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Review Article

Strategy construction to minimize the limitation of respiratory viral vaccine development

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ABSTRACT

Recent outbreak by the coronavirus SARS-CoV-2 is a major global public threat. Similarly, for several years other coronaviruses, RSV or Influenza viruses are also equally showing risk to the worldwide population. Therefore, several countries have been given tremendous efforts to generate an effective vaccine against respiratory viral infections. It is very important to understand the attributes of a protective mucosal antiviral immune response for the development of a vaccine for respiratory viral infections. Characteristics of the mucosal immune system and evolution of the mucosal vaccine play an important role in protection against respiratory viral infection. Memory CD8 T cell populations play a crucial role in making high levels of gamma interferon and tumour necrosis factor may be essential for protection. Whereas developed vaccines of respiratory infections continue to fail in effectively generating long-lived protective immunity. Hence, memory CD8 T cell can elicit long-lived immunity, and immunostimulatory adjuvants such as OX40, OX40L or IL12 can enhance the memory CD8 T cell. Viroporin on the other hand use as a vaccine candidate to avoid viral mutation, as a result, the present review work was constructed for a novel combination i.e., immune adjuvant with newly viral antigenic gene or vaccine candidate that can fulfill the limitation of vaccine development for respiratory infection.

1. Introduction

Respiratory viral (RV) vaccines development is very much challenging due to lacking long-term immunity, diverse antigenicity or regular mutation and different adverse reactions after vaccination. There are two RV vaccines already commercially available one for the SARS-CoV-2 vaccine and the other one is the influenza vaccine. However, some observations were found by researchers [1,2] that decline in influenza vaccine-specific antibodies and the antigenic drift of influenza viruses over time consequently necessitates annual revaccination. On the other hand, vaccination of different SARS-CoV-2 vaccines is going on an emergency basis. Thus, the actual result after immunization is not clear, however, every manufacturer claim that these vaccines are effective against disease outbreak. Whereas many articles were showed that the vaccines have some adverse reaction and death also occurred after vaccination. Different studies showed that lacking long-term immunity in this respiratory vaccine is the key problem.[3,4] Vaccines also need to be generated against respiratory infections like RSV or a common cold, so different studies are going on to generate a new potential vaccine.

Here, we collected the different evidential literatures for discussion to established novel hypothesis- 'use of immune-stimulatory components or adjuvants with viroporin as a vaccine candidate against viral lung infection for the improvement of vaccine efficacy'.

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2. Requirement of Long-term Immunity for RV Vaccine

The mucosal surface of the respiratory system serves favourable condition for both innocuous environmental antigen and human pathogens because the system is ideal for gaseous exchange due to the physiological requirement of the body. For this reason, the mucosal surface contains a complex array of immune regulatory mechanisms which protect healthy individuals, a quiescent and non-inflammatory environment that maintains optimal tissue function.[5–7] Vaccines to be successfully used for boosting and long- term immunological protection characteristics against bacterial respiratory infection such as Bordatella pertussis, Corynebacterium diphtheriae, Haemophilus influenzae type b (Hib), and Streptococcus pneumonia.[8,9] Whereas different viral respiratory vaccine development is quite problematic and also in maximum cases the approach of vaccines has failed towards the pathogens like the influenza virus, parainfluenza virus, RSV, meta-pneumonia virus, SARS-CoV, rhinovirus, measles, and adenovirus.[7] The continuous mutation is the major difficulty to develop an effective vaccine against many intracellular pathogens and resulting in immunological escape from any induced specific immunoglobulin response. The solution to this dilemma is to develop effective vaccines i.e., a combination of immunoglobulin and a long-lasting memory CD8+ T-cell response must be generated.[10,11] When vaccine needs to develop based on long-lasting memory CD8+ T-cell then automatically importance of memory subset (T_{CM} vs T_{EM}) frequency and longevity of memory subsets. Route of immunization also is raised as a big priority for a long lusting vaccine. It was observed in several studies that in most cases protective T cell immunity does not survive for more than a couple of years after the resolution of natural infection or vaccination. Immunity response against boosting up when re-exposure to the same pathogen or cross-reactive antigens is happened.[7] Studies also showed in the case of Influenza or Sendai viral infection in an animal model that the efficacy of protection going to decline form rapidly within three to six months against secondary infection. The secondary infection is always generated after primary infection. Virus-specific memory CD8 T cells in the lymphoid tissues (T_{CM} cells) may not respond, expand, or relocate (to the lung) sufficiently as well as quickly to provide immediate protection against disease caused by reinfection or live pathogen challenge or post-vaccination.[12,13] Some findings demonstrated that the number of T_{CM} cells can be sustained in the absence of both specific antigen and MHC molecules.[14,15] Another fact is that T_{EM} cell populations in the lung airways are maintained in an antigen-independent manner by continual recruitment of new cells from the circulation.[16,17] After infection or vaccination phenotypic and functional changes within the circulating Ag-specific CD8 T cell pool have happened. After that rare naïve CD8 T cells that identified cognate Ag and robustly proliferate. Finally, give rise to an effector CD8 T cell population. Studies reported the properties of cells comprising the Ag-specific CD8 T cell pool, including expression of phenotypic markers. Hence subset representation, showing an ability to localize within tissues and execute the effector functions. The circulating Ag-specific memory CD8 T cell population is also containing a more homogeneous population of Tcm cells and with enhanced proliferative capacity.[18]

Whenever influenza virus infection can migrate to the airways researcher showed that a small number of circulating memory CD8 T cells generated. Therefore, from a T cell-mediated point of view, memory T cell recruitment to the lung airways is controlled by both antigen-independent and antigen-dependent mechanisms immediately following virus clearance. After residual antigen clearance the number of memory CD8 T cells localized within the lung airways stabilizes at a low level therefore, antigen-independent processes are required to maintain this population over time.[19] An interesting fact is that based on this evidence the use of conventional non-replicating attenuated vaccine strains will fail to induce a robust and long- lived T_{EM} population and therefore have limited application in the design of future vaccines.[7]

3. Effectiveness Comparison between different types of Vaccine

While study[20] showed that live attenuated vaccine is much more immunogenic other than modern different kind of vaccine, therefore, the lowest concentration of immunogens of live attenuated vaccine per dose is required.[21] Describe that some drawbacks of live-attenuated vaccine can exist. Viruses with a high mutation rate are unsuitable candidates for such a vaccine type, as reversion may occur during vaccine production. So, the potentially reduced replication of attenuated virus strains in cell culture, which would lower process yields. Comparatively vector-based vaccine technology is already applied in some respiratory vaccine development because recombinant vaccines allow the safety requirements (Table 1), but are often less immunogenic and therefore require higher antigen concentrations per dose.[21,22]

4. Viroporin act as Vaccine Candidate or Antigenic Site of Vaccine

Table 2 represented the description and function of different respiratory viral viroporin. Viroporin of such respiratory viruses (Table 3) can be identified as a new antigenic site to develop a vaccine.[1,4] From the study of,[23] it can be established that mutations in the transmembrane region of viroporin (E protein) of SARS-CoV-2 keeping the PDZ-binding motif (PBM) intact could provide the basis for

Table 1: Effectiveness comparison between different types of vaccine

Type of vaccine	Principle	Immunogenicity level	Comment	Production platform
Live-attenuated vaccine	Attenuation during multiple passages or under non-physiological conditions. Whole virus replicates at low level in vaccinated patients	Very High	Risks of reversion Immunocompromised patients may develop infection Often requires cold chain storage. Details of attenuation often unknown	Mammalian and avian cells
Inactivated Vaccine	Whole live virus is chemically inactivated	High	Risk of incomplete inactivation Highly pathogenic live viruses require increased biosafety Often requires booster/multiple doses Inactivation may have a negative impact on antigenic structures	Mammalian and avian cells
Recombinant Vector Vaccine	Attenuated or recombinant viral vectors express viral antigens in the vaccinated person, Chimeric vectors display recombinant viral epitopes on their surface	High	Low biosafety risk as antigen is expressed by non-replicating vectors Vector antigens can boost immune response First human vaccines in clinical trials	Mammalian, avian, and insect cells
Recombinant subunit vaccine	Recombinant expression of viral genes to produce viral proteins or virus-like particles	Medium	No infection risk for patients due to absence of viral genome Absence of some viral elements may reduce immunogenicity	Mostly insect cells and CHO
Split sub unit vaccine	Whole live viruses are disrupted by detergents (split vaccines) and further purified (subunit vaccines)	Low	No infection risk due to virus split Highly pathogenic live viruses require increased biosafety Disruption of viruses may negatively affect the structure of antigens	Mammalian and avian cells

the development of live-attenuated vaccine and inactivated vaccine. Maximum studies try to build up the vaccine of SARS-CoV-2 based on the Spike or viral RNA-polymerases but also some studies are there to make a center of attention in another way to resolve the limitations of vaccine or improve the potentiality of vaccine that involve viroporin E as a vaccine or drug target candidate.[4,24] Depend on the in-silico studies[25,26] interesting facts were coming out like a high degree of identity (94.7%) between the sequences of the E proteins of SARS-CoV and SARS-CoV-2 and the finding strengthened the hypothesis that the two proteins have a conserved function, as proteins with high sequence identity that is why high structural similarity are likely to possess functional similarity with evolutionary relationships.

Based on the immunological point of view through immuno-informatics analysis[4] predicted that four discontinuous B-cell epitopes along with 1 linear B-cell epitope and 11 T-cell epitopes were found to fulfill the criteria of safety and effectiveness for vaccine design. Some other finding also strongly supports for the E protein as an effective vaccine because T-cell epitopes are much more reliable than that of B-cell prediction mainly because the majority of B-cell epitopes are conformational, and thus they cannot be isolated from the protein structure.[28]

Limitations of Influenza vaccines that are found in a maximized way such as vaccines have narrow specificity and may not have cross-reactivity, even within a single subtype. The significant interest therefore developed is that, to generate a novel kind of vaccine which can fulfill the

Table 2: Summary of respiratory viral viroporins and their proposed functions in the virus life cycle [27]

Virus	Viroporin	Function	Role of viroporin in activation of NLRP3
Influenza A virus (IAV)	M2	In endosomes, it imports hydrogen ions (H+) into the virions and helps to release viral ribonucleo capsid to the cytosol. Neutralizes the trans-Golgi network pH and prevents hemagglutinin from becoming fusogenic.	Ion channel activity of M2 enables H+ export from acidified Golgi, and such activity provides the second signal required for the activation of NLRP3. M2 affects ROS production and K+ efflux which affects IL-1β production and can be blocked by high concentration of extracellular K+ or by adding ROS inhibitors.
Rhino virus	2B	Forms homomultimers that create pores in ER and Golgi complex membranes, thereby reducing the levels of Ca2+ and H+ in the lumens of these organelles in infected cells.	IL-1β was not inhibited by inhibitors of mitochondrial ROS and cathepsin B, which effectively blocks ATP- and Alum induced IL-1β secretion
Respiratory syncytial virus (RSV)	SH	SH gets localized in the cell membranes and intracellular organelle membranes, and changes permeability by disrupting membrane architecture. SH protein is important for viral infectivity, its exact role during viral infection is not clear. Some studies suggest an ancillary role in virus-mediated cell fusion.	Accumulates in the Golgi network within lipid raft structures, forming ion channels selective for monovalent cations (Na+ and K+), which triggers the translocation of NLRP3 from the cytoplasm to the Golgi network and its subsequent activation.
Corona virus	E protein 3a protein	E protein generates an oligomeric structure that forms an ion conductive pore in planar lipid bilayers. 3a protein modulates virus release; however, this protein is not essential for virus viability.	Causes cations imbalances that can be sensed by NLRP3 inflammasome

limitations. The vaccine would have a longer and wider spectrum of action.[29] To avoid the annual revision of the vaccine is also a critical criterion to the development of vaccine thus studies showed that conserved viroporin (M2) maybe act as a vaccine candidate for influenza.[1] Lamb and Choppin were first described and characterized about the M2 protein in the year 1985.[30] The transmembrane M2 protein mainly translated from spliced variant mRNA coding for the matrix protein M1. Already many studies reported that recombinant M2 viroporin dependent vaccine and some of them describe the mechanism of vaccine preparation through partially purified an M2-containing membrane fraction derived from a recombinant insect-cell expression system.[31] In addition to this, it is also a fact that more recent vaccination studies using full-length M2-protein were either based on gene vaccination or viral vectors encoding M2 and, thereby, not only circumvent the difficult task of purifying membrane-anchored M2 for use as a vaccine antigen, but also induce cellular immune responses.[32,33]Table 3 showed the overall studies of M2 based vaccine.

5. Immunostimulatory Adjuvant

Immunostimulatory adjuvant (e.g., OX40, OX40L, or IL12) has the potential to boosting immunity or plays a major role in long lasting immunity after vaccination. If this hypothesis will get successful or applicable in SARS-CoV-2 and influenza then it will become a novel form of immune intervention that specifically targets the activated T cells alone. As the well-known reality that antibodies can provide protection against viruses but it is also an obvious picture with the aim of T cells much more important for limiting infection because cellular memory

Table 3: Use of viroporin and immune-stimulatory adjuvant in different vaccine development trial

Viroporin as vaccine Antigenic site	Influenza Vaccine type	References	Immuno-stimulatory Adjuvant	Vaccine Type	References
M2	Protein DNA Vaccine Adenoviral Vector	1	αOX40	Combination of a Sindbis-SARS-CoV-2 spike vaccine	34
M2e	Peptide		OX40	1. live-attenuated Plasmodium sporozoites for malaria infection 2. Recombinant Rabies vaccine 3.poxvirus/ vaccinia/sindbis vector-based RV vaccine	35,36
M2e	Virus like particle		IL12	1. Plasmid DNA vector-based vaccine against SARS-CoV-2 infection	37
M2e	Protein		Interleukin (IL)	1. Use as an adjuvant against Influenza A virus	38
M2e	Synthetic				
M2e	DNA				

provided by T cells. Present research supports the idea of more centralized memory T cells that circulate throughout secondary lymphoid organs will not respond, expand in number, or relocate sufficiently as well as quickly to provide immediate protection against disease caused by reinfection. Based on the hypothesis memory T cell that populates peripheral organs, such as the lung and gut.[39,40]

5.1. IL12

Mucosal immunity playing as a crucial role to prevent respiratory infection. Mucosal memory T cell immunity is very much significant for successful RV vaccine. Initially, the innate defense system including mucosal tissue as well as alveolar macrophages rather than lymphocytes is reacted against respiratory infection. The upper respiratory system has two major tissues such as nasal-associated lymphoid tissue (NALT) / bronchus-associated lymphoid tissue (BALT) which are make primary protection against infectious agents.[41] Interleukin such as IL 6 and IL 12 support either Th1 (IL-12)- or Th2 (IL-6)-type responses to generate instant mucosal immunity as well as a memory cell. This is the sportive evidence that IL 6 and or IL 12 can serve as an adjuvant for enhancement of mucosal immune responses.[42] Different microorganisms forced to induction the instant immunity i.e., IL-12 is quickly produced by antigen-presenting cells.[43,44] Many shreds of evidence provide the support that IL 12 has the influence on T-dependent (TD) antibody responses and also on T-independent (TI) Antigen's responses. IL 12 particularly goes through the two ways for influencing the B-cell. In the first step where IL12 stimulates Th1 and NK cells to secrete large amounts of IFN-γ which then causes B cells to switch to γ2a and γ3. Then, to produce increased amounts of antibodies in an IFN-γ-independent manner through stimulating the post-switched cells. Where IL-12 binds to receptors expressed on plasmablasts and enhances the production of the switched isotypes.[41] An interesting observation was found by some researchers[45–47] that because of direct stimulation of B cells after the post-switch signal activated murine and human B cells bind IL-12 and express transcripts for both β1 and β2 chains of the IL-12 receptor.

T-independent antibody responses where IL-12 may be capable of influencing humoral immunity in the absence of T cells. The process is only completed whenever activation of NK cells and subsequent secretion of IFN-γ, or by direct stimulation of specific B cells through binding to the B cell IL-12R. Researchers also described that NK cells can activate B cells to produce IgG2a antibodies to TI antigens.[48,49]

In secondary lymphoid organs at the time of CD8 T cell generate IL 12 has been shown to promote strong effector function and memory development and also enhance TCR - induced proliferation, IFN-γ production, or the cytotoxicity of T cells.[50,51] A very interesting observation was found that IL 12 increases the CD8 T cell response to TCR stimulation after previous exposure. Additionally, it was also noted that the IL-12-mediated increase of human activated CD8 T cells responses to further TCR stimulation required pre-treatments with IL-12 of at least 24 h.[52]

These all findings suggested that the IL12 can enhance the T cell dependent and T cell- independent including

CD8 T cell immunity. Therefore, IL12 may act as an adjuvant for minimizing viral respiratory infection. Based on this hypothesis recently CORVax12 is made to prevent the SARS-CoV-2 infection. The vaccine candidate mainly depends on plasmid DNA vector based where spike protein and IL12 encoded gene are composed with plasmid. It is also well informed that the vaccine is under clinical trial (Phase1) and results showed very safe and effective against SARS-CoV-2 infection.[37,38] reported that Interleukin can be used as an adjuvant against the Influenza A virus. Another successful study was performed by[53] where a vaccine was used with the adjuvant IL12 against tuberculosis.

5.2. OX40 (CD134) and OX40L (CD252)

OX40 (also known as ACT35, CD134, TNFRSF4) is a type 1 transmembrane protein of 249 amino acids, with a 49 amino acid cytoplasmic tail and a 186 amino acid extracellular region. Whereas OX40's ligand (OX40L, also known as gp34, CD252, TNFSF4) is a type II glycoprotein with a 23 amino acid cytoplasmic tail and a 133 amino acid extracellular domain.[54] Study[55] gave clear confirmation that the primary cell that is quoted most often as expressing OX40 is the activated T cell. It also stated that through old studies OX40 was T cell specific or restricted to CD4 T cells only and on occasion restricted to Th2 cells. According to[56] the basic principle of OX40 (CD134) is absent in naïve T cells but it became activated after 1-2 days of antigen activation. OX40–immunoglobulin fusion proteins block the interaction of OX40 with its ligand on antigen-presenting cells and eliminate weight loss and cachexia without preventing virus clearance. Reduced proliferation and enhanced apoptosis of lung cells accompanied the improved clinical phenotype. Manipulation of this late costimulatory pathway has clear therapeutic potential for the treatment of dysregulated lung immune responses.

On the other hand, Researchers also showed that APC cells can promote OX40L, including signals through CD40, membrane B cell receptor, and several Toll-like receptors (TLR2, 4, 9), as well as inflammatory cytokines such as TSLP (thymic stromal lymphopoietin) and IL-18.[54,57,58] nicely described based on several studies that for the strong impact of OX40 in regulating CD4 T cells through intracellular molecule those are engaged and targeted by OX 40. Different adaptor molecules such as TRAF 2 and 3 which are activated the both the canonical (IKKβ-dependent) NF-κB1 pathway as well as the noncanonical (NIK/ IKKα-dependent) NF-κB2 pathway. The TRAF 2 and 3 are the combination of TNFR-associated factors and also bind with OX 40 which being the principal molecule that can be recruited to the cytoplasmic tail via a QEE motif that is present in other family members. Studies also showed that OX40 strongly contributes to the overall level of NF-κB1 activation in T cells and transfection systems of NF-κB1 activity after crosslinking OX40 in which CD4 T cells respond to antigen. The opposite point of view was established where CD4 T cells that lack OX40 never keep high levels of several antiapoptotic Bcl-2 family members including Bcl-2, Bcl-xL, and Bfl-1, and the finding directly correlates with reduced NF-κB1 activity.[59,60] Fascinatingly, CD4 T cells that cannot receive OX40 signals when decreased activity of Akt (protein kinase B) and NF-κB1 has occurred and forced expression of an active version of Akt restored defective expansion and survival of OX40-deficient T cells when responding to antigen.[35] Antigen-independent signal could be provided by sources of OX40L which might include LTi cells, B cells, and responding T cells themselves for the support of T cell survival at the late effector phase of immune responses. Simultaneously it can visualize that in presence of antigen ligation of OX 40 provides additional signals and the signal are completely dependent on TCR signaling. It indicates that one of which is focused on enhancing activation of the Akt pathway. After Akt activation enhancement would be provided initial antigen-driven proliferative signals that further synergize with NF-κB1-driven growth and survival signals.

The activity showing directly OX40-OX40L interactions in regulating the CD4 and CD8 T cells along with the insights into augmentation of NK and NKT cell activity. Therefore, clearly[54] suggested that OX40 has a potential ability to act as an adjuvant that could be used as a target in vaccination strategies or therapeutic applications to promote protection against pathogens. Several clinical finding directly support this theory i.e. pulmonary growth of Cryptococcus neoformans, treatment with a stimulatory OX40L.Ig fusion protein promoted fungal clearance, in case of killing the Leishmania donovani through Immunotherapy with OX40L. Ig in combination with anti-CTLA4 enhanced CD4 T cell responses and very importantly notified during vaccination where several vector based vaccines (e.g- Recombinant Rabies vaccine, vaccination with the attenuated malaria parasite, poxvirus/ vaccinia/sindbis vector-based RV vaccine) used with costimulatory OX40 alone or with another TNFR family member to expressing a nominal antigen strongly enhanced memory T cell responses to that antigen[34–36] showed on their animal model studies that overexpression of OX40L and increase the immunogenicity because of costimulatory activity with a vaccine against Rabies virus is occurred. Therefore, they recommended improving the vaccine potentiality that OX40L can be used as an adjuvant. Same as we had seen when on study,[36] that they immunized live-attenuated Plasmodium sporozoites for malaria infection and used OX40 as a costimulatory immunotherapeutic agent to enhance T cell. Their result showed that not only sporozoites specific antibody Ig was increased parallel picture also shown that antigen-experienced effector ($CD11a^{hi}CD44^{hi}$) CD8+ and CD4+ T cells in the liver and spleen and also increased IFN-g and TNF producing CD4+T cells in the

liver and spleen. Other than these studies if we focus only on viral respiratory infection here some interesting studies exist where OX40 and OX40L play a crucial role to increase immunogenicity with a vaccine.

6. Conclusion

This review indicated that based on several pieces of evidence the necessity of long-term immunity from vaccination is very crucial to overcome the limitation of vaccine generation against different viral respiratory infections. Therefore, to achieve the above-mentioned necessity we also discussed the solution where different studies proposed that some modifications should be required in vaccine production. Thus, the modification may be defined as viroporin act like an antigenic site or vaccine candidate and use of costimulatory immune adjuvant such as OX40/ OX40-OX40L or IL12 also require for vaccine formulation. Although it is established here with the support of different studies that Live-attenuated, Inactivated, or Vector based vaccine whatever suitable for viral respiratory infection this type of modification or formulation may be applied to get the long- term immunity.

7. Conflict of Interest

The authors have no conflicts of interest.

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