The production of human parvovirus capsid proteins in *Escherichia coli* and their potential as diagnostic antigens

F. B. Rayment,1 E. Crosdale,2 D. J. Morris,2 J. R. Pattison,3 P. Talbot1 and J. J. Clare1*

1Department of Molecular Biology, Wellcome Biotech, Langley Court, Beckenham, Kent BR3 3BS, 2North Manchester Regional Virus Laboratory, Charlestown Road, Manchester M9 2AA and 3Department of Medical Microbiology, University College and Middlesex School of Medicine, University Street, London WC1E 6JE, U.K.

We have expressed a number of polypeptides derived from the capsid proteins of the human parvovirus B19 in *Escherichia coli*. These include native VP1 (84K) and VP2 (58K) proteins and also fusions to β-galactosidase containing differing amounts of the amino terminus of the VP1/2 polypeptide. Although each of these was expressed at high levels and the majority were produced as full-length proteins, only one was soluble. This soluble polypeptide, p132, is a β-galactosidase fusion protein that includes 145 amino acids from B19 which are entirely derived from the region unique to VP1. Despite containing such a small portion of VP1, which itself constitutes only 4% of total capsid protein, p132 reacted with all our known anti-B19 IgM-positive human serum samples. We conclude that this region contains epitopes which must be prominently exposed on the intact virus. We have demonstrated the use of this recombinant antigen in a simple diagnostic assay for B19-specific antibodies which can be used for initial screening of human serum samples. In a survey of 103 serum specimens, our ELISA positively identified all samples (19/19) which were positive by IgM antibody capture radioimmunoassay. The recombinant p132 antigen is efficiently produced and readily purified from *E. coli*, and its use as a diagnostic antigen should increase the availability of routine clinical testing for human parvovirus infection.

Introduction

The human parvovirus, B19, was first discovered in 1975 during screening of sera for hepatitis B virus by counter-immunoelectrophoresis (Cossart et al., 1975). It was not known to cause any disease in humans until 1981 when it was shown to be the causative agent of aplastic crises in patients suffering from sickle cell anaemia (Pattison et al., 1981; Sergeant et al., 1981; Anderson et al., 1982). Aplastic crises due to B19 infection have also been reported in patients with hereditary spherocytosis (Kelleher et al., 1983), pyruvate kinase deficiency (Duncan et al., 1983), β-thalassaemia intermedia (Rao et al., 1983) and hereditary erythrocytic multinuclearity (West et al., 1986). B19 also causes erythema infectiosum (Anderson et al., 1983) and a transient arthritis (Reid et al., 1985). When it occurs during the early part of pregnancy it leads to an increased risk of second-trimester abortion (Hall et al., 1990) and is also a cause of hydrops fetalis (Brown et al., 1984; Porter et al., 1988).

Like other autonomous parvoviruses, B19 has a very limited genome and is dependent on rapidly dividing host cells for its replication. Individual virions encapsidate either a positive or negative sense genome of 5.4 kb. The organization of this genome is similar to that of other paroviruses, with the left side encoding the non-structural proteins and the right side encoding the structural proteins, VP1 and VP2. These capsid proteins are encoded by a single open reading frame (ORF) but are translated from overlapping transcripts derived by alternative splicing of a common mRNA precursor (Ozawa et al., 1987). The smaller structural protein VP2 (58K) forms 96% of the capsid protein; the remainder is VP1 (84K) (Ozawa & Young, 1981). This striking difference in the levels of capsid proteins is due to differing abundances of the mature mRNAs and also to suppression of VP1 translation by multiple upstream AUG codons, which are removed during splicing of the VP2 transcript (Ozawa et al., 1988).

The potentially serious consequences of a B19 infection highlight the necessity for reliable diagnostic testing, particularly as other viruses (e.g. rubella; Best & Banatvala, 1989) may cause similar symptoms. Currently, the most widely used test for B19 infection is an IgM capture radioimmunoassay (MACRIA) which utilizes intact virus prepared from viraemic human serum as antigen (Cohen et al., 1983). The lack of an efficient *in vitro* propagation system for B19 places severe restric-
sections on the availability of viral antigen for this test and therefore limits diagnostic testing for human parvovirus. For this reason we have begun to investigate the potential of recombinant B19 proteins as antigens in alternative diagnostic tests for B19. Here we describe the expression of several B19 capsid-related proteins in *Escherichia coli* and demonstrate the use of one of these in a simple assay for B19-specific antibodies in human serum samples.

**Methods**

**Purification and cloning of B19 DNA.** Viral DNA was prepared from a human viraemic serum sample obtained from an asymptomatic individual. This was identified by counter-immunoelectrophoresis screening of blood donations, carried out in 1986 at University College, London to obtain a source of antigen for MACRIA. The serum was centrifuged for 5 min in an Eppendorf centrifuge at 14000 r.p.m. to remove debris. The supernatant was then layered on a 10% (w/v) sucrose cushion and centrifuged at 50000 r.p.m. in a Beckman SW50.1 swinging-out rotor for 3 h to pellet virus particles. The pellet was taken up in 130 μl of 1% SDS and incubated at room temperature for 90 min to disrupt particles. Viral protein was digested by the addition of 3 μl 1 M-Tris–HCl pH 7.6, 60 μl 100 mM-EDTA, 30 μl 1 M-NaCl, 47 μl distilled water and 30 μl Proteinase K (10 mg/ml), followed by incubation at room temperature for 15 min. DNA was then purified from the mixture by phenol extraction, ether extraction and ethanol precipitation. During this purification procedure the independently packaged positive and negative strands annealed to form dsDNA which was partially digested with *AflII* and *NdeI*. The 4-5 kb fragment produced in this digestion was then ligated into the vector pDEV39 to give pDEV73 (Fig. 1).

**Plasmid constructions.** The vector used for *E. coli* expression of B19 DNA sequences was pDEV19 (Fig. 1). This is a lacZ fusion vector for the expression of ORF DNA which uses the lac promoter (de Boer et al., 1983) to regulate expression. It is similar to pXY410 (Winther et al., 1986) but has the following modifications. Firstly, the lac gene has been inserted in place of lacY to give efficient repression of the lac promoter in any *E. coli* host strain. Secondly, synthetic DNA sequences carrying the transcription terminator from the *trpA* gene (Christie et al., 1981) have been inserted downstream of both lacZ and lacI. Lastly, pDEV19 has the same deletion of the 705 bp *HaeII* fragment as pAT153, leading to increased plasmid copy number (Twigg & Sherratt, 1980).

Each of the B19–lacZ gene fusions was constructed initially in pXY410 and then transferred to pDEV19 for expression analysis using *EcoRI* and *BamHI* sites which flank the B19 sequences (see Fig. 1 and 2). The hybrid gene in pDEV44 was made by isolating the *lacZ* fragment from pDEV73, which contains the VP1 gene (Shade et al., 1986) but has the following modifications. Firstly, the lac gene has been inserted in place of lacY to give efficient repression of the lac promoter in any *E. coli* host strain. Secondly, synthetic DNA sequences carrying the transcription terminator from the *trpA* gene (Christie et al., 1981) have been inserted downstream of both lacZ and lacI. Lastly, pDEV19 has the same deletion of the 705 bp *HaeII* fragment as pAT153, leading to increased plasmid copy number (Twigg & Sherratt, 1980).

![Fig. 1. (a) Map of pDEV73, the *AflII*-NdeI B19 clone used in this study. (b) The vectors used to construct B19–lacZ fusions, and native VP1 and VP2 expression vectors.](image)

Fig. 1. (a) Map of pDEV73, the *AflII*-NdeI B19 clone used in this study. (b) The vectors used to construct B19–lacZ fusions, and native VP1 and VP2 expression vectors.

![Fig. 2. Diagram showing B19–lacZ hybrid genes. The full-length VP1 and VP2 genes are shown in the upper part with the hybrid lacZ genes aligned below. The coordinates given are with respect to the full-length B19 genome (Shade et al., 1986). In each of the B19–lacZ fusions, the original VP1 start codon is converted to CTG and translation initiates within the vector, three codons upstream. The predicted size of the polypeptide encoded by each gene is indicated on the right. The M, of β-galactosidase is 116K.](image)

The fusion corresponding to pDEV50 was made by transferring the *EcoRI*-HindII fragment from pDEV44, which contains the VP1 gene up to nt 2881, into *EcoRI*- and *SmaI*-digested pXY410. The fusions contained in pDEV57 and pDEV60 were made in two steps. First, the VP1 reading frame up to the Nde1 site at nt 4681 was fused to the same translational start codon as described above by ligating the 1288 bp *AflII*-NcoI fragment from pDEV44 into pDEV73, giving the
plasmid pDEV74. Then the NdeI sites at nt 3604 and nt 4681 of B19 were fused in frame to the lacZ gene by inserting the corresponding fragments from pDEV74 into EcoRI-BamHI-digested pXY410 using synthetic NdeI-BamHI adapter oligonucleotides.

To express the full-length VP1 and VP2 genes, a synthetic DNA fragment containing the last portion of the VP1/2 reading frame was inserted at the 3' end of our original B19 AorII–NdeI clone in pDEV73. The DNA sequence of this fragment was taken from published data (Shade et al., 1986) and extends from the NdeI site (nt 4681) to the termination codon (nt 4789) followed by a SalI site to facilitate cloning. The VP2 gene was then transferred to pDEV19, giving the expression vector pDEV134, using this SalI site and the PstI site (nt 3146) just downstream of the VP2 start codon. Synthetic oligonucleotide linkers were used to complete the 5' end of the gene and to fuse it to the ATG codon of pDEV19, resulting in an amino acid substitution (Thr→Asn) at the second codon of VP2 (Fig. 3). pDEV135, which contains the complete VP1 gene, was constructed from pDEV134 by replacing the region between the EcoRI site and the Ncol site (nt 3381) with the VP1-containing EcoRI–Ncol fragment from pDEV44.

Induction of E. coli cultures and expression analysis. Duplicate cultures of E. coli strain MC1061 transformed with B19 capsid expression vectors were grown in 5 ml of Luria broth at 37°C in a shaking incubator for 16 h. One of each pair of cultures was induced by the addition of 20 ml of Luria broth containing 75 μg/ml isopropylthiogalactoside (IPTG), whereas the control culture had 20 ml of Luria broth alone added; both were incubated as before for a further 5 h.

To prepare total cell extracts, 1 ml of each culture was centrifuged in an Eppendorf microfuge to pellet the cells and the pellet was resuspended in 125 μl distilled water. The cells were lysed by the addition of 125 μl of 2 × SDS gel loading buffer (0.125 M-Tris·HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.02% bromophenol blue) (Laemmli, 1970) and boiled for 5 min. After cooling, 10 μl of each sample was run on SDS-polyacrylamide gels (Laemmli, 1970) and reacted with B19-specific human antiserum using standard procedures (Towbin et al., 1979). Solubility of expressed proteins was assessed using the lysozyme freeze/thaw procedure described by Makoff et al. (1989).

Antisera. Human anti-B19 polyclonal serum samples were obtained from patients convalescent from a B19 infection. The presence of anti-B19 antibodies in these sera was confirmed by MACRIA (Anderson et al., 1983).

Rabbit antiserum was raised against the putative B19 VP2 expressed from pDEV134. After SDS-PAGE of the insoluble pellet fraction from pDEV134 extracts, the putative VP2 band was excised from the gel. The gel slice was macerated by passing it through a 25-gauge needle and mixed 50/50 (v/v) with Freund’s incomplete adjuvant before subcutaneous injection into rabbits. The animals were boosted 14 days after the initial injection and serum samples taken after a further 14 days.

Purification of the p132 antigen. Induced cells were pelleted from the medium by centrifuging at 8000 g for 10 min at 4°C. The pellet was then weighed and ground with 2.5 × the pellet weight of alumina and 5 volumes of buffer (0.2 M-Tris·HCl pH 7.6, 0.2 M-NaCl, 0.01 M-MgCl₂·6H₂O, 5% glycerol). Alumina and cell debris were removed by centrifugation at 8000 g for 10 min at 4°C. Antigen was purified from the supernatant by precipitation with ammonium sulphate at 33% saturation. The precipitate was pelleted at 8000 g for 10 min at 4°C, taken up in TNE buffer (50 mM-Tris·HCl pH 8.0, 50 mM-NaCl, 1 mM-EDTA) and dialysed against TNE buffer. Purity of the preparation was assessed by gel-scanning. The protein was quantified using the Bio-Rad protein assay and then freeze-dried. Stored antigen was reconstituted by dissolution in 50 mM-Tris·HCl pH 8.0, 50 mM-NaCl, 50 mM-EDTA to give a protein concentration of 1 μg/ml.

Fig. 3. Vectors used to express full-length native (a) VP1 (pDEV135) and (b) VP2 (pDEV134). The nucleotide sequence at the beginning of each gene is shown.
Anti-B19 IgM ELISA. The wells of a plastic Falcon microtitre plate (Becton Dickinson) were coated overnight with 100 µl reconstituted B19 antigen [diluted 1:10 in carbonate buffer pH 9.6 (0.03 M-Na₂CO₃, 0.07 M-NaHCO₃)]. The next day, unabsorbed antigen was aspirated and the wells were washed three times with phosphate-buffered saline (PBS)/0.1% Tween-20. Test serum samples, a MACRIA-positive and a MACRIA-negative control serum sample were diluted 1:50 in diluent (PBS/1% bovine serum albumin/4% normal goat serum) and an aliquot of each was added to one well before incubation for 30 min at 37 °C. After washing as before, 100 µl of diluent was added to each well to block unoccupied protein-binding sites on the plastic. The wells were aspirated before detection of anti-B19 IgM binding by successive addition of 100 µl optimally diluted biotin-labelled goat anti-human IgM (Sigma), 100 µl optimally diluted peroxidase-labelled avidin (Sigma) and 100 µl o-phenylenediamine [0.404% with 0.5% (v/v) H₂O₂ in citrate phosphate buffer pH 5.0 (0.005 M-citric acid, 0.1 M-Na₂HPO₄)]. The optimal dilution (typically 1:400) of each batch of biotin-labelled antibody and peroxidase-labelled avidin was determined by checkerboard titration using a MACRIA-positive serum. The absorbances for each well were read at 492 nm, and the result was considered positive if it exceeded half the sum of the absorbances of the positive and negative control serum samples. In an attempt to remove rheumatoid factor (RF) from ELISA-positive MACRIA-negative serum specimens, 0.2 ml of a 1:20 dilution of serum in diluent (see above) was incubated for 18 h at 4 °C with 0.2 ml human IgG hyperimmune serum (RF-absorbent; Behringwerke), and the mixture was then centrifuged at 500 g for 5 min before 100 µl supernatant was retested by ELISA. In an attempt to remove anti-E. coli antibodies from similar serum specimens, 100 µl of a 1:100 dilution of serum was incubated at 4 °C for 18 h with 250 µg of control protein extract prepared from an induced culture of E. coli strain HB101 harbouring the β-galactosidase-expressing plasmid pDEV19; the mixture was retested by ELISA.

Results

The B19 genome was cloned by isolating viral DNA from a human viraemic serum sample. This B19 clone, pDEV73, contained 90% of the genome between nt 102 and 4680 (Fig. 1). DNA sequencing of the VP1/2 ORF showed that this isolate contained 13 differences compared to the published sequence (Shade et al., 1986). Most of these nucleotide differences gave no change in protein sequence; however, amino acid substitutions were found at the following positions in VP1, Lys→Gln at position 4 and Ser→Thr at position 476. Fragments containing regions of the VP1/2 ORF from this B19 isolate were expressed in E. coli under the control of the tac promoter using the high copy number ORF-expression vector pDEV19 (Fig. 1).

Expression of native B19 capsid proteins in E. coli

To express the native B19 capsid proteins the 109 nt DNA sequence missing from the 3' end of the VP1/2 ORF in our B19 clone was chemically synthesized and the entire VP1 and VP2 genes were then subcloned into pDEV19 to give the vectors pDEV135 and pDEV134, respectively.

Total cell lysates from induced cells containing pDEV134 and pDEV135 were analysed in SDS–polyacrylamide gels and proteins were visualized by staining with Coomassie blue as shown in Fig. 4a. Induced protein bands were seen which correspond in size to authentic VP1 (84K) and VP2 (58K). To determine whether these bands were recombinant B19 capsid proteins, gels were blotted onto nitrocellulose and the filters were then reacted with human convalescent serum. The induced 84K band from pDEV135 extracts gave a clear positive signal (Fig. 4b), confirming that the protein was VP1. However, no reaction was observed with the 58K induced band from pDEV134 extracts. The same result was obtained in Western blots using another five human serum samples, each shown to contain B19-specific antibodies by MACRIA using intact virus as the diagnostic antigen (data not shown). Further investigation of this phenomenon by immunoprecipitation was hampered because both VP1 and the putative recombinant VP2 were found to be in an insoluble form when expressed in E. coli (Fig. 4). To determine the identity of the 58K induced band in pDEV134 extracts, antibodies against this protein were raised in rabbits. As can be seen in Fig. 5, this serum cross-reacted strongly with the recombinant VP1 protein in pDEV134 extracts but not with control extracts from E. coli transformed with the vector pDEV19, showing that the 58K recombinant protein was indeed VP2.

Expression of B19 capsid-lacZ gene fusions in E. coli

The native B19 capsid proteins were insoluble when expressed in E. coli and so we explored the production of VP1/2–β-galactosidase fusion proteins for evaluation as
potential diagnostic antigens. Four hybrid B19–lacZ genes were constructed (Fig. 2). These contained varying amounts of the VP1/2 gene, beginning at the first codon of VP1 and extending to different points within the coding region. VP1/2 sequences were fused to the lacZ gene using restriction sites at nt 2881 (pDEV50), nt 3604 (pDEV78), nt 3896 (pDEV44) and nt 4681 (pDEV80). Where necessary, the reading frame was adjusted to give in-frame fusions using Bal31 deletions or synthetic oligonucleotide linkers as described in Methods. Fig. 6(a) shows a Coomassie blue-stained SDS–polyacrylamide gel loaded with protein extracts from cells transformed with each of the four B19–lacZ hybrid genes. Induced protein bands were seen in each case. In pDEV50 extracts (lane 4) the Mr of the major induced band corresponded approximately to the predicted size (132K). In addition, minor induced bands of similar Mr to β-galactosidase (116K) were visible, which were presumably due to proteolytic cleavage. A similar pattern is seen in extracts from pDEV44 (lane 6) and pDEV80 (lane 7), which encode fusion proteins of 167K and 198K, respectively, although with pDEV80 the full-length product is present at a lower level than the shorter cleavage products. Western blotting showed that the full-length fusion proteins in each of these extracts reacted with B19-specific human antiserum (Fig. 6b, lanes 4, 6 and 7). With pDEV78, which is predicted to give a fusion protein of 159K, no full-length product was observed (Fig. 6a, lane 5) but a major induced band of about 100K was seen which retained reactivity with B19 antiserum (Fig. 6b, lane 5). Thus, fusion proteins containing B19 capsid epitopes were produced with each of the four hybrid genes expressed. As a preliminary examination of the suitability of these recombinant proteins for diagnostic use, their solubility was determined. The 132K fusion protein expressed from pDEV50 alone was found to be soluble (Fig. 6). In an attempt to overcome the insolubility of the other fusion proteins, the same VP1/2 DNA sequences were expressed on the carboxy terminus of β-galactosidase. No change in solubility was observed (data not shown).
The soluble 132K VP1–β-galactosidase fusion protein (p132), which was expressed as about 10% of total cell protein, was tested for use as an antigen in an anti-B19 IgM-specific ELISA. The protein was isolated from the soluble fraction of cell extracts to a purity of about 80% by a single precipitation with ammonium sulphate (Fig. 7). An ELISA was established using this purified antigen by binding it to the wells of plastic microtitre plates and incubating with known MACRIA-positive and -negative human serum samples. Positive reactions were detected using biotin-labelled goat anti-human IgM, peroxidase-labelled avidin and o-phenylenediamine. The final protocol used for the ELISA is detailed in Methods and was chosen after optimizing the antigen concentration and reducing the background reactivity of MACRIA-negative samples by the introduction of a blocking layer of diluent. After optimization of the assay, it was found that enough purified antigen was obtained from 1 ml of induced culture to coat 650 wells. Titration of a MACRIA-positive sample indicated that the optimized ELISA could detect as little as 3 MACRIA units of B19-specific IgM antibody. This ELISA was then evaluated in comparison with MACRIA by testing unknown human serum samples.

Using ELISA, 103 serum specimens submitted for diagnosis of current B19 infection were tested before referral for MACRIA testing. The results are shown in Table 1. The sensitivity of the ELISA was excellent, since 19 out of 19 (100%) samples which were positive by MACRIA also gave positive reactions in the ELISA. The specificity was low, however, because only 59 out of 84 (70%) MACRIA-negative samples were also negative by ELISA. Of 25 specimens giving false reactions in the ELISA, 19 were retested after pretreatment with RF-absorbent, and 13 (68%) were then negative.

To reduce further the background of false positives, a control protein extract prepared from induced pDEV19-containing E. coli was used to pretreat the samples. Of 15 false positive samples tested in this way, 14 (93%) were then negative in the ELISA. Of these 14 specimens, 10 were samples in which false reactivity could also be removed by pretreatment with RF-absorbent whereas the false reactivity of the other four samples could not be so removed. Thus, simply by pretreating samples with the control E. coli extract, the specificity of the ELISA was greatly increased to a level similar to that of the MACRIA.

### Discussion

Diagnostic testing for parvovirus infection in humans is normally carried out by MACRIA. At present the extent of testing is restricted by difficulties in obtaining sufficient quantities of intact virus for use as antigen. This problem arises because patients with viraemia are usually asymptomatic. Other diagnostic assays have been described but these also rely on a supply of intact virus (Anderson et al., 1986; Schwartz et al., 1988). It is possible to propagate B19 in bone marrow cells (Ozawa & Young, 1987) but the limited availability of such cells and the low yield of virus prohibits large-scale antigen production using this approach. Recently, empty B19 capsids have been produced in genetically engineered Chinese hamster ovary cells (Kajigaya et al., 1989). Capsid proteins have also recently been expressed in COS-7 cells transfected with B19–SV40 hybrids (Beard et al., 1989). These approaches have more potential as general antigen production systems although the yield

### Table 1. Comparison of the p132-based ELISA with MACRIA

<table>
<thead>
<tr>
<th>MACRIA</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>19</td>
</tr>
<tr>
<td>-</td>
<td>25*</td>
</tr>
</tbody>
</table>

* Of 19 of these sera retested by ELISA after pretreatment with RF-absorbent, 13 were negative. Also, 14 out of 15 of these sera were negative by ELISA after pretreatment with control E. coli protein extract.
was no greater than that from infected bone marrow cells. In this paper we have described a diagnostic assay for B19 using a recombinant B19 capsid antigen which is efficiently produced and readily purified from E. coli, and have demonstrated the effectiveness of this test for initial screening of human serum samples.

Recombinant B19 proteins have previously been expressed in E. coli. However, except in the report of Morinet et al. (1989), these proteins have not been fully exploited for diagnostic use, probably owing to their insoluble nature. Cotmore et al. (1986) did not investigate their potential as diagnostic antigens, whereas Sisk & Berman (1987) used Western blotting, a technique not suitable for large-scale screening. Morinet et al. (1989) obtained soluble expression of a Protein A fusion protein containing the same B19 sequences as that found in our β-galactosidase fusion protein encoded by pDEV44. Although an IgM ELISA was developed, the yield of protein in this system was very low and the product consisted mainly of breakdown fragments.

We have expressed a number of B19 capsid-related polypeptides in E. coli both as mature VP1 and VP2 proteins and also as fragments fused to β-galactosidase. Although most of these proteins were expressed at high levels and as full-length polypeptides, all except one were insoluble. This soluble β-galactosidase fusion protein, p132, which is expressed by the vector pDEV50, contained the shortest segment of B19 polypeptide tested. Despite this, p132 was found to be broadly reactive with human anti-B19 antisera in Western blots, suggesting that it was suitable for use in a general screening assay for B19 antibody. We have shown this to be the case because all anti-B19 IgM-positive serum samples were shown to have reactivity against this antigen in the ELISA. These findings are remarkable because the only B19 sequences present in the pDEV50 fusion protein are unique to VP1, which constitutes only 4% of total capsid proteins. Clearly this region contains prominent epitopes which must be exposed on the intact virus. Previous studies have suggested that antibodies to VP2 appear before VP1 antibodies, which are produced only later in parvovirus infections (Schwartz et al., 1988; Kurtzman et al., 1989). This is based on the identification of IgM-positive serum samples, taken very early in infection, that apparently lacked antibodies to VP1. However, we found that all the MACRIA-positive sera tested reacted in our VP1-specific ELISA. The lack of detection of early VP1 antibodies in previous studies may be due to limitations of the detection method employed. The presence of B19 antibodies was determined by reactivity in Western blots with whole virus preparations. This procedure presumably has greater sensitivity for the detection of antibodies to VP2 than VP1 because VP2 is by far the predominant antigen.

In a direct comparison our recombinant-antigen IgM ELISA detected B19 antibodies in all samples (19 out of 19) which were found to be positive by MACRIA. Although 25 samples gave apparently false positive reactions in our ELISA, at least 13 of these contained RF. Pretreatment with a control E. coli protein extract was, however, more successful than pretreatment with RF-absorbent in removing false reactivity from sera, suggesting that this reactivity mostly reflected the presence of anti-E. coli antibody rather than RF. Using this modification, the specificity of the recombinant-antigen ELISA was similar to that of the MACRIA. Further validation is required to develop the assay fully but our initial data suggest that the recombinant-antigen anti-parvovirus IgM ELISA is useful for first-line screening of diagnostic specimens. Only positive sera need then be referred for confirmation by MACRIA, thus greatly diminishing the requirement for intact virus and providing an opportunity for more widespread clinical screening for B19 than has hitherto been possible. Our assay demonstrates the potential for diagnostic use of recombinant parvovirus antigens produced in E. coli and we are currently developing other diagnostic assays, using both the pDEV50 fusion protein, p132, and other recombinant antigens, which may remove altogether the current dependence on intact B19 virus.

We gratefully acknowledge the contribution of Dr S. J. Richmond to the initiation and execution of our evaluation of the ELISA. We wish to thank Miss U. Ayliffe for supplying us with B19-positive serum samples and Miss T. Silva for typing this manuscript.

References


