Novel vaccine strategies against emerging viruses

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One of the main public health concerns of emerging viruses is their potential introduction into and sustained circulation among populations of immunologically naïve, susceptible hosts. The induction of protective immunity through vaccination can be a powerful tool to prevent this concern by conferring protection to the population at risk. Conventional approaches to develop vaccines against emerging pathogens have significant limitations: lack of experimental tools for several emerging viruses of concern, poor immunogenicity, safety issues, or lack of cross-protection against antigenic variants. The unpredictability of the emergence of future virus threats demands the capability to rapidly develop safe, effective vaccines.We describe some recent advances in new vaccine strategies that are being explored as alternatives to classical attenuated and inactivated vaccines, and provide examples of potential novel vaccines for emerging viruses. These approaches might be applied to the control of many other emerging pathogens.

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Introduction

Emerging diseases affecting livestock and humans represent an important threat to the world's economy and public health. Several factors including increasing urbanization, international travel and commerce, or climate change increase the likelihood that the threat of emerging pathogens will continue, if not worsen, in the future.

When a virus emerges in an infection-free area, or jumps into a new species, the susceptible host population will likely have no or little pre-existing immunity to the pathogen. Lack of herd immunity can result in fast dissemination and in more virulent consequences of infection. Providing protective immunity through vaccination can be the most powerful and cost-effective strategy to prevent and control emerging infectious diseases.

Developing a vaccine against an emerging virus might face several challenges [1,2] (summarized in Box 1). Although conventional vaccination strategies, based on inactivated virus or on the use of life attenuated strains, have been instrumental in the control and even eradication of some important animal and human infectious diseases, in many other cases they fail to deliver the required levels of immunogenicity, safety, cross-protection across the pathogens antigenic variability, or even exacerbate disease. Therefore, new strategies have been explored to obtain safer more effective vaccines (Table 1).

Vaccines based on immunologically relevant viral antigens rather than on the whole virus could satisfy many of the challenges summarized in Box 1. However, individual antigens without the context of a viral infection are poorly immunogenic and therefore expression/delivery methods, as well as adjuvants (reviewed in [3,4••]) must be carefully designed to reach protection.

In this article we discuss some recent advances in the use of novel vaccine strategies for the control of emerging viruses. For simplicity, we focus on a few relevant examples, but these vaccine approaches or vaccine platforms might be applied to the development of safer and more effective vaccines against a number of emerging viruses.

Recombinant proteins and synthetic peptides

A safe strategy to induce immune responses is to deliver a viral antigen produced by recombinant methods or chemical synthesis. In addition to safety, recombinant protein vaccines can have additional advantages: First, production does not require the manipulation of the pathogen, avoiding the risk of accidental escape and the hurdles of high bio-safety and bio-containment requirements. Second, vaccine candidates can be designed even when there is limited information about the pathogen. Third, also, subunit vaccines can be used to overcome the natural immuno-dominance of highly variable epitopes and direct the immune responses against conserved and broadly protective epitopes. Fourth, since individual antigens elicit responses that are different from the response induced by natural infection these vaccine strategies could be used as DIVA (Differentiating

Table 1

Novel strategies applied to the development of vaccines against emerging viruses

Strategy	Advantages	Disadvantages	Example
Recombinant protein and synthetic peptides	Safe: no viral replication. Can direct the response to conserved epitopes.	Poor immunogenicity. Might require the use of potent adjuvants or boosts.	HeV glycoprotein [5]. IAV M2e [9•]. IAV long-α-helix [10•].
Virus-like particles, nanoparticles and multimeric peptides	Multimeric presentation of the antigen in native conformation. Safe, no viral replication.	Production yields, cost and purification can be limiting.	IAV head-less HA particles [13]. CHIKV VLPs [22].
Recombinant viral vectors	Elicit humoral and T cell responses. High level of antigen expression. Several vector platforms with different profiles available.	Pre-existing immunity to the vector can decrease efficacy.	NDV expressing RVFV glycoproteins [27,28]. VSV expressing EBOV glycoprotein [30••]. YFV attenuated strain expressing DENV glycoproteins [39•].
Recombinant bacteria	Adjuvant effect of the vector. Low cost, mass production.	Limited experimental information, no clinical trials.	*L. lacti* expressing SARS-coronavirus N protein [44].
Nucleic acid vaccines	Safe: no viral replication. Elicit humoral and T cell responses. Replicons have increased immunogenicity.	Poor immunogenicity (but can be enhanced by adjuvants and heterologous prime-boost strategies).	DNA vaccine against WNV in horses [47].

HeV: Hendra Virus; IAV: Influenza A Virus; CHIKV: Chikungunya Virus; NDV: Newcastle Disease Virus; RVFV: Rift Valley Fever Virus; VSV: Vesicular Stomatitis Virus; EBOV: Ebola Virus; YFV: Yellow Fever Virus; DENV: Dengue Virus; WNV: West Nile Virus.

Infected from Vaccinated Animals) vaccines with the accompanying serological test.

The main disadvantage of subunit vaccines is that isolated proteins or peptides are usually poor immunogens because they fail to be recognized as Pathogen-Associated Molecular Patterns (PAMPs) and activate innate immune responses, which are required for the full development of acquired immunity. To increase the responses against conserved epitopes they must be presented in an immunogenic conformation and/or accompanied by potent adjuvants.

Recently, a vaccine candidate based on the envelope glycoprotein of the BSL-4 pathogen Hendra Virus (HeV) (family *Paramyxoviridae*, genus *Henipavirus*) has been shown to induce complete protection in a ferret model [5].

Recombinant protein immunization has also been used to induce broadly reactive antibodies against Influenza A Virus (IAV) conserved epitopes. Vaccines that provide long-lived protection across several IAV subtypes, also known as 'universal influenza vaccines' would avoid the need for annual vaccination, and continuous re-formulation of the vaccine to match the circulating strains, and would protect against animal IAVs and future pandemic strains [6••,7,8]. A recombinant protein vaccine (STF2.4xM2e) containing the highly conserved extracellular domain of the IAV M2 protein (M2e) has demonstrated safety and immunogenicity in a Phase I clinical trial. To increase immunogenicity, the M2e sequence was expressed in four tandem copies and fused to flagellin, a TLR5 ligand acting as adjuvant [9•]. Wang and colleagues

Figure 1

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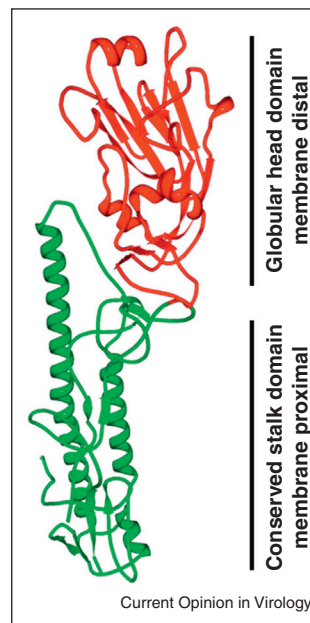
Monomer of the influenza A virus hemagglutinin (HA) protein. The membrane distal globular head (red) mediates receptor binding, is highly variable and induces strain specific neutralizing antibodies. The conserved stalk region (green) mediates membrane fusion facilitating virus entry. Antibodies directed against the head domain are mainly strain specific, while stalk antibodies can offer cross-protection against different subtypes.

Box 1 Potential challenges in the development of vaccines against an emerging virus.

- Incomplete information: our knowledge about an emerging pathogen can be limited for several reasons: it is a recently discovered virus; outbreaks are uncommon and there are few reported natural outbreaks; important immunological parameters such us correlates for protection, antigenic variability or immunodominant antigens are unknown. In addition, for highly variable viruses, the particular strain that will cause the next outbreak or pandemic might be unpredictable.

- Virulence and bio-safety requirements: some emerging viruses have high mortality rates, with no treatment or prophylaxis available, and must be manipulated under maximum (BSL-4) bio-safety conditions. For this type of viruses, using life attenuated strains or inactivated vaccines (that require the growth of large amounts of virus) are not acceptable options. Similar concerns apply to veterinary vaccines against highly contagious viruses to be used in disease-free countries.

- Lack of appropriate animal models: vaccine candidates need to be pre-clinically tested in animal models for safety, immunogenicity and efficacy (protection). The ideal animal model should have a well-known immune system and similar susceptibility and immune responses to the pathogen as the natural host. Other important considerations are prize, size, possibility to use large numbers, or ethical considerations.

- Possibility to Differentiate between Infected and Vaccinated Animals (DIVA or marker vaccines) is highly desired in veterinary vaccines against pathogens that are subject to trade restrictions.

- The time required to develop, validate, mass-produce and deliver a new vaccine should ideally be as short as possible. This could be facilitated by the use of well-known vaccine platforms that have already been tested and validated against similar pathogens.

- Vaccines to be used to control an ongoing outbreak should be stockpiled in advance or produced very rapidly, and induce protection after a single administration and short time after inoculation.

used a synthetic peptide from the conserved stalk region of the IAV hemagglutinin (HA) protein (Figure 1), coupled to the carrier protein keyhole limpet hemocyanin (KLH). Vaccination with two doses of peptide induced cross-reactive antibodies and protected mice against lethal challenge with different subtypes of IAV [10•]. Other approaches include the fusion of the antigen to dendritic cell targeting/activating molecules [11]. While this strategy should result in delivery of the antigen to an antigen presenting cell in the appropriate stimulatory context, further investigation is required to understand the best targeting sequences resulting in high immunogenicity and lack of reactogenicity.

Virus-like particles and multimeric presentation of viral antigens

In the virion, structural proteins are usually arranged in tight and well-ordered conformation, which is believed to be recognized as a PAMP. Therefore, one way to increase the immunogenicity of viral antigens is to deliver them in multimeric conformation and as virus-like particles (VLPs) (reviewed in [12]). VLPs based on both enveloped and non-enveloped viruses can be used to immunize against the homologous virus or engineered to incorporate epitopes from a different pathogen.

In addition to better immunogenicity, VLPs are considered very safe, because they contain no genetic material. VLP preparations do not require the use of inactivating agents (i.e. formalin) that might destroy immunologically relevant (conformational) epitopes.

The VLP approach is been applied in the research toward a universal IAV vaccine (reviewed in [8]). Steel *et al*. prepared headless HA VLPs by co-expressing a deleted

HA protein lacking the highly variable head domain (Figure 1) along with the HIV Gag protein. Mice immunized with these headless HA VLPs induced broadly reactive antibodies against the conserved stalk region of HA and were protected from challenge with a lethal dose of the homologous virus [13]. The influenza virus M2e antigen has been conjugated to the hepatitis virus core protein which self assembles into VLPs (ACAM-FLU-A [14]) and demonstrated safety and immunogenicity in Phase I clinical trials. A similar approach was used to fuse the M2e sequence to the norovirus capsid protein that self-assembles into VLPs. Interestingly, the chimeric VLPs induced responses against both IAV and norovirus [15]. M2 containing VLPs were also obtained by co-expressing the full length M2 protein with IAV M1 protein [16]. Alternative to VLPs, several groups have described multimeric M2e strategies that increase its immunogenicity [17•,18–20].

A VLP vaccine candidate against the Chikungunya Virus (CHIKV, family *Togaviridae*, genus *Alphavirus* [21]) was obtained by expressing the virus' structural proteins in a human cell line. Intramuscular inoculation induced neutralizing antibodies and completely protected from experimental infection in a nonhuman primate model [22].

Replication competent viral vectors

Recombinant viruses have been used for several decades as vectors for protein expression and for vaccination. The list of virus families that are being explored as vectors for vaccination is too broad to be described in detail, and the topic has been reviewed recently elsewhere [23,24,25•]. Viruses to be used as vaccine vectors can be manipulated to enhance their safety and immunogenicity by eliminating virulence factors; changing tropism by replacing

envelope proteins; and increasing coding capacity by eliminating non-essential genes.

One general advantage of viral vectored vaccines is that the antigen is expressed in the context of an actual viral infection, which activates innate immune responses required for the full development of adaptive humoral and T cell-mediated immunity [23]. Possible disadvantages are the competition of immuno-dominant antigens from the vector, or the loss of efficacy in the presence of pre-existing immunity against the vector. In some cases, safety issues derived from the pathogenesis of the vector itself are also required to be considered.

Of interest for vaccination against emerging viruses, many characteristics of a virus-vectored vaccine — including the type and intensity of the immune response, safety considerations, or manufacturing techniques — are determined mainly by the vector and not by the pathogen. Therefore, developing and testing a vaccine against a newly discovered virus can be significantly shortened by the use of a viral vector platform with an extensive record of safety and efficacy.

In addition to the virus families that have been historically used as vectors, such as poxviruses and adenoviruses, attractive new vector candidates are being developed. Newcastle Disease Virus (NDV) is an avian paramyxovirus that does not infect mammals. Attenuated NDV strains are used for vaccination in poultry (including a dual vaccine against NDV and H5N1 avian IAV [26]. In mammals, NDV vectored vaccines present two major advantages: first, there is no pre-existing immunity against the vector and second, the virus is not able to block the innate immune response in mammalian cells, which results in increased safety and immunogenicity. Recently, recombinant NDVs expressing the Rift Valley Fever Virus (RVFV) envelope proteins (Gn and Gc) have been shown to induce complete protection in mice and sheep [27,28].

Viral vectors have also been used to develop vaccines against highly pathogenic emerging viruses. Ebola Viruses (EBOV) are zoonotic Filoviruses that cause hemorrhagic syndromes with very high mortality rates. Several vaccine strategies have shown induction of specific immune responses and protection against lethal challenge in non-human primates [29]. In 2009 an experimental vaccine candidate was used as post-exposure emergency prophylaxis on a researcher, after accidental puncture with a needle containing Zaire EBOV. After consultation with experts from several countries, the chosen vaccine was a recombinant Vesicular Stomatitis Virus (VSV) expressing the EBOV envelope glycoprotein (GP) and it was administered 48 hours after exposure. The person developed antibodies against the vector and the GP protein, but not against other EBOV proteins. In addition, vector but not EBOV RNA was detected in the patient's serum [30••]. A replication defective recombinant adenovirus (rAd5) expressing EBOV GP elicits complete protection after a single inoculation in non-human primates [31] and has shown safety and immunogenicity in humans [32•]. A possible caveat of this strategy is that vaccine efficacy might be affected by pre-existing antibodies against the vector.

The availability of reverse genetics techniques to directly manipulate the genome of many viruses, along with the increased knowledge about their molecular biology, has opened the opportunity to create a new generation of attenuated vaccine strains with increased safety and immunogenicity. Good examples are the ΔNS RVFV vaccine candidates.

The non-structural protein NSs is a major virulence factor that modulates the host's immune response, but is not required for replication in cell culture. Applying reverse genetics to the existing attenuated strains, several groups have obtained viruses lacking NSs and demonstrated safety and immunogenicity in mice and lambs [33–36].

Another promising strategy to create attenuated vaccines is the exchange of sequences from the pathogen in the genome of a less virulent, closely related virus. Examples of this strategy are the flavivirus vaccine candidates based on the backbone of the attenuated Yellow Fever Virus strain YF-17D containing the genes of the envelope proteins prM and E2 from Japanese Encephalitis Virus (ChimeriVax-JE [37], currently licensed in Australia); and West Nile (ChimeriVax-WN02 [38]) and Dengue Virus (CYD 1–4 [39•]), which have both completed Phase II clinical trials; and the pestivirus chimeras combining the genomes of Classical Swine Fever Virus and Bovine Viral Diarrhea Virus [40].

Recombinant bacteria as vaccine vectors

In addition to being extensively used to produce recombinant subunit vaccines, bacteria can also serve as vectors for the *in vivo* delivery of antigens or DNA. Potential advantages of this platform are the low cost and easy to scale-up production, the availability of well characterized attenuated strains, the activation of the innate immunity by the vector and the efficient delivery to antigen presenting cells. Several genera are being explored as vaccine vectors, including *Listeria*, *Salmonella* [41], *Lactococcus* [42] and *Bordetella* [43]. Recombinant bacteria can be used as life attenuated vaccines, inactivated or even as cytoplasm-depleted bacterial ghosts. Recombinant *Lactococcus lacti* expressing the N protein of SARS-coronavirus have been shown to induce antibodies in mice [44]. Li *et al.* reported that a recombinant *Bordetella pertusis* expressing the influenza virus eM2 induces high titers of specific antibodies in mice, but failed to elicit protection in mice [45].

Nucleic acid vaccines

Inoculation of cDNAs encoding viral antigens can lead to uptake and expression of the cDNA by antigen presenting cells and initiation of immune responses [46]. DNA vaccines have many potential advantages for vaccination against emerging viruses: plasmids expressing a viral antigen can be produced rapidly, even when only partial sequence information from the pathogen is available. Antigen is expressed *in vivo* and induces both humoral and cell-mediated immune responses. Large quantities of DNA can be produced in short time at a reduced cost, and DNA preparations are more stable than other types of vaccines, both very desirable properties for a vaccine that must be used in remote areas. Furthermore, DNA vaccines are considered very safe, they are suitable for DIVA applications and they are not affected by anti-vector immunity. The main limitation in the development of DNA vaccines is their intrinsic low immunogenicity. Therefore, great research effort has been invested in the improvement of immunogenicity by more efficient delivery approaches, such as gene gun, skin tattooing, or electroporation; targeting to immune effector cells and the use of potent adjuvants, either co-administered with the vaccine or encoded in the same plasmid. DNA vaccines are also frequently used in combination with other vaccine platforms in prime-boost strategies.

A DNA vaccine is currently licensed to immunize horses against WNV [47] and has undergone Phase I clinical trials in humans [48]. DNA vaccines have been evaluated as candidates against many emerging viruses, including EBOV [49], RVFV [50], Dengue Virus [51], CHIKV [52].

Replicon vaccines are based on defective RNA genomes that are able to undergo replication and express encoded proteins, but cannot produce infectious viral particles. Viral RNA replication is a strong inducer of the innate immunity and, therefore, replicon vaccines may have superior immunogenicity than the equivalent DNA vaccines [53]. Replicon vaccine candidates can be generated by removing essential structural genes from the genome of the pathogen such us West Nile Virus [54] or RVFV [55•,56], or by inserting in a replicon heterologous genes encoding antigens from a pathogen. By far, most heterologous replicon vaccines use alphavirus derived replicons (reviewed in [57]). Replicon vaccines can be delivered as propagation-defective replicon particles, or as plasmids containing the whole replicon sequence under the control of the appropriate promoter.

Conclusion

Emerging infectious diseases can present many challenges for vaccine development. Several novel vaccination strategies that have been developed in recent years can specifically address these challenges. Subunit vaccines, containing only part of the pathogen's antigens, can elicit protective responses that are different from those induced in the infected animal. Because they contain no infectious pathogen, there is no need for high bio-safety measures, risk of accidental escape during production, residual pathogenesis, or reversion to virulence in the vaccinated individuals. The use of well-defined vaccine platforms, with an extensive record of safety and efficacy against similar pathogens can speed-up the process of development, validation and production of vaccines against new emerging and potentially emerging viruses. However, many challenges still lay ahead. Specifically, each vaccine platform has advantages and disadvantages mainly related to their balance between safety and immunogenicity, and ability to be used multiple times. Studies that compare multiple platforms in humans are still lacking. Future research will be needed for the improvement of the safety and immunological characteristics of vaccination strategies.

Conflict of interest

The Icahn School of Medicine at Mount Sinai owns intellectual property in the influenza virus vaccine field, with AG-S being one of the inventors.

Acknowledgements

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- •• of outstanding interest

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