

Measuring cell-mediated immunity against bacterial infection

There is an increasing focus on characterisation of the cell-mediated immune responses (CMI) against bacterial infections in both human and veterinary medicine. Quantification of CMI responses may be important for diagnostic discrimination of cross-reactive antibody-mediated immune responses, but with some infections also holds promise to provide an earlier diagnosis than what can be attained with antibodies, and for vaccine development assessment of both quantity and quality of the CMI response may be a crucial indicator of the desired type of immune response. However, since CMI responses in vivo are tightly regulated by cell-cell communication, and often require various co-stimulatory signals, in vitro assays are often performed with supplementation of recombinant cytokines or other stimulatory molecules. One of the easiest assays for a direct CMI read-out is the interferon-gamma (IFN- γ) assay where de novo synthesized IFN- γ in response to relevant antigen-TCR activation in whole-blood or PBMC culture is quantified by ELISA. Co-stimulatory signals for IFN- γ production by CD4⁺ T cells in vivo are IL-12 and IL-18 and antigen-specific CMI responses in the IFN- γ assay can be significantly enhanced by the addition of these cytokines. We have successfully applied recombinant IL-12 to sustain the antigen-specific IFN- γ production in cultures of day-old cattle samples for assessment of exposure to paratuberculosis. In pigs, T cell survival is much shorter and day-old samples can't be rescued, but with addition of IL-12 or IL-18 to cultures we significantly enhanced Ag-specific IFN- γ production by CD4⁺ T cells to identify CMI responses to infections with *Lawsonia intracellularis* and *Mycoplasma hyosynoviae* where the responses without co-stimulation are of low level.

A simple measurement of IFN- γ production does, however, not necessarily provide the level of information that is required to assess the quality of a CMI response. In evaluations of novel vaccines much focus has recently been focused on the development of polyfunctional CD4⁺ T cells capable of antigen-specific production of the three key cytokines IFN- γ , TNF α and IL-2. This phenotype reflects central memory cells and appears to be much more correlated with vaccine induced protection than the more differentiated monofunctional cell types. Polyfunctional T cells are quantified by multicolour flow cytometry after stimulation with antigen and co-stimulation with anti-CD28 (and sometimes anti-CD49d). In veterinary science, we are (as so often seen before) hampered by a limited reagent tool-box compared to that available for human and mice immunologists. To my knowledge, no anti-CD28 antibodies have been described to activate bovine or porcine T cells. For cattle, anti-IL-2 antibodies are not available and staining with anti-TNF α antibodies produces a high level of surface staining not seen in mice, humans or pigs. In pigs, staining with anti-IL-2 produces a high background level which also makes it difficult to perform reliable identification of the rare polyfunctional T cells. In characterization of vaccine induced CMI responses, we have therefore investigated into alternative protocols of cytokine co-stimulation and use of IFN- γ fluorescence intensity as an approximation of polyfunctional T cells as these cells have been shown to make up to 10-fold more IFN- γ on a per-cell basis compared to monofunctional CD4⁺ T cells.