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Augmentation of natural immune response by orally administered cytokines expressed in transgenic plants

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Cytokines play a major role in the homeostatic maintenance of the mucosal immune system where foreign antigens including several infectious agents are encountered for initiation of immune response. It is therefore considered that administration of cytokines onto the mucosal sites should be effective to prevent infectious diseases because these cytokines would mimic the natural immune response against pathogens invading via the mucosal sites.

At present, cytokines with potential pharmaceutical values are produced as recombinant proteins in *Escherichia coli*, insect or mammalian cell culture. However, plant expression systems have large advantages over other *in vitro* expression systems in terms of low production costs and low risk of contamination of animal pathogens such as viruses and bacterial endotoxins. In addition, recombinant products expressed in edible plant tissues can be orally administered to human and animals.

In this study, genes encoding cytokines were introduced into plants, and the usefulness and applicability of obtained transformants to enhance natural immune response are discussed using *Listeria monocytogenes* infection in mice as a model.

At first, human interferon (HuIFN) -α cDNA was introduced into potato plant (*Solanum tuberosum*) using *Agrobacterium tumefacience*-mediated transformation. Successful expression of biologically active HuIFN-α in transgenic potatoes {560 international unit (IU) /g plant tissue} revealed that biologically active cytokines with potential pharmaceutical value could be expressed in transgenic potato plants.

Although bioactive HuIFN-α was expressed in plants, it was not detected by western blotting analysis due to low expression levels (6 ng/g plant tissue). Therefore, to enhance translation efficiency of mammalian genes and accumulation of recombinant proteins in plant cells, HuIFN-α fused with a signal sequence of seed storage protein and an endoplasmic reticurum retention signal was expressed in tobacco plants (*Nicotiana tabacum*) under the control of the e35S promoter {cauliflower mosaic virus (CaMV) 35 S core promoter with the CaMV enhancer sequence and tobacco mosaic virus (TMV) Ω sequence}. The expression level of the fused HuIFN-α in tobacco plant reached 1.2μg/g plant tissue.

In order to further determine whether the translation efficiency is improved in the fused gene construct, HuTNF-α and the fused HuTNF-α gene construct were introduced into potato plants. HuTNF-α expressed in po-

tato plants was biologically active. TNF-α should be a homotrimeric form to exert its functions. Successful expression of biologically active TNF-α in the potato plant cells indicates that recombinant products, which should be multimeric to exert their functions, can be expressed in this expression system. Furthermore, expression of HuTNF-α at the high levels (15μg/g plant tissue) revealed that cytokines could be expressed using plant expression systems at higher level (more than μg per g plant tissue).

Type I IFN (IFN-α/β) possesses not only 'classical' anti-viral and tumorcidal effects, but also play a key role in the regulation of the systemic immune response. Therefore, it was examined whether orally administered HuIFN-α can augment natural immune response against systemic bacterial infection using *L. monocytogenes* infection model in mice. Daily (6 days) oral administration of HuIFN-α reduced bacterial burden in spleen and liver from *L. monocytogenes* infected mice. This protective effect was observed in the middle phase of *L. monocytogenes* infection, but not in the early phase of the infection, it was considered that orally administered HuIFN-α should contribute to rapid elimination of *L. monocytogenes* from infected organs.

Finally, the potential of orally administered HuIFN-α expressing potato plant to enhance natural immune response was examined using *L. monocytogenes* infection model. Oral administration of extracts of the HuIFN-α expressing potato plant decreased *L. monocytogenes* burden in the spleen, compared with mice which were treated with control plant extracts. Even low concentration of HuIFN-α in the extracts (20IU/mouse/day) exerted the protective effect, compared with that achieved with PBS-diluted HuIFN-α when administered to mice. That may be due to 'bioencapsulation' of HuIFN-α by plant components.

In conclusion, this study revealed that transgenic plants expressing cytokines can be used as feed and/or feed additives in order to enhance natural immune response.

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Studies on porcine interleukin-18 and interleukin-1β converting enzyme.

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In the present study, porcine interleukin-18 (IL-18) and IL-1β converting enzyme (ICE) were cloned and characterized, and the expression of their recombinant proteins using baculovirus expression systems was stated. The production and utilization of monoclonal antibodies to porcine IL-18 were also described. The conclusions obtained by this study are summarized as follows.

A cDNA encoding porcine IL-18 was cloned and its recombinant protein was expressed as both precursor and mature forms by baculovirus systems. The porcine mature IL-18 induced IFN-γ production from porcine