

## DETECTION OF THE HUMAN PARVOVIRUS B19 IN A BLOOD DONOR PLASMA IN RIO DE JANEIRO

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Human parvovirus B19, first described in a blood donor plasma in 1975 (Cossart et al., 1975, *Lancet*, 1: 72-73), is now recognized as causing erythema infectiosum (fifth disease) in healthy children (Anderson et al., 1983, *Lancet*, 1: 1378). In those with genetic haemolytic disease, B19 infection may precipitate an aplastic crisis (Duncan et al., 1983, *Lancet*, 2: 14-16; Kellher et al., 1983; *J. Pediatr.*, 98: 930-932; Pattison et al., 1981, *Lancet*, 1: 664-665). Other outcomes of infection include asymptomatic infection, fever, purpura, vasculitis, arthritis (Reid et al., 1985, *Lancet*, 1: 422; Shneerson et al., 1980, *Brit. Med. J.*, 280: 1580; Simpson et al., 1984, *Science*, 223: 1425) and in pregnant women the virus can have various adverse effects including spontaneous abortion, hydrops fetalis and stillbirth (Brown et al., 1984, *Lancet*, 2: 1033; Knott et al., 1984, *Brit. Med. J.*, 289: 1660; Weiland et al., 1987, *Lancet*, 1: 682-683). Erythema infectiosum can be misdiagnosed as another exanthem, especially rubella, and reliable data were accumulated only after the development of a specific diagnosis (Anderson et al., 1982, *J. Hyg. Camb.*, 88: 309).

The occurrence of B19 infection was first noted in Rio de Janeiro by Yoshida & Gaspar in 1983 (personal communication) who found B19 antibodies by counterimmunoelectrophoresis (CIE) in blood donors. IgG antibodies were also found by radioimmunoassay in three sera from pregnant women submitted for rubella diagnosis in 1985 (Nogueira, personal communication). The infection is widespread in the city of Rio de Janeiro, where the IgG antibody prevalence is 52% for adults aged between 20 and 30 years (Nascimento et al., 1988, *Abst. IV Cong. Bras. Virol.*: 108). A recent report from Belém (Freitas et al., 1988, *Bol. Epidemiol.*

XX: 1-4) showed the presence of B19 specific IgM by RIA in a serum sample collected from a child with clinical symptoms related to infectious erythema.

There are difficulties in diagnosing of B19 infections using IgG or IgM detection because of the shortage of antigen. B19 virus has only been cultivated in fresh human bone marrow cells with a low titer virus production and this is not suitable for use as antigen (Ozawa, et al., 1986, *Science*, 233: 883-886; Srivastava & Lu, 1988, *J. Virol.*, 62: 3059-3063). Therefore all the specific serological tests described until now use as antigen B19 particles purified from infected plasma obtained from blood donors as antigen (Cohen et al., 1983, *J. Hyg.*, 91: 113-130; Anderson et al., 1982, *J. Hyg.*, 88: 309; Anderson et al., 1986, *J. Clin. Microbiol.*, 24: 522-526).

In order to obtain B19 antigen to use in serological diagnosis in the Oswaldo Cruz Institute, we searched for the virus in the blood of healthy donors using the facilities at the National Cancer Institute - Rio de Janeiro (INCa-RJ). This program started in July 1987 on plasma bags received from the General Hospital of the Federal University of Rio de Janeiro (HU-UFRJ) and was extended in December 1987 to INCa.

The method used was counterimmunoelectrophoresis (CIE) using anti-B19 positive human sera and control B19 antigen kindly supply by B. J. Cohen from Central Public Health Laboratory, UK and by A. M. Courouce, from National Center for Blood Transfusion, France. The assay was done with a 0,1% agar/0,4% agarose gel in Tris-acetate 0,05M buffer, pH 8.6. The reactants were placed in well-against-well disposition which was used both to demonstrate the presence of specific B19 antibodies and to detect human parvovirus



Immunocomplex of parvovirus particles eluted from the gel and negative stained by 2% PTA, pH 7.2. Note one empty particle (237,000x; bar = 50 nm).

antigen. Any sera producing any line of precipitation at CIE against the reference sera was repeated once. If the result was confirmed, the immunocomplex was eluted from the gel and examined by electron microscopy (Fig.). We tested 300 plasma bags received during 1987 for the presence of B19 antigen and antibody and we could select four strongly antibody positive. One of these (HU 67130) was used as the source of B19 IgG for the plasma specimens processed during 1988 at INCA/RJ, which were screened only for the presence of antigen. Six thousand four hundred plasmas were submitted to CIE before a positive plasma was found. That plasma was collected on 7 October, 1988 from a 22 years old male living in the city of Cabo Frio in Rio de Janeiro state. After electron microscopy examination one sample was sent to VRL/CPHL/London where the presence of B19 antigen was confirmed by radioimmunoassay with monoclonal antibodies and dot-blot DNA hybridization.

It should be noted that we found the virus during springtime, and it was found in several other countries that erythema infectiosum outbreaks occur mainly during late winter and spring (Anderson & Pattison, 1984, *Arch. Virol.*, 82: 137-148). B19 virus is rarely found

in blood donors (Mortimer & Cohen, 1988, *Abstr. XX Congr. Int. Soc. Blood Transf.*: 272) and there are reports of negative results in tests on over 11,400 sera (Putland et al., 1988, *Aust. Microbiologist*, 9: 214). Nevertheless transmission of B19 by infusion of untreated clotting factor concentrates of plasma pools has been described (Mortimer et al., 1983, *Lancet*, 2: 482-484).

With the reagents obtained we are now able to set up an enzyme-linked immunoassay for the detection of both IgM and IgG anti-B19 antibodies as well as to distribute our HU67130 reference serum among other hospital blood banks that would be able to cooperate in the search of B19 antigen.

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