From Eye to Insight

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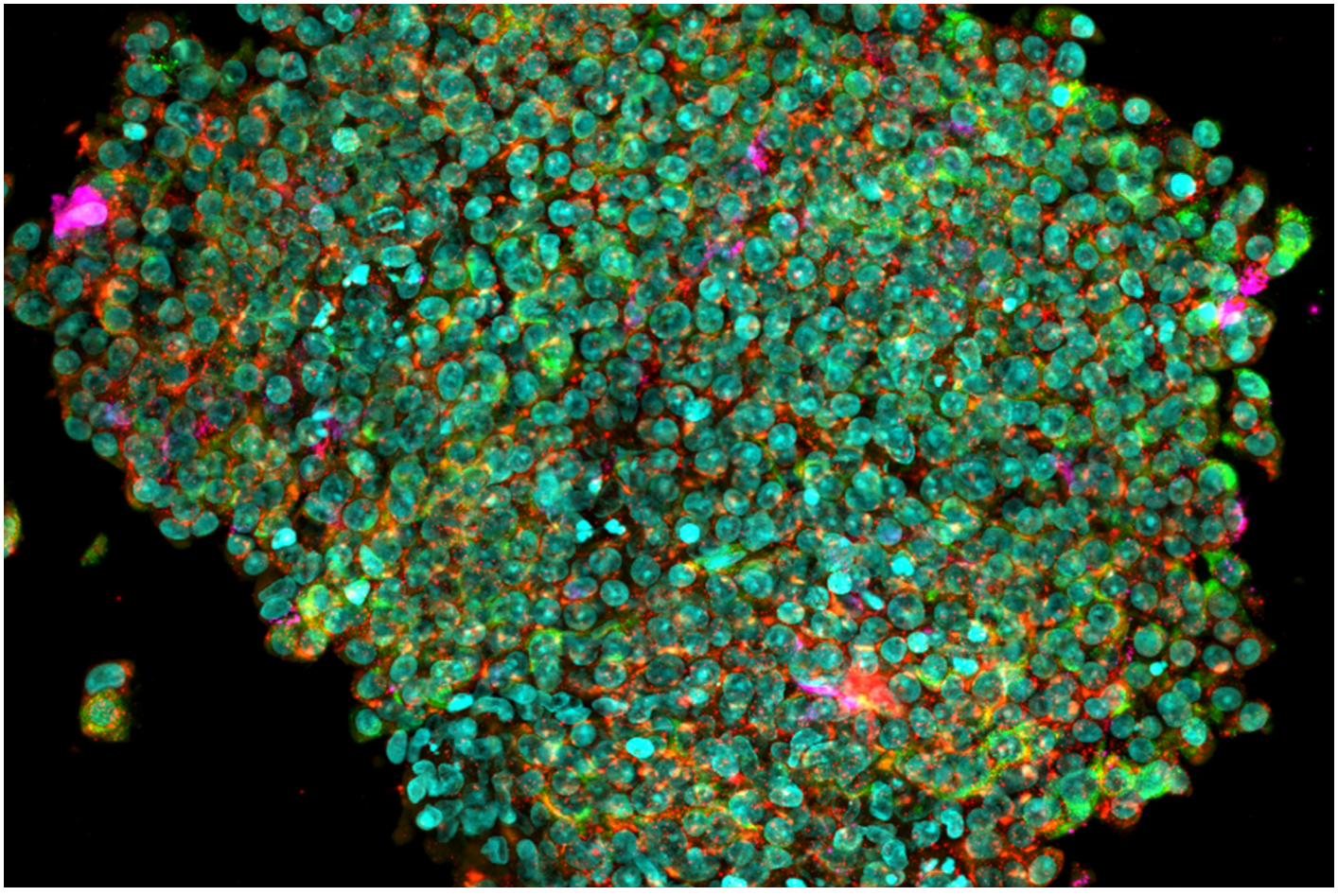
UNDERSTANDING BETTER THE ONSET OF DIABETES

Examining the expression of Interleukin-17 (IL-17) proinflammatory cytokine proteins in human pancreatic islet tissue



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Abstract

The investigation of an Interleukin-17 (IL-17) proinflammatory cytokine protein in human pancreatic islet specimens via high-contrast, rapid imaging is discussed in this article. Achieving a better understanding of the pathogenesis of diabetes, i.e., how it starts, is key for the development of new therapies. Insulin-producing pancreatic beta cells are attacked by the immune system in sufferers of type 1 diabetes (T1D), an autoimmune disease. Knowing more about cytokines in the pancreas of a T1D patient can contribute to the development of better therapies and treatments.

Investigating pancreatic tissue and diabetes

A better understanding of how type 1 diabetes (T1D) starts is the first step toward potentially developing new and improved therapies capable of preventing or permanently reversing T1D [1]. Due to the inaccessibility to human pancreatic tissue, our knowledge of the disease in humans is limited. The network for Pancreatic Organ donors with Diabetes (nPOD) was established with the idea of providing valuable tissues from healthy and diabetic donors to answer basic questions about T1D pathogenesis [2]. One interest of scientists in this field is to identify cytokine proteins, such as those of the Interleukin-17 (IL-17) family of proinflammatory cytokines [3,4], in pancreatic tissue specimens of T1D cases. Because T1D is an autoimmune disease, where insulin-producing pancreatic beta cells are attacked by the immune system, understanding the cytokine milieu in the pancreas of T1D patients will help lead to more insights about the T1D pathogenesis and contribute towards improved therapies.

Challenges when imaging islets

Imaging of these pancreatic islets for this type of research is typically done using confocal microscopy to characterize their cytokine expression. It is extremely time consuming as they are commonly imaged using a 63x objective and extensive z-stacks. In the past, widefield microscopy would be insufficient to image these islets due to the inherent haze associated with widefield imaging, especially when dealing with thicker specimens [5,6].

Methods

Human islets were obtained from a non-diabetic cadaveric donor through IIDP (Integrated Islet Distribution Program) [7]. Isolated human pancreatic islet tissue was used for this study to experimentally examine the expression of an IL-17 cytokine protein. The tissue was labelled with the following markers: AF488 (green) indicates insulin, AF555 (red) glucagon, AF647 (magenta) IL17, and Hoechst (blue) nuclei. The pancreatic tissue was imaged with a THUNDER Imager 3D Assay using a 63x,1.4 NA (numerical aperture) oil-immersion objective. Instant Computational Clearing (ICC) was also applied [5,6]. Images were acquired at the speed of widefield microscopy. Extended depth of field (EDoF) projections were reconstructed from a 92-step, 18-µm Z stack after ICC. Tile scans of an entire human pancreatic islet were also obtained.

Results

THUNDER images with highly enhanced contrast and resolution are seen in figure 1 below. ICC removed the out-of-focus blur or haze seen in the raw image [5,6]. Insulin producing cells (green) are easily resolved and the other markers are readily visualized.

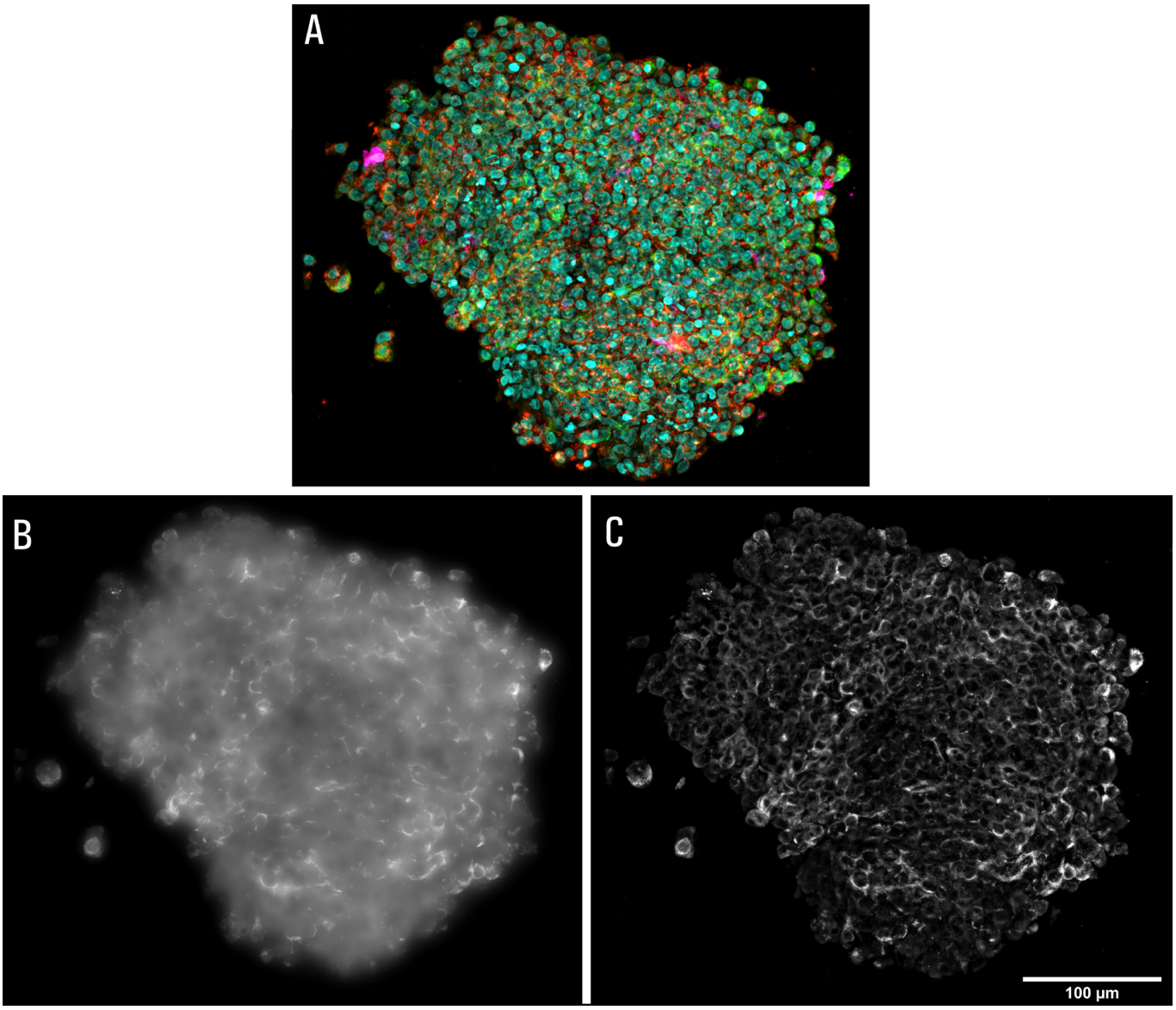

Figure 1. EDoF reconstruction images of a whole human pancreas islet: A) EDoF reconstruction showing all fluorescence signal channels (green = insulin, red = glucagon, magenta = IL17, and blue = nuclei), B) raw widefield image showing just the insulin channel, and C) THUNDER image after ICC showing the insulin channel. Image data courtesy of the Matthias von Herrath Lab, La Jolla Institute of Immunology, California, USA.

Conclusion

The fluorescence signals of IL-17 cytokine proteins, as well as insulin and glucagon, in human islet tissue were more clearly revealed in images attained with a THUNDER Imager 3D Assay and Instant Computational Clearing (ICC) compared to conventional widefield microscope images.

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