Acta Bot. Neerl. 40(1), March 1991, p. 41–52

The use of immunochemical techniques and monoclonal antibodies to study the viral coat protein structure of potato virus A, potato virus Y and beet necrotic yellow vein virus

P. M. BOONEKAMP[1], H. POMP, G. C. GUSSENHOVEN[2] and A. SCHOTS

Laboratory for Monoclonal Antibodies, Wageningen, The Netherlands, [1]Bulb Research Centre, Lisse, The Netherlands, [2]Research Institute for Plant Protection, Wageningen, The Netherlands

SUMMARY

The production and characterization of monoclonal antibodies (mAbs) to the potato viruses Y and A (PVY and PVA, potyviruses), and to beet necrotic yellow vein virus (BNYVV, a furovirus) are presented. These examples illustrate the value but also some pitfalls of the use of mAbs in virological research. The pitfalls are clearly shown by the studies on PVA and BNYVV. If an incorrect selection procedure was used, mAbs were selected, which reacted only with an epitope of PVA introduced during purification of the virus and which was not present on the virion *in situ*. Monoclonal antibodies were selected against continuous and discontinuous epitopes to BNYVV. The discontinuous epitopes were easily destroyed by harsh assay conditions. The mAbs to PVY could be used to locate distinct epitopes on the coat protein of the virus. All mAbs proved very helpful in revealing changes in viral epitopes either during purification or after transfer to various host plants.

Key-words: beet necrotic yellow vein virus, detection, monoclonal antibodies, potato virus A, potato virus Y, viral epitopes.

INTRODUCTION

Viruses are a major threat to agricultural crops. For many years, seed material from vegetatively produced crops are strictly inspected for the absence of viruses. Since the development in the early eighties of the ELISA technique with polyclonal antibodies (pAbs), the number of samples annually assayed by the Dutch inspection services has been increased to more than six million. Although the first monoclonal antibodies (mAbs) to plant viruses were produced 9 years ago and although it took only 5 years to produce

Correspondence: P.M. Boonekamp, Bulb Research Centre, P.O. Box 85, 2160 AB, Lisse, The Netherlands.
Abbreviations: BNYVV = beet necrotic yellow vein virus; ELISA = enzyme-linked immunosorbent assay; (I)ACP-ELISA = (indirect) antigen coated plate-ELISA; (I)DAS-ELISA = (indirect) double antibody sandwich-ELISA: IB = immunoblotting; IEM = immuno-electronmicroscopy; mAbs = monoclonal antibodies; pAbs = polyclonal antibodies; PBS = phosphate buffered saline; PVA = potato virus A; PVY = potato virus Y; RAM-AP = rabbit anti-mouse immunoglobulins conjugated to alkaline phosphatase; SDS-PAGE = sodiumdodecylsulphate-polyacrylamide gel electrophoresis.

mAbs to representatives of most virus groups (Halk & De Boer 1985), no mAbs are used yet for routine virus detection in The Netherlands. The reason is that pAbs still perform very well. They react with many viral epitopes and contain heterologous populations of antibodies that are not very sensitive to experimental conditions and therefore, ensure reliable detection of the viruses. Until now mAbs have been mostly used to analyse the epitope repertoire and the coat protein structure of viruses. As mAbs react with a single epitope they proved to be invaluable to the understanding of the organization of the coat protein to the study of the viral behaviour in various hosts and to the improvement of the classification of viruses (Shukla & Ward 1989).

In this study the production and characterization of mAbs to potato virus Y (PVY), potato virus A (PVA) and to beet necrotic yellow vein virus (BNYVV) are discussed. PVY and PVA belong to the potyviruses, the largest of more than 30 virus groups and form a major threat to the potato crop. Potyviruses are transmitted by aphids. The virion is a single rod and consists of about 2000 copies of a coat protein, encapsulating a single-stranded RNA genome. Recent immunochemical analyses showed that the N- and C-termini of the coat protein regions are surface-exposed, immunodominant, and contain the virus-specific epitopes (Shukla *et al.* 1988). Monoclonal antibodies against many potyviruses have been produced which react only with the virus strain, but not with the viruses from other virus groups (Jordan & Hammond 1988). On the other hand, the core protein regions of the coat proteins contain conservative epitopes (Shukla & Ward 1989). It has been found that the cross-reacting fraction of pAbs obtained against many potyviruses, consist of antibodies that react with common epitopes on all the tested potyviruses (Shukla *et al.* 1989). Monoclonal antibodies against these epitopes have been produced, which indeed cross-react with more than 100 tested potyvirus isolates (Jordan 1990). Recently a conservative epitope on the core protein has been sequenced (Jarvekulg & Saarma 1990).

BNYVV belongs to the furovirus group and is a major threat to sugar beet production. It is transmitted in a complex way by the soil-borne fungus *Polymyxa betae*. BNYVV contains four types of virions, encapsulating four types of single-stranded RNA molecules of different length. It has been found that at least one RNA molecule plays an essential role in the transmission of the virus from *P. betae* to the root, and another in the subsequent spread of the disease (Tamada & Abe, 1989). Immunochemical characterization of the capsid proteins has been performed using pAbs and mAbs (Boonekamp *et al.* 1988; Torrance *et al.* 1988), and some epitopes have been located along the virion particle using immunoelectronmicroscopical studies (Koenig *et al.* 1990; Lesemann *et al.* 1990).

In this study some data will be presented on the production and the use of mAbs to characterize epitopes on the capsid proteins of PVY, PVA and BNYVV. Some of the pitfalls encountered during the selection of mAbs (with various ELISA protocols) will be shown as well as the use of well-selected and incorrectly selected mAbs in understanding the behaviour of viral epitopes during purification of the virus, or after transfer of the virus to another host plant.

MATERIALS AND METHODS

Production and characterization of mAbs

Virus purification. PVA (isolate 613) and PVY-N (isolate 605), isolated from potato, were obtained from Dr P. Gugerli, Switzerland, and were propagated in *Nicotiana rustica*. Purification of PVA was performed according to Gugerli (1979), and of PVY-N according

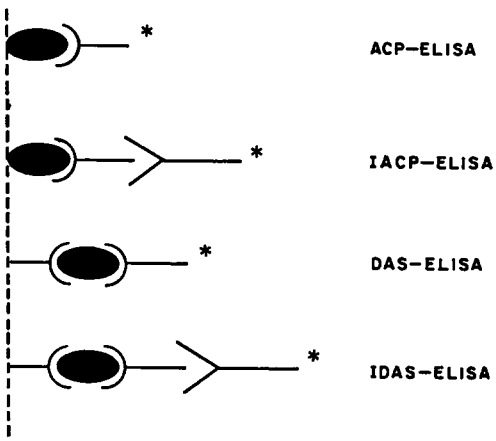

Fig. 1. Schematic representation of the four different ELISA formats used for the detection of the virus. ACP-ELISA = antigen-coated plate-ELISA; IACP-ELISA = indirect antigen-coated plate-ELISA; DAS-ELISA = double antibody sandwich ELISA: IDAS-ELISA = indirect antibody sandwich ELISA. (●) virus, ( )–) mAb or pAb, (>–) RAM, (★) antibody conjugated to alkaline phosphatase. Details are outlined in Materials and Methods.

to Baum & Barnett (1981) and Yang *et al.* (1983). BNYVV was purified by and obtained from Dr D. Peters, Agricultural University, Wageningen, The Netherlands, after first transferring the virus from beet to *Chenopodium quinoa.*

Monoclonal antibody production. Balb/c mice were immunized i.p. with 100 μg purified virus in Freund's complete adjuvant, and 3 weeks later with 100 μg virus in Freund's incomplete adjuvant and finally after more than 8 weeks with 100 μg virus in phosphate buffered saline, pH 7·2 (PBS). Three days after the last injection, spleen cells were fused with SP 2/0 myeloma cells; hybrid cells were cultured and culture media assayed in ELISA for antibody activity with rabbit anti-mouse immunoglobulins conjugated to alkaline phosphatase, RAM-AP (Boonekamp *et al.* 1990).

Rabbit antisera to PVA and PVY were prepared by Maat (1981) and antisera to BNYVV purchased from Sanofi, France. Antisera were conjugated to alkaline phosphatase (pAbs-AP), according to Tobias *et al.* (1982).

ELISA procedures. Details of the ELISA procedures have been described (Boonekamp *et al.* (1990) and are outlined in Fig. 1. Four ELISA formats are shown.

(a) Antigen-coated plate ELISA (ACP-ELISA): plates were coated with virus which was directly detected by conjugated pAbs-AP.

(b) Indirect antigen-coated plate ELISA (IACP-ELISA): plates were coated with virus which was detected by mAbs followed by conjugated RAM-AP.

(c) Double antibody sandwich ELISA (DAS-ELISA): plates were coated with pAbs, virus was trapped and subsequently detected by conjugated pAbs-AP.

(d) Indirect double antibody sandwich ELISA (IDAS-ELISA): plates were coated with pAbs, virus was trapped and subsequently detected by mAbs followed by conjugated RAM-AP.

Gel electrophoresis (SDS-PAGE) and immunoblotting (IB). Polyacrylamide gel electrophoresis was performed in the presence of sodium dodecylsulphate (SDS-PAGE) under

Table 1. Reactions of mAbs-A-1–A5 and of pAbs with purified and non-purified PVA

	Reaction in (I)DAS-ELISA		
	Purified PVA	PVA-infected sap	Virus-free sap
mAb-A-1	+++	−	−
mAb-A-2	+++	−	−
mAb-A-3	++	++	−
mAb-A-4	++	++	−
mAb-A-5	++	++	−
pAbs	++	++	−

To assay the binding of mAbs IDAS-ELISA was used and for pAbs DAS-ELISA. The final concentration of purified PVA was 5 μg ml^{-1}. Virus-infected sap was diluted to obtain a similar ELISA reading (E405 nm) as found for pAbs with purified PVA. The same dilutions were used for the experiments with mAbs. Data are presented as E405 nm readings: <0·1 = −, 0·1 to 1·0 = +, 1·0 to 2·0 = ++, >2·0 = +++.

reducing conditions according to Laemmli & Favre (1973). A 5% stacking gel and a 12·5% separating gel were used. Low-molecular weight standards (Bio-rad) and purified virus were diluted 5–20 times in sample buffer. Virus-infected sap was clarified by centrifugation at 10 000 *g* in the presence of 10% chloroform. The supernatant fraction was diluted five times in sample buffer. Conditions for electrophoresis, gel-staining with Coomassie brilliant blue and immunoblotting (IB) were as described previously (Boonekamp *et al.* 1990). During IB the nitrocellulose-bound viral capsid proteins were detected with mAbs followed by conjugated RAM-AP.

RESULTS

Potato virus A (PVA)

Details on the production of mAbs to PVA have been published (Boonekamp *et al.* 1990). As a result of two fusion experiments, mAbs-A-1 and A-2 were obtained whilst mAbs-A3, A-4 and A-5 resulted from a third fusion experiment. For the selection of mAbs in fusion experiment one and two, ACP-ELISA with purified PVA was used; IDAS-ELISA with PVA-infected plant sap was used for fusion experiment three. The reactivity of the mAbs in ELISA is summarized in Table 1. The data show that mAb-A-1 and A-2 only react with purifed PVA and not with PVA in infected sap. Monoclonal antibodies-A-3, A-4 and A-5 react with purified PVA as well as with PVA in infected sap. During purification of PVA a new epitope is introduced (Boonekamp *et al.* 1990). The discrepancy of mAb-A-1 and A-2 versus mAb-A-3, A-4 and A-5 can be explained as shown in Fig. 2.

Mice immunized with purified PVA give an immunological response to all epitopes including the newly introduced epitopes. The latter was highly exposed on purified PVA in ACP-ELISA. As purified PVA in ACP-ELISA was used for the selection of mAbs in fusions one and two, and as the new epitope appeared to be very immunogenic (Boonekamp *et al.* 1990), it is not surprising that only mAbs against the new epitope were

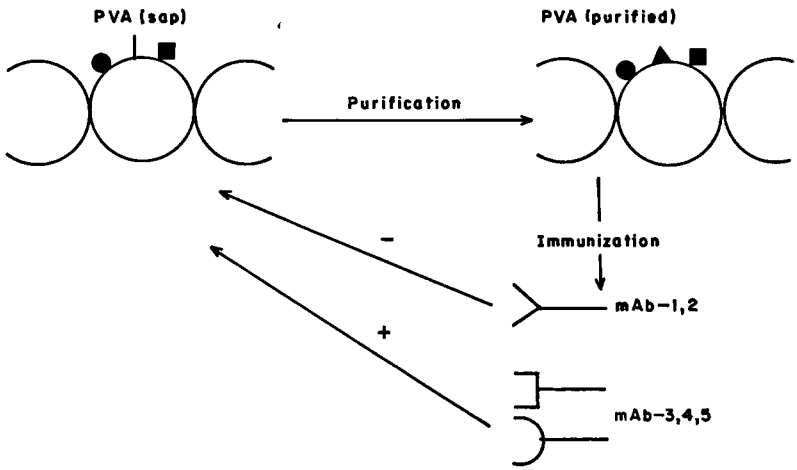

Fig. 2. Model showing the introduction of an epitope on PVA during the purification of the virus. A few capsid proteins with epitopes are indicated. After immunization pAbs will be raised against all epitopes. However, if conditions in ELISA are favourable to select mAbs against the new epitope (▲), such mAbs will only react with the purified PVA but not with PVA in infected sap.

Table 2. Reactions of mAb-Y-1–Y-5 and of pAbs with purified and with non-purified PVY

	Reactions in (I)DAS-ELISA		
	Purified PVY	PVY-infected sap	Virus-free sap
mAb-Y-1	+	++	−
mAb-Y-2	+++	++	−
mAb-Y-3	++	+	−
mAb-Y-4	++	++	−
mAb-Y-5	++	++	−
pAbs	++	++	−

To assay the binding of mAbs IDAS-ELISA was used and for pAbs DAS-ELISA. The final concentration of purified PVY was 5 μg ml^{-1}. Virus-infected sap was diluted to obtain a similar ELISA reading (E405 nm) as found for pAbs with purified PVA. The same dilutions were used for the experiments with mAbs. Data are presented as E405 nm readings: <0·1 = −, 0·1 to 1·0 = +, 1·0 to 2·0 = ++, >2·0 = +++.

selected (mAb-A-1 and A-2). Only when PVA-infected sap, that did not contain the new epitope, was used for selection could mAbs against other epitopes be selected (mAbs-A-3 to A-5). The latter mAbs react with PVA-infected sap. As pAbs contain antibodies to all epitopes of PVA, they will also react with PVA-infected sap.

Potato virus Y (PVY)

PVA and PVY are both potyviruses and were purified in a similar way. When in ELISA, purified PVY was used to select mAbs, all mAbs (Table 2) reacted with purified PVY as

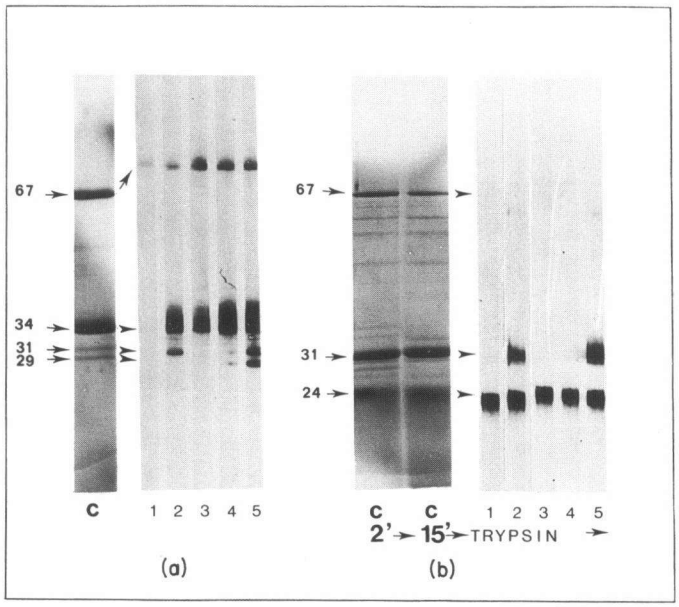

Fig. 3. The reaction of mAb-Y-1 to Y-5 with purified PVY after SDS-PAGE-IB, before (a) and after trypsin treatment of the virions for 15 min, (b). c = Gel stained with Coomassie, note the similar degradation of the capsids after 2 or 15 min treatment of the virion with trypsin. Arrows indicate M_r in kilodaltons.

well as with PVY-infected sap. Apparently, in contrast to PVA, PVY appeared less susceptible to the introduction of new epitopes during purification. The epitopes for mAb-Y-1 to Y-5 are present on purified PVY as well as on PVY in infected sap. SDS-PAGE-IB experiments were performed to define the location of the epitopes on the PVY capsid protein. After Coomassie staining, data show (Fig. 3a) that PVY capsid proteins are present in three forms: a non-degraded 34-K protein and two partially digested proteins of 31 K and 29 K, respectively. After IB these bands could be visualized with mAbs, but the 34-K band appeared as a broader band, (34–38 K). It is often found that bands in IB are broader than bands after Coomassie staining. Despite much speculation, general explanations are not available.

Monoclonal antibody-Y-5 reacted with all three proteins, mAb-Y-2 with 34 and 31-K proteins, mAb-Y-3 and Y-4 only with 34 K protein and mAb-Y-1 did not react at all. The virions were not completely degraded to monomeric capsid proteins (34 K) but a small fraction of the dimeric form (67 K) remained after SDS-PAGE. All mAbs reacted strongly with this band, except mAb-Y-1 which reacted relatively weakly.

In subsequent experiments intact PVY virions were partially digested with trypsin prior to SDS-PAGE-IB, (Fig. 3b). Intact PVY-virions were used to assure that only surface-exposed proteolysis can occur. It has been observed that limited proteolysis of potyviruses might remove N- and C-terminal regions which are surface exposed (Shukla *et al.* 1989). All capsid proteins were degraded to 31-K proteins by trypsin treatment (Fig. 3b), as shown after Coomassie staining. The immunoblotting experiments show that mAb-Y-5 and mAb-Y-2 continued to react with trypsin-degraded proteins but that mAb-Y-3 and Y-4 lost reactivity. Controls (not shown) indicated that the reaction with the 24-K band is due to non-specific binding of the RAM-AP conjugate. These data can be fitted into a model, (Fig. 4).

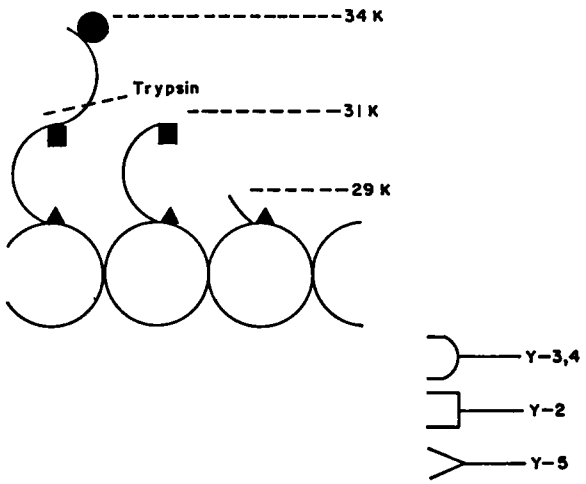

Fig. 4. Model showing the location of epitopes on PVY, based on the SDS-PAGE-IB experiments. A few capsid proteins with epitopes are indicated. Some capsids are partially degraded from 34 to 31 K or 29 K and apparently lost peptides with epitopes. With mAb-Y-2 to Y-5 three epitopes were detected on the 34-K protein, two epitopes on the 31-K protein and one epitope on the 29-K protein. Surface-exposed peptides were removed by trypsin treatment containing the mAb-Y-3 and Y-4 specific epitope. From these data the location of the mAb-Y-2 to Y-5 specific-epitopes can be indicated. mAb-Y-1 does not react at all in SDS-PAGE-IB and may be specific to a discontinuous epitope on intact virons only.

The 34-K protein can occur as partially degraded units of 31 and 29 K, from which the surface-exposed epitopes for mAb-Y-3 and -4, and for mAb-Y-2, respectively, have been removed. Apparently after treating the virions with trypsin, the terminal part of the capsid proteins with the epitopes for Y-3 and Y-4 were removed, but the epitopes for Y-2 and Y-5 remained intact. These epitopes and the putative trypsin cleavage site are indicated in Fig. 4.

In SDS-PAGE-IB mAb-Y-1 did not react with separated PVY capsid protein, and only weakly with dimeric capsid proteins. In IDAS-ELISA with intact PVY virions mAb-Y-1 showed a strong reaction (Table 2). Apparently, the mAb-Y-1-specific epitope on PVY is destroyed after degradation of the virion in separated subunits. The presence of such discontinuous (labile) epitopes has been demonstrated for many viruses (Al-Moudallal *et al.* 1984, Tremaine *et al.* 1985).

In extensive studies on tobacco mosaic virus, discontinuous and continuous (stable) epitopes have been classified (Van Regenmortel 1982) and even visualized in immuno-EM (Dore *et al.* 1988). It has been published that the surface epitopes are virus-specific and that the inner core contains more group-specific epitopes (Jordan & Hammond 1988). Monoclonal antibodies have been produced that cross-react with a large panel of potyviruses (Jordan 1990). In accordance, we observed that mAbs reacting with a core-epitope (mAb-Y-5) cross-reacted with other potyviruses, while against a surface-exposed epitope mAb-Y-2 was more specific for PVY (Boonekamp 1988).

Beet necrotic yellow vein virus (BNYVV)

BNYVV was purified from infected *Chenopodium quinoa* (p-BNYVV), prior to the immunization of mice. BNYVV-infected sap from *Chenopodium quinoa* (c-BNYVV) was used in ELISA to select mAbs. The reactions of mAbs with p-BNYVV, c-BNYVV and b-BNYVV (BNYVV from infected beet sap) are summarized in Table 3. The data show

Table 3. Reactions of mAb-NY-1–mAb-NY-4 with purified BNYVV, (p-BNYVV), BNYVV-infected sap from beet, (b-BNYVV), and BNYVV-infected sap from *Chenopodium quinoa*, (c-BNYVV)

	p-BNYVV ACP-ELISA	IDAS-ELISA	SDS-PAGE-IB	b-BNYVV IDAS-ELISA	c-BNYVV IDAS-ELISA	I-EM
mAb-NY-1	+++	++	++	+	+	Entire
mAb-NY-2	−	+	−	+	±	Extreme
mAb-NY-3	+	+	+	+	+	Entire
mAb-NY-4	−	+	−	+	±	Entire ±

To assay the binding of mAbs, ACP-ELISA or IDAS-ELISA was used. The final concentration of purified BNYVV was 5 μg ml^{-1} approx. Immuno-electronmicroscopy (I-EM) data are from Lesemann *et al.* (1990). Immunogold-labelled antibodies were used to study the distribution of the mAb-binding sites along the virus particles. The cell clones producing mAbs are 3H12 (NY-1), 6D8 (NY-2), 4F11 (NY-3) and 17G2 (NY-4). ELISA data are presented as E405 nm readings: <0·1 = −; 0·1 to 1·0 = +; 1·0 to 2·0 = ++; >2·0 = +++. I-EM data are: entire = binding over entire length of the virus; entire ± = binding unevenly over entire length of the virus; extreme = binding at one extremity of the virus.

that four mAbs could be selected from cell clones 3H12 (mAb-NY-1), 6D8 (mAb-NY-2), 4F11 (mAb-NY-3), and 17G4 (mAb-NY-4).

The mAbs were selected with c-BNYVV in IDAS-ELISA. Monoclonal antibodies NY-1 and NY-3 showed a strong reaction with c-BNYVV but NY-2 and NY-4 reacted only weakly. The I-EM experiments (Lesemann *et al.* 1990) showed that NY-1 and NY-3 bound strongly along the entire length of the virus particle, but that NY-4 bound unevenly along the entire length and that NY-2 bound only at one extreme end. All mAbs reacted with b-BNYVV or p-BNYVV in IDAS-ELISA. However, in ACP-ELISA and after SDS-PAGE-IB, mAb-NY-2 and -4 completely lost reactivity while the reactivity of mAb-NY-1 and -3 even increased. These and some recent studies (Koenig *et al.* 1990) indicate that mAb-NY-1 to NY-4 react with different epitopes on BNYVV.

Monoclonal antibody-NY-1 and NY-3 bind to a continuous epitope, present on each capsid protein along the entire length of the virion. This epitope is stable and not sensitive to degradation of the virion in separated capsid proteins (ACP-ELISA experiments), or even to denaturation of the capsid proteins (IB experiments). To study the location of this epitope on the capsid protein, SDS-PAGE-IB was performed with p-BNYVV. The reaction of mAb-NY-1 and NY-2 with p-BNYVV is shown (Fig. 5). After Coomassie staining data show that p-BNYVV contains 20-K capsids, but also a degraded 18-K band, depending on the isolate (Fig. 5a and b). Monoclonal antibody-NY-1 reacted not only with the 20 K, but also strongly with an additional 25-K band not shown with Coomassie staining. The nature of this compound is unknown. As a control, mAb-NY-2 did not react with the continuous epitope, nor with the 25-K band. It appeared that the continuous epitope is on the capsid protein (20 k) but not on the partly degraded capsid (18 K).

It has been recently shown (Schots & Pomp 1990) that NY-1 and NY-3 react with an epitope on an expressed fusion protein containing the amino acids 104–188 of the C-terminal part of the BNYVV coat protein. As we found no reaction with the 18-K protein, this indicates that the continuous epitope can now be located more precisely at the extreme C-terminus (final 20 amino acids).

Monoclonal antibodies-NY-2 and NY-4 react with discontinuous (labile) epitopes, which are present at one extreme end (NY-2) or are unevenly distributed (NY-4).

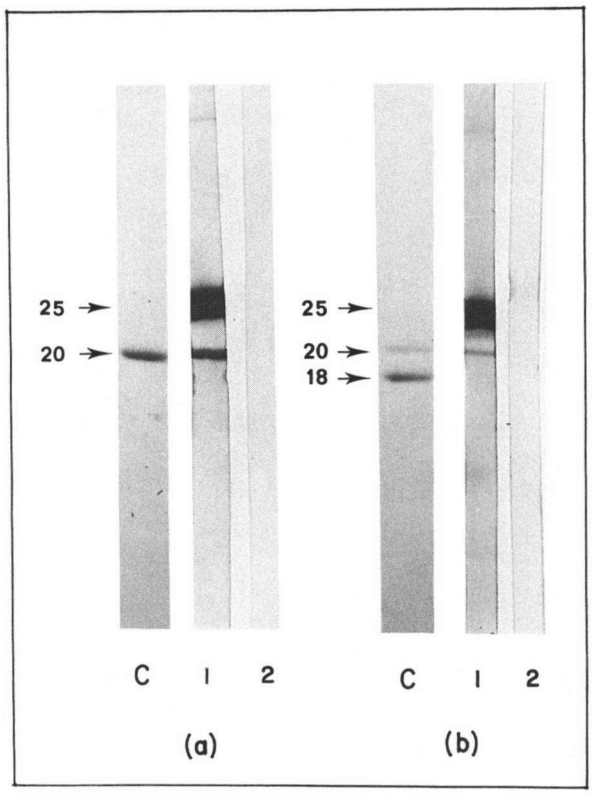

Fig. 5. The reaction of mAb-NY-1 and NY-2 with p-BNYVV in SDS-PAGE-IB. Two batches of purified BNYVV were used containing only intact 20-K capsids (a) or intact and partly degraded capsids of 20-K and 18-K respectively (b). Note that mAb-NY-1 (lane 1) reacts with the 20-K protein but also strongly with a 25-K band, not shown after staining with Coomassie (indicated as lane c). Monoclonal antibody-NY-2 (lane 2) shows no binding at all. Arrows indicate M_r in kilodaltons.

Evidently SDS-PAGE-IB degrades virions in separated capsid proteins. In addition, it has been reported that many viruses degrade to separate capsid proteins upon binding to plastic ELISA plates (ACP-ELISA). Virions stay intact when bound via antibodies to plastic in (I)DAS-ELISA (Al-Moudallal *et al.* 1984, Tremaine *et al.* 1985). This explains why the discontinuous NY-2 and NY-4 specific epitopes are only exposed when mild assay conditions are used, namely (I)DAS-ELISA and I-EM with antibody-coated grids. It was found that both discontinuous epitopes were expressed more in intact b-BNYVV than in c-BNYVV (Table 3).

DISCUSSION

The presented examples show that mAbs are very important tools for virological research. With mAbs not only can the structure of the capsids be studied but also which parts are most important for the characteristics of the virus. Monoclonal antibodies have already proven to be of great use in the classification and identification of viruses (Shukla & Ward 1989). Monoclonal antibodies against conserved epitopes show much cross-reactivity with

other potyviruses (Boonekamp 1988; Jordan & Hammond 1988), and new potyviruses could already be identified using such mAbs (Jordan 1990). Since the majority of antibodies from pAbs are directed to surface-exposed virus-specific epitopes, pAbs do not often cross-react with other potyviruses. Therefore pAbs are not suitable to identify new unknown potyviruses. With regard to the carlaviruses, another important virus group, recently conserved epitopes have been detected by mAbs (Wieczorek & Stace-Smith 1990), indicating a wider application for mAbs against conserved epitopes.

Monoclonal antibodies are also very useful for studying the virus–host–plant interaction. Using mAbs to PVA, it was shown that epitopes can be introduced during purification, probably by oxidative processes in the plant sap (Boonekamp *et al.* 1990). With pAbs it was not possible to distinguish the new epitope from the existing epitopes. Such information is very important as many studies on explaining virus characteristics *in situ* are performed with purified viruses. It is not known yet if similar changes to epitopes occur in other viruses during purification. The data on BNYVV show that the host–plant effects the expression of epitopes.

The discontinuous epitopes reacting with mAb-NY-2 and NY-4 are expressed more in BNYVV-infected sap from beet than from *C. quinoa*. It might be that a slightly altered capsid protein folding leads to better exposure of this epitope in beet. The data on PVY and BNYVV also show that mAbs are invaluable to distinguish the number and the location of epitopes along virions. Such studies are not possible with pAbs.

For mAbs to PVY it could be shown that not all the capsid proteins are the same size, but that degradation at the N- and C-termini may occur *in situ*. *In situ* degradation has also been observed in potexviruses (Koenig *et al.* 1978; Koenig & Torrance 1986). For potyviruses it has been found that the coat protein regions at the N-terminus contain the virus-specific epitopes (Shukla *et al.* 1988).

Potyviruses are highly specialized and virus strains only infect a narrow host range. Infection is a complicated process including transmission by aphids, multiplication of the virus in the plant cell, and subsequent spread to other cells and tissues. Monoclonal antibodies may help to study this process.

Selected virus strains with altered infection behaviour, and virus strains modified in their capsid structure (with genetic enineering or protease treatment of intact virons), can be characterized with mAbs and used to study the infection process. In this way the role of the various parts of the capsid proteins in the subsequent steps in the infection process can be studied. Recently anti-idiotypic mAbs have been produced against a mAb which had been produced against barley yellow dwarf virus (BYDV). Since such anti-idiotypic mAbs react with the binding site of the mAb against BYDV, its own binding site must possess conformational similarities to the BYDV epitope. Therefore anti-idiotypic mAbs can be used to block virus receptor sites in aphid salivary glands and in plant cells and to study the virus transmission and infection processes (Hu and Rochow, 1988). Knowledge of these processes is essential if ways to prevent crops from infection are to be found.

Monoclonal antibodies are not widely used for the routine detection of viruses in commercial crops, although mAbs are available against most viruses. In The Netherlands more than six million samples are annually assayed in ELISA for the presence of viruses, but, as yet, only pAbs are used. A reason is that for routine use, mAbs have some disadvantages compared to pAbs.

An important drawback can be that mAbs have been selected, which react poorly or not at all with the virus strains *in situ*. An extreme example is illustrated by mAb-A-1 and A-2 against PVA. These mAbs react with epitopes which are present only on purified viruses

but not on viruses *in situ*. Polyclonal antibodies react per definition with all exposed epitopes; both those introduced during purification and those already existing epitopes.

A second problem with assays based on mAbs is their sensitivity to experimental conditions, illustrated by the mAbs against the discontinuous epitopes of BNYVV (mAb-NY-2 and NY-4) or PVY (mAb-Y-1). Such epitopes stay intact only under mild assay conditions, which may be absent in plant extracts used for assaying. As pAbs react with discontinuous as well as with continuous epitopes, this problem does not occur. Also the binding of mAbs is sensitive to the experimental conditions as has been reported many times. Monoclonal antibodies are homogeneous antibodies all with the same affinity for the virus. This affinity is in general lower than the affinity of the best class of antibodies in pAbs. Relatively small changes in binding conditions in the sample (pH, temperature, salt concentrations) might further decrease the affinity of mAbs, leading to less specific binding and/or a higher background reaction. Modification (e.g. conjugation) is reportedly detrimental to the affinity of mAbs to a virus in plant sap (Martin & Stace-Smith 1984). As pAbs always contain a class of antibodies with the highest affinity to an epitope, these problems have not been observed in assays with pAbs. A way to avoid the problems with mAbs is to select and use only mAbs with a very high affinity for an epitope.

If mAbs are carefully selected, they can be very valuable for routine applications. The main advantage over pAbs is the possibility of standardizing the assays. This is very important for the certification of exported crops. Good virus-free quality can be guaranteed and verified, which helps to avoid trade frictions and claims. Another advantage is that mAbs can be used to detect a virus, even if the virus could not be purified for antibody production. For instance, the mAbs produced by Jordan & Hammond (1988) against a conservative epitope on a potyvirus, can be successfully used to detect some potyviruses in horticultural crops (A. Derks, personal communication). However, the main application of mAbs for routine assays will not be the detection of viruses but the detection of other plant pathogens such as bacteria, fungi and nematodes. In host–plants these pathogenic and non-pathogenic organisms have too many epitopes in common, which makes it impossible to produce pathogen-specific pAbs. For some of these pathogens, pathogen-specific mAbs could be selected. Only with such mAbs, can serological assays be developed to detect bacteria, fungi or nematodes on a routine basis.

ACKNOWLEDGEMENTS

We are greatly indebted to Dr P. Gugerli from the Swiss Federal Agricultural Research Station in Nyon, Switzerland for providing the potyvirus strains, to Dr D. Peters from the Department of Virology at the Agricultural University in Wageningen, The Netherlands for providing the purified BNYVV, and to Ing D. Z. Maat from the Research Institute for Plant Protection in Wageningen, The Netherlands for providing purified potyviruses and rabbit antisera.

REFERENCES

Al-Moudallal, Z., Altschuh, D., Briand, J.P. & van Regenmortel, M.H.V. (1984): Comparative sensitivity of different ELISA procedures for detecting monoclonal antibodies. *J. Immunol. Meth.* **68**: 35–43.

Baum, R.H. & Barnett, O.W. (1981): Peptide map analysis of cyanogen bromide cleaved capsid proteins of clover yellow vein and bean yellow mosaic virus. *Phytopathology* **71**: 859.

Boonekamp, P.M. (1988): Case study on potato virus Y. In: Boonekamp, P.M. (ed.). *Monoclonal Antibodies and Immunological Techniques to Detect Plant Pathogens*. 75–79. Pudoc, Wageningen, The Netherlands.

—, Pomp, H. & Gussenhoven, G.C. (1988): Monoclonal antibodies against beet necrotic yellow vein virus (BNYVV). In: *Fifth International Congress of Plant Pathology, Kyoto, Japan.* 20–27 August, 1988. Abstract of papers, 49.

—& — (1990): Production and characterization of monoclonal antibodies to potato virus A. *J. Phytopathol.* **128:** 112–124.

Dore, I., Weiss, E., Altschuh, D. & van Regenmortel, M.H.V. (1988): Visualisation by electron microscopy of the location of tobacco mosaic virus epitopes reacting with monoclonal antibodies in enzyme immunoassays. *Virology* **162:** 279–289.

Gugerli, P. (1979): Potato virus A and potato leafroll virus: purification, antiserum production and serological detection in potato and test plants by enzyme-linked immunosorbent assay (ELISA). *Phytopathol. Z.* **96:** 97–107.

Halk, E.L. & De Boer, S.H. (1985): Monoclonal antibodies in plant-disease research. *Ann. Rev. Phytopathol.* **23:** 321–350.

Hu, J.S. & Rochow, W.F. (1988): Anti-idiotypic antibodies against an anti-barley yellow dwarf virus monoclonal antibody. *Phytopathology* **78:** 1302–1307.

Jarvekulg, L. & Saarma, M. (1990): Localisation of the group-specific epitope of potyviruses with monoclonal antibodies. In: *VIIIth International Congress of Virology, Berlin, FRG,* 26–31 August, 1990. Abstract of papers, 473.

Jordan, R.L. (1990): Mapping of potyvirus-specific and group-common coat protein antigenic determinants defined with monoclonal and polyclonal antibodies. In: *VIIIth International Congress of Virology, Berlin, FRG,* 26–31 August, 1990. Abstract of papers, 128.

—& Hammond, J. (1988): Epitope-specificity of strain-, virus-, subgroup-specific and potyvirus group cross-reactive monoclonal antibodies. *Phytopathology* **78:** 1600.

Koenig, R., Lesemann, D.-E., Commandeur, U. & Burgermeister, W. (1990): Antigenic analyses of the coat protein of beet necrotic yellow vein virus by means of monoclonal antibodies. In: *VIIIth International Congress of Virology, Berlin, FRG,* 26–31 August, 1990. Abstract of papers, 83.

— & Torrance, L. (1986): Antigenic analysis of potato virus X by means of monoclonal antibodies. *J. Gen. Virol.* **67:** 2145–2151.

—, Tremaine, J.H. & Shepard, J.F. (1978): *In situ* degradation of the protein chain of potato virus X at the N- and C-termini. *J. Gen. Virol.* **38:** 329–337.

Laemmli, U.K. & Favre, M. (1973). Maturation of the head of bacteriophage T4. I. DNA packaging events. *J. Mol. Biol.* **80:** 575–599.

Lesemann, D.-E., Koenig, R., Torrance, L. *et al.* (1990): Electron microscopical demonstration of different binding sites for monoclonal antibodies on particles of beet necrotic vein virus. *J. Gen. Virol.* **71:** 731–733.

Maat, D. Z. (1981): *Annual Report Research Institute for Plant Protection.* Wageningen, The Netherlands, 33.

Martin, R.R. & Stace-Smith, R. (1984): Production and characterization of monoclonal antibodies specific to potato leaf roll virus. *Can. J. Plant Pathol.* **6:** 206–210.

Schots, A. & Pomp, H. (1990): A review on five years Laboratory for Monoclonal Antibodies in Wageningen, The Netherlands. In: Schots, A. (ed.). *Monoclonal Antibodies in Agriculture.* 7–13. Pudoc, Wageningen, The Netherlands.

Shukla, D.D., Jilka, L., Tosic, M. & Ford, R.E. (1989): A novel approach to the serology of potyviruses involving affinity-purified antibodies directed towards virus-specific N-termini of coat proteins. *J. Gen. Virol.* **70:** 13–23.

—, Strike, P.M., Tracy, K., Gough, K.H. & Ward, C.W. (1988): The N- and C-terminal of the coat proteins of potyviruses are surface-located and the N-terminus contains the major virus-specific epitopes. *J. Gen. Virol.* **69:** 1497–1508.

—& Ward, C.W. (1989): Identification and classification of potyviruses on the basis of coat protein sequence data and serology. *Arch. Virol.* **106:** 171–200.

Tamada, T. & Abe, H. (1989): Evidence that beet necrotic yellow vein virus RNA4 is essential for efficient transmission by the fungus *Polymyxa betae. J. Gen. Virol.* **70:** 3391–3398.

Tobias, I., Maat, D.Z. & Huttinga, H. (1982): Two Hungarian isolates of cucumber mosaic virus from sweet pepper (*Capsicum anuum*) and melon (*Cucumis melo*): identification and antiserum preparation. *Neth. J. Pl. Pathol.* **88:** 171–183.

Torrance, L., Pead, M.T. & Buxton, G. (1988): Production and some characteristics of monoclonal antibodies against beet necrotic yellow vein virus. *An. Appl. Biol.* **113:** 519–530.

Tremaine, J.H., Ronald, W.P. & MacKenzie, D.J. (1985): Southern bean mosaic virus monoclonal antibodies: reactivity with virus strains and with virus antigen in different conformations. *Phytopathology* **75:** 1208–1212.

Van Regenmortel, M.H.V. (1982): Antigens and antigenic determinants. In: *Serology and Immunochemistry of Plant Viruses.* Chap. 1: 1–17. Academic Press, New York.

Wieczorek, A. & Stace-Smith, R. (1989): A conserved region in carlaviruses detected by a monoclonal antibody. *Phytopathology* **79:** 912.

Yang, L., Reddick, B. & Slack, S.A. (1983): Results of experiments on the purification of potato virus Y. *Phytopathology* **73:** 794.