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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

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Abstract Although idiopathic humoral immunodeficiencies are arbitrarily classified into specific antibody deficiency (SAD) or common variable immunodeficiency (CVID), this distinction does not accurately predict the risk of the bronchiectasis, one of the major long-term clinical complications in these patients. In this study, clinical complications were compared with laboratory markers of cellular and humoral immunity in fifty-five consecutive patients (27 children and 28 adults) attending regional immunology clinics in Manchester, United Kingdom. Reduced $CD19^{+}CD27^{+}IgD^{-}$ B cell percentage but not serum immunoglobulin levels or classification of patients into SAD and CVID was associated with a significantly higher prevalence of bronchiectasis (OR 0.4 (0.2–0.8), $P = 0.001$), splenomegaly (OR 0.2 (0.1–0.5), $P = 0.001$) and autoimmunity (OR 0.4 (0.2–0.7), $P = 0.003$). We conclude that in patients with idiopathic humoral immunodeficiencies assessment of B cell switching more accurately predicts clinical prognosis than either classification of patients into SAD and CVID or serum immunoglobulin concentrations.

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Introduction

Suboptimal antibody production due to defects in B lymphocyte maturation and function is the most common feature of human primary immunodeficiency disease. As a consequence of defective humoral immunity, these patients are prone to respiratory infections with encapsulated organisms such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type B. Chronic lung disease and bronchiectasis caused by recurrent and persistent infections with these microorganisms are leading causes of death [1]. Non-respiratory complications include inflammatory and lymphoproliferative disorders, often associated with splenomegaly [2–4]. The reason why certain patients develop clinical complications despite adequate immunoglobulin replacement and antibiotic prophylaxis is becoming clearer with the recent advances in our understanding of the molecular and cellular bases of this heterogeneous group of conditions [5,6].

In about one fifth of patients with defective antibody production, the underlying molecular basis is known and includes mutations in genes important for (i) early B cell development and maturation within the bone marrow (e.g., Btk [7], μ-chain [8], lambda 5/14.1 [9] and BLNK [10]), (ii) later stages of B cell maturation, activation and immunoglobulin isotype switching within the lymphoid organs (e.g., CD40 [11], AID [12], UNG [13], DNA methyltransferase 3B [14], BAFF receptor [6] or TACI [15,16]), and (iii) NK/T cell maturation and function leading to defective T–B cell interaction or secondary B cell loss (e.g., CD40 ligand [17], ICOS [18], 22q11 chromosomal microdeletions [19], SAP [20]). Patients with defects in B cell activation and primary NK/T cell disorders are most at risk of inflammatory and lymphoproliferative complications [21]. The relative risk of chronic lung damage in each of these disorders is unclear.

In the remaining patients where the molecular basis for the humoral immunodeficiency is unknown, the condition is either termed specific antibody deficiency (SAD) if serum immunoglobulin levels are normal or common variable immunodeficiency (CVID) if serum immunoglobulin levels are low [22,23]. This classification does not always predict which patients will have a relatively benign course and which patients will develop bronchiectasis or inflammatory complications, dying prematurely despite regular prophylactic antibiotics or immunoglobulin replacement [3,24]. From the information gained through the study of humoral immunodeficiencies where the molecular defects are known, one would expect patients with defective B cell switching to have more complicated clinical courses than those that do not. Although immunoglobulin isotype switching phenotypes were first used over a decade to subclassify adults with idiopathic CVID [25], it is only during the last few years that the methodology has been simplified by immunophenotyping using flow cytometry [26,27]. In adults with CVID, low IgM but not IgG memory switched B cells are associated with recurrent lower respiratory tract infections and abnormalities on chest CAT scan [28], and low IgG memory switched B cells are associated with splenomegaly [26,27] and inflammatory complications [29].

This is the first study to use these same laboratory techniques to examine the association between immune phenotypes and clinical complications of both children and adults with SAD, as well as children with CVID. Rather than attempting to fit clinical phenotypes into predefined immunological subgroups, this study sets out to determine the immunological markers that most reliably predict which patients with idiopathic humoral immunodeficiencies have a favorable or complicated clinical course.

Patients, materials, methods

Selection of patients and data collection

We studied fifty-five consecutive patients (27 children and 28 adults) attending regional primary immunodeficiency clinics in Manchester, United Kingdom, in 2004–5 with specific antibody deficiency (SAD) or common variable immunodeficiency (CVID) as defined by the IUIS scientific committee report on primary immunodeficiency diseases [23]. Patients with SAD were defined as having normal IgG, IgM, and IgA levels, but poor responses to two doses of Prevnar and a subsequent dose of the 23 valent pneumococcal vaccine and/or Hib vaccine. Patients with primary immunodeficiencies due to defined underlying causes (e.g., Bruton's agammaglobulinaemia, CD40 ligand deficiency, ataxia telangectasia) and immunodeficiencies secondary to other diseases (chromosomal abnormalities, lymphoma, thymoma, asplenia, protein-losing enteropathy, nephrotic syndrome) or drugs were excluded. Patients with TACI mutations were not excluded, as at the time of recruitment this entity had not yet been described. Memory switched B cell percentages of nineteen patients seen in the immunology clinic who on detailed immunological assessment had normal serum immunoglobulins and protective antibody titers after pneumococcal and Hib vaccines are presented for comparison (hospital non-antibody deficient control group). Clinical and laboratory data were obtained from the patients' hospital records. All immunology results are those obtained from blood samples taken prior to immunoglobulin replacement therapy. Approval for the study was obtained from the local research ethics committee and informed consent given by the patients or their parents.

Antibody measurements

Serum IgG, IgA and IgM, as well as IgG subclasses were measured by rate nephelometry (Beckman Image Immunochemistry System, High Wycombe, United Kingdom). Assessment of specific antibody responses to *H. influenzae*, type B and Pneumococcus IgG antibodies were assayed using a commercial ELISA kit (The Binding Site Ltd., Birmingham, United Kingdom). Pneumococcal antibodies against the 23 serotypes present in Pneumovax were assayed by ELISA. ELISA plates were coated with a 1:100 dilution of Pneumovax. Diluted, unadsorbed sera were then added, and bound antibody was detected with monoclonal antibodies directed against IgG (all four subclasses, IgG whole), IgG1 or IgG2. The standards, controls and samples were diluted serially from 1:10 to 1:1280. Optical densities obtained were compared with those from a standard pooled serum and a titer calculated [30]. National or international calibrant material is available for the determination of pneumococcal specific antibody levels; therefore, reference values were

generated 'in house' by testing control sera from healthy adults (n = 42). Serotype-specific Pneumococcal IgG antibodies against 9 serotypes were measured by ELISA. Test sera are preadsorbed with capsular polysaccharide (5 μg/ml) to remove non-specific antibodies. Sera are then incubated on serotype-specific capsular polysaccharide coated plates. The assay is developed using a goat anti-human IgG alkaline phosphatase conjugate and p-nitrophenyl phosphate substrate. Standard curves are generated using an international reference serum (89-S, FDA) and concentrations obtained for unknowns are in μg/ml [31].

Flow cytometric analysis of lymphocyte subsets

Analysis was performed with a Beckman Coulter flow cytometer. Anti-coagulated blood with EDTA or heparin (10 IU/ml) was drawn from patients and processed within 24 h. The lysed whole blood method using the Coulter Q Prep workstation and Immunoprep reagents was used. The following Cyto-Stat TetraChrome reagents were used: CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 = Reagent A, CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 = Reagent B (IgG1 mouse monoclonal antibodies, from Beckman Coulter); Flow count beads (Beckman Coulter, Immunotech, Hamburg, Germany); CD19-PC5, CD27-PE, CD40-PE (all mouse IgG1, from Immunotech, Marseille, France); IgD-FITC (mouse IgG2a from Biosciences Pharmingen, Erembodegem, Belgium); and negative control (mouse IgG2a-FITC, IgG1-PC5, IgG1-PE, IgG1-FITC, from Biosciences Pharmingen). Four color FACS analysis was performed to determine absolute counts and percentage of CD3, CD3/CD4, CD3/CD8, CD19, CD40 (only percentages were measured) and CD56. Three color FACS analysis was performed by gating on ($CD19^+$) B lymphocytes looking at the percentages of naïve B cells ($CD19^+CD27^-IgD^+$), non-switched memory B cells ($CD19^+CD27^+IgD^+$) and switched memory B cells ($CD19^+CD27^+IgD^-$) (Fig. 1).

In vitro lymphocyte proliferation assays

Thirty-four of the patients had lymphocyte transformation testing to assess the proliferation of T and B lymphocytes in response to phytohemagglutinin (PHA) and pokeweed mitogen (PWM). Using aseptic techniques and fresh blood

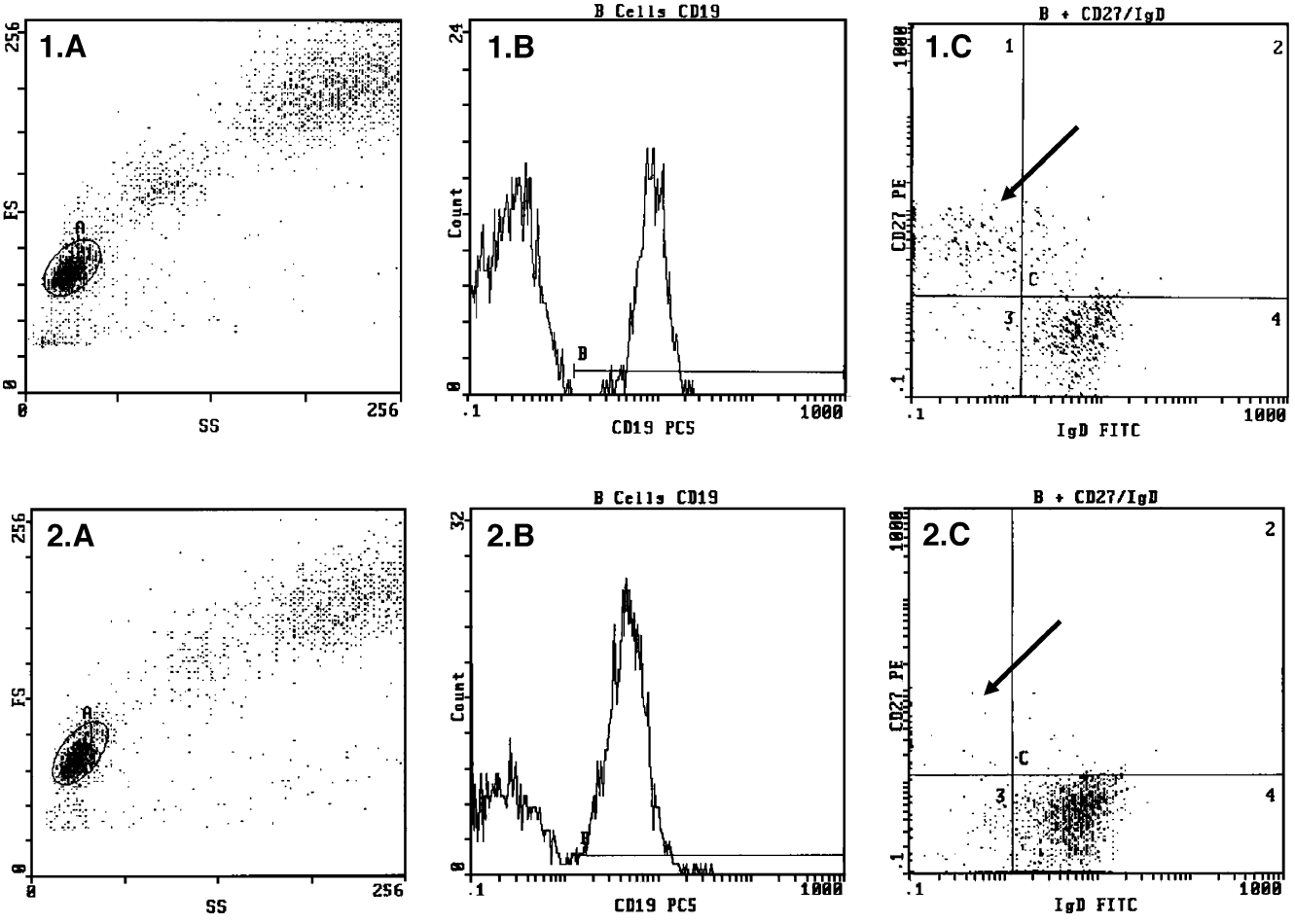

Figure 1 Representative flow cytometry plots illustrating technique of gating and methods of calculating percentage of memory switched B cells in total B cell population. Top panels, (1) patient with normal B cell switching; bottom panels, (2) patient with defective B cell switching. Panel A is a scatterplot gating on lymphocytes from whole blood using forward and side-scatter parameters. Panel B is a histogram of $CD19^+$ B cells frequency in the sample. Panel C: after gating on $CD19^+$ B cells, this histogram illustrates the percentages of B cell subclasses defined by the expression of CD27 and/or IgD (C1 = $CD19^+CD27^+IgD^-$ memory switched B cells (arrow); C2 = $CD19^+CD27^+IgD^+$ memory non-switched B cells; C2 = $CD19^+CD27^-IgD^+$ naive B cells).

samples, the fraction of peripheral blood mononuclear cells (PBMC) was isolated by Ficoll-Hypaque density gradient centrifugation (Histopaque-1077, Sigma) and washed twice with phosphate-buffered saline. After resuspending in RPMI medium with 10% fetal calf serum, PBMC were cultured with PHA and PWM. Cultures were then harvested and pulsed with ^{3}H thymidine (Amersham International, Amersham, United Kingdom), and the proliferative response was determined by estimating the amount of ^{3}H thymidine incorporated into newly synthesized DNA, using a Beta-counter (Packard Tri-Carb 300, Packard Instruments, Downers Grove, IL, USA).

Gene sequencing

Genomic DNA was extracted from peripheral blood leukocytes with proteinase K, sodium dodecyl sulfate and a series of phenol chloroform extractions. The five exons of *AID*, the six exons of *UNG1/UNG2* and the five exons of *TACI* gene were amplified by polymerase chain reaction using primers already reported by us [12,13] and others [16]. PCR products were separated by electrophoresis and purified (Quiagen GmbH, Hilden, Germany). Exons and adjacent intronic regions were sequenced with the dRhodamine dye terminator cycle sequencing kit (ABI prism) and analyzed with the ABI prism 377 genetic analyzer from Perkin Elmer.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS version 12.0) (SPSS Inc, Chicago, USA) was used to analyze the data. As data were not normally distributed, non-parametric statistics were used throughout (Mann—Whitney *U* Test, Spearman correlation coefficient). *P* values are quotes as results of two-tailed tests. Using Bonferroni's post hoc adjustment for multiple comparison (adjusted *P* value = *P* value × total number of comparisons) differences between groups in relation to B cell switching numbers and percentages remained statistically significant. Multivariant analysis using the Cox regression was used to adjust for the possible confounding effects of age and is quoted as odds ratio (OR) (95% confidence interval (CI)).

Results

Clinical characteristics of study population

Fifty-five patients with primary antibody deficiency, 27 (49%) of whom were children (2 to 16 years) and 28 (51%) adults (22 to 65 years) took part in the study. Twenty-eight (51%) were male, and 42 (76%) were Caucasian. Thirty-four (61%) patients had CVID and the rest SAD. Of the 21 SAD patients, 52% had suboptimal *H. influenzae* type b antibody titers post-vaccination (<1.0 μg/ml), all had low pneumococcal IgG and/or IgG2 titers and 75% had suboptimal responses (<0.3 μg/ml) to 4 or more of the 7 Prevnar-related pneumococcal serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) after two doses of Prevnar and a dose of Pneumovax. The nineteen patients (10 (52%) male) who presented with infections but on immunological testing did not have SAD, CVID or any other recognized immunodeficiency were aged 2 to 63 years, which not significantly different from the other two groups studied (Chi square statistic 0.95; *P* = 0.6).

All but one of the patients had a history of ear and chest infections and eight (5 children, 3 adults) had clinical symptoms of bronchiectasis confirmed by high-resolution thoracic CAT scan. Of the 21 patients in the SAD group, all had a history of recurrent episodes of broncho or lobar pneumonia with or without recurrent otitis media and sinusitis particularly in the adults. Three had a history of pneumococcal empyema, one had *H. influenzae* type b pericarditis, one pneumococcal peritonitis and two osteomyelitis. Thirteen patients, 7 children and 6 adults, had autoimmune manifestations (5 enteropathy, 4 cytopenias, 2 arthritis, 2 insulin-dependent diabetes). Eight patients (4 children and 4 adults) had splenomegaly confirmed by abdominal ultrasound scan. Thirty eight patients (18 (65%) children, 21 (75%) adults) were on immunoglobulin replacement therapy, and 35 patients were on prophylactic antibiotics (21 (75%) children, 14 (50%) adults). Prophylactic antibiotics were used as an adjunct to immunoglobulin therapy in patients who had IgG trough levels >8.0 g/l but continued to have recurrent bacterial sinus or ear infections. Antibiotics were also used as primary preventative therapy in a subgroup of patients with SAD, although immunoglobulin replacement was added in those who continued to suffer from recurrent infections during an initial 3- to 4-month trial of this antibiotic monotherapy.

Comparison of clinical and immunological features of patients with SAD and CVID

Table 1 shows the characteristics of the patients with problems of antibody production classified into two groups based on whether their serum immunoglobulin levels were normal (SAD) or low (CVID). Apart from the expected differences in total immunoglobulin levels between the two groups, there were no significant differences in any of the other immune parameters, including numbers of naïve and class switched B cells, as well as absolute numbers of $CD3^{+}$, $CD4^{+}$, $CD8^{+}$ and $CD56^{+}$ lymphocytes, percentages and proliferative responses to PHA and PWM (data not shown), even after adjusting for potential confound effects of age by multi-variant Cox regression analysis. Median (inter-quartile range) percentage of $CD19^{+}CD27^{+}IgD^{-}$ memory switched B cells of the non-antibody immunodeficient controls was 6.0 (3.0—12.0)%. Even after adjusting for any minor effect of age by multi-variant analysis, this was not significantly different to the two other groups studied (SAD 5.0 (2.5—16.1)% (OR (95% CI) 1.0 (0.5—1.9), *P* = 1.0; CVID group 4.6 (3.0 7.3)% (OR 95% CI) 1.7 (0.9—3.1), *P* = 0.1). There was also no significant difference in the number of patients with bronchiectasis, autoimmunity or splenomegaly between the two groups.

Immunology of patients with bronchiectasis, splenomegaly and autoimmunity

The eight patients with bronchiectasis had the same distribution of age and gender as the remainder of the cohort (Table 2). The delay in diagnosing the immunodeficiency in patients with bronchiectasis was not significantly

Table 1 Age, gender, B cell surface markers and serum immunoglobulin concentrations for patients with SAD and CVID

	SAD	CVID	OR (95% CI)	*P* value
Number	21	34		
Age (years)	16 (5–38)	25 (8–51)	1.4 (0.8–2.5)	0.2
Males (%)	11 (52%)	25 (73%)	2.6 (0.8–8.6)	0.1
CD19 (cells/μl)	290 (190–568)	175 (82–565)	0.8 (0.4–1.4)	0.4
$CD19^+CD27^-IgD^+$ %	76 (63–81)	80 (60–87)	2.2 (1.0–4.3)	0.1
$CD19^+CD27^+IgD^+$ %	4.0 (3.6–7.3)	4.8 (2.2–9.8)	0.9 (0.5–1.7)	0.8
$CD19^+CD27^+IgD^-$ %	5.0 (2.5–16.1)	4.6 (3.0–7.3)	0.7 (0.4–1.3)	0.3
$CD19^+CD27^+IgD^-$ (cells/μl)	23 (9–37)	6 (2–19)	0.6 (0.3–1.1)	0.1
Serum IgM (g/l)	0.83 (0.48–1.21)	0.33 (0.16–0.61)	0.5 (0.3–0.8)	0.01
Serum IgG (g/l)	6.36 (5.88–9.23)	2.19 (0.56–3.54)	0.2 (0.1–0.3)	<0.001
Serum IgA (g/l)	0.50 (0.38–1.68)	0.11 (0.00–0.28)	0.3 (0.1–0.5)	<0.001

Figures are given as the number (percentage) for discrete variables and median (inter-quartile range) for continuous variables. Cox regression analysis (odds ratio (OR) (95% confidence interval (95% CI)), with age as covariant for laboratory parameters, is used to compare the SAD and CVID groups.

longer than those without this complication. There were no significant differences in total serum immunoglobulin levels between the groups even after adjusting for possible confounding effect of age by multi-variant Cox regression analysis. Absolute numbers of $CD3^+$, $CD4^+$, $CD8^+$ and $CD56^+$ lymphocytes, and proliferative responses to PHA and PWM were also not statistically different (data not shown). Patients with bronchiectasis did however have two-fifth of the percentage of $CD19^+CD27^+IgD^-$ switched memory B cells (OR (95% CI): 0.4 (0.2–0.8)) of patients without bronchiectasis, and this difference was independent of age (Fig. 2A). The absolute number of switched memory B cells was also significantly lower in the group of patients with bronchiectasis.

The immunology of the patients with and without splenomegaly was similar to the patients with and without bronchiectasis, even though only three patients were common to both groups (Table 3). Patients with splenomegaly had one-fifth of the number of $CD19^+CD27^+IgD^-$ switched memory B cells (OR (95% CI): 0.2 (0.1–0.5)), even after adjusting for the effect of age (Fig. 2B). All other immune parameters, including absolute numbers of class switched B cells, were not significantly different between the two groups even after adjusting for age by multi-variant analysis. One adult with low switched memory B cells had undergone splenectomy and histology had showed extensive granulomata.

Patients with autoimmune manifestations had significantly lower percentages of $CD19^+CD27^+IgD^-$ switched memory B cells (OR (95% CI): 0.4 (0.2–0.7)), even after adjusting for the effect of age (Fig. 2C). Although there was a similar trend for absolute numbers of these cells (OR (95% CI): 0.5 (0.3–1.0)), this failed to reach significance ($P = 0.07$).

Table 4 summarizes the clinical and relevant immunological characteristics of the 13 patients with bronchiectasis, splenomegaly or both, illustrating the variability in serum immunoglobulin levels from near normal to complete absence. Patient 11 who had both bronchiectasis and splenomegaly died recently of non-Hodgkin B cell lymphoma. The remainder are still alive.

Table 2 Age, delay in diagnosis, gender, B cell surface markers and serum immunoglobulin concentrations for patients with or without bronchiectasis

	No bronchiectasis	Bronchiectasis	OR (95% CI)	*P* value
Number	47	8		
Age (years)	16 (8–42)	14 (8–49)	0.8 (0.3–1.7)	0.7
Delay in diagnosis (years)	2 (1–5)	5 (4–10)	0.5 (0.2–1.2)	0.1
Males (%)	27 (57%)	6 (75%)	2.0 (0.4–11.3)	0.4
CD19 (cells/μl)	280 (162–562)	115 (25–588)	1.0 (0.5–2.4)	0.9
CD19 $CD27^-IgD^+$ %	75 (60–83)	87 (81–90)	2.2 (1.0–4.9)	0.9
CD19 $CD27^+IgD^+$ %	4.7 (3.1–8.5)	2.5 (2.0–7.6)	0.6 (0.3–1.4)	0.1
CD19 $CD27^+IgD^-$ %	5.2 (2.2–10.3)	0.7 (0.4–4.0)	0.4 (0.2–0.8)	<0.001
$CD19^+CD27^+IgD^-$ (cells/μl)	13 (5–35)	2 (0–19)	0.4 (0.2–0.8)	0.01
IgM (g/l)	0.51 (0.30–0.91)	0.66 (0.18–1.87)	1.5 (0.6–3.4)	0.3
IgG (g/l)	3.61 (1.21–6.36)	5.16 (1.97–6.87)	1.4 (0.6–3.0)	0.4
IgA (g/l)	0.28 (0.12–0.60)	0.04 (0.00–0.79)	0.7 (0.3–1.5)	0.4

Figures are given as the number (percentage) for discrete variables and median (inter-quartile range) for continuous variables. Cox regression analysis (odds ratio (OR) (95% confidence interval (95% CI)), with age as covariant for laboratory parameters, is used to compare the two groups.

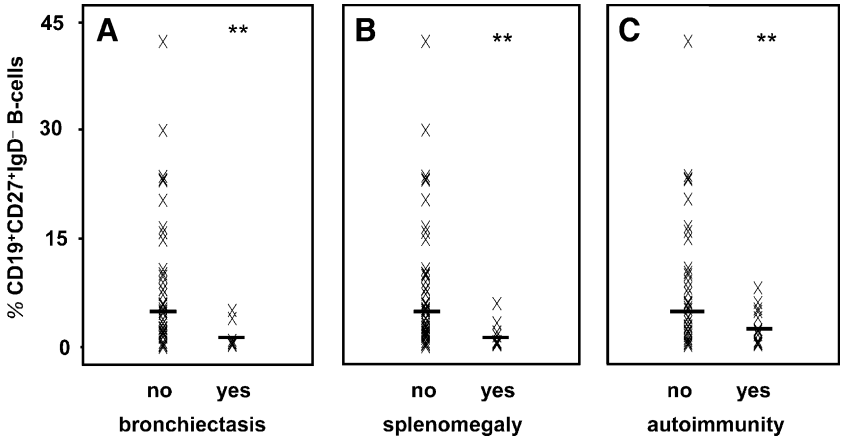

Figure 2 Percentage of memory switched B cells ($CD19^+CD27^+IgD^-$) (Y axis) in patients with or without (A) bronchiectasis, (B) splenomegaly and (C) autoimmunity. Bar represents median. $^{**}P \leq 0.01$ using Mann–Whitney *U* Test.

Gene mutation screening of patients for defects in B cell switching

AID and UNG gene mutations were excluded by gene sequencing in eight patients with $CD19^+CD27^+IgD^-$ B cell percentages less than 2% and in whom there were normal or elevated serum IgM levels but low IgG and IgA suggesting a form of Hyper IgM syndrome. Since the publication of two papers linking mutations in *TACI* to CVID in adults [15,16], the *TACI* gene has been sequenced in eight patients who presented with splenomegaly and/or autoimmunity. Three of these eight patients were found to have missense mutations in the gene leading to amino acid substitutions (Table 5). One, a 16-year-old child, affected with SAD and splenomegaly, had a heterozygous missense mutation in *TACI* gene (V220A), already reported as a rare polymorphism [16]. Another patient, a 12-year-old child presenting with CVID, insulin-dependent diabetes and idiopathic chronic urticaria, had a heterozygous missense mutation (P251L), not yet reported in the literature and not found in 28 healthy controls sequenced at the same time, but its direct role in the CVID phenotype remains unclear. An adult with CVID and autoimmune cytopenias had two missense compound mutations, the P251L and the C104R already reported [15,16]. None of these three male patients had bronchiectasis.

Discussion

This study introduces a number of new concepts concerning the classification of idiopathic humoral immunodeficiencies and clinical outcome. Firstly in this cohort of both children and adults, we find that classification of the humoral immunodeficiency diseases based on whether or not the immunoglobulin class concentrations are normal (SAD) or not (CVID) does not predict which patients develop bronchiectasis, splenomegaly or autoimmunity. We show that the only laboratory immunological parameter associated with a significantly higher frequency of these clinical complications in this cohort of patients is the percentage of $CD19^+CD27^+IgD^-$ memory switched B cells. In both children and adults, these clinical complications are associated with 20–40% of memory switched B cells of the remainder of the cohort. This is the first study to demonstrate this association in children with SAD and CVID and adults with SAD. These findings are in keeping with previous studies that have a

Table 3 Age, gender, B cell surface markers and serum immunoglobulin concentrations for patients with or without splenomegaly

	No splenomegaly	Splenomegaly	OR (95% CI)	*P* value
Number	45	8		
Age (years)	27 (8–44)	31 (8–48)	1.0 (0.5–2.2)	0.9
Males (%)	30 (54%)	6 (75%)	2.0 (0.4–11.3)	0.4
CD19 (cells/μl)	280 (135–575)	230 (100–570)	1.1 (0.5–2.4)	0.8
$CD19^+CD27^-IgD^+$ %	76 (62–83)	83 (77–89)	1.6 (0.8–3.5)	0.2
$CD19^+CD27^+IgD^+$ %	4.8 (2.9–7.6)	3.7 (2.2–9.4)	0.8 (0.4–1.8)	0.6
$CD19^+CD27^+IgD^-$ %	5.1 (2.4–10.5)	1.1 (0.4–3.0)	0.2 (0.1–0.5)	<0.001
$CD19^+CD27^+IgD^-$ (cells/μl)	14 (5–32)	4 (0.4–3.0)	0.6 (0.3–1.4)	0.2
IgM (g/l)	0.51 (0.31–0.95)	0.47 (0.06–1.18)	1.1 (0.5–2.5)	0.8
IgG (g/l)	3.79 (1.99–6.42)	2.04 (0.32–9.01)	0.9 (0.4–2.1)	0.9
IgA (g/l)	0.28 (0.12–0.85)	0.04 (0.00–0.39)	0.5 (0.2–1.1)	0.1

Figures are given as the number (percentage) for discrete variables and median (inter-quartile range) for continuous variables. Cox regression analysis (odds ratio (OR) (95% confidence interval (95% CI)), with age as covariant for laboratory parameters, is used to compare the two groups.

Table 4 Details of clinical manifestations and immunity of patients with bronchiectasis and/or splenomegaly

No.	Age	Gender	Bronch	Spleen	Auto	B cells	switched	IgM	IgG	IgA
1	5	F	–	+	+	540	1.5	0.58	3.93	0.47
2	6	M	+	+	+	580	0.6	4.09	11.20	0.00
3	7	M	+	–	–	0	–	0.84	4.42	0.00
4	11	M	+	–	–	2330	2.0	2.16	6.48	0.61
5	13	F	+	+	+	90	0.4	0.00	1.90	0.00
6	16	M	+	–	+	590	4.0	0.98	5.89	0.85
7	16	M	–	+	–	260	2.0	1.38	10.70	0.73
8	34	M	–	+	+	1320	6.0	0.54	1.28	0.16
9	34	M	–	+	+	200	0.3	0.00	0.00	0.00
10	40	M	+	–	+	40	0.9	0.11	0.88	0.00
11	52	M	+	+	–	20	0.7	0.39	2.19	0.07
12	52	M	–	+	–	130	3.3	0.30	0.00	0.00
13	65	F	+	–	+	140	5.1	0.48	7.00	1.62

No. = patient number; Age (years); Gender: M = male, F = female; Bronch = bronchiectasis; spleen = splenomegaly; Auto = autoimmunity; B cells = $CD19^+$ B cells (cells/μl); switched = $\%CD19^+CD27^+IgD^-$ B cells; IgM IgG and IgA all g/l.

shown similar association between switched memory B cells and splenomegaly [26,27], and autoimmunity [29] in adults with CVID. Carsetti et al. [28] found a similar trend between IgG switched memory B cells and bronchiectasis in his study of adults with CVID but the trend did not reach significance possibly because of the smaller cohort size. They did however find a significant reduction in IgM switched memory B cells as well as pneumococcal IgM titers and bronchiectasis. Although we did not specifically look for the levels of these IgM antibodies, we found no evidence of reduced numbers of memory, non-switched B cells in patients with bronchiectasis. This may be because of the difference in selection criteria between the two studies and the fact that the majority of our patients had had recurrent symptomatic sinopulmonary infections.

We could not find any genetic defect underlying the complications of these humoral immunodeficiencies. Even in patients presenting with normal or increased IgM levels, the involvement of AID or UNG deficiency was ruled out. *TACI* gene mutations (2 compound heterozygous mutations in 1 case and 1 heterozygous mutation in another) were observed in 2 patients who present autoimmunity but no bronchiectasis.

From recent advances in our understanding of humoral immunodeficiencies secondary to known molecular defects [5–20], it is clear that inflammatory and lymphoproliferative complications are more frequent in patients with later defects in B cell maturation and switching than in patients with early defects in B cell development [32]. Impaired B cell switching may be primary, or secondary to T cells dysfunction [33]. Inflammatory and lymphoproliferative complications may be caused via two immune mechanisms. Firstly, in primary B cell switching disorders, e.g., AID deficiency, excessive hyperplasia of unswitched B cells proximal to the block in differentiation lead to lymphadenopathy, splenomegaly and auto-immunity [12]. If the block is complete autoimmune phenomena are much less common than if it is partial such as in TACI deficiency [15,16], where presumably some autoreactive IgG secreting B cell clones can develop [34].

Secondly, impaired B cell switching may be a consequence of defective maturation or dysfunction of T cells [33]. T cell rather than B cell dysfunction causes the granulomatous or non-granulomatous inflammatory reactions to microorganisms which normally live in symbiosis with the host. An example par excellence is the inflammatory bowel disease which occurs in CVID, where intestinal microorganisms in the large intestine trigger T cell induced inflammation [35,36]. Although there is currently little evidence, it is possible that excessive T-cell-induced inflammation also contributes to the development of bronchiectasis in some patients. Macrolide antibiotics are currently in vogue for the treatment of this complication not only because of their anti-bacterial activity but also their anti-inflammatory properties [37]. In our study, there was no significant association between T cell mitogen responses and clinical complications, indicating that subtle abnormalities

Table 5 Patients with mutations in the TACI gene

Age	B cells	Switched B cells	IgM	IgG	IgA	Spleen	Autoimmunity	TACI mutation
12	290	2.0	0.33	6.41	0	0	++	752C > T → 251P > L
16	260	3.8	1.38	11.0	0.7	++	0	659T > C → V220A
43	190	2.2	0.5	7.2	0.3	0	++	251P > L/310C > T → 104C > R

Age (years), B cells (cells/μl), switched B cells (percentage $CD19^+CD27^+IgD^-$ B cells), immunoglobulins (g/l), spleen = splenomegaly, TACI mutation: nucleotide → amino acid substitution, all three patients were male.

in T cell function are possibly involved and that more specific laboratory tests of T cell function are required to detect these abnormalities.

In conclusion, we find that except for serum immunoglobulin classes, other laboratory parameters and clinical outcome measures of adults and children with SAD and CVID are similar and possibly parts of a spectrum involving a similar pathogenesis and clinical course. Furthermore, as we demonstrate that the most important prognostic marker in terms of bronchiectasis and splenomegaly is the percentage of $CD19^{+}CD27^{+}IgD^{-}$ memory switched B cells. We suggest that this marker should be assessed in all patients with primary antibody deficiency with normal B cell counts, not just those patients with CVID, as a low memory switched B cell percentage may indicate that more intensive monitoring and treatment is required to prevent clinical complications.

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