Indomethacin sensitizes resistant transformed cells to macrophage cytotoxicity

Hana Totary-Jain[a,*], **Ronit Vogt Sionov**[b], and **Ruth Gallily**[c]

[a]Department of Molecular Pharmacology and Physiology, Morsaní College of Medicine, University of South Florida, Tampa, FL, USA

[b]Department of Developmental Biology and Cancer Research, IMRIC, The Hadassah Medical School-Hebrew University of Jerusalem, Israel

[c]The Lautenberg Center for General and Tumor Immunology, The Hadassah Medical School-Hebrew University of Jerusalem, Israel

Abstract

Activated macrophages are well known to exhibit anti-tumor properties. However, certain cell types show intrinsic resistance. Searching for a mechanism that could explain this phenomenon, we observed that the supernatant of resistant cells could confer resistance to otherwise sensitive tumor cells, suggesting the presence of a secreted suppressor factor. The effect was abolished upon dialysis, indicating that the suppressor factor has a low molecular weight. Further studies showed that prostaglandin E_2 (PGE_2) is secreted by the resistant tumor cells and that inhibition of PGE_2 production by indomethacin, a cyclooxygenase (COX) inhibitor, eliminated the macrophage suppression factor from the supernatant, and sensitized the resistant tumor cells to macrophage cytotoxicity. This study emphasizes the important role of tumor-secreted PGE_2 in escaping macrophage surveillance and justifies the use of COX inhibitors as an adjuvant for improving tumor immunotherapy.

Keywords

Indomethacin; PGE_2; Tumor cell resistance; Macrophage cytotoxicity; TNFα

1. Introduction

Activated macrophages have the propensity to kill tumor cells both *in vitro* and *in vivo* in virtue of their ability to produce tumor necrosis factor-α (TNFα), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and nitric oxide [1–3]. Usually the macrophages need to be activated in order to kill tumor cells. An exception is the ability of macrophages to eliminate teratocarcinoma cells and embryonic stem cells without the need for an external

*Corresponding author.: totaryjainh@health.usf.edu (H. Totary-Jain), ronit.sionov@mail.huji.ac.il (R.V. Sionov), ruthg@ekmd.huji.ac.il (R. Gallily).

Conflict of interest

The authors declare no conflict of interest.

activation signal [4]. Several activation agents can increase the tumoricidal activity of macrophages, including the cytokines IFN7, IL-12 and TNFα, whole bacteria such as *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) and mycoplasma, and bacterial and yeast components such as lipopolysaccharide (LPS), zymosan and β-glucans [2,5–7].

The tumor microenvironment is comprised of a variety of non-malignant cells such as fibroblasts, stromal cells, endothelial cells, pericytes and a variety of immune cells that can make up to 90% of the total tumor volume [8]. Within the tumor microenvironment, the macrophages are exposed to a wide range of tumor-secreted factors as well as cytokines and chemokines secreted by immune cells, which modulate macrophage functions. Among these factors, TGFβ has drawn much attention, being produced by the stimulated macrophages themselves and various tumor cells. TGFβ promotes macrophage polarization from an anti-tumor M1 to a pro-tumor M2 phenotype [9,10]. Other factors that can contribute to this polarization are the cytokines IL-4, IL-13 and IL-10 [10,11] and repeated activation of macrophages, which leads to loss of TNFα secretion and acquisition of high iNOS activity (our unpublished data). Indeed, the central macrophage product TNFα promotes the generation of immature myeloid-derived suppressor cells (MDSCs) [12], providing a negative feedback mechanism to tune the immune response. Alternatively activated M2 macrophages have a strikingly different gene expression profile compared with M1 macrophages and express a different combination of surface receptors (e.g., CD163), cytokines (e.g., IL-10), tumorgrowth factors (e.g., EGF, FGF1,TGFβ1), pro-angiogenic factors (e.g., VEGF), matrix remodeling factors (e.g., fibrin and matrix metallopeptidases) and chemokines (e.g., CCL17/TARC, CCL22/MDC and CCL24/Eotaxin-2) [10,11]. In addition, M2 macrophages produce lower levels of ROS, but express higher levels of arginase I and indoleamine 2,3-dioxygenase (IDO) [10,11]. Additional subtypes of macrophages have also been identified [13], suggesting a broad spectrum of macrophage activation stages [6].

We have observed that while some tumor cells are susceptible to macrophage cytotoxicity, others are resistant. The aim of our study was to characterize the mechanisms involved in conferring macrophage resistance upon tumor cells. This study shows that a low molecular weight factor secreted by tumor cells, defined as prostaglandin E_2 (PGE_2), prevents macrophage activation required for tumor cytotoxicity. Inhibition of PGE_2 production, using the non-steroid anti-inflammatory drug (NSAID) indomethacin, not only restored macrophage activation, but also conferred sensitivity of the otherwise resistant tumor cells to macrophage cytotoxicity.

2. Material and methods

2.1. Mice

C57BL/6 mice were obtained from the Animal Breeding Farm, Hebrew University-Hadassah Medical School of Jerusalem. All experiments involving animals were approved by the Hebrew University's Institutional Animal Care and Use Committee.[23]

2.2. Cell cultures

Bone-marrow-derived macrophages (BMMΦ) were obtained from bone marrow cells (usually ~30 ×10^6 cells per mouse) harvested from the femur and tibia of 6–8-week old female C57BL/6 mice, which were cultivated in DMEM supplemented with 15% heat-inactivated fetal calf serum (FCS), 5% heat-inactivated horse serum, 30% L929 cell conditioned medium (LCM), 2 mM L-glutamine, 10 mM HEPES, 100U/ml penicillin and 100 μg/ml streptomycin. The macrophages were cultivated on 9 cm diameter bacteriological grade culture dishes (Miniplast, Ein Shemer, Israel) and were used as effectors 10–21 days after bone marrow seeding. LCM was prepared by seeding 10^6 L929 cells in 20 ml DMEM supplemented with 10% FCS in a 75 cm^2 tissue culture flask (Nunclon, Denmark). Following 4–5 days incubation, when a monolayer had been reached, the supernatant was collected and sterile filtered.

A9 fibrosarcoma cells (a C$_3$H fibrosarcoma derived from L929 cells), L929 fibrosarcoma cells, NIH3T3 mouse embryonic fibroblast-like cells and M109 Madison lung carcinoma cells were grown in DMEM supplemented with 5% heat-inactivated FCS, 2 mM L-glutamine, 10 mM HEPES and antibiotics. The FCS used was selected from batches that did not pre-activate macrophages. All cell cultures were incubated at 37°C in a humidified incubator containing 5% CO$_2$. All cultures were routinely tested for being mycoplasma-free.

2.3. Cytotoxicity assay

Cytotoxicity assay was performed as described previously [4]. Briefly, target cells in the log phase of growth were pulsed with 1 μCi/ml of [^{3}H]thymidine (sp. Act 5 Ci/mM; American Radiolabeled Chemicals, Inc.) for 24 h, washed in PBS, trypsinized and resuspended in DMEM with 10% FCS, 2 mM L-glutamine, and 10 mM HEPES. Ten thousand target cells were added to 1×10^5 BMMΦ in 96 flat-bottomed microwells (Nunc, Denmark) in 300 μl DMEM supplemented with 10% heat-inactivated FCS in the absence or presence of 1 μg/ml LPS *(Escherichia coli o55:B5*, Bacto®, Difco). Following 72 h, the supernatants (300 μl) were harvested from the microwells, diluted with Insta-Gel (Packard, Downers Grove, Illinois) and the radioactivity counted. The samples were kept at 4°C for 24 h prior to counting. Percentage specific cytolysis was calculated by the following formula: %Cytolysis=[(E-SR)/(T-SR)] × 100% where E is the d.p.m. of the supernatant from co-culture of target cells and macrophages, SR the spontaneous release in d.p.m. of an equal number of target cells in medium without macrophages, and T the total d.p.m. uptake of target cells.

For determination of target cell sensitivity to TNFα, 5000 [^{3}H]thymidine-labeled cells were seeded in 100 μl DMEM with 5% FCS in each well of a 96-well plate. At the following day, 50 μl of various concentrations of TNFα (Genentech Inc., San Francisco) were added, followed by a 3-day incubation at 37°C. The extent of cell death was determined by measuring the released radioactivity as described above. Control wells got 50 μl of medium. Alternatively, cells were incubated with TNFα in the presence of 2 μg/ml Actinomycin D (Sigma), and the extent of cell death determined 18 h later.

2.4. Production of TNFα by activated macrophages and determination of TNFα titer

One hundred thousand BMMΦ were added to each of the 96 flat-bottomed microwells in 100 μl DMEM with 10% heat-inactivated FCS, 18 h prior to activation. The activation step was performed by changing the medium to DMEM without FCS or cell culture supernatant in the same medium, either in the absence or presence of μg/ml LPS, followed by incubation for 24 h. Macrophage supernatants were assayed for TNFα by bioassay as described previously [4]. Briefly, 4×10^4 Cl-7 cells were plated per 96 flat-bottomed microwell in 100 μl DMEM with 5% FCS. On the following day, 3fold dilutions of test supernatants and control media were made in the wells, followed by immediate addition of actinomycin D (Sigma; 2 μg/ml, final concentration). The cultures were incubated for 20 h at 37°C, and the survived Cl-7 cells were stained for 10 min with crystal violet (0.2% in 2% ethanol), washed with running tap water and allowed to dry. The destruction of the Cl-7 monolayer was determined by the amount of light (at 550 nm) absorbed by the residual stained cells in the wells using a Dynatech MicroElisa Reader (Artek, Farmingdale, NY). The S_{50} titer of TNFα was defined as the reciprocal of the dilution of the test solution required to destroy 50% of the target cell monolayer, as compared to control samples.

2.5. Conditioned medium of cultured cells

2×10^5 cells were seeded per well in a 24-well culture plate (Nunclon, Denmark) in 1.5 ml DMEM with 5% FCS. After 24 h, the medium was exchanged to fresh medium, and the supernatant collected 24 h later.The supernatants were centrifuged at 1200 rpm for 15 min, and kept at 4°C until use.

2.6. Surface TNF receptor binding assay

The assay was performed in accordance to Holtmann & Wallach [14]. TNFα was labeled with ^{125}I by the chloramine-T method to a specific radioactivity of 1500 Ci/mmol. One million target cells were seeded in growth medium in tissue culture plates the day before assay. On the following day, the cells were washes and incubated on ice for 2h with 0.5 nM ^{125}I-TNFα in the absence or presence of excess unlabeled TNFα (20 μM) in PBS containing 140 mM NaCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.7 mM KCl, 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$, 0.5% bovine serum albumin (BSA) and 15 mM sodium azide. Thereafter, the cells were washed three times in the binding buffer, detached in Ca^{2+}- and Mg^{2+}-free PBS containing 5 mM EDTA and transferred to vials for radioactivity measurements. Specific binding of TNFα was calculated by subtracting the values of binding observed in the presence of an excess of unlabeled TNFα from the value of binding observed with ^{127}I-TNFα alone.

2.7. Determination of prostaglandin E$_2$ (PGE$_2$) concentration

The PGE$_2$ concentrations in cell supernatants were determined by radioimmunoassay. 100 μl of sample, buffer alone or PGE$_2$ standard (0.15–10 ng/ml; Sigma) were mixed with 100 μl 0.01 M sodium phosphate buffer pH 7.4 containing 0.15 M NaCl, 0.1% BSA, 0.1% NaN_3 and 500 μl anti-serum to PGE$_2$ (Bio-Makor; diluted 1:10). Following a 30min incubation at 4°C, 100 μl of [5,6,8,11,12,14,15-^{3}H(N)]-PGE$_2$ (0.1mCi/ml, Amersham, England) was added at a dilution giving 50,000–100,000 d.p.m. After 60 min incubation at 4°C, 200 μl of

a dextran coated charcoal solution [1% charcoal with 0.1% dextran (MW 35,000–45,000; Sigma)] in sodium phosphate buffer were added, except for samples intended for total radioactive read. After vigorous mixing, the samples were incubated for 10 min at 4°C, followed by centrifugation at 3000 rpm for 15 min at 4°C. 250 µl of the supernatant was mixed with 3 ml of Instagel, and the radioactivity was measured in a β-counter. No antibody was added to the blank samples. The percentage of bound radioactive PGE_2 was calculated according to $[(S - B)/(T - B)] \times 100\%$, where S is the d.p.m of the sample, B the d.p.m of the blank sample and T the total radioactive amount. The limitation of this assay was 5 pg/ml PGE_2.

2.8. Statistical analysis

Experiments were repeated 3–5 times. The arithmetic average of all experiments performed is given. Statistical significance was assessed by the one-tail distribution-free Mann-Whitney *U* test. Error bars represent standard error. Differences were considered significant when the p value was 0.05 or less.

3. Results

3.1. Differential sensitivity of transformed cells to macrophage cytotoxicity

The aim of this study was to understand why some transformed cells are resistant to macrophage-mediated killing. We observed that M109 lung carcinoma cells and NIH3T3 fibroblast-like cells were relatively resistant, whereas A9 and L929 fibroblast-like cells were highly sensitive to killing by activated macrophages (Fig. 1A). None of these cells were killed by non-activated macrophages, as expected. The resistance could be due to intrinsic resistance to TNFα-mediated killing, the major mediator of macrophage cytotoxicity, or the secretion of a macrophage suppressor factor by the tumor cells. We first analyzed whether the NIH3T3 and M109 cells express TNF receptors by using the TNFα binding assay [14]. We found that NIH3T3 and M109 bound even more [127]I TNFα per cell than the macrophage-sensitive L929 cells (Table 1), thus excluding the lack of TNF receptors as the reason for their resistance to macrophage cytotoxicity. It could be that the cells are resistant to the cytotoxic effect of TNFα. To test this possibility, the cells were incubated in various dilutions of activated macrophage-conditioned medium, which contained an active TNFα S50 titer of 99,170 when analyzed on Cl-7 cells in the presence of actinomycin D. This assay shows that both NIH3T3 and M109 responded to the macrophage-conditioned medium by cell death (Fig. 1B).

We next studied whether the resistant tumors secrete a factor that prevents macrophage activation. For this purpose, macrophages were incubated with the conditioned medium of the transformed cells in the absence or presence of 1 µg/ml LPS, and following a 24 h-incubation at 37°C, the amount of TNFα secreted by the macrophages was determined. We observed that, while the conditioned medium of A9 cells did not interfere with the macrophages' ability to secrete TNFα in response to LPS, the conditioned media of NIH3T3 and M109 cells almost completely blocked TNFα secretion by macrophages (90–98% inhibition; Fig. 1C). These data suggest that the transformed cells indeed secrete a macrophage inhibitory factor.

In order to study whether the inhibitory factor has a low or high molecular weight, we dialyzed the cell conditioned media or control medium against PBS for 48 h and then against DMEM to restore essential nutrients using a dialysis tube with an MW cutoff of 10,000 Da. The dialyzed conditioned media were analyzed for their effects on LPS-induced TNFα secretion by macrophages. After dialysis, the conditioned media of NIH3T3 and M109 had lost their ability to inhibit TNFα secretion, and they even enhanced its production (Fig. 1D). This indicates the presence of a low molecular weight inhibitory factor in the conditioned media.

3.2. Identification of PGE$_2$ as the macrophage inhibitory factor

Since it has been reported that the low-molecular weight biochemical compound prostaglandin E$_2$ (PGE$_2$) can inhibit TNFα secretion from macrophages [15], we wondered whether our inhibitory factor is PGE$_2$. We first analyzed the presence of PGE$_2$ in the conditioned media, and observed that both NIH3T3 and M109 cells produced high levels of PGE$_2$, while PGE$_2$ couldn't be detected in the conditioned media of A9 cells (Table 2). We then analyzed the effect of various concentrations of PGE$_2$ on TNFα secretion by macrophages, and surprisingly observed a dose-dependent effect where high PGE$_2$ concentrations (from 1 nM-1 μM) strongly inhibited TNFα secretion, while low concentrations (especially at 0.1–1 pM) strongly enhanced it (Fig. 2A). The synergistic effect of low PGE$_2$ concentrations on TNFα secretion might explain why the dialyzed conditioned medium even enhanced its secretion by LPS-stimulated macrophages (Fig. 1D). In order to validate that the PGE$_2$ secreted from NIH3T3 and M109 is the factor that inhibited macrophage activation, the cells were treated with the cyclooxygenase (COX) inhibitor indomethacin, and the resulting conditioned medium was analyzed for their ability to affect TNFα production. Indeed, indomethacin abolished the macrophage inhibitory effect of the conditioned medium and even enhanced TNFα secretion (Fig. 2B). The PGE$_2$ concentration in the conditioned medium was negligible in the presence of indomethacin (undetectable in the NIH3T3 conditioned media, while 10 ng/ml in the M109 conditioned media). In light of these encouraging data, it was intriguing to determine whether indomethacin could sensitize the resistant cells to macrophage cytotoxicity. For this purpose, NIH3T3, M109 and A9 cells were incubated with macrophages with LPS in the absence or presence of 50 μM indomethacin, and the extent of cell killing was measured after 3 days of co-cultivation. Indeed, indomethacin sensitized the resistant tumor cells to macrophage cytotoxicity (Fig. 2C). Further studies showed that when the macrophage-sensitive A9 cells were incubated with macrophages that have been pre-incubated with the resistant NIH3T3 or M109 cells, the cell killing was reduced by 75% (Fig. 2D). This reduction in cell killing could be reversed by treating the co-cultures with indomethacin (Fig. 2D), further emphasizing the central role of PGE$_2$ in mediating the macrophage inhibitory effect. This is further manifested by the suppression of A9 cell killing when PGE$_2$ was added to the co-culture of A9 and macrophages (Fig. 2E). Maximum inhibition was obtained at 1–10 nM PGE$_2$ (47–51% inhibition; Fig. 2E).

4. Discussion

Our pioneering study, performed in the late 1980's, is fully relevant today in light of the current recognition that PGE_2 is a key player in the carcinogenesis of colon cancer and several other cancer cell types [16–19], along with the introduction of aspirin in the clinics for the prevention of colon cancer [20,21]. During the years, it has been repeatedly shown that PGE_2 suppresses diverse macrophage functions [22–26] and may even promote the shift towards the M2 phenotype [27–30] as well as the appearance of myeloid-derived suppressor cells with tumor-promoting function [31]. Other studies have demonstrated PGE_2 production by various cancer cells [27,32,33,34–36], besides being produced by the macrophages themselves [37,38]. Our study combines these two issues showing that tumor-secreted PGE_2 protects the tumor cells from macrophage cytotoxicity, a tumor immune escape mechanism that can be overcome by the drug indomethacin. Indomethacin and aspirin belong to the same group of non-steroid anti-inflammatory drugs (NSAIDs), and their benefits as adjuvants in cancer therapy seem thus to be of a dual nature. Namely, by preventing PGE_2 production in the tumor cells, these drugs may directly inhibit PGE_2-dependent tumor cell growth, and, simultaneously, increase macrophage cytotoxicity towards the tumor cells (Fig. 3). Both mechanisms contribute to the reduction of the tumor cell mass. Our study suggests a mechanistic explanation for the anti-tumor effects observed for indomethacin in various tumor models (e.g., [39–41]).

The dose-dependent effects of PGE_2 observed by us on macrophage secretion of TNFα has also been observed by another research group [42]. That group observed that the differential effects of PGE_2 are mediated by changes in the intracellular cAMP/cGMP ratio [42]. A dose-dependent effect of PGE_2 on macrophage adhesion and migration has also recently been documented [43]. An interesting fact is that reducing the PGE_2 level by indomethacin may not only prevent the inhibitory effect of PGE_2 on macrophages, but may even lead to such low levels that the tumoricidal effect of macrophages is enhanced. Indomethacin even increased the tumoricidal effect of activated macrophages on A9 cells that barely produce PGE_2 (Fig. 2C). This might be due to inhibition of PGE_2 production by the macrophages themselves during co-cultivation, where the negative feedback mechanism triggered upon macrophage activation is interrupted.

Of note, we could only reach up to 50% inhibition of A9 cell killing by macrophages when adding PGE_2, even though the higher concentration (10 nM) completely blocked TNFα secretion. This might be due to the induction of another tumoricidal factor, such as the nitric oxide radical by PGE_2 [44]. Nitric oxide has been shown to be involved in macrophage killing of L929 fibrosarcoma cells [3]. This scenario might also explain how PGE_2 can induce tumoricidal activity of resident macrophages on L929 cells in the absence of any other activation signal [45]. Thus, PGE_2 may support some anti-tumor macrophage activities despite abolishing TNFα production. This duality of PGE_2 action might be important for maintaining essential macrophage functions under conditions where excessive immune responses are suppressed.

Altogether, our study sheds new light on the tumor cellmacrophage interrelationship, where macrophage tumoricidal activity can be regained by preventing excessive PGE_2 production

using the NSAID drug indomethacin. Tumor-secreted PGE_2 likely acts in concert with other immune suppressive factors such as TGFβ [9], based on the observation that indomethacin couldn't reverse macrophage killing of A9 cells when co-cultured in the presence of resistant transformed cells to a level similar to that observed in their absence (Fig. 2D). Our data would therefore suggest a potential use of indomethacin as an adjuvant agent in cancer immunotherapy [28]that ought to be combined, for instance, with a TGFβ inhibitor.

Acknowledgments

Abbreviations

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COX	cyclooxygenase
PGE_2	prostaglandin E_2
M Φ	macrophages
NSAID	non-steroid anti-inflammatory drug
TNF	tumor necrosis factor α

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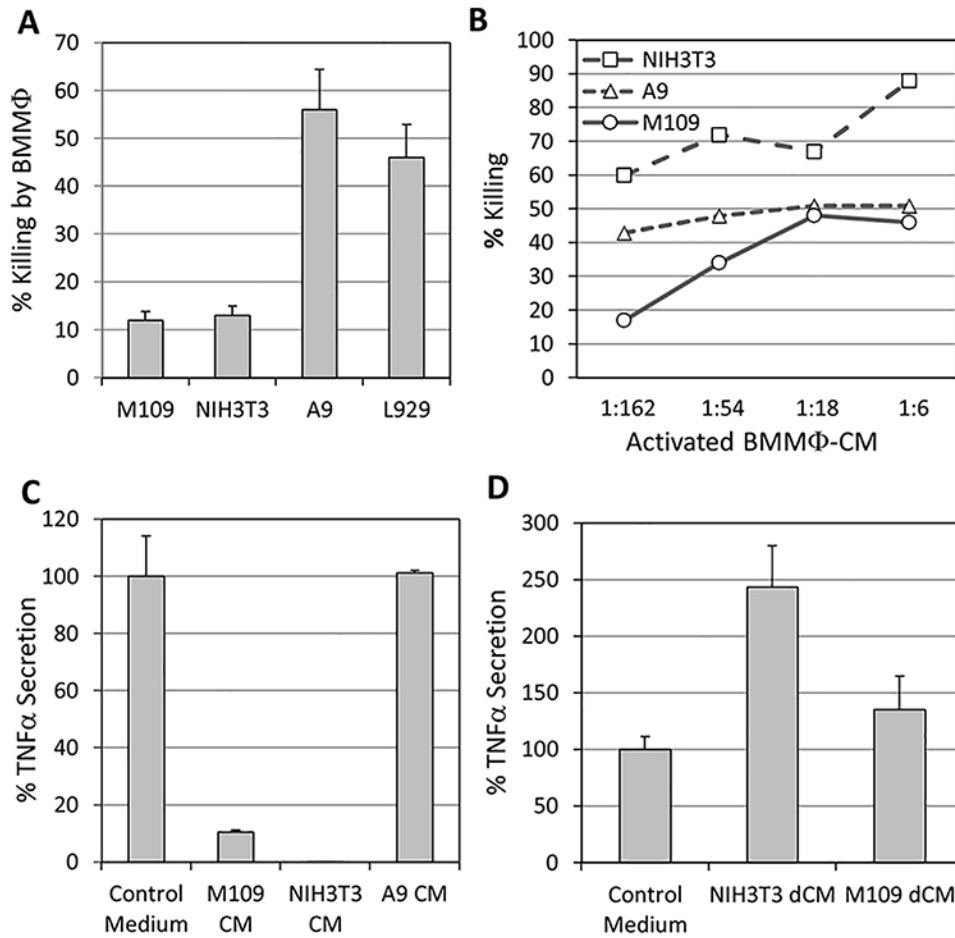
Fig. 1.

(A) The sensitivity of various transformed cells to activated macrophage cytotoxicity. 1×10^4 target cells were incubated with 1×10^5 BMMΦ in the presence of 1 μg/ml LPS for 3 days. In the absence of LPS, there was no killing of any cells. p<0.05 for M109/NIH3T3 versus A9/L929. (B) Cell sensitivity to TNFα. NIH3T3, M109 and A9 cells were incubated in various dilutions of activated macrophage-conditioned medium (BMMΦ-CM) in the presence of 2 μg/ml actinomycin D, and the extent of cell death determined 18 h later. (C) Inhibition of TNFα secretion by the conditioned media of transformed cells. One hundred thousand macrophages were incubated in serum-free medium or in conditioned media (CM) collected from NIH3T3, M109 or A9 cells, in the absence or presence of 1 μg/ml LPS for 24 h, and the amount of active TNFα secreted was determined as described in the Section 2.4. The relative TNFα secretion by macrophages in the presence of conditioned medium is presented in comparison to control medium. p<0.05 for M109 CM and NIH3T3 CM versus control medium and A9 CM. (D) The suppressor factor is lost upon dialysis of the conditioned media. Conditioned media were dialyzed for 48 h against PBS, and then against DMEM, using a dialysis tube with a cut-off of 10,000 Da. The effect of the dialyzed conditioned media (dCM) on LPS-induced TNFα secretion by macrophages was analyzed. The data are presented as relative TNFα secretion by macrophages in the presence of
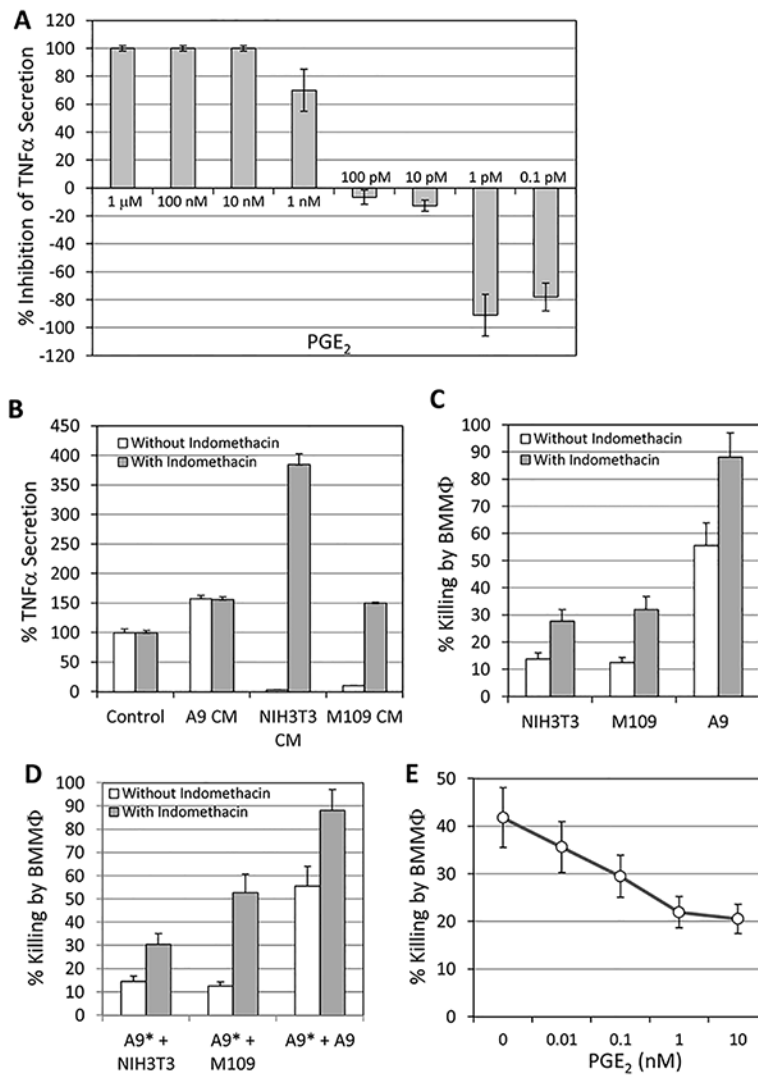
dialyzed conditioned medium in comparison to dialyzed control medium. p<0.05 for NIH3T3 dCM and M109 dCM versus dialyzed control medium.

Fig. 2.
(A) Effect of PGE_2 on TNFα secretion by LPS-stimulated macrophages. Hundred thousand macrophages that have been seeded in each well of a 96-flat bottomed tissue culture plate, were exposed to 1 μg/ml LPS in the absence or presence of various concentrations of PGE_2 as indicated. The amount of TNFα secreted was analyzed 24h later. 1 nM corresponds to 352.4ng/ml PGE_2. $p < 0.05$ for 1 nM-1 μM and 0.1–1 pM PGE_2 versus control medium. (B) Indomethacin treatment of N1H3T3 and M109 cells abolished the macrophage inhibitory effect of conditioned medium. Hundred thousand macrophages were incubated in control medium or conditioned media from untreated or indomethacin (50 μM, 24h)-treated M109, N1H3T3 and A9 cells in the presence of 1 μg/ml LPS, and the amount of TNFα secreted was determined 24h later. The data are presented as relative TNFα secretion by macrophages under each treatment condition in comparison to that of control medium. $p < 0.05$ for N1H3T3 CM and M109 CM in the absence of indomethacin versus control and A9 CM; and $p < 0.05$ for N1H3T3 CM and M109 CM in the presence versus in the absence of indomethacin. (C) Indomethacin sensitized the resistant tumor cells to macrophage cytotoxicity. N1H3T3, M109 and A9 cells were incubated with macrophages in the presence

of 1 μg/ml LPS with or without 50 μM indomethacin for 3 days, and the extent of tumor cell killing determined. $p < 0.05$ for cells in the presence versus in the absence of indomethacin. (D) The presence of resistant cells prevented macrophage cytotoxicity on sensitive cells that could be reversed by indomethacin. Five thousand [^{3}H]-thymidine-labeled A9* cells were addedto 1×10^5 macrophagesthat have been pre-incubated with 5×10^3 unlabeled N1H3T3, M109 or A9 cells for 24 h. The extent of A9 cell killingwas determined after 3 days co-incubation. $p<0.05$ forkilling of A9 cells inthe presenceversus inthe absence of indomethacin. (E) PGE$_2$ suppressed macrophage-mediated cytotoxicity of A9 cells. PGE$_2$ was added at 10pM, 100pM, 1 nM and 10 nM to the co-culture of A9 cells and macrophages in the presence of 1 μg/ml LPS, and the extent of cell killing determined 3 days later. $p<0.05$ forcell killing in the presence versus in the absence of PGE$_2$.
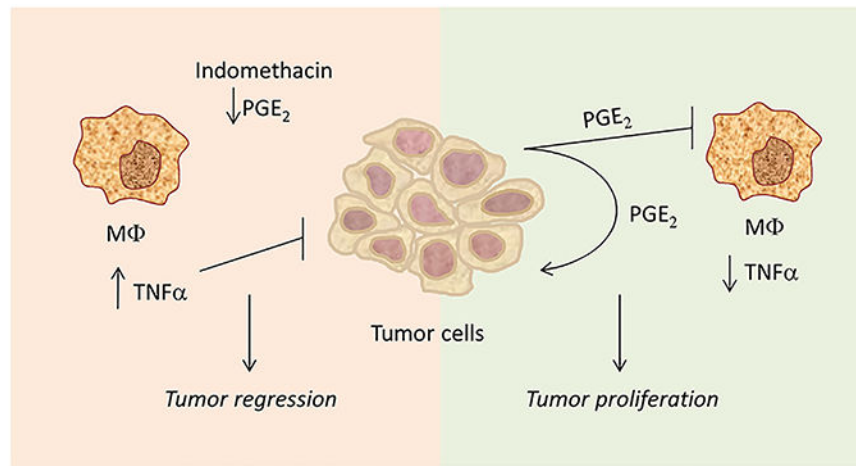
Fig. 3.
The Tumor-PGE_2-Macrophage Cross-Talk. Tumor cells produce PGE_2 that is sometimes necessary for tumor autonomous cell growth, and at the same time suppresses macrophage activation required for anti-tumor function. Interruption of this cross talk by indomethacin, restores the tumoricidal macrophage function that contributes to limit tumor growth.

Table 1

Binding of ^{125}I-TNFα to the different cell lines. The numbers represents the amount of radioactive TNFα bound to 1×10^6 cells after subtraction of the non-specific radioactive binding observed in the presence of excess unlabeled TNFα. The input ^{125}I-TNFα was 2×10^5 cpm.

Cell lines	Specific binding (cpm)
NIH3T3	10,427
M109	12,778
L929	4045

Table 2

Production of PGE_2 by NIH3T3 and M109 cells. The PGE_2 concentrations in the conditioned medium (CM) of the indicated cell lines was determined by radioimmunoassay as described in the Section 2.7. Conditioned media were collected 24 h after seeding 2×10^5 cells in 1.5 ml DMEM with 5% FCS in a 24-well culture plate.

Cell lines	PGE_2 conc in CM
NIH3T3	502pg/ml (1.4nM)
M109	397 pg/ml (1.1 nM)
A9	n.d.
MEF	185 pg/ml (0.5 nM)

MEF: mouse embryonic fibroblasts. n.d.: non detectable.