Arch Pharm Res Vol 25, No 6, 910-916, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Comparative Study of the Endotoxemia and Endotoxin Tolerance on the Production of Th Cytokines and Macrophage Interleukin-6: Differential Regulation of Indomethacin

Byeong Suk Chae

College of Science and Engineering, Woosuk University, Samrae-Up, Jeonbuk, 565-701, Korea

(Received October 14, 2002)

Endotoxin tolerance reduces the capacity of monocytes to produce proinflammatory cytokines, results in cellular immune paralysis, and down-regulates the production of helper T (Th)1 type cytokines with a shift toward a Th2 cytokine response. Prostaglandin (PG)E_2 in the immune system also results in macrophage inactivation and the suppression of Th1 activation and the enhancement of Th2 activation. However, the inhibitory effects of PGE_2 on the altered polarization of the Th cell and macrophage interleukin (IL)-6 production characterized in part by cellular immune paralysis in a state of endotoxin tolerance is unclear. This study was undertaken, using indomethacin, to investigate the role of endogenous PGE_2 on the Th cytokines and macrophage IL-6 production in a state of endotoxin tolerance compared to those with endotoxemia mice, wherein, in this latter case, the increased production of proinflammatory cytokines and PGE_2 is exhibited. Endotoxemia was induced by injection of lipopolysaccharide (LPS; 10 mg/kg in saline) *i.p.* once in BALB/c mice, and endotoxin tolerance was induced by pretreatment with LPS (1 mg/kg in saline) injected *i.p.* daily for two consecutive days and then with LPS 10 mg/kg on day 4. Splenocytes or macrophages were obtained from endotoxemia and endotoxin tolerance models pretreated with indomethacin, and then cytokine production was induced by Con A-stimulated splenocytes for the Th cytokine assays and LPS-stimulated macrophages for the IL-6 assay. Our results showed that endotoxemia led to significantly reduced IL-2 and IL-4 production, to significantly increased IL-6 production, whereas interferon (IFN)-γ production was not affected. Indomethacin in the case of endotoxemia markedly attenuated IFN-γ and IL-6 production and didnt reverse IL-2 and IL-4 production. Endotoxin tolerance resulted in the significantly reduced production of IL-2 and IFN-γ and the significantly increased production of IL-4 and IL-6. Indomethacin in endotoxin tolerance greatly augmented IL-2 production, significantly decreased IL-4 production, and slightly attenuated IL-6 production. These findings indicate that endogenous PGE_2 may mediate the suppressed Th1 type immune response, with a shift toward a Th2 cytokine response in a state of endotoxin tolerance, whereas endotoxemia may be regulated differentially. Also, endogenous PGE_2 may mediate macrophage IL-6 production in the case of endotoxemia to a greater extent than in the case of endotoxin tolerance.

Key words: Endotoxin tolerance, PGE_2, Indomethacin, IL-2, IL-4, IL-6, IFN-γ

INTRODUCTION

Endotoxin, a component of the outer cell membrane of gram-negative bacteria, plays a central role in the pathogenesis of gram-negative sepsis through the action of proinflammatory cytokines. Even though it triggers the inflammatory cascade associated with sepsis, including the production of proinflammatory cytokines and PGE_2, complicated mediators induced by endotoxin lead to macrophage activity being enhanced and helper T cells being attenuated. Consequently, endotoxin has been used as a T-independent antigen promoting humoral immune response (Morrison and Ryan, 1979; Diao *et al.*, 2002).

Endotoxin tolerance reduces the capacity of monocytes to produce proinflammatory cytokines known to induce endotoxemic lethality, including tumor necrosis factor (TNF)-α and IL-1 (Munoz *et al.*, 1991), and enhances protection

Correspondence to: Byeong Suk Chae, College of Science and Engineering, Woosuk University, Samrae-Up, Jeonbuk, 565-701, Korea
E-mail: cbse@core.woosuk.ac.kr

against endotoxic lethality (Lehner *et al.*, 2001). However, endotoxin tolerance induces a state of cellular immune paralysis characterized by the suppressed production of Th1 cytokines and the reduced production of IL-12, which can lead to fatal blunting of immunological responses to subsequent infections in survivors of septic shock (Karp *et al.*, 1998; Volk *et al.*, 2000; Wysocka *et al.*, 2001).

A Th1-mediated response is known to enhance cell-mediated immunity, while a Th2-mediated response is associated with the humoral immunity that is elevated IgE levels and eosinophilia (Abbas *et al.*, 1996). The Th1/Th2 balance plays an important role in resistance to certain infections, in transplantation rejection, and in susceptibility to autoimmunity (Mureille and Leo, 1998). Endotoxin tolerance attenuates the production of Th1 cytokines, such as IL-2 and IFN-γ, induces an alteration of the Th1/Th2 balance (IFN-γ/ IL-4), and up-regulates the production of IL-4 which is responsible for the inflammation of IgE-mediated allergic disease (Castro *et al.*, 1998; Lauw, *et al.*, 2000). Endotoxin tolerance was found to decrease production of IL-6 in peripheral blood mononuclear cells (Dinarello *et al.*, 1991). In contrast, Chen *et al.* (1994) and Erroi *et al.* (1993) reported that IL-6 production was maintained or even increased in endotoxin tolerant macrophages.

On the other hand, PGE_2 plays a predominant role in the inflammatory response caused by gram-negative infection or LPS. PGE_2 is related to immune-suppression including T-lymphocytopenia, dysfunction of T lymphocytes, monocyte inactivation, and metastases in cancer patients (Choudhry, *et al.*, 1995; Menetrier-Caux *et al.*, 1999). PGE_2 results in the decreased capability of lymphocytes to produce Th1 cytokines, with a shift toward a Th2 cytokine response and the production of the immunoglobulin class tending to switch to IgE (Roper *et al.*, 1995; Betz and Fox, 1991). PGE_2 attenuates the activation and capability of T lymphocytes to produce Th1 cytokines with a shift toward a Th2 cytokine response, enhances the production of IL-4 and IL-5, and incites the production of the immunoglobulin class switching to IgE (Betz and Fox, 1991; Minakuchi *et al.*, 1990; Roper *et al.*, 1995). Endogenous PGE_2 has been reported to augment IL-6 production (Hinson *et al.*, 1996; Williams and Schacter, 1997).

On the production of PGE_2 during endotoxin tolerance, Rogers *et al.* (1986) reported that stimulation of prostaglandin and thromboxane B2 synthesis by endotoxin was significantly less in endotoxin tolerant macrophages compared to controls. Howes *et al.* (1985) demonstrated that, in rabbits tolerant to LPS, LPS failed to elevate PGE_2 and thromboxane B2 in the aqueous humor. Chemo *et al.* (1997) demonstrated that endotoxin tolerance is independent of the hypothalamic production of PGE_2, induced in response to the repeated administration of LPS. Chen *et al.* (1994) reported that endotoxin tolerance also attenuated the production of arachidonic acid metabolites as well as proinflammatory cytokines. In contrast, Li *et al.* (1994) reported that the induction of LPS tolerance significantly augmented IL-1 and PGE_2 release. Hafenrichter *et al.* (1994) reported that Kupffer cells, in the case of endotoxin tolerance, produced significantly more PGE_2 and IL-6 than nontolerant Kupffer cells.

The role of endogenous PGE_2 on the altered polarization of the Th cell, which is characterized in part by a state of cellular immune paralysis associated with endotoxin tolerance, remains unclear. Therefore, this study was carried out to investigate the inhibitory effect of PGE_2 synthesis using indomethacin, a potent PGE_2 synthesis inhibitor and anti-inflammatory agent, on Th cytokines and macrophage IL-6 production, in a state of endotoxin tolerance, as compared to their production in a state of endotoxemia, wherein, in this latter case, the increased production of proinflammatory cytokines and PGE_2 is exhibited.

MATERIALS AND METHODS

Animals

Female BALB/c mice (6 weeks of age) weighing 17-21 g were used. The animals were maintained on a 12-h light/dark cycle at 22 ± 2 and 50 ± 10% relative humidity throughout the whole experimental period, and held for a minimum of 4 days before treatment. Mice were given animal chow (Jeil Ind. Ltd., Korea) and provided tap water *ad libitum,* but were deprived of animal chow for 16 hr prior to sacrifice.

Induction of endotoxemia and endotoxin tolerance

To induce endotoxin tolerance, the BALB/c mice were injected intraperitoneally (*i.p.*) daily for 2 consecutive days with a dose of 1 mg/kg LPS (*Escherichia coli* Serotype 0127:B8, Sigma Co., Ltd., U.S.A.) diluted in saline, and they received *i.p* a challenge dose of LPS 10 mg/kg on day 4. To create the endotoxemia group (hereafter referred to as endotoxemia mice), endotoxemia was induced by *i.p.* treatment of LPS 10 mg/kg once and controls received saline with the same regimen.

Preparation of lymphoid cells and induction of cytokines

Splenocyte suspensions were prepared from saline-injected control, endotoxemia and endotoxin tolerant mice using Hanks' balanced salt solution (HBSS; Gibco Co., Grand Island, N.Y., U.S.A.). Erythrocytes in the single cell suspension were lysed by brief treatment with sterile 0.83% (wt/vol) ammonium chloride solution. Subsequently, the cells were washed three times with HBSS and resuspended into a suspension of 3×10^7 cells/ml with RPMI 1640 complete medium. The culture medium consisted of

RPMI 1640 (Gibco Co., Grand Island, N.Y., U.S.A.) supplemented with 10% fetal bovine serum, 100 U/ml penicillin G, 100 μg/ml streptomycin, 1 mM HEPES buffer, and 0.3 % sodium bicarbonate. The cell viability was determined by trypan blue exclusion and consistently exceeded 90%.

To induce cytokine release, 100 μl splenocyte suspensions (3×10^6 cells/well) were previously distributed into each well of a 96 well plate (Falcon 3047; Becton Dickinson, Franklin Lakes, NJ) and incubated in the presence or absence of 1 μM indomethacin (Sigma Co., Ltd., U.S.A) for 12 h at 37°C in a 5% CO_2 humidified incubator, and then stimulated with a final concentration 1 μg/ml concanavalin A (Con A: Sigma Co., Ltd., U.S.A.) for 48 h. The culture supernatants were harvested and stored at -70°C until IL-2, IL-4, and IFN-γ assays.

Preparation of macrophages and induction of IL-6

Peritoneal exudate macrophages from control, endotoxemia and endotoxin tolerant mice were harvested by peritoneal lavage with ice-cold sterile physiological saline 3 days after the *i.p.* injection of the mice with 3 ml of sterile 3% thioglycollate broth. Cells were washed, and resuspended in RPMI 1640 complement medium. Macrophages were allowed to adhere for 2 h at 37°C, 5% CO_2 incubation, the nonadherent cells were removed by washing three times with HBSS, and the macrophages were resuspended in fresh culture medium. Macrophages were plated at a density of 3×10^5 cells/100 μl in flat-bottom 96-microwells in the presence or absence of indomethacin (1 μM) with or without addition of PGE_2 (200 nM) for 12 h, and then the cells were stimulated with 1 μg/ml LPS for 24 h. The culture fluid was stored at -70°C until IL-6 analysis.

Cytokine detection

Cytokine levels in the supernatants were determined using ELISA (enzyme-linked immunosorbent assay) with commercial ELISA kits (R & D systems Inc., M.N., U.S.A.). All samples were assayed in duplicate. The results were measured in picograms per milliliter at 450 nm with correction at 540 nm using an ELISA microplate reader (Molecular Devices Co., Ltd., U.S.A.).

Statistical analysis

All data are expressed as means ± standard error (S.E.). Experiments were always run in duplicate and repeated at least twice. All data were examined for their statistical significance of differences with students' *t*-test.

RESULTS

Effects of indomethacin on the production of IL-2 in a state of endotoxin tolerance

These experiments were carried out to investigate the influence of the *in vitro* administration of indomethacin on the production of Th cytokines by Con A-stimulated splenocytes obtained from endotoxin tolerant mice compared to those with endotoxemia. As shown in Fig. 1, splenic IL-2 production was greatly reduced in both the endotoxemia and endotoxin tolerant mice compared to controls. Indomethacin markedly augmented the suppressed production of IL-2 in endotoxin tolerant mice, but did not affect the production of IL-2 in the endotoxemia mice.

Effects of indomethacin on the production of IL-4 in a state of endotoxin tolerance

As shown in Fig. 2, splenic IL-4 production by the Con A-stimulated splenocytes was significantly reduced, by 36.8%, in the endotoxemia mice compared to controls, while IL-4 production was greatly augmented, by 32.5%, in the endotoxin tolerant mice. Indomethacin did not affect the decreased production of IL-4 in the endotoxemia mice, but markedly attenuated, by 37.6%, the increased production of IL-4 in endotoxin tolerant mice.

Effects of indomethacin on the production of IFN-γ in a state of endotoxin tolerance

As shown in Fig. 3, splenic IFN-γ production was not altered in the endotoxemia mice compared to controls, while IFN-γ production was greatly reduced in the endotoxin tolerant mice. Indomethacin greatly down-regulated IFN-γ

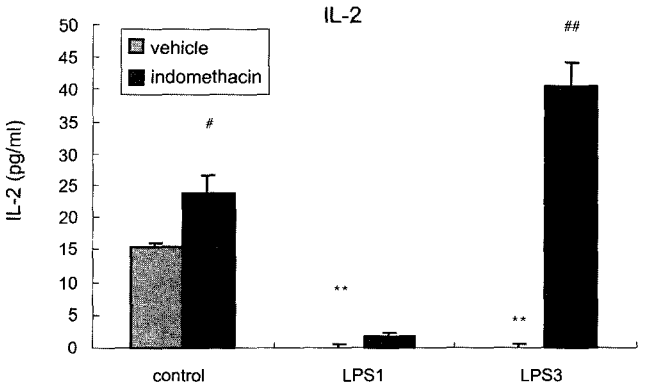

Fig. 1. IL-2 production by Con A-stimulated splenocytes following pretreatment with indomethacin. LPS1: Endotoxemia group induced by *i.p.* injection of LPS 10 mg/kg once. LPS3: Endotoxin tolerant group injected *i.p.* daily for 2 consecutive days with a dose of 1 mg/kg LPS diluted in saline and received *i.p* a challenge dose of LPS 10 mg/kg on day 4. Control: Control group received saline *i.p.* with same regimen. To induce cytokines, 100 μl splenocyte suspensions (3×10^6 cells/well) were incubated in the presence or absence of indomethacin (1 μM) for 12 h, and then stimulated with a final concentration 1 μg/ml Con A for 48 h. Cytokine levels in splenic supernatants were obtained using ELISA. Each value represents the mean ± S.E. of 10 mice. **($P<0.01$): Significantly different from the value in vehicle-treated controls. #($P<0.05$) and ##($P<0.01$): Significantly different from the value in each vehicle-treated group.

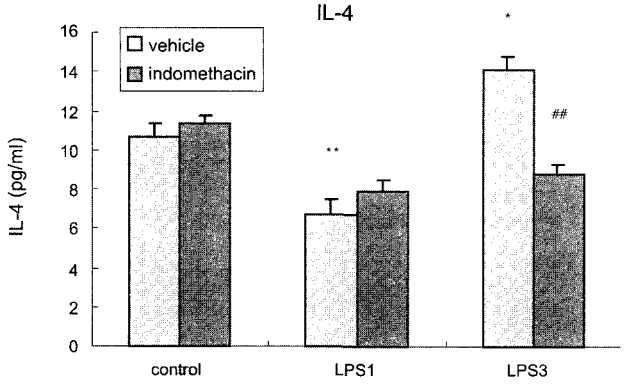

Fig. 2 IL-4 production by Con A-stimulated splenocytes following pretreatment with indomethacin. Each value represents the mean ± S.E. of 10 mice. Other legends and methods are the same as in Fig. 1. *(P<0.05) and **(P<0.01): Significantly different from the value in vehicle-treated controls. ##(P0.01): Significantly different from the value in each vehicle-treated group.

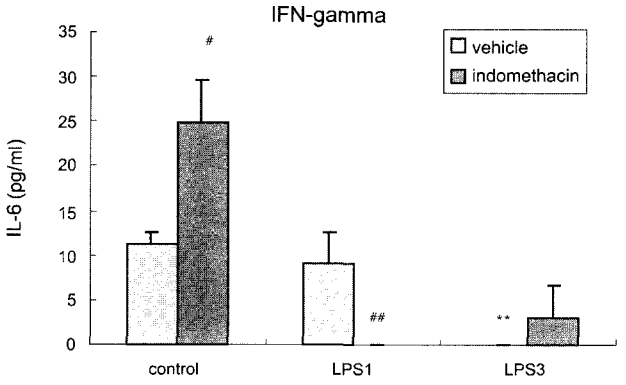

Fig. 3 IFN-γ production by Con A-stimulated splenocytes following pretreatment with indomethacin. Each value represents the mean ± S.E. of 10 mice. Other legends and methods are the same as in Fig. 1. **(P<0.01) Significantly different from the value in vehicle-treated controls. #(P<0.05) and ##(P<0.01): Significantly different from the value in each vehicle-treated group.

production in the endotoxemia mice, and insignificantly enhanced the decreased production of IFN-γ in endotoxin tolerant mice.

Effects of indomethacin on the LPS-stimulated production of IL-6 by macrophages obtained from endotoxin tolerant mice

This experiment was performed to determine the effects of indomethacin on the LPS-stimulated production of IL-6 by macrophages from endotoxin tolerant mice. LPS-stimulated production of IL-6 was not altered in the endotoxin tolerant mice compared to those with the endotoxemia mice (Fig. 4). Indomethacin significantly decreased by approximately 38.9% IL-6 production by LPS-stimulated macrophages in the endotoxemia mice, while it slightly attenuated such production in the endotoxin tolerant model.

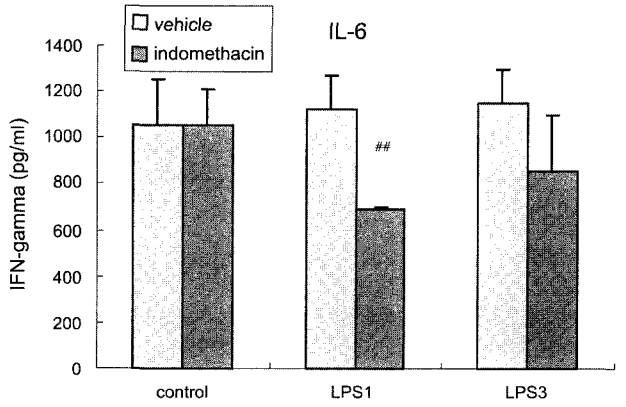

Fig. 4. Effects of indomethacin on the LPS-stimulated production of IL-6 by macrophages. Peritoneal exudate macrophages from control, endotoxemia and endotoxin tolerant mice were harvested by peritoneal lavage with ice-cold sterile physiological saline 3 days after *i.p.* injection of mice with 3 ml of sterile 3% thioglycollate broth. 100 μl macrophage suspensions (3×10^5 cells/well) incubated in the presence or absence of indomethacin (1 μM) for 12 h, and then stimulated with 1 μg/ml LPS for 24 h. Cytokine levels in macrophage supernatants were obtained using ELISA. Each value represents the mean ± S.E. of 10 mice. ##(P< 0.01): Significantly different from the value in each vehicle-treated group.

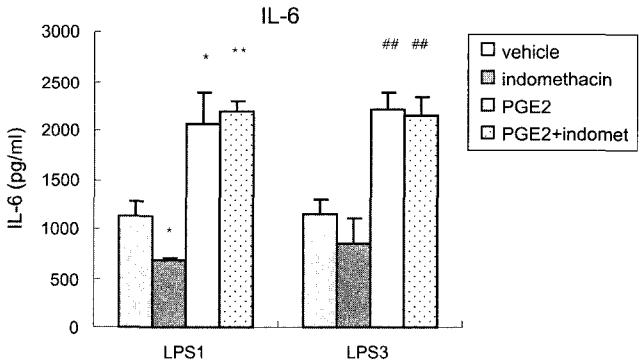

Fig. 5. Effects of exogenous PGE_2 on the indomethacin-reduced production of IL-6 by macrophages stimulated by LPS. LPS1: Endotoxemia group induced by *i.p.* injection of LPS 10 mg/kg once. LPS3: Endotoxin tolerant group injected *i.p.* daily for 2 consecutive days with a dose of 1 mg/kg LPS diluted in saline and received *i.p* a challenge dose of LPS 10 mg/kg on day 4. 100 μl peritoneal macrophage suspensions (3×10^5 cells/well) from control, endotoxemia and endotoxin tolerant mice were incubated in the presence or absence of indomethacin (1 μM) with/without 200 nM PGE_2 addition for 12 h, and then stimulated with 1 μg/ml LPS for 24 h. Each value represents the mean ± S.E. of 10 mice. Other legends and methods are the same as in Fig. 4. *(P< 0.05) and **(P<0.01): Significantly different from the value in vehicle-treated LPS1. ##(P<0.01): Significantly different from the value in vehicle-treated LPS3.

Effects of exogenous PGE_2 on the indomethacin-reduced production of IL-6 by macrophages stimulated by LPS

This experiment was performed to investigate the effects of exogenous PGE_2 on the indomethacin-reduced produc-

tion of IL-6 by macrophages stimulated by LPS. The addition of PGE_2 significantly increased by 83.4% and 92.2% IL-6 production by the LPS-stimulated macrophages obtained from endotoxemia and endotoxin tolerant mice, respectively (Fig. 5). Exogenous PGE_2 synergistically reversed the indomethacin-reduced production of IL-6 in both the endotoxemia and endotoxin tolerance groups.

DISCUSSION

This study was undertaken to investigate the role of PGE_2 using indomethacin on the cellular immune paralysis induced during periods of endotoxin tolerance, and which is characterized by the suppression of LPS-induced Th1 cytokine production. These results show that the induction of endotoxemia led to significantly reduced splenic IL-2 and IL-4 production, to significantly increased macrophage IL-6 production, and to unchanged splenic IFN-γ production, while the administration of indomethacin in endotoxemia induced mice markedly attenuated IFN-γ and IL-6 production and didn't reverse IL-2 and IL-4 production. Endotoxin tolerance results in the significantly reduced production of IL-2 and IFN-γ and the significantly increased production of IL-4 and IL-6, whereas the administration of indomethacin in the case of endotoxin tolerance greatly augmented IL-2 production, significantly decreased IL-4 production, and slightly attenuated IL-6 production.

IL-2 is a Th1 cytokine produced primarily by activated T cells and T cell growth factor. As mentioned above, both endotoxin tolerance and PGE_2 result in the attenuation of IL-2 production by Th1 cells (Benz and Fox, 1991; Choundhry *et al.*, 1995). As shown by our results (Fig. 1), splenic IL-2 production was significantly decreased in both the endotoxemia and endotoxin tolerant mice compared to controls. Indomethacin didn't reverse the suppressed production of IL-2 in the endotoxemia mice, but did bring about such a reversal in the case of endotoxin tolerance. Choudhry *et al.* (1999) demonstrated that PGE_2 released during sepsis induces T cell IL-2 down-regulation. Therefore, our findings suggest that the decreased capacity of Th1 cells to produce IL-2 in a state of endotoxin tolerance may be mediated by endogenous PGE_2, while endotoxemia may enable IL-2 to be regulated by other pathway.

IL-4, a Th2 cytokine, plays an important role in B lymphocyte activation and promotes immunoglobulin synthesis by B lymphocytes. IL-4 promotes the development of Th2-like CD4 T cells and inhibits the development of Th1-like T cells (Swain *et al.*, 1990). IL-4 also induces IL-6 production to sequentially switch first to IgG1 and then to IgE (Howells *et al.*, 1991). Our results showed that IL-4 production was significantly reduced in endotoxemia mice compared to controls, while IL-4 production was significantly enhanced in endotoxin tolerant mice (Fig. 2). However, indomethacin didn't reverse the suppressed production of IL-4 in the endotoxemia mice, but did significantly down-regulate the increased production of IL-4 in the endotoxin tolerant animals. Perez-Santos and Talamas-Rohana (2001) reported that the immune response to infection is associated with a increased Th2 type cellular immune response (IL-10 and IL-4) and that indomethacin increased IL-12 and IFN-γ production, while the IL-10 and IL-4 levels were not changed. Kaur *et al.* (1999) reported that PGE_2 promotes the development of a Th2 response by enhancing IL-4 production. Also, endotoxin tolerance tends to polarize the immune system toward a Th2 type response (Castro *et al.*, 1998; Lauw, *et al.*, 2000). Therefore, our results indicate that endogenous PGE_2 may mediate the increased production of IL-4 by Th2 cells in the case of endotoxin tolerance, but may not contribute to the decreased production of IL-4 in the case of endotoxemia. As shown in Fig. 1 and Fig. 2, our findings also indicate that a down-regulated Th1 type immune response with a shift toward a Th2 cytokine response may be mediated by endogenous PGE_2 in a state of endotoxin tolerance.

IFN-γ, which is produced in Th1 cells, natural killer cells, and macrophages, is associated with the ability to facilitate cytotoxic lymphocyte responses, and attenuates IL-4 production by inhibiting the proliferation of Th2 cells (Coffman and Carty, 1986). Endotoxin is a potent activator of mococytes/macrophages and upregulates the production of IFN-γ in T cells (Morrison and Ryan, 1979), while endotoxin tolerance leads to the suppressed production of IL-12 due to dysfunction of monocytes and the decreased production of IFN-γ by Th1 cells (Karp *et al.*, 1998; Lauw *et al.*, 2000). PGE_2 reduces IFN-γ production by Th1 cells, and potently inhibits production of IL-12, which promotes IFN-γ production (Betz and Fox, 1991; van der Pouw Kraan *et al.*, 1995; Harizi *et al.* 2002). The results of the present study (Fig. 3) support the hypothesis that the down-regulated production of IL-12 during periods of endotoxin tolerance results in the decreased production of IFN-γ (Karp *et al.*, 1998; Lauw *et al.*, 2000). In our study, indomethacin significantly down-regulated IFN-γ production in the case of endotoxemia, but slightly enhanced IFN-γ production in the endotoxin tolerant model. Therefore, our results indicate that the decreased production of IFN-γ in the case of endotoxin tolerance may be more related to the reduced production of IL-12 than to the presence of endogenous PGE_2. Further studies are needed to determine the exact mechanism by which indomethacin influences the decreased production of IFN-γ in the case of endotoxemia.

IL-6 mediates the acute phase response by acting as an endogenous pyrogen, is an important co-factor in IL-4 dependent IgE synthesis (Howells *et al.*, 1991), and has also been found to be involved in the pathogenesis of cancer

cachexia (Barton, 2001). Endotoxin has been reported to augment IL-6 production (Chai *et al.*, 1996; Hinson *et al.*, 1996) Endotoxin tolerance has been reported to suppress IL-6 production (Coffman and Carty, 1986; Schade *et al.*, 1999) However, our results demonstrated that endotoxemia promoted the production of IL-6 by LPS-stimulated macrophages, while inducing endotoxin tolerance had no effect on these results compared to those with the endotoxemia animals (Fig. 4). These data support the report of Chen *et al.* (1994) that in endotoxin-tolerant macrophages, IL-6 production is maintained or even increased. It has also been reported that endogenous PGE_2 augmented IL-6 production by peritoneal macrophages (Chai *et al.*, 1996; Hinson *et al.*, 1996; Williams and Shacter, 1997), and that PGE_2 upregulated IL-6 release during endotoxemia (Roper *et al.*, 1995). In the present study, indomethacin significantly decreased the LPS-induced production of IL-6 in the case of endotoxemia, while slightly decreased it in endotoxin tolerance (Fig. 4). Therefore, these observations indicate that both endotoxemia and endotoxin tolerance result in an enhancement of the LPS-stimulated production of IL-6 by macrophages, and that endogenous PGE_2 may mediate macrophage IL-6 production in the case of endotoxemia, while endotoxin tolerance may result in IL-6 production being regulated differentially.

In our study, exogenous PGE_2 significantly enhanced the indomethacin-reduced production of IL-6 in both endotoxemia and endotoxin tolerant mice compared to controls (Fig. 5). Iwahashi *et al.* (2000) reported that PGE_2 pretreatment synergistically stimulated the LPS-induced expression of IL-6 genes in mouse macrophages. Meyer *et al.* (1994) reported that endotoxin and PGE_2 interacted synergistically to stimulate IL-6 release from intestinal epithelial cells but that indomethacin blunted the effect of endotoxin on IL-6 production. These findings suggest that PGE_2 synergistically enhances the LPS-induced production of IL-6 by macrophages, and that indomethacin may not affect the PGE_2-induced production of IL-6 by macrophages obtained from mice having either endotoxemia or endotoxin tolerance.

In conclusion, these findings suggest that endogenous PGE_2 may mediate the suppressed Th1 type immune response, with a shift toward a Th2 cytokine response in a state of endotoxin tolerance, whereas endotoxemia may be regulated differentially. Also, endogenous PGE_2 may mediate macrophage IL-6 production in the case of endotoxemia to a greater extent than in the case of endotoxin tolerance.

ACKNOWLEDGMENTS

This research was supported by a grant from Woosuk University.

REFERENCES

Abbas, A. K., Murphy, K. M. and Sher, A., Functional diversity of helper T lymphocytes. *Nature,* 383, 787-793 (1996).

Barton, B. E., IL-6-like cytokines and cancer cachexia: consequences of chronic inflammation. *Immunol. Res.*, 23(1), 41-58 (2001).

Betz, M. and Fox, B., Prostaglandin E_2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J. Immunol.*, 146, 108-113 (1991).

Castro, A., Bemer, V., Nobrega, A., Coutinho, A. and Truffa-Bachi, P., Administration to mice of endotoxin from gram-negative bacteria leads to activation and apoptosis of T lymphocytes. *Eur. J. Immunol.*, 28 488-495 (1998).

Chai, Z., Gatti, S., Toniatti, C., Poli, V. and Bartfai, T., Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1ß: a study on IL-6-deficient mice. *J. Exp. Med.*, 183, 311-316 (1996).

Chemo, A., Fraifeld, V., Adramovich, L., Sod-Moriah, U. A. and Kaplanski, J., Tolerance to lipopolysaccharide is not related to the ability of the hypothalamus to produce prostaglandin E_2. *Life Sci.,* 61(8), 813-818 (1997).

Chen, H., Halushka, P. V. and Cook, J. A., Endotoxin tolerance: effects on lethality and macrophage thromboxane (B_2) and interleukin 6 production. *Shock,* 1, 366-371 (1994).

Choudhry, M. A., Ahmad, S. Ahmad, Z. and Sayeed, M. M., Prostaglandin E_2 down-regulation of T-cell IL-2 production is independent of IL-10 during gram-negative sepsis. *Immunol. Lett.* 67(2) 125-130 (1999).

Choudhry, M. A., Ahmad, S. and Sayeed, M. M., Role of Ca^{2+} in prostaglandin E_2-induced T-lymphocyte proliferative suppression in sepsis. *Infect. Immun.*, 63(8), 3101 (1995).

Coffman, R. L. and Carty, J., A T cell activity that enhances polyclonal IgE production and its inhibition by interferon. *J. Immunol.*, 136, 949-954 (1986).

Diao, H., Kohanawa, M. Y., Nakajima, H., Sato, Y., Minagawa, T. and Nakane, A. Y., Lipopolysaccharide triggers invasive streptococcal disease in mice through a tumor necrosis factor-alpha-dependent mechanism. *Immunology,* 105(3), 344-349 (2002).

Dinarello, C. A., Cannon, J. G., Mancilla, J., Bishai, I., Lees, J. and Coceani, F., Interleukin-6 as an endogenous pyrogen: induction of prostaglandin E_2 in brain but not in peripheral blood mononuclear cells. *Brain Res.*, 562, 199-206 (1991).

Erroi, A., Fantuzzi, G., Mengozzi, M., Sironi, M., Orencole, S. F., Clark, B. D., Dinarello, C. A., Isetta, A., Gnocchi, P., Giovarelli, M., et al., Differential regulation of cytokine production in lipopolysaccharide tolerance in mice. *Infect. Immun.*, 61(10), 4356-4359 (1993).

Hafenrichter, D. G., Roland, C. R., Mangino, Mj. and Flye, M. W., The Kupffer cell in endotoxin tolerance: mechanisms of protection against lethal endotoxemia. *Shock*, 2(4), 251-256

(1994).

Harizi, H., Juzan, M., Pitard, V., Moreau, J. F. and Gualde, N., Cycloxygenase-2-issued prostaglandin e(2) enhances the production of endogenous IL-10, which down-regulateds dendritic cell functions. *J. Immunol,* 168(5), 2255-2263 (2002).

Hinson, R. M., Williams, J. A. and Shacter, E., Elevated interleukin 6 is induced by prostaglandin E_2 in a murine model of inflammation: possible role of cyclooxygenase-2. *Proc. Natl. Acad. Sci. U.S.A.,* 93, 4885-4890 (1996).

Howells, G., Pham, P., Taylor, D., Foxwell, B. and Feldmann, M., Interleukin 4 induces interleukin 6 production by endothelial cells: synergy with interferon-gamma. *Eur. J. Immunol.*, 21(1), 97-101 (1991).

Howes, E. L., Jr, Goldyne, M. E., Perez, H. D., Goldstein, I. M. and Rosenbaum, J. T., Lipopolysaccharide tolerance inhibits eye inflammation. I. Reduced immune complex or lipopolysaccharide effects. *Arch. Ophthalmol.*, 103(2), 257-260 (1985).

Iwahashi, H., Takeshita, A and Hanazawa, S., Prostaglandin E_2 stimulates AP-1-mediated CD14 expression in mouse macrophages via cyclic AMP-dependent protein kinase A. *J. Immunology,* 164, 5403-5408 (2000).

Karp, C. L. M., Wysocka, M., Ma, X., Marovich, M., Factor, R. E., Nutman, T., Armant, M., Wahl, L., Cuomo, P. and Trinchieri, G., Potent suppression of IL-12 production from monocytes and dendritic cells during endotoxin tolerance. *Eur. J. Immunol.*, 28, 3128 (1998).

Kaur, K., Harris, S. G., Padilla, J., Graf, B. A. and Phipps, R. P., Prostaglandin E_2 as a modulator of lymphocyte mediated inflammatory and humoral responses. *Adv. Exp. Med. Biol.*, 469, 409-411 (1999).

Lauw, F. N., Ten Hove, T., Dekkers, P. E. P., D. E. Jonge, E., Van Deventer, S. J. H. and Van Der Poll, T., Reduced Th1, but not Th2, cytokine production by lymphocytes after *in vivo* exposure of healthy subjects to endotoxin. *Infection and immunity,* 68(3), 1014-1018 (2000).

Lehner, M. D., Ittner, J., Bundschuh, D. S., van Rooilen, N., Wendel, A. and Hartung, T., Improved innate immunity of endotoxin-tolerant mice increases resistance to *Salmonella enterica* Serovar Typhimurium infection despite attenuated cytokine response. *Infection and Immunity,* 69(1), 463-471 (2001).

Li, M. H., Seatter, S. C., Manthei, R., Bubrick, M. and West, M. A., Macrophage endotoxin tolerance: effect of TNF or endotoxin pretreatment. *J. Surg. Res.*, 57(1), 85-92 (1994).

Menetrier-Caux, C., Bain, C., Favrot, M. C., Duc, A. and Blay, J. Y., Renal cell carcinoma induces interleukin 10 and prostaglandin E_2 production by monocytes. *Br. J. Cancer,* 79(1), 119 (1999).

Meyer, T. A., Noguchi, Y., Ogle, C. K., Tiao, G., Wang, J. J., Fisher, J. E. and Hasselgren, P. O., Endotoxin stimulates interleukin-6 production in intestinal epithelial cells. A synergistic effect with prostaglandin E_2. *Arch. Surg.*, 129(12), 1290-1295 (1994).

Minakuchi, R., Wacholtz, M., Davis, L. and Lipsky, P., Delineation of the mechanism of inhibition of human T cell activation by PGE_2. *J. Immunol.*, 145, 2616-2625 (1990).

Morrison, D. C. and Ryan, J. L., Bacterial endotoxins and host immune responses. *Adv. Immunol.*, 28, 293-304 (1979).

Munoz, C., Carlet, J., Fitting, C., Misset, B., Bleriot, J. P. and Cavaillon, J. M., Dysregulation of *in vitro* cytokine production by monocytes during sepsis. *J. Clin. Investig.*, 88, 1747-1754 (1991).

Mureille, E. and Leo, O., Revising the Th1/Th2 paradigm. *Scand. J. Immunol.*, 47, 1-9 (1998).

Perez-Santos, J. L and Talamas-Rohana, P., In vitro indomethacin administration upregulates interleukin-12 production and polarizes the immune response toward a Th1 type in susceptible BALB/c mice infected with Leishmania mexicana. *Parasite immunol.* 23(11) 599-606 (2001).

Rogers, T. S., Halushka, P. V., Wise, W. C. and Cook, J. A., Differential alteration of lipoxygenase and cyclooxygenase metabolism by rat peritoneal macrophages induced by endotoxin tolerance. *Prostaglandins*, 31(4), 639-650 (1986).

Roper, R., Brown, D., and Phipps, R. Prostagalndin E_2 promotes B lymphocyte Ig isotype switching to IgE. *J. Immunol.*, 154, 162-170 (1995).

Schade, F. U., Flash, R., Flohe, S., Majetschak, M., Kreuzfelder, E., Dominguez-Fernandez, E., Borgermann, J., Reuter, M. and Obertacke, U., Endotoxin tolerance. *In Endotoxin in health and disease*. Brade, H., Opal, S. M., Vogel, S. N. and Morrison, D. C., eds Marcel Dekker, New York, p 50 (1999).

Swain, S. L., Weinberg, A. D., English, M. and Huston, G., IL-4 directs the development of Th2-like helper effectors. *J. Immunol.*, 145(11), 3796-806 (1990).

van der pouw Kraan, T. L., Boeije, L., Smeenk, R., Wijdenes, J. and Aarden, L., Proataglandin-e2 is a potent inhibitor of human interleukin 12 production. *J. Exp. Med.*, 181, 775-779 (1995).

Volk, H. D., Reinke, P. and Docke, W. D., Clinical aspects: from systemic inflammation to "immunoparalysis." *Chem. Immunol.*, 74, 162 (2000).

Williams, J. A. and Shacter, E., Regulation of macrophage cytokine production by prostaglandin E_2: distinct roles of cyclooxygenase-1 and 2. *J. Biol. Chem.*, 272 (41), 25693-699 (1997).

Wysocka, M., Robertson, S., Riemann, H., Caamano, J., Hunter, C., Mackiewicz, A., montaner, L. J., Trinchieri, G. and Karp, C. L., IL-12 suppression during experimental endotoxin tolerance: Dendritic cell loss and macrophage hyporesponsiveness. *J. Immunol.*, 166, 7504-7513 (2001).