New and improved influenza vaccine

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1. Introduction

While influenza has been suspected to be a cause of millions of death throughout the centuries, the 1918-1919 pandemic is particularly notable. This "Spanish flu", one of the early historically recorded influenza outbreak, killed about 20million people worldwide in the space of 4 months. The most recent influenza pandemics were recorded in 1968(Hong Kong influenza) and in 1977. More frequent type of influenza infection, influenza epidemics, continue to afflict many people, causing widespread morbidity especially in the young and the elderly, with a significant economic impact.

The severity of the influenza infection greatly accelerated studies on the pathogenicity and immunity of the viral infection. One of the factors that causes difficulties in controlling the disease is genetic variation: The antigenic changes of two viral surface antigens, the haemagglutinin(HA) and neuraminidase (NA) outperform the immune surveillance of the infected host. The variation is known to occur either as a result of genetic mutation and selection(antigenic drift) or by complete replacement of the HA and NA gene with those of another virus(antigenic shift).

Influenza vaccine has been available for the protective immunity against the infection, but with limited efficacy. To increase the cross-protection, influenza vaccine is formulated by combining two different influenza A strains and one B strain. Currently available vaccines are made either by formalin-inactivation of whole influenza virus or by purification of the surface antigens(subunit vaccine). These vaccines provide only variable degree of protection against the circulating virus strains. Therefore, efforts are being made to augment the immunogenicity of these vaccines either by a good selection of adjuvants or by developing a live attenuated vaccine. Development of live virus vaccine will clearly improve the vaccine efficacy inducing a better humoral and cell-mediated immune response providing a longer lasting protection against pathogenic viruses. Recent advances in reverse genetics of the influenza virus have made a step forward to this exciting possibility[1,2].

2. Rational Design of Novel Influenza Virus Vaccines

With a powerful tool now available for genetic manipulation, developing a live influenza vaccine by guided mutation has become a real possibility. Specific mutation into the pathogenic signals will, in theory, lead to attenuation of the virus, which will be suitable for human immunization. Using an ingenious selection

protocol[3], the 3' and 5' non-coding sequences derived from an influenza B strain was transplanted into the NA(neuraminidase)

RNA of an influenza A strain[4]. This A/B chimeric virus was found to be significantly attenuated and protected the mice against a virulent challenge virus. Further analysis revealed that the NA-specific RNA was significantly lowered relative to the other seven RNAS, suggesting that the non-infectious particles lacking the NA segment are the cause for attenuated infection[5]. Further dissection of the mutation showed that base pair(s) in the panhandle domain was responsible for the attenuation.

Interestingly, this mutation is located ust in-between the canonical promoter element and the poly (U) stretch where polyadenylation occurs. It s likely that the segment specific nucleotides(usually 3-5) which bridge the promoter and the termination signal may be important for a coordinate control for initiation and termination of the particular RNA segment. Mutations in this region might have subtle effects on the overall dynamics of the RNA synthesis of the particular segment. This would cause the attenuation of the pathogenicity without significantly impairing the viral growth. This attenuation procedure, i.e., by generating defective particles, may provide a novel way of producing a live influenza vaccine with increased efficacy.

All influenza RNA genomes carry conserved sequence elements at both the 3' and the 5' end of VRNA. Strong conservation of these sequences may be important for transcription, replication and encapsidation of virion RNA. Recently, an interesting mutant virus was generated which carried a C4 $\Rightarrow$ U4 single base mutation at the 3' end of the NA VRNA. As compared to the wild type, the mutant showed an interesting change in temporal regulation of influenza RNA synthesis . This virus also showed an elevated ratio of NA/HA on the surface of the virion as compared to the wild type[7]. Therefore, it will be interesting to test if the mutant elicit different spectrum of immune repertoires and protective immunity as compared to the wild type.

3. Prospects

The recent advances in reverse genetics facilitated the generation of mutant viruses with altered phenotypes. This could serve as a strategy for developing a live attenuated vaccine against a variety of diseases. There are some key issues that should be addressed before using the genetically attenuated virus for human vaccination. irst, the genetic modification should be stable enough to prevent a reversion to cause disease. Second, the attenuation should not cause an impaired growth of the virus in the eggs where current influenza vaccine strains are grown. More research activities will be focused on these issues to engineer the virus with a desired degree of attenuation and immunogenicity.

4. Summary

Despite continued efforts to improve vaccines and antivirals, influenza virus remains an essentially uncontrolled infectious agent causing frequent outbreaks of epidemics and pandemics. To better understand and curb the disease, a good experimental system has been in need to investigate the parameters that control the pathogenicity of the virus. Unlike DNA viruses or positive-sense RNA viruses, the genomes of the negative-sense RNA viruses such as influenza are not infec-

tious by themselves, and therefore, have been refractory to genetic analysis.

A breakthrough in genetic analysis and engineering of the negative-sense RNA virus has been achieved with the recent advances in reconstituting the influenza ribonucleoprotein(RNP) complex in vitro from the influenza polymerase proteins and the negative-sense virion RNA. By transfection of the in vitro assembled RNP complex, genetically modified RNA genome could be introduced and rescued into the infectious virus particle. This combined in vitro and in vivo approach not only enables the elucidation of the sequence and structural features of RNA signals for gene replication and encapsidation but contributes to a better design of antiviral compounds. Similar 'reverse genetics' approach is being actively pursued in related negative-sense RNA viruses.

The influenza vaccines currently in use are chemically inactivated to eliminate their pathogenic effect. There has always been an interest in live vaccine for reasons of better immunoprophylaxis against influenza infection. Now, the current genetic technology to introduce an "attenuation character" into the virus by gene rescue should provide a precise means to develop a live influenza vaccine. Furthermore, the ability to generate chimeric viruses with foreign epitopes grafted onto the viral surface antigen should be promising in using the influenza virus as a vaccine vector for other infectious diseases.

Reference

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