Adjuvant Activity of Polyacetal Carboxylate for Antibody Induction in Mice and Guinea Pigs (35635)

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It has been described that polyanions of different chemical composition can potentiate stimulation of the development of circulating antibody or of cellular immunity by different antigens. Specifically, Braun *et al.* (2) found that certain oligodeoxyribonucleotides and ribonucleotide homopolymers, especially the complexed double-stranded ones, can act as potent stimulators of the immune mechanism (1, 2). This was confirmed in a study by Woodhour *et al.* (3) who found that poly-I:C and poly-A:U "hyperpotentiated" influenza vaccines administered in oil adjuvant. It was also demonstrated that polyriboinosinic–ribocytidylic acid can accelerate immunologic maturation of newborn mice (4) and can stimulate cellular immunity (5).

The present study was undertaken to investigate the possible adjuvant effect of chlorite-oxidized oxyamylose (COAM) on the induction of circulating antibody. COAM is an interferon inducing polyacetal carboxylate which has antiviral properties (6, 7) comparable to those of polyacrylic acid (8, 9) or pyran copolymer (10). The latter one was recently reported to enhance early antibody induction in mice by SRBC (11). The advantage of COAM over polyacrylate or pyran copolymer is its possible biodegradability due to the presence of oxygen atoms in the backbone.

Materials and Methods. Female NMRI-mice (20–25 g) and Hartley guinea pigs (Animal Virus Diseases Research Institute) were obtained from the "Centrum voor Selectie en Kweek van Laboratoriumdieren" of the University of Leuven.

Chlorite-oxidized oxyamylose (COAM) was synthetized by Mr. P. Claes (Rega Institute, Dept. of Chemistry, University of Leuven) as described earlier (6). Polyriboinosinic and polyribocytidylic acids were purchased from PL-Biochemicals Inc., Milwaukee, Wisc., and annealed into double-stranded polyriboinosinic–ribocytidylic acid (poly-I:C) as described earlier (7). Commercially prepared inactivated influenza vaccines, Influvac and Duvaxyn, (Philips-Duphar, Weesp, The Netherlands) were used. Influvac contained influenza viruses A_2Hk and B/Neth. Duvaxyn contained influenza viruses A equi/1 and A equi/2.

Mice were bled at the orbital sinus and blood was immediately diluted 1:5 in phosphate buffered saline. Antibody was determined in diluted serum harvested after clotting. Guinea pigs were bled by heart puncture and antibody was determined on whole serum. All sera were heated at 56° for 30 min before analysis. Sheep red blood cell agglutinins were determined in Kahn tubes. Hemagglutination-inhibition (HAI) antibody against influenza virus were performed using the Microtiter system (Cooke Instruments A-G, Zurich, Switzerland), using 4 hemagglutinating units of influenza virus. Antigens used were live viruses propagated by allantoic inoculation of 11-day-old chick embryos. The strains used were A_2Hk, B/Neth., and A equi/1. Statistical methods used consisted of variance analysis as described by Croxton (12).

Results. 1. Potentiation of hemagglutinin induction in mice. Groups of 5 to 8 mice were given intraperitoneal injections of either SRBC alone, or SRBC suspended in PBS containing COAM at a dosage level of 4 mg/mouse. A group was also included which received COAM (4 mg/mouse) intraperitoneally 3 days before immunization with sheep red blood cells. At times indicated in

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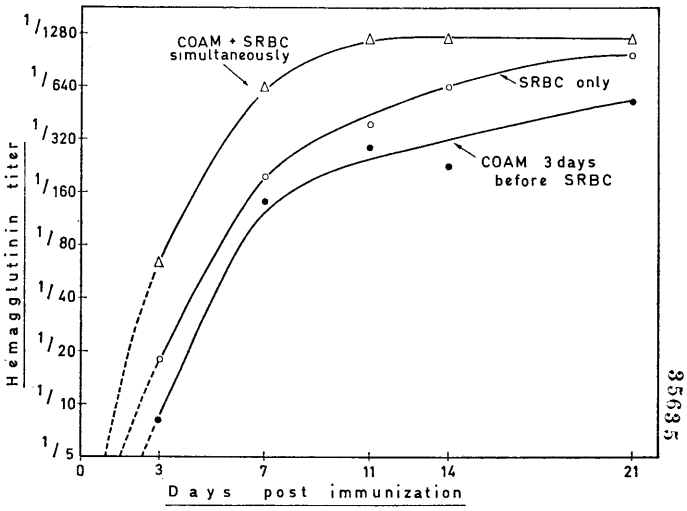

FIG. 1. Influence of COAM (4 mg ip) on the induction of circulating hemagglutinins in mice immunized with sheep red blood cells (0.5 ml of a 10% suspension, ip).

Fig. 1, blood samples were taken at the orbital sinus and hemagglutinin was determined. As shown, during the first 11 days after stimulation, average antibody titers were about 3 times higher in mice treated with COAM than in the controls. By 14 days, antibody levels stabilized in COAM-treated mice while they continued to rise in control animals so that 3 weeks after vaccination the antibody concentrations in treated and control animals were about equal. By variance analysis the observed differences between control and treated mice were significant at the level of $p<0.001$. Mice which received COAM 3 days before immunization developed less antibody than the controls ($p<0.001$).

2. Effect of COAM on induction of influenza HAI-*antibody.* Adjuvant effects for the induction of HAI antibody against influenza was studied both in mice and in guinea pigs. Groups of 12 mice were injected intraperitoneally with either of the following vaccine formulations: (i) vaccine only; (ii) vaccine + poly-I:C; (iii) vaccine + COAM; (iv) vaccine + incomplete Freund adjuvant (IFA); (v) vaccine + IFA + poly-I:C; (vi) vaccine + IFA + COAM.

The dosage schedule was as follows: vaccine containing A_2Hk and B/Neth. strains, 0.0625 ml/mouse; poly-I:C, 25 μg/mouse; COAM, 4 mg/mouse; and IFA, 0.125 ml/mouse. The experiment was done on three sets of mice, which were bled at 7, 14, and 28 days postinjection. Antibody titers were determined on individual samples. Average antibody titers are represented in Fig. 2. The data confirm the results of Woodhour *et al.* (3) in that IFA and poly-I:C acted synergistically in potentiating both influenza antigens. When COAM was included in the vaccine either alone or in combination with IFA, antibody titers were as high as, or higher than, with IFA and poly-I:C.

To facilitate multiple bleedings on one animal, further studies on enhancement of influenza vaccine were done on guinea pigs. In a first experiment we determined whether adjuvant effect could be obtained after intramuscular as well as after intraperitoneal injection. The effect of dose of COAM was also studied: one group of guinea pigs received the same formulation as the mice, *i.e.*, 4 mg of COAM/dose of vaccine. A second group was given 40 mg of COAM, *i.e.*, a dose comparable to that given to mice but calculated on a weight basis. It was reasoned that this should give an indication as to whether adjuvant activity is due to local or systemic effects. Groups of 10 guinea pigs were used. They were given 0.5 ml of vaccine containing A equi/1 antigen. Antibodies were determined at 3, 6, 9, and 12 weeks after vaccina-

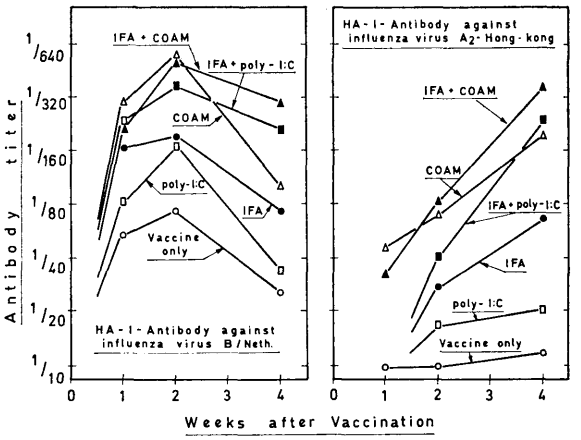

FIG. 2. Influence of COAM (4 mg), poly-I:C (25 μg), and incomplete Freund adjuvant (IFA, 0.125 ml) on induction of HAI antibody by influenza vaccine. All adjuvants were mixed with vaccine and given ip.

tion. Average values are summarized in Table I. Adjuvant activity was present both with intramuscular and intraperitoneal treatments ($p<0.001$). However, a small dose of COAM only stimulated early induction of antibody (3 weeks) as the effect waned at 6, 9, and 12 weeks (interaction between bleeding time and treatment significant at level $p<0.001$).

The requirement for a higher dose in guinea pigs than in mice might point to a systemic rather than a local mechanism. Therefore, in a last experiment, we investigated whether COAM would have adjuvant activity when injected at another site than

TABLE I. Adjuvant Effect of COAM on Antibody Induction by Influenza Vaccine in Guinea Pigs.

Vaccination schedule[a]	Antibody titers[b] (weeks): 3	6	9	12
Expt. 1. Intramuscular vaccination				
Vaccine only	96	80	85	30
Vaccine + COAM, 4 mg	216	108	112	60
40 mg	368	270	182	112
Intraperitoneal vaccination				
Vaccine only	120	158	91	28
Vaccine + COAM, 4 mg	209	138	98	37
40 mg	446	364	295	98
Expt. 2 Intramuscular vaccination				
Vaccine only	52	—	—	—
Vaccine + COAM, 40 mg	195	—	—	—
40 mg ip	64	—	—	—
Intraperitoneal vaccination				
Vaccine only	64	—	—	—
Vaccine + COAM, 40 mg	182	—	—	—
40 mg im	80	—	—	—

[a] Formulations were such that the animals received 0.5 ml of A equi/1 vaccine, mixed with 0.5 ml of either saline or COAM at the indicated doses.

[b] Serum samples were assayed individually. Values shown are reciprocals of geometric means of end-point dilutions.

the vaccine. Groups of 7 guinea pigs were given A equi/1 vaccine intraperitoneally or intravenously. COAM (40 mg/dose) was included or given separately at a distant site. As Table I shows, COAM had adjuvant activity only when given at the same injection site as the vaccine. It seems unlikely therefore that it acts by generalized stimulation of immunity mechanisms.

Discussion. Several polyanions have been found to enhance antibody stimulation when given in conjunction with antigen (1–5). The same or related polyanions are also known to stimulate interferon production or release (10, 13–16), and to impart on the experimental animals a state of resistance to viral (6–10, 13–15), bacterial (17–20), and protozoal (21–24) infections. The relationships between these various effects, as well as the basic mechanisms underlying each of them, are only partially understood.

Interest in the possible adjuvant activity of polyacetal carboxylic acids was stimulated by their low toxicity and apparent biodegradability (6, 7). Such properties would give to these substances an advantage over double-stranded ribonucleic acids, which were shown to exert endotoxin-like activity (25) and over other synthetic carboxylates, such as polyacrylic acid and pyran copolymer which are not biodegradable. In the present study we showed that one of the polyacetal carboxylic acids (COAM) synthetized in our laboratory enhances the formation of antibodies against sheep red blood cells and influenza virus hemagglutinin.

Recently we demonstrated that the antiviral effect of COAM and other interferon-inducing polyanions in mice infected with Mengo virus, is only partly due to the presence of interferon in the tissues, and is largely mediated by the retention of input challenge virus in the peritoneal cavity (26). It was proposed that peritoneal cells, presumably macrophages, are activated by the polymers, so that phagocytosis and intracellular "killing" of the virus by these cells is increased. A similar mechanism might account for increased reaction of mononuclear cells involved in the phagocytosis and processing of antigens, and might thus explain enhancement of antibody stimulation. The adjuvant effect of polyanions would thus resemble that of BCG or bacterial lipopolysaccharides which act by overall stimulation of reticuloendothelial cell activity. In this context it was unexpected to find that COAM had adjuvant activity only when given simultaneously with and by the same injection route as the antigens. This is not so for the antiviral effect of COAM: intraperitoneally administered polymers give good and prolonged protection against intravenously injected vaccina virus (8). Conceivably, antibody induction mechanisms are less sensitive to the RES-stimulating capacity of polyanions so that the adjuvant effects are only realized when macrophages at the site of immunization are put into contact with a high concentration of polymers.

When COAM was given intraperitoneally 3 days before sheep red blood cells, a slight depression of antibody formation was observed. A similar inhibition was reported in comparable experimental conditions using endotoxin and poliovirus or sheep red blood cells as antigens (27); under other experimental conditions lipopolysaccharides enhance antibody formation. Such two-sided effects may reflect biphasic responses on phagocytic activity of reticuloendothelial cells as reported both for lipopolysaccharides (28) and pyran copolymer (19).

An interesting possibility for application of adjuvant activity of polyanions was suggested by Winchurch and Braun (4) who found that poly-I:C accelerates immunologic maturation in newborn mice. It was proposed that polyanions might be used to prevent immune tolerance in young animals towards vertically transmitted oncogenic viruses or virus-induced tumor antigens. Studies in our laboratory demonstrated that COAM, when administered at birth, can prevent development of spontaneous mammary carcinoma in C3H-mice (our unpublished results).

Summary. Chlorite-oxidized oxyamylose (COAM), a polyacetal carboxylic acid, enhances the formation of antibodies against sheep red blood cells and influenza virus hemagglutinin. The effect was present only when the polymer was included in the immunizing antigen injection (intraperitoneally or intravenously), not when it was given at a

time interval before the antigen or by a different administration route. This indicates that an interaction occurs at the site of injection between antigen and polymer, or between polymer and host cells which are involved in antigen processing.

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