Selective Immunoglobulin M Deficiency in an Adult With *Streptococcus pneumoniae* Sepsis and Invasive Aspergillosis

R Hong, S Gupta

Division of Basic and Clinical Immunology, University of California, Irvine, California, USA

Abstract

Primary selective immunoglobulin (Ig) M deficiency usually presents early in life with recurrent or severe infections caused by encapsulated and gram-negative organisms. Primary selective IgM deficiency in adults is rare and is usually associated with autoimmune diseases or malignant neoplasm. We performed an extensive immunological analysis of innate and adaptive immunity in an adult patient with possible primary selective IgM deficiency who presented with life-threatening *Streptococcus pneumoniae* septic shock and invasive *Aspergillus fumigatus* infection. The patient had no evidence of autoimmune disease or malignant neoplasm. Serum IgG, IgA, and IgE were normal; however, serum IgM levels and specific antibody titers against all 14 pneumococcal polysaccharide serotypes were consistently low. Complement CH50, C3, C4, and neutrophil phagocytosis and oxidative burst were normal. Toll-like receptor expression on monocytes was also normal. Therefore, adult patients with serious life-threatening and unusual infections should be investigated for possible selective primary IgM deficiency.

Key words: Selective IgM deficiency. Primary immunodeficiency. Unusual infections. Toll-like receptors. Autoantibodies. Aspergillosis. Complement. Immune complexes.

Resumen

La insuficiencia de IgM selectiva primaria se manifiesta habitualmente en una edad temprana con infecciones recurrentes o graves causadas por bacterias gram-negativas encapsuladas. La insuficiencia de IgM selectiva primaria es muy poco frecuente en adultos y normalmente se asocia con enfermedades autoinmunes o neoplasias malignas. Se llevó a cabo un análisis inmunológico extensivo de la inmunidad innata y adaptativa en un paciente adulto con una posible insuficiencia de IgM selectiva primaria que presentó un choque septicémico de *Streptococcus pneumoniae* que podía poner en peligro su vida y una infección por *Aspergillus fumigatus* invasiva. El paciente no mostraba signos de ninguna enfermedad autoinmune ni de neoplasia maligna. La IgG, IgA e IgE séricas eran normales, no obstante, las concentraciones de IgM séricas y los títulos de anticuerpos específicos frente a los 14 serotipos de polisacáridos neumocócicos fueron bajos. Los complementos CH50, C3, C4, y la fagocitosis de neutrófilos y la explosión oxidativa fueron normales. La expresión de los receptores toll-like en los monocitos también fue normal. Por lo tanto, hay que descartar que los pacientes adultos que presentan infecciones graves potencialmente mortales y poco comunes no tengan una posible insuficiencia de IgM selectiva primaria.

Palabras clave: Insuficiencia de IgM selectiva. Inmunoinsuficiencia primaria. Infecciones poco comunes. Receptores toll-like. Autoanticuerpos. Aspergilosis. Complemento. Complejos inmunes

Introduction

Selective immunoglobulin (Ig) M deficiency is a rare form of primary immunodeficiency with a reported prevalence of 0.03% to 3% [1]. Selective IgM deficiency can be asymptomatic or present symptomatically with infections caused by encapsulated bacteria and viruses, some of which can be serious and even life-threatening. These vary from pneumonia to septicemia and meningitis [2-4]. Selective IgM deficiency is a heterogeneous disorder with no known genetic component, and may occur as a primary or a secondary condition. Secondary selective IgM deficiency is often associated with malignant neoplasm or autoimmune diseases [5-8].

Case Description

A 49-year-old previously healthy man with an unremarkable clinical history presented with fevers, chills, malaise, and a generalized petechial rash. He quickly developed septic shock and respiratory failure, and was admitted to the intensive care unit where he was intubated and received multiple vasopressors, aggressive intravenous fluid hydration, and parenteral antibiotics. Multiple blood cultures grew *Streptococcus pneumoniae*. His hospital stay was prolonged and complicated. He suffered acute renal failure requiring dialysis and disseminated intravascular coagulation with deep vein thrombosis of the inferior vena cava and bilateral iliac veins for which an inferior vena cava filter was implanted. He also developed acute infectious purpura fulminans involving over 30% of his body. This progressed from the initial petechial rash to larger confluent ecchymotic areas, some of which became necrotic and gangrenous, and necessitated amputation of his right leg below the knee and multiple excisional débridements and skin grafts. The right leg stump became infected with vancomycin-resistant *Enterococcus faecium* and *Candida*

Table. Immunologic Studies

	Patient Result		Reference Range	
White blood cell count, $10^3/\mu L$	15.6		4.0-10.5	
Hemoglobin, g/dL	9.3		13.5-16.9	
Platelets, $10^3/\mu L$	96		150-400	
Absolute neutrophil count, $10^3/\mu L$	14		2.0-8.1	
Absolute lymphocyte count, $10^3/\mu L$	0.8		0.9-3.3	
Absolute monocyte count, $10^3/\mu L$	0.5		0.0-0.8	
Absolute eosinophil count, $10^3/\mu L$	0.3		0.0-0.54	
HIV ELISA	Negative		Negative	
Adaptive immunity				
Lymphocyte subsets, No./µL (%)				
CD3 + T cells	1265	(81)	619-1847	(62-84)
CD3 + CD4 + T cells	1062	(68)	338-1194	(31-61)
CD3 + CD8 + T cells	156	(10)	85-729	(10-38)
Ratio of CD4/CD8		(6.8)		(0.9-3.7)
CD3-CD19 + B cells	219	(14)	51-473	(5-26)
CD3-CD56 + NK cells	62	(4)	12-349	(1-17)
In vitro lymphocyte proliferative response (cpm)				
Phytohemagglutinin	198 871		114 881-289 206	
Concanavalin	174 387		131 199-252 925	
Pokeweed mitogen	21 399		20 171-78 728	
Mumps antigen	3763		2052-26 495	
Candida albicans antigen	4957		13 249-60 917	
Tetanus toxoid	907		6092-94 539	
Tuberculin antigen	1324		-545-2580	
Serum immunoglobulins				
IgM, mg/dL	18		65-263	
IgA, mg/dL	180		68-378	
IgE, mg/dL	124		10-150	
IgG, mg/dL	1060		694-1618	
IgG1, mg/dL	660		239-1083	
IgG2, mg/dL	219		123-548	
IgG3, mg/dL	28		27-134	
IgG4, mg/dL	9		8-88	
Autoantibodies				
ANA	1:160 (Nucleolar)		<1:40	
Anti-dsDNA antibody	Negative		Negative	
ANCA	Negative		Negative	
RF	Negative		Negative	
Anti-Smith	Negative		Negative	
Anti-RNP	Negative		Negative	
Lymphocyte apoptosis (% cells)[a]				
Spontaneous	18%		10%-20%	

Abbreviations:ANA, antinuclear antibodies; ANCA, antineutrophil cytoplasmic antibodies; cpm, counts per minute; dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbent assay; HIV, human immunodeficiency virus; Ig, immunoglobulin; RF, rheumatoid factor; RNP, ribonucleoprotein antibodies

[a] Apoptosis was measured by Annexin V binding

Table. Immunologic Studies (continued)

	Patient Result		Reference Range
Adaptive immunity (continued)			
Specific antibodies			
Tetanus toxoid antibody (IgG), IU/mL	0.22		>1
Pneumococcal antibody (IgG µg/mL)	Pre[a]	Post[b]	
Serotype 1	0.34	0.65	
Serotype 3	0.11	0.12	
Serotype 4	0.19	0.18	
Serotype 5	0.59	0.98	
Serotype 6B	0.56	0.46	
Serotype 7F	0.21	0.60	
Serotype 8	0.12	0.38	
Serotype 9N	0.13	0.40	
9Serotyope V	0.13	0.32	
Serotype 12F	0.49	0.50	
14, µg/mL	0.66	1.00	
18C, µg/mL	0.23	0.68	
19F, µg/mL	0.45	0.92	
23F, µg/mL	0.07	0.42	
Innate immunity			
C3, mg/dL	128		88-201
C4, mg/dL	32		16-47
CH50 U/mL	340		101-300
Raji immune complex µgE/mL	9		0-25
C1q binding assay µgE/mL	2.4		0-3.9
C reactive protein, mg/dL	>20		0-0.7
Reactive oxygen species (index)	2.2		2-18
Phagocytosis assay (% phagocytosis)	38.4		25-45
TLR2 + CD14 + monocytes	74.4		19-76

Abbreviations: Ig, immunoglobulin; TLR2, toll-like receptor 2
[a] Pre = IgG > 1 µg/mL
[b] Post = pre x4

albicans, and an additional course of antibiotics was necessary. Five weeks after presentation, necrotic tissue was observed in his nostrils. He was diagnosed with acute invasive fungal sinusitis following nasal biopsy and right maxillary aspirate cultures, which grew *Aspergillus fumigatus*: subsequent débridement was performed. Physical examination revealed post-surgical changes in his nose and the amputation stump. Skin examination revealed multiple erythematous and necrotic rashes on his arms and legs.

Lymphocyte subsets and toll-like receptor (TLR) expression on monocytes were determined by multicolor flow cytometry using direct fluorochrome-conjugated antibodies against CD3, CD4, CD8, CD19, CD16/CD56, CD14/TLR2, and isotype controls. Lymphocyte proliferation was measured by culturing mononuclear cells (2×10^5/well) in triplicate in round-bottom tissue culture plates at 37°C in the presence or absence of optimal concentrations of mitogens and antigens and by incorporation of 3H-thymidine. Data were expressed as net counts per minute.

Phagocytosis of neutrophils was performed on 100 µL of whole blood, which was incubated with phycoerythrin-conjugated yeast or unlabeled yeast for 15 minutes at room temperature. Samples were incubated with 2 mL of fluorescence-activated cell sorting (FACS) lysing solution for 15 minutes and washed with 2 mL of phosphate-buffered saline. Flow cytometry analysis was performed with a FACSCalibur flow cytometer (Becton Dickinson, San Jose, California, USA). Reactive oxygen species (ROS) as a measure of oxidative burst was generated on 100 µL of whole blood, which was incubated at 37°C with oxidation-dependent fluorescence dihydrorhodamine 123 (2.5 µg/mL) for 15 minutes, then stimulated with phorbol-12-myristate-13 acetate (2.5 µg/mL) for an additional 15 minutes. The samples were acquired on the FACSCalibur. All flow cytometry data were analyzed using Simulset (Becton Dickinson, San Jose, California, USA) software.

Comprehensive immunologic evaluation is presented in the Table. The white blood cell count was high with an elevated neutrophil percentage. T cells, T cell subsets, B cells, and natural killer cells were within normal limits. Lymphocyte transformation of phytohemagglutinin, concanavalin A, pokeweed mitogen, mumps antigen, and purified protein derivative antigen was normal. However, low lymphocyte transformation to *C albicans* and tetanus toxoid was observed, suggesting a T cell functional defect. The patient had normal quantitative serum IgG and IgG subclasses, IgA, and IgE. However, serum IgM levels were low at 18 mg/dL and the patient failed to make an antibody

response to all 14 pneumococcal polysaccharide serotypes (even in the presence of *S pneumoniae* sepsis) and to tetanus toxoid. Furthermore, the patient made no antibodies following Pneumovax vaccination. Repeated determinations showed low levels of IgM, and the patient remained IgM-deficient even after 1 year from the initial diagnosis. Neutrophil phagocytic capacity and generation of ROS were normal. TLR2 on monocytes was normal. C-reactive protein (CRP) was markedly elevated. Circulating immune complexes were negative. The antinuclear antibody test result was positive, although the results of testing for anti-dsDNA antibody, antineutrophil cytoplasmic antibody, rheumatoid factor, anti-Smith antibody, and antiribonucleoprotein antibody were negative.

Discussion

Primary selective IgM deficiency is a rare disorder in children and has no known genetic component. However, familial cases [4] and autosomal dominant inheritance [9] have been suggested. Primary selective IgM deficiency in children may present with severe life-threatening infections, whereas in adults it is usually associated with autoimmune diseases and malignant neoplasm [5-8]. A few cases of possible primary selective IgM deficiency in adults with no evidence of autoimmunity or malignant neoplasm have been reported [9,10]. These patients usually present with mild infections. Our patient, who was previously healthy with no evidence of autoimmunity or neoplasm, represents a possible case of primary selective IgM deficiency in adults with severe life-threatening and unusual infections. Numbers of B cells, especially with surface IgM, are generally normal, high, or decreased [5,9,10]. Our patient also had normal proportions of CD19+ B cells. Although levels of IgG and IgG subclasses were normal, our patient had decreased specific IgG antibody response to tetanus toxoid and to all 14 serotypes of pneumococcal polysaccharides. Guill et al [11] also reported decreased specific antibody response to tetanus toxoid and pneumococcal polysaccharide, and decreased IgM response to immunization with φX174 in children with selective IgM deficiency. Proportions and numbers of CD4+ T cells and CD8+ T cells and CD4+/CD8+ T cell ratios have been reported to be normal, low, or high [5,9]. In our patient, the CD4+/CD8+ T cell ratio was abnormally high.

The pathogenesis of primary selective IgM deficiency is unknown. A number of defects have been reported, including intrinsic B cell defect in plasma cell differentiation [6], increased T cell suppressor activity, which may be specific to IgM isotype [5,7,9] or isotype-nonspecific [9], and decreased helper T cell activity [10]. This suggests that selective IgM deficiency is a heterogeneous disorder, which requires further studies to elucidate the predominant mechanisms involved in its pathogenesis.

Our patient is unique in that he had severe infections with 2 organisms, *S pneumoniae* and *A fumigatus*, both containing polysaccharide antigens. *S pneumoniae* is an important pathogen in humans, and both adaptive and innate immune mechanisms provide protection from infection. Although protective anticapsular antibodies can be produced following immunization, the innate immune responses are clearly important in controlling infections in the nonimmune host. Our patient failed to produce protective anticapsular antibodies against any of the 14 serotypes tested. Several components of the innate immune system that play a role in defense against *S pneumoniae* include CRP and signaling via TLRs. CRP provides protection against *S pneumoniae* in both a complement-dependent and complement-independent manner. Our patient had increased CRP and complement levels were normal. Therefore, it appears unlikely that a defect in a CRP-dependent mechanism was responsible for *S pneumoniae* sepsis. TLRs are important in initiating innate responses to a wide variety of pathogens [12]: TLR2 recognizes lipoteichoic acid, lipoarabinomannan, and lipopeptides, whereas TLR4 recognizes lipopolysaccharide. In our patient, TLR2 expression on CD14+ monocytes was normal.

The first line of defense against *A fumigatus* is provided by innate immunity, predominantly by macrophages via secretion of tumor necrosis factor-α (TNF-α). Various mechanisms of TNF–α-mediated resistance against *A fumigatus* include upregulation of phagocytosis and augmentation of antibody-dependent cytotoxicity and of oxidative burst to kill A fumigatus [13,14]. Our patient had normal polymorphonuclear cell phagocytosis. Although a role for specific antibodies in defense against *A fumigatus* has not been described, it is possible that specific antibody responses against the polysaccharide antigen galactomannan may play a role in late defense response, and, therefore, a polysaccharide antibody defect in our patient may be responsible for the development of invasive aspergillosis.

The take-home messages from this patient are as follows: (a) adult patients with serious *S pneumoniae* infection should be investigated for possible selective IgM deficiency; (b) both children and adults with possible primary selective IgM deficiency are susceptible to life-threatening and often unusual infections; and (c) taking into consideration the history of serious and unusual infection in this patient with selective IgM deficiency and various host defense mechanisms against these organisms, patients with selective IgM deficiency should also be investigated for innate immune response.

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Sudhir Gupta, MD, PhD

Medical Sciences I, C240
University of California, Irvine
Irvine, CA 92697, United States
Phone: (949) 824-5818
Fax: (949) 824-4362
E-mail: sgupta@uci.edu

