Archiv für die gesamte Virusforschung 40, 168—170 (1973)

Comparison of Neutralizing Activity in Nasal Secretion and Serum of Ferrets in Response to Infection with Influenza A Viruses

Brief Report

By

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Received August 29, 1972

Nasal secretory antibody is more closely correlated with protection against at least certain viral infections of the nasopharynx than is serum antibody (1, 7). Also, intranasal immunization with inactivated influenza viruses has stimulated higher levels of antibody in respiratory secretions than has subcutaneous immunization (3, 8). Thus, interest has developed in intranasal immunization with inactivated and attenuated influenza viruses.

Experience with parenteral influenza vaccines has led to the conclusion that the antibody response, based on serum assays, is homotypic or anemnestic, so that new serotypes must be included in a current vaccine to provide efficacy (2). However, it has been reported that aerosol immunization with inactivated A2/Taiwan/64 led to heterotypic neutralizing antibody against A2/Hong Kong/68 in respiratory secretions but none in serum (9). If broader neutralizing activity in nasal secretion than in serum can be regularly anticipated following intranasal administration of influenza A viruses, then this route of application assumes a new dimension of interest for vaccine development. The ferret, because of its high degree of susceptibility to influenza, is ideally suited for laboratory studies of this concept. We report their response to infection with influenza A and A1 in terms of homotypic and cross neutralizing activity in serum and nasal secretion.

Eight to 10-week old male ferrets were infected intranasally without anesthesia with 10^5 ferret ID_{50} of influenza A/PR8/34 or influenza A1/Philadelphia/53 (6). Serum was obtained from blood drawn by cardiac puncture. Nasal turbinate homogenates were prepared as previously described (4). Nasal wash was obtained by injecting 2 ml of PBS, pH 7.6, into the posterior nares of anesthetized ferrets with a blunt cannula and collecting the effluent from the anterior nares by gravity. The wash fluid was recycled through the nasal passages for greater removal of mucous. Virus content of turbinates was determined by egg infectivity (4). Neutralization tests were carried out by hemadsorption-inhibition in BSC-1 cells (5). Expressed titers are geometric mean values from 6—12 ferrets.

The maximum titer of each virus in turbinates was observed one day after infection: 7.3 and 5.8 EID_{50}/0.2 ml for A and A1, respectively. These titers diminished similarly over a 10 day period.

Homologous and cross neutralizing activity responses in serum and nasal wash are shown for both infections (Table 1). Homologous serum titers rose to uniformly high levels. The maximum titers achieved after infection with A were higher than those to A1, presumably reflecting the greater replication of A. All cross neutralizing titers in serum were below the baseline for measurement. In nasal wash homologous neutralizing activity was detected following A infection to titers of

Table 1. *Neutralizing Activity in Ferret Nasal Wash and Serum Following Infection with Influenza A or A1*

Infecting virus	Material assayed	Indicator virus	Reciprocal neutralizing titer /0.2 ml			
			Days after infection			
			0	7	14	21
A	Serum	A	<10	1,280	2,560	≥ 5,120
		A 1	<10	<10	<10	<10
	Nasal Wash	A	< 2	64	32	≥64
		A 1	< 2	< 2	< 2	< 2
A 1	Serum	A	<10	<10	<10	<10
		A 1	<10	16	256	512
	Nasal Wash	A	< 2	2	4	4
		A 1	< 2	2	4	2

1:64 or greater; lower titers were obtained after infection with A1, again correlated with the lesser extent of A1 replication. As in serum, no cross neutralizing activity was observed in nasal wash following A infection. But A1 infection consistently elicited neutralizing activity to A in titers comparable to those obtained against the homologous virus.

The broader neutralizing activity response in nasal wash than in serum following A1 infection suggests a difference in the biological reactivity of antibody from these sources. Thus, the observation by WALDMAN *et al.* (9) using inactivated influenza A2 has been extended to a live influenza virus of another serotype. However, the fact that the reciprocal cross gave a homotypic response only shows that this is not a uniform occurrence which can necessarily be used to practical advantage for intranasal immunization with influenza.

Acknowledgements

The technical assistance of J. R. Wagner and B. Zabko-Potapovich is greatly appreciated.

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