

## Difference in Receptor Specificity among Influenza A Viruses from Different Species of Animals

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Influenza A viruses have been isolated from a variety of animals including humans, pigs, horses, mink, sea mammals, and birds [12]. Although interspecies transmission of influenza A viruses has been shown to occur [5, 6, 8, 10, 17, 19, 20], host range restriction does exist among these viruses [7, 12]. For example, replication of avian influenza A viruses in squirrel monkeys is restricted. Similarly, mammalian influenza A viruses poorly replicate in ducks [9, 20]. Although molecular basis of such host range restriction is poorly understood, the hemagglutinin (HA), the major surface glycoprotein of influenza viruses, has been shown to be involved [4, 13].

The HA functions in binding to host cell sialyloligosaccharide receptors. This binding is crucial for the initiation of an influenza viral infection. Comparisons of influenza viruses from various animal species have shown differences in the viruses' ability to recognize particular terminal sialic acid sequences [2, 14, 16]. The variation in receptor specificities among human, avian and equine influenza A viruses suggests that the presence of different receptors in different host animals and the ability of the HA to bind to these receptors is factors associated with host range and tissue tropism of the viruses.

The difference in receptor specificity among influenza A viruses described above was examined using erythrocyte preparations enzymatically modified to contain sialyloligosaccharides of a defined sequence [i.e. N-acetyl neuraminic acid  $\alpha$ 2, 6 galactose (NeuAc2, 6 Gal) or N-acetyl neuraminic acid  $\alpha$ 2, 3 galactose (NeuAc2, 3 Gal)] [16]. Such a simplified assay may preclude detection of the differences in receptor specificity among the viruses, because a variety of sialyloligosaccharides that differ in the type of sialic acids, linkages, and/or core structures exists on the cell surface. Thus, the aim of the present study is to further examine the differences in receptor specificity of influenza A viruses using erythrocytes from different animals and different animal sera for inhibiting

hemagglutination.

Influenza A viruses used in this study were isolated in embryonated eggs. Whenever possible, isolates passaged less than five times in eggs were used without cloning. When hemagglutination of influenza A viruses was compared using erythrocytes from different animal species, differences in receptor specificity was evident (Table 1). Although all of the viruses tested agglutinated chicken erythrocytes, hemagglutination of other erythrocytes differed among the virus strains. Human and swine viruses were similar in their hemagglutination patterns but differed in their activities with hamster erythrocytes. Similarly, differences in the preference of hemagglutination with different erythrocytes were observed with equine and avian viruses, although both agglutinated all the erythrocytes used.

Naturally occurring nonspecific inhibitors of hemagglutinating activity of influenza viruses have been demonstrated in different animal sera [1, 3, 11]. Among those, heat stable inhibitors are sialylated glycoproteins that inhibit hemagglutination as receptor analogs. Receptor specificity was then examined using heat inactivated (56°C, 30 min) sera from different animals to inhibit hemagglutination of chicken erythrocytes by the viruses. Horse serum inhibited hemagglutination by human and swine viruses but not by equine and avian viruses (Table 2) as described before [16]. All the other sera except duck serum inhibited a human virus, although differing in activity. In contrast, the other viruses were not inhibited to the extent seen with the human virus with the exception of the swine virus by rabbit serum.

Human and swine influenza viruses are shown to preferentially bind to oligosaccharides containing NeuAc2, 6 Gal than to those containing NeuAc2, 3 Gal using erythrocyte preparations enzymatically modified to contain sialyloligosaccharides of a defined sequence [16]. In the present study, it was demonstrated that the receptor specificity of these viruses was different. Similarly, even though avian and equine influenza A viruses are shown to

Table 1. Hemagglutination of influenza A viruses with erythrocytes from different animals

Animal species of erythrocytes	A/Udorn/307/72 (H3N2)	A/swine/Tennessee/55/77 (H1N1)	A/equine/California/831/82 (H3N8)	A/ruddy turnstone/65/85 (H7N3)
Chicken	512	16	1024	128
Horse	<1	<1	256	256
Sheep	256	16p <sup>a</sup> )	512	512
Ox	<1	2p	2048	512
Hamster	256	<1	>2048	512

a) p: partial hemagglutination.

Table 2. Hemagglutination-inhibition of influenza A viruses with different animal sera<sup>a)</sup>

Animal species of serum	A/Udorn/307/72 (H3N2)	A/swine/Tennessee/55/77 (H1N1)	A/equine/California/831/82 (H3N8)	A/ruddy turnstone/65/85 (H7N3)
Horse	>512	>512	<1	<1
Ox	>512	<1	16p <sup>b)</sup>	4p
Pig	16	<1	<1	<1
Whale	64	<1	<1	<1
Seal	8	4p	2p	16p
Rabbit	>512	8	8p	8p
Ferret	>512	4p	16p	8p
Chicken	16	<1	<1	<1
Duck	16p	2p	<1	16p

a) Hemagglutination inhibition titers represent the reciprocal of the highest dilution inhibiting 4 hemagglutination units of virus.

b) p: partial inhibition.

preferentially recognize oligosaccharides containing NeuAc2, 3 Gal than to those containing NeuAc2, 6 Gal [16], their hemagglutinating patterns with different erythrocytes varied each other (Table 1). These findings indicate that not only the type of linkage of the sialic acid to galactose but the carbohydrate structure proximal to the cell membrane of sialyloligosaccharides also determine receptor specificity of influenza A viruses.

Hemagglutination was not inhibited by the serum of an animal species from which the virus was isolated (Table 2). This is probably due to selection during replication of the viruses in their hosts, indicating that inhibitors in animal serum and/or in body fluid may be involved in natural protection against influenza viruses and their host range.

Recently we have shown that glycoprotein inhibitors in different animal sera (i.e. horse, pig, and rabbit) are distinct from each other [18]. This explains why horse serum inhibited both human and swine influenza viruses but most of the other sera inhibited only a human virus. The inhibitor in horse serum has been shown to be an alpha-2 macroglobulin [15]. The nature of the inhibitors in other sera is not known. Purification of these inhibitors is in progress in our laboratory, which will provide further understanding of receptor specificity of influenza viruses.

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