IDENTIFICATION OF RUBELLA VIRUS STRUCTURAL PROTEINS BY IMMUNOPRECIPITATION

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Immunoprecipitation of [3H]amino acid labelled virus with monoclonal or human convalescent rubella sera and subsequent analysis by electrophoresis and fluorography, revealed three structural proteins of rubella virus: VP₁: 59,000; VP₂: 44,800; and VP₃: 33,000.

rubella virus structural proteins

INTRODUCTION

Rubella virus is a small spherical enveloped particle, 55–60 nm in diameter and is the only member of the genus Rubivirus of the family Togaviridae. The virus contains a single positive stranded 42S RNA molecule (Hovi and Vaheri, 1970 a,b) and only one serotype is known. Rubella infection in humans is generally a mild disease characterised by fever, coryza and malaise followed by atypical rash consisting of round, slightly raised, discrete macules. Its medical importance stems from its capacity to induce malformations in the foetus if the mother is infected during the first trimester of pregnancy (Fleet et al., 1974; Preblud et al., 1980).

Since the first isolation of the virus in the early 1960s, numerous papers have appeared on the characterisation of rubella structural proteins and intracellular virus-induced polypeptides (Chantler, 1979; Chantler and Tingle, 1980; Ho-Terry and Cohen, 1980; Liebhaber and Gross, 1972; Payment et al., 1975; Trudel et al., 1981, 1980; Vaheri and Hovi, 1972; Van Alstyne et al., 1980). Despite these reports there is still no consistent data on the exact number of structural proteins present in the intact virion or their molecular weights. This reflects the difficulties encountered in the production and purification of intact rubella virions (Corbeil et al., 1977; Trudel and Payment, 1980). By immunoprecipitation of ³H-labelled virus with a rubella neutralising monoclonal antibody or human convalescent sera followed by subsequent electrophoresis and fluorography we were able to demonstrate by this novel approach that rubella virions have only three structural
proteins: VP$_3$: 59K, VP$_2$: 44.8K and VP$_1$: 33K, as had already been stated by others (Ho-Terry and Cohen, 1980; Vaheri and Hovi, 1972) and ourselves (Payment et al., 1975; Trudel et al., 1980).

Rubella virus, strain M-33 (ATCC VR-315), was grown in Vero cells (a continuous heteroploid line of African green monkey kidney cells) produced in a TM-5 multiple tube tissue culture propagator (Corbeil et al., 1977) (Belco Glass Co., Vineland, NJ, U.S.A.). Confluent cell cultures were infected at a multiplicity of 1 plaque forming unit (p.f.u.)/cell. When hemagglutinating activity (HA) was detectable in the supernatant 5 $\mu$Ci/ml of a [$^3$H]amino acid mixture (New England Nuclear: net-250) was added to the culture medium and cultures were incubated for 48 additional hours at 37°C. One liter of supernatant with a viral titer of 32 HAU/25 µl (HAU: hemagglutination units) was concentrated to 50 ml by hollow-fiber ultrafiltration (Trudel and Payment, 1980) on an Amicon Model DH-4 concentrator (Amicon Corporation, MA, U.S.A.) equipped with a H$_1$-100 hollow-fiber cartridge having a molecular weight cut-off of 100,000. Efficiency of concentration, based on hemagglutination activity was 77.5%. Rubella hemagglutination assays were performed in microtitration plates in Auletta’s buffer as described previously (Trudel et al., 1979). The concentrated viral suspension was brought to 10 mM with EDTA to disaggregate the virions. Large cellular debris were removed by differential centrifugation at 10,000 $\times$ g for 15 min and smaller vesicles and membranes were removed by centrifugation at 70,000 $\times$ g for 10 min. The supernatant had an activity of 256 HAU/25 µl and 51,757 cpm/25 µl. Figure 1 shows intact rubella virus particles after concentration.

Monoclonal antibodies to rubella virus were produced by fusion of immune mouse spleen cells to mouse myeloma cells (Köhler and Milstein, 1975). Briefly, a BALB/c mouse was immunized intraperitoneally with 2,000 HAU of sucrose-purified (Trudel and Payment, 1980) virus emulsified in complete Freund’s adjuvant. After 4 wk, a booster dose of 2,000 HAU was given and 3 days later, the mouse was killed and the spleen removed. Hemagglutination inhibition titer (HAI) of the immunized mouse serum was 1:1,024. Spleen cells were fused with the Ag3-X63-653 mouse myeloma cell line (Kearney et al., 1979) in a ratio of 10:1 in the presence of 50% polyethylene glycol (M.W.: 1000; Koprowski et al., 1977). Hybrid cells were selected in hypoxanthine/aminopterin/thymidine (HAT) medium (Lillefield, 1964: approximately 300 spleen cells in 100 µl were seeded in each well of Linbro microplates (Flow Laboratories, VA, U.S.A.). Positive hybrid cells were recloned and transferred into 25 cm$^2$ tissue culture flasks (Flow Laboratories).

Supernatants were screened for anti-rubella antibodies by a modified enzyme-linked immunosorbent assay (Gravell, 1977; Leinikki et al., 1978). Six cultures found positive were further assayed for HAI (Stewart et al., 1967) and neutralisation activity (Furesz et al., 1969). Only one of these monoclonal antibodies showed neutralising activity at a titer of 1:4 and an ELISA titer of 1:16. This monoclonal antibody had no demonstrable anti-hemagglutinin in activity.

Structural proteins were analysed by immunoprecipitation (Ho-Terry and Cohen,
Van Alstyne et al., 1981) and electrophoresis. $^3$H-labelled rubella virus (1 ml) was incubated with 10 μl of a rubella human convalescent serum with an HAI titer of 1:512 or with monoclonal antibody. Tritiated virus and antiserum were incubated for 2 h at 37°C and 16 h at 4°C under constant mild agitation. This suspension was further incubated with 200 μl of protein-A-sepharose CL 4B (Pharmacia Fine Chemicals, Canada) containing approximately 260 μg of protein-A for 2 h at 37°C and 16 h at 4°C under mild agitation. Immunoprecipitates were then washed 3 times in NTE buffer (0.5 M NaCl, 0.05 M Tris HCl, 0.01 M EDTA, pH 7.4). Immunoprecipitates obtained with the human positive serum contained 8,000–10,000 cpm and those obtained with negative serum 3,000–4,900 cpm.

The immunoprecipitates were analysed on 10% polyacrylamide gels in the presence of SDS and prepared by the method of Laemmli (1970). Radioactive proteins were detected by fluorography (Bonner and Laskey, 1974).

Figure 2 illustrates the results of an experiment performed with a negative and a convalescent rubella serum. Radioactivity associated with the negative antiserum was 4,900 cpm (track C) while the positive serum bound 9,400 cpm (track B). A high level of radioactivity was associated with the negative antiserum immunoprecipitate because non specifically bound proteins were not removed by washing with detergent buffer as is usually the case with immunoprecipitates. Detergent was not added because it would have
solubilized the unbound viral membrane proteins rendering impossible the analysis of all the structural proteins of the virus particle. Fluorograms revealed three structural proteins with approximative molecular weights of 61K, 47K and 35K. Track A illustrates the position of the $^{14}$C molecular weight markers (92.5K, 46K, 30K and 12.3K). Table 1 lists the results of 4 experiments with rubella-convalescent antisera and one experiment

**TABLE 1**

Rubella virus structural proteins: SDS-page identification after Immunoprecipitation

<table>
<thead>
<tr>
<th>Structural proteins</th>
<th>Convalescent serum</th>
<th>Monoclonal antibodies</th>
<th>Mean molecular weight</th>
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<tr>
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<td>VP$_2$</td>
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$^a$ Molecular weights are expressed in multiple of 1,000 (K)
### TABLE 2

Summary of the reported rubella virus structural proteins

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**a** SDS-page = Sodium dodecylsulfate polyacrylamide gel electrophoresis.

**b** LSC = Liquid scintillation counting.
with rubella-specific monoclonal antibody. The mean molecular weight of the three structural proteins was $59,000 \pm 1,800$ for VP$_3$, $44,800 \pm 3,400$ for VP$_2$ and $33,000 \pm 1,600$ for VP$_1$.

The study of rubella virus structural proteins has been complicated by the fact that this virus is difficult to produce in large amounts because purification leads to viral inactivation and denaturation. In sucrose gradients, the virus comigrates and equilibrates with cellular membranes that contaminate the preparation rendering the interpretation of electrophoresis patterns ambiguous as can be judged by the differences in the number of rubella structural proteins and their molecular weights reported in the literature (Table 2). The present report of three structural proteins agrees with the work of Vaheri and Hovi (1972), Ho-Terry and Cohen (1980), and with data already published by our group (Payment et al., 1975; Trudel et al., 1980, 1981) but is in contradiction with the studies of Liebhaber and Gross (1972), Chantler (1979), Chantler and Tingle (1980) and Van Alstyne et al. (1981). Taking advantage of the specificity of antibodies we were able to determine the molecular weight of the three structural proteins: 59K (VP$_3$), 44.8K (VP$_2$) and 33K (VP$_1$). Earlier studies have shown that VP$_3$ and VP$_2$ are glycosylated and are the membrane proteins responsible for the hemagglutinating activity while VP$_1$ is shown to be associated with the RNA and the nucleocapsid (Ho-Terry and Cohen, 1980; Payment et al., 1975; Vaheri and Hovi, 1972). These results are similar to the work of Ho-Terry and Cohen (1980), who also had used immunoprecipitation but are in contradiction with Van Alstyne et al. (1981).

We were able to show for the first time that a neutralising monoclonal antibody to rubella virus specifically binds to intact virions composed of the three structural proteins as revealed by electrophoretic analysis of immunoprecipitated $^3$H-labelled virus. The fact that this monoclonal antibody does not inhibit the hemagglutinating activity of the virus could suggest that there are different antigenic sites for hemagglutination inhibiting and neutralising antibodies.

This report should help clarify the rather confused situation on rubella virus structural proteins and help in the further elucidation of the molecular biology of this medically very important virus. Knowledge of the fine molecular structure of the rubella virus and specifically its antigenic determinants are indispensable for the preparation of an efficient subunit vaccine against this virus and the characterisation of the rubella structural proteins is a first step toward reaching that goal.

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