5.0. CONCLUSION

This study has contributed towards the molecular understanding of the mechanism of action of the probiotic *E. faecium* SF68 in swine. Our study, in line with previous reports, showed the biological effect of *E. faecium* SF68 in piglets, which is the reduction in the CD8+ lymphocytes. In addition the study gave first time information on the ability of *E. faecium* SF68 to up-regulate the mRNA expressions of TLR9, the tetraspanin molecule, CD9, and TGF-ß that are associated with anti-inflammatory action. Patterns of immune alteration due to the probiotics may be interpreted in the context of the clinical effects observed, so that eventually such patterns alone would be able to indicate possible beneficial or deleterious effects of probiotics.

Although the application of probiotics shows some promising results, the yet not clearly understood mechanisms of actions and the specificity of action of probiotics makes the use of certain probiotic strains difficult. Unfortunately, only a few studies report on possible adverse effects of ingesting probiotics. It would be most appropriate to compare the specific immune effects attributed to a probiotic treatment with the clinical outcomes of the disease models, in order to conclude whether the immune effects noted are beneficial or rather deleterious. Our *in vivo* study used a disease model, where the sows and the piglets were free of the pathogen at the start of the challenge experiments with *Salmonella typhimurium*. The biological effect of *E. faecium* SF68 to decrease the CD8+ cells has been reported to be associated with a beneficial effect in piglets infected with *E. coli* O141, when the pathogen was already established in the herd. In contrast, in the present infection model, where both the piglets and their sows were free of *Salmonella* at the start of the experiment, the decrease in the CD8+ lymphocytes seems to have favoured an increase in the number of *Salmonella* in the piglets of the probiotic group (Szabó et al., 2009). Our data is the first to show the immune modulatory effect of *E. faecium* SF68 at the gene level through the up-regulation of the mRNA expressions of the anti-inflammatory genes for TLR9, CD9 and TGF-ß. However, this effect appeared to be not strong enough to down-regulate the *Salmonella typhimurium* DT104-induced expressions of genes associated with inflammation.

Further research to analyse mRNA expression patterns of various genes in the pure CD4+ and CD8+ T-lymphocytes collected from spleen, proximal PP and IEL of piglets of both probiotic and control groups using new technologies, such as cDNA-chip technology, would provide more information on the molecular effects of *E. faecium* SF68 both locally and systemically

during infection with *S. typhimurium* DT104. And finally, the comparison of effects of different types of probiotics will provide a first step in understanding the balance between positive and negative effects.

Our *in vitro* study shows that type II cells represent a useful pig intestinal epithelial cell model for studies using TGEV. The presence of microvilli covering the cells and the expression of the pan-cytokeratin marker in both type I and type II cells indicated that the cells are of epithelial nature. Furthermore, the sensitivity of type II cells to TGEV, which is known to specifically infect epithelial cells of the villous (Delmas et al., 1990), together with the expression of MHC class II genes in these cells, indicates that type II cells possibly originate from the villous epithelium. In addition to the expression of MHC class II genes, type II cells share similar morphological features with M cells. These are for instance, the absence of clear cell-to-cell connective junctions and the presence of certain organelles, such as the ER and Golgi apparatus, just above the nucleus. The resistance of type I cells to TGEV, the absence of expression of MHC class II genes in these cells and the occurrence of sparse organelles in the cytoplasm could indicate that the origin of type I cells could possibly be the crypt epithelium. However, further histochemical and molecular characterizations are mandatory to know the possible origin of the cells from the pig intestine. Defining the biological significance of type I cells to be used in infection models would make the further characterization of the cells crucial. However, the sensitivity of type II cells to TGEV and the higher virus titres produced by these cells in comparison to the titres reached in the known model cells for studies on TGEV; i.e. ST cells, qualify type II cells as a pig intestinal epithelial cell model suited very well for *in vitro* studies on TGEV.

Furthermore, the constitutive expression of genes for various cytokines by type II cells and the significant difference in the levels of expressions of inflammatory cytokines induced by the pathogen, TGEV, and the probiotic, *E. faecium* SF 68, strengthen the hypothesis that bacterial signaling at the mucosal surface may depend on epithelial-immuno cross-communication, which appears to be responsible for the innate reaction that can distinguish between pathogenic and non-pathogenic microorganisms. This study also provided molecular evidence on the beneficial effect of the probiotic strain *E. faecium* SF68 on intestinal immune homeostasis, since the pre-treatment of the epithelial monolayers with this strain down-regulates virus-induced cytokine expression. Our results support recent data on the role of IEC in processing non-pathogenic and pathogenic microorganisms-derived signals to the mucosal immune system by secreting soluble mediators, such as cytokines/chemokines

(Dahan et al., 2007; Shaykhiev and Bals, 2007). Application of well-reflected *in vitro* models, involving co-cultures of IEC and other cells of the immune system, could provide more information on the fine-tuning of mucosal responses, which may depend not only on IEC.

Finally, further investigations are required to understand the underlying mechanisms of the observed effects of the probiotic. The available knowledge on the effect of probiotics in the modulation of the immune system of pigs will contribute to the understanding of their mechanisms of actions in human beings.