

Production of hybrid-IgA plantibody against Shiga toxin

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Shiga toxin 1 (Stx1) is a virulence factor of enterohemorrhagic *Escherichia coli* strains serotype O157:H7. In the intestine, secretory immunoglobulin A (SIgA) is a major factor of immune defense against pathogens such as O157:H7. SIgA consists of dimeric IgA (two IgA monomers covalently linked by a J chain) and a secretory component. If Stx-specific SIgA can be expressed in plants, therapeutic or preventive effects would be expected by eating these plants containing “plantibody.” We have established hybrid-IgA having variable regions of the IgG specific for the carbohydrate binding subunit of Stx1 (Stx1B). And then, this hybrid-IgA genes and the J chain gene were introduced into *Arabidopsis thaliana*. To make transgenic edible plants that express hybrid-IgA specific for Stx1B, we took two approaches.

First, we examined whether the hybrid-IgA expressed in *A. thaliana* were able to neutralize cytotoxicity of Stx1. Vero cells were incubated with Stx1, and then cell viability was measured by a colorimetric cell viability assay. When Stx1 was pre-treated with protein extract from a transgenic *A. thaliana* that contains dimeric hybrid-IgA, the cytotoxic effect of Stx1 toward Vero cells was inhibited. Pretreatment of Stx1 with plantibodies prevented a 180 base pair DNA ladder pattern observed in Vero cells after exposure to Stx1, indicating the inhibition of Stx1-induced apoptosis. We also demonstrated the inhibition of Stx1-induced apoptosis of Ramos cells by the plantibodies. They inhibited the appearance of annexin V-positive apoptotic Ramos cells after exposure to Stx1 by means of flow cytometry.

Second, we tried to make transgenic lettuce. Hybrid-IgA genes and the J chain gene were introduced into lettuce (*Lactuca sativa* cv. green wave) by an *Agrobacterium* method. Regenerated shoots were obtained after growing on selection and regeneration-medium (MS medium containing kanamycin and plant hormones).

In conclusion, we revealed that hybrid-IgA expressed in plant retained Stx1 neutralization activity, and we were able to obtain hybrid-IgA transgenic lettuce shoots. These results indicate that edible plants containing hybrid-IgA against Stx1B can be a possible measure for immunotherapy against Stx1-caused food poisoning.