

Severe warm autoimmune haemolytic anaemia due to anti-Jk^a autoantibody associated with Parvovirus B19 infection in a child

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Dear Sir,

Autoimmune haemolytic anaemia (AIHA) is an uncommon clinical condition in which endogenous antibodies are directed against the patient's own red blood cells coated by immunoglobulin and/or complement. Anaemia appears when the destruction of red blood cells overwhelms the bone marrow's capacity to produce new cells. People of all ages can be affected, although some reports suggest that, in childhood, secondary cases are more common than idiopathic forms, and viral or bacterial agents are frequently the only recognisable stimuli. Indeed, AIHA follows viral infection or vaccination much more often in children than in adults. However, in the majority of cases of AIHA the pathogen remains undefined. Parvovirus B19 infections have frequently been implicated as a trigger of several forms of autoimmune diseases both in children and adults. Here we report the case of a 5-year old girl who was referred to our hospital because of anaemia and mild jaundice.

The patient was previously healthy until 15 days prior to admission, when she developed a fever, weakness, lack of appetite, diarrhoea and vomiting for about 4 days. On admission, the main clinical signs and symptoms noted during a general physical examination were pallor, jaundice and tachycardia (heart rate: 150 bpm). Haematological tests showed a haemoglobin (Hb) level of 4.1 g/dL, mean corpuscular volume 83 fL, reticulocyte count $147 \times 10^9/L$ and normal leucocyte and platelet counts. Marked polychromasia with spherocytosis and nucleated red blood cells were noted on the peripheral blood smear, without atypical cells. The serum lactate dehydrogenase (LDH) was raised at 1,047 IU/L, total bilirubin was 2.61 mg/dL, direct bilirubin 0.61 mg/dL, haptoglobin 10 mg/dL, C-reactive protein 10.8 mg/L, aspartate amino transferase 68 IU/L, alanine amino transferase 24 IU/L and ferritin level 354 ng/mL. Tests for anti-nuclear, anti-double-stranded DNA, and anti-smooth muscle antibodies and anti-phospholipids were negative. Abdominal ultrasonography revealed hepatosplenomegaly.

An immunohaematological study was performed. A direct antiglobulin test (DAT) was performed with a broad-spectrum antiserum and with monospecific anti-IgG, -IgA, -IgM, -C3d and -C3b antisera, in

liquid phase and by column agglutination (reagents from Ortho Clinical Diagnostics, Raritan, New Jersey, USA and Diamed, Cressier sur Morat, Switzerland). Eluate testing was performed by Rubin's method and with low pH glycine buffer using a commercial kit (ELU-KITTM II, Immucor, Norcross, Georgia, USA). An indirect antiglobulin test (IAT) with untreated and treated (ficin/papain) homologous red blood cells (Resolve C - Ortho Clinical Diagnostics and ID-Diamed Panel- DiaMed) was also performed. On admission, the DAT was strongly positive for an IgG autoantibody which was also present in the patient's serum. Both the eluate and the serum, investigated using a broad panel of reagent red blood cells, showed an anti-Jk^a antibody. Kidd typing of the erythrocytes, performed using a monoclonal IgM reagent (Ortho Clinical Diagnostics), showed a Jk(a) positive, Jk(b) negative phenotype so the anti-Jk^a antibodies found in the blood of the patient were presumed to be autoantibodies.

AIHA was diagnosed and therapy was started with intravenous methylprednisolone (20 mg/kg/die) and folic acid (20 mg/die). From the fifth day the steroid treatment was continued in the form of oral prednisone (2 mg/kg/die). Due to severe, symptomatic anaemia the child was transfused with a compatible unit (150 mL) of Jk(a) negative, Jk(b) positive red blood cells. Bacterial culture of stools for *Campylobacter*, *Rotavirus*, *Yersinia*, and *Escherichia coli* were negative, as was the search for lactate-positive, coagulase-negative *Enterobacteria*, *Staphylococcus* and *Enterococci spp.* Other causative agents were investigated: cytomegalovirus, Epstein-Barr virus, hepatitis B and C viruses, human immunodeficiency viruses 1/2, herpes simplex virus 1-2 were negative, while Parvovirus B19 IgM and IgG were found.

The patient's clinical conditions gradually improved and she was discharged from hospital. About 2 weeks after admission the immunohaematological tests became negative and the patient had a Hb level of 11.5 g/dL; LDH levels returned to the normal range. One month after the onset, the child developed a transient thrombocytopenia (platelet count of $96 \times 10^9/L$ and $106 \times 10^9/L$ on two different occasions). The prednisone

treatment was maintained at a dose of 2 mg/kg/die for 15 days, and then gradually reduced to 2.5 mg/die and discontinued 40 days after the initial presentation because the patient had normal haematological findings. She remains healthy at follow-up (6 months).

In paediatric patients AIHA is often associated with infections or organ-specific autoimmune diseases¹⁻⁴. Human Parvovirus B19 may cause a widespread benign and self-limiting disease in children and adults, but this viral infection has also been associated with the production of autoantibodies against many autoantigens (nuclear antigens, neutrophil cytoplasmic antigens, phospholipids) and with rheumatoid factor². It has previously been shown that chronic B19 infection can induce anti-viral antibodies with autoantigen-binding properties. The autoimmune sequelae have a multifactorial and complex origin: the involvement of molecular mimicry between self-antigens and viral proteins, the induction of enhanced cytokine production via the viral transactivator protein NS1 and the phospholipase A2-like activity of the capsid protein VP1 seem to contribute to the induction of autoimmune diseases³. Parvovirus B19 interacts extensively with human red blood cells; *in vitro* studies have shown changes in capsid conformation following B19 binding to red blood cells, leading to exposure of a region (VP1 "unique region") that seems to play a central role in the induction of autoimmune processes. Antibodies derived from the exposed VP1 "unique region" would not neutralise free infectious particles in the blood, but would instead target receptor-attached virus⁴.

An interesting finding in our case was the rarely occurring specific complement-binding warm auto-antibodies against the Jk(a) antigen. Generally autoantibodies with single specificity are produced against Rh system antigens. Warm anti-Jk^a autoantibodies have been rarely described, in association or not with haemolysis; most of the cases reported in the literature were in patients with autoimmune disorders, such as ulcerative colitis or systemic lupus erythematosus. In our patient the simultaneous disappearance of the anti-Jk^a autoantibodies and the haemolysis strongly suggests that the anti-Jk^a was responsible for the haemolysis. It is noteworthy that the first manifestations of infection in our patient were in the gastrointestinal system and no infectious agent was identified other than the B19 virus. The gastrointestinal symptoms were followed 2 weeks later by acute haemolysis. Antigen sharing between the gastrointestinal tract and red blood cells has been described by Hinoue *et al.*⁵, who identified and characterised a Kidd antigen/UT-B urea transporter expressed in the human colon, encoded by the *Slc14A1* gene, with a sequence identical to that reported for the Kidd/UT-B present on the red blood cells. In the light

of these data, we can hypothesise a cross-reactivity of autoantibodies between autoantigens of the colon and the red blood cells. The severe, transient haemolysis observed in our patient occurred after the acute phase of infection. Our patient also showed a transient mild thrombocytopenia, while her leucocyte count remained normal. A meta-analysis that included 516 cases of childhood autoimmune thrombocytopenia and 246 healthy controls showed that human B19 infection is closely associated with childhood autoimmune thrombocytopenia. Parvovirus B19, which can be a primary cause of reticulocytopenic post-infection anaemia in childhood, is strongly suspected to have been responsible for the haemolytic anaemia in our patient.

This case is unusual and interesting because of the association of B19 infection with warm AIHA and because of the rare specificity of the autoantibodies. Besides its scientific interest, the authors recommend identification of antibody specificities in AIHA since this will play an important role in the appropriate transfusion therapy if the severity of the clinical course is such as to impose this therapeutic strategy.

The Authors declare no conflicts of interest.

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Arrived: 5 September 2012 - Revision accepted: 16 January 2013

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