Effects of Rooibos Tea Extract on Antigen-specific Antibody Production and Cytokine Generation in Vitro and in Vivo

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Rooibos tea contains a large amount of flavonoids and acts as a potent antioxidant. In this study, we examined the effects of Rooibos tea extract on antigen-specific antibody production and cytokine generation in vitro and in vivo. The primary in vitro anti-ovalbumin (anti-OVA) or sheep red blood cell (SRBC) antibody production in murine splenocytes was markedly stimulated by the addition of the tea extract at concentrations of 1-100 μg/ml. On the other hand, a nonspecific antibody response elicited with lipopolysaccharide (LPS) in purified splenic B-cells was not modified by the extract. Rooibos tea extract caused an increase in the generation of interleukin 2 (IL-2) both in OVA- and anti-CD3-prime splenocytes at concentrations ranging from 10 μg/ml to 1000 μg/ml. In contrast, this tea extract suppressed the generation of interleukin 4 (IL-4) in OVA-prime splenocytes. Moreover, the reduction of OVA-induced antibody production in serum of the cyclosporin A (CyA) -treated rats can be significantly restored, and the IL-2 generation in murine splenocytes was stimulated, following oral administrations of Rooibos tea extract. Thus, our findings suggested that Rooibos tea extract may facilitate the antigen-specific antibody production through selective augmentation of IL-2 generation both in vitro and in vivo. Collectively, Rooibos tea intake may be of value in prophylaxis of the diseases involving a severe defect in Th1 immune response such as cancer, allergy, AIDS, and other infections.

Key words: Rooibos tea; antibody production; IL-2 generation; murine splenocytes

The immune system plays an important role in maintenance of the body homeostasis by eliminating endogenously formed mutated cells such as virus-infected or tumor cells as well as exogenous invading microorganisms. The impairment of the immune responses is known to cause autoimmune diseases and allergy. Thus, so far many immunomodulators have been developed, some being widely used in clinics. Representative immunomodulators are lentinan,2,3 cyclosporin,4 tacrolimus,4,5 d-penicillamin,6 gold salts,7 and ascorbate derivatives.8,9 One might expect the advent of novel immunostimulators, because safe and effective drugs are of critical importance in control of infectious diseases, tumor development, and various types of allergic diseases.

The current studies indicate that there is a functional interaction between immune and nervous systems, namely stimulation of the immune system can modify brain functions and vice versa, in which common factors such as IL-2 mediate.10,11,12

Rooibos (Aspalathus linearis), used to make a kind of herbal tea, grows on the slopes of the Cederberg mountain range in Cape Province, Republic of South Africa. This tea has been taken as a health drink for more than a century in the Republic of South Africa and in Europe.13,14 It is known that Rooibos tea contains abundant flavonoids15 and has a potent antioxidative activity,16 but interestingly contains no caffeine, no alkaloids, and low contents of tannins.17 It is reported that Rooibos tea has diverse physiological and pharmacological actions such as a suppressive effect on the induction of chromosome aberrations,18 radioprotective effect in x-ray irradiated mice,19 inhibitory effect on age-related accumulation of lipid peroxides,19 and anti-HIV activity in vitro.13,20 Moreover, some attempts have been made to discover whether Rooibos tea is effective in control of allergic diseases,21 because many antioxidants inhibit histamine release from mast cells.22 However, so far, little is known about the immunological properties of this tea.
Thus, in this work we studied the effects of Rooibos tea extract on antigen-specific antibody production and cytokine generation in vitro and in vivo, and we showed that this tea extract increased the primary antigen-specific antibody production, with an increase in IL-2 generation.

Materials and Methods

Experimental Animals. Specific-pathogen-free female BALB/c mice (6 weeks old) were purchased from Charles River Japan (Kanagawa). These mice were kept in our animal facility for at least 1 week before use. All mice were used at 8 to 12 weeks of age. Wistar/ST female rats (5 weeks old) were purchased from SLC Japan (Hamamatsu).

They were housed in a room with controlled temperature (23-25°C), humidity (50-60%), and a preset light-dark cycle (12 h, 12 h). Okayama University’s guide for the care and use of laboratory animals was followed in this study.

Reagents. RPMI-1640 medium was purchased from ICN Biomedicals Japan (Tokyo). Eagle’s MEM was from Nissui Pharmaceutical (Tokyo). FBS was from Gibco BRL (Gaithersburg, MD). Goat antimouse IgM and purified mouse IgM were from Organon Teknika (Durham, NC). Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgM was from Kirkegaard & Perry Lab. (Gaithersburg, MD). HRP-conjugated streptavidin was from Zymed Lab. (San Francisco, CA). 2,2′-Azino-bis-(3-ethylbenz-thiazone-6-sulfonic acid) (ABTS), N-2-hydroxyethylpiperadine-N'-2-ethane sulfonic acid (HEPES), Escherichia coli LPS, bovine serum albumin (BSA) (Fraction V), CyA, OVA (grade VI), and 3,3′-diaminobenzidine tetrahydrochloride were purchased from Sigma Aldrich Japan (Tokyo). Anti-Thy 1.2 antibody (clone; F7D5) was from Serotec (Oxford, England). Low toxicity rabbit complement was from Cederlane Lab. (Ontario, Canada). Penicillin G was from Banyu Pharmaceutical (Tokyo). Streptomycin was from Meiji Seika (Tokyo). Rat anti-mouse IL-2, rat anti-mouse IL-4 antibody, biotin-conjugated anti-mouse IL-2, biotin-conjugated anti-mouse IL-4 antibody, and hamster anti-mouse CD3e monoclonal Ab were from Pharmingen (San Diego, CA). Recombinant murine IL-2 and IL-4 were from Genzyme Corp. (Cambridge, MA). SRBC was purchased from Japan Lamb ( Hiroshima).

Extraction of Rooibos tea. The crude extracts were prepared by boiling Rooibos leaves (3.25 g) with 200 ml distilled water for 15 min. After filtration and centrifugation, the supernatant was lyophilized without any purification and kept until the in vitro experiments. For in vivo experiments, the tea (3.25 g) was extracted with 1000 ml of boiled water for 15 min and the crude extract was ingested into rats or mice.

Culture conditions. Cells were cultured in RPMI-1640 medium with 10% heat-inactivated fetal bovine serum (FBS), 50 μM 2-mercaptoethanol (2-ME), 100 U/ml penicillin G, and 100 μg/ml streptomycin at 37°C under 5% CO₂ and 95% air.

Antigen-specific antibody production and cytokine generation in murine splenocytes. For antigen-specific antibody production, spleen cells (1.25 × 10⁶ cells/200 μl/well), in which red blood cells had been lysed by adding ammonium chloride solution (NH₄Cl; 144 mM, Tris; 16.5 mM, pH 7.2), were cultured with antigen (OVA or SRBC) and varying concentrations of Rooibos tea extract in 96-well round-bottom microplates (NUNC; 163320). On day 3 of the culture, cells were collected from each well, washed twice with minimum essential medium (MEM), and then the culture was continued in the presence of the tea extract under the antigen-depleted condition for another 7 days. Cultured cells were collected for cell counts, and the culture medium was collected and frozen at -30°C for IgM enzyme-linked immunosorbent assay (ELISA).

For cytokine generations, spleen cells (2 × 10⁶ cells/200 μl/well), of which red blood cells had been lysed, were cultured with antigen (OVA) or anti-CD3e Ab and varying concentrations of Rooibos tea extract in 96-well flat-bottom microplates (NUNC; 167008) for 3 days. The culture medium was collected and frozen at -30°C for cytokine analysis.

Purification of murine splenic B cells. Splenic B-cells were prepared from BALB/c mice by the method of Kehry and Hudak. Erythrocytes were lysed in Tris-buffered ammonium chloride, and adherent cells were removed by incubating the splenocytes on plastic petri dishes (Falcon; 1005A) in 10 mM HEPES (pH 7.2) buffered MEM with 10% heat-inactivated FBS at 37°C for 90 min, and lastly T-cells were removed by treatments with 1/500 diluted anti-Thy 1.2 Ab (F7D5) at 4°C for 30 min and then with 1/15 diluted low toxicity rabbit complement at 37°C for 40 min.

Antibody production in LPS-stimulated splenic B cells. Purified B-cells (2 × 10⁶ cells/200 μl/well) were cultured in RPMI-1640 medium with LPS (10 μg/ml) and varying concentrations of Rooibos tea extract in 96-well round-bottom microplates for 7 days. Cells were counted, and the culture medium was frozen at -30°C for IgM ELISA.

Experiment on antigen-specific antibody production in vivo. Wistar/ST female rats were injected intraperitoneally with 1 mg/body of OVA. CyA
(10 mg/kg) was administrated intravenously 24 h before OVA challenge. Rooibos tea extract was ingested *ad libitum* (ca. 30 ml/day) starting from one week before OVA challenge to end of the experiment for 3 weeks. Sample sera were frozen at -30°C until assay of anti-OVA IgM.

**Experiment of IL-2 generation in vivo.** BALB/c mice were injected intraperitoneally with 100 μg/body of OVA for generation of IL-2. IL-2 secreting cells in murine splenocytes were detected by enzyme-linked immuno-spot assay (ELISPOT assay) two days after OVA challenge. Rooibos tea extract was ingested *ad libitum* (ca. 4 ml/day) starting from 3 weeks before the ELISPOT assay.

**Measurement of the antigen-specific and total IgM levels.** The antigen-specific and total IgM levels were measured by sandwich ELISA.26 For measurement of antigen-specific IgM, 96-well flat-bottom plates (NUNC; POLYSOAP 475094) were coated with 50 μl of OVA or SRBC (20 μg/ml) at 4°C overnight, and washed with 7.5 mM PBS containing 0.05% Tween 20, and then blocked with 100 μl of 2% BSA in 7.5 mM PBS containing 0.05% Tween 20 at 37°C for 2 h. After washing, the coated plates, with 50 μl of culture media or appropriately diluted standard sera added were incubated at 4°C overnight. After washing, the plates with 50 μl of HRP-conjugated anti-mouse IgM (100 ng/ml) added were incubated for 2 h at 37°C. After washing, peroxidase activity was evaluated in 100 μl of 0.1 M citrate buffer (pH 4.0) containing 2.5 mM ABTS and 0.17% H2O2 in 30 min at room temperature with an ELISA autoreader (Sanko Junyaku, Tokyo) at 405 nm. Pooled anti-OVA serum (160000 U/ml) obtained from OVA-immunized BALB/c mice was used as standard.

For total IgM measurement, 96-well flat-bottom plates (NUNC; MAXISOAP 442404) were coated with 50 μl of anti-mouse IgM Ab (10 μg/ml) at 4°C overnight, and washed with 15 mM phosphate buffered saline (PBS), and then blocked with 100 μl of 2% BSA in 15 mM PBS at 37°C for 2 h. After washing, the coated plates with 50 μl of culture media or standard IgM added were incubated at 4°C overnight. The following process was the same as described above.

**Measurement of cytokines.** Cytokines were measured by sandwich ELISA.25-28 Flat-bottom plates (96-well, MAXISOAP) were coated with 50 μl of anti-mouse IL-4 or anti-mouse IL-2 Ab (2 μg/ml) at 4°C overnight, and washed with 7.5 mM PBS containing 0.05% Tween 20, and then blocked with 100 μl of 1% BSA in 7.5 mM PBS containing 0.05% Tween 20 at 37°C for 2 h. After washing, the coated plates, with 50 μl of culture medium or standard IL-2 or IL-4 added, were incubated at 4°C overnight. After washing, the coated plates with 50 μl of biotin-conjugated anti-mouse IL-2 or anti-mouse IL-4 Ab were incubated at 37°C for 2 h. After washing, the plates with 50 μl of HRP-conjugated streptavidin added were incubated at 37°C for 1 h. After washing, peroxidase activity was evaluated in 100 μl of 0.1 M citrate buffer (pH 4.0) containing 2.5 mM ABTS and 0.17% H2O2 in 30 min at room temperature with an ELISA autoreader at 405 nm.

**Detection of IL-2 secreting cells.** IL-2 secreting cells were detected by ELISPOT assay. Briefly, 24-well culture plates (NUNC,143982) were coated with 350 μl of anti-mouse IL-2 Ab (2 μg/ml) at 4°C overnight, and washed with 7.5 mM PBS, and then blocked with 400 μl of 1% BSA in 7.5 mM PBS at 37°C for 2 h. After washing, the coated plates, with splenocytes (1 × 107 cells/well) from control or Rooibos tea-ingested mice added, were incubated for 3 days. After washing, the coated plates with 350 μl of biotin-conjugated anti-mouse IL-2 added were incubated at 37°C for 2 h. After washing, the plates with 350 μl of HRP-conjugated streptavidin added were incubated at 37°C for 1 h. After washing, 400 μl of 15 mM phosphate buffer (pH 7.4) containing 2 mg/ml 3,3’-diaminobenzidine tetrahydrochloride, 1% agarose, 0.06% intensifier (3% H2O2, 3% NiCl2, and CoCl2) was added, and the plates were incubated in the dark for more than 8 h at 37°C. The black spots that appeared in the well were counted under a stereoscopic microscope.

**Results**

**Effects of Rooibos tea extract on the antigen-specific antibody production in murine splenocytes in vitro**

Rooibos tea is known to have diverse biological activities,13-20 but the immunological properties of this tea were little known. Thus, we examined the effects of Rooibos tea extract on the in vitro antigen-specific antibody responses of murine splenocytes. As shown in Fig. 1, the primary anti-OVA antibody production was markedly increased by Rooibos tea extract at concentrations from 1 μg/ml to 100 μg/ml. At a higher concentration (1000 μg/ml), the antibody production was suppressed by this extract. In the primary response against the particulate antigen SRBC, similar augmentation was also observed (Fig. 2).

Under the same conditions as Fig. 1, another experiment showed that viable cell numbers were improved by treatment of Rooibos tea extract at concentrations from 1 μg/ml to 100 μg/ml and much decreased at 1000 μg/ml. The cell numbers were counted by trypsin blue dye exclusion assay, the numbers were 1.32 ± 0.03 (none), 1.64 ± 0.04 (1 μg/ml), 1.96 ± 0.03 (10 μg/ml), 2.18 ± 0.05 (100 μg/ml), and 0.46 ± 0.01 (1000 μg/ml). These results suggested...
that an improvement in cell numbers contributes in some degree to the increase of the antibody produc-

**Fig. 1.** Effects of Rooibos Tea Extract on the Anti-OVA Antibody Production in Cultured Murine Splenocytes.

Splenocytes (1.25 × 10⁶ cells/200 μl/well) were cultured with OVA (500 ng/ml) and various doses of Rooibos tea extracts in 96-well culture plates, under 95% air – 5% CO₂ atmosphere for 3 days, and the culture was continued for another 7 days in the antigen-depleted condition. Anti-OVA IgM level was measured by ELISA. Values are mean ± SD for triplicate cultures. Statistical significance (*) was determined by a Student’s t test and a value of P<0.05 compared with the control was considered to be significant.

**Fig. 2.** Effects of Rooibos Tea Extract on the Anti-SRBC Antibody Production in Cultured Murine Splenocytes.

Splenocytes (1.25 × 10⁶ cells/200 μl/well) were cultured with SRBC (8 × 10⁶ cells/well) and various dose of Rooibos tea extracts in 96-well culture plates under 95% air – 5% CO₂ atmosphere for 3 days, and the culture was continued for another 7 days in the antigen-depleted condition. Anti-SRBC IgM level was measured by ELISA. Values are mean ± SD for triplicate cultures. Statistical significance (*) was determined by a Student’s t test and a value of P<0.05 compared with the control was considered to be significant.

**Fig. 3.** Effects of Rooibos Tea Extract on the Total IgM Production in LPS-stimulated Splenic B Cells.

B cells (2 × 10⁶ cells/200 μl/well) were cultured with LPS (10 μg/ml) and various concentrations of Rooibos tea extracts in 96-well culture plates under 95% air – 5% CO₂ atmosphere for 7 days. Total IgM level was measured by ELISA. Values are mean ± SD for triplicate cultures.

**Effect of Rooibos tea extract on the nonspecific B cell response elicited with LPS in purified splenic B cells**

We have examined whether Rooibos tea extract potentiates increases in the polyclonal IgM antibody production elicited with LPS, a polyclonal B cell mitogen, by purified splenic B cells. It was found that Rooibos tea extract was without effect on the LPS-elicited antibody production by splenic B cells (Fig. 3). Additionally, we observed that the cell viability and both IgE and IgG productions were not affected by the addition of this tea extract at concentrations from 1 μg/ml to 100 μg/ml (data not shown). Cell viability was decreased at 1000 μg/ml. These findings suggest that the increase in the antigen-specific antibody production by Rooibos tea extract is due to the indirect stimulation of B-cells.

**Effects of Rooibos tea extract on the cytokine generation in murine splenocytes in vitro**

Because a variety of cytokines support the antibody responses, and IL-2 and IL-4 are the representative cytokines controlling the balance of Th1 and Th2 cell functions, we examined whether Rooibos tea extract increases IL-2 and IL-4 generations in OVA and anti-CD3 Ab-primed murine splenocytes. The levels of the cytokines were estimated by ELISA. Rooibos tea extract dose-dependently increased the IL-2 secretion by OVA-primed murine splenocytes between 10 and 1000 μg/ml concentrations (Fig. 4). Anti-CD3e Ab-induced IL-2 secretion was also dose-dependently increased by this tea extract (Fig. 6).
Effects of Rooibos Tea on Antibody and Cytokine Production

Fig. 4. Effects of Rooibos Tea Extract on IL-2 Production in OVA-stimulated Splenocytes.
Splenocytes (2 × 10^6 cells/200 μl/well) were cultured with OVA (500 ng/ml) and various concentrations of Rooibos tea extracts in 96-well culture plates under 95% air–5% CO₂ atmosphere for 3 days. IL-2 level was measured by ELISA. Values are mean ± SD for triplicate cultures. Statistical significance (*) was determined by a Student’s t test and a value of P<0.05 compared with the control was considered to be significant.

Fig. 5. Effects of Rooibos Tea Extract on the IL-4 Generation in OVA-stimulated Splenocytes.
Splenocytes (2 × 10^6 cells/200 μl/well) were cultured with OVA (500 ng/ml) and various concentrations of Rooibos tea extracts in 96-well culture plates under 95% air–5% CO₂ atmosphere for 3 days. The IL-4 level was measured by ELISA. Values are mean ± SD for triplicate cultures. Statistical significance (*) was determined by a Student’s t test and a value of P<0.05 compared with the control was considered to be significant.

These findings indicate that the increase of IL-2, which is a key cytokine for the differentiation and proliferation of B-cells, is partly responsible for the augmentation of the antigen-specific antibody production by Rooibos tea extract. Rooibos tea extract seems to act on Th-cells to increase IL-2 secretion, since anti-CD3 Ab directly acts on T-cells. On the other hand, IL-4 secretion from OVA-primed murine splenocytes was strongly inhibited by Rooibos tea extract as shown in Fig. 5. Thus, our findings suggest that Rooibos tea extract may facilitate the antigen-specific antibody production through selective augmentation of IL-2 produced by Th1-type cells, and simultaneous reduction in IL-4 produced by Th2-type cells.

Restoration of the reduced antigen-specific antibody production by Rooibos tea ingestion in the immunosuppressive rats

We examined whether Rooibos tea stimulates the antigen-specific antibody and cytokine production in vivo. First, the in vivo effect of Rooibos tea on the antigen-specific antibody production was tested. Wistar/ST rats were sensitized with OVA. CyA was used as an immunosuppressant, and administered i.v. the day before antigen challenge. Rooibos tea extract was given from one week before the antigen challenge to the end of the experiment. OVA-specific antibody production was inhibited by the treatment of CyA. The ingestion of Rooibos tea largely restored the reduced OVA-specific antibody production (IgM) in CyA-treated rats (Fig. 7). The mean values of the three groups (OVA, OVA+CyA, and OVA+CyA+Rooibos tea) were 17266 ± 4163, 4248 ± 1940, and 9923 ± 3925 U/ml, respectively. The total IgM level was also higher than that of CyA-
treated control rats (data not shown).

**Effects of Rooibos tea extract on the antigen-induced IL-2 generation by murine splenocytes ex vivo**

We investigated the effects of Rooibos tea on the IL-2 generation ex vivo. BALB/c mice were given Rooibos tea extract for three weeks, and then the number of IL-2 secreting cells in splenocytes was counted by ELISPOT assay. OVA was injected i.p. two days before ELISPOT assay. It was found that the ingestion of Rooibos tea increased the IL-2 secreting cells (Fig.8), although there was no significant difference between the two groups; the mean values of OVA- and OVA + Rooibos tea-treated groups were 520 ± 152 and 760 ± 265 (mean ± SD), respectively. The serum IL-2 level was not detected. These results show that Rooibos tea increased antigen-specific antigen production and cytokine generation both in vitro and in vivo.

**Discussion**

Rooibos tea has been claimed to be a wonder health drink that has broad physiological and pharmacological effects. However, little is known about its immunomodulating activity. Thus, in this study we examined the effects of this tea extract on antigen-specific antibody production in vitro and in vivo. Our results showed that the tea extract increased the antibody responses against SRBC and OVA, at concentrations ranging from 1 μg/ml to 100 μg/ml as same as those which have a pharmacological response.

IL-2 is a lymphokine, synthesized and secreted from T-lymphocytes activated by antigen or mitogen. It is known that IL-2 is mainly involved in the proliferation and differentiation of T cells and B cells. As shown in Fig.4 and 5, IL-2 secretion from OVA-stimulated splenocytes was markedly increased by Rooibos tea extract, while IL-4 secretion was not increased, but decreased dose-dependently by the same concentrations. These findings suggest that the augmentation by Rooibos tea is attributed to the stimulation of IL-2 generation from T cells.

Rooibos tea extract improved the cell survival at concentrations from 1 μg/ml to 100 μg/ml. There are at least two possible explanations for this observation. Firstly, IL-2 increased by the tea extract may promote cell proliferation and inhibit apoptosis, since IL-2 stimulates the expansion of antigen-specific T or B cell clones, and inhibits cell apoptosis via the induction of bcl-2. Secondly, a strong antioxidative activity of Rooibos tea extract may protect lymphocytes against oxidative stress, and consequently inhibit their apoptosis. The former explanation is supported by the result that Rooibos tea extract has no effect on the survival of the purified splenic B cells, and that treatment with anti-IL-2 antibody eliminated the of improvement of cell viability (data not shown). Thus, it seems that Rooibos tea extract improves cell survival via the stimulation of IL-2 generation, not via the antioxidative activity. Unexpectedly, Rooibos tea extract decreased IL-2
production at lower doses such as 1 and 10 μg/ml although it increased the antigen-specific antibody production, suggesting that the IL-2-induced expression of IL-2 receptors on T cells exceeded the increment of IL-2 generation, and consequently the IL-2 level in culture medium was decreased by IL-2 consumption.

The active components in Rooibos tea extract are not identified, and the mode of action of the extract is also unclear. Rooibos tea extract contains many kind of flavonoids, such as quercetin, and saccharides in large amounts, which are known to have diverse bioactivities.\textsuperscript{13-20} We found that the stimulating effect on the antigen-specific antibody production of Rooibos tea extract \textit{in vitro} was diminished by the removal of phenolic compounds (data not shown). This indicates that the phenolic compounds such as flavonoids are responsible for the biological activities of Rooibos tea extract. Moreover, the dialysis does not affect the stimulatory activity of Rooibos tea extract (data not shown). Thus, these findings suggested that the active compounds of Rooibos tea extract may be phenolic compounds, probably of high molecular weight (M.W. \textgreater{} 12000) such as polymeric flavonoids. Actually, some antioxidants are known to have immunopotentiating effects. Yamamoto \textit{et al.} have showed with a stable vitamin C derivative, AA-2G, that ascorbic acid as an antioxidant augments the antigen-specific antibody production and IL-2 secretion.\textsuperscript{99} Furthermore, it is also reported that the addition of H$_2$O$_2$ as an oxidant results in marked reduction in IgM production,\textsuperscript{95} and that the cytokine pattern from activated T cells is governed by the glutathione levels in antigen-presenting cells.\textsuperscript{96} These reports support our results, although there are some reports suggesting that antioxidants inhibit IL-2 generation.\textsuperscript{37,38} Additionally, Meyer \textit{et al.} reported that NF-κB and AP-1, which are transcriptional factors and play a critical role in the immune response, are controlled by redox regulation.\textsuperscript{99} On the other hand, Rooibos tea extract contains various kinds of saccharides in large amounts, which are known to stimulate macrophages in general. For example, lentigin, which is a saccharide containing in shiitake mushrooms, is used in the immunotherapy of cancer with its macrophage-stimulating activity.\textsuperscript{1,2} The main roles of macrophages are to phagocytize antigens, present them to helper T cells, and generate various cytokines. Therefore, it seems that Rooibos tea extract would increase antigen-specific antibody production via the activation of macrophage.

In recent years, type I allergic diseases, which relate to the bias toward the Th2 immune response, have increased markedly. Our results showed that Rooibos tea extract induced the increase of IL-2 and the decrease of IL-4, suggesting a transition from Th2 to Th1. Therefore, Rooibos tea extract can be applicable to the suppression of type I allergic diseases as well as the defense against infectious diseases. Since the natural products having such pharmacological features are very few, further studies on Rooibos tea would be valuable for clinical application.

Many kinds of tea containing different ingredients exist in the world, and show diverse biological effects. For example, green tea, which is the representative of tea in Japan, contains catechins and vitamin C abundantly and has anti-cancer effects.\textsuperscript{40} Psidium tea and Oolong tea are also known to be effective for the cure of allergic diseases and the suppression of accumulation of neutral fat, respectively.\textsuperscript{41} Besides the diverse effects of Rooibos tea as described by other investigators,\textsuperscript{13-20} our study here also showed that Rooibos tea extract stimulated the antigen-specific antibody production and induced the transition from Th2 to Th1 \textit{in vitro}. We also demonstrated that Rooibos tea extract acts as an immunopotentiator \textit{in vivo}. These results indicate that the immunological activities of Rooibos tea extract are clinically useful. Routine intakes of the tea extract can act gently without side-effects. In conclusion, we propose a novel medical remedy, which is a combination therapy of Rooibos tea with some chemical drugs.

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Effects of Rooibos tea on Antibody and Cytokine Production


