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Immunization against influenza A virus: Comparison of conventional inactivated, live-attenuated and recombinant baculovirus produced purified hemagglutinin and neuraminidase vaccines in a murine model system

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Abstract

To simulate the 2003–2004 influenza season and compare available vaccination methods, immunologically naive mice were immunized with: influenza A virus hemagglutinin (rHA) and neuraminidase (rNA) from A/Panama/2007/99 H3N2 or A/Fujian/411/2002 H3N2 expressed by recombinant baculovirus, chromatographically purified, either as single antigens (rHA or rNA) or in combination (rHArNA); conventional inactivated monovalent (CIV) vaccines from each heterotypic strain; or a live-attenuated influenza (LAV) vaccine derived from the A/Panama/2007/99 strain. HA containing vaccines were highly immunogenic for the HA antigen, with no statistically significant differences among groups in the amount of homotypic anti-HA antibody induced. Little cross-reactive anti-HA antibody was induced by any vaccine, including LAV. Statistically, the greatest amount of anti-NA antibody was induced by the purified NA alone or in combination with purified HA; the least amount of anti-NA antibody was found in mice immunized with LAV or CIV. Immunization with vaccines immunogenic for both HA and NA resulted in an immune response to both surface glycoproteins that suppressed homotypic, closely related heterotypic infection and had a greater reduction in mPVT following an infectious challenge by a distantly related heterotypic strain. These studies suggest that vaccines immunogenic for both HA and NA offer an increased level of protection from influenza.

Keywords: Influenza virus; Vaccine

Introduction

Influenza remains a pervasive public health problem in spite of the wide availability of two currently licensed vaccines against influenza: conventional inactivated virus vaccine (CIV) and live-attenuated vaccine (LAV). Conventional inactivated influenza vaccines are derived from formalin inactivated high-yield reassortant viruses whose internal genes are from a A/PR/8/34 high-yield donor parent (Hocart et al., 1995; Johansson et al., 1989; Kilbourne, 1980). Similarly, the live-attenuated influenza vaccine is derived from a master donor virus (MDV) strain containing temperature sensitive (ts), cold-adapted (ca) and attenuation (att) mutations in several genes coding for internal proteins (Belshe, 1995; Couch, 1993; Maassab et al., 1990). Each of these vaccines possesses the hemagglutinin (HA) and neuraminidase (NA) of recently prevalent influenza A and B viruses predicted to cause widespread infection. When either of these vaccines are

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effective, illness is prevented usually by preventing infection (Hobson et al., 1973). Both the conventional inactivated and live-attenuated influenza vaccines are effective when the HA of the vaccine strain closely antigenically matches the HA of the wild-type infecting virus (Couch et al., 1974; Johansson et al., 1989; Kilbourne, 1980); the HA antigen component of these vaccines is chosen prior to each influenza season based on predictions of what viral strain will be the predominant circulating wild-type virus. The inactivated conventional influenza vaccine (i.e., Fluzone, Fluvirin), live-attenuated influenza vaccine (Flumist) and a recombinant baculovirus produced single-antigen purified HA vaccine (FluBlok) currently under development will require annual change in vaccine formulation to meet the continual antigenic drift of the wild-type influenza HA. Vaccine failure can occur as a result of antigenic differences that may accumulate in the HA (antigenic drift) during the time the vaccine strain is chosen and infection occurs or if the predicted vaccine strain is not the prevalent wild-type strain, as occurred in the 2003–2004 influenza season with A/Panama/2007/99 H3N2 and A/Fujian/411/2002 H3N2.

Immunity to both surface antigens engenders a more protective immunity to both a homotypic and heterotypic infectious challenge (Chen et al., 1999; Johansson, 1999; Johansson and Kilbourne, 1993; Kendal et al., 1980). Immunizing mice (Johansson et al., 1998) or humans (Kilbourne et al., 1995; Schiff et al., 2000) with conventional monovalent H3N2 vaccine supplemented with N2-NA chromatographically purified from influenza virions, conventional trivalent influenza vaccine supplemented with recombinant baculovirus produced N1- and N2-NA (Johansson et al., 2002), H3-HA and N2-NA produced from recombinant baculovirus (Johansson, 1999) or with DNA plasmids encoding for HA and NA (Chen et al., 1999) produced an immunity that had a balanced response to both surface antigens. This immunity suppressed homotypic viral infection and significantly reduced viral replication in the lungs of mice challenged with either a closely or more distantly antigenically related heterotypic viral strains (Chen et al., 1999; Johansson, 1999; Johansson et al., 1998, 2002) and significantly reduced duration and frequency of viral shedding in humans (Kilbourne et al., 1995; Schiff et al., 2000).

The present study reports a comparison of the relative immunogenicity of and protection against homotypic and heterotypic infectious challenge induced by a monovalent live-attenuated H3N2 vaccine, monovalent inactivated H3N2 vaccine, purified baculovirus recombinant produced H3-HA and N2-NA vaccines and a combined purified H3-HA and N2-NA vaccine, utilizing the A/Panama/2007/99 H3N2 (the H3N2 strain in the vaccine) and A/Fujian/411/2002 H3N2 (the circulating wild-type strain) strains in an attempt to simulate the antigenic choices of the 2003–2004 influenza season in a murine model system.

Results

Serologic response to immunization

All mice initially immunized and subsequently boosted with a vaccine containing HA (i.e., Groups 1, 2, 4–8) had an anti-HA antibody response consistent with a secondary immune response as measured by HI assay (Table 1). No HI antibody was detected in animals immunized with NA only (Group 3) or mock-immunized (Group 9). There were no statistically significant differences among groups immunized with an HA containing vaccine in anti-HA homotypic antibody titers. However, little cross-reactive heterotypic anti-HA antibody was induced by any vaccine containing HA, including the live-attenuated vaccine (Group 8). Overall, there was no evidence of suppression of the immune response to either HA in any group including groups that contained antigenically equivalent amounts of NA (Groups 4, 5) and both heterotypic HA strains (Group 5).

Anti-NA antibody was produced in all mice immunized with vaccines containing NA (i.e., Groups 3–8) as measured by NI assay. No anti-NA antibody was detected in groups immunized with HA only (Groups 1, 2) or in the mock immunization group (Group 9). Animals immunized with purified NA (Group 3) and each of the combined

Table 1
Serologic response to vaccination

Group	Hemagglutinin inhibition titers		Neuraminidase inhibition titers	
	A/Panama[a]	A/Fujian	A/Panama	A/Fujian
1) H3/Panama[b]	6.3	2.4	<4	<4
2) H3/Fujian[c]	2.6	6.1	<4	<4
3) N2/Panama[d]	<1	<1	8.9	7.1
4) H3/P+ N2/P[e]	6.0	2.2	8.4	6.4
5) H3/P+ H3/F+ N2/P[f]	6.2	6.5	8.2	6.6
6) H3N2/P[g]	5.4	2.1	4.1	3.3
7) H3N2/F[g]	2.6	5.9	3.1	3.9
8) Live H3N2/Panama[h]	5.9	3.1	2.1	<4
9) PBS[i]	<1	<1	<1	<1

[a] Test antigen: purified HA or NA from egg grown virus. Numbers are geometric mean titers of duplicate assays with 15 mice per group. Significance among groups tested by ANOVA ($P < 0.001$) and Tukey test subsequent to ANOVA.

[b] 10 μg of purified recombinant baculovirus produced H3 A/Panama/2007/99.

[c] 10 μg of purified recombinant baculovirus produced H3 A/Fujian/411/2002.

[d] 10 μg of purified recombinant baculovirus produced N2 A/Panama/2007/99.

[e] 10 μg of HA and 10 μg of NA of A/Panama/2007/99 each purified recombinant baculovirus produced.

[f] 10 μg of each purified recombinant baculovirus produced H3 A/Panama/2007/99 and A/Fujian/411/2002 (total H3 protein 20 μg) and 10 μg of purified recombinant baculovirus produced N2-NA from A/Panama/2007/99 (Total N2 protein 10 μg).

[g] Monovalent conventional vaccine: H3N2; P: A/Panama/2007/99, F: H3N2 A/Fujian/411/2002; 10 μg of HA.

[h] Live non-mouse-adapted H3N2 A/Panama/2007/99 virus.

[i] Phosphate buffered saline.