Lipopolysaccharide and dose of nicotine determine the effects of nicotine on murine bone marrow-derived dendritic cells

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Abstract. The reported effects of nicotine on dendritic cells (DCs) are controversial. To investigate the factors which determine the effects of nicotine on DCs, immature dendritic cells (imDCs) induced from murine bone marrow were treated with different doses of nicotine with or without lipopolysaccharides (LPS). The morphology and expression of the co-stimulatory molecules CD80, CD86, CD40 and CD54 were observed and determined by microscopy and flow cytometry, respectively. The results showed that, firstly, nicotine treatment promoted the development of DC precursors into imDCs with a semi-mature phenotype revealed by a higher expression of CD11c and more branched projections. Secondly, lower doses of nicotine (16.5 ng/ml), but not higher (200 µg/ml), up-regulated the expression of the co-stimulatory molecules CD80, CD40 and CD54 on imDCs. Co-administration of LPS and nicotine revealed differential effects of expression on imDCs. Thirdly and importantly, treatment with lower doses of nicotine (16.5 ng/ml) did not augment expression of the CD80, CD86, CD40 and CD54 molecules in mature DCs. Fourthly and interestingly, high doses of nicotine (more than 165 µg/ml) revealed pro-apoptotic activity but lower doses of nicotine (16.5-0.165 ng/ml) achieved an anti-apoptotic effect on imDCs. All data presented here indicate that the controversial effects of nicotine on DCs may be due to the LPS of the nicotinic environment and the dose of nicotine used.

Introduction
Nicotine, a major component of cigarette smoke, unequivocally, has positive effects on illnesses as diverse as neurodegenerative diseases, ulcerative colitis and Tourette syndrome (1-3). Although the expression of nicotinic acetylcholine receptor (nAChR) has been demonstrated in many types of non-neuronal cells such as dendritic cells (DCs), epithelial and endothelial cells (4), the effect of nicotine on immune cells is incompletely characterized. Aicher et al found that nicotine activates DCs and augments their capacity to stimulate T cell proliferation and cytokine secretion, which may contribute to the progression of atherosclerotic lesions (5). Our previous studies further demonstrated that nicotine has stimulatory effects on immature dendritic cells (imDCs), which reveal anti-tumor effects on lymphoma development (6), lung and liver cancer (7). Nouri-Shirazi et al reported that nicotine exerts immunosuppressive effects on immune surveillance through functional impairment of the DC system (8). In parallel with differential expression of costimulatory molecules CD80 and CD86 and lack of IL-12, nicotine-stimulated DCs displayed profoundly reduced Th1-promoting capacity (4), which recently confirmed that the presence of nicotine in the microenvironment promoted the development of mouse bone marrow-derived DC precursors into DCs with a semi-mature phenotype revealed by higher expression of costimulatory molecules CD80 and CD86 and MHC II (9). Investigators have shown that nicotine promotes immune cell activation (5-7), whereas others have suggested that nicotine may have immunosuppressive effects on DCs (4,8,9). Since the biological effect of nicotine on lymphocytes is dependent on dose and duration of exposure (10), the controversial effects of nicotine on DCs may be attributed to differences in experimental design, species, duration of exposure, particularly the nicotine concentration used in these experiments. Hence, further studies are needed to explore the factors which dictate the effects of nicotine on DCs.

In the present study, we first found that nicotine treatment up-regulated CD11c expression on imDCs in the absence of LPS, and secondly that lower and higher doses of nicotine used in previous reports up- or down-regulated the expression of co-stimulatory molecules on imDCs. Co-administration of LPS and nicotine revealed differential effects of expres-
sion of the co-stimulatory molecules on imDCs. Thirdly and importantly, lower doses of nicotine treatment did not augment expression of CD80, CD86, CD40 and CD54 molecules on mature DCs. Fourthly and interestingly, high doses of nicotine (more than 165 µg/ml) revealed pro-apoptotic activity and lower doses of nicotine (16.5-0.165 ng/ml) achieved an anti-apoptotic effect on imDCs. These data presented here indicate that the controversial effects of nicotine on DCs may be due to the nicotinic environment and the dose of nicotine used.

Materials and methods

Reagents. Nicotine and lipopolysaccharides (LPS) were obtained from Sigma-Aldrich (St. Louis, MI, USA). Mouse GM-CSF and IL-4 were obtained from R&D (Minneapolis, MN, USA). Fluorescent-conjugated antibodies were from eBioscience (San Diego, CA, USA). Annexin-V apoptosis detection kit was obtained from Promega (Madison, WI, USA). RPMI-1640 medium, Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum were purchased from Hyclone (Logan, UT, USA).

Animals. Pathogen-free C57BL/6 mice (female, 6-8 weeks old) were purchased from Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (China) and kept at the Animal Center of Xiamen University. All animal studies were approved by the Review Board of the Medical College of Xiamen University.

Bone marrow-derived murine DCs. Bone marrow-derived DCs were prepared as previously described (11). Briefly, bone marrow mononuclear cells were prepared from bone marrow suspensions by depletion of red cells, and then cultured at a density of 1x10^6 cells/ml in RPMI-1640 medium with 10 ng/ml of GM-CSF and 1 ng/ml of IL-4. Non-adherent cells were gently washed out on day 4 of culture; the remaining loosely adherent clusters were used as imDCs. Both imDCs and mature (ma)DCs (1x10^6 cells) were firstly starved in RPMI-1640 medium + 0.5% FCS for 6 h and exposed to nicotine (16.5 ng/ml) for 12 h. After washings, the cells were used as nicotine-treated DCs. imDCs were cultured for a further 4 days in the presence of 10 ng/ml LPS and used as maDCs.

Flow cytometric measurement. Expression of cell surface molecules was determined by flow cytometry according to the methods described previously (11). Before staining with relevant Abs, imDCs were incubated for 15 min at 4°C with an antibody to CD11c/CD32 at a concentration of 1 µg per 1x10^6 cells for blockade of Fc receptors. Staining was performed on ice for 30 min and then cells were washed with ice-cold PBS, containing 0.1% NaN3 and 0.5% BSA. Flow cytometry was carried out with FACSCalibur, and data were analyzed with CellQuest software.

Cell apoptosis assay. Cell apoptosis assay was determined by flow cytometry according to the method described previously (5). For detection of cell apoptosis, DCs were collected from PBS or nicotine-treated imDCs. Cell suspension was washed in PBS and resuspended in binding buffer containing Annexin V-FITC and propidium iodide (PI) for 20 min at room temperature. The samples were analyzed on FACSCalibur and data were analyzed with CellQuest software.

Statistical analysis. All data were expressed as the average of experimental data points, and standard error means were determined using the calculated standard deviation of a data set divided by the number of data points within the data set. Statistical significance was tested using the Student’s t-test and one-way ANOVA test by Prism software. Differences were considered to be statistically significant at p<0.05.

Results

Nicotine treatment promotes differentiation of DC precursors into DCs. When cultured in the presence of GM-CSF with IL-4, DC precursors in the bone marrow differentiated into imDCs, expressing CD11c (12). In order to explore the role of nicotine on DC differentiation, imDCs derived from murine bone marrow were stimulated with nicotine, and the morphology and expression of CD11c were observed by inverted microscopy and flow cytometry, respectively. The results showed that imDCs induced on day 6 grew more branched projections compared to those on day 4, and the expression of CD11c was also increased from 11.19 to 25.00% (Fig. 1A). When imDCs of day 4 were stimulated by nicotine (16.5 ng/ml), more branched projections on imDCs were observed, and the expression of CD11c was up-regulated from 11.19 to 25.68% (Fig. 1A). Compared to imDCs on day 6, imDCs on day 4 stimulated with nicotine had more CD11c molecular expression (Fig. 1B, p=0.0002, imDC day 4 vs. imDC day 4 + Ni; p=0.005, imDC day 4 vs. imDC day 6; p=0.0013, imDC day 4 + Ni vs. imDC day 4; Fig. 1C, p=0.0009, imDC day 4 vs. imDC day 4 + Ni; p<0.0001, imDC day 4 vs. imDC day 6; p=0.0154, imDC day 4 + Ni vs. imDC day 6). Since CD11c is a marker of DCs, the up-regulation of CD11c by nicotine indicated that nicotine enhanced DC differentiation from DC precursors.

Lower doses of nicotine up-regulate the expression of co-stimulatory molecules on imDCs. Several reports have described the controversial effects of nicotine on the expression of DC co-stimulatory molecules (4-9,13). To investigate the effects of nicotine on the expression of co-stimulatory molecules in DCs, imDCs on day 4 were treated with different doses of nicotine (16.5 ng/ml, 25 and 200 µg/ml), and the expression levels of CD80, CD86, CD40 and CD54 were determined by flow cytometry. The results showed that 16.5 ng/ml of nicotine stimulation obviously increased CD80, CD86 and CD54 molecular expression but decreased CD86 expression on imDCs (Fig. 2B, p=0.0256, imDC control vs. Ni 16.5 ng/ml; Fig. 2D, p=0.0098, imDC control vs. Ni 16.5 ng/ml; Fig. 2F, p=0.0240, imDC control vs. Ni 16.5 ng/ml; Fig. 2H, p=0.0013, imDC control vs. Ni 16.5 ng/ml); 25 µg/ml of nicotine treatment also up-regulated CD86 and CD40 molecular expression (Fig. 2D, p=0.0011, imDC control vs. Ni 25 µg/ml; Fig. 2F, p=0.0459, imDC control vs. Ni 25 µg/ml). When 200 µg/ml of nicotine was used to stimulate imDCs, down-regulation of both CD80 and CD54, as well as up-regulation of CD40 were observed (Fig. 2B, p=0.0004, Ni 16.5 ng/ml vs. Ni 200 µg/ml; Fig. 2D, p=0.0010, Ni 25 µg/ml vs. Ni 200 µg/ml; Fig. 2F,
Nicotine (16.5 ng/ml) and LPS co-administration obviously up-regulate co-stimulatory molecules on imDCs. LPS was found to promote DC maturation and to up-regulate co-stimulatory molecules (9). Burgdorf et al. reported that LPS activates the TLR4 pathway and increase DC cross-presentation (14). In order to explore the effects of nicotine and LPS on co-stimulatory molecule expression, imDCs on day 4 were stimulated with LPS and nicotine, and the expression of CD80, CD86, CD40 and CD54 was determined by flow cytometry. The results showed that, in the presence of LPS, 16.5 ng/ml nicotine obviously increased the expression of the co-stimulatory molecules CD80, CD86, CD40 and CD54 was determined by flow cytometry. The results showed that, in the presence of LPS, 16.5 ng/ml nicotine obviously increased the expression of the co-stimulatory molecules CD80, CD86, CD40 and CD54 on imDCs (Fig. 3A, p<0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, Ni 16.5 ng/ml vs. Ni 25 μg/ml; Fig. 3B, p=0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, Ni 16.5 ng/ml vs. Ni 25 μg/ml; Fig. 3C, p<0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, Ni 16.5 ng/ml vs. Ni 25 μg/ml; Fig. 3D, p<0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, Ni 16.5 ng/ml vs. Ni 25 μg/ml; Fig. 3E, p<0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, Ni 16.5 ng/ml vs. Ni 25 μg/ml; Fig. 3F, p<0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, Ni 16.5 ng/ml vs. Ni 25 μg/ml; Fig. 3G, p<0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, Ni 16.5 ng/ml vs. Ni 25 μg/ml; Fig. 3H, p<0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, Ni 16.5 ng/ml vs. Ni 25 μg/ml; Fig. 3I, p<0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, Ni 16.5 ng/ml vs. Ni 25 μg/ml; Fig. 3J, p<0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, Ni 16.5 ng/ml vs. Ni 25 μg/ml). In contrast to 16.5 ng/ml of nicotine stimulation, 200 μg/ml of nicotine obviously decreased CD80, CD86 and CD54 expression; however, increased CD40 expression on imDCs in the presence of LPS was noted (Fig. 3A, p<0.0001, imDC control vs. Ni 200 μg/ml; p<0.0001, Ni 25 μg/ml vs. Ni 200 μg/ml; Fig. 3B, p=0.0032, Ni 25 μg/ml vs. Ni 200 μg/ml; Fig. 3C, p=0.0004, imDC control vs. Ni 200 μg/ml; p=0.0040, Ni 16.5 ng/ml vs. Ni 200 μg/ml; p=0.0252, Ni 25 μg/ml vs. Ni 200 μg/ml; Fig. 3D, p<0.0001, imDC control vs. Ni 200 μg/ml).

Nicotine has little effect on co-stimulatory molecule expression in mature DCs. With DC maturation, the
co-stimulatory molecules were up-regulated accordingly. Mature immunogenic DCs were found to induce Th1 and Th2 cell differentiation, and/or CTL priming, depending on the nature of the maturation signal they received, as well as the constraints imposed by ontogeny and/or environment modifiers (10). Although nicotine increased co-stimulatory molecule expression on imDCs, its effects on co-stimulatory molecule expression of mature DCs is little known. To investigate the effects of nicotine on mature DC co-stimu-

Figure 3. Lower doses of nicotine up-regulate the expression of surface molecules on imDCs in the presence of LPS. imDCs were treated with 16.5 ng/ml, 25 and 200 µg/ml of nicotine in the presence of 100 ng/ml LPS for 12 h on day 4, and then the expression levels of CD80, CD86, CD40 and CD54 on DCs were determined by flow cytometry. (A) CD80 expression on imDCs with different doses of nicotine and LPS stimulation (p<0.0001, imDC control vs. Ni 16.5 ng/ml; p=0.0001, Ni 16.5 ng/ml vs. Ni 25 µg/ml; p<0.0001, Ni 25 µg/ml vs. Ni 200 µg/ml). (B) CD86 expression on imDCs with different doses of nicotine and LPS stimulation (p<0.0001, imDC control vs. Ni 16.5 ng/ml; p=0.0001, Ni 16.5 ng/ml vs. Ni 25 µg/ml; p=0.0001, Ni 25 µg/ml vs. Ni 200 µg/ml). (C) CD40 expression on imDCs with different doses of nicotine and LPS stimulation (p<0.0001, imDC control vs. Ni 16.5 ng/ml; p=0.0001, imDC control vs. Ni 25 µg/ml; p=0.0001, imDC control vs. Ni 200 µg/ml). (D) CD54 expression on imDCs with different doses of nicotine and LPS stimulation (P<0.0001, imDC control vs. Ni 16.5 ng/ml; p<0.0001, imDC control vs. Ni 25 µg/ml; p<0.0001, imDC control vs. Ni 200 µg/ml). A representative flow cytometric analysis out of 3 is shown; Student's t test. Ni, nicotine.

Figure 4. The doses of nicotine determine the expression of surface molecules on maDCs. At day 4, imDCs induced from murine bone marrow were treated with 10 ng/ml LPS for a further 4 days and were considered as mature DCs (maDCs). maDCs were stimulated with 16.5 ng/ml, 25 and 200 µg/ml of nicotine for 14 h, and the expression levels of CD80, CD86, CD40 and CD54 were determined by flow cytometry. (A) Histogramic presentation of CD80 expression on maDCs. (B) CD80 expression on maDCs (p=0.0256, imDC control vs. Ni 16.5 ng/ml; p=0.0004, Ni 16.5 ng/ml vs. Ni 200 µg/ml). (C) Histogramic presentation of CD86 expression on maDCs. (D) CD86 expression on maDCs (p<0.0001, imDC control vs. Ni 16.5 ng/ml; p=0.0011, imDC control vs. Ni 25 µg/ml; p=0.0437, imDC control vs. Ni 200 µg/ml; p=0.0010, Ni 25 µg/ml vs. Ni 200 µg/ml). (E) Histogramic presentation of CD40 expression on maDCs. (F) CD40 expression on maDCs (p<0.0240, imDC control vs. Ni 16.5 ng/ml; p=0.0459, imDC control vs. Ni 25 µg/ml; p<0.0001, imDC control vs. Ni 200 µg/ml; p<0.0010, Ni 25 µg/ml vs. Ni 200 µg/ml). (G) Histogramic presentation of CD54 expression on maDCs. (H) CD54 expression on maDCs (p=0.00013, imDC control vs. Ni 16.5 ng/ml; p=0.001, Ni 16.5 ng/ml vs. Ni 25 µg/ml; p<0.0001, imDC control vs. Ni 200 µg/ml). A representative flow cytometric analysis out of 3 is shown; Student's t test. Ni, nicotine.
Contrary to Nouri-Shirazi et al. (6,7) that nicotine-treated DCs exhibit anti-tumor effects, nicotine has stimulatory effects on imDCs, but also confirmed DCs. Our previous studies not only characterized that nicotine has stimulatory effects on DCs (4-9). Aicher et al (13) also acquired similar results of co-stimulatory molecule expression on DCs. Kawashima et al reported that short-term exposure to nicotine enhanced lymphocyte c-fos gene expression, but long-term exposure down-regulated nAchR mRNA expression (15). In a fetal thymus organ culture model, Middlebrook et al found that low levels of nicotine (10^-18-10^-14 M) increased the number of immature T cells, but a higher dose (>10^-4 M) inhibited T cell development (16). The controversy regarding the effects of nicotine on DCs may be attributed to the differences in experimental design, species, duration of exposure and particularly the nicotine concentration used in these experiments.

In the present study, we demonstrate that different doses of nicotine have obviously different effects in inducing DC apoptosis. High concentrations of nicotine (1.65 mg/ml and 165 µg/ml) were found to be toxic, leading to low cell viability (Fig. 5). When 1.65 mg/ml of nicotine was used to stimulate imDCs, nearly all cells were undergoing apoptosis. There was no surprise to find that 200 µg/ml of nicotine decreased the expression of CD80 and CD86 on imDCs (Fig. 2) and suppressed the proliferation of DC-mediated T cells (8). Aicher et al and our previous studies treated DCs with nicotine 16.5 ng/ml for 12 h, while Nouri-Shirazi et al stimulated DCs with nicotine at a final concentration of 200 µg/ml for 48 h, respectively, approximately 10,000-fold higher compared to the concentration of 16.5 ng/ml (5-8). With 10 µg/ml of nicotine treatment, Vassalo et al (13) also acquired similar results of co-stimulatory molecule expression to Aicher's data. Actually, Vassalo et al found that nicotine as opposed to CSE, failed to inhibit DC-induced T cell priming, to suppress the inflammatory up-regulation of co-stimulatory molecules and the expression of chemotactic cytokine receptor 7 (CCR7) on either imDCs or LPS-matured DCs (13). Since serum nicotine levels in smokers are usually within the range of 10-100 ng/ml, never exceeding...
the amount 100 µg/ml \textit{in vivo} (17), the physiological relevance that nicotine suppresses certain DC responses remains uncertain (5).

Immunity requires DC maturation induced by microbial endotoxins such as LPS, which increase the expression of costimulatory molecules on the DC surface (18,19). Our present study showed that lower doses of nicotine influenced DC maturation and differentiation as revealed by the up-regulation of costimulatory molecules CD80, CD40 and CD1c. In the presence of LPS, in contrast to the 200 µg/ml of nicotine, 16.5 ng/ml of nicotine stimulation obviously up-regulated the expression of molecules CD80, CD40, CD40L and CD54 (Fig. 3). Consistent with our results, Aicher et al reported that nicotine strongly activates DC-mediated adaptive immunity (5). They demonstrated that mouse bone marrow-derived competent DCs exposed to 16.5 ng/ml of nicotine alone express higher levels of MHCs and costimulatory molecules compared to the control DCs and have a greater capacity to stimulate ovalbumin (OVA)-specific \textbf{T} cell proliferation (5). Conversely, Nouri-Shirazi et al reported that nicotine-treated human DCs display an increased capacity for antigen uptake, fail to fully up-regulate MHCs, hardly express CCR7 and display profoundly reduced Th1 promoting capacity (8). But, interestingly, their further studies showed that while the presence of nicotine in the microenvironment has no direct effect on competent mouse bone marrow-derived DC function, it promotes the development of mouse bone marrow-derived DC precursors into DCs with a semi-mature phenotype revealed by higher expression of costimulatory molecules CD80, CD86, CD40 and MHC II molecules and CCR7, and supports the proliferation and differentiation of OVA-specific naïve T cells into effector memory cells (9). The differences in the DC preparations and treatments might account for the discrepancies observed between ours and their studies. It is worth mentioning that, when human monocyte-derived imDCs (day 6) and murine bone marrow-derived imDCs (day 4) were used by Nouri-Shirazi et al to study the effects of nicotine on DC-mediated T cell priming, the conclusions were obviously different. From our results, it appears that all of the factors of the nicotinic microenvironment, nicotine doses used in the experiment and DC maturation status, affect the functions of DCs.

The data presented in this report offer new information regarding the immunological alterations associated with nicotine, particularly at the level of mouse DC differentiation. This finding is important as it provides a rationale for further investigation of the mechanisms by which nicotine influences DCs \textit{in vivo} and consequently hosts immunity using animal models.

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References

1. Avila J and Diaz-Nido J: Tangling with hypothermia. Nat Med 10: 460-461, 2004.
2. Mandavilli A: Nicotine fix. Nat Med 10: 660-661, 2004.
3. Libert C: Inflammation – a nervous connection. Nature 421: 328-329, 2003.
4. Guinet EK, Yoshida M and Nouri-Shirazi: Nicotinic environment affects the differentiation and functional maturation of monocytes derived dendritic cells (DCs). Immunol Lett 95: 45-55, 2004.
5. Aicher AC, Heeschken M, Mohanpt JP, Cooke AM, Zeihe AM and Dimmelser S: Nicotine strongly activates dendritic cell-mediated adaptive immunity: potential role for progression of atherosclerotic lesions. Circulation 107: 604-611, 2003.
6. Gao FG, Wan DF and Gu JR: Ex vivo nicotine stimulation augments the efficacy of therapeutic bone marrow-derived dendritic cell vaccination. Clin Cancer Res 13: 3706-3712, 2007.
7. Gao FG, Li HT, Li ZJ and Gu JR: Nicotine stimulated dendritic cells could achieve anti-tumor effects in mouse lung and liver cancer. J Clin Immunol 31: 80-88, 2011.
8. Nouri-Shirazi M and Guinet E: Evidence for the immunosuppressive role of nicotine on human dendritic cell functions. Immunology 109: 365-373, 2003.
9. Nouri-Shirazi M, Tinajero R and Guinet E: Nicotine alters the biological activities of developing mouse bone marrow-derived dendritic cells (DCs). Immunol Lett 109: 155-164, 2007.
10. Hanna SF: Nicotine effect on cardiovascular system and ion channels. J Cardiovasc Pharmacol 47: 348-358, 2006.
11. Zhang MH, Tang H, Guo ZH, An HZ, Zhu XJ, Song WG, Guo J, Huang X, Chen TY, Wang JL and Cao XT: Splenic stroma drives mature dendritic cells to differentiate into regulatory dendritic cells. Nat Immunol 5: 1124-1133, 2004.
12. Shortman K and Liu YJ: Mouse and human dendritic cell subtypes. Nat Rev Immunol 2: 151-161, 2002.
13. Vassallo R, Tamada K, Lau JS, Kroening PR and Chen L: Cigarette smoke extract suppresses human dendritic cell function leading to preferential induction of Th-2 priming. J Immunol 175: 2684-2691, 2005.
14. Burgdorf S, Kautz A, Böhnert V, Knolle PA and kurts C: Distinct pathways of antigen uptake and intracellular routing in CD4 and CD8 T cell activation. Science 316: 612-616, 2007.
15. Kawashima and Fujii KT: The lymphocytic cholinergic system and its contribution to the regulation of immune activity. Life Sci 74 : 675-696, 2003.
16. Middlebrook AJ, Martina C, Chang Y, Lukas RJ and Deluca D: Effects of nicotine exposure on T cell development in fetal thymus organ culture: arrest of T cell maturation. J Immunol 169: 2915-2924, 2002.
17. Matsunaga K, Klein TW, Friedman H and Yamamoto Y: Involvement of nicotinic acetylcholine receptors in suppression of antimicrobial activity and cytokine responses of alveolar macrophages to \textit{Legionella pneumophila} infection by nicotine. J Immunol 167 : 6518-6524, 2001.
18. Abele R and Tamp R: The ABCs of immunology: structure and function of TAP, the transporter associated with antigen processing. Physiology (Bethesda) 19: 216-224, 2004.
19. Ackerman AL and Cresswell P: Cellular mechanisms governing cross-presentation of exogenous antigens. Nat Immunol 5: 678-684, 2004.