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IMMUNOGENICITY AND TOLERABILITY OF INACTIVATED FLU VACCINE IN HIGH RISK AND HEALTHY CHILDREN

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Abstract We conducted this open study to evaluate the immunogenicity and safety of the inactivated influenza vaccine, Imovax Gripe® in 154 children between 6 and 36 months of age at high risk of influenza-related complications, and in a reference group of 64 healthy children. The study was conducted over two flu seasons, in which the vaccine contained the same A strains but different B strains. The results for the A/H3N2 and A/H1N1 strains from the two flu seasons were pooled, but those for the B strains were not. Anti-hemagglutinin (HA) antibody titers were determined before, and one month after each vaccination, and safety was evaluated based on diary card reporting any adverse event observed, either included or not in the list of "solicited events". Within each group of vaccines, the seroconversion rates, seroprotection rates, and ratio of post- to prevaccination geometric mean titers (GMTR) for the A/H3N2 and the A/H1N1 strains fulfilled all requirements of the criteria of the European Union Committee for Proprietary Medicinal Products (CPMP). The immune responses in high-risk and in healthy children were similar, and consistent with those observed in previous studies conducted in healthy children. The vaccine was equally well tolerated by all study groups. Reactogenicity was low and similar in both high-risk and healthy children. Overall from 9.5% to 15.4% of at-risk children and 12% of healthy children reported a solicited local reaction; 23.0 to 28.8% of high-risk and 25.3% of healthy children reported a solicited systemic reaction. The study results provide support for vaccination of children at high-risk of influenza related complications.

Key words: inactivated influenza vaccine, immunogenicity, safety, high-risk, children

Resumen ***Inmunogenicidad y tolerancia de la vacuna inactivada anti-influenza en niños en alto riesgo y en controles sanos.*** Se realizó un estudio clínico abierto para evaluar la inmunogenícidad y la seguridad de la vacuna inactivada anti-influenza, Imovax Gripe®, en 154 niños entre 6 y 36 meses de edad con alto riesgo de complicaciones ligadas a la influenza, y en un grupo de referencia de 64 niños sanos. El estudio fue conducido en dos temporadas de gripe, durante las cuales la vacuna utilizada contenia las mismas cepas A pero diferentes cepas B. Los resultados para las cepas A/H3N2 y A/H1N1 de las dos temporadas de gripe fueron combinados (*pool* de datos), pero no los de las cepas B. Los títulos de anticuerpos anti-hemaglutinina (HA) fueron determinados inmediatamente antes y un mes despues de cada vacunación, y la seguridad o tolerancia fue evaluada según la información de efectos adversos notificados, en cartillas para llenado diario, que incluían todos los eventos, figuraran o no en la lista de los "eventos solicitados". En cada grupo, las tasas de seroconversion y de seroprotección, y la razón de la media geométrica de títulos post-/ pre-vacunación (GMTR) para las cepas A/H3N2 y A/H1N1 cumplieron con todos los requisitos del Comité de Especialidades Farmacéuticas (CPMP) de la Unión Europea. Las respuestas inmunes fueron similares en los niños con alto riesgo y en los sanos, y consistentes con los resultados observados en los estudios anteriores en los niños sanos. La vacuna fue bien tolerada y la reactogenicidad fue baja y similar en los dos grupos de niños estudiados. Las reacciones locales listadas en la solicitud, fueron observadas en el 9.5 a 15.4% y en el 12% de niños con alto riego y sanos respectivamente; mientras que los síntomas sistémicos solicitados fueron observados en el 23.0 a 28.8% y el 25.3% de niños respectivamente. Los resultados de este estudio proveen informatión adicional a favor de la vacunación de niños con alto riesgo de complicaciones relacionadas con influenza.

Palabras clave: vacuna inactivada anti-influenza, inmunogenicidad, tolerancia, alto riesgo, niños

In young children, influenza is usually self-limiting, but it may be associated with severe complications, particularly in children with chronic medical conditions. Common complications include exacerbation of underlying pulmonary or cardiopulmonary diseases, or bacterial pneumonia**.** Individuals younger than 2 years of age are at substantially higher risk of hospitalization to treat lower respiratory tract infection, non-specific febrile illness, or central nervous system complications following influenza infection[1-4]. During influenza epidemics, attack rates can be over 40% in preschool children and 30% in school

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age children, with the highest rates of severe influenza-related illness occurring among infants 6-12 months of age[3]. Indeed, some reported rates of influenza-related hospitalization in children younger than 1 year of age are similar to or even higher than those reported for the elderly with high-risk medical conditions[4].

Vaccination is the primary means of protection against illness caused by influenza. Based on the epidemiology of the disease, the World Health Organization recommends annual influenza vaccination for children 6 months of age and older who have at least one specific medical condition such as chronic cardiac, pulmonary, kidney and metabolic disorders that places them at risk of severe complications of infection[5]. The Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention (CDC) and the American Academy of Pediatrics (AAP) in the United States, recommend flu vaccination of all children between 6 and 23 months of age[6, 7].

In our institution, the Hospital Nacional de Niños, San José, Costa Rica, oxygen-dependent infants have routinely been administered influenza vaccine, leading to a significant reduction of influenza-associated hospitalizations for those patients. The hospital performs viral immunofluorescence testing in all patients presenting with an upper or lower respiratory tract infection. In the year 2000 we detected a rise in influenza virus circulation in our high-risk patients that began in March (late summer), with a maximum number of cases in May (early rainy season) and a subsequent decrease in June. Most studies on the immunogenicity and safety of flu vaccines in children have been conducted in healthy individuals. Few data are available on the immunogenicity of flu vaccines in at-risk children, such as those in our institution[8]. Therefore, to provide additional support for vaccination, we evaluated the immunogenicity and tolerability of the inactivated influenza vaccine Imovax Gripe® (Sanofi Pasteur, Lyon, France) in children, 6 to 36 months of age, at high risk for developing respiratory complications of influenza, and in a control group of healthy children.

Methods

Study design

This was an open, controlled study performed from March 2000 to March 2002 at one pediatric centre in Costa Rica. The study was performed according to Good Clinical Practice guidelines, the Declaration of Helsinki and ICH regulatory guidelines. Full ethical approval was obtained from the "Unidad de Bioética e Investigación del Hospital Nacional de Niños" (San José, Costa Rica). Informed written consent was obtained from each child's parent or legal guardian as well as an independent witness as required by the current local regulations before enrolment in the study.

Children of either sex, from 6 to 35 months of age were assigned in equal numbers to a high risk group (Group A) or a control group of healthy children (Group B). The high risk group included subjects with chronic disorders of the pulmonary or cardiovascular systems, including asthma, and conditions requiring regular medical follow-up or hospitalization during the previous year due to chronic metabolic diseases, renal dysfunction, hemoglobinopathies or immunosuppression. Premature, oxygen-dependent children were also eligible for inclusion in Group A. Children with a known or suspected allergy to any of the vaccine components, a previous history of therapy with cadaveric pituitary derived human growth hormone, or having already received flu vaccination for the current season were excluded. The study was carried out over two influenza seasons (2000-2001 and 2001-2002).

Vaccine

The vaccine used in this study was the inactivated split virion influenza vaccine Imovax Gripe (licensed in many countries under the name Vaxigrip®). A pediatric dose of 0.25 ml, which is half of the adult dose, was given by intramuscular injection into the right deltoid muscle or into the right thigh. High-risk children with no history of flu infection or vaccination (Subgroup A1) and healthy children (group B) received two doses of flu vaccine with a one month interval. High-risk children who had been vaccinated against flu in the previous years or had a confirmed history of flu infection (Subgroup A2) received one dose of vaccine. On enrolment, subjects were given an inclusion number that indicated the chronological order of enrolment, their group allocation, and whether they were to receive one or two doses of vaccine.

The study vaccine formulation for both seasons contained the same A strains, A/Moscow/10/99 an A/Panama/2007/99 (H3/N2) analogous strain, and A/New Caledonia/20/99 (H1N1), but different B stains. The 2000-2001 formulation incorporated a B/Yamanashi/166/98 analogous strain: B/Beijing/184/93 and in the 2001-2002 season a B/ Johannesburg /5/99 analogous strain: B/Sichuan /379/99. Each 0.25 ml pediatric dose contained 7.5 µg of the hemagglutinin of each virus strain. The 2001-02 vaccine was thiomersal free.

Safety and tolerability

Each subject was observed for 30 minutes for the occurrence of immediate reactions to vaccination. Parents or guardians were given a diary card to record the date of onset, date of resolution and severity of any local or systemic event occurring during the 30 days period between the vaccination and follow-up visit were these events included in a list of "solicited events" or not. Solicited local events were pain redness, induration, edema, ecchymosis and pruritus. Solicited systemic events were fever (axillary temperature ≥ 37.1 °C), asthenia, headache, arthralgia, myalgia, shivering and sweating. Serious adverse events (SAEs) were recorded throughout the study.

Immunogenicity

The primary objective was evaluation of the immunogenicity of the vaccine one month after vaccination of 6 to 35 month-old children with a high risk of influenza complications. Evaluation of the immune response in healthy subjects, who served as a reference group in case of an impaired response in high risk infants, was a secondary objective. No statistical hypothesis was tested.

Sera prepared from blood samples collected from each subject immediately prior to, and 30 days after each vaccination, were stored between -20 °C to -80 °C and posted to a World Health Organization (WHO) reference center laboratory

at Rockefeller University in Lyon, France. Anti-hemagglutinin (HA) antibody titers were determined using the WHO haemagglutination inhibition reference technique, and expressed as an inverse dilution (1/dil).[9] The analysis was observer-blinded.

The immune response to the three vaccine viral strains was assessed by calculation of the geometric mean titers (GMT) of anti-HA antibodies (with 95% confidence intervals), geometric means of post- to pre-vaccination antibody titer ratios (GMTR), seroprotection rates (percentage of subjects with a titer ≥ 40 IU/ml on day 30), seroconversion rates (percentage of subjects with a pre-vaccination titer < 10 IU/ml achieving a titer ≥ 40 IU/ml by day 30) or significant increases in anti-HA levels (at least a four-fold rise by day 30, whatever the pre vaccination titer). European Agency for the Evaluation of Medicinal Products/Committee for Proprietary Medicinal Products (CPMP) immunogenicity criteria for licensed influenza vaccines specify 70% seroprotection or 40% seroconversion, or a GMTR of 2.5 for individuals between 18 and 60 years of age. There are no specific criteria for those younger than 18 years. A GMT ≥ 40 IU/ml is considered to be protective against influenza infection[10].

Statistical evaluation

This was a descriptive study based on the use of 95% confidence intervals (CI), with no statistical hypothesis testing being performed. As no statistical hypothesis testing was performed, the sample size calculation was based on the expected precision of the results (extent of the 95% CI) and on the European recommendations for studies evaluating influenza vaccines to be used during each influenza season (at least 50 subjects per group). Therefore, 60 subjects were planned to be recruited in each subgroup, a total of 180 children. Subjects were excluded from the immunogenicity analysis if an injection was missed or if a blood sample was not collected within the 21 to 40 days following injection specified in the study protocol. Tolerability was analyzed for all subjects who received at least one dose of vaccine. Subgroups A1 and A2 were evaluated separately.

Results

Subject disposition

A total of 218 subjects were enrolled in the study (84 in Subgroup A1, 54 in Subgroup A2, and 80 in Group B). Of these, 94 high-risk children, (60 in Subgroup A1, and 34 in Subgroup A2) and 60 healthy children were recruited in the first inclusion period from 1 March to 29 May during the 2000-2001 influenza season. An additional 64 subjects (44 high risk children: 24 in Subgroup A1, and 20 in Subgroup A2; and 20 healthy children) entered in the second inclusion period, from 11 October to 25 February, during the 2001-2002 season. All enrolled subjects received the first vaccination. A total of 23 subjects discontinued the study, 19 subjects during the first inclusion period; of which four were lost to follow-up, 14 were withdrawn by parents, and one by the investigator. Four subjects discontinued during the second inclusion period. One was lost to follow-up and the parents withdrew 3 subjects. Subject demographics are summarized in Table 1. The majority of at risk children (n=51) had chronic disorders or malformations of the respiratory tract, or a history of pulmonary infections; cardiovascular diseases of malformations of the heart (n=21); or gastrointestinal disorders (N=15), for the most part gastroesophageal reflux.

Immunogenicity

Immunogenicity results are summarized in Tables 2 and 3 for all vaccine strains and treatment groups. The re-

TABLE 1.– *Subject demographics and reasons for discontinuation (both inclusion periods)*

	At risk				Healthy		All	
	Subgroup A1.(2 inj)		Subgroup A2.(1 inj)		Group B.(2 inj)			
N included	84		54		80		218	
Male n (%)	48 (57.1%)		38 (70.4%)		46 (57.5%)		132 (60.6%)	
Female n (%)	36 (42.9%)		16 (29.6%)		34 (42.5%)		86 (39.4%)	
Male/Female ratio	1.33		2.38		1.35		1.53	
Age (mo) Mean±SD*(range)	14.5±7.89 (6-36)		24.5±6.97 (10-35)		17.3±9.29 (6-36)		18±9.09 (6-36)	
	n	%	n	%	n	%	n	%
Subjects completed	74	88.1	50	92.6	71	88.8	195	89.4
Subjects withdrawn	10	11.9	4	7.4	9	11.3	23	10.6
Lost to follow up	3	30.0	1	25.0	1	11.1	5	21.7
By parents	6	50.0	3	75.0	8	88.9	17	69.6
By investigator	1	20.0	0	0	0	0	1	8.7

**SD = Standard deviation; inj: injection*

TABLE 2.– *Geometric mean titers, seroconversion and seroprotection rates, and geometric means of post-to prevaccination ratios of anti-hemagglutinin antibodies before, and 30 days after each vaccination in high-risk children*

	Subgroup A1 (High Risk, 2 doses)			Subgroup A2 (High Risk, 1 dose)	
Influenza Vaccine Strains	Pre-vaccination	Post-1st dose	Post-2nd dose	Pre-vaccination	Post-vaccination
A/Panama/2007/99 (A/H3N2)	N = 62	N = 59*	N = 62	N = 45	N = 45
GMT (1/dil)	12.1	54.1	206.9	34.8	233.4
95% CI[7.4;19.8]	[34.2;85.6]	[151;283]	[18.6;65.3]	[142;385]	
SC: n/N (%)		33/62 (53.2)	55/62 (88.7)		28/45 (62.2)
SP: n/N (%)	10/62 (16.1)	39/62 (62.9)	61/62 (98.4)	19/45 (42.2)	38/45 (84.4)
GMTR		4.5	17.1		6.7
A/New Caledonia/20/99 (A/H1N1)	N = 62	N = 60*	N = 62	N = 45	N = 45
GMT (1/dil)	5.7	20.1	100	7.5	82.5
95% CI	[4.9;6.6]	[13.6;29.7]	[74.8;134]	[5.7;10.0]	[49;139]
SC: n/N (%)		18/62 (29)	55/62 (88.7)		30/45 (66.7)
SP: n/N (%)	2/62 (3.2)	18/62 (29)	55/62 (88.7)	4/45 (8.9)	32/45 (71.1)
GMTR		3.5	17.5		11
B/Yamanashi/166/98	N = 41	N = 38*	N = 41	N = 27	N = 27
GMT (1/dil)	5.9	9.9	48.2	7.7	36.1
95% CI	[5.1;6.9]	[6.63;14.8]	[34.7;67]	[5.2;11.5]	[21.5;60.7]
SC: n/N (%)		5/41 (12.2)	26/41 (63.4)		11/27 (40.7)
SP: % n/N (%)	1/41 (2.4)	5/41 (12.2)	26/41 (63.4)	4/27 (14.8)	14/27 (51.9)
GMTR		1.7	8.1		4.7
B/Johannesburg/5/99	N = 21	N = 21	N = 21	N = 18	N = 18
GMT (1/dil)	10.3	44.9	89.8	7.6	27.2
95% CI	[6.5;16.4]	[15.2;133]	[44.6;181]	[4.6;12.8]	[12.6;58.7]
SC: n/N (%)		9/21 (42.9)	16/21 (76.2)		7/18 (38.9)
SP: n/N (%)	4/21 (19)	9/21 (42.9)	17/21 (81)	2/18 (11.1)	8/18 (44.4)
GMTR:		4.3	8.7		3.6

**3 subjects for A/H3N2 and B/Yamanashi strains and 2 for A/H3N2 strains had no titer available*
GMT = Geometric mean titer
GMTR = Ratio of pre - to post vaccination GMT
Seroprotection rate (SP): proportion of subjects with a titer ≥ 40 (1/dil), one month after immunization
Seroconversion rate (SC): percentage of subjects with a pre-vaccination titer < 10 (1/dil) and a post-vaccination titer ≥ 40 (1/dil). If pre-vaccination titer was ≥ 10 (1/dil), SC was defined as at least a four-fold increase in titer one month after vaccination.

sults for the A/H3N2 and A/H1N1 strains from the two flu seasons are pooled, but the results for the B strain could not be pooled because the vaccine strains in the two enrolment periods were different. Immune responses in the high risk and healthy children were similar. For each group of vaccines, the seroconversion rates, seroprotection rates, and mean-fold increase in GMT (GMTR) for the A/H3N2 and the A/H1N1 strains met all the CPMP criteria. Influenza A seroprotection rates in the high-risk children given two doses (subgroup A1) and in the healthy children (group B) 30 days after the second dose were from 88.7 to 98.4% and 79.7 to 94.9%, respectively. In high risk children given one dose (subgroup A2), the A strain seroprotection rates were 71.7 and 84.4%.

All children receiving two doses of vaccine met all CPMP immunogenicity criteria for the B/Johannesburg vaccine virus strain and two of the three criteria for B/Yamanashi. High risk children who received one dose of vaccine (subgroup A2) met the seroconversion and GMTR criteria for B/Yamanashi and the GMTR criterion for B/Johannesburg. All the children who were given a single dose of vaccine (subgroup A2) had a history of influenza infection or previous vaccination, but the group GMT at enrolment was seropositive (≥ 10 IU/ml) only in the case

TABLE 3.– *Geometric mean titers, seroconversion and seroprotection rates, and geometric means of post-to prevaccination ratios of anti-hemagglutinin antibodies before, and 30 days after each vaccination in healthy children*

Influenza Vaccine Strains	Group B (Healthy children) Pre-vaccination	Post-1st dose	Post-2nd dose
A/Panama/2007/99			
(A/H3N2)	N = 60	N = 60	N = 60
GMT (1/dil)	13.4	57.6	158.1
95% CI	[8.3;21.6]	[35;94.8]	[113;222]
SC: n/N (%)		28/59 (47.5)	52/59 (88.1)
SP: n/N (%)	14/60 (23.3)	32/59 (54.2)	56/59 (94.9)
GMTR		4.2	12.4
A/New Caledonia/20/99			
(A/H1N1)	N = 60	N = 60	N = 60
GMT (1/dil)	6.2	17.2	64
95% CI	[5.1;7.5]	[11.2;26.4]	[46.7;87.7]
SC: n/N (%)		13/59 (22)	47/59 (79.7)
SP: n/N (%)	4/60 (6.7)	13/59 (22)	47/59 (79.7)
GMTR		2.7	10.2
B/Yamanashi/166/98	N = 42	N = 42	N = 42
GMT (1/dil)	5.2	7.1	31.7
95% CI	[4.8;5.5]	[5.64;8.87]	[23.8;42.4]
SC: n/N (%)		1/41 (2.4)	23/42 (54.8)
SP: % n/N (%)	0/42 (0)	1/41 (2.4)	23/42 (54.8)
GMTR		1.4	6.1
B/Johannesburg/5/99	N = 18	N = 18	N = 18
GMT (1/dil)	6.1	10.6	44.3
95% CI	[4.6;8.1]	[5.57;20.1]	[29.8;65.9]
SC: n/N (%)		3/18 (16.7)	13/17 (76.5)
SP: n/N (%)	1/18 (5.6)	3/18 (16.7)	13/17 (76.5)
GMTR:		1.7	7.8

GMT = Geometric mean titer
GMTR = Geometric mean titer of post/pre-vaccination titer ratios
Seroprotection rate (SP): proportion of subjects with a titer ≥ 40 (1/dil), one month after immunization
Seroconversion rate (SC): percentage of subjects with a pre-vaccination titer < 10 (1/dil) and a post-vaccination titer ≥ 40 (1/dil). If pre-vaccination titer was ≥ 10 (1/dil), SC was defined as at least a four-fold increase in titer one month after vaccination.

of the A/H3N2 virus strain (Table 2). Note that the small number of children receiving each of the B strains, 27 in 2000-2001 and 18 in the following season, makes it difficult to evaluate vaccine immunogenicity.

Safety

Three subjects experienced an immediate reaction following vaccination, two of which were considered related to the vaccination: one child experienced fever (37.8 °C), and another experienced moderate local redness lasting 1 day. A third child, at high-risk because of a history of neonatal pneumonia due to adenovirus and respiratory synticial virus, and with oxygen dependence, had a cough and mild respiratory disease of 1-day duration at the time of his vaccination. He experienced an exacerbation of his respiratory symptoms, cough and acute respiratory distress, within 30 minutes within the vaccination. The child was hospitalized and bronchial hyperreactivity reaction was diagnosed. The child recovered with treatment and was discharged 3 days later. According to the investigator and the sponsor, the event could have been associated with the pain and/or stress caused by the injection.

TABLE 4.– *Subjects reporting solicited local reactions within 30 days following vaccination*

	High risk children				Healthy children	
	2 injections (A1)		1 injection (A2)		2 injections (B)	
	n Sj	%	n Sj	%	n Sj	%
After 1st vaccination						
Any reaction	11	13.8	8	15.4	9	12.0
Pain	6	7.5	3	5.8	3	4.0
Redness	6	7.5	2	3.8	3	4.0
Induration	2	2.5	2	3.8	2	2.7
Oedema	3	3.8			2	2.7
Ecchymosis	2	2.5	1	1.9	2	2.7
Pruritus	1	1.3			1	1.3
After 2nd vaccination						
Any reaction	7	9.5			7	9.7
Pain	3	4.1			2	2.8
Redness	4	5.4			1	1.4
Induration	3	4.1			1	1.4
Oedema	3	4.1			2	2.8
Ecchymosis	2	2.7			5	6.9
Pruritus	1	1.4			1	1.4

Sj: Subjects

There were no notable between-group differences in solicited local or systemic reactions. All the reported local reactions were solicited reactions appearing within the 3 days of vaccine administration. Overall, from 13.8% to 15.4% of at risk children and 12% of healthy children reported a local reaction, most being cases of mild pain at the injection site. Moreover, 33 of the 40 reactions reported during the first inclusion period and 18 of 29 after the second inclusion period resolved within 3 days. Only two severe local reactions were reported during the first inclusion period: one case of pain after the first injection in subgroup A1 and one case of edema after the second injection in group B. The incidence of local reactions decreased following the second dose in both subgroup A1 and group B. Solicited local reactions are summarized in Table 4.

After the first dose, 27.5% to 28.8% of high-risk (Groups A1 and A2) and 25.3% of healthy children reported a solicited systemic reaction judged related to vaccination (Table 5). The incidence was 23% following the second dose in high risk children (Group A1), but was 45.8% in healthy children. This increase resulted primarily from an increase in reported fever (axillary temperature ≥ 37.1 °C), the most frequently reported systemic reaction in each study group after either the first or the second injection (Table 5). Most systemic reactions, 95/131 (72.5%) reported in the first inclusion period and 66/84 (78.6%) during the second inclusion period, occurred within the 3 days following injection. Most had resolved within 3 days. Fourteen severe systemic reactions were reported during the first inclusion period and 8 during the second inclusion period.

Altogether, 23 subjects experienced a total of 26 serious adverse events (SAEs). The majority of the SAEs were respiratory or gastrointestinal system events, related to the health status of the children. Five SAEs were considered to be possibly related to vaccination by the investigator and the sponsor: one bronchial hyperreactivity reaction, one bronchopneumonia, one case of dysentery diarrhea and distended abdomen), one case of worsening of bronchopneumonia, and one case of increase in respiratory secretions. All the children fully recovered with treatment. No subject was withdrawn because of an adverse event.

Discussion

This study was conducted to assess the immunogenicity and safety of the inactivated, split-virion influenza vaccine Imovax Gripe in high risk and healthy 6 to 35-month-old children with the aim of providing additional support for influenza vaccination of high risk children. Despite recommendations for annual influenza vaccination of children with high-risk conditions, studies show low rates of vaccine coverage in these children, with estimates of about 5 to 30% for children in the United States[11]. The low influenza vaccine coverage in children is in sharp contrast to the much higher estimated coverage for per-

TABLE 5.– *Subjects reporting solicited systemic reactions related to vaccination within 30 days following injection*

	High risk children				Healthy children	
	2 injections (A1)		1 injection (A2)		2 injections (B)	
	n Sj	%	n Sj	%	n Sj	%
After 1st vaccination						
Any reaction	22	27.5	15	28.8	19	25.3
Fever (axillary ≥ 37.1 °C)	20	25.0	11	21.2	13	17.3
Asthenia	9	11.3	5	9.6	3	4.0
Headache	1	1.3	3	5.8	0	0
Arthralgia	1	1.3	2	3.8	0	0
Myalgia	2	2.5	1	1.9	1	1.3
Shivering	6	7.5	0	0	0	0
Sweating	5	6.3	2	3.8	2	3.8
After 2nd vaccination						
Any reaction	17	23.0			33	45.8
Fever (axillary ≥ 37.1 °C)	15	20.3			23	31.9
Asthenia	3	4.1			4	5.6
Headache	1	1.4			2	2.8
Arthralgia	0	0			1	1.4
Myalgia	0	0			3	4.2
Shivering	2	2.7			1	1.4
Sweating	0	0			2	2.8

sons 65 years of age or older or to national coverage for routine childhood immunizations[11].

As there were inclusion periods in both the 2000-2001 and the 2001-2002 flu seasons, two vaccine formulations were used in this trial. The vaccines contained the same A strains, but different B strains, with a B/Yamanashi/166/98 analogous strain, B/Beijing/184/93, in the 2000-2001 formulation, and a B/Johannesburg analogous strain, B/Sichuan /379/99 in 2001-2002. Also, the preservative, thiomersal, was present in the 2000-2001 formulation but not in 2001-2002. A total of 94 high-risk subjects and 60 healthy subjects received the first formulation and 44 high-risk subjects and 20 healthy subjects received the second formulation. A total of 23 children did not complete the study.

The seroprotection, seroconversion, and mean-fold increases in GMT to all three vaccine strains met all immunogenicity criteria in both the high risk and the healthy children who received two doses of vaccine containing the B/Johannesburg analogous strain during the 2001-2002 season. The anti-influenza virus antibody GMTs against each vaccine virus strain in both the healthy and high risk study groups were also above the 40 IU/ml titer considered to be protective against infection.

In children given one dose of vaccine, the response to both vaccine A strains met all three criteria, but the response to B/Johannesburg was weaker, with only the GMTR being met. It should be noted that although these children had confirmed histories of either influenza infection or previous influenza vaccination, the prevaccination GMT for this group was below the 10 IU/ml titer threshold of seropositivity. A second dose might have resulted in achievement of the recommended seroconversion and seroprotection rates. The response to the B/Yamanashi analogous strain in the 2000-2001 season met two of the three immunogenicity criteria, mean-fold increase in GMT and seroconversion (HAI titers ≥ 40 IU/ml). The slightly reduced response, seen in each of the study groups, may simply have resulted from a somewhat lower immunogenicity of the B/Yamanashi-like strain relative to the B strain used in the succeeding season.

The vaccine tolerability was very satisfactory in all treatment groups. The incidence of solicited local reactions ranged from approximately 12% to 15% following the first injection, and was under 10% in both high risk and healthy children. The incidence of solicited systemic reactions was similar for high risk and healthy children following the first injection, 28.8% and 25.3%, respectively, with fever being the most frequently reported. Solicited systemic reactions occurred less frequently following the second vaccination in high risk children, 23% versus 45.8%, with the between group difference resulting from a differ-

ence in the incidence of fever. The relatively higher incidence of fever in the healthy children was most likely the result of the definition, an axillary temperature ≥ 37.1 °C.

The immunogenicity of *Imovax Gripe* observed in this study is consistent with that observed in earlier studies in healthy children. In an open uncontrolled study, Gonzalez et al evaluated the immunogenicity of 2 doses of Imovax Gripe (Vaxigrip) (0.25 ml/dose) in 65 children, 6 months to 3 years of age during the March to June 1996 influenza season in Montevideo, Uruguay[12]. Geometric mean titers increased 9.8-fold over pre-immunization concentrations for the A (H3N2) strain, 13.3-fold for the A (H1N1) strain, and 4.1-fold for the B strain. Seroprotection rates were 91.8% for the A/H3N3 and 81.6% for the H1N1 strains, respectively, and 93.9% for the B strain, exceeding CPMP immunogenicity criteria for young adults[10]. The incidence of solicited local (9%) and systemic (28%) reactions reported by Gonzalez is similar to that observed in our study population.

In a series reported by Lina et al, Imovax Gripe (Vaxigrip) administered to 42 children, ranging in age from 8 to 10 years, during three influenza seasons in France, exceeded the CPMP criteria for seroconversion, seroprotection, or GMTR during all 3 seasons[13]. Seropro-tection rates of 83% and 93% were observed for the A/H3N2 and A/H1H1 strains, respectively, and 100% for B/Harbin/7/94 in response to a single 0.5 ml dose, but the pre-vaccination GMT for the B strain was much higher than in the children vaccinated in this study, 54.3 IU/ml versus 5.2 to 10.3 IU/ml.

In a previous study conducted in adults in Colombia, Imovax Gripe induced seroprotective levels of antibodies against the three vaccine strains in 94-99% of subjects from 18 to 60 and in 88-97% of those older than 60 years of age[14].

Finally, our results add to those reported in other groups of young high-risk children.[15-17] Similar immune responses to those seen here were reported in a study of 52 high-risk children younger than 5 years of age, with chronic lung or congenital heart diseases. Both the A/Taiwan and B/Panama analogous strains used in that study vaccine met all the immunogenicity criteria, and the A/Shandong strain met the seroconversion and GMTR requirements. The incidence of solicited local (23%) and systemic (48%) reactions were slightly higher than those reported in our study. The vaccine response rates (seroconversion) and GMTs were higher in our study population than reported for a group children with chronic pulmonary disease, of similar ages to those in our study who had been pre-term infants[16] and in another study of children between 6 and 18 months with bronchopulmonary dysplasia or congenital heart disease[17]. In those studies, however, control groups of heathy children were not available for comparison. It is noteworthy that the available reactogenicity data for the influenza vaccines evaluated in the studies in high-risk children shows it to be low.

In conclusion, it is important to show that both the immune response to vaccination and the tolerability are satisfactory in children at high risk for severe influenza complications because of the potential of vaccination to reduce the incidence of serious complications in such children. The immune responses to the inactivated influenza vaccine used in this study in high-risk children aged 6 and 36 months were similar to those seen in the reference group of health children. The immunogenicity results were consistent with responses that have been reported in other recent studies, and the vaccine was equally well tolerated by all study groups. These study results thus provide additional support for the vaccination of children at high risk of influenza related complications.

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En 1859, mientras observaba al microscopio una gota de solución de azúcar que sufría la fermentación butírica, vio Pasteur que los microorganismos existentes perdían los movimientos en los bordes de la gota, mientras que los del centro se mantenían activamente móviles. Esta observación actuó como una chispa […]. Estaba convencido de que la fermentación alcohólica […] dependía de la vida de la levadura, pero sabía también que la producción del alcohol a partir del azúcar no incluía la participación del oxígeno. Esto indicaba que, en ciertas circunstancias, la vida podía desarrollarse sin oxígeno, una conclusión en conflicto con la doctrina universal aceptada de que el oxígeno era el propio aliento de la vida. Cuando Pasteur vio que los organismos de la fermentación butírica se volvían inmóviles a medida que se aproximaban al borde de la gota, imaginó inmediatamente que resultaban inactivados por el contacto con el aire. […] Pronto demostraron los experimentos que no se multiplicaban en medio oxigenado, mientras que crecían abundantemente cuando se suprimía el oxígeno del ambiente. En este caso, fue un hecho aparentemente trivial, que llevó a Pasteur a concluir que: (a) existe la vida sin oxígeno; (b) las fermentaciones en general son reacciones metabólicas por las cuales una célula puede obtener su energía de ciertas sustancias orgánicas en ausencia del oxígeno; (c) la producción de alcohol es sólo un caso particular del proceso de fermentación y es la reacción por la cual obtiene la levadura energía en condiciones anaerobias.

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Pasteur (Tomo 2). Barcelona: Salvat, 1985, p 341