

The loss of IgM memory B cells correlates with clinical disease in common variable immunodeficiency

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Background: Recurrent lower respiratory tract infections caused by encapsulated bacteria might cause permanent organ damage in patients with common variable immunodeficiency (CVID). Despite the profound hypogammaglobulinemia, some patients do not experience bacterial pneumonia. We have shown that IgM memory B cells and natural antibodies play an important role in the defense against encapsulated bacteria.

Objective: In this study we addressed the question of whether the apparent paradox of patients with severe hypogammaglobulinemia but no increased frequency of respiratory infections can be explained by the presence of IgM memory B cells and anti-pneumococcal polysaccharide (anti-PnPS) IgM.

Methods: We measured the frequency of memory B cells and the levels of anti-PnPS IgM antibodies in 26 patients with CVID with recurrent bacterial pneumonia and bronchiectasis (group 1) and 22 who never had pneumonia and showed no lung lesions (group 2). An additional 6 patients had a clinical history of recurrent pneumonia without lung abnormalities at computed tomographic scanning.

Results: Patients of group 1 lacked IgM memory B cells and failed to produce anti-PnPS IgM antibodies, and those of group 2 had a normal frequency of IgM memory B cells and produced anti-PnPS IgM antibodies.

Conclusions: IgM memory B cells and anti-PnPS IgM antibodies protect patients with CVID from bacterial pneumonia. Evaluation of these 2 parameters discriminates patients with low or high risk of recurrent infections caused by encapsulated bacteria and low or high risk of bronchiectasis.

Identification of high-risk individuals at diagnosis might help in the planning of a more effective therapeutic strategy and prevent permanent organ damage (J Allergy Clin Immunol 2005;115:412-7.)

Key words: Common variable immunodeficiency, IgM memory B cells, bacterial pneumonia, bronchiectasis, natural antibodies, capsular polysaccharide, pneumococcal vaccine

Common variable immunodeficiency (CVID) is a heterogeneous immune disorder of unknown cause characterized by reduced serum levels of all immunoglobulin isotypes and inability to mount protective antibody responses in the presence of normal numbers of circulating B cells. CVID is the most common symptomatic humoral immunodeficiency, with a prevalence of 1 in 50,000.^{1,2} Upper and lower respiratory tract infections caused by encapsulated bacteria, such as *Haemophilus influenzae* and *Streptococcus pneumoniae*, are the most important clinical manifestations leading to chronic lung disease, bronchiectasis, and eventually death.^{2,3} This clinical observation suggests that a defective antibody response against capsular polysaccharide antigens is the major factor contributing to lung disease in patients with CVID. The use of regular parenteral immunoglobulin substitution therapy reduces the occurrence of acute pulmonary infections. Despite correct treatment,²⁻⁶ bronchiectasis and fibrosis are still common in patients with primary defects of antibody production (CVID and X-linked agammaglobulinemia). There are, however, patients with CVID who do not have recurrent pneumonia, and a minority of these patients are completely asymptomatic. The reason for these exceptions is unknown.

The immune response against bacterial polysaccharides is T-cell independent. Activated B cells rapidly produce antibodies, mostly of an IgM isotype, directed against capsular polysaccharides. These antibodies are of key importance in the initial phase of infection because they opsonize the pathogen and favor its phagocytosis by macrophages.^{7,8}

In the human peripheral blood the B-cell compartment is composed of mature and memory B cells, which can be distinguished on the basis of the expression of CD22, CD27, IgM, and IgD.⁹⁻¹² Mature B cells are CD22⁺CD27⁻, and memory B cells are CD22⁺CD27⁺. Memory B cells can be further subdivided into IgM memory (IgM^{bright}IgD^{dull}) and switched memory B cells

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Supported by a program grant from the Italian Ministry of Health N. 3AI/F3 to R. Carsetti, EC project QL-G-CT-2001-01536 to A. Plebani, Cofin Murst 2002, Facoltà la Sapienza 2002-2003 to F. Aiuti, M. M. Rosado is supported by a Marie-Curie Fellowship by the European Community (QLK2-CT-2001-51093). V. Guazzi and S. Donnanno are supported by a Doctor in Research on "Science of immunological Therapies" grant, University of Rome, "La Sapienza."

Received for publication June 18, 2004; revised September 29, 2004; accepted for publication October 4, 2004.

Available online January 10, 2005.

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0091-6749/\$30.00

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doi:10.1016/j.jaci.2004.10.048

Abbreviations used

CT: Computed tomography
CVID: Common variable immunodeficiency
IVIG: Intravenous immunoglobulin
PnPS: Pneumococcal polysaccharide

(IgM⁺IgD⁺).⁹⁻¹² IgM memory B cells can be generated in the absence of germinal centers¹³ and play a major role in the protection against encapsulated bacteria. We have shown that the absence of IgM memory B cells is correlated with low-absent anti-polysaccharide IgM antibodies and increased susceptibility to pneumococcal infection in infants and in splenectomized and asplenic patients.¹¹

Our preliminary results suggested an inverse correlation between the frequency of IgM memory B cells and susceptibility to bacterial lower respiratory tract infections.^{11,12}

On the basis of these observations, we investigated whether the frequency of IgM memory B cells and the presence of anti-pneumococcal polysaccharide (anti-PnPS) antibodies can explain the different susceptibility to encapsulated bacterial pneumonia of patients with CVID.

METHODS

Study cohort

Fifty-four patients affected by CVID were given diagnoses according to the criteria established by the European Society for Immunodeficiencies/Pan-American Group for Immunodeficiency group of experts.¹ All patients were receiving regular intravenous immunoglobulin (IVIG) substitution therapy, with preinfusion IgG levels constantly greater than 500 mg/dL. As control subjects, we recruited 27 age-matched healthy donors. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by our institutions' human research committees.

The 54 patients with CVID were divided into 3 groups according to their clinical history of recurrent lower respiratory tract infections and on the basis of lung computed tomographic (CT) scan findings. The latter were evaluated by a radiologist blinded to the patients' clinical histories. Group 1 included 26 patients with recurrent pneumonia and positive CT scan results, and group 2 was composed of 22 patients with no clinical history of pneumonia or chronic bronchitis and negative CT scan results. Group 3 contained 6 patients with recurrent pneumonia but a negative CT scan result, and thus they lacked objective evidence of sequelae of lower respiratory tract infection. The 3 groups were comparable for current mean age, age at onset of symptoms, age at diagnosis, time of IVIG therapy, and mean delay of diagnosis. The clinical characteristics of the patients are summarized in Table I (see also Table E1 in the Journal's Online Repository at www.mosby.com/jaci). We focused on patients of group 1 and 2, who represent the 2 extremes of the disease in regard to susceptibility to lower respiratory tract infections. Data relative to group 3 patients are shown in Table II (see also Tables E1 and E2 in the Journal's Online Repository at www.mosby.com/jaci) but could

not be statistically analyzed because of the low number of patients and will be discussed in a separate paragraph in the Results section.

Flow cytometric analysis

PBMCs purified as described previously were stained with appropriate combinations of fluorescein, phycoerythrin, allophycocyanine, cychrome, or biotin-labeled antibodies, followed by streptavidin-Red670, streptavidin-allophycocyanine (Caltag, San Francisco, Calif). Monoclonal HIB22 (anti-CD22), M-T271 (anti-CD27), G20-127 (anti-IgM), and IA6-2 (anti-IgD) antibodies were from Pharmingen (San Diego, Calif), and anti-IgM, Fc5 μ fragment-specific antibody was obtained from Jackson Immuno Research Laboratories (West Grove, Pa). All analyses were performed on a FACSCalibur (Becton and Dickinson, Sunnyvale, Calif) interfaced to a Macintosh CellQuest computer program. One hundred thousand events per sample were analyzed.

The frequency of total, IgM, and switched memory B cells was analyzed in 27 adult blood donors to establish the range of normality. The frequency of total memory B cells varied between 18% and 48% of all B cells. Similarly, there were large differences in the frequency of IgM and switched memory B cells (both between 6% and 37% of the B cells), explaining the high SD. For each frequency, we considered the mean minus 1 SD to be the cutoff value. Thus 6% was the cutoff value for switched memory B cells ($13.8\% \pm 7\%$), and 9% was the cutoff value for IgM memory B cells ($16.2\% \pm 7\%$; Table II and Fig 1, D and E).

ELISA

ELISA for the quantitation of serotype-specific anti-PnPS IgM was according to the protocol established by the Vaccines, Immunization, and Biologicals Department of the World Health Organization in Geneva, Switzerland. Briefly, plates were coated with 1 μ g/mL PnPS types 1, 4, or 14 (PnPS; American Type Culture Collection, Manassas, Va). Serum samples and standard serum (human anti-pneumococcal reference serum, lot 89-SF, kindly provided by Dr Goldblatt) were mixed with an absorbent containing 5 μ g/mL C-polysaccharide (252130; Statens Serum Institute, Copenhagen, Denmark) and 10 μ g/mL 22F capsular PS to neutralize antibody binding to C-polysaccharide and other common contaminants present in the PnPS-coating antigens. Serial dilutions were incubated for 18 hours at room temperature, and the binding to polysaccharide-coated plates was revealed with an alkaline phosphatase-conjugated anti-human IgM μ -specific F(ab)₂ fragment (Sigma, St Louis, Mo). Optical density was measured at 405 nm, and concentrations were calculated by means of interpolation with the standard curve. Test sensitivity was 15 ng/mL.

CT scanning

Chest radiography and high-resolution computed tomographic scans have been performed for all patients to assess lung damage.

Statistical analysis

The Mann-Whitney *U* test (*P* values) was used for unpaired comparison of results obtained in group 1 and 2 patients and healthy individuals. A simple regression (Spearman rank correlation coefficient, *r* values) was used to correlate the group characteristics. The independence between each clinical sign (recurrent pneumonia, splenomegaly, and autoimmunity) and immunologic parameter (IgM anti-PnPS and IgM memory B cells) was tested by using the exact Fisher test. A *P* value of less than .05 and an *r* value of greater than 0.4 were considered significant. Statistical analyses were performed by using StatView 5.0 software (SAS Institute, Cary, NC).

TABLE I. Summary of clinical data of group 1 and group 2 patients with CVID

	No. of patients	Sex (M/F)	Actual age (y)	Age at onset (y)	Age at diagnosis (y)	Mean delay of diagnosis (y)	Time of IVIG therapy (y)	No. of patients with splenomegaly	No. of patients with autoimmunity*
Group 1	26	13/13	37.3 ± 12.2	17.9 ± 10.5	24.9 ± 10.6	6.9 ± 7.2	12.4 ± 7.8	14 (54%)	9 (35%)
Group 2	22	17/5	42.1 ± 17.3	17.9 ± 15.4	29.6 ± 18.4	11.9 ± 13.1	12.4 ± 7.9	6 (27%)	7 (32%)

All values represent the mean ± 1 SD.

*Thyroiditis, autoimmune anemia, or thrombocytopenia.

TABLE II. Summary of immunologic data of group 1, group 2, and group 3 patients with CVID

	No. of patients	Total IgM (mg/dL)	IgM anti-PnPS 1 (ng/mL)	IgM anti-PnPS 4 (ng/mL)	IgM anti-PnPS 14 (ng/mL)	B cells (%)	Mature B cells (%)*	Memory B cells (%)*	IgM memory B cells (%)*	Switched memory B cells (%)*
Group 1	26	15.8 ± 19.6	14.2 ± 31.2	10 ± 25.6	14.5 ± 28.9	11.6 ± 13.7	93.5 ± 5.7	6.4 ± 5.7	3.8 ± 6.3	0.3 ± 0.7
Group 2	22	29.7 ± 28.5	102.2 ± 192.3	60.3 ± 67.8	99.2 ± 124.7	9.6 ± 6.6	65.6 ± 21.5	34.9 ± 22.3	29.9 ± 19.5	5.3 ± 5.2
Group 3	6	10.8 ± 8.9	88.5 ± 170.2	27.2 ± 42.2	56.2 ± 94.1	16 ± 16.2	83.2 ± 14.9	18.5 ± 16.3	14.3 ± 18.1	1.3 ± 2.1
Control subjects	19	142 ± 24	1547.7 ± 416.9	892.1 ± 447.2	721.7 ± 392.4	10.5 ± 5.2	69.6 ± 11	30.4 ± 11	16.2 ± 7.8	13.8 ± 7

All values represent the mean ± 1 SD.

*Expressed as percentage of B cells (B cells = 100%).

RESULTS

B-cell subset analysis

The percentage of total B cells, identified by the expression of the B-cell marker CD22 (Fig 1, A), was comparable in group 1 (11.6% ± 13.7%) and group 2 (9.6% ± 6.6%) patients (Fig 2, A) and not different (P = not significant) from that seen in healthy control subjects (10.6% ± 1.7%; Table II and Table E2 in the Journal's Online Repository).

In contrast, the frequency of memory B cells was dramatically reduced in group 1 (6.5% ± 5.7%, P < .0001; Fig 1, A, upper panels; Fig 2, C) compared with not only healthy adults (30% ± 9%) but also group 2 patients (34.9% ± 22.3%).

Switched memory B cells were significantly reduced in both groups: 0.3% ± 0.7% (P < .0001) in group 1 and 5.3% ± 5.2% (P < .0001) in group 2 (Figs 1, B, and 2, D and Table II). They were virtually undetectable in all group 1 patients, but 7 patients of group 2 had a normal number. However, group 2 patients were protected from lower respiratory tract infections, regardless of whether they had switched memory B cells.

On the contrary, IgM memory B cells were significantly reduced only in group 1 (3.8% ± 6.3%, P < .0001 vs control subjects and group 2), whereas in patients of group 2, their frequency was higher than in healthy control subjects (29.2% ± 19.5% vs 16.2% ± 7%, P < .02; Table II and Fig 2, E). The defect of IgM memory B cells was correlated with the clinical history of bacterial infections of the lower respiratory tract (P < .0001) but not with other clinical signs, such as splenomegaly and autoimmunity (P = not significant for both signs). A more detailed analysis of the data showed that of the 26 patients of group 1, 22 completely lacked or had very few IgM memory B cells, and only 4 had a normal frequency. In contrast, of the 22 patients of group 2, 20 had a normal

frequency of IgM memory B cells. The reduction of IgM memory B cells in patients with recurrent infections is highly significant. The exceptions, however, suggest that this parameter alone is not sufficient to predict the risk of increased susceptibility to pneumonia.

Anti-PnPS IgM

The concentration of IgM to PnPS type 14 (Table II and Table E2 in the Journal's Online Repository) was remarkably lower in group 1 compared within group 2 (14.5 ± 28.9 vs 99.2 ± 124.7 ng/mL, P < .0003; Fig 3, A). In both groups, however, the value was significantly lower than in healthy control subjects (721.7 ± 392.4 ng/mL, P < .0001 for both groups). Similar results were obtained for IgM anti-PnPS types 1 and 4 (Table E2 in the Journal's Online Repository). The higher concentration of IgM anti-PnPS in group 2 compared with that seen in group 1 was not related to a higher level of total IgM, which was similarly low in both groups (29.7 ± 28.5 vs 15.9 ± 19.6 mg/dL, P = not significant). It is noteworthy that 17 of the 26 patients of group 1 and only 2 patients of group 2 had undetectable levels of IgM anti-PnPS.

Correlation between IgM memory B cells and anti-PnPS IgM

A significant correlation between the level of IgM anti-PnPS types 1, 4, and 14 and the frequency of IgM memory B cells (P = .004 and r = 0.43 for anti-PnPS type 14) was found.

Twenty-two of the 26 patients of group 1 lacked IgM memory B cells (<9%) and had undetectable (17/22) or very low levels (5/22) of anti-PnPS type 14 IgM (≤15 ng/mL). The remaining 4 patients had a normal frequency of IgM memory B cells but lacked PnPS IgM antibodies. In contrast, most patients (20/22) in group 2 had a frequency of IgM memory B cells of 9% or greater, and 17 of 22

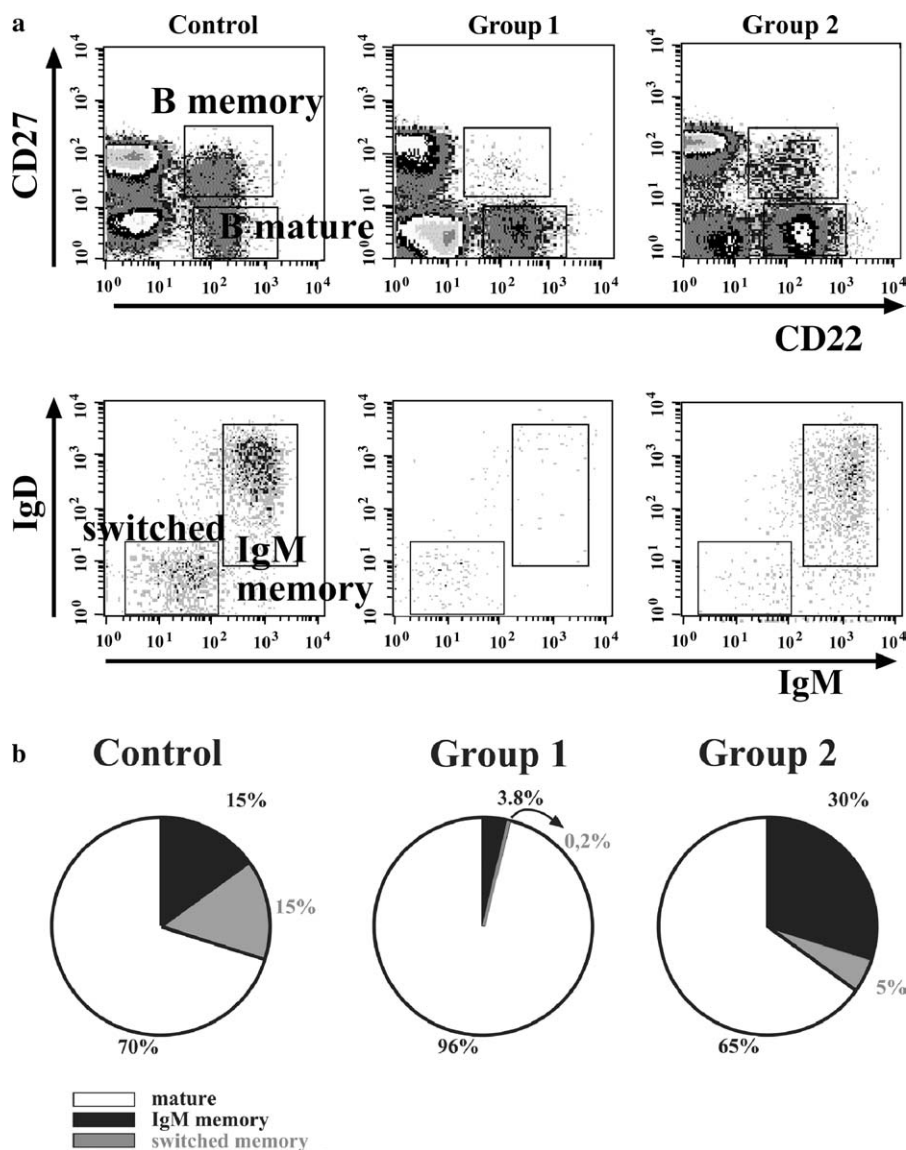


FIG 1. Identification of mature, IgM memory, and switched memory B cells by means of 4-color fluorescence. **A**, The expression of CD22 and CD27 discriminates CD22⁺CD27⁻ mature B cells and CD22⁺CD27⁺ memory B cells (top panels). Memory B cells can be divided into IgM memory (IgM^{bright}IgD^{dull}) and switched memory (IgM⁻IgD⁻) B cells. **B**, Distribution of B-cell subsets in control subjects and patients with CVID shown as a pie graph: mature (white), IgM memory (black), and switched memory (gray) B cells.

patients produced anti-PnPS IgM antibodies (Fig 3, B, and Table E2 in the Journal's Online Repository).

Group 3 patients

Six patients with recurrent pneumonia but a negative CT scan result were included as group 3. Two patients had no IgM memory B cells and no anti-PnPS IgM. Two had a normal frequency of IgM memory B cells but did not secrete anti-PnPS IgM. The remaining 2 had few IgM memory B cells and secreted anti-PnPS IgM. Switched memory B cells were reduced in all 6 patients (Table II). On the basis of our immunologic parameters, the first 4 patients appear to be similar to those of group 1 and the last 2 appear to be similar to patients of group 2. Because all

patients of group 3 had recurrent infections, it is possible that disease evolution will lead to signs of lung damage and to reclassification of these patients into group 1. Indeed, if group 3 patients were included in group 1 now, the differences in IgM memory B cells and anti-PnPS type 14 IgM levels in comparison with group 2 would still be highly significant ($P < .0001$ and $P < .0002$, respectively).

Effect of vaccination

Six patients, 3 of group 1 and 3 of group 2, were immunized with a 23-valent PnPS vaccine. The IgM antibody response to polysaccharides 1, 4, and 14 was evaluated after 4 weeks. Only patients of group 2 had an increase of at least 2 times in the IgM antibody titer (data

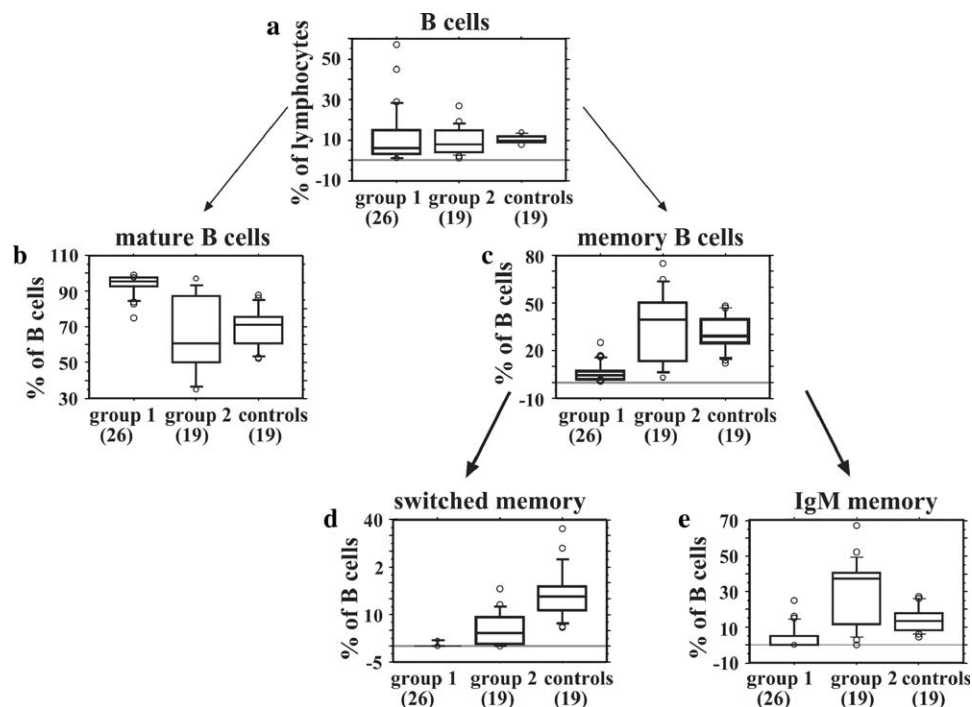


FIG 2. Percentage of total B cells (**A**). The relative frequency of each subset within the B-cell compartment is shown as a box plot for mature (**B**), memory (**C**), switched (**D**), and IgM memory (**E**) B cells. Data are shown as box plots. Boxes represent values between the 25th and 75th percentiles. The horizontal line corresponds to the median.

not shown), suggesting that they were able to respond to polysaccharide vaccination.

DISCUSSION

CVID is the most frequent symptomatic humoral immunodeficiency. Affected patients are prone to bacterial respiratory tract infections that often result in pulmonary damage. However, a long-term clinical follow-up of patients with CVID has led to the observation that, despite the severe hypogammaglobulinemia, some patients with CVID do not have recurrent lower respiratory tract infections, and a minority of these patients are almost asymptomatic. The aim of our study was to find the immunologic basis of this different susceptibility.

We recently demonstrated that infants and splenectomized patients, 2 groups of individuals with increased susceptibility to encapsulated bacterial infections, have a remarkable reduction of IgM memory B cells and suggested that this subset might play a crucial role in the IgM response against polysaccharide antigens. We have now investigated whether the presence and function of IgM memory B cells could explain the different susceptibility of patients with CVID to bacterial pneumonia.

We found that a severe deficiency of switched memory B cells¹⁴⁻¹⁶ was a feature of most patients with CVID, irrespective of whether they had a history of recurrent lower respiratory tract infections and lung damage (group 1) or not (group 2). In contrast, the reduction of IgM

memory B cells and the absence of anti-PnPS IgM are distinctive features of patients with CVID with recurrent lower respiratory tract infections. Thus the apparent paradox of patients with CVID with a severe hypogammaglobulinemia but no increased incidence of pneumonia might be explained by a residual B-cell function, resulting in the differentiation of IgM memory B cells and the production of detectable levels of anti-PnPS IgM. This concept is further supported by the observation that a residual capacity to produce IgM anti-polysaccharide antigens is preserved in the majority of group 2 patients. In animal models the protective effectiveness of antibodies depends on a threshold affinity and, if this is reached, on a minimal serum concentration.¹⁷ The baseline level of anti-PnPS IgM was lower than normal in group 2 patients but higher than in group 1 patients, suggesting that in human subjects a threshold antibody concentration might be necessary and sufficient to ensure protection.

Our study suggests that a complete lack of B-cell function is associated with the clinical features of group 1 patients, whereas the residual activity of IgM memory B cells explains the less severe disease of group 2.

Because IgM memory B cells are also found in patients with X-linked hyper-IgM syndrome, their presence and function does not depend on signaling through CD40–CD40 ligand.¹³ In contrast, the interaction between CD40 and its ligand might be indispensable for the development of switched memory B cells.¹³ The finding of the reduction of this pool in most patients of both group 1 and 2 excludes a relevant protective function of this subset

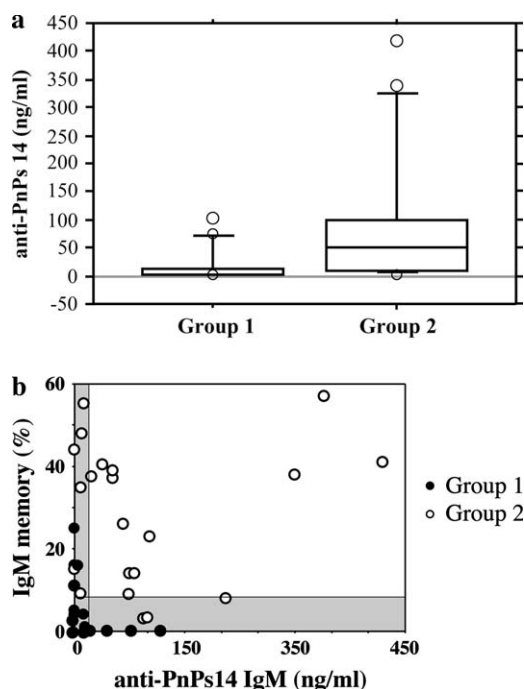


FIG 3. Serum concentrations of IgM anti-PnPS type 14 (A). Plot showing the level of IgM anti-PnPS and the frequency of IgM memory B cells in group 1 (black dot) and 2 (white dot) patients (B). Note that dots representing patients with identical values overlap.

against encapsulated bacterial infections and supports our hypothesis of the crucial role of the T cell-independent antibody response of IgM memory B cells. In agreement, several T-cell abnormalities have been reported in patients with CVID,¹⁸ but no association with increased susceptibility to bacterial pneumonia^{19,20} was demonstrated.

The observation that, despite early immunoglobulin substitution therapy, immunodeficient patients have chronic lung disease and bronchiectasis^{4,5} could be partly explained by the consideration that IVIG only replace serum IgG, corresponding to the high-affinity antibodies produced by switched memory B cells. They cannot replace the function of IgM antibodies.

In conclusion, the measurements of 2 parameters, the frequency of IgM memory cells and the level of anti-PnPS IgM antibodies, might help to identify patients with a high risk of recurrent bacterial pneumonia and eventually bronchiectasis (Fig 3, B). These patients could take advantage of a more aggressive therapeutic strategy, such as prophylaxis of infectious episodes with antibiotics and respiratory rehabilitation.

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