
MEETING REPORT

Highlights of the Society for Medicines Research Symposium held March 6, 2008, at the National Heart and Lung Institute, London, United Kingdom.

Biotherapeutics

by Katherine Brown
and David Fox

The opening presentation was given by Dr. Colin Longstaff of the National Institute for Biological Standards and Control (NIBSC), providing the audience with an overview of the role played by this public body, funded by the U.K. government, in the development and monitoring of biotherapeutics. In particular, NIBSC develops standards and test methods to ensure the quality and safety of biological products used in medicine. NIBSC is a global generator and repository for WHO International Standards including many blood products, vaccines and recombinant drug products of all classes. Major projects include studies on variant Creutzfeld-Jacob disease and reagents for influenza and HIV/AIDS, and they also house the U.K. Stem Cell bank. They act at the interface between leading edge scientific research, product development, regulation and policy. At the national level, they work closely with the Medicines and Healthcare products Regulatory Agency (MHRA) in the licensing and batch release of biological products and the testing of problem batches of drugs. At the European level they work with the European

Summary

This Society for Medicines Research symposium was held on March 6, 2008, at the National Heart and Lung Institute, London, U.K. The meeting, organized by Kate Brown and David Fox reviewed the state-of-the-art in biotherapeutics. This is an area that is acquiring increasing importance in drug discovery and development portfolios, offering new and alternative ways of treating a wide range of conditions as well as opening up new and exciting areas of science and technology. Presentations ranged from the therapeutic potential of monoclonal and polyclonal antibodies, vaccines and oligonucleotides, through to the scientific basis of the emerging area of RNA interference. In addition, some of the technical aspects of delivering novel therapies and ensuring their quality were also discussed. © 2008 Prous Science, S.A.U. or its licensors. All rights reserved.

Medicines Agency (EMA) providing experts to represent the U.K. on a number of working parties. NIBSC has strong links with the European Directorate for the Quality of Medicines as the official medicines control laboratory for biologicals for the U.K. and also representing the U.K. on specialist groups setting policy on drug testing and drafting monographs for the European Pharmacopoeia. Further afield, they also interface with the FDA, the Center for Biologics Evaluation and Research and the WHO.

Dr. Longstaff went on to describe issues regarding the standardization of biological products, pointing out that their activity is currently not measured by physicochemical means unlike small-molecule drugs. Recent observations with **streptokinase**, a plas-

minogen-binding protein from *Streptococcus* bacteria used as thrombolytic treatment for myocardial infarction, were used to highlight problems arising from the production of biosimilars. Small changes in the N-terminal sequence were found to lead to major differences in potency that were dependent on assay format. The format used will be set down in the drug's license and may refer to the method in the local pharmacopoeia. Thus, it is possible that the potency of a product could change three-fold depending on the test method.

NIBSC was also called in to assist the MHRA with the investigation into the Northwick Park clinical trial of **TGN-1412**, a humanized antibody for

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the treatment of chronic lymphocytic leukemia and rheumatoid arthritis which was withdrawn from human clinical trials because of life-threatening indications. NIBSC received sample sets of the antibody from the clinical trial along with reference material. They concluded that there was nothing wrong with the material, that the protein concentration was as expected and there was no evidence of abnormal toxicity or contamination. Hence, they could conclude that the drug had met the release specification. Earlier safety studies by the company developing the antibody had failed to pick up any potential dangers for a number of reasons. Significantly, animal models, including primate immune cells did not respond in the same way as human immune cells explaining why preclinical tests in primates did not predict the adverse events in humans. Furthermore, *in vitro* studies with human cells were able to induce a "cytokine storm" but required correct presentation of the antibody in an immobilized form. It was also observed that there was a complicated relationship between dose and stimulation and that the apparent low dose given in the phase I trial was in fact close to the dose that might be predicted to induce the maximum effect *in vivo*. These findings were incorporated into the Duff report¹ and recommendations regarding testing and the future conduct of clinical trials of potentially dangerous new products may be found in EMEA/CHMP/SWP/28367/2007. In terms of future trends, Dr. Longstaff suggested that we will witness an increase in the use of monoclonal antibodies for the treatment of cancer. His take-home message was that standardization is important at an early stage of biological product development in order to reproducibly measure activities and that this standardization must be done well in advance of planning a licensing application. The NIBSC staff are always happy to engage in discussion with R&D organizations to share their expertise and help identify and resolve any issues ahead of discussions with international regulatory bodies.

Oligonucleotides

Dr. Eugen Uhlmann (Coley Pharmaceutical Group) provided the audience with a full account of the therapeutic potential of oligonucleotides, starting with a historical perspective on the evolution of the field and then focusing on oligodeoxynucleotides (ODNs), in particular those targeting immune stimulatory Toll-like receptors (TLRs).

There are three main types of oligonucleotide-based therapeutics:

- Triplex-forming oligonucleotides that inhibit transcription (no clinical development candidates yet);
- Antisense/ribozymes/small interfering (siRNA) that target messenger RNA and inhibit translation;
- Oligonucleotides, including aptamers, immunostimulatory RNA, CpG ODN that target proteins, and decoy ODNs (double-stranded DNA molecules that bind to transcription factors and block transactivation).

The antisense approach involves the binding of oligonucleotides to RNA resulting in duplex molecules that are subsequently cleaved by the ribonuclease degrading enzyme RNase H. This enzyme is structurally related to Ago2 which cleaves target duplexes in siRNA-based approaches. Chemically modified antisense RNA (which is more stable) does not always recruit RNase H effectively and alternative RNases may be used instead. For example, by incorporating 2'5'(A)₄, the duplex will recruit RNase L, whereas inclusion of an external guide sequence (which introduces secondary structure into the antisense molecule) recruits RNase P.

Ribozymes, on the other hand, are RNA molecules with intrinsic catalytic activity. Some have been tested in the clinic, but efficacy is generally low. The major challenge is the need to balance sufficient binding affinity to achieve cleavage with a low enough dissociation constant to allow recycling of the ribozyme.

Dr. Uhlmann highlighted the range of existing development programs involving oligonucleotides, including **mipomersen sodium** (ISIS Pharmaceuticals), **oblimersen sodium** (Genta) and **TPI-ASM-8** (Topigen) (Table I).

In addition, there are two products that have been approved, both delivered by intravitreal injection:

- **Fomivirsen sodium** (ISIS-2922; *Vitravene*[®]; ISIS Pharmaceuticals) antisense for cytomegalovirus retinitis. Launched 1998;
- **Pegaptanib sodium** (*Macugen*[®]; Pfizer and OSI EyeTech) aptamer, vascular endothelial growth factor (VEGF) antagonist for macular degeneration. Launched 2005.

There are significant challenges associated with the development of oligonucleotide agents. First, they are very susceptible to nuclease cleavage; 3'-exonucleases cleave the 3' hydroxyl on DNA-based oligonucleotides and RNA molecules can also be cleaved by 2'-exonucleases on the 2' hydroxyl rendering them less stable than their DNA counterparts. One strategy to overcome instability is to remove the hydroxyl group, but for immunostimulatory ODNs, it is required for activity. Chemical modification can increase stability and sometimes can enhance binding. Such modifications can include phosphorothioates, sugar replacements, locked nucleic acids, introduction of 2'-fluoro or 2'-*O*-methyl groups, base modifications (e.g., 7-deaza, 2,6-diamino) and lipophilic end modifications (e.g., conjugating to cholesterol). Additional technical challenges include cell uptake/delivery since the site of action is the cytoplasm (siRNA and antisense) or the endosome (immunostimulatory ODNs). Aptamers, however, target extracellular proteins and cell penetration is not an issue. Once the oligonucleotide is in the right compartment, it still needs to access the target and this can be difficult if the agent has low binding affinity or if the RNA target has a high degree of sec-

TABLE I. ONGOING OLIGONUCLEOTIDE DEVELOPMENT PROGRAMS

AGENT	MECHANISM	COMPANY	PHASE	CONDITION
Mipomersen sodium (ISIS-301012)	Apolipoprotein B expression inhibitor	ISIS Pharmaceuticals	Phase III	Hypercholesterolemia
Oblimersen sodium (Genasense)	Bcl2 expression inhibitor	Genta	Phase III	Melanoma
TPI-ASM-8	Antisense therapy	Topigen	Phase I/II	Asthma
ALN-RSV01	siRNA	Alnylam Pharmaceuticals	Phase II	Respiratory syncytial virus (RSV) infection
ARC-1779	Aptamer targeting van Willebrand factor	Archemix	Phase II	Thrombotic thrombocytopenic purpura; percutaneous coronary intervention
Agatolimod sodium (PF-3512676)	Toll-like receptor 9 agonist	Coley Pharmaceutical, Pfizer	Phase II	Cancer
HYB-2055 (IMO-2055)	Toll-like receptor 9 agonist	Idera	Phase II	Cancer
AVT-01	Decoy oligodeoxynucleotide	Avontec, AnGes	Phase II	Asthma, psoriasis

ondary structure. Beyond this, there are potential issues to consider including specificity, toxicity, pharmacokinetics, manufacturing and analysis.

The TLRs are a large family of pattern recognition receptors comprising at least 12 different TLR subtypes (1–12). Some TLRs are present on the cell surface (TLR1, -2, -4, -5 and -6) and the remainder are endosomal (TLR3, -7, -8 and -9). To date, only the TLR3 crystal structure has been solved, but modeling predicts that TLR9 has a very similar structure. Among the best-investigated immune stimulators in this field are CpG ODNs. In particular, unmethylated CpG ODNs mimic bacterial DNA as opposed to vertebrate DNA (where CpGs are less common and are methylated). Following administration, CpGs that target TLR9 are taken up by an endocytic pathway that delivers them to the endosome which is their site of action through two complementary pathways. Activation of TLR9 causes direct activation of B cells causing them to proliferate. In addition, agonism of TLR9 expressed in plasmacytoid dendritic cells (pDCs) produces interferon which activates monocytes and natural killer cells. Activation of pDCs also leads to T cells being primed.

The team at Coley have described three types of CpG ODNs:

- A class: associated with high interferon production and little B-cell proliferation. G-tetrads at their ends cause them to multimerize.
- B class: associated with low interferon production and strong B-cell proliferation. Structurally these are linear phosphorothioates and have no secondary structure.
- C class: high interferon production and strong B-cell proliferation. Their structure includes a 3'-palindrome that causes them to form dimers.

Structure–activity relationship (SAR) data for CpGs can be generated in an analogous way to small molecules,² although sequence-dependent differences in activation of human compared to mouse TLR9 can lead to confusion and misinterpretation. **Agatolimod sodium** (PF-3512676) is an example of a B class CpG ODN that has demonstrated increased efficacy over paclitaxel alone in two metastatic cancer models (Lewis lung carcinoma and Renca renal cell cancer model). There is no effect in nude mice, confirming that for this agent, efficacy is dependent on T cells. CpGs have been studied in a range of diseases including infectious diseases, cancer and allergy/asthma where they

re-direct the immune response from Th2 to Th1.

RNA interference

A comprehensive overview of the emerging area of RNA interference was given by Prof. Dr. Achim Aigner (Dept. Pharmacology and Toxicology, Philipps-University, Marburg, Germany). In less than the 10 years since the discovery of RNA interference (RNAi), novel RNAi therapeutics have already made it to clinical trials. These RNAi effector molecules are double-stranded RNA oligonucleotides, typically of between 21–25 base pairs, with sequences specific to the target of interest. RNAi has the potential to silence any gene/gene product and in so doing has opened the door to interventions for targets previously considered undruggable.

As with any drug discovery process there are clearly challenges as well as new opportunities. These challenges—specificity, safety and delivery—are familiar to those involved in conventional small-molecule approaches. RNAi molecules with complete sequence homology to the target of interest may drive off-target effects in genes with partial homology. Certain sequence motifs are known to trigger the host cell immune response and larger oligonucleotides also have a propensity for immune

stimulation. A mouse study demonstrated the fatal consequences of oversaturation by RNAi precursors in the microRNA system.³ Probably the greatest ongoing challenge for RNAi therapeutics is developing clinically appropriate delivery vehicles that ensure sustained delivery to the required site of action. To date, there has been a high propensity for proof-of-principle studies to focus on liver-based therapies, where the siRNA is delivered directly to the liver.⁴ A number of strategies for formulation to support clinical delivery were highlighted including protection by cholesterol or aptamers, encapsulation by liposomes or polyethylenimine and antibody conjugation.

Prof. Aigner's review also highlighted similarities with conventional drug discovery programs. Design processes are actively employed to drive identification of molecules with improved efficacy; these combine bioinformatic approaches with the ever-necessary empirical testing.

There are, however, clear distinctions from small-molecule approaches. Multiple biological processes are involved in generating the end-stage inhibitory effect, reported as simple potency of an RNAi molecule. Defining the SARs, conventionally so important in influencing design strategies, will clearly be more complex than molecule potency reflecting target sequence k_d . There is a very limited knowledge base to support predictions of *in vivo* efficacy from *in vitro* data. Different delivery vehicles required for the *in vitro/in vivo* settings may invoke differing immune response and hence differing effects. Species differences in biological processes will also need to be understood. The potential to incorporate multiple target RNAi molecules in a single delivery vector may, however, offer new opportunities such as combating the resistance liabilities of existing antiviral therapeutics.

Intellectual property associated with RNAi methodology is complex

and is still largely unresolved with many of the filed patents not effectively challenged as yet. Despite this, there are currently three clinical trials ongoing against well-established targets: age-related macular degeneration, respiratory syncytial virus (RSV) infection and acute renal failure. The number of new trials scheduled highlights the current enthusiasm to fully exploit this rapidly developing technology for novel therapeutics.

Therapeutic antibodies

The first of two talks on therapeutic antibodies was given by Dr. John Haurum (Symphogen) and focused on the opportunities presented by polyclonal antibodies. The talk began by providing some historical perspective on the evolution of antibody-based approaches. Dr. Haurum described how the development of the hybridoma technique allows the production of pure highly specific monoclonal antibodies (mAbs) *in vitro*. More than 200 antibodies are in different stages of clinical development and more than 15 have been approved for market. The ones that are in the market are expected to generate revenues higher than USD 12 billion and the more advanced are in the cancer area with only one, palivizumab (*Synagis*[®]) for RSV, in the antivirals area. Despite their clear potential, mAbs do have disadvantages that relate to their mono-specific nature. Specifically, mAbs do not cover full spectrum of effectors and are therefore less effective in treating diseases in which the targets are complex. A third generation of antibodies, polyclonal antibodies, can be applied to complex diseases such as infectious diseases and cancer and can be highly specific and effective against mutating antigens. The polyclonal approach attempts to mimic the complex immunoglobulin system in the plasma by having a mixture of antibodies targeting multiple epitopes. In this way, synergy is achieved, sensitivity to immune escape can be reduced and a broader reactivity towards variant targets is expected.

Symphogen has a new platform, called *Symplex*[™], that allows them to clone, express and identify human antibodies from blood donors that have natural immunization, or have been vaccinated for a particular target. The antibodies are isolated from the blood donor and sequences of the heavy and light chains of the antibodies are reverse-transcribed using the *Symplex*[™] PCR technology, retaining the sequence diversity found in the donor blood. The repertoire is then cloned and subsequently expressed in *Escherichia coli* and/or in Chinese hamster ovary cells. Symphogen has also patented a production technology, *Sympress*[™], which uses Chinese hamster ovary cells and site-specific integration technology that enables the creation of cell lines from which polyclonal antibodies can be isolated in a reproducible manner. Examples of this technology for the production of antibodies targeting tetanus, influenza and vaccinia have been documented.⁵ The results showed that the efficacy is higher for polyclonal mixtures than for monoclonal mixtures. Consistency is one of the difficulties with this technology due to the cell stability in a mixed environment. Some preliminary data were presented on **Sym001**, an anti-Rhesus D therapeutic containing 25 distinct recombinant polyclonal antibodies, which is in development for idiopathic thrombocytopenia purpura and prophylaxis of hemolytic disease of the newborn. As part of the development program, a method of producing batches of Sym001 with low compositional diversity has successfully been achieved. The data showed that the required dose was significantly lower for Sym001 (300 µg) compared to the monoclonal product.

The second talk on therapeutic antibodies was given by Dr. Richard Pither (UCB-CellTech) who described some of the technology platforms available to support the discovery of novel antibody-based therapeutics ranging from the development of novel antibody formats to address specific disease targets, through to novel engineering and biophysical ap-

proaches to optimize product characteristics. Dr. Pither highlighted how the antibody market is quickly growing by presenting data that showed an increase in the market from USD 10.3 billion in 2003 to USD 14 billion in 2005. These relatively expensive therapies have to compete with small-molecule drugs, and five products in particular are driving the antibody market, accounting for 80% of revenues. For the future, there will be a clear need to align innovation (from research through to development and commercialization) with medical needs in key areas including arthritis, multiple sclerosis, psoriasis, systemic lupus and oncology.

An important tool in meeting this ambitious challenge is the ability to identify suitable antibodies through high-throughput screening of arrays of prospective candidates that differ from one another in the composition of the variable region. The rule of thumb is that for every 1 billion B cells screened, one antibody with good potency will be isolated. Once the antibody is identified, a process of antibody fragment engineering (for examples, see ⁶) delivers optimized antibodies with the appropriate pharmacokinetic properties (tissue penetration/volume of distribution). The pharmacokinetic properties of antibody fragments (fAbs) can also be improved through PEGylation. Dr. Pither then went on to describe the profile of a univalent and Fc-free mAb (**certolizumab pegol**, *Cimzia*[®]) that is being evaluated for rheumatoid arthritis. This mAb avoids Fc-mediated depletion *in vivo* and, as a result, shows rapid accumulation and more extended exposure relative to marketed mAbs. In terms of manufacture, it has been shown that thermal stability (compared to denaturation and chemical degradation) is a key factor in driving expression levels and the ease of processing.⁷ In conclusion, although antibodies are expensive to develop (USD 1.2 billion for new product development), as well as being non-orally available and posing potential immunogenicity risks, they have an

important role to play in helping to target disease-related proteins that have traditionally been viewed as undruggable. One possible future avenue proposed by the speaker was that the structural information generated with high-affinity fAbs (e.g., through epitope mapping) could be used to build pharmacophore models to drive subsequent small-molecule drug development approaches.

Delivery of biotherapeutic agents

Each of the talks during the day identified as a key challenge the delivery of biotherapeutic agents to the site of action. Dr. Pierandrea Esposito (Esteve) brought together some of the main themes and outlined where future opportunities for improved delivery might be exploited. The talk began by giving a sense of how biotherapeutics has evolved in the last decade. While the number of small-molecule drugs reaching development has decreased steadily over the past 10 years, the number of biotherapeutics reaching development has stayed fairly constant. In 2005, approximately 800 biotherapeutics entered development, with about 200 eventually being marketed. Development phases in the biotherapeutics market are increased, driven by growing safety concerns and focus upon long-term therapies. The market value of biotherapeutics is growing (USD 33 billion in 2005) and there has been a shift in the market focus from small peptides (e.g., human growth hormone) to large molecules, such as monoclonal antibodies and cytokines, which are often delivered in high doses (>300 mg). Furthermore, new delivery technologies can increase the product life cycle of older biotherapeutics.

The shift in focus to larger protein molecules and increased dosage provides technical challenges, since the delivery options for high protein concentrations are limited. Limiting factors include solubility of the protein, viscosity of the solution, volume of injection and cost of goods, among others. Improving therapeutic effica-

cy, patient compliance, pushing protein delivery towards long-acting or sustained-release injectables or non-parental administration (pulmonary, transdermal, intranasal, etc.) are other key challenges.

The structural complexity of the biotherapeutic in question, as well as the biophysical properties of the protein (solubility, etc.), need to be understood as they strongly influence the bioanalytical methods that can be employed during formulation. The injectability of concentrated protein solutions presents several challenges. Concentrated solutions are highly viscous, affecting their ability to be administered using a syringe, although this can be overcome by using colloidal suspensions. The physical state of the solution can be altered to aid delivery, such as through the use of microparticles to pack the solution into a low volume (1 ml). *Crystallomics*[™] utilizes high-protein crystals to deliver biotherapeutics. An example of a crystalline biotherapeutic is **crystalline human growth hormone**, which has been shown to have sustained release, increasing the duration of action of a single injection.

In formulation, there is a strong need to improve and innovate systems; examples are partially aqueous processes, such as *Biosphere*[™], and totally aqueous processes such as *Octadex*[™]. New, better performing materials such as *Polyactive*[™] and polyorthoesters are also being developed. Additionally, microencapsulation of some biotherapeutics returns 100% activity and provides sustained protein release.

The importance of anticipating issues during clinical development was highlighted using **Rec-h protein** as a case study. This biotherapeutic has a high molecular mass (50 kDa), is highly glycosylated and is highly viscous in solution, with a limited volume for injection. In its favor, it has good solubility and stability and a long plasma half-life. The predicted doses and injection frequency meant that the

administration regimen and delivery conditions needed to be optimized. Selective monoPEGylation of an N-terminal residue maintained product homogeneity and preserved the surface charge of the native molecule, while retaining binding capacity and biological activity. The pharmacokinetic profile of Rec-h protein was greatly improved with monoPEGylation, meeting the objectives of reducing injection frequency and volume.

Anticipating life cycle management by using different delivery options to differentiate products was also discussed. This was demonstrated by analyzing the pipeline of glucagon-like peptide-1 analogue peptides, where long-acting formulations (e.g., *Exenatide LAR*[®]) are quickly following the current leading product (*Byetta*[®]).

Vaccine-based therapies

The final two sessions of the day focused on vaccine-based therapies. The first of these, given by Professor Richard W. Titball (University of Exeter) began with a historical overview of the area, reminding the audience of the success of vaccines against several pathogens and diseases including smallpox and diphtheria.

Vaccines fall into two broad classes: living and nonliving. The former includes live-attenuated and live-vector vaccines. They are often the most effective vaccines as they mimic disease caused by the pathogen. However, concerns about their safety in some individuals (particularly those who are immunocompromised) is directing attention towards the development of nonliving vaccines. These include killed pathogens, subunits (proteins, lipopolysaccharide, conjugate) and naked DNA vaccine.

The suitability of these various vaccine types depends on the nature of the disease. Some bacteria (e.g., *Streptococcus pneumoniae* and *Neisseria meningitidis*) are extracellular pathogens while others have an intracellular lifestyle (obligate like *Rickettsia*

or facultative like *Mycobacterium tuberculosis*). Protection from extracellular pathogens can often be achieved by humoral immunity while protection against intracellular pathogens typically requires cellular immunity, characterized by CD4⁺/CD8⁺ T, natural killer and $\gamma\delta$ T4 cell involvement. Most vaccines are able to induce good antibody responses. Cellular immunity, involving CD8⁺ T cells and leading to a cytotoxic response, can be achieved with living and naked DNA vaccines. Currently, few vaccines are available against intracellular bacteria including tuberculosis and typhoid fever vaccines. However, their efficacy is questionable and a key challenge remains the identification of safe and effective vaccines against intracellular pathogens.

The remainder of the talk focused on Rick Titball's experience at the Ministry of Defence R&D Agency at Porton Down where new vaccines for plague have been developed. *Yersinia pestis* is the pathogen responsible for plague epidemics during history. There are two forms plague: the bubonic form and the pneumonic form. Bubonic plague is caused by flea bite and is associated with swelling of the lymph nodes where the bacteria replicate. It is often treatable with antibiotics and most people recover. In contrast the pneumonic form, caused by inhalation of the bacteria, is rarer and is generally lethal within 1–2 days. Plague is a present-day disease with ~3000 cases reported to the WHO each year and it is also recognized as a potential biowarfare agent.

Two plague vaccines exist:

- a killed whole cell vaccine that has been widely used in Asia including Vietnam. However, it requires a 6-month immunization regimen and is highly reactogenic. It does not protect against pneumonic plague.
- a live attenuated vaccine that was in the past used in Madagascar and in the former Soviet Union. It requires a single dose vaccination and is protective against both forms although

it is associated with a high rate of severe systemic reactions that can lead to death.

The search for a **new subunit-based vaccine** for plague involved 10 years of research and vaccine development is ongoing. The antigens selected for inclusion in a subunit vaccine were the F1 antigen (capsular antigen) and the V antigen of the type III secretion pathway system. The operon encoding expression, export and surface assembly of F1 antigen was expressed in *E. coli*. The V antigen was expressed as a GST (glutathione *S*-transferase) fusion protein. Purification was performed on GST and the fusion was digested on column using a protease itself fused to the GST. Either antigen provided some protection against plague but a combination of F1 and V antigens provided high-level protection against bubonic and pneumonic plague. The role of antibody in protection was demonstrated in SCID/beige mice.

In a further study involving primates, serum from immunized cynomolgus macaques was transferred into mice that were then shown to be protected against plague. This demonstrates that the macaques had generated a protective immune response. This passive transfer procedure can also be used to evaluate whether immunized humans have developed protective immunity. The vaccine is formulated in an alhydrogel adjuvant and a two-dose schedule (day 1 and day 21) delivers 40 μ g of each antigen in 0.5 ml per dose. The phase II study will involve ~3000 subjects and a license for use in protection against plague is expected in the U.S. and Europe in 2010.

PMEDTM DNA vaccines

The final talk by Dr. Peter Loudon (PowderMed) gave an account of the needle-free particle-mediated epidermal delivery (*PMED*TM) technology for administration of DNA vaccines. Central to the device is the formulation of DNA to microscopic gold beads (1–3 μ M diameter) which are then

delivered to the nucleus of epidermal cells. The DNA is expressed and stimulates antigen-specific immune responses. It is important to note that the epidermis is rich in antigen-presenting cells such as Langerhans cells and potent immune responses can be generated here by small quantities of DNA. The safety and efficacy of first-generation PMED DNA vaccines was reported in 2001⁸ where a clinical study demonstrated that administration of microgram quantities of DNA was safe and well-tolerated and could stimulate both antibody and T-cell responses to single antigens. Second-generation PMED DNA vaccines seek to demonstrate improved efficacy against target diseases that require immunization with multiple antigens or for therapy against chronic disease. For these indications, PowderMed have developed an upgraded, disposable, hand-held delivery device (ND10) which is suitable for commercial scale manufacture and delivers a single shot of DNA.

Dr. Loudon went on to describe a **PMED-based influenza vaccine**. Here the DNA vaccine is easily produced in *E. coli* rather than by having to grow attenuated virus in eggs. A standard backbone vector is used for DNA expression, containing a cytomegalovirus promoter and a gene for the antigen (in the case of the H3 panama strain of influenza this is hemagglutinin). The vaccine was administered as a single dose (1, 2 and 4 µg) in 12 different cohorts. After 21 days, a protective response was demonstrated with the 4 µg dose (40% seroconversion and increase in geometric mean titer) and in partnership with NIBSC it was shown that cross-reactivity for at least five strains was similar to those of licensed vaccines. In more difficult-to-treat T-cell-dependent conditions, optimization of the codon usage of the DNA vaccine has proven to be important. In the case of human papillomavirus (HPV) which causes genital warts (HPV6 and 11), codon optimization of the E1 and E2 proteins leads to significantly improved protection in an *in vivo* canine mouth infection model.

The remainder of the talk described the use of the DNA-encoded immunostimulator-labile toxin (DEI-LT, an enterotoxin from *E. coli*) as an adjuvant for PMED vaccines. For example, in an H5N1 influenza challenge study carried out in collaboration with NIBSC, using influenza H5 DNA in mice, the protective responses at day 21 were higher when DEI-LT was used as an adjuvant. Furthermore, at day 72, only the mice immunized with a combination of DEI-LT/H5 survived. The same adjuvant has also been studied in combination with PMED vaccines for genital herpes. More recently, calreticulum has emerged as an effective adjuvant for T-cell-mediated responses in combination with PMED DNA vaccines⁹ although its precise mechanism of action remains unclear.

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