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Regulation of TXB_2 and PGE_2 production by TGF-β_1 in *in vitro* silica dust-exposed rat alveolar macrophage

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We investigated the effect of transforming factor factor-β_1 (TGF-β_1) on thromboxane B_2 (TXB_2) and prostaglandin E_2 (PGE_2) production in *in vitro* silica dust-exposed rat alveolar macrophages (AM). In the presence of 5 μg of anti-TGF-β_1 antibodies, TXB_2 production decreased, but PGE_2 production increased. Addition of 2 ng of TGF-β_1 to the culture medium potentiated TXB_2 production, but PGE_2 production apparently did not change. At 50 ng of TGF-β_1, TXB_2 production decreased, and PGE_2 production varied. Our data suggest that in rat AM: (1) both endogenous and exogenous TGF-β_1 regulate TXB_2 production; and (2) in the absence of endogenous TGF-β_1 the liberation of PGE_2 increases; however, exogenous TGF-β_1 does not have a regulatory effect on PGE_2.

Key words: Rat alveolar macrophages, PGE_2, TGF-β_1, TXB_2

Introduction

Chronic airway inflammation and lung fibrosis are common features of prolonged exposure to mineral particles of silica in humans and in rodents.[1] Alveolar macrophages (AM) have an essential role in lung clearance and defence mechanisms against inhaled particles.[2] They fulfil these functions via inflammatory mediators produced upon macrophage activation. Among the many mediators are arachidonic acid metabolites such as thromboxane A_2 (TXA_2) and prostaglandin E_2 (PGE_2). Thromboxane A_2 (of which TXB_2 is the stable metabolite) is a very potent platelet aggregating factor and pulmonary vasoconstrictor. A recent study of the regulatory effect of TXB_2 on the proliferation of vascular smooth muscle cells from rats demonstrated a rapid build-up of cytoskeletal protein in these cells in hypertension, suggesting that TXB_2 may play a role in the remodelling of the vascular wall in hypertension.[3,4]

Prostaglandin E_2 is one of the key inflammatory mediators with multiple functions. In the lung, it induces bronchodilation and causes an increase in epithelial cell chloride secretion.[5] PGE_2 induces vasodilatation in unventilated foetal lung.[6]

Another mediator that is produced by alveolar macrophages is transforming growth factor-β_1 (TGF-β_1). TGF-β_1 is a multifunctional peptide. TGF-β_1 can influence cell proliferation or differentiation and other functions in various types of cells, some of which are related to inflammation and tissue fibrosis. For example, TGF-β_1 increases the rate of fibronectin gene transcription,[7] can down-regulate interleukin-1β receptors,[8] and inhibits interleukin-6 release from fibroblasts.[9] The most profound effect of TGF-β_1 is its ability to enhance the formation of extracellular matrix. TGF-β_1 promotes the formation of collagen and fibronectin in fibroblasts of human, rat and mouse origin. From *in vivo* studies, it has been shown that TGF-β_1 can stimulate the formation of the typical granulation tissue found in tissue repair.

Although pulmonary macrophage-derived mediators have a pivotal role in lung inflammation and fibrosis, the interrelationship between these mediators is just beginning to be understood. We have shown the very early release (15 min) of TGF-β_1 from silica dust-exposed rat alveolar macrophages.[10] The amount of TGF-β_1 released at this time was the greatest over 24 h kinetic studies, and preceded the production of TXB_2 and PGE_2. To further unravel the interrelationship between eicosanoids and TGF-β_1 produced by alveolar macrophage, the modification of TXB_2 and PGE_2 production by TGF-β_1 in *in vitro* silica-exposed rat alveolar macrophages was investigated. We observed decreased production of TXB_2 in the presence of anti-TGF-β_1 antibodies, or high concentration (50 ng) of TGF-β_1. TXB_2 production increased in the presence of a low concentration of TGF-β_1 (2 ng) in the culture medium. PGE_2 production increased in

the presence of anti-TGF-β_1 antibodies. Exogenous TGF-β_1 at low or high concentration did not have any effect on PGE_2 production in rat alveolar macrophages.

Materials and Methods

Materials: Hanks' solution and Dulbecco's phosphate buffered saline (DPBS), and HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) buffer were prepared in our laboratory. Six-well tissue culture plates were obtained from Falcon Laboratory Products (Beckton and Dickinson Labware, Lincoln Park, NJ). DMSO (dimethylsulfoxide) was obtained from Calbiochem (San Diego, CA). Allopurinol, oxypurinol, allopurinol riboside, Penn/Strep, M199 medium (without phenol red), and human serum albumin were all obtained from Sigma (St. Louis, MO). Human recombinant and polyclonal anti-TGF-β_1 antibody were obtained from R&D Systems (Minneapolis, MN). RIA reagents for TXB_2 and PGE_2 assay were purchased from Advanced Magnetics, Inc. (Boston, MA).

Isolation and culture of rat alveolar macrophages: Sprague–Dawley rats weighing approximately 225 g were lavaged *ex vivo*. Rats were given an intramuscular injection of sodium pentabarbital (10 mg) and the rib cage was opened to expose the lungs. An incision halfway through the trachea was made and the lavage tube of the trachea cannula was inserted into the incision. The lungs were washed five times each with 10 ml of lavage fluid (phosphate-buffered saline containing 0.1% EDTA, at 37°C). The collected lavage fluid was filtered through sterile Nitex gauze and centrifuged at 250 × ***g*** for 10 min. The supernatant was discarded, and the cells resuspended in M199, 10 mM HEPES 0.3% human serum albumin, 100 U/ml penicillin and 100 µg/ml streptomycin (culture medium). Cells from all rats were pooled and centrifuged again at 250 × ***g*** for 10 min. Cells were enumerated in a haemocytometer. Viability was determined by the exclusion of trypan blue and cells were plated at 1×10^6/ml of culture medium in 6-well culture plates. The cells were preincubated for 1 h at 37°C in a humidified atmosphere at 5% CO_2 in air. The nonadherent cells were removed by aspiration. Fresh culture medium (37°C) was placed in the wells and unlabelled arachidonic acid was added at a final concentration of 3 µM.[10] This incubation with arachidonic acid was conducted in order to replenish endogenous arachidonic acid utilized during the exaggerated eicosanoid production which occurs during cell isolation and attachment.[11] The cells were incubated at 37°C for 24 h. After incubation the medium containing arachidonic acid was removed and fresh culture medium was placed in the wells. Macrophages were activated with a microcrystalline form of silica dust (Min-U Sil, 2–7 µm diameter, provided by Dr Peter Bolsitis, MIT) at a final concentration of 200 µg/ml, at 37°C for 24 h, in the absence or presence of anti-TGF-β_1 antibodies, or TGF-β_1. Nonspecific antibody as an isotype-matched control for anti-TGF-β_1 antibody was tested. Media were collected into silianized tubes for the determination of eicosanoids.

Radioimmunoassay for TXB_2 and PGE_2: Following incubation, the culture medium was removed and acidified to pH 3.0 with 1 N HCl. Eicosanoids were extracted twice with 2 ml ethyl acetate/cyclohexane (1:1). The extracts were stored at −20°C until analysis of eicosanoids by specific radioimmunoassay, as described previously.[12] The antibodies utilized in the assay of TXB_2 or PGE_2 showed less than 1% cross-reactivity with other eicosanoids.

Cell viability test: The lactate dehydrogenase (LDH) activity in rat culture conditions was measured using a Sigma kit for LDH. There was no detectable LDH enzyme activity in the culture medium from rat alveolar macrophages.

Data analysis: Results are presented as means ± S.E. of a number of independent experiments.

Results

The effect of TGF-β_1 on TXB_2 production in rat alveolar macrophages: Table 1 shows the effect of anti-TGF-β_1 antibodies, or low or high concentrations of TGF-β_1, on TXB_2 production in silica-exposed rat alveolar macrophages. In the presence of 5 µg of anti-TGF-β_1 antibodies in the culture medium, during the process of macrophage activation with silica, the production of TXB_2 was inhibited. The TGF-β_1, at 2 ng concentration in the culture medium, potentiated TXB_2 production. At 50 ng of TGF-β_1 in the culture medium, the production of TXB_2 was inhibited.

The effect of TGF-β_1 on PGE_2 production in rat alveolar macrophages: Table 2 shows the effect of anti-TGF-β_1 antibodies, or low or high concentrations of TGF-β_1, on PGE_2 production in silica-exposed rat alveolar macrophage. In the presence of 5 µg of anti-TGF-β_1 antibodies in the culture medium, the production of PGE_2 was

Table 1. The effect of TGF-β_1 on TXB_2 production by rat alveolar macrophages exposed to silica dust

Treatment	Experiment 1		Experiment 2		Experiment 3	
	TXB_2 (pg/ml)	% Control	TXB_2 (pg/ml)	% Control	TXB_2 (pg/ml)	% Control
Control	62 ± 2		179 ± 5		79 ± 3	
Silica	132 ± 2	213	369 ± 2	206	172 ± 9	218
Silica + 5 µg anti-TGF-β_1	60 ± 5	100	313 ± 2	175	103 ± 7	130
Silica + 2 ng TGF-β_1	ND	ND	491 ± 3	274	219 ± 2	277
Silica + 50 ng TGF-β_1	47 ± 5	76	69 ± 0	39	158 ± 3	200

Alveolar macrophages at 1 × 10^6 cells were activated with 200 µg of silica dust in the absence or presence of anti-TGF-β_1 antibodies, or TGF-β_1 added at the time of cell activation, and incubated at 37°C for 24 h. TXB_2 was assayed by the RIA as described in Methods. $n=2$ in each experimental group. ND = not done.

Table 2. The effect of TGF-β_1 on PGE_2 production by rat alveolar macrophages exposed to silica dust

Treatment	Experiment 1		Experiment 2		Experiment 3	
	PGE_2 (pg/ml)	% Control	PGE_2 (pg/ml)	% Control	PGE_2 (pg/ml)	% Control
Control	445 ± 6		207 ± 9		411 ± 10	
Silica	939 ± 3	211	241 ± 10	116	438 ± 10	107
Silica + 5 µg anti-TGF-β_1	1137 ± 75	256	330 ± 9	159	783 ± 20	191
Silica + 2 ng TGF-β_1	ND	ND	253 ± 3	122	457 ± 2	111
Silica + 50 ng TGF-β_1	840 ± 91	189	227 ± 7	110	504 ± 20	123

Alveolar macrophages at 1 × 10^6 cells were activated with 200 µg of silica dust in the absence or presence of anti-TGF-β_1 antibodies, or TGF-β_1 added at the time of cell activation, and incubated at 37°C for 24 h PGE_2 was assayed by the RIA as described in Methods. $n=2$ in each experimental group. ND = not done.

potentiated. The addition of 2 ng of TGF-β_1 to the culture medium had no apparent effect on PGE_2 production in either of two experiments performed. At 50 ng of TGF-β_1, the production of PGE_2 varied.

Discussion

This study was designed to evaluate the regulation of TXB_2 and PGE_2 production by TGF-β_1 in alveolar macrophages. The reason for these studies was to supplement the limited information in the literature on the interrelationship between the inflammatory mediators released by a specific inflammatory cell. TXB_2, PGE_2 and TGF-β_1 are the principal inflammatory mediators released by alveolar macrophages during the lung's defence process against pathogens or foreign matter. We have demonstrated in earlier studies[10] that these three mediators are released from rat alveolar macrophages upon exposure to silica dust. In those studies we observed a striking difference between the kinetics of release of TGF-β_1 (maximum release at 15 min post-silica dust) and TXB_2 (plateau release at 6 h post-silica dust), or PGE_2 release (plateau release at 6 h post-silica dust). Because it is thought that TGF-β_1 has a central regulatory role in vascular physiology and pathology, we formulated a hypothesis that perhaps TGF-β_1 has an impact on the production of either TXB_2 or PGE_2, or both, by alveolar macrophage upon silica dust-exposure.

We observed a positive effect of TGF-β_1 on TXB_2 production in rat alveolar macrophages which had been exposed to silica dust. In the absence of TGF-β_1 (plus anti-TGF-β_1 antibodies), the quantity of TXB_2 released from rat alveolar macrophages to the culture medium in response to silica exposure was significantly smaller when compared to the response with silica alone. Exogenous TGF-β_1, at low concentration, potentiated the action of silica dust, and the amount of TXB_2 produced by rat alveolar macrophages was significantly greater than the amount of TXB_2 released by alveolar macrophages activated by silica alone. High concentration of TGF-β_1, however, inhibited TXB_2 production in rat alveolar macrophages; in response to silica, the amount of TXB_2 released was smaller than the amount of TXB_1 produced in rat alveolar macrophages activated with silica alone, or in the absence of TGF-β_1 (in the presence of anti-TGF-β_1 antibodies). This observation suggests that upon exposure to silica dust a homeostatic autocrine loop for TGF-β_1, that regulates the production of TXB_2, appears to function in rat alveolar macrophages. Our data also suggest the role for concentration of TGF-β_1 in the regulation of TXB_2 production; depending on the concentration, this growth factor appears to be either stimulatory or inhibitory to rat alveolar macrophage, and to TXB_2 production.

At the same time, however, the effect of TGF-β_1 on the generation of another eicosanoid,

namely PGE_2, in silica-exposed rat alveolar macrophages appeared to be very different to that observed for TXB_2. In the absence of TGF-β_1 (plus anti-TGF-β_1 antibodies), the amount of PGE_2 produced in silica-exposed alveolar macrophages was greater than when compared to silica alone. When anti-TGF-β_1 antibodies were present in the culture medium during the exposure of macrophages to silica, we observed that (1) these macrophages liberated greater amount of PGE_2 compared to silica alone; and (2) there was a stimulation of PGE_2 production by alveolar macrophages which did not produce PGE_2 after exposure to silica. Furthermore, the presence of exogenous TGF-β_1, at low or high concentrations, had either no apparent effect, or a variable effect, respectively, on PGE_2 production in silica-exposed rat alveolar macrophages when compared to silica alone. Our data suggest, therefore, a possible distinct biological role for endogenous, but not exogenous TGF-β_1 to down-regulate PGE_2 production in rat alveolar macrophages after exposure to silica.

Data presented in this manuscript provide evidence of the regulatory role of TGF-β_1 in the liberation of inflammatory eicosanoids, TXB_2 and PGE_2, in rat alveolar macrophages, when the cells are exposed to silica dust. In support of our observation are the reports of Datta *et al.*, where the authors demonstrate that TGF-β_1 inhibits lipoxygenase and epoxygenase eicosanoid production by osteosarcoma cells,[13] or eicosanoid metabolism in osteogenic osteosarcoma cells.[14] The regulation of eicosanoid liberation by TGF-β_1, or the regulation of TNF-α production by TGF-β_1[13] suggests that the mechanism of action of TGF-β_1 on tissues may not necessarily be direct, but rather it requires actions of other mediators. The interrelationship between TGF-β_1 itself and all the inflammatory mediators directed by TGF-β_1 in inflammation appears to be very intriguing.

In summary, our data suggest that in silica-exposed alveolar macrophages, both endogenous and exogenous TGF-β_1 regulate TXB_2 production. Our data suggest that endogenous, but not exogenous TGF-β_1 has a regulatory role in PGE_2 production. Because both TXB_2 and PGE_2 were measured in the same samples, and because there is a consistent increase in silica-stimulated PGE_2 synthesis in the presence of anti-TGF-β_1 antibodies in the absence of an effect of authentic TGF-β_1, it appears that in silica-exposed alveolar macrophages the regulatory effects of TGF-β_1 are different between the different components of the arachidonic acid pathway.

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