Development of a bovine/ovine cytokine 15-plex assay for immunoprofiling of the cellular response in ruminants

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ABSTRACT BOOK

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been limited compared to humans and mice. To address this gap, several Immunological Toolbox initiatives (from 2003 onwards; largely funded in the UK and USA) aimed to prioritise new reagent development against research gaps. Today, all these efforts have produced thousands of reagents and associated methods, but a global overview of availability and applicability is lacking.

After feedback from several national and international workshops, we have developed a searchable and highly scalable veterinary immunology reagent database, accessed through a website; immunologicaltoolbox.co.uk. This provides detailed knowledge on existing reagents, as well as reagents that are being developed. As such, we aim to highlight gaps in capability and avoid the duplication of effort, which itself can provide strong evidence to support investment in specific research and development. Information presented will include cross-reactivity between species and associated applications alongside a comments and rating feature to facilitate continual community scrutiny and improvement. Frameworks to facilitate collaboration, exchange or purchase from the owner or company will also be available.

The website also links directly to the practical components of the Pirbright/Roslin Toolbox. This includes the generation of new reagents based on community priorities, and the capability to recombinantly express and engineer monoclonal antibodies. Consequently, this first version of the website has largely been driven by the information available from Pirbright and Roslin antibody repositories. However, the quality and quantity of future information will rely on community engagement. Discussions at an international level will be extremely valuable in helping to remove barriers and promote veterinary immunological research.

O42

Development of a bovine/ovine cytokine 15-plex assay for immunoprofiling of the cellular response in ruminants

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Evaluation of the immune status must be assessed in highly controlled conditions to be amenable for reproducibility. Furthermore, whereas the cellular response plays a major role, immunity has been measured for a long time through antibody production. For the purpose of assessing immune cell responsiveness in ruminants, we developed and validated a whole blood assay coupled to a high-throughput multiplex cytokine assay using the Luminex technology. A custom Milliplex assay was developed with MERCK-Millipore company for the measurement of 15 bovine/ovine cytokines of both the innate and adaptive immunity, and chemokines. Whole blood samples were collected from 110 dairy cows in an INRA experimental unit, and immediately stimulated during 24 hours with heat-killed bacteria (*Escherichia coli, Staphylococcus aureus, and Streptococcus uberis*), Toll-like receptor ligands (FSL-1 for TLR2, LPS for TLR4, and Gardiquimod for TLR7-8), or soluble anti-CD3/CD28 monoclonal antibodies. Cytokine secretion was determined and compared with a sample prepared in the same condition but without any stimulant. Using ANOVA and data mining methods (PCA, clustering…), we showed that the

protocol is able to detect differences between bacteria, with *S. uberis* being the most potent to induce a response, compared to *S. aureus* and *E. coli* that were distinct but more closely related to each other. Similarly, TLR ligands could be distinguished, and Gardiquimod was significantly different from the MyD88-associated TLR2/4-activating ligands. LPS and *E. coli* provided very similar response profiles confirming previous data indicating that a large part of the *E. coli* response is mediated through LPS signaling. A high variability of response was detected amongst cattle suggesting environmental and genetic variations of the cytokine response. Altogether, results indicate that cytokine profiling in cattle is achievable and can be used in further work to delineate more precisely the responsiveness of cattle in various situations, including variability of the genetic background.

O43

CD40-targeted immunization as a method for rapid antiserum production and epitope mapping

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The CD40 receptor is expressed on all professional antigen presenting cells and is integral to the activation and subsequent production/affinity-maturation of antibodies. Previously published data from our laboratories demonstrated that targeting an antigen to chicken CD40 receptor induced more rapid and robust antibody production specific against the antigen. Using this method, a panel of oligopeptides of varying lengths ranging from 9 to 23 amino acids were designed to span an entire target toxin were synthesized for the purpose of epitope mapping. The target, *Clostridium perfringens* alpha toxin, possesses two enzymatic functions: hemolytic activity and phospholipase C activity. By loading this panel of peptides onto the CD40-targeting platform and immunizing chickens by subcutaneous injection (equivalent to 0.33ug of peptide antigen dosage per chicken), a panel of high titer peptide-specific antiserum samples was produced in a week. Peptide-specific antibody responses measured a minimum mean of 4.6-fold higher than pre-immune baseline, and as high as 13-fold higher. These serum samples were used to screen for the ability to neutralize the alpha toxin's enzymatic functions. Results demonstrated multiple candidates for hemolytic neutralization, but not against phospholipase C. With this successful proof-of-concept, the CD40-targeted immunization method was able to produce functional antiserum for *in vitro* laboratory usage a week after a single immunization. This targeted method is more advantageous than the traditional style of antiserum production, which requires multiple immunizations over a span of several weeks, necessitating long-term animal care/housing and consumption of more antigen. Furthermore, because this platform uses chickens as a host, there is an increased chance of response against conserved mammalian domains. Additionally, this method of rapid antiserum production is a useful technique for cheaper, faster epitope mapping and has since been successfully used for candidate selection in development of a necrotic enteritis vaccine for poultry.