Criteria For Live Virus Vaccines Against Respiratory Infections

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SUMMARY

A live virus vaccine against respiratory diseases should ideally stimulate:

(1) persistent local and systemic immunity even if local antibodies have the same specificity as the serum antibodies;
(2) persistent systemic and local immunological memory;
(3) rapid onset of immunity and a broad long-term protection.
(4) It should have genetic markers linked to the attenuation.

In the light of the most recent discoveries in the field of immunology, one can try to define the criteria which should be met by a live vaccine against respiratory infections.

1. Immunity against respiratory infections is based on complex mechanisms involving both the local and the systemic immune systems.

   Although the relative importance of these mechanisms is still a matter of debate, the protective benefit derived from the stimulation of both immune systems is fully acknowledged (Couch, 1974; Tyrrell, 1974).

   Whilst, on the one hand, the effect of high doses of antigen on the appearance of the systemic immune response has been described by Waldman et al. (1972) and Nash et al. (1973), on the other, the local administration of an antigen is more likely to induce a local immune response with secretory IgA predominating than parenteral immunization and, overall, simultaneous stimulation of both systems can best be achieved by topical administration of live vaccine. The multiplication of live organisms at the mucosal surface provides an adequate antigenic mass for this purpose.

   Furthermore, the administration of multiple component live vaccines does not preclude the achievement of both local and systemic responses. Studies in calves using a trivalent vaccine (Imuresp r-a-p, Smith Kline Animal Health Products, Philadelphia) containing IBR, PI-3 and BAV-3 viruses, administered by the nasal route stimulated both nasal and systemic antibody responses to all components in all the calves (tables 1 and 2).
2. Studies such as these led us to conclude that the secretory immune system possesses attributes of *immunologic memory* based on the following findings:

   (a) an earlier specific antibody response following restimulation,
   (b) higher peak antibody levels,
   (c) persistence of antibodies for a longer time following recall, which are illustrated in table 3.

   All animals became positive one week after re-exposure to the vaccine, whereas none, or few, animals developed antibody in the nasal secretions two weeks after the first administration of the vaccine.

   The mean titres reached were higher after the secondary inoculation of the vaccine. Persistence of local antibodies was also improved following this secondary inoculation. This was striking for all three antigens although it differed in extent from one component to the other.

   The development of a local immunological memory at the respiratory mucosal surfaces was thus achieved with a live vaccine. This is at variance with the data reported by Ogra and Karzon (1969) who used inactivated poliovirus intranasally. However Buscho and his colleagues (1972) found evidence for immunological memory in the respiratory secretory system of man following nasal exposure to inactivated rhinovirus, and studies in mice and in calves using respectively a tetanus toxoid (Gerbrandy and Van Dura, 1972) or a live PI-3 virus (Marshall and Frank 1971) suggest that the secretory immune system may have immunological memory at mucosal surfaces with live viruses or with inactivated antigens in various species.

   Clearly, because of its extreme importance in the mobilization of the host response to disease, the stimulation of an immunological memory is a cri-

Table 1

SERUM ANTIBODY RESPONSE FOLLOWING A SINGLE INTRANASAL EXPOSURE TO TRIVALENT PI_3, IBR BAV_3 COMBINED VACCINE

Treatment	Number of animals	Geometric (log_2) mean antibody titre and percentage of seroconversion ( ) 6 weeks after vaccination					
		PI_3 (HI)		BAV_3 (SN)		IBR (SN)	
		Pre	Post	Pre	Post	Pre	Post
Vaccinated	10	2.2	5.6 (100)	0	4.4 (90)	0	1.4 (100)
Controls	3	1.7	1.0 (0)	0	3.7 (100)	0	0.0 (0)

Table 2

NASAL ANTIBODY RESPONSE FOLLOWING PRIMARY AND SECONDARY INOCULATION WITH A TRIVALENT PI-3, IBR, BAV-3 COMBINED VACCINE

Component	Inoculation	Time post exposure (weeks) Number positive/number tested (mean NI of positives)					
		0	1	2	3	4	5
PI-3	1st	0/14 (Neg)	*	3/4 (2.3)	4/4 (2.25)	5/10 (2.45)	*
	2nd	2/4 (2.5)	4/5 (3.1)	*	10/10 (3.25)	*	10/10 (3.25)
BAV-3	1st	0/14 (Neg)	*	1/4 (1.25)	*	10/11 (3.4)	2/4 (2.25)
	2nd	3/3 (3.5)	3/3 (4.3)	*	10/10 (4.3)	*	8/8 (3.8)
IBR	1st	0/14 (Neg	*	*	*	1/14 (1.6)	*
	2nd	0/4 (Neg)	3/4 (1.8)	*	10/10 (1.6)	*	4/9 (1.6)

* not tested

Table 3

RESULTS OF INTRANASAL VACCINATION IN CALVES PREVIOUSLY EXPOSED TO TRIVALENT PI-3, IBR, BAV-3 VACCINE

Period since last exposure (months)	Increase in neutralizing antibodies in nasal washings (≥4-fold) number positive calves/total number vaccinated					
	1 week			3 - 4 weeks		
	IBR	PI-3	BAV-3	IBR	PI-3	BAV-3
0	0/8	0/3	0/5	0/8	3/3	5/6
1.5	3/4	4/4	3/3	10/10	10/10	10/10
10 - 16	7/10	0/8	2/6	10/11	11/11	10/11

Table 4

PERSISTENCE OF LOCAL AND SYSTEMIC ANTIBODIES EVOKED BY TRIVALENT PI-3, IBR, BAV-3 COMBINED VACCINE

. . . month after 1st administration	Nb positive/Nb tested					
	IBR		BAV-3		PI-3	
	local	systemic	local	systemic	local	systemic
2	10/10	10/10	10/10	10/10	10/10	10/10
4	4/9	10/10	8/8	10/10	10/10	10/10
6	0/10	10/10	9/10	10/10	8/10	10/10
12	0/3	3/3	4/4	4/4	2/4	4/4
18	0/8	5/8	1/7	7/7	0/7	6/7

terion which should be fulfilled by all vaccines. In addition, such a memory must have *prolonger persistence.*

Results published by Buscho and co-workers show that a secondary type reaction of the secretory immune system could be demonstrated 420 days following the first inoculation of a vaccine.

Our data with the live bovine respiratory vaccine (table 3) show that memory persisted for various periods of time depending on the antigen but was still detectable 16 months after the previous administration of the vaccine for the IBR and BAV-3 components.

3. *The duration of protection* is an important consideration in evaluating vaccines. It is measured either by:
   a) the persistence of antibodies, which is the most common method, or
   b) by evaluating the protective capacity of a vaccine against artificial or natural challange.

a) *Persistance of antibodies.*

Serum antibody levels to respiratory virus infections fall only slightly, six months after infection (Waldman & Ganguly, 1974).

Our results following intranasal vaccination with the live vaccines show that similar persistence can be obtained following immunization (table 4).

Circulating antibodies persisted at least twelve months for all three viruses, whereas local antibodies were detected for shorter periods but were still present for BAV-3 and PI-3 one

Table 5

Protection Study against the Respiratory Disease due to the RSB virus

Farms	Animals		Natural infection: days post 1st Vacc.	Animals presenting respiratory symptoms : Percentage		Mean score/animal		Serological evidence of infection by RSB virus	Protection %
	V	C		V	C	V	C		
1	10	14	76	10	93	0.3	1.4	+	89
2	20	26	116	0	100	0	0.3	+	100
3	4	5	89	100	100	1.2	1	0	-
4	30	15	72	3.3	100	2	1.8	+	96.7
5	6	7	118	0	100	0	2	+	100
6	30	32	92	0	56	0	1.7	+	100
7	50	33	90	0	100	0	1.5	+	100
8	27	13	54	100	100	1	1	0	-
9	25	14	54	20	43	2	2.7	+	53 N.S.
10	25	2	75	12	0	0.5	0	0	-(1)

V = vaccines C = controls

N.S. : Test X_1^2 (p 0.05) : no significant difference in percentages between diseased vaccinates and controls, but a positive economic balance has nevertheless been observed in favor of the vaccinated groups.

(1) The number of the animals used as controls is not representative.

Table 6

CLINICAL SYMPTOMS FOLLOWING NATURAL CHALLENGE IN ANIMALS VACCINATED INTRANASALLY WITH RLB 106

Experiment	Quarantine period	Number of animals vaccinated	Number of animals controls	% of animals with symptoms vaccinated	% of animals with symptoms controls
A	none	113	129	10.7	58
B	none	80	17	19	76
C	5 days	138	19	0	100

Table 7

VIRUS ISOLATION ATTEMPTS FROM TISSUES OF CALVES INOCULATED 5 TO 7 DAYS EARLIER WITH RLB STRAIN OF IBR

Calf n°	Inoculum virus strain	Clinical symptoms	Post-mortem respiratory lesions	Virus from turbinates	Virus from trachea	Virus from lungs
1	ts	–	–	+	–	–
2	ts	–	–	+	–	–
5	ts	–	–	+	–	–
4	wild	+	+	+	+	+
6	wild	+	+	+	+	–

year after vaccination. Local antibodies to IBR were no longer detectable at that time but the local memory which is present may account for the fact that protection still existed.

b) *Duration of protection.*
Table 5 shows the duration of protection afforded by a respiratory syncytial (RS) virus vaccine against a natural RS challenge occurring 2 to 4 months after vaccination.
The results show that in 7 farms where there was disease caused by RS virus, protection was conferred by vaccination. Moreover, in farms 2 and 5 where the natural RSB virus challenge followed the first by a somewhat longer period, no vaccinated animal presented symptoms, whilst 100% of the controls were sticken with the disease. Vaccination thus ensured protection for at least 118 days after first vaccination.

4. Special attention must be given to the *rapid onset of protection* after vaccination. This is of the utmost importance in face of an outbreak. Live vaccines administered by the nasal route may afford protection as early as three days after vaccination (Todd et al., 1971) (table 6). This suggests the involvement of non-immune defence factors such as interferon.
In field trials with a thermospecific mutant of IBR (Zygraich et al., 1974a) we found that the vaccine was able to afford complete protection against a natural challenge five days after vaccination and that significant protection could be achieved even if the vaccines were exposed to the challenge directly after vaccination.
5. Live virus vaccines should also possess *virological merkers, indicators of attenuation.*
Recently, attenuated strains have been obtained by oriented selection of mutants or recombinants. These vaccines have laboratory characteristics which can be correlated with attenuation for the natural host. Thermospecific viruses are examples of physiological mutants. The attenuation can be predicted on the basis of their cut-off temperature. In vivo studies in animal models (Zygraich et al., 1972) and in the natural host (Zygraich et al., 1947b; Murphy, 1975; Murphy et al., 1973) have demonstrated that the attenuation resulting from a ts mutation is a site specific attenuation (table 7).
The table shows the limitation of the multiplication of an IBR ts mutant. Growth restriction to the upper respiratory tract is responsible for attenuation because the main target organs, like the trachea and the lungs, remain uninfected.
6. The possession of genetic markers is a prerequisite for a live attenuated vaccine. It is useful to control the genetic stability of the strain during its in vivo and in vitro multiplication (table 8). This table shows the stability of the RLB 106 strain of IBR following in vivo replication. These markers are also particularly useful when investigating the spread of virus from infected vaccines to susceptible contacts.
7. The question of the *substrate* used for vaccine production requires consideration. In order to standardize the production of the vaccine by providing well controlled substrates two approaches exist: on the one hand, the use of a tissue from SPF animals and, on the other hand, the use of cell lines. Bovine respiratory vaccines can be produced in cell lines which have well defined karyotypes, the absence of adventitious agents and the lack of tumorogenic and oncogenic potential.
8. The rational for the use of *multiple component vaccines* is based on the fact that several etiologic agents may be involved in respiratory diseases.

Table 8

TS CHARACTERISTICS OF VIRUS REISOLATED FROM THE NOSE

Sample from calf n°	Days between vaccination and sampling	Titre expressed in log_{10}/0.1 ml at temperature		
		37°	38°	39°
72	10	4.7	4.5	0
74	9	4.3	3.3	0.3
88	6	4.3	4.3	0.2
Inoculum	—	4.3	4.3	0.5

Combined vaccines must be as efficacious as the individual component vaccines given separately at different times. Thus, there must be no interference between the viruses and the antibody response of all the components must be adequate. This has been achieved for several vaccines in both the human and veterinary fields.

From our experience with combined vaccines, it appears that interference can be overcome by adjusting the proportions of the different components of the vaccine. Consquently, the viruses replicate simultaneously and the titres of the reisolated viruses and their excretion period are in agreement with those of monovalent vaccines, as shown in fig. 1 which displays the simultaneous replication in the nasal mucosa of three ts mutants of IBR, PI-3 and BAV-3.

9. Finally a live vaccine against respiratory diseases must be *fully attenuated* and any discomfort, inconvenience or adverse reactions associated with administration of the vaccine must be minimal and certainly considerably less than the discomfort associated with the disease.

This review was aimed at defining the profile of the product one attempts to obtain, in order to orient the laboratory and clinical work in a way that provides clear answers to the questions raised.

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