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VARIOUS TRIGGERS FOR AUTOIMMUNE HEMOLYTIC ANEMIA IN CHILDHOOD

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Autoimmune haemolytic anaemia (AIHA) is a diverse spectrum of disease entities that trigger the production and deposition of autoantibodies and/or a fixation of complement components on the target red blood cells (RBC). The final result is the intravascular and/or extravascular destruction of RBCs. Where immunological response is concerned there are three clinical entities of AIHA: with cold reacting, warm reacting or biphasic antibodies. Ethiologicaly, it is known that AIHA may occur in children after viral infection, immunization or it can be associated with immune connective tissue disease. In this report similar putative triggers linked with AIHA such as EBV, Mycoplasma pneumoniae, adeno virus infection, immunization and immune connective tissue disease are described in four children. All the cases described were treated with corticosteroides, three of them successfully, while one patient is still on therapy (three years period) due to persistent and severe AIHA coupled with putative autoimmune hepatitis. However, it seems that the prolonged course of disease in the three cases described here may be connected with HLA DR 2 and HLA B27.

Descriptors: ANEMIA, HEMOLYTIC, AUTOIMMUNE-immunology, etiology; AUTOANTIBODIES-analysis; AGGLUTININS-analysis

INTRODUCTION

Autoimmune hemolytic anemia (AIHA) includes a diverse spectrum of disorders triggering the production and deposition of autoantibodies fixing on complement components on the target red blood cells (RBCs). The result of their action is a shortening of RBC survival in vivo and the evidence of host antibody reactivity with autologous RBCs in vitro (1). AIHA results in hemolysis (anemia, hemoglobinemia and hemoglobinuria) and with direct autoagglutination of RBCs (acrocyanosis, vascular lesions). The key laboratory tests for the diagnosis of AIHA include the demonstration of immunoglobulin and/or complement components on a RBC surface, a positive direct antiglobulin test (DAT) and the presence of serum-free IgG or cold reacting autoantibodies (high titre over a broad temperature range). According to the thermal properties of RBC autoantibodies three clinical AIHA entities are possible: forms with cold-reacting, warm-reacting or biphasic autoantibodies (2). Warm-reactive autoantibodies, best active at 37°C, usually facilitate the sequestration of sensitised RBCs by macrophages in the spleen and liver, whereas cold-reactive antibodies cause immediate intravascular hemolysis of sensitised RBCs by a complement-mediated mechanism or their sequestration by macrophages (1).

Mixed AIHA has been defined as a condition with the presence of both warm and cold antibodies. In the syndrome involving biphasic autoantibodies the patient's serum contains a cold complement-fixing antibody that produces hemolysis when the blood is first cooled (to allow the binding of an antibody) and then warmed (to provide optimal condition for complement-mediated hemolysis). AIHA may occur in either primary (idiopathic) or secondary form. The secondary form arises due to the cold-reacting antibodies and it is often seen during infections with Mycoplasma pneumoniae and Epstein-Barr virus (EBV).

In children transient hemolytic anemia can arise after viral infections, immunization or may be linked with immune connective tissue disorders. Following an infection with Mycoplasma pneumoniae there is a common transient increase of the titre and the thermal range of cold, usually harmless anti-I agglutinins. When the thermal range is high enough, the child may develop an episode of AIHA, which may be severe. In infectious mononucleosis anti-i of the same I/i system is frequently present as a transient phenomena as well. From a review of published cases, there are some reports that underline the presence anti-i in up to 50% of patients with the infectious mononucleosis (3).

Transient warm-reacting AIHA in children may also occur after a viral infections, immunization and secondary to other systemic immune diseases (4).

Although AIHA in childhood is a rather rare syndrome, it should be considered at the time of the first patient admission, due to the occasionally fulminant and life-threatening hemolysis.

In establishing the diagnosis of AIHA one should bear in mind the special characteristics of childhood AIHA, especially the possible triggers. Here we pre-

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sent four consecutive patients with AIHA, who were treated at the Pediatric Hematology Department, of the University Hospital, Split.

MATERIAL AND METHODS

A polyspecific direct antiglobulin test (DAT) was carried out using Anti-human globulin polyspecific in tube agglutination technique (TAT) (Biotest, Dreieich, Germany) and Biovue Anti-Human Globulin Anti-IgG, Anti-C3d in gel agglutination technique (GAT) (Ortho, Raritan, USA). Monospecific DAT has was carried out using monospecific Anti-IgG, Anti-IgM, Anti-IgA and Anti-C3d reagent (Dr Molter, Neckargemund, Germany) in TAT and ID-DAT Screening I (Diamed, Cressier sur Morat, Switzerland) in GAT. Antibody elution was performed using DiaCidel (Diamed, Cressier sur Morat, Switzerland). The indirect antiglobulin test (IAT) and antibody identification were performed using Anti-human globulin polyspecific (Biotest, Dreieich, Germany) in TAT, LISS-Coombs 37°C (Diamed, Cressier sur Morat, Switzerland) and Biovue Anti-Human Globulin Anti-IgG, Anti-C3d (Ortho, Raritan, USA) in GAT.

The test on paroxysmal nocturnal hemoglobinuria was done using ID-PNH (Diamed, Cressier sur Morat, Switzerland) in GAT.

RESULTS

Patient #1

At admission a 13-year-old girl manifested clinical signs of mild AIHA (pallor, jaundice, and weakness, lost of appetite and abdominal pain). Monospecific DAT was positive with anti IgG and negative with anti C3 reagent (TAT and GAT). Antibody elution from the patient's RBCs as well as the serum studies revealed non-specific IgG autoantibodies. The same results have persisted, but with a small fluctuation in reactivity between monthly controls. Peripheral blood analysis revealed the following findings: ESR 84 mm/l; RBC 4. 13 x 10^{12} cells/L; HCT 35%; HGB 13.2 g/dl; LDH 316 U/L; Haptoglobin (Hp) < 69 mg/l; Ferritin 20.6 μg/L; Iron 19 μmol/L; UIBC 49 μmol/L; TIBC 68 μmol/L; Bilirubin 76.6 μmol/L, Bilirubin indirect 67.2 μmol/L. Antinuclear antibodies (ANA) were positive while cryoglobulins (114 mg/l), circulated immune complexes (4.4 μg/ml) and ANCA MPO (19.6 Iμ/ml) were increased. LE cells were found positive. Further viral serology showed: negative anti-adenovirus IgM and positive anti-adenovirus IgG (1: 300). Serology on CMV, EBV and Toxoplasma were neg. HLA tipization revealed: HLA-A 25 (10); B 15, 27; Cw 1, 2; Bw 4, 6; DR 2; DQ 1; DRw 51. Corticosteroid therapy (methylprednisolone) 2mg/kg was introduced eight days after aggravation of a haemolytic crisis continuing with peroral therapy for five months. In spite of the therapy, we observed several attacks of clinically manifested haemolysis during this period. During the therapy the patient developed signs of hepatitis and a new attack of AIHA. Moreover, distinct liver enlargement and dysfunction (hypoproteinemia, hypofibrinogenemia, ascites) were observed. Serologic markers for hepatitis B and C (HBsAg, anti-HBs, anti-HBc, anti-HCV) were negative. Finally, the patient was diagnosed as AIHA due to the warm-reacting non-specific IgG autoantibodies with a prolonged course of repeated haemolysis attacks. The course of the disease was complicated by probably autoimmune hepatitis. The patient has been on corticosteroid therapy combined with azathioprine for almost three years. However, signs of haemolysis and hepatitis are present.

Patient #2

An 8-year-old boy presented with clinical signs of mononucleosis and severe AIHA (jaundice, pallor). Polyspecific DAT was found negative as well as monospecific anti-IgG, anti-IgM, and anti-IgA, anti-C3 DAT, both by TAT and GAT. IAT was found negative, but screening of irregular antibodies was positive on 4°C autoantibodies titre 1:128 and izo-antibodies titre 1:128. The Donath-Landsteiner test and a test for paroxysmal nocturnal hemoglobinuria were found negative. Viral serology showed IgM anti-EBV-VCA (60 Au/ml), IgG anti-EBV-VCA (19o Au/ml), IgM anti EBNA negative and IgG anti EBNA negative; IgM anti-CMV positive (80 Au/ml), IgG anti-CMV positive (35 Au/ml). Circulated immune complexes were elevated (4.68 μg/ml), ANCA PRO3 were elevated (26 Ij/ml), while cryoglobulins (167 mg/ml) were increased. Peripheral blood analysis revealed: ESR 55 mm/l; RBC 1.5 x 10^{12}cells/L; HTC 15%; HGB 50 g/dl; LDH 678 IU/l; Haptoglobin < 0.06 g/l; Ferritin 463.8 μg/ml; Iron 36 μmol/l; UIBC 30 μmol/l; TIBC 66 μmol/l; Bilirubin total 35.6 μml/l, Bilirubin indirect 27.8 μmol/l. HLA tipization revealed: HLA-A 2; B 44 (12); Cw 2, 7; Bw 4, 6; DR 16(2); DQ 5(1); DRw 51.

Corticosteroid therapy (methylprednisolone 2mg/kg) was introduced for two months then gradually decreasing. Maintenance therapy was 0.5 mg/kg per os. After ten months of therapy the patient reached a complete stabile remission, lasting to the present day (four years and six months). As other causes of haemolysis (hereditary or microangiopathic) were excluded, the patient was considered to have "DAT negative AIHA". EBV and CMV infection can be considered as a possible pathogenic trigger for AIHA development.

Patient #3

A 10-month-old boy manifested severe AIHA 3 days after immunization against diphtheria, poliomyelitis, pertusis and tetanus. Polyspecific DAT was positive, as well as monospecific anti-C3; anti-IgG, anti-IgM, anti-IgA. DAT was negative, both by TAT and GAT. Antibody elution from the patient's RBCs gave a negative result. Furthermore, IAT were negative, while screening for irregular antibodies were positive at 4°C and 22°C. due to the anti P1 alloantibodies. The patient was typed as P1 negative.

Circulated immune complexes were elevated (4. 68 μg/ml), as well as ANCA PRO3 (26 Ij/ml), while cryoglobulins (167 mg/ml) were increased.

Cold agglutinins titres were found as follows: anti-I negative at 4°C, 22°C and 37°C, anti-i was positive (titre was 32 at 4°C, 4 at 22°C and 2 at 37°C). Peripheral blood analysis revealed: ESR 24 mm/l. RBC 2.5 x 10^{12}cells /L; HTC 18%; HGB 63 g/dl; LDH 1104 IU/l; Haptoglobin < 0. 06 g/l; Ferritin 210.0 μg/ml; Iron 14 μmol/l; UIBC 50 μmol/l; TIBC 64 μmol/l; bilirubin 10, 4 μml/l. HLA genotyping revealed: HLA-B 49 (21); Cw 6, 7; Bw 4, 6; DR 7.

Corticosteroid therapy (methylprednisolone 2 mg/kg) was introduced for 43 days. After one month of therapy the patient reached complete stabile remission.

lasting to the present day (two years and four months). Two months after the first presentation detectable cold-reacting anti-i antibodies completely disappeared from the patient's serum. To conclude, we believe that the patient developed AIHA with cold-reacting anti-i antibodies reacting up to 37°C. However, the broad temperature range of anti-i reactivity can be influenced by immunisation 3 days before AIHA manifestation.

Patient #4

A 15-month old girl manifested bronchopneumonia for 9 days and signs of anaemia that appeared 3 days prior to admission. Analysis of peripheral blood showed the following results: ESR 140 mm/l; RBC 2. 18x 10^{12}cells /L; HGB 72 gd/l; HTC 18 %; MCV 86 f/ll; MCH 33 pg; MCHC g/dl 38. 4; PLT 135x10^9/L; WBC 11. 9x10^9/L; RTC 38‰; Bilirubin total 10 μmol/l; LDH 589 μ/l; Feritin 226 mg/ml. Polyspecific DAT was positive, as well as monospecific anti-C3 and anti-IgM. Anti-IgG was weakly positive while anti-IgA was negative, both by TAT and GAT. Antibody elution from the patient's RBCs was negative. Furthermore, IAT was negative, but screening of irregular antibodies was positive at 22°C and 37°C. Identification of antibodies showed non-specific cold anti-I antibodies. Nevertheless anti-I titer was found 8 at 4°C, and 4 at 22°C and 37°C. Viral serology revealed: IgM anti Mycoplasma pneumoniae positive 1:150, IgG anti Mycoplasma pneumoniae 1:200, IgA anti Mycoplasma pneumoniae neg; adenovirus IgM 1:200 and IgG anti adenovirus 1:390 positive; EBV antibodies (IgG and IgM) VCA, EBNA and Parvo B 19 were negative. The lymphocyte autocrossmatch reaction was also positive.

The cardiolipin IgM antibodies (37 IU/ml) and circulated immune complexes (2. 24 μg/ml) were elevated. HLA genotyping revealed: HLA-A 1, 32 (19); B 52 (5), 61 (40); Cw 2; DR 15 (2), 17 (3); DQ 6 (1), 2; DRw 51, 52.

Corticosteroid therapy (methylprednisolone) 2 mg/kg/day was introduced immediately then continued for fourteen days, and tapering to 1.5 mg/kg over three weeks. After almost two months the patient reached complete stabile remission, lasting three years and seven months. One month following the first admission the anti-I antibodies did not react at 22°C and 37°C and reacted at 4°C with a titre of 4. Monospecific anti-C3, anti-IgG, IgM and anti-IgA was positive. One month after therapy all monospecific DAT analysis were negative both by TAT and GAT, with an anti-I reactivity solely at 4°C, and a titer of 4. Seven months after reaching stabile remission for the AIHA the lymphocytes autocrossmatch reaction was negative.

The serology finding of IgM, C3 and IgG on RBC indicated AIHA of mixed cold-warm type, with a predominant cold component.

The presumed cause of clinically significant hemolysis was considered to be anti-I of broad temperature range, triggered probably by Mycoplasma pneumoniae infection.

DISCUSSION

Autoimmune haemolytic anaemia (AIHA) constitutes of a diverse spectrum of disease entities that trigger the production and deposition of an autoantibody and/or a fixation of complement components on the target cell RBCs. The result of their action is a shortening of RBC survival in vivo and evidence of host antibody reactive with autologous RBC in vitro (1). Three general clinical entities of AIHA exist - with cold reacting, warm reacting or biphasic autoantibodies, according to the thermal properties of the RBC autoantibody (2). Mixed AIHA has been defined as the presence of both warm and cold antibodies. In addition, the presence of symptoms of cold agglutinin disease, or low-titer and high thermal amplitude cold agglutinin (CA) might be necessary for the diagnosis of mixed AIHA (5).

Warm-reactive antibodies usually facilitates the sequestration of sensitised RBCs by macrophages in the spleen and liver whereas cold-reactive antibodies cause either immediate intravascular haemolysis of sensitised RBCs by complement-mediated mechanism or their sequestration by macrophages (1).

The key laboratory findings for the diagnosis of AIHA are the demonstration of immunoglobulin and/or complement components on a RBC surface (positive DAT), a long with the presence of serum-free IgG, high-titer or broad temperature range cold-reacting autoantibodies. AIHA in infancy is rare (6). In children transient AIHA may occur following a viral infection, immunization or secondary to immune connective tissue disease (4). Reporting our 4 cases we confirm that immunisation might induce the AIHA in infancy. Autoimmune haemolytic anaemia is rarely caused by IgM warm autoantibodies, and it is sometimes difficult to diagnose (7).

Various triggers of AIHA: EBV infection, mycoplasma pneumoniae infection, immunization and immune connective tissue disease were confirmed in our report. In this report we also describe the clinical course and response to corticosteroid therapy. Good response to corticosteroid treatment has already been pointed out (8). In three cases described in this work anaemia resolved completely with steroid therapy during the first episode.

Some authors such as Meyer suggest successful pulsed high dose dexamethasone treatment in chronic autoimmune haemolytic anaemia of the warm type (9).

This procedure may be useful in the treatment of prolonged courses of AIHA and autoimmune hepatitis in case#1 we presented.

However, only a few authors investigated the linkage between AIHA and possible HLA antigen (10). Even though we analysed only four patients with AIHA it seems that the prolonged course of disease in at least three cases may be connected with HLA DR 2. We also noticed that one patient with AIHA and autoimmune hepatitis expressed HLA B27 loci as well as HLA DR 2 antigens.

CONCLUSION

Various triggers of AIHA such as EBV infection, immunization and immune connective tissue disease was confirmed in our report. We believe that the prolonged course in cases 1 and 3 is probably connected with HLA DR 2 locus.

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Sažetak

RAZLIČITI UZROCI AUTOIMUNE HEMOLITIĆKE ANEMIJE U DJETINJSTVU

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Autoimune hemolitičke anemije obuhvaćaju različiti spektar bolesti kod kojih se stvaraju i odlažu autoprotutijela i/ili vežu komponente komplementa na crvene krvne stanice. Posljedica je intravaskularno i/ili ekstravaskularno uništavanje crvenih krvnih stanica. Prema imunosnom odgovoru razlikujemo tri klinička entiteta bolesti: s hladnim, s toplim ili bifazičnim protutijelima. Poznato je da se AIHA može pojaviti kod djece nakon virusne infekcije, imunizacije i može biti povezana s imunosnim bolestima vezivnog tkiva. Prikazali smo češće uzroke AIHA kao što su EBV, Mycoplasma pneumoniae, adeno virus, imunizacija ili vezano uz imunološku bolest vezivnog tkiva. Svi opisani bolesnici liječeni su kortikosteroidima, troje uspješno. Četvrti se bolesnik kod kojeg je AIHA udružena sa autoimunim hepatitisom nakon tri godine još liječi. Čini se da je produženi tijek bolesti kod naša tri bolesnika povezan sa HLA DR 2 i HLA B27.

Deskriptori: AUTOIMUNA HEMOLITIČKA ANEMIJA-imunologija, etiologija; AUTOANTITIJELA-analiza; AGLUTININI-analiza

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