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Secretory immune response after nasal vaccination with live attenuated influenza viruses

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SUMMARY

Immunoglobulins and virus-specific antibody in nasal secretions were measured in 18 volunteers before and after intra-nasal vaccination with two live-attenuated type A influenza virus strains. Specific serum antibody rises occurred in 14 out of 18 vaccinees ; of those, 11 and 10, respectively, showed siginificant increase of IgA concentration and of virus-specific antibody in nasal secretions collected 2-4 weeks after vaccination. In one case, a volunteer showed appreciable increase of virus neutralizing antibody in nasal secretions without increase of local IgA concentration and of serum HI antibody. This data indicate that administration of live virus vaccines by upper respiratory route confers effective immunity associated with local antibody synthesis.

A major contribution to immune resistance to influenza is given by secretory antibody in the respiratory tract; in fact, local immunity is thought to be responsible for the greater resistance to infection induced by natural influenza compared with parenteral immunization by inactivated virus vaccines (Couch et al., 1971; Kilbourne et al., 1974; Potter et al., 1979; Schulman, 1975). Several studies prove that inactivated influenza virus vaccination stimulates levels of serum neutralizing antibody, while responses in terms of secretory antibody are rather low. Indeed, protective efficacy of the inactivated virus vaccine is not considered to be fully satisfactory, even though immunogenicity of vaccine preparations may be very high (Kilbourne, 1978; Sabin, 1976).

An alternative approach to prevention of illness is to administer a live attenuated influenza virus (LAV) vaccine at the portal of entry in order to induce the local production of secretory antibody normally elicited by natural infection. Live virus vaccines, if administered by mass immunization, not only provide clinical protection (Rocchi et al., 1979) but also offer the possibility to break the chain of transmission of epidimic influenza strains, since LAV vaccines, when challenged with wild virus, do not shed in the majority of cases demonstrable amounts of virus (Beare et al., 1973; Donnikian et al., 1977; Sabin, 1976).

In order to contribute to studies on local immune response to influenza virus infection, we investigated on the amount of immunoglobulins and of virus-

specific antibody in nasal secretions before and after local immunization with two live attenuated influenza virus vaccine strains.

MATERIAL AND METHODS

Volunteers. Eighteen healthy students, between the ages of 20 and 26 years, volunteered to participate in the study, a complete explanation of nature and benefits of which was given to participants in order to obtain consent. Each participant received a single dose of vaccine. Vaccinations were carried on in April 1977, when 110 volunteers received A(H_3N_2) influenza virus vaccine, and in April 1978, when 8 volunteers received A(H_1N_1) influenza virus vaccine.

Vaccination. Two attenuated influenza virus preparations were kindly provided by Recherche et Industrie Thérapeutique, Belgium. Attenuated strains were recombinants of A/Puerto Rico/8/34 (H_0N_1) with A/Victoria/3/75 (H_3N_2) or A/URRS/90/77 (H_1N_1). Virus suspensions were given to vaccinees by dropping 0.25 ml into each nostril, i.e., an individual doses of 10^7 EID_{50}.

Antibody study. Specimens of blood serum and of nasal secretion were collected before vaccination and 24 days, and 15 and 30 days thereafter from the recipients of A-Victoria and A-URSS virus vaccines, respectively. Nasal secretions were obtained by absorption on cotton pads introduced into nostrils after surface pantocaine anesthesia. The samples were stored frozen at −20°C. Serum antibody titer was determined by a standard hemagglutination-inhibition (HI) micromethod (Sever, 1962) employing 4 units of virus antigen. Influenza virus specific IgA antibody in nasal secretions was demonstrated by indirect immunufluorenscence (IF) test performed on A-Victoria virus infected African green monkey kidney cell cultures; anti-IgA conjugate was purchased from DAKO (Copenhagen, Denmark). Neutralizing (Nt) antibody in nasal secretions was titrated after incubation of the sample with 25–50 TCD_{50} of virus at 34°C (one hour) and at 4°C (two hours); the suspensions were then inoculated onto African green monkey kidney tissue cultures; the cultures were tested for hemadsorption on the third day after inoculation. IgA in nasal secretion was determined by standard immunodiffusion technique; IgA concentration was considered to be significantly increased when concentration in the sample obtained after vaccination exceeded that of the prevaccinal sample by 25% or more.

RESULTS

No clinically significant response was observed after vaccination with A-Victoria LAV; moderate rhinorrhoea, horseness and cough, lasting three days, were reported by one individual who received A-URRS LAV. The incidence of serum antibody rise following vaccination was about 75%, since 8/10 and 6/8 subjects receiving A-Victoria or A-URRS LAV, respectively, showed significant increase

Table 1. Serum and nasal secretion immune response in volunteers receiving A/Victoria/3/75 (H_3N_2) or A/URSS/90/77 (H_1N_1) live attenuated vaccines.

A-Victoria vaccine					A-URSS vaccine				
	time of samples collection	immune responses				time of samples collection	immune responses		
		serum	nasal secretion				serum	nasal secretion	
volunteer	(days)	HI titres**	virus-specific IgA antibody (IF)	IgA (mg%)	volunteer	(days)	HI titres**	virus Nt antibody titres**	IgA (mg%)
R.R.	0*	10	–	39.3	G.G.	0*	40	2	52.6
	24	40	++	61.9		15	40	8	52.6
P.R.	0	5	–	31.0		30	20	8	43.7
	24	40	–	71.3	R.S.	0	20	8	39.3
A.L.	0	10	–	27.3		15	160	16	61.9
	24	10	–	31.9		30	80	16	52.6
E.G.	0	5	–	35.1	A.F.	0	5	2	43.7
	24	20	+	52.6		15	10	16	57.2
F.P.	0	10	±	31.0		30	10	4	57.2
	24	80	±	39.3	A.M.	0	5	2	39.3
M.M.	0	5	±	48.1		15	20	2	57.2
	24	10	+	87.0		30	20	16	48.1
G.G.	0	5	–	<27.0	R.P.	0	10	2	43.7
	24	5	–	76.7		15	20	4	66.8
E.P.	0	5	±	39.3		30	40	4	57.2
	24	20	±	57.2	L.A.	0	5	4	27
R.D.	0	10	±	48.1		15	10	4	61.9
	24	40	+++	27.0		30	10	4	52.6
S.M.	0	5	±	43.7	L.B.	0	5	2	27.3
	24	20	±	48.1		15	80	8	71.6
						30	80	8	66.8
					E.M.	0	5	2	43.7
						15	20	8	71.6
						30	20	16	43.7

*: sample collected before vaccination
**: reciprocal of serum dilution

of HI reactivity in the serum sample collected 24 or 30 days after vaccination, compared with the sample collection before.

The IgA concentration in the nasal secretions obtained from the two groups of volunteers before and after secretion is shown in Table 1. After A-Victoria LAV administration, increase of IgA concentration was detected in nasal secretion of 6 out of 10 vaccinees; in this group, only one post-vaccinal nasal secretion sample was collected 24 days after vaccination. Among the A-URRS LAV recipients, 6 individuals showed secretory IgA increase in the sample collected 15 days after vaccination; in the third nasal secretion sample, which was collected in this group 30 days after vaccination, IgA concentration showed appreciable decline with respect to the values of the second one. In each group of vaccinees, the increase in IgA concentration in nasal secretions occured, as a rule, among individuals showing significant virus-specific HI serum antibody response.

Indirect immunofluorescence test detected the appearance or the increase of A/Victoria/3/75 virus-specific IgA antibody in the post-vaccinal nasal secretion sample of 4 out of 8 volunteers which presented serum antibody responses following vaccination with A-Victoria LAV (Table 1).

After A/URSS/90/77 LAV vaccination, virus-specific neutralizing antibody reactivity was significantly increased in the nasal secretions of 7 volunteers; six of those showed HI serum antibody response (Table 1).

DISCUSSION

The serum HI antibody rise after vaccination demonstrated the immunogenicity of the live attenuated influenza virus preparations used in this study. The results presented here indicate the occurence of local immune responses in terms of increase in IgA concentration and in virus-specific antibodies.

Local immune responses occurred in the majority of individuals showing serum antibody rise. One individual among the A-URSS LAV recipients showed an appreciable increase of virus neutralizing antibody in nasal secretion without increase of local IgA concentration and of serum HI antibody. This descrepancy could either be explained through the occurrence of non-specific virus inhibitors in the nasal secretions, or through the occurrence of an isolated local antibody response (Sabin, 1976).

In this study, the development of virus specific local antibody in the IgA fraction, proven by indirect immunofluorescence test, indicates that influenza vaccination with live virus at the portal of entry is able to mimic imunogenic effects of natural infection. This evidence together with the data of the local increase of IgA concentration, which became evident shortly after vaccination, seems to be of special interest since it has been recently found that recovery from and, possibly, resistance, to respiratory virus infections is related with specific reactivity in this class of immunoglobulins (Mills et al., 1971; Ogra et al., 1977).

Our data support the generally accepted knowledge that administration of live virus vaccines by respiratory route may confer an effective immunity associated with local antibody synthesis.

RÉSUMÉ

On a mésuré la quantité d'immunoglobulines et d'anticorps spécifiques dans les sécretions nasales de 18 voluntaires, avant et après une immunisation avec deux souches de vaccin à virus vivant attenué; 11 et 10 vaccinés, respectivement, sur les 14 qui ont présenté une séroconversion, ont montré, 2-4 semaines après la vaccination, une élevation des IgA et des anticorps spécifiques dans les sécretions nasales. Dans un cas, un volontaire a présenté une élevation des anticorps neutralisants dans la sécretion nasale sans une correspondante élévation des IgA et du niveau sérique d'anticorps spécifiques. Ces résultats confirment que la vaccination par voie nasale avec virus vivant attenué produit une réponse immunitaire efficace associée avec une synthèse locale d'anticorps.

REFERENCES

1. Beare, A. S., Habershan, R. B., Tyrrell, D. A. J. and Hall, T. S., 1973: Recombinant live influenza vaccine virus; tests of transmissibility in man. J. Biol. Standard, 1, 233.
2. Couch, R. B., Douglas, R. G., Rossen, A., Kasel, J. A., in: Dayton, D. H., Small, P. A., Chanock, R. M., Kanfman, H. E., Tomassi, T. B.: The secretory immunologic system. pp. 93-112, U.S. Government printing office, Washington, D.C., 1971.
3. Donikian, M. A., McKee, J. and Greene, L. C., 1977: Challenge versus natural infection as an index of protection after influenza immunization. Develop. Biol. Standard, 39, 149.
4. Kilbourne, E. D. 1978: Influenza as a problem in immunology. J. Immunol., 120, 1447.
5. Kilbourne, E. D., Chanock, R. M., Choppin, P. W., Davenport, F. M., Fox, J. P., Gregg, M. P., Jackson, G. G., Porkman, P. D., 1974: Influenza vaccines - Summary of influenza workshop. J. Infect. Dis., 129, 750.
6. Mills, J., Knopf, H. L. S., Vankirk, J., Chanock, R. M.: Significance of local respiratory tract antibody to respiratory syncytial virus, in : Dayton, D. H., Small, P. A., Chanock, R. M., Kaufman, H. R., Tomassi, T. B.: The secretory immunologic system, pp. 149-165, U.S. Government printing office, Washington, D.C. 1971.
7. Ogra, P. L., Morag, A., Ogra, S. S., Beutner, K. R., 1971: Host defense mechanisms in resistance to airways and pulmonary infections in infants and children. Pediat. Res., 11, 231.
8. Potter, C. W., Oxford, J. S., 1979: Determinants of immunity to influenza infection in man. Brit. Med. Bull., 35, 69.
9. Rocchi, G., Carlizza, L., Andreoni, M., Ragona, G., Piga, C., Pelosio, A., Volpi, A., Muzzi, A., 1979: Protection from natural infection after live influenza virus immunization in an open population. 82, 231.

10. Sabin, A. B., 1976: Vaccination at the portal of entry of infectious agents. Develop. Biol. Standard., 33, 3.
11. Schulman, J. L.: Immunology of influenza in: The influenza viruses and influenza. Ed. by Kilbourne E. D., Acad. Press., N.Y., 1975.
12. Sever, J. L., 1962: Application of a microtechnique to viral serological investigations. J. Immunol., 88, 320.

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