Natural Product Sciences
16(4) : 271-279 (2010)

Bamboo Culm Extract Attenuates Early Development of Systemic Inflammation in Pristane-Primed Lupus Mice

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Abstract – Systemic lupus erythematosus (SLE) is characterized by systemic inflammation through production of inflammatory mediators and signaling abnormalities between T- and B- cells, leading to autoantibody production and multiorgan injuries. This study was investigated whether bamboo culm extract (BC) attenuates development of lupus systemic inflammation in the early stage in pristane-induced lupus mice. The pristane-induced lupus mice were administrated with BC 0.5 ml/kg or PBS and healthy mice with PBS orally once a day for 14 days. Our results showed that BC remarkably attenuated levels of serum TNF-α, IL-6, IL-10, IFN-γ, PGE_2, and VEGF, production of macrophages IL-6 and PGE_2 and expression of macrophages IL-6 and COX-2 mRNA in the presence or absence of LPS in pristane-induced lupus mice. Also, BC remarkably reduced expression of CD40L on the splenic T cells and CD80 on the splenic B cells and upregulated the reduced apoptosis of splenic T cells and CD4+ T cells in pristane-induced lupus mice. Therefore, these findings suggest that BC may attenuate early development of lupus systemic inflammation via downregulation of inflammatory mediators and amelioration of abnormal signaling between T cells and B cells.
Keywords – Bamboo culm, PGE_2, lupus, proinflammatory cytokine, VEGF, costimulatory molecule

Introduction

Systemic lupus erythematosus (SLE) is a T cell-dependent autoimmune disease characterized by humoral autoimmunity and inflammatory injuries of multiorgan. (Reininger *et al*., 1996; Hoffman, 2004; Pollard *et al*., 2004; Kyttaris *et al*., 2005; Takeuchi *et al*., 2005). Hyperactivity of lupus Th cells is known to be associated with over-reactive B cells leading to production of autoantibody. Th 1 cytokines, such as IL-6, IL-10, and IFN-γ, has been reported to contribute to autoantibody production, organ damage and high mortality in lupus (Llorente *et al*., 1995; Theofilopoulos *et al*., 2001; Ishihara *et al*., 2002; Chun *et al*., 2007). It has been reported that TNF-α apparently plays a significant role in the inflammatory process in lupus (Aringer and Smolen, 2003). PGE_2, an IL-6 inducer, is also a novel therapeutic target on the lupus inflammation (Hinson *et al*., 1996; Akaogi *et al*., 2006; Chae *et al*., 2008).

The signaling abnormalities between T- and B- cells and decreased apoptosis of activated T cells are associated with over-reactive B cells and lupus inflammation in lupus. The decreased spontaneous apoptosis of T cells in SLE leads to potentially decreased regulatory function (Anderson and Harken, 1990). Abnormal expression of CD40L, CD80 and CD86 on lupus lymphocytes or antigen presenting cells (APCs), including B cells, plays an important role in most features of systemic lupus, including autoantibody production and organ inflammation (Wu *et al*. 1995; Kinoshita *et al*., 2000).

Bamboo has been used as a traditional folk remedy for many centuries in the Orient. Bamboo has anti-fatigue activity, anti-oxidative capacity, anti-inflammatory effect, neuroprotective activity, and anti-tumor activity (Zhang *et al*., 2006; Ito *et al*., 2007; Lee *et al*., 2008; Seki *et al*., 2008). Bamboo culm extract has been reported to attenuate cell adhesion molecule expression and NFkappaB activity through suppression of the oxidative stress (Lee *et al*., 2008). Recently, our previous study demonstrated that bamboo culm extract downregulated activation of NKT- and B- cells and production of IL-6 in the late stage in pristane-induced lupus mice (Chae and Park, 2009). However, whether Bamboo culm extract (BC) attenuates early development of systemic inflammation in lupus remains not clear.

We observed that BC attenuated serum proinflammatory cytokines, PGE_2 and VEGF and ameliorated abnormal costimulation of T- and B- cells in the early phase in

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pristane-induced lupus mice.

Experimental

Animals – Adult female BALB/c mice at 3-4 weeks of age were purchased from the Dae-Han Experimental Animal Center (Taejeon, Korea), and had been maintained in our animal facility on a regular 12-h light-dark cycle under a temperature of 22 ± 2 °C and relative humidity of 55 ± 5% with water and food available *ad libitum*. Mice were received *i.p.* a single injection of 0.5 ml of pristane (Sigma Chemical Co., St., Louse, MO) or PBS (phosphate-buffered saline), and then, later 2 weeks, were used as a pristane-induced lupus model or healthy controls for experiment.

Preparation of plant extracts – Bamboo culm extract (Bambusae Caulis in Liquamen) was obtained from Damyang Jeonnam (Korea). The bamboo culm was washed and broken into about 5 cm fragments. The mineral material mixture that was composed of elvan, germanium, sericite, jade, and amethyst was heated at 250 - 300 °C to induce far-infrared radiation. The bamboo culm fragments were exposed to the mineral material mixture heated by the far-infrared radiation for 6 h and then extracted. The bamboo culm extract (BC) was used as a stock solution, which was yielded 4.8 ml from fresh bamboo culm 1.2 kg. The specimen (WSU-08-07) is deposited at the herbarium of the college of pharmacy, Woosuk University.

Administration of BC – The stock solution was diluted with PBS for administration of BC solution. The pristane-induced lupus mice were administrated with BC 0.5 ml/kg or PBS orally once a day for 14 days and healthy mice with PBS.

Preparation of serum – Bloods were harvested from hearts under anesthetics in BC-treated lupus, lupus control, and healthy mice. The blood was allowed to clot for 2 h at room temperature, and then centrifuged for 20 min at 2000 × g. The sera were collected and stored at ≤ –20 °C for cytokines, PGE_2, and VEGF assays.

Preparation of lymphoid cells – Splenocyte suspensions were prepared from BC-treated lupus, lupus control, and healthy mice using Hanks' balanced salt solution (HBSS: Gibco Co., Grand Island, N.Y., U.S.A.). Erythrocytes in the single cell suspensions were lysed by brief treatment with sterile red blood cell lysing buffer solution (Sigma). Subsequently, the cells were washed with HBSS and resuspended in RPMI 1640 complete medium supplemented with 10% fetal bovine serum (FBS) and penicillin (10 U/ml)-streptomycin (10 μg/ml) at density of 1×10^7 cells/ml.

Preparation of macrophages – Peritoneal macrophages from BC-treated lupus, lupus control, and healthy mice were harvested by peritoneal lavage with ice-cold sterile physiological saline 3 days after *i.p.* injection of the mice with 2 ml sterile 3% thioglycollate broth. Cells were washed and resuspended in complete RPMI 1640 medium. Macrophages were allowed to adhere for 2 h at 37 °C, 5% CO_2 incubation. Nonadherent cells were removed by washing with PBS, and the macrophages were resuspended in fresh culture medium.

Cell culture – Peritoneal macrophages (0.5×10^6 cells/ml) from BC-treated lupus, lupus control, and healthy mice were cultured in complete RPMI 1640 medium for 6 h in the presence or absence of LPS 10 μg/ml (Sigma Chemical Co., St., Louse, MO) at 37 °C, and 5% CO_2. The cell supernatants were then harvested and stored at –70 °C for IL-6 assay.

Cytokine assay – The concentrations of TNF-α, IL-6, IL-10, and IFN-γ in plasma or/and peritoneal macrophage supernatants were determined by using cytokine monoclonal antibodies (BD Biosciences Pharmingen, U.S.A.). All measurements were carried out in duplicate. The results were measured in picograms per milliliter at 450 nm using an ELISA microplate reader (Molecular Devices Co., Ltd., U.S.A.). The lower limit of sensitivity for each of the ELISA was equal to or smaller than 5 pg/ml.

PGE_2 immunoassay – Peritoneal macrophages (0.5×10^6 cells/ml) from lupus control and healthy mice were cultured in complete RPMI 1640 medium for 24 h in the presence or absence of LPS 10 μg/ml (Sigma Chemical Co., St., Louse, MO) or final concentration of BC 10^{-5} μl/ml at 37 °C, and 5% CO_2. The cell supernatants were then harvested and stored at –70 °C for PGE_2 assay. PGE_2 concentration in plasma and peritoneal macrophage supernatants was determined by using a monoclonal antibody/enzyme immunoassay kit from Cayman Chemical, according to the manufacturer's instruction. Concentrations of PGE_2 were measured at 405 nm using ELISA.

VEGF immunoassay – Serum VEGF concentrations in BC-treated lupus, lupus control, and healthy mice were determined using mouse VEGF monoclonal antibodies (BD), according to the manufacturer's instruction. Concentrations of VEGF were measured at 450 nm using ELISA reader.

Flow cytometry analysis – Splenocytes from BC-treated lupus, lupus control, and healthy mice were harvested, washed, and preincubated with anti-Fc receptor monoclonal antibody (MAb) 2.4G2. The cells (1×10^6 cells/0.1 ml) were directly stained with fluorescein isothiocyanate (FITC)-labelled anti-CD3 (Pharmingen),

FITC-labelled anti-CD80, FITC-labelled anti-CD86, PE-labelled anti-CD40L, and PE-labelled anti-CD45R/B220. The cells were incubated for 30 min in the dark, washed, and fixed with 1% paraformaldehyde until analysis. Cells were acquired (10,000 events per group) and analyzed for two-parameter immunofluorescence using flow cytometry (Coulter, EPICS/ML).

TUNEL assay – Splenocytes from BC-treated lupus, lupus control, and healthy mice were cultured for 24 h at 37 °C, and 5% CO_2. The cells were harvested, washed, and preincubated with anti-Fc receptor MAb 2.4G2. The cells (1.0×10^6 cells/0.1 ml) were stained with FITC-labelled anti-CD3 or FITC-labelled anti-CD4, incubated for 30 min in the dark, and washed. The cells were then fixed in 4% paraformaldehyde for 10 min, washed in PBS, and then permeabilized in 1% saponin for 2 min on ice. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay was carried out for apoptotic cell detection by labeling free 3'-OH DNA ends with fluorescein-labeled dUTP, using the enzyme terminal deoxynucleotidyl transferase (TdT) (*In situ* cell death detection kit, Fluorescein, Roche, U.S.A.). The cells were then incubated at 37 °C for 1 h in the dark, washed and resuspended in PBS for flow cytometery analysis (Coulter, EPICS/ML).

Total RNA isolation and RT-PCR – The peritoneal macrophages (2×10^6 cells/ml) obtained from BC-treated lupus, lupus control, and healthy mice were harvested after incubation for 4 h in the presence or absence of LPS 10 μg/ml at 37 °C, and 5% CO_2. Total RNA was extracted from the peritoneal macrophages using an RNA purification kit (QIAGEN) according to the manufacturer's instructions and quantitated spectrophotometrically at 260 nm. cDNA synthesis from total RNA (2 μg) was performed with QuantiTect® Reverse Transcription kit (QIAGEN). PCR was performed in a 20 μl final volume containing 2 μl of the first strand cDNA, 1 μM of sense and antisense primers (BIONEER, Kor.), and 10 μl of 400 nM of QuantiTect® SYBR Green PCR Master Mix (QIAGEN) using a MultiGene PCR (Labnet International Inc.). With a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as an internal control, was amplified by PCR at the same time. Amplification was performed for 15 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s in a thermocycler (GeneAmp 9600-R, Perkin-Elmer, Wellesley, MA). The primers were as follows: IL-6 (sense 5'-CATAGCTACCTGGAGTACATGA-3' and antisense 5'-CATTCATATTGTCAGTTCTTCG-3'); COX-2 (sense 5'-GGCCATGGAGTGGACTTAAA-3' and antisense 5'-ACCTCTCCACCAATGACCTG-3'). GAPDH (sense 5'-GCCAAGGTCATCCATGACAAC-3' and antisense 5'-AGTGTAGCCCAAGATGCCCTT-3') was used as positive control. The amplified PCR products were analyzed by electrophoresis on a 1.2% agarose gels and visualized by ethidium bromide staining.

Statistical Analysis – All data were expressed as means ± standard error (S.E.). Experiments were always run in duplicate and repeated at least twice. Analysis of variation and Students' *t*-test were used to determine statistical significance, and $p < 0.05$ was considered to be statistically significant.

Results and Discussion

BC attenuated *in vivo* and *in vitro* IL-6 in pristane-induced lupus mice – Abnormal expression of key signaling molecules in lupus appears to be the axis of T-lymphocyte dysfunction, which is potential drug targets (Hoffman, 2004; Fernandez *et al*., 2006). IL-6 has been shown to be with the disease activity in chronic inflammation and autoimmune diseases including lupus (Ishihara *et al*., 2002). IL-6 has been reported to be a biomarker in SLE and a therapeutic target in the lupus pathogenesis (Liang *et al*., 2006; Chun *et al*., 2007). Pristane-induced lupus BALB/c mice are characterized by slightly elevated levels of plasma IL-6, which is caused by pristane-stimulated peritoneal macrophages (Shacter *et al*., 1992). Recently, bamboo culm extract has been reported to downregulate the late inflammatory autoimmunity via decreased production of IL-6 in pristane-induced lupus mice (Chae and Park, 2009). However, whether bamboo culm extract (BC) attenuates early production of systemic IL-6 in lupus remains unknown. Therefore, this study was designed to investigate effect of BC on the overproduction of IL-6 in the early stage in pristane-induced lupus mice. In the present study, sera were obtained from BC-treated lupus, lupus control, and healthy mice. The peritoneal macrophages obtained from in BC-treated lupus, lupus control, and healthy mice were cultured for 6 h for IL-6 production and for 4 h for IL-6 mRNA expression. As shown in Fig. 1, our results demonstrated that BC remarkably decreased levels of serum IL-6. Also, IL-6 production and IL-6 mRNA expression by peritoneal macrophages without LPS were increased but not with LPS in pristane-induced lupus mice, indicating that LPS 10 μg/ml may be too high concentration to examine effect of BC on the production of IL-6 in pristane-induced lupus mice. Elevated IL-6, a B cell differentiation factor, is correlated with induction of

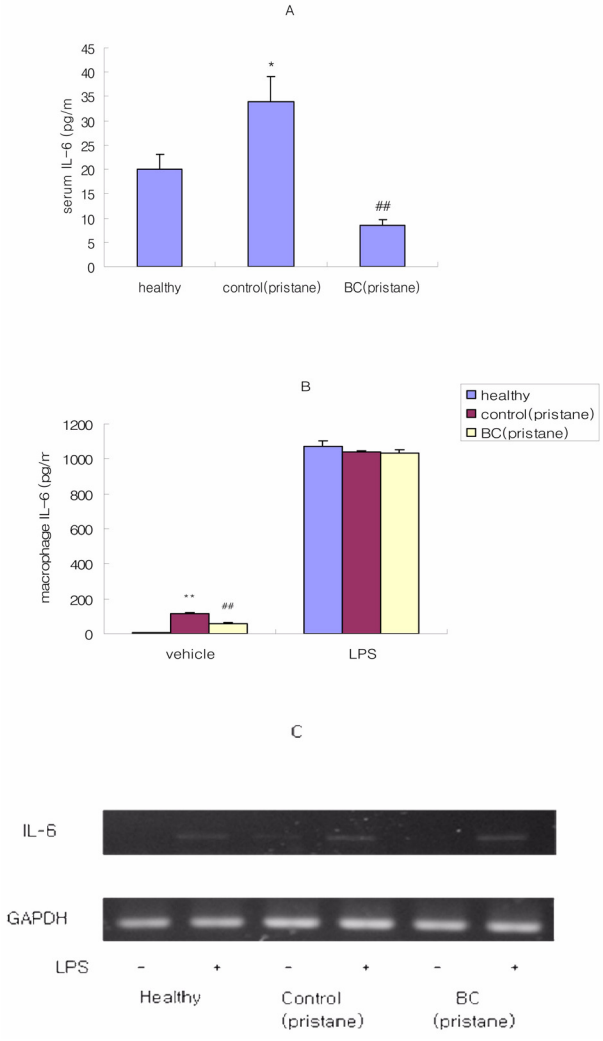

Fig. 1. Inhibitory effect of BC on the production of IL-6 in pristane-induced lupus mice. Bamboo culm extract (BC) or PBS was administrated orally once a day for 14 days in the lupus and healthy mice. Bloods were harvested from hearts under anesthetics in BC-treated lupus, lupus control, and healthy mice. Bloods were allowed to clot for 2 h at room temperature and then centrifuged for sera. The peritoneal macrophages obtained from in BC-treated lupus, lupus control, and healthy mice were cultured for 6 h for IL-6 production and for 4 h for IL-6 mRNA expression in the presence or absence of LPS. Concentrations of cytokines were measured at 450 nm using ELISA. All measurements were carried out in duplicate. Each value represents the mean ± S.E. * ($p < 0.05$) and ** ($p < 0.01$): Significantly different from the value in each healthy control. $^{\#\#}$ ($p < 0.01$): Significantly different from the value in pristane-primed controls.

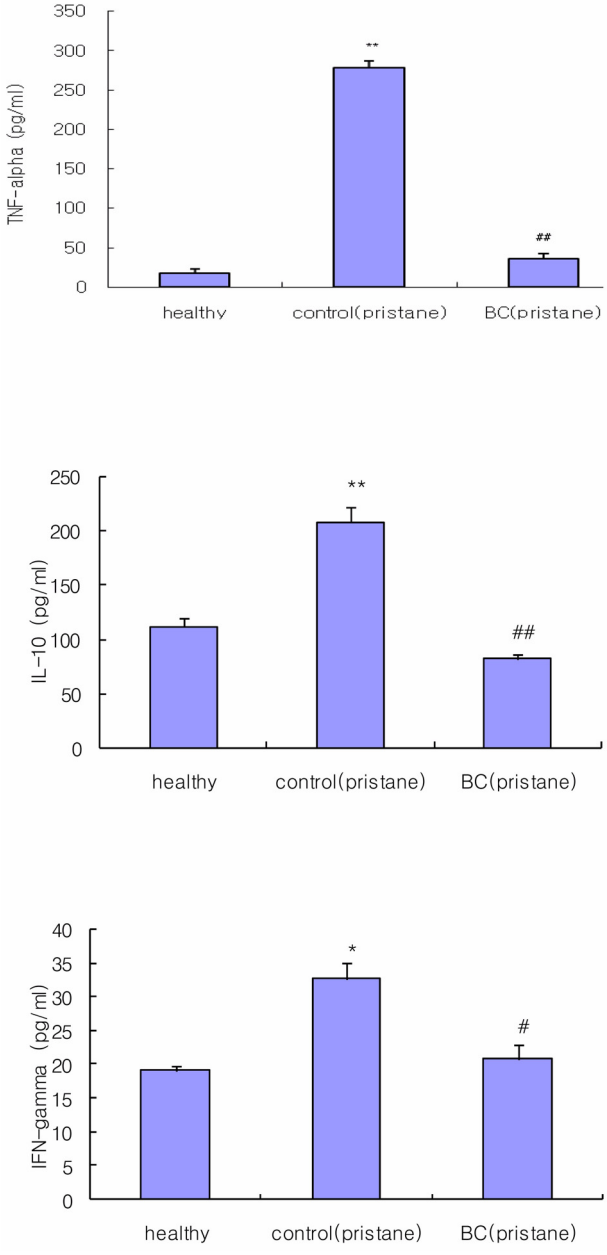

Fig. 2. Inhibitory effect of BC on the levels of serum proinflammatory cytokines in pristane-induced lupus mice. All measurements were carried out in duplicate. Each value represents the mean ± S.E. Other legends and methods are the same as in Fig. 1. * ($p < 0.05$) and ** ($p < 0.01$): Significantly different from the value in each healthy control. $^{\#}$ ($p < 0.05$) and $^{\#\#}$ ($p < 0.01$): Significantly different from the value in pristane-primed controls.

differentiation to autoantibody-forming cells, and decreased apoptosis of lymphocytes in lupus.[24] Therefore, our findings indicate that BC may attenuate early activation of lupus autoimmunity and inflammation via inhibition of IL-6 production in lupus.

BC attenuated levels of serum proinflammatory cytokines in pristane-induced lupus mice – Overproduction of the Th1 and Th2 cytokines, such as IL-10 and IFN-γ contributes to pathogenesis and high mortality in lupus (Kyttaris *et al*., 2005). IFN-γ, Th 1 cytokine, has been reported to be associated with promotion of polyclonal B cell activation, lymphoaccumulation, and pathogenesis of tissue injury in SLE (Balomenos *et al*.,

1998; Haas *et al*., 1998). IL-10, Th 2 cytokine contributes to autoantibody production and high mortality in lupus (Llorente *et al*., 1995). It has been reported that pristane-induced lupus mice had immunoregulatory abnormalities of T cells and hyperreactivity of B cells in the *ex vivo* immune responses (Chae and Shin, 2007). It has been reported that TNF-α apparently plays a significant role in the inflammatory process in lupus (Aringer and Smolen, 2003). In this study, we evaluated whether BC reduces levels of serum TNF-α, IL-10 and IFN-γ in pristane-induced lupus mice. These results demonstrated that BC remarkably attenuated levels of serum TNF-α, IL-10 and IFN-γ in pristane-induced lupus mice (Fig. 2), indicating that BC may attenuate hyperreactivity of B cells associated with autoantibody production and systemic inflammation via down-regulation of production of TNF-α, IL-10 and IFN-γ.

BC inhibited production of PGE_2 in pristane-induced lupus mice – PGE_2 plays a pivotal role in development of local inflammatory responses and tissue injuries in lupus. It has been reported that inhibition of prostaglandin synthesis resulted in suppression of renal functional reserve in lupus nephritis (Herrera-Acosta *et al*., 1987) and that PGE_2 mediated dysregulation of proinflammatory cytokine production in pristane-induced lupus mice (Chae *et al*., 2008). It has also been reported that elevated PGE_2 receptors EP2 and EP4 contributed to upregulation of IL-6 in pristane-treated mice (Akaogi *et al*., 2006). Therefore, PGE_2 is thought to be a novel therapeutic target in the lupus pathogenesis. We examined whether BC inhibits production of PGE_2 in pristane-induced lupus mice. Peritoneal macrophages obtained from BC-treated lupus, lupus control, and healthy mice were cultured for 24 h for PGE_2 production and for 4 h for COX-2 mRNA expression in the presence or absence of LPS 10 μg/ml. As shown in Fig. 3, these results showed that BC remarkably attenuated levels of serum PGE_2 in pristane-induced lupus mice and significantly downregulated PGE_2 production and slightly COX-2 mRNA expression by peritoneal macrophages in pristane-induced lupus mice. Endogenous PGE_2 has been reported to augment IL-6 production during inflammation (Hinson *et al*., 1996). Some evidence demonstrated that PGE_2 attenuates the activation and capability of T cells to produce Th 1 cytokines with a shift toward Th 2 cytokine responses (Betz *et al*., 1991). Therefore, our findings suggest that BC may inhibit lupus inflammation through downregulation of PGE_2 production in lupus.

BC reduced levels of serum VEGF in pristane-induced lupus mice – Systemic inflammation promotes

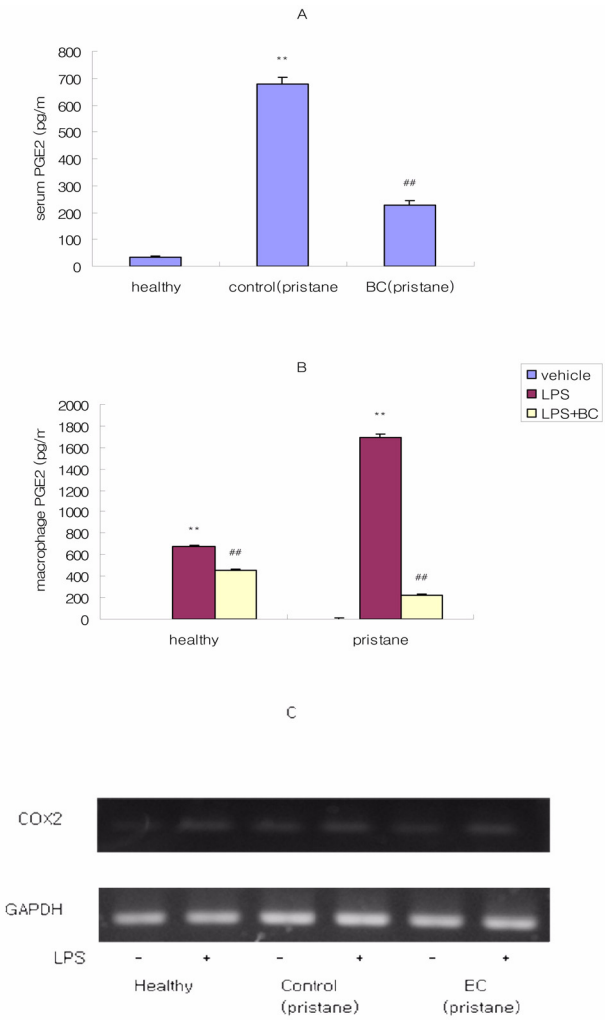

Fig. 3. Inhibitory effect of BC on the production of PGE_2 in pristane-induced lupus mice. Peritoneal macrophages from BC-treated lupus, lupus control, and healthy mice were cultured for 24 h for PGE_2 production and macrophages for 4 h for COX-2 mRNA expression in the presence or absence of LPS. Concentrations of PGE_2 were measured at 405 nm using ELISA. Each value represents the mean ± S.E. Other legends and methods are the same as in Fig. 1. ** ($p < 0.01$): Significantly different from the value in each healthy control (A) or each vehicle-treated control (B). $^{\#\#}$ ($p < 0.01$): Significantly different from the value in pristane-primed controls (A) or LPS-treated immune cells in pristane-primed controls (B).

multiple organ failure through the induction of diffuse microvascular leak (Anderson and Harken, 1990). VEGF (vascular endothelial growth factor) is shown to act as an inflammatory marker, induce vascular permeability and mediate inflammation. Elevated levels of serum VEGF has been reported to be associated with disease activity and destructive change in inflammatory rheumatoid arthritis patients (Ballara *et al*., 2001) and be high in lupus patients (Ciprandi *et al*., 2008). Here, we observed that

levels of serum VEGF remarkably was enhanced in pristane-induced lupus mice compared to healthy mice, supporting previous studies that VEGF can also be a target for lupus therapy. As shown in Fig. 4, these data also demonstrated that BC significantly downregulated levels of serum VEGF in pristane-induced lupus mice. Anti-IL-6 receptor antibody therapy has been reported to reduce VEGF production in rheumatoid arthritis (Nakahara *et al*., 2003). Here, BC was shown to downregulate IL-6 production in pristane-induced lupus mice in Fig. 1. Therefore, our findings indicate that BC may attenuate lupus activity through downregulation of serum VEGF in lupus, which may be associated with inhibitory effect of BC on the production of IL-6 in lupus

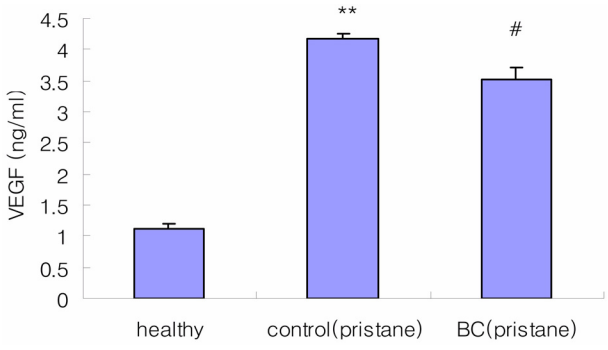

Fig. 4. Effect of BC on the levels of serum VEGF in pristane-induced lupus mice. Serum VEGF concentration in BC-treated lupus, lupus control, and healthy mice was determined at 450 nm using ELISA reader. Each value represents the mean ± S.E. Other legends and methods are the same as in Fig. 1. ** ($p < 0.01$): Significantly different from the value in each healthy control. # ($p < 0.05$): Significantly different from the value in pristane-primed controls.

Regulatory effect of BC on the CD40L expression on the splenic T cells in pristane-induced lupus mice – CD40 ligand (CD40L), which is expressed on the activated T cells, rapidly inducts a costimulatory activity on B cells and is a powerful regulator of T cell-dependent B cells function, including B cells survival (Wu *et al*. 1995; Yin *et al*., 1999). Abnormal interaction of CD40L with its receptor, CD40 on B cells, in patients with SLE has been reported to contribute to autoantibody production and lupus organ injuries (Yazdany and Davis, 2004). Abnormal expression of CD40L on activated T cells is thought to be a potential target for therapy in SLE (Wu *et al*., 1995). In this study, we measured whether BC attenuates CD40L overexpression on the splenic T cells in pristane-induced lupus mice. As shown in Fig. 5, our observation showed that CD3+CD40L+ expression on splenocytes was enhanced in pristane-induced lupus mice compared to healthy mice. Also, these results demonstrated that BC strongly downregulated CD3+CD40L+ expression on the splenocytes in pristane-induced lupus mice. Therefore, our data indicate that the BC may attenuate T cell-dependent B cells hyperactivity via downregulation of abnormal expression of CD40L on the activated T cells in lupus.

Regulatory effect of BC on the CD80 and CD86 expression on the splenic B cells in pristane-induced lupus mice – The CD80 and CD86 antigens are markers of APC cell activation including B cells. CD80 and CD86 found only on antigen-presenting cells (APCs) play important roles in most features of systemic lupus, including autoantibody production and renal pathology (Kinoshita *et al*., 2000). Treatment with both CD80 and

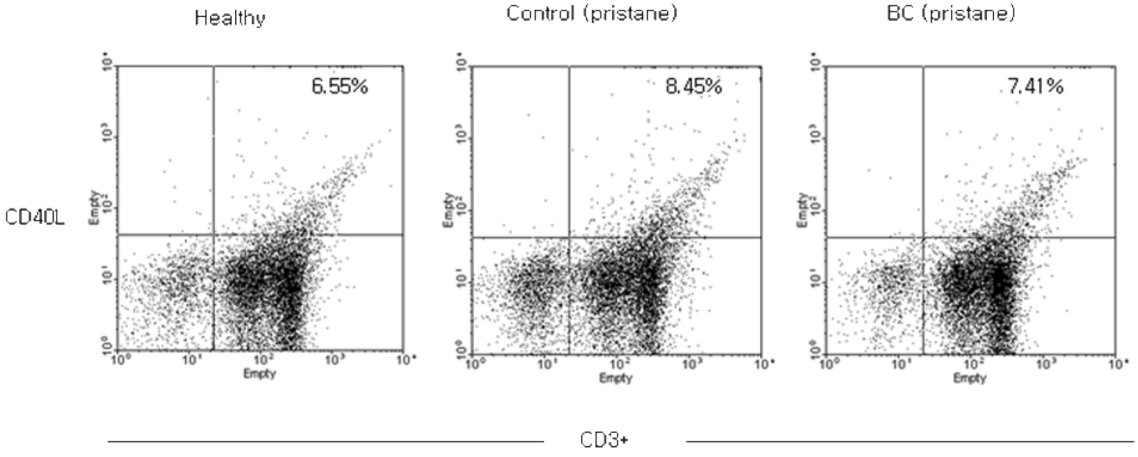

Fig. 5. Regulatory effect of BC on the CD40L expression on the splenic T cells in pristane-induced lupus mice. The harvested splenocytes from BC-treated lupus, lupus control, and healthy mice were preincubated with anti-Fc receptor MAb 2.4G2. The cells were stained with FITC-labelled anti-CD3 and PE-labelled anti-CD40L. The cells were incubated for 30 min in the dark, fixed with 1% paraformaldehyde, and analyzed using flow cytometry.

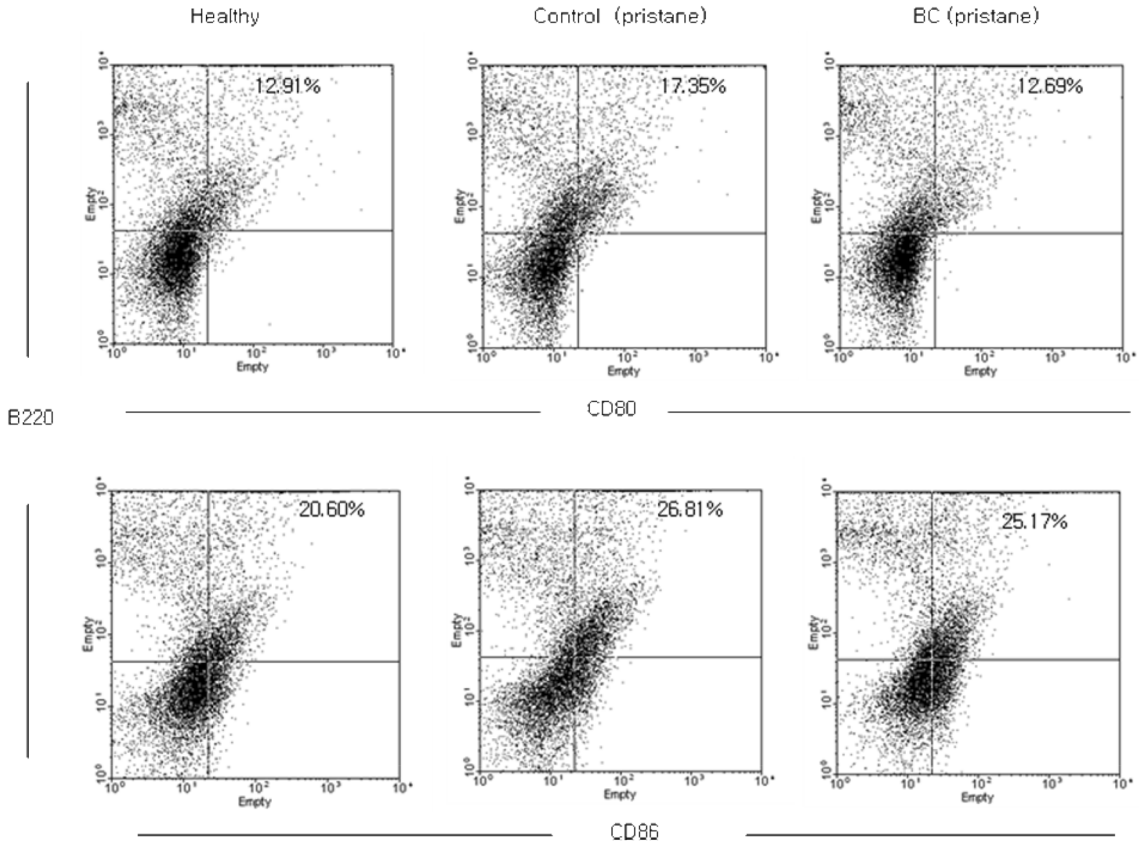

Fig. 6. Regulatory effect of BC on the costimulatory molecule expression on the splenic B cells in pristane-induced lupus mice. The splenocytes from BC-treated lupus, lupus control, and healthy mice were stained with FITC-labelled anti-CD80 or FITC-labelled anti-CD86 and PE-labelled anti-CD45R/B220. Other legends and methods are the same as in Fig. 5.

CD86 monoclonal antibodies after the onset of lupus resulted in a significantly prolonged survival with reduction of autoantibody production (Nakajima *et al*., 1995). Our previous results demonstrated that BC reduced expression of CD86 in splenic B cells but not CD80 in the late stage in pristane-induced lupus mice (Chae and Park, 2009). In the present study, we investigated whether BC attenuates early expression of CD80 and CD86 on the splenic B cells in pristane-induced lupus mice. As shown in Fig. 6, these results showed that BC remarkably reduced splenic CD80+CD45R/B220+ expression and slightly CD86+ CD45R/B220+ in pristane-induced lupus mice. Therefore, these data suggest that BC may attenuate B cell overreaction via downregulation of abnormal expression of CD80 rather than CD86 in the early stage on the B cells in lupus.

Effect of BC on the decreased apoptosis of splenic T cells in pristane-induced lupus mice – Activated T cells in lupus have been reported to be resistant to apoptosis (Xu *et al*., 2004; Chae *et al*., 2008). Down-regulation of spontaneous apoptosis and activation-induced cell death of T cells in SLE resulted in enhanced help to B cells and loss of potentially regulatory function. We measured apoptosis of splenic T cells in BC-treated lupus, lupus control, and healthy mice using the TUNEL assay. In the present study, our results demonstrated that BC upregulated the decreased apoptosis of CD3+ and CD4+ T cells in splenocytes in pristane-induced lupus mice (Fig. 7). Human lupus T cells have been reported to resist inactivation and escape death by upregulating COX-2 (Xu *et al*., 2004). Therefore, our results indicate that BC may attenuate abnormal function of Th in lupus via upregulation of apoptosis of T cells.

In conclusion, our findings indicate that BC may attenuate early stage-development of lupus systemic inflammation and autoimmunity via inhibition of inflammatory mediators and amelioration of abnormal costimulation between T cells and B cells.

Acknowledgements

This work was supported by Woosuk University (2010).

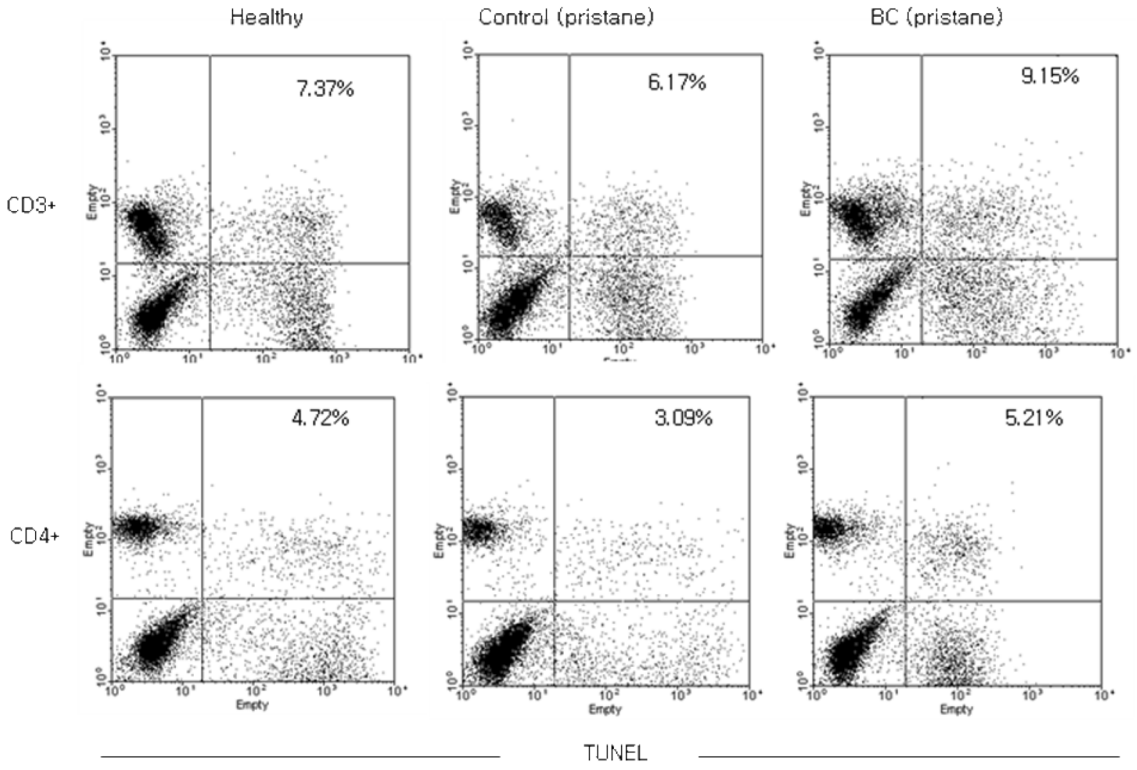

Fig. 7. Effect of BC on the decreased apoptosis of splenic T cells from pristane-induced lupus mice. Splenocytes from BC-treated lupus, lupus control, and healthy mice were cultured for 24 h at 37 °C, 5% CO_2 incubation. The harvested cells were preincubated with anti-Fc receptor MAb 2.4G2. The cells were stained with PE-labelled anti-CD3 or anti-CD4, incubated for 30 min in the dark, fixed in 4% paraformaldehyde for 10 min, and permeabilized in 1% saponin. Apoptosis of CD3+ and CD4+ cells was measured using TUNEL assay. Other legends and methods are the same as in Fig. 5.

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Received October 13, 2010
Revised December 6, 2010
Accepted December 9, 2010