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STUDIES OF IMMUNE RESPONSE IN A PATIENT WITH SELECTIVE COMPLETE C1q DEFICIENCY*

*A. İzzet Berkel MD***

SUMMARY: Berkel Aİ. (Immunology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Studies of immune response in a patient with selective complete C1q deficiency. Turk J Pediatr 35: 221-226, 1993.

Immune response in a six-year-old girl with a rare complement defect, namely selective complete C1q deficiency, was studied. Her cell-mediated immune response (delayed hypersensitivity skin tests, E, EAC rosettes, in vitro lymphocyte transformation with phytohemagglutinin), isohemagglutinin titers, serum immunoglobulin levels, antibody titers to tetanus, Epstein-Barr virus, keyhole limpet hemocyanin, pneumococcus and bacteriophage ø x 174 were all normal. However, she had abnormal kinetics of the antibody response to bacteriophage following primary and secondary immunizations. Her antibody titers reached a peak four weeks after primary immunization and three weeks after secondary immunization, while the titers of controls peaked at two weeks and one week, respectively. In this study we could not prove the hypothesis that defective antibody response caused recurrent infections in this patient. An alternative hypothesis is the possible role of defective clearance of immune complexes in the development of severe infections. Patients with selective complete C1q deficiency form immune complexes with bacterial or viral antigens, thus activating the classical complement pathway. As a result, very low C1q levels decrease even further, leading to a severe immunodeficiency and recurrent infections. Future studies in this area may help to explain the mechanism(s) responsible for infections in this rare type of complement defect. *Key words: immune response, complement deficiency, C1q deficiency.*

Complement defects are associated with the impairment of complement-related functions which result in decreased host resistance, leading to infections[1]. Selective complete C1q deficiency is a rare complement defect associated with vasculitis, lupus-like syndrome, infections and renal disease. Symptoms include malar rash, chronic skin infections, lesions of mucous membranes, fever and recurrent infections[2]. Most patients have circulating immune complexes and develop glomerulonephritis. Many die of either renal failure or serious infections, including sepsis and / or meningitis[3,4].

The aim of this study was to determine the immune response in a six-year-old girl with selective complete C1q deficiency who died of a sepsis-like illness, and to

* From the Imunology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

** Professor of Pediatrics and Pediatric Immunologist, Hacettepe University Faculty of Medicine.

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ascertain whether her infection was caused by an abnormal antibody response due to a complement defect. Our studies have shown that the patient's cellular and humoral immunity were normal except for abnormal kinetics of the antibody response to bacteriophage ø x 174. Her alternate pathway activity was intact[5].

Previously described methods were used to assess serum and surface membrane immunoglobulins (SmIg), isohemagglutinins, autoantibodies [smooth muscle (SMA), antimitochondrial (AMC), antinuclear (ANA)], antibody titers to Epstein-Barr virus (EBV), pneumococci and tetanus, E and EAC rosettes, delayed hypersensitivity responses [purified protein derivative (PPD), Candida albicans, streptokinase-streptodornase (SKSD), phytohemagglutinin (PHA)] and lymphocyte transformation (with PHA)[6]. Antibodies to bacteriophage ø x 174 and keyhole limpet hemocyanin (KLH) were determined by administering 2×10^9 plaque-forming units of bacteriophage / kg of body weight intravenously and a 1000 µg KLH (0.4 ml) intramuscularly[7,8]. The same dosage of bacteriophage was repeated six weeks later with the aim of detecting secondary antibody response. The patient was immunized against pneumococcus by a single 0.5 ml dose of pneumococcal vaccine (Pneumovax, Merck Sharp and Dohme, Westpoint, PA) which contained 14 pneumococcal serotypes, each one 50 µg. Serum samples were obtained at various intervals for antibody studies and were frozen and stored at −70 °C until assayed. Written consent from the parents was obtained. All antibody studies were performed four months prior to her death, while the patient was on maintenance prednisone therapy.

Case Report

The patient's clinical findings were described previously[5]. She had been in good health until 4.5 years of age, when an erythematous malar rash and oral ulcerations developed. At the age of five, she suffered from episodes of fever, gingivitis, stomatitis, pyodermia and had a gluteal abscess. Following immunologic evaluation, a diagnosis of selective complete C1q deficiency was established on the basis of the absence of hemolytic complement (CH_{50}) and C1q activity. After a four-week-course of treatment with prednisone (2 mg / kg / day), the skin lesions and fever disappeared. However, they recurred each time corticosteroid therapy was discontinued. The patient was therefore put on maintenance prednisone therapy (alternate daily doses of 5 mg and 10 mg prednisone). The following laboratory tests were either normal or negative on several occasions: urinalysis, renal and liver functions, serum elctrolytes, LE cell, cryoglobulins, direct Coombs, hepatitis B surface antigen (HB_s Ag), anti HB_s antibody, antistreptolysin-O (ASO) titer, C reactive protein (CRP), erythrocyte sedimentation rate, nitroblue tetrazolium test (NBT), antidesoxyribonucleic acid (anti DNA), antiribonucleic acid (anti RNA), and anti-extractable nucleic acid (anti ENA) antibodies. Serum immune complexes were positive on several determinations. Two and one half years after

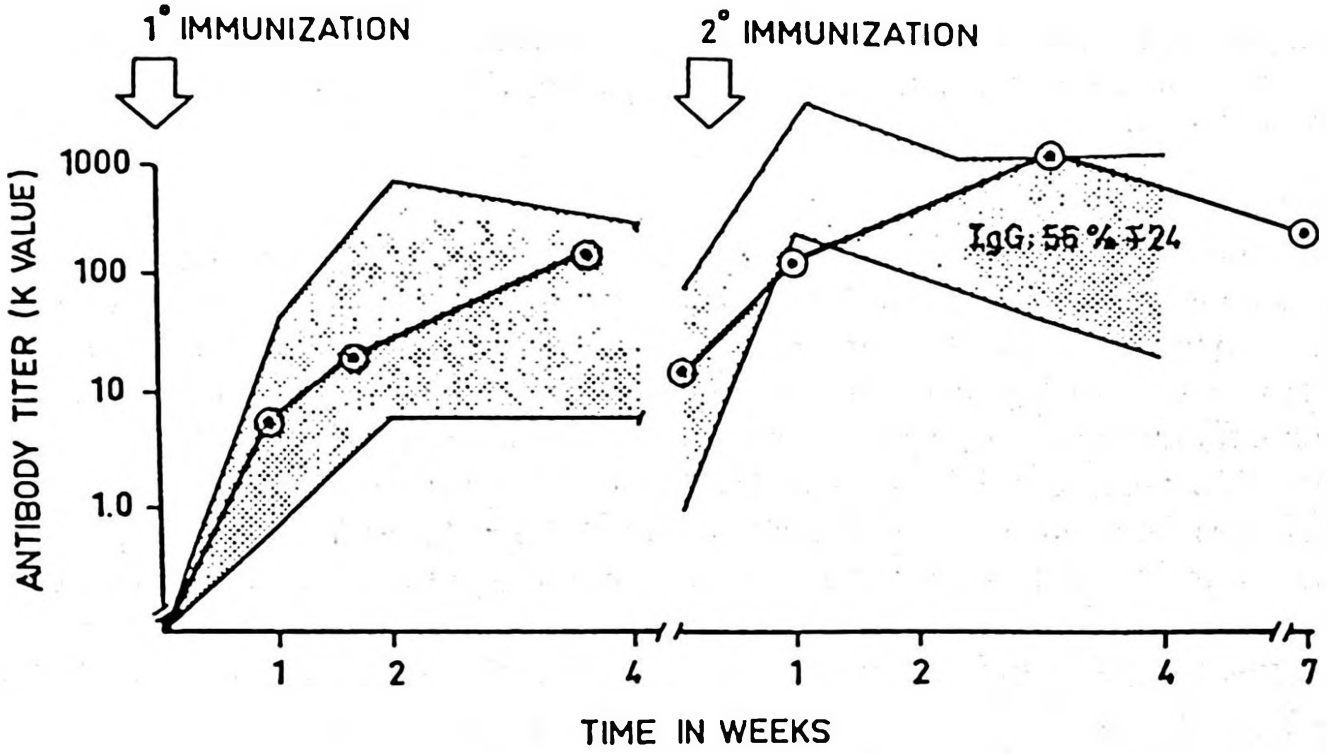
diagnosis, the patient died at home of a sepsis-like illness. Consent was not given for a postmortem examination. The family history for the disease was negative.

Results

Cell-mediated immunity studies revealed positive delayed hypersensitivity skin reactions to two (PHA and SKSD) of four antigens tested. The percentage of E rosette formation was 52% (normal 65 ± 8%). In vitro transformation response to PHA was normal (patient 62.186 cpm; normal 28.989-116.682). The results of tests for humoral immunity revealed that 15.6 percent of circulating lymphocytes had C3b receptors (EAC rosettes) (normal 17.5 ± 6.1%) and 17.5 percent had surface membrane immunoglobulins (normal 14.6 ± 4.2%). The isohemagglutinin titer was normal (the patient was blood group A and the serum anti B titer was 1:32). Serum immunoglobulin levels were normal (IgG value was 1400 mg / dl; normal 680-1270 mg / dl, IgA 155 mg / dl; normal 33-148 mg / dl and IgM 155 mg / dl; normal 75-206 mg / dl). Antibody determinations revealed ANA to be positive while ASM and APC antibodies were negative.

Antibody response to tetanus toxoid showed a four-fold increase from the initial value of one unit rose to 16 units which comprised the IgG type (normal is four-fold increase or more). Antibody titers of the IgG type against various antigens of EBV were normal: anti-viral capsid antigen (VCA) 1:80 (normal $> 1:10$), anti-early antigen (EA) diffuse (D) $< 1:10$, restricted (R) $< 1:10$ (normal $> 1:10$ in acute infection), anti-nuclear antigen (EBNA) 1:80 (normal $> 1:10$). Antibody response to eight out of 12 tested pneumococcal serotypes (types 3,4,7,8,14,18,19,23) was normal (more than a 400 ng / ml increase from the pre-immunization level). The response was low for serotypes 1,6A,9 and 12, although the patient had protective levels (over 200 ng / ml) for serotypes 9 and 12. The patient had a good IgM and IgG antibody response to KLH when compared with the serum titer of the immunized healthy controls. IgM antibody titer was positive for seven successive serum dilutions starting with 1:50 (normally positive for fewer tubes in serial dilutions). IgG antibody titer was positive in diluted serum (normally positive in serial dilutions).

The patient's antibody response to bacteriophage ø x 174 is seen in Figure 1. She had a normal clearance of phage and showed a near normal primary response, the highest titer being observed at about four weeks while the peak titer in the normal controls was reached at two weeks. Her secondary response showed a high antibody titer of 1023, which was reached at day 22. The peak secondary response of the normal controls was at day 7. She was able to produce IgG. The sample obtained 22 days after the secondary immunization contained 22% IgG (Fig. 1).



Responses to primary (1°) and Secondary (2°) immunization with Bacteriophage øx 174 in normal controls (shaded areas) and in the patient with selective complete C1q Deficiency (0 circles). Primary and secondary responses were present in the patient, but were rather delayed. Usually the peaks of the primary and secondary antibody responses are at two weeks and one week respectively, while these peaks in the patient were at four weeks and three weeks. In the controls 56 ± 24 percent (mean ± 1 S.D.) of the antibody in the secondary response consisted of IgG whereas the patient had a value of 22%.

Discussion

Complement plays an important role in host defense against viral and bacterial infections. In many instances, complement deficiencies have been associated with increased susceptibility to infection[1]. This was also observed in the patients with selective complement C1q deficiency whom we have followed. Three of these patients died of sepsis or meningitis and one of bronchopneumonia and renal failure[2,4]. The role played by antibody deficiency in susceptibility to infection has not been investigated due to the limited number of individuals with this defect.

Complement defects which occur naturally provide an opportunity to evaluate the role of complement on antibody response. Previous studies have suggested that normal function of the early components of the classical complement pathway has an important role in the induction of T dependent antibody production. A reduced antibody response to T dependent antigens has been observed in C_4 deficient men and animals, and also in mice following complement depletion with the cobra venom factor[9-11]. In vivo complement depletion suppresses the production of IgG and other T dependent antibody classes much more than the

relatively T dependent production of IgM[11]. Once normal priming has occurred, depletion during secondary immunization has a less marked suppression effect on the antibody response[11].

In our patient, we found no abnormality in cellular or humoral immunity except for abnormal kinetics of the antibody response to bacteriophage. The titers increased continuously during the primary response (in controls, antibody titers persistently peaked at two weeks post phage injection) and after secondary immunization, the peak was observed at three weeks rather than at one week (Fig. 1).

The C1q level in patients with selective complete C1q deficiency is very low but not zero (about one percent of normal)[3], and the half life of C1q is short (3.4 hours)[2]. The low C1q level may be sufficient to ensure formation of enough antigen-antibody-complement (C3b) complexes to stimulate antibody production and induce maturation of antigen specific memory cells. Therefore this study did not confirm our hypothesis of a defective antibody response as the mechanism for recurrent infections in this patient. An altenate hypothesis is the possible role of defective opsonization in the development of sepsis or other severe infections in this deficiency. Patients with complement defects may have defective clearance of antigen-antibody complexes by the reticuloendothelial system, due to failure of opsonization of immune complexes resulting from complement deficiency[12]. In our patient, circulating immune complexes were positive on several occasions. It has also been shown that early components of complement are sufficient for viral neutralization[13,14], and C1q may react directly with viral particles[15]. It is conceivable that our patient formed immune complexes with some viral antigens utilizing a small amount of C1q which results in the activation of the classical complement pathway and brings the already low C1q level to an even lower or maybe undetectable level leading to severe immunodeficiency and recurrent infection. In addition to normal cellular and humoral immunity, activation of the alternative complement pathway may also compensate for this. In order to prove the latter hypothesis, comprehensive immunologic studies in patients with selective complete C1q deficiency are necessary and may offer some clues for the mechanism(s) responsible for infections in this rare type of complement defect.

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