

### Thymic output markers indicate immune dysfunction in DiGeorge syndrome

To the Editor:

Patients with DiGeorge syndrome (DGS) have characteristic facial abnormalities, conotruncal cardiac defects, thymic hypoplasia, hypocalcemia, and immunodeficiency.<sup>1</sup> The spectrum of immunodeficiency in DGS ranges from profound T-cell deficiency (complete DGS) to normal T-cell numbers and function.<sup>2,3</sup> The current understanding is that the immunologic abnormalities occur as a result of disrupted T-cell development caused by thymic tissue hypoplasia or absence. No study to date, however, has linked the degree of thymic hypoplasia to immunologic function in DGS.

The amount of thymic tissue can be evaluated radiographically by magnetic resonance imaging or computed tomography scan. However, these methods are difficult to quantify and may fail to detect accessory or aberrantly located thymic tissue. A more effective method is the measurement of biological markers such as naive T-cell subsets, thymulin level, and T-cell receptor excision circle (TREC) count. A majority of T cells newly derived from the thymus have CD45RA and CD62L markers and are called *naive T cells*. Quantifying naive T cells and the ratio of naive to memory T cells may approximate thymic output. Thymulin is a zinc-coupled nonapeptide hormone secreted by thymic epithelial cells that is essential for thymocyte maturation. Low thymulin levels are found in patients with DGS, presumably because of thymic hypoplasia.<sup>4</sup> TREC counts represent a nonduplicating population of episomal DNA fragments that form as T cells' variable (diversity) joining [V(D)J] recombination events excise intervening stretches of DNA. TREC counts reflect the number of recent thymic emigrants.<sup>5</sup> We hypothesized that naive T cells, thymulin, and TREC counts are useful clinical makers that correlate with cell-mediated and humoral immune function. To test this hypothesis, we evaluated selected thymic markers and immune functions in patients with DGS.

In this cross-sectional study, 39 patients age 6 months to 18 years with a diagnosis of definite or probable DGS were recruited. DGS was defined by clinical phenotype and/or fluorescent in situ hybridization-confirmed microdeletion of chromosome 22q11.2 or 10p13-14.

Thymic markers and cell-mediated and humoral immune functions were performed at the time of recruitment. Extended flow cytometry was performed according to established methods for the following: CD3, CD4, CD8, CD56, CD19, CD45RA, and CD45RO. Rosette inhibition assay of thymulin hormone was performed using Dardenne and Bach's method. Lymphocyte proliferation assays to mitogens (phytohemagglutinin, concanavalin A, and pokeweed) and to antigens (tetanus toxoid, *Staphylococcus* protein A, and *Candida albicans*) were performed on children older than 9 months. The concentrations of mitogens and antigens were as follows: 3

separate dilutions of phytohemagglutinin and concanavalin A at final concentrations of 5, 2.5, and 1.25  $\mu\text{g/mL}$  and 50, 25, and 12.5  $\mu\text{g/mL}$ , respectively; pokeweed, 50  $\mu\text{g/mL}$ ; tetanus toxoid, 0.5, 0.3, and 0.1 IU/mL; *Staphylococcus* protein A (SpA), 3.125, 0.31, and 0.03 mg/mL; and *Candida albicans*, 50, 10, and 2  $\mu\text{g/mL}$ . Lymphocyte transformation to antigens (LTA) and lymphocyte transformation to mitogens (LTM) stimulation indices (SIs) were calculated as a ratio of all mitogens or antigens to background. Mean SIs of greater than 3 for LTA and greater than 10 for LTM were considered normal. TREC counts were measured by real-time PCR and reported as number of copies per  $10^6$  PBMCs. Quantitative immunoglobulin levels (IgG, IgA, IgM) were measured in children older than 1 year by nephelometry using age-appropriate normal references. Antidiphtheria antibodies were measured after subjects received at least 2 vaccine doses, and a normal response was considered 0.15 IU/mL or greater. All immunological tests, which were previously standardized, were performed at the All Children's Hospital Immunoparameters Laboratory. Statistical analysis was performed using SPSS version 13 (SPSS, Chicago, Ill) for descriptive statistics and 2-tailed Pearson correlations with  $P < .05$  as significant.

Of 39 patients studied, 30 patients had chromosome 22q11.2 microdeletion, and 1 had 10p13-14 microdeletion. The remaining 8 patients were diagnosed clinically by the presence of 3 of the 4 following characteristics: characteristic facial abnormalities, conotruncal heart defects, hypocalcemia, or decreased T-cell counts. The majority of patients were white (74.7%) and female (56.4%) and had either aortic (38.5%) or pulmonic (43.6%) dominant cardiac lesions with at least 1 episode of hypocalcemia (71.8%). Median patient age was 3.0 years.

TREC counts correlated to mean LTA SI (Fig 1, A;  $r = 0.48$ ;  $P = .01$ ). When TREC counts were analyzed for correlation to SI of an individual antigen, low TREC counts correlated with poor SI for *Candida* ( $P = .018$ ) and staphylococcal ( $P = .036$ ) but not tetanus antigens ( $P = .107$ ). TREC did not correlate with either mean LTM SI ( $P = .10$ ) or antidiphtheria antibodies ( $P = .26$ ). Thymulin levels correlated with diphtheria antibodies (Fig 1, B; Table I;  $P = .03$ ;  $r = 0.42$ ). CD4 naive cell count correlated to antidiphtheria antibodies (Table I;  $P = .04$ ). CD4 as well as CD8 naive to memory cell ratio correlated with antidiphtheria antibodies (Fig 1, C and D;  $P = .005$ ,  $r = 0.507$ ; and  $P = .03$ ,  $r = 0.41$ , respectively). Thymulin, TREC count, mean LTA SI, and mean LTM SI did not correlate with patient age. However, naive to memory CD4 and CD8 ratios negatively correlated with age (data not shown).

Subgroup analysis for other outcome measurements was performed between subjects with and without chromosome 22q11 microdeletion; between subjects with and without hypocalcemia; and among subjects with various

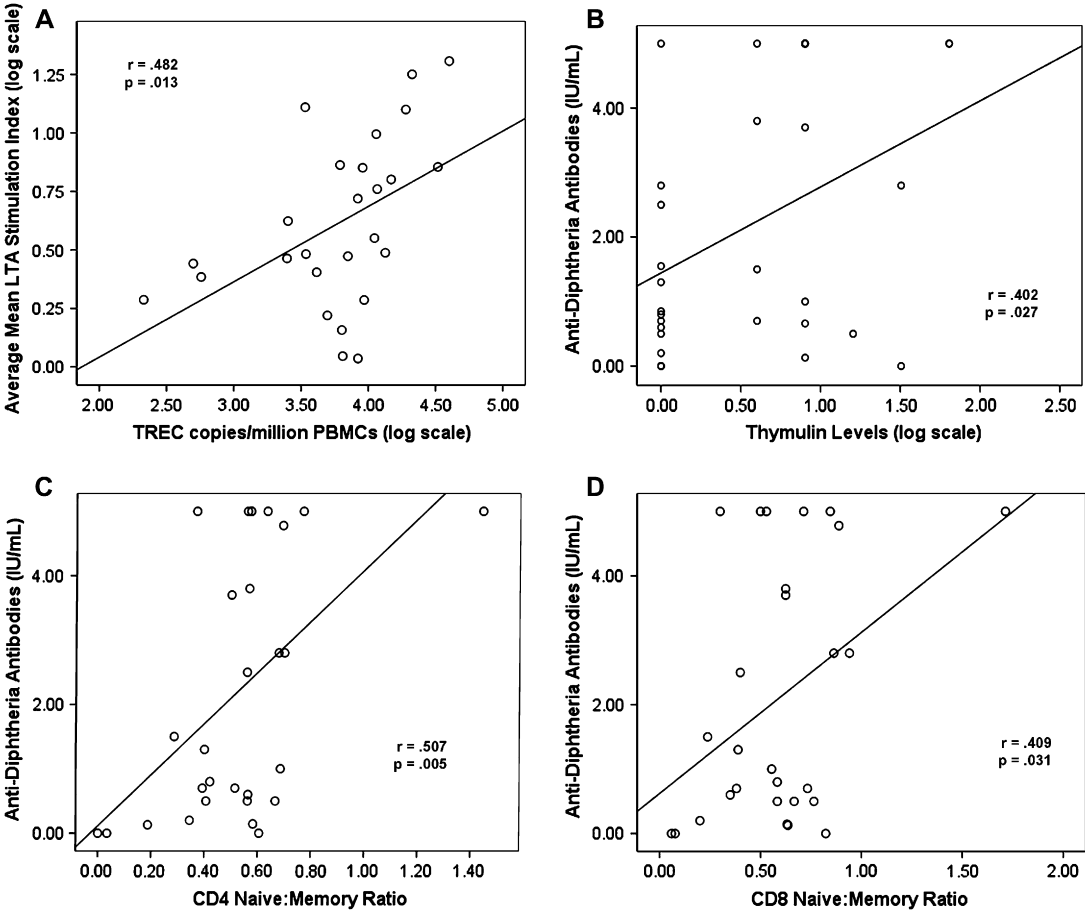


**FIG 1.** Correlation between thymic output markers and immunologic function. Pearson correlations are shown for TREC and mean LTA SI (A), thymulin and antidiphtheria antibodies (B), CD4 naive to memory T-cell ratio and antidiphtheria antibodies (C), and CD8 naive to memory T-cell ratio and antidiphtheria antibodies (D).

**TABLE I.** Correlation between thymic output and cell-mediated and humoral immune function markers

|                        | Mean ± SD       | Cell-mediated functions* |             | Humoral function* |
|------------------------|-----------------|--------------------------|-------------|-------------------|
|                        |                 | LTA                      | LTM         | Diphtheria Ab     |
| TREC count             | 9923.1 ± 9445.6 | 0.01**                   | 0.10        | 0.26              |
| Thymulin level         | 15.8 ± 32.1     | 0.87                     | 0.45        | 0.03**            |
| CD4 %                  | 31.4 ± 8.0      | 0.75                     | 0.46        | 0.11              |
| CD4 count              | 902.8 ± 400.6   | 0.35                     | 0.61        | 0.33              |
| CD4 naive %            | 15.6 ± 9.8      | 0.72                     | 0.56        | 0.06              |
| CD4 naive count        | 479.9 ± 333.5   | 0.14                     | 0.49        | 0.04**            |
| CD4 naive:memory ratio | 0.5 ± 0.3       | 0.75                     | 0.94        | 0.00**            |
| CD8 naive %            | 7.6 ± 4.1       | 0.22                     | 0.45        | 0.63              |
| CD8 naive count        | 231.2 ± 183.6   | 0.14                     | 0.10        | 0.36              |
| CD8 naive:memory ratio | 0.6 ± 0.3       | 0.94                     | 0.96        | 0.03**            |
| Mean ± SD              |                 | 5.8 ± 4.6                | 78.5 ± 54.9 | 2.2 ± 2.0         |

\*P value calculated using 2-tailed Pearson correlation.

\*\*Statistically significant (P < .05).

cardiac defects. No significant difference on TREC, thymulin, or mean LTA SI was observed among these subgroups. None of the 39 patients had significant immunoglobulin deficiency or abnormal antibody response to

diphtheria or tetanus vaccination. No correlation was found between any thymic output marker and antitetanus antibodies. TREC and thymulin did not correlate significantly.

In this study, TREC counts to mean LTA SI correlation was the strongest among the thymic markers analyzed, exceeding that of CD4 or CD8 absolute, naive, and memory T-cell numbers. All patients with reduced TREC counts (<10,000 copies per 10<sup>6</sup> PBMCs) also had a low mean LTA SI. This contrasts with the 40% of patients who had normal TREC counts and low mean LTA SI. Therefore, TREC counts may offer an alternative, earlier, rapid, and cost-effective approach to assessing T-cell function in patients with DGS.

Rapid peripheral T-cell expansion and maturation may play a role in the lack of correlation between CD4 and CD8 naive or memory cell populations and mean LTA SI. We found significant correlation between ratio of naive and memory CD4 and antidiphtheria antibodies. However, this may be a result of the fact that both factors decreased naturally with age. Our data confirm previous studies that patients with DGS have normal antibody response to diphtheria vaccination. It may be important to consider repeat vaccination against diphtheria in those patients with DGS who do not respond to routine vaccination.

Low thymulin levels in these patients with DGS confirm observations in previous studies.<sup>6</sup> It is provocative that thymulin levels did correlate, independent of age, with antidiphtheria antibodies, indicative of the relationship between thymic mass and humoral immune function. We found no correlation between thymic markers and mean LTM SI. This may be a result of the fact that none of our patients had abnormal mean LTM SI. It is possible that LTM may not be a sensitive enough marker of thymic function to be clinically useful in predicting cellular or humoral immune function. This is consistent with the finding that patients with DGS typically normalize LTM responses by 3 years of age.<sup>7,8</sup>

In summary, we present clear evidence that patients with DGS and abnormal TREC levels have thymic hypoplasia leading to abnormal cellular immune function as determined by LTA. These data support this approach and warrant future longitudinal studies to investigate TREC counts' function in clinical decision-making such as the discontinuation of prophylactic antibiotics and timing of live viral vaccine administration.

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## REFERENCES

- Perez E, Sullivan EK. Chromosome 22q11.2 deletion syndrome (DiGeorge and velocardiofacial syndromes). *Curr Opin Pediatr* 2002;14:678-83.
- Markert ML, Hummel DS, Rosenblatt HM, Schiff SE, Harville TO, Williams LW, et al. Complete DiGeorge syndrome: persistence of profound immunodeficiency. *J Pediatr* 1998;132:15-21.
- Chinen J, Shearer WT. Basic and clinical immunology. *J Allergy Clin Immunol* 2005;116:411-8.
- Iwata T, Incefy GS, Cunningham-Rundles C, Smithwick E, Geller N, et al. Circulating thymic hormone activity in patients with primary and secondary immunodeficiency diseases. *Am J Med* 1981;71:385-94.
- Kong F, Chen CH, Cooper MD. Thymic function can be accurately monitored by level of recent T cell emigrants in the circulation. *Immunity* 1998;8:97-104.
- Kornfeld SJ, Zeffren B, Christodoulou CS, Day NK, Cawkwell G, Good RA. DiGeorge anomaly: a comparative study of the clinical and immunologic characteristics of patients positive and negative by fluorescence in situ hybridization. *J Allergy Clin Immunol* 2000;105:983-7.
- Chinen J, Rosenblatt HM, Smith EO, Shearer WT, Noroski LM. Long-term assessment of T-cell populations in DiGeorge syndrome. *J Allergy Clin Immunol* 2003;111:573-9.
- Barrett DJ, Ammann AJ, Wara DW, Cowan MJ, Fisher TJ, Stiehm ER. Clinical and immunological spectrum of DiGeorge Syndrome. *J Clin Lab Immunol* 1981;6:1-6.

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## Clinical reactivity to hazelnut in children: Association with sensitization to birch pollen or nuts?

To the Editor:

In areas where birch trees are endemic, hazelnut allergy is often the result of primary sensitization to cross-reactive birch pollen allergen Bet v 1, usually resulting in mild oral allergy syndrome (OAS) in both adults and children.<sup>1,2</sup> On the other hand, sensitization to hazelnut in early childhood has been related to sensitization to other tree nuts and peanut,<sup>3,4</sup> which can all cause serious reactions.<sup>5</sup> Children can be sensitized to hazelnut at an early age.<sup>3</sup> Remarkably, a large proportion has never to their knowledge ingested hazelnut, and most allergic reactions to nuts in childhood occur after the first known exposure.<sup>6</sup>

This is the first study to determine the clinical relevance of hazelnut sensitization and eliciting doses (EDs) in childhood by double-blind placebo-controlled food challenge (DBPCFC). Furthermore, we investigated the relationship between hazelnut allergy and sensitization to birch pollen and other species of nut and peanut.

Twenty-eight sensitized children (8 female, 20 male; age 4-16 years) with suspected allergy to hazelnut were recruited. All children were atopic, with atopic dermatitis the most frequently reported condition (86%). Sixteen children (57%) had seasonal rhinoconjunctivitis. Asthma-related symptoms were reported by 13 children (47%).

Sensitization was re-evaluated before DBPCFC by a skin prick test (SPT) with commercial hazelnut extract (ALK-Abelló, Nieuwegein, The Netherlands) and specific IgE levels using the CAP system FEIA (Pharmacia Diagnostics, Uppsala, Sweden) to hazelnut, birch pollen,