ANIMAL IMMUNIZATION FOR ANTIBODY PRODUCTION

OVERLOOKED ..... 

...... AND OBSOLETE 

Dr Alison Gray
WHY SHOULD WE BE CONCERNED ABOUT ANIMAL BASED ANTIBODY PRODUCTION?

• Animal based antibody production requires immunization
• Includes hybridoma (monoclonal), polyclonal and (immunized) recombinant methods
• Subsequently appear as *in vitro* tests

• Welfare issues: 100’s of thousands of animals, mistreatment and undesirable procedure

• 3Rs: Replacement methods exist

• A myriad of misconceptions and unawareness about antibody production

.....Lack of enforcement of Directive 2010/63/EU on the protection of animals used for scientific purposes
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WELFARE ISSUES: STATISTICS

• Multibillion dollar global industry

• Only two Member State countries published this information (up to 2013 - now ceased)

<table>
<thead>
<tr>
<th>Actual animal numbers reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK 2013</td>
</tr>
<tr>
<td>The Netherlands</td>
</tr>
</tbody>
</table>

ESTIMATES

• Not taking into account academic use ↑, import ↓, repeat production ↑ or rebranding ↓

EU: from 833 thousand to 1.25 million animals per year

World wide: 5 fold increase and limitless new possibilities.....
WELFARE ISSUES: MISTREATMENT, UNDESIRABLE PROCEDURE

Antibody Making and Animal Welfare

The US Department of Agriculture and to cease selling research antibodies, which are used to identify and treat diseases in animals.

Assessment of side effects induced by injection of different adjuvant/antigen combinations in rabbits and mice

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National Institute of Public Health and Environmental Health, The Netherlands, 2Department of Animal Sciences and Biotechnology, University of Agricultural Sciences, The Netherlands, 3Department of Veterinary Medicine, University of Agricultural Sciences, The Netherlands

Summary

We evaluated the side effects induced by injection of different adjuvant/antigen combinations in rabbits and mice. The side effects included swelling, erythema, and pain at the injection site, as well as fever, weight loss, and decreased appetite. The side effects were more severe in rabbits than in mice, and the severity of the side effects was dependent on the adjuvant/antigen combination used.

Characterization and mapping of monoclonal antibodies against the Sleeping disease virus, an aquatic alphavirus

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2Laboratoire d'études et de recherches en pathologie des poissons, AgroParisTech, 29280 Plouzané, France

Sleeping disease virus (SDV) is an emerging pathogen in salmonid aquaculture, the impact of which is underestimated due to the lack of efficient diagnostic tools. To better characterize this new aquatic alphavirus and to make molecular tools available, a panel of monoclonal antibodies (mAbs) was generated.

E1 glycoprotein were selected. Some of these mAbs were further produced as ascites fluids. Because the first attempt to immobilize mice against nsP2, nsP4 and E2 led to an anaphylactic shock of mice, immunizations were repeated with a new batch of the recombinant protein contaminated with bacterial proteins. In the final batch, the time of induction of the anaphylaxis was increased.
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AFAs : ANIMAL FRIENDLY AFFINITY REAGENTS

• Antibodies

• Non-antibody scaffolds or mimetics

  • Instead of a constant supply of animals, we create a library of AFAs in a tube: equivalent to a life time’s supply of animals

  • All AFAs formats have the characteristic of having an affinity for / binding to a unique target / antigen

  • Produced in micro-organisms

  • No animal use, including immunization

  • More than 40 AFA solutions available, various stages of development, some commercialized
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### MISCONCEPTIONS / UNAWARENESS

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>TARGET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostics and health</td>
<td>genetic testing, markers of infectious, chronic and sexually-transmitted disease, oncology</td>
</tr>
<tr>
<td>Therapeutics</td>
<td>rheumatoid arthritis, multiple sclerosis, Alzheimer’s disease, Ebola and different types of cancers</td>
</tr>
<tr>
<td>Substances of abuse testing</td>
<td>MDMA, methadone, digoxin, cocaine, caffeine, ketamine, marijuana, methamphetamine and barbiturates</td>
</tr>
<tr>
<td>Anti doping programs for competitive sport</td>
<td>Erythropoietin, human growth hormone and human chorionic gonadotrophin</td>
</tr>
<tr>
<td>Fertility, ovulation and pregnancy testing</td>
<td>human chorionic gonadotrophin, luteinizing hormone, progesterone</td>
</tr>
<tr>
<td>Food safety and environmental contaminants in air, water, soil, packaging, processing / cooking and naturally occurring</td>
<td>Radionuclides, polycyclic aromatic hydrocarbons, arsenic, heavy metals, pesticides, natural toxins, lubricants, cleaning agents, carcinogens, Processing contaminants, hormones, veterinary drugs, allergens</td>
</tr>
</tbody>
</table>
LACK OF AWARENESS AND THEREFORE ENFORCEMENT AT MEMBER STATE / LEC LEVEL
"...Antibody production without the use of animals has not been shown to produce the range of antibody specificities and affinities required in this project....." (200 mice and 30 rats over 5 years)

"...whilst antibodies can be made without immunisation, antibodies that are generated as a result of immunisation have a higher specificity and higher affinity....." (4000 mice and 500 rabbits over 5 years)

"...although antibodies can be synthesized in vitro, these are of low affinity and are unable to perform the highly demanding requirements we need from our antibodies....." (900 mice)

"...Antibodies are produced in the laboratory animals. There are no convenient alternatives for the production of antibodies. A known procedure of production of antibodies in bacterial cells still requires the use of laboratory animals in order to obtain the gene that encodes this antibody from spleens of the animal. This technique is too labour intensive, prone to errors, time consuming and costly. ....."

"... Mouse is by far the best model for producing antibodies. Alternative approaches are being developed, but none of them has become as reliable as the mouse model. We have tried one of the best alternatives, but could not make any antibody....."

"... Animals produce antibodies with the highest affinity. New technologies are available which produce antibodies in-vitro, however, these are low affinity antibodies and although we have tried to use them in our work, none have been successful....."
Antibodies are produced by a living immune system involving activation of specific cells in response to antigens. This means that laboratory animals of excellent health status and known genetic background are required...

We are investigating more modern methods of producing antibodies in the laboratory, using bacteria, but research in these areas is still at an early stage and is not yet ready for use.

There are still applications for which animal derived antibodies are essential, e.g. antivenom production, where antibodies to all of the epitopes of the toxins and peptides are required: 10s to 100s...

Donkeys need to be used to produce the secondary reagents...

Ig has an on-going research programme with partners to develop methods for... phage display antibodies through purification of peripheral blood lymphocytes from hyper-immunised sheep... (with) the potential to reduce...

only the whole animal immune response is adequate... to provide cells for in-vitro selection and antibody production
WHY IS DIRECTIVE 2010/63/EU NOT BEING ENFORCED?

• No EU led expert guidance / recommendation report available

• Project license applications not adequately interrogated by LECs – massive generic applications granted: 5 year duration, 100’s-1000’s animals

• The assumption that the technology can not be ‘ready’ if scientists are not using it

• Lack of expertise / motivation by users of traditional methods and a propensity toward familiar methods due to economics /time factor / implementation

• Accessibility to commercial Abs limited (but improving) –focus on pharma, high prices, royalties, few catalogue AFAs.

• Scientific misconceptions block uptake of AFAs including that technology still in its infancy, poor affinity, immunization required etc ....

• Criticisms come from scientists who are expert in animal derived antibody production, NOT phage display
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ANIMAL FRIENDLY AFFINITY REAGENTS (AFAS)

Current Status

• AFAs do not necessitate the use of animals at any stage of production.

• Many AFAs are mature and available to replace ALL animal use:
  
  • As a method to adopt in the lab
  
  • As a commercialised reagent from a supplier (catalogue or custom)

• AFAs are structurally equivalent, wide range of applications, equal or greater specificity and affinity to a huge repertoire of antigens and offer greater control over their properties, generation time and cost

• Even the production method is similar: from combinatorial diversification to selection

• This justifies the replacement of animal immunization methods in accordance with Directive 2010/63/EU.
AFABILITY is striving to replace animal derived antibody (ADA) production methods with Animal Friendly Affinity-reagents (AFAs) that do not require the use of animals, thereby significantly reducing the numbers and suffering of animals in the biomedical sciences.

AFABILITY challenges the enforcement of Directive 2010/63/EU, improves accessibility of replacement methods and creates awareness of the use of animal derived antibody production methods by all scientific disciplines.
**ACTIVITY HIGHLIGHTS**

- **AFABILITY (2013)** publicly launched in July 2016
- Website [www.afability.com](http://www.afability.com), facebook page and LinkedIn discussion forum
- Papers released July 2016 : open access
- Winner of Beagle Freedom Science prize 2016
- Winner of Lush public awareness prize 2017
- Ongoing program of antibody (AFA) development at University Nottingham
- EU/ECVAM invited expert and advisor, member of ECVAM stakeholder forum (ESTAF)
- Collaborations with FRAME and others
RECOMMENDATIONS

• Antibody production methods that use animal immunization should be replaced in EU Member States

• Manufacturers outside the European Union should be required to adhere to European standards to qualify for import to Member States

• An expert working group should be established to set up a roadmap for replacement.

• Programs should be implemented to ensure that animal-friendly antibody producers are fully supported

• EU Statistics on the number of animals used for experimental and other scientific purposes should include data on the use of animals for antibody production as an independent category

• These actions should be reinforced through international cooperation and national agencies that can execute government regulation and prevent outsourcing to regions where animal welfare is less well regulated.
FOCUS AREAS

• Commitment by all relevant EU authorities (medicines, IVD, food and environment, microbiology, agriculture, pharma, cosmetics, chemicals...), including regulation of imported commercial products

• Prior to a project authorization for animal derived antibody production, clear guidelines must be applied:
  
  ➢ Project license applicants should demonstrate steps taken to produce AFAs
  
  ➢ Independent centres of excellence should be part of the application process
  
  ➢ Where animal derived Ab production should be last resort and demonstrated on case by case basis

• Working toward cosmetics style ban on EU production and import
EU SCIENCE HUB
The European Commission's science and knowledge service

European Commission > EU Science Hub > Science update > EURL ECVAM renews its Scientific Advisory Committee

News & events

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EURL ECVAM renews its Scientific Advisory Committee

New ESAC will meet in December to review affinity reagents produced using animal-free technologies and methods for skin sensitisation.

The JRC's EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) is delighted to announce the renewal of the EURL ECVAM Scientific Advisory Committee (ESAC) whose members were formally appointed on the 16th of April 2018.

Details of the new ESAC including its members can be found on the Register of Commission Expert groups.

Renewed every three years, ESAC is composed of external scientists who are appointed on the basis of their scientific expertise and who act independently in the public interest. ESAC advises EURL ECVAM on scientific and technical issues related to the protection of animals used for scientific purposes. The new ESAC will meet in Ispra, Italy, on 3-5 December 2018 at the European Commission's Joint Research Centre and preparations are currently underway to mandate ESAC to deliver opinions on the scientific validity of alternative methods to assess chemicals for their skin sensitisation potential, and antibodies and new generation affinity reagents produced using animal-free technologies.
GREAT NEWS FOR THE TECHNOLOGY THAT HAS REVOLUTIONIZED ANTIBODY PRODUCTION WITHOUT THE NEED FOR ANIMALS!

The 2018 Chemistry Laureates

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2018 with one half to Frances H. Arnold "for the directed evolution of enzymes" and the other half jointly to George P. Smith and Sir Gregory P. Winter "for the phage display of peptides and antibodies".

Read the press release
Thank you!

With thanks and gratitude to the supporters of the AFABILITY mission including the Lush prize team, Carl Borrebaeck, Sachdev Sidhu, Coenraad Hendriksen and Charu Chandrasekera, to my colleagues Kevin Gough, Ben Maddison and Jon Owen at the School of Veterinary Sciences, University of Nottingham, the staff and the trustees at FRAME, the Beagle freedom prize and LFVALTF