Lack of coronary aneurysms during Kawasaki Disease (KD) correlates with higher levels of autoantibodies to both full form and spliced variant of immune regulator EDIL-3 (Del-1)

R. Ross Welliver MD, Aviraag Vijaya Prakash, Sanjiti Mirmire, Sarah Baron, and Mark D Hicar MD, PhD

Introduction

The causative etiology of Kawasaki Disease (KD) and its feared sequela, coronary aneurysm, is as yet unknown; proposed pathophysiology includes infectious, postinfectious and autoimmune causes. A recent study of individuals with KD (Consiglio *et al., Cell*, 2020, Figure 1) demonstrated enriched autoantibody response to EDIL3 (also known as DEL-1), a structural glycoprotein expressed by both macrophages and vascular endothelium. This presents an attractive potential target for investigation, as EDIL3 is known to modulate inflammation via leukocyte binding and infiltration of the vessel wall (Kourtzelis *et al., Nat. Immunol.*, 2019).

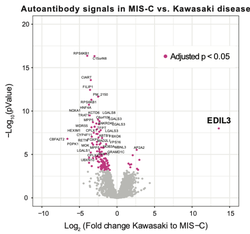


Figure 1: From Consiglio *et al.,* 2019. Volcano plot shows fold change increase in antibody binding in KD vs MIS-C, purple targets p<0.05.

EDIL3 regulates neutrophil mediated inflammation; potentially mediated by autoantibodies

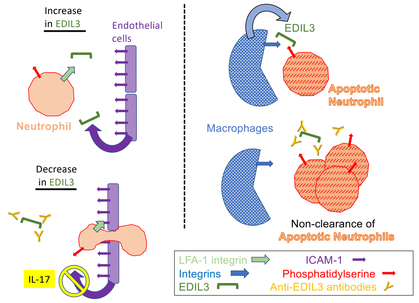


Figure 2: EDIL3 (also called Del-1) regulates inflammation at a variety of locations. (Hajishengallis and Chavakis, 2019). Endothelial cells produce EDIL3 to downregulate neutrophil extravasation. Proinflammatory IL-17 downregulates this production, leading to increase in tissue inflammation. After neutrophils are apoptotic, EDIL3 is instrumental in macrophage targeting and clearance. Autoantibodies could interfere with these processes potentially leading to increase in inflammation.

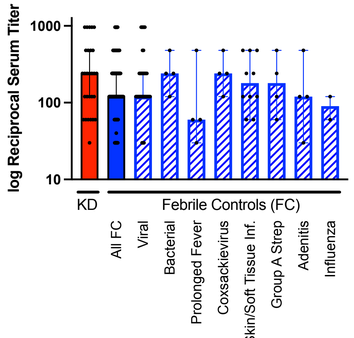
Methods

- Screened >230 serum samples isolated from febrile children, including 58 KD samples, against EDIL3 via direct ELISA.
- Human EDIL3 / DEL1 Protein (Recombinant 6His, C-terminus) was obtained from LSBio.
- 96-well ELISA plates pre-coated with 100 uL of 10 ng/mL EDIL3 in PBS for at least 24 hours before use.
- Serum samples initially diluted 1:30, then serially diluted 1:2 to a final dilution of 1:960 or 1:5360, as indicated. Washed and blocked appropriately, then incubated with TMB-Ultra (ThermoFisher) to initiate the color reaction.
- Reaction allowed to proceed until the background/blank color increase, terminated with 50 uL of 1N sulfuric acid.
- Absorbance was read at 450 nm using an automated plate reader.

Results

EDIL3 autoantibodies do not distinguish KD from Febrile controls (FC)

Figure 3: Elisa results of EDIL3 direct binding by plasma from children with KD (28) and FC (123). A difference in the median titer was shown, but this was not statistically significant.
Columns represent median and 95% CI are shown. Subgroups of FC are shown as hatched blue.
Intriguing prolonged febrile children showed the least activity.



Lower EDIL3 autoantibody binding in children with aneurysms

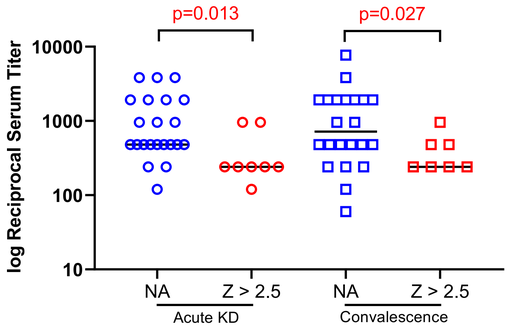


Figure 4: Serum titers of anti-EDIL3 binding in acute and convalescent KD in children with (Z >2.5) and without (NA) coronary aneurysms. Aneurysm formation defined by Z score > 2.5 using Boston scoring system (n=8, non-cardiac group n=22). Horizontal lines represent median reciprocal serum titer. Numerical values represent p values in Mann-Whitney test.

Autoantibodies do not distinguish the major splice forms (both lower in those with Z scores >2.5).

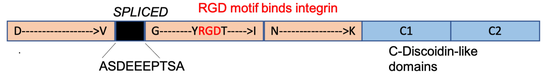


Figure 5: Schematic of full-length EDIL3 and splice variant site. The full protein encompasses 480 amino acids, the black box represents a 10 amino acid region that is affected by splice variation, located between EGF domains 1 and 2.

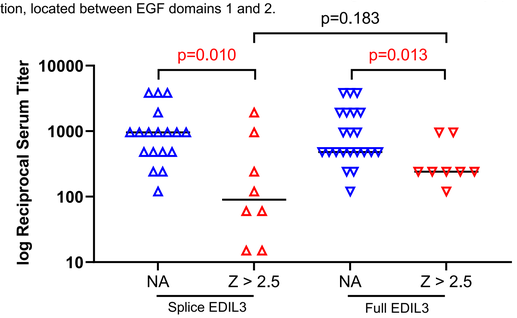


Figure 6: Serum titers of autoantibody binding to splice variants of EDIL3 in acute KD children with and without coronary aneurysms. Splice variant EDIL3 on left, full length protein on right. Horizontal lines represent median reciprocal serum titer. Numerical values represent p values in Mann-Whitney test.

IVIG administration abrogates anti-EDIL3 binding differences

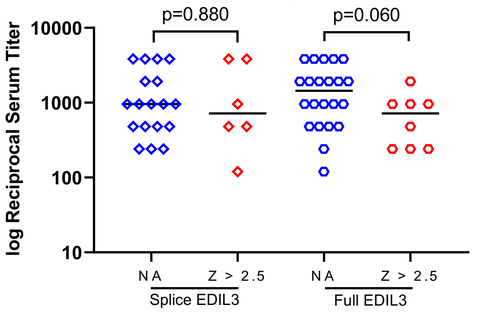


Figure 7: Serum titers of antibodies against EDIL3 splice variants following IVIG administration in children with and without coronary aneurysms. Numerical values represent p values in Mann-Whitney test.

Low EDIL3 autoantibody binding shows high specificity for aneurysm formation

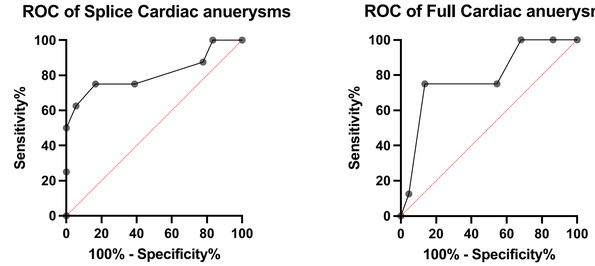


Figure 8: Receiver Operator Curve (ROC) analyses for optimal reciprocal titer cut-offs in splice and full variants of EDIL3. Reciprocal titer value <180 for splice variant EDIL3 had an 80.9% chance of detecting an aneurysm (p = 0.013, 62.5% sens, 94.4% spec.). Reciprocal titer value <360 had a 78.7% chance of predicting aneurysm formation in full-length EDIL3 (p=0.018, 75% sens, 86.4% spec.). No significant difference between models.

Conclusions

- Lower anti-EDIL3 binding correlates with coronary aneurysm formation
- Anti-EDIL3 antibodies do not distinguish KD from febrile children
- IVIG abrogates EDIL3 binding differences, supporting ubiquitous autoantibodies

Future Directions

- Development of anti-EDIL-3 antibodies into point of care test to assign high risk
- Correlation to circulating EDIL-3 levels
- Cloning of anti-EDIL-3 antibodies to define targeted epitope and assess specificity for pathology

University at Buffalo The State University of New York

Department of Pediatrics
Division of Infectious Diseases
Jacobs School of Medicine and Biomedical Sciences
buffalo.edu