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The distribution and function of immunocompetent cells during development and weaning in the jejunal pig intestine

Summary

In industrialised meat production piglets are removed abruptly from their nursing mother animal at 25-28 days old and weaned onto piglet feed. This results in qualitative and quantitative changes to the antigens found in the ecosystem of the lumen of the pig gut. In addition to the normal growth processes, these influence the epithelium of the gut wall and with it also the mucosal immune system. The Peyer's patches (PP) of the jejunal areas of the intestine were examined in comparison with the neighbouring villi areas. The T-cells circulating in the organism can enter the lamina propria of the small intestine, reach the PP with the interfollicular zones and react to environmental signals in situ in the intraepithelial cells in the intestinal epithelium as well as in the regions of the dome and villi areas. They can leave these areas again via the intestinal lymph system. In order to examine the permanent alterations in the mucosa, the four compartments dome epithelium (DE), villi epithelium (ZE) and, on each side of the basal membrane, the profoundly situated compartments of the subepithelial dome (SED) as well as villi lamina propria (LP) were differentiated and the target cells were counted separately. Against this background, the following tests were performed.

In Part 1, using three pigs as an example, a qualitative - semi-quantitative analysis of the colocalisation of M-cells and immunocompetent cells was performed. In this part of the work, ten different cell markers were established and used to illustrate the different cell populations using multiple fluorescence markings. For both the qualitative and quantitative assessment, photographic multiple fluorescent images were created. In a complex procedure the individual cell populations were marked "by hand" and the cell count was established per area. Thus, for all the examined cell populations, comparable cell counts per 10,000μm^2 could be compiled, pig-specific and very accurate for each jejunal intestinal section. These data were statistically evaluated using comparison of means, t-tests and posthoc-tests.

In Part 2 a qualitative-quantitative analysis of the CD3, CD4 and CD8 positive T-cell populations in the compartments of five different pig groups were performed, each with four animals. For this purpose an appropriate immunohistochemical stain was established for each marker. The assessment was carried out as for Part 1, additionally multiple linear regressions were performed, in order to be able to evaluate for the individual compartments the influence of the variables: age, feed, sow milk, as well as the effectiveness of a pathogenic *E. coli* strain on the cell population.

In Part 3 a qualitative-functional analysis of the cytokine production at a transcriptional level using non-radioactive in-situ hybridisation was performed by means of RNA-probes for each of two typical Th1 and Th2 cytokines (Th1: IL2 and IFNγ; Th2: IL4 and IL10). From the analyses in this work, the cell count for the examined cell types in the differentiated compartments could be precisely specified. The detected changes in the cell concentrations in the compartments are significant and the regression model shows that the variable feed plays the greatest role in the alteration of the cell population in the compartments. The effects are greatest in the epithelial compartments. The factor sow milk was more effective on the epithelium; the influence of the pathogenic *E. coli* strain manifested itself particularly in the deeper situated compartments of the SED and villi LP. The relationships of the influencing variables with each other are different between the markers and the compartments. The obvious differences in the proportion of immunocompetent cells in the compartments can be complemented on a functional level to only a limited extent by the representation of cytokine-mRNA. The highest activity at a transcriptional level, apart from few interfollicularly-situated positive cells, could be mainly established in the basal and lateral zones of the follicle mantel. The regions of the follicle mantel with their cytokine-mRNA positive cells never form a closed ring, but are always open to the dome. Strong positive signals for all the tested cytokines, clear and in each available animal, could only be found in the dome area of the oldest pigs (150 days old). A functionally prevalent immunological situation (Th1/Th2; cellular/humoral; inflammatory/suppressive) could not, however, be finally deduced from these investigations.

The focus of this work was directed towards the reactions evoked through altered environmental conditions in the directly affected areas of the intestinal mucosa. **In situ** these could be understood, pictured, quantitatively documented and functionally classified at the level of the cells involved.