The Effect of Antibody on Rubella Virus Infection in Human Lymphoid Cells

By J. K. CHANTLER* AND M. A. DAVIES

Division of Medical Microbiology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

(Accepted 8 January 1987)

SUMMARY

The effect of rabbit anti-rubella virus (RV) serum on the course of RV infection of human lymphoid cells has been examined. The antibody was found to abolish viral replication such that no virus progeny could be detected either extracellularly (after removal of the antiserum) or intracellularly, in the treated cells. Viral protein synthesis was also found to be totally inhibited in the presence of anti-RV but resumed immediately on removal of the antibody block at levels suggesting that viral RNA had been accumulating in the cell. Infectious focus assays indicated that the effect could be explained in part by the restriction by antiviral antibody of cell-to-cell spread in the indicator monolayer. Thus by 48 h when 100% of lymphoid cells infected with virus alone were capable of forming infectious foci, only 40 to 50% of cells infected in the presence of antiviral antibody produced foci. However this restriction imposed by antibody did not adequately explain the total inhibition of viral protein synthesis that occurred under these conditions. Pretreatment of RV with excess neutralizing antiserum reduced the virus titre >99.9% but did not eliminate infectious virus. The ‘non-neutralized’ fraction (10^2 to 10^3 p.f.u./ml) brought about a productive infection with final yields of 10 to 25% of control titres (>10^5 p.f.u./ml). Incubation of virus with both anti-RV and goat anti-rabbit Ig eliminated viral infectivity, indicating that the ‘non-neutralized’ fraction was in the form of infectious immune complexes. The results suggest two major modes of action for anti-RV antibody, one in neutralizing extracellular virus, the second exerted on the infected cells, totally inhibiting viral translation and thus preventing the virus from completing its replicative cycle.

INTRODUCTION

Specific antiviral antibody, induced following virus infection in vivo, is known to effect protection by a number of different mechanisms. Classically, the role of antibody was believed to be primarily neutralization of extracellular virus, preventing further spread of the agent and also reinfection by the virus. Antibody also has a role in destroying virus-infected cells, directly, in concert with complement, and also by antibody-dependent cellular cytotoxicity mediated by killer cells (Perlmann, 1984). In addition to these protective effects, antibody may under certain circumstances actually enhance viral replication by allowing infectious virus–antibody complexes to enter cells lacking virus receptors through binding to cellular receptors for the Fc portion of immunoglobulin (Halstead & O’Rourke, 1977; Peiris & Porterfield, 1979) or in some cases to complement receptors (Cardosa et al., 1983). Antibody may also be involved in the initiation of persistent infections by certain viruses, by stripping virus-induced membrane antigens from the cell surface, thus on the one hand preventing maturation of progeny virions and on the other hand protecting the cell harbouring viral genome from immune surveillance (Fujinami & Oldstone, 1984).

In the case of rubella virus (RV), the virus is known to persist in humans both in infants infected congenitally and in adults, mostly women, who develop rubella-associated arthritis following natural infection or immunization (Cooper & Buimovici-Klein, 1985; Chantler et al., 1987).
were incubated in the presence or absence of a 1:10 dilution of anti-RV. At various times the cells were counted

in PBS. Virus titres were determined in triplicate by assessing microfocus development in a standard plaque

assay in RK13 cells (Kouri et al., 1979). In measles virus infections \textit{in vitro}, antibody has been shown to exert two effects, causing modulation or 'capping' of viral antigens at the cell surface (Oldstone & Tishan, 1978; Gorman & Lachmann, 1982), and also a differential inhibition of the synthesis of certain measles virus proteins as measured by $[^{35}S]$methionine incorporation (Fujinami & Oldstone, 1979, 1980). This suggests a further possible mechanism of antibody action levelled at intracellular events. In this paper, studies on the effect of anti-RV antibody on RV replication in cultured lymphoid cells are reported. Antibody was found to inhibit viral translation totally, although 40 to 50\% of the cells were infected. The inhibition was reversible and synthesis of RV polypeptides could be detected 4 to 6 h after removal of anti-RV antibody from the medium. This lends further support to an intracellular site of action for antiviral antibody presumably directed by antibody acting at the cell surface.

METHODS

Cells and virus. Human peripheral blood mononuclear cells (PBMC) were prepared by Ficoll–Hypaque gradient centrifugation of venous blood from normal adults as described previously (Chantler & Tingle, 1980). Human lymphoblastoid cell lines Raji, CCRF-CEM and Jijoye were obtained from the American Tissue Culture Collection. They were cultured in RPMI medium supplemented with 10\% foetal calf serum + 1\% antibiotic/antimycotic (Gibco).

Two strains of RV have been used in these experiments. Strain 1B2 is a wild isolate which has been plaque-purified in this laboratory. FIN is a derivative of the Therien strain obtained from Dr A. Salmi, University of Alberta, Edmonton, Canada, which had previously been plaque-purified at the University of Turku, Finland. Both strains are extremely similar in polypeptide composition and biological properties and cause productive infections in both freshly isolated PBMC and human lymphoblastoid cell lines (M. A. Davies & J. K. Chantler, unpublished observations). Following infection with either strain, cells were incubated in RPMI supplemented with heat-inactivated foetal calf serum.

RV-specific antisera. Two RV-specific antisera have been tested for their ability to modulate RV infections. In most experiments, the antiserum used was prepared in rabbits against 1B2-infected RK13 cell sonicates. The serum was adsorbed with fixed and unfixed RK13 cells and PBMC, and gave no detectable background staining in immunoperoxidase reactions of control RK13 cells. Its neutralization titre was >99\% inhibition at a 1:100 dilution. Some experiments have been repeated with rabbit antisera prepared against partially purified supernatant virus instead of infected cells, with identical results.

Heat-treated sera were prepared by incubating whole serum at 50°C for 1 h to destroy complement components. An IgG fraction was purified by ammonium sulphate precipitation (final concentration 40\%) followed by chromatography on DEAE-Sepharose (Hudson & Hay, 1980).

Two monoclonal antibodies with anti-haemagglutinin activity (anti-HA1 and anti-HA2) were kindly supplied by Dr R. Tedder, London, U.K. In addition three further monoclonal antibodies, one against each major structural protein of the virus (anti-E1, anti-E2 and anti-C) were provided by Dr J. Wolinsky, Houston, Tx., U.S.A.

Virus titration in modulated cells. To examine the effects of anti-RV antibody on RV replication, virus was first adsorbed to the cells for 2 h, and the inoculum was then replaced with medium containing anti-RV where appropriate. All experiments reported were carried out this way, except the experiment to determine the effect of pretreatment with neutralizing antiserum. Titration of infectious virus production in cells treated (or not treated) with anti-RV was carried out in two ways. To assess the ability of the modulated cells to release infectious virus, the medium of infected cells (with or without anti-RV) was removed and the cells were washed once in phosphate-buffered saline (PBS) to remove residual anti-RV. One ml of RPMI medium with 2\% heat-inactivated foetal calf serum was then added to the cells and the virus released over the next 2 h was collected and titrated. In addition, intracellular titres of infectious RV were assayed by preparing sonicates of modulated or unmodulated infected cells in PBS. Virus titres were determined in triplicate by assessing microfocus development in a standard plaque assay in RK13 cells (Kouri \textit{et al.}, 1974).

Infectious focus assay. The percentage of cells infected with RV under various conditions was determined by an infectious focus assay. PBMC or Raji cells were infected at high multiplicity, and following a 2 h adsorption period were incubated in the presence or absence of a 1:10 dilution of anti-RV. At various times the cells were counted
Antibody in rubella virus infection

Table 1. Inhibition of RV (strain 1B2) replication in PBMC by specific antibody

<table>
<thead>
<tr>
<th>Titre of virus (p.f.u./ml)</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>120 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV-infected (no additions), supernatant*</td>
<td>3.2 × 10^4</td>
<td>1.5 × 10^5</td>
<td>7.9 × 10^5</td>
<td>2.1 × 10^5</td>
</tr>
<tr>
<td>RV-infected (no additions), cell-associated</td>
<td>1.2 × 10^3</td>
<td>8.8 × 10^3</td>
<td>2.7 × 10^4</td>
<td>1.4 × 10^4</td>
</tr>
<tr>
<td>RV-infected (+ anti-RV), supernatant*</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>RV-infected (+ anti-RV), cell-associated</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

* Titre determined of virus released during a 2 h collection period after removal of antibody from modulated cells.

and appropriate dilutions made in order to plate out 50, 100, 250, 500 or 1000 cells per Petri dish in duplicate. The dishes were then centrifuged to deposit the lymphoid cells on to the cell sheet, the inoculum was carefully removed and replaced with RPMI medium containing 2% agar. Infectious foci were recorded after 7 days and had a 'microfocus' appearance as described by Kouri et al. (1974).

Immunoprecipitation and PAGE analysis. Immunoprecipitations were carried out as follows. 35S-labelled cell pellets were solubilized in 1% NP40 in TNE buffer (0.1 M-Tris-HCl pH 7.2, NaCl, EDTA) in 1.5 ml Eppendorf tubes. Cell debris was removed by centrifugation for 2 min at 12000 g in an Eppendorf microfuge. Rabbit anti-RV serum was added to a 1:50 dilution and the solution was incubated for 2 to 4 h at 0 °C. One-hundred μl of 10% heat-killed and formalin-fixed Staphylococcus aureus Protein A (Calbiochem, Pansorbin) was added per ml of buffer and incubation continued for 1 to 2 h at 0 °C. The immunoprecipitates were then pelleted in the microfuge and washed thoroughly three times in 1% NP40, 0.1% SDS, 0.5% sodium deoxycholate. The final pellet was resuspended and boiled for 2 min in SDS solubilization buffer (0.125 M-Tris pH 6.8, 2% SDS, 1% 2-mercaptoethanol) and analysed by polyacrylamide gel electrophoresis using 5 to 25% gradient slab gels and the discontinuous buffer system described by Laemmli (1970). The gels were run for 5 h at a constant current of 32 mA. They were stained in 0.5% Coomassie Brilliant Blue in 50% TCA and destained in 7% acetic acid, 25% methanol, 2% glycerol. They were dried using a Bio-Rad model 483 gel dryer and exposed to Kodak XRPI X-ray film for approximately 10 days. The films were developed in a Kodak RP X-Omat processor.

RESULTS

Incubation of RV-infected cells with neutralizing antiserum

The effect of neutralizing antiserum on RV replication in human PBMC was examined by comparing the titres of virus released from cells which had been incubated either in the presence or absence of anti-RV serum for various lengths of time. For example, at 24, 48, 72 and 120 h post-infection, the culture fluid was replaced with a small volume of RPMI medium (without anti-RV antibody) and the virus released during the next 2 h was collected and titrated as shown in Table 1. The results show that high titres of virus were released by 48 h in human PBMC incubated in medium alone, and that virus continued to be released in large quantities over the next 72 h. However cells which had been incubated in neutralizing antiserum did not release any virus during the 2 h collection period after removal of the antibody block. The effect was not due to residual neutralizing antibody remaining after the cells had been washed, prior to the collection period, as antiserum could be added to normal 48 h infected cells and washed out after 15 min without an appreciable reduction in virus titre. Similar results were obtained by determining the amounts of intracellular virus in cells incubated in the presence or absence of anti-RV. No infectious virus was detected in sonicates of cells incubated in medium containing anti-RV. Thus the effect of specific antibody appeared to be not only neutralization of released virus, but at some earlier stage in the replicative cycle.

Intracellular effects of neutralizing antiserum on RV replication

The effect of RV-specific antiserum on the synthesis of RV-induced proteins in infected PBMC was examined. PBMC were infected at high multiplicity with RV strain 1B2 (5 to 10 p.f.u./cell) and were incubated in the presence or absence of anti-RV following a 2 h adsorption period. They were labelled from 72 to 78 h post-infection when viral protein synthesis was normally maximal. In addition further samples of infected cells were released from the antibody
Fig. 1. Intracellular polypeptide synthesis in modulated RV-infected cells. Autoradiogram of a 5 to 20% slab gel showing the pattern of intracellular polypeptide labelling in mock- or RV-infected PBMC (strain 1B2). (a) Mock-infected cells without anti-RV; (b) RV-infected cells without anti-RV; (c) RV-infected cells with anti-RV; (d) RV-infected cells labelled from 0 to 24 h after release from antibody block; (e), as (d) but labelled from 24 to 48 h; (f), as (d) but labelled from 48 to 72 h.

block on day 3 post-infection and were labelled 0 to 24 h, 24 to 48 h or 48 to 72 h post-release. The pattern of intracellular polypeptide labelling is shown in Fig. 1. In lane (b), the major virus-induced proteins including the three structural proteins VP1 (52K to 55K), VP2 (40K to 42K) and VP3 (32K) can be identified in addition to several high molecular weight species, ICPs 86, 75 and 66 previously characterized in RV-infected cells (Chantler, 1979; Chantler & Tingle, 1980). Host protein synthesis was substantially inhibited in human PBMC by 72 h post-infection as can be seen by comparison of lane (b) with the mock-infected cell pattern in lane (a). However
when infected cells were incubated in the presence of neutralizing antiserum no viral proteins could be identified and the labelling pattern was identical to, although less intense than that of mock-infected cells (lane c). Thus viral protein synthesis appeared to be totally inhibited by the presence of anti-RV in the culture fluid. In contrast, when the anti-RV was removed from the growth medium and the cells were labelled after release from the antibody block, viral protein synthesis was found to resume, and a large amount of progeny virus was released into the medium. In addition the amount of the individual viral proteins synthesized was much greater than was normally detected in infected PBMC, particularly in the first 24 h post-release, a time period when very little, if any, viral proteins can be identified in a normal infection (Chantler & Tingle, 1980). This suggests that viral RNA had accumulated in the infected cells incubated with anti-RV serum, such that when the antibody block was removed large amounts of the viral proteins could be translated immediately.

**Immunoprecipitation of RV polypeptides from infected cells incubated in the presence or absence of anti-RV**

Infected PBMC were incubated for 3, 5 or 7 days in medium containing anti-RV and the cells were then labelled for 6 h on each of these days with [35S]methionine. A second sample of cells was released from the antibody block on each day and labelled from 48 to 54 h later, to determine how long the viral genome could be conserved under conditions preventing its expression. The cell pellets were immunoprecipitated with anti-RV serum to reduce the background of cellular protein in modulated infected cells and the results are shown in Fig. 2. In lane (b), a clear pattern of RV-induced polypeptides found in infected cells at 72 h post-infection is shown. There was a little background precipitation from mock-infected cells of several host proteins which adsorb to Protein A in the absence of antibody (lane a). In lanes (c), (e) and (g) the polypeptides labelled in the presence of anti-RV on days 3, 5 and 7 respectively are shown. No viral polypeptides were detectable, indicating that viral RNA translation had been totally prevented. On both days 3 and 5, removal of the antibody block enabled viral replication to resume, resulting in the synthesis of viral polypeptides (lanes d and f) and the release of $10^5$ to $10^6$ p.f.u./ml of infectious virus 48 h later. On day 7 post-infection, the viability of the human PBMC was very low (> 80% dead cells) and reversal of the antibody block resulted in only low levels of viral protein synthesis and viral progeny release ($10^2$ to $10^3$ p.f.u./ml). The relatively short life-span of human PBMC in culture has restricted the time period over which persistence of the viral genome can be studied under conditions of an antibody block. Recent studies conducted in continuous lines of human lymphoblastoid cells have indicated that RV can persist for over 6 months when the medium is supplemented with anti-RV serum (M. A. Davies & J. K. Chantler, unpublished observations).

**Characterization of the active component in serum in inhibiting viral replication**

The initial experiments on the effects of specific antibody on RV replication in human lymphoid cells were conducted using crude serum. To characterize the effector of viral restriction, the serum was first heat-treated or ammonium sulphate-precipitated, and the IgG fraction was purified by DEAE-Sepharose column chromatography. The result is shown in Table 2. Both heat-treated serum and purified IgG fraction were active in viral restriction indicating that the effect was due to antibody without a requirement for complement components. PAGE analysis of intracellular viral protein synthesis similarly showed that purified IgG could mimic the inhibition found with crude serum (not shown).

**Effect of host cell on antibody restriction of viral replication**

The ability of anti-RV antibody to prevent viral replication in different cell types was tested using separated populations of lymphocytes or monocytes and three human lymphoblastoid cell lines Raji (B line), Jijoye (B line) and CCRF-CEM (T line). A monocyte-rich fraction was prepared by plastic adherence, and infected on the day of isolation. The lymphocyte-rich fraction remaining in the supernatant was treated with phytohaemagglutinin for 3 days and similarly infected. Both cultures were incubated in the presence or absence of antiserum and labelled for viral protein synthesis 72 to 78 h post-infection. The results are shown in Fig. 3 and
Fig. 2. Immunoprecipitates of RV-infected PBMC. Autoradiogram showing the pattern of RV-specific (strain 1B2) polypeptides immunoprecipitated from mock- or RV-infected cells. Cells were incubated for 3, 5 or 7 days with rabbit anti-RV and $^{35}$S-labelled either in the continued presence of antiserum or 48 h after release from the antibody block. (a) Day 3, mock-infected PBMC labelled 72 to 78 h p.i.; (b) day 3, RV-infected PBMC labelled 72 to 78 h p.i.; (c) day 3, RV-infected PBMC + anti-RV labelled 72 to 78 h p.i.; (d), as (c) but labelled 48 to 54 h after release from antibody block; (e) day 5, RV-infected PBMC + anti-RV labelled 120 to 126 h p.i.; (f), as (e) but labelled 48 to 54 h after release from antibody block; (g) day 7, RV-infected PBMC + anti-RV labelled 168 to 175 h p.i.; (h), as (g) but labelled 48 to 54 h after release from antibody block.

Table 2. Characterization of active component in serum

<table>
<thead>
<tr>
<th></th>
<th>Titre of intracellular virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV-infected PBMC</td>
<td>5.9 × 10^6</td>
</tr>
<tr>
<td>RV-infected (+ anti-RV)</td>
<td>0</td>
</tr>
<tr>
<td>RV-infected (+ heat-treated anti-RV)</td>
<td>0</td>
</tr>
<tr>
<td>RV-infected (+ purified IgG)</td>
<td>0</td>
</tr>
</tbody>
</table>

48 h after release from block

<table>
<thead>
<tr>
<th></th>
<th>48 h after release</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV-infected PBMC</td>
<td>2.2 × 10^4</td>
</tr>
<tr>
<td>RV-infected (+ anti-RV)</td>
<td>7.3 × 10^4</td>
</tr>
<tr>
<td>RV-infected (+ heat-treated anti-RV)</td>
<td>7.3 × 10^4</td>
</tr>
<tr>
<td>RV-infected (+ purified IgG)</td>
<td>1.1 × 10^5</td>
</tr>
</tbody>
</table>
Figure 3. RV restriction by specific antibody in separated monocytes and lymphocytes. Autoradiogram showing 35S-labelled polypeptides immunoprecipitated from RV-infected PBMC, or separated monocytes and lymphocytes, which had been incubated with anti-RV IgG. (a) PBMC, RV-infected, labelled 72 to 78 h p.i.; (b) monocytes, RV-infected + anti-RV, labelled 72 to 78 h p.i.; (c) monocytes, RV infected + anti-RV, labelled 48 to 54 h after release from antibody block; (d) lymphocytes, RV-infected + anti-RV, labelled 72 to 78 h p.i.; (e) lymphocytes, RV-infected + anti-RV, labelled 48 to 54 h after release from antibody block.

Table 3. Both monocyte-rich preparations and lymphocytes (in particular T cells) have previously been shown to support viral replication (Chantler & Tingle, 1980), and the permissiveness of lymphoblastoid cell lines has also been established (Chantler, 1981). In the presence of specific antibody (IgG fraction), no RV protein synthesis was detected in either the monocyte-rich or the lymphocyte-rich fraction (Fig. 3b and d). Moreover cells incubated in anti-RV IgG did not contain infectious virus progeny intracellularly (Table 3). In each case the viral
Table 3. Effect of antibody (IgG fraction) on RV infection in different host cells

<table>
<thead>
<tr>
<th></th>
<th>Mock-infected, 72 h p.i.*</th>
<th>Infected, 72 h p.i.*</th>
<th>Infected + IgG, 72 h p.i.*</th>
<th>Infected, 48 h after release from antibody block†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>0</td>
<td>1 x 10⁶</td>
<td>0</td>
<td>8.8 x 10³</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0</td>
<td>2.7 x 10⁴</td>
<td>0</td>
<td>5.1 x 10²</td>
</tr>
<tr>
<td>Raji (B line)</td>
<td>0</td>
<td>1.6 x 10⁶</td>
<td>0</td>
<td>2.0 x 10⁶</td>
</tr>
<tr>
<td>Jijoye (B line)</td>
<td>0</td>
<td>1.2 x 10⁵</td>
<td>0</td>
<td>6.0 x 10³</td>
</tr>
<tr>
<td>CCRF-CEM (T line)</td>
<td>0</td>
<td>1.4 x 10⁶</td>
<td>0</td>
<td>1.6 x 10⁶</td>
</tr>
</tbody>
</table>

* Virus titres (p.f.u./ml) in sonicated cell pellets.
† Virus titre (p.f.u./ml) in supernatant medium.

Table 4. Infectious focus assay in lymphoblastoid cell lines

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Raji - IgG</th>
<th>Raji + IgG</th>
<th>CCRF-CEM - IgG</th>
<th>CCRF-CEM + IgG</th>
<th>Jijoye - IgG</th>
<th>Jijoye + IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.i.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>40-60</td>
<td>10-25</td>
<td>50-55</td>
<td>18-25</td>
<td>25-40</td>
<td>10-15</td>
</tr>
<tr>
<td>48</td>
<td>&gt;100*</td>
<td>25-40</td>
<td>&gt;100</td>
<td>25-40</td>
<td>&gt;100</td>
<td>15-25</td>
</tr>
<tr>
<td>72</td>
<td>&gt;100</td>
<td>40-45</td>
<td>&gt;100</td>
<td>30-45</td>
<td>&gt;100</td>
<td>25-40</td>
</tr>
</tbody>
</table>

* Infectious foci after 24 h p.i. are frequently >100% due to the rapid and continuing release of infectious virus particles despite careful washing procedures.

The effect of monoclonal antibodies on RV replication

Five monoclonal antibodies with anti-haemagglutinin activity against RV have been tested for their ability to mimic the effect of the polyclonal rabbit anti-RV IgG in restricting viral replication. The monoclonal antibodies were added at a 1:50 dilution of purified ascites (HI titres are given in Table 5) immediately following a 2 h adsorption period and intracellular virus titres were assessed 48 h later. The results are shown in Table 5. Two- to fivefold reductions in virus titre were found with several of the monoclonal antibodies but titres of >10⁵ infectious particles/ml were still released, and one monoclonal antibody actually increased the virus yield (anti-HA₂). Intracellular polypeptide labelling patterns of infected cells incubated with two of the monoclonal antibodies are shown in Fig. 4. Viral polypeptides are indicated on the right-hand side and in the immunoprecipitations shown in lanes (g) and (h) it can be seen that the genome was conserved and viral RNA translation and assembly ensued after removal of the antibody block (Fig. 3 c and e, Table 3). Similar results were found in the three lymphoblastoid cell lines (Table 3).

Infectious focus assay on infected cells restricted by neutralizing antibody

To determine the part played by prevention of virus spread in the restriction of RV replication, infectious focus assays were performed on lymphoblastoid cells incubated in the presence or absence of anti-RV IgG. The results are shown in Table 4. In the absence of anti-RV, 25 to 60% of cells in the Raji, CCRF-CEM or Jijoye cultures were infected by 24 h and this rose to >100% by 48 h. By this time, infectious virus was being released in large amounts and the number of foci was often greater than the number of cells estimated, despite careful washing procedures. In contrast, the percentage of cells forming foci in cultures incubated with anti-RV was 15 to 25% at 24 h post-infection and rose to 25 to 45% by 72 h, possibly indicating that some spread of the viral genome occurred in the absence of viral assembly in the modulated cells. As one third to a half of the cells in each lymphoblastoid cell culture were infected by 72 h, the absence of detectable viral protein synthesis in these cells can not be explained by insufficient numbers of cells having been infected.
Antibody in rubella virus infection

Fig. 4. Polypeptides synthesized in PBMC (strain FIN) modulated by monoclonal anti-HA antibodies. Autoradiogram showing $^{35}$S-labelled patterns of total intracellular proteins (a to e) or immunoprecipitates (f to h) of RV-infected cells treated with polyclonal anti-RV or anti-HA$_1$ and anti-HA$_2$ monoclonal antibodies. (a) Mock-infected whole cell proteins; (b) RV-infected whole cell proteins; (c to e) RV-infected whole cell proteins treated with (c) anti-RV, (d) anti-HA$_1$ or (e) anti-HA$_2$; (f to h) RV-infected immunoprecipitates from treatments with (f) anti-RV, (g) anti-HA$_1$ or (h) anti-HA$_2$.

Table 5. Monoclonal antibodies in RV infection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HI titre of undiluted ascites</th>
<th>RV titre at 48 h (p.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV-infected PBMC (no anti-RV)</td>
<td>–</td>
<td>$2.1 \times 10^6$</td>
</tr>
<tr>
<td>RV-infected PBMC + anti-RV IgG (1:10)</td>
<td>NT*</td>
<td>0</td>
</tr>
<tr>
<td>RV-infected PBMC + anti-HA$_1$ (1:50)</td>
<td>&gt;10000</td>
<td>$4.1 \times 10^5$</td>
</tr>
<tr>
<td>RV-infected PBMC + anti-HA$_2$ (1:50)</td>
<td>4096</td>
<td>$6.8 \times 10^6$</td>
</tr>
<tr>
<td>RV-infected PBMC + anti-E$_1$ (1:50)</td>
<td>4096</td>
<td>$1.0 \times 10^6$</td>
</tr>
<tr>
<td>RV-infected PBMC + anti-E$_2$ (1:50)</td>
<td>0</td>
<td>$1.4 \times 10^6$</td>
</tr>
<tr>
<td>RV-infected PBMC + anti-C (1:50)</td>
<td>0</td>
<td>$1.8 \times 10^6$</td>
</tr>
</tbody>
</table>

* NT, Not tested.
major virus-induced proteins were still synthesized in cells incubated with the anti-HA1 and anti-HA2 antibodies. However the amounts in lane (g) (anti-HA1) were considerably less than in either lane (h) (anti-HA2) or in the infected control (lane b). In lane (f) the lack of synthesis of viral proteins in the presence of polyclonal anti-RV can again be seen, and only small amounts of host proteins precipitated by adsorption to Protein A can be seen.

In the whole cell infected patterns shown in lanes (c), (d) and (e) an interesting observation was made. In (c), in the presence of rabbit anti-RV, no viral proteins were made, and there was only a slight inhibition of host cell macromolecular synthesis. In (d), the reduced level of viral protein synthesis found on incubation with anti-HA1 was associated with normal inhibition of host translation by 72 h post-infection and the pattern was very similar to the corresponding immunoprecipitate in (g). In (e), however, the much higher level of viral protein synthesis in the presence of anti-HA2 was associated with very little if any inhibition of cellular translation. This result raises questions as to the mechanism of switch-off of host translation (unlikely therefore to be a new viral translation product) and again emphasizes that incubation with antibody can have effects on internal events during the infectious process. This pattern of normal inhibition of cellular translation on incubation with anti-HA1, but continuing cellular translation with anti-HA2 has been observed repeatedly.

**Pretreatment of RV with neutralizing antiserum prior to infection**

The effect of pretreatment of RV with anti-RV IgG prior to adsorption to human lymphoid cells was examined. Virus was incubated with various concentrations of neutralizing antiserum for 15 to 30 min and a sample was then removed for titration and the remainder was added to human PBMC. Following adsorption the cells were incubated without antiserum and the titres of virus released at 72 h determined (Table 6, b). The rabbit antiserum reduced the titre of stock virus by over 99% at a dilution of 1:100 (Table 6, a). However even at a 1:10 dilution, a small amount of infectious virus remained in the inoculum and brought about a productive infection of PBMC. This small infectious or 'non-neutralized' fraction could not be further reduced by increasing the antibody concentration, a finding also described for other viruses (Mandel, 1979). Although less than 1% of the stock virus, the 'non-neutralized' fraction brought about a productive infection with final yields of over 10^5 p.f.u./ml (about 10 to 25% of the control; Table 6, b). Only if anti-RV IgG at the same concentration was also included in the medium after virus adsorption, was viral replication inhibited (column c). Under these conditions, even a 1:100 dilution of rabbit antiserum prevented the virus from completing its replicative cycle. These results clearly indicate a role for antibody, in addition to the inhibition of extracellular virus, directed at some intracellular event.

In further experiments, RV was treated with both anti-RV IgG and goat anti-rabbit Ig. This inoculum was then allowed to adsorb to PBMC and incubated for 72 h. The results (Table 6) show that the addition of a secondary antibody was capable of eliminating the non-neutralized fraction indicating that it was in the form of infectious virus-immune complexes.
Antibody in rubella virus infection

The effect of incubating RV with very low concentrations of anti-RV has also been examined for possible enhancement of viral infectivity. Such antibody-dependent enhancement has been described for a number of other viruses including members of the Togavirus group (Porterfield & Cardosa, 1985). RV was pre-incubated with dilutions of antibody ranging from 1:500 to 1:100000, and the virus was then used to infect mixed populations of PBMC. Virus titres and number of infectious foci were determined at 48 h. The antibody was found to be inhibitory at the low dilution (1:500) reducing both the final virus titre and the number of infectious foci approximately 10-fold. At higher dilutions (1:100000) no effect was observed.

DISCUSSION

Effects of virus-specific antibody on viral translation when added to the medium of infected cells have previously been described by Fujinami & Oldstone (1980) with measles virus. In their studies, differential inhibition of certain viral polypeptides, in particular F1, P and M relative to the HA or NC proteins, was found when cells were treated with anti-measles virus antibody prepared from human convalescent serum. The effect could be mimicked with a monoclonal antibody to the viral haemagglutinin (Fujinami & Oldstone, 1980). In contrast, our results with RV show a total inhibition of viral translation and assembly on incubation of the infected cells with polyclonal anti-RV serum or an IgG fraction. The inhibition of viral replication by exogenous antibody may in part be due to the prevention of virus spread in the cell culture. Infectious focus assays on lymphoblastoid cell lines infected at high multiplicity indicate that 10 to 25% of exponentially growing cells take up infectious virus during the first 6 h post-infection. If antibody is added to the medium, virus spread is limited but by 72 h post-infection approximately 40% of cells are infected (Table 4). Thus the total inhibition of intracellular viral protein synthesis in the studies reported here can not be explained by lack of infection of sufficient cells in the culture. Indeed, pretreatment of virus with neutralizing antibody does not prevent establishment of a productive infection (Table 6) indicating that whereas antibody has an important role in reducing the titre of extracellular virus, its maximal effect is on the infected cell at which level viral replication can be totally blocked, although the viral genome persists.

Preliminary evidence suggests that the point of restriction of the infective process by antibody is after viral RNA replication. On removal of the antibody block, high levels of viral protein synthesis commence immediately and the major viral polypeptides can readily be identified on gels 4 to 6 h later. In contrast, during the first 24 h of a normal infection, very little synthesis of viral proteins can be detected (Chantler & Tingle, 1980). The possibility that the incoming viral RNA is replicated but not translated, and therefore accumulates in the modulated cell is currently under investigation. This pool of viral RNA would therefore be available for immediate translation on removal of the antibody block. Interestingly, the highest levels of viral polypeptides we have detected in infected PBMC have been in cells incubated for 48 h post-infection in antibody and then released from the block and labelled 24 to 48 or 48 to 72 h later (see Fig. 1, lanes e and f). At these times in the absence of antibody (i.e. 72 to 96 h and 96 to 120 h post-infection) viral polypeptide synthesis is dropping to low levels as the host cells die off.

In conclusion the present results suggest an additional role for antibody in combating certain viral infections, exerted on intracellular events in the viral replicative cycle. The inhibition can be reversed by removing antibody from the medium and washing the cells, indicating that the antibody is acting at some site on the cell surface from which it can be dislodged by washing procedures. Antibody greatly reduces the titre of extracellular virus but the 'non-neutralized' fraction is capable of initiating a productive infection, and only by exerting an effect on the infected cell as well can antibody inhibit the infectious process.

This work was supported by the Medical Research Council of Canada and the Arthritis Society of Canada.

REFERENCES


(Received 21 October 1986)