Stimulation of IL-8 Production by Aralia cordate Lectin in Human Colon Carcinoma Caco-2 Cells

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Some lectins are known to stimulate interleukin-8 (IL-8) productions in human colon carcinoma Caco-2 cells. Since IL-8 may cause deleterious effects, we examined this stimulatory activity of Aralia cordate lectin (ACL) and Wasabia japonica lectin (WJL), both of which we isolated recently. The results indicate that ACL exhibited strong stimulatory activity for IL-8 protein production, while WJL showed marginal activity. The activity of ACL was associated with high enhancement of IL-8 gene expression. The effect of ACL was abolished almost completely in the presence of brefeldin A, indicating that internalization into cells is necessary for ACL to exert activity. The findings suggest that ingestion of a large amount of raw vegetable Aralia cordate might cause unfavorable effects on the colon.

Key words: lectin; interleukin-8; Aralia cordate; Wasabia japonica; Phaseolus vulgaris

Lectins are proteins or glycoproteins of non-immune origin with specific binding affinity for carbohydrate moiety of glycoconjugates. They are found in many food items, are frequently resistant to degradation by cooking and digestive processes, and are present in an active form in colonic contents after digestion. Studies have shown that many dietary lectins are resistant to breakdown during gut passage and are bound and endocytosed by epithelial cells. Some lectins are powerful exogenous growth factors for the small intestine and can induce dramatic shifts in bacterial flora. They can also modulate hormone and immune functions in vivo.

Recently, it was found that Phaseolus vulgaris agglutinin (PHA), Lens culinaris agglutinin, and Vicia faba agglutinin specifically trigger interleukin-8 (IL-8) production by the human colon carcinoma cell line Caco-2. These lectins did not trigger IL-1, IL-6, or monocyte chemoattractant protein-1 production. IL-8 is a potent chemoattractant for neutrophils which, when activated, contribute to local tissue destruction and inflammation. It is conceivable that long-lasting administration of lectins might in the end cause a state of chronic inflammation and tissue damage.

Recently, we isolated Aralia cordate lectin (ACL) and Wasabia japonica lectin (WJL) from the vegetables udo (Aralia cordate) and wasabi (Wasabia japonica) respectively, but their biological activities remain to be explored. In the present study we examined their IL-8 production-stimulatory activity to study possible deleterious effects when ingested in the raw state.

Caco-2 cells were obtained from Riken Gene Bank, Ibaraki, Japan, and cultured in 10% fetal bovine serum in Dulbecco’s minimum essential medium containing 50 U/ml penicillin, 50 μg/ml streptomycin, 2.5 μg/ml amphotericin B, and 50 μg/ml gentamycin at 37°C under 5% CO2. ACL and WJL were prepared by affinity chromatography with porcine plasma proteins immobilized on agarose and 0.5 M methyl α-mannoside and 0.2 M lactose as eluants, respectively, according to the method described previously. PHA-E4 and brefeldin A were products of Sigma (St. Louis, MO, U.S.A.). Caco-2 cells (1 × 104) were cultured to subconfluence in a 48-well microculture plate. On the day of stimulation the medium was changed to 200 μl of Dulbecco’s minimum essential medium and incubated with lectins. After 24 h of culture, the protein concentration of IL-8 in the lysate of whole cell culture was determined using an IL-8 Human ELISA Biotrac System (Amersham Biosciences, Tokyo, Japan).

The mean ± SD from three experiments for IL-8 production by unstimulated cells was 8.4 ± 1.7 pg/ml. When PHA at 50 μg/ml was used as a positive control experiment, IL-8 production was 155 ± 16 pg/ml, indicating stimulatory activity, as reported before. ACL at 50 μg/ml exhibited strong activity to induce IL-8 production of 282 ± 32 pg/ml. By contrast, IL-8 produced in the presence of WJL at 50 μg/ml was 16 ± 2.5 pg/ml, suggesting very low activity, if any.

To determine how these lectins affect IL-8 gene expression, reverse transcription-polymerase chain re-

Abbreviations: ACL, Aralia cordate lectin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-8, interleukin-8; PHA, Phaseolus vulgaris agglutinin; RT-PCR, reverse transcription-polymerase chain reaction; WJL, Wasabia japonica lectin

Note

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action (RT-PCR) was performed. Caco-2 cells were cultured in a 25 cm² cell culture flask (Corning, NY, U.S.A.) and stimulation with lectins in Cosmedium 001 (Cosmo Bio, Tokyo, Japan) was done. Total RNA was prepared from 1 × 10⁶ cells cultured with or without lectin using Catrimox-14 RNA Isolation Kit ver. 2.11 (Takara Bio, Tokyo, Japan), followed by digestion with deoxyribonuclease (RT grade, Nippon Gene, Tokyo, Japan) according to the manufacturer’s instructions. RT-PCR was performed using extracted total RNA and Ready-To-Go RT-PCR Beads (Amersham Biosciences). Amplified DNA was subjected to electrophoresis in 2% agarose gels, stained with SYBR Green I, and imaged and calculated using FluorImager, as described previously. The primers for IL-8 designed according to GenBank Accession no. NM 000584 were 5'-AGGAACCATCTCACTGTGTG-3' and 5'-ATTTGGGGTG-GAAAGGTT-3', and those for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (GenBank Accession Number NM 002046) were 5'-AAGGTCTATCCCTGA-GCTGAA-3' and 5'-CCCCTCTTCAAGGGGTCTAC-3', respectively. The expected lengths of PCR products for IL-8 and GAPDH were 169 bp and 495 bp, respectively.

The results indicated that PHA-E4 and ACL each at 50 µg/ml induced increases in the IL-8 mRNA level (Fig. 1), while no significant increase was observed for WJL at 50 µg/ml (data not shown). These results are compatible with the findings for stimulatory effects on IL-8 protein production. Stimulation with PHA-E4 and ACL was dose-dependent, and ACL exhibited much higher activity than PHA-E4 (Fig. 1). ACL caused significant stimulation of IL-8 mRNA expression even at 0.05 µg/ml, while PHA-E4 at 10 µg/ml showed no significant activity.

Previously, several lectins have been shown to exert biological activity after internalization2,8,9 that could be inhibited by brefeldin A. Since brefeldin A also inhibits protein secretion,9 it appears not to be suitable to measure IL-8 protein levels. Hence, we examined the effects of brefeldin A on mRNA expression. Caco-2 cells were cultured in Cosmedium 001 with brefeldin A at 100 ng/ml in ethanol or with the vehicle for 1 h, and then stimulated with either PHA-E4 or ACL for 24 h to examine the gene expression of IL-8. The results indicate that brefeldin A blocked almost completely the increase stimulated by these lectins (Fig. 2), indicating that internalization of PHA-E4 or ACL is necessary for the stimulatory activity for IL-8 production in Caco-2 cells. A similar observation has been reported for the highly toxic lectin ricin.9 In this case, the lectin even at 0.1 ng/ml stimulated IL-8 mRNA expression, and 100 ng/ml brefeldin A almost completely abolished the effect.

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**Fig. 1.** Dose-Dependent Effects of Lectins on IL-8 mRNA Expression in Caco-2 Cells. Caco-2 cells stimulated with lectins at the indicated concentrations for 24 h were examined for mRNA expression by RT-PCR. Representative results of agarose gel electrophoresis from three experiments are shown (A). Fluorescence intensity was determined with FluorImager, and mRNA levels normalized to GAPDH mRNA are expressed as relative to the control (100%). Results are given as means ± SD of three experiments (B). *Significant difference from the control value at p < 0.05 (Student’s t-test). **Significant difference from the control value at p < 0.01.
Our preliminary experiments indicate that ACL and WJL each at 100 µg/ml showed no inhibition for cell proliferation when tested for Caco-2 cells and human leukemia U937 cells, suggesting that these lectins have no cytotoxicity, unlike ricin. We also reported that PHA-E4 at 50 µg/ml exhibited no inhibitory activity for cell proliferation of U937 cells. Therefore it appears that the cytotoxicity and induction of IL-8 production are not related.

When formalin-fixed and non-permeabilized Caco-2 cells were stained with biotinylated ACL and fluorescein isothiocyanate-conjugated streptavidin essentially ac-
According to the method described previously, the cell surfaces were fluorescence-labeled (Fig. 3A). Inclusion of 0.5 M methyl α-mannoside in the staining caused a decrease in cell surface fluorescence (Fig. 3B), suggesting that surface mannose residues of Caco-2 cells are involved in the binding of ACL. Thus ACL belongs to the mannose-specific lectins, as described previously. On the other hand, WJL is galactose-specific and ricin is also galactose-specific. PHA-E4 has a high affinity for the galactose-N-acetylglucosamine sequence carrying inner core sugars with mannose residues. Therefore it appears that the carbohydrate-specificity of lectin is not related to this stimulatory activity for IL-8 production, as suggested previously. A mechanism by which these lectins trigger IL-8 production should be clarified in future studies. Since ACL has very strong activity to induce gene expression of IL-8 as compared with PHA-E4, this lectin may be more suitable to study the regulation of IL-8 production than PHA as proposed previously.

Udo is often eaten in a raw state as in salads. The present results suggest that ingestion of large amounts of raw vegetable udo may cause unfavorable effects in the colon.

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References


