Differentiation of Rubella Virus Strains by Neutralization Kinetics

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SUMMARY

The neutralization of rubella virus was investigated and shown to proceed by first order kinetics over the first 10 to 15 min. A comparison of the rate constant of neutralization (K) for six strains of rubella virus was carried out over this period of the reaction. Two particularly antibody-sensitive isolates were detected. It was noted that the three strains isolated from in utero infections were poorly neutralized by heterologous antisera when compared to postnatal strains.

Although several highly sensitive serological tests have been used successfully for the assay of rubella virus antibodies, including radio-immunoassay (Meurman et al. 1977), enzyme immunoassay (Gravell et al. 1977) and enhanced plaque neutralization (Sato et al. 1979b), none are readily adaptable for the characterization of antigenic differences between various rubella virus isolates or strains.

Simple haemagglutination inhibition has been applied to this type of analysis (Banatvala & Best, 1969), but neither this nor the kinetic haemagglutination inhibition test devised by Best & Banatvala (1970) could detect a difference in the antigenic make-up of the various strains of rubella virus examined. The neutralization test is, however, highly suitable for comparative studies (Mandel, 1978); Oxford (1969) was able to demonstrate small antigenic differences between strains of rubella virus using a 50 % infectious dose assay of surviving virus. In the same year Fogel & Plotkin (1969) demonstrated some antigenic differences by cross-neutralization tests employing a plaque reduction method. The development of a highly reliable and reproducible method of plaque assay in GL-RK13 cells (Gould et al. 1972), has made detailed investigation of the neutralization of the virus possible. This report presents an examination of the neutralization kinetics of a standard strain and a comparison of the rates of neutralization of six other strains of known history.

Six strains of rubella virus of varied history were selected for study (Table 1). Stocks of virus were grown in Vero cells and stored at -70 °C. The standard virus used was a pool of the RA27/3 strain grown in WI-38 cells, lyophilized and stored at -20 °C. Vero cells were obtained from the Public Health Laboratory Service at Colindale, U.K., and GL-RK13 cells from the National Institute of Health, Bethesda, U.S.A. The growth medium for both cell cultures consisted of Eagle's MEM containing 0.11 % NaHCO3 and 5 % foetal calf serum, while maintenance medium contained 0.22 % NaHCO3 and 28 % foetal calf serum. Details of the plaque assay are given elsewhere (Gould et al. 1972).

California strain rabbits received four successive intravenous inoculations of undiluted virus at 7 to 10 day intervals and were bled out from the heart 13 weeks after the first inoculation. Rabbits receiving virulent virus were housed separately from those receiving the attenuated strains and untreated rabbits housed with both failed to produce detectable antibody to rubella virus at any time. Sera were separated and stored at -20 °C. The standard pool of hyperimmune serum used was produced against the Edmunds strain of rubella virus (Banatvala & Best, 1969).

Dilutions of antiserum (2 ml) were mixed with equal volumes of virus suspension containing approx 1033 p.f.u. The mixture was incubated in a sealed Bijoux bottle in a water
Table 1. *K* values for replicate tests on six systems of homologous antiserum and antigen mixture

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Origin</th>
<th>Passage history</th>
<th>Mean <em>K</em> value</th>
<th>Mean NK*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesley</td>
<td>Adult with rubella</td>
<td>MK(3)</td>
<td>2.94 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>HPV-77</td>
<td>Adult with rubella</td>
<td>MK(78)</td>
<td>2.72 ± 0.05</td>
<td>100 ± 1.63</td>
</tr>
<tr>
<td>Cendehill</td>
<td>Adult with rubella</td>
<td>YRK(51)</td>
<td>1.60 ± 0.03</td>
<td>100 ± 1.63</td>
</tr>
<tr>
<td>Dunning</td>
<td>Rubella syndrome infant</td>
<td>RK13(4)</td>
<td>2.50 ± 0.14</td>
<td>100 ± 3.26</td>
</tr>
<tr>
<td>Thomas</td>
<td>Rubella syndrome infant</td>
<td>RK13(3)</td>
<td>2.55 ± 0.18</td>
<td>100 ± 4.31</td>
</tr>
<tr>
<td>RA27/3</td>
<td>Rubella-infected conceptus</td>
<td>HEK(4)</td>
<td>1.60 ± 0.13</td>
<td>100 ± 8.16</td>
</tr>
</tbody>
</table>

*Mean normalized *K* value.

Table 2. Normalized *K* values for each antiserum assayed separately with the six different antigenic types

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>RA27/3</th>
<th>HPV-77</th>
<th>Cendehill</th>
<th>Dunning</th>
<th>Thomas</th>
<th>Lesley</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA27/3</td>
<td>100</td>
<td>109.5</td>
<td>46</td>
<td>53</td>
<td>57.5</td>
<td>111</td>
</tr>
<tr>
<td>HPV-77</td>
<td>53.3</td>
<td>100</td>
<td>55.5</td>
<td>51</td>
<td>66</td>
<td>218</td>
</tr>
<tr>
<td>Cendehill</td>
<td>73</td>
<td>135</td>
<td>100</td>
<td>88</td>
<td>61.5</td>
<td>145.5</td>
</tr>
<tr>
<td>Dunning</td>
<td>67</td>
<td>80</td>
<td>70</td>
<td>100</td>
<td>27</td>
<td>210</td>
</tr>
<tr>
<td>Thomas</td>
<td>66</td>
<td>81</td>
<td>102.5</td>
<td>91</td>
<td>100</td>
<td>125</td>
</tr>
<tr>
<td>Lesley</td>
<td>30</td>
<td>117</td>
<td>56</td>
<td>50</td>
<td>44</td>
<td>100</td>
</tr>
</tbody>
</table>

bath at 32.5 °C. Samples (0.1 ml) were withdrawn at intervals and titrated in monolayer cultures of GL-RK13 cells. Mixtures of pre-immune serum with virus and standard serum with standard virus were similarly treated. The surviving virus was plotted on a logarithmic scale against time on a linear scale and the rate of neutralization per minute, the *K* value, was calculated from the initial slope of the curve where the reaction was apparently first order, i.e. a straight line passing through the origin (Dulbecco *et al.* 1956).

A comparison of the neutralization of one antigen with different antisera is only possible if the *K* values are normalized (McBride, 1959), because the antibody content of each pool of antiserum cannot be standardized. Normalized *K* values represent the rate at which a particular serum neutralized a heterologous virus as compared to the rate at which it neutralized the homologous virus, which is taken as 100. In any one test, one serum was assayed against all the strains of rubella virus to minimize variations between separate experiments.

It was found that a dilution of 1/8 of the standard antiserum (Edmund) gave a reaction curve with a standard pool of RA27/3 virus in which first order kinetics were observed for approximately the first 10 to 15 min. The results for thirteen replicate tests showed a good level of reproducibility, with a standard deviation of ±6.920 for the normalized *K* value. For all other investigations of the test sera, a dilution of 1/8 was also used and the *K* values determined for the first 10 min of reaction time. The *K* values for all six homologous reactions, which were done in triplicate, showed very little variation between replicates (Table 1).

The normalized *K* values for each antiserum determined with all the antigens under study are given in Table 2 and each value represents the mean of two experiments. Two strains of virus, HPV-77 and Lesley, appeared to be particularly sensitive to heterologous antibody. Thus, the normalized *K* values for heterologous antisera with Lesley were, in all cases,
higher than the homologous value. For HPV-77 virus, only two antisera, that is Dunning and Thomas gave normalized K values less than 100. With the other strains of virus, RA27/3, Cendehill, Dunning and Thomas, heterologous antisera often gave normalized K values which were much less than 100. In one instance with Thomas antiserum there was evidence of identity with a heterologous antigen, Cendehill, but the relationship was not reciprocal. The virus strains originating from cases of intra-uterine infection, RA27/3, Dunning and Thomas, showed a reaction of identity only with homologous antisera whereas the other strains, all isolated from post-natal infections were efficiently neutralized by some or all of the heterologous antisera.

The mechanism and nature of the neutralization test is still unresolved (Mandel, 1978), but results reported here are in agreement with Rawls et al. (1967), in that, for rubella virus, neutralization proceeds by first order reaction over the first 10 to 15 min. On this premise, it is concluded that the varying K values recorded for different rubella virus isolates imply some antigenic diversity and the most interesting feature of this diversity is the differentiation between viruses isolated from intra-uterine and post-natal infections. The apparent differences between viruses isolated from in utero and post-natal infections deserves further examination, if only to determine whether the prolonged in vivo growth of the former types might result in the selection of variants sufficiently different antigenically from the parental strain to account for the persistence of the virus. Such antigenic drift has already been reported for equine infectious anaemia virus (Kono et al. 1973) and Visna virus (Narayan et al. 1977).

There is no doubt that prolonged in vitro cell passage of rubella virus in cell culture may lead to alterations in certain properties such as virulence of vaccine strains for man (Parkman et al. 1966; Plotkin, 1969), loss of ability of RA27/3 to exhibit intrinsic interference (Kleiman & Carver, 1977) and diminished plaque size in two fresh isolates (Gould et al. 1972). In this regard, however, Sato et al. (1979a) found plaque characteristics of the vaccine strains preserved after five serial subcultures, which confirmed a similar observation made by Gould et al. (1972). The unique properties of Cendehill virus, such as the unusual pattern of antigenicity for rabbits and monkeys (Oxford & Potter, 1970; Zygraich et al. 1971), the delayed antibody response in man (Moffat et al. 1972) and the characteristic plaque morphology (Gould et al. 1972; Sato et al. 1979a), may be due to the prolonged passage history in rabbit kidney cells. This has not been investigated, however, but it would be of great interest to compare the serological and other properties of the vaccine strains with the original isolates from which they were developed.

Whether or not antigenic changes develop during passage of the virus in cell cultures has not been shown although it is also worth noting that extensive passage of virus isolates in cell culture did not seem to influence antigenic specificity or diversity, since Lesley virus, with only three subcultures, was heterospecific as was HPV-77 virus after 83 subcultures and, incidentally, both were from post-natal infections. Furthermore RA27/3 virus, isolated from an intra-uterine infection and which had undergone 32 subcultures, was just as homospecific as Thomas virus, which also originated from an intra-uterine infection, with only five subcultures.

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REFERENCES


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