provaxs | GHENT UNIVERSITY

Service offer

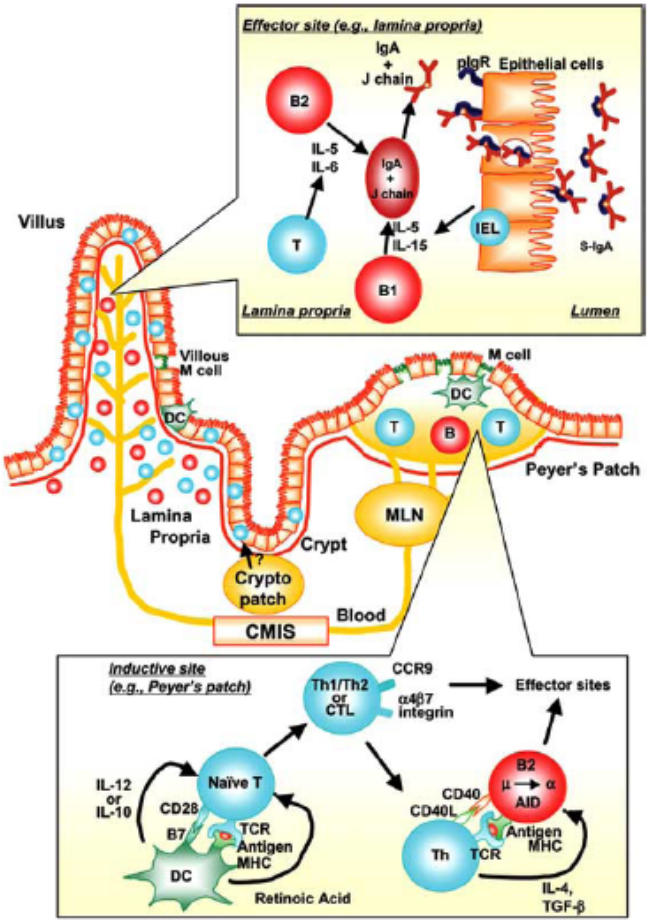
Model system for the screening of feed compounds that improve gut health

Introduction

One of the strategies to maintain optimal gut health in animal production in the absence of antibiotic growth promoters is to support the active immunity of the animal. Immunity at the level of the digestive system is concentrated in the mucosal immune system of the gut associated lymphoid tissue (GALT). Therefore, the GALT is the obvious target if we want to increase intestinal immunity. As illustrated in figure 1 the local immune response in the gut is only possible via a collaboration of several cell types.

Figure 1 : Generation of a specific immune response in the intestinal mucosa

Current Molecular Medicine, 2005, Vol. 5, No. 6 *Kunisawa et al.*



M cells and dendritic cells sample the intestinal contents and bring pathogens in close contact with macrophages, B and T cells in specialized regions in the intestinal epithelium like e.g. the Peyer's patches (inductive site). Macrophages destroy the

Service offer

pathogen and stimulate the B and T cells to launch an immune response that is specific to this pathogen. Depending on the pathogen and the co-stimulatory signals this response can be the activation of cellular (cytoxic T lymphocytes (CTL)) or humoral (antibodies) immunity. Antibodies (IgA) are secreted at the effector site and transported through the epithelial cells to neutralize the pathogens already in the intestinal lumen. Macrophages thus act at the first line and stimulate a whole cascade of events leading to final antibody production.

The intense contact between intestinal contents and immune cells at the mucosa offers opportunities to modulate intestinal immunity via the feed. While immunostimulating activities are claimed on many feed additives like phytoproducts, organic acids, chelates, pre- and probiotics and many others these additives mostly have only bacteriostatic effects or indirect effects on the immune system. On the contrary it has been widely demonstrated that β glucans have a direct immunostimulating effect. More specifically they bind to a receptor on macrophages and increase their activity towards stimulation of B and T cells. As a consequence, increased antibody titers after vaccination and better protection after challenges with a pathogen have been observed when β glucans were incorporated in the feed. However, not all β glucans are equally effective and their interaction with the receptor on the macrophage depends on their primary and secondary structure (degree of polymerization (DP) and degree of substitution (DS)).

Since immunization and challenge trials are laborious, time consuming and therefore very costly we propose to develop a model system based on in vitro assays that can be used to evaluate the immunomodulating property of feed additives. β glucans with different purity, DP and DS can thus be compared.

Most if not all sources of β glucans that are used in feed are impure or semi-purified compounds that contain a variety of oligosaccharides including mannan-oligosaccharides or MOS. These molecules can interfere with the attachment of pathogenic bacteria (e.g. enterotoxic Escherichia coli or ETEC) to the intestinal epithelial thus reducing their colonization. Next to immunomodulation this is another aspect of the possible positive effect of these compounds on gut health. In our lab, interference of attachment of bacteria to the intestinal epithelium can also be quantified in vitro.

provaxs | GHENT UNIVERSITY

Service offer

Rationale and proposed tests

As outlined above, once a pathogen is sampled out of the intestinal contents a local immune response in the intestinal mucosa is generated in different steps (Table 1). At first macrophages and other phagocytic cells (like dendritic cells (DC on Fig.1) ; non-specific immunity) take up the pathogen. They destroy the pathogen with enzymes and O-radicals and process it for presentation to local T cells. Phagocytic cells also secrete pro-inflammatory cytokines that stimulate the activity and proliferation of those T and B cells that are most fit to attack the type of pathogen that was presented by the phagocytic cell (specific immunity). Finally the B cells will produce antibodies (IgA) that can be secreted in the intestinal lumen to neutralize the pathogens.

Table 1 : Different steps in a local immune response in the intestinal mucosa and corresponding tests

	Immune response	Test
Innate	Phagocytosis	Superoxide and O-radicals
	Pro-inflammatory signals	Cytokines
Adaptive	Stimulation and multiplication of B and T cells	B and T cell proliferation

For each step in this sequence we have developed an in vitro test that can be used to analyze the immunomodulating capacity of glucans.

Step 1 : Phagocytosis
Test 1 : Production of superoxide and O-radicals

Macrophages and neutrophils are two major categories of phagocytic cells that carry dectin1 (the glucan receptor) on their outer membrane. For this test, monocytes and neutrophils are isolated separately out of blood of control pigs (not stimulated with glucans). Monocytes are the cells that will differentiate to macrophages once they have migrated out of the blood into the tissues. Both cell types are then transferred to microplates (monocytes and neutrophils in separate wells) where we can analyze their production of O-radicals in vitro. The amount of O-radicals is a measure of the non-specific defense against pathogens. By addition of glucans to the cell medium at different concentrations we can measure their stimulatory effect on the production of O-radicals.

Step 2 : Pro-inflammatory signals
Test 2 : Cytokines interleukin 1 & 6 (IL1, IL6) and tumor necrosis factor α (TNFα)

IL1, IL6 and TNFα are pro-inflammatory cytokines that are released at the beginning of an immune response. Most adjuvantia that are used in vaccines increase the production of these cytokines. We already demonstrated that some glucans could increase the production of these cytokines by phagocytic cells in vitro. Isolated immune cells (macrophages, monocytes, B and T cells) are incubated in a control

Service offer

medium or a medium including glucans. The effect of the glucans on the cells will then be analyzed by measurement of the expression of IL1, IL6 and TNFα.

Step 3 : Stimulation and multiplication of B and T cells
Test 3 : B and T cell proliferation

Macrophages/monocytes, B and T cells are isolated and proliferation of B and T cells is induced at a basal level in vitro. This proliferation can be followed by the addition of radioactive nucleotides in the medium. When cells multiply, these radioactive nucleotides are incorporated in their DNA, which can be measured at the end of incubation in a counter. We already demonstrated that some glucans could stimulate this basal level of B and T cell proliferation.

Test 4 : Inhibition of attachment to the intestinal epithelium
4.1. Inhibition of adhesion by binding to the F4 fimbriae (bacteria)

The principle of this assay is that adhesion of the F4+ ETEC to the villous brush borders is inhibited by binding of feed components to the bacterial fimbriae.

The F4+ ETEC are pre-incubated with the feed additives. Subsequently, the F4+ ETEC are incubated in vitro with villi on the brush border. The percentage adhesion after pre-incubation with one of the feed additives compared to the control (no additives added) is a measure of the inhibition of attachment.

provaxs | GHENT UNIVERSITY

Service offer

Benefits and value

- the activity of all the samples within each test will be **comparable**
- the results will give an **activity profile** of each sample, indicating at what level the immunological response is most probably modulated when the sample is included in the feed
- the capacity of a sample to interfere with **attachment** of E coli (test 5) is a measure of protection against attachment and colonization of the intestinal epithelium in vivo
- **final validation** of the results of the in vitro screening towards gut health and protection against pathogenic challenges will have to be performed by in vivo tests
- **flexibility** to make a selection of the tests that have to be performed in function of your needs : e.g. use all the tests to compare the activity of different products and use only the test with the highest response for your main product for QC

Contact

Sven ARNOUTS - +32 495 707 334 – Sven.Arnouts@UGent.be