Non-permissiveness of synovial membrane cells to human parvovirus B19 in vitro

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The ability of cultured human synovial cells derived from synovial membrane and cartilage to support the replication of human parvovirus B19 was assessed. No viral DNA synthesis nor viral antigens were detected suggesting that B19 virus is not capable of replicating in synovial cells. The significance of this finding in relationship to the pathogenesis of parvovirus arthritis is discussed.

Human parvovirus B19 is a small (20 to 25 nm), non-enveloped virus containing a linear ssDNA genome of 5-5 kb (Summers et al., 1983). Although B19 is an autonomous parvovirus, it is extremely fastidious. Replication is restricted to mitotically active cells and has only been demonstrated in erythroid progenitor cells from the bone marrow (Mortimer et al., 1983; Ozawa et al., 1987; Srivastava & Lu, 1988) and foetal liver (Yaegashi et al., 1989; Brown et al., 1991). Although prenatal infection is associated with widespread viral infection of a variety of tissues including liver, heart and lung (Salimans et al., 1989), the virus is generally found in the erythroid progenitor cells which are widely distributed in the developing foetus. Such prenatal infections frequently cause spontaneous abortion or hydrops foetalis (Brown, 1989; Anand et al., 1987).

Post-natal infection with human parvovirus is associated with several clinical manifestations including erythema infectiosum (EI) or fifth disease (Anderson et al., 1983), and a transient aplastic crisis in patients with pre-existing haemolytic disease (Saarinen et al., 1986). EI is a mild exanethematous disease in children but is frequently associated with post-infectious polyarthritis in adults (Reid et al., 1985). This parvovirus-associated arthropathy is very similar to the joint manifestations found following rubella infection or immunization (Editorial, 1985; Anderson et al., 1985) with which it is commonly confused. It presents as a symmetrical polyarthritis of sudden onset in peripheral joints and is normally short-lived. As with rubella arthritis, joint symptoms are more common in adults than in children, and in women than in men (Ford et al., 1988). Moreover, parvovirus arthropathy, like rubella-associated arthritis, can persist for months or even years in certain individuals (White et al., 1985; Reid et al., 1985) and has been associated with rheumatoid arthritis in a few cases (Cohen et al., 1986; Stierle et al., 1987; Simpson et al., 1984). The acute joint symptoms induced by human parvovirus have been suggested to be immune-complex-mediated as they are usually coincident with the appearance of specific antiviral antibodies in serum. Whether infection of joint tissue occurs is not known as the presence of viral antigen or DNA in joint tissue has rarely been tested. However, human parvovirus has been detected in the synovial fluid of a patient with B19 virus-associated arthritis (Djikmans et al., 1988).

In view of the close similarity in the joint symptoms produced by human parvovirus and rubella virus and our previous evidence that, in vitro, cells and tissue derived from human foetal joints are highly permissive to rubella virus (Miki & Chantler, 1992; Huppertz et al., 1991), we examined the ability of B19 virus to replicate in mitotically active synovial membrane and cartilage (SMC) cultures.

Human parvovirus in the form of a high-titre patient’s serum was provided by Dr Peter Tattersall, Yale University, New Haven, Conn., U.S.A., who also supplied us with rabbit polyclonal antisera to the B19 virus non-structural and fusion proteins. Human foetal SMC cells were prepared as described previously (Miki & Chantler, 1992). Bone marrow (BM) cells from a patient with chronic myelogenous leukaemia was obtained from Dr K. Humphries (Terry Fox Laboratory, Vancouver, Canada). BM cells were cultured in Iscove’s modification of Dulbecco’s MEM (DMEM) containing 20% foetal bovine serum, 5% agar, lymphocyte-conditioned medium plus 1.5 units/ml erythropoietin.

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Fig. 1. Detection of B19 virus DNA in infected (lanes 1 and 2) or mock-infected (lanes 3 and 4) SMC in comparison with infected (lanes 5 and 6) or mock-infected (lanes 7 and 8) BM cells. Lanes 1, 3, 5 and 7 represent cell-associated DNA and lanes 2, 4, 6 and 8 show the result for supernatant fluid. SMC cultures (a) were examined for B19 virus DNA at (i) 0, (ii) 17 and (iii) 96 h post-infection, BM cultures (b) at (i) 8, (ii) 48 and (iii) 96 h post-infection. (c) M-11, a source of infectious B19 virus was used as the sample. Tenfold dilutions of extracted DNA served to determine the limit of detection and were equivalent to (lane 1) $5 \times 10^5$ to (lane 5) $5 \times 10^1$ genomes.

The ability of parvovirus B19 to replicate in SMC cells was determined by quantifying intracellular and supernatant virus DNA levels at three time-points post-infection. The BM cells infected in parallel were used as a permissive system to provide a positive control. In addition, attempts to detect viral antigen by immunoperoxidase staining were carried out.

In Fig. 1, the results of dot-blot analysis for viral DNA are shown. For this experiment, SMC and BM cells were infected at an m.o.i. of 10 to 20, and were incubated for various periods up to 96 h post-infection. At the time of harvest, both the culture medium and the cells were examined for virus DNA. The medium was treated with polyethylene glycol 3500 (to a final concentration of 10%) to precipitate supernatant virus which was then pelleted by centrifugation at 7800 g for 20 min. DNA was extracted from the viral pellet and cells by a modified Hirt procedure (Rosenthal et al., 1983). The extracted DNA was washed in 70% ethanol, vacuum-dried and dissolved in distilled water. The samples were denatured by heating to 70 °C for 30 min in 250 mm-NaOH. They were neutralized with an equal volume of 2 M-ammonium acetate, and applied by Hybond N nylon (Amersham) using a 96-well dot-blot apparatus. After air-drying the DNA was cross-linked to the membrane by exposure to u.v. light (312 nm) for 5 min. The probe to detect the B19 virus DNA consisted of a 700 nucleotide fragment of B19 virus DNA cloned into plasmid pAT153, obtained from Dr M. J. Anderson (University College, London, U.K.). The viral insert was excised with PstI and purified by electrophoresis in a 1-2% agarose gel, followed by isolation using Gene Clean (BioCan). The purified insert was labelled with digoxigenin 11-dUTP using the random priming method. Prehybridization (2 h at 42 °C) and hybridization (18 h at 42 °C) were carried out in 5 × SSC, 0.1% sodium lauryl sarcosine, 0.02% SDS and 0.5% blocking reagent (Boehringer Mannheim). Denatured labelled probe (50 ng) was added to the hybridization buffer. Following hybridization, the filters were washed and bound probe was quantified with an indirect immunodetection system using alkaline phosphatase and nitroblue tetrazolium, and 5-bromo-4-chloro-3-indolylphosphate as substrates.

The results shown in Fig. 1 indicate that B19 virus DNA replication in SMC could not be detected (lanes 1 and 2). A small amount of B19 virus DNA was seen in the cell-associated sample (lane 2, i) at 1 h post-infection, but this did not increase in subsequent samples taken at later time-points. It is presumed therefore to represent non-specific adsorption of viral particles to the cell surface, or uptake of virus which is unable to replicate and is degraded intracellularly. In contrast to the results for SMC, B19 virus DNA replication was detected in the permissive BM cells (lane 6). An increased amount of B19 virus DNA was found at 48 and 96 h post-infection relative to that found at 8 h post-infection. Low levels of B19 virus DNA were also detected in the culture fluid of these cells, equivalent to approximately $10^4$ infectious particles derived from $2 \times 10^3$ cells/culture. This indicates that complete replication and release of B19 virus was occurring in BM cells although at very low yield as has been described by others (Ozawa et al., 1986).

Attempts to detect B19 virus antigen in SMC cells infected for 24 to 96 h were also carried out by immunoperoxidase staining. Polyclonal antisera to either B19 virus capsid or non-structural proteins, provided by Dr P. Tattersall, were used as primary antibodies for the reactions. No specific viral antigen staining was obtained (data not shown), confirming the lack of B19 virus replication in SMC cells.

Our conclusion is that SMC cells, derived from normal human foetal joint tissue, are non-permissive to the replication of B19 virus. This contrasts with our results for a number of other arthritogenic viruses which were found to replicate in both cell and organ cultures of human joint tissue (Huppertz et al., 1991; Miki & Chantler, 1992). In particular, rubella virus, which is similar to parvovirus B19 in its clinical association with joint symptoms, was found to replicate to high titre in SMC cells and to penetrate deeply into the synovial membrane in organ culture (Huppertz et al., 1991; Miki & Chantler, 1992). Mumps virus, adenovirus and varicella-zoster virus also replicated to a limited extent in SMC, whereas coxsackie B virus was found to be totally restricted (Huppertz & Chantler, 1991; J. K. Chantler,
N. P. H. Miki & H. I. Huppertz, unpublished results). Ross River virus, a togavirus associated with epidemic polyarthritis in Australia, has also been shown to replicate in synovial cell cultures (Cunningham & Fraser, 1985a) although persistent infections which occur with rubella virus were not established (Miki & Chantler, 1992; Cunningham & Fraser, 1985b).

These results indicate that local replication and persistence of virus in synovial membrane cells may be involved in the pathogenesis of arthritis induced by certain viruses, particularly rubella virus and mumps virus. However the complete restriction of replication in joint tissue of parvovirus B19 reported here, and of coxsackie B virus described previously (Huppertz et al., 1991) suggests that immunopathological mechanisms alone may be involved in the joint inflammation observed in association with infection by these viruses. Parvovirus B19 arthritis has been suggested to be immune complex-mediated as the joint symptoms coincide with the appearance of antiviral antibody (White et al., 1985; Editorial, 1985). This is largely speculative however as analysis of synovial fluid for B19 virus antigen or DNA has rarely been carried out. In one study of 76 patients with rheumatoid or inflammatory arthritis, six patients with evidence of recent B19 virus antigen or DNA has rarely been carried out. In one study of 76 patients with rheumatoid or inflammatory arthritis, six patients with evidence of recent B19 virus infection were identified (as determined by the presence of IgM antibody specific for the virus). However neither B19 virus antigen nor DNA was detected in their synovial fluid (Cohen et al., 1986). In a contrasting study, Dijkmans et al. (1988) found evidence for B19 virus DNA in the inflammatory exudate of a 33-year-old woman with acute B19 virus-associated arthritis. Clearly the role of B19 virus in joint tissue inflammation of the majority of patients with parvovirus infection needs to be further investigated. Moreover, detection of viral antigen or nucleic acid in synovial samples is not indicative of viral replication in the tissue unless foci of infected cells containing intracellular B19 virus DNA and protein are identified. In cases of chronic parvovirus arthropathy, it would seem likely therefore that viral persistence occurs in an extra-articular site and that the prolonged symptoms are generated by immunopathological mechanisms (Anderson, 1987).

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