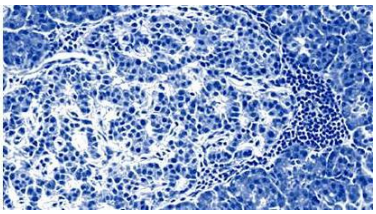
nPOD
Network for Pancreatic Organ Donors with Diabetes



Development of an Insulin-specific B Cell ELISPOT assay for clinical evaluation of the insulin-specific B cell deletional therapeutic, AKS-107

AUTHORS

David Alleva, Thomas Lancaster, Sylaja Murikipudi, Thillainayagam Sathiyaseelan, Andrea Delpero, Ramya Ragupathy, and Todd Zion. Akston Biosciences Corporation, Beverly, MA 01915

PURPOSE

Insulin-specific B cells (IBCs) are associated with autoimmune diabetes in mouse models and in human subjects with type 1 diabetes (T1D). While anti-insulin antibodies (iAbs) produced by such IBCs are not known to mediate destruction of insulin-producing pancreatic islet beta-cells, the antigen-presenting capacity of IBCs appears to be an important factor in activating pathogenic autoreactive T cells that mediate such beta-cell destruction. We developed an IBC-specific deletional Fc-fusion protein therapeutic, AKS-107 (two inactivated insulin moieties fused to human Fc), designed for T1D prevention in at-risk prediabetic subjects that are biomarker-positive for IBCs and insulin autoantibody titers. Therefore, an IBC-specific ELISPOT assay was developed to measure the frequency of IBCs in peripheral blood from T1D subjects.

METHODS

The assay is based on the ability of ex vivo activated IBCs to produce iAbs prior to culturing in the ELISPOT assay. The iAb-spot detection system required significant optimization of novel critical reagents to achieve appropriate sensitivity, accuracy, and precision. The optimized assay format consists of generating antibody secreting plasma cells via activation of B cells in PBMC cultures (from frozen samples) with IL-2 and the TLR7/8 agonist, R848, for 4 days, followed by cell harvesting and culturing on PVDF ELISpot plates coated with goat anti-human IgG-Fc capture Ab for 18 hr. Bound insulin-specific Ab spots were detected via a 3-step detection/amplification process with biotin-labeled AKS-107 Fab'2 fragment, followed by anti-biotin-FITC Ab, followed by anti-FITC-HRP Ab, and finally developed with ultra-sensitive TMB to visualize the iAb-specific spots.

SUMMARY OF RESULTS
Eleven PBMC samples from subjects with established T1D receiving insulin therapy were evaluated in which six samples showed significant and reproducible insulin-specific spots above background, and were confirmed to be insulin-specific by competitive inhibition via pre-incubation with AKS-107.

CONCLUSIONS
In addition, a correlation was observed between elevated iAb titer and elevated IBC ELISPOT frequency, a feature that could be used as an inclusion criterion for the AKS-107 first-in-human clinical safety study in subjects with established T1D receiving insulin therapy.