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Prostaglandin E_2 down-regulates viable Bacille Calmette–Guérin-induced macrophage cytotoxicity against murine bladder cancer cell MBT-2 *in vitro*

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SUMMARY

The regulatory effect of prostaglandin (PG) E_2 and a cyclooxygenase (COX) inhibitor on Bacille Calmette–Guérin (BCG)-induced macrophage cytotoxicity in a bladder cancer cell, MBT-2, was studied *in vitro*. BCG stimulated thioglycollate-elicited murine peritoneal exudate cells (PEC) to induce cytotoxic activity and to produce cytokines such as interferon (IFN)-γ, tumour necrosis factor (TNF)-α and PGE_2. NS398, a specific COX-2 inhibitor, and indomethacin (IM), a COX-1 and COX-2 inhibitor, enhanced viable BCG-induced cytotoxic activity and IFN-γ and TNF-α production of PEC. However, NS398 and IM did not enhance these activities induced by killed BCG. Enhanced cytotoxicity was mediated by increased amounts of IFN-γ and TNF-α. Exogenous PGE_2 reduced cytotoxic activity and IFN-γ and TNF-α production of PEC. These results suggest that PGE_2 produced by BCG-activated macrophages has a negative regulatory effect on the cytotoxic activity of macrophages. Accordingly, a PG synthesis inhibitor may be a useful agent to enhance BCG-induced antitumour activity of macrophages.

Keywords BCG bladder cancer cytotoxicity prostaglandin E_2 macrophage

INTRODUCTION

Bacille Calmette–Guérin (BCG) has proved to be effective in immunotherapy for bladder cancer [1,2], but its antitumour mechanism is not fully understood. There have been several reports that BCG stimulates cytotoxic activity of macrophages, T cells and NK cells [3,4]. The activated effector cells kill target cells by both non-specific soluble factors and direct cell to cell contact. It is reported that patients who failed in BCG immunotherapy showed a higher antibody response to BCG heat-shock proteins in their sera [5] and higher levels of interleukin (IL)-6 and/or IL-10 in the urine of patients [6]. These studies indicate that Th2 immune responses are more easily induced than Th1 immune responses during BCG-immunotherapy and suppress cellular immune responses. It is reported that prostaglandin (PG) inhibits the production of Th1 type cytokines such as interferon (IFN)-γ and IL-12, favouring the production of Th2-type cytokines such as IL-4 and IL-5 by human lymphocytes [7]. PGE_2 suppressed the activation of NK cells, lymphokine-activated killer cells and cytotoxic T cells by inhibiting IL-2 receptor expression on effector cell surfaces [8]. Recently, we also found that PGE_2 suppressed IFN-γ and IL-12 production by macrophages (9).

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PGs are synthesized from cell membrane phospholipids by way of arachidonic acid. Two types of enzyme, cyclooxygenase (COX)-1 and -2, are known to mediate this pathway [10]. COX-1 is an enzyme that is constitutively expressed in many types of tissues and is not significantly up-regulated by external stimuli. On the other hand, COX-2 is an inducible enzyme that is up-regulated by several internal or external stimuli such as lipopolysaccharides [11] and IL-1β [12]. Therefore, COX-1 and -2 are considered to play an important role in the production of PGs at inflammation sites.

Little is known about the expression of COX and the production of PGs in BCG-stimulated macrophages. To investigate the role of PGs in BCG-induced macrophage activities we used NS398, a COX-2 specific inhibitor, and indomethacin (IM), a COX-1 and COX-2 inhibitor. This study demonstrates that PGs regulate BCG-induced macrophage antitumour activity negatively, while COX-1 and COX-2 inhibitors augment its activity.

MATERIALS AND METHODS

Reagents

The culture medium was RPMI-1640 (Nissui Pharmaceutical Co., Tokyo, Japan) containing 10% heat inactivated fetal calf serum (FCS), L-glutamine (2 mM) and penicillin-G (100 U/ml) (Grand Island Biological Co., Grand Island, NY, USA). PGE_2 and IM were purchased from Sigma Chemical Co., St Louis, MO, USA.

NS398 was purchased from Cayman Chemical Co., Ann Arbor, MA, USA. Anti-Pan-NK cell monoclonal antibody (MoAb), clone DX5 and anti-IFN-γMoAb, clones R4–6A2, XMG1·2, were purchased from Pharmingen Co., San Diego, CA, USA. Anti-TNF-α mAb, clone G281-2626, was purchased from Dainihon Pharmaceutical Co., Tokyo, Japan. Anti-Thy1·2 MoAb, clone F7D5, was purchased from Serotec Ltd, Oxford, UK. Low toxic rabbit complement was purchased from Cosmobio Co., Tokyo, Japan.

Animals

Female C3H/HeN mice were purchased from Seac Co., Ohita, Japan, maintained for at least 1 week in our laboratory and were then used for experiments at 6–8 weeks of age. All animal experiments were performed according to the guidelines for the care and use of animals approved by the University of Occupational and Environmental Health, Japan.

BCG and culture medium

BCG (Tokyo 172 strain) were kindly supplied by Japan BCG Production Co., Tokyo, Japan and were grown to the mid-log phase in Middlebrook 7H9 broth (Difco Laboratories Inc., Detroit, MI, USA) supplemented with the following enrichment: 10% albumin-dextrose-catalase (ADC) (Difco Laboratories Inc.), 0·2% glycerol and 0·5% Tween 80. The grown bacteria were washed and suspended in phosphate buffered saline (PBS) pH 7·4. The concentration of the bacteria suspension was adjusted spectrophotometrically at a 590-nm wave length. Killed BCG was prepared by heating the viable bacteria at 121°C for 30 min. After extensive washing of killed bacteria with PBS, the concentration was adjusted equal to that of viable BCG. Viability was tested by culturing on 7H10 agar and Ziehl–Neelsen staining confirmed that the structure of bacteria was intact.

Target cell for cytotoxicity

The target cell used in this study was N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT)-induced transitional cell carcinoma, MBT-2, from C3H/HeN mice [13].

In vitro culture of peritoneal exudate cells (PEC)

PEC were harvested from C3H/HeN mice that had been injected i.p. with 2 ml of 5% thioglycollate (Difco Laboratories Inc.) medium 3 days previously. Cells were collected by washing out the peritoneal cavity with PBS. PEC were composed of more than 90% macrophages and less than 10% lymphocytes by flow cytometric analysis. After washing several times with PBS, the cells were suspended in RPMI 1640–10% FCS medium. One hundred μl of PEC (1×10^5 cells/well) suspension were cultured in 96-well flat-bottomed microtitre culture plates (Falcon #3072, Becton Dickinson Co., Frankline Lake, NJ, USA). After 2 h incubation at 37°C in 5% CO_2 and 95% air, BCG (1×10^5 bacilli/well) and/or other agents were added, and final volumes in wells were adjusted to 200 μl. The culture supernatants were harvested after 12 h (for TNF-α) or 24 h (for IFN-γ). As a preliminary experiment, we confirmed that these culture periods were best for the assay of each cytokine. The amounts of IFN-γ in the culture supernatants were assayed by an enzyme-linked immunosorbent assay (ELISA) using capture MoAb, biotinylated detection MoAb, streptavidin-conjugated alkaline phosphatase and p-nitrophenyl-phosphate as a substrate (Zymed Laboratory Inc., San Francisco, CA, USA). PGE_2 was assayed with an enzyme immunoassay (EIA) kit (Cayman Chemical Co.) [9]. TNF-α was measured by a L929 bioassay [14].

Depletion of T cells and NK cells from PEC

PEC (1×10^5 cells/well) were seeded in 96-well flat-bottomed culture plates (Falcon #3072) at 37°C for 1 h in 5% CO_2 and 95% air. After that, anti-Thy-1 and/or anti-NK MoAb with complement were added to each well and cultured for a further 2 h. Non-adherent cells and dead cells were removed by aspirating the media. These treated PEC, composed of more than 98% Mac-1(+) cells and less than 2% T cells and NK cells as detected by a flow-cytometry, were used for the following experiments.

Cytotoxicity assay

One hundred μl of PEC (1×10^5 cells/well) suspension were seeded in 96-well flat-bottomed microtitre culture plates. After 2 h culture, BCG (1×10^5 bacilli/well) and/or other agents were added, and final volumes in wells were adjusted to 200 μl and incubated at 37°C for 12 h in 5% CO_2 and 95% air. MBT-2 murine bladder cancer cells were radiolabelled with 100 μCi of [^{51}Cr]sodium chromate (NEN Life Science Co., Tokyo, Japan) at 37°C for 12 h in 5% CO_2 and 95% air. After incubation, the cells were washed three times with PBS, detached by 0·25% trypsin (Difco Laboratories Inc.) and suspended at a concentration of 1×10^5/ml in RPMI-10% FCS medium. Fifty μl of ^{51}Cr-labelled target cells (5×10^3 cells/well) suspension were added to 96-well flat-bottomed microtitre culture plates containing 200 μl of effector cells which had been incubated with several agents for 12 h. After a further 20 h incubation, the supernatants were harvested and released ^{51}Cr was counted with a gamma counter.

For the experiment using culture inserts, PEC (5×10^5 cells/well) were cultured in 1 ml of medium in 24-well flat-bottomed microtitre culture plates (Falcon #3047) and ^{51}Cr-labelled target cells ($2{\cdot}5 \times 10^4$ cells/well) in 250 μl medium were added in the presence or absence of a cell culture insert (Falcon #3095). All assays were performed in triplicate. Spontaneous ^{51}Cr release was determined by incubating radiolabelled target cells in the absence of PEC. Maximal ^{51}Cr release was determined by incubating the same amount of target cells in 1% Triton X-100 (Sigma Chemical Co.). The percentage of specific cytotoxicity was calculated as follows:

$$\%(\text{cytotoxicity}) = [(\text{experimental release} - \text{spontaneous release}) / (\text{maximal release} - \text{spontaneous release})] \times 100$$

Statistical analysis

All determinations were made in triplicate and each result was expressed as the mean ± standard deviation (s.d.). Statistical significance was determined by paired Student's *t*-test. A *P*-value of 0·05 or less was considered significant [15].

RESULTS

Cytotoxic activity and cytokine production of PEC stimulated with BCG plus a COX inhibitor

Since PG is reported to have suppressive activity on cellular immunity, we studied the effect of inhibitors of PG synthesis, NS398 and IM, on BCG-induced PEC cytotoxicity. As shown in Fig. 1, BCG enhanced PEC-mediated cytotoxicity. Viable BCG enhanced PEC-mediated cytotoxicity more efficiently than killed BCG. In addition, NS398 and IM further enhanced viable BCG-

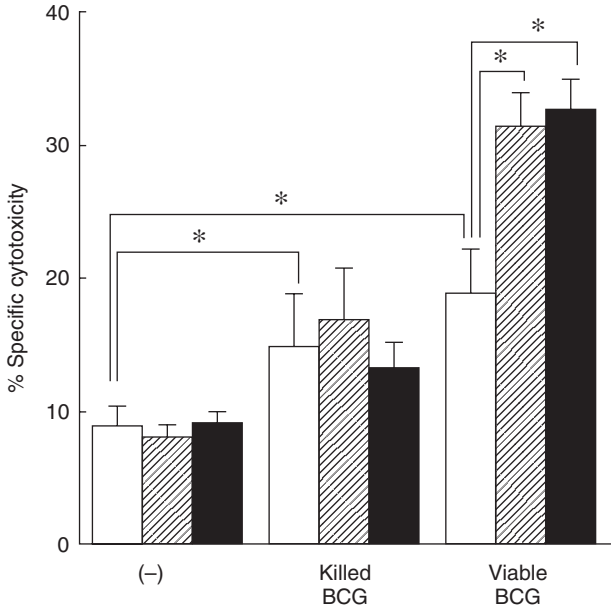

Fig. 1. Effect of NS398 or IM on cytotoxicity of PEC stimulated with BCG against MBT-2 cells. PEC (1×10^5) were treated with killed or viable BCG (1×10^5) in the absence or presence of NS398 or IM (1 μM) for 12 h, cultured with ^{51}Cr-labelled MBT-2 for a further 20 h, and then the released ^{51}Cr in the supernatants was counted. The effector/target ratio was 20/1. The results are expressed as the percentage specific killing of MBT-2 cells ± s.d. of triplicate cultures. *Significantly enhanced. □, (–); ▨, NS398; ■, IM.

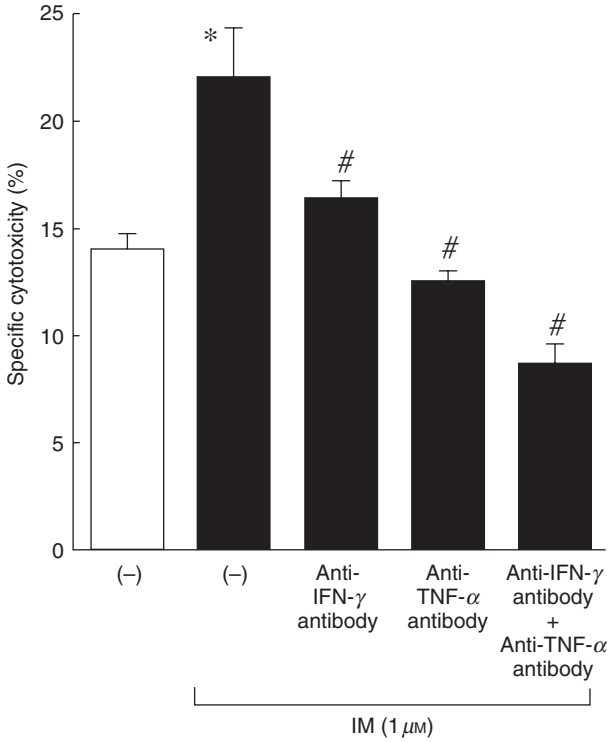

Fig. 2. Effect of anti-IFN-γ and/or anti-TNF-α antibody on cytotoxicity of PEC stimulated with BCG and a COX inhibitor. PEC (1×10^5) were stimulated with viable BCG (1×10^5) plus IM (1 μM) in the presence or absence of anti-IFN-γ (5 μg/ml) and/or anti-TNF-α (5 μg/ml) antibody for 12 h in 96-well plates, and then ^{51}Cr-labelled MBT-2 cells were seeded onto the effector cells. After a further 20 h incubation, released ^{51}Cr in the supernatants was counted. The effector/target ratio was 20/1. *Significantly increased from IM(–) group. #Significantly decreased from antibody (–) group.

induced cytotoxicity. There was no significant difference between the enhancing effect of NS398 and IM. Interestingly, NS398 and IM did not enhance killed BCG-induced cytotoxicity. Neither NS398 nor IM alone enhanced PEC-mediated cytotoxicity without BCG. The amount of PGE_2 in the culture supernatant is shown in Table 1. PEC without stimulators did not produce a detectable amount of PGE_2. However, BCG stimulated PEC to produce PGE_2. Viable BCG stimulated PEC more efficiently to produce PGE_2 than killed BCG. NS398 and IM (1 μM) completely diminished PGE_2 production. This suggests that PGE_2 produced by PEC stimulated with both killed and viable BCG depends on both COX-1 and COX-2.

To study the mechanism of COX inhibitor-induced enhancement of BCG-induced cytotoxicity, we measured cytokines in the culture supernatant. As shown in Table 1, viable BCG markedly stimulated PEC to induce IFN-γ and TNF-α. NS398 and IM further enhanced viable BCG-induced cytokine production. PEC stimulated with killed BCG only slightly induced IFN-γ, but not TNF-α production in the presence and absence of NS398 and IM. NS398 and IM alone did not enhance these productions.

Anti-IFN-γ and/or TNF-α antibodies reduced cytotoxicity of PEC stimulated with BCG plus a COX inhibitor

To determine more directly the participation of IFN-γ and TNF-α in COX inhibitor-induced enhancement of cytotoxic activity, we added anti-IFN-γ and/or anti-TNF-α antibody to the culture with BCG plus a COX inhibitor. As shown in Fig. 2, anti-IFN-γ, and especially anti-TNF-α antibody, significantly reduced the cytotoxicity of PEC against MBT-2. The combination of both

Table 1. Cytokine and prostaglandin production of PEC stimulated with killed and viable BCG and COX inhibitors[1]

Stimulator	Agents (1 μm)	IFN-γ (pg/ml)	TNF-α (U/ml)	PGE_2 (nM)
(–)	(–)	<32	<100	<0·01
	NS398	<32	<100	<0·01
	IM	<32	<100	<0·01
Killed BCG	(–)	118 ± 9	<100	2·2 ± 0·4*
	NS398	211 ± 22*	<100	<0·01
	IM	183 ± 17*	<100	<0·01
Viable BCG	(–)	967 ± 31	1028 ± 21	4·8 ± 0·7*
	NS398	2117 ± 124*	1200 ± 105*	<0·01
	IM	2429 ± 64*	1448 ± 172*	<0·01

[1]PEC were cultured with killed or viable BCG in the presence or absence of NS398 or IM for 12 h (for TNF-α) or for 24 h (for IFN = γ and PGE_2), and cytokines and PGE_2 in the culture supernatant were assayed. The results are expressed as mean ± s.d. of thriplicate cultures. *Significantly increased from the control group.

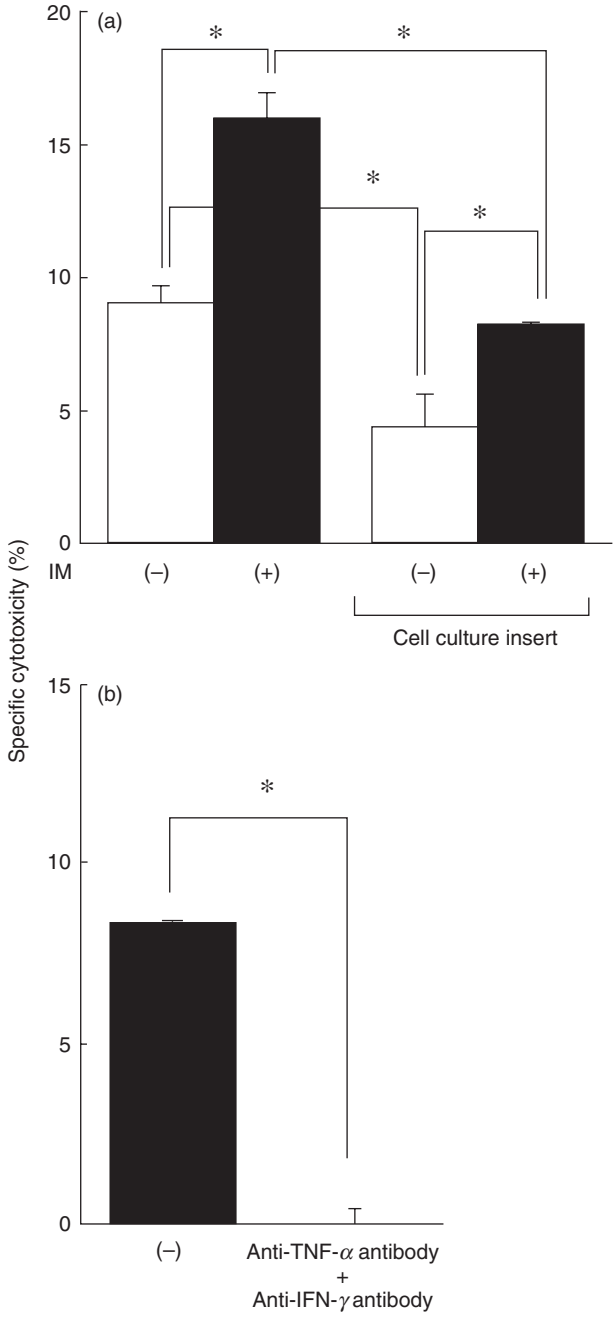

Fig. 3. Cytotoxic activity of PEC stimulated with BCG and a COX inhibitor in the presence of the culture insert. (a) PEC (5×10^5) were cultured with viable BCG (5×10^5) plus IM (1 μM) for 24 h in 24-well plates, and then ^{51}Cr-labelled MBT-2 cells were seeded onto the effector cells directly or indirectly using the cell culture insert. (b) PEC (5×10^5) were cultured with viable BCG (5×10^5) plus IM (1 μM) in the presence or absence of anti-IFN-γ (5 μg/ml) and/or anti-TNF-α (5 μg/ml) antibody for 12 h in 24-well plates, and then ^{51}Cr-labelled MBT-2 cells were seeded into the cell culture insert. After a further 20 h incubation, released ^{51}Cr in the supernatants was counted. The effector/target ratio was 20/1. *Significantly different.

antibodies completely inhibited the cytotoxicity to below the control level. Both IFN-γ and TNF-α in the culture supernatant were also diminished by these two antibodies (data not shown). These results indicate that the enhanced cytotoxicity of PEC induced

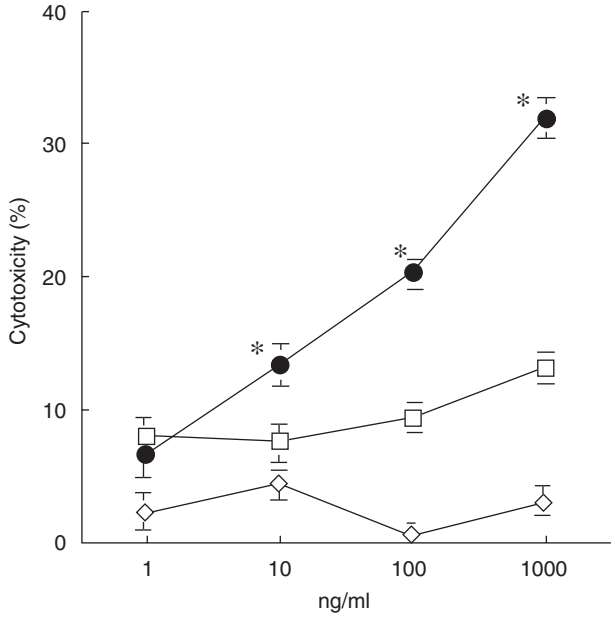

Fig. 4. Cytotoxic activity of IFN-γ and TNF-α on MBT-2 cells. ^{51}Cr-labelled MBT-2 cells (5×10^5) were cultured with various concentrations of IFN-γ, TNF-α or IFN-γ/TNF-α for 20 h and released ^{51}Cr in the supernatant was counted. *Significantly killed. □, IFN-γ, ◇, TNF-α; ●, IFN-γ+TNF-α.

with a COX inhibitor is mediated by enhanced production of IFN-γ and TNF-α.

To clarify the role of soluble factors in COX inhibitor-enhanced cytotoxicity, we used a cell culture insert to separate target cells from effector cells and assayed its cytotoxicity. As shown in Fig. 3a, in the presence of the cell culture insert the cytotoxic activity was diminished by about 50%. This means that BCG-induced cytotoxicity depends on both soluble factors and direct cell-to-cell killing. Cytotoxicity was again enhanced by BCG with a COX inhibitor both in the absence and presence of the cell culture insert. As shown in Fig. 3b, COX inhibitor-enhanced cytotoxicity in the presence of the cell culture insert was completely inhibited by the combination of anti-IFN-γ and anti-TNF-α antibody. These results suggest that BCG-induced cytotoxicity enhanced by a COX inhibitor was mediated mainly by soluble factors such as IFN-γ and TNF-α. In fact, exogeneous IFN-γ and TNF-α killed MBT-2 directly in a dose-dependent manner, while the cytotoxic activity of IFN-γ or TNF-α alone was marginal (Fig. 4).

Cytotoxic activity in T cells and NK cells-depleted PEC stimulated with BCG and a COX inhibitor

We depleted T cells and NK cells by antibody plus complement treatment, to study the participation of T cells and NK cells in BCG and COX inhibitor-induced cytotoxicity of PEC. The depletion of T cells decreased the cytotoxicity about by 30%, but the depletion of NK cells had no effect on the cytotoxicity in the presence or absence of a COX inhibitor (Fig. 5). Furthermore, the production of IFN-γ and TNF-α was also reduced by T cell depletion, but not by NK cell depletion. These results suggest that T cells play some roles in the expression of cytotoxicity of PEC induced with BCG and a COX inhibitor.

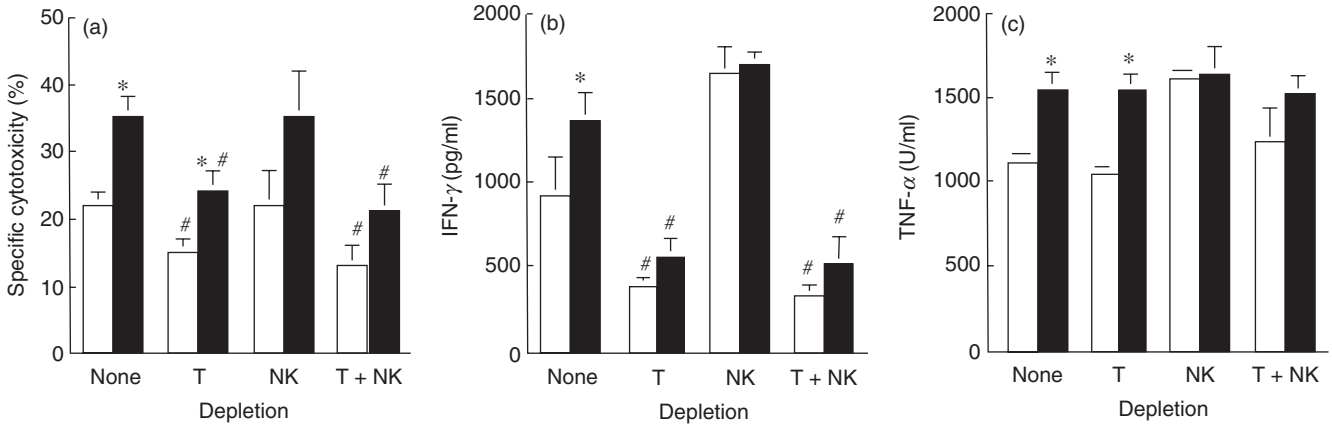

Fig. 5. Cytotoxic activity and cytokine production of T cell and/or NK cell-depleted PEC. (a) PEC (1×10^5) were cultured in 96-well culture plates for 1 h, treated with anti-Thy-1·2 and/or anti-NK antibody with a complement for 2 h, then the media were removed and replaced with fresh media. These treated PEC were stimulated with BCG (1×10^5) in the presence (■) or absence (□) of IM (1 μM) for 12 h, and then ^{51}Cr-labelled MBT-2 cells were added. After a further 20 h incubation, released ^{51}Cr in the supernatants was counted. (b) IFN-γ production in the supernatant was assayed 24 h after BCG stimulation. (c) TNF-α production in the supernatant was assayed 12 h after BCG-stimulation. *Significantly increased from IM (–) group. #Significantly decreased from non-depleted group.

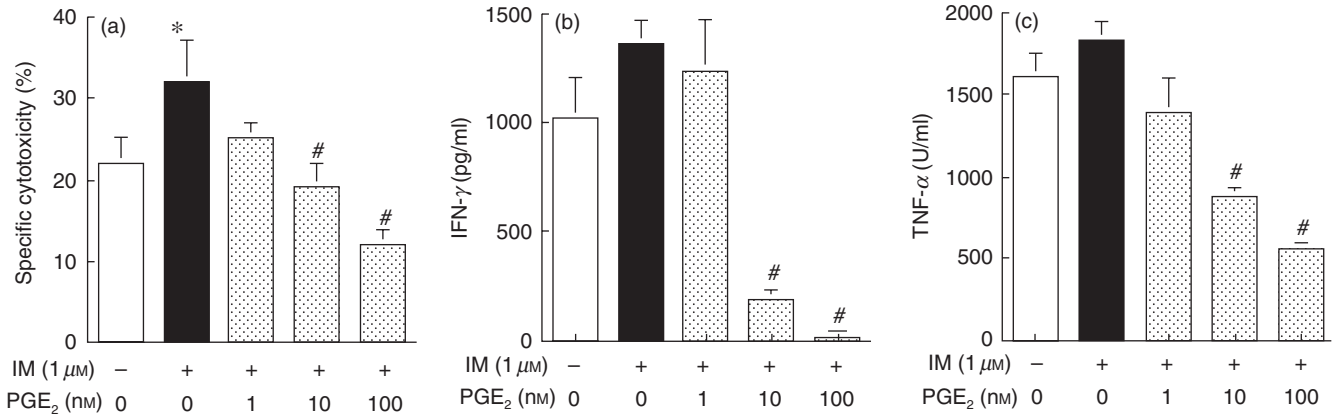

Fig. 6. Effect of exogenous PGE_2 on cytotoxicity and cytokine production of PEC stimulated with viable BCG against MBT-2 cells. (a) PEC (1×10^5) were treated with viable BCG (1×10^5) in the absence or presence of IM (1 μM) and various concentrations (1–100 nM) of exogenous PGE_2 for 12 h, incubated with ^{51}Cr-labelled MBT-2 for a further 20 h, and then the released ^{51}Cr in the supernatants was counted. The effector/target ratio was 20/1. (b) IFN-γ production in the supernatant was assayed 24 h after BCG-stimulation. (c) TNF-α production in the supernatant was assayed 12 h after BCG-stimulation. *Significantly increased from IM (–) group. #Significantly decreased from PGE_2 (–) group.

Exogenous PGE_2 suppresses BCG-induced cytotoxicity and cytokine production

To elucidate further the role of PGE_2 in viable BCG-induced cytotoxicity, the effect of exogenous PGE_2 on the cytotoxicity of PEC stimulated with viable BCG was studied. As shown in Fig. 6a, IM again enhanced cytotoxicity of BCG-treated PEC, while PGE_2 reduced the cytotoxicity of IM and BCG-treated PEC in a dose-dependent manner. Exogenous PGE_2 also suppressed IFN-γ and TNF-α production by IM and BCG-treated PEC in a dose-dependent manner (Fig. 6b,c).

DISCUSSION

It is reported that infection with *Mycobacterium tuberculosis* or *M. bovis* BCG induces PGs in human monocytes [16]. PGs inhibited immune effector cell functions such as the activity of cytotoxic T cells, NK cells and macrophages in a *Mycobacterium* infection model [17]. Other groups reported that arachidonic acid metabolites had immunosuppressive effects on mouse splenic macrophages infected with *Mycobacterium* [18]. In particular, PGE_2 produced at the early stage of infection inhibited TNF-α production in macrophages [19]. To elucidate the role of PGE_2 in BCG-induced macrophage cytotoxicity, we studied the effect of COX-1 and COX-2 inhibitors on the cytotoxic activity of BCG-activated macrophages against murine bladder cancer cells *in vitro*.

We found that two COX inhibitors, NS398 and IM, enhanced the cytotoxic activity of PEC stimulated with viable BCG, but did not enhance the cytotoxicity induced with killed BCG (Fig. 1). There was no significant difference in the enhancing activity on

BCG-induced cytotoxicity between NS398 and IM. PEC stimulated with viable BCG produced large amounts of PGE_2. However, the amount of PGE_2 produced by killed BCG-treated PEC was about half that of viable BCG-treated PEC (Table 1). Exogenous PGE_2 reduced viable BCG-induced cytotoxicity in a dose-dependent manner. These results suggest that the endogenous PGE_2 produced by BCG-activated macrophages down-regulates BCG-induced macrophage cytotoxicity.

In a previous paper we have reported that BCG-induced macrophage-mediated cytotoxicity was regulated by soluble factors such as IFN-γ and TNF-α [20]. We also found that IFN-γ and TNF-α production by PEC were down-regulated by PGE_2 and up-regulated by COX inhibitors (Fig. 6 and Table 1). The enhanced cytotoxicity of BCG-treated PEC by COX inhibitors was also reduced by the depletion of T cells and NK cells (Fig. 5). The participation of T cells and NK cells in this experiment seems to be mediated by cytokines such as IFN-γ, because cytokine production was also reduced by T cells and NK cells depletion and MBT-2 cells were not susceptible for T cells and NK cells.

It is reported that IL-12 is produced mainly by macrophages, playing an important role in the induction of IFN-γ production by T cells and NK cells. Furthermore, it was reported that PGE_2 was a potent inhibitor of IL-12 production [21,22]. In our study, IL-12 production by BCG-treated macrophages was down-regulated by exogenous PGE_2 as well as IFN-γ and TNF-α, but not enhanced by COX inhibitors (data not shown).

The important finding in this study is the difference between viable and killed BCG for the induction of macrophage-mediated cytotoxicity and PGE_2. Viable BCG induced macrophage-mediated cytotoxicity and produced IFN-γ, TNF-α and PGE_2 more efficiently than killed BCG. This seems to be caused by the longer survival of BCG in macrophages which can stimulate macrophages continuously.

PGE_2 plays an important role in the induction of inflammation and regulates negatively immune responses. Accordingly, depletion of PGE_2 results in the diminution of inflammation and the enhancement of immune responses such as cytokine production, as shown in this report. When PEC were stimulated with killed BCG, PGE_2 was also produced, but its amount was about half that induced with viable BCG. Killed BCG did not stimulate IFN-γ and TNF-α production significantly (Table 1), but antitumour activity was almost the same as viable BCG (Fig. 1). However, NS398 and IM did not enhance antitumour activity and cytokine productions of killed BCG-treated PEC.

In a previous paper we have reported that antitumour activity of BCG-treated PEC was mediated by both direct cell-to-cell contact killing and cytokines such as IFN-γ and TNF-α [20]. In fact, MBT-2 were susceptible to the killing activity of IFN-γ and TNF-α (Fig. 4). In the experiment using cell culture insert, the COX inhibitor seems to up-regulate both cytokines and cell-to-cell contact killing. However, killed BCG seem to induce only cell-to-cell contact killing in the presence or absence of a COX inhibitor, because killed BCG enhanced only small amounts of IFN-γ and TNF-α. Therefore, the suppressive effect of PGE_2 seems to be on cytokine production which enhances antitumour activity.

There are some reports that cancer cells produce PGE_2 [23,24]. We also studied the effect of PGE_2 and COX inhibitors on the growth of MBT-2 cells. We found that MBT-2 (1×10^5/ml) produced 1·9 nM of PGE_2. However, the growth of MBT-2 was not influenced by 1 μM of a COX inhibitor and exogeneous addition of PGE_2 (data not shown). Thus, the effect of PGE_2 and COX inhibitors seemed to be on PEC, but not on tumour cells directly, in this report.

In conclusion, PGE_2 produced by BCG-stimulated macrophages has a suppressive effect on macrophage-mediated cytotoxic activity and IFN-γ and TNF-α production, and COX inhibitors enhances these activities. Therefore, inhibitors of PG synthesis may be useful agents to reduce inflammatory responses and enhance BCG-immunotherapy.

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