Inula Britannica flower modulates type 1 and type 2 T cell responses in BALB/c mice

Qing-Hua SONG, Takao KOBAYASHI, Tie HONG and Jong-Chol CYONG*

Department of Bioregulatory Function, Graduate School of Medicine, University of Tokyo

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Abstract

We have already reported that Inula Britannica flower (IB, 旋覆花, Xuan-fu-hua) induced Th2 type cytokines and showed preventive effects on experimental acute hepatitis and autoimmune diabetes in mice, which had been induced by a Th1-dominant immune response. In the present study, we investigated the effects of Inula Britannica flower on the production of antibodies against ovalbumin and the phenotypes of B and T lymphocytes in BALB/c mice. The oral administration of Inula Britannica flower decreased the production of IgG1 in the primary response, but increased the proportion of total B cells and activated B cells. Inula Britannica flower reduced the proportions of IFN-γ-producing cells in splenic $CD4^+$ T lymphocytes and the ratio of IFN-γ/IL-4 produced by lymphocytes collected from an inguinal lymph node of the immunized leg. On the other hand, the intraperitoneal administration of Inula Britannica flower decreased antibody production and the proportion of IFN-γ-producing cells, and increased B cells, activated B cells and the proportion of IL-4-producing cells. Furthermore, IB suppressed the production IFN-γ and IL-6, but induced that of IL-4 from lymph node cells.

These data suggested that Inula Britannica flower increased B cells and activated B cells, suppressing the production IFN-γ and increaing that of IL-4, however, IB also suppressed IL-6, and the production of antibodies.

Key words Inula Britannica flower (旋覆花, Xuan-fu-hua), BALB/c mice, cytokine, T helper (Th1), Th2.

Introduction

Diseases mediated by the immune system such as atopic dermatitis and allergies, which are called the modern sicknesses, have recently show a tendency to increase caused by the destruction of the environment and atmospheric pollution.[1] Hormones are used for the treatment of these diseases. However, the side-effects are serious,[2,3] and decisive methods of therapy have not yet been established. Kampo medicines, on the other hand, seem to have the advantage of being slightly more effective than hormonal agents, and they can also be selected according to the constitution of each patient, and more readily used for long-term treatment because of fewer adverse effects. It has been reported that Kampo medicines are useful in clinical practice.[4,5]

Inula Britannica flower (IB) is believed to promote diuresis and decrease edema. The therapy for atopic dermatitis prescribes that the promotion of diuresis and decrease of edema is indispensable, according to the theory of Kampo medicine. IB is expected to be used on the therapy of atopic dermatitis. In this study, we investigated the effects of IB on primary and secondary immune responses, B cells and the balance between Th1 and Th2.

Materials and Methods

Experimental animals: BALB/c female mice were obtained from Clea Japan (Tokyo, Japan) at 6

*〒113-8655 東京都文京区本郷 7-3-1
東京大学医学部生体防御機能学講座 [illegible] 宗鐵
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

weeks of age, and were provided with commercial pellets (CE2 Clea Japan) through the whole experiment and tap water *ad libitum* until the start of the treatment.

Crude drugs: The flower of *Inula Britannica* L. subsp *japonica* KITAM. was used as Inula Britannica flower (IB) in present study. IB was obtained from Uchida Co. (Tokyo, Japan). 100 g of the crude herb was boiled with 1000 ml of distilled water until the volume was reduced to 500 ml. The supernatant fluid was filtered and centrifuged. The yield was 15 % of the original herb weight. In the orally administered IB group (IB(p.o.)), the herbal extract was given as drinking water continuously for five weeks. The concentration of the extract was adjusted to 2.5 g/kg/day as original herb weight (375 mg/kg/day as extract). In the intraperitoneally administered IB group (IB (i.p.)), the herbal extract was given as an intraperitoneal injection of 500 mg/kg/day as original herb weight (75 mg/kg/day as extract) once a week for five weeks.

Immunizations: One week after the beginning of administration, mice were immunized with 1 μg of ovalbumin (OVA) emulsified in 20 μl of incomplete Freund's adjuvant (FIA) by injection into the footpad. Two weeks after the first immunization, 50 μl of blood was collected from the fundus vein, diluted with 200 μl of PBS(-) containing 100 units/ml heparin, and centrifuged. The supernatant was collected and stored at -20°C for assay (primary response). On the next day of bleeding, mice were boosted with additional OVA as in the first immunization. Two weeks after the second immunization, blood was collected from the trunk, allowed to clot for 1 hour and centrifuged at 1000×g for 15 min at 4°C. Serum was stored at -20°C for assay (secondary response).

Antibodies: Antibodies used in this study were hamster anti-mouse CD3ε IgG (145-2C11), hamster anti-mouse CD28 IgG (37.51), Cy-Chrome (CC)-conjugated rat anti-mouse CD4 IgG2a (RM4-5), fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse IFN-γ IgG1 (XMG1.2), R-phycoerythrin (PE)-conjugated rat anti-mouse IL-4 IgG2b (BVD4-1D11), horse radish peroxidase (HRP)-conjugated anti-mouse IgG2a (R15-19), PE-conjugated rat anti-mouse CD45R/B220 IgG2a (RA3-6B2), FITC-conjugated rat anti-mouse CD25 (IL-2 Receptor, αChain) IgM (7D4) monoclonal antibodies (Pharmingen, San Diego, CA) and HRP-conjugated rabbit anti-mouse IgG1 (Organon Teknika).

Measurement of antibody production: Anti-OVA IgG1 and IgG2a antibodies in the serum were measured by the ELISA method. The 96-well plates were coated with 4 μg/ml OVA in bicarbonate buffer, pH 9.6, at 4°C. Wells were blocked with 1 % BSA (Fraction V, Calbiochem) for 2 hours at room temperature. Diluted serum or plasma was applied to the wells and incubated for 2 hours at 37°C. Bound antibodies were detected by incubation with HRP-conjugated anti-IgG1 or HRP-conjugated anti-IgG2a for 1 hour at 37°C. These reactions were developed with 2, 2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) for 0.5 hours and optical density (OD) at 405 nm was read with a plate reader (BioRad).

Flowcytometric analysis: At autopsy, the spleen was immediately removed and pressed with slide glasses in PBS (-). The cell suspension was passed through #200 a metal sieve and washed three times with PBS (-). Spleen cells (5×10^6/ml in PBS(-)) were incubated with 1 μg of FITC-conjugated anti-CD25 and PE-conjugated anti-CD45R/B220 antibodies for one hour at 4°C. Fluorescence-activated cells were washed with PBS (-) and analyzed by an EPICS XL flow cytometer (Coulter Cytometry Co., Hialeah, FL). A fluorescence histogram of at least 5,000 counts was collected for each sample.

Cytokine expressions of splenic T lymphocytes: Spleen cells were washed and prepared as described above, resuspended at 5×10^6/ml in RPMI1640 medium containing 10 % fetal calf serum (FCS, Bioscience), stimulated for 18 h with anti-CD3 (2 μg/ml) and anti-CD28 antibodies (2 μg/ml) and cultured for the final 6 h in a medium containing 3 μM monensin (Sigma). The cells were stained with 0.5 μg of Cy-Chrome-conjugated anti-CD4, fixed, permeabilized and then stained with 0.1 μg of FITC-conjugated anti-IFN-γ and PE-conjugated anti-IL-4 antibodies by using a cell fixation/permeabilization kit (Cytofix/Cytoperm, Pharmingen) according to the manufacturer's instructions. Fluorescence-activated cells were analyzed by an EPICS XL flow cytometer (Coulter Cytometry Co., Hialeah, FL). A fluorescence histo-

gram of at least 5,000 counts was collected for each sample.

Cytokine production : Lymphocytes were collected from an inguinal lymph node of the immunized leg. Lymphocytes were prepared as described above, washed and resuspended at 2×10^6 cells/ml in RPMI 1640 medium containing 10 % FCS and stimulated with 1 μg/ml anti-CD3 antibody or 10 μg/ml LPS. After incubation for 36 hours, the supernatant was collected, and IFN-γ, IL-4 and IL-6 levels in the supernatant were measured by the ELISA method using an Immunoassay Kit (Bio Source).

Statistical analysis : Data were analyzed by Student's t-test to determine significance.

Results

Effect on spleen weight

The spleen weights of the control group tended to increase compared with the intact group. However, the weights of the group receiving oral IB (IB(p.o.) group) were significantly suppressed compared with those of the control group. No effects of intraperitoneal administration of Inula IB (IB(i.p.) group) were observed (Fig. 1).

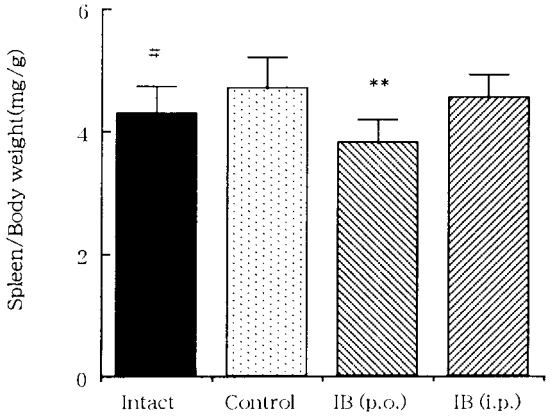

Fig. 1 Effects of Inula Britannica flower on the spleen weight in OVA-immunized mice. In the IB (p.o.) group, Inula Britannica flower extract was orally administered at a dose of 375 mg/kg/day as extract continuously for five weeks, and in the IB (i.p.) group, it was intraperitoneally administered at a dose of 75 mg/kg/day once a week for five weeks. Each value is the mean±S.E. of 6 mice. # or **: $p<0.1$ or 0.01 vs. Control.

Effect on production of antibodies in OVA-immunized mice

The production of IgG1 in the primary response significantly decreased in the IB (i.p.) group and tended to decrease in the IB (p.o.) group, compared with control group (Table I). The production of IgG1 and IgG2a, and the ratio of IgG2a/IgG1, were significantly decreased in the IB (i.p.) group in the secondary response (Table I).

Effect on the B cell phenotype

Since the production of antibodies is closely associated with B cells, we analyzed B cell phenotypes in the spleen, using flow cytometry. The proportion of B lymphocytes ($B220^+$) in the spenic lymphocytes of the control group did not significantly change compared with the intact group. Those in the IB (p.o.) group and the IB (i.p.) group were significantly increased compared with the control group.

The proportion of activated B cells (CD25B220) in the splenic lymphocytes of the control group showed no significant difference from that of the intact group. Significant increases were observed in the IB (p.o.) and IB (i.p.) groups compared with the control group (Fig. 2).

The proportion of activated B cells in total B cells showed a tendency to increase in the IB (p.o.) group, and a significant increase in the IB (i.p.) group, compared with the control group (Fig. 2).

Table I Effects of Inula Britannica flower on antibody productions. (Mean±S.E., n=6)

	Primary response			Secondary response		
	IgG1 (OD)	IgG2a (OD)	IgG2a/IgG1	IgG1 (OD)	IgG2a (OD)	IgG2a/IgG1
Control	0.29±0.01	0.52±0.27	1.81±0.92	0.44±0.01	1.47±0.28	3.30±0.58
IB (p.o.)	0.27±0.01#	0.34±0.18	1.31±0.67	0.45±0.01	1.29±0.30	2.87±0.66
IB (i.p.)	0.25±0.02*	0.32±0.09	1.41±0.41	0.28±0.09*	0.61±0.01*	1.44±0.63*

In the IB (p.o.) group, Inula Britannica flower extract was orally administered at a dose of 375 mg/kg/day consecutively for five weeks, and in the IB (i.p.) group, it was intraperitoneally administered at a dose of 75 mg/kg/day once a week for five weeks. * or # different from the control at $p<0.05$ or 0.1, respectively.

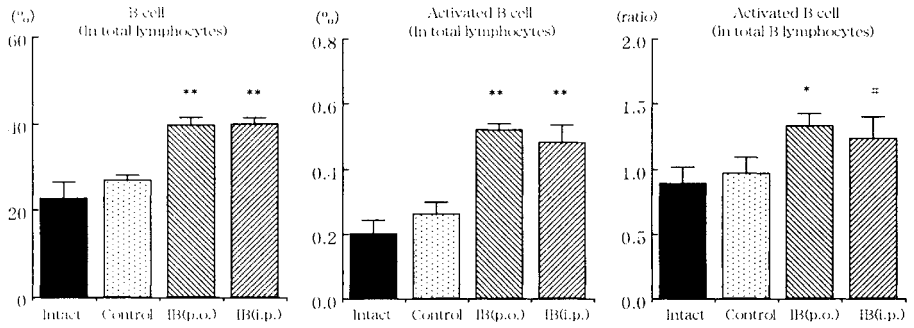

Fig. 2 Effects of Inula Britannica flower on B cell phenotypes in OVA-immunized mice. In the IB (p.o.) group, Inula Britannica flower extract was orally administered at a dose of 375 mg/kg/day as extract continuously for five weeks, and in the IB (i.p.) group, it was intraperitoneally administered at a dose of 75 mg/kg/day once a week for five weeks. Each value is the mean±S.E. of 6 mice. #, * or **: $p<0.1$, 0.05 or 0.01 vs. Control.

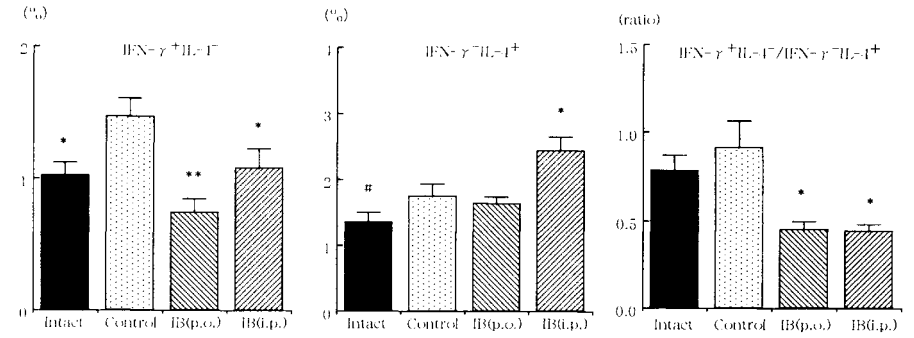

Fig. 3 Effects of Inula Britannica flower on cytokine productions of $CD4^+$ T cells stimulated by anti-CD3/CD28 antibody in OVA-immunized mice. In the IB (p.o.) group, Inula Britannica flower extract was orally administered at a dose of 375 mg/kg/day as extract continuously for five weeks, and in the IB (i.p.) group, it was intraperitoneally administered at a dose of 75 mg/kg/day once a week for five weeks. Each value is the mean±S.E. of 6 mice. #, * or **$p<0.1$, 0.05 or 0.01 vs. Control.

Effects on the production of cytokines in splenic Th cells

In the control group, the proportion of IFN-γ^+ IL-4^- cells in $CD4^+$ splenic lymphocytes was significantly increased compared with that in the intact group. They significantly suppressed in IB (p.o.) group and IB (i.p.) group compared with control group. The proportion of IFN-γ^-IL-4^+ tended to increase in the control group compared with that in the intact group. It was significantly increased in the IB (i.p.) group compared with the control group (Fig. 3). The ratio of IFN-γ^+IL-4^-/IFN-γ^-IL-4^+ in the control group was not significantly different from that in the intact group. However, in the IB (p.o.) and IB (i.p.) groups, the ratio was significantly reduced compared with the control group (Fig. 3).

Effect on production of cytokines in lymphocytes of lymph node

We measured the cytokine production of lymphocytes from a lymph node stimulated with 1 μg/ml anti-CD3 antibody. IB (i.p.) tended to decrease IFN-γ production and significantly increased IL-4 production compared with the control group. The ratio of IFN-γ/IL-4 was clearly, but not significantly reduced in the IB (p.o.) group, and significantly decreased in the IB (i.p.) group compared with the control group (Fig. 4).

We also measured the production of IL-6 in

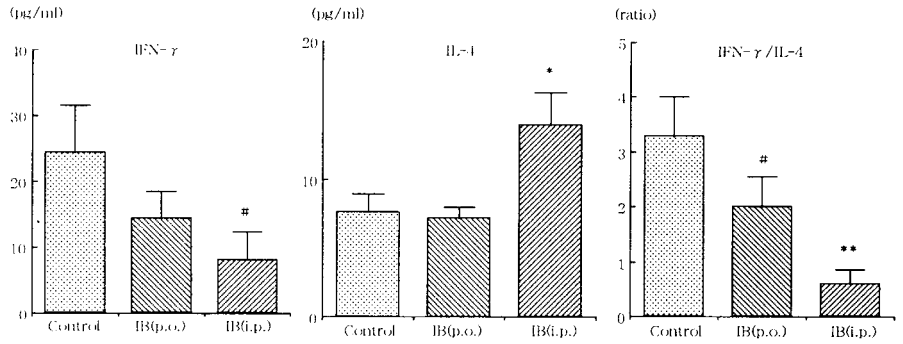

Fig. 4 Effects of Inula Britannica flower on cytokine production of lymphocytes stimulated by anti-CD3 antibody in OVA-immunized mice. In the IB (p.o.) group, Inula Britannica flower extract was orally administered at a dose of 375 mg/kg/day as extract continuously for five weeks, and in the IB (i.p.) group, it was intraperitoneally administered at a dose of 75 mg/kg/day once a week for five weeks. Each value is the mean±S.E. of 6 mice. # or * $p<0.1$ or 0.05 vs. Control.

lymphocytes from a lymph node stimulated with 5 μg/ml LPS. IL-6 production in the IB (p.o.) group was lower than in the control group, but there was no statistically significant difference. Production tended to be reduced in the IB (i.p.) group, compared with the control group (Fig. 5).

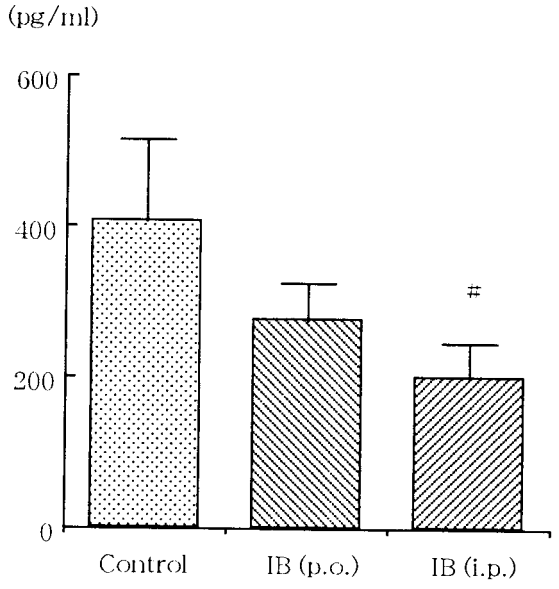

Fig. 5 Effects of Inula Britannica flower on IL-6 production of lymphocytes collected from an inguinal lymph node from immunized leg, stimulated by LPS in OVA-immunized mice. In the IB (p.o.) group, Inula Britannica flower extract was orally administered at a dose of 375 mg/kg/day as extract continuously for five weeks, and in the IB (i.p.) group, it was intraperitoneally administered at a dose of 75 mg/kg/day once a week for five weeks. Each value is the mean±S.E. of 6 mice. #$p<0.1$ vs. Control.

Discussion

Inula Britannica flower (IB) as used in Kampo medicines is composed of the flower heads of several Compositae plants, such as *Inula Britannica* L. subsp. *japonica* KITAM. and *I. Serrata* BUR. var. *hupehensis* LING. Chlorogenic acid and caffeic acid, the components of IB, promote small intestine peristalsis.[8] It has also been reported that taraxastery acetate, a component of IB, can protect from experiment hepatitis.[9] It is recently reported that a component extracted from IB has a cytotoxic action against human cancer cells.[10]

We have reported that IB showed a preventive effect against experimental hepatitis induced by *Propionibacterium acnes* and lipopolysaccharide (LPS) in mice.[9] In that model for human hepatitis, T cells, especially Th1 cells, are essential for the induction of the final effector cells which are surrogated by *P. acnes* priming and LPS boosting.[11] Our further study using mice with experimental hepatitis showed that IB inhibited the IFN-γ production of splenic $CD4^+$ T cells in mice *in vivo* and IL-12 production by peritoneal macrophages *in vitro*.[6] We have also investigated the effects of IB on autoimmune diabetes and found that it suppressed the increase of blood glucose, reduced insulitis and destruction of β-cells and inhibited the increase in IFN-γ production of stimulated splenic T lymphocytes and the increase of IFN-γ-production cells in $CD4^+$ cells.[7] These data suggest

that IB may induce a shift of T cell activity from the Th1 to the Th2 type and have a preventive effect on Th1-dependent experimental hepatitis and autoimmune diabetes. However, it is possible that Th2 -like diseases such as allergy and asthma, may be aggravated by IB.

Ovalbumin (OVA) is known as an intense allergy antigen. The induction of cytokine production[12] and effects on the balance of Th cells[13] have been reported after immunization by OVA. Th2 cells are dominant in BALB/c mice[14] and also play a dominant role in immune sensitization by OVA.[15] It is thus considered that OVA-sensitized BALB/c mice are inclined to be doubly in Th2 dominant.[16] We therefore investigated the effects of IB on immune responses against OVA.

In this study, an increase of spleen weight was observed after OVA immunization. IB inhibited this increase of spleen weight. IB showed a preventive effect on spleen swelling induced by OVA immunization. Futhermore, we found that IB can reduce the production of IgG1 in the primary response, and of IgG1 and IgG2a in the secondary response. The ratio of IgG2a/IgG1 was also suppressed, which showed that the suppression of IgG2a was stronger than that of IgG1. IgG2a production is promoted by IFN-γ and IgG1 production is promoted by IL-4.[17,18] The balance of IgG2a/IgG1 can also be seen as the balance of Th1/Th2. IB may affect this balance.

Since antibody production is closely associated with B cells, we analyzed the B cell phenotypes, using flowcytometry. The proportions of B cells and activated B cells were increased by IB.

We investigated the effects of IB on the cytokine expression of splenic T lymphocytes, using flow cytometry. IB decreased the proportion of IFN-γ^+ IL-4^- cells, increased that of IFN-γ^-IL-4^+ cells, and reduced the ratio of IFN-γ^+IL-4^-/IFN-γ^-IL-4^+. Moreover, we examined the effects of IB on the production of cytokines in lymphocytes collected from an inguinal lymph node from the immunized leg. IB suppressed the production of IFN-γ, promoted that of IL-4 and decreased the ratio of IFN-γ/IL-4. IL-4 is known as a B cell growth factor.[19,20] IFN-γ and IL-4 restrict each other. IB activated B cells, decreasing IFN-γ and the proportion of IgG2a/IgG1, and increasing IL-4, B cells and activated B cells. All these data suggest that IB can promote Th2 dominance and are consistent with the results of antibody production in the present study and in our previous reports.[6,7]

Although IB accelerated the Th2-dominant response and induced B cell activation, the production of antibodies was reduced. It was suggested that other factors might be associated. IL-6 induces antibody production in B cells.[21,22] We measured the production of IL-6 in lymphocytes from a lymph node stimulated with 5 μg/ml LPS and confirmed that IL-6 production was suppressed by IB. It is considered that the decrease of antibody production was due to a decrease in the inducing factor of antibody production, in spite of the increase in both B cells and activated B cells. Though IB induced the production of Th2 dominant cytokines, and increased B cells, at the same time it also suppressed IL-6. It was therefore suggested that IB suppressed the guidance of plasma cells, and decreased the response of humoral immunity and that Th2-like diseases such as allergy and asthma may possibly be aggravated by IB.

IB is multicomponent drug, since it is a crude drug. Although some kinds of active components of IB have been clarified,[8,9] it is also reported that some actions of IB are recognized, but the active components have not been determined.[6,23] The data in this study showed the possibility that the active principle of IB is not a single component. In the present study, we established one dose only, and the administration for mice was 10-20 times of the dose for human adults. It is necessary to investigate the doses of IB relation to its effects, furthermore all the active components of IB.

Acknowledgement

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和文抄録

現在まで，我々は旋覆花 (Inula Britannica flower, IB) が Th2 優位な免疫反応を誘導することにより，Th1 依存的な疾患として知られている炎症急性肝炎及び自己免疫糖尿病の発病を抑制することを報告してきた。本実

験ではBALB/cマウスにおけるovalbumin (OVA) の免疫に対する特異的抗体産生能，B細胞，T細胞の分化に対する旋覆花の影響を検討した。経口投与では旋覆花は一次応答のIgG1の産生の抑制作用，B細胞増殖および活性化作用が観察された。また，脾臓のTh細胞のIFN-γ^+IL-4$^-$産生細胞の抑制作用，IFN-γ^+IL-4$^-$/IFN-γ^-IL-4$^+$の比の抑制作用が認められた。リンパ節のリンパ細胞のサイトカイン産生能について，脾臓Th細胞のサイトカイン産生細胞と同様に旋覆花は，IFN-γ/IL-4の比の抑制作用が観察された。旋覆花の腹腔投与では同様にIgG産生の抑制作用，B細胞，活性化B細胞の増加作用，また，脾臓Th細胞のIFN-γ^+IL-4$^-$産生細胞の抑制，IFN-γ^-IL-4$^+$産生細胞の促進作用が観察された。さらに，リンパ節のリンパ細胞のサイトカイン産生能について，IFN-γ産生の抑制作用及びIL-4産生の促進作用，またはIL-6産生の抑制作用が認められた。

これらの成績から，旋覆花の作用はIFN-γを抑制し，IL-4を増強することによって，IL-4のB細胞増殖因子としての作用を活性化することから，B細胞の割合も活性化B細胞の割合も増加させ，B細胞増殖因子として作用することが示唆された。しかし，抗体産生誘導因子のIL-6の抗体が抑制されたため，抗体の産生は抑制された。

References

1) Businco, L., Bruno, G. and Giampietro, PG.: The atopic child and the environment. *Curr Probl in Dermatol.* **28**, 161-172, 1999.
2) Toyoshima, K.: Adverse reaction and safety of inhalative corticosteroid in the long term management of asthmatic children. *The Allergy in Practice.* **18**, 800-804, 1998.
3) Kurihara, N., Honjoh, M., Wakui, F., Chino, K., Hara, H. and Morishima, T.: Necrotizing fasciitis associated with atopic dermatitis after the cessation of strongest steroid ointment therapy. *Rinsho Derma* (Tokyo). **38**, 147-150, 1996.
4) Nyunt, A.K., Takeuchi, Y., Yokomuro, K. and Miyanaga, Y.: Comparative studies on the antiallergic effects of Kampo medicines used for the therapy of respiratory diseases. *Jpn. J. Allergil.* **44**, 503-512, 1995.
5) Onda, H.: The treatment of atopic dermatitis by Kampo medicine. *J Traditional Med.* **14**, 245-251, 1997.
6) Song, Q H., Kobayashi, T., Iijima, K., Hong, T. and Cyong, J-C.: Hepatoprotective effects of *Inula Britannica* on hepatic injury in mice. *Phytotherapy Res.* **14**, 180-186, 2000.
7) Kobayashi, T., Song, Q-H., Hong, T., Kitamura, H. and Cyong, J-C.: Cytokine mediated preventive effect of *Inula Britannica* on multiple low doses of Streptozotocin-induced autoimmune diabetes in mice. *Phytotherapy Res.* (submitted)
8) Gzok, V.G. and Lang, K.: Chlorgensaure-Wirkungen am Magen-Darmkanal. *Arzneimittel-Forschung.* **11**, 545-549, 1961.
9) Iijima, K., Kiyohara, H., Tanaka, M., Matsumoto, T., Cyong, J-C. and Yamata, H.: Preventive effect of Taraxasteryl Acetate from *Inula britannica subsp. japonica* on experimental hepatitis in vivo. *Planta Med.* **61**, 50-53, 1995.
10) Park, EJ. and Kim, J.: Cytotoxic sesquiterpene lactones from Inula britannica. *Planta Med.* **64**, 752-754, 1998.
11) Tanaka, Y., Takahashi, A., Watanabe, K., Takayama, K., Yahata, T., Habu, S. and Nishimura, T. A.: pivotal role of IL-12 in Th1-dependent mouse liver injury. *Int Immunol.* 8, 569-576, 1996.
12) Horino, A., Taneichi, M., Naito, S.: Cytokine production by spleen cells from mice with ovalbumin-specific, IgGE-selective unresponsiveness induced by ovalbumin-liposome conjugate. *Allergol Int.* **46**, 249-253, 1997.
13) Vinuesa, M.A.,Tanaka, Y., Hakugawa, J.: In situ expression of interleukin-4, -5 and 6 in Peyer's patch from ovalbumin-sensitized BALB/c mice after oral challenge. *Allergol Int.* **46**, 243-247, 1997.
14) Heinzel, F.P., Sadick, M.D., Holaday, B.J., Coffman, R.L. and Locksley, R.M.: Reciprocal expression of interferon ν or interleukin 4 during the resolution or progression of murine leishmaniasis. *J. Exp. Med.*, **169**, 59-72, 1989.
15) Lim, YS., Kang, BY., Kim, EJ., Kim, SH., Hwang, SY., Kim, KM. and Kim, TS.: Vaccination with an ovalbumin/interleukin-4 fusion DNA efficiently induces Th2 cell-mediated immune responses in an ovalbumin-specific manner. *Arch Pharmacal Res.* **21**, 537-542, 1998.
16) Vinuesa, M.A., Tanaka, Y., Hakugawa, J., Bae, S.J. and Katayama, I.: *In situ* expression of interleukin-4, -5 and -6 in Peyer's patch from ovalbumin-sensitized BALB/c mice after oral challenge. *Allergol Int.* **46**, 243-247, 1997.
17) Takatsu, K.: Cytokines involved in B-cell differentiation and their sites of action. *Proc Soc Exp Biol Med.* **215**, 121-133, 1997.
18) Yoshino, S. and Sagai, M.: Induction of systemic Th1 and Th2 immune responses by oral administration of soluble antigen and diesel exhaust particles. *Cell Immunol.* **192**, 72-78, 1999.
19) Carballido, JM., Schols, D., Namikawa, R., Zurawski, S., Zurawski, G., Roncarolo, MG. and de Vries, JE.: IL-4 induces human B cell maturation and IgE synthesis in SCID-hu mice. Inhibition of ongoing IgE production by in vivo treatment with an IL-4/IL-13 receptor antagonist. *J. Immunol.* **155**, 4162-4170, 1995.
20) Punnonen, J., Kainulainen, L., Ruuskanen, O., Nikoskelainen, J. and Arvilommi, H.: IL-4 synergizes with IL-10 and anti-CD40 MoAbs to induce B-cell differentiation in patients with common variable immunodeficiency. *Scand J Immunol.* **45**, 203-212, 1997.
21) Bonig, H., Packeisen, J., Rohne, B., Hempel, L., Hannen, M., Klein-Vehne, A., Burdach, S. and Korholz, D.: Interaction between interleukin 10 and interleukin 6 in human B-cell differentiation. *Immunol Invest.* **27**, 267-280, 1998.
22) Rieckmann, P., Tuscano, JM. and Kehrl, JH.: Tumor necrosis factor-alpha (TNF alpha) and interleukin-6 (IL-6) in B lymphocyte function. *Methods.* **11**, 128-132, 1997.
23) Iijima, K., Tanaka, M., Kiyohara, H., Matsumoto, T., Cyong, J-C. and Yamada, H.: Effects of *Inula Britannica* on immune hepatic injury in mice. *J Med & Pharm Soc for WAKAN-YAKU.* 8, 346-347, 1991.