

Decreased CD27+ Memory B cells in DiGeorge Anomaly

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ABSTRACT

Patients with DiGeorge anomaly (DGA) have a variety of B-cell immune deficiencies, including IgA deficiency and impaired polysaccharide antibody responses. CD27+ memory B cells have been found to be decreased in a variety of B-cell immunodeficiencies, such as selective antibody deficiency (SAD). We evaluated for a correlation between reduced CD27+ memory B cells and impaired polysaccharide antibody responses in patients with DGA. A retrospective analysis of DGA patients referred for recurrent sinopulmonary infections was performed. Twenty-one patients with DGA (62% male and median age was 5 ± 0.8 years) were evaluated. Six patients had reduced CD3+ T cells, and two patients had reduced CD4+ T cells, but none had opportunistic infections. B-cell numbers and percentages were normal in all patients, but the percentages of memory CD27+ B cells were decreased compared to normal values, $12 \pm 2\%$ versus 34%. CD27+ B cells were $<15\%$ in 86% of patients. Eighty-one percent of patients had reduced protective antibody responses to $<70\%$ of *Streptococcus pneumoniae* serotypes. Immunoglobulin levels were normal in all patients except one with selective IgA deficiency. Seventy-five percent of the patients had both decreased CD27+ B cells and decreased antibody responses to *S. pneumoniae* immunizations. In addition, patients who were treated with intravenous immunoglobulin (IVIG), had CD19+ B cells $\leq 7\%$, and 12 of 14 patients treated with prophylactic antibiotics had CD19+ B cells $\leq 14\%$. There was an association of anti-*S. pneumoniae* antibody responses and percentages of CD27+ B cells in 86% of patients. The percentages of CD27+ memory B cells and antibody responses to *S. pneumoniae* were associated in patients with DGA. Decreased memory B cells may be involved in the pathogenesis of SAD, as seen in patients with DGA, and may be predictive of increased susceptibility to infections.

INTRODUCTION

DI GEORGE ANOMALY (DGA) is considered a field defect of the third and fourth pharyngeal pouches that occurs in the first trimester of fetal development and affects facial, parathyroid, aortic arch, and thymic structures.¹⁻⁴ This leads to the clinical syndrome of unusual facies, hypocalcemia, conotruncal cardiac anomalies, and congenital T-cell immunodeficiency. In addition, speech and development abnormalities frequently

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occur in patients with DGA. Most patients with DGA anomaly have a heterozygous microdeletion of chromosome 22.q11.2. Recent studies have suggested that TBX1 is the gene defect that results in DGA.⁵

Thymic aplasia leads to profound T-cell immunodeficiency, complete DGA.¹ Fortunately, most patients with DGA have some to normal thymic tissue and sufficient T cells to protect against opportunistic infections.^{4,6,7} More commonly, patients with DGA have recurrent otitis media and sinusitis.⁷ Anatomic and physiologic reasons related to velopharyngeal insufficiency may account for this increased susceptibility. B-cell numbers, serum IgG, IgA, and IgM levels, and antibody function are typically normal in DGA; although there is increased frequency of IgA deficiency and functional antibody responses, especially to bacterial polysaccharide antigens, are frequently impaired in patients with DGA who have recurrent sinopulmonary infections.⁷⁻¹² As a potential mechanism, Haire et al.¹³ reported that in DGA patients with recurrent infections, B-cell repertoire and diversity were decreased due to decreased T-cell help.

Recently, investigators have reported that CD27+ memory B cells are reduced in a variety of B-cell immunodeficiencies, including common variable immunodeficiency (CVID), hyper-IgM syndromes (HIGM), and selective antibody deficiency (SAD).¹⁴⁻¹⁷ Because SAD frequently occurs in patients with DGA, we examined whether there was an association of reduced CD27+ memory B cells and impaired antibody responses to pneumococcal polysaccharide antigens in patients with DGA.

MATERIALS AND METHODS

Patient population

This is a retrospective analysis of patients with DiGeorge anomaly who were referred to the Pediatric Allergy and Immunology Clinic at St. Louis University Health Sciences Center for evaluation of recurrent sinopulmonary infections. All children received standard childhood immunizations, as well as unconjugated *Streptococcus pneumoniae* (Pneumovax®) immunizations.

Immunologic studies

T, NK, and B cell enumeration studies were performed on whole blood as previously described in the Pathology Laboratory by cytofluorographic analyses.¹⁸ The CD27+ subpopulation is expressed as the percentage of gated CD19+ B cells. In this study, we examined total CD27+ B cells, which in adults represent 25% to 45% of B cells,^{19,20} and defined decreased CD27+ B cells as <15% of CD19+ B cells. Serum IgG, IgA, and IgM quantification was performed by the Clinical Laboratory in the Department of Pathology using nephelometry.¹⁸ Antidiphtheria and antitetanus antibody titers were measured by Quest Laboratories. *S. pneumoniae* antibody concentrations to 12 or 14 serotypes were measured at Quest Laboratories. We defined a normal response to unconjugated *S. pneumoniae* immunization as an antibody level of ≥ 2.0 $\mu\text{g/mL}$ to $\geq 70\%$ of the serotypes tested, as recommended by Bonilla et al.²¹ In previous studies, we defined normal anti-*S. pneumoniae* antibody responses $>50\%$ of serotypes; in addition, we observed that patients with more severe infections and were treated with intravenous immunoglobulin (IVIG) had protective antibody responses to $\leq 25\%$ of the serotypes.¹⁸ Landesman et al.²² and Douglas et al.²³ demonstrated that by 12 to 17 months of age protective levels of antibody were produced to 11 of 12 pneumococcal serotypes and by 2 years of age all serotypes induced protective levels. We did not choose to use a ≥ 4 -fold rise in antibody titer following vaccination because this standard has not been as well studied, and in our experience the presence of a protective antibody level (in the absence of a ≥ 4 -fold postvaccination rise in antibody titer) is not associated with an increased risk of infection. All antibody studies were performed prior to initiation of IVIG therapy.

Statistics

Multiple linear regressions were performed on CD27+ B cells and percentages of protective antibody responses to *S. pneumoniae* serotypes.

RESULTS

Patient characteristics

As seen in Table 1, there were 21 patients with DGA. All patients were identified to have a heterozygous 22q11.2 microdeletion. The median age was 5 ± 0.8 years old, and there were 13 males and 8 females. A variety of cardiac anomalies were present in 67% of the patients, including tetralogy of Fallot (three patients), ventricular septal defect (four patients), interrupted aortic arch (one patient), pulmonary atresia (one patient), anomalous pulmonary venous return (one patient), and patent foramen ovale (one patient). Hypoparathyroidism and treatment for hypocalcemia was present in only 19% of the patients. Speech delay and speech therapy were present in 76% of the patients, and varying severity of developmental delay was present in 62%. Recurrent sinopulmonary infections were frequent in these patients, consisting primarily of sinusitis in 48%, otitis media in 62%, and pneumonia in 24%. The susceptibility to recurrent infections was managed predominantly by prophylactic antibiotics in 67% of the patients, and by IVIG treatments in 14% of the patients.

T-cell immunity

As seen in Table 2, CD3+ T-cell numbers and percentages were normal in the majority of patients. Six patients had reduced CD3+ T cells, 356, 683, 1,012, 1,075, 1,285, and 1,334 CD3+ cells/mm³; however, none of the patients had opportunistic infections. In the majority of patients, the percentages and numbers of CD4+ T cells were normal, but were decreased in two patients, 308 and 472 CD4+ cells/mm³. Furthermore, naive CD4+CD45RA+ T cells were also reduced in a minority of patients.

B-cell immunity

The numbers of B cells were normal in patients with DGA, 892 ± 141 cells/mm³. However, the percentages of CD27+ B cells were reduced compared to published adult normal levels,^{24,25} $12 \pm 2\%$ versus 34% (range 20–45%), and CD27+ B cells were $<15\%$ in 18 patients (86%). Serum IgG, IgA, and IgM levels were normal in all of the patients, except for one patient with selective IgA deficiency (Table 2). Functional antibody responses to protein antigens were normal in all of the patients to diphtheria toxoid, 80% of patients to tetanus toxoid, and in 80% of patients to *Hemophilus influenzae* type B immunizations.

We stratified the antibody responses to *S. pneumoniae* serotypes into quartiles of positive antibody responses to 0–25% of serotypes, 26–50% of serotypes, 51–75% of serotypes, and 76–100% of serotypes. Nineteen per-

TABLE 1. CLINICAL CHARACTERISTICS OF PATIENTS WITH DiGEORGE ANOMALY

Characteristic	Percent of patients (21)
Age, yrs	5 ± 0.8
Male/female, %	62/38
22q11.2 microdeletion, %	100
Cardiac disease, %	67
Hypocalcemia, %	19
Speech delay, %	76
Developmental delay, %	62
Recurrent infections, %	
Sinusitis	48
Otitis media	62
Pneumonia	24
Prophylactic antibiotics, %	67
IVIG, %	14

DGA, DiGeorge anomaly; IVIG, intravenous immunoglobulin. Data presented as Mean $\pm$ SE for age and percentage of patients.

MEMORY B CELLS IN DIGEORGE ANOMALY

TABLE 2. IMMUNOLOGIC FINDINGS IN PATIENTS WITH DiGEORGE ANOMALY

Study	Patient (21)	Normal for age 2–5 years old
ALC	3,514 $\pm$ 353	2,700–11,900
CD3 cells/mm ³	1,864 $\pm$ 224 (6)	1,400–8,000
CD4 cells/mm ³	1,158 $\pm$ 154 (2)	900–5,500
CD8 cells/mm ³	658 $\pm$ 76 (0)	400–2,300
CD19 cells/mm ³	892 $\pm$ 141 (0)	600–3,100
CD56 cells/mm ³	488 $\pm$ 83 (0)	100–1,400
CD4+CD45RA+, %	74 $\pm$ 4 (2)	63–91
CD4+CD45RO+, %	55 $\pm$ 4	7–20
CD19+CD27+, %	12 $\pm$ 2 (18)	34 (25–45) ^b
IgG, mg/dL	828 $\pm$ 56 (1)	508–1,359
IgA, mg/dL	147 $\pm$ 37 (1)	21–129
IgM, mg/dL	92 $\pm$ 13 (0)	31–126
IgE, IU/dL	19 $\pm$ 4	9–69
antidiphtheria toxoid, μ g/mL	1.0 $\pm$ 0.3 (0)	$\geq$ 0.05
anti-tetanus toxoid, μ g/mL	2.6 $\pm$ 0.5 (4)	$\geq$ 0.50
anti-HiB, mcg/ml	4.3 $\pm$ 0.9 (4)	$\geq$ 1.00
anti- <i>S. pneumoniae</i> antibody responses ^c		
0–25% of serotypes	33%	
26–50% of serotypes	38%	
51–75% of serotypes	10%	
76–100% of serotypes	19%	

Data presented as mean $\pm$ SE. In parenthesis, number of patients with decreased levels.

^aFrom Comans-Bitter et al.³⁷ and ^bBrouet et al.¹⁹

^cPercentage of patients that had protective anti-*S. pneumoniae* antibody levels to 0–25% of serotypes, 26–50% of serotypes, 50–75% of serotypes, and 76–100% of serotypes.

cent of patients with DGA produced protective antibody responses to $\geq$ 70% of the serotypes; whereas 81% of patients had reduced antibody responses to $<$ 70% of the serotypes (Fig. 1). When CD27+ B cells percentages (absolute numbers of CD27+ B cells were similar) and *S. pneumoniae* antibody responses were correlated, 16 patients (75%) had both decreased antibody responses to *S. pneumoniae* to $<$ 70% of serotypes and decreased CD27+ B cells $<$ 15%, and 2 patients (10%) had both normal percentages of CD27+ B cells and antibody responses to *S. pneumoniae* (Fig. 1). On the other hand, two patients (10%) had decreased CD27+ B cells but normal antibody responses to *S. pneumoniae*, and one patient (5%) had normal CD27+ B cells but decreased *S. pneumoniae* antibody responses. However, multiple linear regression was not significant ($R^2 = 0.082$). Thus, in 86% of the patients there was an association of anti-*S. pneumoniae* responses and percentages of CD27+ B cells. Three patients who were treated with IVIG had CD27+ B cells of 1, 4.3, and 7%,

CD27+ Memory B-cells and *Streptococcus pneumoniae* Antibody Responses

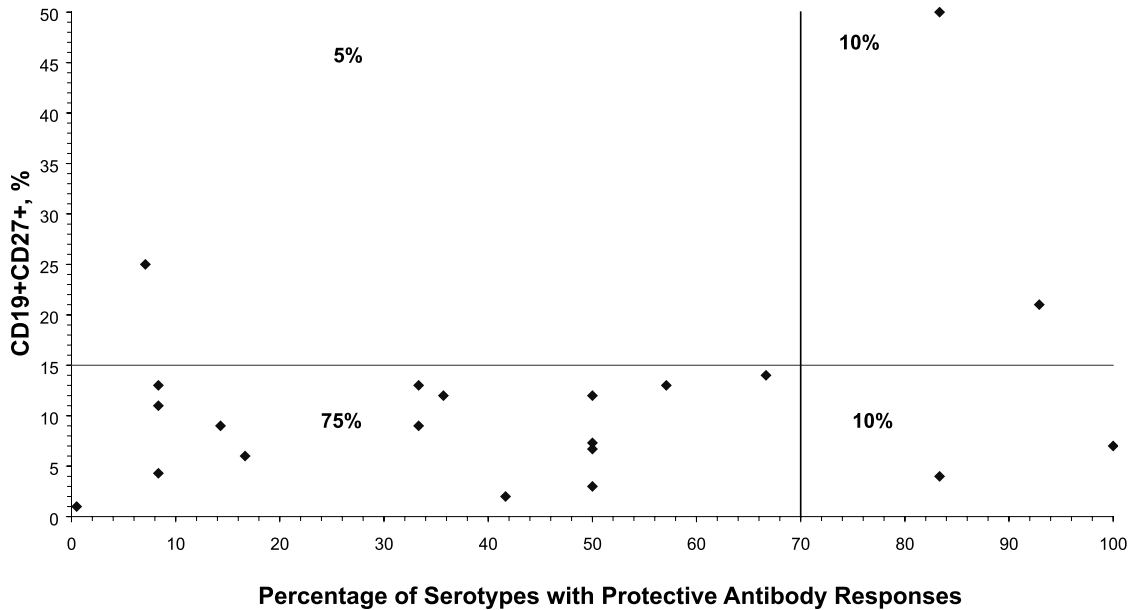


FIG. 1. Correlation of CD27+ memory B cells and anti-*S. pneumoniae* antibody responses. In the majority (75%) of patients, there was an association of decreased CD27+ memory B cells and decreased antibody responses to *S. pneumoniae*, and in 10% of patients there were normal CD27+ memory B cells and normal antibody responses to *S. pneumoniae*. Multiple linear regression was not significant ($R^2 = 0.082$).

and 12 of 14 patients treated with prophylactic antibiotics had CD27+ B cells $\leq 14\%$. Only 2 of 14 patients treated with prophylactic antibiotics had normal percentages of memory B cells.

DISCUSSION

A variety of immune abnormalities may be present in patients with DGA. Patients with complete DGA have low percentages of T cells and develop severe infections caused by opportunistic infections. In patients with partial DGA, the T cell numbers and function vary widely based on the amount of thymic tissue present. Abnormalities in the humoral immune response are seen in about 10–15% of patients with DGA.^{4,26} Decreased percentages of CD27+ memory B cells have been widely reported in other immunodeficiencies,^{14–16} but only one other study found decreased memory B cells in patients with DGA.²⁷

The majority of patients in this study had normal percentages of T cells. However, 28% of patients had decreased CD3+ T cells, but none of the patients developed opportunistic infections. CD4+ T cells and naive CD4+ CD45RA+ T cells were reduced in 9% of our patients. Other studies have also reported decreased T cells in patients with DGA. Jawad et al.⁷ found decreased average T-cell numbers compared with controls, and Chinen et al.²⁸ found lower median T cells in 45 patients with DGA compared with controls. Pierdominici et al.²⁹ evaluated 14 patients with 22q11.2 deletion and found that they had decreased CD3+ and CD8+ T cells when compared with controls, but there was no significant difference in CD4+ T cell numbers when compared with controls. Three years after the initial study, they found spontaneous improvement in all T cell populations, which is in contrast to other studies evaluating T-cell subtypes.^{7,28}

Our patients had normal numbers and percentages of B cells. Immunoglobulin levels were normal except in the one patient with selective IgA deficiency. Other studies have also reported normal im-

munoglobulin levels in DGA, especially in older subjects.^{7,30} On the other hand, hypogammaglobulinemia has been observed,^{9–12,31} which may be the result of a decreased T helper activity for the production of immunoglobulins.^{11,12}

The majority of our patients had reduced pneumococcal antibody responses to *S. pneumoniae* immunizations. Selective antibody deficiency to polysaccharide antigens has been previously reported in patients with DGA.^{9,10} This antibody deficiency may lead to recurrent sinopulmonary infections in patients with DGA. Haire et al.¹³ postulated that T cells play an important role in the generation of diversity within the B-cell repertoire, as evidenced by reduced IgM VH somatic mutation in one patient with complete DGA, but normal in a patient with partial DGA. Whether this plays a role in decreased CD27+ memory B cells in DGA is unclear.

In our patients, the antibody responses to *S. pneumoniae* were stratified into quartiles to allow for evaluation of correlation between antibody function and CD27+ memory B-cell percentages. Unfortunately, we did not analyze subpopulations of CD27+ B cells. CD27+ B cells can be further delineated into IgM+IgD+CD27+ (approximately 40% of CD27+ B cells), IgM+IgD-CD27+ IgM memory (approximately 20% of CD27+ B cells), and IgM-IgD-CD27+ class-switch B cells (approximately 40% of CD27+ B cells).¹⁷ CD27+IgD-switched memory B cells are typically $\geq 7\%$; thus, when CD27+ B cells and anti-*S. pneumoniae* antibody responses were correlated, we used a conservative value of 15% as normal percentages of CD27+ memory B cells. The majority of our patients had reduced antibody titers to *S. pneumoniae*, indicating a selective antibody deficiency. Seventy-five percent of the patients had both decreased antibody titers and decreased CD27+ B cells. In contrast, 10% of the patients had both protective responses to $\geq 70\%$ of the serotypes and normal CD27+ B cell percentages. Clinically, CD27+ B cells were typically decreased in DGA patients who were treated with either IVIG and/or prophylactic antibiotics. Therefore, an association between antibody responses to *S. pneumoniae* and CD27+ memory B cells was present in most of the patients. While few other studies have reported the incidence of decreased memory B cells in patients with 22q11.2 deletion, this phenomenon has been well reported in patients with CVID,^{16,19,20,32–35} which may help to increase our understanding of this finding in DGA patients.

Decreased memory B cells are common in CVID patients, and the Frieberg classification was developed to characterize these patients based on numbers of memory B cells.³² Group I includes patients with $<0.4\%$ memory B cells. This group can be further subdivided into group Ia with increased numbers of memory (CD19+CD21-) B cells and group Ib with normal numbers of memory B cells. Group II includes patients with normal numbers of memory B cells, and group III consists of patients with a total B cell count of $<1\%$. This classification of CVID patients has been accepted by other investigators.^{24,25,32} In an evaluation of patients with CVID, Agematsu et al.¹⁶ found that all of these patients had decreased memory B cells. These investigators also found that Ig V region genes showed no or few changes of nucleotides/amino acids in patients with low memory B cells.

Alachkar et al.¹⁴ evaluated patients with SAD and CVID and found that the median percentages of memory B cells in these patients were not significantly different than controls, but that patients with splenomegaly, bronchiectasis, and autoimmune disease had significantly decreased memory B cells. Similarly, Ko et al.³⁶ found that patients with the least amount of memory B cells have the lowest levels of IgG, decreased vaccine responses, and higher frequencies of autoimmune and granulomatous disease. These studies indicate that CD19+CD27+ B cell numbers should be monitored in patients with primary antibody deficiencies and normal B-cell counts to alert the physician that extensive monitoring and treatment may be required to prevent further complications.

One study found that IgM+IgD+CD27+ (nonswitched) memory B cells in the tonsils are protective against pneumococcal infection,¹⁷ so decreases in these cells could lead to increased infections. A subset of CVID patients has been identified with defective activation of TLR9 and low memory B cells.³⁵ Another investigator has found that TLR9 is required in the conversion of transitional B cells to IgM memory B cells, which are important in antibody responses to polysaccharide antigens. These studies indicate that dysfunctional TLR9 may also lead to abnormal antibody responses to *S. pneumoniae* and increased pneumococcal infections.

The many studies evaluating memory B cells in SAD and CVID may be helpful in evaluating and managing patients with DGA. Many patients with DGA have abnormalities in their humoral immune responses. Patients with DGA and a history of recurrent infections should have a complete B and T cell immune evaluation. This assessment should include analysis of memory B cell numbers. Based on our findings there was an asso-

ciation between pneumococcal antibody responses and memory B cells in patients with DGA. In addition, decreased memory B cells were also associated with patients who were treated with IVIG and/or prophylactic antibiotics. Unfortunately, we did not examine switched memory B cells, IgD-CD27+ B cells, which may be a better subpopulation of memory B cells to examine for anti-*S. pneumoniae* antibody responses. In addition, we did not have age-adjusted normal values of CD27+ B cells, which also needs to be determined in future studies. In conclusion, further studies evaluating CD19+CD27+ B cells and subpopulations of memory B cells in patients with DGA should provide a better understanding of this immune deficiency.

DISCLOSURE STATEMENT

The authors declare there are no competing financial interests.

REFERENCES

1. Ochs HD, Nelson DL, Stiehm ER. Other well-defined immunodeficiency syndromes. In: Stiehm ER, Ochs HD, Winkelstein A, eds. Immunologic disorders in infants and children, 5th edition, New York: Elsevier Sanders, 2004, pp. 505–579.
2. Ryan AK, Goodship JA, Wilson DI, et al. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: A European collaborative study. *J Med Genet* 1997; 34:798–804.
3. McDonald-McGinn DM, Kirschner R, Goldmuntz E, et al. The Philadelphia story: The 22q11.2 deletion: Report on 250 patients. *Genet Couns* 1999; 10:11–24.
4. Sullivan KE, Jawad AF, Randall P, et al. Lack of correlation between impaired T cell production, immunodeficiency, and other phenotypic features in chromosome 22q11.2 deletion syndromes. *Clin Immunol Immunopathol* 1998; 86:141–146.
5. Yagi H, Furutani Y, Hamada H, et al. Role of TBX1 in human del22q11.2 syndrome. *Lancet* 2003; 362:1366–1373.
6. Sullivan KE, McDonald-McGinn D, Driscoll DA, Emanuel BS, Zackai EH, Jawad AF. Longitudinal analysis of lymphocyte function and numbers in the first year of life in chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). *Clin Diagn Lab Immunol* 1999; 6:906–911.
7. Jawad AF, McDonald-McGinn DM, Zackai E, Sullivan KE. Immunologic features of chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). *J Pediatr* 2001; 139:715–723.
8. Smith CA, Driscoll DA, Emanuel BS, McDonald-McGinn DM, Zackai EH, Sullivan KE. Increased prevalence of immunoglobulin A deficiency in patients with the chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). *Clin Diagn Lab Immunol* 1998; 5:415–417.
9. Gennery AR, Barge D, O'Sullivan JJ, Flood TJ, Abinun M, Cant AJ. Antibody deficiency and autoimmunity in 22q11.2 deletion syndrome. *Arch Dis Child* 2002; 86:422–425.
10. Schubert MS, Moss RB. Selective antibody deficiency in familial DiGeorge syndrome. *Ann Allergy* 1992; 69:231–238.
11. Etzioni A, Pollack S. Hypogammaglobulinaemia in DiGeorge sequence. *Eur J Pediatr*. 1989; 49:143–144.
12. Mayumi M, Kimata H, Suehiro Y, et al. DiGeorge syndrome with hypogammaglobulinaemia: A patient with excess suppressor T cell activity treated with fetal thymus transplantation. *Eur J Pediatr* 1989; 148:518–522.
13. Haire RN, Buell RD, Litman RT, et al. Diversification, not use, of the immunoglobulin VH gene repertoire is restricted in DiGeorge syndrome. *J Exp Med* 1993; 178:825–834.
14. Alachkar H, Taubenheim N, Haeney MR, Durandy A, Arkwright PD. Memory switched B cell percentage and not serum immunoglobulin concentration is associated with clinical complications in children and adults with specific antibody deficiency and common variable immunodeficiency. *Clin Immunol* 2006; 120:310–318.
15. Litzman J, Vlkova M, Pikulova Z, Stikarovska D, Lokaj J. T and B lymphocyte subpopulations and activation/differentiation markers in patients with selective IgA deficiency. *Clin Exp Immunol* 2007; 147:249–254.
16. Agematsu K, Futatani T, Hokibara S, et al. Absence of memory B cells in patients with common variable immunodeficiency. *Clin Immunol* 2002; 103:34–42.

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17. Takizawa M, Sugane K, Agematsu K. Role of tonsillar IgD⁺CD27⁺ memory B cells in humoral immunity against pneumococcal infection. *Hum Immunol* 2006; 67:966–975.
18. Wolpert J, Knutsen AP. Natural history of selective antibody deficiency to bacterial polysaccharide antigens in children. *Pediatr Asthma Allergy Immunol* 1998; 12:183–191.
19. Brouet JC, Chedeville A, Fermand JP, Royer B. Study of the B cell memory compartment in common variable immunodeficiency. *Eur J Immunol* 2000; 30:2516–2520.
20. Jacquot S, Macon-Lemaître L, Paris E, et al. B cell co-receptors regulating T cell-dependent antibody production in common variable immunodeficiency: CD27 pathway defects identify subsets of severely immuno-compromised patients. *Inter Immunol* 2001; 13:871–876.
21. Bonilla FA, Bernstein IL, Khan DA, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *Ann Allergy Asthma Immunol* 2005; 94:S1–S63.
22. Landesman SH, Schiffman G. Assessment of the antibody response to pneumococcal vaccination in high risk patients. *Rev Infect Dis* 1981; 3S:184–196.
23. Douglas RM, Paton JC, Duncan SJ, Hansman DJ. Antibody response to pneumococcal vaccination in children younger than 5 years of age. *J Infect Dis* 1983; 148:131–137.
24. Vlková M, Thon V, Sárkyová M, et al. Age dependency and mutual relations in T and B lymphocyte abnormalities in common variable immunodeficiency patients. *Clin Exp Immunol* 2006; 143:373–379.
25. Horn J, Thon V, Bartonkova D, et al. Anti-IgA antibodies in common variable immunodeficiency (CVID): Diagnostic workup and therapeutic strategy. *Clin Immunol* 2007; 122:156–162.
26. Goldmuntz E. DiGeorge syndrome: New insights. *Clin Perinatol* 2005; 32:963–978.
27. Finocchi A, Di Cesare S, Romiti ML, et al. Humoral immune responses and CD27⁺ B cells in children with DiGeorge syndrome (22q11.2 deletion syndrome). *Pediatr Allergy Immunol* 2006; 17:382–388.
28. Chinen J, Rosenblatt HM, O'Brian Smith E, Shearer WT, Noroski LM. Long-term assessment of T cell populations in DiGeorge syndrome. *J Allergy Clin Immunol* 2003; 111:573–579.
29. Pierdominici M, Marziali M, Giovannetti A, et al. T cell receptor repertoire and function in patients with DiGeorge syndrome and velocardiofacial syndrome. *Clin Exp Immunol* 2000; 121:127–132.
30. Junker AK, Driscoll DA. Humoral immunity in DiGeorge syndrome. *J Pediatr* 1995; 127:231–237.
31. Chien YH, Yang YH, Chu SY, Hwu WL, Kuo PL, Chiang BL. DiGeorge sequence with hypogammaglobulinemia: a case report. *J Microbiol Immunol Infect* 2002; 35:187–190.
32. Warnatz K, Denz A, Dräger R, et al. Severe deficiency of switched memory B cells (CD27⁺IgM⁺IgD⁺) in subgroups of patients with common variable immunodeficiency: A new approach to classify a heterogeneous disease. *Blood* 2002; 99:1544–1551.
33. Ferry BL, Jones J, Bateman EA, et al. Measurement of peripheral B cell subpopulations in common variable immunodeficiency (CVID) using a whole blood method. *Clin Exp Immunol* 2005; 140:532–539.
34. Piqueras B, Lavenu-Bombled C, Galicier L, et al. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. *J Clin Immunol* 2003; 23:385–400.
35. Cunningham-Rundles C, Radigan L, Knight AK, Zhang L, Bauer L, Nakazawa A. TLR9 activation is defective in common variable immune deficiency. *J Immunol* 2006; 176:1978–1987.
36. Ko J, Radigan L, Cunningham-Rundles C. Immune competence and switched memory B cells in common variable immunodeficiency. *Clin Immunol* 2005; 116:37–41.
37. Comans-Bitter WM, deGroot R, van den Beemd R, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. *J Pediatr* 1997; 130:388–393.

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