

Analysis of Switched Memory B Cells in Patients with IgA Deficiency

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Key Words

Switched memory B cells • IgA deficiency • Bronchiectasis

Abstract

Background: Selective IgA deficiency (SIGAD) is the most common primary antibody deficiency, characterized by significant decreased serum levels of IgA in the presence of normal IgG and IgM. Despite several investigations into the nature of the disease, the exact pathophysiology of SIGAD is still unknown. **Methods:** In this study, switched memory B cells (CD19+/CD27+/IgD– cell population) of 28 patients with SIGAD and 28 matched healthy controls were investigated using flow cytometry. **Results:** The percentage of switched memory B cells in all healthy controls was more than 0.4%. In SIGAD patients, who were classified as group I, the percentage of switched memory B cells was less than 0.4% (0.34 ± 0.06) in 7 patients (25%). The remaining 21 patients were designated as group II ($1.74 \pm 0.12\%$). The mean concentration of IgG in group I was significantly lower than in group II ($1,014 \pm 278$ vs. $1,388 \pm 406$ mg/dl, $p = 0.028$). Comparison of clinical features between the 2 groups revealed that episodes of pneumonia during the course of disease were significantly higher in group I than in group II ($p = 0.002$). Autoimmune diseases in group I (57.1%) were also significantly higher ($p =$

0.01) than in group II (23.8%). The prevalence of bronchiectasis was 57% in group I, while only 1 patient (4.7%) in group II developed bronchiectasis ($p = 0.006$). Specific antibody deficiency in group I was documented in 5 patients and in group II in 4 patients ($p = 0.01$). **Conclusions:** The classification of SIGAD patients by assessment of switched memory B cells could help physicians with the clinical prognosis for these patients, whereas the patients with reduced switched memory B cells are prone to severe phenotypes.

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Introduction

Selective IgA deficiency (SIGAD) is the most common primary antibody deficiency [1]. It is defined by decreased serum levels of IgA (<5 mg/dl) in the presence of normal IgG and IgM in a subject older than 4 years [2, 3]. Most affected persons are asymptomatic, whereas approximately one third of patients suffer from recurrent mucosal infections, allergies, and autoimmune diseases [4, 5]. The respiratory and gastrointestinal tracts are the main affected organs [6–8]. IgA-deficient patients may be frequently affected by allergies, the most common of which is asthma followed by allergic rhinitis, conjunctivitis, ur-

ticaria, atopic dermatitis and food allergies [6, 9]. Auto-immune diseases associated with SIGAD include immune thrombocytopenic purpura, autoimmune hemolytic anemia (AIHA), rheumatoid arthritis, lupus erythematosus, thyroiditis and vitiligo [10, 11].

The fundamental defect of SIGAD is still unknown; however, two recent studies reported that coding variants in the *TNFRSF13B* gene, which encodes the transmembrane activator and CAML interactor (TACI), could be associated with common variable immunodeficiency (CVID) and SIGAD [12, 13]. Mutations in the TNFR family member TACI, which mediates isotype switching in B cells, were found to be present in 5% of patients with CVID. Mutations in TACI were also found in relatives of patients with CVID who suffered from SIGAD as well as in a patient with SIGAD [14]. B cells from individuals with TACI mutations cannot produce IgG and IgA in response to the TACI ligand APRIL (a proliferation-inducing ligand), probably reflecting impaired isotype switching [15].

Memory B cells are of a B cell subtype that is formed following primary infection. Memory B cells, characterized by cell surface expression of CD27, rapidly generate immunoglobulins of all isotypes during secondary immune responses [16]. Switched memory B cells were first found to be absent in X-linked hyper-IgM syndrome [17]. Subsequently, a number of reports have described a reduction in CD27+ memory B cells in peripheral blood from patients with CVID [18–22]. Warnatz et al. [18] divided CVID patients into 2 groups based on switched memory B cells in peripheral blood lymphocytes (PBLs).

Based on the common genetic basis for SIGAD and CVID [23, 24], we designed this study to check whether patients with SIGAD have any defect in the switching of B cells to IgA-producing plasma cells. In the study we examined the frequency of memory B cells with the flow-cytometric method in Iranian patients with SIGAD. We related this to their laboratory and clinical characteristics to segregate the SIGAD phenotypes.

Patients and Methods

Patients

Diagnosis of SIGAD was based on a low serum IgA level (<5 mg/dl) and normal IgG and IgM levels in patients older than 4 years. Other immunodeficiency diseases such as ataxia-telangiectasia and drug-induced IgA deficiency were excluded. The clinical features of each patient were recorded in a designed questionnaire. This study was approved by the Ethics Committee of the Tehran University of Medical Sciences and informed consent was obtained from patients or their parents.

Immunoglobulin Levels and Antibody Responses

Serum immunoglobulin levels of IgG, IgA, IgM, IgE, and IgG subclasses were measured by nephelometry (Behring Nephelometer; Behringwerke, Marburg, Germany) and the antibody responses against diphtheria and tetanus toxoids were measured using an enzyme-linked immunosorbent assay (ELISA). All patients were fully immunized according to the national vaccination program including diphtheria and tetanus toxoids. All patients received unconjugated pneumococcus polysaccharide vaccine (PNEUMO 23® 123; Aventis Pasteur, France). Specific antibodies against whole pneumococcal antigen were measured using the protocol of the third-generation ELISA assay format before and 4 weeks after vaccination [25].

Cell Preparation

A sample of 2–3 ml of whole blood was obtained from patients with SIGAD and age-sex matched healthy controls and was treated with ethylenediaminetetraacetic acid.

Antibodies and Flow Cytometry

One hundred microliters of whole blood from each subject was incubated with an appropriate concentration of different monoclonal antibodies (phycoerythrin-Cy5-conjugated anti-CD19, fluorescein isothiocyanate-conjugated anti-IgD, and phycoerythrin-conjugated anti-CD27) for 30 min at 4°C. All antibodies were obtained from Dako (Glostrup, Denmark). After lysing red blood cells with Uti-Lyse Erythrocyte Lysing Reagent (Dako), three-color data acquisition was performed using the Partec flow cytometry system (Nuremberg, Germany). Data analysis was performed using Flomax flow cytometry analysis software (Partec). Two-step gating was applied for enumerating different memory B cell subsets. First, PBLs were gated according to their forward-scatter and side-scatter properties and afterwards 2,000–4,000 CD19+ B cells from gated PBLs were selected for the evaluation of surface IgD and CD27 expression. Three-color isotype-matched antibodies with irrelevant specificity were used as a negative control to determine the nonspecific background staining.

Classification Based on Memory B Cell Populations

The population of memory B cells (CD19+CD27+) was determined in PBLs from all the subjects. Afterwards, patients were subdivided into 2 groups based on flow-cytometric results: group I had less than 0.4% switched memory B cells (CD19+CD27+IgD–) in PBLs, while in group II, switched memory B cells represented more than 0.4% of PBLs [18, 26]. All the measurements were conducted in conjunction with age- and sex-matched control samples.

Pulmonary Evaluation

Pulmonary function was evaluated using a computerized pneumotachograph (Jaeger, Germany) in patients of 6 years and older who could cooperate. Forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), FEV₁/FVC and maximal midexpiratory flow were recorded. In those with chronic pulmonary infections, a high-resolution computed tomography scan was performed to confirm the presence of bronchiectasis.

Statistical Analysis

Statistical comparisons of quantitative data were made using the paired Student t test for matched case-control and the unpaired Student t test for the 2 groups. When the data did not fulfill

Table 1. Comparison of immunological data in 2 groups of patients with SIGAD

	IgG, mg/dl	IgM, mg/dl	IgA, mg/dl	CD3 % of lymphocytes	CD4 % of lymphocytes	CD8 % of lymphocytes
GI	1,014.2 ± 278	89.2 ± 14	1.5 ± 0.7	61.6 ± 14	27.8 ± 10	30.2 ± 16
GII	1,388.6 ± 406	104.4 ± 25	4.3 ± 2.5	57.9 ± 11	32.8 ± 7	22.1 ± 4
C	1,437 ± 621	117.3 ± 44	45.6 ± 12.5	60.2 ± 14	33.4 ± 2	30.6 ± 7
p: GI to GII	0.031	0.22	0.23	0.43	0.37	0.09
p: GI to C	0.022	0.17	<0.001	0.70	0.21	0.34
p: GII to C	0.12	0.60	0.01	0.53	0.64	0.11
p value of ANOVA analysis	0.028	0.53	<0.001	0.57	0.28	0.24

GI = Group I; GII = group II; C = controls.

Table 2. Percentage of B lymphocyte subpopulations in 2 groups of patients with SIGAD

	CD19+ % of PBLs	CD27–, IgD+ naïve % of PBLs	CD27–, IgD+ naïve % of B cells	CD27+, IgD+ IgD memory % of PBLs	CD27+, IgD+ IgD memory % of B cells	CD27+, IgD– switched % of PBLs	CD27+, IgD– switched % of B cells
GI	5.4 ± 1.7	3.5 ± 1.7	45.6 ± 18.3	0.8 ± 0.5	13.2 ± 4.5	0.34 ± 0.06	4.31 ± 0.4
GII	13.8 ± 4.2	6.5 ± 4.0	92.3 ± 23.8	3.6 ± 1.3	42.7 ± 16.2	1.74 ± 0.12	15.6 ± 9.4
C	13.4 ± 4.5	6.2 ± 2.5	87.4 ± 20.3	2.4 ± 2.1	27.3 ± 13.4	1.6 ± 0.8	14.7 ± 7.2
p: GI to GII	0.001	0.13	0.09	0.12	0.07	<0.0001	<0.001
p: GI to C	0.004	0.17	0.12	0.47	0.29	<0.0001	<0.001
p: GII to C	0.69	0.95	0.75	0.36	0.23	0.68	0.52
p value of ANOVA analysis	0.001	0.12	0.11	0.96	0.21	<0.0001	<0.001

We assigned the patients to the classifications suggested by Warnatz et al. [18]. PBLs of group I SIGAD patients comprise less than 0.4% of class-switched memory B cells (CD19+CD27+IgD–), while in group II, like the controls, this population accounts for more than 0.4%. Results are expressed as means ± SD. GI = Group I; GII = group II; C = controls.

the criteria for applying parametric tests, nonparametric statistics were used (Mann-Whitney U test, Spearman correlation coefficient). Nominal data were evaluated by the Fisher exact test. Differences between groups were considered significant at $p < 0.05$.

Results

Patients' Characteristics

Twenty-eight SIGAD patients (16 males and 12 females from unrelated families), aged from 4 to 31 years (median = 12), were included in the study. Patients were followed up for a total of 116 patient-years with a mean follow-up of 4.4 years (SD ± 1.5) per patient. Table 1 shows the general characteristics, serum immunoglobulin concentrations, and distributions of lymphocyte subtypes in the studied patients.

Memory B Cells in Patients with SIGAD

The patients with SIGAD had lower percentages of both memory B cells and switched memory B cells compared to their matched control subjects (1.47 ± 0.5 vs. 1.67 ± 0.8 ; $p < 0.001$; Student t test). A difference was established ($p = 0.001$) between the percentages of CD19+ B cells of group I (mean $5.4 \pm 1.7\%$), group II (mean $13.8 \pm 4.2\%$) and healthy controls (mean $13.4 \pm 4.5\%$), using the ANOVA statistical test (table 2).

In all healthy controls, more than 0.4% of PBLs belonged to the CD19+CD27+IgD– cell population. In contrast, the percentage of switched memory B cells in 7 patients (25%) was less than 0.4% (mean $0.34 \pm 0.06\%$); they were classified as group I. No reduction in the percentage of switched memory B cells was found in the remaining 21 patients (75%) designated as group II (mean $1.74 \pm 0.12\%$).

Fig. 1. Peripheral B cells in 2 patients with selective IgA deficiency. Representative histograms of group I (left) and group II (right) patients are shown. A total of 4,000 CD19+ B cells were scanned for each patient. The group I patient had switched memory B cells accounting for 0.34% of peripheral B cells. The example from group II had switched memory B cells accounting for 10% of peripheral B cells. IgD = Immunoglobulin D; PE = phycoerythrin; FITC = fluorescein isothiocyanate.

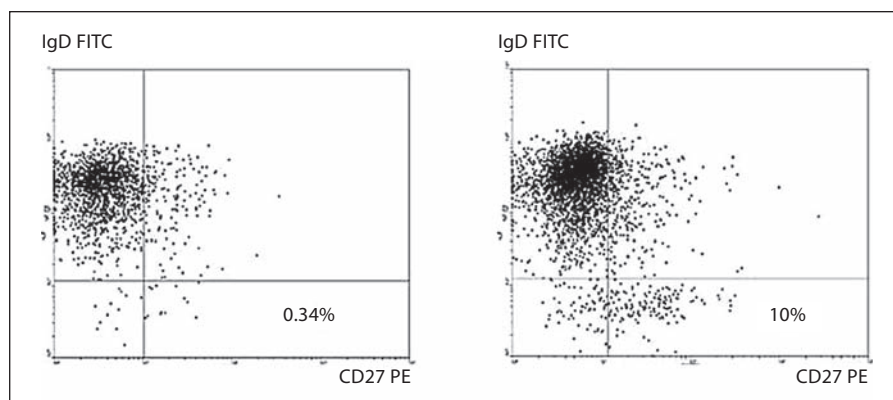


Table 3. Comparison of clinical manifestations and complications in 2 groups of patients with SIGAD

	Group I (7 patients)	Group II (21 patients)	p value
Parental consanguinity	3 (42.8%)	4 (19%)	0.2
Age of diagnosis	7.7 ± 2.1	14.3 ± 2.8	0.04
Number of episodes of pneumonia during course of disease per person-year	3.3 ± 0.5	0.5 ± 0.3	0.002
Splenomegaly	3 (42.8%)	0	0.011
Lymphadenopathy	5 (71.4%)	2 (9.5%)	0.004
Autoimmunity	4 (57%)	5 (23.8%)	0.01
Developing CVID	2 (28.5%)	0	0.05
Restrictive pattern of PFT	3 (42.8%)	0	0.011
Obstructive pattern of PFT	0	8 (38%)	0.06
Mixed pattern (obstructive- restrictive) of PFT	4 (57%)	0	0.002
Bronchiectasis	4 (57%)	1 (4.7%)	0.006

Immunoglobulin Production

Serum immunoglobulin concentrations (IgG and IgM) were evaluated at the time of diagnosis and before initiation of immunoglobulin replacement by the ANOVA test between groups. The mean concentration of IgG was significantly lower in group I ($1,014 \pm 278$ mg/dl; $p = 0.028$) than in group II ($1,388 \pm 406$ mg/dl). Likewise, the mean serum concentration of IgM was lower in group I (89 ± 14 mg/dl) than in group II (104 ± 25 mg/dl), but this difference was not significant ($p = 0.53$) (table 2). Evaluation of IgG subclasses showed IgG2 deficiency in only 2 patients and IgG3 deficiency in only 1 patient in group I.

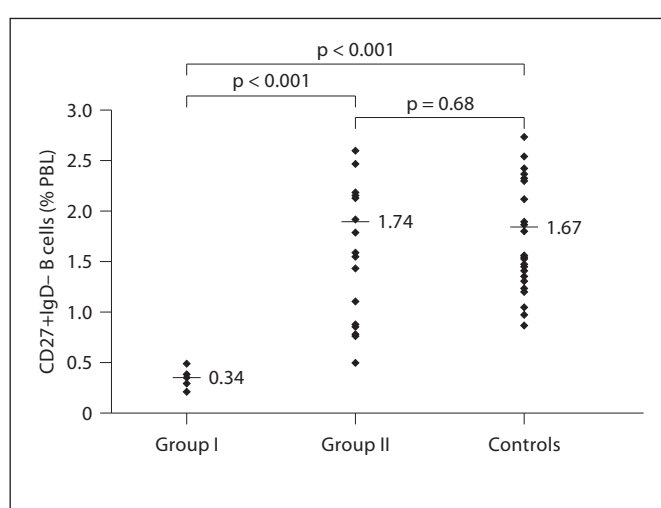


Fig. 2. Profound decrease of switched memory B cells in the SIGAD patients of group I comparing them to group II and controls. Statistical analysis was performed by the ANOVA test, and p values are given.

Antibody responses against tetanus and diphtheria proteins and polysaccharide antigen were measured in all patients to check specific antibody deficiency (SAD). SAD was documented in 5 patients in group I, while 4 patients in group II had a low response to specific antigens ($p = 0.01$).

Clinical Manifestations

In 3 families (42.8%) of 7 patients in group I, parents were consanguine first cousins, while just 4 families (19%) in group II had first-cousin parental consanguinity. No significant difference was found between groups I and II in the patients' ages, except for the age of diagnosis

which was significantly lower in group I (7.7 ± 2.1 vs. 14.3 ± 2.8 ; $p = 0.04$; Student *t* test analysis).

The most prevalent presenting symptoms were recurrent upper and lower respiratory and gastrointestinal infections, occurring in 5 (83.3%) subjects in group I and 20 (90%) subjects in group II. Autoimmunity in the remaining cases of group I and allergy in the remaining cases of group II were the main manifestations presented. Episodes of pneumonia during the course of disease were significantly higher in group I than in group II ($p = 0.002$; Student *t* test analysis).

Complications

Splenomegaly was documented in 42.8% of patients in group I, but not seen in any patient of group II ($p = 0.011$; Fisher exact test analysis). Furthermore, lymphadenopathy was seen in 71.4 and 0.9% of patients in groups I and II, respectively ($p = 0.004$; the Fisher exact test analysis). Autoimmune diseases during the course of disease were found in 4 patients in group I (myasthenia gravis/AIHA/hypothyroidism in 1 patient, vitiligo in 1 patient, AIHA/rheumatoid arthritis in 1 patient, and AIHA/hypothyroidism in 1 patient) and 5 patients in group II (alopecia areata in 2 patients, hypothyroidism in 2 patients, and myasthenia gravis/vitiligo in 1 patient) which was significantly more common in group I ($p = 0.01$; χ^2 test analysis).

Intravenous immunoglobulin (IVIG) was prescribed for 4 patients in group I, including 2 patients who developed CVID and 2 patients with subclass deficiency and SAD that needed IVIG because of severe infections.

Two patients from group I who developed CVID had some special characteristics. One of them was a 22-year-old female, presenting first with myasthenia gravis and SIGAD, who subsequently developed CVID over time and died due to uncontrolled pulmonary failure [24]. The second patient was a 26-year-old male, who presented first with respiratory infections and SIGAD, but subsequently developed bronchiectasis and received a diagnosis of CVID. A higher frequency of severe infections and hospitalization was recorded in both patients. They both experienced a similar type of autoimmunity (AIHA and hypothyroidism).

Paraclinical Findings

Apart from 3 children in group II who were under 6 years old, 25 patients cooperated to perform a pulmonary function test (PFT) using spirometry. In group I all the patients had an abnormal PFT, which included a restrictive pattern in 3 patients and a mixed pattern (obstruc-

tive-restrictive) in 4 others. On the other hand, only 8 patients in group II had an obstructive pattern, and the remaining patients were normal. Chest radiography and high-resolution computed tomography scans were performed in all the patients to assess structural lung damage. The prevalence of bronchiectasis was 57% in group I, while only 1 patient in group II developed bronchiectasis ($p = 0.006$; Fisher's exact test analysis).

Discussion

A maturation defect in B cells to produce IgA is the most frequent type of primary immunodeficiency in humans, with an incidence rate of 1:394 to 1:15,000 in different regions [6, 7, 27, 28]. The prevalence of SIGAD among healthy Iranian blood donors is estimated to be about 1 in 651 individuals [29]. Although IgA had the highest daily synthesis and commonest isotype, which present in mucosal immunity [30], only approximately one third of the SIGAD cases are susceptible to bacterial infections [4, 5]. Occasionally, patients associated with the IgG subclass and SAD may be more susceptible to infections [31].

The molecular basis of SIGAD is unknown; however, defects at several steps which involve the stem cells may be suggested [32], including structural gene defects, impaired switching, failure of differentiation of the surface IgA+ B cells to plasma cells and defects at the transcriptional and/or at the posttranscriptional level of secretory mRNA [33]. Up to now, there has been no documenting of homozygous gene deletions causing an IgA class deficiency in the constant $\alpha 1$ and $\alpha 2$ genes, except various rare segment deletions in chromosome 14 [34, 35].

Memory B cells are usually formed after primary infection. In the wake of first-encounter (primary response) infections in the germinal centers involving a particular antigen, the responding naïve cells proliferate to produce a colony of cells, most of which differentiate into effector B cells, and the rest persist as the memory cells that can survive for years. These responses persist for providing highly efficient defense against reinfection. Thus, the memory B cells with stronger and more specific antibody production were stuck in a secondary antibody response as a memory of the immune system.

According to this study, most patients with SIGAD had lower percentages of both memory B cells and class-switched memory B cells compared to the healthy controls; these patients can be subsequently classified according to the percentages of memory B cells and switched

memory B cells. Regarding this classification, SIGAD patients with less than 0.4% of PBLs, belonging to the CD19+CD27+IgD⁻ cell population, showed specific characteristics. A higher rate of pneumonia, autoimmunity, hepatosplenomegaly and restrictive and mixed patterns of PFT were documented in this subgroup of SIGAD patients. Furthermore, specific antibody and IgG subclass deficiencies were more frequent in group I than in other SIGAD patients.

Therefore, our observations suggest that the underlying defect of the regulatory mechanisms, possibly at the level of switching, is an etiology of SIGAD, especially in the patients of group I. A switching defect in a SIGAD patient may be due to lack of a specific switch recombinase, polymorphism or abnormalities in the cytokine network, especially in IL-21 and TACI (ligand for BAFF and APRIL) mutations [15, 36, 37].

Two patients from group I went from SIGAD to CVID, which is concurrent with the idea that SIGAD shares

many features with CVID. The variation in the inheritance patterns, the lack of an identified genetic defect, the coincidence of SIGAD and CVID in one family [23, 38, 39] and the progression from SIGAD to CVID suggest common underlying genetic defects [1]. There is some evidence of mutations in the gene *TNFRSF13B* encoding TACI [14, 40, 41] and an increased frequency of some extended MHC haplotypes such as HLA B8, DR3 [23, 42–45], HLA A28, B14 and HLA A1, B8 [46–50] in both SIGAD and CVID. In addition, they may share a common immunopathology, especially in the switching step [36].

In summary, the classification of SIGAD patients by the assessment of switched memory B cells represents an important biomarker for the clinical prognosis of these patients, since the severe reduction of switched memory B cells is associated with a more severe clinical manifestation of infection and immune dysregulation.

References

- ▶ 1 Hammarström L, Vorechovsky I, Webster D: Selective IgA deficiency (SigAD) and common variable immunodeficiency (CVID). *Clin Exp Immunol* 2000;120:225–231.
- ▶ 2 Bonilla FA, Bernstein IL, Khan DA, Ballas ZK, Chinen J, Frank MM, Kobrynski LJ, Levinson AI, Mazer B, Nelson RP Jr, Orange JS, Routes JM, Shearer WT, Sorensen RU: Practice parameter for the diagnosis and management of primary immunodeficiency. *Ann Allergy Asthma Immunol* 2005;94:S1–S63.
- ▶ 3 Conley ME, Notarangelo LD, Etzioni A: Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). *Clin Immunol* 1999;93:190–197.
- ▶ 4 Burrows PD, Cooper MD: IgA deficiency. *Adv Immunol* 1997;65:245–276.
- ▶ 5 Hanson LA, Bjorkander J, Carlsson B, Robertson D, Soderstrom T: The heterogeneity of IgA deficiency. *J Clin Immunol* 1988;8:159–162.
- ▶ 6 Schaffer FM, Monteiro RC, Volanakis JE, Cooper MD: IgA deficiency. *Immunodef Rev* 1991;3:15–44.
- ▶ 7 Cunningham-Rundles C: Physiology of IgA and IgA deficiency. *J Clin Immunol* 2001;21:303–309.
- ▶ 8 Burgio GR, Duse M, Monafò V, Ascione A, Nespoli L: Selective IgA deficiency: clinical and immunological evaluation of 50 pediatric patients. *Eur J Pediatr* 1980;133:101–106.
- ▶ 9 Sloper KS, Brook CG, Kingston D, Pearson JR, Shiner M: Eczema and atopy in early childhood: low IgA plasma cell counts in the jejunal mucosa. *Arch Dis Child* 1981;56:939–942.
- ▶ 10 Strober W, Sneller MC: IgA deficiency. *Ann Allergy* 1991;66:363–375.
- ▶ 11 Edwards E, Razvi S, Cunningham-Rundles C: IgA deficiency: clinical correlates and responses to pneumococcal vaccine. *Clin Immunol* 2004;111:93–97.
- ▶ 12 Castigli E, Wilson SA, Garibyan L, Rachid R, Bonilla F, Schneider L, Geha RS: TACI is mutant in common variable immunodeficiency and IgA deficiency. *Nat Genet* 2005;37:829–834.
- ▶ 13 Salzer U, Chapel HM, Webster AD, Pan-Hammarström Q, Schmitt-Graeff A, Schlesier M, Peter HH, Rockstroh JK, Schneider P, Schaffer AA, Hammarström L, Grimbacher B: Mutations in *TNFRSF13B* encoding TACI are associated with common variable immunodeficiency in humans. *Nat Genet* 2005;37:820–828.
- ▶ 14 Castigli E, Geha RS: TACI, isotype switching, CVID and IgAD. *Immunol Res* 2007;38:102–111.
- ▶ 15 Yel L: Selective IgA deficiency. *J Clin Immunol* 2010;30:10–16.
- ▶ 16 Tangye SG, Liu YJ, Aversa G, Phillips JH, de Vries JE: Identification of functional human splenic memory B cells by expression of CD148 and CD27. *J Exp Med* 1998;188:1691–1703.
- ▶ 17 Agematsu K, Nagumo H, Shinozaki K, Hoki-bara S, Yasui K, Terada K, Kawamura N, Toba T, Nonoyama S, Ochs HD, Komiyama A: Absence of IgD-CD27(+) memory B cell population in X-linked hyper-IgM syndrome. *J Clin Invest* 1998;102:853–860.
- ▶ 18 Warnatz K, Denz A, Dräger R, Braun M, Groth C, Wolff-Vorbeck G, Eibel H, Schlesier M, Peter HH: 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.
- ▶ 19 Brouet JC, Chedeville A, Ferman 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, Kobata T, Tanaka Y, Morimoto C, Schlossman SF, Tron F: B cell co-receptors regulating T cell-dependent antibody production in common variable immunodeficiency: CD27 pathway defects identify subsets of severely immuno-compromised patients. *Int Immunol* 2001;13:871–876.
- ▶ 21 Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevrete S, Debre P, Schmitt C, Oksenhendler E: Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. *J Clin Immunol* 2003;23:385–400.

- ▶ 22 Ferry BL, Jones J, Bateman EA, Woodham N, Warnatz K, Schlesier M, Misbah SA, Peter HH, Chapel HM: Measurement of peripheral B cell subpopulations in common variable immunodeficiency (CVID) using a whole blood method. *Clin Exp Immunol* 2005;140:532–539.
- ▶ 23 Schroeder HW Jr, Zhu ZB, March RE, Campbell RD, Berney SM, Nedospasov SA, Turetskaya RL, Atkinson TP, Go RC, Cooper MD, Volanakis JE: Susceptibility locus for IgA deficiency and common variable immunodeficiency in the HLA-DR3, -B8, -A1 haplotypes. *Mol Med* 1998;4:72–86.
- ▶ 24 Aghamohammadi A, Mohammadi J, Parvaneh N, Rezaei N, Moin M, Espanol T, Hammarström L: Progression of selective IgA deficiency to common variable immunodeficiency. *Int Arch Allergy Immunol* 2008;147:87–92.
- ▶ 25 Aghamohammadi A, Moin M, Karimi A, Naraghi M, Zandieh F, Isaiean A, Tahaei A, Talei-Khoei M, Kouhi A, Abdollahzade S, Pouladi N, Heidari G, Amirzargar AA, Rezaei N, Sazgar AA: Immunologic evaluation of patients with recurrent ear, nose, and throat infections. *Am J Otolaryngol* 2008;29:385–392.
- ▶ 26 Vodjgani M, Aghamohammadi A, Samadi M, Moin M, Hadjati J, Mirahmadian M, Parvaneh N, Salavati A, Abdollahzade S, Rezaei N, Srrafnejad A: Analysis of class-switched memory B cells in patients with common variable immunodeficiency and its clinical implications. *J Investig Allergol Clin Immunol* 2007;17:321–328.
- ▶ 27 Kanoh T, Mizumoto T, Yasuda N, Koya M, Ohno Y, Uchino H, Yoshimura K, Ohkubo Y, Yamaguchi H: Selective IgA deficiency in Japanese blood donors: frequency and statistical analysis. *Vox Sang* 1986;50:81–86.
- ▶ 28 Rezaei N, Aghamohammadi A, Moin M, Pourpak Z, Movahedi M, Gharagozlou M, Atarod L, Ghazi BM, Isaiean A, Mahmoudi M, Abolmaali K, Mansouri D, Arshi S, Tarash NJ, Sherkat R, Akbari H, Amin R, Alborzi A, Kashef S, Farid R, Mohammadzadeh I, Shabestari MS, Nabavi M, Farhoudi A: Frequency and clinical manifestations of patients with primary immunodeficiency disorders in Iran: update from the Iranian Primary Immunodeficiency Registry. *J Clin Immunol* 2006;26:519–532.
- ▶ 29 Aghamohammadi A, Cheraghi T, Gharagozlou M, Movahedi M, Rezaei N, Yeganeh M, Parvaneh N, Abolhassani H, Pourpak Z, Moin M: IgA deficiency: correlation between clinical and immunological phenotypes. *J Clin Immunol* 2009;29:130–136.
- ▶ 30 Mestecky J, McGhee JR: Immunoglobulin A (IgA): molecular and cellular interactions involved in IgA biosynthesis and immune response. *Adv Immunol* 1987;40:153–245.
- ▶ 31 Preud'homme JL, Hanson LA: IgG subclass deficiency. *Immunodef Rev* 1990;2:129–149.
- ▶ 32 Hammarström L, Lonnqvist B, Ringden O, Smith CI, Wiebe T: Transfer of IgA deficiency to a bone-marrow-grafted patient with aplastic anaemia. *Lancet* 1985;1:778–781.
- ▶ 33 Islam KB, Baskin B, Nilsson L, Hammarström L, Sideras P, Smith CI: Molecular analysis of IgA deficiency: evidence for impaired switching to IgA. *J Immunol* 1994;152:1442–1452.
- ▶ 34 Suzuki H, Kaneko H, Fukao T, Jin R, Kawamoto N, Asano T, Matsui E, Kasahara K, Kondo N: Various expression patterns of alpha1 and alpha2 genes in IgA deficiency. *Allergol Int* 2009;58:111–117.
- ▶ 35 Smith CI, Hammarström L: Detection of immunoglobulin genes in individuals with immunoglobulin class or subclass deficiency: evidence for a pretranslational defect. *Monogr Allergy* 1986;20:18–25.
- ▶ 36 Borte S, Pan-Hammarström Q, Liu C, Sack U, Borte M, Wagner U, Graf D, Hammarström L: Interleukin-21 restores immunoglobulin production ex vivo in patients with common variable immunodeficiency and selective IgA deficiency. *Blood* 2009;114:4089–4098.
- ▶ 37 Migone N, Feder J, Cann H, van West B, Hwang J, Takahashi N, Honjo T, Piazza A, Cavalli-Sforza LL: Multiple DNA fragment polymorphisms associated with immunoglobulin mu chain switch-like regions in man. *Proc Natl Acad Sci USA* 1983;80:467–471.
- ▶ 38 Vorechovsky I, Zetterquist H, Paganelli R, Koskinen S, Webster AD, Bjorkander J, Smith CI, Hammarström L: Family and linkage study of selective IgA deficiency and common variable immunodeficiency. *Clin Immunol Immunopathol* 1995;77:185–192.
- ▶ 39 De la Concha EG, Fernandez-Arquero M, Martinez A, Vidal F, Vigil P, Conejero L, Garcia-Rodriguez MC, Fontan G: HLA class II homozygosity confers susceptibility to common variable immunodeficiency (CVID). *Clin Exp Immunol* 1999;116:516–520.
- ▶ 40 Castigli E, Wilson S, Garibyan L, Rachid R, Bonilla F, Schneider L, Morra M, Curran J, Geha R: Reexamining the role of TACI coding variants in common variable immunodeficiency and selective IgA deficiency. *Nat Genet* 2007;39:430–431.
- ▶ 41 Pan-Hammarström Q, Salzer U, Du L, Bjorkander J, Cunningham-Rundles C, Nelson DL, Bacchelli C, Gaspar HB, Offer S, Behrens TW, Grimbacher B, Hammarström L: Reexamining the role of TACI coding variants in common variable immunodeficiency and selective IgA deficiency. *Nat Genet* 2007;39:429–430.
- ▶ 42 Ploski R, Ek J, Thorsby E, Sollid LM: On the HLA-DQ(alpha 1*0501, beta 1*0201)-associated susceptibility in celiac disease: a possible gene dosage effect of DQB1*0201. *Tissue Antigens* 1993;41:173–177.
- ▶ 43 Candore G, Lio D, Colonna Romano G, Caruso C: Pathogenesis of autoimmune diseases associated with 8.1 ancestral haplotype: effect of multiple gene interactions. *Autoimmun Rev* 2002;1:29–35.
- ▶ 44 Behan PO: Immune disease and HLA associations with myasthenia gravis. *J Neurol Neurosurg Psychiatry* 1980;43:611–621.
- ▶ 45 Stary J, Sedlacek P, Vodvarkova S, Gasova Z, Bartunkova J: Development of common variable immunodeficiency in a patient with Evans syndrome treated by autologous stem cell transplantation. *Pediatr Allergy Immunol* 2003;14:334–337.
- ▶ 46 Schaffer FM, Palermos J, Zhu ZB, Barger BO, Cooper MD, Volanakis JE: Individuals with IgA deficiency and common variable immunodeficiency share polymorphisms of major histocompatibility complex class III genes. *Proc Natl Acad Sci USA* 1989;86:8015–8019.
- ▶ 47 Cobain TJ, French MA, Christiansen FT, Dawkins RL: Association of IgA deficiency with HLA A28 and B14. *Tissue Antigens* 1983;22:151–154.
- ▶ 48 Hammarström L, Axelsson U, Bjorkander J, Hanson LA, Moller E, Smith CI: HLA antigens in selective IgA deficiency: distribution in healthy donors and patients with recurrent respiratory tract infections. *Tissue Antigens* 1984;24:35–39.
- ▶ 49 Heikkila M, Koistinen J, Lohman M, Koskimies S: Increased frequency of HLA-A1 and -B8 in association with total lack, but not with deficiency of serum IgA. *Tissue Antigens* 1984;23:280–283.
- ▶ 50 Mohammadi J, Ramanujam R, Jarefors S, Rezaei N, Aghamohammadi A, Gregersen PK, Hammarström L: IgA deficiency and the MHC: assessment of relative risk and microheterogeneity within the HLA A1 B8, DR3 (8.1) haplotype. *J Clin Immunol* 2010;30:138–143.