

Promises and disappointments in studies of cell mediated immune responses against bacterial infections in blood samples from pigs

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Measurements of cell-mediated immune responses (CMI) are important for diagnostic discrimination of cross-reactive antibody-mediated immune responses and in studies of pathogenesis and vaccine efficacy to ascertain the degree of CMI involvement following infection or vaccination. *In vitro* CMI can be measured by antigenic stimulation of lymphocytes followed by measurement of induced proliferation or interferon-gamma (IFN- γ) production. In the past couple of years we have studied CMI in several porcine bacterial infections and experienced both promising and disappointing results.

Flow cytometry analysis of antigen-specific lymphocyte proliferation has been measured by incorporation of the thymidine analogue BrdU into DNA of proliferating cells followed by intracellular staining with an anti-BrdU monoclonal, or by incorporation of the fluorescent CFSE dye into cell-membranes and subsequent analysis of fading fluorescence with each cell division. There are pros and cons for each method, but the CFSE technique appears to be more sensitive and is more easily combined with assays for other parameters such as apoptosis.

Measurement of antigen-specific IFN- γ production in culture supernatant (IFN- γ test) or numbers of antigen-specific IFN- γ producing cells (ELISpot) have also been used as indirect measures of CMI. In experimental infections with *Brucella suis* a very high and consistent IFN- γ response was observed in whole-blood cultures following overnight incubation with antigen. This assay was able to overcome problems with false-positive sero-reactors due to infection with *Yersinia enterocolitica* serotype O:9. While prominent IFN- γ responses were observed in *Brucella* infected pigs it was not possible to obtain any significant IFN- γ response to stimulation with *Yersinia* YOP proteins in the *Yersinia* infected pigs, illustrating how CMI are only induced in infections with a Th1-type immune pathogenesis.

There are strong indications that CMI are important for the protection against infections with *Lawsonia intracellularis*, *Mycoplasma hyosynoviae* and *M. hyopneumoniae*, but the CMI in these infections are not as evident as with *B. suis*. In an attempt to enhance the antigen-specific response we have investigated the value of adding the co-stimulatory cytokines IL-12 and IL-18 to the cultures. In contrast to our observations in cattle, IL-12 addition did not result in increased IFN- γ production with pig lymphocytes. However, addition of recombinant porcine IL-18 specifically enhances the antigen-specific IFN- γ production allowing for measurements of subtle CMI in less potent Th1 inducing infections. Another important observation regarding the performance of the whole-blood IFN- γ assay is the need to stimulate fresh samples. This was illustrated by a more than 40-fold decrease in mean *Brucella* specific IFN- γ when positive samples were cultured one day after sampling.