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# expressing antigenic proteins in mice Balb-C: a model of edible vaccines for oedema disease

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***RIASSUNTO*** – Somministrazione a topi Balb-C di semi di tabacco transgenici per proteine batteriche ad attività antigenica: vaccini edibili per la prevenzione della malattia degli edemi – *L'allevamento suinicolo intensivo richiede strategie innovative volte al controllo delle forme morbose. Nell'ambito delle* "medical molecular farming" *si stanno sviluppando ricerche volte alla produzione di piante geneticamente modificate per l'espressione di antigeni vaccinali, aprendo le frontiere verso un innovativo sistema di vaccinazione orale. In questo lavoro abbiamo testato su modello murino la capacità di evocare una risposta immunitaria locale da parte di semi di tabacco (somministrati per via orale) ingegnerizzati per l'espressione di proteine antigeniche di sierotipi di* Escherichia coli *responsabili della Malattia degli Edemi del suino.*

**KEY WORDS:** Oedema disease, transgenic plants, IgA, oral vaccination.

**INTRODUCTION** – Oedema disease of pigs is an enterotoxaemia affecting pigs aged 4 to 12 weeks and responsible of considerable economic losses. The oedema disease is caused by the extra-intestinal effects of Shiga-like toxin II variant, SLT-Iiv or VT2e. SLT are bipartite molecules composed of a single enzymatic intracellularly active A-subunit and a pentamer of B-subunit (Bertschinger and Gyles, 1994), which trigger attaching to the specific gastrointestinal receptor. Moreover pathogenic *Escherichia coli* bacteria possess one or more virulence factors which affect the capability of the germ to cause the disease (Imberechts *et al.*, 1992). The F18 fimbriae are present as long flexible filamentous structures related to verocitotoxic *E.coli* strains. Intestinal colonization with a live *E.coli* strains resulted in significantly increased level of anti-fimbrial F18 antibodies, especially IgA, in serum and intestinal wash fluids. Numerous studies have shown that viral epitopes and subunits of bacterial toxins can be expressed and correctly processed in transgenic plants (Rossi *et al.*, 2002a). At present there is a great interest in developing vaccination strategies aiming to lead mucosal immunity, with production of IgA rather than IgG. The dissection of pathogens into their various components allows to develop specific subunit vaccines which offer a safer alternative with the same efficacy as the whole pathogen vaccines (Mason *et al.*, 1992). When orally administered with feed such antigens can induce an immune response and in some cases can protect against a subsequent challenge with the pathogen (Koprowsky, 2001). We focused our attention on transformed tobacco plants for seed specific expression of genes encoding F18 fimbriae and VT2e-B subunit of Shiga-like toxin (Rossi *et al.*, 2002b). Transgenic plant lines were obtained by agroinfection using an expression cassette with 7S basic globulin promoter (GLOB) that is able to induce protein expression in seeds. The aim of this study has been to evaluated if tobacco seeds administered in

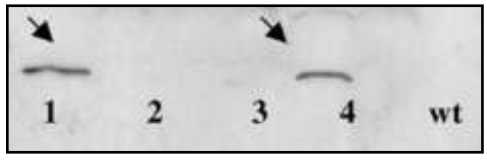
feed was an efficient way to perform a vaccinal strategy. Secondly our objective has been to test immunogenicity of tobacco seeds expressing bacterial proteins after oral administration in mice Balb-C model.

**MATERIAL AND METHODS** – Transgenic tobacco seeds were obtained by agroinfection, as previously described (Rossi *et al.*, 2002a). We evaluated the presence of F18 fimbria and VT2e-B subunit protein by Western blot analysis using specific policlonal serum. The amount of eterologous protein was estimated through comparison with a specific marker, in order to formulate the experimental feed. In order to give the mouse no chance to choose, the treatment diets were prepared as pellets containing respectively 20% non transgenic tobacco seeds (control group), 10% F18+ plus 10% VT2e-B subunit tobacco seeds for treatment (group 1), 10% F18+ plus 10% VT2e-B subunit tobacco seeds and 10μg of B subunit of Cholera toxin (group 2), 10% F18+ plus 10% VT2e-B subunit tobacco seeds and 10mg saponin (group 3).

25 female Balb-C mice (4 weeks old) were allotted in cages with 7 mice each in treatment groups and 4 mice for control group. Mice, fasted for 12 hours were administered one of four treatment diets on days 0,5,8,14,19,23, and on the remaining days were administered commercial basal diet (CP 19%). Individual body weights and feed intake were recorded weekly. After 27d the animals were sacrificed. The entire small intestine (duodenum, jejunum, and ileum) were collected. The samples were fixed in 4% paraformaldehyde in 0.01M phosphate buffered saline, pH 7.4 for 24 h at 4°C, dehydrated in alcohols, and embedded in paraffin. Serial microtome sections of the three intestinal tracts (4 μm thick) were stained with hematoxylin/eosin. Other sections were processed for visualization of IgA-forming cells, by immunostaining with a policlonal antibody against IgA, (Dako, Italy). The data were analyzed by ANOVA using the GLM procedure of the SAS Institute, Inc. (1985).

**RESULTS AND CONCLUSIONS 0** – The amount of transgenic proteins in tobacco seeds was estimated by western blotting, about 35μg/g of seeds (Fig. 1).

Figure 1. Western blotting with specific serum to detect VT2e-B subunit of shiga-like toxin on soluble proteins of tobacco seeds.



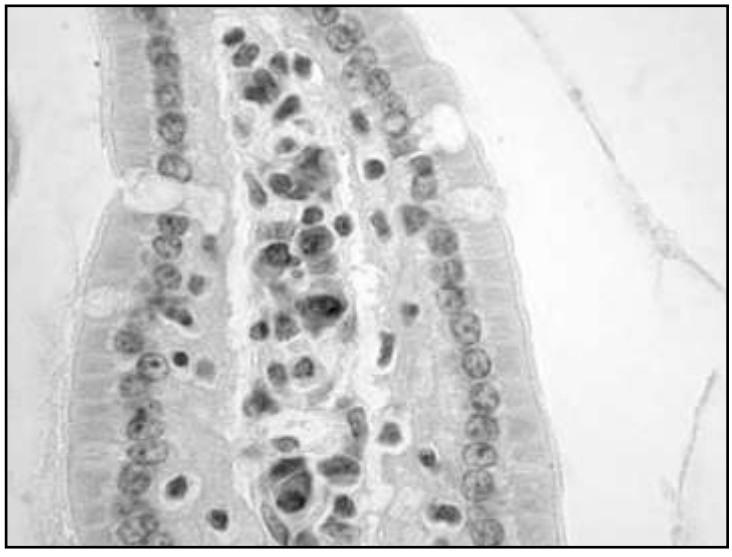

The seeds of lines positive for the presence of f18fimbria and VT2-B subunit of Shiga-like toxin were used for pellet production.

Dry matter intake (DMI) and growth parameters (weight, and average daily gain) didn't show any significant differences in all experimental groups, according to the standard performances of Balb-C mice. In the experiment we didn't observe any adverse reaction to tobacco seeds.

Histological examinations showed that the oral treatment with transgenic tobacco seeds, either itself or in association with F18 fimbria or VT2e-B subunit, did not result in altered microscopic structure of the small intestine within the groups, indicating that supplementations did not produce toxic effects on these tissues.

Immunostaining of the small intestine showed that the B subunit of Cholera toxin group promotes an increase in the number of IgA-forming plasma cells in the tunica propria. (Fig. 2)

Figure 2. Positive immunostaining of the IgA-forming plasma cells (arrows) in the ileum of the B subunit of Cholera toxin group (1000x).



It is possible that this treatment enhances the quantity of secretory immunoglobulins within the mouse small intestine, increasing in this species the local defence capacities towards the toxic lumenal content.

Considering that the different treatments have not altered the structure of the mouse small intestinal mucosa and that one of the chosen treatments seems to increase IgA-forming plasma cells, we can hypothesise that our ongoing work may in the future obtain results which will be applied to the swine.

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