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Control of Influenza: Live Vaccine Development

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Despite various efforts on improving vaccines and antivirals, influenza epidemics continue to afflict many people, causing widespread morbidity and mortality in the young and the elderly. Since the discovery of the unusual 'cap-stealing' mechanism of transcription, significant advances were made on molecular aspects of influenza gene regulation. This provides new insights for developing new antiviral compounds. Reverse genetic technologies have also been advanced for generating recombinant chimeric viruses suitable for designing live vaccine.

Influenza virus belongs to segmented negative -sense RNA virus, and uses RNA as replication intermediate. During virus infection , three different processes are temporally regulated: (i) transcription of (-)vRNA into (+)mRNA, (ii) replication of (-)vRNA into (+)cRNA, and (iii) replication of (+)cRNA into (-)vRNA. All these processes are catalysed by the RNA-dependent RNA polymerase, which consists of three different subunits, PB1, PB2, and PA. Recent breakthroughs on reverse genetic systems provides experimental setting by which trans- and cis-acting signals required for influenza genome replication and transcription could be analysed in vitro and in vivo. The promoter and terminator elements have been defined, and the model for RNA transcription is now proposed. The ability to dissect gene replication in two different modes of RNA synthesis, both at initiation and elongation, would facilitate screening and design of new antivirals at the step of gene replication.

Within the sequence motif conserved at the extreme ends of the influenza vRNAs, a unique natural variation, U or C, is observed at position 4 of the 3' end. Our result suggests that this position 4 nucleotide is a genetic determinant for the repertoire of surface antigen and their ratio could be changed without detrimental effects on virus growth. The results could be usefully applied in designing genetically engineered influenza virus for vaccination. The

observed down-regulation of transcription by C4 nucleotide is consistent with its potential role in segment-specific regulation of influenza gene expression, especially of PB1, PB2 and PA proteins, during influenza infection.

Since both HA and NA are involved in protective immunity of influenza infection, the ability to change the repertoire of the two surface antigens could be usefully applied to improve the immunogenicity of influenza vaccine. Through conventional repeated passage at low temperature, a master strain of live influenza vaccine was developed. It is possible to tranfer the cis-acting signal that controls the repertoire of surface antigen unto the cold adapted master strain through transfection of influenza RNP complex. This genetically engineered influenza virus is expected to present higher amount of the HA protein on the attenuated virus, and will be an ideal candidate for an improved live vaccine against influenza infection. Based upon these technical platforms, strategies are emerging for utilizing influenza virus as vaccine carrier for other infectious diseases.

Although trivalent subunit vaccine has been available, the influenza vaccine has been under-utilized because of cumbersome route of vaccination and low level of protection. Therefore, there has always been a great need to develop live attenuated influenza vaccine which can be administered through nasal route and elicit better immunogenicity. Through conventional repeated passage at low temperature, a live influenza vaccine carrier could be established. By reassortant formation between the 'cold-adapted' vaccine carrier and virulent strains, a prototype of trivalent live influenza vaccine is developed. Moreover, live vaccine with different repertoire of HA and NA proteins could also be generated by transfection of HA and mutant NA genes into the live attenuated master strain.

The strategy, when applied to chimeric virus carrying foreign viral epitopes grafted unto the influenza surface antigens, will be useful to augment immunogenicity of the chimeric vaccine. The second generation influenza vaccine has to meet new regulatory requirements on safety issues, especially on genetic stability of attenuation phenotype. Other issues should also be considered: growth properties, potential recombination among virulent viruses, and the ratio of three live viral strains to optimize the cross-protective vaccine efficacy. Considering these issues, current status and the prospect of developing live influenza vaccine will be discussed.