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PAPER

Honeybee royal jelly inhibits autoimmunity in SLE-prone NZB × NZW F1 mice

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Royal jelly (RJ) is a gelatinous secretion from young nurse worker bees (*Apis mellifera*), which serves as the sole food for the queen bee. Because of its pleiotropic functions for queen bees, RJ has also been used as a dietary supplement with various health benefits for humans. Because RJ is being indicated to have immunomodulatory potential for humans, we undertook the study to determine whether the oral administration of RJ could alter the development of systemic autoimmunity in New Zealand Black (NZB) × New Zealand White (NZW) F1 mice that genetically exhibit many manifestations similar to human systemic lupus erythematosus (SLE). We herein reported that mice administered with RJ showed a significant delay in the onset of the disease, as manifested by decreased proteinuria and a prolongation of lifespan. In addition, RJ administration after the onset of the disease significantly improved the renal symptoms, leading to an extended lifespan. RJ administration to mice caused a significant decrease in the serum level of IL-10, and in the autoantibodies against ssDNA, dsDNA and erythrocytes, as well as a reduction in the number of splenic autoreactive B cells. In conclusion, our data suggest that the use of RJ may be beneficial in the prevention of the early onset of SLE and in the control of the active progression of the manifestations of SLE. *Lupus* (2009) **18,** 44–52.

Key words: autoantibodies; autoreactive B cells; NZB × NZW F1 mice; royal jelly; systemic lupus erythematosus

Introduction

Royal jelly (RJ) is the milky-white gelatinous substance secreted from the cephalic glands of nurse worker bees (*Apis mellifera*) for the sole purpose of stimulating the growth and development of the queen bee. The queen bee fed RJ can develop superior characteristics of size, strength, stamina and longevity. The queen bee can live for 5–7 years, whereas the worker bees live for only about 35–40 days.

Because of the presence of a variety of biologically active compounds, such as free amino acids, simple sugars, proteins, short chain hydroxy fatty acids, trace elements and vitamins, RJ is believed to be a functionally active supplement, not only for the bees but also for humans.[1–3] In addition to its use as a dietary supplement, RJ has been reported to be associated with many health-promoting properties,[4–9] such as cancer prevention, cholesterol reduction and wound healing, as well as anti-fatigue, antihypertensive and antibacterial effects. In addition to these physical effects, several reports have also interestingly postulated that RJ has immunomodulatory activities. For instance, the immunomodulatory properties of RJ through the stimulation of antibody production and immunocompetent cell proliferation in mice have been described.[10] Kohno, *et al.*[11] reported that the anti-inflammatory action of RJ was generated through the inhibition of proinflammatory cytokine production by macrophages. The fatty acids in RJ were suggested to modulate the immune responses in rats through affecting their dendritic cells.[12] Reports providing experimental evidence of RJ suppression of type-I allergic reactions have also been published. Another report showed that the suppression of allergic reactions by RJ was associated with the restoration

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of macrophage function and Th1/Th2 cytokine responses.[13] The oral administration of RJ is also known to inhibit the development of atopic dermatitis–like lesions in mice.[14] However, almost nothing is known about the influence of RJ on autoreactive allergic responses.

Systemic lupus erythematosus (SLE) is a multisystem chronic autoimmune disorder characterised by circulating antigen–antibody complexes. A number of self-antigens, such as dsDNA (double-stranded DNA), ssDNA (single-stranded DNA), histones, ribonuclear proteins, erythrocyte membrane antigens and so on, are considered to participate in the immune complex formation. The conventional treatment of SLE with potent immunosuppressive drugs is accompanied by hazardous side-effects. Therefore, it is suggested that alternative strategies be developed to allow a reduction in the dosage and/or to diminish the toxicity of the medications used in the treatment of SLE. Additional studies to explore effective new alternative approaches are anticipated. The present study aims to address the effects of RJ on autoimmunity, using a genetically SLE-prone mouse model (NZB × NZW F1), which exhibits many manifestations similar to human SLE, including a female predominance of onset, immune complex–mediated glomerulonephritis with proteinuria and antinuclear autoantibody production.

Methods

Mice

SLE-prone (NZB × NZW) F1 female mice, 6 weeks old, were purchased from Japan SLC Inc. (Shizuoka, Japan). The mice are known to be SLE model mice, which spontaneously develop SLE-like manifestations, and almost all mice die within a year.[15] The mice were maintained under specific pathogen-free conditions during the experiments. The experiments were performed according to the ethical principles and guidelines established by the University of the Ryukyus for the care and use of experimental animals.

RJ and administration regimens

Native RJ produced by Yamaguchi's organic beeculture[16] was provided by Japan Royal Jelly Co. Ltd (Tokyo, Japan). The RJ was diluted in phosphate buffered saline (PBS) and 30 μL of diluted RJ solution containing 2.0 mg of protein was orally administrated to each mouse with administration regimens of daily, or twice or thrice weekly. The control mice were treated by the same regimens with RJ-free PBS.

Urinary protein measurement

Proteinuria was measured semiquantitatively by impregnating woolpaper test strips with urine (Uropaper-III, Eiken Chem. Co. Ltd, Tokyo, Japan). According to the manufacturer's instructions, the colour change of the strip infiltrated with the urine sample was visually judged against a standard strip and scored from 0 to 4+. The urinary protein content was graded according to the score as follows: 0 = <15 mg/dL, 1+ = 30 mg/dL, 2+ = 100 mg/dL, 3+ = 300 mg/dL and 4+ = 1,000 mg/dL. The mice that showed proteinuria of over 100 mg/dL on two successive occasions in a week were considered to have developed nephritis.

Serum collection and cell preparation from spleen

Tail snip blood samples were collected from each mouse into capillary tubes and the sera were separated for the estimation of autoantibodies. The splenocytes, used to measure autoreactive B cells by enzyme-linked immunospot assays (ELISPOT), were prepared as follows. The mice were killed by total bleeding through a cardiac puncture and the spleens were excised from the mice. The splenocytes were harvested in a cold Eagle's Minimum Essential Medium (MEM) by mincing and forcing through a 200-gauge stainless steel mesh. The resulting cell suspension was centrifuged and the cell pellet was resuspended in a haemolysing solution consisting of 155 mM NH_4C1, 10 mM $KHCO_3$, 1.0 mM EDTA-Na and 170 mM Tris (pH 7.4) to remove the erythrocytes. After washing twice, the cells were suspended in Eagle's MEM to adjust to concentrations of 5×10^6 and 2×10^6 cells/mL.

Single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) ELISA

The serum IgG antibodies, reactive with ssDNA and dsDNA, were determined by ELISA kits purchased from Alpha Diagnostic International (San Antonio, Texas, USA). All ELISAs were performed according to the manufacturer's instructions. Briefly, 100 μL of each of the diluted serum samples, as well as the negative and positive controls, were added to the ssDNA/dsDNA pre-coated wells of a plastic microplate. The plate was then incubated for 30 min at room temperature (approximately 23 °C). After washing three times with the washing buffer, 100 μL of horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG was added to each well and the plate was further incubated

for 30 min. After incubation, the wells were washed five times and 100 μL of a given substrate solution (3,3′,5,5′-tetramethylbenzidine base) were added to each well. The colour was allowed to develop for 15 min at room temperature and the reaction was stopped by adding 100 μL of stop solution to each well. The absorbance was measured at 450 nm using a microplate reader (Bio-Rad, model 550, Shinagawa, Tokyo, Japan). The antibody index for the ssDNA and dsDNA autoantibodies was calculated as follows: (absorbance of the test sample minus absorbance of the negative control divided by absorbance of the positive control minus absorbance of the negative control) × 100. The indices for the ssDNA and dsDNA autoantibodies were prepared for the confirmation as to the positivity of the test samples. The minimum threshold value for an index was calculated according to the manufacturer's instructions. The antibody indices greater than 5.9 and 6.4 were considered positive for the ssDNA and dsDNA binding autoantibodies, respectively. The positive and negative controls were supplied with the kit. However, the sources of the positive and negative controls were not stated in the manufacturer's instructions.

ELISA for detection of anti-erythrocyte autoantibody

For the preparation of the erythrocyte ghost membrane antigens, heparinized blood samples were collected from NZB × NZW F1 female mice by cardiac puncture. After three washing steps with an excess of cold PBS, the pellet containing anucleated and nucleated blood cells was resuspended in Eagle's MEM. The cell suspension was then passed through a MEM-equilibrated column packed with fibrous cellulose powder (CF11, Whatman International Ltd, Maidstone, Kent, England), and the pure erythrocytes collected as elutes were used for ghost membrane preparation after washing twice with cold PBS. The pellet of the erythrocytes (0.5 mL) was then suspended in 45 mL of a cold haemolysing buffer (5.0 mM/L sodium phosphate, 0.5 mM/L EDTA, pH 8.0) and allowed to undergo lysis for 20 min at 4 °C. The ghost membranes obtained were washed 6 times with the same buffer at 4 °C and the pellet was passed through a 22-gauge needle to assist the lysis of any residual red pellet materials between the two washing steps. Finally, the ghost membranes were treated repeatedly by rapid freezing at −80 °C and thawing to elute the antigens. The crude antigens prepared as above were used as the coating antigens for the anti-erythrocyte ELISA.

For ELISA to detect the anti-erythrocyte IgG antibodies, flat-bottomed microplates (Sumilon, Sumitomo Bakelite Co. Ltd, Tokyo, Japan) were coated with 100 μL/well of erythrocyte ghost membrane antigens (equivalently adjusted to 25 μg/mL of protein content in 0.1 M sodium carbonate buffer, pH 9.6) overnight at 4 °C. After blocking with 1% bovine serum albumin (BSA)–Tris buffered saline (TBS, pH 7.4) for 1 h at room temperature and washing with TBS containing 0.05% Tween 20, 100 μL of the serum samples of each dilution was allowed to react in each well for 2 h at room temperature. After washing three times with the wash buffer, 100 μL of alkaline phosphatase (AP)–conjugated goat anti-mouse IgG (Sigma, St. Louis, MO, USA) was added to each well at a dilution of 1:5000, and the plates were further incubated for 1 h at room temperature. The plates were finally rinsed five times and 100 μL of substrate solution (0.1% *p*-nitrophenyl phosphate: Sigma Fast™; Sigma) was added to each well and allowed to develop colour for 20 min. The reaction was terminated by adding 100 μL of stop solution and the colour absorbance was measured at 405 nm with a plate reader. The pooled sera from 4-week-old NZW mice, which do not produce anti-erythrocyte autoantibodies, were used as the negative controls, whereas those from 60-week-old NZB mice, which spontaneously produce increasing levels of anti-erythrocyte autoantibodies with ageing, were used as the positive controls. Although it was possible to calculate the indices of the test samples, it seemed to be inappropriate to calculate the minimum threshold index for the confirmation as to the positivity of the test samples. Therefore, unlike the anti-ssDNA and anti-dsDNA autoantibodies, we just showed optical density (OD) values for anti-erythrocyte antibodies rather than an index.

IL-10 ELISA

Serum IL-10 was determined by a DuoSet® ELISA Development kit (R & D, Minneapolis, Minnesota, USA). The assay was performed according to the manufacturer's instructions. Briefly, the 96-well microplate was coated with capture antibody diluted in PBS and was incubated overnight at room temperature. Following incubation, the plate was washed and blocked by adding 300 μL of reagent diluent to each well for 2 h. After washing with the wash buffer, 100 μL of sample or standards in reagent diluent were added per well and the plate was incubated for 2 h at room temperature. The plate was then washed and each well was filled with 100 μL of the detection antibody, diluted in reagent diluent. After incubation for 2 h at room temperature, 100 μL of the working dilution Streptavidin–HRP was added to each well. The plate was incubated in the dark for 20 min at

room temperature. After washing, 100 μL of substrate solution was added to each well. After 20 min of incubation, 50 μL of stop solution was added to each well. Finally, the OD of each well was determined using a microplate reader set to 450 nm (Bio-Rad, model 550). The IL-10 concentrations were calculated by creating a computer generated standard curve.

Preparation of dsDNA

Plasmid DNA (pBR322; Takara, Chuo, Tokyo, Japan) was linearised for an ELISPOT assay by digestion with restriction enzymes (Pst1, EcoR1, BamH1 and Nde1) and purified by a plasmid DNA purification kit (Labo-PaSS™ Mini; Hokkaido System Science, Hokkaido, Japan). The linearised dsDNA of plasmid origin was used for coating the microwell ELISPOT plates for the detection of anti-dsDNA antibody secreting B cells.

ELISPOT assay

The ELISPOT assays were performed to measure the autoantibody secreting B cells against ssDNA, dsDNA and erythrocyte membrane antigen. The (Polyvinylidene Fluoride), PVDF-bottomed-well ELISPOT plates (MultiScreen HTS™; Millipore Co., Massachusetts, Bedford, USA) were pre-activated with 15 μL/well of 70% ethanol for 10 min and then coated with purchased salmon testes ssDNA (Sigma) and dsDNA prepared as described above. The erythrocyte membrane antigens prepared for ELISA were also used for the ELISPOT assay. The antigens, diluted in sterile carbonate buffer, pH 9.6, or PBS were applied to each well and incubated overnight at 4 °C. After washing three times with 0.05% Tween 20-TBS, the plates were blocked with 100 μL/well of 1.0% BSA solution in TBS for 1 h at room temperature. Subsequently, the plates were washed and 100 μL of splenic cell suspension containing 2×10^5 or 5×10^5 cells was applied to each well in triplicate followed by incubation for 3 h at 37 °C in a 5% CO_2 atmosphere. After vigorously washing five times with the washing buffer, the plates filled with AP-conjugated goat anti-mouse IgG were further incubated overnight at 4 °C. Following the addition of the one component membrane substrate BCIP/NBT (5-bromo-4-chloro-3-indolyl-phosphate/*p*-nitro blue tetrazolium chloride) and additional 20 to 30-min incubation, the plates were prepared for the counting of the blue spots microscopically.

Statistical analysis

The statistical significance of the data was determined by Student's *t*-test using a computer software program (GraphPad Software, Inc., San Diego, CA, USA). All the *P* values reported were two-sided, and statistical significance was defined as a *P* value below 0.05. The Kaplan–Meier method was used to compare the survival distributions.

Results

Effect of RJ on survival of NZB × NZW F1 mice

To determine whether the oral administration of RJ could alter the survival time of the mice, the Kaplan–Meier survival curves were monitored. RJ was administered orally twice weekly to 8-week-old mice. As shown in Figure 1, the survival of the mice administered with RJ was significantly prolonged in comparison with that of the control mice administered with PBS. For the control mice, the first death was observed on week 31 followed by the deaths of all mice by week 39. However, the first death among the mice administered with RJ was observed on week 39, and it required 44 weeks for all deaths to occur. The mean survival time (40.4 ± 2.2 weeks) of the mice receiving RJ was significantly ($P < 0.005$) longer than that (35.2 ± 2.7 weeks) of the control mice.

In the next investigation, we further determined whether RJ administration after the onset of the disease could affect the survival time of the mice.

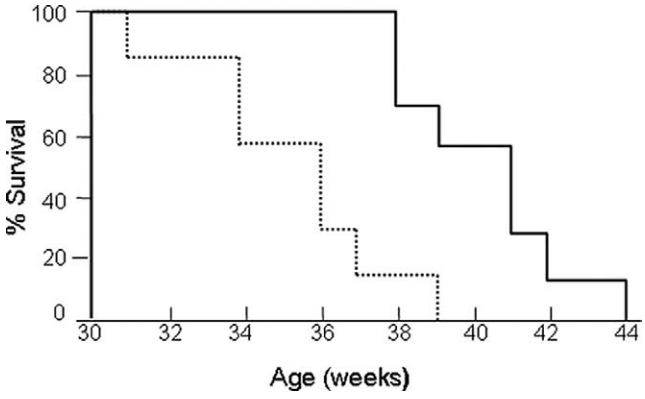

Figure 1 Royal jelly (RJ) administration delayed the onset of disease and prolonged the lifespan of NZB × NZW F1 mice. Two groups of seven mice were orally given 30 μL of RJ solution containing 2 mg of protein twice per week. RJ treatment was initiated when the mice reached 8 weeks of age and was continued until all the mice succumbed to death. As a control, RJ-free PBS was administered with the same regimen. The results were expressed as percentages of survival to number of mice treated. The solid line represents the mortality of the RJ-treated mice and the dashed line represents that of the control mice. A comparison between the mean survival time of the control mice (35.2 ± 2.7 weeks) with that of the RJ-treated mice (40.4 ± 2.2 weeks) showed statistical significance with a *P* value less than 0.005.

For the experiment, RJ administration was started as a daily regimen after the appearance of a proteinuria score of 2+ or more in each mouse, and the mean survival times were calculated from the week starting the treatment (week 0). The results are indicated in Figure 2. The prolongation of the survival time of the RJ mice was also significantly showed in the experiment. The mean survival time of the mice in the RJ-treated group was 13.7 ± 6.4 weeks, which was about 4.2 times longer ($P < 0.005$) than that (3.3 ± 1.9 weeks) of the control mice.

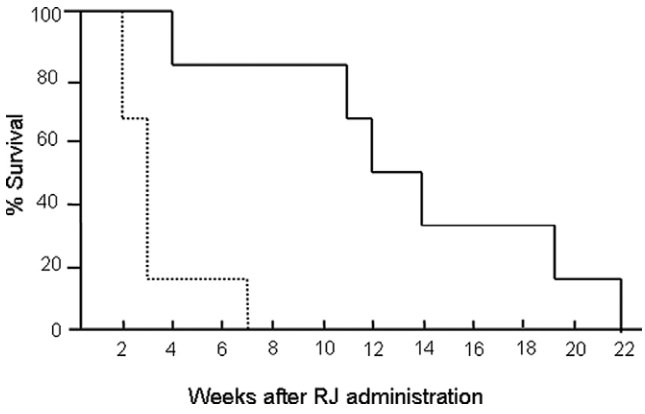

Figure 2 Royal jelly (RJ) administration after the onset of SLE extended the lifespan of NZB × NZW F1 mice. Two groups of seven mice were treated daily with 30 μL of RJ solution containing 2 mg of protein. In the experiment, RJ administration was started after the onset of the disease, when the mice started showing a proteinuria score of 2+ or more in two successive occasions in a week. Another criterion on which we conducted the study was that the mice were of the same age at the start of the treatment. However, the start of the treatment was not the same for the entire group. The solid line represents the mortality of the RJ-treated mice and the dashed line represents that of the control mice. The difference in the mean survival times between the RJ-treated mice and the PBS controls (13.7 ± 6.4 weeks vs 3.3 ± 1.9 weeks, respectively) was statistically significant with a P value less than 0.005.

Impact of RJ on the degree of proteinuria

Because the degree of proteinuria may serve as an index of the progression and severity of kidney disease, we investigated whether RJ administration could affect the degree of proteinuria in the NZB × NZW F1 mice. The proteinuria was monitored for the mice as mentioned in Figure 1, and the urinary protein levels were scored as described in Figure 3. It was apparent that RJ depressed the manifestation of proteinuria in the SLE-prone mice. The inhibition of proteinuria was more apparent even after the onset of renal dysfunction, when the daily administration of RJ was started after the appearance

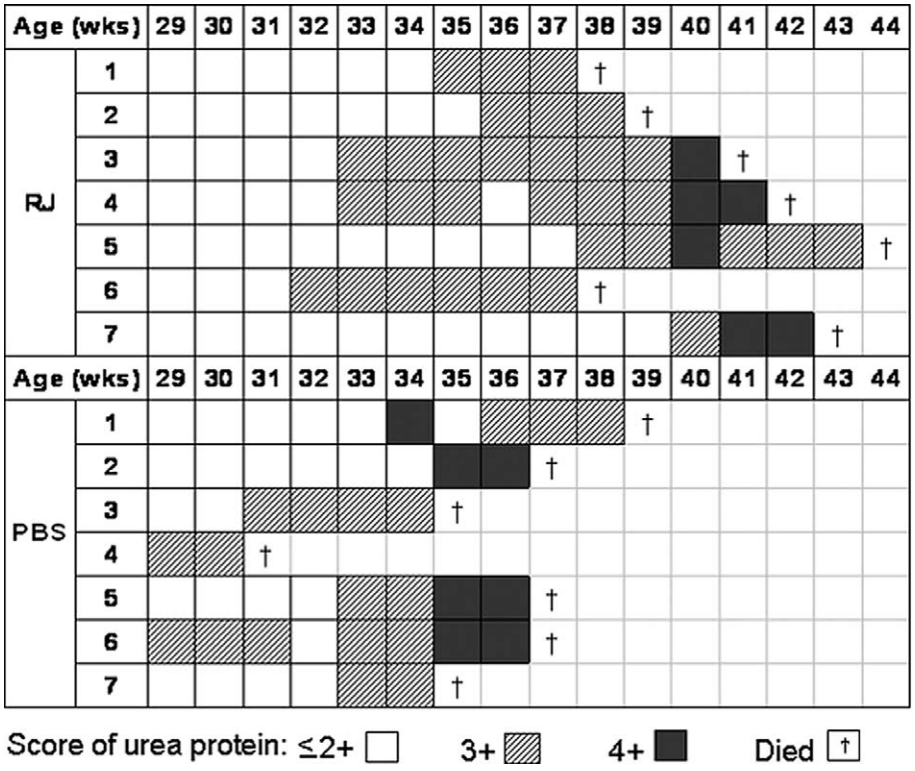

Figure 3 Diagrammatic illustrations showing a delayed increase in urinary protein excretion by RJ administration to NZB × NZW F1 mice. The scores of the urinary protein concentrations of the same mice as described in Figure 1 were transitionally monitored at the indicated times until all deaths occurred.

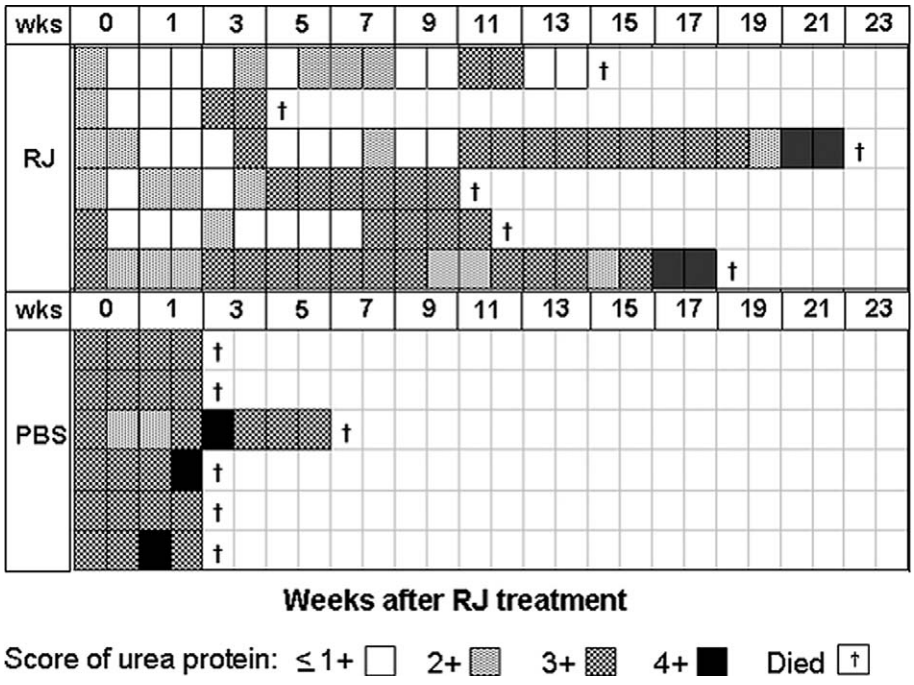

Figure 4 Diagrammatic illustrations showing the effect of RJ in the well-balanced maintenance of proteinuria after the onset of SLE. The scores of the urinary protein of the same mice in Figure 2 are represented. In the experiment, RJ administration was performed daily after the manifestation of urinary protein scores over 2+ on two successive occasions in a week. The scores are represented on the indicated times (weeks) after the initiation of the treatment.

of 2+ or greater proteinuria (Figure 4). The proteinuria score improved tentatively in some mice after RJ administration, and showed a tendency to be maintained at a low level in the mice.

Effect of RJ on autoantibody production

The SLE-afflicted mice are known to produce a variety of autoantibodies. Among the autoantibodies, we determined the presence of serum autoantibodies against nuclear DNA and erythrocyte surface antigen. As to the anti-nuclear autoantibodies, the control mice showed elevated autoantibody levels against both dsDNA and ssDNA. However, anti-dsDNA and anti-ssDNA autoantibody levels were lower in the RJ-mice on weeks 23 and 27, although the difference was not significant in the anti-dsDNA autoantibody levels. However, no difference was observed after 31 weeks of age for both the autoantibodies (Figure 5A,B). The results for anti-erythrocyte autoantibodies were similar to those of the autoantibodies to ssDNA (Figure 5C).

To further confirm the above results, the number of splenic autoreactive B cells to dsDNA, ssDNA and erythrocyte antigens were determined by ELISPOT assays in the mice treated thrice weekly for 13 weeks with RJ. As indicated in Figure 6, the number of anti-dsDNA, anti-ssDNA and anti-erythrocyte autoantibody–producing B cells were lower in the RJ mice than were those of the control mice. The differences were statistically significant for each antigen-specific autoantibody secreting cell, indicating that RJ administration inhibited the autoimmune responses at the cellular level.

Effect of RJ on serum IL-10 levels

IL-10 is an important cytokine that is known to be implicated in the development of SLE. We analysed whether RJ administration could alter the serum IL-10 production in female NZB × NZW F1 mice. At 27 weeks of age, the control NZB × NZW F1 mice produced 476 ± 62 pg/mL, whereas the RJ-fed animals produced only 334 ± 62 pg/mL of IL-10 (Figure 7). That is, RJ administration could reduce the serum levels of IL-10 significantly ($P < 0.005$). The findings suggest that the inhibition of the SLE symptoms in NZB × NZW F1 mice by RJ administration might be mediated through down-regulation of IL-10.

Discussion

The present results report for the first time that the oral administration of RJ prolongs the lifespan of

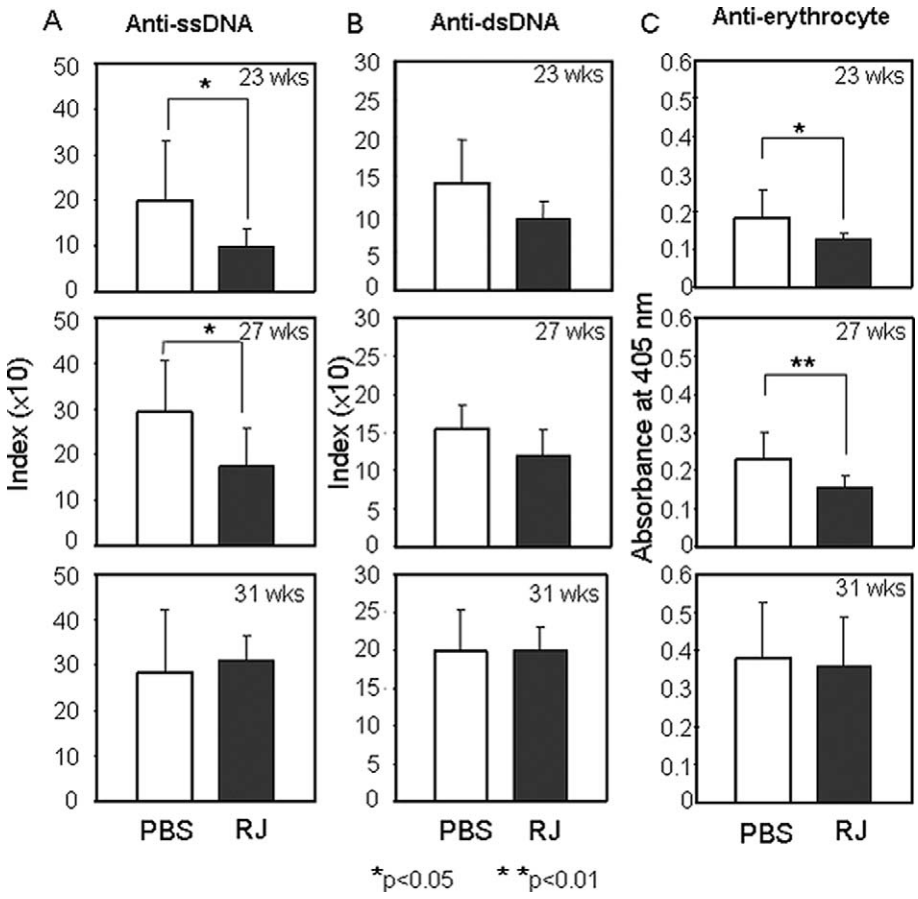

Figure 5 RJ administration caused a decrease in serum IgG autoantibody levels. Sera were collected on weeks 23, 27 and 31 from the mice in Figure 1 and analysed individually for the autoantibodies to ssDNA (A), dsDNA (B) and erythrocyte surface antigen (C) in the mice. Each vertical wide bar represents the mean value of the mice and the vertical lines on the wide bars represent the standard deviations (SD). A *P* value less than 0.05 was considered to be significant.

SLE-prone NZB × NZW F1 mice. The prolongation of the lifespan by RJ was evident not only by the early initiation of administration before the onset of the disease but also by the late treatment after the disease developed.

In these mice, the prolongation of the lifespan was closely associated with the development of proteinuria. The appearance of proteinuria was apparently delayed by the early administration of RJ before the onset of the disease. In addition, RJ was effective in suppressing the progression of disease symptoms even when it was administered after the onset of the disease. The long-term maintenance of moderate levels of proteinuria by RJ resulted in a prolonged survival of the mice. The results show that RJ is effective not only in the prevention of the onset and the progression of the disease but also in the improvement of its manifestations.

The disease in the mouse model is known to be related to the high level of production of various autoantibodies. Our present data showed that the inhibitory effect of RJ on the onset or progression of the disease might be due to a significant decrease in the levels of different types of serum autoantibodies, especially the anti-ssDNA and anti-erythrocyte autoantibodies. Using the ELISPOT assays, we identified the spleen as the major organ for generating the autoreactive B-cell response in murine SLE and also found that RJ could result in a significant reduction in the number of autoreactive cells in the spleen, indicating that RJ administration inhibited the autoimmune response, not only in the serum autoantibody level but also in the number of autoreactive B cells. The inhibition of autoantibody production, however, was limited to the early stage, before the onset of the disease. No difference was observed in the late stage when the mice were over 30 weeks of age. Obviously, we cannot rule out the possibility that the decrease in autoantibody production could be the result of a reduction in hypergammaglobulinemia as a consequence of kidney damage because we previously observed a decreased serum γ-globulin level in the late stage of the disease with serious nephritis in the

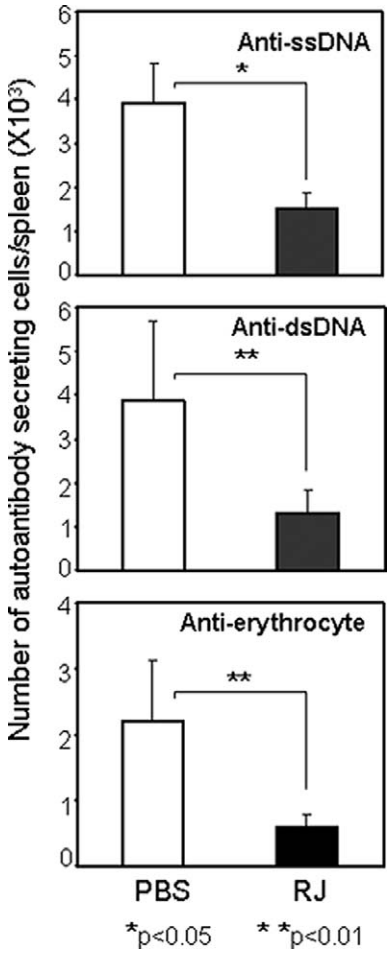

Figure 6 RJ administration caused a decrease in the number of splenic autoreactive B cells. Groups of five NZB × NZW F1 female mice aged 10 weeks received RJ thrice weekly for 13 weeks. The mice were then killed to collect splenocytes for an ELISPOT analysis. The absolute number of autoreactive splenic cells to ssDNA, dsDNA and erythrocyte surface antigen were calculated. The data are represented as the means ± SD of the mice in each group. A *P* value less than 0.05 was considered to be significant.

model mouse.[17] However, the significant inhibition of splenic autoreactive B-cell responses to ssDNA, dsDNA and erythrocytes by RJ treatment may provide a possible explanation as to why autoantibody production was inhibited in the RJ-treated mice. Namely, the inhibitory effect of RJ on the production of autoantibody was truly because of its action on the regulation of antibody secreting B-cell responses, but it was not because of a reduction in hypergammaglobulinemia caused by nephritis. Our findings are consistent with the previously published report showing suppression of T-cell–mediated antibody production by RJ in rats.[18]

However, although the number of autoreactive B cells for all tested autoantibodies was reduced significantly in the RJ-treated mice, it was not statistically reflected in the serum level of anti-dsDNA IgG autoantibody. Therefore, we speculated that other mechanisms might also be involved in the RJ-mediated inhibition of SLE symptoms. With this end in view, we also measured the serum IL-10 levels. It was found that RJ administration caused a significant decrease in the serum IL-10 levels, which are gradually elevated with an increase in age in the NZB × NZW F1 mice. Because IL-10 has been reported to be associated with the development of SLE, the decrease in IL-10 levels by RJ administration may contribute to the amelioration of the disease symptoms.

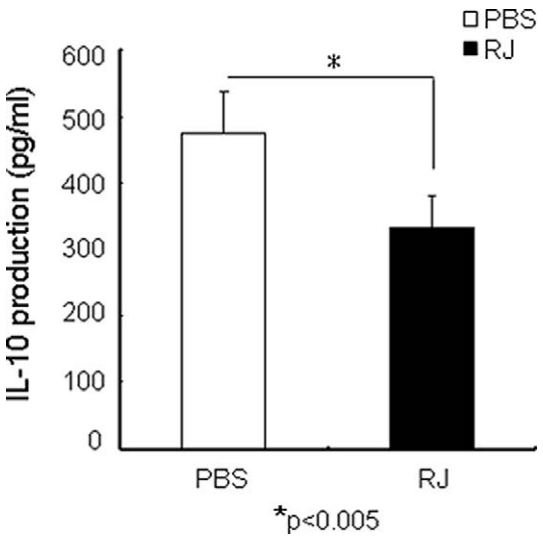

Figure 7 Royal jelly (RJ) inhibited serum IL-10 levels in NZB × NZW F1 mice. IL-10 levels were measured by ELISA and the differences in the serum IL-10 levels between the RJ-treated mice and the control mice were compared. Sera were collected from 27-week-old mice. The white bars indicate the results for the control group and the solid black bars indicate those of the RJ-treated group. The results represent the mean ± SD of seven mice. A *P* value less than 0.05 was considered to be significant.

At any rate, the findings thus provide new evidence that RJ may provide benefit to persons at risk for SLE, as well as those in the early stages of the disease. That is, RJ may be beneficial as a dietary supplement for those who are at risk of developing SLE, or as an adjunct to conventional medications for the treatment of SLE. The beneficial effects of RJ on the clinical manifestations of SLE may have a possible association with the down-regulation of the levels of different cytotoxic autoantibodies, as well as IL-10. The therapy of SLE with conventional treatment approaches requires high doses of immunosuppressive agents given over prolonged time-periods and is sometimes questionable in terms of safety issues and the degree of variability in the results. Therefore, it is suggested that

alternative strategies are required to reduce the dosage and/or diminish the toxicity of the potent immunosuppressive drugs.[19] This is why alternative treatment methods, such as the use of vitamins, fatty acids and some Chinese herbal medicines, have attracted a great deal of recent attention.[20–22] In this respect, the present study may deserve the attention of the clinicians who treat patients with SLE.

RJ has been reported to possess several pharmacological activities including anti-allergic, anti-inflammatory and immunomodulatory properties. Thus, the protective action of RJ against autoimmune diseases may be attributable to the effect of several ingredients of RJ on the immune system. The anti-inflammatory action of major RJ protein 3 (MRJP3) through the inhibition of proinflammatory cytokines, such as TNF-α, IL-6 and IL-1 has been reported.[11] Although several case reports demonstrating the role of RJ in the induction of asthma and anaphylaxis have been reported,[23,24] reports providing experimental evidence on the effect of RJ in the suppression of allergic reaction through the restoration of macrophage function and Th1/Th2 cytokine responses have also been published.[13] In addition, a recent report showed that purified RJ fatty acids, namely 10-hydroxy-2-decanoic acid (10-HAD) and 3,10-dihydroxy-decanoic acid (3,10-DDA) possessed immunomodulatory activities *in vitro* manifested generally by the DC-dependent inhibition of allogeneic T-cell proliferation and IL-2 production, as well as the suppression of the antigen-specific immune response *in vivo*.[12] Conversely, RJ has been reported to be associated with immunostimulatory functions through the induction of proinflammatory cytokine production.[25] On the basis of the contents of the previously published reports and our findings in this study, we therefore hypothesise that the protection of mice against SLE by RJ, as manifested by a prolongation of lifespan associated with the amelioration of disease activities, may be a balancing action of different RJ ingredients favouring the host.

Acknowledgements

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References

1 Hove, SR, Dimick, PS, Benton, AW. Composition of freshly harvested and commercial royal jelly. *J Apic Res* 1985; **24**: 52–61.
2 Lercker, G, Capella, P, Conte, LS, Ruini, F, Giordani, G. Componets of rotal jelly I. Identification of the organic acids. *Lipids* 1981; **16**: 912–919.
3 Lercker, G, Capella, P, Conte, LS, Ruini, F, Giordani, G. Componets of rotal jelly II: the lipid fraction, hydrocarbons and sterols. *J Apic Res* 1982; **21**: 178–184.
4 Tamura, T, Fujii, A, Kuboyama, N. Antitumor effects of royal jelly (RJ). *Nippon Yakurigaku Zasshi* 1987; **89**: 73–80 [article in Japanese].
5 Vittek, J. Effect of royal jelly on serum lipids in experimental animals and humans with atherosclerosis. *Experientia* 1995; **51**: 927–935.
6 Fujii, A, Kobayashi, S, Kuboyama, N, *et al.* Augmentation of wound healing by royal jelly (RJ) in streptozotocin-diabetic rats. *Jpn J Pharmacol* 1990; **53**: 331–337.
7 Kamakura, M, Mitani, N, Fukuda, T, Fukushima, M. Anti-fatigue effect of fresh royal jelly in mice. *J Nutr Sci Vitaminol (Tokyo)* 2001; **47**: 394–401.
8 Tokunaga, KH, Yoshida, C, Suzuki, KM, *et al.* Antihypertensive effect of peptides from royal jelly in spontaneously hypertensive rats. *Biol Pharm Bull* 2004; **27**: 189–192.
9 Fujiwara, S, Imai, J, Fujiwara, M, Yaeshima, T, Kawashima, T, Kobayashi, K. A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. *J Biol Chem* 1990; **265**: 11333–11337.
10 Okamoto, I, Taniguchi, Y, Kunikata, T, *et al.* Major royal felly protein 3 modulates immune responses in vitro and in vivo. *Life Sci* 2003; **73**: 2029–2045.
11 Kohno, K, Okamoto, I, Sano, O, *et al.* Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. *Biosci Biotechnol Biochem* 2004; **68**: 138–145.
12 Vucevic, D, Melliou, E, Vasilijic, S, *et al.* Fatty acids isolated from royal jelly modulate dendritic cell-mediated immune response in vitro. *Int Immunopharmacol* 2007; **7**: 1211–1220.
13 Oka, H, Emori, Y, Kobayashi, N, Hayashi, Y, Nomoto, K. Suppression of allergic reactions by royal jelly in association with the restoration of macrophage function and the improvement of Th1/Th2 cell responses. *Int Immunopharmacol* 2001; **1**: 521–532.
14 Taniguchi, Y, Kohno, K, Inoue, S, *et al.* Oral administration of royal jelly inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. *Int Immunophamacol* 2003; **3**: 1313–1324.
15 Andrews, BS, Eisenberg, RA, Theofilopoulos, AN, *et al.* Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *J Exp Med* 1978; **148**: 1198–1215.
16 Yamaguchi, K. Innovation of natural beekeeping and quality control of bee products for promising apiculture. 1. Current problems of beekeeping and recurrence of natural bee-culture for resolution of the problems. *J Yunan Agric Univ* 2004; **19**: 322–329.
17 Morshed, SRM, Mannoor, K, Halder, RC, *et al.* Tissue-specific expansion of NKT and $CD5^+$ B cells at the onset of autoimmune disease in $(NZB \times NZW)F_1$ mice. *Eur J Immunol* 2002; **32**: 2551–2561.
18 Sver, L, Orsolic, N, Tradic, Z, Njari, B, Valpotic, I, Basic, I. A royal jelly as a new potential immunoregulator in rats and mice. *Comp Immunol Microbiol Infect Dis* 1996; **19**: 31–38.
19 Patavino, T, Brady, DM. Natural medicine and nutritional therapy as an alternative treatment in systemic lupus erythematosus. *Altern Med Rev* 2001; **6**: 460–471.
20 Maeshima, E, Liang, XM, Goda, M, Otani, H, Mune, M. The efficacy of vitamin E against oxidative damage and autoantibody production in systemic lupus erythematosus: a preliminary study. *Clin Rheumatol* 2007; **26**: 401–404.
21 Duffy, EM, Meenagh, GK, McMillan, SA, Strain, JJ, Hannigan, BM, Bell, AL. The clinical effect of dietary supplementation with omega-3 fish oils and/or copper in systemic lupus erythromatosus. *J Rheumatol* 2004; **31**: 1551–1556.
22 Yap, HK, Ang, SG, Lai, YH, Ramgolam, V, Jordan, SC. Improvement in lupus nephritis following treatment with a Chinese herbal preparation. *Arch Pediatr Adolesc Med* 1999; **153**: 850–852.
23 Thien, FC, Leung, R, Baldo, BA, Weiner, JA, Plomley, R, Czarny, D. Asthma and anaphylaxis induced by royal jelly. *Clin Exp Allergy* 1996; **26**: 216–222.
24 Takahama, H, Shimazu, T. Food-induced anaphylaxis caused by ingestion of royal jelly. *J Dermatol* 2006; **33**: 424–426.
25 Majtan, J, Kovacova, E, B□likova, K, Simuth, J. The immunostimulatory effect of the recombinant apalbumin 1-major honeybee royal jelly protein on TNF-alpha release. *Int Immunopharmacol* 2006; **6**: 269–278.