correlates with impaired in vitro migration and defective CCR7 signaling in response to CCL21 in aged DCs. Interestingly, vaccination of young mice using old OVA peptide6257–264–pulsed DCs (OVA PP-DC) resulted in impaired activation of OVA-specific CD8+ T cells in vivo. Effector functions of these T cells, as determined by IFN-γ production and cytotoxic activity, were similar to those obtained from mice vaccinated with young OVA PP-DCs. A decreased influx of intratumor CD8+ T cells was also observed. Importantly, although defective in vivo migration could be restored by increasing the number of old DCs injected, the aging defect in DC tumor surveillance and OVA-specific CD8+ T-cell induction remained. Taken together, our findings suggest that defective T-cell stimulation contributes to the observed impaired DC tumor immunotherapeutic response in aging. [Cancer Res 2008:68(15):6341–9]

Introduction

Increased susceptibility to malignancies, infections, autoimmune diseases, and a poor response to immunization in the elderly have been taken as indicative of declining immune function in aging. Immunosenescence, the progressive deterioration in immune function that accompanies aging, results from the alteration of both adaptive and innate immunity. With advancing age, T cells undergo major changes including a shift from the naive to memory phenotype, a reduction in the proliferation response, and impaired cytolytic activity (1, 2). However, the recognized changes in T lymphocyte function may not completely explain the defect in immune responsiveness observed in old age, and other members of the immune system may play an equally important role in contributing to immunosenescence.

Dendritic cells (DC) form a distinct heterogeneous hematopoietic lineage of antigen-presenting cells (APC) with unique abilities to stimulate naïve T lymphocytes (3, 4). DCs are derived from bone marrow progenitors and reside in peripheral tissues or in circulation as phagocytic immature precursors. DCs acquire a terminally mature phenotype (iDC) on uptake of antigen and in response to stimuli such as cytokines, necrotic cells, and microbial products, enabling them to migrate to secondary lymphoid tissues where they present antigen to T cells and induce antigen-specific responses. Due to their central role in immunology, DCs represent a potent adjuvant for use in tumor vaccination protocols (5, 6). Because the elderly are preferentially affected by diseases targeted by DC-directed immunotherapy, it will be critical to understand how aging affects DC functions as well. Data on qualitative and quantitative alterations of human and murine DCs in aging have recently been summarized (7–9) and have shown that the results of such studies are often contradictory and difficult to compare because the origin of the cells, their culture conditions, and maturation protocols vary greatly.

Recent studies comparing the efficacy of young bone marrow–derived DC vaccines in young and old mice suggested that the aging microenvironment affects the DC antitumor response (10, 11). Using young hosts, we reported that ovalbumin peptide–pulsed DCs (OVA PP-DC) from old C57BL/6 mice were less effective than their young counterparts in inducing the regression of B16 melanomas expressing OVA (B16-OVA; ref. 12). This implies that an intrinsic functional defect(s) also exists in aged DCs. Effective DC vaccination involves first the appropriate uptake and processing of tumor-associated antigens, then DC trafficking to regional draining lymph nodes, and finally interaction and activation of antigen-specific CTLs. In the present study, we therefore examined the effect of aging on these three specialized DC functions.

Materials and Methods

Animals. Young (3–6 mo) and old (18–20 mo) C57BL/6 mice were purchased from Harlan Laboratory, and OVA-specific MHC-I–restricted, T-cell receptor–transgenic young C57BL/6 mice (OT-I mice) were from The Jackson Laboratory.

Generation and purification of DCs. Erythrocyte-depleted bone marrow cells flushed from femurs and tibiae of mice were cultured at 1 × 106/mL in the presence of 20 ng/mL murine recombinant granulocyte macrophage colony-stimulating factor and interleukin (IL)-4 (R&D Systems) in complete RPMI 1640 as before (13). DCs were purified from 5-d cultures using CD11c+ microbeads (Miltenyi Biotec). To generate mature cells, DCs were replated with 1 μg/mL lipopolysaccharide (LPS; Sigma) for 24 h.

FITC-OVA uptake. DCs (5 × 104) were loaded with 0.1 and 1 mg/mL FITC-OVA protein (Sigma) or 10 μg/mL FITC-OVA-peptide6257–264 (University of Michigan Protein Facility Core, Ann Arbor, MI) for the indicated time at 37°C or 4°C (background). Cells were stained with phycoerythrin.
(PE)-anti-MHC class I antibody (BD PharMingen) and analyzed by flow cytometry.

**Analysis of DC migration in vivo.** Young and old tDCs were stained with CMPTX or carboxyfluorescein diacetate succinimidyl ester (CFSE) dyes (Molecular Probes) according to the manufacturer’s instructions. Equal number or an indicated ratio of cells from each age group was mixed together and injected s.c. into the footpads of young mice. Draining lymph nodes were harvested 24 h later, embedded in optimum cutting temperature compound (Sigma), and snap-frozen. Serial 5-μm sections were made and the frequency of stained cells assessed in a blinded fashion by fluorescence microscopy (Olympus BX61 microscope). Results are expressed as the percent of injected DCs present per microscope field.

**Chemotaxis assay.** DCs (2.5 × 10^5 in 0.1 mL) were plated in the upper chamber of a 5-μm pore size transwell (Costar). Indicated concentrations of recombinant mCCL21 (R&D Systems) were added to the lower wells of the chamber. Plates were incubated at 37°C for 2 h, and 10^5 15-μm beads (Bangs Laboratories) added to standardize the results. Cells and beads were quantitated by flow cytometry, and the number of cells in each sample calculated using the formula (number of cell events / number of bead events) × 10^5. The chemotaxis index was calculated as the ratio between the cell number migrated in response to CCL21 and the cell number migrated to the medium alone.

**Cell surface analysis.** Analysis of DC surface markers was done with a FACSCalibur (Becton Dickinson) as previously described (12) using the antibodies I-A<sup>+</sup>-PE, CD40-PE, CD86-PE, CD54-FITC, CD80-PE, CCR7-PE, and DC-SIGN-PE-antibodies (BD Biosciences) and FACS analyzed for immune infiltrate using the following antibodies: CD45-PerCP, CD4-FITC, CD8-FITC, CD11c-FITC, Ly-6G-FITC, NK1.1-PE, and CD14-PE (all from BD Biosciences). Fifteen-micrometer beads (5 × 10^5/mL) were added to all samples and 100,000 events acquired. The number of infiltrating cells per tumor was determined by the following equation: (number of double-stained events / number of bead events) × (5 × 10^5 beads/mL) × cell sample volume (in mL). Because the tumors were of different sizes, the data were normalized to the tumor weight by dividing the total number of infiltrating cells in each sample by their respective tumor weight.

**Statistical analysis.** Results are expressed as means ± SE. Statistical analyses were done using Student’s t test, and P ≤ 0.05 was taken as statistically significant. For multiple comparisons, two-tailed Student’s t test was used.

**Results**

Aged DCs have impaired ability to stimulate CD8<sup>+</sup> T-cell proliferation. We have previously reported that old DCs were less effective than young DCs in stimulating the proliferation of OVA-specific CD4<sup>+</sup> T lymphocytes (12). The B16-OVA tumor model suggests that old DCs may also be less capable of supporting cytotoxic CD8<sup>+</sup> T-cell function. We therefore compared the proliferation response of OVA-specific T cells from OT-I mice to OVA peptide<sub>257–264</sub> PP-DCs derived from young and old mice. Figure 1A shows that young DCs induce a greater T-cell proliferation than old DCs (P < 0.0025), confirming that the capacity of DCs to stimulate OVA-specific CD8<sup>+</sup> T cells is also impaired in aging.

DC-dependent CD8<sup>+</sup> T-cell response depends on the interaction of processed antigen with the T-cell receptor in the context of MHC class I molecules and the interaction of costimulatory molecules with their respective cell surface receptors. We first examined the effect of aging on tumor antigen uptake and presentation in the context of MHC class I molecules using whole OVA protein and OVA peptide<sub>257–264</sub>. As shown in Fig. 1B, phagocytosis of FITC-OVA protein was equally efficient in young and old DCs. Using FITC-OVA peptide<sub>257–264</sub> we also found that both young and old DCs express similar number of MHC I-peptide complexes on their cell surface, with 50.27 ± 9.99% and 53.51 ± 5.96% double-positive cells, respectively (Fig. 1C). Similar results were obtained using FITC-OVA protein (data not shown), suggesting that aging does not significantly affect DC presentation of OVA peptide in the context.
of MHC class I molecules. Next, we analyzed DCs for the expression of markers relevant to presentation of antigen to T cells. Overall, there was little or no significant difference between young and old OVA PP-DCs in MHC class I, CD80, CD86, CD54, and CD40 expression (Fig. 1D). Interestingly, a decrease in DC-SIGN (CD209) expression was observed in aging (P < 0.01). This finding is consistent with our previous report showing that decreased DC-SIGN expression in aging may contribute to impaired CD4+ T-cell proliferation in the OT-II model (12).

**DC in vivo trafficking to draining lymph nodes is affected by aging.** Migration of DCs to the secondary lymphoid organs is essential for the cells to exert their T-cell regulatory function. To determine in vivo DC trafficking, young and old tDCs were labeled with CMPTX and CFSE, respectively, and equal number of cells mixed together before injection into the footpads of young mice to exclude the influence of the aged microenvironment. Twenty-four hours later, the draining popliteal lymph node was harvested, and fluorescence assessed by fluorescent microscopy. Figure 2A shows representative images, with young DCs in red and old DCs in green. We found that half as many aged DCs were present in the popliteal lymph nodes (P < 0.025; Fig. 2B). Interestingly, the number of old DCs migrating is the same as young DCs in animals injected with two times more old DCs than young DCs (Fig. 2C). No additional beneficial effect was observed when three times more old DCs were injected.

**Aged DCs have impaired CCR7 signaling and function.** Engagement of CCR7 chemokine receptor on DCs to its ligands CCL19 (ELC/MIP-3β) and CCL21 (SLC/6Ckine) in lymph nodes has been identified as the critical event in DC lymphoid homing (16, 17). To determine whether the impaired migratory function in aged DCs correlates to a decrease in CCR7 function, the chemotactic response of young and old tDCs to CCL21 was determined. Figure 3A shows that CCL21 induced a weaker chemotactic response by old DCs compared with young DCs (P < 0.025). We next examined CCR7 expression of young and old tDCs by FACS. Somewhat surprisingly, the age-dependent reduced CCR7 migration response did not correspond to any significant change in CCR7 surface expression on old tDCs, with 60.2 ± 5.48 positive cells as compared with 53.4 ± 7.6 for their young counterparts (Fig. 3B). Comparison of DC CCR7 signaling revealed that CCL21 stimulation
induces a greater level of tyrosine phosphorylation of proteins of molecular weight 46 to 97 kDa in young tDCs as compared with old tDCs (Fig. 3C), suggesting that the age-dependent impaired CCR7 response may be, in part, secondary to the DC signaling defect in aging.

**Aged DCs stimulate a weaker T-cell immune response.** We next examined the immune function of T cells in mice treated with DCs. Mice with a 7-day-old B16-OVA tumor were inoculated with either young or old OVA PP-DCs as described previously (12). Spleens were harvested on day 14 for immune function analysis. First, we measured the OVA peptide–specific CD8+ T-cell frequency by flow cytometry. For this purpose, splenocytes were depleted of CD19+ cells, as the presence of B cells has been shown to interfere with the T-cell response (13). The percentage of OVA-specific CD8+ T cells was calculated as the ratio of OVA tetramer-positive cells to total CD8+ T cells. As shown in Fig. 4A, OVA-specific CD8+ T cells were detectable in both groups of immunized mice. However, the frequency of those T cells decreased by 2.5-fold in mice that received the old PP-DC vaccine, compared with mice receiving the young PP-DC vaccine (mean percent double-positive cells from three independent experiments, 0.39 ± 0.1 versus 1.04 ± 0.22; P < 0.025).

Cytotoxic assays using splenocytes obtained from the same vaccinated mice were next done. As shown in Fig. 4B, CTLs generated from the spleens of young PP-DC–immunized mice showed 68% cytotoxic activity against the B16-OVA cells at an effector/target ratio of 25:1. In contrast, CTLs obtained from the spleens of old PP-DC–immunized mice showed only 26% cytotoxic lysis (P < 0.025). These results confirm that the enhancement of the antitumor effect in mice immunized with young PP-DC vaccine corresponds to augmentation of the cytolytic activity. Interestingly, assessment of cytokine release of activated CTL cells in response to tumor stimulation revealed that T cells from mice receiving the young DCs produced greater amounts of IFN-γ (P < 0.005), TNF-α (P < 0.001), IL-10 (P < 0.05), and IL-6 (P < 0.001; Fig. 4C).

**Decreased ability to induce OVA-specific T cells but not impaired migration leads to deficient antitumor immunity.** The results presented in Fig. 4 suggest that old DCs stimulate a weaker T-cell response when compared with young DCs. The experiments in Fig. 5 were designed to determine if the lack of proper immune response after stimulation with old DCs is a direct consequence of their inability to migrate to the lymph nodes, and if it reflects a difference in the quality rather than in the quantity of the T cells after young and old DC stimulation. We have shown in Fig. 3C that the in vivo age-associated defect in DC migration could be restored by injecting animals with two times more old DCs. We investigated if the age-associated defect in DC tumor surveillance and T-cell induction could be restored by increasing the number of DCs injected. Young tumor-bearing mice were immunized with Dulbecco’s PBS, 2 × 10^6 young or old PP-DCs as before, or 4 × 10^6 old PP-DCs. We found that mice immunized with two times more old DCs had similar tumor size (Fig. 5A) and similar number of OVA-specific CD8+ T cells (Fig. 5B) as those receiving 1 × old DCs. Those values remain significantly lower than those observed in mice receiving young DCs. The results suggest that the defective antigen-specific T-cell induction after stimulation with old DCs is not a consequence of impaired migratory properties of those cells but is, at least in part, related to a qualitative effect on T-cell stimulation.

To further test this hypothesis, we wanted to see if the weaker T-cell response we observed, as defined by reduced cytotoxic activity and IFN-γ production (Fig. 4B and C), was due to decreased OVA-specific CD8+ T-cell induction by old DCs. As shown in Fig. 5B, total splenic CD8+ T cells obtained from mice immunized with old PP-DCs and restimulated in vitro with OVA peptide have lower IFN-γ production as measured by intracellular staining, with a frequency of 1.46 ± 0.16% IFN-γ positive cells as compared with...
3.03 ± 0.35% in CD8\(^+\) T cells from mice immunized with young PP-DCs. However, when we assessed IFN-\(\gamma\) production among OVA-specific CD8\(^+\) T cells by gating on OVA-Pent\(^+\) CD8\(^+\) T cells (elliptic gate in Fig. 5B), we found no age difference in the ability of those cells to produce IFN-\(\gamma\).

Becker and colleagues (18) have shown that IFN-\(\gamma\)^+ T cells, but not IFN-\(\gamma\)/C0 T cells, from tumor-immunized mice were cytolytic and mediate tumor rejection on adoptive transfer. The same authors showed that those cells could be enriched using an IFN-\(\gamma\) capture assay. Using the same technique, we recovered IFN-\(\gamma\)-producing CD8\(^+\) T cells from 6-day cultures of splenocytes from mice immunized with young and old PP-DC vaccines and repeated the cytotoxic assays using same numbers of those cells. We found that the ability of IFN-\(\gamma\)-producing CD8\(^+\) T cells obtained from mice immunized with young DCs to induce lysis of B16-OVA cells was only slightly better (not statistically different) compared with those obtained from mice immunized with old DCs (Fig. 5D). This result suggests that the quality of the T cells after old DC stimulation on a per cell basis is largely intact and that the defective cytokine production and cytotoxic activity presented in Fig. 4B and C are mainly due to the fact that old DCs induce half as many OVA-specific T cells than young DCs and, therefore, less OVA-specific T cells were added to the assays. In both experiments, increasing the number of old DCs injected did not have any effect on T-cell functions.

Reduced number of intratumor CD8\(^+\) T cells in mice receiving OVA peptide–pulsed old DCs. Tumor rejection is initiated by CD8 CTLs that infiltrate solid tumors. We analyzed by flow cytometry single-cell suspensions from B16-OVA melanoma for the presence of infiltrating leukocytes (CD45\(^+\) cells) at 7 days after the initiation of treatment with young and old OVA PP-DCs. For comparison, we also analyzed tumors from mice treated with Dulbecco’s PBS. Figure 6A shows the typical data obtained from single untreated, young OVA PP-DC (1x^\(-\) ), old OVA PP-DC (1x^\(-\) ), and old OVA PP-DC (2x^\(-\) )–treated mice. Figure 6B summarizes the tumor data from a total of six experiments. Nearly half of the immune cells contained in OVA PP-DC–treated tumors were T cells, whereas in Dulbecco’s PBS–treated tumors NK1.1^+ cells were the most abundant immune cell subset represented. Interestingly, although both young and old OVA PP-DC treatments could induce the influx of T cells, young OVA PP-DC administration resulted in higher numbers of infiltrating CD8\(^+\) T cells compared with old OVA PP-DC–treated mice (\(P < 0.005\)). Approximately 66% of the T-cell infiltrates were CD8\(^+\) cells in the young DC–immunized group whereas the proportion of CD8\(^+\) cells decreased by 20% in the old DC–immunized group, with no change in the total number of lymphocytes. Increasing the number of old DCs injected had no significant effect on the tumor infiltrate composition. Both DC-treated groups also induced an influx of CD11c^+...
cells to the tumor sites, although the results did not reach statistical significance.

Discussion

Despite numerous recent advances in the molecular and cell biology of DCs, only very few groups have addressed the topic of DC function and aging. Some studies have reported no age-related differences between DC numbers, phenotype, morphology, and maturation in human monocyte-derived DCs from young and aged subjects (7, 19, 20). Others, however, have indicated that migration and phagocytosis of the same DC subset were impaired with aging (21). A progressive loss of circulating plasmacytoid DCs numbers and a decreased density of Langerhans cells with aging have also been reported (22–24). These reports suggest that the aging-associated changes in DCs may vary with the subset of DCs studied, their tissue of residence, and environmental signals. Examining different in vivo models may provide important insight into the effect of age on DC function.

The ultimate goal of tumor DC immunotherapy is to induce strong tumor-specific T-cell-mediated immunity that can block the growth and metastasis of malignant tumor cells in tumor-bearing hosts. In most cases, the development of antitumor immunity requires strategies capable of stimulating CD8+ CTLs. Using the B16-OVA melanoma tumor model, we recently showed that old DCs loaded with specific tumor antigens are less able to control tumor growth. The scope of the present study was to investigate the underlying mechanism responsible for the defective DC antitumor function observed in aging.

The tumor model requires the antigen-loaded DCs to migrate to the lymphoid tissue to stimulate T lymphocytes. Evidence for impaired DC migration in aging includes a recent report showing that recruitment of airway DCs to draining mediastinal lymph nodes may diminish in aged mice (25). Similarly, the ability of Langerhans cells to migrate to regional lymph nodes declines with age (26, 27). In the present study, we reported that the vigor of DC migration and subsequent influx of DCs into draining lymph nodes is also affected by aging. We have also shown that aged DCs have impaired capacity to migrate in vitro in response to the CCR7 ligand CCL21. This was not due to a defect in CCR7 protein expression but rather by a defect in signal transduction, as shown by comparison

![Figure 4. Effects of young and old DCs on T-cell functions. A, in vivo detection of OVA-specific CD8+ T cells by MHC pentamer staining. Day 7 tumor-bearing B6 mice were vaccinated with young or old OVA PP-DCs. Seven days later, total splenocytes were harvested, depleted of CD19+ cells, and stained for CD8-RPE-Alexa Fluor 647 and OVA-Pent-PE. Representative figure of FACScan plots depicting the percentage of OVA-specific CD8+ T cells. B, CTL activity of splenocytes from mice receiving young and old DC vaccines. Spleens isolated from DC-vaccinated or control mice (Dulbecco’s PBS (DPBS)--treated tumor-bearing mice) were stimulated for 6 d in vitro with mitomycin C--treated B16-OVA tumor cells and cytotoxic activities against B16-OVA target cells were measured. Points, mean specific lysis of quadruplicate values (%) from three independent experiments; bars, SE. C, cytokine release of activated CTL cells in response to tumor stimulation. Splenocytes from vaccinated mice were cocultured with irradiated B16-OVA cells. After 48 h, supernatants were collected for cytokine analysis by Cytometric Bead Array. Columns, average amount of cytokine secreted from three experiments; bars, SE. *, P < 0.001; **, P < 0.005; ***, P < 0.05, young PP-DCs versus old PP-DCs.]
with the pattern of tyrosine phosphorylation using young and old DCs stimulated by CCL21. This is consistent with the recent article from the Gupta group, who reported a decreased in vitro migration toward CCL19, another CCR7 ligand, of monocyte-derived LPS-stimulated DCs from elderly subjects as compared with young subjects, despite similar levels of expression of CCR7 (21). They attributed their results to defects in the downstream signaling pathway, possibly in the phosphatidylinositol 3-kinase pathway. However, the exact mechanism is unknown and further studies will be needed to unravel it. We found that although the impaired in vivo migration of aged DCs can be restored to a level comparable to that seen in young DCs by increasing the quantity of cells injected, the age-associated impairment in tumor surveillance, as defined by tumor growth and antigen-specific T-cell induction, remains, suggesting that DC migration through CCR7-CCL21 interaction is not the primary mechanism for the observed aging defects.

In mouse models using T cells reactive to defined tumor antigens, tumor regression correlated with an early and sustained influx of CD8+ T cells. We found that the frequency of splenic antigen-specific CD8+ T cells is altered during aging, with a significant decrease in the influx of CD8+ T cells into tumors 7 days after injection of old PP-DCs, as compared with young PP-DC–injected mice. Importantly, effector functions of these antigen-specific T cells, as determined by IFN-γ production and cytotoxic activity, were similar to those obtained from mice vaccinated with young OVA PP-DCs on a per cell basis. This suggests that the quality of those T cells is intact and that decreased T-cell number per se is sufficient to explain the reduced inhibition of tumor growth observed in mice vaccinated with old PP-DCs. One possible explanation could be the impaired in vivo T-cell induction by aged DCs that we described.

For efficient antigen presentation and induction of an immune response by DCs, the number and stability of MHC I-peptide complexes are crucial (28). Our results showed that there is no age effect on the uptake and surface expression of OVA peptide/MHC I complexes. Because others have shown that aged APCs may require longer periods of contact with CD8+ T cells than young APCs to initiate the same antigen-driven response (29), we performed the same experiments after 24-hour culture and obtained similar results (data not shown). Altogether, the results suggest that difference in the formation and kinetics of MHC class I-OVA peptide complexes on DCs is not responsible for the observed defective DC-mediated antitumor response. Despite intact peptide presentation and no significant change in MHC class I and the classic costimulatory molecules, the proliferation of OT-I T cells

Figure 5. Effect of increasing number of old DCs on tumor growth and antigen-specific induction and function. Mice bearing 7-d established s.c. B16-OVA tumors were treated with 2 x 10^6 young PP-DCs (1x), 2 x 10^6 old PP-DCs (1x), 4 x 10^6 old PP-DCs (2x), or saline (Dulbecco's PBS), and tumors and spleens harvested 7 d later. A, graph depicts tumor size (n = 6 tumors per treatment group). B, splenocytes were stimulated for 6 h with OVA peptide, labeled with OVA-Pent-PE, CD8-FITC, and IFN-γ-PE-Cy7, and analyzed by flow cytometry. Dot plots show OVA-Pent staining and intracellular IFN-γ production by splenic CD8+ T cells. The indicated number shows the percentage of the gated population within the total CD8+ live cells. C, the percentage of IFN-γ+ cells among OVA-specific CD8+ T cells (determined using the elliptic gate in B) was assessed in three independent experiments. Horizontal columns, mean; bars, SE. D, IFN-γ CD8+ T cells were purified from spleens as described in Materials and Methods and assessed for cytotoxicity using B16-OVA tumor cells as targets. Points, mean of three independent experiments; bars, SE.


Research.
was impaired in old DCs. Recently, Geijtenbeek and colleagues (30) identified DC-SIGN as a novel adhesion receptor on DCs that is essential in several key functions throughout the life cycle of DCs, including interactions between DCs and T cells. They showed that DC-SIGN binds intercellular adhesion molecule 3 (ICAM-3) with high affinity and that this cellular interaction establishes the first molecular interaction between DCs and resting cells (31). In another study, van Gisbergen and colleagues investigated the behavior of DC-SIGN in synapse formation. Using a DC-SIGN deletion mutant, they found that DC-SIGN can recruit lymphocyte function-associated antigen 1 (LFA-1) to the contact site and shift from an initial transient DC-SIGN-ICAM-3 interaction to a more stable LFA-1-ICAM-3 interaction (32). Gijzen and colleagues (33) have shown the relevance of DC-SIGN in DC-induced T-cell proliferation by showing that antibodies against human DC-SIGN inhibit DC-induced proliferation of resting T cells. It is clear from our data that the capacity of DCs to induce T-cell proliferation in vitro is reduced with aging and that this correlates with a selective reduced DC-SIGN expression. Future studies will be needed to establish the exact relationship between those two observations.

We are currently in the process of breeding DC-SIGN knockout mice. These mice will help unravel the precise role of DC-SIGN not only in DC-T-cell interaction but also in other important processes in DC biology in which DC-SIGN has been shown to be involved, such as migration and antigen uptake. Additionally, focused microarray experiments that include genes involved in DC antigen uptake, processing, and presentation will enable us to discover other genes that may potentially play a role in these processes.

In summary, we here report a reduced ability of aged DCs to support the induction of antitumor T-cell responses at multiple levels. Treatment of tumors with old OVA PP-DCs eliciting a modest antitumor effect resulted in a substantial decrease in the frequency, but not potency, of antigen-specific CD8+ T cells and a far less pronounced CD8 T-cell infiltration into the tumor mass. Defects affecting DC antigen presentation to T cells could factor into the observed impairment in antitumor immunity, a finding that has important implications for using DC-based immunotherapy in older subjects.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

Received 10/9/2007; revised 5/27/2008; accepted 5/28/2008.

Grant support: NIH grants 1R01AG020628-01A2 and 3R01 AR42525 (R.L. Yung), the Ann Arbor VA Health System VA Merit Review (R.L. Yung), and NIH National Institute on Aging grant AG024824, University of Michigan Claude Pepper Older Americans Independence Center (A. Grolleau-Julius).

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