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Immunomodulating Activity of a Fucoidan Isolated from Korean *Undaria pinnatifida* Sporophyll

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A fucoidan, isolated from Korean *Undaria pinnatifida* spoprophyll (UP-F), was investigated for its immunomodulating activity on murine macrophages and splenocytes, and its activity was compared with that of fucoidan from *Fucus vesiculosus* (FV-F). Treatment of UP-F resulted in inhibition of the growth of murine macrophage RAW 264.7 cells, but its cytotoxicity was not observed in normal murine splenocytes. FV-F was shown to be highly cytotoxic to both immune cells, and its cytotoxic activity was higher than that of UP-F. Treatment of UP-F induced TNF-α in a dose-dependent manner from two types of macrophages, RAW 264.7 cells and murine peritoneal macrophages. The TNF-α-inducing activity of UP-F was higher than that of FV-F. UP-F also actively induced chemokines (RANTES and MIP-1α) from RAW 264.7 cells. Furthermore, treatment of UP-F gave rise to activation of murine splenocytes to produce cytokine (IL-6) and chemokines (RANTES and MIP-1α), showing significantly higher activity than that of FV-F. These results indicate that UP-F is less cytotoxic to immune cells than FV-F, and possesses immunomodulating activity to produce cytokines and chemokines from macrophages and splenocytes.

Key Words: fucoidan, immunomodulating activity, *Undaria pinnatifida* sporophyll

INTRODUCTION

Fucoidans, a group of marine sulfated polysaccharides of the cell-wall matrix of brown algae, contain large proportions of L-fucose and sulfate, together with minor amounts of other sugars like xylose, galactose, glucose, mannose, uronic acids, and rhamnose (Berteau and Mulloy 2003; McCandless and Craigie 1979). Fucoidans have the main skeleton of α1,3-linked-L-fucose-4-sulfate (Patankar *et al.* 1993), but a repeating structure of alternating α(1->3) and α(1->4) glycosidic bonds is also frequently found depending on the algal species (Daniel *et al.* 1999). It was shown that these acidic polysaccharides possess a variety of physiological and biological activities such as anti-inflammatory (Ostergaard *et al.* 2000), antiviral (Beress *et al.* 1993; Hoshino *et al.* 1998), anticoagulant (Nishino and Nagumo 1991; Kim *et al.* 2007) and antitumor (Zhuang *et al.* 1995) activity. These biological functions of fucoidans led many investigators to apply these polysaccharides to human and veterinary health care, and thus production and applications of fucoidans as therapeutic agents have been increasingly important topics of intensive researches.

Macrophages play an important role in regulating innate immunity as well as adaptive immune responses by production of cytokines such as IL-1β, IL-6, TNF-α and IFN-γ, and various types of chemokines such as RANTES, MCP-1, MIP-1α, TARC etc (Goerdt *et al.* 1999; Le Page *et al.* 2000). Splenocytes, mainly composed of many types of immune-related cells such as T cells, B cells, dendritic cells and macrophages, regulate humoral and cellular immune responses against foreign antigens like bacteria and viruses, and endogenous antigen like tumors. The immunomodulating activity by splenocytes is partly associated with production of cytokines and chemokines (Fan *et al.* 2006). Therefore, it is likely that active substances to enhance the ability of these immune cells to produce cytokines and chemokines can act as potent immunomodulators.

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To date, several different fucoidan preparations from various algal species, including *Fucus vesiculosus* (Choi *et al.* 2005; Oomizu *et al.* 2006) and *U. pinnatifida* (Maruyama *et al.* 2003), have been reported for their immunostimulating activity. However, fucoidan from Korean *U. pinnatifida* sporophyll have not been clearly demonstrated for this activity. The structures of fucoidan vary from their algal source species to species, and the variation may give rise to difference in the extent of most biological activities, including immunomodulating activities (Berteau and Mulloy 2003).

Previously, we reported the purification and composition of a fucoidan from Korean *Undaria pinnatifida* spoprophyll and its anticoagulating activity (Kim *et al.* 2007). In this study, we examined the immunomodulating activity of this fucoidan (UP-F) to induce cytokines and chemokines from two major immune-related cells, macrophages and splenocytes, and compared its ability with a commercial fucoidan of *Fucus vesiculosus* (FV-F).

MATERIALS AND METHODS

Isolation and characterization of fucoidans

The cultured *Undaria pinnatifida* sporophyll used in this study as a source of algal fucoidan was collected from a southern coastal area of Wando, Korea, and was kindly provided from HarimBio Co. Ltd. (Wando, Korea). The extraction of fucoidan and further purification by DEAE-cellulose column chromatography were performed as described by Kim *et al.* (2007). The presence of fucoidan in the fraction during purification was identified by the presence of L-fucose after the monosaccharide composition analysis by high performance anion-exchange chromatography (HPAEC) using Bio-LC system (Dionex, USA) as described previously (Lee *et al.* 2006). As a control sample, commercial fucoidan of Fucus vesiculosus (FV-F) was purchased from Sigma (St. Luis, USA).

Collection of peritoneal macrophages and splenocytes

Balb/c mice (7 weeks old) were injected intraperitoneally with 1 ml of 2% thioglycollate (Difco, USA) and peritoneal exudative cells were harvested 4 days after injection by the method described previously (Watanabe *et al.* 1999). Briefly, the cells suspended in 0.5 ml of RPMI-1640 supplemented with 10% fetal calf serum, vitamin solution, sodium pyruvate, nonessential amino acids and L-glutamine were plated into tissue culture plates. After 2 h-incubation, adherent macrophages were obtained by washing the plate with PBS to remove non-adherent cells. Splenocytes were aseptically isolated from the spleen of Balb/c mice.

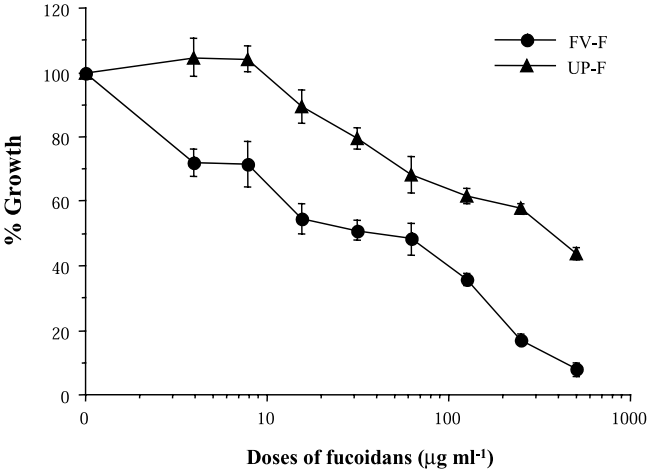

Fig. 1. Cytotoxic activity of UP-F and FV-F to RAW 264.7 macrophages. RAW 264.7 cells (5×10^4 well^{-1}) were co-incubated with the indicated doses of UP-F or FV-F for 24 h. The viability of the cells was determined using Cell Counting Kit.

Cell culture and cytotoxicity assay

RAW 264.7 cells, peritoneal macrophages and normal splenocytes were maintained as monolayer cultures in complete RPMI medium. RAW 264.7 cells (5×10^4 well^{-1}), peritoneal macrophages (5×10^5 well^{-1}) and splenocytes (5×10^5 well^{-1}) in 96-well plates were incubated with various doses of each fucoidan (0-500 μg ml^{-1}) at 37°C for 24 h respectively. The cultures were added by 10 μl well^{-1} of Cell Counting Kit (Allexis, MA, USA) solution, and incubated for 2 h before termination. Cytotoxic activity was determined spectrophotometrically at 450 nm.

In vitro activation of cells

Assay for activation of RAW 264.7 cells, murine peritoneal macrophages and splenocytes was carried out as described below. RAW 264.7 cells (5×10^4 well^{-1}), peritoneal macriophages (5×10^5 well^{-1}) and splenocytes (5×10^5 well^{-1}) were treated with the indicated doses of each fucoidan for 24 h, and the supernatants of cell cultures were used for cytokine and chemokine determination. The level of cytokines and chemokines was measured by an ELISA kit (BD Bioscience, USA).

Statistical analysis

The statistical significance of differences between the

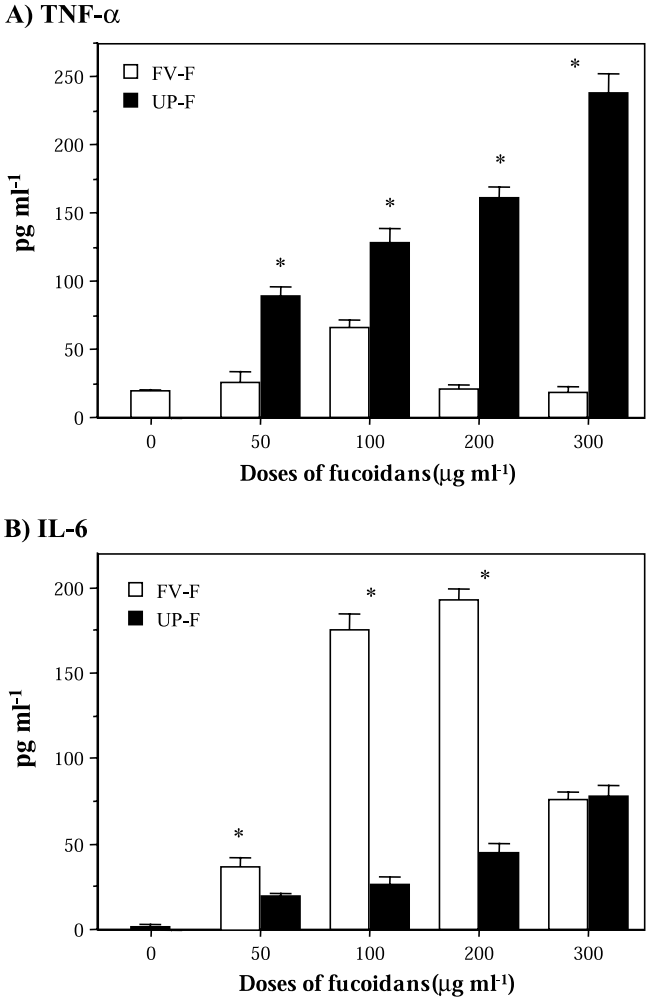

Fig. 2. Effect of UP-F and FV-F on cytokine production from RAW 264.7 macrophages. RAW 264.7 cells were co-incubated with the indicated doses of each fucoidan for 24 h. The level of TNF-α (A) and IL-6 (B) in the supernatants of the cultures was determined by ELISA kit. *p < 0.001, compared with FV-F-treated group by Student two-tailed *t* test.

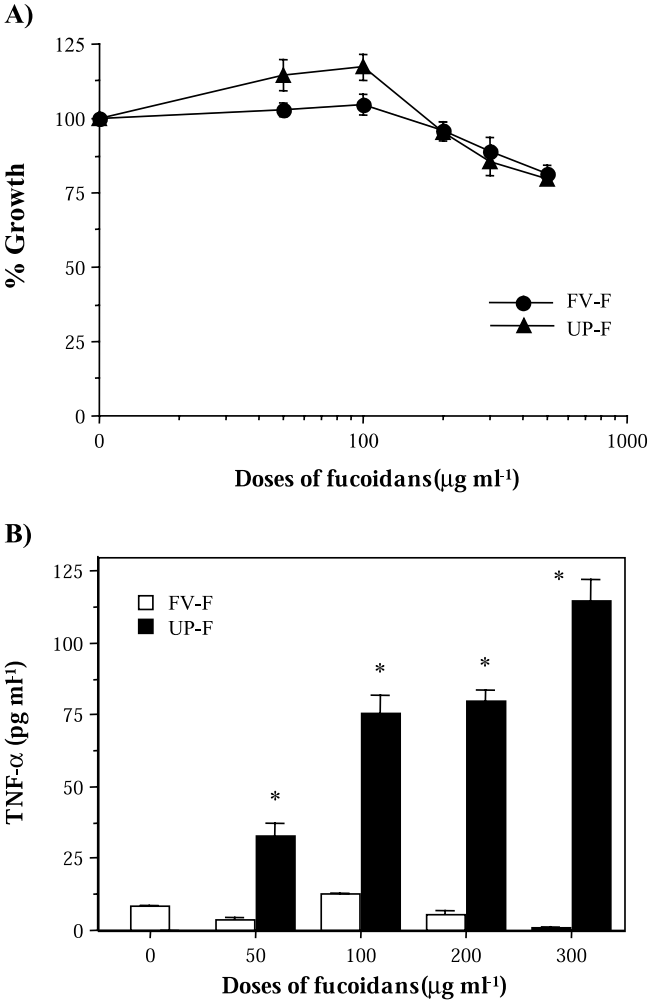

Fig. 3. Effect of UP-F and FV-F on the growth and TNF-α induction in peritoneal macrophages. Peritoneal macrophages (5×10^5 well^{-1}) were treated with the indicated doses of UP-F or FV-F for 24 h. The viability (A) and the level of TNF-α in the supernatants of the cultures (B) were determined by Cell Counting Kit and ELISA kit, respectively. *p < 0.001, compared with FV-F-treated group by Student two-tailed *t* test.

groups was determined by applying the Student's two-tailed *t* test.

RESULT AND DISCUSSION

Activation of macrophages by UP-F

In order to evaluate and compare biological properties of two fucoidans, UP-F and FV-F, to RAW 264.7 macrophages, cytotoxic activity of these polysaccharides was examined. When RAW 264.7 cells were co-incubated with varying concentrations of UP-F or FV-F for 24 h, both fucoidans inhibited the growth of RAW 264.7 cells in a dose-dependent manner (Fig. 1). In terms of 50% inhibitory concentration (IC_{50}), UP-F (> 350 μg ml^{-1}) showed more than 10-times higher value than that of FV-F (35 μg ml^{-1}), suggesting that UP-F is less cytotoxic to RAW 264.7 cells than FV-F.

Treatment of various doses of UP-F markedly induced TNF-α from RAW 264.7 macrophages, and its TNF-α-inducing activity was higher than that of FV-F (Fig. 2A). Especially, the activity of UP-F to induce TNF-α increased at least up to the dose of 300 μg ml^{-1} whereas FV-F caused rapid reduction in TNF-α production at over 200 μg ml^{-1}. Presumably, this was because FV-F is more cytotoxic to RAW 264.7 cells than UP-F as shown in Fig. 1. In contrast to TNF-α production, FV-F showed higher activity to produce IL-6 than that of UP-F (Fig. 2B). Coincident with TNF-α production, IL-6 production by FV-F was decreased at the dose of 300 μg ml^{-1}, but

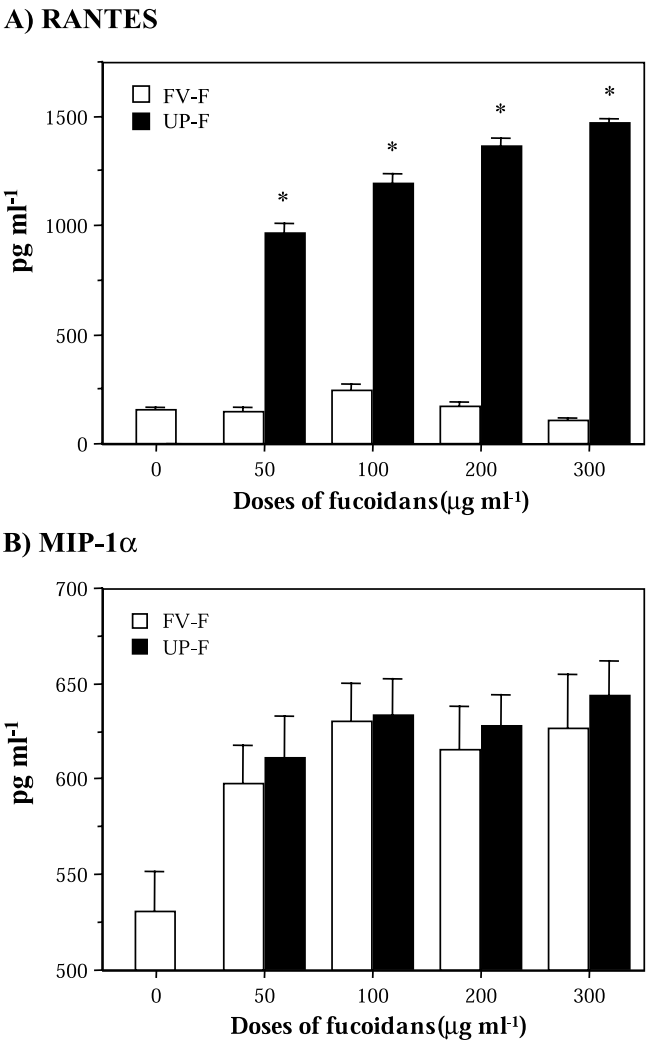

Fig. 4. Chemokine induction by UP-F and FV-F from Raw 264.7 macrophages. RAW 264.7 cells were treated with the indicated doses of UP-F or FV-F for 24 h. The level of RANTES (A) and MIP-1α (B) in the supernatants of the cultures was determined by ELISA kit. *p < 0.001, compared with FV-F-treated group by Student two-tailed *t* test.

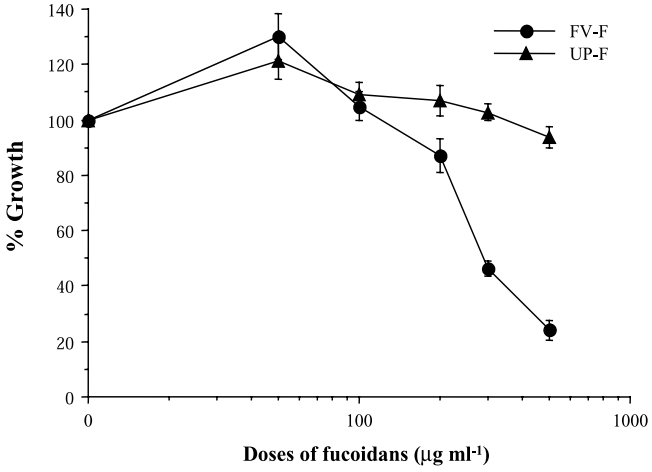

Fig. 5. Cytotoxic activity of UP-F and FV-F to murine splenocytes. Splenocytes (5×10^5 well^{-1}) were co-incubated with the indicated doses of each fucoidan for 24 h. The viability of the cells was determined using Cell Counting Kit.

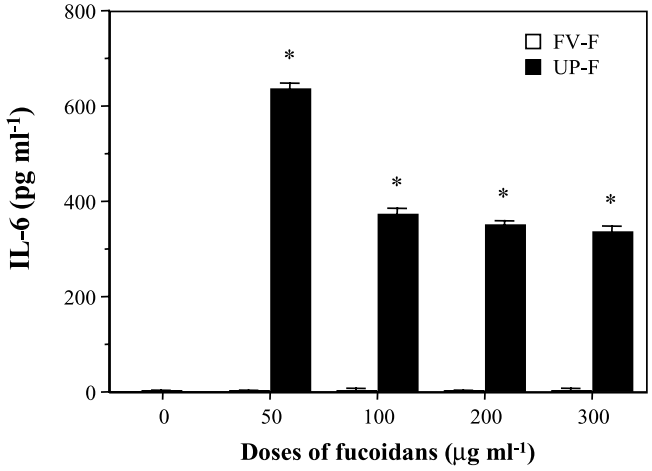

Fig. 6. Effect of UP-F on IL-6 production from splenocytes. Splenocytes were treated with the indicated doses of UP-F or FV-F for 24 h. The level of IL-6 in the supernatants of the cultures was determined by ELISA kit. *p < 0.001, compared with FV-F-treated group by Student two-tailed *t* test.

UP-F gradually increased IL-6 production in a dose-dependent manner. However, these fucoidans did not induce IFN-γ from RAW 264.7 cells (data not shown). In an experiment for activation of the primary macrophages by UP-F and FV-F, these fucoidans induced TNF-α production from peritoneal macrophages in a similar pattern with the result of Fig. 2A, showing that UP-F was more effective than FV-F. Interestingly, cytotoxic activity to peritoneal macrophages by both UP-F and FV-F appeared lower than that on RAW 264.7 cells (Fig. 3A). The activity of UP-F to induce cytokines was also observed in production of chemokines (RANTES and MIP-1α) from RAW 264.7 cells (Fig. 4). FV-F also actively induced MIP-1α, but not RANTES. These results indicate that UP-F is able to activate macrophages to produce cytokines (TNF-α and IL-6) as well as chemokines (RANTES and MIP-1α), and UP-F is less cytotoxic to macrophages than FV-F.

Activation of splenocytes by UP-F

The interaction of the two fucoidans with splenocytes was examined by their cytotoxicity to the cells and activity to induce cytokines and chemokines. UP-F showed no detectable cytotoxic activity up to the dose of 500 μg ml^{-1}, but FV-F inhibited the growth of splenocytes in a dose-dependent manner (Fig. 5). Treatment of UP-F, not FV-F, induced IL-6 from splenocytes (Fig. 6).

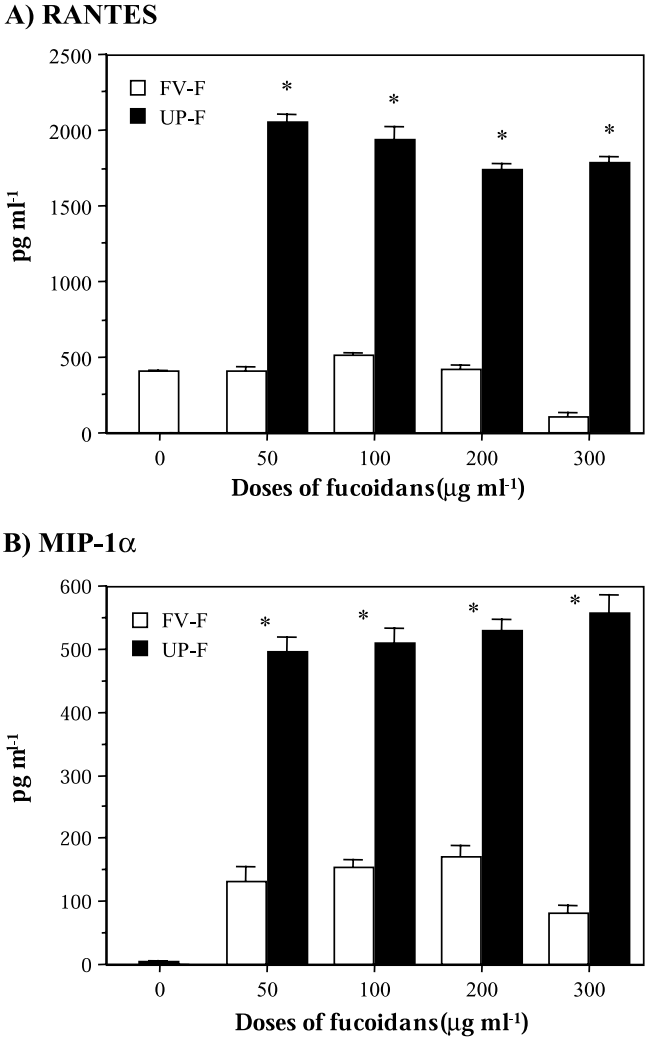

Fig. 7. Chemokine induction by UP-F and FV-F from murine splenocytes. The level of RANTES and MIP-1α in the supernatants obtained from the experiment of Fig. 6 was determined by ELISA kit. *p < 0.001, compared with FV-F-treated group by Student two-tailed *t* test.

Unexpectedly, however, both UP-F and FV-F had no effects on the level of IL-2, IFN-γ and TNF-α that might be secreted from activated T cells (data not shown). In chemokine assay, UP-F was shown to induce high level of RANTES and MIP-1α from splenocytes, but FV-F induced only MIP-1α (Fig. 7). The activity of UP-F to induce MIP-1α from splenocytes was significantly higher than that of FV-F. These results indicate that UP-F has a lower cytotoxicity to splenocytes than FV-F, and this fucoidan is able to activate splenocytes to induce some types of cytokines and chemokines.

Taken together, although more detailed research is needed, the results obtained in this study suggest that the fucoidan of Korean *Undaria pinnatifida* sporophyll has distinct biological activities from the commercial *Fucus vesiculosus* fucoidan, and this fucoidan may be a promising candidate for the development of a potent immunomodulating agent. Further study to elucidate the precise mechanisms related to the interaction of this fucoidan with immune cells is now under way.

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