Infectivity Difference Between the Two Phenotypes of Autographa californica Nuclear Polyhedrosis Virus: Importance of the 64K Envelope Glycoprotein

By B. ANDREW KEDDIE* AND LOY E. VOLKMAN
Department of Entomology and Parasitology, University of California, Berkeley, California 94720, U.S.A.

(Accepted 15 January 1985)

SUMMARY

Two phenotypes of Autographa californica nuclear polyhedrosis virus (AcNPV), occluded virus and budded virus (BV), are responsible for causing disease in Trichoplusia ni. Virus released from occlusion bodies by alkali (LOVAL) is more infectious in the gut than in the haemocoel whereas BV is more infectious in the haemocoel than in the gut. Reduction of infectivity of BV in the haemocoel by the monoclonal antibody AcV1 to a level comparable to LOVAL clearly implicates its target, a 64K phosphoglycoprotein abundant in the BV envelope but not detected in LOVAL, as being involved in BV's greater infectivity in that location. Homologous antiserum reduces the infectivity of LOVAL in the gut to that of LOVAL in the haemocoel, suggesting an analogous envelope component may account for its greater infectivity in the gut.

Two phenotypes of Autographa californica nuclear polyhedrosis virus (AcNPV) are involved in causing disease in susceptible lepidopteran hosts. One phenotype, occluded in a protein matrix forming a polyhedron, is released from the protein matrix upon contact with the midgut contents of a feeding larva following ingestion of polyhedra. This virion phenotype, artificially released from polyhedra in the laboratory by exposure to alkali, is described in this report as larvae-occluded virus, alkali liberated (LOVAL), and consists of single or multiple nucleocapsids within an envelope. Numerous studies indicate that this phenotype of AcNPV and other baculoviruses initiates infection specifically in columnar epithelial cells by fusion of the virion envelope and the microvillar membrane of these cells (Kawanishi et al., 1972; Tanada et al., 1975). Nucleocapsids pass through the cytoplasm to the nucleus where virus replication occurs. Some of the progeny nucleocapsids pass into the cytoplasm of these cells and are released by budding through the basement membrane into the haemocoel. The nucleocapsids bud singly rather than in groups and gain loose-fitting envelopes containing peplomers at the anterior termini (Adams et al., 1977; Granados & Lawler, 1981). This virion phenotype, described here as budded virus (BV), invades the insect systemically and infects numerous tissues, although the mechanism of entry into these tissues has not been clearly established. Several cell types have been shown to produce BV both in vivo and in vitro (Summers & Volkman, 1976; Hess & Falcon, 1977; Adams et al., 1977). Later in infection, cells (other than midgut cells) switch from BV to polyhedra production. Nucleocapsids that remain in the nucleus become enveloped either singly or in groups and are subsequently occluded within a protein matrix to form polyhedra (Volkman et al., 1976). Eventually the polyhedra are released by cytolysis, and after insect death contaminate the substrate which may be ingested by another susceptible insect. For detailed descriptions recent reviews are available (Granados, 1980; Faulkner, 1981; Tanada & Hess, 1984).

The two phenotypes (LOVAL, BV) which have different roles in infection have been distinguished on the basis of morphology, infectivity (Dougherty et al., 1975; Summers &

In this paper we report on the effect on infectivity of LOVAL and BV in vivo (assayed both per os and intrahaemocoelically) of homologous and heterologous antisera as well as a monoclonal antibody, AcV₁, previously demonstrated to have neutralizing activity against AcNPV BV in vitro (Hohmann & Faulkner, 1983). Homologous antisera neutralized each phenotype in its normal location (LOVAL in the gut, BV in the haemocoel) in a host insect. The monoclonal antibody AcV₁ neutralized BV in the haemocoel reducing its infectivity in that location to a level comparable to LOVAL. Since the target antigen of AcV₁ is known to be a 64K phosphoglycoprotein present in the BV envelope but not in the envelope of LOVAL (Volkman et al., 1984; Volkman & Goldsmith, 1984), these results suggest that the 64K protein is an important factor in BV's greater infectivity in the haemocoel.

Insects (Trichoplusia ni) obtained from L. E. Caltagirone (University of California, Berkeley) and Chevron Chemical Co. (Ortho Division, Richmond, Ca., U.S.A.) were used to establish a colony. They were reared for 25 generations in our facilities without apparent endogenous viral infections. A common RNA virus contaminant of these insects was not detected using ELISA (Morris et al., 1981). During the course of this study two diet formulations were utilized. Generations 1 to 10 were fed a diet adapted by replacing cotton-leaf meal with alfalfa leaf meal and adding linseed oil (Ignoffo, 1963); generations 11 to 25 were fed a diet modified by replacing formaldehyde and methyl-p-hydroxybenzoate with mycostatin (0.08 g/100 ml diet) and aureomycin by tetracycline (0.02 g/100 ml diet) (Tanada & Chang, 1968).

The E2 variant of AcNPV was originally supplied by M. D. Summers (Texas A & M University, College Station, Tx., U.S.A.) as culture-derived polyhedra (Smith & Summers, 1978). These polyhedra were used to infect laboratory-reared Spodoptera exigua larvae. Polyhedra from these larvae were the source of LOVAL. Virus was released and gradient-purified as described previously (Volkman et al., 1976).

Infectious haemolymph from AcNPV-infected T. ni larvae was used to inoculate IPLB-SF-21 cells (Vaughn et al., 1977). After the second passage BV was collected from the culture medium and purified as described elsewhere (Volkman & Goldsmith, 1983).

Virion concentrations were determined using optical density readings (Beckman DB Spectrophotometer) with a predetermined extinction coefficient of 10. Both LOVAL and BV were diluted in BML/TC10 (Gardiner & Stockdale, 1975) and aliquots stored at −70 °C in 1 ml capped polystyrene tubes.

Two hybridoma clones, designated AcV₁ and AcV₄, were obtained from P. Faulkner (Queen's University, Kingston, Ontario, Canada) (Hohmann & Faulkner, 1983). These clones were used to generate monoclonal antibodies which were prepared from culture supernatant fluid. Aliquots were stored at −20 °C. Antiserum to BV and LOVAL were made in New Zealand white rabbits as previously described (Volkman, 1983). These antisera, designated α-BV and α-LOVAL, were stored at −20 °C.

The infectivities of both virion phenotypes were determined by intrahaemocoelic injection and peroral inoculation. Early to mid-fourth instar T. ni larvae were selected for all experiments. Larvae were anaesthetized by exposure to a CO₂-enriched atmosphere for 1 to 2 min. For intrahaemocoelic injections 5 μl of test mixture was injected through the planta of the first left abdominal proleg using a 33-gauge needle (Hamilton, 1/2 inch, point 4) fitted to a 1 cm³ tuberculin syringe (Becton-Dickinson, Yale glass Luer tip, Blue Plunger). The peroral inoculation procedure was similar to the intrahaemocoelic injection except that the needles were 33-gauge blunt tips (Hamilton, 1/2 inch, point 3, electro-tapered and polished). The needle was inserted through the mouth as far as the foregut–midgut region which can be discerned through the dorsal integument of the fourth instar larvae when the needle is inserted.

Multiple controls were used initially for both routes of injection. Positive controls consisted of larvae injected with (i) virus and preimmune rabbit serum, and (ii) virus and BML/TC10. Negative controls consisted of larvae which received (i) no treatment, (ii) CO₂ anaesthetic only, (iii) preimmune serum, and (iv) antibody preparations.
Table 1. Infectivity of virion phenotypes

<table>
<thead>
<tr>
<th>Virion phenotype</th>
<th>Location</th>
<th>$LD_{50}$ (pg/insect)</th>
<th>95% Fiducial limits</th>
<th>Standard error</th>
<th>Degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOVAL</td>
<td>Per os</td>
<td>1.5</td>
<td>0.76-2.7</td>
<td>0.06</td>
<td>8.7</td>
</tr>
<tr>
<td>Haemocoel</td>
<td></td>
<td>17.7</td>
<td>6.9-45</td>
<td>0.14</td>
<td>12.9</td>
</tr>
<tr>
<td>BV</td>
<td>Per os</td>
<td>$3.7 \times 10^{-4}$</td>
<td>2.0-7.8</td>
<td>0.14</td>
<td>1.9</td>
</tr>
<tr>
<td>Haemocoel</td>
<td></td>
<td>$6.5 \times 10^{-2}$</td>
<td>4.5-9.3 $\times 10^{-2}$</td>
<td>0.15</td>
<td>20.5</td>
</tr>
</tbody>
</table>

Twelve to 24 insects were used for each dilution (four to seven dilutions per experiment) with experiments replicated a minimum of three times. The total number of insects used was in excess of 6000. After injection larvae were placed individually in numbered, 1 oz plastic cups filled with sufficient diet to complete growth. Test insects were reared in an incubator at 23 to 25 °C in constant darkness except for the daily examination period. Insects that died in the 24 h post-treatment period were excluded from the sample since they were judged to have succumbed to injection trauma.

For neutralization studies, antibody preparations were initially diluted 25:1 with BML/TC10 and filtered (0.45 μm Acrodisc). Virus samples were diluted with prefiltered BML/TC10. Virus dilutions were either 5:1 or 10:1 with four to seven in a series. Equal volumes of the diluted virus suspension and the diluted antibodies were mixed (Vortex-Genie Mixer); thus the overall antibody dilution was always 50:1. The virus–antibody mixtures and controls were incubated at 37 °C for 1 h in 1 ml capped polystyrene tubes, then injected either per os or into the haemocoel of fourth instar T. ni larvae as previously described.

The assays were evaluated by probit analysis using a computer program (Robertson et al., 1980). This program generated $LD_{50}$ values in terms of pg virus per insect. The neutralizing effect of an antibody was determined by computing a ratio between the $LD_{50}$ of a virus dilution series incubated with a test antibody and the $LD_{50}$ of the same virus dilution series incubated with a control serum or diluent. The ratio was converted to a log$_{10}$ value and referred to as the neutralization index (NI) log$_{10}$, e.g. haemocoel ($LD_{50}$BV AcV$_1$/$LD_{50}$ BV) = ($1.6 \times 10^{1}$/ $6.5 \times 10^{-2}$) = 246 NI; log$_{10}$246 = 2.4.

The results of infectivity studies found in Table 1 demonstrate that each viral phenotype is more infectious in that location in which it normally occurs within an insect: LOVAL in the gut and BV in the haemocoel. Overall BV is the more virulent, requiring only $6.5 \times 10^{-2}$ pg per $LD_{50}$ in the haemocoel. However, BV is $10^4$-fold less infectious in the gut than in the haemocoel. In comparison LOVAL, although more infectious in the gut than BV, is only 11-fold less infectious in the haemocoel than in the gut. Thus not only is there a difference of infectivity between the two phenotypes, but switching locations within the insect has considerably different effects on their respective infectivities. These results agree substantially with the results of a previously published study in the same system (Volkman & Summers, 1977) and with those of two other studies in different insect–NPV systems (Kawarabata & Aratake, 1978; Stairs, 1980).

Neutralization experiments with constant antibody concentrations and serial dilutions of virus have the advantage of allowing comparisons of the relative activities and specificities of antibodies tested against homologous and heterologous viruses (Martignoni et al., 1980). In this study the technique was applied to two phenotypes (i.e. LOVAL, BV) of the same virus using heterologous and homologous antisera as well as two monoclonal antibodies, and assayed by injection in two locations within the insects. Of the results listed in Table 2, only three are considered significant (NI log$_{10}$ ≥ 1.7) (Casals, 1967).

In the first analysis of the three significant treatments listed in Table 2, one can say that LOVAL antiserum neutralizes LOVAL, and BV antiserum and the antibody AcV$_1$ neutralize BV, demonstrating that these antibodies react with homologous virions (antigens) and interfere with their infectivity. Significantly, the neutralization is location-specific, i.e. LOVAL antiserum neutralizes LOVAL in the gut only while BV antiserum and AcV$_1$ act against BV in
### Table 2. Neutralization

<table>
<thead>
<tr>
<th>Virion phenotype</th>
<th>Location</th>
<th>Antibody</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (pg/insect)</th>
<th>95% Fiducial limits</th>
<th>Slope</th>
<th>Standard error</th>
<th>χ²</th>
<th>Degrees of freedom</th>
<th>NI log&lt;sub&gt;10&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOVAL Per os</td>
<td>α-LOVAL</td>
<td>68.9</td>
<td>34</td>
<td>144</td>
<td>0.8</td>
<td>0.08</td>
<td>18</td>
<td>10</td>
<td>1.7*</td>
</tr>
<tr>
<td></td>
<td>α-BV</td>
<td>1.0</td>
<td>0.3</td>
<td>2.1</td>
<td>0.7</td>
<td>0.10</td>
<td>1.8</td>
<td>4</td>
<td>-0.2</td>
</tr>
<tr>
<td></td>
<td>AcV₁</td>
<td>1.2</td>
<td>0.3</td>
<td>3.4</td>
<td>0.8</td>
<td>0.17</td>
<td>1.0</td>
<td>2</td>
<td>-0.1</td>
</tr>
<tr>
<td></td>
<td>AcV₂</td>
<td>2.5</td>
<td>1.0</td>
<td>6.4</td>
<td>0.9</td>
<td>0.19</td>
<td>1.8</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Haemocoeil</td>
<td>α-LOVAL</td>
<td>10.2</td>
<td>4.8</td>
<td>21</td>
<td>1.0</td>
<td>0.16</td>
<td>7.9</td>
<td>10</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>α-BV</td>
<td>5.1</td>
<td>1.9</td>
<td>13</td>
<td>1.7</td>
<td>0.17</td>
<td>0.7</td>
<td>2</td>
<td>-0.5</td>
</tr>
<tr>
<td></td>
<td>AcV₁</td>
<td>42.8</td>
<td>22</td>
<td>83</td>
<td>1.0</td>
<td>0.15</td>
<td>0.7</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>AcV₂</td>
<td>19.9</td>
<td>11</td>
<td>36</td>
<td>1.5</td>
<td>0.13</td>
<td>3.9</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>BV Per os</td>
<td>α-LOVAL</td>
<td>2.4 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>1.1 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>6.2 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.7</td>
<td>0.14</td>
<td>10.6</td>
<td>7</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>α-BV</td>
<td>1.1 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.7 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.0 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.2</td>
<td>0.19</td>
<td>0.8</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>AcV₁</td>
<td>2.3 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.2 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4.8 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.1</td>
<td>0.18</td>
<td>9.4</td>
<td>8</td>
<td>-0.2</td>
</tr>
<tr>
<td></td>
<td>AcV₂</td>
<td>4.4 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.4 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>9.5 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.4</td>
<td>0.19</td>
<td>2.2</td>
<td>4</td>
<td>-0.9</td>
</tr>
<tr>
<td>Haemocoeil</td>
<td>α-LOVAL</td>
<td>9.4 x 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>4.6 x 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>1.9 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1.8</td>
<td>0.16</td>
<td>1.4</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>α-BV</td>
<td>1.1 x 10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.5 x 10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>8.5 x 10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.0</td>
<td>0.15</td>
<td>4.1</td>
<td>3</td>
<td>3.2*</td>
</tr>
<tr>
<td></td>
<td>AcV₁</td>
<td>1.6 x 10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.4 x 10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.4 x 10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.6</td>
<td>0.16</td>
<td>1.2</td>
<td>2</td>
<td>2.4*</td>
</tr>
<tr>
<td></td>
<td>AcV₂</td>
<td>5.3 x 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>2.6 x 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>1.1 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1.1</td>
<td>0.16</td>
<td>4.4</td>
<td>5</td>
<td>-0.1</td>
</tr>
</tbody>
</table>

*Significant.
the haemocoel. Since conditions in the gut and haemocoel are quite different, especially with respect to pH and enzymes, the observation that neutralization occurs in both locations suggests that the lack of neutralization in either location is not the result of simple degradation or uncoupling of the antigen–antibody complex. The observation that AcV₁ demonstrates neutralizing activity against BV in the haemocoel is significant because the target antigen has been identified and characterized (Volkman et al., 1984; Volkman & Goldsmith, 1984). The AcV₁-reactive antigen is an exposed envelope 64K phosphoglycoprotein with an isoelectric point of 3.15. This protein is abundant in BV envelopes but has not been detected in LOVAL.

The infectivity of BV treated with AcV₁ was reduced approximately 240-fold in the haemocoel but remaining infectivity was nearly equivalent to that of LOVAL in the haemocoel. This result is consistent with a hypothesis that the difference of infectivity between BV and LOVAL in the haemocoel is influenced by the presence of the phosphoglycoprotein found in the envelope of BV but not in the envelope of LOVAL.

Similarly, an envelope component of LOVAL recognized by anti-LOVAL in this phenotype but not in BV, as indicated by neutralization, may be involved in the infectivity of LOVAL in the gut. The infectivity of neutralized LOVAL in the gut was similar to that of treated or untreated LOVAL in the haemocoel. These two results suggest that an envelope component of LOVAL may account for its greater infectivity in the gut compared to BV in that location.

This research was supported by U.S. Department of Agriculture Competitive Research Grant 83-CRCR-1-1244.

REFERENCES


(Received 18 September 1984)