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Aging affect the anti-tumor potential of dendritic cell vaccination, but it can be overcome by co-stimulation with anti-OX40 or anti-4-1BB☆

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Abstract

It has been well established that there is a decline in immune function with age resulting in a diminished capacity to respond to infections or tumors. Although many studies have demonstrated the efficacy of autologous dendritic cells (DC) vaccines in stimulating an anti-tumor immune response in the young, almost none of these reports consider the effect that aging has on the immune system or test whether DC-vaccination is effective in old hosts. In this study we compared the efficacy of DC-vaccination in young and old mice. Our results showed that DC-vaccination in young animals induced an anti-tumor response resulting in ~60% tumor growth inhibition, while minimal protection was observed in old animals. DC vaccination plus rIL-2 further enhanced the anti-tumor response in young animals (~70–75% inhibition), while ineffective in old animals. In contrast, co-administration of anti-OX-40 or anti-4-1BB mAbs vigorously enhanced the anti-tumor immune response in both young (~85–90% inhibition) and old mice (~70–75% inhibition). Our data indicate that although old mice have a decline in immune function, they have the capacity to develop strong anti-tumor responses as long as they are provided with efficient co-stimulation.

Keywords: Aging; Co-stimulation; Dendritic cells; Anti-OX40; Anti-4-1BB

1. Introduction

Prostate cancer is the most common malignancy in American men (Fleshner, 2005). Eighty percent of diagnosed prostate cancer occurs in patients 60 years old or older. Sixty percent of new cases have disseminated tumors to other sites, which are difficult to detect, treat and the prognosis for these patients is poor. Taking into consideration that chemotherapy or radiation has high toxic side effects, especially in the elderly population, immunotherapeutic intervention is an attractive strategy that could be potentially effective in more frail elderly cancer patients. However, experimental and clinical studies have demonstrated declines of immune function during aging (Solana and Pawelec, 1998; Malaguarnera et al., 2001) including: decreased proliferation of T cells (Song et al., 1993); decreased secretion of pro-inflammatory cytokines and chemokines (Engwerda et al., 1996); reduced cytotoxic activity of $CD8^+$T cells (Bloom et al., 1990); and qualitative deficiency of B lymphocytes with a reduced response to exogenous antigens (LeMaoult et al., 1997).

The dendritic cells (DCs) constitute the most potent antigen presenting cells (APCs), and could function as important initiators and modulators of a specific and lasting immune response against tumor antigens. It has been shown that APCs from the aged animals have reduced TLR and cytokines expressions, and also express reduced level of co-stimulatory molecules (Plowden et al., 2004). It is still not clear how APC function is affected in vivo by these changes occurring during the aging process. Some studies have shown that DC function is unaltered in the aged (Lung et al., 2000), while other reports, indicate that APCs from aged mice are less effective in stimulating cytotoxic T cells (Plowden et al., 2004; Donnini et al., 2002). The aged T cell defects include inefficient generation of effector cells and reduced IL-2 levels, contributed mainly by CD4 T cells (Haynes et al., 2004), while aged CD8 T cells are thought not to be intrinsically deficient in clonal expansion and generating anti-tumor response (Plowden et al., 2004; Norian and Allen, 2004). Though aged CD8 T cells require longer period of contact with aged APCs to initiate the same degree of antigen driven clonal expansion, but this is due to the defects in APC function by virtue of their low expression

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of co-stimulatory molecules and cytokines (Plowden et al., 2004). Therefore, it is unclear whether alterations on APC function or whether it is the deficiency in the T cells compartment of the immune system that contribute to the lack of an effective immune response in the aged.

Even though many laboratories are evaluating DC-based vaccines pulsed with different prostate antigenic determinants to induce anti-tumor immune responses against prostate tumors (Rini, 2004; Degl'Innocenti et al., 2005), none of these laboratories have taken into consideration the effect that aging has on the anti-tumor immune response. Several reports have demonstrated that immunotherapeutic intervention could be effective in young animals, but that the same therapy is not effective in old animals (Flood et al., 1998; Provinciali et al., 2003). It is still unclear if anti-tumor efficiency of immunotherapy protocols is a function of age. Since prostate cancer is mainly a disease of the aged, a successful vaccine should be customized to be effective in both the young and the old. Present study is an attempt in this direction.

TRAMP (transgenic adenocarcinoma of the mouse prostate) is prostate tumor model of mice that closely resemble the human disease. TRAMP mice express the SV40 large T antigen under the control of the prostate-specific rat probasin promoter (Greenberg et al., 1995; Gingrich et al., 1996) and exhibit well-differentiated prostate tumors by 24 weeks of age. Since TRAMP mice do not survive more than a year and 1-year-old mice are not considered as old mice, we took advantage of the tumor cell line TRAMP-C2 derived from TRAMP mice which is tumorogenic in C57BL/6 mice (Gingrich et al., 1996). We evaluated whether there were differences in the anti-tumor immune responses between young (2-month-old) and old (20-month-old) C57BL/6 animals transplanted with TRAMP-C2 cells after immunization with DCs pulsed with apoptotic TRAMP-C2 tumor cells. We had previously demonstrated that the absence of anti-tumor immune response in old mice appears to be due to the lack of clonal expansion of the specific tumor reactive T cells (Lustgarten et al., 2004). However, others and we have shown that co-stimulation through the members of the TNFR family restores the immune response in old mice (Lustgarten et al., 2004; Bansal-Pakala and Croft, 2002). Given the potential benefit of anti-4-1BB and anti-OX40 mAb in enhancing the immune responses, we tested if combination of these co-stimulatory molecules with DC-based vaccine results in a better anti-tumor response in the aged.

2. Materials and methods

2.1. Mice, cell lines and reagents

Young (2-month-old) and old (20-month-old) C57BL/6 mice were purchased from the National Institute of Aging (NIA, Bethesda, MD) and housed under specific pathogen-free conditions. The TRAMP-C2 tumor cell line derived from the TRAMP transgenic mouse was obtained from Dr Norman Greenberg (Fred Hutchinson Cancer Research Center, Seattle, Washington). MC57 is a fibroblast cell line derived from C57BL/6 mouse. All cell lines were maintained in complete RPMI medium (RPMI 1640) supplemented with 10% FCS, 2 mM glutamine, 5×10^{-5} M 2-mercapethanol (ME) and 50 μg/ml gentamicin. Anti-OX40 (OX86) mAb was obtained from the European Cell Culture Collection (Wiltshire, UK) and the anti-4-1BB (3H3) was obtained from Dr Robert Mittler (Emory University, Atlanta, GA). Recombinant human IL-2 (rIL-2) was obtained from the Biological Resource Branch, National Cancer Institute, Bethesda, MD.

2.2. Generation of dendritic cells

Dendritic cells were derived from bone marrow as described by Inaba et al. (Inaba et al., 1992). Briefly, bone marrow cells were depleted of lymphocytes utilizing magnetic beads conjugated with antibodies against CD4, CD8 and B220 (Dynal, Oslo, Norway). The remaining cells were cultured in complete RPMI medium containing 3% GM-CSF containing media (supernatant from J558L cells transfected with murine GM-CSF gene, obtained from Dr R Steinman, Rockefeller University). Media was changed every second day, each time applying fresh complete RPMI medium containing 3% GM-CSF. On day 8, DCs were collected and phenotype confirmed by flow cytometry.

2.3. Apoptosis induction of tumor cells

The TRAMP-C2 tumor cells were exposed to UVB irradiation (2 mj/(cm^2/s)) and incubated for 18 h in medium without serum at 37 °C for the induction of apoptosis. DCs and apoptotic cells were collected and co-cultured in complete RPMI medium containing GM-CSF overnight at a ratio of 2 apoptotic cells per 1 DC (5×10^5 DCs/well). The exposure of DCs to apoptotic tumor cells results in uptake of apoptotic tumor cells causing their maturation.

2.4. Generation of CTL bulk cultures and cytotoxic activity

The young and old C57BL/6 mice were immunized subcutaneous (s.c.) with their respective DCs (10^6) pulsed with apoptotic TRAMP-C2 tumors alone or in combination with anti-OX40 or anti-4-1BB mAbs. The responder spleen cells from primed animals were re-stimulated in vitro with DCs pulsed with apoptotic TRAMP-C2. Cytotoxic activity was measured in standard ^{51}Cr release assay. TRAMP-C2 and MC57 cells were incubated with 150 μCi of ^{51}Cr sodium chromate for 1 h at 37 °C. Cells were washed three times and re-suspended in complete RPMI medium. For the CTL assay, ^{51}Cr labeled target cells (10^4) were incubated with varying concentrations of effector cells in a final volume of 200 μl in U-bottomed 96-well microtiter plates. Supernatants were recovered after 6 h of incubation at 37 °C and analyzed for ^{51}Cr released by cell lysis. The percent of lysis was determined by the formula: percent specific lysis = 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release).