Choosing and using immune parameters in animal science studies

For a more detailed discussion, please see Selvaraj et al., 2016.

The growing interest in animal health and the relationship with nutrition and/or feed additives mean that those not trained in immunology are frequently including immune parameters in their work. There are, however, an array of immune parameters that can be assessed, all of which may give different indications of 'status', and so it is important to appreciate the strengths and weaknesses of the assays available. This technical note will provide a short overview of some of the traditional immune-related assays available to animal scientists. A very brief overview of the immune system, for reference purposes, was provided in a previous technical note (Immunomodulation – what are we trying to achieve?; August 2017).

Cell-related assays

1. Delayed-type hypersensitivity (DTH) assay

The DTH assay invariably involves intradermal injection of phytohemagglutinin (PHA), a plant lectin, and subsequent (48 – 72 h post-injection) measurement of localised swelling. PHA is a T cell mitogen and thus initiates T cell proliferation without the involvement of antigen-presenting cells (APC) (Ceuppens, 1998). This, along with a reduced swelling response to PHA following thymectomy, led to the DTH response being interpreted as an indicator of acquired immune function and disease resistance. However, PHA causes a local inflammatory response, involving the recruitment of innate immune cells, and thus any early swelling measurements (<48 h post-injection) are likely to represent the initial inflammatory phase rather than significant T cell infiltration.

2. Lymphocyte proliferation assay (LPA)

The LPA is the *in-vitro* equivalent of the DTH assay and assesses the proliferative response of lymphocytes to various mitogens (e.g. PHA, concavalin A, etc.). The assay requires mononuclear cells to be harvested, typically from blood, spleen or lymph nodes, before exposure to the selected mitogen(s). However, such harvesting inevitably yields mixed cell populations (e.g. including other immune cells) of varying proportions, while pure lymphocyte populations are not compatible with the assay. The assays to actually measure cell

proliferation can also influence interpretation of results. The LPA has generally been used to assess immune deficiencies, adequacy and/or enhancement. However, given the drawbacks outlined, as well as the actual functional significance of greater (than say 'normal') proliferation, the value of LPA, certainly on its own, maybe questionable.

3. Immune cell proportions

Measurement of immune cell populations, based on established cell surface markers, typically using flow cytometry, is frequently employed in nutritional studies investigating immune influences. Flow cytometric analysis is, however, a specialism and the procedures require appropriate control measures and interpretation of data. However, fluctuations in immune cell numbers/proportions, interpreted correctly, can provide insight as an immune (status) indicator.

4. Gene expression

mRNA extracted from various body compartments/tissues can be utilised to investigate the expression of numerous immune-related genes using real-time PCR. These samples would, without prior cell sorting, contain mixed cell populations, thus providing a general overview of responses, thus limiting the opportunity to interrogate the contribution of individual cell types. Alternatively, purified cell populations (or single cells) can be studied but the wider picture may then be lost. However, even within a particular cell type, with similar cell size and surface markers, the magnitude of gene expression can vary greatly even to the same stimuli and not all cells within a population may respond to the stimulus (Selvaraj et al., 2016), demonstrating the challenge of interpretation. Furthermore, gene transcription does not necessarily equate to functional protein due to various processes that silence or destroy mRNA.

Humoral-related assays

1. Antibody assays

B cells produce antigen-specific antibodies. Affinity (strength of single combining site-antigen interaction) and avidity (overall strength of antibody-antigen interaction) of an antibody indicate its ability to neutralise the antigen/pathogen. The affinity and avidity of antibodies increases during the course of an infection and are greater during secondary immune responses (Steiner and Eisen, 1967). Therefore, measurement of total antibodies, without assessing changes in affinity and avidity could miss important differences in antibody responses. Various factors can influence an individual animal's normal antibody ranges and thus it's important to measure basal levels on commencing an experiment and any antibody changes considered in the context of normal ranges, and bodily distribution, to interpret biological significance.

Natural antibodies are those found circulating in (healthy) individuals without exposure to (exogenous) antigenic stimulation and are typically measured through the haemolysis-hemagglutination assay, which typically utilises red blood cells from another species (or keyhole limpet haemocyanin). Natural antibodies are not considered to be that sensitive to short-term factors/influences and each individual (based on genotype) has a different

repertoire and thus may assay differently depending on the particular antigen (tested), thereby limiting the usefulness of natural antibody analysis.

2. Acute phase protein (APP) assay

Various (acute phase) proteins are produced by the liver in response to inflammatory cytokines following infection or tissue injury. Serum concentrations of APP peak 24 to 48 h post-challenge and can typically be analysed by ELISA. Measurement of only one APP is usually not particularly informative and thus it's often proposed to assay multiple APPs to calculate an APP index (Ceron et al., 2005). In addition, other factors (e.g. feed intake) can influence APP production and thus need to be considered when assessing APP responses.

Conclusion

A more holistic approach to the promotion of efficient animal production means that the disciplines of nutrition and immunology are becoming ever more integrated. It is, therefore, helpful for the animal nutritionist/scientist to have a fundamental understanding of immunology and to consider the use of appropriate immune-related assays, data from which is correctly interpreted. This technical note gives a brief overview of frequently used assays and highlights some of the limitations of each. In almost all instances, it is not appropriate to use a single assay/measure to indicate (shifts in) immune status. Appropriately combining suitable immune parameters, sample numbers and timepoints is much more likely to yield meaningful and informative data.

References

Ceron, J. J., P. D. Eckersall, and S. Martynez-Subiela. 2005. Acute phase proteins in dogs and cats: current knowledge and future perspectives. Vet. Clin. Pathol. 34:85–99.

Ceuppens, J. L., M. L. Baroja, K. Lorre, J. Van Damme, and A. Billiau. 1988. Human T cell activation with phytohemagglutinin. The function of IL-6 as an accessory signal. J. Immunol. 141:3868–3874.

Selvaraj, R. K., R. Shanmugasundaram, and R. Rengasamy. 2016. The use and abuse of immune indices in nutritional immunology studies. J. Appl. Poult. Res. 25:284–291.

Steiner, L. A., and H. N. Eisen. 1967. Sequential changes in the relative affinity of antibodies synthesized during the immune response. J. Exp. Med. 126:1161–1183.