Natural Product Sciences
13(4) : 322-327 (2007)

Regulatory Effect of Fresh Rehmanniae Radix Extract on the *in Vitro* Production of Proinflammatory Cytokines in Pristane-Induced Lupus Mice

Byeong Suk Chae* and Jae Heon Yang

College of Pharmacy, Woosuk University, Wanju, Jeonbuk 565-701, Korea

Abstract – Fresh Rehmanniae radix is known as a traditional medicine with anti-inflammatory and antioxidant activities. However, whether Rehmanniae radix attenuates autoimmune inflammation in lupus models characterized by T cell-dependent autoimmune disease including overproduction of proinflammatory cytokines, loss of T cell tolerance, and B cell hyperactivity remains unclear. We investigated the effect of fresh Rehmanniae radix methanol extracts (RGMeOH) on the *in vitro* overproduction of proinflammatory cytokines by immune cells from pristane-induced lupus BALB/c mice. These results showed that RGMeOH remarkably attenuated Con A-increased overproduction of proinflammatory cytokines, such as IL-2, IFN-γ, IL-6 and IL-10 by splenocytes from pristane-induced lupus mice. RGMeOH greatly reduced LPS-induced production of TNF-α by splenic macrophages from pristane-induced lupus mice, while significantly enhanced LPS-induced production of IL-10 but did not alter IL-6 by splenic macrophages and splenocytes. These findings suggest that RGMeOH may ameliorate lupus systemic inflammatory autoimmunity via down-regulation of TNF-α and T cell-dependent cytokine production.
Keywords – fresh Rehmanniae radix, TNF-α, IL-6, IL-10, IL-2, IFN-γ, pristane, lupus

Introduction

Systemic lupus erythematosus (SLE) is a T cell-dependent, inflammatory autoimmune disease that is characterized by overactive B cells, loss of T cell tolerance, and overproduction of proinflammatory cytokines, which contribute to immune-mediated inflammation by autoantibody production and multiple organ injuries in lupus (Kyttaris, *et al*., 2005; Takeuchi, *et al*., 2005). Lupus pathogenesis exhibits different pattern of cytokine production from healthy subjects or other autoimmune diseases. Proinflammatory cytokines, in particular, such as TNF-α, IL-6, IL-10 or IFN-γ, may play a major role in propagating the inflammatory processes responsible for tissue damage (Uhm, *et al*., 2003). These cytokines are overexpressed both systemically and locally. IL-6 and IL-10 contribute to B cell hyperactivity and autoantibody production in lupus pathogenesis (Llorente, *et al*., 1995; Richards, *et al*., 1998). TNF-α and IFN-γ apparently also play an important role in the inflammatory organ injuries in lupus pathogenesis (Fan and Wuthrich, 1997; Aringer and Smolen, 2003). Levels of serum IL-2 were higher in 50% of active lupus patients (Haung, *et al*., 1988). Therefore, these cytokines have been targeted for novel therapies of lupus (Aringer and Smolen, 2004).

Rehmannia glutinosa Libosch has been used widely as a herbal medicine in Korea, Japan, and China. *Rehmannia glutinosa* is known to have antioxidant and hypoglycemic activities (Kiho, *et al*., 1992; Kubo, *et al*., 1994). According to the reports about its anti-inflammatory effect and immunity, *Rehmannia glutinosa* ameliorated renal function in acute renal failure rats (Kang, *et al*., 2005) and inhibited the secretion of both IL-1 and TNF-α from mouse astrocytes (Kim, *et al*., 1999). Steamed root of *Rehmannia glutinosa* dose-dependently inhibited compound 48/80-induced allergic reaction and anti-DNP IgE-induced production of TNF-α by rat peritoneal mast cells (Kim, *et al*., 1998). Recently, fresh Rehmanniae radix methanol extracts has been reported to attenuate *in vitro* inflammatory responses via down-regulation of TNF-α and up-regulation of IL-10 in C57BL/6 healthy mice (Chae and Shin, 2006). However, whether fresh Rehmanniae radix attenuates T cell-dependent lupus inflammation remains unclear.

Pristane induces a lupus-like syndrome in nonautoimmune mice characterized by the development of glomerulonephritis and lupus-associated autoantibodies (Richards, *et al*., 1998). Recently, it has been reported that upregulated production of IL-2, IFN-γ, IL-6 and IL-10 exhibited *in vivo* and *in vitro* in pristane-induced lupus

*Author for correspondence
Fax: +82-63-290-1812; E-mail: cbse@woosuk.ac.kr

mice compared to healthy mice (Chae and Shin, 2007). We investigated the effect of fresh Rehmanniae radix methanol extracts (RGMeOH) on the *in vitro* overproduction of proinflammatory cytokines by immune cells from pristane-induced lupus BALB/c mice. In the present study, our results demonstrated that RGMeOH remarkably attenuated *in vitro* production of TNF-α and T cell-dependent cytokines, such as IL-2, IFN-γ, IL-6 and IL-10 in 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 (Daejeon, 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*. The mice were received *i.p.* a single injection of 0.5 mL of pristane (Sigma Chemical Co., St., Louse, MO, U.S.A.) or PBS (phosphate-buffered saline). PBS-treated and pristane-primed 6 to 10-mo-old BALB/c female mice were used as a normal or lupus model for experiment.

Plant material extraction – The crude extract was obtained from fresh roots of Rehmanniae radix using 99% methanol. The Rehmanniae radix methanol extract (RGMeOH) was dried and quantified for the total amount of crude extract. A stock solution was prepared at 100 mg of solid per ml in dimethyl sulfoxide (DMSO, Sigma) and was further diluted with RPMI 1640 immediately before treatment of the cells to achieve the final concentration of 0.10 mg/mL.

Preparation of lymphoid cells – Splenocyte suspensions were prepared from normal and pristane-induced lupus 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 into a suspension of 1×10^7 cells/mL with complete RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and penicillin (10 U/mL)-streptomycin (10 μg/mL).

Preparation of macrophages – Splenic macrophages in splenocyte suspension from normal and pristane-induced lupus mice were allowed to adhere for 2 h at 37 °C, 5% CO_2 incubation, and then the nonadherent cells were removed by washing with PBS, and the macrophages were resuspended in fresh culture medium.

Cell culture – Splenocytes (1×10^6 cells/mL) or splenic macrophages (1×10^6 cells/mL) from normal and pristane-induced lupus mice were cultured in complete RPMI 1640 medium for 6 h, 24 h, or 48 h in the presence or absence of LPS 10 μg/mL (Escherichia coli Serotype 026: B6, Sigma) or Con A 2 μg/mL at 37 °C, 5% CO_2 incubation. The cell supernatants were then harvested and stored at −70 °C for cytokine assay.

Cytokine assay – The concentrations of TNF-α, IL-2, IL-6, IL-10, and IFN-γ in supernatants of splenocytes and splenic macrophages were determined using ELISA with cytokine monoclonal antibodies (BD Biosciences Pharmingen, San Diego, CA, 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.

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 Student's *t*-test were used to determine statistical significance, and $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Effect of RGMeOH on the production of TNF-α by splenic macrophages from pristane-induced lupus mice – Recently, Chae and Shin (2006) demonstrated that RGMeOH 0.10 mg/mL decreased *in vitro* production of TNF-α but increased IL-2 and IFN-γ in C57BL/6 mice. Therefore, we expected that RGMeOH at 0.10 mg/mL concentration might also ameliorate inflammatory pathogenesis via regulation of proinflammatory cytokine production in pristane-induced lupus mice. In the present study, we carried out *in vitro* concentration of RGMeOH at 0.10 mg/mL in this lupus model.

TNF-α is highly proinflammatory and is potentially detrimental in lupus organ disease (Aringer and Smolen, 2003). TNF-α is associated with inflammatory injuries in organs including kidneys in lupus animal models and lupus patients with renal inflammation (Herrera-Esparza, *et al*., 1998). Levels of the serum TNF-α were also higher in lupus patients with active pathogenesis than inactive (Davas, *et al*., 1999). However, anti-TNF-α or TNF-α inhibitors protected organs from inflammatory injuries even in presence of autoantibody in SLE (Aringer and Smolen, 2004). Therefore, blocking TNF-α may be interesting in focusing on a therapy for treating SLE inflammatory organ disease. Recently, RGMeOH signifi-

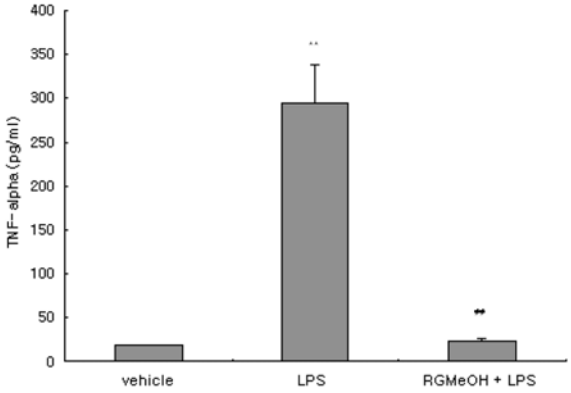

Fig. 1. Effect of RGMeOH on the LPS-induced production of TNF-α by splenic macrophages from pristane-induced lupus mice. RGMeOH : fresh *Rehmannia glutinosa* Libosch methanol extracts. Splenic macrophages (1×10^6 cells/well) from pristane-induced lupus mice were harvested and then incubated with vehicle or RGMeOH 0.10 mg/mL in the presence or absence of 10 μg/mL LPS for 6 h. Cytokine levels were measured using ELISA method. Each value represents the mean ± S.E. ** ($p < 0.01$): Significantly different from the value in each vehicle-treated group. $^{\#\#}$ ($p < 0.01$): Significantly different from the value in each LPS-treated group.

cantly attenuated LPS-induced production of TNF-α by peritoneal macrophages in healthy C57BL/6 mice (Chae and Shin, 2006). In the present study, we investigated whether RGMeOH might reduce LPS-induced production of TNF-α by splenic macrophages from pristane-induced lupus mice. Splenic macrophages from pristane-induced lupus mice were cultured for 6 h for TNF-α in the presence of LPS 10 μg/mL 30 min after 0.10 mg/mL of RGMeOH treatment. In Fig. 1, our observation showed that RGMeOH remarkably attenuated the LPS-increased production of TNF-α by splenic macrophages from pristane-induced lupus mice. Therefore, these observations indicate that RGMeOH may attenuate locally or systemically active lupus inflammation via inhibition of TNF-α production.

Overproduction of proinflammatory cytokines by mitogen-treated immune cells from pristane-induced lupus mice – *In vivo* overexpression of proinflammatory cytokines is thought to promote effector function for autoimmunity development in lupus pathogensis. Proinflammatory cytokines such as IL-6 and IL-10 are associated with induction of overactive B cells that play a critical role in autoantibody production in lupus (Llorente, *et al*., 1995; Richards, *et al*., 1998). Also, IFN-γ contributes loss of T cell regulation that helps B cells produce autoantibody in lupus, resulting in lupus pathogenic autoimmunity and inflammatory organ damage (Fan and Wuthrich, 1997). However, the *in vitro* production of cytokines to antigenic stimuli in lupus models compared with healthy control remains controversial. It has recently been reported that mitogen-induced *ex vivo* production of IL-6, IFN-γ, IL-4, and IL-10 by immune cells were enhanced in all SLE patients regardless of their difference in disease activity (Lit, *et al*., 2006). Also, upregulated production of IL-2, IFN-γ, IL-6 and IL-10 exhibited *in vivo* and *in vitro* in pristane-induced lupus old mice compared to healthy control mice (Chae and Shin, 2007). In contrast, several reports demonstrated that the *in vitro* responses of lupus immune cells to mitogen as well as the *in vivo* cellular responses of lupus patients to antigen were deficient (Takeuchi, *et al*., 2005).

Therefore, we investigated pattern of *in vitro* production of proinflammatory cytokines in this experimental condition. In this study, splenocytes (1×10^6 cells/mL) from normal or pristane-induced lupus 6 to 10-mo-old BALB/c female mice were cultured for 48 h in the presence of Con A 2 μg/mL or for 24 h in the presence of LPS 10 μg/mL. Here, we demonstrated that mitogen-stimulated immune cells from pristane-induced lupus mice have greatly upregulated production of proinflammatory cytokines such as IL-2, IFN-γ, IL-6, and IL-10, compared to normal mice (Fig. 2A and 2B). These results indicate that *in vitro* overproduction of proinflammatory cytokines, such as IL-2, IL-6, IL-10, and IFN-γ in pristane-induced lupus mice, may be targeted for novel therapies.

Effect of RGMeOH on the Con A-induced production of splenic cytokines in pristane-induced lupus mice – Overproduction of cytokines by Th1 and Th2 lymphocytes may determine lupus immune-mediated production of autoantibodies and pathogenic inflammation in lupus (Reininger, *et al*., 1996). Also, overproduction of proinflammatory cytokines that may result in Th1 and Th2 hyperreactivity in lupus was shown in Fig. 2A and 2B. In the present study, we investigated whether RGMeOH regulates T cell-dependent production of cytokines in pristane-induced lupus mice. Con A has been used as a mitogen to activate T lymphocytes. Splenocytes (1×10^6 cells/mL) from normal or pristane-induced lupus mice were cultured for 48 h in the presence of Con A 2 μg/mL 30 min after RGMeOH treatment. Our results were observed that RGMeOH remarkably attenuated Con A-induced overproduction of splenic IL-2, IFN-γ, IL-6, and IL-10 (Fig. 3A and 3B). It is well-known that IL-2 plays an important role in T cell proliferation and differentiation and IFN-γ induces cell-mediated immune responses and inhibits production of Th2 cytokines and Th2 cytokines. Increased production of IL-2 is associated with activated T cells and T cell growth factor. IFN-γ has also reported to trigger T cell-dependent polyclonal B cell activation (Funauchi, *et al*., 1991) and to be an important

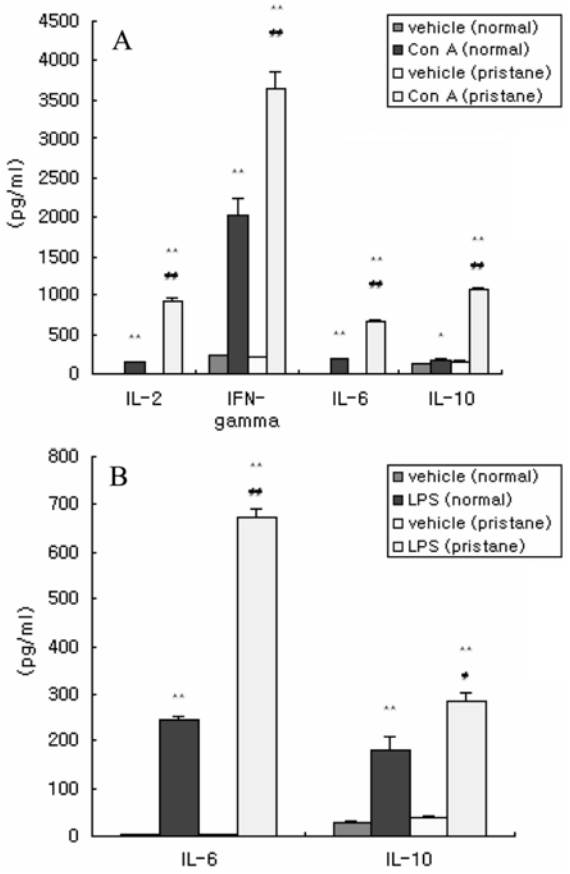

Fig. 2. Overproduction of proinflammatory cytokines by mitogen-treated immune cells from pristane-induced lupus mice.
Splenocytes (1×10^6 cells/mL) from normal or pristane-induced lupus mice were cultured for 48 h in the presence of Con A 2 μg/mL (Fig. 2A) or for 24 h in the presence of LPS 10 μg/mL (Fig. 2B). Other legends and methods are the same as in Fig. 1. Each value represents the mean ± S.E. * ($p < 0.05$) and ** ($p < 0.01$): Significantly different from the value in each vehicle-treated group. $^{\#\#}$ ($p < 0.01$): Significantly different from the value in each LPS- or Con A- treated normal group.

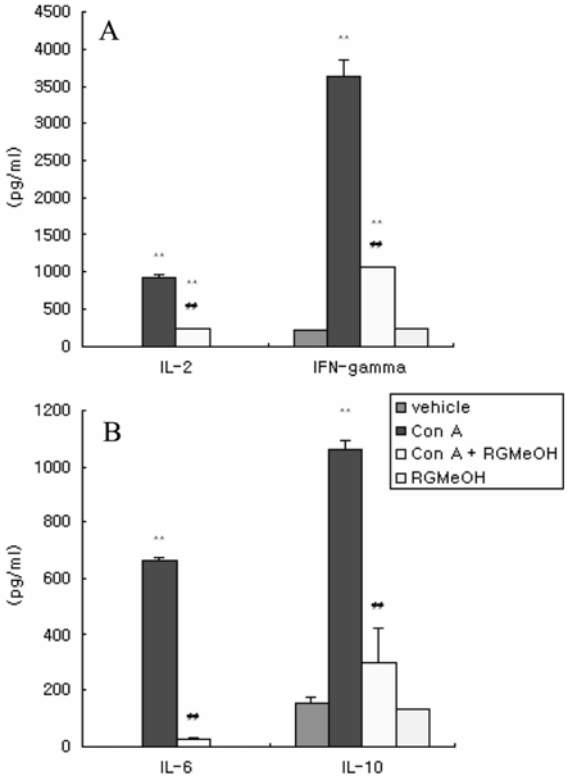

Fig. 3. Effect of RGMeOH on the Con A-induced production of splenic cytokines in pristane-induced lupus mice.
Splenocytes (1×10^6 cells/mL) from normal or pristane-induced lupus mice were cultured for 48 h in the presence of Con A 2 μg/mL 30 min after RGMeOH treatment. Other legends and methods are the same as in Fig. 1. Each value represents the mean ± S.E. ** ($p < 0.01$): Significantly different from the value in each vehicle-treated group. $^{\#\#}$ ($p < 0.01$): Significantly different from the value in each Con A-treated normal group.

factor on development of lupus rental inflammation (Haas, *et al*., 1998; Theofilopoulos, *et al*., 2001). Therefore, *in vitro* overproduction of IL-2 and IFN-γ in pristane-induced lupus mice may be involved in dysregulation of activated T cells and loss of T cell tolerance. IL-6 and IL-10 maintain B cell activity, promote Th2 differentiation, inhibit Th1 differentiation, and stimulate antibody production by B cells (Abbas, *et al*., 1996). IL-6 and IL-10 contribute to B cell hyperactivity and autoantibody production in lupus pathogenesis (Llorente, *et al*., 1995; Richards, *et al*., 1998). Anti-IL-6 and anti-IL-10 antibodies attenuated rental injury or autoimmunity in lupus animal models (Llorente, *et al*., 2000; Liang, *et al*., 2006). Therefore, these data indicate that RGMeOH may attenuate hyperreactivity of T cells and B cells through inhibition of T cell-dependent production of proinflammatory cytokines, such as IL-2, IFN-γ, IL-6, and IL-10, in lupus.

Effect of RGMeOH on the LPS-induced production of splenic IL-6 and IL-10 in pristane-induced lupus mice – In this study, splenocytes and splenic macrophages (each 1×10^6 cells/mL) from normal or pristane-induced lupus mice were cultured for 24 h in the presence of LPS 10 μg/mL 30 min after RGMeOH treatment. Our results showed that RGMeOH significantly enhanced LPS-induced production of IL-10 but did not alter IL-6 by splenic macrophages (Fig. 4A) and splenocytes (Fig. 4B) from pristane-induced lupus mice. These results were different from the observation obtained by using Con A, T cell-activating mitogen (Fig. 3B). However, the data in this lupus model almost consist with the report (Chae and Shin, 2006) that RGMeOH enhanced LPS-induced production of IL-10 but not IL-6 by peritoneal macrophage in healthy mice. LPS is well-known as a T cell-

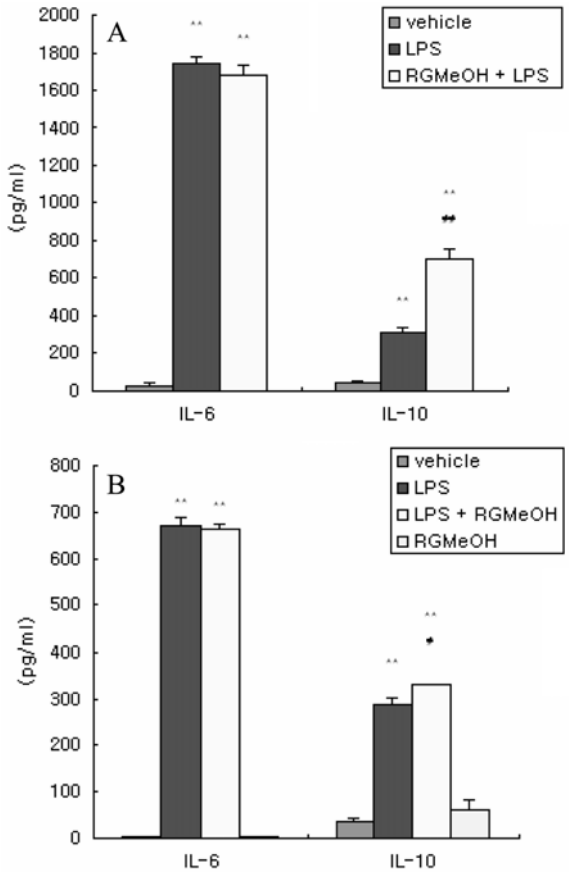

Fig. 4. Effect of RGMeOH on the LPS-induced production of splenic IL-6 and IL-10 in pristane-induced lupus mice.

Splenocytes and splenic macrophages (each 1×10^6 cells/mL) from normal or pristane-induced lupus mice were cultured for 24 h in the presence of LPS 10 μg/mL 30 min after RGMeOH treatment. Other legends and methods are the same as in Fig. 1. Each value represents the mean ± S.E. ** ($p < 0.01$): Significantly different from the value in each vehicle-treated group. $^{\#}$ ($p < 0.05$) and $^{\#\#}$ ($p < 0.01$): Significantly different from the value in each LPS-treated normal group.

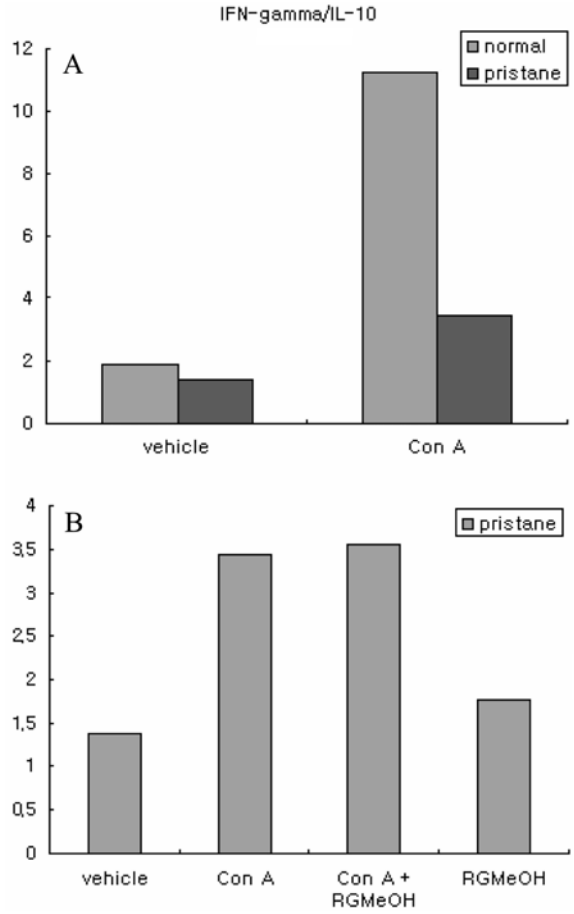

Fig. 5. Effect of RGMeOH on the ratio of splenic IFN-γ to IL-10 in pristane-induced lupus mice.

Splenocytes (1×10^6 cells/well) from PBS-treated normal or pristane-induced lupus BALB/c mice were incubated with vehicle or RGMeOH 0.10 mg/ml in the presence or absence of Con A 2 μg/ml for 48 h. Other legends and methods are the same as in Fig. 1. Each value represents the mean.

independent and inflammatory antigen that induces activation of polyclonal B cells as well as antigen presenting cells. Therefore, these findings indicate that RGMeOH may trigger T cell-independent production of IL-10 but not IL-6 in lupus inflammatory pathogenesis. Further studies are necessary to clarify the exact mechanism by which RGMeOH attenuated Con A-increased production of IL-10 by immune cells from pristane-induced lupus mice, while enhanced LPS-induced production of IL-10.

Effect of RGMeOH on the ratio of splenic IFN-γ to IL-10 in pristane-induced lupus mice – Lupus is characterized by Th-dependent B cell hyperactivity leading to autoantibody production, severity of which depends on ratio of Th2 to Th1 immune responses (Viallard, *et al*., 1999). The decreased ratio of Th1/Th2 is related to disease activity in lupus (Chen, *et al*., 2000). Here, we investigated effect of RGMeOH on the imbalance of Th1/Th2 cytokines with T cell activation with Con A in pristane-induced lupus mice compared to normal mice. As shown in Fig. 5A, we observed that IFN-γ/IL-10, Con A-induced ratio of Th1 to Th2 cytokines by splenocytes, was 11.25 and 3.44 in normal mice and pristane-induced lupus mice, respectively, indicating that Th1 responses exhibit a strong shift toward Th2 responses in pristane-induced lupus mice compared to normal mice. RGMeOH slightly increased Con A-induced ratio of IFN-γ/IL-10 about 8.72% in pristane-induced lupus mice compared to normal mice (Fig. 5B). Therefore, these results suggest that RGMeOH may ameliorate ration of Th1/Th2 through slight suppression

of Th2 type immune responses with a shift toward Th1 responses in pristane-induced lupus mice.

In conclusion, our findings suggest that RGMeOH may attenuate lupus systemic inflammatory autoimmunity via down-regulation of TNF-α and T cell-dependent cytokine production.

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (the Center for Healthcare Technology Development, Chonbuk National University, Jeonju 561-756, Republic of Korea).

References

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

Aringer, M. and Smolen, J.S., SLE-Complex cytokine effects in a complex autoimmune disease: tumor necrosis factor in systemic lupus erythematosus. *Arthritis Res. Ther.* **5**, 172-177 (2003).

Aringer, M. and Smolen, J.S., Tumour necrosis factor and other proinflammatory cytokines in systemic lupus erythematosus: a rationale for therapeutic intervention. *Lupus* **13**(5), 344-347 (2004).

Chae, B.S. and Shin, T.Y., Effect of fresh Rehmanniae radix methanol extracts on the production of cytokines. *Yakhak Hoeji* **50**(3), 184-190 (2006).

Chae, B.S. and Shin, T.Y., Immunoregulatory abnormalities of T cells and hyperreactivity of B cells in the *in vitro* immune response in pristane-induced lupus mice. *Arch. Pharm. Res.* **30**(2), 191-198 (2007).

Chen, S., Hu, D., Shi, X., Shen, N., Gu, Y., and Bao, C., The relationship between Th1/Th2-type cells and disease activity in patients with systemic lupus erythematosus. *Chin. Med. J. (Engl)* **113**(10), 877-880 (2000).

Davas, E.M., Tsirogianni, A., Kappou, I., Karamitsos, D., Economidou, I., and Dantis, P.C., Serum IL-6, TNFalpha, p55 srTNFalpha, p75srTNFalpha, srIL-2alpha levels and disease activity in systemic lupus erythematosus. *Clin. Rheumatol.* **18**(1), 17-22 (1999).

Fan, X. and Wuthrich, R.P., Upregulation of lymphoid and renal interferon-gamma mRNA in autoimmune MRL-Fas(lpr) mice with lupus nephritis. *Inflammation* **21**(1), 105-112 (1997).

Funauchi, M., Sugishima, H., Minoda, M., and Horiuchi, A., Effect of interferon-gamma on B lymphocytes of patients with systemic lupus erythematosus. *J. Rheumatol.* **18**(3), 368-372 (1991).

Haas, C., Ryffel, B., and Le Hir, M., IFN-gamma receptor deletion prevents autoantibody production and glomerulonephritis in lupus-prone (NZB x NZW)F1 mice. *J. Immunol.* **160**(8), 3713-3718 (1998).

Herrera-Esparza, R., Barbosa-Cisneros, O., Villalobos-Hurtado, R., and Avalos-Diaz, E., Renal expression of IL-6 and TNF-alpha genes in lupus nephritis. *Lupus* **7**(3), 154-158 (1998).

Huang, Y.P., Perrin, L.H., Miescher, P.A., and Zubler, R.H., Correlation of T and B cell activities in vitro and serum IL-2 levels in systemic lupus erythematosus. *J. Immunol.* **141**(3), 827-833 (1988).

Kang, D.G., Sohn, E.J., Moon, M.K., Lee, Y.M., and Lee, H.S., Rehmannia glutinose ameliorates renal function in the ischemia/reperfusion-induced acute renal failure rats. *Biol. Pharm. Bull.* **28**(9), 1662-1667 (2005).

Kiho, T., Watanabe, T., Nagai, K., and Ukai, S., Hypoglycemic activity of polysaccharide fraction from rhizome of Rehmannia glutinosa Libosch. f. hueichingensis Hsiao and the effect on carbohydrate metabolism in normal mouse liver. *Yakugaku Zasshi*. **112**(6), 393-400 (1992).

Kim, H.M., An, C.S., Jung, K.Y., Choo, Y.K., Park, J.K., and Nam, S.Y., Rehmannia glutinosa inhibits tumour necrosis factor-á and interleukin-1 secretion from mouse astrocytes. *Pharmacol. Res.* **40**(2), 171-176 (1999).

Kim, H.M., Lee, E.H., Lee, S.J., Shin, T.Y., Kim, Y.J., and Kim, J.B., Effect of Rehmannia glutinosa on immediate type allergic reaction. *Int.. J. Immunopharmacol.* **20**, 231-240 (1998).

Kubo, M., Asano, T., Shiomoto, H., and Matsuda, H., Studies on rehmanniae radix. I. Effect of 50% ethanolic extract from steamed and dried Rehmanniae radix on hemorheology in arthritic and thrombosic rats. *Biol. Pharm. Bull.* **17**(9), 1282-1286 (1994).

Kyttaris, V.C., Juang, Y.T., and Tsokos, G.C., Immune cells and cytokines in systemic lupus erythematosus: an update. *Curr. Opin. Rheumatol.* **17**(5), 518-522 (2005).

Lit, L.C., Wong, W.C.K., Tam, L.S., Li, E.K.M., and Lam, C.W.K., Raised plasma concentration and ex vivo production of inflammatory chemokines in patients with systemic lupus erythematosus. *Annals of the Rheumatic Diseases* **65**, 209-215 (2006).

Liang, B., Gardner, D.B., Griswold, D.E., Bugelski, P.J., and Song, X.Y., Anti-interleukin-6 monoclonal antibody inhibits autoimmune responses in a murine model of systemic lupus erythematosus. *Immunology* **119**(3), 296-305 (2006).

Llorente, L., Richaud-Patin, Y., Garcia-Padilla, C., Claret, E., Jakez-Ocampo, J., Cardiel, M.H., Alcocer-Varela, J., Grangeot-Keros, L., Alarcon-Segovia, D., Wijdenes, J., Galanaud, P., and Emilie, D., Clinical and biologic effects of anti-interleukin-10 monoclonal antibody administration in systemic lupus erythematosus. *Arthritis Rheum.* **43**(8), 1790-800 (2000).

Llorente, L., Zou, W., Levy, Y., Richaud-Patin, J., Wijdenes, J., Alcocer-Varela, J., Morel-Fourrier, B., Brouet, J. C., Alarcon-Segovia, D., Galanaud, P., and Emilie, D., Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J. Exp. Med.* **181**, 839-844 (1995).

Reininger, L., Santiago, M.L., Takahashi, S., Fossati, L., and Izui, S., T helper cell subsets in the pathogenesis of systemic lupus erythematosus. *Ann. Med. Interne. (Paris)* **147**(7), 467-471 (1996).

Richards, H.B., Satoh, M., Shaw, M., Libert, C., Poli, V., and Reeves, W.H., Interleukin 6 dependence of anti-DNA antibody production: evidence for two pathways of autoantibody formation in pristane-induced lupus. *J. Exp. Med.* **188**(5), 985-990 (1998).

Takeuchi, T., Tsuzaka, K., Abe, T., Yoshimoto, K., Shiraishi, K., Kameda, H., and Amano, K., T cell abnormalities in systemic lupus erythematosus. *Autoimmunity* **38**(5), 339-346 (2005).

Theofilopoulos, A.N., Koundouris, S., Kono, D.H., and Lawson, B.R., The role of IFN-gamma in systemic lupus erythematosus: a challenge to the Th1/Th2 paradigm in autoimmunity. *Arthritis Res.* **3**(3), 136-141 (2001).

Uhm, W.S., Na, K., Song, G.W., Jung, S.S., Lee, T., Park, M.H., and Yoo, D.H., Cytokine balance in kidney tissue from lupus nephritis patients. *Rheumatology (Oxford)* **42**(8), 935-938 (2003).

Viallard, J,F., Pellegrin, J.L., Ranchin, V., Schaeverbeke, T., Dehais, J., Longy-Boursier, M., Ragnaud, J. M., Leng, B., and Moreau, J. F., Th1 (IL-2, interferon-gamma (IFN-gamma)) and Th2 (IL-10, IL-4) cytokine production by peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* **115**(1), 189-195 (1999).

(Accepted November 13, 2007)