

Response to Comment on: Korpos et al. The Peri-islet Basement Membrane, a Barrier to Infiltrating Leukocytes in Type 1 Diabetes in Mouse and Human. *Diabetes* 2013;62:531–542

Éva Korpos and Lydia Sorokin

We are responding to the letter by Simeonovic and Parish (1). All previous studies on the extracellular matrix (ECM) of the pancreatic islet were acknowledged (2). The claim that “limited information exists on the nature of the ECM of the pancreas and, in particular, on the composition of peri-islet capsule” is based on the fact that the entire ECM of the peri-islet capsule was studied, which includes not only the peri-islet basement membrane (BM), but also the subjacent interstitial matrix. Twenty-two ECM molecules (and five ECM receptors) were investigated. Although some of these ECM molecules had been studied previously, none had been studied in relation to each other both in healthy and diabetic pancreata—this is our interpretation of a comprehensive study. Further, as taken from the review of our article, “distinction between islets with a continuous versus a discontinuous BM seems difficult to ascertain objectively. Oblique sections of the BM or contrast enhancement of images may result in focal areas of low intensity staining that may be interpreted as ‘gaps’....” It is therefore impossible to define whether the BM/ECM layers are intact by staining of individual thin sections, as performed in all studies concerning ECM of the pancreas; rather, serial sectioning is required or analyses of whole mount stained samples, as performed in Korpos et al. (2).

Although Irving-Rodgers et al. (3) studied individual sections of prediabetic and diabetic pancreata, they did not provide an assessment of the inflammatory status of the organ (ratio of healthy islets, peri-insulinitis, and invaded islets) and therefore do not provide direct and quantitative evidence for a correlation between the integrity of the peri-islet BM and disease progression. Korpos et al. performed stereological analyses of pancreata from 5-, 14- (normoglycemic), and 21-week-old (diabetic) NOD mice. Organs were sectioned completely, and every 10th section was immunofluorescently stained with pan-laminin antibody to label BMs, CD45 to assess leukocyte infiltration, and insulin. Three hundred to four hundred islets were analyzed per mouse. Only in this way was it possible to definitively demonstrate loss of peri-islet ECM at sites of leukocyte infiltration (Fig. 2A, B, and D and Supplementary Video 2

of ref. 2). Additionally, the integrity of the peri-islet BM was quantified by measuring the mean fluorescence intensity of the pan-laminin immunosignal surrounding healthy versus invaded islets (Fig. 2C and Supplementary Figure 1 of ref. 2).

Ziolkowski et al. (4) identified heparanases at sites of mononuclear cell infiltration, as defined solely by hematoxylin-eosin staining and showed reduced incidence of diabetes in mice treated with heparanase inhibitor; however, no correlation was made to specific immune cell types nor to loss of BM components (Fig. 4B and C of ref. 4). Korpos et al. used immune cell markers to determine their localization in relation to the peri-islet ECM, and showed that macrophages colocalize with cathepsins, identified to be upregulated only in infiltrated islets by laser capture microdissection and subsequent gene-chip analysis, at sites of loss of ECM.

References to published data on the human pancreas appear on pages 536 and 537 of our article (2). As for NOD samples, BM and interstitial matrix ECM components were analyzed in relation to each other and to immune cell infiltrates, which had not been previously studied in human samples. We analyzed rare pancreas samples from early diagnosed diabetic patients, which permitted the unique opportunity to show a similar loss of several ECM components at sites of CD45⁺ infiltrating leukocytes into the islets as observed in NOD samples, demonstrating the relevance of our data for the human disease, something that others working on the ECM of the pancreas have not done.

ACKNOWLEDGMENTS

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

REFERENCES

1. Simeonovic CJ, Parish CR. Comment on: Korpos et al. The peri-islet basement membrane, a barrier to infiltrating leukocytes in type 1 diabetes in mouse and human. *Diabetes* 2013;62:531–542 (Letter). *Diabetes* 2013;62:e13. DOI: 10.2337/db13-0470
2. Korpos É, Kadri N, Kappelhoff R, et al. The peri-islet basement membrane, a barrier to infiltrating leukocytes in type 1 diabetes in mouse and human. *Diabetes* 2013;62:531–542
3. Irving-Rodgers HF, Ziolkowski AF, Parish CR, et al. Molecular composition of the peri-islet basement membrane in NOD mice: a barrier against destructive insulinitis. *Diabetologia* 2008;51:1680–1688
4. Ziolkowski AF, Popp SK, Freeman C, Parish CR, Simeonovic CJ. Heparan sulfate and heparanase play key roles in mouse β cell survival and autoimmune diabetes. *J Clin Invest* 2012;122:132–141

From the Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, Münster, Germany.

Corresponding author: Éva Korpos, korpos@uni-muenster.de.

DOI: 10.2337/db13-0543

© 2013 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. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.