IJIR. 2019;(3)1:RA1 DOI: 10.15305/ijir/v3i1/279

REVIEWS

Acute inflammation and influenza: Innovative nanoparticle vaccination studies in a pig model

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Abstract

According to the reports of World Health Organization (WHO), around three to five million people are infected with seasonal influenza annually and approximately 250,000 to 500,000 people die worldwide. The risk of influenza associated complications are due to severe inflammatory reactions in the respiratory tract, and it is common in children <5 years, pregnant women, elderly individuals and people with chronic medical conditions. Influenza A virus (IAV) subtypes H1N1 and H3N2 are the major currently circulating viruses among humans. Vaccination is the most effective way to prevent influenza in humans. Influenza virus primarily infects epithelial cells lining the respiratory tract mucosa, and nasal virus shedding forms the means of viral transmission. Hence, induction of strong mucosal antibody and cell-mediated immune responses are beneficial for efficient protection during influenza epidemics and pandemics. But protection from currently used influenza vaccines varies from 10 to 60%, and it induces poor mucosal immune responses. Since inflammation associated with IAV infection is a 'double edge sword', control of infection-associated morbidity is essential. It is possible only through the use of potent intranasal influenza vaccines, which induce robust mucosal immune response and alleviates inflammation in the respiratory tract. This review article summarizes inflammatory responses triggered by IAV infection causing severe pulmonary disease, and the novel mucosal vaccination strategies, tested in a pig model, that have been showing promise to mitigate influenza induced immunopathology.

History of flu pandemics, swine flu human sufferings

IAV infection is a serious respiratory tract problem responsible for a great number of deaths and hospitalizations worldwide. This year marked the 100th anniversary of the famous 'Spanish flu' outbreak. It has been considered as the most devastating viral pandemic in human history responsible for death of over 50 million people.[1, 2, 3] After the 1918 outbreak, four pandemic IAV infections have been witnessed until now: 1957 Asian flu virus (H2N2), 1968 Hong Kong flu virus (H3N2), 1977 Russian flu virus (H1N1) and 2009 swine-origin flu virus (H1N1).[4, 5] It is apparent and has been accepted universally that swine is an intermediate 'mixing vessel' host, as it plays a central role in the reassortment of viruses of human, avian and swine origin, which eventually recombine to form pandemic virus.[6, 7] For example, the 1918 Spanish flu and the 2009 pandemic swine IAV (SIV) strains were present in swine for a very long period of time, before their emergence in humans.[1, 4] Thus, prevention of SIV transmission requires high priority. [8, 9]

Pathogenesis of influenza and inflammation

IAV primarily infects the epithelial cell lining of the entire respiratory tract in humans, some mammalian species and birds. The infection happens rapidly after exposure to virus, within few hours to a couple of days, depending on the pre-existing immune status of the host, releasing loads of viruses that reinfect other naïve cells.[10] The acute burst of virus in infected cells leads to the rapid simultaneous activation of many signal transduction pathways, resulting in robust inflammatory responses. Though such inflammation is required to attract the immune cells to the site of virus infection and activate the adaptive immunity for the efficient control of virus proliferation, the sudden burst of secreted cytokines and chemokines cause severe discomfort, lung damage, morbidity and even death. Many studies have reported a strong association between inflammation and severe cases of IAV infection.[12-14] Once inside the epithelial cells of the respiratory tract, IAV is recognized by the pattern recognition receptors (PRRs) of innate immune cells. The PRRs sense the pathogen-associated molecular patterns (PAMPs) present in

microorganisms including IAV. There are three types of PRRs that recognizes the danger signal of IAV infection: toll-like receptors (TLRs), nod-like receptors (NLRs) and retinoic acid-inducible gene-I-like receptors (RIG-I). All these receptors are activated during the IAV infection and replication, and act synergistically to activate transcription factors, including NF-kB and IRF3/7, resulting in rapid onset of proinflammatory response and local antiviral state.[15-17] Furthermore, respiratory tract epithelial cells constitutively express TLR3, which is activated during IAV infection, contributing to the production of pro-inflammatory chemokines and cytokines.[18] The NLRs are responsible for the cleavage of pro-caspase-1 in its active form, which cleaves pro-IL-1b and pro-IL-18 into IL-1b and IL-18 that contribute to the inflammatory response following IAV infection.[19] Overall, the signaling pathways, activated by all classes of PRRs, lead to the transcription of pro-inflammatory genes and production of large amounts of cytokines and chemokines that orchestrate the inflammation in the lungs during IAV infection. The equilibrium of the inflammatory response in the lungs is the determinant for the outcome of IAV infection.

Balance of adaptive immunity and 'cytokine storm' due to influenza

Proinflammatory cytokines, such as interferons, interleukins, chemokines and tumor necrosis factor, are the key molecules controlling the lung environment during IAV infection.[20] These molecules are responsible for the communication between immune and non-immune cells, and can drive several important response steps against IAV, including epithelium activation, leukocyte recruitment, cell proliferation and differentiation and development of adaptive immunity.[20] Proinflammatory cytokines and chemokines activate and recruit leukocytes into the lungs and airways, and these cells can produce large amounts of these molecules in a positive feedback loop. If this cycle is not controlled, it can lead to the exacerbation of the inflammatory response. The systemic presence and large levels of these signaling molecules lead to an event known as 'cytokine storm', which is one of the causes of increased mortality during severe IAV infections.[21-23] The cytokine storm is correlated with the emergence of severe clinical symptoms, including alveolar hemorrhage, acute pneumonia, extensive pulmonary edema, acute respiratory distress syndrome and death.[12]

Current flu vaccines and promising nanotechnology-based vaccines

Currently used flu vaccines induce virus strain-specific immunity against homologous virus infections. They are delivered by intramuscular route and thus provide weak mucosal immunity in the respiratory tract. Moreover, every year we need to match the dominant circulating virus strains in the vaccine formulation. If there is any mismatch in the vaccine virus, it can lead to severe inflammatory consequences especially in children, aged people and individuals with immune deficiencies. Therefore, developing a potent intranasal mucosal vaccine, which provides local immunity in the respiratory tract and increases the breadth of cross-protective immunity is highly warranted.

Nanotechnology is an important endeavor of the 21st century. Nanometer scale materials have favorable physicochemical properties for mucosal vaccine delivery, as their size, shape, charge and composition could be designed.[24-27] Several biodegradable and biocompatible natural and synthetic polymers are approved by US Food and Drug Administration and European Medical Agency for drug delivery.[26-31] Soluble vaccine antigens are poorly immunogenic, but it can be made highly immunogenic by entrapping in polymer-based nanoparticles.[32,33] Induction of immunity by nanoparticle-delivered (<500 nm) vaccine antigens is mediated through its particulate nature, efficient internalization, processing and presentation of antigens by professional antigen presenting cells such as dendritic cells, macrophages and B cells. [34-37]

Promising candidate influenza nanoparticle vaccines studies in a pig model

Similar to humans, pigs are natural hosts for influenza and get infected by similar IAV subtypes such as H1N1, H3N2 and H1N2. Vaccines promoting protective immunity in pigs could help reduce/block the transmission of SIV to humans. The pig lung has marked similarities to that of humans in terms of tracheobronchial-tree structure, airway morphology, abundance of airway submucosal glands, and in producing cytokines and chemokines.[38-40] The functions and electrophysiological properties of the pig airway epithelium and submucosal glands resemble to that of humans.[41-43] The pig genome and protein sequence share high homologies (>80%) with the human counterparts, in contrast to mice (<10%). Porcine immune responses more closely resemble human responses than mouse responses with >80% of parameters studied. Mice showed only <10% similarity to humans.[44, 45] The availability of swine genome sequence and genetically modified pigs has further increased the use of the pig model in biomedical research.[46-51] Like humans, pigs are outbred species, and pig models have been in use in research on several human

diseases.[45-61] In summary, because of the anatomical, genetic and immunological similarities between pig and humans, pig can recapitulate pathogenesis of flu and specific mucosal immunity in the respiratory tract of humans.[61-63] To demonstrate cross-reactive immunity and reduce inflammation in the lungs, we developed and evaluated a few biodegradable and biocompatible polymer nanoparticle-based vaccine delivery platforms in the pig model.

Influenza virus-conserved peptides have the potential to elicit increased breadth of immunity. But without the help of potent adjuvant and delivery system, they are poorly immunogenic. Biodegradable polylactic-co-glycolic acid (PLGA) nanoparticle-based vaccine was shown to enhance cross-presentation of antigens to CD8+ T cells by dendritic cells.[36] In a study, norovirus P particle containing SIV M2e (extracellular domain of the matrix protein 2) chimera and highly conserved two each of H1N1 peptides of 2009 pandemic and classical human influenza viruses were entrapped in PLGA nanoparticle. Pigs vaccinated with this vaccine formulation and challenged with a virulent and zoonotic SIV H1N1 had no clinical fever and any signs of flu. This candidate vaccine significantly increased the frequency of antigen-specific IFN-γ secreting CD4 and CD8 T cells in the lung lymphocytes.[65] Thus, our initial study in pigs demonstrated that influenza H1N1 conserved peptides cocktail entrapped in biodegradable nanoparticle, delivered intranasally as mist, induced epitope specific effector and memory T cell responses. However, it failed to induce the mucosal and systemic antibody response.

In another study, with a goal to improve the antibody response, we used killed SIV H1N2 entrapped in PLGA nanoparticle instead of peptides and delivered as intranasal mist to pigs.[66] In vaccinated and virulent heterologous SIV H1N1 challenged pigs, clinical flu signs were absent. This was associated with reduced gross and microscopic inflammatory lung pathology and reduced viral antigenic mass in the lung sections with clearance of infectious challenge virus in most of the nanoparticle vaccinated pig lung airways. Immunologically, our candidate PLGA-based vaccine, irrespective of not boosting the mucosal antibody response, augmented the frequency of IFN-Ɣ secreting total T cells, T-helper and cytotoxic T cells against both the vaccine and challenge SIV. Both these studies using PLGA nanoparticle delivery system demonstrated that intranasal vaccination using potent particle flu vaccine could induce strong cytotoxic T cell response and cross-protection against IAV-induced lung inflammation. Since the robust cytotoxic T cell response is capable of providing heterosubtypic immunity in influenza infections, PLGA-based vaccination approach forms an ideal platform to use the pig model for translation of particulate candidate flu vaccine to effectively control flu-induced inflammation in humans. However, upper respiratory tract infection and nasal virus shedding was not reduced due to the lack of induction of mucosal secretory IgA by PLGA particle delivery system. In yet another study with the goal to augment mucosal IgA response against IAV antigens, we adapted the novel mucoadhesive chitosan nanoparticle vaccine delivery platform to administer killed SIV H1N2 as intranasal mist.[67] We evaluated the immune response and cross-protective efficacy. Interestingly, pigs vaccinated with chitosan particle vaccine enhanced both serum IgG and robust mucosal secretory IgA antibody response in the nasal passage and in the lungs reactive to homologous (H1N2), heterologous (H1N1) and heterosubtypic (H3N2) IAV strains. In animals challenged with a zoonotic and virulent heterologous SIV H1N1 reduced severity of macroscopic and microscopic influenza-associated inflammatory pulmonary lesions was noted. Importantly, the infectious SIV titers in nasal passage and lungs were significantly reduced. In addition to B cell response, the T cell response was also elicited in pigs received chitosan particles system, but it was not as robust as PLGA particle delivery system. The increased T-cell response was detected especially in the lung draining tracheobronchial lymph nodes of pigs. Thus, our study revealed that chitosan-based influenza nanovaccine may be an ideal candidate vaccine platform, and pig is a useful animal model for preclinical testing of particulate intranasal human influenza vaccines. This vaccination strategy may help to mitigate inflammation in the respiratory tract and transmission of genetically variant emerging field viruses to susceptible people in the real field scenario.

Conclusion

Influenza-induced acute inflammation leading to severe morbidity and mortality can be mitigated using novel intranasal vaccine delivery platforms, which induce robust local mucosal immunity in the respiratory tract. Swine is an ideal animal model system to conduct intranasal flu vaccine preclinical trials to successfully translate the novel human flu vaccine formulations.

Competing interests

The author declares that he has no competing interests.

Citation

Renukaradhya GJ. Acute inflammation and influenza: Innovative nanoparticle vaccination studies in a pig model. IJIR. 2019;3(1):RA1.

Submitted: 5 September 2018, **Accepted:** 25 September 2018,
Published: 28 January 2019

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