Stimulation of Rabies Vaccine in Mice by Low Doses of Polyadenylc:Polyuridylic Complex

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Addition of 100 µg of polyadenylc:polyuridylic (poly A:U) complex to each dose of inactivated rabies vaccine increased immunity to rabies challenge in mice. Stimulation was also observed after addition of 10 µg of poly A:U to the vaccines. Mixtures of rabies vaccine and poly A:U lost their stimulatory properties after storage at 37 or 4°C for 1 month. However, these data are encouraging for practical use of poly A:U as an adjuvant to viral vaccines.

Polyadenylc:polyuridylic (poly A:U) homoribopolymer had a potent adjuvant effect on immunity against Brucella abortus challenge in mice. Poly A:U enhanced immunization in rendering low amounts of Brucella antigens immunogenic. Increased mouse protection was not accompanied by production of circulating antibodies (G. Renoux, M. Renoux, and R. Branche, submitted for publication). The present work extended these investigations to the adjuvant effects of poly A:U upon a viral vaccine. Particularly, we believed it of some interest to see whether any adjuvant effect could be obtained by doses of poly A:U complex which were demonstrated insufficient to stimulate production of antibodies to a variety of antigens (2, 3). Rabies vaccine was chosen because of the practical significance of its possible stimulation by poly A:U.

MATERIALS AND METHODS

Vaccine. Batch no. 8C 100 of Rabifia (Iffa-Merieux, Lyon, France), a lyophilized vaccine from inactivated rabies virus cultured on newborn hamster cells (1), was used throughout this study.

Poly A:U. Polyadenylc acid [poly(A)], batch 11.60.301, and polyuridylic acid [poly(U)], batch 11.62.308, were purchased from Miles Inc. (Elkardt, Indiana). Equimolar ratios of poly(A) and poly(U) were mixed in phosphate buffer, 0.006 M, with 0.15 M NaCl (pH 7.0) at room temperature, to form a double helix, poly A:U. Formation of that complex was verified by spectrometric changes at 260 nm. A stock solution that contained 1 mg/ml was kept at −30°C until use.

Vaccinations. Groups of 3- to 4-week-old female SPF mice (Iffa-Credo, St. Germain sur l’Arbresle, France) weighing 12 to 15 g were employed. Vaccinations consisted in three subcutaneous injections, at 1-week intervals, of 0.6 ml of the chosen vaccine, i.e., 0.5 ml of rabies vaccine and 0.1 ml of either poly A:U suspension (treated groups) or buffered saline (control groups). Stock rabies vaccine was serially diluted (1:5 ratio) from 1:5 to 1:3,125 in buffered saline, pH 7.5. Five groups of mice were vaccinated by these dilutions (control vaccines). Five groups were treated with the same dilutions, each containing 10 µg of poly A:U in a mouse dose ("10" vaccines). Five additional groups were vaccinated by these diluted rabies vaccines that contained 100 µg of poly A:U per mouse dose ("100" vaccines). Stability of the mixtures of poly A:U and rabies vaccines was estimated by vaccinating mice with "100" vaccines kept either at 4°C or at 37°C for 1 month. Protection thus obtained was compared to that given by mouse vaccination with liquid rabies vaccine, kept in the same time and temperature conditions and to immunization following vaccination with rabies vaccine of the same batch left in the lyophilized state until use.

Tests for appraisal of vaccine activity. One week after the third injection, protection induced by rabies vaccines, with or without poly A:U, was tested according to WHO Recommendations, as based on NIH mouse potency test (4). Results were expressed as the ratio of the fraction of mice which survived in each group. These figures permitted calculation (5) of the reciprocal of that dilution of vaccine which protected 50% of the mice against 100 LD₅₀ of CVS strain of rabies virus contained in a 0.03-ml intracerebral inoculation. CVS was a gift from T. J. Witktor (Wistar Institute, Philadelphia, Pa).

RESULTS

Stimulatory effects of 10 or 100 µg of poly A:U added to rabies vaccines. The results disclosed a stimulatory effect on anti-rabies protection following addition of poly A:U to the vaccines (Table 1). A significant difference, at P level = 0.95, was observed between "100" vaccine and stock vaccine. After addition of 100 µg of poly A:U to each mouse dose of rabies vaccine, mouse potency test revealed a 4.3-fold increase of the protection index (confidence limits from 1.4 to 13) when compared with control groups injected by rabies vaccine alone. However, there was
no significant difference between the "10" vaccine and the "100" vaccine. Indeed, when 10 μg of poly A:U was added to rabies vaccines, mouse protection index was multiplied by 3.4, this mean figure being included between 1.1 and 10.

Storage of mixtures of poly A:U and rabies vaccines. After 1 month at 4 or 37°C, stock reconstituted liquid rabies vaccines alone and vaccines that contained 100 μg of poly A:U per mouse dose, were compared to the same batch, no. 8C 100, of vaccine that had been kept lyophilized until use (Table 2). Observed differences are not significant at \( P = 0.95 \). All tested vaccines revealed a similar mouse protection index. In these experimental conditions, poly A:U does not remain in a double-stranded state.

**DISCUSSION**

Our data indicate a fourfold enhancing effect on immunization of mice against rabies, following addition of 100 μg, or even 10 μg, of poly A:U to each dose of rabies vaccine. These findings are in contrast to publications where antibody stimulation was evidenced only after administration of 300 to 1,250 μg of the homoribopolymer together with antigens (2, 3). An adjuvant effect on rabies immunization of low doses of poly A:U seems to indicate, as pointed out elsewhere (Renoux, Renoux, and Branche, submitted for publication), that there is no direct relationship between antiviral or antibacterial immunizations and the production of circulating antibodies. Induction of cellular immunity (macrophages and T cells) might respond to stimuli other than those needed to stimulate antibody-forming cells. Under our experimental conditions, 100 μg of poly A:U added to rabies vaccine, and kept at 37 or 4°C for 1 month, lost most of its stimulatory activity. Nevertheless, these preliminary data appear encouraging. More tests employing poly A:U in viral vaccines, perhaps at higher concentrations than in the present work, clearly are called for.

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**LITERATURE CITED**


