

# Selective antipolysaccharide antibody deficiency associated with peripheral blood CD5+ B-cell predominance

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**Background:** Primary humoral deficiencies vary from complete absence of B cells and/or serum immunoglobulin to lacunar deficits involving specific antibody responses to polysaccharides.

**Objectives:** We compared the B-cell CD5 expression in patients with selective antipolysaccharide antibody deficiencies (SPADs), common variable immunodeficiency (CVID), and IgG subclass deficiency and in normal control subjects.

**Methods:** Five patient populations were evaluated: (1) patients with severe SPAD (no protective serologic postvaccine response to any of 12 polysaccharide antigens tested); (2) patients with intermediate SPAD (diminished response to polysaccharide antigens and adequate response to 1 to 3 of 12 serotypes tested); (3) patients with IgG subclass deficiency; (4) patients with CVID; and (5) age-matched control subjects. Blood was collected from all patients and evaluated by using flow cytometry. Results were compared by using the Student *t* test.

**Results:** Patients with severe SPAD deficiencies had a marked predominance of CD5+ B cells in the peripheral blood (93% to 97% of total B cells, *n* = 2). The intermediate SPAD group had a mean CD5+ B-cell percentage that was significantly higher than that of the age-matched control group (87.4%, *n* = 7, vs 52.5%, *n* = 20; *P* = .007). Patients with CVID and IgG subclass deficiency had mean CD5+ B-cell percentages that were similar to those of the age-matched control subjects.

**Conclusions:** Our studies demonstrate that patients with SPAD had a markedly increased percentage of CD5+ B cells in the peripheral blood as compared with age-matched control subjects and patients with other humoral deficiencies. This observation suggests that an association may be present between CD5+ B-cell predominance and SPAD. (*J Allergy Clin Immunol* 1999;103:637-41.)

**Key words:** Selective antipolysaccharide antibody deficiency, CD5+ B cells

An intriguing disorder of lacunar antibody deficiencies has been reported, which involves responses to poly-

## Abbreviations used

APC: Allophycocyanin

CVID: Common variable immunodeficiency

PE: Phycoerythrin

SPAD: Selective antipolysaccharide antibody deficiency

saccharide antigens.<sup>1-7</sup> Although precise clinical and laboratory diagnostic criteria are debated, selective antipolysaccharide antibody deficiency (SPAD) is typically defined as an absent or diminished antibody response to polysaccharide capsular antigens in polyvalent pneumococcal polysaccharide vaccine in a patient with normal immunoglobulin and IgG subclass levels.<sup>5,7</sup> The pathogenesis of SPAD is unknown, although the nature of the antibody response to polysaccharide antigens is T-cell independent, suggesting a B-cell defect. We demonstrate that there is a significant increase in peripheral blood CD5+ B cells in patients with severe and intermediate SPAD as compared with normal control subjects and patients with other humoral deficiencies such as common variable immunodeficiency (CVID) and IgG subclass deficiency.

## CASE PRESENTATIONS

Patient 1 (a 5-year-old white boy) and patient 2 (an 8-year-old white boy) were first seen for evaluation of recurrent infection. The parents of both patients reported recurrent and frequent episodes of otitis media, sinusitis, and pneumonia since the age of 5 months despite prophylactic antibiotics and tympanostomy tubes. At age 2 years, patient 1 experienced an episode of severe hemorrhagic varicella that required hospitalization. Later that year, he had an acute onset of hemiplegia that resolved over a period of 3 to 4 months (normal computed tomography and magnetic resonance imaging scan results). Patient 2 had alopecia totalis and experienced recurrent episodes of laryngotracheobronchitis. Their growth and development had been normal, and there was no family history of immunodeficiency or consanguinity. Their physical examination was essentially unremarkable. Immune evaluation revealed a normal total white blood cell count, with a normal number of T cells, natural killer cells, and B cells (Table I). Quantitative serum immunoglobulin evaluation consistently showed no major abnormalities (Table I). T-cell responses to mitogens and antigens were normal. There were no autoantibodies detected. Serologic evidence of HIV, EBV, and cytomegalovirus was absent. The patients demonstrated a normal antibody response to protein antigens (tetanus and diphtheria) but no protective titers to 12 serotypes of pneumococcus after immunization with two 23-valent pneumococcal polysaccharide vaccines (Table II). Both patients had low initial levels of anti-

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TABLE I. Immunologic profile

|                      | Patient 1 | Patient 2 | Normal range               |
|----------------------|-----------|-----------|----------------------------|
| Serum immunoglobulin |           |           |                            |
| IgM                  | 101       | 55        | 26-170 mg/dL               |
| IgG                  | 518       | 558       | 546-1170 mg/dL             |
| IgA                  | 53        | 190       | 43-208 mg/dL               |
| IgG1                 | 401       | 379       | 313-961 mg/dL              |
| IgG2                 | 67        | 119       | 102-456 mg/dL              |
| IgG3                 | 33        | 19        | 16-140 mg/dL               |
| IgG4                 | 17        | 17        | 1-70 mg/dL                 |
| Blood leukocytes     |           |           |                            |
| WBC                  | 7.5       | 8.6       | 4.5-13 $\times 10^9$ /mL   |
| T cells (CD3+)       | 2.48      | 1.73      | 0.71-4.1 $\times 10^9$ /mL |
| B cells (CD19+)      | 1.40      | 0.34      | 0.07-0.9 $\times 10^9$ /mL |
| NK cells (CD16+)     | 0.45      | 0.11      | 0.0-0.9 $\times 10^9$ /mL  |

Serum immunoglobulins, IgG subclasses levels, and blood leukocyte numbers demonstrate a lack of quantitative deficiency.  
NK, Natural killer.

bodies to *Neisseria meningitidis* A and C; patient 2 had protective titers only to group A. There were no detectable antibody titers to *Haemophilus influenza* b (<0.44  $\mu$ g/mL), parainfluenza 1,2,3 (<1:8), influenza a, b (<1:8), polio 1,2,3 (<1:8), or respiratory syncytial virus (<2.0 SD) in either patient. Further evaluation of the B-cell phenotype demonstrated that approximately 97% and 93% of the B cells express surface CD5 (Fig 1).

## METHODS

### Patient population

Age-matched normal control patients were enrolled during routine medical visits. Blood was drawn at the time of blood collections for routine screening studies.

Immunodeficient patients were evaluated during periods of good health. Immunodeficient control subjects were gathered from the immunology clinic at Rainbow Babies and Children's Hospital, Cleveland, Ohio. Because of our clinic population, CVID was defined as being present in adults with IgG of less than 200 mg/dL, IgA of less than 20 mg/dL, and IgM of less than 20 mg/dL (as determined by University Hospital Laboratory, Cleveland, Ohio).<sup>8</sup> Additionally, because of our clinic population, IgG subclass deficiency was defined as being present in children with decreased levels of IgG1 (<2 SD below mean for age) and/or IgG2 (<2 SD below mean for age), with normal antibody responses to polysaccharide and tetanus antigens (IBT Laboratory, Lennox, Kan).<sup>9</sup> Severe SPAD was defined as being present in patients with normal levels of immunoglobulins, subclasses, and serum antibody responses to tetanus but an absent protective postserologic response (>1.8  $\mu$ g/mL) to 12 serotypes of pneumococcus 1 month after immunization with the 23-valent pneumococcal polysaccharide vaccine (Pneumovax7; Merck and Co, West Point, Pa). Moderate SPAD was defined as being present in those patients with protective serologic responses of greater than 1.8  $\mu$ g/mL to no more than 3 serotypes of pneumococcus after Pneumovax immunization. Serologic responses were verified by Dr Ricardo Sorenson in both patients (Tulane University, New Orleans, La). Similar antibody responses were performed with the meningococcal vaccine (Connaught).

### Flow cytometry

Four-color direct immunofluorescent staining with mAbs was performed on aliquots of heparinized peripheral blood collected from each patient group. Blood was processed within 12 hours of collection.

All mAbs used in this study were purchased from Becton Dickinson Immunocytometry Systems (BDIS; San Jose, California), except antibodies against CD9 and CD24 (Coulter, Miami, Fla); CD37, CD40, CD80, and CD86 (Pharmingen, San Diego, Calif); IgM (Leinco Technologies, Ballwin, Mo); and CD19 allophycocyanin (APC)-conjugate (Immune Source, Los Altos, Calif). Antibody staining was performed on 200  $\mu$ L of whole blood as described by the manufacturers. In all assays 4-color analysis was performed. Anti-CD5 phycoerythrin (PE)-conjugated and anti-CD19 APC-conjugated antibodies were used to maximize the sensitivity of detecting CD5+ and CD19+ cells. The percentage of CD5+ B cells was determined from 4-quadrant dot plots by dividing the percent of CD5+CD19+ dual-positive cells by the percent of total CD19+ cells. For surface antigen analysis of CD5+ B cells, cells were stained with anti-CD19 APCs and anti-CD5 PE in addition to an FITC-conjugated antibody. In some instances anti-CD5 FITC was required because of the use of a PE-conjugated antibody. Anti-CD45 PerCP was used in all assays to facilitate lymphocyte gating. In general, 10,000 lymphocyte-gated cells were analyzed on a FACScalibur (BDIS) flow cytometer by using CELLQUEST (BDIS) and PAINT-A-GATE software. Nonspecific staining was assessed by the use of fluorochrome-conjugated monoclonal mouse immunoglobulins (BDIS).

## RESULTS

We identified 2 patients with severe SPAD (see Case presentations section). Table II shows the antibody response to 12 serotypes of pneumococcus in the 2 patients after pneumococcal capsular polysaccharide vaccination. The complete lack of protective postvaccination antibody responses (>1.8  $\mu$ g/mL) to any of the 12 serotypes of pneumococcus is characteristic of the disorder. Neither patient showed a response to group C of the meningococcal vaccine. Only patient 2 demonstrated a significant response to group A. Each patient lacked antibody titers to *H influenza* despite having received all the required vaccination with Hib. The unconjugated *H influenza* vaccine was not available for this study.

Previous reports indicated that increased quantities of CD5+ B cells were present in the peripheral blood in patients with autoimmune disorders and in clinical settings in which there were poor antipolysaccharide antibody responses, such as during infancy and after bone marrow transplant.<sup>10-13</sup> We therefore assessed, through multicolor flow cytometry, the percentage of B cells (defined as CD19+ cells) that expressed surface CD5 in the 2 individuals with severe SPAD. As shown in Fig 1, an extremely high percentage of B cells in both patients expressed CD5 (97% and 93%) compared with age-matched control subjects (Table III). Having noted the high percentage of CD5+ B cells in the peripheral blood in these 2 patients, we next addressed whether these cells were phenotypically similar to CD5+ B cells in normal patients. Therefore we extensively immunophenotyped the peripheral blood CD5+ B cells in the 2 patients with severe SPAD with a panel of mAbs by using 4-color FACS analysis. The cell surface expression pattern of CD9, CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD45, CD62L, CD80, CD86, CD95, HLA-Dr, and surface immunoglobulin did not differ sig-

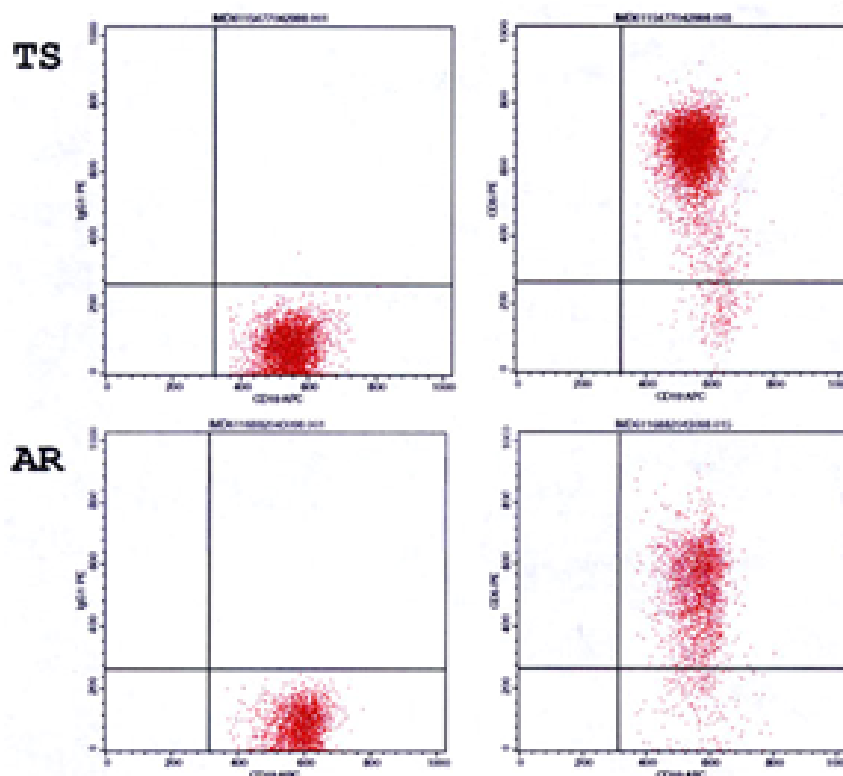


**FIG 1.** Four-quadrant dot plot flow cytometric analysis of CD5 (*y axis*) versus CD19 (*x axis*) in patients 1 and 2. *Left panels* show negative controls for each patient. *Right panels* show increased percentage of CD5+CD19+ cells in patients 1 (97%) and 2 (93%).

**TABLE II.** Antibody responses to polysaccharide antigens

| Serotypes            | Patient 1 |        |        | Patient 2 |        |        | Normal range   |
|----------------------|-----------|--------|--------|-----------|--------|--------|----------------|
|                      | Before    | After* |        | Before    | After* |        | After<br>μg/mL |
|                      |           | First  | Second |           | First  | Second |                |
| Pneumococcus         |           |        |        |           |        |        |                |
| Type 1               | <0.3      | <0.3   | <0.3   | <0.3      | <0.3   | <0.3   | >1.8           |
| Type 4               | <0.3      | <0.3   | <0.3   | <0.3      | <0.3   | <0.3   | >1.8           |
| Type 6/26            | <0.3      | <0.3   | <0.3   | <0.3      | <0.3   | <0.3   | >1.8           |
| Type 8               | <0.3      | <0.3   | <0.3   | <0.3      | <0.3   | <0.3   | >1.8           |
| Type 9               | <0.3      | <0.3   | <0.3   | <0.3      | 1.2    | <0.3   | >1.8           |
| Type 12              | <0.3      | <0.3   | <0.3   | <0.3      | <0.3   | <0.3   | >1.8           |
| Type 14              | <0.3      | <0.3   | <0.3   | <0.3      | <0.3   | <0.3   | >1.8           |
| Type 19              | <0.3      | <0.3   | <0.3   | <0.3      | <0.3   | <0.3   | >1.8           |
| Type 23              | <0.3      | <0.3   | <0.3   | <0.3      | <0.3   | <0.3   | >1.8           |
| Type 51              | <0.3      | <0.3   | <0.3   | 0.9       | 1.1    | 0.7    | >1.8           |
| Type 56              | <0.3      | <0.3   | <0.3   | <0.3      | <0.3   | <0.3   | >1.8           |
| Meningococcus        |           |        |        |           |        |        |                |
| Type A               | 98        | 106    | —      | 64        | 715    | —      | >200 AU/mL     |
| Type C               | <1.0      | <1.0   | —      | <1.5      | <1.0   | —      | >5.0 AU/mL     |
| <i>H Influenza</i> b | <0.44     | —      | —      | <0.44     | —      | —      | >1.0 μg/mL     |

Serologic data show a lack of protective antibody responses to 12 serotypes of *Streptococcus pneumoniae* and *Neisseria meningitidis* after challenge with the corresponding vaccine.

\*Determined 1 month after vaccination

nificantly from that of normal control subjects. In addition the cells in both patients were polyclonal (normal  $\lambda/\kappa$  ratio), indicating a neoplastic process was not present. The only significant difference seen between the 2 patient's B

cell counts was the high percentage of CD25+ B cells present in patient 1 compared with patient 2 and normal control subjects. The significance of this finding was unclear, but it does suggest B-cell activation in this patient.

**TABLE III.** Mean percentages of CD5+CD19+ cells in various patient and control groups

| Disease state | n  | Age (y) | CD5+ CD19+ cells (% mean $\pm$ SD) | Significance |
|---------------|----|---------|------------------------------------|--------------|
| CVID          | 6  | 25-42   | 55.5 ( $\pm$ 25.6)                 | .415         |
| Control*      | 23 | 22-54   | 47.2 ( $\pm$ 20.4)                 |              |
| IgG subclass  | 6  | 2-10    | 49.1 ( $\pm$ 13.5)                 | .345         |
| Control*      | 20 | 2-10    | 52.5 ( $\pm$ 17.3)                 |              |
| SPAD          | 7  | 6-10    | 87.4 ( $\pm$ 12.0)                 | .007         |
| Control*      | 20 | 2-10    | 52.5 ( $\pm$ 17.3)                 |              |

Mean B1a cell percentage of total B-cell population in CVID, IgG subclass, and intermediate SPAD compared with age-matched control subjects showing significantly elevated mean CD5+ B-cell percentage in SPAD only.

\*Age matched.

To determine whether the peripheral blood predominance of CD5+ B cells extended to other B-cell immunodeficiencies, we compared the percentage of CD5+ B cells in patients with intermediate SPAD, CVID, and IgG subclass deficiency with that of normal age-matched control subjects (Table III), again using multicolor flow cytometry. The mean CD5+ B-cell percentage in both CVID and IgG subclass deficiency did not vary significantly from the normal age-matched control subjects; however, patients with intermediate SPAD showed a statistically significant increase in mean CD5+ B-cell percentage when compared with control subjects.

## DISCUSSION

The clinical entity known as SPAD has been reported previously but is poorly understood.<sup>1-7</sup> The most commonly used test for defining SPAD involves the determination of antibody response to 4 to 12 serotypes of pneumococcus 1 month after vaccination with a 23-valent pneumococcal capsular polysaccharide vaccine in a patient greater than 2 years of age.<sup>7</sup> Most immunologists have defined SPAD as being present in patients with recurrent respiratory tract infections, normal quantitative serum immunoglobulin levels, normal antibody responses to protein immunizations, and a complete absence of protective response to unconjugated polysaccharide vaccinations.<sup>1-6</sup> The mechanism of this deficient response remains to be defined, although the T-cell independence of antibody responses to polysaccharides suggests that B cells may be implicated as the site of the abnormality.

The B-cell compartment may be divided into 2 populations on the basis of the surface expression of CD5.<sup>14,15</sup> CD5+ B cells predominate in the fetal circulation and decrease in adulthood, have a limited antibody repertoire, and produce low-affinity antibodies of the IgM isotype.<sup>11,14</sup> In humans controversy exists as to whether these 2 subpopulations represent separate lineages or different stages of B-cell activation or differentiation.<sup>14-18</sup> An association with CD5+ B-cell predominance and poor polysaccharide responses has been seen during normal ontogeny in infants and during the reconstitution stage of bone marrow transplantation.<sup>12-14</sup> Additionally,

Barrett et al<sup>19</sup> showed that in humans, CD5+ B cells do not form antibodies to pneumococcal polysaccharide antigens, but rather this function was specifically found in the CD5- B-cell subgroup.<sup>19</sup>

The published mean percentage of peripheral blood CD5+ B cells and that of our age-matched control subjects (who presumably have a normal response to polysaccharides) ranged from 20% to 60%.<sup>11,14,18</sup> The intermediate SPAD group demonstrated a mean CD5+ B-cell percentage significantly greater than that of normal control subjects (87.4% vs 52.5%,  $P = .007$ ). The 2 patients with severe SPAD showed a striking predominance of CD5+ B cells (93% to 97%). Although the antibody response to polysaccharide antigens in patient 1 (97%) is completely absent, it appeared that some antibody responses to a few polysaccharide antigens were present in patient 2 (93%), suggesting a disease spectrum. These findings suggested that there may be an inverse relationship between the magnitude of the polysaccharide response and CD5+ B-cell percentage.

Our data also suggests that these 2 patients do not have proliferation of abnormal cells, but instead have an overabundance of normal CD5+ B cells and a lack of normal CD5- B cells. These patients also do not appear to have a block in B-cell development, but rather they may have either a defect in CD5- B-cell generation or a shift toward CD5+ B-cell preference. On the basis of our data, we do not believe that this abnormal B-cell phenotype represents malignancy or activation.

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