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which are not yet examined. To understand epidemiology of theileriosis, studies on vector

tick distribution should be carried out.

## Cloning of genes encoding animal cell growth factors and the expression of these genes in transgenic plants

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Epidermal growth factor (EGF) is known to naturally enhance the intestinal mucosal immunity, while transforming growth factor- $\beta$  (TGF- $\beta$ ) plays a role in immunoglobulin (Ig) class switch to IgA, and may reduce intestinal inflammatory response. Therefore, oral administration of those factors, which could enhance the intestinal mucosal defenses, would benefit the prevention and treatment of gastrointestinal infections or the autoimmune diseases.

Expression of proteins with potential medical applications in transgenic plants has advantages over other expression systems because of low costs for production and of the possible application of the recombinant products for edible drugs. However, the major drawback at the present time is the inability to achieve the high amount of protein expression in transgenic plants. In this study, molecular cloning of dog and cat EGF was performed since EGF has been known to promote gastrointestinal maturation, and thus could be useful for the prevention of gastrointestinal infections. In addition, aiming at higher level expression of the recombinant products in transformed plants, bovine TGF- $\beta$ 1 gene fused with the legumin signal gene which can heighten the transgene expression in plants

was introduced into tobacco.

For the cloning of dog and cat EGF genes, polymerase chain reaction (PCR) was carried out using specific primers designed from a region of the EGF gene conserved among other species. The resultant PCR products were sequenced, and identified as dog and cat EGF genes based on their similarities to known EGF sequences. The open reading frames of dog and cat EGF genes encode proteins with 1,216 and 1,210 amino acids, respectively, and showed very high homology to human, mouse and rat EGF genes.

Bovine TGF- $\beta$  gene was introduced into tobacco using the *Agrobacterium tumefaciens*-mediated transformation system. The insertion of the TGF- $\beta$  gene into tobacco genomic DNA was confirmed in all of the selected transformants by PCR. Only 2% of the transformants were confirmed to express detectable levels of the recombinant protein, determined by ELISA. No significant difference was observed in the frequency of transformants positive for protein expression between legumin-positive and-negative transformants.

However, the levels of protein expression were increased by the addition of the legumin signal.

By improving the expression levels of pro-

teins of veterinary importance, such as cytokines and vaccines, transgenic plants will be-

come major suppliers of inexpensive biopharmaceuticals in the future.

## The development of a tick vaccine using the tick-derived proteins of HL 34 and HL 35

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Tick feeding activities and the diseases they transmit constitute the largest source of disease-control-related monetary losses in the livestock industry. As the importance of tick is their role as vectors for animal pathogens, suppression of the tick vector population is the most effective way to control many tick-borne diseases, particularly protozoan diseases. At the present time, ticks can be effectively controlled by the use of acaricides which have many disadvantages, and hence it is necessary to develop alternative tick-control methods which are more friendly to natural environment. Immunological control of ticks is currently a major sustainable and practical alternative method. In an earlier study, a recombinant 29 kDa (p 29) protein associated with the tick salivary glands induced partial anti-tick immunity in rabbits. Thus, the objective of this study was to obtain additional antigen in order to enhance the recombinant p 29 (rp 29)-based vaccine.

Two cDNAs, here in named asHL 34 and HL 35, were cloned from a tick cDNA library, and expressed as recombinant proteins in *Es-*

*cherichia coli*. Vaccination of rabbits with rHL 35 did not induce protective anti-tick immunity, while immunization with rHL 34 resulted in 14.7 and 29.1% mortality of nymph and adult ticks, respectively. Additionally, some of the dead ticks from HL 34-immunized rabbits were red in their body colors, suggesting that HL 34-induced immunity is effective from the early stage during tick feeding. By Western blot analysis, it was found that expressions of both HL 34 and HL 35 were not limited to the salivary glands, and that HL 34 was expressed in both immature and mature ticks. Northern blot analysis revealed that HL 35 was expressed only during feeding, 4 days post-infestation, while HL 34 appeared to be upregulated just before the completion of feeding. This may be the reason for the difference in the protection efficacy observed between HL 34 and HL 35. From these results, it was suggested that HL 34 is a potential candidate antigen for the tick vaccine. It will be of interest to examine the effectiveness of the cocktail vaccine of rHL 34 and rp 29 against ticks.