

Lymphocyte Stimulation Test for the Diagnosis of Non-IgE-Mediated Cow's Milk Allergy: A Step Closer to a Noninvasive Diagnostic Tool?

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As the incidence of milk allergy appears to be increasing, intestinal symptoms associated with cow's milk in neonates and infants, mostly in a non-IgE-mediated manner, are also more frequently encountered [1]. The diagnosis of cow's milk allergy (CMA) in these cases is less straightforward due to the delayed nature, mildness, and nonspecificity of the symptoms. Vomiting, regurgitation, irritability, feeding difficulties, bloody stools, persistent crying, or colic can be part of nonallergic disorders but can also be attributed to an immunological reaction to cow's milk proteins [2]. Therefore, a major problem and clinical challenge in the management of such reactions is the lack of a validated, reliable diagnostic test. To date, the main diagnostic procedure is an exclusion diet followed by rechallenge. Nevertheless, hydrolyzed milk formulae that substitute cow's milk in an exclusion diet may also improve some of the above mentioned symptoms independently of immunological mechanisms. Frequently, rechallenge with cow's milk after remission is not performed, resulting in an overestimation of milk allergy diagnosis followed by breast-feeding discouragement and extensive (and expensive) exclusion diets [3, 4].

The assessment of cellular immune responses to cow's milk proteins would certainly improve the focus of etiologic treatment of intestinal symptoms in the very young.

In this issue, Kimura et al. [5] propose that a lymphocyte stimulation test (LST) for κ -casein can be a useful diagnostic test for intestinal CMA in infants.

LSTs, also termed lymphocyte proliferation assays or lymphocyte transformation tests, measure the ability of lymphocytes to undergo clonal proliferation when stimulated *in vitro* by a foreign antigen. The consensus method usually involves isolating peripheral blood mononuclear cells, placing them in tissue culture with and without various stimuli, and measuring cell proliferation 5–7 days later [6–8]. The result, which is a function of the number of lymphocytes that are stimulated by a given antigen to produce a proliferative response, can be interpreted as the number of dividing cells and/or as the percentage of stimulated cells (stimulation index).

Although LSTs have been widely used for decades in the research and diagnosis of several immunologically mediated diseases (immunodeficiencies, cancer, aging, malnutrition, substance abuse, stress, surgery, shock, infections, and autoimmune diseases), there is conflicting evidence regarding the diagnostic value of this assay in the field of allergy and more specifically CMA [9, 10]. The controversial results of these studies may be due to variable protocols, variability in clinical presentation, and incomplete understanding of the pathogenic mechanisms

involved in CMA. As a result, each study is valid under its own experimental design but is not directly comparable to the rest.

In the study by Kimura et al. [10], patients presenting with intestinal symptoms were grouped after challenge and IgE measurement into a CMA IgE(–) group, a CMA IgE(+) group, and a nonspecific intestinal symptoms group. Controls with no symptoms were included as well. Sixty-two of 72 (86.1%) patients with non-IgE-mediated CMA showed positive LSTs compared with only 20% among the nonspecific-symptoms group. The observed difference is strong enough to suggest that such a test may prove diagnostically useful in this patient group. A negative LST would be a strong argument to convince parents to reintroduce cow's milk in an infant's diet sooner rather than later.

It will of course be necessary to reevaluate this approach in larger and different populations. Selection bias from inclusion, geography, and other factors may influ-

ence the results. Nevertheless, the study by Kimura et al. [10] provides strong arguments, reigniting the interest in and discussion on LSTs and pointing out a specific population, selected through clinical presentation, that this method may be useful in.

An interesting point of the procedure was that the optimal stimulating antigen in this context was κ -casein. This antigen may be preferentially recognized by T-cells in children with non-IgE CMA. However, the test could not discriminate between IgE and non-IgE cases.

More studies will be required to position LST within the diagnostic armamentarium of the allergy clinic. Points of interest would be the comparison of LST with atopy patch tests or any additive prognostic value of LST to atopy patch tests in non-IgE CMA. Higher cutoff values might increase the specificity of the results. Finally, changes in LST might be associated with CMA remission and should be investigated in relation to the appropriate time to reintroduce cow's milk into the patient's diet.

References

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