CASE REPORT
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Successful Management of Neutropenia in a Patient with CD40 Ligand Deficiency by Immunoglobulin Replacement Therapy

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ABSTRACT

Hyper-IgM syndromes are characterized by profound reduction of serum IgG, IgA, and IgE levels with normal or increased concentrations of serum IgM. CD40 ligand deficiency is X-linked form of the disease, which results in a lack of immunoglobulin class switching from IgM to IgG in B cells. In addition to the recurrent infections, a number of patients suffer from neutropenia. There are some evidences indicating the effect of G-CSF in combination with intravenous immunoglobulin (IVIG) in improvement of neutrophil counts, which has become the most common procedure to control neutropenia.

In this report we present a 6 year-old patient of CD40 ligand deficiency, who suffered from chronic, severe neutropenia. Administration of IVIG was started for him when the diagnosis was made at the age of 1.5 years and he was on the regular IVIG therapy after that time untill now for a period of 4.5 years. IVIG and prophylactic antibiotic therapy, despite cessation of granulocyte colony-stimulating factor, injection after one month, corrected the severe neutropenic state of this patient.

It seems that regular administration of sufficient doses of IVIG can be useful in the management of neutropenia in CD40 ligand deficiency, which results in better quality of life with decreasing occurrence of infection.

Keywords: Antibody Deficiency; CD40 Ligand; Hyper IgM Syndrome; Intravenous Immunoglobulin; Neutropenia

INTRODUCTION

Hyper-IgM syndromes are rare immuno-deficiency diseases, first described in 1961;[1] these syndromes are characterized by profound reduction of serum IgG, and IgE levels with normal or elevated concentrations of serum IgM and IgD.[2-5] CD40 ligand deficiency is X-linked form of the disease, which is caused by mutations in the gene located at Xq26.3-27 [6-10] for CD40 ligand, which suspends immunoglobulin class switching from IgM to IgG in B cells.[11] Patients with CD40 ligand deficiency are susceptible to recurrent infections with pathogenic and opportunistic microorganisms including *Pneumocystis carinii*.[12] In addition to the recurrent infections, about one-third[13] to

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two-thirds[14] of these patients suffer from episodic, cyclic, or chronic neutropenia; which result to secondary complications.[2,5] The occurrence and persistence of neutropenia despite intensive therapy suggests that the etiology in this group of patients is related to the CD40 ligand (CD154) defect. Several documented studies showed the effect of granulocyte colony-stimulating factor (G-CSF) alone or in combination with intravenous immunoglobulin (IVIG) in improvement of neutrophil counts.[5, 11, 15, 16] It is still controversial whether sufficient doses of IVIG are capable to increase the neutrophil counts to normal levels. In this report we present a case of CD40 ligand deficiency who suffered from chronic, severe neutropenia. Administration of IVIG and prophylactic antibiotic in addition to only one month G-CSF injection corrected the severe neutropenic state of the patient resulting in considerable diminution of infection episodes.

CASE REPORT

The patient is a 6 year-old boy, the third child of unrelated parents with no family history of suggestive X-linked immunodeficiency. He was fully immunized with viral and bacterial vaccines without complications and was well until the age of 6 months, when he developed pneumonia he requiring hospitalization. During the age of 6 months to 1.5 years, he had recurrent episodes of infections requiring 7 times hospitalization for: pneumonia (3 episodes), septic arthritis (1 episode), urinary tract infection (1 episode), and febrile convulsion (2 episodes). At the age of 1.5 years because of a pyogenic infection (suspicious of *Pneumocystis carinii*) he was referred to the department of immunology for further evaluations. Growth and developmental assessment revealed mild retardation. Immunological investigation showed low serum IgG and IgA accompanied by increased IgM (IgG=90, IgA=0, IgM=245 mg/dl), with normal number of circulating B cells with diminished neutrophils (WBC= 12000, PMN=1%, Lymphocytes =89%, Monocytes=9%, Eosinophil= 1%). Flowcyto-metry was performed in order to determine lymphocyte subsets, The percent of lymphocytes sub-sets in this patients were found to be: CD3+ 82%, CD4+ 41%, CD8+ 14% and CD19+ 11%. Since the blood group of this patients was AB+, he lacked isohemagglutinin in his serum. The diagnosis of Hyper-IgM syndrome was made for this patient according to the clinical and laboratory findings. Bone marrow aspiration showed severe maturation arrest in myeloid lineage, but there was no histological or microbial evidence of infection.

In order to make definitive diagnosis in this patient, genomic DNA was extracted from peripheral blood using a modified salting-out method. Mutation detection for CD40L gene was initially performed by polymerase chain reaction (PCR) amplification of genomic DNA using CD40L -specific primers and single strained conformation polymorphism (SSCP) analysis. PCR-SSCP analysis demonstrated that he had an abnormally mobilized band of exon 2 compared with controls. DNA sequencing indicated one nucleotide deletion (83Tdele), which resulted in a frame shift and stop codon (V30deleX36) in CD40L gene. At the age of 1.5 year IVIG (400 mg/kg every 3 weeks), GCSF (7µg/kg/day for one month) and antibiotic (co-trimoxazol 20mg/kg/day TDS) were started for him. He continued to be on the regular IVIG therapy after that time untill now. No side effect (i.e. rash, medullary pain, splenomegaly) and no elevated levels of leukocyte alkaline phosphatase were detected during G-CSF administration period. The patient responded to the therapy with a resolution of his infection and rising count of neutrophils to 3%. After one month the treatment was continued with IVIG every three-week and daily antibiotic without G-CSF administration; which resulted in neutrophil count of 23% with markedly decreased episodes of infection without any need for hospitalization during follow up period. The only complaint that recurred untill now is a frequent swelling of the right knee which resolved with IVIG injection. At the age of 3 years, again his complete blood cell count showed severe neutropenia (WBC= 11000, PMN=1%, Lympho-cytes= 86%, Monocytes= 9%, Eosinophil=3%). So, we increased the IVIG dose of the patient to 500mg/kg every 3 weeks; which was again effective in elevating the neutrophil count (Figure 1). At subsequent visits the boy looked well and is thriving as expected. He is a 6 year-old boy now and under control without any further complications.

DISCUSSION

The CD40 ligand deficiency is a X-linked primary immunodeficiency disorder characterized by normal or increased levels of IgM and markedly reduced levels of IgG and IgA. The results reported by Levy et al.[14] suggest that opportunistic infections or persistent

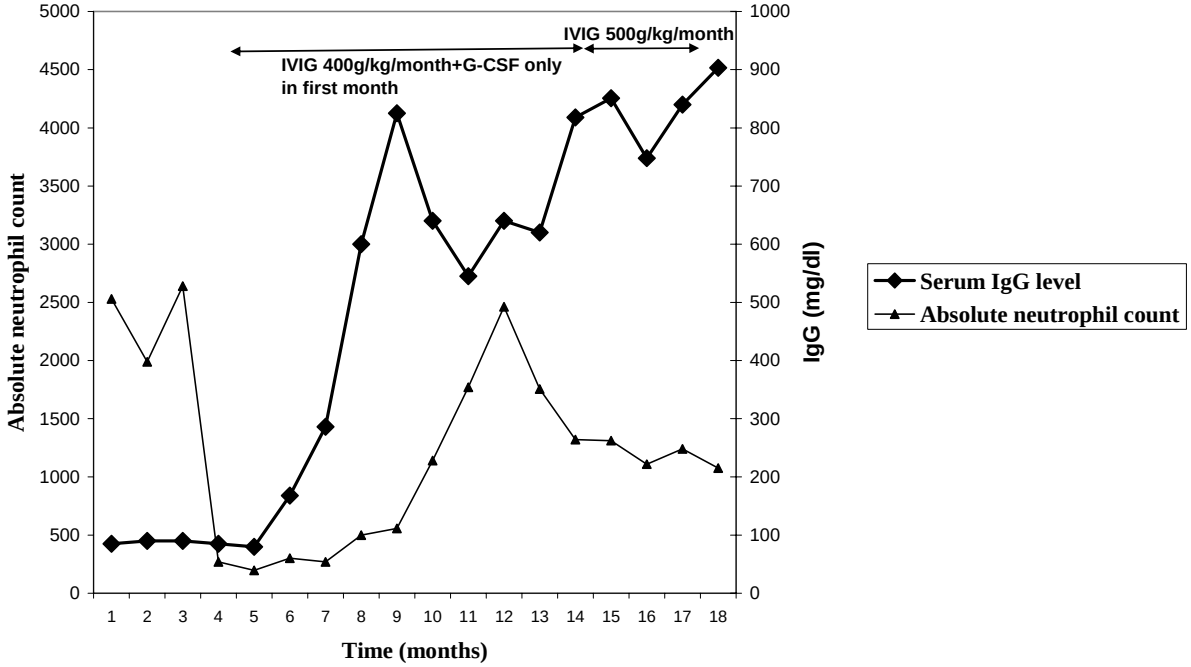

Figure 1. Absolute neutrophil counts and IgG serum levels in 18 laboratory test series of patient with CD40 ligand deficiency.

neutropenia in a male patient with hypogammaglobulinemia and normal numbers of T cells and B cells should raise a suspicion for hyper-IgM syndrome. Because of advances in molecular techniques and development of mutational analysis allowed definitive diagnosis of Hyper IgM syndromes using mutational analysis. Most of the mutations described for hyper-IgM syndromes have been point mutations, leading to simple amino acid substitutions, premature stop codons or splicing defects that cause aberrant transcription and these all result in the expression, if any, of non-functional CD40L proteins.[17]

Chronic neutropenia is associated with CD40 ligand deficiency in about half[2, 5] to two-thirds[14] of patients. The underlying basis of this association remains almost unclear. The condition may result from the presence of autoantibodies to neutrophils or from defective myeloid differentiation which cause maturation arrest[2] or from a defect in release of mature granulocytes from the marrow to the peripheral blood.[5] Attempts to find neutrophil antibody were unsuccessful.[2, 5]

The presence of neutropenia and elevated IgM level indicated that he might be an X-linked HIGM (HIGM1). In this patient, nucleotide deletion, resulted in a framshift and stop codon in CD40L gene. The interaction of CD40L on T-cells and CD40 on B-cells are essential for class-switching recombination and somatic hypermutation. Mutations in CD40L gene prevent class-switching recombination and germinal center formation as well as affecting T-cell function.[18]

Mori et al.[5] have suggested that interaction between CD40 on stromal cells and the CD40 ligand on T cells in the bone marrow may stimulate synthesis of G-CSF, which subsequently induces release of mature neutrophils from the marrow pool to peripheral blood. The defect of CD40 ligand therefore may reduce local G-CSF production, resulting in severe neutropenia in patients with CD40 ligand deficiency.

It is controversial whether IVIG, in sufficient doses, can remedy the neutrophil counts to normal levels. Some, reported no consistent effect of IVIG on controlling neutropenia,[2,5] while others acknowledged the usefulness of IVIG in controlling the neutropenic state of the patients.[12] The mechanism for improvement in the neutrophil count may be the modulation of an autoimmune process, but may also be the result of preventing infections causing secondary neutropenia.[12]

Continuing administration of G-CSF in addition to high doses of IVIG every 4 weeks and prophylactic antibodies, improved the neutropenic state in some

studies,[5,11] resulting in less frequent episodes of grave infection. In our patient the absolute neutrophil counts (ANC) was raised by administration of IVIG and prophylactic antibiotics despite cessation of G-CSF injection after one month. It seems that regular administration of sufficient doses of IVIG would be useful in the management of these patients, which results in better quality of life with less occurrence of grave infection. CD40 ligand deficiency is characterized by a high incidence of opportunistic infection, cancer and poor survival rate.[14] Rigorous use of IVIG therapy, *Pneumocystis Carinii* pneumonia prophylaxis with trimethoprim-sulfamethoxazole, accurate monitoring of gastrointestinal manifest-tations (including cancer), and management of neutronpenia reduce morbidity and mortality rates.

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