

Refinement in vaccine research at CAMR

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Summary

This manuscript reports current work and future plans at CAMR to refine, reduce and replace animal experimentation in the course of vaccine research. Refinement should not simply be restricted to reducing the suffering of animals used in animal experimentation, but it should also include development of *in vitro* assays that more closely reflect the host–pathogen interaction and clinical reality. We are therefore implementing two approaches to achieve this objective: refinement of necessary *in vivo* work; and development and analysis of alternative *in vitro* immunoassays and bioassays. We are seeking collaborations to expand our expertise in fields such as flow cytometry, and are looking for partners to develop and test new potency tests that are more relevant surrogate markers of protection than existing *in vivo* tests.

In vivo refinement

In almost all vaccine research studies, potency testing has involved experimental challenge of animals with the agents of infectious human disease in which the endpoint was death. In common with other institutes and according to UK Home Office regulations, we have introduced and implemented endpoints that are based on observed clinical signs in mice. The condition of the fur and behavioural characteristics have been found to be suitable endpoints in challenge studies with the organisms *Streptococcus pneumoniae* and *Neisseria meningitidis*. Where an animal is found to be displaying the appropriate combination of clinical signs of distress, it is humanely killed thus eliminating unnecessary suffering in these types of experiment. This has led to significant reduction in the suffering of animals used in research aimed at finding new vaccines against human infectious diseases at CAMR. We will continue to assess these types of humane endpoints in vaccine and therapeutic research at CAMR and implement them wherever possible.

In vitro alternatives

Whilst we find that there is currently no scientific alternative to *in vivo* potency tests in primary vaccine research, there has been considerable effort invested into finding more appropriate *in vitro* alternatives that do not require experimental infection of animals. We started assessing *in vitro* alternatives to experimental *in vivo* challenge assays several years ago (Robinson & Funnell 1992) when we were developing a new acellular *Bordetella pertussis* (whooping cough) vaccine.

During this development period, with the assistance of a grant from the UK Home Office, we assessed the applicability of tissue culture adhesion inhibition assays. Although we found that *Bordetella pertussis* adhered well to tissue culture cell lines, these assays tended to introduce bias by being very sensitive to the effects of certain virulence components (filamentous haemagglutinin) and yet less sensitive to others (fimbriae). This was due to the fact that these rather simple clonal cell cultures were not closely modelling all components of the human respiratory tract (Funnell & Robinson 1993,

Funnell 1995). This highlights the importance of ensuring that model systems are not over-simplified.

Humoral immune responses are commonly assessed by enzyme linked immunosorbent assays (ELISA) and, subsequently, a great deal of emphasis is placed on ELISA titres of serum antibodies in vaccine trials. The biological significance or protective potency of an antibody response is not, however, always proportional to ELISA titre. This is because some types of antibody are more effective than others in initiating complement cascades and cellular mechanisms of antimicrobial host defences.

We have proposed that a number of immunological and functional assays, carefully selected and developed to represent most arms of the immune system's response to vaccination, could represent a valuable *in vitro* alternative to *in vivo* potency testing. We mean to develop several bioassays that can be easily standardized and set up throughout a network of interested laboratories. The bioassays under consideration are listed below.

Bacterial antibody agglutination

Antibody-mediated agglutination is often part of an effective adaptive immune response to bacterial infection. In fact, agglutination was found to be a good correlate of protection in early clinical trials of whole cell pertussis vaccines (MRC 1958, Cameron 1988). Cross-linkage of bacteria is mediated by bispecific antibody (agglutinins) that effectively immobilize free bacteria and may also initiate other cascades of the immune response. Assays for the detection of agglutinins are simple to perform but, classically, have been difficult to standardize and automate. Our research indicates that these problems may be overcome with the assistance of new developments in detection and automation. We have commercial partners but are looking to extend our research to include agglutination in a coordinated refinement research programme. Agglutination is an important aspect of the immune response that is currently under-rated due to the difficulties in large-scale assessment but

this is likely to change as the technology is developed.

Bactericidal assays

Bactericidal assays directly assess the ability of serum to kill target microorganisms. As with agglutination, bactericidal antibody assessment suffers from difficulties in reproducibility, chiefly due to technical difficulties in analysis of results and lack of standardized reagents. We are considering utilizing flow cytometry in combination with viability staining for automated bactericidal assay analysis. In combination with agglutination assays, this would greatly increase the data available to vaccine researchers on the quality of immune response mounted in human volunteers to experimental vaccines.

Opsonophagocytosis

Some types of antibody (opsonins), when bound to their target, provide a signal to phagocytic cells to engulf the coated microorganism or target cell. This antibody-mediated enhancement of phagocytosis is termed opsonophagocytosis. Classically, opsonophagocytosis assays have also suffered from technical difficulties in both reagent control and methodology. Standard methods use peripheral blood cells removed from volunteers mixed with test sera and bacteria grown in the laboratory. These reagents inevitably introduce variability due to differences between individual volunteers and the variability of batch cultures of some types of microorganism.

We have pioneered new techniques to ease these difficulties with the use of modern technical advances in flow cytometry and immortalized cell culture (Leech *et al.* 1998). Immortalized cell lines offer a standard reagent that can be easily distributed between countries from a central resource such as the European Collection of Animal Cell Cultures (at CAMR). Other reagents such as vaccine-coated fluorescent beads could also be manufactured and distributed to many laboratories in different countries, hence avoiding the variability of bacterial

growths used in the classic assays (Funnell *et al.* 1998a). This type of integrated and standardized approach using cell lines and coated particles still needs development before it can be used to replace or reduce animal infection studies currently used in potency testing throughout Europe and the rest of the world.

Cellular immunity

The importance of cellular immunity to vaccines is widely acknowledged, but most assays that assess this arm of the immune response are difficult to perform and standardize. This is another area which could be developed and used in combination with those listed above to complete a panel of assays that may in future reduce or replace animal experimentation.

The discriminatory power of modern flow cytometers has unleashed the ability to carry out bioassays for agglutination, bactericidal activity, opsonophagocytosis and cellular immunity in small volume samples all on the same machine. There is now a remarkable opportunity for groups of scientists to develop a standard set of immunoassays that could measure several important surrogate markers of protection in the human host for use in potency testing. Additional benefits of these types of assay would be the relatively easy technology transfer required to standardize techniques between laboratories using the same equipment and reagents and this is especially true for opsonophagocytosis.

A prerequisite for research and development in these types of assays is the availability of appropriate human clinical material. Recruiting human patients for inclusion in refinement research studies is likely to be more difficult than other types of clinical study. We have experience in initiating the required network for obtaining such samples. CAMR has recently initiated a collaborative study with the Public Health Laboratory Service (PHLS) funded by the UK Department of Health on pneumococcal vaccine research (Funnell *et al.* 1998b) in which acute and convalescent sera are obtained from culture-confirmed cases of invasive pneumococcal infection presenting

at nine regional hospitals. This has required considerable effort to set up from both logistic and ethical perspectives. This type of clinical networking will be required for the elucidation of important new surrogate markers of protection and will be an essential part of clinically relevant refinement research.

Conclusion

It is hoped that this review will help to launch new collaborations between European laboratories to consolidate the project outlined above and to increase the chances of funding from bodies like the European Fifth Framework or commercial partners. We believe that clinically relevant infectious disease refinement research will lead to the development of a panel of assays that predict the potency of vaccines with two major benefits over existing methods: a greatly reduced requirement for animal usage and suffering; and less extrapolation leading to increased accuracy.

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