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Recent advances in veterinary applications of structural vaccinology

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The deployment of effective veterinary vaccines has had a major impact on improving food security and consequently human health. Effective vaccines were essential for the global eradication of Rinderpest and the control and eradication of foot-and-mouth disease in some regions of the world. Effective vaccines also underpin the development of modern intensive food production systems such as poultry and aquaculture. However, for some high consequence diseases there are still significant challenges to develop effective vaccines. There is a strong track record in veterinary medicine of early adoption of new technologies to produce vaccines. Here we provide examples of new technologies to interrogate B cell responses and using structural biology to improve antigens.

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Protective antibody responses are an essential component of the responses induced by effective vaccines for a number of pathogens. Antibodies can recognise short peptides that contain key residues that form the epitope, but these peptides might lack additional residues that are outside of the linear epitope, so they do not represent the structures recognised by antibodies in immunised or infected animals. Currently, quantitative rules linking sequence, structure, immunogenicity, and protection are still lacking. The induction of robust protective immune responses in livestock species still relies on the presentation of antibody epitopes in the context of the whole pathogen or individual proteins. Therefore, the immediate application of structural vaccinology to enhance protective antibody responses will be the engineering of proteins to maintain epitopes rather than the use of isolated epitopes [1]. The use of structural information in vaccine design will allow proteins to be modified to produce more stable vaccine antigens to ensure the structure of key epitopes are maintained during vaccine production, distribution and application. There are two examples of the application of structural vaccinology for vaccine design that have been validated by *in vivo* proof of concept studies in the livestock target species, these are FMD virus like particle (VLP) vaccines and bovine RSV (bRSV) fusion (F) protein.

Structure-based vaccine design as a route to broadly protective PRRS vaccines

PRRS continues to be the key economically important pig disease worldwide. The causative PRRS viruses (PRRSV) are rapidly evolving, as most dramatically illustrated by the emergence of highly pathogenic variants in Southeast Asia and Eastern Europe [2–4]. Thus, more efficacious control strategies are urgently sought. Current vaccines can confer protection from disease but show variable efficacy against challenge with heterologous strains [5–12]. A variety of experimental subunit approaches have been evaluated as potential next-generation vaccines but have at best conferred only limited protection [13–18]. The limitations of both existing and experimental vaccines support the proposition that a new approach is required to design immunogens capable of providing broad protection against this hypervariable pathogen. Neutralising antibodies (nAbs) can provide immunity against PRRSV as demonstrated by a dose-dependent protective effect conferred by passive transfer of homologous PRRSV-neutralising sera [19]. However, the PRRSV-specific Ab responses measurable from 7 days post-infection is non-neutralising, and nAb responses are often not observed until at least four weeks post-infection and titres, when measurable, are often lower than those elicited by other viral infections [20,21]. Collectively, these observations suggest that PRRSV has evolved strategies to modulate the B cell response to evade the induction of protective nAbs via the glycan shielding of neutralising epitopes and/or the promotion of responses against non-neutralising decoy epitopes [22]. These data also suggest that vaccination strategies that induce high-titre nAbs would be efficacious. The development of such a strategy would benefit from an improved understanding of the neutralising epitopes on PRRSV that confer protection. Since a lack of cross-protection is a major constraint in the development of PRRSV vaccines, the identification of conserved epitopes is of paramount importance.

Linear nAb epitopes have been identified on GP2, GP3 and GP4 [23,24] of PRRSV-1 and GP5 of both PRRSV-1 and PRRSV-2 [25–27]. The complexity of the nAb response to PRRSV and the limitation of current understanding was illustrated by recent studies investigating the cross-neutralisation of field strains [28,29]. Evaluation of the neutralisation of PRRSV-1 isolates by a panel of hyperimmune sera revealed significant differences in the sensitivity of PRRSV strains to neutralisation; however, no correlation was observed with known linear nAb epitopes or N-linked glycosylation sites [29]. Interestingly, 10% of sera exhibited significant neutralising activity against all isolates, suggesting that these sera contain nAb specific for conserved epitopes that may be poorly exposed and consequently weakly immunogenic in most strains. Whilst it may be suggested that differences in residues outside the described linear epitopes altered accessibility, an alternative explanation is the presence of as-yet-unidentified conformational nAb epitopes. In support of this, a study of PRRSV-2 infected pigs identified a single animal with a broadly nAb response that even neutralised PRRSV-1 [30•]. The deletion of a single non-conserved amino acid in the M protein conferred resistance to this response, although it remains to be determined whether this mutation is altering a conformational epitope or blocking access to a linear epitope. Evidence for the natural occurrence of broadly nAbs was presented in a recent study of two US sow herds with multiple exposures to PRRSV that revealed sera capable of neutralisation of strains spanning both PRRSV species [31•]. The identification of nAb epitopes and particularly the conserved viral structures recognised by these broadly nAbs would lay the solid basis on which to rationally develop a much-needed second generation vaccine to prevent and control PRRS.

Identification of the epitopes recognised by broadly nAbs is an area of intense recent research in the context of a number of highly variable human viruses [32–35]. Central to this effort are methods to generate and analyse the specificity of naturally occurring monoclonal antibodies (mAbs). Recent advances in methodologies to analyse antigen-specific B cells and their immunoglobulin genes are now providing large numbers of human mAbs for application both as therapeutics and in the design of novel immunogens [36]. We are pursuing two complementary approaches to isolate broadly neutralising mAbs from a cohort of pigs hyper-immunised by sequential challenge infections with heterologous PRRSV strains. The first approach involves the use of a retroviral vector to constitutively express the B cell lymphoma-6 (Bcl-6) transcription factor and the anti-apoptotic Bcl-2-like protein 1 (Bcl-xL) in memory B cells [37••]. With co-stimulation, transduced cells proliferate, secrete their Abs and retain surface immunoglobulin expression. These cells are therefore amenable to both enrichment/cloning by antigen-baiting and direct analysis of Ab specificity in cell culture supernatants. This approach has been successfully deployed to isolate human mAbs capable of broadly neutralising human parechovirus [38], RSV [37••] and influenza A virus [39], to eventually identify epitopes as vaccine targets. Significantly, this approach has been used to successfully immortalise B cells from rabbits, mice, llamas and non-human primates [37••,40]. The second approach, which builds on our experiences in isolating FMDV-neutralising bovine mAbs, is using high-throughput sequencing of immunoglobulin genes from plasma B cells. Bioinformatic analysis will identify over-represented clonal families that will be expressed as recombinant mAbs using an established high-throughput protein expression pipeline. Cross-neutralising mAbs will be identified by *in vitro* screening epitopes elucidated by cryo-EM structural analysis of mAb complexed to purified PRRSV virions. We hope that the isolation of these mAbs will contribute to both our understanding of the nAb response to PRRSV and allow epitopes to be resolved that may be engineered as immunogens to induce cross-protective immunity.

Structural vaccinology approaches to induce enhanced immune responses against FMDV and RSV in cattle

FMD is endemic in large parts of South America, Africa, The Middle East and Asia and is, globally, the most economically important infectious disease of livestock, affecting cattle, pigs, sheep, goats and other artiodactyl species (Figure 1). In endemic countries, FMD not only affects national and international trade, but also impacts on the whole livestock industry with damaging consequences for local farmers and, invariably, loss of income. Livestock health is clearly linked to human health and prosperity, hunger, malnutrition, and poor health are widespread and stubborn development challenges. Routine vaccination programmes are employed in the endemic regions of the world but regular immunisation is required. Improved vaccines, in terms of stability and protection against emerging FMDV, are essential for disease control and to establish and maintain FMD-free status in many regions of the world. The current FMD vaccines are inactivated whole virus preparations which contain an adjuvant to enhance the immune response. There are 7 FMDV serotypes: O, A, Asia1, C and three strains circulating predominately in sub-Saharan Africa, SAT (South Africa Territories) 1, 2 and 3.

There is currently a massive shortfall in the availability of vaccines, most strikingly in Africa. There is a significant impediment to building new high disease containment facilities to increase the production of conventional killed virus vaccines because of the high initial and on-going costs. A particular difficulty for FMDV is that effective vaccination requires the presence of intact inactivated virus or VLPs. Individual proteins or peptides have