Swine Influenza Virus Vaccine: Potentiation of Antibody Responses in Rhesus Monkeys

Abstract. *Polyriboinosinic·polyribocytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose [poly(ICLC)] enhances the antibody response in rhesus monkeys immunized with swine influenza virus subunit vaccine. Monkeys given the vaccine-adjuvant combination had earlier and significantly ($P < .05$) higher titers by 14 days compared to those that received vaccine alone. The potentiation of the antibody response of young monkeys given a split-virus vaccine in combination with poly(ICLC) suggests that this vaccine-adjuvant combination may similarly provide a potentially useful alternative approach to the immunization of pediatric and young adult age groups against swine influenza.*

Parkman *et al.* (*1*) have stated that single doses of the influenza A/NJ/76 virus vaccines are less than satisfactory for immunization of persons below age 25 against swine influenza. Alternative approaches to immunizing the pediatric age group include the use of lower doses of the whole-virus vaccine, split-virus vaccines with more antigen, and two-dose sequences (*1*). Another alternative would be to use an adjuvant to increase the potency of the vaccine. Hilleman (*2*) reported that a complex of polyriboinosinic and polyribocytidylic acids [poly(I)·poly(C)] only weakly potentiated the antibody response in monkeys to ordinary aqueous influenza vaccine. Poly(I)·poly(C), however, is only minimally effective in primates as an interferon inducer, possibly because of the presence in primate serum of high concentrations of nucleases that hydrolyze the compound. A complex of poly(I)·poly(C) with poly-L-lysine and carboxymethylcellulose [poly(ICLC)] has been shown to be a much more effective interferon inducer in primates than the parent compound (*3*). In addition, poly(ICLC) significantly enhances the antibody response of rhesus monkeys to formalin-inactivated Venezuelan equine encephalomyelitis virus vaccine (*4*). Here we present data to show that poly(ICLC) potentiates the antibody response to a monovalent influenza subunit antigen prepared from the A/NJ/76 (New Jersey; swine) strain of virus, when tested in monkeys.

Monovalent influenza virus subunit vaccine, designated A/Swine X-53 (*5*), was used. The dosage of poly(ICLC), prepared as described previously (*3*), was 100 or 300 μg/kg in the first study and 10, 30, or 100 μg/kg in the second study. Poly(ICLC) was combined with the vaccine just prior to immunization and given in the femoral muscle mass. Hemagglutination inhibition (HAI) titers were measured by the method of Robinson and Dowdle (*6*), adapted for microtiter technique. The antigen used in the HAI tests was prepared from the A/Swine X-53 strain of influenza virus provided by the Center for Disease Control (*6*). Sixteen healthy, well-conditioned young adult male or female rhesus monkeys (*Macaca mulatta*) weighing 4 to 7 kg were used in the first study, and placed into four groups of four monkeys each. One group was used as vaccinated controls. In the other groups, 0.5 ml of vaccine [200 chick cell agglutinating (CCA) units per monkey] was mixed with either 0.3 ml of poly(ICLC) (100 or 300 μg/kg) or an equivalent volume of saline, so that each monkey was injected intramuscularly with a total volume of 0.8 ml. A negative control group was given 0.8 ml of saline alone without vaccine. In the second study, 20 well-conditioned, young male or female rhesus monkeys weighing less than 2.0 kg were allocated into five groups of four monkeys each. Those in the unvaccinated control group were each given 100 μg/kg of poly(ICLC). Monkeys given the vaccine-adjuvant combinations were given 10, 30, or 100 μg of poly(ICLC) per kilogram. The monkeys were bled prior to inoculation and on days 7, 14, 28, 42, 56, and 105 after vaccine inoculation for antibody determinations. In the calculation of geometric mean HAI antibody titers, negative responses were assigned values that were one-half of the lowest detectable titer of 1:10. Rectal temperatures were recorded twice each day. Temperatures above 39.7°C were considered a febrile response.

Monkeys given either 100 or 300 μg of poly(ICLC) per kilogram in combination with vaccine had significantly greater ($P < .05$, Students' t-test) antibody responses at 14, 28, 42, and 56 days after inoculation than monkeys given the vaccine alone (Table 1). Only one of four monkeys given vaccine alone had detectable HAI antibody by day 14 (titer of 1:20); whereas eight of eight monkeys given vaccine with poly(ICLC) had titers of 1:20 or greater and six of eight had titers of 1:40 or greater. By day 28, eight of eight monkeys treated with poly(ICLC) had titers greater than or equal to 1:40. There was no difference between adjuvant doses in their effect on the antibody response of monkeys.

When young rhesus monkeys were given 200 CCA units of the vaccine alone (Table 2), no HAI antibody was detectable until day 42. Nine of 12 monkeys given the vaccine-adjuvant combination had detectable antibody by day 7, and all monkeys given the combination had significantly greater ($P < .05$) antibody responses by day 42 than monkeys given the vaccine alone.

Fever was not observed in monkeys given either saline or vaccine alone. In older monkeys given vaccine in combination with poly(ICLC), three of eight monkeys had rectal temperatures greater

Table 1. Hemagglutination inhibition (HAI) titer response of monkeys given 200 CCA units of influenza vaccine (A/Swine X-53) with or without poly(ICLC) as an adjuvant ($N = 4$). The < signs indicate reciprocal HAI titers below the lowest detectable value of 1:10. Values in parentheses represent group geometric means.

Poly (ICLC) (μg/kg)	HAI titer by days after vaccination						
	0	7	14	28	42	56	105
	<	<	<	<	20	10	20
	<	<	<	<	20	10	40
	<	<	<	20	20	20	40
	<	<	20	40	160	80	80
			(7)	(12)	(34)	(20)	(40)
100	<	<	20	40	160	80	20
100	<	<	40	40	320	320	640
100	<	10	80	160	160	640	160
100	<	20	640	320	80	160	160
		(8)	(80)*	(95)*	(160)*	(226)*	(48)
300	<	<	20	40	160	80	40
300	<	<	40	160	160	80	80
300	<	<	40	160	320	160	160
300	<	20	160	160	640	320	40
		(7)	(48)*	(113)*	(269)*	(135)*	(68)

*Group geometric mean titers in parentheses are significantly different ($P < .05$) when compared with the vaccinated group of monkeys that received no poly(ICLC).

Reprinted from SCIENCE, 23 September 1977, volume 197, pages 1289-1290

405 037

Table 2. Hemagglutination inhibition (HAI) titer response of young monkeys given 200 CCA units of influenza vaccine (A/Swine X-53) with or without poly(ICLC) as an adjuvant ($N = 4$). The < signs indicate reciprocal HAI titers below the lowest detectable value of 1:10. Values in parentheses represent group geometric means.

Poly (ICLC) (μg/kg)	HAI titer by days after vaccination						
	0	7	14	28	42	56	105
	<	<	<	<	10	<	10
	<	<	<	<	10	10	20
	<	<	<	<	<	10	20
	<	<	<	<	10	10	20
					(8)	(8)	(17)
10	<	<	<	<	160	80	160
10	<	40	160	320	20	40	20
10	<	40	80	80	40	40	40
10	<	20	80	40	20	20	20
		(20)*	(48)*	(48)*	(40)*	(40)*	(40)
30	<	20	160	80	160	40	40
30	<	20	20	80	160	80	<
30	<	20	40	160	160	80	80
30	<	40	320	40	20	40	40
		(24)*	(80)*	(80)*	(74)*	(57)*	(48)*
100	<	<	20	40	20	40	40
100	<	<	20	20	20	20	20
100	<	40	160	80	160	80	40
100	<	40	320	80	40	40	80
		(14)	(67)*	(48)*	(40)*	(40)*	(40)*

*Group geometric mean titers in parentheses are significantly different ($P < .05$) when compared with the vaccinated group of monkeys that received no poly(ICLC)

than 39.7°C (40.0, 40.0, and 40.3°C) only at 24 hours after vaccination. Two of the three febrile monkeys were given 300 μg (per kilogram) of the poly(ICLC). By 48 hours after injection, none of these monkeys had fever. On the other hand, no fever was recorded in the young monkeys given poly(ICLC) at doses of 10, 30, or 100 μg/kg at any time following vaccination. There was no induration or erythema at the injection site in any of the vaccinated monkeys.

Potentiation of the weakly immunogenic subunit influenza virus vaccine, in addition to potentiation of killed Venezuelan equine encephalomyelitis whole-virus vaccine (*4*), suggests that poly(ICLC) may have potential for widespread use as an immunological adjuvant for weakly antigenic vaccines. In some circumstances inactivated virus vaccines, though less antigenic, may offer advantages over live virus vaccines, such as increased safety, reduced adverse reactions, greater stability, and improved control of production methodology.

Poly(ICLC) has been given experimentally to human patients and is known to cause moderate febrile responses (*7*). Unstabilized poly(I)·poly(C) was shown by Adamson and Fabro (*8*) to be embryotoxic in rabbits when given subcutaneously (1000 μg kg^{-1} dose^{-1}) on days 8 and 9, or 11 and 12, of pregnancy. These data have not been extended to other species, and the dose was at least 100-fold greater than the minimal effective dose of poly(ICLC) used in the present studies. Lefkowitz *et al.* (9) recently showed that nontoxic doses of adenine arabinoside and a poly(I)·poly(C) given in combination resulted in synergistic mortality in mice; their dose of poly(I)·poly(C) was approximately 50-fold greater than the minimal effective adjuvant dose of poly(ICLC) in our study. Possible adverse drug interactions with other medications that might be used on a routine basis in patients, such as coumarin anticoagulants, cardioglycosides, or other compounds, must be considered with any clinical use of this compound. Robinson *et al.* (*10*) gave poly(I)·poly(C) to 26 patients with leukemia or solid tumors. One patient had clear laboratory evidence of disseminated intravascular coagulation and two had moderately severe hypersensitivity reactions. The hypersensitivity reactions were anaphylactoid in nature. No anaphylactoid reactions were elicited in guinea pigs given repeated injections of poly(ICLC) (*11*). In addition, Steinberg *et al.* (*12*) have shown that mice given poly(I)·poly(C) develop antibodies against the complex and, further, that poly(I)·poly(C) accelerates the autoimmune disease in NZB mice.

Many of the potential problems associated with the use of high doses of poly(I)·poly(C) might be avoided if as little as 10 μg of poly(ICLC) per kilogram is effective as an adjuvant as shown by these data. This dose is below that required to induce circulating interferon and fever in monkeys. Since there was no significant difference between the antibody responses of monkeys given the 10, 30, or 100 μg/kg doses of poly (ICLC), it seems reasonable to assume that lower doses of the complex may also be effective, thus reducing the possibility of untoward side effects. Sammons *et al.* (*13*) gave 6.0 mg/kg of poly(ICLC) intravenously to rhesus monkeys daily for ten consecutive days, with inappetence and fever being the primary clinical evidence of toxicity. In contrast, in recent studies three of four cynomolgus monkeys given poly(ICLC) (3.0 mg/kg per injection) died after 12 injections (*14*), possibly suggesting that cynomolgus monkeys are less resistant to the toxicity of poly(ICLC) than rhesus monkeys.

Since influenza vaccines are frequently inadequate, the adjuvant approach reported here could, conceivably, extend to other influenza vaccines or other weakly immunogenic vaccines. If present adjuvant studies are extended to include man, the lowest possible dosage of poly(ICLC) should be used to decrease the volume required for an injection and to minimize the potential for nonspecific febrile reactions.

E. L. STEPHEN
D. E. HILMAS, J. A. MANGIAFICO
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701
H. B. LEVY
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014

References and Notes

1. P. D. Parkman, G. J. Galasso, F. H. Top, G. R. Nobel, *J. Infect. Dis.* 134, 100 (1976).
2. M. R. Hilleman, *Arch. Intern. Med.* 126, 109 (1970).
3. H. B. Levy, G. Baer, S. Baron, C. E. Buckler, C. J. Gibbs, M. J. Iadarola, W. T. London, J. Rice, *J. Infect. Dis.* 132, 434 (1975).
4. W. E. Houston, C. L. Crabbs, E. L. Stephen, H. B. Levy, *Infect. Immun.* 14, 318 (1976).
5. Lot No. 17061, kindly supplied by Wyeth Laboratories.
6. R. O. Robinson and W. R. Dowdle, in *Diagnostic Procedures for Viral and Rickettsial Infections*, E. H. Lennette and N. J. Schmidt, Ed. (American Public Health Association, New York, ed. 4, 1969), pp. 414–433.
7. K. Champney, H. B. Levy, A. M. Lerner, *Clin. Res.* 24, 451A (1976).
8. R. H. Adamson and S. Fabro, *Nature (London)* 223, 718 (1969).
9. E. Lefkowitz, M. Worthington, M. A. Conliffe, S. Baron, *Proc. Soc. Exp. Biol. Med.* 152, 337 (1976).
10. R. A. Robinson, V. T. DeVita, H. B. Levy, S. Baron, S. P. Hubbard, A. S. Levine, *J. Natl. Cancer Inst.* 57, 599 (1976).
11. H. B. Levy, unpublished data.
12. A. D. Steinberg, S. Baron, N. Talal, *Proc. Natl. Acad. Sci. U.S.A.* 63, 1102 (1969).
13. M. L. Sammons, E. L. Stephen, H. B. Levy, S. Baron, D. E. Hilmas, *Antimicrob. Agents Chemother.* 11, 80 (1977).
14. E. L. Stephen, unpublished data.
15. We thank W. R. Beisel, R. Edelman, D. G. Harrington, and H. Rozmiarek for criticism of the manuscript.

1 April 1977; revised 9 May 1977