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Pathogenesis of Lethal H5N1 Hong Kong Influenza Virus

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Influenza virus pandemic is occurring in the interval of about 30 years. It is well known that the genomes of avian influenza viruses play an important role in creating human pandemic viruses. Avian influenza viruses continuously threaten our human lives. In the late of February 2003, avian H5N1 influenza viruses of which genomes are very similar to those of H5N1 influenza viruses circulating in wild birds were transmitted to humans. Meanwhile, Avian (H5N1) influenza A viruses transmitted directly from chickens to humans in 1997 claimed the lives of six of the 18 people infected. The cause of these viruses' virulence in humans is not known. In humans infected with circulating influenza viruses, virus replication peaked 48 hours after infection, and little virus was shed after 6 to 8 days. The decline of viral replication corresponded closely to the level of circulating interferon. Although both innate and induced immune responses are thought to play a role in virus clearance, interferons and TNF-α . are the first line of defense against the replication of influenza viruses. We therefore investigated the inhibitory effect of these cytokines on lethal H5N1 influenza viruses directly transmitted from chickens to humans in 1997.

Highly pathogenic H5N1 influenza viruses are resistant to anti-viral cytokines. When porcine lung epithelial cells were treated with anti-viral cytokines such as IFN-α, IFN-γ, or TNF-α, The replication of the H5N1/97 influenza viruses was not affected by pretreatment with IFN-α, IFN-γ, or TNF-α The titers of the H5N1/97 viruses were comparable in treated and untreated cells, whereas the replication of the control human, swine, and avian influenza viruses was effectively blocked.

The dose of cytokine does not alter the resistance of H5N1 viruses. To investigate the dose effect of the cytokines on the replication of H5N1/97 influenza viruses, we pretreated SJPL cells with 300 to 900 ng/ml of recombinant swine IFN-α, IFN-γ, or TNF-α before infecting them with A/HK/156/97 (H5N1). The replication of H5N1/97 influenza viruses was not inhibited even at the highest concentration of cytokines.

The NS gene of H5N1 viruses is required for resistance to anti-viral cytokines. The NS1 protein encoded by influenza viruses is known to attenuate the host response mediated by interferons α and β. To investigate the role of the NS gene in the resistance of H5N1/97 viruses to cytokines, we used reverse genetics to create a recombinant A/PR/8/34 (H1N1) virus that contained the NS gene of A/HK/156/97 (H5N1). We designated the recombinant virus RecPR8-NS(H5N1/97). Pretreatment with 200 ng/ml of IFN-α, IFN-γ, or TNF-α did not inhibit the replication of the recombinant virus but effectively inhibited replication of the wild-type A/PR/8/34 virus.

Glutamic acid at position 92 of the NS1 molecule is necessary for resistance to anti-viral cytokines. To determine what residue of NS1 is responsible for H5N1 viruses' resistance to the interferons and TNF-α, we compared the NS1 amino acid sequences of different influenza viruses. Surprisingly, we found glutamic acid at position 92 of the H5N1/97 NS1 molecule. We then created a recombinant A/PR/8/34 (H1N1) virus that encoded the mutant NS1. The recombinant virus, designated RecPR8-NS(E92D)(H5N1/97), was susceptible to the antiviral activity of IFN-α, IFN-γ, and TNF-α.

The NS gene confers cytokine resistance to H5N1/2001 viruses. The H5N1/97 viruses disappeared from Hong Kong's live poultry markets after the wholesale slaughter of birds in 1997. In 2001, however, multi-reassortant H5N1 influenza viruses containing the hemagglutinin (HA) gene of H5N1/97 were isolated there. We investigated whether the H5N1/2001 influenza viruses are susceptible to the antiviral activity of IFNs and TNF-α. The replication of these viruses was not inhibited in SJPL cells pretreated with 200 ng/ml of INF-α, IFN-γ, or TNF-α.

The HA molecule is not implicated in H5N1 viruses' resistance to cytokines. One of the unique features of the H5N1/97 influenza viruses is their inclusion of multiple basic amino acids at the cleavage site of the HA protein. To investigate the role of the HA protein in resistance to cytokine activity, we created two recombinant viruses by using A/Teal/HK/W312/97 (H6N1), whose genome is nearly identical to that of H5N1/97 with the exception of the HA gene. We replaced the HA gene of this virus with that of the lethal A/Goose/HK/437-4/99 (H5N1) influenza virus, which is similar to that of H5N1/97 viruses. This recombinant was termed RecTeal-HA(H5N1). We then created a second recombinant virus by replacing the NS gene of RecTeal-HA(H5N1) with the NS gene of A/PR/8/34 (H1N1). This virus was designated RecTeal-NS(PR8)-HA(H5N1). After pretreatment with IFN-γ, IFN-α, or TNF-α, the replication of RecTeal-NS(PR8)-HA(H5N1) was almost completely blocked in pretreated SJPL cells.

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