The Differential Growth of Virulent and Avirulent Strains of Rinderpest Virus in Bovine Lymphocytes and Macrophages

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SUMMARY

Rinderpest virus (RV) grew readily in cultures of purified bovine peripheral blood lymphocytes and udder macrophages. The growth of three strains of RV was compared and there appeared to be a relationship between increasing virulence and increased ability to infect lymphocytes and macrophages. The proportion of infected cells as determined by the presence of virus antigens was a better indicator of affinity between a strain and cell type than production of new infectious virus. RV grew better in populations of predominantly T lymphocytes than in T-depleted cultures. Although RV could infect 100% of cells in macrophage monolayers, it did not appear to infect more than about 30% of cells in lymphocyte cultures. Virulent RV grew more readily in bovine than caprine or ovine lymphocytes, whereas virulent peste des petits ruminants virus (PPRV) grew better in lymphocytes from sheep and goats. There was no marked difference in the growth of either virus in lymphocytes from uninfected or recently convalescent animals.

INTRODUCTION

It is well established that there are different strains of rinderpest virus (RV) which vary in their virulence for cattle and other susceptible species but are indistinguishable by other techniques (Plowright, 1968). The recent epidemics of rinderpest in Africa (Nawathe et al., 1983; Rossiter et al., 1983) have involved more than one strain with varying virulence and have encouraged renewed attempts to distinguish between strains and, possibly, to determine which characteristics may be associated with virulence.

In infected cattle, RV grows primarily in lymphoid tissues with subsequent spread to epithelia in which necrosis is responsible for the typical clinical signs. There is evidence that virulent RV grows to higher titre than avirulent RV in lymphoid tissues in vivo (Taylor & Plowright, 1965) and, consequently, it seemed possible that differences between strains of varying virulence would be reflected in their differential growth in cultured lymphocytes and macrophages. This report describes investigations to test this hypothesis and also whether another member of the morbillivirus group, peste des petits ruminants virus (PPRV), which preferentially infects sheep and goats, shows a differential growth pattern in lymphocytes from cattle compared to lymphocytes from ovines.

METHODS

Viruses. Virulent RV (RVY), which had caused 100% mortality in 33 experimentally infected British cattle, was isolated from a cow in the Yemen Arab Republic (W. P. Taylor, unpublished) and used after 3 passages in bovine kidney (BK) cells. A mildly virulent strain (RBT/1), which was isolated from an ox in Tanzania, caused <5% mortality in cattle (Plowright, 1963) and was used after 8 passages in BK cells. Avirulent RV was the tissue culture vaccine strain (TCRV), which was used after 96 passages in BK cells (Plowright & Ferris, 1962).

A virulent strain of PPRV (PPRV-Melilq) recently isolated from a sick goat in Sudan (El Hag Ali & Taylor, 1984) was used after 6 passages in sheep kidney and BK cells. All virus stocks were prepared from the same batch of BK cells and frozen at -70 °C until used.
Cultures. Bovine, ovine and caprine peripheral blood lymphocytes and bovine udder macrophages were prepared as described previously (Wardley et al., 1976; Rouse et al., 1976). Populations of T-depleted and T-enriched lymphocytes were produced by incubation with nylon wool (Rouse et al., 1976) and differential centrifugation after rosetting with sheep red blood cells (Parish et al., 1974). Immunofluorescence for receptors for surface immunoglobulin and peanut agglutinin were used as markers for B and T lymphocytes, respectively (Pearson et al., 1979). ‘Immune’ lymphocytes were prepared from the blood of cattle, sheep and goats which had recovered 5 to 10 days previously from experimental infections with RV or PPRV.

Lymphocytes were cultured as 1 x 10^6 to 2 x 10^6 cells in 2 to 3 ml RPMI 1640 medium plus 10% foetal calf serum (FCS) in loosely stoppered plastic tubes at 37 °C in 5% CO₂ in air and were stimulated with predetermined optimal doses of mitogens (Sigma), 10 mg/ml phytohaemagglutinin (PHA), 10 mg/ml concanavalin A (Con A) or 0.6 mg/ml pokeweed mitogen (PWM), 48 to 96 h prior to infection of cultures. Incorporation of [³H]thymidine overnight was used to quantify the effectiveness of the mitogenic responses in one experiment.

Lymphocytes were infected by pelleting and resuspension in 1 ml of virus at 37 °C for 1 h, after which they were washed 5 times and recultured. Duplicate tubes were harvested daily for preparation of cytocentrifuge smears for morphology and antigen studies and the remaining cells and medium stored at −70 °C until assayed for infectivity at the end of the experiment. Macrophages were cultured in RPMI 1640 medium plus 10% FCS in 15 mm diam. plastic well plates containing sterile glass coverslips. Cultures were infected by substituting growth medium with 1 ml of virus for 1 h at 37 °C, followed by washing, reculturing and harvesting of coverslips and infected medium as for lymphocyte cultures. In comparative experiments with different strains of virus, cultures were infected with theoretically identical multiplicities of infection as close to 1:0 as possible.

Techniques. Virus infectivity was assayed in BK cells in a microtitre system (Rossiter & Jessett, 1982). Samples were diluted in half-log increments and six-well replicates were used to assay each dilution. Virus antigens were detected in acetone-fixed cells by indirect immunofluorescence, using rabbit anti-RV serum and goat anti-rabbit immunoglobulin–fluorescein isothiocyanate conjugate. Cell morphology and virus cytopathic effects (c.p.e.) were detected in methanol-fixed cultures stained by the May–Grünwald–Giemsa technique. Significant differences were tested for in paired Student's t-tests.

RESULTS

The growth of virulent and avirulent RV in different lymphocyte cultures is shown in Fig. 1. After an eclipse phase of 18 to 24 h, new virus was produced rapidly in mitogenically stimulated cultures to a peak at about 72 h after infection. Infectivity of both RV strains was produced to highest titre in PWM-treated cultures. The virus grew to significantly (P < 0.01) higher titre in PWM- and Con A-treated cultures and apparently, but not significantly (P > 0.05), higher titre in PHA cultures in comparison with controls. There was no significant difference in the infectivity produced by the virulent or attenuated infected cultures, although the amount of infectivity retained at time 0 h in RVY-infected cultures was significantly (P < 0.01) higher than in TCRV-infected cultures.

Virulent RV infected a significantly (P < 0.01) higher proportion of cells than avirulent virus, as shown by the percentage of antigen-bearing cells (Fig. 1). The highest numbers of antigen-bearing cells were detected in mitogen-treated cultures between 48 and 96 h after infection, after which the proportion fell. The highest proportion, up to 23%, of antigen-positive cells was detected in virulent RV-infected Con A-treated cultures 48 h after infection. Only very low numbers of antigen-bearing cells were detected in avirulent RV-infected control lymphocytes.

Virus antigen was rarely seen in small lymphocytes and was restricted to medium and large lymphocytes and syncytia in later stages of culture. Antigen first appeared as discrete intracytoplasmic particles (Fig. 2a) which increased in number and size to fill the cytoplasm (Fig. 2b). During later stages of infection, intranuclear fluorescence was detected and large, strongly staining masses were evidently in the cytoplasm (Fig. 2c). Syncytia seen in cultures 3 to 4 days after infection always contained virus antigen (Fig. 2d). [³H]Thymidine incorporation indicated that the Con A-, PWM- and PHA-treated cultures were approximately 25, 6 and 2 times more mitotically active than untreated controls. Morphological studies, however, indicated that, although small lymphocytes constituted 95% of control cultures, between 30 and 70% of all mitogen-stimulated cultures were large lymphocytes or lymphoblasts at the time of infection.
Rinderpest virus in bovine leukocytes

The first evidence of c.p.e. was small multinucleate cells 1 day after infection of stimulated cultures. These increased in number and size, especially in PWM-treated cultures, being readily visible as syncytia with up to 10 nuclei. Intracytoplasmic inclusions were visible in most syncytia and occasionally in large lymphocytes. Healthy small lymphocytes were detected in cultures for up to 15 days after infection.

The growth of virulent and avirulent RV in T lymphocyte-enriched and -depleted subpopulations is shown in Table 1. Both viruses grew to higher titre and infected more cells in T-enriched than in the T-depleted cultures. Again, highest titres of infectivity and antigen-bearing cells were produced in PWM-treated cultures. Titres of virulent virus were generally higher than those of avirulent virus.

The growth of the three RV strains was compared in Con A-treated cultures, with the proportion of antigen-bearing cells being determined 3 days after infection (Table 2). The two virulent strains infected significantly ($P < 0.05$) more cells than the avirulent strain, and RVY consistently, but not significantly ($P > 0.05$), infected more cells than RBT/1.

The comparative growth of RVY, TCRV and PPRV in lymphocytes from different species is shown in Table 3. RVY grew most readily in bovine cells, whereas PPRV preferred ovine and caprine lymphocytes. The proportions of antigen-bearing cells gave a better indication of differential growth in cell culture than the levels of infectivity produced. There was no consistent difference in growth of RV or PPRV in lymphocytes from non-immune or recently recovered animals.

The comparative growth of three strains of RV in macrophages is shown in Table 2. In udder macrophages virulent RV grew more readily than avirulent virus, producing high titres of new infectivity within 3 days (Fig. 3), whereas similar levels were only reached after 6 days with avirulent virus. Virulent virus also infected more cells during the initial stages of culture and grew steadily to involve 100% of the monolayer after 7 days, whereas avirulent virus had involved only about 60% of the cell sheet by that time. The development of c.p.e. and virus antigens in macrophage cultures was essentially the same as that described for RV in other adherent cells (Liess & Plowright, 1963; Plowright, 1968).
Fig. 2. Development of rinderpest virus antigens detected by indirect immunofluorescence in concanavalin A-stimulated bovine lymphocyte cultures. (a) Discrete focus of cytoplasmic antigen in a medium-sized lymphocyte 12 h after infection. (b) Masses of virus antigen clearly visible in the cytoplasm and nucleus of large lymphocytes and lymphoblasts 36 h after infection. Uninfected small lymphocytes are also visible (arrow). (c) Two lymphoblasts 36 h after infection. Strongly staining masses of cytoplasmic antigen, probably inclusions (arrows) and intranuclear antigen (arrowhead) are visible. (d) Antigen throughout a syncytium 72 h after infection. Attached to the syncytium are a lymphoblast in the early stages of infection with cytoplasmic antigen (arrowhead) and two non-antigen-bearing smaller lymphocytes (arrows). All bar markers represent 10 µm.

Table 1. Growth of rinderpest virus in lymphocyte subpopulations*

<table>
<thead>
<tr>
<th>Cell population</th>
<th>Surface marker (°)</th>
<th>Avirulent</th>
<th>Virulent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PNA</td>
<td>sIg</td>
<td>0 h</td>
</tr>
<tr>
<td>T-depleted</td>
<td>30-40</td>
<td>60-70</td>
<td>3.0</td>
</tr>
<tr>
<td>T-depleted + Con A</td>
<td>3.9</td>
<td>4.0  (10)</td>
<td>3.7</td>
</tr>
<tr>
<td>T-depleted + PWM</td>
<td>3.3</td>
<td>4.1  (5-10)</td>
<td>3.3</td>
</tr>
<tr>
<td>T-enriched</td>
<td>90-95</td>
<td>1-5</td>
<td>2.8</td>
</tr>
<tr>
<td>T-enriched + Con A</td>
<td>3.4</td>
<td>4.0  (20)</td>
<td>3.1</td>
</tr>
<tr>
<td>T-enriched + PWM</td>
<td>3.4</td>
<td>5.1  (35)</td>
<td>3.6</td>
</tr>
</tbody>
</table>

* Abbreviations: Con A, concanavalin A; PWM, pokeweed mitogen; PNA, peanut agglutinin; sIg, surface immunoglobulin.
† Virus infectivity titre is expressed as TCID₅₀/ml/10⁶ cells. Figures in parentheses indicate percentage of antigen-bearing cells.
Table 2. *Proportions (\(\%\)) of virus antigen-bearing cells in bovine lymphocytes and macrophage cultures infected with three strains of rinderpest virus*

<table>
<thead>
<tr>
<th>Virus inoculum</th>
<th>Dose (TCID_{50})</th>
<th>Con A-treated lymphocytes</th>
<th>Udder macrophages</th>
</tr>
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<tbody>
<tr>
<td>TCRV</td>
<td>5.85 ± 0.26†</td>
<td>3 ± 1†</td>
<td>15 ± 4‡</td>
</tr>
<tr>
<td>RBT/1</td>
<td>5.78 ± 0.20</td>
<td>10 ± 6</td>
<td>37 ± 12</td>
</tr>
<tr>
<td>RVY</td>
<td>5.85 ± 0.32</td>
<td>14 ± 8</td>
<td>45 ± 13</td>
</tr>
</tbody>
</table>

* TCRV, Tissue culture rinderpest virus vaccine; RBT/1, rinderpest bovine Tanzania/1; RVY, rinderpest virus Yemen.
† Mean ± standard deviation of three separate experiments in each of which triplicates of 3-day cultures were examined.
‡ Mean ± standard deviation of two separate experiments.

Table 3. *Growth of rinderpest and peste des petits ruminants viruses in bovine, caprine and ovine lymphocytes*

<table>
<thead>
<tr>
<th>Species of lymphocyte</th>
<th>Immune status</th>
<th>RVY</th>
<th>TCRV</th>
<th>PPRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>Non-immune*</td>
<td>1.45 (11.5)</td>
<td>1.25 (3)</td>
<td>0.85 (2.0)</td>
</tr>
<tr>
<td></td>
<td>Immune*</td>
<td>1.85 (14.0)</td>
<td>1.15 (2.5)</td>
<td>0.75 (2.5)</td>
</tr>
<tr>
<td>Caprine</td>
<td>Non-immune†</td>
<td>1.22 (3.3)</td>
<td>1.15 (2.6)</td>
<td>1.12 (12.7)</td>
</tr>
<tr>
<td></td>
<td>Immune†</td>
<td>0.97 (4.3)</td>
<td>1.06 (3.0)</td>
<td>1.50 (10.8)</td>
</tr>
<tr>
<td>Ovine</td>
<td>Non-immune*</td>
<td>1.15 (4.5)</td>
<td>ND§</td>
<td>1.25 (8.5)</td>
</tr>
<tr>
<td></td>
<td>Immune*</td>
<td>1.08 (5.0)</td>
<td>ND</td>
<td>1.25 (8.5)</td>
</tr>
</tbody>
</table>

* Mean of two different animals.
† Mean of three different animals.
§ Increase in infectivity titre (TCID_{50}/ml) between 0 and 72 h after culture infection. Figures in parentheses are the percentage of antigen-bearing cells.
§ ND, Not done.

Fig. 3. Development of infectivity (a) and antigen-bearing cells (b) in bovine udder macrophages infected with virulent (●) and avirulent (○) rinderpest virus.
Measles virus has been shown to grow more readily in mitogen-stimulated than unstimulated lymphocytes (Joseph et al., 1975; Sullivan et al., 1975). The results reported here show that this is also true for RV, which infected more cells and grew to higher titre in mitogen-stimulated cells compared to control lymphocytes. Specific virus antigens were virtually always detected in large lymphoid cells, including the control cultures in which the low background level of lymphoblastoid transformation provided a small population of cells that accounted for the low degree of virus proliferation. Although PHA has proved to be a successful mitogen for assisting measles virus proliferation in lymphocytes (Sullivan et al., 1975; Zweiman et al., 1979), it was far less effective in this study than Con A or PWM. This may reflect the use of a suboptimal dose of PHA rather than selection of specific subpopulations of lymphocytes since the [3H]thymidine incorporation of PHA cultures in this study was only twice that of controls, despite morphological evidence of a high proportion of blast cells.

Since the virus never appeared to infect more than 30% of the cells in a culture, it might be suggested that RV only infects certain subpopulations of bovine lymphocytes, compared with measles virus, which infects B, T helper and T suppressor human lymphocytes (Joseph et al., 1975; Huddlestone et al., 1980). Certainly, RV grew better in T-enriched as opposed to T-depleted cultures despite the use of PWM, which probably stimulates B cells as well as T cells (Usinger et al., 1981). It is of interest to note that the addition of mitogens after infection appears to increase the percentage of cells showing virus antigen and this technique could be used to resolve whether RV grows in B cells (Huddlestone et al., 1980).

Highest virus infectivity titres were produced in PWM-stimulated cultures, whereas the highest proportions of cells bearing RV antigen were usually found in Con A-treated cultures, suggesting that the effects of these mitogens differ with respect to producing lymphoblasts suitable for virus growth.

The growth of RV in udder macrophages was similar to that seen with measles virus and canine distemper virus (CDV) in macrophage cultures (Poste, 1971; Appel, 1978) and RV in adherent cells derived from blood monocytes (Tokuda et al., 1962; Pigoury et al., 1967). In both lymphocytes and macrophages the dynamics of avirulent and virulent RV production were essentially the same as in BK cells (Plowright, 1964), an eclipse phase of less than 24 h being followed by rapid growth for 2 to 3 days, after which titres remained steady or declined slowly. Bovine macrophages produced a similar amount of RV as mitogen-stimulated lymphocytes, whereas with measles virus previous studies had shown that macrophages produced 10 to 20% less virus than lymphocytes.

A further interesting feature of this study was the difference in the degree of replication of the strains of RV. Although virulent RV only produced slightly higher titres of infectivity than the avirulent virus in stimulated lymphocytes, it consistently infected more than twice as many cells. A comparative trial using three strains of RV confirmed this and indicated a positive correlation between virulence and ability to infect and grow in lymphoblasts, and essentially the same relationship was seen with the growth of these strains in macrophages. An earlier report (Poste, 1971) described increased cytopathology in alveolar macrophages infected with virulent as opposed to avirulent CDV, although this effect was not seen in macrophages derived from three other tissues. More recently, Appel (1978) showed that attenuated CDV reverted to virulence following 10 passages in dog alveolar macrophages and concluded that the ability to infect macrophages might be associated with virulence, a phenomenon previously discussed for several viruses (Allison, 1974).

The results also show that virulent RV and virulent PPRV grow more readily in lymphocytes from species for which they are naturally attenuated, a feature which could be used to distinguish between these isolates and further supports the hypothesis of the ability to infect macrophages being associated with virulence. If this increased ability to infect and grow in lymphocytes and macrophages is involved in RV virulence, it might affect the host. Virulent strains would infect macrophages and lymphocytes more rapidly and in greater numbers than avirulent strains, thus swiftly producing high titres of virus which spreads via the blood to infect epithelial tissues in the alimentary and upper respiratory tracts. Avirulent strains including
attenuated vaccines, however, infect fewer lymphocytes and macrophages; viraemia develops later and to a lesser degree and is therefore less able to cause infection in epithelial tissues before the development of the protective immune response. The low levels of viraemia and swift onset of antibody production following inoculation of avirulent virus indicate that this may be so (Taylor & Plowright, 1965; Plowright, 1968).

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REFERENCES


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