The makings of a killer

The 1997 flu in Hong Kong infected only 18 patients, but killed 6 of them. Now, reverse genetics experiments have pinpointed the NS1 gene as a primary culprit.

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In 1997 a highly lethal influenza virus made the leap from birds to humans in Hong Kong. It killed 6 out of a total of 18 patients before culling of the local poultry prevented the possibility of additional transmission to humans. Fortunately, the virus did not transmit efficiently from human to human, and no additional illnesses were reported. In this issue, Seo *et al.*[1] explain how this virus got its lethal edge. They examine the ability of these Hong Kong viruses to counteract the antiviral effects of secreted tumor necrosis factor (TNF) and interferons (IFNs)—cytokines that have a critical role in the body's defense against viral infection. They find that viral aggression is associated with the viral NS gene, which encodes the NS1 protein. Their data indicate that the Hong Kong viruses possess NS genes that more effectively counteract the host IFN/TNF response than those of less virulent strains. In addition, the introduction of the Hong Kong virus's NS gene into the 'PR8' virus, an otherwise avirulent influenza virus strain, enhanced pathogenicity in pigs. Because viruses similar to the 1997 Hong Kong viruses continue to circulate in wild birds in and around Hong Kong, the reemergence of such a virus in the human population continues to be a possibility. A more complete understanding of the NS gene's virulence characteristics could help us size up this threat.

The innate immune response of the cell is the first line of defense against viruses. In 1998, García-Sastre *et al.*[2] showed that the type I IFN system, a major component of the innate antiviral immune response, has an important role in influenza virus virulence, at least in mice. These studies suggest that highly virulent influenza viruses might have developed efficient means to inhibit this antiviral cytokine. Subsequent studies found that an influenza virus lacking the NS1 gene grew poorly in cells having an intact type I IFN system[3]. Surprisingly, this highly attenuated virus was able to grow in cells deficient in IFN synthesis and even kill knockout mice lacking the signal transducer and activator of transcription-1 (STAT-1), a protein needed for IFN signaling[3]. A recent report by Seo and Webster identified TNF as an additional host-cell factor that can exert a potent anti-influenza virus effect[4]. The new study by Seo *et al.*[1] implicates TNF in addition to IFN as a key target of the NS1 protein. Together, these findings suggest NS1 is required to overcome the innate antiviral cytokine response of the host, and that the degree of virulence might depend on the sequence of the gene encoding NS1. Thus, influenza virus strains encoding a 'strong' NS1 protein would be expected to efficiently down-modulate innate immunity, replicate quickly and be particularly virulent (Fig. 1).

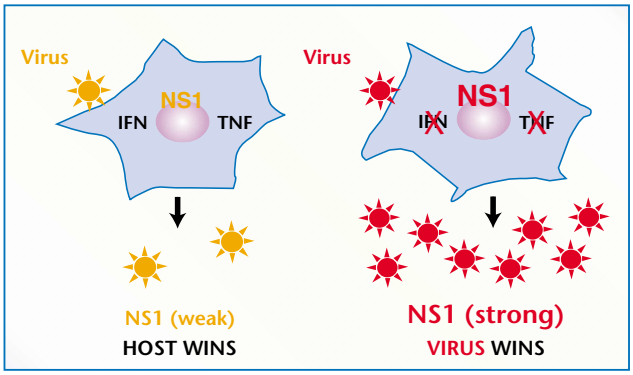

Fig. 1. IFN antagonists in viral pathogenesis. Studies on several viruses, including the influenza A virus, have demonstrated that full virulence requires viral-encoded mechanisms to counteract the host IFN response[3]. Influenza virus infection can induce antiviral host defense mechanisms, including those mediated by IFN and TNF. An influenza virus encoding a weak NS1 protein is unable to disarm this host antiviral response; this results in decreased viral replication, as in the left panel (host wins). However, an influenza virus encoding a strong IFN-antagonist NS1 protein efficiently counteracts the host cell IFN and TNF response and viral replication proceeds relatively unimpeded, as in the right panel (virus wins).

The carefully performed study by Seo *et al.*[1] takes advantage of reverse genetics techniques that allow alteration of the influenza virus genome. The Hong Kong NS1 protein is characterized by the presence of a glutamic acid at position 92. The mere change of this glutamic acid to an aspartic acid, as found in the NS1 of most influenza virus strains, disarmed the virus. In porcine lung cells, a virus with this single amino-acid change no longer showed enhanced resistance to IFNs and TNF. The authors also found that Hong Kong NS1-associated virulence was maintained in engineered viruses possessing different hemagglutinin molecules and even in a virus possessing an otherwise entirely different gene constellation. Most impressive is the finding that although the PR8 strain of influenza virus is innocuous in pigs, five-week-old pigs infected with a genetically engineered PR8 virus containing the Hong Kong NS gene grew seriously ill. These data clearly implicate the Hong Kong NS gene as a virulence factor.

In any discussion of the virulence of influenza viruses, the pandemic of 1918 must be considered (Fig. 2). The 1918 virus wreaked unprecedented havoc—killing more than 40 million people worldwide, according to some estimates. The threat that another pandemic strain may emerge makes understanding the pathogenesis of highly virulent viruses such as the 1918 virus or the 1997 Hong Kong viruses all the more urgent. Earlier, in an attempt to study the contribution to virulence of the gene encoding NS1 in the virus strain of 1918, influenza viruses containing this gene were constructed. They were compared, under high containment conditions, with otherwise isogenic viruses with a wild-type NS1 gene[5,6]. Results in mice showed that viruses with the 1918 NS1 gene were actually less virulent than the corresponding wild-type control virus[5]. However, subsequent studies using human A549 cells and an extensive microarray analysis suggested that a virus containing the 1918 NS1 gene was able to downregulate expression of several genes involved in the IFN signaling pathway. This downregulation occurred more efficiently than with an isogenic counterpart containing the NS1 gene of the influenza WSN virus[6]. These results agree with the finding by Seo *et al.*[1] and suggest that in the right host cell, an NS1 protein can have a pronounced effect on

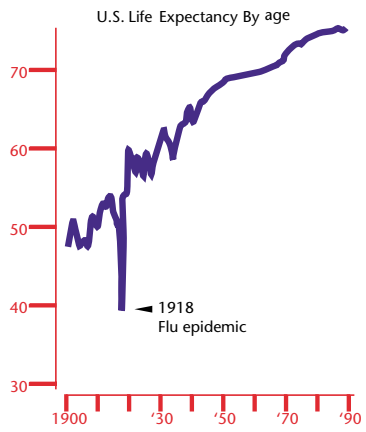

Fig. 2 The 1918 influenza pandemic reduced life expectancy in the US by approximately 10 years. Average life expectancy in the US (*y* axis) is plotted versus the indicated years (1900–1990) (*x* axis). The large drop in life expectancy in 1918 was due almost entirely to the influenza pandemic (indicated by the arrow). Adapted from Joshua Lederberg.

the innate immune response.

The cumulative data suggest that an influenza virus with a strong NS1, like that from the Hong Kong virus (or the 1918 pandemic virus), will be able to efficiently overcome the host cytokine (IFN) response (Fig. 1). In contrast, replication of viruses encoding a 'weak' NS1 will be inhibited by the host cytokine response and, as a consequence, these viruses may be less likely to cause serious disease (Fig. 1). Whether strong NS1 proteins show increased stability, as Seo *et al.* propose, remains to be determined[1]. In addition, data from the mouse experiments using viruses with the 1918 NS gene[5] suggest that this antagonist activity may be species-specific and that a particular NS1 protein may be a killer in humans but not in mice. In this respect, the pig model as used by Seo *et al.*[1] may more closely mirror human influenza virus infections than the mouse system.

What is the mechanism by which the NS1 gene exerts such a crucial influence on the host's antiviral cytokine response? Previous studies have shown that an influenza virus lacking the NS1 gene fails to prevent activation of the interferon regulatory factors IRF-3 (ref. 7), IRF-7 (ref. 8) and nuclear factor-κB (ref. 9) in infected cells. The activation of these transcription factors is essential to the initial production of IFN. These proteins therefore have a critical role in the early antiviral response of an infected host cell. Viruses lacking the NS1 gene or having mutations abrogating its activity were also unable to prevent the activation of the dsRNA-dependent protein kinase (PKR) (refs. 10,11), one of the most important effector antiviral molecules induced by IFNs. These data may also provide an explanation for the resistance to IFN seen in the viruses having the strong Hong Kong NS gene. It is as yet unclear how these observations connect with resistance to the antiviral effects of TNF.

Clearly, virulence of influenza and of other viruses is polygenic. For instance, the genes encoding HA and PB2 have been implicated as determinants of virulence for Hong Kong influenza viruses in mice[12]. Pathogenicity is also dependent on the immune status of the infected host. Seo *et al.*[1] have shown, however, that the NS gene can exert a powerful effect on the suppression of the innate immune system and that the IFN (cytokine) antagonist activity of the NS1 protein can substantially contribute to the disease-causing potential of influenza viruses. Interestingly, the expression of IFN-antagonist proteins is not a property unique to influenza viruses. Rather, it seems to be the norm among all types of viruses, including highly pathogenic viruses such as smallpox and Ebola viruses[13]. It is thus likely that virally encoded IFN antagonist proteins have a major role in viral pathogenicity, and that they may be novel targets for antiviral drug intervention.

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Limitations in brain repair

Stroke and irradiation can cause severe brain damage, with consequences for neuronal replacement. The results of two new studies may help us understand the barriers to effective therapies to restore injured brain tissue (pages 955–962 and 963–970).

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Two papers in this issue address the problem of neuron birth and replacement in the adult brain following injury[1,2]. The old saw, 'the devil is in the details', was never as true and of such importance to patients and practitioners as in dealing with the prospects of brain repair. Given how radically the stem-cell concept has altered our formerly deterministic view of the central nervous system (CNS), it is not unexpected that the public and even the scientific community have become intoxicated by an almost unbridled euphoria over the prospects of cell rebirth. But now we must deal with the fine print in our contract with developmental biology.

Though ostensibly unrelated, or even contradictory, both studies should be viewed as voicing cautionary notes on the ease and efficiency with which neuron replacement may be accomplished. Taken together, the reports alert us to the limitations of neuron replacement. One study places blame for actual pathology on the neural progenitor cell