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Short communication

Route of administration is the prime determinant of IgA and IgG2a responses in the respiratory tract of mice to the cold-adapted live attenuated influenza A donor strain A/Leningrad/134/17/57

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Abstract

Serum antibody and antibody secretory cell (ASC) responses to the cold-adapted (CA) live attenuated influenza A donor strain A/Leningrad/134/17/57 in BALB/c mice were determined in the lungs and mediastinal lymph nodes after administration by the intranasal, subcutaneous and intramuscular routes. Both types of response were greatest when an inoculum consisting of $10^{6.5}$ 50% egg infectious doses (EID_{50}) was administered twice intranasally at an interval of 3 weeks. Serum responses by the intramuscular route were much higher than by the subcutaneous route but, at doses of $10^{6.5–7.5}$ EID_{50}, were still lower than that obtained with two doses of an intranasal inoculum of $10^{6.5}$ EID_{50}. Virus-specific ASC responses for IgA and IgG2a were obtained in the lungs and mediastinal lymph nodes of mice inoculated with $10^{6.5}$ EID_{50} by the intranasal route. However, ASC responses after inoculation by either the subcutaneous or intramuscular routes were barely detectable, even at doses as high as $10^{7.5}$ EID_{50}. These results confirm that intranasal administration of live vaccines induces far higher virus-specific IgA and IgG2a responses in the respiratory tract of mice than can be achieved by parenteral administration and that serum antibody levels induced by parenteral vaccination are unrelated to the respiratory ASC response.

Keywords: IgA; A/Leningrad/134/17/57; Influenza A donor

1. Introduction

Evidence has accumulated over many years, principally from studies in mice, that immunity to influenza is mediated by both cellular and humoral responses—especially those involving IgA, and that responses induced by the direct administration of live viruses to the respiratory tract are superior to those obtained by parenteral routes [1–4]. Such findings have coincided with major efforts over the past 20 years to develop live attenuated vaccines for use in humans. In the US vaccines, prepared as reassortants of the cold-adapted (CA) influenza A and B donor strains A/Ann Arbor/6/60 and B/Ann Arbor/1/66, have undergone advanced clinical testing and have been shown to be safe and efficacious [5–8]. In Russia, efficacy has been demonstrated for vaccines prepared from the CA donor strains, A/Leningrad/134/17/57, A/Leningrad/134/47/57 and B/USSR/60/69, which have been used for many years in mainly pediatric populations [9–11]. CA vaccines have been shown in clinical trials to induce superior systemic cytotoxic T-cell and mucosal antibody responses in the upper respiratory tract to those induced by inactivated vaccines [3,12,13], although very little information is available concerning responses in the human lower respiratory tract. Apart from a recent comparative study in mice [14], there have been even fewer immunological studies on Russian CA vaccines.

Earlier challenge studies by Fazekas de St. Groth and Donnelly [15], Schulman and Kilbourne [16] and Tannock et al. [17], in which endpoints were determined according to protection afforded against death or by the capacity of immunized mice to clear virus from the lungs following challenge with homologous virulent virus, have also clearly indicated that immunization by the respiratory route is a far more efficient means of inducing protection than by parenteral routes. In mice superior responses to challenge have been shown to be associated with increases in levels of local IgA and serum IgG2a antibodies [18] and probably results from the amplification and presentation of critical protective epitopes to immune cells [19]. We describe in the present communication virus-specific IgA and IgG2a antibody

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secreting cell (ASC) responses in the lungs and mediastinal lymph nodes (MLN), which drain the lower airways of mice, after administration with two doses of the Russian CA donor strain A/Leningrad/134/17/57 (Len/17) by the intranasal (i.n.), intramuscular (i.m.) and sub-cutaneous (s.c.) routes. Virus-specific ASC responses were compared with serum antibody responses. Previous studies in mice have shown that two doses, each of 5–6 $\log_{10}$ 50% egg infectious doses (EID_{50}) of the CA donor strain A/Ann Arbor/6/60 or reassortants prepared from A/Ann Arbor/6/60 and administered at an interval of 3 weeks, are necessary to induce a maximum immunogenic response [20]. Similar findings for the Russian CA donor strains have been made by Romanova et al. [21].

2. Materials and methods

2.1. Mouse experiments

Groups of 4–6-week-old male BALB/c mice from the Animal Resources Centre (Murdoch, WA, Australia) were anaesthetized with Ketamine/Xylazine (50–150 mg/kg) and inoculated by the i.n., s.c. or i.m. routes with 50 μl of Len/17 diluted in phosphate-buffered saline (PBS). Len/17 was obtained from Professor G.I. Alexandrova of The Research Institute of Experimental Medicine, Russian Academy of Medical Sciences under The Australia–Russia Agreement for Collaboration in Medical Research and Public Health. Individual mice inoculated by the i.n. route received two doses, each of $10^{6.5}$ EID_{50}, at an interval of 3 weeks. Virus-specific ASC responses in the lungs and MLN were investigated as these are the primary sites of infection where viral antigens are presented to T and B cells via antigen processing cells [22]. Control mice were inoculated twice with the same volume of normal allantoic fluid. Higher i.n. doses resulted in respiratory distress and death, indicating the presence of some residual virulence for Len/17 in mice, and were not used in the study. However, mice inoculated by the s.c. and i.m. routes with $10^{6.5}$, $10^{7.0}$ or $10^{7.5}$ EID_{50} showed no signs of distress. Seven days after the last inoculation, all mice were euthanased by increasing the normal anaesthetizing dose by a factor of 2.5.

2.2. Serum antibody assays

Euthanased mice were heart bled and the sera heated at 56 °C for 30 min to inactivate thermolabile non-specific inhibitors. Thermostable inhibitors were inactivated by adding an equal volume of 0.05 M potassium periodate to each serum and incubating the mixture at room temperature for 2 h. The reaction was stopped by the addition of an equal volume of 5% (w/v) glucose. Influenza-specific serum antibodies were detected using a standard hemagglutination inhibition (HI) assay in 96-well V-microtiter plates using 0.5% chicken erythrocytes [23].

2.3. ASC analysis

Single cell suspensions of lymphocytes from the lungs and MLN of mice were prepared, depleted of erythrocytes, pooled and resuspended in T-cell medium (TCM), as described by Baumgarth and Kelso [24]. Aggregates of cell debris from lung suspensions were first allowed to settle and the suspended cells retained for counting and analysis. Influenza-specific ASCs were enumerated using an ELISPOT assay described by Wareing et al. [14].

3. Results

Table 1 shows that geometric mean titers (GMTs) of 132, 12 and 4 were obtained in response to two doses of an inoculum consisting of $10^{6.5}$ EID_{50} of Len/17 by the i.n., i.m. and s.c. routes, respectively. Increasing the s.c. immunizing dose to $10^{7.0}$ and $10^{7.5}$ EID_{50} did not result in an increase in antibody titer. GMTs obtained following two i.m. doses of an inoculum of $10^{7.0}$ or $10^{7.5}$ EID_{50} of Len/17 were 47 and 64, less than those obtained with two i.n. doses of $10^{6.5}$ ED_{50}.

Two i.n. doses of $10^{6.5}$ EID_{50} induced high numbers of virus-specific IgG2a and IgA ASCs in both the MLN (569 ± 141 and 1069 ± 397) and lungs (721 ± 340 and 1053 ± 486), respectively. Few, if any, ASCs could be detected in response to two doses of the same i.m. or s.c. inocula (Table 2). Increasing the immunizing dose of Len/17 to $10^{7.0}$ or $10^{7.5}$ EID_{50} resulted in no significant increase in levels of virus-specific ASCs for either the i.m. or s.c. routes.

4. Discussion

Protection of the upper respiratory tract to influenza infection is primarily due to local mucosal IgA responses [6,25,26]. IgG2a, as well as IgG1, have been shown to be the major IgG subclasses involved in virus neutralization in mice [27] and IgG2a is also an efficient activator of complement

Table 1
Serum HI antibody responses in mice inoculated with Len/17 by the s.c., i.m. and i.n. routes

Inoculum ($\log_{10}$ EID_{50})	Route of inoculation		
	s.c.	i.m.	i.n.
Control	4[a]	4	4
6.5	4	12	132
7.0	4	47	ND[b]
7.5	4	64	ND

All control mice had titers of <4.

[a] Sera from groups of three mice inoculated with two doses consisting of $10^{6.5}$, $10^{7.0}$ or $10^{7.5}$ EID_{50} of Len/17 were pooled. Serum HI antibody responses were expressed as geometric mean titres from three independent experiments.

[b] ND: not determined.

Table 2
Virus-specific ASC responses in the lungs and MLN of mice administered Len/17 by different routes

Ig-isotype	Inoculum $\log_{10}$ EID_{50}	MLN			Lung		
		s.c.	i.n.	i.m.	s.c.	i.n.	i.m.
IgA	6.5	0	1069 ± 397[a]	0	0	1053 ± 486	0
	7.0	0	ND	0	0	ND[b]	6.3 ± 6.3
	7.5	0	ND	0	0	ND	3.7 ± 3.7
IgG2a	6.5	0	569 ± 141	0	0	721 ± 340	1.3 ± 1.3
	7.0	34.7 ± 34.7	ND	0	2.0 ± 1.0	ND	0
	7.5	0	ND	8.7 ± 8.7	0	ND	2.7 ± 1.3

[a] Groups of three mice were inoculated with two doses consisting of $10^{6.5}$, $10^{7.0}$ or $10^{7.5}$ EID_{50} of Len/17 by the i.n., i.m. and s.c. routes. Lymphocytes from mice from each group were pooled. Data are mean ± S.E.M. ASC response from three independent experiments.
[b] ND: not determined.

[27,28]. Live influenza infection has been shown to provide the most effective protection against homologous challenge and is accompanied by increases in local IgA and serum IgG2a antibody in mice [18]. Relatively poor responses have been reported for subunit influenza vaccines, compared with live vaccines, for very young and (presumably) unprimed children [12,29].

From the present study, s.c. inoculation with Len/17 did not elicit significant serum antibody or ASC responses in either the lungs or MLN (Tables 1 and 2). Similarly, i.m. inoculation induced only very low numbers of ASCs, although a serum antibody response could be detected. An increase in the size of the i.m. dose neither led to an increase in the numbers of ASCs detected nor to a proportionate increase in HI titer (Tables 1 and 2). Intravenous injection of live virus has been shown to induce low ASC responses in the lungs but undetectable levels of virus-specific IgA in lung washings [4,30]. The s.c. inoculation with inactivated virus in the presence of an adjuvant produced higher serum antibody responses than live virus administered by intravenous injection or by aerosol [4], indicating a role for adjuvants in the modulation of immune responses [18,28,31].

Justewicz et al. [19] have shown that ASCs accumulate in the spleens of mice after intraperitoneal inoculation with an influenza subunit vaccine. When live virus was administered by the i.n. route, accumulation occurred mainly in the lungs [32]. In humans, inactivated split virus vaccines administered by the intramuscular route have been shown to induce circulating IgG, IgA and IgM ASCs, as well as IgA- and IgM-specific ASCs in the tonsils [33] but there is no information concerning ASC activity in the human lower respiratory tract. Low serum IgA levels induced by inactivated vaccines are probably unrelated to levels of virus-specific IgA ASCs, which are probably recruited to the mucosal surfaces of the respiratory tract from the circulatory system. However, migration of IgA ASCs to the mucosal surfaces appears to be dependent upon replication in the respiratory mucosa [34], which may explain the poor ASC response in the MLN and lungs of mice following i.m. administration with CA viruses, despite the presence of a detectable serum antibody response. Tannock et al. [20] showed that CA viruses were restricted in their capacity to grow in the respiratory tract of mice and that a single i.n. dose of CA virus to mice induced a serum antibody response, but relatively little capacity to clear a respiratory virus challenge consisting of wild-type viruses with the same surface antigens. These findings are consistent with those of the present study, which also suggests that, at least for mice, protection against respiratory infection is not directly related to levels of serum antibody.

References

[1] Bruhl P, Kerschbaum A, Kistner O, Barrett N, Dorner F, Gerencer M. Humoral and cell-mediated immunity to vero cell-derived influenza vaccine. Vaccine 2000;19:1149–58.
[2] Webster RG, Askonas BA. Cross-protection and cross-reactive cytotoxic T cells induced by influenza virus vaccines in mice. Eur J Immunol 1980;10:396–401.
[3] Blazevic V, Trubey CM, Shearer GM. Comparison of in vitro immunostimulatory potential of live and inactivated influenza viruses. Hum Immunol 2000;61:845–9.
[4] Liew FY, Russell SM, Appleyard G, Brand CM, Beale J. Cross-protection in mice infected with influenza A virus by the respiratory route is correlated with local IgA antibody rather than serum antibody or cytotoxic T cell reactivity. Eur J Immunol 1984;14:350–6.
[5] Mendelman PM, Cordova J, Cho I. Safety, efficacy and effectiveness of the influenza virus vaccine, trivalent, types A and B, live, cold-adapted (CAIV-T) in healthy children and healthy adults. Vaccine 2001;19:2221–6.
[6] Belshe RB, Gruber WC, Mendelman PM, et al. Correlates of immune protection induced by live, attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. J Infect Dis 2000;181:1133–7.
[7] Longini IM, Halloran ME, Nizam A, et al. Estimation of the efficacy of live, attenuated influenza vaccine from a 2-year, multi-center vaccine trial: implications for influenza epidemic control. Vaccine 2000;18:1902–9.
[8] Belshe RB, Gruber WC, Mendelman PM, et al. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. J Pediatr 2000;136:168–75.
[9] Khan AS, Polezhaev F, Vasiljeva R, et al. Comparison of US inactivated split-virus and Russian live attenuated, cold-adapted trivalent influenza vaccines in Russian schoolchildren. J Infect Dis 1996;173:453–6.
[10] Kendal AP. Cold-adapted live attenuated influenza vaccines developed in Russia: can they contribute to meeting the needs for influenza control in other countries? Eur J Epidemiol 1997;13:591–609.

[11] Rudenko LG, Slepushkin AN, Monto AS, et al. Efficacy of live attenuated and inactivated influenza vaccines in schoolchildren and their unvaccinated contacts in Novgorod, Russia. J Infect Dis 1993;168:881–7.
[12] Johnson PR, Feldman S, Thompson JM, Mahoney JD, Wright PF. Immunity to influenza A virus infection in young children: a comparison of natural infection, live cold-adapted vaccine, and inactivated vaccine. J Infect Dis 1986;154:121–7.
[13] Clements ML, Murphy BR. Development and persistence of local and systemic antibody responses in adults given live attenuated or inactivated influenza A virus vaccine. J Clin Microbiol 1986;23:66–72.
[14] Wareing MD, Watson JM, Brooks MJ, Tannock GA. Immnunogenic and isotype-specific responses to Russian and US cold-adapted influenza a vaccine donor strains A/Leningrad/134/17/57, A/Leningrad/134/47/57, and A/Ann Arbor/6/60 (H2N2) in mice. J Med Virol 2001;65:171–7.
[15] Fazekas de St. Groth S, Donnelly M. Studies in experimental immunology of influenza. IV. The protective value of active immunization. Aust J Exp Biol Med Sci 1950;28:61–75.
[16] Schulman JE, Kilbourne ED. Induction of partial specific heterotypic immunity in mice by a single infection with influenza A virus. J Bacteriol 1965;89:170–4.
[17] Tannock GA, Wark MC, Smith LE, Sutherland MM. A clearance test in mice using non-adapted viruses to determine the immunogenicity of influenza strains. Arch Virol 1981;70:91–101.
[18] Ben Ahmeida ET, Jennings R, Erturk M, Potter CW. The IgA and subclass IgG responses and protection in mice immunised with influenza antigens administered as ISCOMS, with FCA, ALH or as infectious virus. Arch Virol 1992;125:71–86.
[19] Justewicz DM, Morin MJ, Robinson HL, Webster RG. Antibody-forming cell response to virus challenge in mice immunized with DNA encoding the influenza virus hemagglutinin. J Virol 1995;69:7712–7.
[20] Tannock GA, Paul JA, Barry RD. Relative immunogenicity of the cold-adapted influenza virus A/Ann Arbor/6/60 (A/AA/6/60-ca), recombinants of A/AA/6/60-ca, and parental strains with similar surface antigens. Infect Immun 1984;43:457–62.
[21] Romanova JR, Tannock GA, Alexandrova GI. Protective responses in mice to vaccination with multiply administered cold-adapted influenza vaccine reassortants and wild-type viruses. Vaccine 1997;15:653–8.
[22] Cyster JG. Chemokines and cell migration in secondary lymphoid organs. Science 1999;286:2098–102.
[23] Concepts and Procedures for Laboratory-Based Influenza Surveillance, Immunology Series, vol. 6, Procedural Guide, US Department of Health and Human Services/Public Health Service/Centers for Disease Control, Section 4.2, 1982.
[24] Baumgarth N, Kelso A. Functionally distinct T cells in three compartments of the respiratory tract after influenza virus infection. Eur J Immunol 1996;26:2189–97.
[25] Renegar KB, Small Jr PA. Immunoglobulin A mediation of murine nasal anti-influenza virus immunity. J Virol 1991;65:2146–8.
[26] Renegar KB, Small Jr PA. Passive transfer of local immunity to influenza virus infection by IgA antibody. J Immunol 1991;146:1972–8.
[27] Hocart MJ, Mackenzie JS, Stewart GA. The IgG subclass responses to influenza virus haemagglutinin in the mouse: effect of route of inoculation. J Gen Virol 1989;70:809–18.
[28] Benne CA, Harmsen M, van der Oraaff W, Verheul AF, Snippe H, Kraaijeveld CA. Influenza virus neutralizing antibodies and IgG isotype profiles after immunization of mice with influenza A subunit vaccine using various adjuvants. Vaccine 1997;15:1039–44.
[29] Johnson Jr PR, Feldman S, Thompson JM, Mahoney JD, Wright PF. Comparison of long-term systemic and secretory antibody responses in children given live, attenuated, or inactivated influenza A vaccine. J Med Virol 1985;17:325–35.
[30] Jones PD, Ada OL. Influenza virus-specific antibody-secreting cells in the murine lung during primary influenza virus infection. J Virol 1986;60:614–9.
[31] Lovgren K, Kaberg H, Morein B. An experimental influenza subunit vaccine (iscom): induction of protective immunity to challenge infection in mice after intranasal or subcutaneous administration. Clin Exp Immunol 1990;82:435–9.
[32] Justewicz DM, Doherty PC, Webster RG. The B-cell response in lymphoid tissue of mice immunized with various antigenic forms of the influenza virus hemagglutinin. J Virol 1995;69:5414–21.
[33] Brokstad KA, Cox RJ, Olofsson J, Jonsson R, Haaheim LR. Parenteral influenza vaccination induces a rapid systemic and local immune response. J Infect Dis 1995;171:198–203.
[34] El-Madhun AS, Soreide A, Olofsson J, Cox RJ, Haaheim KR. Is influenza priming necessary to mount a mucosal immune response to parenteral influenza vaccination in small children? Amsterdam: Elsevier; 1996. p. 298–303.