Apolipoproteins are a mediator of tissue-specificity in the foreign body response

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Statement of Purpose: The theory of tissue-based class control, developed by Matzinger[1], suggests that the immune response to a pathogen is not primarily tailored to the pathogen identity but instead the tissue location of that infection. We propose that this theory also plays a role in biomaterials and medical device implantation wherein the tissue microenvironment greatly regulates the response to the implanted material or device. White adipose is associated with pro-fibrotic and inflammatory responses in the context of implanted devices[2].

Methods: 6-8 week old C57BL/6 WT or *Lepr*$^{-/-}$ (db/db) mice were anesthetized and implanted with 500 ul of alginate (1.8% SLG20 crosslinked with $BaCl_2$, diameter = 500um), polyethylene (450-500um, Cospheric) particles, or a sham surgical control. After 4 weeks, mice were harvested for preparation for flow cytometry or histology. We developed a novel whole-body paraffin embedding protocol to keep the abdominal cavity intact wherein the abdomen was formalin fixed with acid-based decalcification before embedding and taking 5 um sections. These were stained with Movat's Pentachrome or IHC for CD3 and B220. Flow cytometry samples were processed as previously published[3]. Isolated cells were split into 2 samples and stained with two 14-color antibody panels for identification and characterization of myeloid and lymphoid cells, respectively. *In vitro* protein adsorption to the surface of polymers was evaluated with mass spectrometry after solubilization in 8M urea.

Results: To determine if this fibrosis was specific to white adipose, we developed a whole peritoneal cross-sectioning method to investigate tissue-specific fibrotic events after intraperitoneal implantation of 500 um biopolymer (alginate) and synthetic polymer (polyethylene) particles. Particles of both classes only fibrosed to the surface of white adipose or pancreatic tissues (Fig 1A). Furthermore, there was a location specific fibrosis that was modulated in the context of obesity, wherein lean mice did not experience particle fibrosis to the liver, but after obesity-associated induction of liver steatosis, particles adhered tightly to the liver (Fig 1B). Immunologically, there was a large difference in the myeloid compartment of the intraperitoneal space after implantation with alginate/PE in lean and obese mice. This includes larger neutrophil and macrophage persistence (Fig 1C). We also detected a larger number of B cells in the peritoneum of obese mice with both alginate and polyethylene implants which were organized in extra-nodal follicle like structures distal from the capsule area. Through analyzing adsorbed proteins, we noted that there was an enrichment of apolipoproteins (ApoE, ApoA-I, ApoA-IV) to the surface of polymers (Fig 1D). Apolipoproteins are associated with fat metabolism and have previously been described as components of the protein corona of nanoparticles. This suggests that apolipoproteins may be a mediator of the foreign body response to intraperitoneal implants, and govern tissue-specific adhesions.

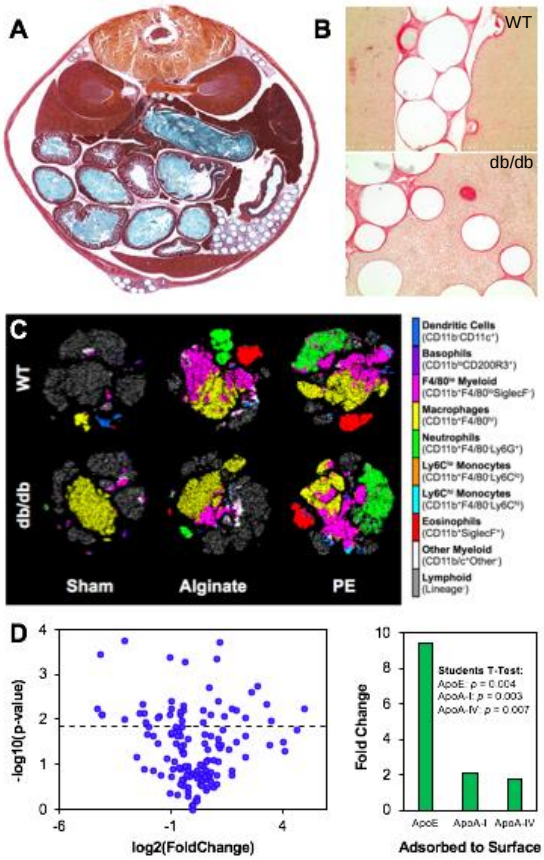

FIG 1: Tissue Specific Adhesions to Fat Correlates with Apolipoprotein adsorption to polymers. (A) Full abdominal cross-section of WT mouse with IP polyethylene implants. (B) Fibrosis to liver induced in obese animals (db/db; bottom image) (C) FACS of myeloid compartment of IP space (D) Protein adsorption to the surface of PE enriches for apolipoproteins.

Conclusions: We have shown that particles in the abdominal cavity experience tissue-specific adhesions to white adipose and the pancreas. Obesity-induced liver steatosis promotes adhesions to the liver. Furthermore, apolipoproteins associated with fat metabolism were found to readily adsorb to the surface of polymers. We hypothesize that tissue-specific adhesions are due in part to the adsorption of apolipoproteins to polymer surfaces. We are currently confirming these observations in knockout mouse strains and developing targeted therapeutics.

References:

[1] Matzinger and Kamala, *Nat Reviews Immunol* 2011
[2] Reid et al. *Journal of Tissue Eng Regen Med* 2013
[3] Sadtler et al. *Science* 2016