J. Hyg., Camb. (1982), **88**, 309
Printed in Great Britain

The development and use of an antibody capture radioimmunoassay for specific IgM to a human parvovirus-like agent

By M. J. ANDERSON, L. R. DAVIS*, S. E. JONES, J. R. PATTISON

Departments of Medical Microbiology and Haematology, King's College Hospital Medical School, Denmark Hill, London SE5 8RX*

AND G. R. SERJEANT

MRC Laboratories (Jamaica), University of West Indies, Mona, Kingston 7, Jamaica, West Indies

(*Received* 15 *October* 1981; *accepted* 31 *October* 1981)

SUMMARY

An IgM-antibody capture radioimmunoassay (MACRIA) was developed for the detection of IgM antibody specific for the human parvovirus-like agent B19. Diagnosis of infection with this agent by either antigen detection or antibody seroconversion had been made by counter-current immunoelectrophoresis (CIE) in 18 cases of aplastic crisis occurring in children with homozygous sickle-cell disease. The MACRIA described here gave positive results in 17 of these 18 cases; in the remaining case only an acute specimen taken from the patient during viraemia and late convalescent specimens taken 184 and 247 days after onset of illness were avaliable.

The test was used to investigate 20 further cases of aplastic crisis in which neither viral antigen nor antibody seroconversion could be detected by CIE. Detection of virus-specific IgM permitted diagnosis of infection with this parvovirus-like agent in 17 of these cases. In the remaining three cases only single serum specimens taken late in convalescence, 82 days or more after the onset of symptoms, were available.

In addition to these 34 cases of aplastic crisis in which primary infection with this agent was diagnosed by MACRIA, seven cases of apparent 'silent' infection detected by CIE were investigated. The test permitted the discrimination between primary infection and re-exposure to the virus in six of these patients.

The use of this assay has added a considerable weight of evidence implicating primary infection with this parvovirus-like agent as an important cause of aplastic crisis in children with sickle-cell disease. Furthermore, MACRIA permits diagnosis of infection when only single serum specimens taken up to ten weeks after infection are available. Thus the use of this test will significantly facilitate the investigation of other clinical syndromes of presumptive infectious aetiology.

INTRODUCTION

Infection of man with a parvovirus-like agent was first reported in 1975 by Cossart *et al.* These workers described the presence of particles with the morphology of parvoviruses in the sera of 11 individuals; nine were healthy blood donors, one had received a renal transplant one week previously, and one was a case of acute hepatitis. The serum of one of the healthy blood donors was designated B19. Subsequent studies indicated that infection with this agent is relatively common; antibody to B19 has been found by both immuno-electron microscopy (Paver & Clarke, 1976) and counter-current immunoelectrophoresis (Cossart *et al.* 1975) in 30–40 % of adults. Despite sporadic reports of B19 viraemia in patients with febrile illness (Schneerson, Mortimer & Vandervelde, 1980), vomiting and myalgia (Paver & Clarke, 1976) this agent has not, until recently, been associated with any clinical syndrome.

Recent work has demonstrated an association between the parvovirus-like agent and aplastic crisis in children with homozygous sickle-cell disease (Pattison *et al.* 1981; Serjeant *et al.* 1981). To date infection with the parvovirus-like agent has been diagnosed in 18 cases of aplastic crisis using counter-current immunoelectrophoresis (CIE), either by detecting virus in acute-phase serum specimens or by demonstrating seroconversion. In children with sickle-cell disease the life span of the erythrocyte is markedly reduced; even so a period of days must elapse between damage to erythroid precursors in the bone marrow and the fall in peripheral blood haemoglobin. It is likely therefore that the period of maximum virus replication and viraemia precedes the clinical presentation in aplastic crisis, after which the viraemia is very short lived. One child attended for a routine visit and was found to have a viraemia seven days before being admitted in aplastic crisis (Serjeant *et al.* 1981). In another patient a viraemia was present on the first day of illness together with a trace of antibody. Three days later no virus could be detected (Pattison *et al.* 1981). Furthermore viraemia was not detectable in any of the patients studied more than three days after the onset of symptoms.

Equally, diagnosis on the basis of seroconversion requires a specimen taken very early in the course of the crisis, or preferably, one taken shortly before the illness. Such specimens are often not available even in closely monitored patients such as children with sickle-cell disease.

The detection of virus-specific IgM is an established means of diagnosing recent infection on the basis of single serum specimens. We have therefore investigated the possibility of adapting the IgM antibody-capture radioimmunoassay (MACRIA) described by Flehmig *et al.* (1979) and Mortimer *et al.* (1981) to the detection of IgM antibody specific for this parvovirus-like agent.

MATERIALS AND METHODS

M-antibody capture radioimmunoassay (MACRIA)

Antigens. Blood donations made at the Lewisham Blood Transfusion Centre were screened by CIE for the parvovirus-like antigen. One unit of blood yielded an agent

https://doi.org/10.1017/S0022172400070169 Published online by Cambridge University Press

(St virus) serologically identical by immunodiffusion with B19 and with the morphology of a parvovirus as revealed by electron microscopy. Some of this antigen was concentrated by centrifugation at 100000 *g* for 2h and the resultant pellet washed in phosphate-buffered saline (Dulbecco A) (PBS) by two further cycles of centrifugation. The final pellet was resuspended in PBS to one-twentieth of the original serum volume.

Serial specimens of serum were collected at approximately two-month intervals over a period of one year from a member of staff. Antibody to B19 and St virus could not be detected by CIE in any of these specimens, indicating that infection had not occured during the year. Therefore a specimen taken in the middle of this series was selected as a control, parvovirus antigen-negative specimen.

Reference sera. Two sera were used throughout this study as anti-parvovirus IgM-positive and negative controls. The reference positive serum was taken from the donor of the virus-containing unit of blood 14 days after the viraemia was detected. This serum was antibody positive by CIE with both B19 and St virus. The reference negative serum was taken from the husband of this donor and was antibody negative to B19 and St virus by CIE. A second specimen from the husband 14 days later was virus antigen positive by CIE and he subsequently seroconverted for antibody. Thus, the first specimen had been taken before infection had occurred and was chosen as the reference negative serum.

Antisera. Dako antiserum to the μ chain of human IgM was purchased from Mercia-Brocades, Weybridge, Surrey.

Anti-parvovirus IgG was prepared from an immune human, a third blood donor whose serum gave a strong line of precipitate by CIE against both B19 and St virus. This serum was fractionated on DE 52 gel (Whatman Ltd, Maidstone, U.K.) by the method of Tedder (1981). This IgG preparation was labelled with ^{125}I in the ratio 1 mCi ^{125}I to 80 μg protein by the iodogen method of Salacinski *et al*. (1979). Free iodine was separated from that bound to the protein by fractionation on a Sephadex G 25 column (Pharmacia Ltd, Hounslow, U.K.). Approximately 60% of the available iodine was bound to the protein. This radio-iodinated protein was adsorbed over human serum proteins linked to Sepharose 4B (Pharmacia Ltd, Hounslow, U.K.) and stored at 4°C for up to 2 months in PBS with 5% bovine serum albumin (BSA).

Assay method. The MACRIA assay was based on that described by Mortimer *et al*. (1981) and involves three steps. In the first an anti-μ-coated solid phase is reacted with the serum under test. In the second stage, the solid phase is reacted with viral antigen, and finally ^{125}I-labelled antiviral antibody is added and bound radioactivity assessed after washing.

Polystyrene beads, 6·4 mm diameter (Northumbria Biologicals, Cramlington, U.K.) were coated with anti-human IgM by gentle agitation in anti-μ diluted 1 in 500 in 1 M-HCl at room temperature for one hour. The beads were held in this solution at 4°C for at least 48 h prior to use. Immediately prior to use, anti-μ-coated beads were washed once in PBS and incubated for 3 h at room temperature in PBS containing 1% BSA.

In the first stage of the test two beads were reacted in the wells of plastic trays

(Abbott) with 200 μl of each test serum, diluted in PBS with 0·05% Tween 20 (Sigma) (PBST) for 3 h at 37 °C. Preliminary tests indicated that a 10^{-1} dilution of test serum in PBST resulted in the best differentiation between the reference positive and negative sera.

Following this reaction the serum was aspirated from the beads and the beads washed three times in PBST. The beads were then incubated in 200μl of parvovirus antigen, diluted in PBST, for 40 h at 4 °C. The preliminary experiments designed to determine the optimum concentration of antigen are described below. Following these, an antigen preparation consisting of the St virus-containing serum diluted 1 in 4 in PBST was used throughout. At the end of this incubation, the antigen was aspirated from the beads which were then washed six times in PBST.

In the third stage of the assay, the beads were reacted with 200 μl volumes of ^{125}I-labelled anti-parvovirus IgG for 3·5 h at 37 °C. Preliminary tests indicated that a dilution of this labelled antiserum such that 200 μl contained 250000 counts per minute provided the best discrimination between the reference positive and negative sera. Following the principles of Mortimer *et al.* (1981), the radio-iodinated protein was diluted in PBS containing 0·2% Tween 20, 10% heat-inactivated rabbit serum (Wellcome Reagents, London, England) and 20% heat-inactivated human parvovirus antibody-negative serum (the reference negative serum).

Following this incubation the radiolabelled antiserum was aspirated from the beads which were then washed nine times in PBST. The radioactivity bound to the beads was measured by counting for 10 min in an NE 1600 gamma counter (Nuclear Enterprises, Edinburgh, U.K.).

Expression of results. Background counts, obtained by counting an empty cassette for ten minutes, were subtracted from the counts obtained for each bead. Following the preliminary studies, described below, in order to obtain results which were directly comparable in spite of the slight day-to-day variation in the performance of the assay, the following calculation was performed for each test serum.

$$\text{binding index (BI)} = \frac{C_t - C_n}{C_p - C_n} \times 100,$$

where C_t, C_n and C_p are the counts obtained with the test serum, reference negative serum and reference positive serum respectively. The BI for each serum tested is thus an indication of the amount bound by the test serum with respect to the amount bound by the reference positive and negative sera.

Statistical significance was calculated using Student's t test applied to log transformation of BI data.

Patients and controls

Test group. A total of 72 sera from 37 cases of aplastic crisis were tested for parvovirus-specific IgM. All these patients had parvovirus antibody detectable by CIE. Fourteen patients were resident in England and the remainder in Jamaica.

In addition paired sera demonstrating seroconversion by CIE, obtained from seven children with homozygous sickle cell disease in whom no haematological

https://doi.org/10.1017/S0022172400070169 Published online by Cambridge University Press

Table 1. *The specificity of the assay*

	Reference serum: antiparvovirus IgM	
	Positive	Negative
Parvovirus antigen		
positive	2705 ± 88*	990 ± 21
negative	838 ± 23	854 ± 17

* Counts per 10 min less background; mean of 2 replicates ± standard deviation.

abnormalities were recorded, were tested. All seven children were resident in S.E. London and regularly attended the sickle cell clinic in the King's Health District (Teaching).

Control groups. Sera from 162 patients considered on epidemiological grounds unlikely to have suffered recent parvovirus infection were collected and tested. These patients comprised (*a*) 64 children with homozygous sickle cell disease who had no history of aplastic crisis in the preceding two years, 29 being resident in Jamaica and 35 in S.E. London; (*b*) 48 normal children, resident in S.E. London, of similar ages and (*c*) 50 U.K. adults, suffering unrelated disease including five patients with laboratory proven recent primary infection with rubella, three with adenovirus and one each with Coxsackie B4 and B5.

RESULTS

Specificity of the assay

The results obtained when beads were reacted in the first stage of the assay with either the reference positive or reference negative serum diluted 10^{-1} and, in the second stage, with either St virus or the antigen negative preparation diluted 1 in 4, are shown in Table 1. Approximately three times as much radiolabelled antiserum was bound to the beads which had been incubated with the reference positive serum and the parvovirus-positive antigen as was bound to beads incubated with any other combination of reference sera and antigen preparation. No significant difference ($P > 0{\cdot}1$) was noted between the radioactivity bound to beads incubated with the IgM-positive serum and parvovirus-negative antigen or the IgM-negative serum and either the parvovirus-positive or -negative antigen preparations.

Sensitivity of the assay

Titration of antigen. Preliminary results (data not given) showed that a dilution of one part crude St virus with three parts PBST gave the best discrimination between the reference positive and negative sera. As the dilution of the antigen preparation was increased, the discrimination between the two decreased. This reduction in the performance of the test was due to the increasing binding of the radiolabel to beads incubated in the first stage with reference negative serum,

https://doi.org/10.1017/S0022172400070169 Published online by Cambridge University Press

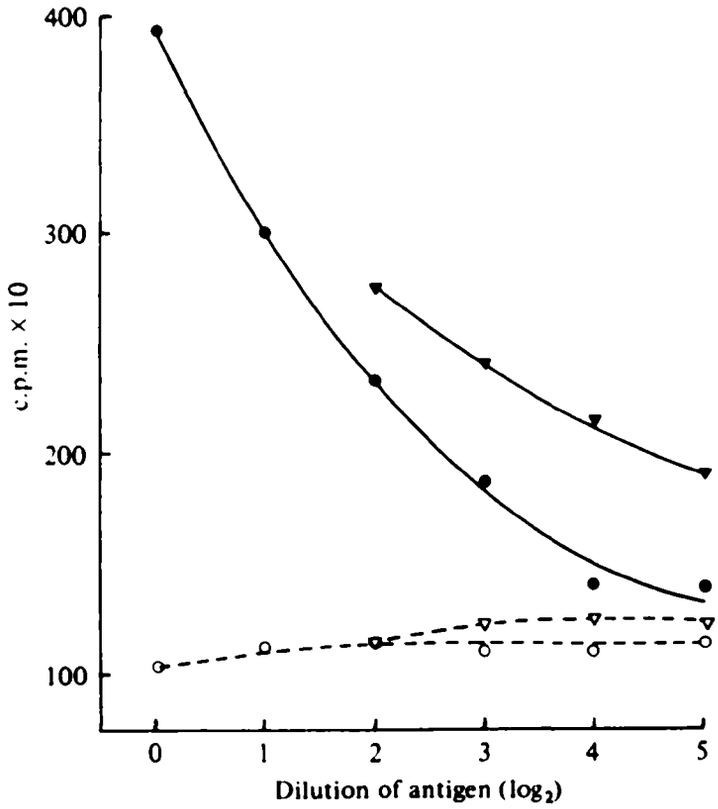

Fig. 1. The counts of ^{125}I-labelled anti-parvovirus globulin bound to beads incubated with the reference positive (●—●; ▼—▼) and negative (○---○; ▽---▽) sera. Two antigen preparations were used; untreated St virus (▼—▼; ▽---▽) and antigen centrifuged and washed in PBS (●—●; ○---○). Each point represents the mean of duplicate determinations.

rather than to a decrease in binding to beads incubated with the reference positive serum. Accordingly, the serum concentration in the present dilution series of antigen was maintained at one part in four.

Following incubation for 3h with either the reference positive or reference negative serum diluted 10^{-1} in PBST, beads were incubated for 40h at 4°C with a range of antigen dilutions. Two antigen preparations were examined. The first consisted of untreated St virus. This was diluted 1 in 4 in PBST and thereafter in $\log_2$ steps in the antigen-negative serum, which had likewise been diluted 1 in 4 in PBST. The second antigen preparation was St virus which had been washed and resuspended to one-twentieth of its original volume in PBS. This concentrated antigen was further diluted 20-fold in a mixture of one part antigen-negative serum with three parts PBST and further twofold dilutions made in the same diluent.

The best discrimination between the reference positive and negative sera was given by the most concentrated preparation of washed antigen examined, i.e. washed antigen diluted to its original serum concentration in a 25% solution of antigen negative serum (Fig.1). With increasing dilution of this antigen, the counts bound to beads incubated with the reference positive serum decreased until at an antigen dilution of 640 there was no significant difference between the counts bound to beads incubated with the reference positive and negative sera. A similar pattern of decreasing binding of labelled antibody with increasing dilution of antigen was

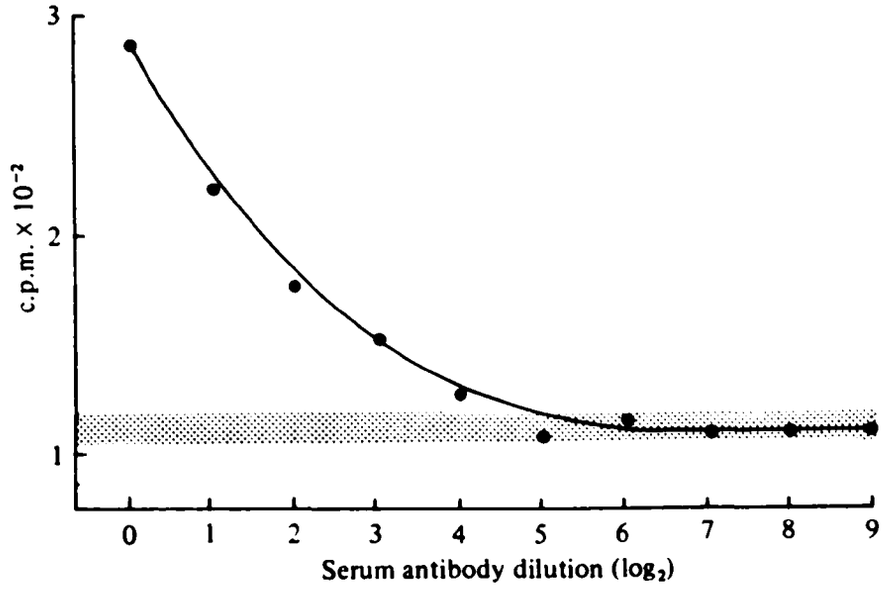

Fig. 2. The mean counts of ^{125}I-labelled anti-parvovirus globulin bound to pairs of beads incubated with serial two-fold dilutions of reference positive serum in reference negative serum. The shaded bar represents the area bounded by the 95% confidence limits of 30 replicate beads incubated with the reference negative serum.

observed with the crude serum antigen, although the rate of decline was less steep than that obtained with the PBS washed antigen preparation. There was no significant difference between the counts bound to beads incubated with the reference negative serum with any of the antigen preparations tested.

Although the most sensitive test incorporated washed, concentrated antigen at a dilution of 1 in 20, this was too expensive in its use of the limited amount of antigen available. Accordingly a compromise was adopted whereby the antigen used in all future tests was untreated St virus diluted fourfold in PBST.

Titration of reference positive serum. To determine the sensitivity of the assay in terms of ability to detect decreasing amounts of virus-specific IgM, twofold dilutions were made of the reference positive serum in the reference negative serum. Each dilution was assayed for virus-specific IgM after a further tenfold dilution in PBST (Fig. 2) Beads incubated with the reference positive serum bind 2½ to 3 times as much radiolabel as beads incubated with the reference negative serum. As the amount of anti-parvovirus IgM is reduced by diluting the reference positive serum in reference serum, the amount of viral antigen and thus radiolabelled antibody binding to the beads decreases. The shaded bar on the figure represents the 95% confidence limits of 30 replicate beads of the radioactivity bound to beads incubated with the reference negative serum. The assay is thus capable of detecting one-eighth the concentration of the parvovirus-specific IgM found in the reference serum.

Control studies

The BIs of the 162 sera considered unlikely to contain parvovirus-specific IgM are shown in Table 2. Sera from Jamaican children suffering from sickle cell disease with no history of aplastic crisis yielded BIs ranging between −9 and 14, with a

https://doi.org/10.1017/S0022172400070169 Published online by Cambridge University Press

Table 2. *Binding indices of control sera*

Control group		Number tested	Mean binding index (range)
Sickle cell anaemia	Jamaica	29	2·3 (−9 to 14)
	England	35	4·5 (−5 to 24)
Normal U.K.	Children	48	0 (−11 to 26)
	Adults	50	0 (−15 to 19)

mean value of 2·3. Comparable sera from sickle cell patients resident in England gave BIs with a somewhat higher mean value of 4·5 (range −5 to 24) although these values were not statistically different ($t = 1{\cdot}7932$, $0{\cdot}05 < P < 0{\cdot}1$, df = 62) from those of Jamaican patients.

Sera from patients with no haematological disease gave lower BIs than those obtained from children with sickle cell disease; the mean values for normal children and adults both being 0, with ranges of −11 to 26 and −15 to 19 respectively. There is no significant difference ($t = 1{\cdot}4000$, $0{\cdot}1 < P < 0{\cdot}2$, df = 96) between the results for haematologically normal adults and children. However, when the results from all the patients with sickle cell disease are compared with all of the haematologically normal patients, the former give significantly higher values ($t = 3{\cdot}4578$, $P < 0{\cdot}001$, df = 160).

Investigation of cases of aplastic crisis

In 18 cases of aplastic crisis infection with parvovirus had been diagnosed on the basis of the detection of viraemia or seroconversion by CIE. Forty sera from these cases were tested in the MACRIA for parvovirus-specific IgM (Fig. 3). Sixteen sera were taken from 15 of these cases in the acute stage of illness within three days of the onset of symptoms. Ten of these sera bound significantly more parvovirus-like antigen than did negative sera, indicating the presence of specific IgM. The remaining six sera which gave BIs within the negative range contained parvovirus-like antigen detected by CIE. Early convalescent sera, taken between four days and four weeks after the onset of symptoms were available from eight of the patients. Each of these eight sera gave a BI above the negative range, and of the order of 100. As the period from the onset of symptoms increased, the BIs of the later convalescent sera decreased; sera taken up to 10 weeks after the onset of symptoms all gave BIs above the negative range while sera taken 130 days or more after the onset of illness all fell within the negative range.

In 17 of these 18 cases, at least one serum specimen gave a BI above the negative range. In the remaining case, neither the acute-phase specimen, which was taken on the first day of illness and contained parvovirus-like particles, nor either of the convalescent specimens, taken 184 and 247 days later, gave BIs above the negative range.

In addition to these 18 cases of aplastic crisis in which parvovirus infection had been diagnosed by CIE, serum specimens were available from 20 further cases of

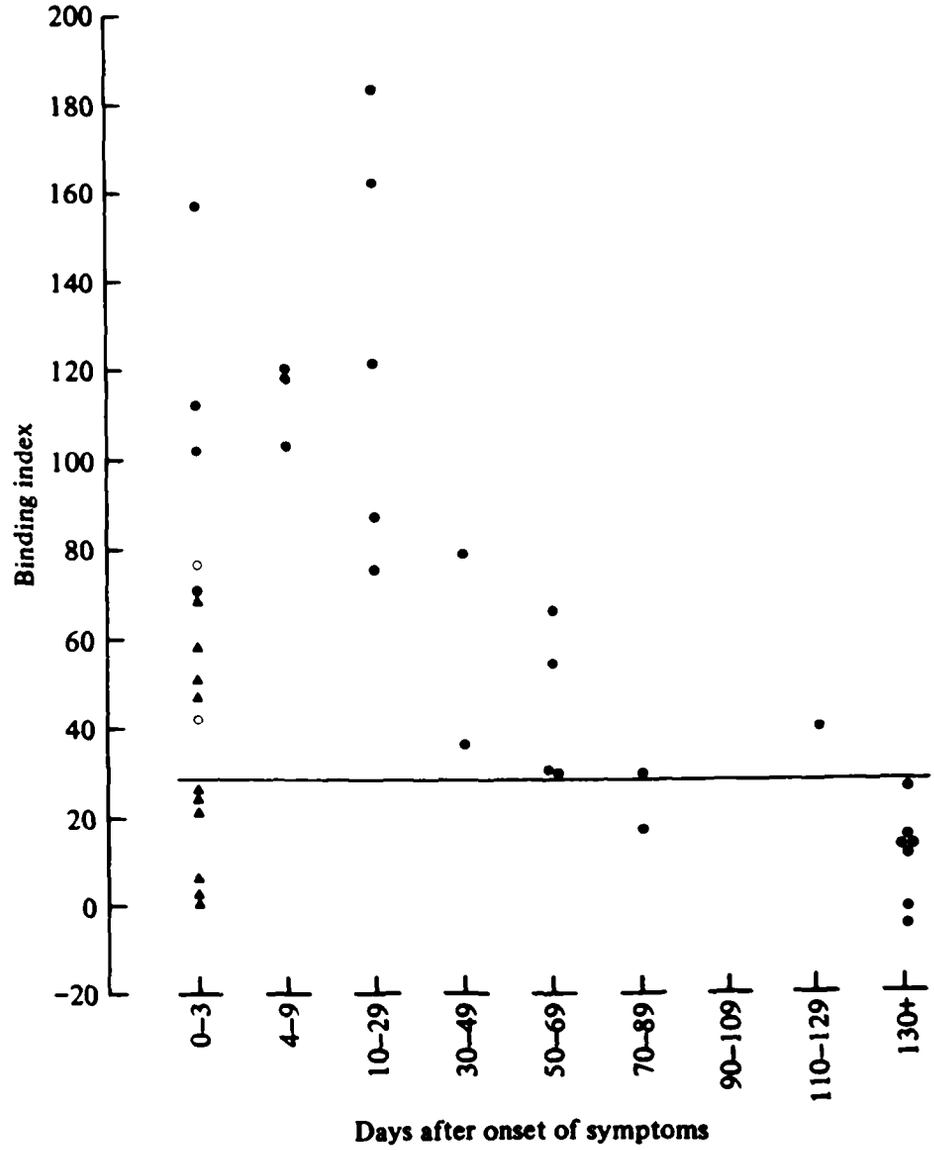

Fig. 3. The binding index of sera taken from 18 cases of aplastic crisis in which parvovirus infection was diagnosed by CIE, by either detection of antigen in acute phase serum or by antibody seroconversion. The symbols indicate antigen positive sera (▲), CIE antibody positive sera (●) and CIE antibody negative sera (○). The horizontal bar represents a BI of 28, which is used as a cut-off value to distinguish positive from negative MACRIA results.

aplastic crisis occurring in the period 1979-80. These 20 cases had been investigated by CIE and all were viral antibody positive. However, detection of antigen in acute sera, or seroconversion for anti-parvovirus antibody could not be demonstrated, and therefore recent infection could not be proved. These cases were examined for evidence of recent infection with the parvovirus-like agent by testing the 32 available sera for virus-specific IgM (Fig. 4).

In 10 cases serum specimens had been taken in the acute phase of illness, within three days of the onset of symptoms. Each of these sera gave BIs significantly above negative levels, of the order of 100, indicating the presence of parvovirus-specific IgM. Sera taken from a further seven of the cases between four days and four weeks after the onset of illness also showed evidence of virus-specific IgM

https://doi.org/10.1017/S0022172400070169 Published online by Cambridge University Press

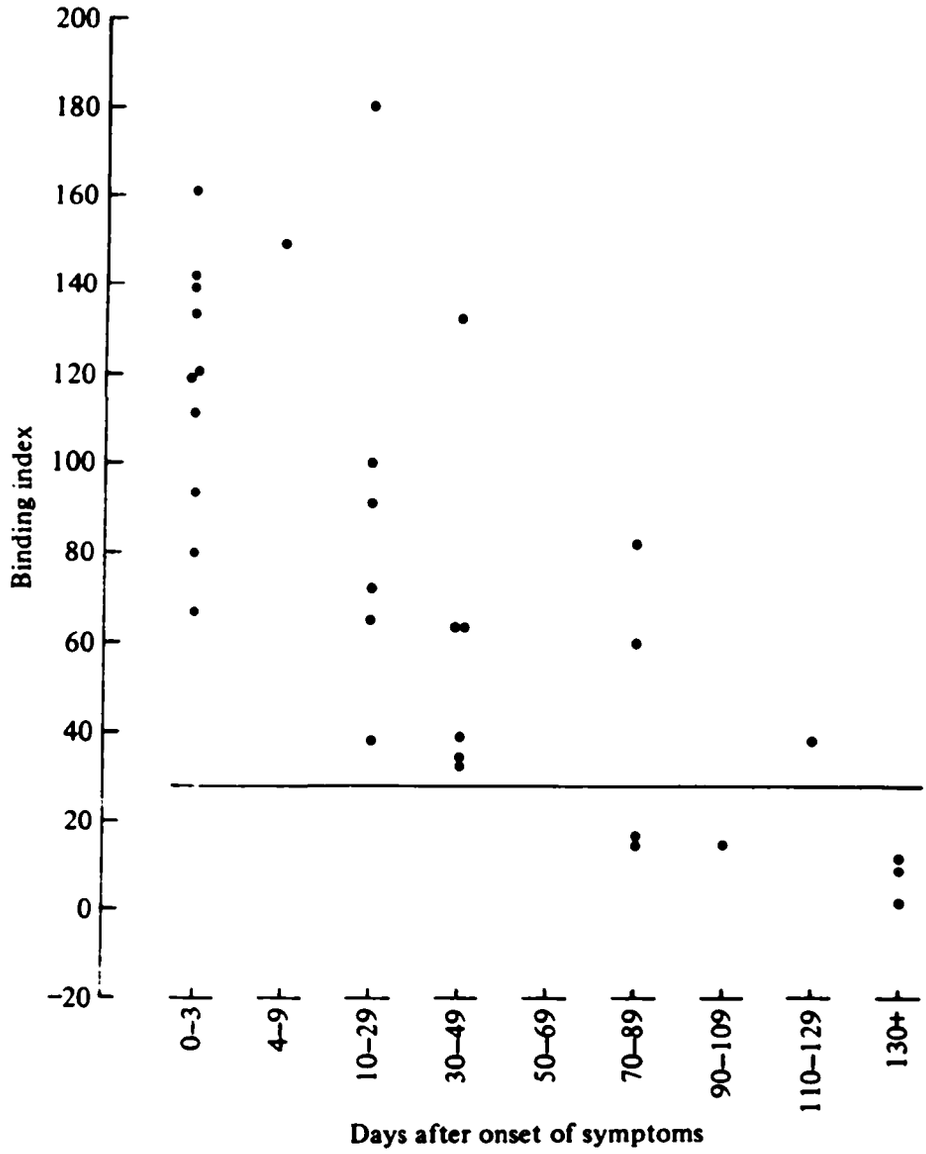

Fig. 4. The binding index of sera taken from 20 cases of aplastic crisis in which parvovirus infection could not be demonstrated by CIE. The horizontal bar represents the cut-off BI of 28.

giving BIs above the negative range. Thereafter, with increasing time following the onset of symptoms the BIs tended to decrease, so that specimens taken 130 days or more after the onset of illness gave BIs within the negative range.

Thus in 17 of 20 cases not diagnosed by CIE, at least one specimen gave a BI of greater than 28 and was considered to contain virus-specific IgM. In the remaining three cases only a single specimen of serum was available taken 82 days, 1 year and 18 months respectively after the aplastic crisis.

Seroconversions without aplastic crises

In addition to diagnosing infection with the parvovirus-like agent in 18 cases of aplastic crisis, CIE has provided evidence of infection in seven children with homozygous sickle cell disease without the occurrence of haematological abnormality. The seven children demonstrated seroconversion to the parvovirus-like agent, and these cases of apparent 'silent' infection were investigated using the parvovirus MACRIA (Table 3).

https://doi.org/10.1017/S0022172400070169 Published online by Cambridge University Press

Table 3. *Seroconversion in children with sickle cell disease, occurring without symptoms*

Case	Binding index: CIE antibody-negative serum	Binding index: CIE antibody-positive serum	Time interval (days)
1	−3	137	21
2	15	96	25
3	−7	97	17
4	17	113	29
5	−3	54	49
6	5	8	13
7	6	−6	190

In cases 1 to 6, the two sera of each pair demonstrating seroconversion were taken within 50 days of each other. In cases 1 to 5, the BI of the second, antibody-containing serum was above the negative range, indicating the presence of virus-specific IgM. In case 6, although the interval was only 13 days both sera gave BIs within the negative range; no virus-specific IgM was detected, indicating that this patient had not suffered a recent primary infection. In case 7 the CIE antibody-positive and -negative specimens were taken at an interval of 190 days. Both specimens gave BIs within the negative range.

DISCUSSION

The use of M-antibody capture radioimmunoassay (MACRIA) for the detection of virus-specific IgM was first described by Flehmig *et al.* (1979) as a means of diagnosing infection with hepatitis A virus. More recently this type of assay has been applied to the diagnosis of rubella (Mortimer *et al.* 1981) and the detection of IgM anti-hepatitis B core antigen (Tedder & Wilson-Croome, 1981). This investigation describes the development of a MACRIA for the detection of IgM antibody specific for the parvovirus-like agent B19, and its use in diagnosing infection with this virus.

Many of the reagents and procedures used in this test are common to other MACRIA systems; the solid-phase polystyrene beads and the method of their preparation are as described previously (Tedder, 1981). The dilution at which the test serum is reacted with the beads varies widely in different test systems; in the MACRIA for antibody to hepatitis antigens, test sera are diluted 1 in 1000 to 1 in 10000, while in the rubella MACRIA Mortimer *et al.* (1981) recommend the lower dilution of 1 in 35. In the assay described here, the low dilution of 1 in 10 of test serum was found to yield the best results; increasing the dilution of test serum reduced the difference in radiolabel binding to IgM-positive and -negative sera. The reasons for these differing optimal dilutions of test sera are unclear although they may relate to a difference in the proportion of IgM which is virus specific following infection with different agents. It is to be expected that the parvovirus MACRIA

will be of lower sensitivity than previously described MACRIAs which use hyperimmune sera as the radiolabels and are able to use relatively high concentrations of their more abundant antigens. Accordingly, a lower dilution of test serum is used to achieve a higher probability of interaction with the relatively sparse antigen, thus providing greater opportunity for binding of the relatively low-potency radiolabelled antiviral antibody.

It is certain that the concentration of antigen used in this assay is suboptimal; the use of more concentrated preparations of the virus markedly enhances the test. The form of the curve relating radioactivity bound to antigen dilution suggests that inclusion of even more virus than could be used in this experiment would result in larger differences between the radiolabel bound to specific IgM-positive and -negative sera. These results are in accord with those of Mortimer *et al.* (1981) who found that at low concentrations of rubella antigen insufficient virus was bound to maximize label binding by test sera with high concentrations of anti-rubella IgM. However, the supply of antigen available for use in the test described here is very limited. To date the only source of the parvovirus-like agent is the serum of persons with an acute-phase viraemia and until a more plentiful source of antigen can be found by, for example, the *in vitro* propagation of the virus, supplies of antigen will continue to be limited. Accordingly the test described here uses the smallest amount of virus consistent with accurate discrimination between specific IgM-positive and -negative sera.

The limitation in antigen availability has also meant that it has not been possible to purify by affinity chromatography the specific anti-parvovirus portion of the total IgG fraction of the immune serum, or raise hyperimmune antisera in animals. The radiolabel used in this assay is thus of relatively low potency compared with the radio-iodinated antiviral globulins used in other MACRIA systems. Although Mortimer *et al.* (1981) reported no significant difference between the sensitivity of rubella MACRIAs utilizing radio-iodinated human antibody and a hyperimmune rabbit IgG it is to be expected that increasing the proportion of specific IgG in the radiolabel must lead to more specific and sensitive assays.

In spite of these limitations the MACRIA described here easily distinguishes between a serum taken 13 days after the viraemia was detected, which is likely to contain high-titre virus-specific IgM, and a serum taken 13 days prior to the antigen-positive specimen. The capacity of the test to detect lower concentrations of specific IgM was investigated by diluting the reference positive serum in the reference negative serum. The difference in radiolabel bound by the IgM-positive and -negative reference sera shows that the test will discriminate between an IgM-negative serum and one containing one-eighth the concentration of parvovirus-specific IgM present in the positive reference serum.

In using a newly developed test to establish a relationship between an infective agent and a clinical syndrome it is essential to avoid false positive results. Therefore stringent criteria must be used in analysing test results. Accordingly, 162 sera considered unlikely to contain parvovirus-specific IgM were tested to establish the range of BIs which could be expected of negative sera. The children with homozygous sickle cell disease who had no history of recent aplastic crisis gave

low BIs with a mean value of 3·2 ranging from −9 to 24. A group of haematologically normal adults and children was also tested. Since there is no clearly defined clinical syndrome associated with infection with the parvovirus-like agent in such individuals it is not possible to be sure that the group does not contain some instances of recent infection. Nevertheless the highest BI was 26. The mean BI (0) was significantly lower than that of the group with sickle cell anaemia (3·2). The reasons for this difference remain to be determined, but since the mean value for the controls with sickle cell anaemia is higher this was used to derive the BI above which results could be considered positive. The mean plus four standard deviations gives a BI of 28, a value which excluded the highest BI obtained with any control serum.

In order to determine whether the MACRIA with this cut-off value of 28 is a useful diagnostic test, sera from 18 cases of aplastic crisis in which parvovirus infection was diagnosed by CIE were tested. At least one serum from each of 17 of these cases was MACRIA positive. Six specimens taken within three days of the onset of illness were MACRIA negative but all these sera contained viral antigen by CIE. The other ten sera taken within three days of the onset of illness were MACRIA positive. Eight of these were CIE antibody positive but two were negative, indicating the greater sensitivity of MACRIA for the detection of antibody at this stage of the infection. Thereafter all sera taken up to ten weeks after the onset of symptoms were MACRIA positive although the amount of specific IgM diminishes with time. Specimens taken later than ten weeks after the illness are again likely to give negative results. This distribution of results is as expected, with an assay for virus-specific IgM. Although other MACRIA tests give consistently positive results up to five months after infection (Mortimer *et al.* 1981) the negative results obtained here with sera taken in the fourth month after infection further indicate differences in sensitivity between tests. The MACRIA failed to diagnose one of the 18 cases. Three sera were available from this patient; the first was taken on the day of onset of symptoms and was viral antigen positive and the other two were taken 184 and 247 days after the onset of illness.

A more important application of IgM antibody tests lies in their application to cases in which the first serum specimen taken is antibody positive. Accordingly the MACRIA was applied to sera taken from 20 cases of aplastic crisis in which diagnosis of infection with the parvovirus-like agent could not be made by CIE. A very similar distribution of results to those found with the previous group was obtained. All sera taken less than ten weeks after the onset of symptoms gave positive results, permitting diagnosis of infection in 16 cases. Sera taken 74 days and 73 days after the onset of symptoms were available from two of these cases, and these specimens were also MACRIA positive. However, a specimen taken 75 days after the onset of illness from another of these 16 cases gave a negative MACRIA result. Therefore single serum specimens taken after the tenth week following the onset of symptoms may not always permit a diagnosis of infection with the parvovirus-like agent. In spite of this a further patient from whom only a single, late convalescent serum specimen was available, taken 111 days after the onset of symptoms, gave a positive result (BI 36). As in the previous group, sera taken

more than 130 days after the onset of symptoms consistently gave negative MACRIA results.

Thus in 17 of the 20 cases of aplastic crisis in which CIE tests for both antigen and antibody seroconversion were negative, a diagnosis of recent infection with the parvovirus-like agent was made by detecting virus-specific IgM. In the remaining three cases, only single serum specimens were available; these specimens were taken late in convalescence at 82 days, 1 year and 18 months after the onset of symptoms respectively. Although two sera taken 111 and 128 days after the onset of illness were found to be positive, virus-specific IgM of this duration seems to be the exception rather than the rule.

Thus MACRIA provides a valuable aid in the diagnosis of infection with this parvovirus-like agent. Infection at or near the time of aplastic crisis could be proved by CIE in only 18 of the 38 cases studied. MACRIA permits identification of primary parvovirus infection with specimens taken up to ten weeks after the onset of symptoms. Sera giving high BI values of the order of 100 are indicative of infection occurring within the preceding four weeks. Application of the MACRIA to sera from the 38 cases of aplastic crisis has permitted the diagnosis of parvovirus infection, closely associated in time with crisis, in 34 of these children. Thus the number of cases of aplastic crisis associated with primary parvovirus infection has been increased from 18 to 35 by the use of this test.

In addition to these cases of aplastic crisis associated with infection with the parvovirus-like agent, a sequential serum antibody study of children with sickle cell anaemia attending King's College Hospital had revealed seven instances of seroconversion to anti-parvovirus antibody detected by CIE. None of these children had experienced aplastic crisis in the interval between the antibody-negative and -positive specimens being taken. When the paired sera demonstrating seroconversion were tested in the MACRIA, five of these cases were found to have anti-parvovirus IgM in the CIE antibody-positive specimen. The reasons for these five instances of asymptomatic primary infection are discussed elsewhere (Anderson *et al.* 1982). However, it is of interest to note that each of these five children had received a transfusion of packed cells during the period of seroconversion. While it is theoretically possible, though unlikely, that CIE had detected passively transferred antibody in the second serum specimen, the detection of virus-specific IgM indicates rather that primary infection had occurred. It is unlikely that each of the transfusions had contained virus-specific IgM. Moreover the relatively small volume of plasma included in these transfusions together with the dilution occurring in the recipient's body would render IgM antibody from this source virtually undetectable, and certainly could not account for the high levels of virus-specific IgM found in these patients.

In the remaining two patients in whom seroconversion had occurred, virus-specific IgM could not be detected in any of the serum specimens. In one of these cases the interval between the CIE antibody-negative and -positive specimens was 13 days. From the data shown, it is obvious that if primary infection had occurred at any time within these 13 days, the specimen containing antibody detected by CIE would have contained detectable virus-specific IgM; in none of the 42 sera

from cases of aplastic crisis taken within 70 days of the onset of symptoms and found to contain antibody by CIE did the MACRIA fail to detect anti-parvovirus IgM. Thus in this case it would appear that re-exposure to the virus had resulted in the serum antibody level being raised to the relatively high level required for detection by CIE. This example illustrates the value of this test in permitting the differentiation between primary infection and re-exposure of immune individuals.

In the second of these IgM-negative cases of seroconversion no insight can be gained into the nature of the infection; the interval between the specimens was 190 days. In none of the 40 cases of primary infection studied has IgM antibody been detected in specimens taken more than 130 days after the onset of symptoms. Thus it is possible that primary infection may have occurred shortly after the first CIE antibody-negative specimen was taken. Alternatively, the patient may have been re-exposed to the virus during this period, causing his serum IgG level to be raised to that detectable by CIE. Work is currently in progress to develop a more sensitive radioimmunoassay for IgG antibody to this parvovirus-like agent so that cases of this kind may be resolved. The use of such an assay will confer the additional advantage of permitting the identification of sickle cell anaemics who are at risk from infection with this agent.

While the technique of CIE in detecting both the parvovirus-like antigen and its antibody has been of value in indicating the relationship between infection with this agent and aplastic crisis in children with sickle cell disease, the use of MACRIA in detecting virus-specific IgM has already markedly reinforced this association. It is hoped that the use of this test in investigating other clinical syndromes of presumptive infectious aetiology will add to our knowledge of the activity of the agent.

We wish to thank, for their co-operation in screening blood donations for parvovirus-like antigen and antibody, Dr Judith Kemp, her staff, all blood donors involved and especially the person who donated the antigen-positive unit and her family. In addition we would like to thank Mrs D. Blewer for help with the typescript. One of us (S.E.J.) is supported by a grant from the National Fund for Research into Crippling Diseases. The authors are grateful to the Rayne Management Committee for providing research facilities.

REFERENCES

ANDERSON, M. J., DAVIS, L. R., HODGSON, J., JONES, S. E., MURTAZA, L., PATTISON, J. R., STROUD, C. E. & WHITE, J. M. (1982). The occurrence of infection with a parvovirus-like agent in children with sickle cell anaemia during a two year period. *Journal of Clinical Pathology*, in press.

COSSART, Y. E., FIELD, A. M., CANT, B. & WIDDOWS, D. (1975). Parvovirus-like particles in human sera. *Lancet* i, 71–73.

FLEHMIG, B., RANKE, M., BERTHOLD, H. & GERTH, H.-J. (1979). A solid phase radioimmunoassay for detection of IgM antibodies to hepatitis A virus. *Journal of Infectious Diseases* **140**, 169–175.

MORTIMER, P. P., TEDDER, R. S., HAMBLING, M. H., SHAFI, M. S., BURKHARDT, F. & SCHILT, U. (1981). Antibody capture radioimmunoassay for anti-rubella IgM. *Journal of Hygiene* **86**, 139–153.

PATTISON, J. R., JONES, S. E., HODGSON, J., DAVIS, L. R., WHITE, J. M., STROUD, C. E. & MURTAZA, L. (1981). Parvovirus infections and hypoplastic crises in sickle cell anaemia. *Lancet* i, 664–665.

PAVER, W. K. & CLARKE, S. K. R. (1976). Comparison of human fecal and serum parvo-like viruses. *Journal of Clinical Microbiology* **4**, 67–70.

SALACINSKI, P., HOPE, J., MCCLEAN, C., CLEMENT-JONES, V., SYKES, J., PRICE, J. & LOWRY, P. J. (1979). A new simple method which allows theoretical incorporation of radioiodine into proteins and peptides without damage. *Journal of Endocrinology* **81**, 131–137.

SCHNEERSON, J. M., MORTIMER, P. P. & VANDERVELDE, E. M. (1980). Febrile illness due to a parvovirus. *British Medical Journal* **2**, 1580.

SERJEANT, G. R., TOPLEY, J. M., MASON, K., SERJEANT, B. E., PATTISON, J. R., JONES, S. E. & MOHAMED, R. (1981). Outbreak of aplastic crises in sickle cell anaemia associated with parvovirus-like agent. *Lancet* ii, 595–597.

TEDDER, R. S. (1981). *Laboratory Investigation of Rubella*. No. 16 PHLS Monograph Series (ed. J. R. Pattison), London: H.M.S.O.

TEDDER, R. S. & WILSON-CROOME, RUTH (1981). IgM-antibody response to the hepatitis B core antigen in acute and chronic hepatitis B. *Journal of Hygiene* **86**, 163–172.