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Opposing Effects of Indomethacin and Nordihydroguaiaretic Acid on Macrophage Function and Tumor Growth

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The effects of indomethacin (INDO) and nordihydroguaiaretic acid (NDGA) were compared on macrophage function and tumor growth. Intraperitoneal injections (5 mg/kg/day, 6, 8 or 9 days) of INDO in C57BL/6 mice stimulated the cytotoxicity of peritoneal macrophages and inhibited growth of subcutaneous B16 melanoma, whereas NDGA injections suppressed macrophage cytotoxicity, increased macrophage prostaglandin-E_2 (PGE_2) release, and enhanced the tumor growth. Further, pretreatment of macrophages *in vitro* with the INDO group serum increased cytostasis against B16 tumor cells, while the use of NDGA group serum reduced it. Incubation of tumor-bearer's macrophages *in vitro* with INDO (10^{-6} *M* and 10^{-7} *M*) or 10^{-6} *M* NDGA for 4 h reduced PGE_2 release, but 10^{-7} *M* NDGA markedly enhanced the PGE_2 release. Our data indicate that INDO and NDGA are able to modulate the macrophage function directly, and produce opposing effects on macrophage function and tumor growth. Inhibitory actions of INDO and NDGA on the cyclooxygenase or lipoxygenase pathway of arachidonic acid metabolism appear to contribute to their effects.

Key words: Opposing effects — Indomethacin — Nordihydroguaiaretic acid — Tumor growth — Macrophage function

A number of investigators have reported an inhibitory effect of indomethacin (INDO), a non-steroidal anti-inflammatory drug (NSAID), on tumor growth in both animals[1–6] and humans.[7–9] The suppression of tumor cell proliferation or stimulation of an immune response, due to inhibition of prostaglandin synthesis in arachidonic acid (AA) metabolism, has been suggested.[6, 10, 11] Details of the mechanism are still under debate. Macrophages are known to be major prostaglandin(PG)-producing cells and also play a critical role in the regulation of immune responses by releasing monokines which affect lymphocyte function and natural killer cell activities.[12–14] We found earlier with C57BL/6 mice bearing subcutaneous (s.c.) tumors and lung metastases of B16 melanoma[15] that excision of the s.c. tumor strongly suppressed cytotoxicity and markedly enhanced PGE_2 release of peritoneal macrophages. Simultaneously, excision significantly accelerated metastatic growth. However, re-injection of tumor cell lysate into the excision wound stimulated the cytotoxicity and inhibited PGE_2 release of macrophages, preventing the acceleration of metastatic growth. These observations raised the possibility that modulation of macrophage function via AA metabolism would lead to changes in tumor growth. In the present study, we have examined the effect of INDO and nordihydroguaiaretic acid (NDGA) on macrophage function and tumor cell growth both *in vivo* and *in vitro*. We have chosen INDO as an inhibitor of the cyclooxygenase pathway and NDGA as an inhibitor of the lipoxygenase pathway, assuming that these inhibitors might produce opposing effects.

MATERIALS AND METHODS

Animals Inbred, virus-free, male C57BL/6 mice (6 to 8 weeks old), were purchased from Jackson Laboratories, Bar Harbor, ME. In conducting this research, the investigators adhered to National Research Council Guidelines for the care and use of laboratory animals.

Tumor cell cultures B16 melanoma F10 syngeneic to C57BL mice was obtained from The National Cancer Institute Tumor Bank. Tumor cells had been cryopreserved at −100°C using complete MEM (CMEM: Eagle's minimum essential medium supplemented with 10% fetal calf serum, penicillin 300 units/ml, streptomycin 300 mg/ml and sodium pyruvate 100 m*M*. Gibco, Grand Island, NY) containing 10% dimethyl sulfoxide. For transplantation to mice, tumor cells subcultured for 3 days in CMEM were harvested by short trypsinization (0.25% trypsin plus 0.02% EDTA). They were washed and suspended in Hanks' balanced salt solution (HBSS). Viability, assessed by trypan blue exclusion, was always greater than 95%.

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Intraperitoneal (i.p.) administration of INDO or NDGA Four experiments were performed. Viable B16 tumor cells (5×10^4) were implanted s.c. into the right gluteal area of the mice on Day 0. On Day 10 (Exp. 1), Day 12 (Exp. 3 and 4), or Day 14 (Exp. 2), the mice were divided into an INDO group, an NDGA group and an ethanol (EtOH) control group. Twenty mg of INDO or NDGA (Sigma Co., St. Louis, MO) was dissolved in 1 ml of EtOH and diluted $\times100$ with phosphate-buffered saline (PBS). EtOH was also diluted $\times100$ with PBS. INDO or NDGA (5 μg/g mouse average body weight) was given intraperitoneally. Control mice were given the diluted EtOH in a volume equal to the injection volume of INDO solution. The injection was given once a day on 2 consecutive days followed by one day of rest, a total of 6 times (Exp. 1 and 2) or 8 times (Exp. 4), or on 3 consecutive days followed by one day of rest, a total of 9 times (Exp. 3). All mice were killed on Day 25. Tumor size was measured in two dimensions at the maximum diameter with calipers and the average was taken as the tumor diameter.

Preparation of peritoneal macrophage suspension Peritoneal cells were obtained from experimental mice by lavage using sterile 0.9% NaCl solution containing 2 units/ml of sodium heparin. Cells pooled for each group were washed with HBSS and suspended in CMEM. The number of macrophages in the cell suspension was counted based on the adherence, morphology, and uptake of neutral red as described previously.[15] The number of macrophages in the suspension was adjusted to 1×10^6 cells/ml.

Macrophage cytotoxicity The cytotoxicity of the macrophages was assayed by the method of Martin *et al.*[16] with slight modifications. Briefly, a suspension of washed peritoneal cells in CMEM (1×10^6 macrophages/ml) was placed in the wells of a 96-well flat-bottomed culture plate (0.1 ml/well) and incubated for 45 min at 37°C. Non-adherent cells were removed by washing with HBSS. Then, B16 cells in CMEM were added on the macrophage monolayer at an effector:target (E:T) ratio of 20:1. After 72 h of incubation at 37°C, the wells were washed with HBSS, fixed with 5% formaldehyde, and stained with 1% methylene blue dissolved in 0.01 *M* borate buffer, pH 8.5. After washing of the wells the dye bound to the residual cells was eluted with 0.1 *N* hydrochloric acid and its absorbance was measured at 630 nm. Each sample was determined in 6 to 16 replicates as indicated in each section. Tumor cell growth was expressed in absorbance values.

PGE_2 released by macrophages Monolayers of macrophages were prepared in a 96-well plate (1×10^5 macrophages/well) as described in the previous section. Wells were washed and 0.2 ml/well of fresh CMEM was added to the macrophage monolayer. The culture was continued for 18 h. The culture supernatants were pooled for each group and their PGE_2 levels were determined in 4 replicates/sample by enzyme immunoassay (PGE_2-EIA kit, Advanced Magnetic Inc., Cambridge, MA). The sample values were corrected for the PGE_2 level of CMEM alone, which was extremely low.

Effects of serum obtained from INDO-, NDGA-, or EtOH-treated mice on normal macrophages Blood samples were obtained from mice of the INDO, NDGA and EtOH groups by cardiac puncture on Day 25. Sera were sterilized by passing them through a cellulose acetate membrane filter and stored at -100°C. Peritoneal cells were obtained from normal mice, and monolayers of macrophages (1×10^5 macrophages/well) were prepared in a 96-well plate as described above. The wells were washed with HBSS, and 0.2 ml of MEM containing 20% of serum sample was added to each of the wells. The culture was continued for 18 h, then the supernatant was removed and the wells were washed. B16 cells in CMEM were added to the monolayer of treated macrophages at an E:T ratio of 20:1. After a 72 h incubation, the growth of target cells was determined by measuring the absorbance as described in the previous section.

Direct effect of INDO, NDGA, or EtOH on the growth of B16 tumor cells INDO and NDGA were each dissolved in 1 ml of ethanol and diluted with CMEM. EtOH was diluted with CMEM in a manner identical to the preparation of the 10^{-6} *M* INDO solution. Aliquots of 0.1 ml of B16 tumor cells suspended in CMEM (5×10^4 cells/ml) were placed into the wells of 96-well plate, then 0.1 ml aliquots of varying concentrations (final 10^{-4} *M*, 10^{-5} *M*, 10^{-6} *M*, and 10^{-7} *M*) of INDO or NDGA or EtOH solutions were added to the wells. After a 72 h incubation, the growth of tumor cells was determined by measuring the absorbance of extracted dye as described in the section on macrophage cytotoxicity assay.

Effect of INDO, NDGA, or EtOH *in vitro* treatment on macrophage PGE_2 production Peritoneal cells were obtained from mice which had borne s.c. B16 melanoma for 14 days, when the tumors were about 10 mm in diameter. Macrophage monolayers (1×10^5 macrophages/well) were prepared in the wells of a 96-well plate as described above. The wells were washed with HBSS, then 0.1 ml of INDO, NDGA (10^{-6} *M* and 10^{-7} *M*), or EtOH solution was added to the macrophage monolayers. The wells were washed twice with HBSS after a 4 h incubation at 37°C, and 0.2 ml/well of fresh CMEM was added. The PGE_2 level of the culture supernatant was measured after an 18 h incubation at 37°C as described earlier.

Evaluation of data The data were statistically analyzed[17] by the one-way analysis of variance (ANOVA). Multiple comparison of data among the groups was performed by the protected least significant difference test (LSD), with $P<0.05$ as the criterion of significance. The difference

Table I. Average Tumor Size at the Start of Therapy

	No. of mice/group	No. of injections	Initial tumor diameter (M±SE mm)		
			EtOH group	INDO group	NDGA group
Exp. 1	6	6	palpable	palpable	palpable
Exp. 2	5	6	10.2±1.7	10.1±0.9	10.3±1.2
Exp. 3	8	9	5.6±0.9	5.6±0.8	5.7±0.9
Exp. 4	7	8	4.9±0.3	5.4±0.3	5.2±0.3

Tumor size was measured at 10 (Exp. 1), 14 (Exp. 2), and 12 days (Exp. 3 and 4) after tumor transplantation. EtOH: ethanol, INDO: indomethacin, NDGA: nordihydroguaiaretic acid.

between the data of two groups was evaluated by using Student's t test (two tailed). The average was expressed as the mean±standard error (M±SE).

RESULTS

Effect of INDO or NDGA administration on growth of s.c. tumor The number of mice, initial tumor size and number of i.p. injections for the four experiments are shown in Table I. As displayed in Fig. 1, growth of s.c. tumor was inhibited in the INDO group (Exp. 1 and 4, $P<0.05$), while the tumor growth of the NDGA group was enhanced when compared with that of the EtOH control group (Exp. 1 and 4, $P<0.05$) and the INDO group (Exp. 1, 2, 3 and 4, $P<0.05$). These changes began after 4 (Exp. 2) or 6 (Exp. 1, 3 and 4) i.p. injections.

Effect of INDO or NDGA administration on macrophage cytotoxicity against B16 tumor cells As shown in Fig. 2, peritoneal macrophages from the INDO group clearly suppressed the growth of tumor cells when compared with those of the control group (Exp. 1, 2, 3 and 4, $P<0.05$). In contrast, the cytostatic activity of the NDGA group macrophages was significantly less than that exhibited by the control group macrophages (Exp. 1, 2 and 4, $P<0.05$) or the INDO group macrophages (Exp. 1, 2, 3 and 4, $P<0.05$). These patterns of macrophage cytotoxicities are concordant with the trends found in the growth of s.c. tumor in each group (Fig. 1).

Effect of INDO or NDGA administration on macrophage PGE_2 production The levels of macrophage PGE_2 production determined from Exp. 3 and 4 are shown in Fig. 3. The amount of PGE_2 found from CMEM alone was extremely low (less than 0.01 ng/ml, n=6). The INDO group macrophages produced a significantly lower PGE_2 than the control group macrophages ($P<0.05$) in Exp. 4, but it was higher in Exp. 3, though the difference was not statistically significant. On the other hand, the NDGA group macrophages produced a significantly higher ($P<0.05$) level of PGE_2 than those of the control group in both experiments.

Effect of sera of INDO- or NDGA-treated mice on normal macrophages Fig. 4 shows the results obtained with the serum samples for Exp. 2 and Exp. 3. Macrophages pretreated with the INDO group serum inhibited tumor cell growth more effectively than those treated with the control group serum (Exp. 3, $P<0.05$). In contrast, macrophages pretreated with NDGA group serum had significantly decreased cytostatic activity compared to those treated with sera of the control group or INDO group (Exp. 2 and 3, $P<0.05$). Therefore, the serum of the INDO-treated mouse contains stimulatory factor(s), while the serum of the NDGA-treated mouse has factor(s) inhibitory to cytostatic activity on macrophages. These results are in good agreement with those observed in the growth of s.c. tumors as well as the cytotoxicity of macrophages obtained directly from the experimental animals described above.

Direct effects of INDO, NDGA, or EtOH *in vitro* treatment on growth of tumor cells As shown in Table II, INDO and NDGA did not inhibit tumor cell growth at low concentrations, but exhibited a significant ($P<0.05$) inhibitory effect at concentrations higher than 10^{-4} M (INDO) or 10^{-5} M (NDGA). The result indicates that the efficacy of NDGA is ten-fold higher than that of INDO in terms of effect on cell growth. Since neither INDO nor NDGA influenced the cell growth in the range of 10^{-6} M to 10^{-7} M, we chose this range of concentration for our *in vitro* studies.

Effect of INDO or NDGA *in vitro* treatment on macrophage PGE_2 production Tumor-bearer's macrophages treated *in vitro* with 10^{-6} M or 10^{-7} M INDO had reduced PGE_2 production compared to that of the control macrophages (Fig. 5). The reduction was significant ($P<0.05$) in macrophages treated with 10^{-6} M INDO. In contrast, macrophages treated with 10^{-7} M NDGA showed significantly increased ($P<0.05$) PGE_2 release compared with control macrophages. However, this was reversed when macrophages were treated with a higher concentration of NDGA (10^{-6} M), reducing PGE_2 release significantly ($P<0.05$). Thus, PGE_2 production of NDGA-treated macrophages showed completely opposite trends depending on NDGA concentration.

Time course of PGE_2 release by macrophages incubated with INDO or NDGA Fig. 6 shows the PGE_2 released

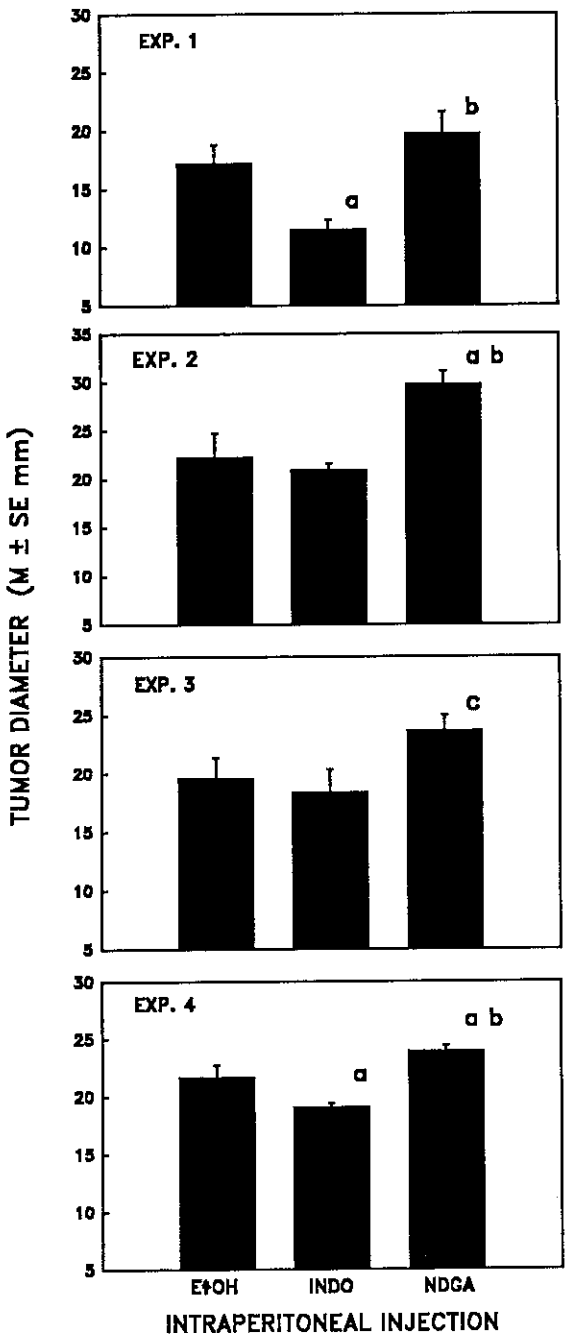

Fig. 1. Growth of subcutaneous tumor in INDO- or NDGA-injected mice. Maximum diameters of s.c. tumor were measured from the indomethacin group (INDO), nordihydroguaiaretic acid group (NDGA) and ethanol control group (EtOH) on Day 25. Tumor size is expressed as M±SE mm. a: different from EtOH group. b: different from INDO group. (both at $P<0.05$ by NOVA and LSD). c: different from INDO group at $P<0.05$ by t test.

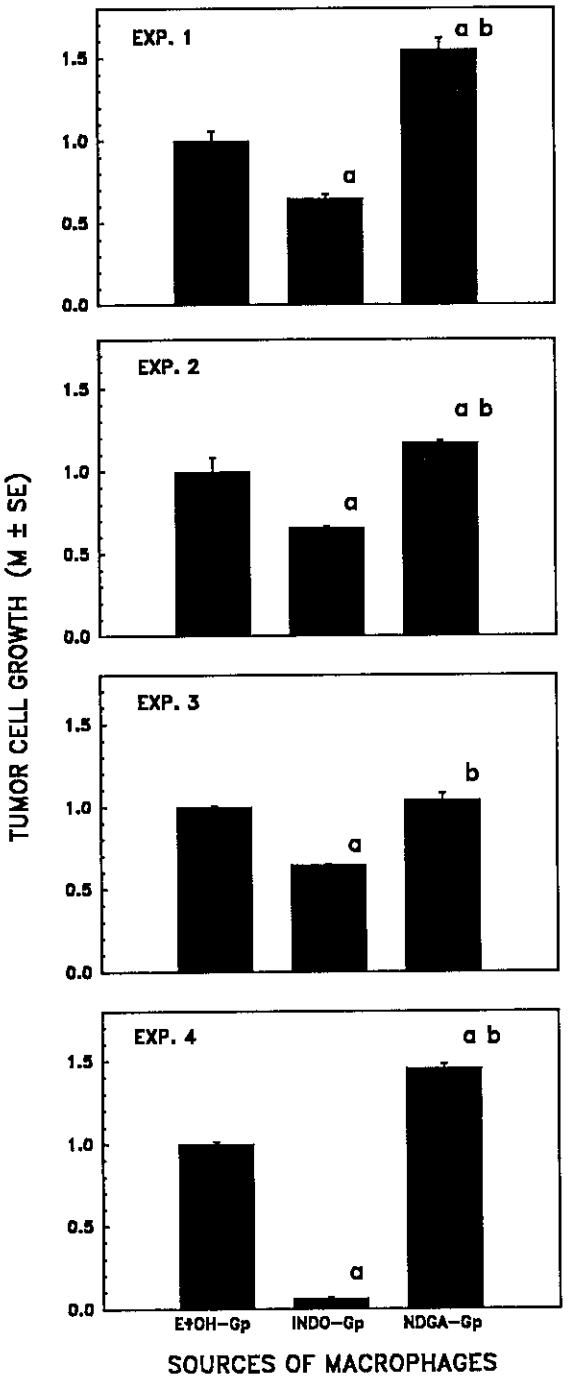

Fig. 2. Effect of INDO or NDGA injection on macrophage cytotoxicity against B16 melanoma cells. B16 melanoma cells were co-cultured with peritoneal macrophages obtained from the indomethacin group (INDO), nordihydroguaiaretic acid group (NDGA) and ethanol group (EtOH) at the E:T ratio of 20:1. Tumor cell growth is expressed as M±SE of absorbance at 630 nm measured from extracts of stained tumor cells. a: different from EtOH group. b: different from INDO group. (All at $P<0.05$ by ANOVA and LSD.)

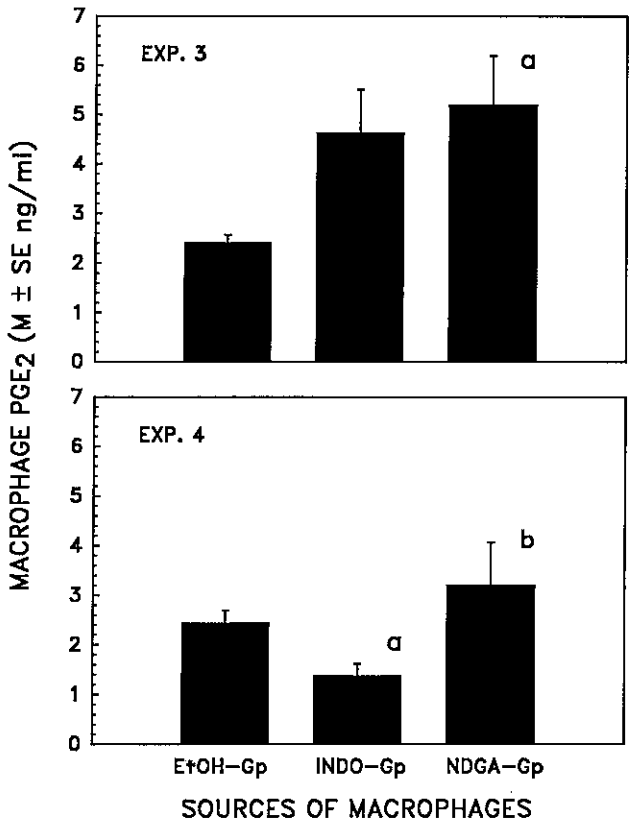

Fig. 3. Effect of INDO or NDGA injection on macrophage prostaglandin-E_2 (PGE_2) production. PGE_2 levels of culture supernatants of peritoneal macrophages obtained from the indomethacin group (INDO), nordihydroguaiaretic acid group (NDGA), and ethanol control group (EtOH) were determined in 4 (Exp. 3) or 6 (Exp. 4) replicates/sample. Data are shown as M±SE ng/ml. a: different from EtOH group. b: different from INDO group. (All at $P<0.05$ by ANOVA and LSD.)

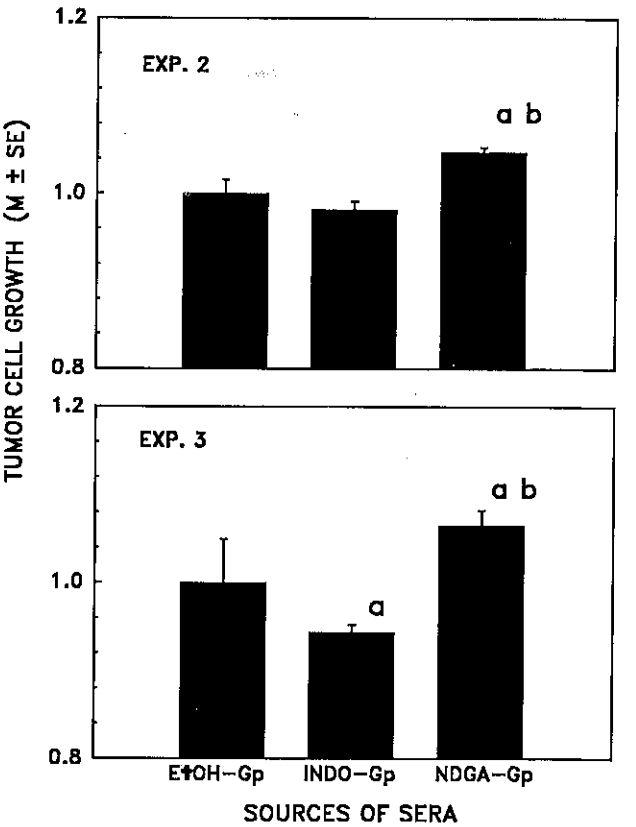

Fig. 4. Effect of sera of INDO- or NDGA-injected mice on normal macrophage function. Sera were obtained from the indomethacin group (INDO), nordihydroguaiaretic acid group (NDGA) and ethanol control group (EtOH) and diluted (20%) with medium. Peritoneal macrophages of normal mice were incubated with the diluted sera for 18 h. B16 melanoma cells were co-cultured with a washed macrophage monolayer (E:T=20:1) for 72 h. Tumor cell growth is expressed as M±SE of absorbance at 630 nm measured from extracts of stained tumor cells. a: different from EtOH group. b: different from INDO group. (All at $P<0.05$ by ANOVA and LSD.)

from tumor-bearer's macrophages after continuous incubation with 10^{-6} *M* INDO or 10^{-7} *M* NDGA for 2, 4, 8 and 24 h. Initially, PGE_2 release was higher in the presence of NDGA, and lower in the presence of INDO than that of the control. By 8 h, PGE_2 levels were increased in both the NDGA-treated and the INDO-treated macrophages, while maintaining their initial difference. On the other hand, PGE_2 release rapidly rose in the EtOH-treated macrophages during the 4 to 8 h period. By 24 h, all had lost their PGE_2-releasing ability.

DISCUSSION

We used diluted EtOH as a control in both *in vivo* and *in vitro* experiments, since this is an essential solvent for the preparation of INDO and NDGA solutions. As shown in "Results," INDO administration inhibited the growth of s.c. tumor, especially if treatment was initiated before measurable tumors developed, whereas NDGA injection invariably accelerated tumor growth. Also, INDO enhanced the cytotoxicity of peritoneal macrophages against B16 melanoma cells, but NDGA suppressed it. These results indicate that macrophage cytotoxicity, altered by INDO or NDGA administration, has a negative association with tumor growth. It is well known that INDO is inhibitory to PGE_2 production by macrophages.[18,19] This inhibitory effect was also demonstrated in our *in vitro* studies and one of the animal

Table II. Effect of EtOH, INDO and NDGA on the Growth of B16 Melanoma Cells

Concentration (M)	n	Medium alone (M±SE)	EtOH[a] (M±SE)	INDO (M±SE)	NDGA (M±SE)
0	12	0.95±0.01	—	—	—
10^{-7}	6	—	0.97±0.02	0.95±0.02	0.91±0.02
10^{-6}	6	—	0.94±0.02	0.91±0.02	0.92±0.01
10^{-5}	6	—	0.96±0.01	0.91±0.01	0.72±0.01[b]
10^{-4}	6	—	0.94±0.02	0.69±0.01[b]	0.11±0.01[b]

Data are expressed as mean±one standard error (M±SE) of absorbance at 630 nm measured from extracts of stained tumor cells.
a) Concentration of ethanol vehicle (EtOH) is identical to that included in the indomethacin (INDO) or nordihydroguaiaretic acid (NDGA) solution.
b) Different from medium alone group at $P<0.05$ by ANOVA and LSD.

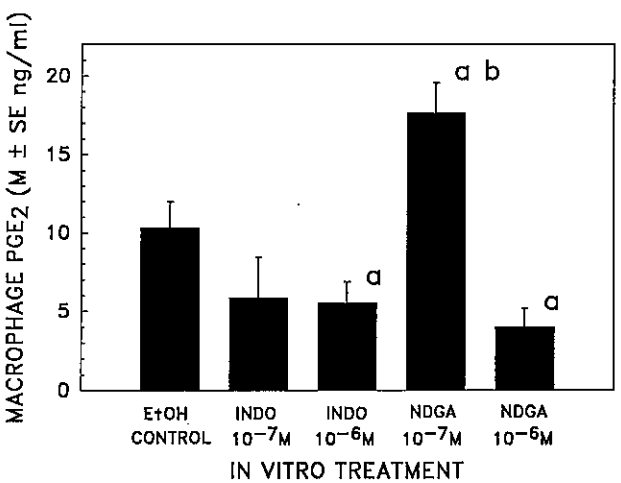

Fig. 5. Effect of INDO or NDGA *in vitro* treatment on macrophage PGE_2 production. Peritoneal macrophages obtained from mice which had borne B16 melanoma for 14 days were pretreated with INDO or NDGA solution (10^{-6} *M* and 10^{-7} *M*/ml), or EtOH for 4 h. The EtOH concentration was identical to that contained in the INDO (10^{-6} *M*) solution. PGE_2 levels determined from the culture supernatants of pretreated macrophages in 6 replicates/sample are shown as M±SE ng/ml. a: different from EtOH group. b: different from INDO 10^{-6} *M*, 10^{-7} *M* and NDGA 10^{-6} *M* group. (All at $P<0.05$ by ANOVA and LSD.)

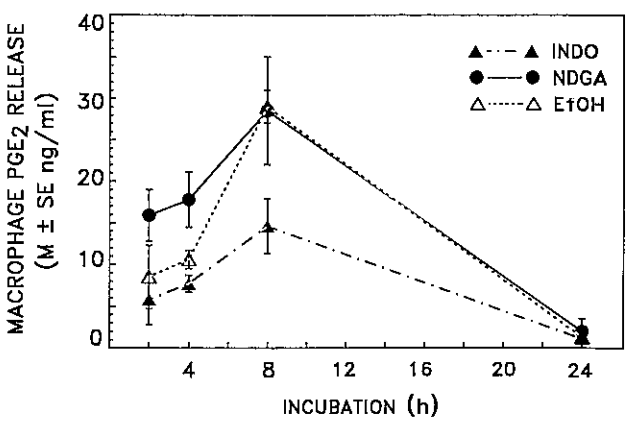

Fig. 6. Effect of duration of INDO or NDGA *in vitro* treatment on macrophage PGE_2 production. Peritoneal macrophages obtained from mice bearing B16 melanoma for 14 days were incubated with INDO solution (10^{-6} *M*) and NDGA solution (10^{-7} *M*)for 2, 4, 8, and 24 h. The EtOH concentration was identical to that contained in the INDO (10^{-6} *M*) solution. PGE_2 levels determined from culture supernatants in duplicate/sample are expressed as M±SE ng/ml.

studies (Exp. 4). However, the results were inconsistent between the two animal experiments, possibly due to the involvement of other factors under the *in vivo* conditions. In contrast, NDGA administration significantly accelerated PGE_2 release by macrophages. We have previously observed with the B16 melanoma-C57BL/6 mouse model[15] that excision of s.c. tumor causes significant acceleration of metastatic growth accompanied with impaired cytotoxicity and a high PGE_2 release by peritoneal macrophages. When transferred, splenic macrophages of such mice exhibited enhanced metastatic growth in recipients, indicating the development of suppressor macrophages. Suppressor macrophages are known to show impaired cytotoxicity and a high PGE_2 release, and to induce suppressor T-lymphocytes through their high PGE_2 release.[20,21] Basides, *in vivo* activation of suppressor T-cells has been reported to be blocked by INDO.[22] Taken together, the results obtained with the NDGA-treated mice, such as enhanced PGE_2 release by macrophages, suppressed macrophage cytotoxicity, and accelerated tumor growth, appear to imply the development of suppressor macrophages after NDGA treatment.

The serum of INDO-injected mice was stimulatory while that of the NDGA-treated mice was inhibitory to the cytostatic activity of macrophages. The trend shown in these results is identical to that exhibited by the macrophages obtained directly from INDO- or NDGA-treated mice. However, the cause-effect relationship of these parameters is not clear at present.

The animal studies clearly demonstrated that INDO or NDGA injection changes macrophage functions, but did not clarify whether these changes were the result of direct or indirect effects of these drugs. Therefore, we conducted *in vitro* studies to examine the direct effect of INDO and NDGA on tumor cells and macrophages of tumor-bearing mice. We found that the ability of NDGA to alter cell growth was about ten-fold greater than that of INDO. Also, to directly change the tumor cell growth, a much higher concentration of INDO (1000-fold) or NDGA (100-fold) and a longer duration of drug contact were necessary as compared with those sufficient to affect the macrophage function. These results suggest that macrophages are much more sensitive than tumor cells to intraperitoneally administered INDO or NDGA. INDO inhibited PGE_2 production of macrophages at both 10^{-7} *M* and 10^{-6} *M*. In contrast, NDGA accelerated macrophage PGE_2 production at 10^{-7} *M*, but inhibited it at the higher concentration (10^{-6} *M*). Although we employed NDGA as an inhibitor of the lipoxygenase pathway, there are a few reports[19, 23, 24] indicating that NDGA can also inhibit the cyclooxygenase pathway. Such inhibition of both pathways may account for the inhibition of PGE_2 release by 10^{-6} *M* NDGA. Compared to the effect of 10^{-6} *M* NDGA, the marked increase of macrophage PGE_2 release caused by the lower NDGA concentration (10^{-7} *M*) seems strange. It is conceivable, however, that this is due to the selective inhibition of the lipoxygenase pathway by the lower concentration of NDGA followed by a shunting of AA metabolism to the cyclooxygenase pathway. To provide evidence for this hypothesis, we examined the levels of leukotriene-B_4 (LTB_4), a lipoxygenase pathway product, using enzyme immunoassay, but the levels detectable from the limited volume of samples were very low and too unreliable for our purpose. Elliott *et al.*[19] found with ionophore-stimulated mouse peritoneal macrophages that 10^{-5} *M* NDGA reduced not only LTB_4 but also a cyclooxygenase pathway product, thromboxane-B_2 (TxB_2). However, a lower concentration of NDGA (10^{-6} *M*) reduced LTB_4 synthesis while it slightly increased TxB_2 synthesis. Conversely, INDO reduced the TxB_2 level but markedly increased the LTB_4 level. These observations support our hypothesis that a selective inhibition of one of these two major pathways would cause a shunting of AA metabolism to another pathway. In our time-course study, the levels of PGE_2 release were clearly different between the EtOH-, INDO-, and NDGA-treated macrophages initially. The PGE_2 level of the EtOH-treated control also rose by the end of 8 h incubation, and finally all had lost the ability to release PGE_2 after 24 h of contact with these agents. In our preliminary studies, macrophages incubated in the medium with or without EtOH for 4 h did not show any significant difference in their PGE_2 production. Therefore, the direct effect of EtOH for 4 h did not appear to account for the elevation of PGE_2 level at 8 h in the EtOH- and NDGA-treated groups, which could be a result of physical stimulation exerted by the procedures of cell preparation.

Many other investigators[19–25] observed that *in vitro* treatment with INDO stimulated macrophage cytotoxicity. Braun *et al.*[26] also reported that the cytotoxicity of γ-interferon stimulated peritoneal macrophages from ovarian cancer patients was accelerated by INDO but was inhibited by NDGA.

Macrophages release a number of monokines and other products which could be involved in influencing tumor growth, including interleukin-1, tumor necrosis factor, active oxygen species, and lysozomal enzyme. The release of these products was inhibited by PGE_2 and promoted by lipoxygenase products.[23, 27–30] Undoubtedly, the functional changes of macrophages caused by INDO or NDGA will consequently alter the activities of these products, cytokines, and other factors. Therefore, not only the direct cytotoxicity of macrophages but also the indirectly altered host defense system could be responsible for the effects of the INDO or NDGA administration.

Recently, a number of studies have indicated that NSAIDs, such as INDO, aspirin, piroxicam, and sulindac, can inhibit the growth of human tumors.[7, 9, 31–33] However, as shown with INDO and NDGA, NSAIDs may produce opposing effects depending upon their inhibitory effects on the cyclooxygenase pathway or lipoxygenase pathway of AA metabolism. Therefore, careful preliminary studies are recommended prior to the clinical use of NSAIDs on cancer patients.

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