Veterinary Immunology Toolbox Meeting

21st – 22nd August 2017

The Pirbright Institute

Contents

Meeting attendees

Armin Saalmüller (AS), University of Veterinary Medicine Vienna
Bill Golde (BG), Moredun
Bryan Charleston (BC), The Pirbright Institute
Carly Hamilton (CH), The Roslin Institute
Don Knowles (DN), United States Department of Agriculture
Efrain Guzman (EG), The Pirbright Institute
Elma Tchilian (ET), The Pirbright Institute
Fiona Tomley (FT), Royal Veterinary College
Gary Entrican (GE), Moredun
Isabelle Schwartz-Cornil (ISC), French National Institute for Agricultural Research (INRA)
Ivan Morrison (IM), The Roslin Institute
Jayne Hope (JCH), The Roslin Institute
Joan Lunney (JL), United States Department of Agriculture
John Hammond (JH), The Pirbright Institute
Kerstin Mair (KM), University of Veterinary Medicine Vienna
Kim Thompson (KT), Moredun
Linda Wooldridge (LW), University of Bristol
Lonneke Vervelde (LV), The Roslin Institute
Madeleine Clark (MC), The Pirbright Institute
Mark Stevens (MS), The Roslin Institute
Nick Juleff (NJ), Bill & Melinda Gates Foundation
Simon Carpenter (SC), The Pirbright Institute
Simon Graham (SG), The Pirbright Institute
Tim Connelley (TC), The Roslin Institute
Tom McNeilly (TM), Moredun

Minutes

1. Introduction to the Meeting - John Hammond, The Pirbright Institute

- Two meetings held in 2015/2016 highlighted the importance of reagent development for veterinary species. Therefore, The Roslin Institute (RI) and The Pirbright Institute (TPI) were encouraged to fund toolbox activities to develop veterinary immunological reagents through their Core Capability grants (CCG).
- These funding bids were successful and RI and TPI are working together to coordinate and develop immune reagents. It was noted that that these activities are not restricted to RI/TPI and that the wider community should be engaged.
- To facilitate community involvement, the Veterinary Immunology Toolbox Meeting was organised. Representatives from different countries, organisations, species and pathogens were invited who either make or use immunological tools, and have an interest in the development of immunological reagents for livestock.
- There are several new and existing activities in this area that may benefit from greater coordination and collaboration.
- Aims of the meeting:
    - identify initial gaps and priorities
    - identify areas lacking resources for future prioritisation
    - identify funding opportunities (how do we build on existing resources to gain further funding and therefore ensure sustainability and expansion)
    - consider commercialisation and provision of reagents
- Outcomes of the meeting:
    - expand the group to include as many relevant researchers/parties as possible
    - CH to write project minutes and distribute to attendee list/edit for publication in a journal, possibly Frontiers in Immunology
    - decide a method to enable communication within this group

2. Background to the Immunological Toolbox: historical activities, funded projects across institutes - Gary Entrican, Moredun

- Contributions of veterinary/comparative immunology: bursa in birds and thymus in sheep therefore there is huge value in looking at comparative immunology. Biomedical models are important, for example, lymphatic cannulation in cattle and sheep as alternatives to mouse models.
- A landmark in immunological reagent development was the development of monoclonal antibody (mAb) hybridomas by Georges Kohler and Cesar Milstein in 1975. Georges Kohler, Cesar Milstein and Niels Jerne were awarded the Nobel Prize in Physiology or Medicine in 1984 "for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies".
- Between 1980 and 1990 there were a number of veterinary immunology reagent development efforts resulting in a wide range of phenotypic and anti-cytokine mAbs in a range of species. However, these efforts were largely conducted by individual organisations and not formally coordinated. Knowledge sharing, dissemination of resources and industry support was minimal.
- The International Union of Immunological Societies (IUIS) implemented a Veterinary Immunology Committee (VIC) and the VIC toolkit was founded at the 6th International

Veterinary Immunology Symposia (IVIS) in Uppsala in 2001. The first meeting of the VIC Toolkit Workshop was held at the 7th IVIS meeting in Quebec City in 2004.

- The aim of IUIS VIC is "to enhance the profile of veterinary immunology through international knowledge exchange and outreach activities that support research into animal diseases and vaccine development for the benefit of global communities".
- Activities of VIC include: promoting veterinary immunology; assisting with the planning and funding of the IVIS; coordinating databases containing information of interest to veterinary immunologists and coordinating the development of a toolkit of veterinary immunological reagents
- Consortium research projects:
    - The BBSRC Immunological Toolbox (2003-2009), coordinated by Jim Kaufman (Institute for Animal Health (IAH)), Gary Entrican (Moredun)
    - BBSRC Immunological Toolbox Strategic Lola (2010), coordinated by Jayne Hope (IAH). Grant failed at the full submission stage - applications including multiple species were not favoured and so funding applications moved to single species grants
    - The route to identification of immunological correlates of protection in ruminants (2012 – 2015), coordinated by Gary Entrican (Moredun). Partners: Moredun, RI, and AbD-Serotec. AbD-Serotec did not renew because of transition with Bio-Rad
    - US Veterinary Immune Reagent Network (VIRN) (2005 – 2010; 2010 – 2015), coordinated by Cynthia Baldwin. Kingfisher were the commercial partner and therefore there was a clear route to market
    - Swine Immune Toolkit (2015 – 2018), coordinated by Joan Lunney (United States Department of Agriculture (USDA)). Partners: USDA-ARS, University of Bristol, KingFisher Biotech
    - National Avian Research Facility (2013 – present), partnership between RI and TPI
- Stakeholders in immune reagent development projects:
    - NADIR (2009-2013)
    - VetBioNet (2017-2022)
    - STAR-IDAZ/SIRCAH (2011-2016, 2017-2022)
    - BBSRC UK Veterinary Vaccinology Network (UK VVN) (2015-2020)
    - GCRF MRC/BBSRC International Veterinary Vaccinology Network (IVVN) (2017-2021)
- Recent BBSRC One Health accelerate vaccine development call
- SWOT analysis:

  <u>Strength</u>
    - Establish global networks
    - Published exemplers
    - Good track record – validated peer review
    - Response mode funding

  <u>Weakness</u>
    - Searchable database
    - Lost valuable reagents
    - Don't generate profit

Opportunities:

- Zoonoses
- Food safety and security
- Biomedicine

Threats:

- Stagnation of database
- Sustainability
- Landscape changes
- Community buy-in

- Avenues for funding
  - TPI and Roslin strategic core funding
  - Research focus moving ahead: transcriptomics, gene products to target for reagents
  - Access and buy in from universities, ministries.
  - Toolbox is also a network – diversity

3. Current funding for immunological toolbox activity - John Hammond, The Pirbright Institute

- There is 1FTE funding at TPI for translating current hybridoma stocks into translatable gene blocks via sequencing:
  - Can build in class switching
  - Calculate current stocks and populate toolbox website
  - This FTE will align with TPI Central Services Unit to be better skilled and have better resources to produce reagents
  - Benefits: secure reagents for the future as they exist as sequences, reduce costs of liquid nitrogen storage, sharing of reagents will be much easier (no need to ship cells or supernatant) and may be possible to engineer antibodies to better suit research needs
- Recombinant antibody pipeline:
  - Collaboration with Ray Owens at Oxford Protein Production Facility
  - Established a sequencing protocol at TPI for mouse and cattle hybridomas and heterohybridomas
  - Designed vector backbones for cattle and mouse to allow ligation of commercially generated antibody gene blocks
  - Co transfection of heavy and light chains into chinese hamster ovary (CHO) cells then harvest supernatant for direct use or purification
  - It was suggested that companies could also do this but cheaper in house
- Immunological toolbox website and database:
  - Money from the BBSRC Tools and Resources Fund will be used to develop an immunological toolbox website and database
  - This will be a community website allowing multiple users to add and edit content, with a developer site and a live site.
  - Requires community buy-in as specific areas of the website will be developed by a specific person in the field – JCH has approached colleagues at RI to aid in the final design/addition of content

- Currently the website is structured around specific antigens however the aim is that the website will not only be focused on antibodies. Top level would be antigen and then go down from there through species, protein, antibody, interacting partners, isoforms etc.
- Worth consulting Harry Dawson re the Swine Immunology website: https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/diet-genomics-and-immunology-laboratory/docs/dgil-porcine-translational-research-database/

4. Current funding for immunological toolbox activity - Jayne Hope, The Roslin Institute

- Immunological toolbox at RI:
  - BBSRC funded project 'Defining correlates of protective immunity in ruminants' funded from 2011-2014. Moredun/Roslin joint project with AbD Serotec
  - Wellcome Trust funding for chicken immunological tool development including database/website funded to 2018 (Pete Kaiser/David Hume)
  - Toolbox activities at The Roslin Institute funded through Institute Strategic Programme Grant from 2012 onwards
- Institute Strategic Programme (ISP) Funding at RI currently funds 1 FTE across 3 posts (2017-2022). This post is included in ISP2: Control of Infectious Diseases, Theme 3: Host responses underlying immunity, Objective 3.4: Generate tools and resources for veterinary vaccinology. Priorities include:
  - development of tools to study macrophage development and function
  - reagents and methods to dissect antibody responses of animals at the single B-cell level to define the Ig repertoire and retrieve desirable antibody specificities
  - recombinant cytokines and chemokines
  - high-throughput typing systems for expressed products of highly polymorphic immune loci (MHC, NK cell receptors etc.)
  - Multiplex platforms for detection of cytokines and immune-related gene expression
  - platforms for screening recombinant antibody libraries, camelid nanobody libraries and/or phage display libraries
- Coordinate activities with the Pirbright Institute ISPG:
  - New reagent development/assays at RI
  - Sequencing of existing antibody secreting cell lines and molecular techniques for expression of recombinant antibodies & class switching at Pirbright (database development)
  - Joint steering committee to define priorities based on requests and input from network/community

5. Immunology Toolbox, US perspective - Joan Lunney, United States Department of Agriculture

- US Veterinary Immune Reagent Network (VIRN) Project directors:
  - Cattle/Ruminant: Cynthia Baldwin
  - Chicken/Poultry: Hyun Lillehoj
  - Swine: Joan Lunney
  - Equine: Bettina Wagner
  - Trout: Erin Bromage and John Hanson
  - Catfish: Melanie Wilson and Eva Bengten

    - Commerical partner: Joanna LaBresh, Kingfisher Biotech
- Why? Need to measure the effectiveness of veterinary species disease and vaccine responses and develop novel biotherapeutics
- Goal of VIRN is to develop sets of public available reagents and new assays
- Because of differences in reagent availability across species, each species set their own priorities. If reagents were already publicly available then there was no need to make them. Unfortunately, not all researchers made their reagents publically available.
- VIRN Strategy:
    - Cloning: easy for mammalian species but more difficult for poultry and fish
    - Expression: expression systems such as yeast, mammalian, bacterial
    - Cell surface antigens: selected targets then predict epitope(s) or full sequencing cloning and expression strategies
    - mAb production: standard mouse immunisations; problem: low productivity, many lost hybridomas
    - Difficulties: failure to express some proteins (e.g. granzyme) or prod mAbs for certain targets (TCRs, poCD19, Igs)
    - Assay development
- Post VIRN strategies:
    - USDA NIFA: funding targeted to individual species with 1-2 species funded per year - 2015 US UK swine immune toolkit and Cornell equine; 2016 BARC chicken; 2017 fish and ruminants
    - Kingfisher Biotech: a reliable source for yeast expressed proteins
    - Changed models: commercial protein expression and immunisations, fusions and mAb production as well as assay development. Evaluate targets based on results for human and rodent reagents
    - Continuation: share priorities for targets globally to prevent duplication of effort
- US UK Swine Immune Toolkit:
    - Goal is to generate priority reagents for swine immune studies with commercial partner Kingfisher Biotech. Proteins are bought from Kingfisher to make hybridomas so no IP issues
    - Specific objectives: 1) clone and express swine immune cytokines and chemokines, IgE and cell surface CD antigens and receptors 2) prepare mAb panels reactive with swine targets 3) use reagents produced to develop new assays for swine immune markers 4) provide the veterinary community with new commercial reagents and up-to-date information and techniques
- Kingfisher Biotech Expression System:
    - Expressed in yeast because of its advantages (expressed to media, properly folded, post-translationally modified and endotoxin free)
    - Never use tags
    - Steps are taken each year to improve the expression system (e.g. electroporation, zeocin selection etc.) and expression (e.g. codon optimisation, vector modification etc.)
    - A good expression system is key and this expression system should not be undervalued. ALL proteins tested to date are active.
- Phage display for mAbs to more complicated targets, for example, anti-CCR3, anti-CCR9 and anti-CCR10 antibodies
- Development of Immune Reagents for Poultry, Hyun Lillehoj:
    - Reagents for poultry generated from Lillehoj's lab (2006-2015)
    - New NIFA grant for poultry immune reagents (2017-2022): development of cytokines/chemokines and cell surface molecules

- Collaborative Immune Reagent Network for Aquacultured Species (CIRNAS) http://biology.unm.edu/cirnas/ :
    - Collaborative network whose goal is to serve the aquaculture community by advancing the availability of immunological resources and knowledge base for fish health
    - Develop monoclonal and polyclonal antibodies and immune based assays for atlantic salmon, rainbow trout, channel catfish and tilapia

6. International Veterinary Vaccinology Network – Tim Connelley, The Roslin Institute

- The IVVN received a £2.1 million grant in July 2017 from the MRC and BBSRC through the GCRF Networks in Vaccines Research and Development initiative for an initial 4-year period.
- Project led by The Roslin Institute and Pirbright Institute, with 25 partners in low-and-middle income countries (LMICs), the UK and other countries (e.g. RVC, ILRI, Jenner Institute).
- The aim of the IVVN is 'to form a multi-disciplinary and inter-connected vaccinology research and development community to address critical bottlenecks in the design and development of vaccines against diseases prevalent amongst the livestock of LMICs'.
- Funded activities:
    - Networking: 1) Annual meeting; with alternating UK/LMIC partners hosting – first in Nairobi, Kenya in October 2017. Four sessions: Vaccines for zoonotic diseases; Veterinary Vaccine production in Africa; Synthetic biology in vaccine development and Livestock vaccine here and now. £20,000 available for scholarships for researchers from LMIC institutes 2) Workshops; convened and hosted by members to focus on specific topics/issues 3) Website; coordination of Network activities, source of relevant information www.intvetvaccnet.co.uk
    - Catalyst: 1) Pump-priming grants; up to 1yr duration and £100K – collaboration of multiple Network partners e.g. 2) Laboratory exchanges; to facilitate technology/skill transfer up to £10K to pay for transport, subsistence etc.
- The broader context of IVVN's activities:
    - Remit for current funding is primarily to support collaboration between UK and LMIC based scientists, however researchers from other countries are encouraged to join the Network (Canada, Australia and US)
    - Focus on early vaccine R&D challenges and laboratory based sciences, however interaction with epidemiologists and other relevant specialists are encouraged
    - Engagement with industry – hosting a 'vaccine producers' session at first annual meeting in October 2017
    - Advocating for veterinary vaccinology: follow on funding from 'pump-priming' grants; establishing a portfolio of collaborative, innovative projects that can be presented to funders
- Immunological Toolbox:
    - Breeds and species of domestic livestock of specific relevance to LMICS e.g. goats, water buffalo, tilapia, Bos indicus cattle; host workshops to look for species cross-reactivity of antibodies etc.; support relevant pump-priming projects
    - Accessibility of immunological reagents to researchers in resource-poor LMIC institutes e.g. a mechanism for making reagents available to researchers that may otherwise have their activities curtailed by access

    - Website as hub for veterinary vaccinology community: to access information about immunological reagents available (host relevant databases) and for community to register requests for immunological reagents required (collation and prioritisation)
- Other GCRF Vaccine Networks:
    - VALIDATE (Vaccine development for complex Intra-cellular neglected pathogens): http://www.validate-network.org/home
    - BactiVac (bacterial vaccines): http://www.birmingham.ac.uk/research/activity/immunology-immunotherapy/research/bactivac/index.aspx
    - IMPRINT (Immunising pregnant women and infants Network)
    - HIC-vac (Human infection challenge vaccine Network)

7. Fish Immunology/Vaccinology – Kim Thompson, Moredun

- Aquaculture is the most rapidly growing sphere of animal food production; production increased from 34.6 to 73.8 m tonnes from 2001 to 2014.Over 600 species of finfish and shellfish cultured globally
- Aquaculture and disease:
    - Significant losses due to disease - pathogens and parasites estimated to be responsible for 5-7% annual losses in finfish aquaculture. Effective disease control is important.
    - A range of commercially important pathogens: bacteria, viruses (RNA and DNA) and parasites
- Vaccines for aquaculture:
    - Each year ~418 million salmon and ~90 million rainbow trout vaccinated globally
    - Vaccination reduces the need for antibiotics and chemicals; reduces problems with antibiotic resistance; reduces environmental impacts; control significant diseases; increases productivity; save costs for farmer and improves animal welfare.
    - Major area for growth in aquaculture
- History of fish vaccinology:
    - First commercial vaccines for aquaculture licensed in USA in 1970's against enteric redmouth disease, vibriosis, and later furunculosis. Made from formalin-killed cultures grown in vitro and administered by immersion
    - Aeromonas salmonicida proved less immunogenic than Yersinia ruckeri and Vibrio anguillarum and Vibrio ordalii. Therefore mixed with adjuvant and administered by injection to improve its immunogenicity
- Commercial vaccines against over 30 bacterial and viral pathogens are available: many are multivalent and most vaccines are available for salmon and trout.
- Increasing number of vaccines for marine fish and many are under development.
- Potential types of vaccines for aquaculture: formalin inactivated pathogen, live attenuated pathogen, tissue culture, purified macromolecules, recombinant, recombinant vector vaccines, synthetic vaccines, DNA vaccines and VLPs.
- Methods of vaccine delivery: injection (most effective but need to anaesthetise, labour intensive, stressful for the fish); immersion (good for mass vaccination of small fish only, does not work for all vaccines); oral (most suitable for mass vaccination but dosage uncertain and sometimes poor potency, less stressful to the fish, most often used as a booster vaccine)
- Adjuvants:

    - Novel vaccine adjuvants to needed to stimulate cell mediated and mucosal immunity. As external surfaces involved in pathogen entry, understanding mucosal immunity is extremely important
- Immune system: some fish depend more on innate defences, whilst the adaptive response is more developed in other species.
- Monoclonal antibodies are limited and more mAb tools needed to evaluate immune response to assess vaccine efficacy and the host response during vaccine development and disease outbreaks.

8. Group Discussion on Current Activities

- B cell sequencing:
    - NJ stated that single B cell sequencing is a priority.
    - JH has done some single B cell sequencing in cattle using ER tracker and surface Ig. Working with memory B cells: planning to take subsets and RNA seq to look at transcription profiles and see if we can identify good B cell markers.
    - The Pirbright institute are currently putting in business case for Dolomite technology to do single cell RNA sequencing: pair read and have it in containment alongside (in depth) Illumina sequencing
    - SG sorting swine plasma cells but constrained by reagents available therefore is important to get good B cell markers.
    - IM explained the difficulties in identifying plasma cells – a small population of responding B cells is present in cattle after three immunisations with antigen. IM involved in a project at RI that is transforming B cells with *T. annulata* to generate B cell clones.
- T cells/MHC:
    - TC sequencing T cell repertoire from water buffalo, sheep, goats etc. – looking at databases for these - databases hosted by CTLGH
    - TC and JH typing cattle MHC (also some goat)
    - MHC pipeline used for cattle can be used elsewhere
    - Have an almost complete MHC repertoire for Holstein cattle (6-8 haplotypes for 80% of animals)
    - There is also a complete package of imbred lines from Babraham pigs: MHC, structures and tetramers. Would like to SNIP the pigs. Cell subsets (mucosal) making tetramers of these
- Porcine reagents (AS and KM):
    - Collaboration between Berhein-Ingleheim and Austrian research Councils (7 years of funding) to derive mAbs for porcine
    - Generate new porcine antibodies to improve vaccine studies by measuring T and B cell memory
    - mAbs against B cell differentiation markers (CD24 CCR6), homing markers and T cell differentiation and polarisation markers (CD69, CCR6, CXCR5, Bcl6 etc.) is the aim
    - Plan to test cross-reactive mAbs on lymphocytes and transfected cells to see if there is high sequence homology. If no cross reactivity then generate antibodies – generate recombinant fusion protein to immunise mice (target specific) or sort differentiated B and T cells then immunise mice with cell lysates (random)
    - This work begins on 1st October 2017
- Ovine reagents:
    - TM made many ovine reagents but not sure how to market them – what do we do with this reagents?

9. Breakout sessions

- Priorities:
  - General agreement that we need new reagents to understand activation/memory responses within B and T cells (define function). Also important to think about other cells populations e.g. NK, gamma deltas. Developing an assay for correlates of protection – we don't always know what we are targeting
  - Transcriptomics and antibody methods wok in conjunction
  - Other tools: cell lines, animals (e.g. making and maintaining KO)
  - Priorities are generally disease/pathogen/locally driven
  - Managing 'orphan' reagents – need to find out what these are
- Toolbox website and database:
  - Strong support for website and database – linking to UK VVN and IVVN, IUIS, VIC etc.
  - As part of the website, could have a section where people can submit requests. Would have to contain details of how users would access the material etc.
  - Community buy in is key as website needs to be accurate, curated and be tracked in real time
  - Intermediate lists of hybridomas etc until the database is ready - on UK VVN/IVVN? First iteration of the website/database in 6 months.
  - Collate information on all the 'networks' and add links to these onto UK VVN and IVVN websites.
  - Tap into international funders USDA/US vaccine network for example and other international resources. Generate add on funding to ISP core capability
- Communication:
  - Hold a workshop through the UK VVN to identify priorities; potentially before UK VVN Conference in Stirling in January 2018.
  - Teleconferences possibly every 6 months (Joan Lunney happy to lead and take notes) including a representative for each species
  - Appoint a steering committee
  - Webinars
  - Use VetImm list for sharing of information
  - Use IVVN as a means of communication and dissemination of information
  - Positional paper on examples of the importance of immunology to veterinary vaccinology
  - Outreach to ODA/LMICs for awareness of resources and how they would be available

Action Points

- Send minutes to meeting attendees and write up minutes as a publication (CH).
- Find out if a workshop could be held before the UK VVN conference in Stirling (MC)
- Pinpoint key people for the steering committee and collate lists of what everyone has in terms of antibodies and hybridomas (Everyone)
- Identify volunteers to represent each species during teleconferences (Everyone).
- Collate information on all the 'networks' and add links to these onto UK and International VVN websites (CH and MC).
- Send any relevant information on existing reagents to CH to be put on the IVVN website (Everyone)