Nicotine Inhibition of Apoptosis in Murine Immune Cells[1]

AMAL HAKKI,* KEITH PENNYPACKER,† SHABNAM EIDIZADEH,† HERMAN FRIEDMAN,* AND SUSAN PROSS*,[2]

*Departments of *Medical Microbiology and Immunology and †Pharmacology and Therapeutics, University of South Florida College of Medicine, Tampa, Florida 33612-4742*

Nicotine, the addictive component of tobacco, is thought to be at least partially responsible for the deleterious effects of smoking such as heart disease and cancer. Evidence shows that nicotine is an immunomodulator and that one of its possible mechanisms is regulation of apoptosis, or programmed cell death, in immune cells. This study examined the effects and the mechanisms of action of nicotine on dexamethasone (DEX)-induced apoptosis in murine immune cells by examining the expression of levels of the 17-kDa active caspase-3, a marker of apoptosis. Thymocytes and splenocytes from adult BALB/c female mice were incubated with concentrations of nicotine correlating to those found in the blood and tissue of smokers (0.01 μg/ml [0.022 μM] and 1 μg/ml [2.2 μM]), concurrently with 100 nM DEX, to induce apoptosis. Cytosolic protein fractions were analyzed by Western blotting with polyclonal antibodies that recognize the active form of caspase-3. The data showed that nicotine significantly blocked the formation of the DEX-induced 17-kDa caspase-3 subunit expression. This downregulation ranged from 65% to 100% of the active caspase-3 expressed in cultures treated with DEX alone. Addition of d-tubocurarine chloride (dTC), a general nicotinic receptor antagonist, inhibited nicotine downregulation of the DEX-induced active caspase-3 expression, providing evidence that this action of nicotine was receptor-mediated. These data support that nicotine is an important immunomodulator at the level of immune cell apoptosis, a process thought to be a contributory mechanism of autoimmunity, cardiovascular disease, and carcinogenesis.

[Exp Biol Med Vol. 226(10):947–953, 2001]

Key words: apoptosis; nicotine; immune; caspase-3; dexamethasone

This research was supported in part by the American Heart Association (grant FL 0051206B).

[1] A.H. and K.P contributed equally to this manuscript.

[2] To whom requests for reprints should be addressed at Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, MDC-10, 12901 Bruce B. Downs Boulevard, Tampa, FL 33612. E-mail: spross@hsc.usf.edu

Received February 12, 2001.
Accepted July 19, 2001.

1535-3702/01/22610-0947$15.00

Although the greatest exposure to nicotine is from the use of tobacco products, the use of nicotine alone has been increasing as people have turned to nicotine replacement therapy such as gums, inhalers, or patches to reduce their tobacco consumption (1). In addition, nicotine and its related compounds have been used to supplement therapy for illnesses including Alzheimer's disease, ulcerative colitis, Parkinson's disease, and Tourette's syndrome (2). However, along with the therapeutic effects, it is reported that the use of nicotine may also have negative consequences on the cardiovascular, pulmonary, gastrointestinal, urogenital, pancreatic, and nervous systems (3–5). Nicotine appears to play a role in the modulation of apoptosis, a process of programmed cell death essential to normal development and cellular homeostasis (6, 7), but controversy exits in terms of how nicotine affects this process. Whereas some studies have demonstrated that nicotine induces apoptosis (8, 9), other studies have demonstrated that nicotine inhibits apoptosis, potentiating the survival of tumor cells (10).

Apoptosis is an active pathophysiological process leading to cell self-destruction by activating intrinsic suicide programs. Complex signaling pathways are sequentially turned on, resulting in the induction of several death genes and *de novo* protein synthesis (11). Multiple agents induce apoptotic-signaling pathways, including glucocorticoid hormones (12). Glucocorticoids affect a variety of tissues and body systems, and their role in the immune system is central for induction of cell cycle arrest and the programmed cell death of both immature thymocytes and peripheral T lymphocytes (13). Dexamethasone (DEX), a synthetic glucocorticoid hormone, is considered a model for induction of apoptosis (14, 15). It induces apoptosis through binding to the glucocorticoid receptors, which upon activation lead to the production of active caspases, the repression of genes necessary for cell proliferation, and the transcriptional upregulation of responsive lysis genes (16).

Apoptosis induced by glucocorticoids or other agents results in induction of a system of cysteine proteases called caspases (17). Activation of caspase-3 involves the proteolytic cleavage from its 32-kDa proenzyme form creating the

17-kDa subunit that is an important biomarker of apoptosis (18). It is this activated caspase-3 that acts early in the apoptotic signaling cascade, cleaving nuclear enzymes involved in the repair of DNA (19), facilitating the downregulation of Bcl-2 (20), and ultimately leading to cell death.

This study focuses on the effects of nicotine on DEX-treated murine thymocytes and splenocytes. Both cell types were exposed to physiological concentrations of nicotine in order to assess the effect of nicotine on DEX-induced apoptosis as measured by active caspase-3 expression (18, 21). Nicotine was found to significantly decrease the DEX-induced apoptosis of thymocytes and splenocytes. Addition of the nicotinic receptor antagonist d-tubocurarine chloride (dTC) blocked this effect. These data demonstrate that nicotine can modulate the immune system via apoptosis and that this effect is receptor-mediated.

Materials and Methods

Experimental Animals. BALB/c female mice (Jackson Laboratories, Bar Harbor, ME) 8 to 12 weeks old were employed in this study. They were housed at the University of South Florida IACUC-approved animal care facilities. Water and mouse chow were provided *ad libitum.* Mice were sacrificed by asphyxiation with CO_2 dry ice.

Cell Preparation. The procedure has been previously described (22). In brief, the spleen or thymus was surgically removed and placed in a sterile plastic bag containing 10 ml of Hanks' Balanced Salt Solution (HBSS, Sigma Chemical Co. St. Louis, MO). The bag was placed in a Stomacher 80 Lab Blender (Tekmar, Cincinnati, OH) for 10 sec to disrupt the tissue into a single cell suspension. Red blood cells were lysed in a solution containing 155 mM ammonium chloride, 10 mM potassium bicarbonate, and 100 μM EDTA (Sigma Chemical Co.). The remaining cells were washed and cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 units penicillin/ml, 100 μg streptomycin/ml, 2 mM glutamine, and 0.5 μM β-mercaptoethanol (Sigma Chemical Co.). Cell viability exceeded 95% by trypan blue exclusion.

Chemicals and Reagents. Nicotine hydrogen bitartrate (Sigma Chemical Co.) was stored in a dessicator jar at room temperature and was shielded from light. Nicotine was freshly prepared as a 10 mg/ml stock solution for each experiment. DEX (Sigma Chemical Co.) was prepared at a concentration of 100 nM. dTC (Sigma Chemical Co.), a nicotinic receptor antagonist, was prepared at a concentration of 100 μM.

Experimental Procedure. Cells, 20 × 10^6/ml thymocytes or splenocytes, were incubated for 3 hr at 37°C in a humid chamber under 5% CO_2 atmosphere with the cells in culture medium serving as the negative control, and with DEX serving as a positive control. In order to assess the impact of nicotine on DEX-induced apoptosis, cells were incubated with nicotine at either 0.01 or 1.0 μg/ml (0.022 or 2.2 μM), representing the concentrations of nicotine found in smokers' blood and tissue, respectively (23, 24). Groups included cells cultured with nicotine alone, as well as those concurrently exposed to DEX (100 nM) and nicotine. To determine if the mechanism of action of nicotine was receptor-mediated in these experiments, dTC (100 μM) was added concurrently to the cultures containing DEX and nicotine.

Protein Determination. In order to extract cytosolic protein, cell pellets collected by centrifugation were lysed in 150 to 200 μl of lysis buffer (25). Cells were then mixed gently and incubated on ice for 20 min.

Western Blot Analysis. The procedure for Western blotting is described (25). Briefly, 30 μg/μl of cytosolic protein extracts from cell lysates were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), electrophoretically transferred to a nitrocellulose membrane, and the resulting blot was blocked with 4% skim milk in cold phosphate-buffered saline (PBS) for 30 min. Immunoblots were probed with primary antibodies that recognize the proenzyme and activated forms of caspase-3 (polyclonal rabbit anti-caspase-3, PharMingen, San Diego, CA) at 1:10,000 dilution, and were incubated on a shaker overnight at 4°C. After washing with cold PBS, the immunoblots were probed with a horseradish peroxidase-labeled secondary antibody (goat anti-rabbit IgG H&L) at 1:20,000 dilution and were incubated on a shaker for 1 hr at room temperature. The enhanced chemiluminescence (ECL) was detected using ECL reagent (Amersham, Arlington Heights, IL).

Statistical Analysis. All groups analyzed represented at least four independent experiments. Data were analyzed by analysis of variance (ANOVA) and Dunnett's test, and a $P < 0.05$ was considered significant.

Results

Apoptosis in Thymocytes. Thymocytes represent a fairly pure population of T lymphocytes. As seen in Figure 1, thymocytes with no treatment (Control), as well as with treatment with nicotine (Nic) at either 0.01 or 1 μg/ml (0.022 or 2.2 μM) showed minimal levels of activated caspase-3 expression. Thymocytes exposed to 100 nM DEX induced approximately 4- to 5-fold the amounts of active caspase-3 relative to the control level. Addition of either 0.01 or 1 μg/ml of nicotine to the DEX-exposed cultures blocked the increased expression of active caspase-3 ($P < 0.05$). The resultant levels of active caspase-3 following the DEX and nicotine combined treatment were not significantly different from either those of the control cultures or cultures treated with nicotine alone, clearly demonstrating that nicotine inhibited the DEX-induced active caspase-3 expression in thymocytes.

Apoptosis in Splenocytes. This treatment regime was applied to cells from the spleen, a secondary lymphoid organ, which is a mixed population of T cells, B cells, and macrophages. The splenocytes were examined for the ef-

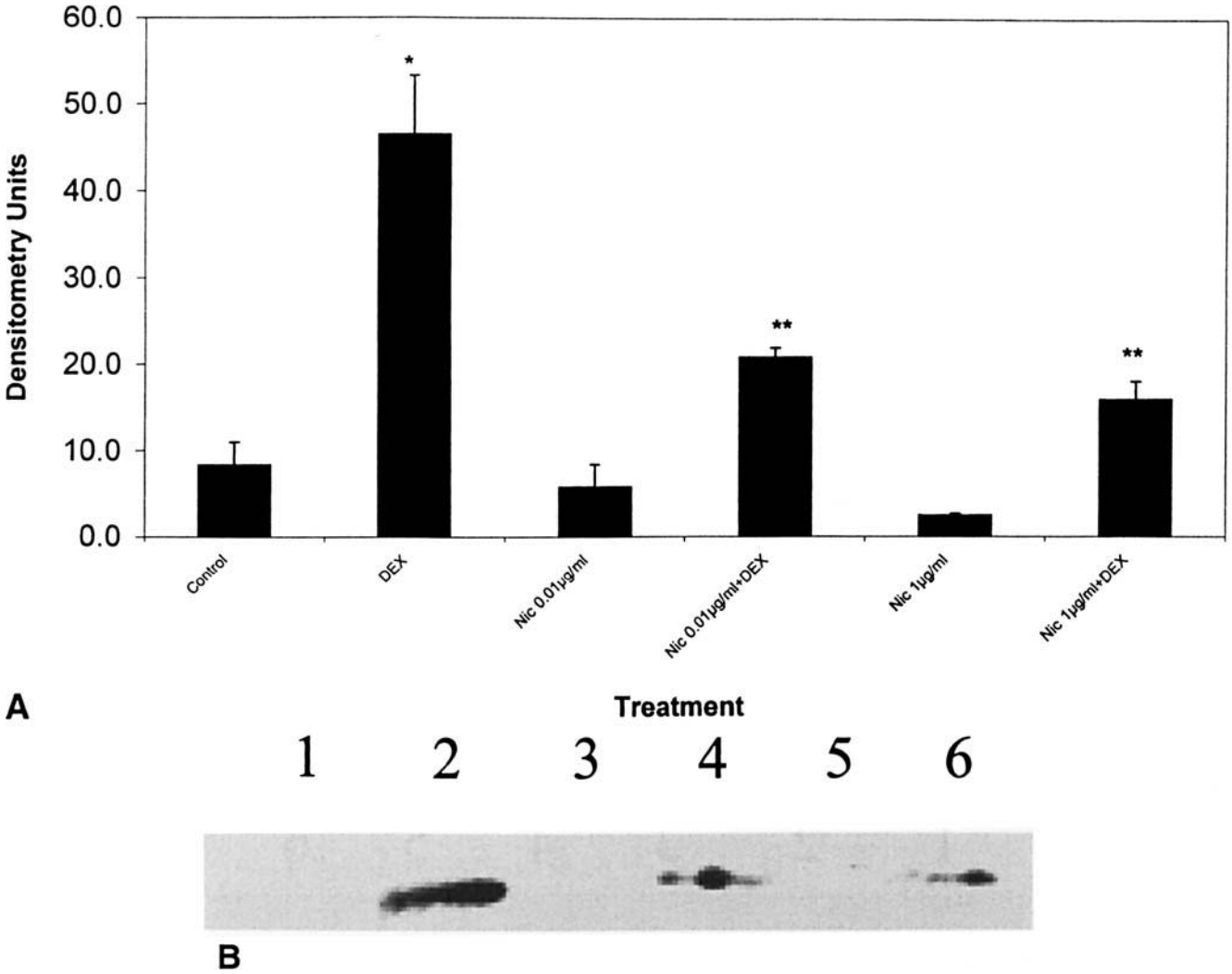

Figure 1. (A) Effect of nicotine on murine thymocytes. Murine thymocytes were incubated for 3 hr in either of six groups: untreated cells (Control), cells with 100 nM DEX, cells with nicotine alone (Nic 0.01 µg/ml or Nic 1 µg/ml [0.022 or 2.2 µM]), and cells with nicotine and DEX (0.01 µg/ml nicotine + DEX, or 1 µg/ml nicotine + DEX). *, Significantly different ($P < 0.05$) compared with the Control; **, significantly different ($P < 0.05$) compared with DEX. The DEX group showed a significantly enhanced expression of active caspase-3 compared with the Control and Nic 0.01 µg/ml and Nic 1 µg/ml groups ($P < 0.05$). The DEX-treated cells showed a significant decrease in expression of active caspase-3 when treated concurrently with nicotine. (B) Expression of the 17-kDa subunit of active caspase-3 detected by Western blot analysis. The bands are strongly evident in the cultures treated with DEX alone (lane 2), compared with the untreated (lane 1), and nicotine-treated cultures (Nic 0.01 µg/ml [lane 3] and Nic 1 µg/ml [lane 5]). Treatment of the DEX-exposed cultures with nicotine at 0.01 µg/ml (lane 4) and nicotine at 1 µg/ml (lane 6) decreased the intensity of this band.

fects of nicotine on DEX-induced active caspase-3 expression (Fig. 2, A and B). As with the thymocytes, spleen cells in medium alone (Control) expressed a low level of active caspase-3. Cultures treated with either 0.01 or 1 µg/ml (0.022 or 2.2 µM) of nicotine alone resulted in 42% and 31% of the control levels, respectively, whereas DEX upregulated expression of active caspase-3 more than 20-fold (Fig. 2, DEX). As with the thymocytes, nicotine treatment significantly blocked the DEX-induced expression of active caspase-3 (Fig. 2). The active caspase-3 expression following 0.01 µg/ml of nicotine and DEX was 5% that of the cultures treated with DEX alone, whereas that of the 1 µg/ml nicotine and DEX-treated cultures was 2% of those cultures treated with DEX alone (Fig. 2).

dTC Treatment. In order to determine if nicotine's action was receptor-mediated, dTC, an antagonist for nicotinic receptors, was added to cell cultures that were treated with DEX and nicotine. Blocking nicotinic receptors with dTC (Fig. 3, A and B) significantly reversed the inhibitory effect of nicotine on the DEX-induced caspase-3 activity ($P < 0.05$). The resultant levels of active caspase-3 were not significantly different from those treated with DEX alone.

Discussion

The use of nicotine continues despite reports correlating nicotine with alterations of the immune system (26–28), potential increases in cancer (7, 29), and contributions to cardiovascular disease (30, 31). Since dysregulation of ap-

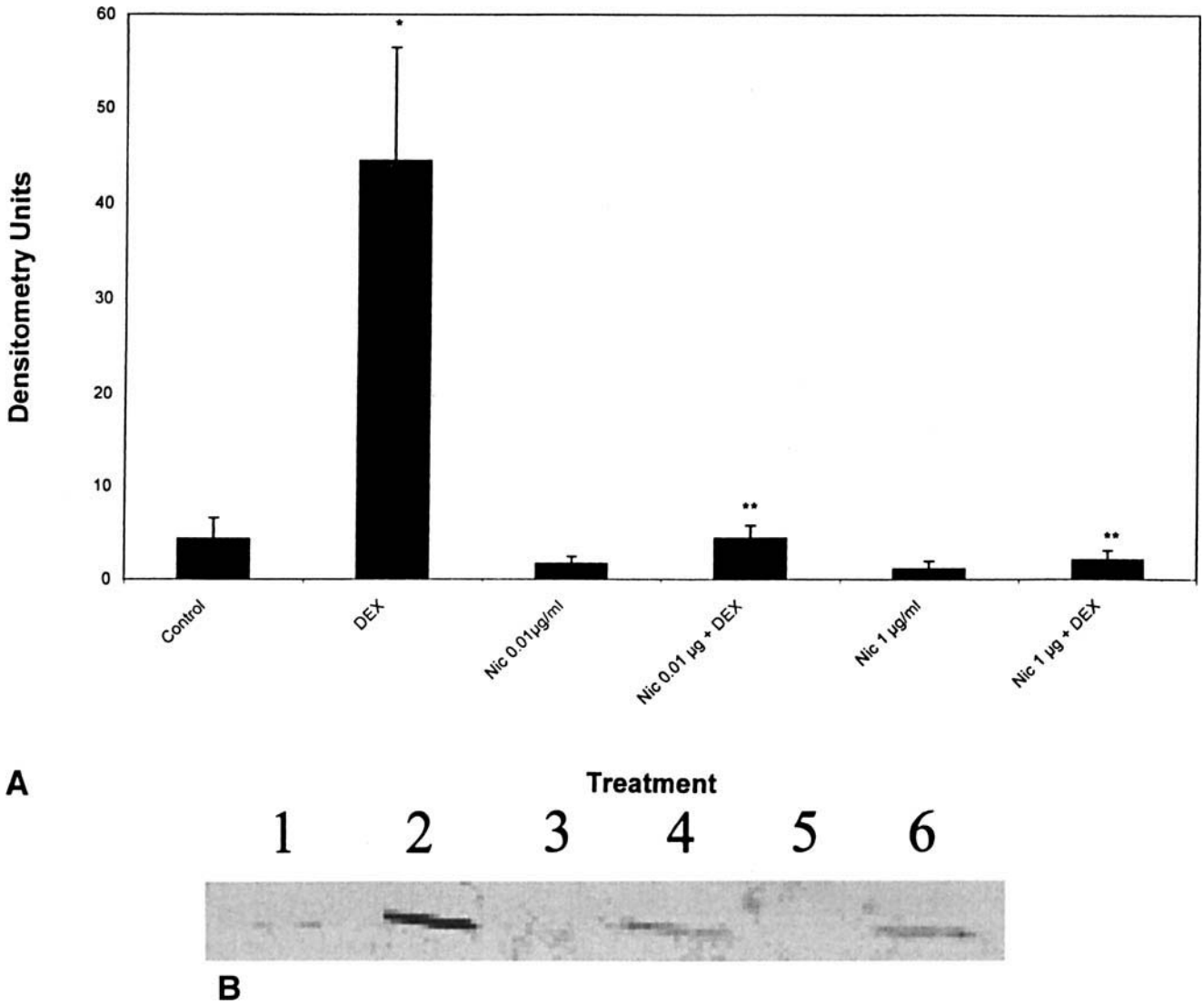

Figure 2. (A) Effect of nicotine on murine splenocytes. Murine splenocytes were incubated for 3 hr in either of six groups: untreated cells (Control), cells with 100 nM DEX, cells with nicotine alone (Nic 0.01 µg/ml or Nic 1 µg/ml [0.022 or 2.2 µM]), and cells with nicotine and DEX (0.01 µg/ml nicotine + DEX, or 1 µg/ml nicotine + DEX). *, Significantly different ($P < 0.05$) compared with the Control; **, significantly different ($P < 0.05$) compared with DEX. Note that either nicotine concentration significantly decreased the expression of active caspase-3 compared with the DEX group. The DEX group showed a significantly enhanced expression of active caspase-3 compared with the Control and Nic 0.01 µg/ml and Nic 1 µg/ml groups ($P < 0.05$). The DEX-treated cells showed a significant decrease in expression of active caspase-3 when treated concurrently with nicotine. (B) Expression of the 17-kDa subunit of active caspase-3 detected by Western blot analysis. The bands are strongly evident in the cultures treated with DEX alone (lane 2), compared with the untreated (lane 1), and nicotine-treated cultures (Nic 0.01 µg/ml [lane 3] and Nic 1 µg/ml [lane 5]). Treatment of the DEX-exposed cultures with nicotine at 0.01 µg/ml (lane 4) and nicotine at 1 µg/ml (lane 6) decreased the intensity of this band.

optosis may be a factor in the development of these pathological conditions (32), this paper investigated the effect of nicotine on DEX-induced expression of active caspase-3, a marker for apoptosis, in murine thymus and spleen cells (33). Nicotine was found to significantly decrease the DEX-induced active caspase-3 immunoactivity of both thymocytes and splenocytes. The concentrations of nicotine at 0.01 or 1 µg/ml are physiologically relevant to the levels found in the serum and tissues of heavy smokers (23, 24). The nicotinic receptor antagonist dTC reversed this action of nicotine, showing that nicotine's actions are receptor mediated.

The potential impact of nicotine on immunity comes from its role as a natural agonist of the nAChRs. Endogenous acetylcholine from the peripheral nervous system exerts its effect on the immune system directly and also acts indirectly through the hypothalamic pituitary adrenal (HPA) axis, which regulates the release of glucocorticoids (34, 35). High levels of glucocorticoids have been shown in many studies to correlate with an increased susceptibility to infections (36, 37), as well as to induce an increase in apoptosis (13). Our data show that nicotine inhibited the effect of DEX, raising the question of whether nicotine could decrease the apoptosis induced by glucocorticoids released

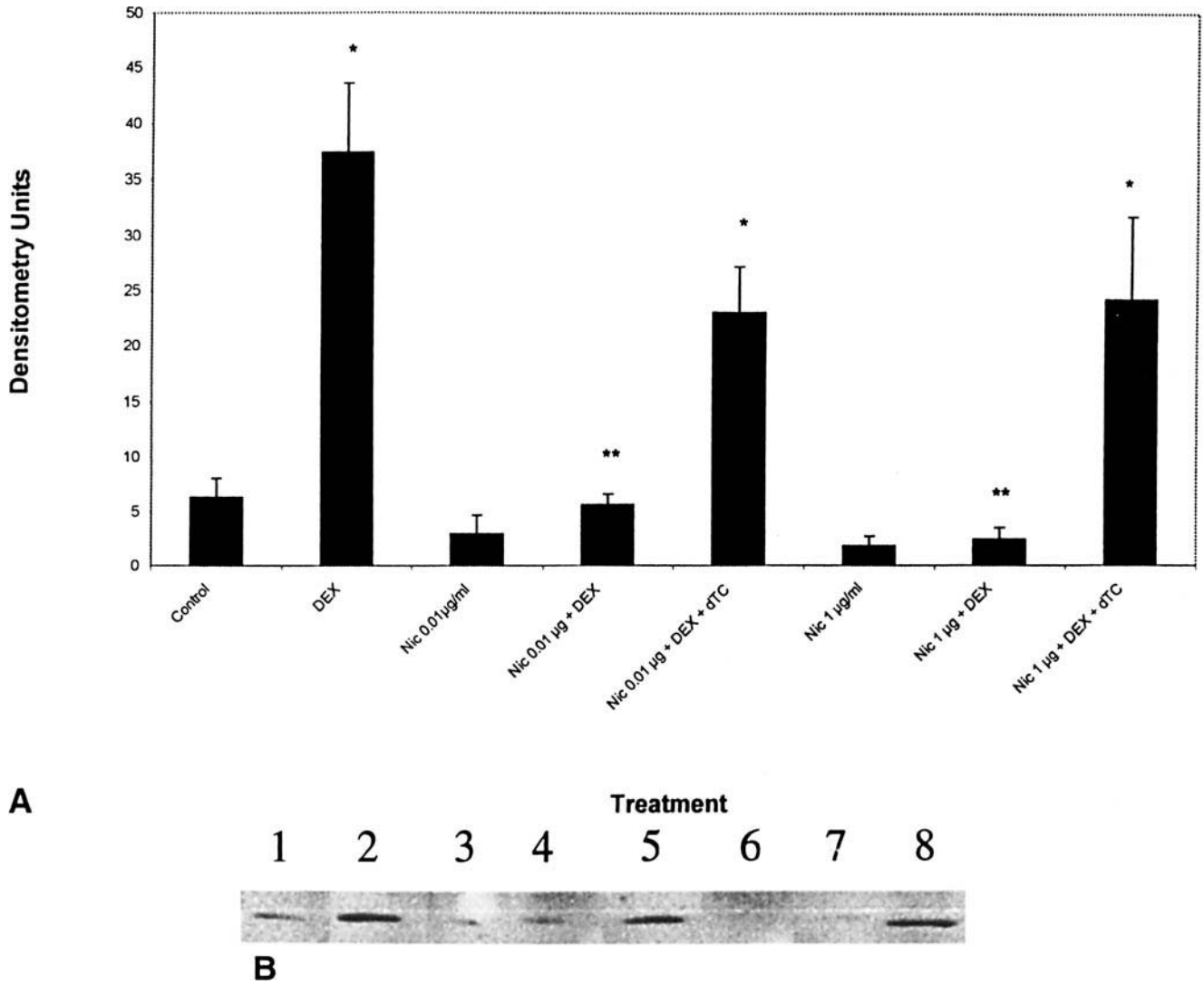

Figure 3. (A) Effect of nicotine on murine splenocytes: the role of receptors. Murine splenocytes were incubated for 3 hr in either of eight groups: untreated cells (Control), cells with 100 nM DEX, cells with nicotine alone (Nic 0.01 µg/ml or Nic 1 µg/ml [0.022 or 2.2 µM]), and cells with nicotine and DEX (0.01 µg/ml nicotine + DEX, or 1 µg/ml nicotine + DEX). *, Significantly different ($P < 0.05$) compared with the Control; **, significantly different ($P < 0.05$) compared with DEX. The DEX group showed a significantly enhanced expression of active caspase-3 compared with the Control and Nic 0.01 µg/ml and Nic 1 µg/ml groups ($P < 0.05$). The DEX-treated cells showed a significant decrease in expression of active caspase-3 when treated concurrently with nicotine. Groups exposed to nicotine, DEX, and the nicotinic receptor antagonist dTC (Nic 0.01 µg/ml + DEX + dTC, or Nic 1 µg/ml + DEX + dTC) had a significantly enhanced expression of active caspase-3 compared with the groups treated with nicotine combined with DEX alone. The expression of the active caspase-3 was not statistically different from the expression seen in cultures treated with DEX alone. (B) Expression of the 17-kDa subunit of active caspase-3 detected by Western blot analysis. The bands are strongly evident in the cultures treated with DEX alone (lane 2), compared with the untreated control (lane 1), and nicotine-treated cultures (Nic 0.01 µg/ml [lane 3] and Nic 1 µg/ml [lane 6]). Treatment of the DEX-exposed cultures with 0.01 µg/ml nicotine (lane 4) and 1 µg/ml nicotine (lane 7) decreased the intensity of this band. Note that the expression of the 17-kDa bands from cultures treated with nicotine, DEX, and dTC (Nic 0.01 µg/ml + DEX + dTC [lane 5] and Nic 1 µg/ml + DEX + TCR [lane 8]) was enhanced compared with the cells treated with nicotine combined with DEX alone.

under stress conditions. Furthermore, in the thymus, where immature T cells are programmed to be immunocompetent, there was a basal level of active caspase-3 activity that was diminished with nicotine alone (Fig. 1, A and B). Similarly, in the spleen, where the body reacts to antigenic challenge, there also was a basal level of active caspase-3 expression that was diminished with nicotine alone (Figs. 2, A and B and 3, A and B). This difference was shown to be statistically significant when the data from the two spleen groups were combined (Figs. 2 and 3). This suggests that while the body is naturally removing cells that are either not needed or are damaged, nicotine may inhibit this effect. Nicotine's inhibition of apoptosis, under basal conditions or under situations that stimulate glucocorticoid actions, conceivably may alter the immune response to infections and impact on the processes involved with vascular damage or cancer.

Apoptosis is a process of paramount importance in maintaining homeostasis, especially in systems where cells are continually being generated and destroyed, such as the immune system (38). Inhibition of apoptosis in antigen-

stimulated immune cells would be predicted to result in difficulties in mounting a full defense against viral or bacterial infection (6), possibly by decreasing the proportion of high-affinity antigen-specific immune cells. Although there are many pathways to induce apoptosis, this action by glucocorticoids is of primary importance in immune cells (13), accounting in part for the impact of glucocorticoids on the immune system (13). The data presented here show that nicotine decreases DEX-induced apoptosis and that this process was receptor-mediated. Therefore, the role of either the direct intake of nicotine or the alteration of natural acetylcholine should be considered in terms of immune function.

Increasing evidence supports the notion that nicotine itself may have some carcinogenic potential, acting either directly as a carcinogen or, more likely, indirectly as a promoter of cancer. Associations of nicotine and cancer have been described in terms of both lung and cervical tumor progression in humans (39). One of the ways such promotion could occur is by inhibiting the process of apoptosis (40, 41). Although the mechanism by which nicotine inhibited the apoptotic induction by DEX was not addressed in this study, an alteration by nicotine in terms of the DEX-induced production of caspases and transcription of relevant genes including *fas* and *bcl-2* should be considered.

This paper demonstrates that nicotine inhibits DEX-induced apoptosis in immune cells, as assessed by increased expression of active caspase-3. In order to gain a more detailed understanding of the biological impact of these findings, these investigations are being extended to include cardiovascular and tumor cell lines. The goal is that the understanding of the intricacies of nicotine action in these cell types will contribute to the development of relevant novel therapies.

1. Fant RV, Owen LL, Henningfield JE. Nicotine replacement therapy. Prim Care **26:**633–652, 1999.
2. Birtwistle J, Hall K. Does nicotine have beneficial effects in the treatment of certain diseases? Br J Nurs **5:**1195–1202, 1996.
3. Goldman J, Klinger M. Nicotine and cardiovascular complications of chronic hypertensive disease. Przegl Lek **55:**74–76, 1998.
4. Kavitharaj NK, Vijayammal PL. Nicotine administration induced changes in the gonadal functions in male rats. Pharmacology **58:**2–7, 1999.
5. Roy TS, Andrews JE, Seidler FJ, Slotkin TA. Nicotine evokes cell death in embryonic rat brain during neurulation. J Pharmacol Exp Ther **287:**1136–1144, 1998.
6. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science **267:**1456–1462, 1995.
7. Heusch WL, Maneckjee R. Signalling pathways involved in nicotine regulation of apoptosis of human lung cancer cells. Carcinogenesis **19:**551–556, 1998.
8. Berger MR, Zeller WJ. Interaction of nicotine with anticancer treatment. Klin Wochenschr **66:**127–133, 1988.
9. Yamamura M, Amano Y, Sakagami H, Yamanaka Y, Nishimoto Y, Yoshida H, Yamaguchi M, Ohata H, Momose K, Takeda M. Calcium mobilization during nicotine-induced cell death in human glioma and glioblastoma cell lines. Anticancer Res **18:**2499–2502, 1998.
10. Maneckjee R, Minna JD. Opioids induce while nicotine suppresses apoptosis in human lung cancer cells. Cell Growth Differ **5:**1033–1040, 1994.
11. Cross TG, Scheel-Toellner D, Henriquez NV, Deacon E, Salmon M, Lord JM. Serine/threonine protein kinases and apoptosis. Exp Cell Res **256:**34–41, 2000.
12. Biola A, Andreau K, David M, Sturm M, Haake M, Bertoglio J, Pallardy M. The glucocorticoid receptor and STAT6 physically and functionally interact in T-lymphocytes. FEBS Lett **487:**229–233, 2000.
13. Planey SL, Litwack G. Glucocorticoid-induced apoptosis in lymphocytes. Biochem Biophys Res Commun **279:**307–312, 2000.
14. Broome HE, Dargan CM, Krajewski S, Reed JC. Expression of Bcl-2, Bcl-x, and Bax after T cell activation and IL-2 withdrawal. J Immunol **155:**2311–2317, 1995.
15. Amos CL, Woetmann A, Nielsen M, Geisler C, Odum N, Brown BL, Dobson PR. The role of caspase 3 and BclxL in the action of interleukin 7 (IL-7): A survival factor in activated human T cells. Cytokine **10:**662–668, 1998.
16. Woronicz J, Calnan B, Winoto A. Death genes in T cells. Curr Top Microbiol Immunol **200:**137–146, 1995.
17. Velier JJ, Ellison JA, Kikly KK, Spera PA, Barone FC, Feuerstein GZ. Caspase-8 and caspase-3 are expressed by different populations of cortical neurons undergoing delayed cell death after focal stroke in the rat. J Neurosci **19:**5932–5941, 1999.
18. Hoshi T, Sasano H, Kato K, Yabuki N, Ohara S, Konno R, Asaki S, Toyota T, Tateno H, Nagura H. Immunohistochemistry of caspase-3/CPP32 in human stomach and its correlation with cell proliferation and apoptosis. Anticancer Res **18:**4347–4353, 1998.
19. O'Brien MA, Moravec RA, Riss TL. Poly (ADP-ribose) polymerase cleavage monitored *in situ* in apoptotic cells. Biotechniques **30:**886–891, 2001.
20. Fujita N, Tsuruo T. Involvement of Bcl-2 cleavage in the acceleration of VP-16-induced U937 cell apoptosis. Biochem Biophys Res Commun **246:**484–488, 1998.
21. Fujikawa K, Shiraki K, Sugimoto K, Ito T, Yamanaka T, Takase K, Nakano T. Reduced expression of ICE/caspase1 and CPP32/caspase-3 in human hepatocellular carcinoma. Anticancer Res **20:**1927–1932, 2000.
22. Hakki A, Hallquist N, Friedman H, Pross S. Differential impact of nicotine on cellular proliferation and cytokine production by LPS-stimulated murine splenocytes. Int J Immunopharmacol **22:**403–410, 2000.
23. Sasson IM, Haley NJ, Hoffmann D, Wynder EL, Hellberg D, Nilsson S. Cigarette smoking and neoplasia of the uterine cervix: smoke constituents in cervical mucus. N Engl J Med **312:**315–316, 1985.
24. Hellberg D, Nilsson S, Haley NJ, Hoffman D, Wynder E. Smoking and cervical intraepithelial neoplasia: Nicotine and cotinine in serum and cervical mucus in smokers and nonsmokers. Am J Obstet Gynecol **158:**910–913, 1988.
25. Pennypacker KR, Yang X, Gordon MN, Benkovic S, Miller D, O'Callaghan JP. Long-term induction of Fos-related antigen-2 after methamphetamine-, methylenedioxymethamphetamine-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine- and trimethyltin-induced brain injury. Neuroscience **101:**913–919, 2000.
26. McAllister-Sistilli CG, Caggiula AR, Knopf S, Rose CA, Miller AL, Donny EC. The effects of nicotine on the immune system. Psychoneuroendocrinology **23:**175–187, 1998.
27. Froen JF, Akre H, Stray-Pedersen B, Saugstad OD. Adverse effects of nicotine and interleukin-1β on autoresuscitation after apnea in piglets: Implications for sudden infant death syndrome. Pediatrics **105:**E52, 2000.
28. Singh SP, Kalra R, Puttfarcken P, Kozak A, Tesfaigzi J, Sopori ML. Acute and chronic nicotine exposures modulate the immune system through different pathways. Toxicol Appl Pharmacol **164:**65–72, 2000.
29. Wright SC, Zhong J, Zheng H, Larrick JW. Nicotine inhibition of

apoptosis suggests a role in tumor promotion. FASEB J **7**:1045–1051, 1993.
30. Benowitz NL. The role of nicotine in smoking-related cardiovascular disease. Prev Med **26**:412–417, 1997.
31. Feuerstein GZ. Apoptosis in cardiac diseases: New opportunities for novel therapeutics for heart diseases. Cardiovasc Drugs Ther **13**:289–294, 1999.
32. Volm M, Koomagi R. The implications of proliferation and apoptosis for lung cancer metastasis. Oncol Rep **6**:373–376, 1999.
33. Fukuzuka K, Rosenberg JJ, Gaines GC, Edwards CK III, Clare-Salzler M, MacKay SL, Moldawer LL, Copeland EM III, Mozingo DW. Caspase-3-dependent organ apoptosis early after burn injury. Ann Surg **229**:851-858; discussion 858–859, 1999.
34. Rosecrans JA, Karin LD. Effects of nicotine on the hypothalamic-pituitary-axis (HPA) and immune function: Introduction to the Sixth Nicotine Round Table Satellite, American Society of Addiction Medicine Nicotine Dependence Meeting, November 15, 1997. Psychoneuroendocrinology **23**:95–102, 1998.
35. Pickworth WB, Fant RV. Endocrine effects of nicotine administration, tobacco and other drug withdrawal in humans. Psychoneuroendocrinology **23**:131–141, 1998.
36. Norbiato G, Bevilacqua M, Vago T, Taddei A, Clerici. Glucocorticoids and the immune function in the human immunodeficiency virus infection: A study in hypercortisolemic and cortisol-resistant patients. J Clin Endocrinol Metab **82**:3260–3263, 1997.
37. Dhabhar FS, McEwen BS. Enhancing versus suppressive effects of stress hormones on skin immune function. Proc Natl Acad Sci U S A **96**:1059–1064, 1999.
38. Hawkins CJ, Vaux DL. The role of the Bcl-2 family of apoptosis regulatory proteins in the immune system. Semin Immunol **9**:25–33, 1997.
39. Waggoner SE, Wang X. Effect of nicotine on proliferation of normal, malignant, and human papillomavirus-transformed human cervical cells. Gynecol Oncol **55**:91–95, 1994.
40. Kawiak J, Hoser G, Skorski T. Apoptosis and some of its medical implications. Folia Histochem Cytobiol **36**:99–110, 1998.
41. Barr PJ, Tomei LD. Apoptosis and its role in human disease. Biotechnology **12**:487–493, 1994.