The Vaccine Renaissance

Nikolai Petrovsky

Flinders Medical Centre/Flinders University and Vaxine Pty Ltd.; Bedford Park, SA Australia

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Approximately 100 delegates drawn from academia, government and industry gathered in Providence, Rhode Island on 20 October 2010, for the Vaccine Renaissance Conference IV. This meeting was the fourth in a series of annual conferences for vaccinologists in the US North-East organized by Prof. Annie De Groot (University of Rhode Island). The conference had a strong academic stream with a focus on early stage vaccine research and development. This year, the day before the main conference, an associated immuno-informatic workshop was held at the University of Rhode Island, which provided training to delegates on use of immuno-informatic tools for rational vaccine design.

A highlight of the Vaccine Renaissance conference is that its scope is much broader than just standard infectious disease vaccines, with many conceptually novel vaccine ideas being presented. By way of example, Vincent Tuohy (Cleveland clinic, USA) presented data on vaccinating against breast cancer by targeting self-proteins expressed by the breast. His prophylactic breast cancer vaccine targets alpha-lactalbumin, which is only expressed by the lactating breast. SWXJ female mice immunised with this lactalbumin vaccine with complete Freund's adjuvant (CFA) were protected against later development of breast cancer. Given concerns about potential for induction of autoimmunity against normal breast tissue it was reassuring that no inflammatory infiltrate was observed in the breast when non-lactating mice were administered the vaccine. The vaccine also inhibited 4T1 tumor cell growth in Balb/C mice, although no effect of vaccination was seen if the mice were inoculated with tumour prior to immunization. Similarly, in MMTV-PyVT mice only a very slight difference in tumor size was observed after vaccination. This suggests the breast cancer vaccine may only work in a prophylactic rather than a therapeutic setting. Both CD4 and CD8 T- cells from immunized mice were able to adoptively transfer protection against breast tumors to naive mice, suggesting a T- cell rather than antibody mediated mechanism of action. This may present a problem for translation of the work to humans, since CFA is too toxic and no other good T- cell adjuvant is currently licensed for human use. The other issue was at what age women would best be immunized, with timing around the menopause being the conservative approach although this would then miss protecting against premenopausal breast cancers.

Another interesting vaccine presented was a vaccine against lipopolysaccharide (LPS) originally designed as a therapeutic vaccine for septic shock. Alan Cross (University of Maryland)

explained the original concept of the J5 vaccine was to generate antibodies to LPS that would bind and neutralize free circulating endotoxin and thereby treat shock due to excessive LPS-induced circulating immune mediators, such as tumor necrosis factor alpha and interleukin 1. The J5 vaccine is based on LPS combined with gram-negative outer membrane protein. Initial phase 1 clinical trials of the J5 LPS vaccine conducted without adjuvant induced only a poor antibody response. A later version of the J5 vaccine developed using various adjuvant formulations showed that whereas CpG increased antibody titers, the combination of aluminium hydroxide and CpG paradoxically reduced antibody titres. Hence a human trial of the J5 vaccine with CpG7909 is planned for the near future. In mice, intranasal administration of J5 vaccine with CpG protected against lethal *Klebsiella pneumoniae* and also *Francisella pneumoniae*. Recent evidence suggests that J5 LPS vaccine protects against lethal Gram-negative infection by targeting the Gram-negative pathogen itself rather than by the original idea of blocking the action of LPS.

A dream of the military is to have vaccines able to simultaneously protect soldiers against multiple bioterrorist threats. Les Dupuy (USAMRIID) discussed the advantage of a DNA vaccine approach for this task, with the idea that multiple plasmids coding for proteins from different organisms can be easily injected at the same time, thereby priming a soldier against multiple different threats before being deployed to the battlefield. The problem of DNA vaccines, as seen with a DNA vaccine against Ebola USAMRIID developed, is that while the DNA vaccines might have been effective in small rodents, e.g., mice, they provided little or no protection in larger animals, such as rabbits. As a consequence, better DNA delivery techniques are being explored including codon-optimized plasmids plus delivery by electroporation. Codon-optimised DNA vaccine gave significantly high neutralizing antibodies than the standard DNA vaccine, although interestingly the antibody titers as measured by ELISA were similar. Injection with electroporation gave a 10-fold higher antibody response than intramuscular injection alone, with monkeys receiving 50–500 ug of codon-optimized DNA vaccine by electroporation producing protective neutralizing antibodies after just one dose of DNA vaccine, which was then boosted by administration of a second dose. Along the same lines, David Weiner (University of Pennsylvania), one of the early pioneers of DNA vaccination, explained why initial DNA vaccine approaches performed so poorly, and how recent improvements meant that DNA vaccines were now close to realizing their true potential. He highlighted improvements in plasmid design including promoter sequences and codon optimisation, molecular adjuvants and better vaccine targeting to

Correspondence to: Nikolai Petrovsky; Email: nikolai.petrovsky@flinders.edu.au
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cells as being the keys to successful DNA vaccination. His group have shown that they can now get 1,000x the level of protein expression after intramuscular injection of a DNA vaccine compared to what they obtained with their initial DNA vaccines over a decade ago. As further proof, their current generation DNA vaccines generate 22x greater vaccine potency in primates as measured by ELISPOT. In a collaboration with Merck and Inovio, their group has been testing prime boost regimes combining DNA vaccination with Adeno5 vector immunization. After the first immunization, the DNA vaccine produced lower immune responses, but was able to boost after each dose where the Adeno5 vector did not show boosting. When the two strategies were combined together, the DNA was capable of boosting the Adeno5 primed response and the Adeno5 vector was able to boost the DNA primed response. A consensus sequence was generated from strains of H5N1 pandemic influenza virus and when administered as a DNA vaccine encoding the influenza hemagglutinin and nuclear protein this was able to induce a neutralizing antibody response in ferrets and protected ferrets against lethal H5N1 challenge; however, there were differences in that the combined vaccine group were completely protected and did not get ill, whereas animals only immunized with DNA encoding nuclear protein still got sick and lost weight before recovering, reinforcing the importance of immune responses against hemagglutinin for influenza protection.

One of the issues in design and testing of DNA vaccines intended to induce cellular immunity is how to best measure this readout. Devon Shadlock (University of Pennsylvania) has been studying the measurement of primate T-cell responses to DNA vaccines. He has found activation marker staining using a combination of antibodies against CD3, CD4, CD8, HLA DR and CD38, to be more sensitive than other techniques, such as T-cell proliferation assays and ELISPOT, for detecting vaccine responses. Niranjana Sardesai (Inovio Pharmaceuticals) presented the latest Collectra DNA vaccine delivery device that is being used in human trials of a human papilloma virus (HPV) vaccine, Inovio VGX-3100EP. A trial was performed in subjects with carcinoma in situ (CIN) grades 2–3 who received three doses of either 0.3 mg or 3 mg of DNA for each of two HPV plasmids. Subjects experienced only minor adverse events with no serious adverse events and dose-dependent antibody responses being observed. Fifteen out of 18 subjects became positive to at least one HPV antigen and antibody responses lasted up to nine months. Cellular immune responses as measured by gamma interferon ELISPOT were observed in 13 of 18 subjects. The tolerability of the intramuscular DNA vaccine delivery device was poor with a pain score of 6.2/10 at the time of the immunization falling to a score of 1.4/10 at 10 min. As a consequence Inovio have now moved to an intradermal electroporation device that is better tolerated and gives a pain score of 2.5/10 at the time of administration reducing to 1/10 at 10 minutes.

Several new vaccine technologies were presented at the conference. Bolyn Hubby (Vaccines Liquidia Technologies Inc.) discussed a nano-etching technology to produce controlled size particles for use in vaccines. The nano-powders produced in this way have been designed to absorb protein onto the surface of the

particle, thereby enhancing protein immunogenicity. Their prototype product, LIQ-001, is made from a biocompatible polymer to which influenza hemagglutinin is absorbed and is currently in Phase 1 clinical trials with 80 subjects having been immunized so far.

A timely review of the state of HIV vaccines was presented by Katharine Kripke (NIH). Over the last 10 years major HIV clinical trials have included the Vaxgen USA trial, the Vaxgen Thailand trial, Thailand Rv144 trial, the STEP trial and HVTN505 trial. To date the only trial showing a statistically significant protective response was the Thailand Rv144 trial where over 16,000 volunteers were immunized with an Alvac-HIV canary pox vector and then boosted with gp120 protein boost where protective efficacy against HIV infection was 31.2% (95% confidence interval = 1.1–52.1). The efficacy looked higher in the first six months of the trial suggesting waning protection over time and overall the vaccine was relatively poorly immunogenic, suggesting that newer more potent HIV vaccine approaches should be able to do even better. Notably, alum, which is recognized as a very weak adjuvant particularly for T-cell immunity, has been the adjuvant almost exclusively used in HIV vaccine trials to date, providing at least one explanation for the poor vaccine immunogenicity efficacy seen in the HIV vaccine studies that included protein antigen arms, such as the Thailand Rv144 trial. Thus, a major area of need within the HIV vaccine field is the development of more effective vaccine adjuvant technologies.

Given the presence at the meeting of Polly Matzinger, the creator of the ‘immune danger model’ as a keynote speaker, it was appropriate that Nikolai Petrovsky (Vaxine Pty Ltd., Australia) throw down the gauntlet by challenging the immune danger signal model as applied to vaccine adjuvants. In particular, he challenged the paradigm that increased adjuvant potency invariably needs to come at the cost of increased vaccine reactogenicity and toxicity, i.e., the consequences of stronger danger signals. He presented data showing the extremely low reactogenicity and yet high adjuvant potency of Advax™, a polysaccharide adjuvant derived from delta inulin, that is currently in advanced clinical development in influenza and hepatitis vaccines. Advax™ despite being a potent human adjuvant does not suffer from the reactogenicity or toxicity of other adjuvants, as highlighted by a recent vaccine adjuvant comparative study in camels conducted independently by the Central Veterinary Research Laboratory in Dubai, United Arab Emirates, which confirmed Advax™ to have the best potency to reactogenicity ratio of any of the tested commercial adjuvants. The tolerability of Advax™ is explained by its mechanism of action not involving induction of a typical inflammatory cytokine profile thought necessary for the generation of danger signals. In a recent HIV vaccine advance, in a partnership with Advanced Bioscience Laboratories (Kensington, USA), Advax™ adjuvant when combined with a gp120 protein boost was shown to significantly enhance the humoral and cellular immune response in mice following initial priming with a DNA vaccine encoding *env*. Of even greater interest, the combination of DNA vaccine priming with sequential adjuvanted protein boosts via both intramuscular and intranasal routes, was found to provide optimal enhancement of both systemic and mucosal immunity

against gp120, which was able to neutralize homologous strains of HIV. The immune responses generated by this novel DNA prime/nasal protein boost/intramuscular protein boost approach utilising Advax adjuvants were long-lived and showed little attenuation even at 26 weeks post-immunization.

Polly Matzinger (NIH) dazzled the audience with an exposé on immune tolerance and the impact of maternal immunity on a neonate's ability to respond appropriately to immunization. Rather than tolerance implying the absence of an immune response, she instead suggested to the audience that what was happening in immune privileged sites such as the anterior chamber of the eye or testes was instead just a deviation of the immune response away from a destructive response, e.g., a Th1 or Th2 response, to an alternative non-destructive response, e.g., a Th3 response characterized by TGFβ and IgA antibody production. Under this model regulatory T cells do not act via suppression but rather by skewing the immune response in an alternative direction. This led into discussion of whether the accepted paradigm of suppression of neonatal vaccine responses by maternally transferred antibody is correct. They have shown using an anthrax vaccine model that maternal immunization and subsequent transfer of anti-anthrax antibody to the neonate via placental transfer and breast feeding, does not suppress the ability of the neonate to itself respond to anthrax immunization. Given the fact that pregnant women and neonates are high-risk groups for mortality from infections such as influenza, this highlights the urgent need for more research into vaccine responses in both mothers and neonates and how these might impact on each other.

The subject of aberrant infant vaccine responses was also addressed by Christine Shaw (Novartis), who has been researching the phenomena of immuno-pathogenesis caused by respiratory syncytial virus (RSV) vaccines. Most human infants get infected with RSV by two years of age and subsequently get reinfected with a subclinical infection every few years, thereby boosting antibody titers until such time as immunity wanes in the elderly and clinical RSV infection again is seen. Protection against RSV correlates with neutralizing RSV antibody titers. Development of an RSV vaccine has been hindered by the results of an early human clinical trial in which a formalin-inactivated RSV vaccine adjuvanted with aluminum hydroxide resulted in more severe disease leading to deaths in some children when they became exposed to RSV. The cotton rat model has been used to study the cause of this immuno-potential. Alveolitis in the cotton rats is a specific marker for enhanced disease, whereas peribronchiolitis is not predictive. The formalin-inactivated RSV vaccine induces only poorly neutralizing, low avidity IgG to RSV in cotton rats when compared to post-infection titers. Whereas unpurified inactivated cell-culture RSV virus induced a high disease score in cotton rats exposed to RSV, more highly purified RSV antigen failed to induce subsequent RSV immuno-potential. In mouse models T-cells are able to transfer RSV immuno-potential and it was found that bovine serum-albumin in the poorly purified inactivated vaccine was the critical mediator of enhanced disease due to immuno-potential such that the addition of bovine serum albumin (BSA) to a highly purified or recombinant RSV antigen was

able to reproduce immuno-potential disease, presumably by causing a Th2 skew in the immune response that is associated with immuno-potential. This suggests that a highly purified recombinant vaccine approach with an appropriate adjuvant that avoids a marked Th2 skew in the immune response may be safe and effective for preventing RSV infections.

Despite a major emphasis on cutting edge vaccine approaches, the meeting did not ignore more conventional vaccine problems. Ted Ross (Pittsburg University) presented data on impaired influenza vaccine responses in the elderly and how these might be overcome with the use of alternatives to current inactivated influenza vaccines, for example using recombinant influenza virus-like particles (VLP). These influenza VLPs are made up of neuraminidase, hemagglutinin and M1 protein and are produced in insect or mammalian cell-lines. The VLP vaccine induced broader hemagglutinin inhibiting antibodies in mice compared with recombinant hemagglutinin alone and was associated with a switch to IgG2a production, whereas recombinant hemagglutinin alone induced predominantly IgG1 antibodies. The VLP vaccine protected when given either intramuscularly or intranasally whereas the recombinant hemagglutinin alone only protected when given intramuscularly. Studies were then conducted in mice aged for 24 months that were given VLPs plus a polyIC adjuvant, intranasally. Although in the elderly mice the VLPs with poly IC could not be shown to enhance anti-influenza antibody production, they still showed improved protection against lethal influenza challenge with the mechanism for this still not known.

The meeting participants acknowledged that the vaccine world should never be complacent as new and old pathogens are constantly emerging or changing to present new vaccine challenges. For example, Mark Buller (Saint Louis University) presented an update on the development and testing of new orthopox vaccines against smallpox, designed to avoid the problems of existing approved vaccines. Another example discussed of a newly emergent problem is the Chikungunya virus that is rapidly spreading around the globe and is a significant cause of morbidity particularly in Asia against which a new DNA vaccine has been developed that has recently been shown to protect against lethal Chikungunya virus infection in mice.

Given the recent declaration by the World Health Organization of the end of the 2009 H1N1 influenza pandemic, it was timely that Manon Cox [Protein Sciences Corporation (PSC)] present a talk entitled "Preparing for the Next Pandemic: Rapid 'Novel' Flu Vaccine Product (Getting the First Recombinant Influenza Vaccine Approved)." Highlighted was the often under-appreciated difficulties in getting any new vaccine technology through the immense regulatory hurdles that are required for a new product like PSC's recombinant hemagglutinin-based influenza vaccine to get onto the market. While recombinant influenza vaccines offer great promise for pandemic use they first must gain FDA approval, with their use being limited to clinical trials until that time. This talk presented salient lessons for those developing even more novel vaccine technologies such as DNA vaccines, as if even a relatively standard recombinant protein vaccine faces such great hurdles in obtaining regulatory approval, then these other

technologies are likely to face even greater challenges when it is their turn to seek approval.

Overall the meeting was extremely positive and provided encouragement that many great developments are happening in the vaccine world. In particular, a key message from this meeting was that DNA vaccines should not be abandoned when they may finally be on the verge of delivering their promised benefits and, similarly, that work on HIV vaccines should not be abandoned just when they might just be starting to deliver positive results with great scope to improve upon previously tested HIV vaccines by incorporating better adjuvants and delivery routes. This optimism was, however, tempered with the realization that the challenges for vaccine developers are enormous and great vaccine science alone cannot generate new vaccines, as every new vaccine must also overcome major

regulatory hurdles in order to gain approval. Hence, major resources and financing need to be made available by funding agencies to support this critical second step in vaccine development without which great vaccine science alone is unable to deliver the benefits the public expects from its vaccine research dollars.

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