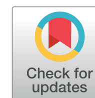

RESPONSE TO COMMENT ON MULULUKUTLA ET AL.

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

Surya N. Mulukutla,[1] Maria Acevedo-Calado,[1] Christiane S. Hampe,[2] Massimo Pietropaolo,[1] and Ashok Balasubramanyam[1]

Diabetes Care 2019;42:e82–e83 | https://doi.org/10.2337/dci19-0007

We thank Dr. Heneberg (1) for the comments regarding our article (2) describing autoantibody responses to the IA-2 extracellular domain in ketosis-prone diabetes (KPD).

We agree that the intracellular domain of the neuroendocrine autoantigen IA-2 (amino acid residues 605–979) represents the immunodominant region of the molecule. In fact, both autoantibody and autoreactive T-cell responses from patients with type 1 diabetes recognize peptides localized within the intracellular domain of IA-2 (IA-2IC) (3). Nonetheless, we identified autoantibody and T-cell responses specifically directed to the IA-2 extracellular domain (IA-2EC) in individuals with or without antibodies against IA-2IC (4). Our recent studies demonstrate that IA-2EC is a target of humoral immune responses in a subgroup of patients with KPD (2). To demonstrate specificity of IA-2EC autoantibodies, we performed competitive autoantibody binding studies according to previously published protocols (4). Preincubation of serum samples of some patients with type 1 diabetes positive for IA-2EC autoantibodies with unlabeled IA-2IC did not inhibit [^{35}S] methionine-labeled IA-2EC binding, whereas complete inhibition of IA-2EC binding occurred following preincubation with either unlabeled IA-2EC or IA-2FL containing epitopes localized in the IA-2 extracellular domain. This competitive binding experiment confirms that there is indeed a specific antibody response directed toward IA-2EC epitopes in patients with autoimmune diabetes.

We have considered Dr. Heneberg's suggestion to remove the transmembrane domain from the full-length IA-2 molecule. It is conceivable that the transmembrane domain may affect folding of the IA-2FL protein in our fluid phase assay, and this may explain the discrepancy in autoantibody binding to the IA-2EC protein compared with the IA-2FL protein (2). Of note, the IA-2FL antibody radiobinding assay used in the current study achieved excellent ratings in the 2016 Islet Autoantibody Standardization Program (IASP), exhibiting 62% sensitivity and 99% specificity.

We acknowledge that IA-2 and its homolog phogrin (IA-2β) are cleaved in neuroendocrine cells and generate a range of polypeptides. In pancreatic β-cells, the cytoplasmic domain of IA-2 is cleaved, traffics to the nucleus, and stimulates transcription of the insulin gene. As a general rule, protein antigens are internalized and processed in antigen-presenting cells (APCs) before presentation of their peptides to CD4^{+} or CD8^{+} T cells that elicit specific T-cell and autoantibody responses (3,5). Proteins that are present in the cytosol of APCs are degraded by proteasomes to yield peptides that are presented on class I MHC molecules, whereas proteins that are internalized from the extracellular environment and sequestered in vesicles are degraded in lysosomes (or late endosomes) to generate peptides that are presented on class II MHC molecules. Hence, the site of proteolysis in the APCs is the key determinant of which molecules the generated peptides will bind to, i.e., to MHC class I or class II. Convincing evidence demonstrates that close interactions between β-cells and APCs in the islet microenvironment initiate the autoimmune process in type 1 diabetes. Even under noninflammatory conditions, pancreatic β-cells transfer insulin-containing vesicles to the APCs, and insulin peptides are then processed and presented to T cells in this microenvironment (6). Thus, it is unlikely that the protein cleavage of IA-2 in neuroendocrine cells affects the types of autoimmune responses against IA-2 peptides.

Funding. This work was supported by the Robert and Janice McNair Foundation, JDRF grant 17-2012-688 (to M.P.), and National Institutes of Health grants R01-DK101411 (to A.B.) and R01-DK53456 (to M.P.).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

References

1. Heneberg P. 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 (Letter). Diabetes Care 2019;42:e81. DOI: 10.2337/dc19-0022

[1]*Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Baylor College of Medicine, Houston, TX*
[2]*Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, University of Washington, Seattle, WA*

Corresponding authors: Massimo Pietropaolo, massimo.pietropaolo@bcm.edu, and Ashok Balasubramanyam, ashokb@bcm.edu

© 2019 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at http://www.diabetesjournals.org/content/license.

2. Mulukutla SN, Acevedo-Calado M, Hampe CS, Pietropaolo M, Balasubramanyam A. Autoantibodies to the IA-2 extracellular domain refine the definition of "A+" subtypes of ketosis-prone diabetes. Diabetes Care 2018;41:2637–2640
3. Peakman M, Stevens EJ, Lohmann T, et al. Naturally processed and presented epitopes of the islet cell autoantigen IA-2 eluted from HLA-DR4. J Clin Invest 1999;104:1449–1457
4. Acevedo-Calado M, James EA, Morran MP, et al. Identification of unique antigenic determinants in the amino terminus of IA-2 (ICA512) in childhood and adult autoimmune diabetes: new biomarker development. Diabetes Care 2017;40:561–568
5. Abbas AKLA, Pillai S. *Cellular and Molecular Immunobiology*. Philadelphia, PA, Elsevier Inc., 2018, p. 117–144
6. Unanue ER, Ferris ST, Carrero JA. The role of islet antigen presenting cells and the presentation of insulin in the initiation of autoimmune diabetes in the NOD mouse. Immunol Rev 2016;272:183–201