Journal of Biomedical Science **12:** 79–89, 2005.
DOI 10.1007/s11373-004-8172-x

The distinct effects of a butanol fraction of *Bidens pilosa* plant extract on the development of Th1-mediated diabetes and Th2-mediated airway inflammation in mice

Cicero Lee-Tian Chang[1,†], Hui-Kai Kuo[1,2,†], Shu-Lin Chang[1,3], Yi-Ming Chiang[1], Tsung-Han Lee[2], Wen-Mein Wu[4], Lie-Fen Shyur[1] & Wen-Chin Yang[1,2,*]

[1]*Institute of BioAgricultural Sciences, Academia Sinica, Taiwan, ROC;* [2]*Department of Life Sciences, National Chung Hsing University, Taiwan, ROC;* [3]*Department of Biological Science and Technology, National Chiao Tung University, ROC;* [4]*Department of Nutrition and Food Sciences, Fu Jen Catholic University, ROC;* [†]*These authors equally contributed to this work*

Received 28 May 2004; accepted in revised form 5 November 2004

Key words: airway inflammation, *Bidens*, diabetes, helper T cells (Th)

Summary

Bidens pilosa is claimed to be useful for immune or anti-inflammatory disorders; however, little scientific evidence has been published concerning its function. In this paper, immune disease mouse models were used to study the function of a butanol fraction of *B. pilosa*. We demonstrated treatment with the butanol fraction of *B. pilosa* ameliorated Th1 cell-mediated autoimmune diabetes in nonobese diabetic (NOD) mice but caused deterioration of Th2 cell-mediated airway inflammation induced by ovalbumin (OVA) in BALB/c mice. We next showed that Th2 cytokines (IL-4 and/or IL-5) increased but Th1 cytokine (IFN-γ) decreased following injections with the butanol fraction of *B. pilosa* in both mouse strains. Accordingly, Th2 cytokine-regulated IgE production in mouse serum increased following treatment with this fraction. Finally, we found that the butanol fraction of *B. pilosa* inhibited Th1 cell differentiation but promoted Th2 cell differentiation. Taken together, the butanol fraction of *B. pilosa* has a dichotomous effect on helper T cell-mediated immune disorders, plausibly via modulation of T cell differentiation.

Introduction

Upon encountering antigens, naive T helper (Th0) cells differentiate into two functionally distinct subsets, namely type I T helper (Th1) cells and type II T helper (Th2) cells, which mediate cellular and humoral immunity, respectively. Cytokines have been demonstrated to be key players in the differentiation of Th0 into Th1 or Th2 cells [1]. Th1 cells are characterized by the secretion of Th1 cytokines including interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-2, and lymphotoxin (LT). Th2 cells are characterized by the secretion of Th2 cytokines (IL-4, IL-5, IL-13 and/or IL-10). Interestingly, Th1 cells and their cytokine, IFN-γ, enhance Th1 generation but inhibit Th2 generation whereas Th2 cells and their cytokine, IL-4, promote Th2 generation but inhibit Th1 generation. Under aberrant conditions, a Th1/Th2 imbalance and various cytokines are thought to cause autoimmune diseases. For instance, Th1 cells and their cytokines can exacerbate Th1-mediated autoimmune diseases such as autoimmune diabetes, rheumatoid arthritis and Crohn's disease but improve airway

*To whom correspondence should be addressed. Fax: +886-2-26515120; E-mail: wcyang@gate.sinica.edu.tw

hypersensitivity and allergy. On the contrary, Th2 cells and their cytokines can antagonize Th1 cells, their cytokines and Th1-modulated disorders. Therefore, skewing T cell differentiation or cytokine administration is frequently used to treat immune diseases [1].

Nonobese diabetic (NOD) mice can spontaneously develop a Th1 cell-mediated autoimmune diabetes with the destruction of pancreatic β cells. This type of diabetes shares many immunopathological features with human type I insulin-dependent diabetes mellitus [2]. T cells ($CD4^+$ and $CD8^+$), B cells, macrophages and NK cells [3] have been shown to be involved in autoimmune diabetes. However, compelling evidence has revealed that $CD4^+$ Th1 cells play a dominant role in the progression of diabetes in NOD mice [3, 4]. In marked contrast, airway hypersensitivity induced by ovalbumin in a mouse model is frequently used as a tool to study Th2 cell-mediated asthma. Overwhelming evidence has suggested that Th2 cells and their IL-4 and IL-5 are the main factors inducing this allergen-induced hypersensitivity in the lung [5–8], and eosinophils, $CD8^+$ T cells, mast cells and B cells are not necessarily required for this disease [9–13].

Bidens pilosa, an Asteraceae plant widely found in tropical and subtropical areas of the world, has been used in various folk medications, including those for stomach illnesses, malaria infections and liver disorders [14–17]. Several constituents isolated from *B. pilosa* have been shown to possess anti-inflammatory, immunosuppressive [18], antibacterial [19], anti-malarial [14] and anti-hyperglycemic activities [20] or exhibit inhibitory effects on the prostaglandin synthesis pathway [17]. However, the role of *B. pilosa* in preventing autoimmune diseases remains unknown.

In this study, we found that a butanol fraction of *B. pilosa* extract attenuated diabetes in NOD mice but aggravated airway inflammation, probably via an ability to control T cell differentiation and cytokine production.

Materials and methods

Chemicals and cells

Phorbol 12-myristate 13-acetate (PMA), phytohemagglutinin (PHA), ionomycin, ovalbumin (OVA), aluminum hydroxide gel, *n*-butanol and methacholine were purchased from Sigma (St Louis, MO, USA). Tacrolimus (FK506) was obtained from Fujisawa Pharmaceuticals (Taipei, Taiwan). Cytokines (IFN-γ, IL-4 and IL-2) and antibodies (anti-IFN-γ and anti-IL-4) were purchased from R&D systems (Minneapolis, MN, USA). RPMI 1640 medium and HBSS (Hanks' balanced salt solution) were purchased from Gibco (Grand Island, NY, USA). Human umbilical cord blood cells were obtained from Taipei Medical University Hospital (Taipei, Taiwan). All chemicals and solvents used in this study were of analytical grade.

Preparation of butanol extracts from B. pilosa

Bidens pilosa Linn. was collected and deposited as a voucher specimen (no. 0211943) at the Herbarium of National Taiwan University, Taipei, Taiwan. Crushed whole plants (10.0 kg) were extracted by boiling in water for 2 h, prior to evaporation, suspension in water (1.0 l) and partition with *n*-butanol (1.0 l × 3 times), yielding two fractions, a water fraction and a butanol fraction (37.7 g). The quality of the butanol fraction of *B. pilosa* (BPB) from batch to batch was similar in terms of the ratios of index compounds using HPLC analysis. The amounts of 2-β-D-glucopyranosyloxy-1-hydroxy-5(*E*)-tridecene-7,9,11-triyne and 3-β-D-glucopyranosyloxy-1-hydroxy-6(*E*)-tetradecene-8,10,12-triyne were 1.5 and 1.1% (w/w) the dry weight of the butanol fraction, respectively, using HPLC analysis [21].

Mice, immunization and drug treatment

NOD mice from the Jackson Laboratory (Bar Harbor, ME, USA) and BALB/c mice from the National Laboratory Animal Center (Taipei, Taiwan) were maintained in the institutional animal facility and handled according to the guidelines of the Academia Sinica Institutional Animal Care and Utilization Committee.

Marked diabetes in female NOD mice started from ~12 weeks of age with a cumulative diabetes incidence ~60% of the total mouse population at 30 weeks of age. Female NOD mice received intra-peritoneal (i.p.) injections of the butanol fraction of *B. pilosa* at 10 mg/kg three times per week starting from 4 weeks of age to

the indicated age. Control mice were treated with phosphate buffer solution (PBS) three times per week from 4 weeks of age to the indicated age (negative control) or with FK506 at 1 mg/kg three times per week from 2–6 weeks of age (positive control).

For mice with airway hypersensitivity induced by ovalbumin, 6–8 week-old female BALB/c mice were primed with an i.p. injection of 50 μg ovalbumin mixed with 2 mg aluminum hydroxide gel on day 0, followed by sequential boosting with inhalation of 5% ovalbumin solution on days 14, 29, 30 and 31. On day 35, mice were sacrificed for histological or other examinations [22]. For the negative control, mice were treated with PBS instead of ovalbumin using the above-mentioned protocol. To evaluate the effects of the butanol fraction of *B. pilosa* on ovalbumin-induced airway hypersensitivity, BALB/c mice receiving the same *B. pilosa* butanol fraction treatment as NOD mice had airway hypersensitivity induced by ovalbumin as previously mentioned. On day 35, mice were sacrificed for further examination [23].

Histological examination

To determine the severity of insulitis, the pancreases from NOD mice were embedded in Optimal Cutting Temperature (OCT) Compound (Sakura, Torrance, CA, USA). Five-micrometer frozen pancreatic sections were cut and stained with hematoxylin and eosin (H&E). More than 20 islets per pancreas were examined for the degree of insulitis. The severity of insulitis was scored as follows: 0, normal; 1, leukocyte infiltration around the islets; 2, <50% leukocyte infiltration; and 3, >50% leukocyte infiltration as previously described [24].

For β cell granulation and leukocyte infiltration, 5 μm frozen pancreatic sections were tested for anti-insulin antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and then stained with alkaline phosphatase-conjugated goat anti-rabbit antibody plus Fast-Red development (Dako Corp., Carpinteria, CA, USA), followed by hematoxylin counterstain. More than 20 islets per pancreas were examined for β cell granulation and leukocyte infiltration. The granulation of total β cells per islet represented the integrity of a β cell islet. The β cell granulation score was as follows: 3, an intact well-granulated islet (100% granulated β cells per islet); 2, 50 to 75% granulated β cells per islet; 1, 20 to 50% granulated β cells per islet; and 0, 0% granulated β cells per islet. The degree of β cell granulation (%) = 100% × (total scores of all β islets per pancreas)/the number of β islets per pancreas. Leukocyte infiltration is also an indication of the destruction of a β cell islet by leukocytes. The leukocyte infiltration score was described as follows: 0, no visible islet-associated mononuclear cells; 1, polar islet-associated infiltrating mononuclear cells; 2, peripheral noninvasive infiltrating mononuclear cells; and 3, extensive invasive infiltrating mononuclear cells. The degree of leukocyte infiltration = total scores of all β islets per pancreas/the number of β islets per pancreas [25]. For lung histology, lungs from BALB/c mice were fixed with 10% buffered formalin and then embedded in paraffin. The 5 μm lung sections were stained with H&E, followed by microscopic examination [22].

Bronchoalveolar lavage fluid (BALF)

BALB/c mice were sacrificed and their tracheas were cannulated and clamped unilaterally. A lung lavage was done using 1 ml HBSS at 37 °C, followed by an additional 1 ml HBSS. The recovered BALF was cooled on ice. Following centrifugation at 1500 rpm for 5 min at 4 °C, the supernatant from the first 1 ml BALF was saved for cytokine analysis. Cells isolated from BALF were resuspended in 1 ml HBSS, counted and stained with Diff-Quick (Kookje Scientific Products, Tokyo, Japan). Half a million cells in 200 μl HBSS were cytospun at 500 rpm for 5 min, followed by Toludine blue staining. Three hundred cells on the slide were counted and differentiated into monocytes, mast cells, eosinophils, neutrophils and lymphocytes. The differentiation of mast cells from eosinophils was confirmed by the existence of blue and eosin-stained granules in the cytoplasm, respectively [23].

Serum antibody and cytokine measurement

The level of serum IgE was determined using an ELISA kit (BETHYL, Montgomery, TX, USA). IFN-γ and IL-4 from the blood or BALF were

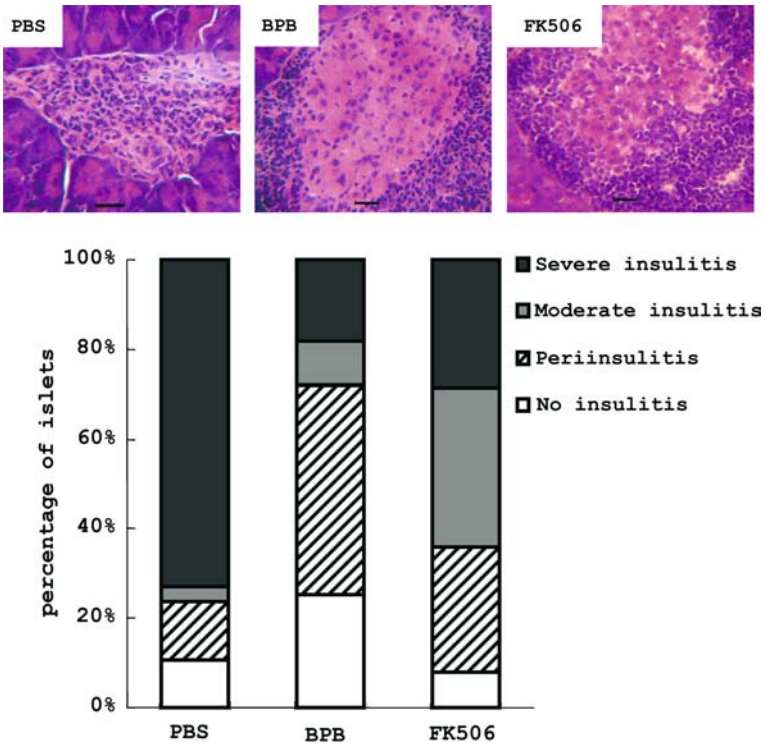

Figure 1. The butanol fraction of *B. pilosa* reduces the severity of autoimmune diabetes in NOD mice. Pancreatic tissues obtained from NOD mice receiving treatment with PBS, a butanol fraction of *B. pilosa* at 10 mg/kg (BPB) or FK506 were stained with hematoxylin and eosin, followed by light microscopy. PBS: microscopic image of 18-week-old pancreatic β islets, BPB: microscopic image of 18-week-old pancreatic β islets, FK506: microscopic image of 30-week-old pancreatic β islets. Bar: 10 μm (upper panel). The degree of insulitis was microscopically examined and graded as normal islet (blank), peri-insulitis (hatched), moderate insulitis (<50% leukocyte infiltration, gray) and severe insulitis (>50% leukocyte infiltration, black) (lower panel).

measured using ELISA kits (eBioscience®, San Diego, CA, USA). The same samples were also quantified using IL-5 and IL-13 ELISA kits (R&D Systems).

T cell differentiation

Human umbilical cord blood $CD4^+$ Th0 cells were purified with a MACS column (Miltenyi, Auburn, CA, USA) and grown in RPMI 1640 medium. Th0 cells (0.5×10^6/ml) were incubated with RPMI medium containing PHA, IL-12 and anti-IL-4 (Th1 conditions) or RPMI medium containing PHA, IL-4 and anti-IL-12 (Th2 conditions). IL-2 was added to the medium 48 h later. The butanol fraction of *B. pilosa* was incubated with differentiating helper Th cells for 24 h on day 5. For intracellular staining of cytokines, IFN-γ and IL-4, T cells were treated with PMA/ionomycin for 4 h plus Golgiplug (BD Biosciences, San Diego, CA, USA) for 2 h. After T cells were fixed and permeabilized, they were stained with FITC-conjugated anti-IFN-γ or anti-IL-4 mAbs and then subjected to fluorescence activated cell sorter (FACS) analysis. Th0 cells were defined as the naive $CD4^+$ T cells which did not secrete IFN-γ and IL-4 following PMA plus ionomycin stimulation. Th1 cells were defined as the differentiated $CD4^+$ T cells secreting IFN-γ but not IL-4 following PMA plus ionomycin stimulation. Th2 cells were defined as the differentiated $CD4^+$ T cells secreting IL-4 but not IFN-γ following PMA plus ionomycin stimulation.

T cell transfection and luciferase assay

Jurkat cells (10×10^6) were electrophorated at 975 μF and 260 V using a Bio-Rad Gene Pulser with pGATA-3-Luc [26], a construct containing

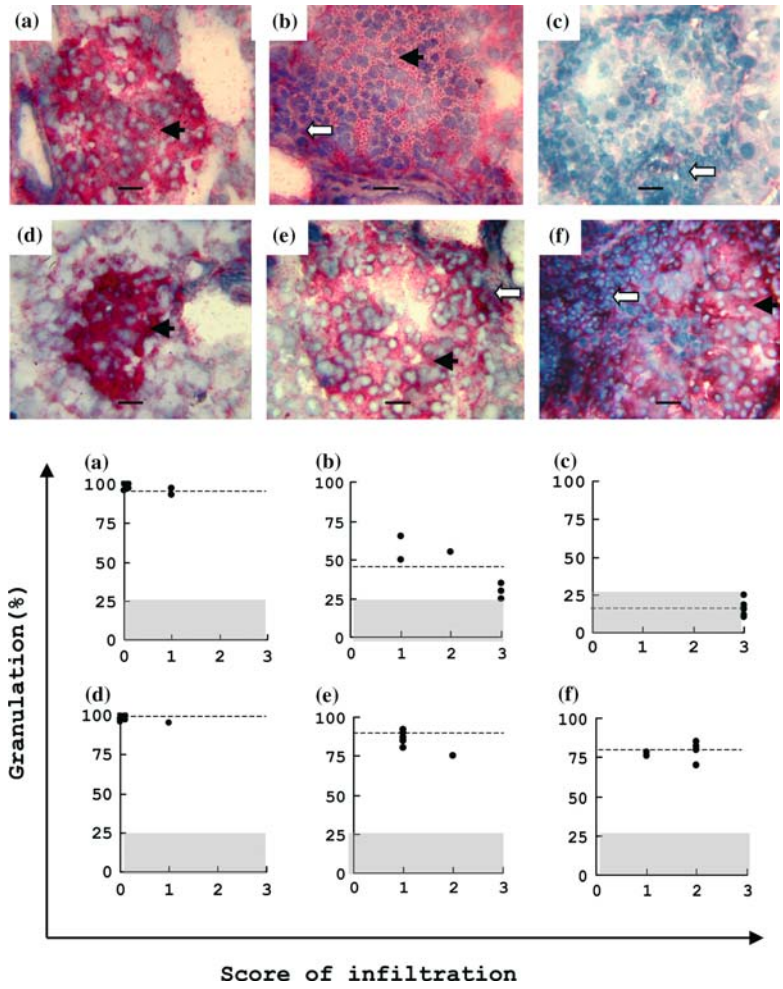

Figure 2. The development of autoimmune diabetes in NOD mice is suppressed by the butanol fraction of *B. pilosa*. Upper panel: pancreatic tissues obtained from NOD mice without treatment (a, b and c) or treated with the butanol fraction of *B. pilosa* (d, e and f) were detected using anti-insulin antibody, alkaline phosphatase-conjugated goat anti-rabbit antibody and Fast-Red development, followed by hematoxylin counterstain. The β cells in red are indicated by a black arrow whereas the islet-associated infiltrated mononuclear cells in blue are indicated by white arrows. a and d: microscopic images of 4-week-old pancreatic β islets; b and e: microscopic images of 15-week-old pancreatic β islets; and c and f: microscopic images of 18-week-old pancreatic β islets. Bar: 10 μm. Lower panel: the β cell granulation (*Y* axis) and leukocyte infiltration (*X* axis) indicate the integrity of β cells in the pancreatic islets and infiltration of leukocytes into the islets, respectively. These scores were calculated from the images in the upper panel, based on the methods described in Materials and methods.

the 2.5 kb GATA-3 promoter region coupled to a luciferase gene, or 1.5 kb pT-bet-Luc, a construct containing the T-bet promoter region coupled to a luciferase gene, together with pRL-TK, a construct containing the Herpes thymidine kinase promoter region coupled to a *Renilla* luciferase gene (Promega, Madison, WI, USA). The cells were incubated for 2 h and then left unstimulated or stimulated for 24 h with a butanol fraction of *B. pilosa* or PMA (Sigma). After the cells were lysed, the postnuclear proteins were quantified by Bradford reagent (Bio-Rad) and subjected to a dual luciferase reporter assay [27].

Statistical analysis

Data from three independent experiments or more are presented as mean ± SE (standard error). Student's *t*-test was used for statistical analysis between various treatment groups in both *in vitro* and *in vivo*

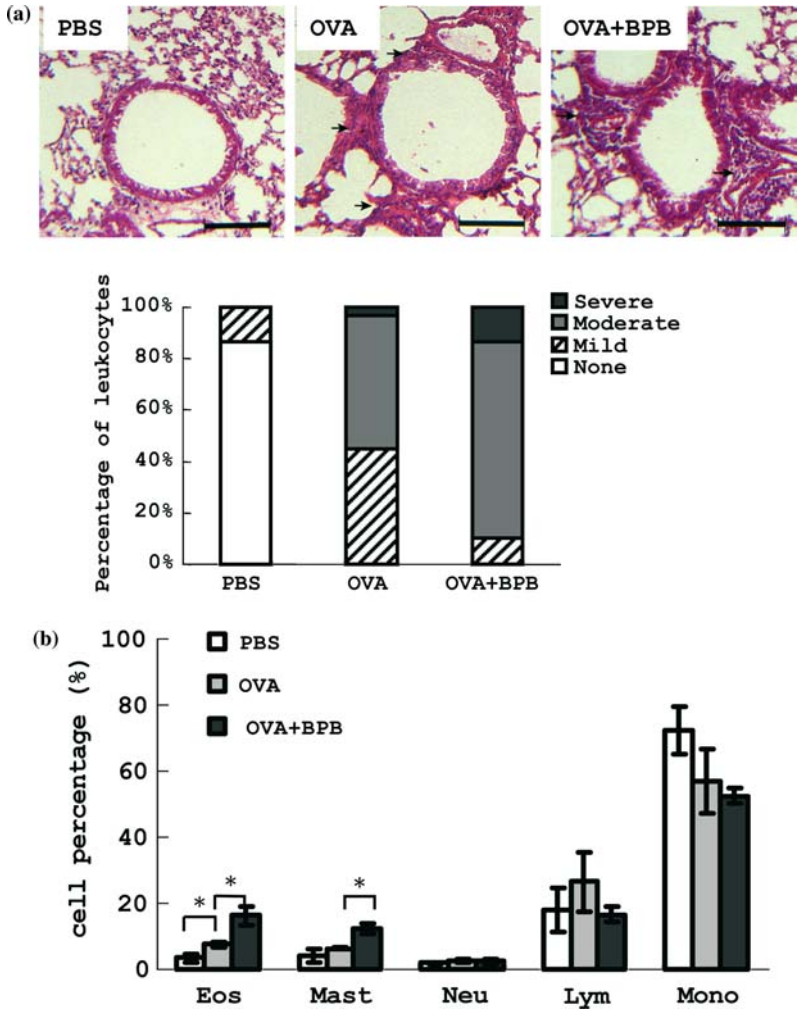

Figure 3. The butanol fraction of *B. pilosa* elevates ovalbumin-induced pulmonary inflammation and leukocyte infiltration. (a) Lung tissues obtained from BALB/c mice receiving PBS treatment (PBS), ovalbumin sensitization (OVA) or ovalbumin sensitization plus butanol fraction of *B. pilosa* treatment (OVA + BPB). The arrows indicate areas of leukocytes infiltration (upper panel; bar: 25 μm). The degree of airway inflammation in different treatments was microscopically examined and graded as none (blank), mild (hatched), moderate (gray) and severe (black) (lower panel). Scoring is described in Materials and methods. (b) BALF cell counts. Percentage of eosinophils (Eos), mast cells (Mast), neutrophils (Neu), lymphocytes (Lym) and monocytes (Mono) from the BALF were determined. *$p < 0.05$ was considered statistically significant.

studies. Statistical probability (p) values less than 0.05 were considered to be statistically significant.

Results

The butanol fraction of B. pilosa *reduces the severity of autoimmune diabetes and suppresses the development of autoimmune diabetes in NOD mice*

In this report, we evaluated the effect of the butanol fraction of *B. pilosa* on Th1- and Th2-mediated immune disorders. Since our preliminary results implied that this fraction could prevent hyperglycemia in NOD mice (data not shown), we first wanted to study whether the butanol fraction of *B. pilosa* could protect NOD mice from diabetes. We found that the butanol fraction treatment in mice helped maintain the normal morphology of pancreatic β islets stained with H&E. These results indicated that this fraction could reduce the severity of insulitis in NOD mice (Figure 1). Next, we investigated whether the butanol fraction of *B. pilosa* could modulate the development of

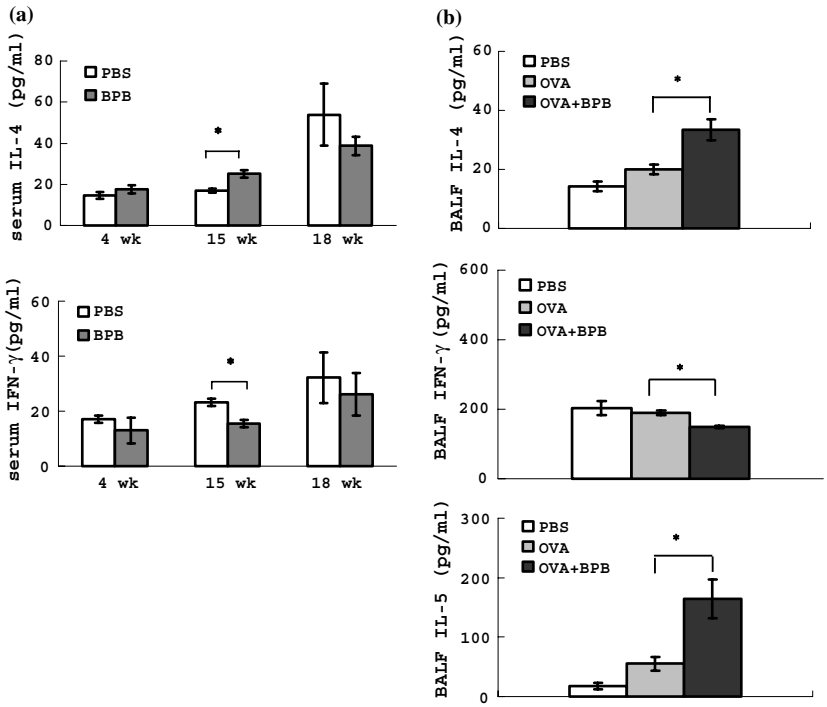

Figure 4. Cytokine profiling of NOD mice and ovalbumin-sensitized mice following treatment with the butanol fraction of *B. pilosa*. (a) The levels of IL-4 and IFN-γ in the blood of NOD mice at 4, 15 and 18 weeks of age were determined by ELISA. (b) The levels of IL-4, IL-5 and IFN-γ in the BALF of BALB/c mice were determined by ELISA. $*p < 0.05$ was considered statistically significant.

autoimmune disease in NOD mice. We confirmed that β cell death and leukocyte infiltration increased during diabetes progression in NOD mice aged 4–18 weeks (Figure 2a–c) as reported elsewhere [28, 29]. Of note, treatment with the butanol fraction of *B. pilosa* inhibited β cell death and leukocyte infiltration in NOD mice aged from 4 to 18 weeks (Figure 2d–f). The inhibition of β cell death and leukocyte infiltration by this fraction persisted for more than 30 weeks (data not shown). Therefore, we conclude that the butanol fraction of *B. pilosa* ameliorates the development of Th1-mediated diabetes in NOD mice.

The butanol fraction of B. pilosa *exacerbates pulmonary inflammation and leukocyte infiltration in BALB/c mice induced by ovalbumin in BALB/c mice*

Since the butanol fraction of *B. pilosa* suppressed Th1 cell-mediated autoimmune diabetes in NOD mice, we wanted to know if it could affect Th2 cell-mediated airway hypersensitivity. We discovered that treatment with the butanol fraction of *B. pilosa* could exacerbate ovalbumin-induced pulmonary inflammation (OVA + BPB, upper and lower panels, Figure 3a) in comparison with normal lung tissue (PBS, upper panel, Figure 3a) or the pulmonary inflammation induced only by ovalbumin (OVA, upper panel, Figure 3a) in BALB/c mice. Consistent with the pulmonary inflammation, the butanol fraction of *B. pilosa* significantly increased the infiltration of eosinophils and mast cells into the airway of the ovalbumin-sensitized BALB/c mice, although other leukocytes in the airway were not much altered (Figure 3b). These data imply that the butanol fraction of *B. pilosa* promotes Th2-mediated pulmonary inflammation induced by allergic ovalbumin in BALB/c mice.

The butanol fraction of B. pilosa *increases production of Th2 cytokines, as well as IgE levels, but decreases Th1 cytokines in NOD mice and ovalbumin-sensitized BALB/c mice*

Cytokines are thought to be crucial for autoimmune diseases [1]. Therefore, we next examined whether

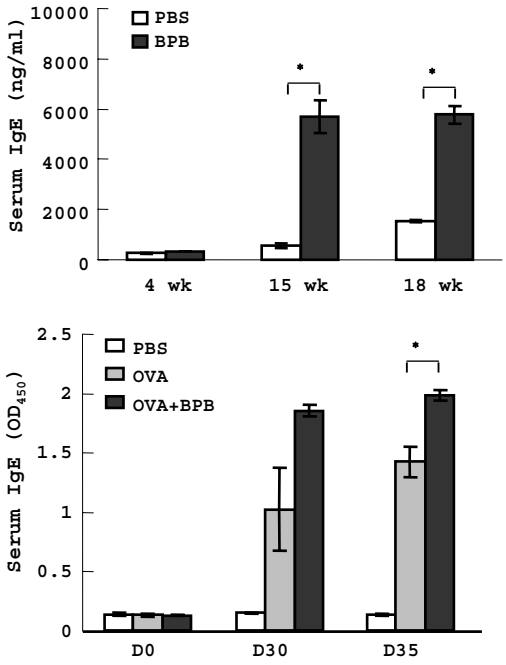

Figure 5. Serum IgE levels in NOD mice and ovalbumin-sensitized mice following treatment with the butanol fraction of *B. pilosa*. Upper panel: blood sera from the NOD mice (4, 15 and 18 weeks) were collected. The sera were incubated on ELISA plates coated with anti-IgE, followed by incubation with anti-IgE and peroxidase-conjugated secondary Ab. The OD_{450} values were obtained using an ELISA reader. The OD_{450} values were converted into ng/ml against a standard curve. Lower panel: blood sera from the BALB/c mice 30 days (D30) and 35 days (D35) post-immunization were collected. The sera were incubated on ELISA plates coated with ovalbumin, followed by incubation with anti-IgE and peroxidase-conjugated secondary Ab. The OD_{450} values were obtained using an ELISA reader. $^{*}p < 0.05$ was considered statistically significant.

treatment with the butanol fraction of *B. pilosa* could affect cytokine levels in the serum of NOD mice or in the BALF of the mice with airway inflammation. We demonstrated that IL-4 production increased whereas IFN-γ production decreased in both mouse models (Figure 4a, b). In addition, IL-5 production increased in the BALF of BALB/c mice (Figure 4b) but was not detectable in the serum of NOD mice (data not shown). The butanol fraction of *B. pilosa* inhibited the production of the Th1 cytokine, IFN-γ, but enhanced that of the Th2 cytokine, IL-4, irrespective of mouse strains; changes in cytokine levels in a local area (e.g. BALF) were more easily detected than in systemic whole-body blood. Since Th2 cytokines, such as IL-4 and IL-5, are important for antibody class switch to IgE class [30], we further checked IgE levels in the serum or BALF. We found that the butanol fraction of *B. pilosa* enhanced IgE production in NOD and BALB/c mice (Figure 5). The level of IgE in the blood or BALF correlated to the level of Th2 cytokines. The levels of IL-5 and IgE in the BALF also correlated to the infiltration of eosinophils and mast cells into the airway (Figure 3), as previously reported [23].

The butanol fraction of B. pilosa *augments Th2 cell differentiation but decreases Th1 cell differentiation* ex vivo

Since IL-4, IL-5 and IFN-γ are primarily produced by different subsets of $CD4^{+}$ helper T cells (namely Th1 or Th2) [1], we subsequently assessed whether the butanol fraction of *B. pilosa* could affect T cell differentiation. We used it to treat differentiating Th1 or Th2 cells *in vitro*. Our results revealed that the butanol fraction of *B. pilosa* suppressed Th1 cell differentiation (and IFN-γ expression) while promoting Th2 differentiation (and IL-4 expression) (Figure 6). To better understand why the butanol fraction of *B. pilosa* preferred Th2 cell differentiation to Th1 cell differentiation, we evaluated if this fraction could affect the expression of T-bet and GATA-3 genes involved in the differentiation of Th1 and Th2, respectively. Accordingly, our results showed that the butanol fraction of *B. pilosa* up-regulated the transcription of GATA-3 instead of T-bet (Figure 7).

Taken together, these results suggest that the butanol fraction of *B. pilosa* regulates T cell differentiation, its cytokines, and autoimmune diseases.

Discussion

In this study, we found that a butanol fraction of *B. pilosa* extract could ameliorate Th1-mediated autoimmune diabetes whilst increasing Th2-mediated pulmonary inflammation. Meanwhile, this fraction preferred Th2 cell differentiation to Th1 cell differentiation. Accordingly, the butanol fraction of *B. pilosa* elevated the transcription of GATA-3 but not T-bet, explaining how this fraction favored Th2 cell differentiation. In good agreement with T cell differentiation, the butanol fraction of *B. pilosa* increased Th2 cytokines but decreased Th1 cytokines. To our knowledge, this is the first report to date evaluating the effect of a *B. pilosa* extract in preventing autoimmune diseases.

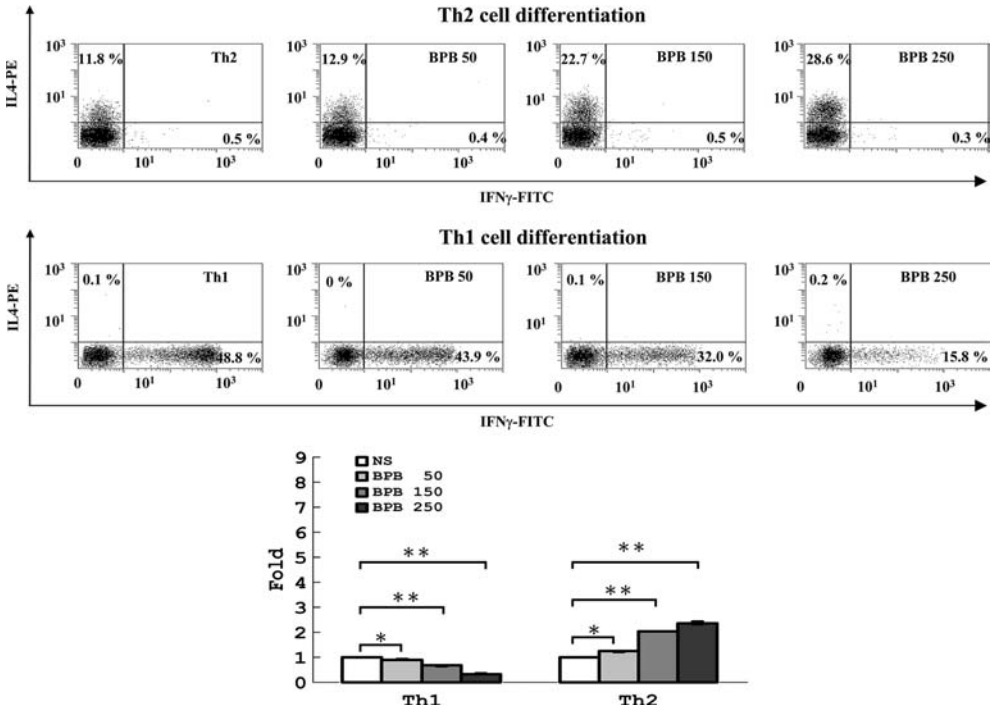

Figure 6. The butanol fraction of *B. pilosa* promotes the differentiation of naive $CD4^+$ T helper cells into Th2 cells but inhibits the differentiation of naive $CD4^+$ T helper cells into Th1 cells. Upper panel: $CD4^+$ cells purified from human umbilical cord blood were treated with vehicle (NS) or the butanol fraction of *B. pilosa* at 50 μg/ml (BPB 50), 150 μg/ml (BPB 150) or 250 μg/ml/ml (BPB 250) under Th1 conditions (PHA, IL-12 and anti-IL-4) or Th2 conditions (PHA, IL-4 and anti-IL-12). T cells were analyzed using FACS and the percentage of IFN-γ or IL-4 producing cells was calculated. Lower panel: data from the upper panel are shown in folds. The decrease in folds of the IFN-γ producing Th1 cell population was calculated based on the following formula: fold = the percentage of the Th1 cell population with BPB treatment/the percentage of Th1 cell population without BPB treatment (NS). Similarly, the increase in folds of the IL-4 producing Th2 cell population was obtained from the following formula: fold = the percentage of the Th2 cell population with BPB treatment/the percentage of Th2 cell population without BPB treatment (NS). * $p < 0.05$ and ** $p < 0.01$ were considered statistically significant.

B. pilosa extracts and a mixture of 2-β-D-glucopyranosyloxy-1-hydroxy-5(*E*)-tridecene-7,9,11-triyne and 3-β-D-glucopyranosyloxy-1-hydroxy-6(*E*)-tetradecene-8,10,12-triyne from *B. pilosa* have been demonstrated to treat diabetes in db/db mice. This result has been partly ascribed to the anorexic effect of both polyacetylenic glucosides [24]. However, the mechanism by which *B. pilosa* extracts or its active polyacetylenic glucosides affect diabetes is unknown. Here, we demonstrated that the butanol fraction of *B. pilosa* can prevent diabetes development in NOD mice. Our previous data showed that both polyacetylenic compounds could inhibit Th1 cell differentiation but promote Th2 cell differentiation [21].

Autoimmune disease is the third largest category of illnesses in the developed world, just behind cardiovascular disease and cancer. So far, treatments for autoimmune diseases rely primarily on immunosuppressants or immune modulators. Immunosuppressants like cyclosporine A can shut down the immune system and prevent inflammation. However, treatment with immunosuppressants may carry a risk of infection or cancer [31, 32]. Immune modulators, which skew T cell differentiation, have been used to treat T cell-mediated disorders. For instance, IL-4 and IL-10 are used for the treatment of Th1-mediated diseases [33–35]. However, a strategy which skews production of Th1 cells into Th2 cells may have adverse effects, such as the induction of Th2-mediated autoimmune diseases. The *B. pilosa* butanol fraction showed potential to prevent Th1-mediated diabetes; however, it also promoted Th2-mediated airway inflammation, similar to IL-4 treatment for Th1 immune disorders [20]. Therefore, administration of the *B. pilosa* butanol fraction provides an

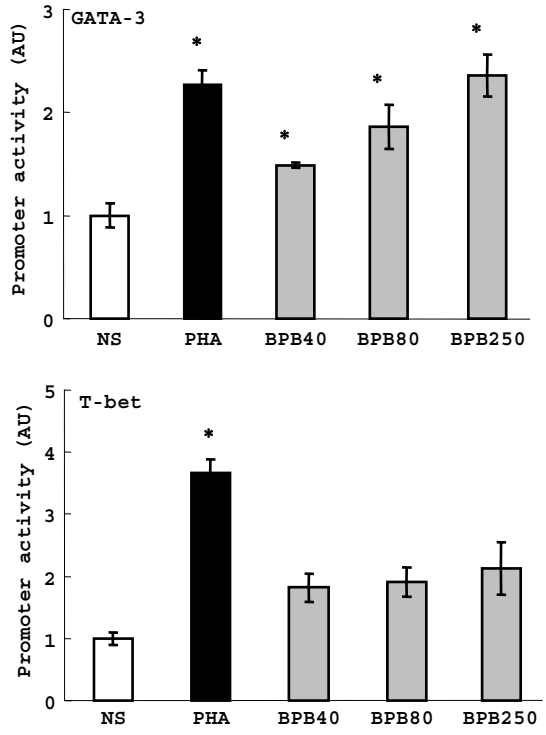

Figure 7. The butanol fraction of *B. pilosa* can promote the transcription of the GATA-3 gene but not the T-bet gene. Jurkat cells, a T cell line, transfected with pGATA-3-Luc or pT-bet-Luc together with pRL-TK were left unstimulated (NS) or stimulated with PHA (positive control) or a butanol fraction of *B. pilosa* at 40 μg/ml (BPB40), 80 μg/ml (BPB80) or 250 μg/ml (BPB250) for 24 h. Ten microgram of cell lysates were subjected to dual luciferase reporter assay. The promoter activities in arbitrary units (AU) were obtained from the ratio of firefly luciferase activity to *Renilla* luciferase activity in the lysates. Data are representative of three experiments. $*p < 0.05$ was considered statistically significant.

alternative in the treatment of Th1-mediated diseases.

Acknowledgements

The authors are indebted to Drs. S.Y. Wang, N.S. Yang, R.H. Hsieh, C.R. Tzeng, M.L. Kuo, T.K. Wu, and H.K. Sytwu for valuable suggestions. We thank Dr. I.C. Ho for kindly providing his reagent. We also want to thank Ms. B.S. Chu and Ms. H.M. Ho for their excellent technical assistance. This work was supported by grants (NSC91-3112-P-001-035-Y, NSC91-2320-B-001-056 and AS91IBAS199) from the National Science Council and Academia Sinica, Taiwan, ROC.

References

1. Abbas A.K., Murphy K.M. and Sher A., Functional diversity of helper T lymphocytes. Nature 383: 787–793, 1996.
2. Castano L. and Eisenbarth G.S., Type-I diabetes: a chronic autoimmune disease of human, mouse, and rat. Annu. Rev. Immunol. 8: 647–679, 1990.
3. Katz J.D., Benoist C. and Mathis D., T helper cell subsets in insulin-dependent diabetes. Science 268: 1185–1188, 1995.
4. Toyoda H. and Formby B., Contribution of T cells to the development of autoimmune diabetes in the NOD mouse model. Bioessays 20: 750–757, 1998.
5. Gavett S.H., Chen X., Finkelman F. and Wills-Karp M., Depletion of murine $CD4^+$ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. Am. J. Respir. Cell. Mol. Biol. 10: 587–593, 1994.
6. Hamelmann E., Oshiba A., Loader J., Larsen G.L., Gleich G., Lee J. and Gelfand E.W., Antiinterleukin-5 antibody prevents airway hyperresponsiveness in a murine model of airway sensitization. Am. J. Respir. Crit. Care Med. 155: 819–825, 1997.
7. Hamelmann E., Takeda K., Haczku A., Cieslewicz G., Shultz L., Hamid Q., Xing Z., Gauldie J. and Gelfand E.W., Interleukin (IL)-5 but not immunoglobulin E reconstitutes airway inflammation and airway hyperresponsiveness in IL-4-deficient mice. Am. J. Respir. Cell Mol. Biol. 23: 327–334, 2000.
8. Hogan S.P., Koskinen A., Matthaei K.I., Young I.G. and Foster P.S., Interleukin-5-producing CD4+ T cells play a pivotal role in aeroallergen-induced eosinophilia, bronchial hyperreactivity, and lung damage in mice. Am. J. Respir. Crit. Care. Med. 157: 210–218, 1998.
9. Gonzalo J.A., Lloyd C.M., Kremer L., Finger E., Martinez A.C., Siegelman M.H., Cybulsky M. and Gutierrez-Ramos J.C., Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and adhesion receptors. J. Clin. Invest. 98: 2332–2345, 1996.
10. Hamelmann E., Takeda K., Schwarze J., Vella A.T., Irvin C.G. and Gelfand E.W., Development of eosinophilic airway inflammation and airway hyperresponsiveness requires interleukin-5 but not immunoglobulin E or B lymphocytes. Am. J. Respir. Cell. Mol. Biol. 21: 480–489, 1999.
11. MacLean J.A., Sauty A., Luster A.D., Drazen J.M. and De Sanctis G.T., Antigen-induced airway hyperresponsiveness, pulmonary eosinophilia, and chemokine expression in B cell-deficient mice. Am. J. Respir. Cell. Mol. Biol. 20: 379–387, 1999.
12. Takeda K., Hamelmann E., Joetham A., Shultz L.D., Larsen G.L., Irvin C.G. and Gelfand E.W., Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. J. Exp. Med. 186: 449–454, 1997.
13. Wills-Karp M., Immunologic basis of antigen-induced airway hyperresponsiveness. Annu. Rev. Immunol. 17: 255–281, 1999.
14. Brandao M.G., Krettli A.U., Soares L.S., Nery C.G. and Marinuzzi H.C., Antimalarial activity of extracts and fractions from *Bidens pilosa* and other Bidens species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds. J. Ethnopharmacol. 57: 131–138, 1997.

15. Chih H.W., Lin C.C. and Tang K.S., Anti-inflammatory activity of Taiwan folk medicine 'ham-hong-chho' in rats. Am. J. Chin. Med. 23: 273–278, 1995.
16. Geissberger P. and Sequin U., Constituents of *Bidens pilosa* L.: do the components found so far explain the use of this plant in traditional medicine? Acta Trop. 48: 251–261, 1991.
17. Jager A.K., Hutchings A. and van Staden J. Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. J. Ethnopharmacol. 52: 95–100, 1996.
18. Pereira R.L., Ibrahim T., Lucchetti L., da Silva A.J. and Goncalves de Moraes V.L., Immunosuppressive and anti-inflammatory effects of methanolic extract and the polyacetylene isolated from *Bidens pilosa* L. Immunopharmacology 43: 31–37, 1999.
19. Rabe T. and van Staden J., Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 56: 81–87, 1997.
20. Ubillas R.P., Mendez C.D., Jolad S.D., Luo J., King S.R., Carlson T.J. and Fort D.M., Antihyperglycemic acetylenic glucosides from *Bidens pilosa*. Planta Med. 66: 82–83, 2000.
21. Chang S.L., Chang C.L., Chiang Y.M., Hsieh R.H., Tzeng C.R., Wu T.K., Sytwu H.K., Shyur L.F. and Yang W.C., Polyacetylenic compounds and butanol fraction from *Bidens pilosa* can modulate the differentiation of helper T cells and prevent autoimmune diabetes in non-obese diabetic mice. Planta Med. 70: 1045–1051, 2004.
22. Iwata A., Nishio K., Winn R.K., Chi E.Y., Henderson W.R., Jr. and Harlan J.M., A broad-spectrum caspase inhibitor attenuates allergic airway inflammation in murine asthma model. J. Immunol. 170: 3386–3391, 2003.
23. Mathew A., MacLean J.A., DeHaan E., Tager A.M., Green F.H. and Luster A.D., Signal transducer and activator of transcription 6 controls chemokine production and T helper cell type 2 cell trafficking in allergic pulmonary inflammation. J. Exp. Med. 193: 1087–1096, 2001.
24. Grattan M., Mi Q.S., Meagher C. and Delovitch T.L., Congenic mapping of the diabetogenic locus Idd4 to a 5.2-cM region of chromosome 11 in NOD mice: identification of two potential candidate subloci. Diabetes 51: 215–223, 2002.
25. Peterson J.D., Pike B., McDuffie M. and Haskins K., Islet-specific T cell clones transfer diabetes to nonobese diabetic (NOD) F1 mice. J. Immunol. 153: 2800–2806, 1994.
26. Hwang E.S., Choi A. and Ho I.C., Transcriptional regulation of GATA-3 by an intronic regulatory region and fetal liver zinc finger protein 1. J. Immunol. 169: 248–253, 2002.
27. Yang W.C., Ching K.A., Tsoukas C.D. and Berg L.J., Tec kinase signaling in T cells is regulated by phosphatidylinositol 3-kinase and the Tec pleckstrin homology domain. J. Immunol. 166: 387–395, 2001.
28. Sreenan S., Pick A.J., Levisetti M., Baldwin A.C., Pugh W. and Polonsky K.S., Increased beta-cell proliferation and reduced mass before diabetes onset in the nonobese diabetic mouse. Diabetes 48: 989–996, 1999.
29. Valorani M.G., Hawa M.I., Buckley L.R., Afeltra A., Cacciapaglia F. and Pozzilli P. The natural history of insulin content in the pancreas of female and male non-obese diabetic mouse: implications for trials of diabetes prevention in humans. Diabetes Metab. Res. Rev. 20: 394–398, 2004.
30. DeKruyff R.H., Rizzo L.V. and Umetsu D.T., Induction of immunoglobulin synthesis by $CD4^+$ T cell clones. Semin. Immunol. 5: 421–430, 1993.
31. Kai N., Motojima K., Tsunoda T. and Kanematsu T., Prevention of insulitis and diabetes in nonobese diabetic mice by administration of FK506. Transplantation 55: 936–940, 1993.
32. Mayer D.F. and Kushwaha S.S., Transplant immunosuppressant agents and their role in autoimmune rheumatic diseases. Curr. Opin. Rheumatol. 15: 219–225, 2003.
33. Kawamoto S., Nitta Y., Tashiro F., Nakano A., Yamato E., Tahara H., Tabayashi K. and Miyazaki J., Suppression of T(h)1 cell activation and prevention of autoimmune diabetes in NOD mice by local expression of viral IL-10. Int. Immunol. 13: 685–694, 2001.
34. Ko K.S., Lee M., Koh J.J. and Kim S.W., Combined administration of plasmids encoding IL-4 and IL-10 prevents the development of autoimmune diabetes in nonobese diabetic mice. Mol. Ther. 4: 313–316, 2001.
35. Trembleau S., Penna G., Gregori S., Gately M.K. and Adorini L., Deviation of pancreas-infiltrating cells to Th2 by interleukin-12 antagonist administration inhibits autoimmune diabetes. Eur. J. Immunol. 27: 2330–2339, 1997.