

Letter to the Editor

Memory B-cell subsets as a predictive marker of outcome in hypogammaglobulinemia during infancy

To the Editor:

Transient hypogammaglobulinemia of infancy (THI) is a transient immunodeficiency caused by a delay in maturation of antibody production and characterized by reduced serum IgG levels in early infancy.¹⁻³ A putative diagnosis is initially made after exclusion of other causes of hypogammaglobulinemia, whereas a definite diagnosis of THI can only be made *a posteriori* in patients with normalization of IgG.

Nowadays, there are no clinical features or diagnostic tests that differentiate self-limiting THI from permanent immune defects. The prevalence of this disorder is uncertain, although a similar prevalence to symptomatic selective IgA deficiency has been reported.⁴ The genetic basis is unknown, but an increased incidence has been documented in family members of patients with other immunodeficiencies.³ This condition probably includes a heterogeneous group of disorders of the immune response, and age plays a significant role in the diagnosis.

In the recent years, we and other authors have investigated immunoglobulin isotype switching phenotypes to subclassify patients with common variable immunodeficiency and specific antibody deficiency.⁵⁻⁷ This led us to evaluate the predictive value of memory B-cell subsets in children with hypogammaglobulinemia of unknown cause (putative THI) over time. In the context of the Immunodeficiency Strategic Committee of the Italian Association of Pediatric Haematology and Oncology and the Italian Primary Immunodeficiency Network

(http://www.aieop.org/stdoc/prot/rec_thi_en_06.pdf), we enrolled 32 patients age 12 to 36 months with suspected THI according to (1) IgG serum levels <2 SDs of age-appropriate normal references, (2) circulating B cells >2%, and (3) exclusion of known causes of hypogammaglobulinemia such as drugs, genetic disorders, chromosomal abnormalities, infectious diseases, neoplasia, and systemic disorders. The median patient age was 28 months. Informed consent was obtained from the patients' parents. The study was approved by the Institutional Review Board. At diagnosis, immunologic evaluation included enumeration of T-lymphocyte and B-lymphocyte subsets using anti-CD3, anti-CD4, anti-CD8, anti-CD19, anti-CD5, anti-CD22, anti-CD27, anti-IgM, and anti-IgD obtained from Pharmingen (San Diego, Calif) and anti-IgM Fc5 μ fragment specific antibody from Jackson Immuno-Research Laboratories (West Grove, Pa). All analyses were performed on a FACSCalibur (Becton and Dickinson, Sunnyvale, Calif) interfaced with a Macintosh CellQuest computer program (Becton and Dickinson). One hundred thousand events per sample were analyzed. Statistical analysis was performed by using the Fisher exact test, and a *P* value less than .05 was considered significant. In our cohort of putative patients with THI, percentages of CD3, CD4, CD8, mature B cells (CD22⁺CD27⁻; 69.5% $\pm$ 6.9%), transitional B cells (CD24^{bright}CD38^{bright}; 10.6% $\pm$ 6.8%), and memory B cells (CD24⁺CD27⁺; 13% $\pm$ 5.3%) were comparable with those found in healthy age-matched controls according to our previous reports.^{6,8} In the human peripheral blood, 2 populations of memory B cells have been identified: IgM memory (IgM^{bright}IgD^{dull}) and switched memory B cells

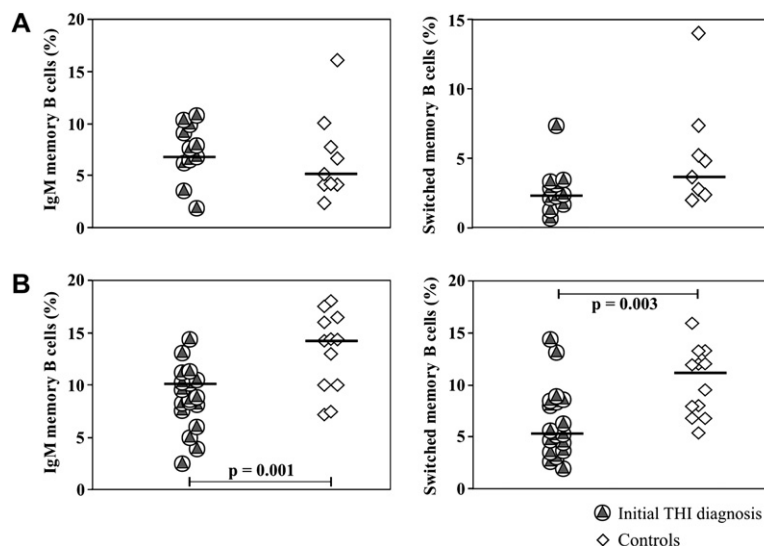


FIG 1. Analysis of memory B-cell subsets in 32 children with initial diagnosis of THI. **A**, IgM memory B cells (IgD⁺IgM⁺CD27⁺) and switched memory B cells (IgD⁺IgM⁺CD27⁺) in group 1. **B**, IgM memory B cells (IgD⁺IgM⁺CD27⁺) and switched memory B cells (IgD⁺IgM⁺CD27⁺) in group 2. Bar represents the median. Statistical analysis was performed by the Student *t* test, and *P* values are given.

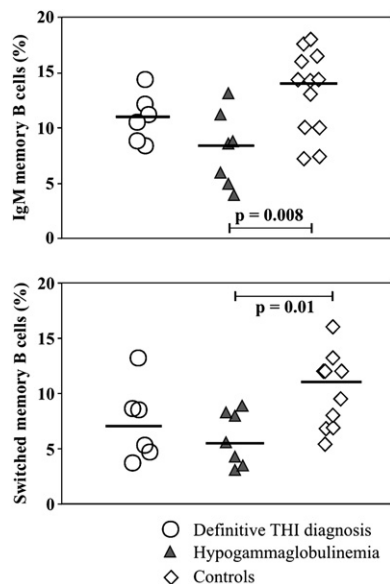


FIG 2. IgM memory B cells and switched memory B cells in 6 children with definitive THI diagnosis and in 7 children with persistent hypogammaglobulinemia after 3 years of follow-up. Bar represents the median. Statistical analysis was performed by the Student *t* test, and *P* values are given.

(IgM⁺IgD⁺); therefore, a more detailed analysis of memory B-cell subsets was performed in children with putative THI. Consistent with our observation (V. Moschese et al, manuscript in preparation, November 2006) that most children with THI normalize their IgG values within 24 months of age, the cohort was stratified by age. Group 1 consisted of 13 patients <24 months and group 2 of 19 patients >24 months. As reported in Fig 1, the frequency of IgM memory B cells and switched memory B cells in group 1 was not significantly different from that seen in age-matched controls. On the contrary, in group 2, a lower frequency of IgM memory B cells ($10.1\% \pm 3\%$) and switched memory B cells ($5.3\% \pm 3.4\%$) than age-matched controls ($14\% \pm 3.7\%$ and $11\% \pm 3.3\%$; $P < .01$ for both) was observed. To assess further the contribution of memory B-cell subsets to the hypogammaglobulinemia state, we investigated 13 patients with an initial diagnosis of THI who had been followed up over a 3-year period. Six had spontaneously normalized their IgG levels with confirmation of THI diagnosis, and 7 had persistent hypogammaglobulinemia. In patients with persistent hypogammaglobulinemia, IgM and switched memory B cells were found to be significantly reduced compared with controls (8.2% and 5% vs 14% and 11% , respectively; $P < .01$), whereas no differences were observed in patients with normalized IgG—that is, patients with a definite diagnosis of THI (Fig 2).

By definition, the diagnosis of THI can only be established with certainty in retrospect, when a child's immunoglobulin values reach age-appropriate levels. Although most children with THI spontaneously recover their IgG values and have a benign clinical course, some of them do not recover from hypogammaglobulinemia and

develop selective IgA deficiency, common variable immunodeficiency, or other dysgammaglobulinemias.^{9,10} To date, no study has allowed the distinction between the relative benign course of THI and other primary immunodeficiencies.

In this study we demonstrate for the first time that a subgroup of children with undefined hypogammaglobulinemia shares common B-cell abnormalities with patients with common variable immunodeficiency. In a limited number of patients, this is further confirmed by our observation that a decreased proportion of IgM and switched memory B-cell subsets is associated with persistent hypogammaglobulinemia.

In summary, our data suggest that, before 2 years of age, a child with a low level of serum immunoglobulins may be cautiously defined as having hypogammaglobulinemia during infancy. Only after 2 years of age, the evaluation of B cell memory subsets can be used to predict the evolution of the disease and discriminate, in the group of children with hypogammaglobulinemia during infancy, those who really were patients with THI from those who are likely to have permanent immunodeficiency later in life.

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REFERENCES

1. International Union of Immunological Societies. Primary immunodeficiency diseases: report of an IUIS Scientific Committee. *Clin Exp Immunol* 1999;118(suppl 1):S1-28.
2. Stiehm ER, Ochs HD, Winkelstein JA. Immunodeficiency disorders: general considerations. In: Stiehm ER, Ochs HD, Winkelstein JA, editors. *Immunologic disorders of infants and children*. 5th ed. London: Elsevier Saunders; 2004. p. 289-355.
3. Bonilla FA, Bernstein L, Khan DA, Ballas ZK, Chinen J, Frank MM, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *Ann Allergy Asthma Immunol* 2005;94(suppl 1):S1-63.

4. Walker AM, Kemp AS, Hill DJ, Shelton MJ. Features of transient hypogammaglobulinemia in infants screened for immunological abnormalities. *Arch Dis Child* 1994;70:183-6.
5. Warnatz K, Denz A, Drager R, Braun M, Groth C, Wolff-Vorbeck G, et al. Severe deficiency of switched memory B cells (CD27(+)IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. *Blood* 2002;99:1544-51.
6. Carsetti R, Rosado MM, Donnanno S, Guazzi V, Soresina AR, Meini A, et al. The loss of IgM memory B cells correlates with clinical disease in common variable immunodeficiency. *J Allergy Clin Immunol* 2005;115:412-7.
7. Alachkar H, Taubenheim N, Haeney MR, Durandy A, Arkwright PD. Memory switched B cell percentage and not serum immunoglobulin concentration is associated with clinical complication in children and adults with specific antibody deficiency and common variable immunodeficiency. *Clin Immunol* 2006;120:310-8.
8. Kruetzmann S, Rosado MM, Weber H, Germing U, Tournilhac O, Peter HH, et al. Human immunoglobulin M memory B cells controlling *Streptococcus pneumoniae* infections are generated in the spleen. *J Exp Med* 2003;197:939-45.
9. McGeady SJ. Transient hypogammaglobulinemia of infancy: need to reconsider name and definition. *J Pediatr* 1987;110:47-50.
10. Tiller TL Jr, Buckley RH. Transient hypogammaglobulinemia of infancy: review of the literature, clinical and immunologic features of 11 new cases, and long-term follow-up. *J Pediatr* 1978;92:347-53.

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