COMMENT ON MULUKUTLA ET AL.

Autoantibodies to the IA-2 Extracellular Domain Refine the Definition of "A+" Subtypes of Ketosis-Prone Diabetes. Diabetes Care 2018;41:2637–2640

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I read with great interest the article by Mulukutla et al. (1) demonstrating that antibodies against the luminal (extracellular) domain of phosphatase IA-2, termed IA-2EC (amino acids 26–577), are expressed by patients with ketosis-prone diabetes. Among patients that were negative for other αIA-2 autoantibodies, the αIA-2EC antibodies were reported in 2% of patients with ketosis-prone diabetes without the presence of autoantibodies (1), 6% (2) or 7% (3) of patients with type 1 diabetes, and 5% of patients diagnosed with type 2 diabetes (3). Despite the fact that the mRNA signal of IA-2 is ubiquitous and protein expression is high in various neuroendocrine cells, the data on αIA-2EC autoantibodies from other neuroendocrine disorders are lacking.

Some patients who reacted with the IA-2EC polypeptide did not react with the full-length (IA-2FL) polypeptide (1) or with its truncated variant IA-2BDC, which lacks the transmembrane domain (Δ557–629) and also lacks 255 amino acids of the N-terminus (2,3). Such observation is surprising and points attention toward the engineering of previously used αIA-2 assays. Besides possible issues with protein folding, cleavage of the antigens might play a role. Molecular research on IA-2 and its homolog phogrin (IA-2β) revealed that it contains a short signal peptide followed by a heavily N-glycosylated ectodomain with a furin-like convertase site and other putative cleavage sites. IA-2 and IA-2β are cleaved to a range of polypeptides, with a half-time of N-terminal fragment cleavage reported to be only 45 min. The cleavage is not restricted to the β-cells but was observed in transfected cells of other origins, including the fibroblasts (4,5).

Despite extensive posttranslational processing of IA-2, the current (1) and previously used diagnostic assays did not reflect the frequently occurring IA-2 cleavage and did not visualize the antigens at the protein level. Only two studies (2,3) provided at least illustrative figures concerning the immunoprecipitation experiments, in which they used the combination of patient sera and antigens that were used for the diagnostic assays. Even these illustrative figures show that the constructs used were cleaved as the sera reacted with both the full-length and cleaved products (although the existence of such products was not discussed). The recent article by Mulukutla et al. (1) suffers from the same issues as it completely adopted the methodology that was used in the above-mentioned studies (2,3).

In conclusion, the IA-2FL construct needs to be engineered in a way that gets rid of its transmembrane part but, in contrast to IA-2BDC, retains the full-length N-terminal part. The cleavage of IA-2FL, IA-2BDC, and similar antigens needs to be tightly controlled, and the integrity of antigens that were bound to the diagnostic plates needs to be reanalyzed. Without having trusted data on the integrity and folding of IA-2FL, IA-2BDC, and similar antigens, the argument for routine testing for αIA-2EC antibodies (1) lacks sufficient evidence-based support, as IA-2EC is unlikely to be superior to properly engineered uncleaved IA-2Δ557–629.

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