Immunological Toolbox Workshop

17th January 2018

University of Stirling

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Meeting attendees

Name	Organisation
Simon Graham	The Pirbright Institute
Gary Entrican	Moredun Research Institute
Jeremy Salt	GALVmed
Timothy Connelley	Roslin Institute
Sandra Adams	University of Stirling
Kim Thompson	Moredun Research Institute
Janina Costa	Moredun Research Institute
Anita Jaglarz	Moredun Research Institute
Abdel Aziz Yassin	Vet serum & vaccine research institute
Ryan Waters	The Pirbright Institute
Carly Hamilton	Roslin Institute
Kerstin Mair	University of Veterinary Medicine Vienna
Bryan Charleston	The Pirbright Institute
Niklas Ahlborg	Mabtech
Maria Forlenza	Wageningen University
Alana Dowling	Royal Veterinary College
Marie-Christine Bartens	Royal Veterinary College
Rhona Macdonald	Moredun Research Institute
Tharangani Herath	Harper Adams University
John Hammond	The Pirbright Institute
Shankar Chandra Mandal	University of Stirling
Christopher Payne	University of Stirling
Savitree Ritchuay	University of Stirling
Khalid Shahin	University of Stirling
Travis Beddoe	La Trobe University
Oliver Rosenwasser	BioRad
Madeleine Clark	The Pirbright Institute
Gustavo Ramirez-Paredes	University of Stirling
Michael Bailey	Bristol University
Anna Raper	Roslin Institute

Minutes

John Hammond (JH) presentation

- This workshop is a follow on meeting from the workshop held at The Pirbright Institute (TPI) in August 2017.
- Overview of the workshop in August:
  - Roslin/TPI's bid was successful, securing 5 years of funding to promote veterinary reagents.
  - A toolbox website would be generated which would cover commercially available and lab based tools/reagents.
  - TPI will sequence existing hybridomas (both at TPI, Roslin and elsewhere as required, which will reduce liquid nitrogen storage, secure the future of reagents and create research opportunities with recombinant antibodies.
  - Roslin will generate new reagents after prioritisation of community need.
  - Joan Lunney suggested 6 monthly, species-specific teleconferences, hosted by IUIS.
  - It was decided that a meeting would be held to decide on gaps and priorities prior to the UK Veterinary Vaccinology Network Conference on 18th and 19th Jan 2018.
- Focus of this workshop:
  - What are the barriers preventing us making better vaccines?
  - Identify initial gaps and priorities
  - Identify areas lacking resources for future prioritisation
  - Identify funding opportunities
  - Consider commercialisation and provision of reagents
- Update on activities at TPI:
  - 1FTE to translate current hybridoma stocks into sequences and transfectable gene blocks depending on agreed priorities.
  - Aligned central services unit to be better skilled and aligned with resources to produce reagents.
  - Outside organisations/individuals will be able to access this expertise.
  - Recombinant pipeline has been set up in collaboration with Prof. Ray Owens (OPPF, Oxford). The anticipated benefits of this are that this is cheaper to do in house and allows bespoke manipulation. TPI has already made mouse and cattle hybridomas and heterohybridomas respectively and are confident the pipeline is working.
  - JH presented flow data that illustrated native and recombinant Gr13.1 (NKp46+ antibody made by Tim Connelley) staining of NKp46+ cells in PBMC.
  - To date, 10 antibodies have been made using this pipeline.
  - JH also presented data showing data on markers for cell subsets – TPI have carried out ER tracker experiments in cattle to identify bovine plasma cells.
- Toolbox website progress:
  - Leveraged money from BBSRC GCRF Tools and Resources fund.
  - Basic design of a simple database backend that allows complex searches from the front end (likely based on antigen/protein).
  - Allows multiple curators to add/edit content with a developer site and a live site.
  - Species-specific volunteers have been identified: Jayne Hope (ruminants); Joan Lunney (pigs); avian and aquaculture representatives still to be identified – preferably out with the UK/USA.

    - This database will not just be about antibodies, idea is to go from genome to antibody and include PCR, isoforms, assays and expressions. There will also be reagents for pathogens as well as host.
    - There is a capacity to have star rating system to 'rate' reagents.
    - There will be an access agreement to use the database through a MTA model.
    - Previous toolbox data is of a high quality and therefore will be the first data to be translated into the database.
    - First preliminary design of the database will be disseminated for comment.
- Update on activities at Roslin:
    - Roslin's main role is to make antibodies – antibody to CD107a was shown as an example of taking a sequence through to the development of successful assays.
    - Roslin reagent request form currently being adapted and any activities will be costed on an individual basis.
    - Steering committee for the Immunological Toolbox have been identified: Eleanor Riley, John Hammond, Jayne Hope, Simon Graham, Mark Stevens and Gary Entrican. Aquaculture representative still to be identified.
- Areas to discuss identified from the August workshop:
    - B cell markers
    - T cell markers
    - Mucosal immune reagents
    - Immune variation
    - Zoonoses

General discussion

Tim Connelley: how many hybridomas will TPI be able to sequence?
JH: Able to sequence hundreds of hybridomas, it is the scale up after this that is costly.

Mick Bailey: how far back are you going to generate sequences and do we know if the hybridomas have changed by somatic hypermutation? E.g. pig CD4 antibody loses affinity periods of time
JH: Go for earliest hybridoma and test, hence why recombinant pipeline is useful as there will be no maturation.

Gary Entrican: what are your yields?
JH: Amounts comparable to hybridoma (or slightly less); they are transient transfections at the moment and last around 4 days.

Bryan Charleston: do we aim to sequence other antibodies e.g. unknown/orphan antibodies?
Gary Entrican: this is an aim of IUIS: to identify and rescue these reagents.
JH: we would hope as part of the toolbox that we could go to Institutes and ask to sequence their known and unknown reagents, especially if there is a danger that they will be lost.
Tim Connelley: could take DNA and a small amount of supernatant from these unknown hybridomas and store in a central area so these reagents are not lost over time; actively go out into the community and request these reagents.
Bryan Charleston: could go to funders and ask for support to preserve these antibodies.
JH: aim is to sequence as many hybridomas as possible and then the steps going forward from this can be demand-led.
Bryan Charleston: could make a list of around 50 priority reagents that we do not want to lose.

Breakout groups

Attendees were divided into three breakout groups, led by Gary Entrican, Simon Graham and Tim Connelley. The following questions were discussed:

- What are the barriers preventing us making better vaccines?
- Identify initial gaps and priorities
- Identify areas lacking resources for future prioritisation
- Identify funding opportunities
- Consider commercialisation and provision of reagents

Group led by Gary Entrican:

- What are the barriers preventing us making better vaccines? Choice of analyte e.g. blood over tissue; need to understand correlates of protection (COP).
- Identify initial gaps and priorities: single cell analysis e.g. for T cell subsets; replacing polyclonals with monoclonals e.g. in some ruminant ELISAs, one of the antibody pairs is polyclonal – need to build on existing reagents; homing receptors.
- Identify funding opportunities: identify companies to put funds into the sustainability of the website/database.
- The group also discussed the importance of community buy-in e.g. the need for sufficient data on the website/database before launching the toolbox to the community.

Group led by Simon Graham:

- What are the barriers preventing us making better vaccines? Limited availability/expertise in adjuvants; most of these in private sector; mucosal adjuvants; need systems to be able to identify vaccine candidate antigens, therefore the need for tools.
- Identify initial gaps and priorities: T cell subsets, B cell subsets; better understanding of the functional properties of antibody classes and subclasses – currently use neutralising antibody as a COP; need to publicise what we are doing to avoid duplication and where cross reactivity does or does not exist.
- Identify areas lacking resources for future prioritisation: must do a gap analysis on what we reagents exist before deciding on prioritisation.
- Funding: BBSRC, EU, BMGF are all interested in veterinary vaccine reagent development.

Group led by Tim Connelley:

- What are the barriers preventing us making better vaccines? COP and immunity.
- Identify initial gaps and priorities: a series of harmonised protocols and guidelines that are consistent and allow comparison across studies. Ruminants: markers for T cell subsets and activation markers, tools to study adjuvants e.g. TLR antibodies. Pigs: lack of reliable B cell markers. Fish: mucosal immunity, salmon have the most immunological tools so would be beneficial to see what is available for salmon first.

Summary

- Consensus that the priorities are B and T cell markers, across all species including fish.
- Group agreed it is key that a gap analysis is carried out first, then need to characterise existing reagents before identifying the priorities going forward.