Current Challenges in the Risk Assessment of Neuraminidase Inhibitor-Resistant Influenza Viruses

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(See the article by Baz et al, on pages 740–745.)

In this issue of the Journal, Baz et al [1] present data on the replicative fitness and virulence of seasonal influenza A(H1N1) viruses and their oseltamivir-resistant variants. The sudden emergence and global spread of oseltamivir-resistant variants of seasonal influenza A(H1N1) viruses carrying the substitution His→Tyr at position 274 (275 by N1 numbering) in the drug-targeted neuraminidase (NA) were hallmarks of the 2007–2008 influenza season. The viruses continued to circulate during the next season and increased in prevalence to become the predominant circulating influenza virus strain in several countries. Moreover, there have been rare reports of the acquisition of the H275Y mutation by the pandemic novel influenza A(H1N1) virus (previously referred to as swine-origin influenza H1N1) that emerged in the spring of 2009. H275Y is the NA mutation that is most frequently associated with oseltamivir resistance in the N1 subtype. The emergence of drug-resistant viruses that retain replicative fitness is a significant obstacle in the effective management of influenza virus infections and poses a threat to pandemic strategies to reduce morbidity and mortality when vaccine is either not available or in limited supply. There is a need to better understand virological and clinical correlates of viral fitness and the methods used to assess viral fitness. The results of previous in vivo studies indicated that mutations conferring resistance to NA inhibitors (NAIs) often lead to a loss of viral fitness [2–5]. This is in contrast with the results of Baz and colleagues, which suggest that the viral fitness and virulence of oseltamivir-resistant influenza A(H1N1) viruses were unimpaired compared with those of oseltamivir-susceptible viruses. The conclusions drawn by Baz and colleagues are consistent with the available (although somewhat limited) epidemiological information [6–9]. Nevertheless, it is essential to analyze in more detail the experiments and experimental design that led to these conclusions.

The influenza virus has 2 glycoproteins on its surface: hemagglutinin (HA) and NA. The HA facilitates viral infection by attaching to cellular receptors containing the terminal neuraminic (sialic) acids, whereas the NA facilitates the spread of viral progeny by removing neuraminic acids from newly synthesized virions and from cellular surfaces. The NA also appears to play a role in facilitating passage of the virus through the mucin layer in the upper respiratory tract. Therefore, both surface glycoproteins interact with the neuraminic acid of the receptor and are essential for viral replication. The structure of the receptors, especially a linkage between the terminal neuraminic acid and an adjacent sugar (α2,3 or α2,6), affects the efficiency of HA binding to the receptor, as well as the efficiency of cleavage by NA [10]. In the upper respiratory tract of humans and ferrets, the linkages are primarily but not exclusively α2,6, whereas in MDCK cells and mice they are primarily but not exclusively α2,3.

One important component of viral fitness is viral replicative capacity [11], which reflects the ability of virus to produce infectious progeny under specific environmental circumstances. As demonstrated by Baz and colleagues, replicative capacity can be measured in in vitro host cell systems (eg, Madin-Darby canine kidney [MDCK] cells, the most commonly used cells for influenza virus isolation and propagation) and in animal models. These host systems differ by more than receptor repertoires, but together they more accurately depict viral replication under various growth conditions. Typically, the replicative fitness of a mutant variant is compared with that of the wild-type virus.
A mutant variant can be generated by means of site-directed mutagenesis, coupled with reverse-genetics techniques, to introduce a specific mutation (eg, H275Y); selected in cell culture in the presence of an inhibitory drug (eg, a NAI); or detected among field isolates. Unless generated by reverse genetics, mutant variants of wild-type viruses typically have additional mutations besides the ones conferring resistance (eg, H275Y), which may affect replicative potential; Baz and colleagues used pairs of wild-type and mutant variant viruses in which the mutant variants arose via several routes. In their article, the ability of viruses and their H275Y mutant variants to produce infectious progeny was compared in MDCK cells and a ferret model. The simplest method of determining relative fitness value is to compare the replicative kinetics for each virus by measuring infectious titers in the culture supernatants. Another approach, also used in the study, allows the assessment of viral spread in cell monolayers by measuring plaque size. The replicative kinetic of the H275Y viral isolate (A/Brisbane/59/2007[H1N1]-like) was compared with that of the related virus, which was also A/Brisbane-like but lacked the mutation in the NA. Of note, there were other amino acid differences, including in HA and NA, between those 2 viruses. The data presented indicated a lack of difference in replication between the wild-type and mutant A/Brisbane-like viruses. In contrast to the A/Brisbane-like viruses, the replicative kinetics in MDCK cells of the laboratory strain A/WSN/33(H1N1) was markedly different from that of its reverse genetics–derived H275Y mutant. The H275Y mutation alone in the A/WSN/33(H1N1) genetic background was responsible for a >10-fold reduction in viral titers during the first 24 h of viral replication. This result is consistent with previously published data by the same authors in which the A/WSN/33(H1N1) virus acquired the H275Y mutation as a result of passage in MDCK cells in the presence of the NA, peramivir [12]. Of note, the viral replication experiments are often performed in 2 different cell lines: the standard MDCK cell line (American Type Culture Collection) and the MDCK cell line (SIAT1, the gene encoding the human sialyltransferase ST6GalI) that is genetically modified to overexpress α2,6 receptors, which better support the replication of contemporary seasonal viruses [13, 14].

It is worth mentioning other reports that have used similar in vitro systems to assess the effect of the H275Y mutation on viral replicative capacity. Rameix-Welti et al [15] reported no significant differences in growth kinetics when a similar set of seasonal viruses was tested. Yen et al [16] introduced the H275Y mutation in the laboratory strain A/Puerto Rico/34(H1N1) and in the highly virulent avian A/Vietnam/1203/2004(H5N1) virus. The H275Y mutation had no apparent effect on the replicative fitness of the A/Puerto Rico/34(H1N1) virus and reduced by ~10-fold the titers of the avian virus during the first 24 h after infection. Finally, most disconcerting is the severe impairment of growth of the H275Y mutant of the A/Texas/36/91(H1N1) virus in MDCK cells compared with wild-type virus reported by Ives et al [17]. These results highlight the differential effect of the H275Y mutation on the replicative fitness of different influenza viruses that could be due to differences in viral genetic background, as well as in cell host systems. Thus, it is difficult not only to interpret and compare the effect of the H275Y mutation in different virus strains but also to predict the effect of the H275Y mutation on the fitness of the future viruses (eg, novel influenza H1N1).

Baz and colleagues reported that both A/Brisbane-like viruses replicated less efficiently in MDCK cells compared with the 2 reference strains from previous seasons, A/New Caledonia/2009/99(H1N1) and A/Solomon Islands/3/2006(H1N1). In contrast, replication of A/Brisbane-like viruses seems to be comparable to that of A/Solomon Island/3/2006 in the other study [15]. It is safe to assume that the clinically obtained A/Brisbane-like viral isolates were passaged fewer times in cell culture than were the reference viruses used for comparison. This may have affected the outcomes, given that extensive passaging is known to improve fitness of the virus in a defined cell host system (MDCK cells, embryonated chicken eggs, and others).

The ferret model is the most representative of human influenza virus infection, although there are some limitations with this model, including the lack of preexisting immunity and incomplete correlation with all human symptoms and complications. The ferret model provides an estimate of viral virulence by both clinical symptoms (eg, fever) and viral titers in nasal wash specimens and/or lungs. One caveat to the ferret model worth mentioning is the importance of the inoculation dose of virus; a very high dose may reduce the ability to differentiate symptoms and viral replication between the viruses. Although Baz and colleagues inoculated ferrets with a relatively high dose of virus (~10⁶ plaque-forming units per ferret), they were able to detect the difference between the wild type and the H275Y mutant. A higher pyretic response among oseltamivir-resistant compared with oseltamivir-susceptible A/Brisbane-like viruses was observed in animals. The viral titers of the mutant were statistically significantly greater (>10-fold) on day 2 after infection; however, the difference was not observed at later time points. In contrast, the viral titers and febrile response were reduced in ferrets infected with the H275Y mutant of A/Texas/36/1991(H1N1) [17]. The H275Y mutant of A/Hanoi/30408/2005(H5N1) also showed reduced viral titers in ferret nasal wash samples compared with titers of wild-type virus [18]. The findings of Baz and colleagues are consistent with a recent comparison of humans infected with either oseltamivir-resistant or oseltamivir-susceptible A(H1N1) [6, 9] and underscore the value of a ferret model for the assessment of viral fitness.

The affinity of HA toward neuraminic acid–containing receptors dictates how
much NA activity is needed for viral spread in cell culture. It is postulated that the functional balance between HA and NA is essential for optimal viral replication. An imbalance, however, may occur when virus propagates in a new host because of a difference in the structure and/or density of receptors or if NA activity is inhibited by a drug (ie, an NAI). Although most of the studies of the interplay between HA and NA have been done with laboratory strains, it is recognized that similar changes in the viral antigens may occur under natural conditions [10]. In 2008, Rameix-Welti et al [15] speculated that the natural evolution of the seasonal influenza A(H1N1) viruses led to the emergence of a new group of viruses whose increased NA affinity for receptors upset the HA and NA functional balance and that the H275Y mutation facilitated its restoration by reducing NA affinity. Many questions regarding viral evolution and other factors that might contribute to the rapid spread of oseltamivir-resistant H275Y viruses remain unanswered, and additional studies are warranted.

In conclusion, Baz and colleagues report findings on viral fitness that are consistent with epidemiologic reports and are, thus, reassuring. However, it is important to remember that these findings do not predict the effect of the H275Y mutation on other virus strains with the N1 antigenic subtype. In addition, it is conceivable that other mutations besides H275Y in the N1 subtype may reduce susceptibility to NAIs [19] without significant loss of viral fitness. Careful monitoring of cell lines and their receptor repertoire, viral passage history, and full-genome sequence analysis of viruses are necessary to extract clinically relevant information from in vitro and animal experiments.

References