Protective efficacy of a live, attenuated, influenza virus vaccine ('Alice' strain)

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Summary

Animal studies have indicated the high degree of efficacy and broad protection of 'Alice' vaccine against various heterologous H₃N₂ influenza virus strains. Similarly, challenge studies carried out in volunteers have confirmed the high degree of efficacy of 'Alice' vaccine versus homologous and heterologous influenza A virus strains.

Introduction

Recent work on influenza immunization has indicated that the most often quoted criterion for successful immunization with a viral vaccine, namely the elevation of circulating serum antibodies, did not correlate completely with protection from overt influenza infection.

In fact, killed influenza vaccines administered parenterally are usually able to induce circulating haemagglutination-inhibition (HI) antibodies, often in close to 100% of cases, whereas their protective efficacy is accepted in the range of 60-80%. On the contrary, the use of live intranasal vaccines has led to somewhat lower seroconversion rates or geometric mean titres and to higher protective efficacy.

This is exemplified in a comparative trial reported by Beare *et al.* (1968), in which ninety-eight volunteers were vaccinated with inactivated influenza B virus parenterally or with live influenza B virus intranasally. Seroconversion was observed in 71% of those given the killed vaccine and in only 51% of those receiving the live vaccine. These subjects were subsequently challenged with live influenza virus, with the results that influenza infection was reduced by 62% and 94% for the killed and live vaccines respectively (Table 1).

In another paper, Beare (1969) observed that some subjects vaccinated intranasally with a live influenza vaccine did not develop HI or neutralizing circulating antibodies and yet could not apparently be re-infected when challenged subsequently. He concluded that the effects of live influenza vaccines were

TABLE 1. Serological response and protection versus challenge

Vaccine type	Serological HI response	Protection	
		Clinical symptoms	'Flu infection (lab. tests)
Killed vaccine*	71%	50%	62%
Live vaccine*	51%	82%	94%

* Influenza B/England/13/65.

evidently not to be measured solely by the stimulation of circulating antibodies.

Mann *et al.* (1968) have also reported that intranasal live influenza virus vaccines seem to be able to afford protection even without stimulation of serum antibody. These results indicate clearly that local infection is superior to parenteral immunization in inducing immunity in respiratory secretions and in providing protection at the site of infection.

It has therefore been postulated that a live attenuated influenza vaccine administered intranasally, by mimicking an asymptomatic natural infection, would stimulate a full immune response and thereby provide a protection similar to that observed after a natural infection.

Material and methods

The protective efficacy of a live attenuated influenza vaccine ('Alice' strain) has been tested in an animal model and in volunteers, by the use of a challenge technique rather than by the measurement of circulating antibodies. 'Alice' strain has been extensively investigated (Huygelen *et al.*, 1973; Peetermans *et al.*, 1973; Lamy *et al.*, 1973; Peetermans, Lamy and Delem, 1975; Waldman *et al.*, 1975; Rubin *et al.*, 1974; Noble *et al.*, 1974; Prévost *et al.*, 1975) and shown to be attenuated, safe and immunogenic.

Results

(1) *Experiments in ferrets*

Groups of ferrets were immunized intranasally with 'Alice' vaccine (one dose containing $10^{6.5}$ – $10^{7.0}$ EID₅₀) and others were kept as controls (Table 2). All animals were challenged 5 weeks later with various influenza virus strains, representative of three recent antigenic drifts.

TABLE 2. Heterologous challenges of ferrets immunized with 'Alice' influenza strain

Vaccine strain	Challenge strains
'Alice' serotype A/England/42/72	A/Dunedin/4/73 (Port Chalmers-like) A/England/635/74 A/Scotland/840/74

The parameters used in evaluating protection in these animals are shown in Table 3. Basically there are three criteria: clinical: body temperature and symptoms; viral excretion; nasal protein increase.

TABLE 3. Parameters of protection evaluation

(1) Body temperature	One or two readings $\geq 40^\circ\text{C}$ at 24–72 hr post-inoculation Two readings $\geq 1^\circ\text{C}$ above the mean pre-inoculation temperature
(2) Symptoms	Depression Dyspnoea Nasal discharge Coughing Conjunctivitis
(3) Viral excretion	Tested 2–4 days post-inoculation
(4) Nasal protein increase on day 7	At least three-fold above the pre-inoculation level

As shown in Table 4, there was a complete protection against A/Dunedin virus (a Port Chalmers-like strain) in all three immunized ferrets, as compared to controls. After challenge with A/England/635 there was an apparent reinfection in two out of three ferrets: one case showing a body temperature increase, another shedding virus on day 2. However, these signs of infection were much less pronounced

than in the controls, for both of these parameters. After challenge with A/Scotland virus, one ferret shed virus on day 2 but at a significantly lower titre than the controls.

The response to challenge with England/635 or Scotland/840 was not significantly different from the response observed with Dunedin/4, indicating that the degree of protection provided by 'Alice' vaccine was about equal against all three H₃N₂ subtypes.

In conclusion, the study in ferrets confirms the broad protection provided by a live influenza virus vaccine (McLarren, Potter and Jennings, 1974).

(2) *Experiment in man*

The results of four artificial challenge studies are summarized in Table 5. In total, twenty-two subjects immunized with 'Alice' strain and twenty-eight controls were infected with an homologous H₃N₂ virus strain, 3–4 months after vaccination. Several parameters were followed:

Illness: on the basis of recorded respiratory symptoms, protection was observed in 88.5% of the cases. However, this clinical evaluation did not take into account that some symptoms might not have been associated with influenza.

Influenza infection: when considering influenza infection as defined either by virus isolation or by a four-fold rise of antibodies, protection against confirmed influenza was observed in 100% of the cases.

Table 6 shows the results of studies still in progress where the challenge virus was either homologous or heterologous (Port Chalmers-like). In both cases protection was complete against virus associated illness.

The evaluation of protective efficacy can also be calculated from natural challenge conditions, i.e. evaluating protection after an influenza outbreak in a community population containing vaccinees and control subjects. Table 7 illustrates the results of such a natural challenge. An outbreak of Port Chalmers influenza occurred in a high-risk population, vaccinated 3–4 months previously. There was no difference in the overall rate of illness among 'Alice' vaccinees, intramuscular vaccinees or controls. However, the

TABLE 4. Results of heterologous challenge of ferrets vaccinated intranasally with the 'Alice' strain

Challenge strains	Group	Body temperature		Symptoms	Viral shedding			Nasal protein mean increase on day 7
		$\geq 40^\circ\text{C}$	$\geq 1^\circ\text{C}$		Day 2	Day 3	Day 4	
A/Dunedin/4/73 (Port Chalmers-like)	Vaccinees	0/3	NT	0/3	0/3	0/3	0/3	NS
	Controls	4/4	NT	4/4	4/4	4/4	4/4	
A/England/635/74	Vaccinees	1/3	0/3	None	1/3	0/3	0/3	0
	Controls	3/3	1/3	None	3/3	3/3	3/3	
A/Scotland/840/74	Vaccinees	0/2	0/2	0/2	1/2	0/2	0/2	NS
	Controls	2/3	2/3	3/3	3/3	3/3	2/3	

NS, not significant; NT, not tested.

TABLE 5. Challenge studies

Study groups	Challenge virus dose/person	Illness	Virus isolation	Serum 4x rise	Nasal 4x rise	Virus associated illness	Protection rate (%)	
							Illness	Virus associated
(1) 'Alice'—2 doses	Udorn BEK 4	1/6	1/6	1/6	1/6	None	69	100
Control	10 ^{2.12} TCID ₅₀	6/11	6/11	3/11	0/11	5/11		
(2) 'Alice'—2 doses	A/Engl/42/72 RIT	0/4	3/4	1/4	—	None	100	100
Control	10 ^{6.8} EID ₅₀	1/5	4/5	1/5	—	1/5		
(3) 'Alice'—2 doses	A/Engl/42/72 RIT	0/6	0/6	0/6	3/6	None	100	100
Control	10 ^{6.8} EID ₅₀	1/6	1/6	4/6	4/6	1/6		
(4) 'Alice'—2 doses	Udorn BEK4E1 RIT	0/6	0/6	0/6	0/6	None	100	100
Control	10 ^{6.12} EID ₅₀	3/6	4/6	4/6	4/6	3/6		
Total 'Alice'		1/22	4/22	2/22	4/18	None	88.5	100
Control		11/28	15/28	12/28	8/23	10/28		

TABLE 6. Artificial challenge in progress with homologous and heterologous viruses

Study	Groups	Challenge virus	Illness	Virus isolation	Serum 4x rise	Virus associated illness	Protection rate (%)	
							Illness	Virus associated
Rytel	'Alice'	Udorn BEK4E1	1/12	0/12	0/12	0/12		
	Control	10 ^{6.72} EID ₅₀ (10 months later)	8/10	10/10	8/10	8/10	89.6	100
Waldman	'Alice'	Dunedin/3/73	0/6	0/6	0/6	0/6		
	Control	10 ^{6.1} EID ₅₀ (± 30 days later)	3/7	3/7	6/6	3/7	100	100

TABLE 7. Response of vaccinees to natural outbreak of 'Port Chalmers' influenza. Rochester, N.Y., March/April, 1974

Group	No. of subjects	No. ill	No. infected			Illness associated with infection
			Virus isolated	Antibody response	Total	
'Alice'	45	17 (37.8%)	0	3 (6.7%)	3 (6.7%)*	2 (4.4%)
Parenteral killed vaccine	52	20 (38.5%)	5 (9.6%)	5 (9.6%)	8 (15.4%)*	7 (13.5%)
No vaccine	14	7 (50%)	2 (14.3%)	6 (42.9%)	6 (42.9%)*	3 (21.4%)

* Chi square, $P < 0.05$; † Chi square, $P < 0.10 > 0.05$.

Protective efficacy

Group	Infection	Illness associated with infection
'Alice'	84.3%	79.4%
Killed parenteral vaccine	64.1%	36.9%

rate of infection was much lower among 'Alice' vaccinees and the difference with the control group was significant at the 5% level. Protection against infection was 84% in the 'Alice' vaccinees and 64% in the killed vaccine group. Protection against virus-associated illness was 79% and 37% for live and killed vaccines respectively. It is of interest to point out that seroconversion rates observed 5 weeks after vaccination (pre-challenge) were 40% for the live

vaccine and 80% for the killed vaccine, confirming once more the lack of direct correlation between seroconversion rate and protection against challenge infection.

Table 8 summarizes the total experience in challenge studies with 'Alice' strain in man, with homologous (A/England/42/72) and heterologous (A/Port Chalmers/1/73) viruses, indicating the high degree of protection obtained with the 'Alice' strain.

TABLE 8. Results from challenge studies in volunteers vaccinated with 'Alice' strain (2 doses)

	Incidence of 'flu illness*		Protection against 'flu illness
<i>Homologous challenge A/England/42/72</i>			
Vaccinees	0/34	0%	100%
Placebos	18/38	47.36%	
<i>Heterologous challenge A/Port Chalmers/1/73</i>			
Vaccinees	2/51	3.92%	86.28%
Placebos	6/25	28.57%	

* Virus associated illness.

Conclusion

In conclusion, a live attenuated vaccine ('Alice' strain), administered intranasally, has been shown to provide a high degree of protection against challenge infection, both in an animal model and in man, and both with homologous and heterologous strains.

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