Ex vivo Nicotine Stimulation Augments the Efficacy of Therapeutic Bone Marrow–Derived Dendritic Cell Vaccination

Feng Guang Gao, Da Fang Wan, and Jian Ren Gu

Abstract

Purpose: To explore the preventive and therapeutic antitumor effects of nicotine-treated immature dendritic cells (imDC).

Experimental Design: First, bone marrow–derived imDCs were stimulated with nicotine *in vitro*, and nicotinic acetylcholine receptor, costimulator molecules, chemokine receptor, and endocytosis ability of imDCs were detected by flow cytometry. Second, the DC-dependent antigen-specific T-cell proliferation, CTL priming, and interleukin-12 secretion were determined by flow cytometry, enzyme-linked immunospot assay, and ELISA, respectively. Finally, preventive and therapeutic antitumor effects of such imDCs were determined by i.p. transfer against tumor challenge or implantation in mice.

Results: Nicotine could up-regulate expression of nicotinic acetylcholine receptor, costimulatory molecules, such as CD80, CD86, and CD40, adhesion molecule CD11b, and chemokine receptor CCR7 and enhance endocytosis ability of imDCs. In addition, nicotine could promote imDC-dependent CTL priming and interleukin-12 secretion *in vitro*. Most importantly, systemic transfer of *ex vivo* nicotine-stimulated imDCs could reveal preventive and therapeutic effect on tumor development.

Conclusions: *Ex vivo* nicotine stimulation can significantly improve the efficacy of imDCs for adaptive therapy of cancer and nicotine-treated imDCs may be considered as a potential candidate for preventive and therapeutic tumor vaccination.

Numerous approaches for antigen-specific active immunotherapy have been developed relying on random encounter of the vaccine with host dendritic cells (DC; refs. 1, 2). A lack of encounter of the vaccine antigen with DCs might result in the absence of an immune response, or alternatively, an inappropriate encounter might lead to silencing of the immune response (3). Therefore, studies are urgently needed based on *ex vivo*–generated autologous DCs loaded with antigen under controlled conditions. Some reports have shown that DCs loaded with antigens could induce therapeutic and protective antitumor immunity in mice (3, 4). But, the efficacy of therapeutic vaccination has been questioned recently (5). In the last decade, several groups have documents that nicotine has positive effects in treatment of neurodegenerative diseases, ulcerative colitis and, Tourette syndrome (6–8). Although the expression of nicotinic acetylcholine receptor (nAChR) has been shown in many types of nonneuronal cells, such as DCs, epithelial cells, and endothelial cells (9), the effect of nicotine on immune cells is incompletely characterized. Some investigators showed that nicotine could promote immune cell activation (10–12), whereas others indicated that nicotine might have immunosuppressive effects on macrophages and DCs (13–15). In the present study, we first characterized that nicotine has stimulatory effects on immature DCs (imDC) and second showed that nicotine stimulation could enhance T-cell priming ability of imDCs. Finally and most importantly, we revealed the *in vivo* preventive and therapeutic effect on tumor development by systemic transfer of *ex vivo* nicotine-stimulated imDCs, indicating that nicotine-DCs may be considered as a potential candidate for preventive and therapeutic tumor vaccination.

Materials and Methods

Reagents. Nicotine and α-bungarotoxin were obtained from Sigma-Aldrich. Granulocyte macrophage colony-stimulating factor and interleukin (IL)-4 were from R&D. BD Trucount Tube and FITC-dextran were from BD Biosciences and Invitrogen, respectively. The H-2K^b CTL peptide of ovalbumin (SIINFEKL) was synthesized by Sangong. Fluorescence-conjugated antibodies were from eBioscience. Mouse IL-12 ELISA kit was obtained from Bender MedSystems, and IFN-γ enzyme-linked immunospot kit was from U-CyTech Biosciences.

Animals. Pathogen-free C57BL/6 mice (female, 6-8 weeks old) were bought from Shanghai Laboratory Animal Center of Chinese Academy of Sciences and kept at the Animal Center of Cancer Institute, Shanghai

Authors' Affiliation: The National Laboratory for Oncogenes and Related Genes, Cancer Institute of Shanghai Jiao Tong University, Shanghai, People's Republic of China

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Requests for reprints: Jian Ren Gu, National Laboratory for Oncogenes and Related Genes, Cancer Institute of Shanghai Jiao Tong University, Shanghai 200030, People's Republic of China. Phone: 86-21-64177401; Fax: 86-21-64177401; E-mail: nlorg@sh163.net.

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Bone marrow–derived murine DCs. Bone marrow–derived DCs were prepared as described previously (16). Briefly, bone marrow mononuclear cells were prepared from bone marrow suspensions by depletion of red cells and then were cultured at a density of 1×10^6 cells/mL in RPMI 1640 with 10 ng/mL granulocyte macrophage colony-stimulating factor and 1 ng/mL IL-4. Nonadherent cells were gently washed out on day 4 of culture; the remaining loosely adherent clusters were used as imDCs. Both imDCs and mature DCs (maDC; 1×10^6 cells) were first starved in RPMI 1640 + 0.5% FCS for 6 h and exposed to nicotine (10^{-7} mol/L) or α-bungarotoxin (10^{-5} mol/L)/nicotine (10^{-7} mol/L) for 12 h. After washes, the cells were used as nicotine-treated DCs. imDCs were cultured for further 4 days in the presence of 10 ng/mL lipopolysaccharide (LPS) and used as maDCs.

Flow cytometric measurement. Expression of cell surface molecules was determined by flow cytometry according to the methods described previously (16). Before staining with relevant antibodies, imDCs were incubated for 15 min at 4°C with antibody to CD16/CD32 at a concentration of 1 μg/1×10^6 cells for blockade of Fc receptors. Staining was done on ice for 30 min and then cells were washed with ice-cold PBS, containing 0.1% NaN_3 and 0.5% bovine serum albumin. To determine the effect of nicotine on imDC maturation, both LPS (10 ng/mL) and nicotine (10^{-7} mol/L) were used to treat imDCs. Flow cytometry was done with FACSCalibur and data were analyzed with CellQuest software.

Endocytosis assay. To assess DC endocytosis ability, FITC-dextran was used according to the method described previously (12). Briefly, nicotine-treated DCs were incubated with FITC-dextran at a final concentration of 1 mg/mL at 37°C for 30 min. After three washes, the fluorescence of FITC-dextran uptaked by DC was determined by flow cytometry.

Antigen-specific T-cell proliferation assay. Antigen-specific proliferation assay was done as described previously (12) and determined by BD Trucount system (16). Briefly, nicotine-treated DCs pulsed with ovalbumin or SIINFEKEL peptide at the final concentration of 1 or 2 μg/mL, respectively. Cells were washed and added to plates containing syngeneic T cell at a ratio of 1:10 in mixed lymphocyte reactions. Following 5 days of coculture, cell number was determined by BD Trucount system.

IL-12 ELISA assay. To detect IL-12 in the suspension of mixed lymphocyte reactions, ELISA was done according to the methods described previously (16).

IFN-γ enzyme-linked immunospot assay. IFN-γ enzyme-linked immunospot assay was done using published method (17). Briefly, plate was coated with anti-IFN-γ antibody and cells were transferred. Cells were stimulated with SIINFEKL peptide at a final concentration of 1 μg/mL and incubated at 37°C for 16 to 20 h. The substrate was added and spots were counted.

Preventive tumor challenge experiments. Preventive tumor challenge experiments were done as described previously (17). Briefly, mice were i.p. transferred with 5×10^5 imDCs, nicotine-treated imDCs pulsed with SIINFEKL peptide (2 μg/mL), or EL4 lysate (1×10^5 cells/mL). Two days later, the mice were challenged with 2×10^6 EG7 or EL4 tumor cells through s.c. injection in the neck scruff. Nine to 14 days after tumor challenge, mice were sacrificed and tumor weights were recorded.

Therapeutic implanted tumor experiments. Briefly, mice were first implanted with 2×10^6 EG7 or EL4 cells. Then, the mice were i.p. transferred with nicotine-treated imDCs pulsed with SIINFEKL peptide (2 μg/mL) or EL4 lysate (1×10^5 cells/mL). For EG7 system, the transfer of 5×10^5 imDCs was done 2 days after tumor implantation. For EL4 system, the transfer was done 3 and 5 days after tumor implantation with 1×10^6 and 5×10^5 imDCs, respectively. Normal C57BL/6 mice were implanted with EG7 or EL4 cells as control. Eleven to 14 days after tumor implantation, mice were sacrificed and tumor weights were recorded.

Statistical analysis. All data were expressed as mean and SE. Statistical significance was tested using the Student's *t* test. Statistical differences were considered to be significant if $P < 0.05$.

Results

α7 nAchR was expressed in bone marrow–derived DC and up-regulated by nicotine. α7 nAchR, one of the most abundant nicotinic receptors, was determined by flow cytometry to explore the role of nicotine and its receptors in imDCs and maDCs. The results showed that α7 nAchR was constitutively

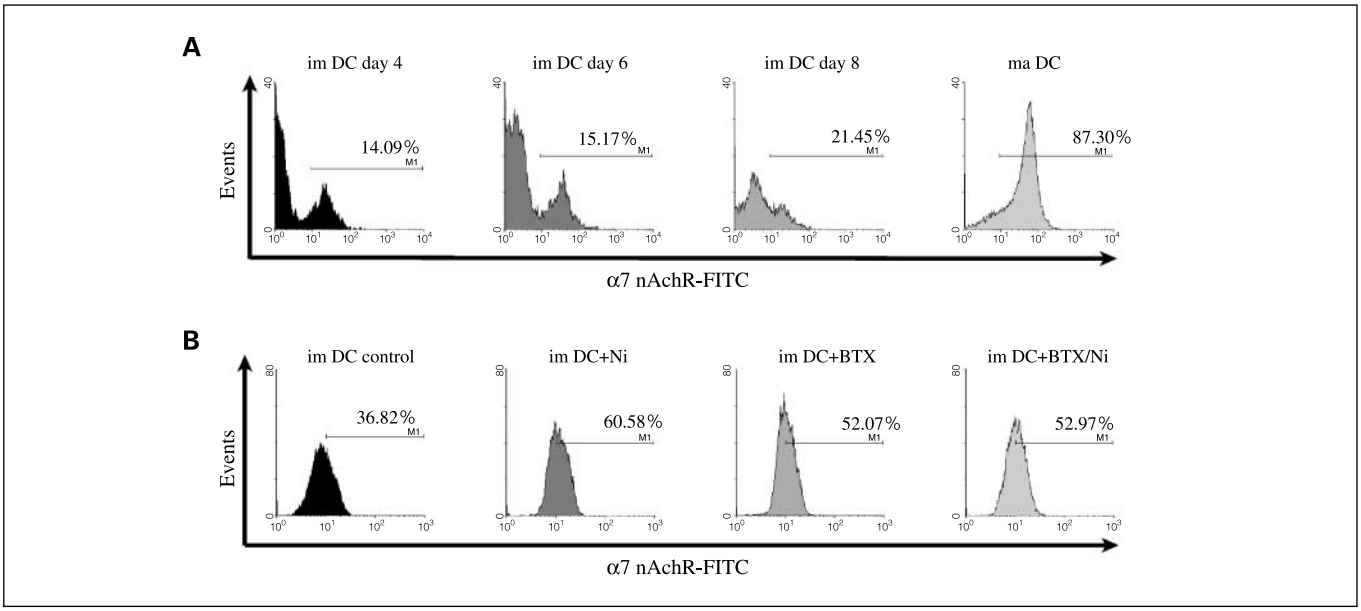

Fig. 1. α7 nAchR was expressed in DCs and up-regulated by nicotine. *A,* α7 nAchR was constitutively expressed in DCs. Granulocyte macrophage colony-stimulating factor and IL-4 induced imDCs, and LPS-matured DCs were stained with α-bungarotoxin-FITC and done with flow cytometry. *B,* α7 nAchR was up-regulated in imDCs under nicotine exposure in the presence of LPS. Representative flow cytometry analysis out of three. $P < 0.05$, imDC control versus imDC+Ni; $P < 0.05$, imDC+Ni versus imDC+BTX/Ni. BTX, α-bungarotoxin; Ni, nicotine.

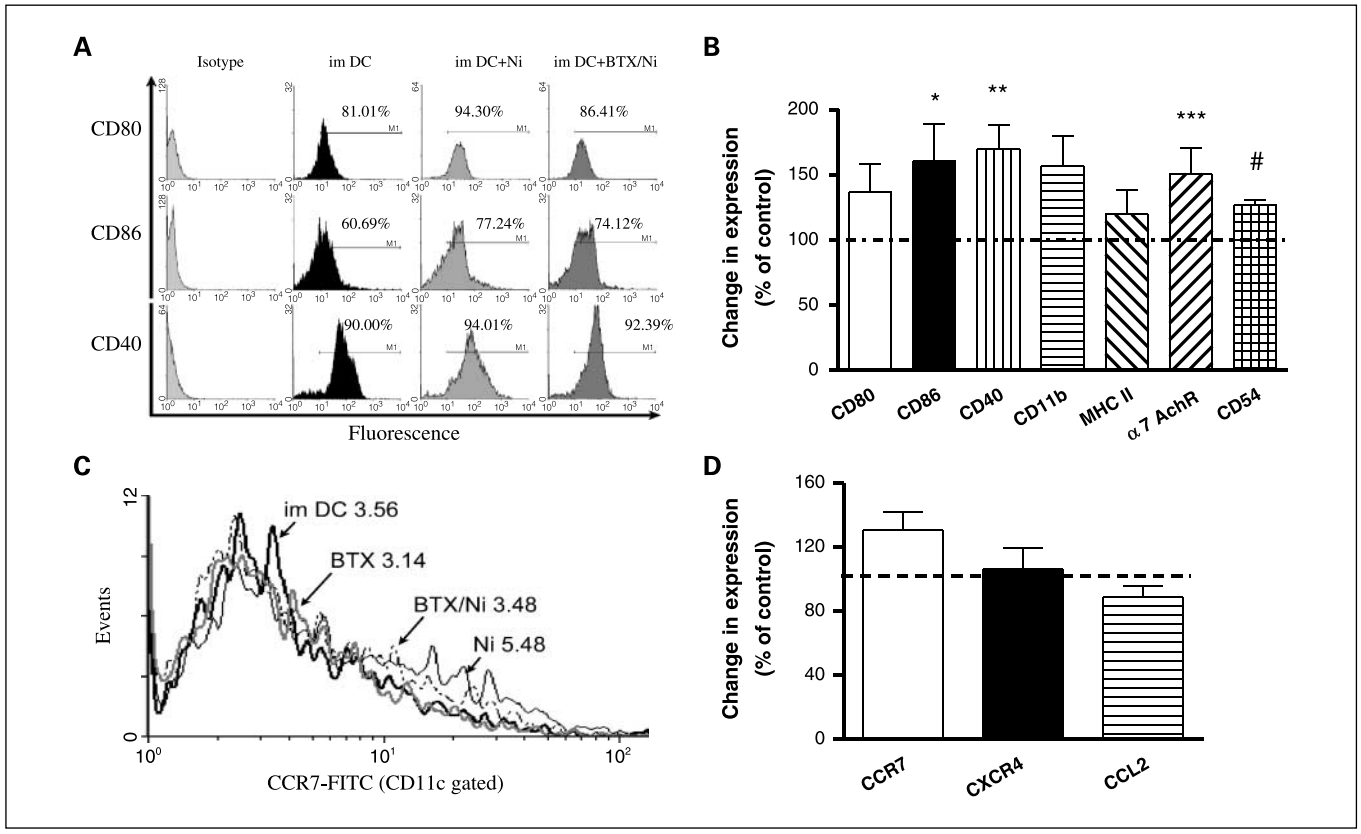

Fig. 2. Costimulator molecules and chemokine receptors in imDCs were up-regulated by nicotine. *A,* CD80, CD86, and CD40 in imDCs were up-regulated by nicotine treatment. *B,* histographic presentation of FACS on expression of surface molecules and nAchR. Columns, mean (n = 4); bars, SE. *, P = 0.0147; **, P = 0.0224; ***, P = 0.0328; #, P = 0.0078, nicotine versus control, paired t test. *C,* CCR7 expression in imDCs was increased under nicotine exposure. Numbers in histogram indicated geometric mean fluorescence of each imDC population. P = 0.0328, nicotine versus imDC. *D,* histographic presentation of chemokine receptors. Columns, mean (n = 3); bars, SE. P = 0.0321, CCR7 versus CCL2. Student's t test.

expressed in both imDCs and LPS-matured DCs, which was up-regulated along with DC maturation (*imDC* versus *maDC*; $P < 0.001$; Fig. 1A). To examine the effect of nicotine on DC maturation, both LPS and nicotine were used. The flow cytometry analysis showed that the α7 nAchR expression was increased in the course of imDC maturation induced by granulocyte macrophage colony-stimulating factor and IL-4 (Fig. 1A); Fig. 1B further showed the effect of nicotine on imDCs α7 nAchR under 12-h exposure of nicotine in the presence of LPS. The higher background of imDC control in Fig. 1B was attributed to the LPS stimulation. However, under LPS and nicotine costimulation, the α7 nAchR level was up-regulated to 60.58% versus 36.82% with LPS alone.

Nicotine induced up-regulation of surface molecules and chemokine receptors in imDCs. To understand the role of nicotine-treated imDCs in T-cell priming, surface molecule expression was determined by flow cytometry. The data showed that expression of costimulator molecules CD80, CD86, and CD40 was obviously up-regulated by nicotine treatment, which was partly abrogated by α-bungarotoxin (Fig. 2A). Contrary to MHC class II molecule, expression of CD11b and α7 nAChR was also up-regulated to ~1.4- and 2-fold, respectively (Fig. 2B). To further characterize nicotinic effect, chemokine receptors were determined by intracellular staining. Our results showed that the geometric mean fluorescence of CCR7 was up-regulated from 3.56 to 5.48 in contrast to the down-regulation of CCL2 (*Ni* versus *imDC*, P = 0.0328; Fig. 2C; *CCR7* versus *CCL2*, P = 0.0321; Fig. 2D). As CCR7 expression would be enhanced along with DC maturation, the effect on CCR7 implicated that nicotine could promote imDC maturation.

Nicotine augmented the ability of imDCs to stimulate T-cell proliferation. As DC endocytosis is a very important step for antigen presentation (4), we further examined the effect of nicotine on cell endocytosis. With nicotine treatment, the geometric mean fluorescence of imDCs and maDCs were increased from 251.78 to 308.41 and 71.43 to 85.49, respectively (*imDC/Ni* versus *maDC/Ni*; $P < 0.001$; Fig. 3A). Compared with maDCs, the nicotine-treated imDCs had more powerful endocytosis ability as shown in Fig. 3A.

To further determine its effect on T-cell priming, nicotine-treated imDCs and maDCs were analyzed with ovalbumin-specific proliferation assay. Compared with vehicle-treated cells, nicotine could significantly enhance the imDC-dependent T-cell priming to ~3-fold, but it has little effect on maDC (*imDC/OVA* versus *imDC/Ni/OVA*, $P < 0.001$; *imDC/Ni/OVA* versus *maDC/Ni/OVA*, P = 0.001; Fig. 3B). In addition, a remarkable increase of IL-12 secretion in nicotine-treated imDCs was observed (1,794-3,157 pg/mL), whereas much less effect was seen in maDCs after the same treatment (1,447-1,847 pg/mL; *imDC/OVA* versus *imDC/Ni/OVA*, P = 0.0003; Fig. 3C). These data indicated that only the imDCs but not the maDCs were sensitive to nicotine stimulation (*imDC/Ni/OVA* versus *maDC/Ni/OVA*; P = 0.0002; Fig. 3C).

***Nicotine increases CTL generation* in vitro.** Because nicotine stimulation facilitated T-cell priming, we further explored if such effect could generate effective CTL. We found that the nicotine-stimulated imDCs could substantially generate more effective CTL than untreated imDCs (*imDC/Ni/OVA* versus *imDC/OVA*; $P = 0.0071$; Fig. 4A). As ovalbumin contains Th and CTL epitope, SIINFEKL peptide representing the CTL epitope was used to exclude the potential Th epitope function. We found the similar effect of nicotine on DC-dependent CTL priming activities (*imDC/GF257* versus *imDC/Ni/GF257*, $P = 0.003$; *imDC/Ni/GF257* versus *imDC/BTX/Ni/GF257*, $P = 0.0412$; Fig. 4B).

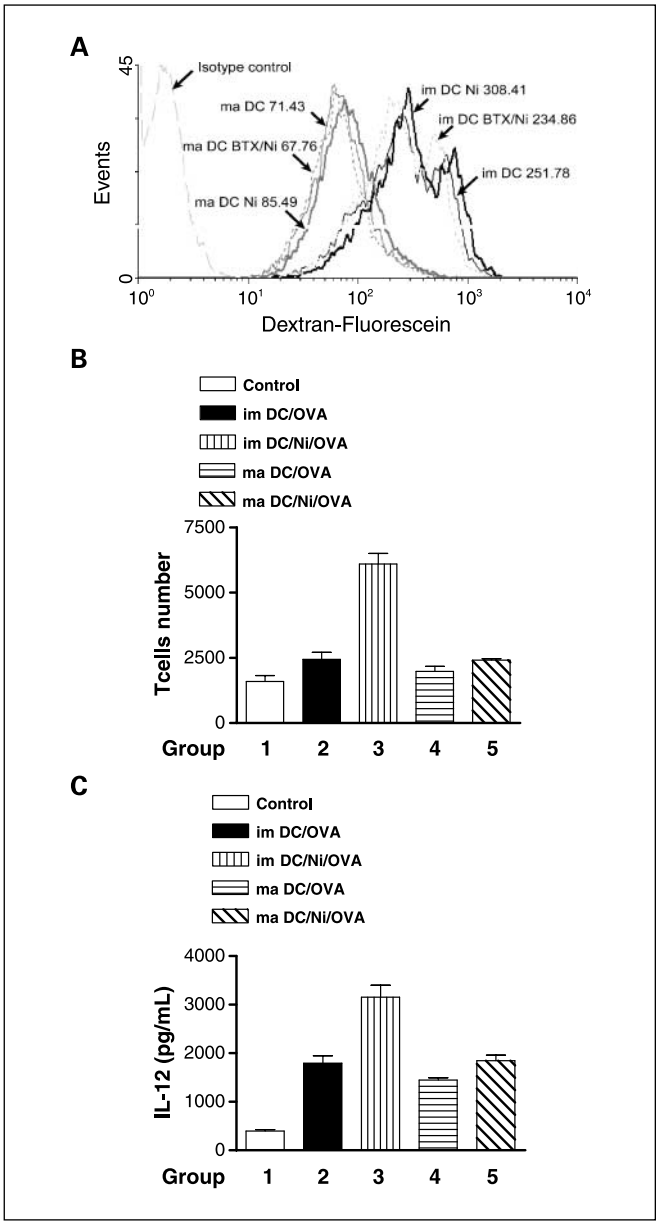

Fig. 3. Nicotine augmented the T-cell priming ability of imDCs. *A,* endocytosis abilities of nicotine-treated imDCs and LPS-matured DCs were determined by flow cytometry with dextran-FITC. Numbers in histogram indicated geometric mean fluorescence of each DC population. $P < 0.001$, imDC/Ni versus maDC/Ni. *B,* nicotine enhanced imDC-dependent T-cell priming. Nicotine-treated DCs pulsed with ovalbumin were used in mixed lymphocyte reactions, and T-cell proliferation was determined by flow cytometry. Columns, mean ($n = 3$); bars, SE. $P < 0.001$, imDC/OVA versus imDC/Ni/OVA; $P = 0.001$, imDC/Ni/OVA versus maDC/Ni/OVA. *C,* IL-12 secretion in mixed lymphocyte reactions was determined by ELISA. Columns, mean ($n = 3$); bars, SE. $P = 0.0003$, imDC/OVA versus imDC/Ni/OVA; $P = 0.0002$, imDC/Ni/OVA versus maDC/Ni/OVA. Student's t test. OVA, ovalbumin.

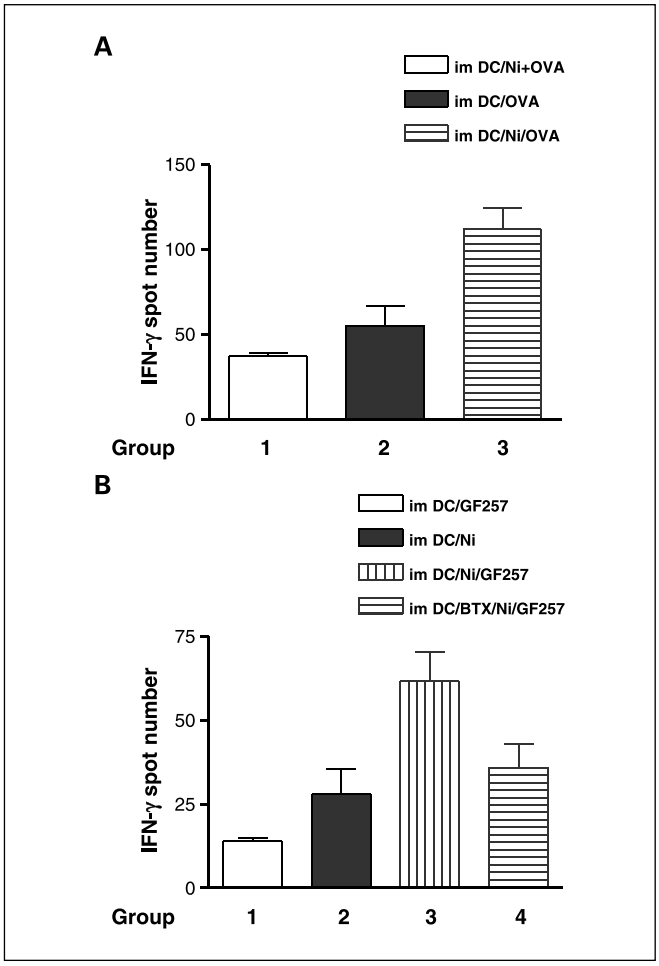

Fig. 4. Nicotine treatment facilitated imDC-dependent CTL priming *in vitro*. *A,* nicotine enhanced imDC-dependent ovalbumin-specific CTL priming. Nicotine-treated imDCs pulsed with ovalbumin or GF257 were used in mixed lymphocyte reactions. CTL priming was determined by enzyme-linked immunospot. $P = 0.0071$, imDC/Ni/OVA versus imDC/OVA. *B,* nicotine enhanced imDC-dependent GF257-specific CTL priming. Columns, mean ($n = 6$); bars, SE. $P = 0.003$, imDC/GF257 versus imDC/Ni/GF257; $P = 0.0412$, imDC/Ni/GF257 versus imDC/BTX/Ni/GF257. Student's t test. GF257, SIINFEKL peptide.

Transfer of nicotine-treated imDCs could achieve preventive effect on tumor challenge. We inoculated mice i.p. with nicotine-treated imDCs to explore if this transfer could achieve preventive effect on tumor challenge in both tumor-specific antigen (TSA) system and tumor-associated antigen (TAA) system. The DC transfer was done 2 days before tumor transplantation. The results showed that the transfer of nicotine-treated imDCs could obviously achieve tumor protection, which inhibits tumor growth at a rate of 96.1% in TSA system and 40.5% in TAA system (*imDC/Ni/GF257* versus *control*, $P = 0.0017$; Fig. 5A; *imDC/Ni/GF257* versus *imDC/GF257*, $P = 0.0352$; Fig. 5A; *imDC/Ni/EL4 lysate* versus *control*, $P = 0.0339$; Fig. 5C). In TSA system, as positive control, the transfer of CTL epitope-pulsed imDCs only exhibited 80.5% inhibitory rate (*imDC/GF257* versus *imDC*; $P = 0.0350$; Fig. 5A). Interestingly, although α-bungarotoxin could partially reverse nicotinic effect, the tumor protection can still be acquired by the transfer of nicotine-treated imDCs (*imDC/*

BTX/Ni/GF257 versus *control*; $P = 0.0046$; Fig. 5A). IFN-γ enzyme-linked immunospot assay excluded the possibility that the reduction of tumor protection was attributed to effective CTL sparsity (*imDC/GF257* versus *control*, $P = 0.0254$; *imDC/Ni/GF257* versus *control*, $P = 0.0334$; Fig. 5B).

***Transfer of nicotine-treated imDCs could achieve antitumor therapeutic effect* in vivo.** Based on the tumor-preventive effect of the nicotine-treated imDCs, we further explored whether the transfer of such imDCs could eradicate preexisting tumor. Our results showed that the transfer of either nicotine-treated or antigen-pulsed imDCs could partially inhibit the tumor growth at the rate of 53% and 72.7%, respectively, in TSA system (*imDC/Ni* versus *control*, $P = 0.0312$; *imDC/GF257* versus *control*, $P = 0.0023$; Fig. 6A). Most importantly, the i.p. transfer with imDCs after combined treatment of nicotine and antigen could exhibit better antitumor effects in mice than those transferred with imDCs with either nicotine or antigen treatment (*imDC/Ni/GF257* versus *imDC/GF257*, $P = 0.0187$; *imDC/Ni/GF257* versus *imDC/Ni*, $P = 0.0118$; Fig. 6A). As encouraged by therapeutic effect in TSA system, we further ploughed to explore if such imDCs could inhibit tumor growth in TAA system, which could be more feasible for clinical cancer immunotherapy. On contrary to nicotine-treated or TAA-pulsed imDCs, the transfer of imDCs after combined treatment of nicotine and TAA could inhibit the tumor growth at a rate of 62.7% (*imDC/Ni/EL4 lγ* versus *control*, $P = 0.0317$; *imDC/Ni/EL4 lγ* versus *imDC/EL4 lγ*, $P = 0.021$; Fig. 6B).

Discussion

In the recent years, we have studied the biological role of neurotransmitters and their receptors in different cells in malignant tissues. Unexpectedly, we found that nicotine could activate bone marrow–derived imDCs. Our further studies revealed that the nicotine-treated imDCs could have potential antitumor effects. First, it was evidenced by the up-regulation of nAChR, costimulatory molecules CD86, CD86, and CD40, and adhesion molecule CD11b in nicotine-treated imDCs. Second, we showed the up-regulation of endocytosis, IL-12 secretion, and CCR7 after nicotine treatment. Third, nicotine could enhance the imDC-dependent CTL priming in antigen-specific proliferation assay. Most notably, we have shown that the transfer of *ex vivo* nicotine-treated imDCs could effectively inhibit the tumor growth both preventively and therapeutically.

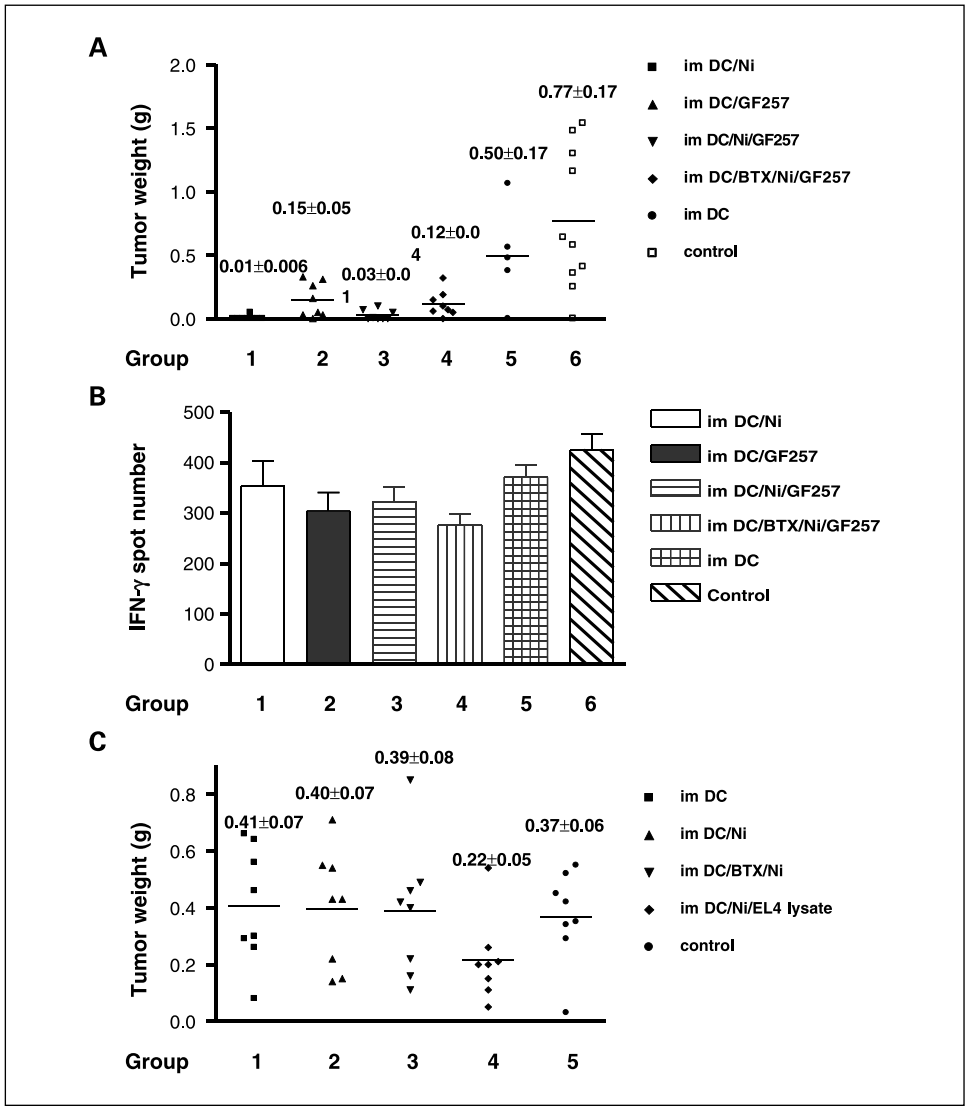

Fig. 5. Transfer of nicotine-treated imDCs could achieve preventive effect on tumor challenge. *A,* nicotine enhanced preventive DC efficacy in TSA system. All mice were challenged with EG7 cells and 2 d later were transferred with imDCs. imDCs were stimulated with nicotine(■), GF257(▲), Ni/GF257(▼), BTX/Ni/GF257(♦), or PBS(●). Control was transferred with PBS (□). Control (□), $n = 10$; imDC (●), $n = 5$; other groups, $n = 8$. $P = 0.0017$, ▼ versus □; $P = 0.0124$, ■ versus ▲; $P = 0.0352$, ▼ versus ▲; $P = 0.0350$, ▲ versus ●; $P = 0.0364$, ▼ versus ♦; $P = 0.0046$, ♦ versus □. *B,* reduction of tumor protection was not attributed to effective CTL scarcity. $P = 0.0254$, imDC/GF257 versus control; $P = 0.0334$, imDC/Ni/GF257 versus control. *C,* nicotine enhanced preventive DC efficacy in TAA system. Mice were challenged with EL4 cells and 2 d later were adoptive transferred with imDCs. imDCs were stimulated with nicotine(▲), BTX/Ni (▼), Ni/EL4 lysate(♦), or PBS(■). Control was transferred with PBS (●). Each group, $n = 8$. $P = 0.0317$, ♦ versus ▲; $P = 0.0339$, ♦ versus ●. Student's t test.

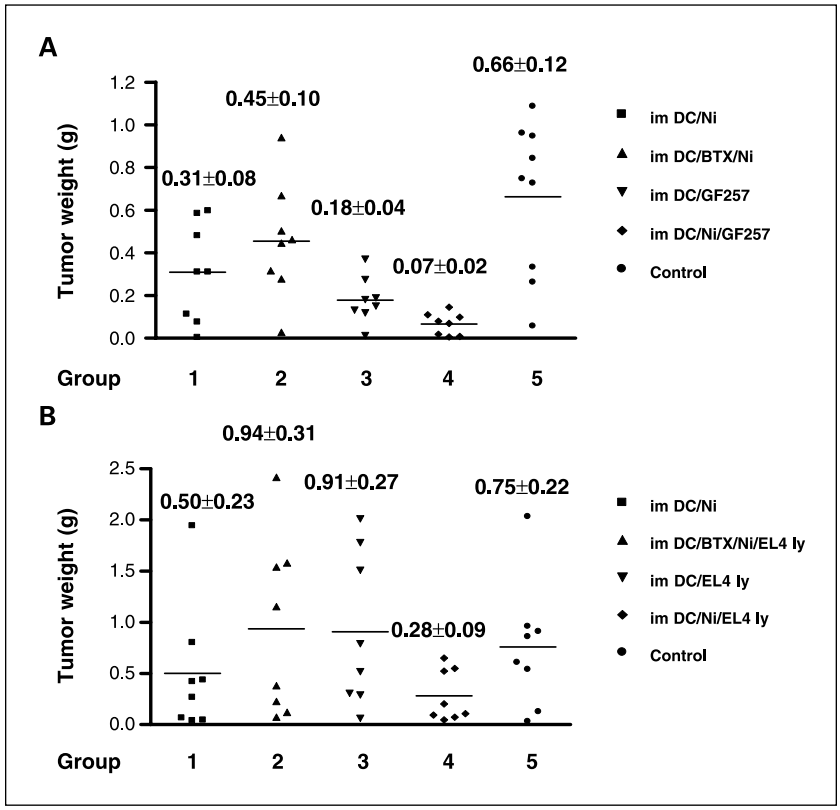

Fig. 6. Transfer of nicotine-treated imDCs could achieve antitumor therapeutic effect *in vivo*. *A,* nicotine enhanced therapeutic DC efficacy in TSA system. Mice were first implanted with EG7 cells and 2 d later were i.p. transferred with 5×10^5 imDCs. imDCs were treated with nicotine(■), BTX/Ni (▲), GF257(▼), and Ni/GF257(♦).Control was transferred with PBS (●). Control (●), $n = 9$; other groups, $n = 8$. $P = 0.0187$, ♦ versus ▼; $P = 0.0312$, ■ versus ●; $P = 0.0118$, ♦ versus ■; $P = 0.0181$,▼ versus ▲; $P = 0.0023$, ▼ versus ●; $P = 0.0014$, ♦ versus ▲; $P = 0.0003$, ♦ versus ●. *B,* nicotine enhanced therapeutic DC efficacy in TAA system. Mice were first implanted with EL4 cells and 3 and 5 d later were i.p. transferred with 1×10^6 and 5×10^5 imDCs, respectively. imDCs were treated with nicotine(■), BTX/Ni/EL4 lysate (▲), EL4 lysate (▼), and Ni/EL4 lysate (♦). Control was transferred with PBS (●). Each group, $n = 8$. $P = 0.0317$, ♦ versus ●; $P = 0.021$, ♦ versus ▼. Student's *t* test. ly, lysate.

In the past years, although several reports documented the biological effect of nicotine on DCs and macrophages (10–15), these articles have not mentioned the role of nicotine-treated imDCs on cancer growth. Furthermore, the previous reports showed that the effect of nicotine on DCs was quite controversial. Nicotine has been reported to decrease levels of proinflammatory cytokines and to reduce the ability of T-cell priming (12), although nicotine has also been described to enhance the costimulatory molecule expression in DCs and facilitate T-cell priming (13). Because the biological effect of nicotine on lymphocyte is dependent on dose and duration of exposure (18), the controversy may be attributed to the differences in experimental design, species, duration of exposure, and especially the nicotine concentration used in these experiments. Short-term exposure to nicotine could enhance lymphocytes c-fos gene expression, but long-term exposure would down-regulate nAchR mRNA expression (19). In addition, in a thymus organ culture model, it had been found that the low concentration of nicotine (10^{-18}-10^{-4} mol/L) increased the number of immature T cells, but the higher dose (>10^{-4} mol/L) could inhibit T-cell development (20). In our studies on stimulatory effect on imDCs and maDCs, we found that the imDCs showed much remarkable response to nicotine in expression of surface molecules, cytokine secretion, endocytic activity, and T-cell priming, which could be blocked by antagonist α-bungarotoxin and D-tubocurarine (Figs. 1-4; Supplementary Materials and Methods). Therefore, we focused our further studies on imDCs to test their potential antitumor activities after *ex vivo* nicotine treatment.

The *ex vivo*–generated, antigen-loaded DCs have been used as vaccine to improve immunity against cancer (3, 21, 22). However, new alternatives, such as IFN-γ DC or IL-15 DC, are much more efficient in inducing antigen-specific CTL priming *in vitro* (23–25). In our study, we show that nicotine can augment the capturing ability of imDC and facilitate effective CTL priming. Systematic transfer of the nicotine-treated imDCs could obviously enhance the efficacy for protection of tumor challenge and therapeutic effect on tumor growth, which was confirmed in both TSA and TAA systems. Although the *ex vivo* nicotine-treated imDCs showed significant antitumor effect, it did not implicate that nicotine itself has the similar biological effect on cancer growth. Because cancer tissues are composed of cancer cells, fibroblasts, vasculatures, and immune cells, the mechanism of nicotine-induced effects on these cells as well as the overall interaction among them are complex events, which remained to be clarified. In addition, to use nicotine as a chemical reagent for *ex vivo* treatment is a completely different event from smoking, which is definitely harmful. Moreover, the molecular pathways of imDCs after nicotine treatment, such as Src, as studied in other cells (26), need further extensive studies.

Taken together, we showed for the first time that nicotine-treated bone marrow–derived imDCs can both prevent tumor development and inhibit tumor growth in mice model, thereby providing new insight into the development of effective antitumor adaptive immunotherapy.

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