

IL-18 potentiated whole blood IFN- γ assay can identify cell-mediated immune responses towards *Lawsonia intracellularis* in experimentally infected pigs.

Ulla Riber, Jeanne Toft Jakobsen, Henriette Cordes, Gregers Jungersen,

Technical University of Denmark, National Veterinary Institute, Bülowsvej 27, 1790 V, Copenhagen (urib@vet.dtu.dk)

Lawsonia intracellularis is an obligate intracellular bacteria causing proliferative enteropathy (PE) in pigs. The infection causes diarrhoea, retarded growth and sudden death in pigs and is one of the most economically important diseases in the swine industry worldwide. The infection is one of the major causes of antibiotics usage in modern pig production. Diagnostic measurement of *L. intracellularis* infection includes detection of *L. intracellularis* in faecal samples by PCR, immunohistopathology examination of intestine and measurement of *Lawsonia*-specific antibodies in serum.

There are strong indications that cell-mediated immune responses (CMI) are important for the protection against infections with *L. intracellularis* and in mice models IFN- γ has been shown to play a key role in the host defence against experimental infections¹². In *L. intracellularis* infected pigs, IFN- γ is only sparsely investigated, but IFN- γ producing cells have been shown by ELISPOT in pigs experimentally infected with *L. intracellularis*³.

At National Veterinary Institute, experimental *L. intracellularis* infection studies have been performed and an IFN- γ assay was developed based on overnight incubation of heparin-stabilised whole blood with SDS-treated *L. intracellularis* (SDS-antigen) in the presence of IL-18. The produced IFN- γ was quantified by ELISA.

Using this IL-18 potentiated IFN- γ assay, high levels of IFN- γ was measured from 2 weeks p.i. in pigs infected at 12 weeks of age, however, pigs infected at 5 weeks of age exhibited a much lower level of IFN- γ response. Thus, age seems to be an important parameter in measurement of IFN- γ in response to *L. intracellularis* infection. In the young pigs antibiotic treatment (from 3 weeks p.i.) cleared the *L. intracellularis* infection. In contrast to the low response observed during the acute primary infection, these pigs mounted, after antibiotic treatment, a high antigen-specific IFN- γ response 7 weeks p.i., even when no signs of *L. intracellularis* infection were detected. Pigs receiving a primary *L. intracellularis* infection at 5 weeks of age followed by a challenge infection at 12 weeks of age showed increased or constant high levels of IFN- γ after challenge. These pigs were protected against *Lawsonia* re-infection in contrast to age matched challenge control pigs, which developed *Lawsonia* infection.

Four-color flow cytometry was used to identify cells producing IFN- γ after co-incubation of PBMCs with SDS-antigen and IL-18. IFN- γ producing cells were mostly identified as CD4⁺, and especially CD4⁺CD8⁺ double positive T-cells also expressing CD25.

¹ Go, Y.Y., Lee, J.K., Ye, J.Y., Lee, J.B., Park, S.Y., Song, C.S., Kim, S.K., Choi, I.S., 2005. Experimental reproduction of proliferative enteropathy and the role of IFN-gamma in protective immunity against *Lawsonia intracellularis* in mice. J. Vet. Sci. 6, 357-359.

² Smith, D.G., Mitchell, S.C., Nash, T., Rhind, S., 2000. Gamma interferon influences intestinal epithelial hyperplasia caused by *Lawsonia intracellularis* infection in mice. Infect. Immun. 68, 6737-6743.

³ Guedes, R.M., Gebhart, C.J., 2003. Onset and duration of fecal shedding, cell-mediated and humoral immune responses in pigs after challenge with a pathogenic isolate or attenuated vaccine strain of *Lawsonia intracellularis*. Vet. Microbiol. 91, 135-145.