Use of the Enzyme-Linked Immunosorbent Assay (ELISA) for the Estimation of Serum Antibodies in an Influenza Virus Vaccine Study

R. Jennings*, T. Smith, and C.W. Potter

Department of Virology, Academic Division of Pathology, University of Sheffield School of Medicine, Beech Hill Road, Sheffield S 10 2RX, England

Abstract. The value of the enzyme-linked immunosorbent assay (ELISA) for determining the serum antibody responses of volunteers following immunisation with various inactivated influenza virus vaccines was assessed, and the incidence of seroconversions, as measured by both haemagglutination-inhibition (HI) and ELISA response of the volunteers determined. ELISA was found to be more sensitive than the HI test in detecting serum antibodies, but was also less specific under the conditions used. With regard to efficacy, the whole virus vaccine proved to be more effective in inducing serum antibody in an unprimed population than either tween-ether split or subunit adsorbed vaccines, but the reverse situation held when the population was primed with respect to the antigen concerned.

Introduction

In volunteer studies, the serological response to influenza virus vaccines is usually assessed using the haemagglutination-inhibiting (HI) and neuramidase-inhibiting (NI) antibody tests, (Pandemic Working Group of the Medical Research Council 1977; Jennings et al. 1978; Murphy et al. 1979; Potter et al. 1980). These tests are widely-used, easy to perform and acceptable, and have been standardised (WHO Technical Report Series 1959; Aymard-Henry et al. 1973). Indeed, the levels of HI antibody in human sera have been correlated with relative susceptibility or resistance to type A influenza virus infection (Meiklejohn et al. 1952; Hobson et al. 1972). However, the HI test is relatively insensitive and does not detect low levels of antibody (Gross and Davis 1979). In recent years new serological tests have been used for the estimation of influenza antibodies including single radial diffusion and single radial haemolysis (Schind et al. 1975), and radioimmuno-precipitation (Six and Kasel 1978). Another newly developed technique, the enzyme-linked immunosorbent assay (ELISA) has been used for the detection of antibodies to several different viruses including rubella (Veijtcorp 1978), mumps (Leinikki et al. 1979), respiratory syncytial virus (Richardson et al. 1978) and herpes simplex virus (Gilman and Docherty 1977), as well as influenza virus (Bishai and Galli 1978). In most of these studies

* Corresponding author
ELISA has proved to be considerably more sensitive for the detection of antiviral antibodies than conventional techniques but has been used primarily to detect antibodies in sera from patients suffering or recovering from virus infection.

In the present studies we have assessed the value of ELISA for determining the serum antibody responses of volunteers following immunisation with various inactivated influenza virus vaccines. Thus, students were immunised with two doses of either whole virus (WV), tween-ether (TE) or subunit adsorbed (SUA) influenza virus vaccines containing A/Texas/1/77 (H3N2), A/USSR/92/77 (H1N1) and B/Hong Kong/73 antigens. Serum from blood samples collected from each volunteer just before, and three weeks after each vaccine dose were tested for HI antibodies to influenza viruses A/Texas/77, A/USSR/77 and B/Hong Kong/73. A random sample of the sera were also tested for antibodies to A/Texas/77 virus using ELISA and the extent of correlation between HI and ELISA antibody titres determined. Antibody responses to immunization as measured by HI and ELISA, and the level and incidence of the antibody responses to each of the three types of influenza virus vaccine included in the study were also compared; the relative efficacy of these vaccines, and the value of ELISA for the measurement of influenza antibody are discussed.

Materials and Methods

Viruses

Influenza viruses A/Texas/1/77 (H3N2), A/USSR/92/77 (H1N1) and B/Hong Kong/8/73 were all obtained from Dr. J.J. Skehel, World Influenza Centre, National Institute for Medical Research, Mill Hill, London, and were grown in fertile hen’s eggs as described previously (Jennings et al. 1976).

Vaccines

All three vaccines were supplied by Philips-Duphar B.V., Weesp, The Netherlands. In their preparation, the virus strains used were grown in fertile eggs, purified by zonal centrifugation and inactivated with β-propiolactone. Aliquots of each whole virus preparation were then split by treatment with Tween (0.01%) and ether, (Jennings et al. 1978), and the whole and split monovalent preparations assayed for haemagglutinin content and blended to produce WV and TE-split trivalent vaccines.

The SUA vaccine consisted of haemagglutinin and neuraminidase subunits obtained from egg-grown virus disrupted with Triton N101. The released subunits were separated, purified and adsorbed to aluminium hydroxide gel as described by Brady and Furminger (1976).

The concentration of virus haemagglutinin in each vaccine was determined at the National Institute for Biological Standardisation and Control, Hampstead, London, using single radial diffusion (Wood et al. 1977). The WV vaccine was found to contain 22.9 µg of A/Texas/77 HA, 15.9 µg A/USSR/77 HA and 17.5 µg of B/Hong Kong/73 HA per dose. The corresponding figures for the TE and SAU vaccines were 15.2, 12.7 and 17.1, and 6.2, 10.7 and 2.5 µg HA per dose respectively.

Study Design

A total of 74 male and female students from the University of Sheffield, volunteered to take part in the study. All were healthy with no history of allergy to eggs or egg