An enzyme-linked immunosorbent assay for the detection of mouse polyomavirus-specific antibodies in laboratory mice

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Summary

An enzyme-linked immunosorbent assay (ELISA) was developed for the detection and quantification of IgM and IgG serum antibodies to mouse polyomavirus (MPV). To evaluate the potential of this ELISA for the screening of laboratory rodents, serum samples from specific pathogen free (SPF) BALB/c RIVM mice, collected after experimental intraperitoneal infection with MPV, were tested by this assay. The results were compared with those obtained from the same sera in an immunofluorescence assay (IFA) and a haemagglutination inhibition assay (HIA). The ELISA proved to be the most sensitive of the 3 assays, allowing the detection of seropositive animals within 7 days post-infection and giving antibody titres that were about 4 to 8 times higher than those found in the IFA and HIA respectively. More than 5000 serum samples from non-infected specific pathogen free laboratory mice and 90 from 10 SPF N:NIH/RIVM mice experimentally infected with K-papovavirus, were negative in this assay, thus confirming the specificity of the ELISA

Keywords Mouse polyomavirus; polyomavirus; enzyme-linked immunosorbent assay; serology

Mouse polyomavirus (MPV), a member of the family Papovaviridae (Matthews 1982), has been shown to cause asymptomatic infections in laboratory and wild mice (Rowe 1961, Eddy 1969a,b). Although under natural conditions MPV infection has not been shown to induce tumours in laboratory or wild mice, MPV-induced tumours can be induced readily experimentally in a variety of animal species (for review see Shah & Christian 1986).

Both for health and virological surveillance programmes of laboratory animals, and for the control of biological products produced or controlled in laboratory rodent cells, fast and reliable serological test procedures are needed. For the serology of MPV infections in mice, an immunofluorescence assay (IFA) and a haemagglutination inhibition assay (HIA) have been described (Eddy *et al.* 1958, Malmgren *et al.* 1968). Both methods have shown their value, but because of their relatively low sensitivity, these assays have their limits in surveillance programmes. Therefore we developed an enzyme-linked immunosorbent assay (ELISA) for the serology of MPV, using a principle that we also use for the serological monitoring of other rodent viruses, including K-papovavirus (KPV) of mice (Groen *et al.* 1989a). This procedure can be incorporated easily in routine screening programmes. To study the potential of this newly developed ELISA, we have tested a panel of more than 5000 serum samples from specific

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pathogen free (SPF) BALB/c mice. Ten of these had been infected intraperitoneally with MPV and 10 others with KPV. These 20 mice were followed for the development of MPV-specific serum antibodies by ELISA, IFA and HIA.

Materials and methods

Virus and antigen preparation

MPV was obtained from the American Type Culture Collection (Rockville, MD, USA) (nr. ATCC VR.252) and cultured in primary mouse embryo cells (MEC) in Eagle minimal essential medium supplemented with 10% fetal calf serum (FCS) as previously described (Groen *et al.* 1989a). This virus was also used as stock virus. When the monolayer showed confluency, the culture medium was discarded and the cells were infected with $10^{2.5}$ $TCID_{50}$/50 μl of MPV. The infected cells were maintained in Eagle minimal essential medium with 2% FCS. Infected monolayers were trypsinized when the first cytopathic changes were observed, the cells were transferred to 10 drop well slides and fixed with −70°C ethanol. Uninfected MEC cells were treated in a similar way and served as negative controls. Virus antigens for use in the ELISA system were prepared by mixing stock virus for 30 sec with 0.5 vol of trichlorotrifluorethane (Arklone®) and centrifugation for 15 min at 2000 rpm. This procedure was repeated once. The supernatant fluid was then layered on top of a 30% CsCl (w/v) cushion and centrifuged in a SW27 Beckman Inc. rotor (Beckman Inc., California, USA) for 4 h at 20 000 rpm. The final pellet was resuspended in a small volume of phosphate buffered saline (PBS), pH 7.2. The protein concentration determined by the Bradford method (Bradford 1976) was adjusted to 450 μg/ml and 1 ml aliquots of this preparation were frozen at −70°C until use as antigen in the ELISA.

Experimental infections and sampling

Five weeks old female SPF BALB/c mice, free from most known murine viruses including MPV and KPV (Groen *et al.* 1989b, Spijkers *et al.* 1990) were obtained from the breeding colony of the National Institute of Public Health and Environmental Protection (RIVM). They were infected intraperitoneally with $10^{2.5}$ $TCID_{50}$ MPV on day 0. Serum samples were collected at regular intervals as indicated in Figs 1 and 2. The animals were also observed daily for the presence of clinical signs. Serum samples of 10 outbred N:NIH/RIVM mice ($n = 90$) from the same source which had been infected experimentally with K-papovavirus in the course of a previous study (Groen *et al.* 1989a) were also used in these studies. From 3000 SPF N:NIH/RIVM outbred mice and 2000 SPF BALB/c mice in the colonies of the same institute, serum samples were obtained in the course of routine screening procedures.

Haemagglutination inhibition assay (HIA)

A previously described HIA (Eddy *et al.* 1958) was used with the following modifications. A 25% Kaolin (Janssen Pharmaceutica, Beerse, Belgium) suspension in PBS was used to remove non-specific serum inhibitors. Serial 2-fold serum dilutions were made and an equal volume of MPV-haemagglutinating antigen purchased from Microbiological Associates Inc. (MBA, Bethesda, MD, USA), diluted in PBS to contain 8 haemagglutinating units, was added to each serum dilution. After 1 h of incubation at room temperature, 0.75% (v/v) guineapig erythrocytes were added and incubated overnight at 4°C. The highest serum dilution giving complete inhibition was taken as the end point of the antibody titration.

Immunofluorescence assay (IFA)

The indirect immunofluorescence test was performed essentially as described (Eddy 1969b) employing 10 well drop slides with MPV infected and uninfected MEC cells, fixed with −70°C ethanol and stored at −70°C until use. After dilution of the mouse sera 1:16 in PBS, they were titrated further in serial 2-fold dilutions. The serum dilutions were spotted on the slides and

incubated for 30 min at room temperature. The slides were washed 3 times for 5 min with PBS and incubated for 30 min at room temperature with fluorescein isothiocyanate (FITC) conjugated goat anti-mouse total Ig (Amersham, Little Chalfont/Bucks, UK) diluted 1:50 in PBS.

Enzyme-linked immunosorbent assay (ELISA) for the detection of MPV—specific IgM and IgG antibodies

For the ELISA to quantify MPV-specific IgM or IgG antibodies, 50 μl of the antigen and cell lysate as control antigen in carbonate buffer (pH 9.6) were coated overnight at 4°C to polystyrene 96 well microtitre plates (no. 2595; Costar, Cambridge, MA, USA). Plates were washed twice with PBS-Tween (0.05% Tween 20 in PBS). Each well was blocked with 50 μl PBS-Tween containing 1% bovine serum albumin (BSA) (PBS-TB). After incubation for 30 min at 37°C, plates were washed as described above. Fifty μl aliquots of serial 2-fold serum dilutions in PBS-TB were added to each well. After incubation for 1 h at 37°C, the plates were washed as described. Fifty μl volumes of a commercially available horse-radish peroxidase-conjugated goat anti-mouse IgM (Cappel Laboratories Cochranville, PA, USA) or sheep anti-mouse IgG (Cappel Laboratories Cochranville, PA, USA) preparation were then added and the plates were incubated for 1 h at 37°C. After washing the wells twice, 50 μl of substrate solution were added to each well. The substrate solution was prepared immediately before use by dissolving 0.1 mg/ml 3,3′ 5,5′ -tetramethylbenzidine (TMB) in 0.11 M NaAc-citric acid buffer (pH 5.5) supplemented with 0.006% H_2O_2. The enzyme-substrate reaction was stopped after 10 min by adding 50 μl vol of 2.0 M H_2SO_4. The absorbance was measured spectrophotometrically at 450 nm in a Titertek Multiskan (Flow Laboratories, McLean, VA, USA). Any reaction above three times the average background values, obtained with a panel of 50 serum samples from SPF mice, was considered positive. The ELISA antibody titre to MPV was determined by calculating the reciprocal of the serum dilution giving an OD_{450} exceeding three times the average background values.

Results

No clinical signs were observed during the observation period in the 10 mice experimentally infected with MPV. Serum samples from MPV-infected, KPV-infected and from non-infected mice, were tested by ELISA, IFA and HIA for the detection of MPV-specific serum antibodies. The development of anti-MPV antibody titres in the sera of the experimentally MPV-infected mice measured in the IgG ELISA, IFA and HIA are shown in Fig 1. Anti-MPV IgG serum antibodies could be demonstrated in the IgG ELISA as early as 7 days after infection in 7 out of 10 animals and in all the 10 animals within 14 days. These IgG serum antibodies reached maximum titres of 65 000 after about 50 days and persisted for the rest of the observation period. By IFA and HIA, antibodies were detectable from day 14 onward, with titres gradually rising to 16 000 and 9000 respectively during the course of the observation period. Anti-MPV IgM serum antibodies were found by ELISA as early as 3 days after MPV infection in 6 out of 10 animals and within 7 days in all the 10 animals (titres $\geqslant$20) (Fig 2). These IgM antibodies reached maximum titres of about 850 and had disappeared about 5 weeks later. As expected, no anti-MPV antibodies were

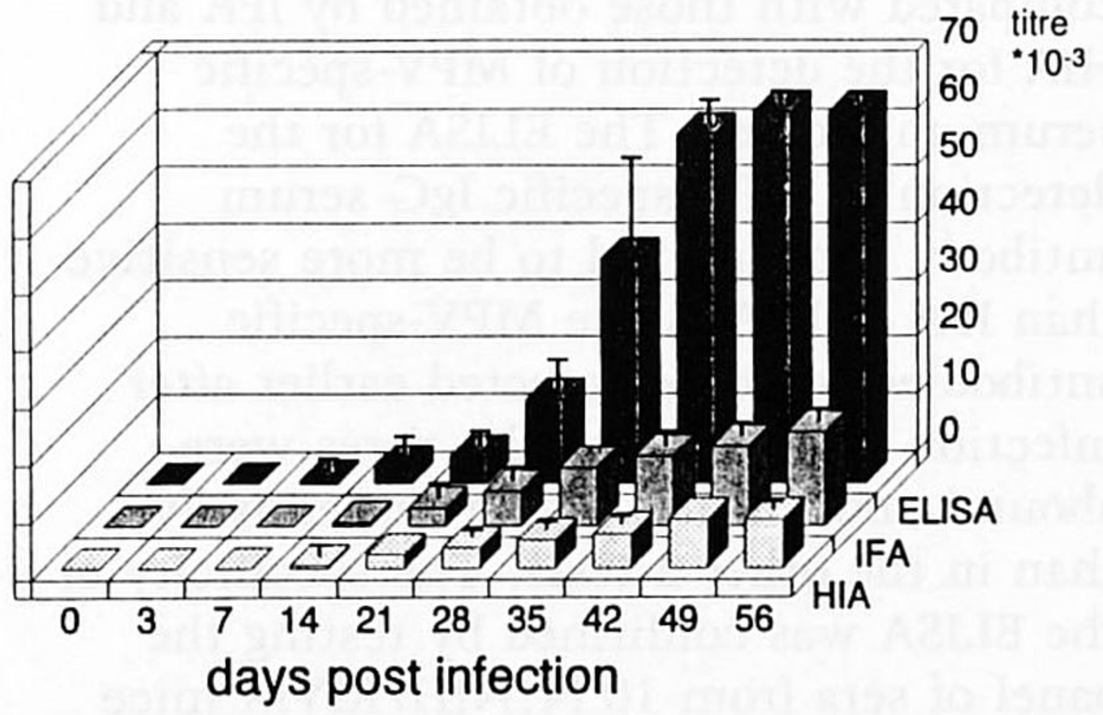

Fig 1 Development of MPV-specific IgG serum antibodies in 10 experimentally infected SPF BALB/c RIVM mice in ELISA, IFA and HIA. Average titres with SD values are shown

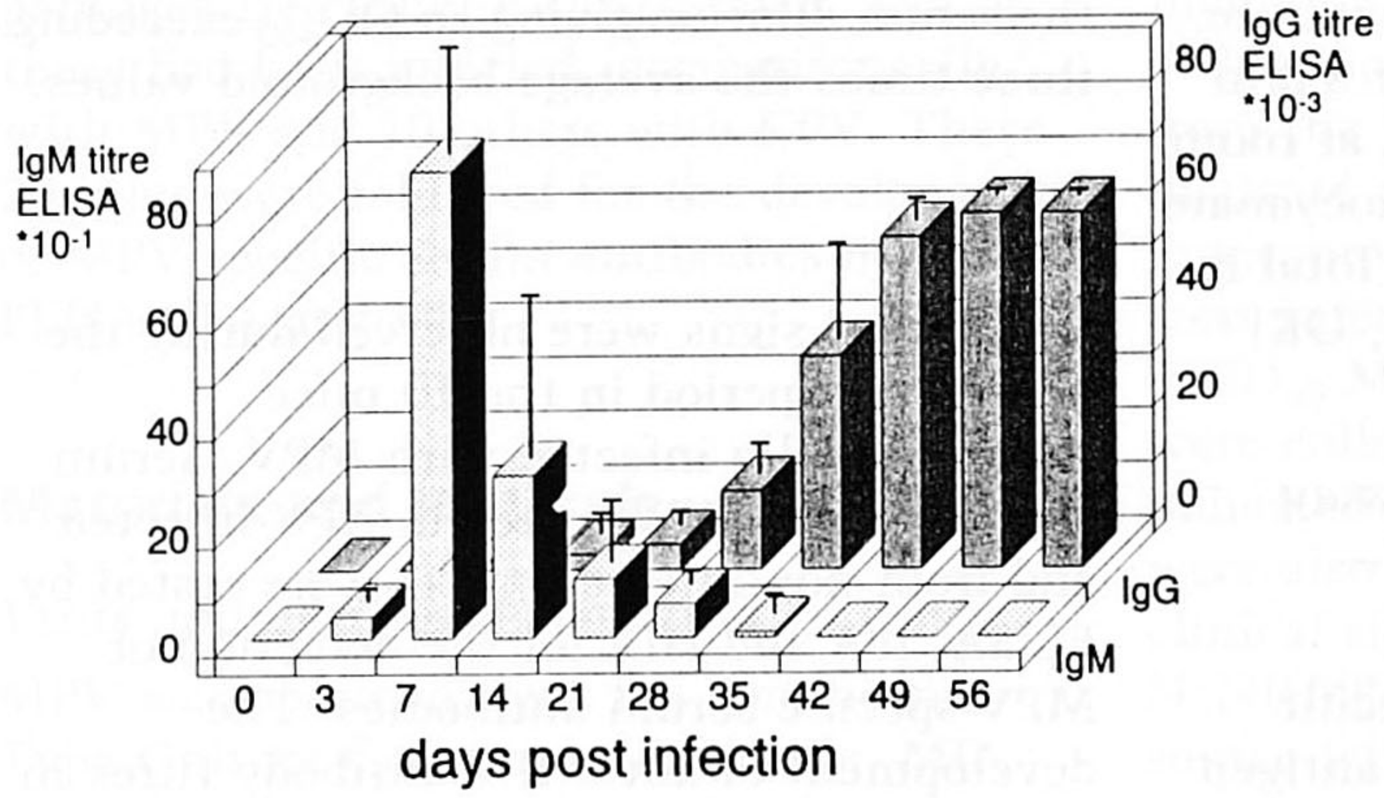

Fig 2 Development of MPV-specific IgG and IgM serum antibodies in 10 experimentally infected SPF BALB/c RIVM mice in ELISA. Average titres with SD values are shown

detected in the sera of the KPV-infected or the non-infected mice in the ELISA, the IFA or the HIA. In the sera of the KPV-infected mice, antibodies were detected in previously described ELISA, IFA and HIA systems for the detection of KPV-specific antibodies (Groen *et al.* 1989a).

Discussion

In the present paper we have evaluated the potential of a newly developed ELISA for the detection of MPV-specific antibodies in the sera of laboratory mice. To this end we have infected 10 BALB/c RIVM mice intraperitoneally with MPV. As expected (Dubensky & Villarreal 1984), none of these animals developed clinical signs during the observation period. The results obtained by ELISA for the detection of MPV-specific IgG antibodies in the sera of the experimentally infected mice were compared with those obtained by IFA and HIA for the detection of MPV-specific serum antibodies. The ELISA for the detection of MPV-specific IgG serum antibody titres proved to be more sensitive than IFA or HIA, since MPV-specific antibodies could be detected earlier after infection by ELISA and the titres were about 4 and 8 times higher respectively than in the other 2 tests. The specificity of the ELISA was confirmed by testing the panel of sera from 10 N:NIH/RIVM mice experimentally infected with KPV, another papovavirus of the mouse which has no antigenic cross-reactivity with MPV (Bond *et al.* 1978). The specificity was further demonstrated by testing the sera from 5000 BALB/c or outbred SPF mice, free from most known murine viruses including MPV and KPV (Groen *et al.* 1989a). All these sera proved to be negative by the newly developed IgG ELISA. The sensitivity and specificity of this ELISA, in which a direct antigen coat to a solid phase is used, offer the opportunity to develop a system in which one serum sample may be tested simultaneously against a variety of antigens coated to the same carrier matrix. As we have also developed this system for the detection of antibodies to other rodent viruses, e.g. KPV (Groen *et al.* 1989a), reoviruses (Spijkers *et al.* 1990), and hantavirus (Groen *et al.* 1989b), it may be of special interest in SPF screening-programmes of rodent colonies.

If, during routine screening procedures, positive samples are found by ELISA or MPV-specific IgG antibodies, additional testing by ELISA for MPV-specific IgM antibodies may be carried out in order to detect recent MPV infections. This may give information about the origin and the spread of the infection in the colony.

It should be noted that although in the IFA an anti-total mouse Ig preparation was used, MPV-specific IgM antibodies could not be detected, probably due to a too low sensitivity of the test.

Taken together, the data presented in this study clearly indicate that the newly developed ELISAs for the detection of MPV-specific IgG and IgM antibodies, are useful tools which can be incorporated in health

and virological quality surveillance programmes of laboratory rodents and in control procedures for biological products produced by rodent cells.

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