Induction of Anti-inflammatory Responses by Dietary *Momordica charantia* L. (Bitter Gourd)

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We assessed the immunomodulatory activity of *Momordica charantia* L. (bitter gourd), a vegetable that has been reported to possess various bioactivities. We examined the effects of bitter gourd on intestinal immunity by monitoring the TGF-β and IL-7 secretion from Caco-2 cells and the IL-10 and IL-12 secretion from THP-1 cells that are used as in vitro models of the intestinal epithelium and monocyte/macrophages, respectively. We also determined the in vivo immunological responses of rats fed on bitter gourd for 3 weeks. We found that bitter gourd induced a decrease in the intestinal secretion of IL-7 and an increase in the secretions of TGF-β and IL-10, these effects reflecting the bitter gourd-induced changes in systemic immunity, i.e., a decrease in the number of lymphocytes, increases in the populations of Th cells and NK cells, and increase in the Ig production of lymphocytes. Dietary bitter gourd may therefore induce both intestinal and also systemic anti-inflammatory responses.

**Key words:** *Momordica charantia* L. (bitter gourd); immunomodulatory activity; IL-7; TGF-β; IL-10

The host defense system is important for maintaining homeostasis. Within the immune system, control is maintained by the interactions of various immune cells and molecules, including cytokines and immunoglobulins (Ig). For example, such cytokines as IL-10 and IL-12 contribute to the immune responses mediated by Th1 and Th2 cells which regulate the balance between cellular and hormonal immunity. Additionally, IL-10 and TGF-β play an important role in downregulating the immune functions of regulatory T cells.1,2

Many environmental factors, including food intake, can affect these interactions. The effects of some vegetable extracts on such immune cell activities as neutrophil accumulation,3 Ig production4 and cytokine production5,6 have been reported. *Momordica charantia* L. (bitter gourd), a cucurbitaceae fruit widely consumed as a vegetable in Asia, has been reported to possess bioactivity, including antidiabetic activity,7,8 antimitagenicity,9,10 antiviral activity11 and cytotoxicity.12

Since intestinal immunity is an important defense against extrinsic factors entering the living body, we studied the effects of bitter gourd consumption on immune responses in the intestinal lamina propria, where intestinal epithelial cells interact with immune cells to maintain the mucosal barrier, and subepithelial macrophages play a key role in inflammation. We examined the effects of the bitter gourd on the IL-7 and TGF-β production of intestinal epithelial cells and the IL-10 and IL-12 production of monocyte/macrophages, using the human colonic adenocarcinoma cell line, Caco-2, as the model of intestinal epithelial cells13 and the human acute monocytic leukemia cell line, THP-1, as the model of monocytes/macrophages. Our study attempts to determine the effects of dietary bitter gourd on intestinal and systemic immunity both in vitro and in vivo.

**Materials and Methods**

**Materials.** *Momordica charantia* L. (bitter gourd) was purchased from a local market in Kyoto, Japan. Freeze-dried powder of the edible portion of bitter gourd (freeze-dried bitter gourd) was presented by the Sun Project (Kumamoto, Japan). Porcine pepsin (975 units/mg of protein), pancreatin (4×, USP) and bile extract were purchased from Sigma (St. Louis, MO, USA). Cellmatrix type 1-A purchased from Nitta Gelatin (Osaka, Japan) was used for the collagen treatment of plates.

**Cell culture.** Caco-2 cells (ATCC HTB-37) were seeded at a density of 1×10⁵ cells/cm² in collagen-treated 12-well plates (Greiner, Germany). To achieve full differentiation, the cells were cultured for 14 days in a medium consisting of DMEM (Nissui Pharmaceuticals, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; JRH, Lenexa, KS,

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USA) and 1% non-essential amino acids (Gibco Laboratories, Grand Island, NY, USA), this medium being changed to a fresh one every 1-2 days. THP-1 cells (presented by Dr. Fumio Amano, National Institute of Infectious Diseases) were grown in DMEM with 10% FBS. The cells were also cultured in the Caco-2-THP-1 co-culture system previously described.\(^{10}\) Caco-2 cells were seeded at a density of \(1 \times 10^5\) cells/0.5 ml/well in apparatus with a 12-mm-diameter semipermeable membrane (0.4-\(\mu\)m pore size; Transwell, Corning Corser, Cambridge, MA, USA). After the Caco-2 cells had reached full differentiation, the Transwell devices were put into 12-well cluster plates. Each lower compartment of the cluster plates (the basolateral compartment of the Caco-2 monolayer) contained the THP-1 suspension (\(1.5 \times 10^5\) cells/1.5 ml/well) in its culture medium.

**Preparation of the sample digested in vitro.** The in vitro digestion procedure described by Glahn *et al.*\(^{15}\) was conducted with minor modifications. Briefly, the homogenized fresh fruit of bitter gourd was acidified (pH 2.0), before adding pepsin to a final concentration of 1.25 mg/ml, and incubated at 37°C in a shaking bath for 1 hr. The pH of the gastric digest was then increased to 6.0 with sodium bicarbonate, and the bile extract and pancreatin were added to provide respective final concentrations of 1.85 mg per ml and 0.3 mg per ml of digest. The pH value was then elevated to 7.0 with 1 mol/ml of sodium hydroxide, before 6-ml aliquots of the partially digested bitter gourd were transferred to dialysis tubing with a 15,000 molecular weight cut-off (Spectra/Per 2.1, Spectrum Medical, Los Angeles, CA, USA) and dialyzed against 25 ml of serum-free DMEM medium at 37°C for 2 hr. The dialysate was sterilized by filtration through a 0.22-\(\mu\)m membrane, and then stored at −80°C until being used as the digested bitter gourd. A control solution was prepared by the same procedure, but without containing the bitter gourd.

**Cytokine assays.** IL-7 and TGF-\(\beta\) were determined in the supernatant of Caco-2 cells that had been cultured for 48 hr in a medium containing the digested bitter gourd and 10% FBS, using ELISA developed by R&D Systems (Minneapolis, MN, USA) and Pharmingen (OptEIA Set; San Diego, CA, USA), respectively. The supernatant of THP-1 cells (\(1 \times 10^5\) cells/ml) cultured for 72 hr in a medium containing the digested bitter gourd and 10% FBS was assayed for its IL-10 and IL-12 contents by ELISA (OptEIA Set; Pharmingen). In the case of the Caco-2-THP-1 co-culture system, the IL-10 and IL-12 contents were measured in the supernatant of THP-1 cells that had been cultured in the lower chamber for 72 hr after the digested bitter gourd solution had been substituted for the culture medium of Caco-2 cells in the upper chamber.

**Measurement of the cell proliferation.** The viability of THP-1 cells was determined by the WST-1 assay (Dojindo Laboratories, Kumamoto, Japan).

**Animals and experimental diets.** Male Wistar rats (4 weeks old) were obtained from Clea Japan (Tokyo, Japan). The rats were given a commercial diet (CE-II, Clea Japan) and water ad libitum in a room maintained at 22±2°C with a 12-hr light/dark cycle. After pre-bleeding the rats for 2 days, they were randomly divided into two dietary groups of 6 rats each: the control diet group (powdered CE-II [Clea Japan]) and bitter gourd diet group (powdered CE-II with 5% freeze-dried bitter gourd). The animals were fed on the experimental diets for 3 weeks.

**Quantification of Ig in rat plasma.** IgA, IgG and IgM in rat plasma were determined by ELISA (rat IgA, IgG and IgM ELISA quantitation kit, Bethyl Laboratories, Montgomery, TX, USA).

**Isolation of Rat Lymphocytes.** The spleens from the respective groups of rats were chopped in PBS, and the resulting cell suspensions were filtered through gauze. These cell suspensions were centrifuged by Lymphocyte-Rat apparatus (Cedarlane Laboratories, Hornby, Ontario, Canada). To eliminate the red blood cells, ammonium chloride (final concentration of 0.83%) was added to the recovered cells.

The lymphocytes from rat plasma were isolated by centrifugation with the Lymphocyte-Rat apparatus.

The number of lymphocytes obtained was measured by counting with a hemacytometer after the cells had been washed three times and suspended in a Hank’s balanced salt solution (HBSS; ICN Biomedicals, Costa Mesa, CA, USA).

**Flow cytometric analysis.** The lymphocytes from the rat blood or spleen were reacted for 30 min at 4°C with FITC-conjugated antibodies (Abs) to rat CD4, R-PE-conjugated Abs to rat CD8, and FITC-conjugated Abs to rat CD161 (Dainippon Pharmaceutical, Osaka, Japan), before being washed with HBSS. After adding 7-aminoactinomycin D (7-ADD) (ICN), the labeled cells were analyzed for their incorporation of 7-ADD and for the population size of effector cell subsets by using a FACScalibur (Becton Dickinson, San Jose, CA, U.S.A.) with CELLQuest software.

**Results and Discussion**

**Effects of bitter gourd on the cytokine secretion by intestinal cells**

We firstly examined the effect of bitter gourd on the cytokine secretion by the intestinal cells. We used...
Caco-2 cells and THP-1 cells as the respective models of intestinal epithelial cells and monocyte/macrophages. Significantly less (p < 0.05) IL-7 was secreted by Caco-2 cells cultured with the digested bitter gourd solution than by Caco-2 cells cultured with the control solution (Fig. 1A). In contrast, significantly more (p < 0.05) TGF-β was secreted by Caco-2 cells cultured with the digested bitter gourd solution than those cultured with the control solution (Fig. 1B).

In the case of THP-1 cells, the digested bitter gourd had no effect on IL-12 production, but did promote IL-10 secretion (p < 0.05; Fig. 2A). The proliferation of THP-1 cells cultured with the digested bitter gourd was 116% of that of the control THP-1 cells (p < 0.01; Fig. 3). However, this increase in proliferation did not totally account for the elevated level of IL-10 secretion, which was 142% of the control value. This result suggested that the digested bitter gourd had also promoted IL-10 secretion by an unidentified mechanism. The function of subepithelial cells may be changed via interaction with the intestinal epithelium. To examine the effect of digested bitter gourd on the cytokine secretion by subepithelial monocyte/macrophages, we used a Caco-2-THP-1 co-culture system. As shown in Fig. 2B, the digested bitter gourd induced THP-1 cells in the co-culture system as well as in the mono-culture system to secrete IL-10.

In brief, the fact that digested bitter gourd induced the production of the down-regulating cytokines,

TGF-β and IL-10, \textit{in vitro} suggests that this preparation acted as an immune down-regulator of intestinal immunity.

\textit{Evaluation of the immune response to a dietary intake of bitter gourd by using an animal model}

The effects of bitter gourd on the intestinal im-

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1}
\caption{Cytokine Secretion by Caco-2 Cells Cultured with Digested Bitter Gourd. A: IL-7; B: TGF-β. Caco-2 cells were incubated with digested bitter gourd for 48 hr. Statistical difference was analyzed by Student’s t-test (**P < 0.05).}
\end{figure}

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\caption{Digested Bitter Gourd Enhanced IL-10 Secretion by THP-1 Cells. A: In a mono-culture system. THP-1 cells were cultured with digested bitter gourd for 72 hr. B: In a Caco-2-THP-1 co-culture system. THP-1 cells were cultured in the lower chamber for 72 hr after digested bitter gourd had been substituted in the upper chamber. Each value is the mean ± SD (n = 3). Statistical difference was analyzed by Student’s t-test (**P < 0.05).}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3}
\caption{Promotion of THP-1 Proliferation by Digested Bitter Gourd. THP-1 cells were cultured with digested bitter gourd for 72 hr. The viability is the mean ± SD (n = 3) and is expressed as a percentage of the control. Statistical difference was analyzed by Student’s t-test (**P < 0.01).}
\end{figure}
Fig. 4. Dietary Intake of Bitter Gourd Decreased the Number of Lymphocytes.
A: number of lymphocytes in the plasma; B: number of lymphocytes in the spleen. Rats were fed on the control diet or bitter gourd diet for 3 weeks. Each value is the mean ± SD for each group. Statistical difference was analyzed by Student’s t-test (*P < 0.05).

Fig. 5. Effects of Dietary Bitter Gourd on the Populations of Effector Cell Subsets.
A: lymphocytes in the plasma; B: lymphocytes in the spleen. Rats were fed on the control diet (△) or bitter gourd diet (●) for 3 weeks. Stained cells were analyzed by flow cytometry to calculate the percentage of CD4+ (helper T) cells, CD8+ (cytotoxic T) cells and CD161+ (NK) cells among lymphocytes in the plasma and spleen. Each value is the mean ± SD for each group. Statistical difference was analyzed by Student’s t-test (*P < 0.05).

Immunity and systemic immunity seem to be similar. In particular, the response of THP-1 cells to bitter gourd may reflect that of monocyte/macrophages in both the subepithelial region of the intestine and in blood. To determine the immunomodulatory properties of bitter gourd in vivo, we measured the numbers of lymphocytes, the distribution of CD4+, CD8+ and CD161+ effector cells, and the Ig production of lymphocytes in the plasma and spleen of rats that had been fed on the control or bitter gourd diet for 3 weeks. We first confirmed that the growth (increase in body weight) of the rats in the two dietary groups was not significantly different (data not shown).

As shown in Fig. 4, the numbers of lymphocytes in the plasma and spleen of the rats fed on the bitter gourd diet were less (although not significantly so) than those in the plasma and spleen of the rats fed on the control diet. Cell survival, division, and death determine the overall number of mature T cells in peripheral lymphoid tissues. IL-7 plays a key role in maintaining T cell homeostasis by controlling proliferation and survival.10 IL-7 in combination with IL-12 synergistically increases T cell proliferation.11 Our in vitro finding of inhibited IL-7 production and unchanged IL-12 production may be involved in the mechanism by which the dietary bitter gourd decreased the numbers of plasma and spleen lymphocytes in vivo.

We next examined the effects of dietary bitter gourd on the effector cell subsets (i.e., helper T [Th] cells, cytotoxic T cells and NK cells) by flow cytometric measurement of lymphocytes in the plasma and spleen (Fig. 5). The dietary intake of bitter gourd significantly increased the percentage of CD4+ (Th) cells in the spleen and increased Th cells in the plasma, although not significantly. Since IL-7 dramatically induces a decrease in the CD4 to CD8 ratio by disproportionately increasing the number of CD8+ T cell,18,19 dietary bitter gourd is suggested to increase the population of Th cells by inhibiting IL-7 production. As shown in Fig. 5A, the percentage of lymphocytes that were CD161+ (NK) cells in the plasma was also increased by the dietary intake of bitter gourd. This increase seems to contradict our finding that bitter gourd promoted the secretion of down-regulating cytokines in vitro. However, NK cells have been proposed to participate in the regulation of humoral immune responses20 and IL-10 enhances NK cell proliferation without affecting IFN-γ production (a promoter of naïve Th cell differentiation into Th1 cells).21 Although we did not assay the concentration of INF-γ in the plasma, the dietary bitter gourd did seem to increase the NK cell population by promoting IL-10 secretion rather than by enhancing cellular immunity.

NK cells have also been observed to secrete TGF-β22 and to induce autologous resting B cells to syn-
synthesize Ig, including the switching of IgM to IgG and IgA.23) The secretion of IgG, IgM and IgA is also promoted by IL-10.20) We then examined the effect of the dietary bitter gourd on the concentration of Ig in the plasma. The concentrations of IgA, IgG and IgM in the plasma of rats that had been fed on the bitter gourd diet were significantly higher than those of the rats fed on the control diet (Fig. 6). This bitter gourd-induced increase in IgM concentration was observed 1 week before the corresponding increases in IgA and IgG. After antigen stimulation, the production of IgM is usually induced first, followed by the production of the other Ig classes. TGF-β induces class switching of IgM → IgD+ precursors to IgA+ B cells.25) Thus, the increase of each Ig in the plasma by the dietary bitter gourd could have been caused by increases in the IL-10 and TGF-β secretion and in the population of NK cells.

We in this study found that bitter gourd induced decreased IL-7 secretion and increased TGF-β and IL-10 secretion in intestinal cells in vitro, and that these results were involved in the effects of dietary bitter gourd on systemic immunity in vivo, i.e., by the decrease in number of lymphocytes, and the increases in Th and NK cell populations and in the Ig production of lymphocytes. These responses (except for the decrease in number of lymphocytes) indicate that dietary bitter gourd probably enhanced Th2 (namely, suppressed inflammatory) responses that boost hormonal immunity.

Bitter gourd is known to contain several bioactive constituents, including charantin, momordin, poly-peptide-P and vicine;26) however, the effective substance induced the responses we observed in this study remains unknown and should be clarified by a further investigation.

Our results both in vitro and in vivo suggest the possibility that a dietary intake of bitter gourd would induce the anti-inflammatory response of both the intestinal immune and systemic immune systems.

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