Letters to the Editor

Severe aplastic anaemia after parvovirus infection in the absence of underlying haemolytic anaemia

Infection with human parvovirus (HPV), the causative agent of erythema infectiosum,¹ is a well recognised precipitant of marrow aplasia in patients with a haemoglobinopathy and may unmask hereditary spherocytosis in otherwise asymptomatic patients. We describe a patient with no red cell abnormalities who has had progressive bone marrow failure after an erythema infectiosum-like illness and HPV seroconversion.

A 20 year old caucasian woman presented with a one month history of a rash on her legs and arthralgia. She was otherwise well and receiving no medication. On examination, bruising and a vasculitic rash on her shins were noted. There was fusiform swelling over the proximal interphalangeal and metacarpophalangeal joints of the hands, but no other abnormal findings. Investigations showed a haemoglobin concentration of 10.8 g/dl, a reticulocyte count of less than $5 \times 10^9/l$, a white cell count of $3.8 \times 10^9/l$ (neutrophils $1.3 \times 10^9/l$), a platelet count of $59 \times 10^9/l$. There was no evidence of haematinic deficiency and the blood film, haemoglobin electrophoresis, osmotic fragility and sucrose lysis test all yielded normal results. There was no evidence of immunological disorder or deficiency. Bone marrow aspirate and a trephine biopsy specimen were hypocellular. Initial and convalescent sera for HPV IgM and IgG titres were consistent with primary virus exposure of two to four weeks before her initial presentation. HPV DNA was not detected in either sample.

Her fiancé's HPV serology was also consistent with recent infection. Over the following 18 months her haemoglobin deteriorated to 7.0 g/dl, white cells to $2.5 \times 10^9/l$ (neutrophils $0.7 \times 10^9/l$), and platelets to $13 \times 10^9/l$. A repeat bone marrow examination again showed hypocellularity. In the absence of an HLA compatible donor for bone marrow transplantation she received treatment with horse anti-lymphocyte globulin and corticosteroids. This led to a transient improvement in the blood counts and she has continued to require blood product support.

Severe aplastic anaemia has an identifiable cause in only 50% of cases and in most of these it is drug related. Infectious causes are rare but there is an association with infectious hepatitis, non-A non-B hepatitis, Epstein-Barr virus, rubella and cytomegalovirus infections. Transient marrow failure is an acknowledged complication of HPV infec-

tion in patients with sickle cell anaemia, β -thalassaemia, pyruvate kinase deficiency, and hereditary spherocytosis, but the course is benign and the virus has not been implicated in severe aplastic anaemia in normal or abnormal people. When healthy adult volunteers underwent intranasal inoculation with HPV the ensuing mild febrile illness was followed by transient reticulocytopenia, mild anaemia, neutropenia and a fall in the platelet count. It was also noted that after two or three weeks three of the four initially seronegative volunteers developed rash and arthralgia consistent with a diagnosis of erythema infectiosum.²

Serum from patients with recent HPV infection has been shown to inhibit normal red cell colony growth in vitro,³ but the association of confirmed recent infection and the development of severe aplastic anaemia does not establish a causative link without the presence of the virus in the marrow. In view of the documented in vivo and in vitro suppression of haemopoiesis being directly related to the virus it is likely that this could occur. Aplasia has been described in immunodeficient patients. A child with combined immunodeficiency with immunoglobulins (Nezelof's syndrome) had a 15 month history of relapsing-remitting marrow failure. HPV DNA was isolated from the patient's serum at each deterioration and HPV capsid protein and DNA were shown in the bone marrow.⁴ Similarly, aplasia was described in a 3 year old boy with acute lymphoblastic leukaemia in remission following an upper respiratory tract infection associated with a papular rash over the face and chest. His serum was positive for HPV antigen and after three months when his blood counts had returned to normal he developed a clinically important anti-HPV IgG titre, having previously been seronegative.⁵ In both these cases impaired host immunity could have permitted the continued presence of HPV with subsequent marrow failure.

These cases and our own suggest that severe aplasia may develop in conjunction with HPV infection and that the marrow failure may be prolonged. We therefore suggest that HPV infection be considered in patients with severe aplastic anaemia even in normal subjects.

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HIV detection by DNA amplification

HIV-1 carriers are routinely detected by the presence of antibodies to HIV-1.¹ The antibody test may give uncertain results in cases where infection has occurred but seroconversion has not, and in neonatal blood samples from infected mothers, where the presence of maternal antibody does not prove that the fetus is affected.

I describe a DNA detection technique of high sensitivity. Evidence of HIV-1 DNA in the peripheral cells in cases of HIV-1 antibody positive haemophiliacs was obtained using the polymerase chain reaction (PCR)² with the heat stable DNA polymerase from *Thermus aquaticus*. As far as I am aware this is the first report of direct detection of HIV-1 DNA from peripheral blood without the use of molecular hybridisation.

Twelve asymptomatic, HIV-1-seropositive haemophiliacs aged between 22 and 47, who were known to have seroconverted between three and five years before sample collection, were studied. DNA was extracted from peripheral blood and amplified using two 20 base primers whose sequence was taken from a region of the HIV-1 pol gene³ conserved over a wide variety of isolates, including more recently isolated African HIV-1 strains. These primers (OLIGO-A2: 5'-CAGTAGTAATACAAGATAAT OLIGO-B3: 5'-ATCCTCATCTGTCTAC-TTG) are complementary to opposite strands of the HIV-1 pol gene separated by 80 bases of DNA, which was the target for

Agarose gel electrophoresis of DNA samples after 50 cycles of amplification. Tracks 1 to 11 show samples from HIV-1 seropositive patients. Track 12 was from a healthy control. Track 14 is a pBR322 Hae III digest.

amplification. DNA (1 µg) was denatured by boiling for five minutes in PCR buffer (Anglian Biotech Ltd) with 4 units of *Thermus aquaticus* DNA polymerase (Anglian Biotech Ltd). Subsequent cycles consisted of denaturation at 95°C (one minute), primer annealing (one minute), and DNA synthesis (three minutes).

The reaction product was analysed by electrophoresis in 4% agarose and stained with ethidium bromide. After photography the DNA was transferred to nylon membranes by native blotting and denatured in situ.⁴ The filters were then probed with two overlapping synthetic 45 base oligonucleotides complementary to 70 bases of the target sequence (5'-GTGACATAAAAGT-AGTGCCAAGAAGAAAAGCAAAAAT-CATTAGGG-3', 5'-CACACAATCATCA-CCTGCCATGTGTTTTTCATATACCC-TAATGAT-3') labelled by primer extension.

After 50 cycles of amplification it was possible to detect a 120 base fragment in 10 of 11 of the seropositive patients (figure). The case that was negative by PCR was one of a batch of blood samples which had been kept in a freezer that failed over a weekend, and resulted in extensive loss of DNA subsequently extracted (tracks 2, 3, and 4). A sample taken from this patient at a later date produced a positive result. Amplification of DNA from 15 negative controls (four normal volunteers and 11 seronegative haemophiliacs) did not produce a similar DNA fragment. The presence of HIV-1 sequences in the 120 base fragment was confirmed by hybridisation.

The PCR followed by "oligomer cleavage" has already been used with the Klenow fragment of DNA polymerase to amplify HIV-1 sequences from peripheral blood,⁵ although these studies did not use

primers from the pol gene and reported a lower (64%) detection rate in seropositive cases than the present study. So far, we have had no negative results from seropositive patients with the exception of one case where the blood sample was spoiled, and who was positive on later retesting.

Increasing the sensitivity of HIV-1 DNA detection by the PCR using Taq polymerase with primers from highly conserved regions of the HIV-1 genome could produce a test with a direct role in the assessment and management of HIV-1 carriers, provided that the increased selectivity of Taq polymerase amplification does not mean that sequence variation would result in false negative results. False positive gel bands can readily be eliminated by the use of a hybridisation probe. Further studies should permit the development of a technique which will be selective enough to eliminate the need for hybridisation.

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Urinary bladder fungus ball

A 71 year old, moderately well controlled insulin dependent diabetic caucasian man, with no previous history of surgery to his urinary tract, had required an indwelling bladder catheter following a cerebrovascular accident in 1984. This was removed after six months following several urinary tract infections. He continued to have intermittent dysuria, frequency, and urinary incontinence, and was referred in June 1985 to the surgeons for investigation.

Intravenous urography showed bilateral hydronephrosis with a faintly calcified laminated filling defect in the bladder (fig 1). Cystoscopy showed congested and inflamed bladder mucosa with a large mobile intravesicle mass measuring 60 × 40 mm. The prostate was not unduly large.

As the patient was not thought fit for surgery, daily bladder washouts with Noxyflex via an indwelling catheter were started. Despite this treatment he continued to have

Fig 1 Intravenous urogram showing bilateral hydronephrosis and faintly calcified laminated opacity in the bladder.