Rapid Baculovirus Detection, Identification, and Serological Classification by Western Blotting-ELISA using a Monoclonal Antibody

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SUMMARY
A hybridoma cell line, called 3D10, has been established which secretes monoclonal antibodies against the 42K protein of Autographa californica nuclear polyhedrosis virus (Ac NPV). Following separation of a complex protein mixture by denaturating SDS-polyacrylamide gel electrophoresis and electrophoretic protein transfer to a nitrocellulose filter, this monoclonal antibody is still able to react with its antigen. The antigen-antibody complex can be stained by indirect enzyme-linked immunosorbent assay (ELISA) using horseradish peroxidase-conjugated rabbit anti-mouse serum. The Western blot-ELISA described is extremely sensitive, being able to detect as little as 10 ng of Ac NPV or $3 \times 10^5$ polyhedral inclusion bodies. Using this method it is possible to find traces of Ac NPV in very crude materials, e.g. impure polyhedra, infectious haemolymph, and larval homogenates. On the other hand, this monoclonal antibody is very specific and reacts only with isolates of Ac NPV and Galleria mellonella NPV. No reactions were found against eleven other NPV isolates. The implications of these findings for baculovirus identification and classification are discussed.

INTRODUCTION
Members of the family Baculoviridae (Matthews, 1979), are being considered as possible microbial insecticides (Summers et al., 1975; Miltenburger, 1978; Tinsley, 1979). Although several baculovirus preparations are already used in integrated pest control there is still a need for considerably more information on their host specificity, safety, and classification. The current nomenclature of baculoviruses (BV) is based on the original insect host's name. Some BV are not species-specific in their host range and therefore it is difficult to estimate the number of different isolates that have been described. Three main methods have been used to characterize BV-isolates. The analysis of viral DNA by restriction enzyme mapping and the investigation of viral protein patterns by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Miller & Dawes, 1978; Smith & Summers, 1978; Summers & Smith, 1978) have usually produced more satisfactory results than immunological methods. The latter require virus preparations which are completely free of inclusion body protein (IBP) (Harrap et al., 1977; McCarthy & Lambiase, 1979) because the IBP in particular may cause serological cross-reactions (Smith & Summers, 1981).

Recent advances in immunology, particularly the development of techniques for the preparation of monoclonal antibodies (Köhler & Milstein, 1975), have increased the possibility of using better defined serological tests. Monoclonal antibodies have already proved useful in animal virus identification (Koprowski et al., 1977, 1978; Wiktor et al., 1980). The application of the Western blot radioimmunoassay (RIA) (Bowen et al., 1980; Renart et al., 1979; Towbin et al., 1979) by Smith & Summers (1981) has highlighted the potential use of such antibaculovirus immunological reagents.

The hybridoma cell line, 3D10 (Roberts & Naser, 1982a, b) secretes monoclonal antibodies against the 42 kdal (42K) protein of Autographa californica nuclear polyhedrosis virus (Ac NPV). This is supposedly a major capsid protein (Smith & Summers, 1981; Summers & Smith, 1978).
This antibody reacts in SDS-PAGE/Western blot-ELISA. We report on the benefits of this method for detecting traces of Ac NPV in crude materials and, because of its specificity, for rapid preliminary BV classification.

METHODS

Monoclonal antibody. The establishment and the basic properties of the hybridoma cell line 3D10 are described in separate paper (Roberts & Naser, 1982a, b). In the following experiments, ascites fluids only were used as a source of monoclonal antibodies.

Virus samples. Polyhedral inclusion bodies (PIBs) of Ac NPV were originally donated by Dr A. Röder, Hoechst AG, Frankfurt, F.R.G. From the same source we also obtained the following preparations: Mamestra brassicae (Mb) NPV, Spodoptera littoralis (Sl) NPV, Lymantria dispar (Ld) NPV, Boarmia bistorta (Bb) NPV and Lymantria monacha (Lm) NPV.

As unknown samples, we received preparations of the following species from Dr J. Huber, Biologische Bundesanstalt Darmstadt, F.R.G.: various samples of Ac NPV, Sl NPV, Galleria mellonella (Gm) NPV, Ld NPV, Malacosoma neustria (Mn) NPV, Plusia acuta (Pa) NPV, Heliothis armigera (Ha) NPV, Heliophilis saxa (Hz) NPV, and Laspeyresia pomonella granulosis virus (Lp GV).

From Hoechst AG we also obtained a sample which was thought to contain Hz NPV (referred to as 'Hz' NPV), because the virus was propagated in Heliothis virescens inapparently infected with Ac NPV. These larvae had been inoculated with Hz NPV.

Polyhedra-like inclusion bodies were isolated from a moribund larva of Gastropacha quercifolia (Gq). This sample was also investigated.

Western blot-ELISA

SDS-PAGE. Protein mixtures were separated by SDS-PAGE in a slab gel apparatus (dimensions 10 x 14 x 0.15 cm) using a 3% stacking gel and an 11% separating gel (Laemmli, 1970; Maizel, 1971).

Blotting procedure. The electrophoretic transfer of proteins onto nitrocellulose filters (0.45 μm; Schleicher & Schüll) was performed overnight, essentially as described by Towbin et al. (1979), using a home-made blotting box and a battery charger as power source.

Immunological detection of the 3D10 antigen. The remaining protein-binding sides on the nitrocellulose filters were blocked by incubating the filters in 3% casein (Merck, Darmstadt, F.R.G.) in phosphate-buffered saline pH 7.4 (PBS) for 45 min at room temperature on a rotary shaker. (Casein gave very satisfactory results and is much cheaper than the bovine serum albumin normally used.) The filters were then washed three times in PBS, incubated with a 1:10000 dilution of ascites fluid from hybridoma 3D10 in PBS for 2 h at room temperature, and subsequently washed as above. The peroxidase-conjugated transfer of proteins onto nitrocellulose filters (0-45 μm; Schleicher & Schüll) was performed overnight, essentially as described by Towbin et al. (1979), using a home-made blotting box and a battery charger as power source.

Preparation of antigen samples. Virus and polyhedra were purified according to Maskos & Miltenburger (1981). Infectious haemolymph was obtained by bleeding fifth-instar Mb larvae which had been infected as fourth-instar with 5 x 107 PIBs. Larval homogenates were made by rapidly freeze-thawing one middle larval segment and washing the crude suspension with PBS. The final pellet, a very crude polyhedra preparation, was then dissolved directly in 200 μl sample buffer (5% 2-mercaptoethanol, 2% SDS, pH 6-8) and 20 μl samples were electrophoresed. To obtain extracts from infected cells, two bottles of half-confluent Mb 0503 cells (Miltenburger et al., 1977) were infected with tissue culture supernatant Ac NPV at an m.o.i. of approx. 1. Two days after infection the cells were harvested, washed twice with PBS, and the final cell pellet was dissolved directly in 500 μl sample buffer.

RESULTS

Optimal dilution of 3D10 ascites fluid

In preliminary experiments 1 μg purified virus was directly bound to nitrocellulose filters and 3D10 was applied in log dilutions ranging from 10⁻² to 10⁻⁸. The 10⁻² to 10⁻⁴ dilutions produced similar results, whereas the 10⁻⁵ dilution gave a slightly less intense reaction. With 10⁻⁶ diluted ascites fluid there was still a slight reaction but with the more dilute antibody solutions no reaction was observable. To be on the safe side, 3D10 was used as a 1 in 10000 dilution for the subsequent assays.
Fig. 1. Determination of the lowest amount of purified Ac NPV (purified virus particles), which can be detected using 3D10 in Western blot-ELISA. As in all the subsequent figures, the samples were prepared in one Eppendorf cup and split into two equal volumes which were then run on two gels in parallel. One gel (a) was stained with Coomassie Brilliant Blue whereas the other (b) shows the result obtained with the Western blot-ELISA on the nitrocellulose filter. The samples analysed were the following. Mol. wt. standard (lane 1): bovine serum albumin, 67000; egg albumin, 45000; chymotrypsinogen, 25000; lanes 2 to 8, Ac virus in the quantities of 3.3 µg, 1 µg, 0.33 µg, 0.1 µg, 33 ng, 10 ng, and 3.3 ng.

Fig. 2. Ac NPV PIBs were directly solubilized in sample buffer and the sensitivity of the Western blot-ELISA using 3D10 was determined with this source of antigen. The samples were 0-1 µg of purified Ac NPV (lane 1) and the following amounts of Ac NPV PIBs: $10^7$ (lane 2); $3.3 \times 10^6$ (lane 3); $10^6$ (lane 4); $3 \times 10^5$ (lane 5); $10^5$ (lane 6); and $3 \times 10^4$ (lane 7).
Fig. 3. Analysis of 18 NPV preparations. Approximately 10⁷ PIBs of the investigated samples (lanes 2 to 19) were directly solubilized in sample buffer and the soluble fractions electrophoresed. Lane 1 contains mol. wt. markers (see Fig. 1); lane 20 contains 2 μg of Ac NPV (purified virus particles). (The full names of the BV samples are given in Methods.)

Sensitivity of the Western blot–ELISA with 3D10

Ac virus was electrophoresed in quantities ranging from 3-3 μg per slot to 3-3 ng per slot (Fig. 1a, b). As can be seen from Fig. 1 (b), the Western blot–ELISA using the monoclonal antibody 3D10 is extremely sensitive. This antibody still produced a reaction when only 10 ng of Ac virus was electrophoresed. This reaction was not very strong, but was clearly visible on the wet nitrocellulose filter. A 33 ng amount of purified Ac NPV was easily detected in this test after drying the filter (Fig. 1). As the 42K protein is probably less than 10% of total Ac NPV protein,
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Fig. 4. Comparison of a sample falsely named Hz NPV with samples of Ac NPV and control Hz NPV. Approximately 10^7 PIBs of each sample were used in this experiment. Arrows on the left side indicate co-migrating bands of Hz and Ac NPVs, whereas the arrows on the right side indicate differences. The overstaining between the 13K and 20K positions in lanes 7 and 8 does not interfere with the effect to be demonstrated.

the test system might detect 3 ng of the 42K protein. When Ac PIBs were solubilized directly in sample buffer and subjected to electrophoresis, it was possible to detect as few as 3 \times 10^5 PIBs (Fig. 2a, b). The blot in Fig. 1(b) shows slight cross-reactions with different Ac NPV proteins. By comparing these bands with the stained protein bands in Fig. 1(a) it can be concluded that 3D10 reacts with a protein of mol. wt. 42000. This is in agreement with results obtained by radioimmunoprecipitation (Roberts & Naser, 1982b).

Potential use of 3D10 in BV identification and classification

Polyhedra as antigen

The results of a representative experiment are presented in Fig. 3(a, b). 3D10 reacted with all the Ac NPV preparations which had been obtained from different sources. There is also a reaction with Gm NPV but none at all with more than ten other BV samples which had been isolated from a broad range of hosts. Even the polyhedra derived from Spodoptera littoralis, a noctuid species closely related to Autographa californica, gave no reaction. The great potential of this assay can be seen from Fig. 3 and 4. A preparation allegedly containing Hz NPV was positive in the Western blot-ELISA (Fig. 3). However, since Heliothis zea and Autographa californica, which are both noctuids, are not closely related, we further investigated both NPV species, which should not be related either (Smith & Summers, 1978). Indeed, when the false Hz ('Hz' in Fig. 3 and 4) was compared with Hz NPV from a different source the latter showed no reaction (Fig. 4b). In addition, the Coomassie Brilliant Blue-stained tracks as seen in Fig. 3(a) and 4(a) show there was a great similarity between the 'Hz' NPV and Ac NPV preparations.
Fig. 5. Detection of Ac NPV in dead Mb caterpillars. Larval homogenate was dissolved in 200 μl sample buffer in the following amounts: 1 mg (lanes 1 and 5); 250 μg (lanes 2 and 6) 60 μg (lanes 3 and 7) and 15 μg (lanes 4 and 8). A 20 μl amount of each sample was investigated. 'Ac' stands for homogenate derived from a Mb larva which had died from Ac NPV, whereas 'Mb' samples were derived from a Mb larva which had been infected by Mb NPV.

Detection of Ac NPV in crude materials

Infectious haemolymph

Ac NPV is not a major component of infectious haemolymph (see below) taken from Mb larvae 5 days after infection with 10⁷ Ac NPV PIBs. However, as can be calculated, for example from the results obtained with Ac NPV PIBs, the amount of Ac NPV contained in 10 μl infectious haemolymph having an m.o.i. of approx. 10⁹ per ml is at the borderline of sensitivity. Thus the visible band can not be really demonstrated on a photograph. However, it should be stated that there was no cross-reaction at all against any other protein present in the haemolymph of Mb larvae.

Dead caterpillars

The very crude material isolated from dead Mb larvae proved to be a good source of antigen. As shown in Fig. 5(a, b), Ac NPV could easily be detected in homogenates of Ac NPV-infected dead caterpillars, whereas no reaction was found with homogenates of Mb NPV-infected larvae. About 1 mg of a deceased larva seems to provide enough antigen for the unequivocal demonstration that Ac NPV is the infectious agent.

Infected tissue culture cells

Fig. 6(a, b) clearly shows that only in Ac NPV-infected cell cultures was there a protein present which reacts in Western blot–ELISA with the monoclonal antibody 3D10. There is no reaction with the 42K protein of Lp GV which obviously does not share the antigenic determinant recognized in infected Mb cell extracts, even though it has the same apparent molecular weight.
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Fig. 6. Detection of Ac NPV in homogenates of the Mb 0503 cell line. A 10 μl amount of each homogenate was analysed. Lanes 1 and 6 contain mol. wt. markers; lane 2, 25 μg Lp GV; lane 3, homogenate of Ac NPV-infected Mb cells; lane 4, homogenate of non-infected Mb cells and lane 5, 1 μg Ac NPV (purified virus particles).

DISCUSSION

Unknown BV isolates are normally identified by a comparison of polyhedral (Maskos & Miltenburger, 1981) or viral (Summers & Smith, 1978; Harrap et al., 1977) protein patterns by PAGE, or by comparing restriction enzyme profiles (Miller & Dawes, 1978; Smith & Summers, 1978), with those from more thoroughly investigated virus species. In other virus systems serological classification is quite common (Altstein & Zhdanov, 1979). With baculoviruses however, difficulties have arisen using immunological methods since the IBPs of all BV seem to cross-react (Smith & Summers, 1981). We can provide some new information concerning this problem. We have monoclonal antibodies, originally directed against Ac NPV polyhedrin, which react with all IBPs tested so far in the indirect ELISA (Roberts & Naser, 1982a, b). The fact that all IBPs contain some similar antigenic determinants, and the difficulty of obtaining virus preparations absolutely free of these proteins (Wood, 1980) might explain some of the problems that investigators have encountered in this field so far. Recently, it has been shown that it is possible to overcome these problems by applying the indirect and the double antibody sandwich ELISA (Langridge et al., 1981; Crook & Payne, 1980).

Smith & Summers (1981) demonstrated the great potential of another new technique, the Western blot–RIA, in baculovirus identification. Using this method they have been able to identify the cross-reacting proteins and have suggested that the main reason for cross-reactions could be conserved antigenic determinants of the major BV capsid proteins. We have combined the Western blot–ELISA with another modern tool in immunology, monoclonal antibodies (Köhler & Milstein, 1975). As can be seen, this combination offers a very sensitive system for the detection of Ac NPV. It can detect 10 ng of purified virus and can identify the presence of the 42K protein in as few as $3 \times 10^3$ polyhedra. This sensitivity is associated with a high degree of specificity. Neither proteins from the haemolymph of NPV-infected Mb caterpillars (not shown), nor the proteins from crude polyhedra preparations (Fig. 3 and 4) derived from Ac NPV
and other BV isolates, nor any protein of the permanent Mb 0503 cell line (Fig. 6) gave significant cross-reactions. Even in crude homogenates of diseased larvae it is possible to demonstrate the presence of the 3D10-specific antigen without ambiguous cross-reactions (Fig. 5).

The monoclonal antibody 3D10 is thus very specific, and reacts exclusively with preparations of Ac NPV, independent of their source, and with Gm NPV. Gm NPV is known to be closely related to Ac NPV (Smith & Summers, 1979) and it is therefore no surprise to find that 3D10 reacts with it. On the other hand, there is no cross-reaction with eleven other BV isolates obtained from a wide range of hosts (Fig. 3a, b). Reproducibly, however, we found cross-reactions with several bands in our Ac NPV preparations when analysing more than 1 μg of purified virus particles (Fig. 1b). There is one stronger reaction with a protein of mol. wt. 85 000, which might be a dimer of the 42K protein, and there are several weak reactions. It is very unlikely that there are several proteins sharing the 3D10 epitope. There is no cross-reaction in any of the other Ac NPV-containing preparations (Fig. 2 to 6). We therefore conclude that our test system demonstrates the degradation of the 42K protein by the alkaline protease which is present in Ac PIBs derived from insects (Wood, 1980).

The great potential of this test system, using the monoclonal antibody 3D10, is apparent from Fig. 3 and 4. A preparation falsely thought to be Hz NPV could be identified as Ac NPV. As can be seen from Fig. 4(a), 9 out of 11 major protein bands migrate identically on SDS–PAGE. It has already been shown by Maskos & Miltenburger (1981) that there are differences in the protein patterns of Ac NPV PIB preparations obtained from different sources.

It will be interesting to test Trichoplusia ni NPV and Rachiplusia ou NPV in our new system since they are thought to be closely related to Ac NPV (Smith & Summers, 1978, 1979).

The described Western blot-ELISA with the monoclonal antibody 3D10 gives positive and unambiguous results over a broad range of antigen concentrations and it does not require pure antigen preparations. It is also a sensitive, easy to apply, and fast, technique, which does not require radioisotopes. We think, therefore, that the method described for virus detection and classification is likely to be useful not only in the field of BV, but also for detection and diagnosis of other viruses.

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REFERENCES


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