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SPECIAL ARTICLE

The emerging story of a human parvovirus-like agent

It is now seven years since Cossart and her co-workers reported finding a novel parvovirus-like agent (PVLA) infecting man. Although this virus was found as a systemic infection in both Dr Cossart's eleven subjects and a further four subjects described subsequently (Paver & Clarke, 1976; Schneerson, Mortimer & Vandervelde, 1980) it was not until 1981 that a clearly defined clinical syndrome was identified as attributable to infection with this agent. Work in the Departments of Microbiology and Haematology, King's College Hospital Medical School and collaboration with the MRC laboratory at Kingston, Jamaica has shown that infection with the PVLA is the major cause of aplastic crisis in children with sickle cell anaemia (Sergeant *et al.* 1981, Anderson *et al.* 1982*a*, *b*).

In the short history of human parvovirus research chance has played two important roles. The first was the discovery of the virus. In testing the serum of donated units of blood for the presence of hepatitis B virus, 'false positive' results were obtained with nine blood donations. These units of blood gave positive reactions when tested for hepatitis B virus surface antigen (HBsAg) by counter immuno-electrophoresis (CIE) using a human serum as a source of antibody. However, when these units of blood were tested by the newer, more sensitive passive haemagglutination test, no HBsAg could be detected. These specimens were then examined by electron microscopy, and were found to contain not the characteristic pleiomorphic particles of HBsAg but smaller, spherical particles with a relatively uniform diameter of 23 nm. The virus recovered from one of these units of blood was designated B19 (Cossart *et al.* 1975). In the same communication these workers reported finding an apparently indentical virus in blood samples from two further persons; one of these was a patient who had received a renal transplant one week earlier, while the second was a patient suffering acute hepatitis. This last observation raised the possibility of the B19 virus being one of the elusive non-A, non-B hepatitis viruses. The absence of jaundice in the other ten persons infected with this virus did not negate this possibility since the majority of infections with the known hepatitis viruses are anicteric, and many are completely subclinical. In common with other laboratories, we have examined the serum of patients suffering non-A, non-B hepatitis for PVLA antigen without success. None of the sera from 40 cases diagnosed at this hospital were found to contain the PVLA antigen, and only some 50% of these patients had specific antibody detectable by CIE. The proportion of non-A, non-B hepatitis patients with antibody to PVLA is consistent with both our own experience and those of Cossart *et al.* (1975) and Paver & Clarke (1976) who report that slightly more than 30% of the population have antibody detectable by either CIE or immune electron microscopy by the age of 16 years. Thus experience of PVLA infection appears no more common among patients with non-A, non-B hepatitis than among the normal population.

Infection with the PVLA is apparently a common event, occurring most often in childhood. Both our own studies and those of Edwards *et al.* (1981) show that the peak of antibody acquisition occurs between the ages of 4 and 6 years, and by the age of 16 one-third of subjects have PVLA antibody detectable by CIE. The true incidence of infection is likely to be higher than this; CIE is recognized as an insensitive assay and indeed two of the children studied sequentially at this hospital have only barely detectable antibody one year after infection. In a third child seroconversion by CIE occurred in the absence of a PVLA specific IgM antibody response, demonstrating that persons with no antibody detectable by CIE may nevertheless have experienced PVLA infection. Thus the 30 % of adults recognized as seropositive by CIE is likely to be an underestimate, constituted by that proportion of immune individuals with high resting titres of antibody.

The PVLA appeared initially somewhat enigmatic, able to infect without causing illness. However, Cant & Widdows (1975) found that three of the four blood donors from Dr Cossart's group of nine who were followed up, became ill shortly after giving blood; two complained of fatigue which was in one individual accompanied by leucopenia, while the third developed a rash. Leucopenia was also reported in two patients studied by Schneerson, Mortimer & Vandervelde (1980). These two young men also suffered fever, malaise and headache, beginning some nine days after being tattooed. Tattooing is a well recognized means of transmission of hepatitis B virus (Cossart, 1977), but although this route may have been responsible for the infection in these two young men, several observations would argue against PVLA being commonly transmitted in this fashion. First, in contrast to hepatitis B virus, no asymptomatic carrier state has thus far been reported for the PVLA. In each of the patients available to follow up in whom PVLA has been detected, the virus has been replaced within a few days or weeks by specific antibody. Thus the capacity of serum virus to act as a reservoir of infection is relatively limited. Second, again in contrast to hepatitis B virus infection in Western Europe and the USA, PVLA antibody is commonly detected in children who have been subjected to none of the traumatic procedures by which hepatitis B virus is transmitted in these cultures.

It is, nevertheless, possible that PVLA may be (and indeed may have been) transmitted in blood or blood products; CIE is no longer used as a method of screening blood donations for HBsAg but has been replaced by the more sensitive techniques of passive haemagglutination and radio-immunoassay (RIA). These methods are not only more sensitive but they are also more specific, so that the chances of detecting 'false positive' reactions due to PVLA have been eliminated. On the other hand, since this PVLA appears to be endemic in Britain, the proportion of patients receiving blood transfusion who are susceptible to PVLA infection will be very much smaller than is the case for hepatitis B virus (less than 70 % compared with more than 95 % for hepatitis B). Furthermore, the frequency of PVLA viraemia in adults is considerably smaller than is found with hepatitis B virus; estimates vary between one in four thousand and one in forty thousand compared with the figure of one in one thousand for HBsAg (Wallace, Milne & Barr, 1972).

If inoculation with infected blood is not the primary route of transmission, how is this virus spread? Two possibilities are worthy of consideration; the faeco-oral route and droplet spread infecting the respiratory tract. The faeco-oral route is at first sight an attractive proposition; during the 1970s a number of viruses such as the Norwalk, Hawai and W agents were described as 'parvovirus-like'. These agents were recovered in stool specimens from cases of acute gastroenteritis, and were able to produce the same symptoms in volunteers who ingested bacteria-free filtrates of these specimens. However, it would appear that these agents were wrongly classified as parvovirus-like, since they are larger in diameter than either the animal parvoviruses (20 nm) or B19 (23 nm). Apart from these colourfully named agents, 'small round viruses' are on occasion seen by electron microscopy in stool specimens from both patients suffering gastroenteritis and healthy individuals. Some of these particles have a diameter of 20–23 nm and may indeed be parvoviruses. The defective adeno-associated parvoviruses are not uncommonly found in faeces, together with their helper, adenovirus. The B19 agent exhibits no antigenic relationship with the adeno-associated parvoviruses (Cossart *et al.* 1975) and has not been reported as occurring together with either adenovirus or members of the alternative 'helper' genus, the herpes viruses; rather it has come to be regarded as a virus fully capable of independent replication. Particles morphologically very similar to B19 have been found in faecal specimens in the absence of helper virus, nevertheless (Paver *et al.* 1973). It would seem, however, that these faecal parvoviruses are distinct from those found in the blood; Paver & Clarke (1976) found no evidence of any antigenic relationship between the parvovirus-like particles found in the serum and those found in faeces. In this laboratory, PVLA has not been detected in any of over 200 faeces from young children examined by CIE. Furthermore, in those serum specimens which have been obtained from patients at the time of or shortly after the excretion of small round viruses, no evidence of recent infection with PVLA was found using a radioimmunoassay for PVLA specific IgM antibody (unpublished observations). Thus it appears that although human parvoviruses may be spread from person to person by the faeco-oral route, these enteric parvoviruses are antigenically distinct from the parvovirus-like agents found in the blood.

Infection accomplished by the inhalation of virus in droplets into the respiratory tract is an important route of transmission for many viruses. This mode of infection appears initially unpromising for the serum PVLA. The agent has not thus far been reported in respiratory secretions, although in the absence of systems for either the *in vitro* isolation of this virus or sophisticated serological tests we are left with the relatively insensitive technique of CIE as a means of virus detection. The only evidence for respiratory tract involvement in PVLA infection comes from studies of children with homozygous sickle cell disease. Four of five children suffering primary infection with this virus who escaped the more common and serious complication of erythroid aplasia of the bone marrow (aplastic crisis) had presented to the sickle cell clinic at King's with respiratory tract symptoms ranging from mild upper respiratory tract infection to chest infection (Anderson *et al.* 1982*a*)

If the route of transmission of PVLA found in serum remains obscure, so equally

did the epidemiology and clinical importance of these viruses until the fortuitous discovery of their association with aplastic crisis in children with sickle cell anaemia. It was in 1981 that chance played its second important role in human parvovirus research. In the pursuit of a research programme investigating the role of virus infections in the production of congenital abnormality, the incidence of PVLA infection was under study. All serum specimens received by the Department of Virology at King's College Hospital Medical School for the investigation of acute illness in children, were examined by CIE for PVLA antigen. After testing over 800 specimens, two were found to give a line of precipitate with anti-B19 antibody, and on examination by immune electron microscopy, parvovirus-like particles were seen. Enquiries of the clinician submitting these specimens for viral diagnostic testing revealed that both of these specimens had been obtained from children with homozygous sickle cell disease who presented for treatment in aplastic crisis.

Children with homozygous sickle cell (SS) disease suffer a chronic haemolytic anaemia, with peripheral blood haemoglobin levels ranging between 7 and 10 g/dl in contrast to the range of 10–15 g/dl in normal children. In an attempt to compensate for the continual lysis of their erythroid cells, the bone marrow of children with SS disease is hyperactive, and the elevated rate of red cell production is reflected in the high percentage of reticulocytes in the peripheral blood. In aplastic crisis, red cell production ceases for a period of some 5–7 days, the bone marrow is hypocellular with respect to erythroid precursors and the number of circulating reticulocytes drops to undetectable levels. Because of the short life span of the erythrocytes (of the order of 10–15 days in contrast to 120 days in a normal child) the cessation of red cell production results in a marked drop in peripheral blood haemoglobin levels and the children present in acute distress. For a number of reasons haematologists have long suspected aplastic crisis of having an infectious aetiology, not least of the reasons being the preponderance of cases among young children. Further, cases of aplastic crisis cluster in both time and space; epidemics of cases have been found to occur with a periodicity of some 3–5 years among the large population of children with SS disease in Jamaica (Sergeant *et al.* 1981), and there is a marked propensity for the condition to occur in family contacts. On circumstantial evidence, many infections have been implicated in the pathogenesis of aplastic crisis so that such episodes had come to be regarded as a non-specific response to infection. However, the fact that only rarely do persons with SS disease suffer more than one aplastic crisis in the course of a lifetime has always suggested the involvement of a single aetiologic agent. It was with interest therefore that we examined sera taken shortly after the onset of aplastic crisis from a further twelve children in the U.K. Six of these were found to contain PVLA antigen and parvovirus-like particles. These children subsequently developed anti-PVLA antibody detected by CIE. Of the remaining six cases, one child demonstrated seroconversion although the first serum specimen collected on admission to hospital did not contain detectable PVLA antigen. In five cases only specimens taken in the convalescent period were available for testing; each of these contained anti-PVLA antibody detected by CIE and examination of these sera for PVLA specific IgM antibody revealed that all five children had suffered recent primary infection with this virus. Thus in all of these 14 cases of aplastic crisis

occurring in the U.K. between 1979 and 1980, primary infection with the PVLA at the time of the crisis had occurred.

During the same period the sickle cell disease clinic at the MRC Laboratories in Jamaica had noted an epidemic of cases of aplastic crisis. In a collaborative study we investigated 28 Jamaican children presenting with aplastic crisis (Sergeant *et al.* 1981; Anderson *et al.* 1982*b*). Our results indicated that 23 of these children had suffered recent primary infection with the PVLA. Of the remainder, two of the children had developed bone marrow aplasia due to other causes (chloramphenicol treatment, and pneumococcal septicaemia) and with the other three only serum specimens taken three months after the aplastic episode were available for testing; each of these three contained anti-PVLA antibody detected by CIE.

Epidemics of infection with the PVLA thus occurred simultaneously in Jamica and the U.K. in 1979–1980, giving an attack rate of some 13% in the susceptible population of children with SS disease in both of these locations. The virus recovered from eight children resident in the U.K. and two in Jamaica is serologically identical with Dr Cossart's B19 virus, giving a line of identity in immunodiffusion. Further, the results to date indicate that these viruses are human parvoviruses, having a diameter of 21 nm, identical with adeno-associated human parvoviruses examined in parallel, and a buoyant density of 1·38–1·45 g/l (unpublished observations), within the range of animal parvoviruses. Definitive inclusion of this virus in the Parvoviridae awaits elucidation of the nature of the nucleic acid of the virus and its polypeptide composition.

The pathogenesis of disease in the only group in whom a specific syndrome has thus far been identified is at present unclear, but the following sequence of events appears likely. The virus gains entry into the body, probably by either the respiratory or alimentary tract. Certainly this is followed by dissemination of the virus around the body, at an early stage with respect to the onset of clinical symptoms since in one patient viraemia was detected in serum taken for routine purposes seven days before the patient presented in aplastic crisis. Studies are in progress to determine whether the depletion of erythroid precursor cells is due directly to their infection with the virus. It would appear that either as a result of infection of this tissue, or simultaneously with it, large quantities of virus are produced; the viraemia at the time of onset of symptoms is almost invariably sufficient to be visualized by direct electron microscopy. One must assume that during this viraemia infection is established in tissues sited to permit onward transmission of the virus to succeeding hosts although again the site of this replication remains to be determined.

Certainly the recognition of a defined clinical syndrome caused by infection with this virus has enabled some elucidation of the epidemiology of the human serum parvovirus. Appreciation of the special role which children with SS disease play in signalling infection in the community has permitted the identification of the cyclic behaviour of parvovirus infection within a given population; the observation of Sergeant *et al.* (1981) of sequential epidemics of aplastic crisis suggests that the PVLA behaves in a similar fashion to rubella, with periods of high incidence of infection occurring every 3–5 years.

Attempts to identify the incubation period between exposure to the virus and

either the onset of symptoms or excretion of the virus could make little progress prior to 1981 due to the vague and non-specific nature of those symptoms that could be attributed to infection with the B19 virus. The fact that the 15 infections documented in the first five years of research into human parvoviruses were discovered by chance bears witness to the difficulties encountered. With the recognition of the acute illness which PVLA infection produces in children with SS disease it has been possible to gain more definite information concerning the incubation period of this infection. Unfortunately our own studies of the epidemic of aplastic crises which occurred during 1979 and 1980 were conducted retrospectively, and examination of haematologically normal contacts for evidence of infection was not possible. However, examination of the dates of onset of symptoms in playmate and sibling contacts reveals two patterns. In the first, acute illness in a child with SS disease is followed by aplastic crisis in contacts within a period of one to seven days; in one example in this group two siblings and their playmate presented in aplastic crisis within 24 h of each other. A second pattern was observed in another pair of siblings; the sister presented in aplastic crisis and was found to have the PVLA in her serum at this time. Seventeen days later, her brother presented in aplastic crisis and the first serum specimen available for testing, obtained four days later, was found to contain PVLA-specific IgM although no virus could be detected. Rapid clearance of the virus from serum, and its replacement with PVLA-specific IgM antibody has been found in each of the cases of aplastic crisis studied, indeed we now regard the onset of symptoms in children with SS disease as heralding the termination of the viraemia. Clearly the period of one to seven days observed in the first pattern is unlikely to be sufficient for the production of disease, and virus excretion in a systemic infection. Rather, case to case intervals of this duration are likely to be the result of infection by a common source. Seventeen days would seem a more likely candidate incubation period. Interestingly, the incubation period predicted by applying the formula

$$T = 2\pi (AD)^{\frac{1}{2}},$$

where T is the interepidemic period, A is the average age at infection and D is the incubation period, proposed by Anderson & May (1982) is 16·7 days for an infection cycling with a periodicity of three years and a peak incidence in children aged five years.

As stated above the duration of the viraemia is, in our experience, relatively short. However, an observation by Paver & Clarke (1976) suggested that it could be of the order of weeks rather than days. These workers reported that in one of their two infected blood donors the virus was not only present to high titre in the donated blood but could also be detected in much lower titre in a second specimen taken 4½ weeks later. Viraemia of this duration seems to be the exception rather than the rule, however. The unit of blood which has provided the virus antigen for our own studies was obtained from a woman whose husband's serum contained neither PVLA antigen nor antibody at the time of his wife's donation. Fourteen days later our donor's serum virus had been replaced by circulating antibody. At

the same time, her husband exhibited a viraemia which was in turn replaced five days later by anti-PVLA antibody. Thus in this man the maximum possible duration of viraemia was 2½ weeks.

Currently, the major restraining influence on research into the PVLA is a shortage of virus. To date the virus has been obtained exclusively from blood donations, and as was noted earlier, the incidence of PVLA viraemia in blood donors is low. Much current research effort is therefore directed at attempts to grow the virus in tissue culture. Success in this field will of course go much of the way to fulfilling the potential for the development of a vaccine to protect children with SS disease from the severe and occasionally fatal effects of infection with the PVLA.

There is no doubt that the plentiful and secure supply of virus that culture will provide will greatly benefit all aspects of the research into the biology of this agent. The development of a system for virus growth in tissue culture will permit full characterization of this virus and its proper classification. Virus free from the contaminating proteins of human serum will facilitate the production of specific antisera and these, together with purified virus, will provide the means to develop more sensitive and specific assays of PVLA antibody. It is to be hoped that these second generation assays will not only provide improved tests for the diagnosis of infection but when applied to large scale population surveys will permit further elucidation of the epidemiology of this virus. A system for the *in vitro* culture of PVLA virus would offer potentially perhaps the most sensitive means of virus detection so that the mode of transmission of the virus could be identified.

Together with this work we may expect to see the role of parvovirus-like viruses in the production of disease more fully investigated. The data thus far available suggest that the PVLA is a true parvovirus so that by analogy with the pathology observed in animal parvovirus infections one may predict that human disease will be found in those tissues comprized of rapidly dividing cells. The bone marrow is just such a tissue and the erythroid aplasia seen in children with SS disease may prove to be a model of typical parvovirus disease. Of course it is only by virtue of the curtailed life span of the erythrocytes in these children that acute and severe disease is manifest and it may well be that significant pathology is only observed in similar organs with a high rate of cell turnover.

Perhaps the most exciting prospect is that to which our research was originally addressed; a copious viraemia and the predilection of the virus for rapidly dividing cells fulfil two requirements for intrauterine infection. The porcine parvovirus is now well recognized as a cause of fetal death and malformation (Mengeling, Paul & Brown, 1980) and the role of the equivalent human virus in the production of congenital abnormality is once more under investigation.

The culture of the human PVLA is perhaps an essential step for the infant field of human parvovirus research to take. It is to be hoped that with this accomplished human parvoviruses will come of age and that the next few years will see the fulfilment of much of the potential of this relatively novel and exciting human virus.

ADDENDUM

Since this article was written we have demonstrated, in collaboration with Dr Rao of Cooke County Hospital, Chicago, acute PVLA infection during aplastic crises in five adult patients (aged 18–23 years) with sickle cell anaemia and in one with thalassaemia intermedia. Moreover infection with PVLA has been demonstrated during an episode of red cell aplasia in a 13-year-old boy with pyruvate kinase deficiency (J. B. Kurtz, personal communication). These latter two cases demonstrate the capacity of PVLA to cause significant disease in any condition associated with increased erythropoiesis as a compensation for chronic haemolytic anaemia.

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