SHORT REPORTS

Alfa-2a recombinant interferon in HIV associated thrombocytopenia

We report a case of thrombocytopenia associated with human immunodeficiency virus (HIV) infection that was treated with interferon.

Case report

A male homosexual (aged 30) presented with bruising and gingival bleeding. His platelet count was $5 \times 10^9/l$ and his bone marrow contained increased numbers of megakaryocytes. An palpable spleen and absence of antinuclear-factor supported a diagnosis of immune thrombocytopenia. Prednisolone 60 mg daily raised the platelet count to $66 \times 10^9/l$, but he became jaundiced with "hepatic" liver function values. He had been a low grade hepatitis B carrier (hepatitis B surface antigen titre on radioimmunoassay 6.25, e antigen negative, and core antigen IgM equivocal) and was positive for HIV antibodies by Western blotting. After six weeks of prednisolone the hepatitis B surface antigen titre had risen to 134 (haemagglutination >8192) and he became positive for e antigen and core antigen IgM, findings consistent with reactivation of hepatitis B. Steroids were discontinued. The platelet count fell but responded ($250 \times 10^9/l$) to intravenous immunoglobulin 0.4 g/kg daily for five days. The effect was short lived and he required repeated intravenous immunoglobulin for a platelet count of less than $35 \times 10^9/l$. After mild trauma he experienced right loin pain and profuse haematuria, indicating renal haemorrhage, which was managed by platelet infusions and intravenous immunoglobulin. Liver biopsy at six months showed chronic active hepatitis.

Recombinant alfa-2a interferon, 3 MU on alternate days, was given subcutaneously. Core antigen IgM fell to equivocal values, e antigen became negative, and e antibody appeared. Liver function values became near normal. The platelet count, measured weekly, rose from 62 (SD 98) $\times 10^9/l$ to 110 (49) $\times 10^9/l$; the number of weeks the platelet count was below $35 \times 10^9/l$ fell from 10 to 0 and the requirement for intravenous immunoglobulin fell from 15 days to four days over this period, being given when the platelet count appeared as if it might reach less than $35 \times 10^9/l$ (figure). Interferon was continued for five months and then stopped; the platelet count fell to less than $35 \times 10^9/l$ and further intravenous immunoglobulin was required. Interferon was restarted after three months. The platelet count rose again, and, although the rise in the mean weekly count was not as pronounced (69 (27) $\times 10^9/l$), he did not require any further intravenous immunoglobulin over the four months, and the count did not fall below $35 \times 10^9/l$.

Platelet associated immunoglobulin was measured before we started the second course of interferon and during interferon treatment using a fluorescence activated cell cytometer (Epics C, Coulter Electronics). Platelets obtained by centrifugation from blood stored in edetic acid and washed in phosphate buffered saline were then fixed by 1% paraformaldehyde to prevent in vitro adsorption of immune complexes. They were then reacted with fluorescein isothionate conjugated antihuman IgG and IgM. Platelet associated immunoglobulin was calculated as % fluorescence using a standard curve. At the start of the second course of interferon, when the platelet count was $28 \times 10^9/l$, concentrations of both platelet associated IgG and IgM were raised (25 ng/ 10^6 platelets (normal <4.85) and 15 ng/ 10^6 platelets (normal <1 respectively). These concentrations fell with the platelet response. Immune complexes were not detected on the platelets or in serum.

Comment

This case illustrates that thrombocytopenia related to HIV infection is not always benign.¹ The cytopenia is thought to reflect an intact immune

Mean (SD) platelet count and platelet associated immunoglobulin concentrations in relation to interferon treatment.

response to HIV, occurring in up to 25-38%² of cases and resolving on progression to the full acquired immune deficiency syndrome (AIDS). The thrombocytopenia may be due to platelet autoantibody production³ or to viral related stem cell damage.⁴ Treatment is not always indicated unless there is bleeding or the count is very low. Treatment with steroids or splenectomy can precipitate Kaposi's sarcoma, opportunistic infections, and metabolic effects, and intravenous immunoglobulin is increasingly being considered as first line treatment.

This man developed chronic active hepatitis, which supports the notion that immunosuppressive manoeuvres should be avoided. The very high levels of platelet associated antibodies, particularly IgM, were at variance with the generally accepted mechanism of circulating immune complexes⁵ in homosexual associated idiopathic thrombocytopenia but agreed with the findings of others⁴ and confirmed a failure of control of the humoral immune system. The immune modulatory and anti-HIV activities of interferon might have been expected to exert an effect in such a patient. The use of interferon in our patient was indeed associated with an improvement in the platelet count, obviated the need for steroids, reduced the requirement for intravenous immunoglobulin, and deferred splenectomy. The reduction in platelet associated immunoglobulin in association with its use supports a causal effect. This approach requires further investigation.

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Regional Department of Infectious Diseases and Tropical Medicine, Monsall Hospital, Manchester M10 8WR

M E ELLIS, MRCP, DCH, consultant physician
K R NEAL, MRCP, registrar
C L S LEEN, MRCP, senior registrar

The London Hospital, Whitechapel, London E1 1EB

A C NEWLAND, MRCP, MRCPATH, senior lecturer in haematology

Correspondence to: Dr Ellis.

Treatment of thrombocytopenia with alfa interferon

Idiopathic thrombocytopenia has traditionally been treated with steroids¹ and immunosuppressive drugs² and, if these fail, by splenectomy. Recently high dose intravenous gammaglobulins have also been used successfully, particularly in children.^{3,4}

Human immunodeficiency virus (HIV) infection is associated with thrombocytopenia,⁵ which may be autoimmune. All the established treatments used in idiopathic thrombocytopenia have disadvantages in patients with HIV infection. Steroids and immunosuppressants may predispose to further infection, and splenectomy carries the risks of surgery. While not immunosuppressive, treatment with intravenous gammaglobulin usually requires admission to hospital and is expensive.

We describe two patients with unrelated causes of thrombocytopenia who received alfa (lymphoblastoid) interferon as part of their treatment for another disease and whose clinical and laboratory features of thrombocytopenia improved.

Case 1

A boy of Indian parents, born in 1974, was well until 1984, when easy bruising and epistaxes were noted. His platelet count was consistently below $30 \times 10^9/l$ and his bone marrow showed plentiful megakaryocytes. Autoimmune thrombocyto-

penia was provisionally diagnosed. He was positive for hepatitis B surface and e antigens at this time. His spleen was noted to be minimally enlarged on ultrasonography. He was suspected of having neonatally acquired chronic hepatitis B infection and was treated by a four week course of high dose steroids followed two weeks after withdrawal by a 12 week course of alfa interferon (Wellferon).

Prednisolone 2 mg/kg was begun and tailed to 0.5 mg/kg before stopping. During this period his platelets rose to within the normal range ($450 \times 10^9/l$) only to relapse when steroids were stopped ($30 \times 10^9/l$). Interferon was then begun at a dose of 2.5 mU/m^2 rising to 10 mU/m^2 thrice weekly and his platelet count again rose, varying between 150 and $300 \times 10^9/l$. When his treatment was discontinued the platelet count fell and bruising and epistaxes recurred. This did not respond to doses of prednisolone below 40 mg/day, on which he became Cushingoid. He was referred for splenectomy, after which his platelet count returned to normal.

Case 2

A homosexual patient born in 1949 was noted to be positive for hepatitis B surface antigen in 1980. In 1984 his platelet count was noted as $32 \times 10^9/l$. In 1985, when he still had thrombocytopenia, he was referred for treatment of his hepatitis B virus carrier state and was noted to be HIV antibody positive. Ultrasound showed no splenic enlargement and the thrombocytopenia was thought to be related to HIV infection. In both this and the previous case antiplatelet antibody testing was refused by the local laboratory because of the risk of infection. His platelet count fell to $1 \times 10^9/l$ and bruising and petechiae were noted. Two courses of intravenous immunoglobulin were given with beneficial but short lasting effect (see figure). A course of danazol was ineffective and was stopped when rising

Case 2. Patient with thrombocytopenia showing transient responses to intravenous gammaglobulin and sustained responses to increasing doses of interferon. Thrombocytopenia was symptomatic below $20 \times 10^9/l$.

transaminase activities suggested hepatotoxicity. After our experience with case 1 a third dose of globulin was administered followed by alfa interferon 2.5 mU/m^2 thrice weekly. On this regimen his platelet count slowly rose and all clinical signs of a bleeding tendency disappeared. The dose was gradually increased to 10 mU/m^2 and on each occasion there was a further rise in the platelet count. He remained well on interferon thrice weekly for one year.

Comment

Thrombocytopenia is a complication of some viral infections and, in particular, has been recognised as a common feature of HIV infection, possibly due to induction of platelet specific autoantibodies. Suppression of these autoantibodies by steroids or splenectomy can be effective but may induce immunosuppression. Interferon is known to be antiviral and immunostimulatory but can also, under some circumstances, inhibit antibody formation. Thus in the two patients described it may have inhibited viral replication or production of antiplatelet antibodies.

In severe thrombocytopenia haematoma formation from subcutaneous or intramuscular treatment is a risk. Intravenous interferon is a possibility in these circumstances. We conclude therefore that interferon may be a useful non-immunosuppressive treatment for thrombocytopenia, including that related to HIV infection.

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Royal Free Hospital of Medicine, London NW3 2QG

ANDREW M L LEVER, MD, MRCP, lecturer in infectious diseases
M GARY BROOK, MB, MRCP, senior registrar
IVY YAP, MB, MRCP, research fellow
HOWARD C THOMAS, PHD, FRCP, professor of medicine

Correspondence to: Dr Lever.

Concentrations of free serum digoxin after treatment with antibody fragments

The injection of antibody fragments (Fab) has been shown to be an effective method of treating digoxin toxicity.¹ During treatment, however, the concentration of digoxin no longer bears any relation to that given in standard therapeutic guidelines; there is a rapid rise in the concentration of total digoxin in the serum, nearly all of which is bound to the antibody fragments and is biologically inactive.^{2,3} We describe a patient who was treated with antibody fragments and consider how the measurement of free digoxin may help in managing similar cases.

Patient, methods, and results

The patient was an 82 year old woman who had been treated with digoxin 0.25 mg daily and frusemide 40 mg daily after a myocardial infarct eight weeks before. She was admitted with severe breathlessness and was found to have a ventricular tachycardia. This did not respond to lignocaine or amiodarone; because the serum digoxin concentration was 5.8 µg/l she was given 120 mg antibody fragments specific for binding digoxin (Digibind, Wellcome Foundation Ltd) intravenously before treatment with direct current shock was attempted. She reverted to sinus rhythm with a single shock of 25 joules and improved without complication. She resumed digoxin 0.0625 mg daily and was discharged seven days after admission.

Digoxin was measured in serum samples collected at intervals after the injection of antibody fragments. Total digoxin was measured using an Abbott TDX analyser. The method included pretreatment of the serum sample with an equal volume of 3% 5-sulphosalicylic acid in 50% methanol to denature the protein and release protein bound digoxin. We found in separate experiments that the addition of a large molar excess of fragments to samples containing digoxin substantially reduced the amount of digoxin obtained in the assay but that the roughly equimolar concentration of antibody fragments used clinically did not affect the measurement. When necessary samples were diluted with isotonic saline before protein denaturation to ensure that the concentration of digoxin was below the upper limit (5 µg/l) of the assay.

Free digoxin was separated from bound digoxin by centrifuging 2 ml of serum at 3500 g and 20°C for 30 minutes in a Centricon-10 microconcentrator (Amicon Corporation), which has a membrane excluding molecules larger than 10 000 daltons and hence retains antibody bound digoxin (approximately 50 000 daltons), while allowing free digoxin to pass through into the ultrafiltrate. Only 0.25 ml of ultrafiltrate was obtained, and this was assumed to have a negligible effect on the equilibrium between bound and free digoxin. The concentration of free digoxin was measured in the same way as total digoxin.

The table shows the concentrations of serum digoxin during the study. The

Assay of serum digoxin after treatment with antibody fragments

Time after injection of antibody fragments (h)	Concentration of serum digoxin (µg/l)		% Free digoxin
	Total	Free	
-1.5	5.8	*	
12	74.2	1.0	1.3
33	28.2	1.2	4.3
56	13.2	1.1	8.3
80	10.5	0.9	8.6
104	3.7	*	
128	2.7	0.5	18.5
152	1.9	0.3	15.8

*Insufficient serum to permit separation by ultrafiltration.