Reducing Auto-inflammation: The Impact of T Cell Deficiency on Atherosclerosis

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Purpose

There is comparatively sparse investigation regarding the precise immune mechanisms implicated in atherosclerosis, especially regarding T cells. T cell mediators associated with the CD40-CD154 inflammatory dyad are found in autoimmune diseases such as T1D and RA and may be a cause of the added atherosclerotic risk which these disease states maintain. This study seeks to provide a model to explore the impact of T cells on plaque formation and composition.

Background

Atherosclerosis is a major comorbid condition implicated in coronary artery disease, peripheral vascular disease, and stroke. It is a progressive, inflammatory disease resulting from the interaction between modified lipoproteins, monocyte-derived macrophages, T cells, and the arterial wall (Figure 1).

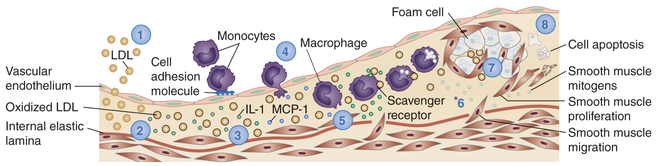

Figure 1. Atherosclerotic Progression

Autoimmune inflammation is directly associated with CD40 expression on T-cells. CD40 is a ubiquitously expressed biomarker that induces production of pro-inflammatory cytokines (IFN-γ, IL-1β, TNF-α) and matrix metalloproteinases (Figure 2). Therapeutic targeting of immune mediators has come into the spotlight in atherosclerosis. This comes from the realization that trials with statins reduce the relative risk of cardiovascular (CV) events by 10-40% leaving a 'residual risk' of 60-90% for which the CANTOS trial (IL-1β inhibition) provided proof of concept that targeting inflammation reduces CV event rates. Nonetheless, this therapy did not prove efficacious for treatment of type 1 diabetes (T1D). This project seeks to explore further pathways by which the inflammatory portion of plaque progression may be targeted for therapy by first delineating the role of T cells in this process.

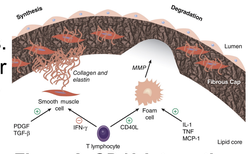

Figure 2. CD40 Interaction

Lilly LS. Pathophysiology of Heart Disease. 6th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2011.

Methods

1) Generate novel T cell-deficient atherosclerotic mouse model
   - Perform congenic back breeding of TCRα-/- and ApoE-/- mice
   - Genotype tail or ear clips using a series of primers
   - Confirm T cell-deficiency via flow cytometry of splenic homogenate stained with CD3 and CD4 antibodies
2) Characterize plaques in terms of volume and content
   - Sacrifice regular-diet mice at 8 months of age
   - Dissect aortic arch with its main branch points (brachiocephalic trunk, left common carotid artery, and left subclavian artery) through the abdominal aorta
     - Section longitudinally
     - Analyze en-face with Sudan IV staining
   - Dissect heart
     - Snap-freeze in OCT and obtain serial sections of aortic cross sections
     - Stain with trichrome and Picrosirius red stains to characterize lesion in terms of area, volume, collagen, and smooth muscle content
   - Statistical analysis via unpaired one-tailed t-tests

Results

Confirmation of ApoE-/- TCRα-/- Model

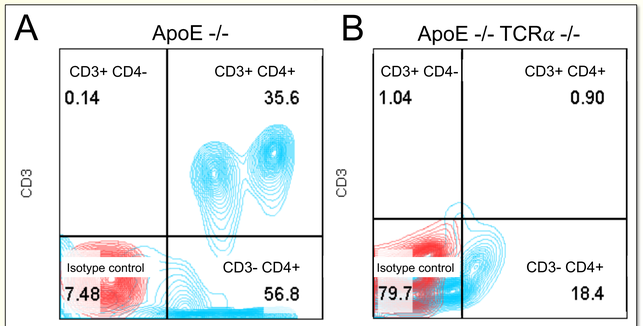

Figure 3. Flow cytometry confirmation of ApoE-/- (A) and ApoE-/- TCRα-/- (B) mouse models, respectively. The percentage of CD3$^+$ CD4$^+$ T cells within the ApoE-/- splenic homogenate was 35.6% (A). This is in drastic comparison to that of the double KO having <1% CD3$^+$ CD4$^+$ T cells (B).

Aortic Valve Staining

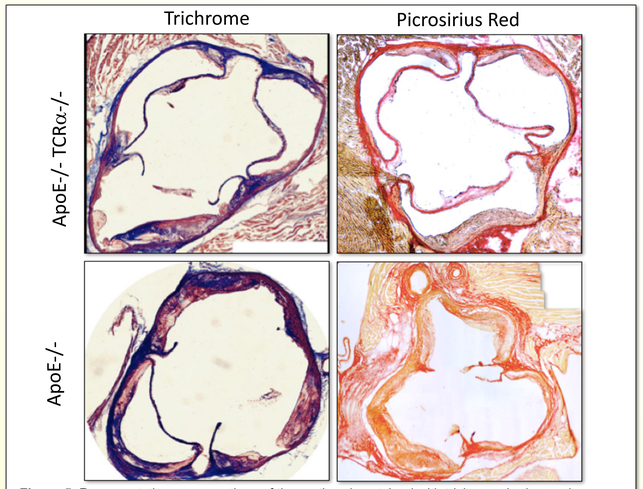

Figure 5. Representative cross sections of the aortic valve stained with trichrome (red=muscle, blue=collagen, black=nucleus) and Picrosirius red (red=collagen, yellow=muscle). The double KO model shows reduced plaque, collagen, and smooth muscle content compared to the ApoE-/-.

Sudan IV Aorta Staining

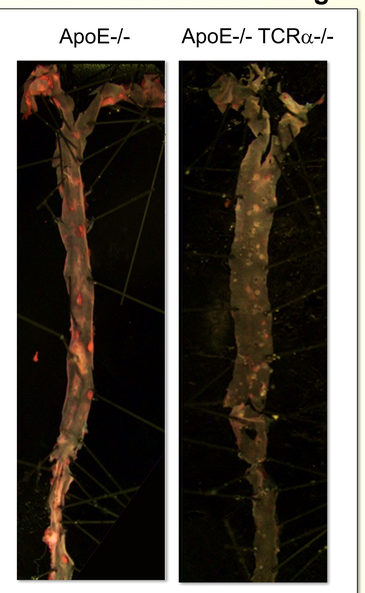

Figure 4. Representative Sudan IV stain of ApoE-/- vs. ApoE -/-TCRa -/- mice with appreciably more lipid deposition in the ApoE-/- model.

Plaque Quantification

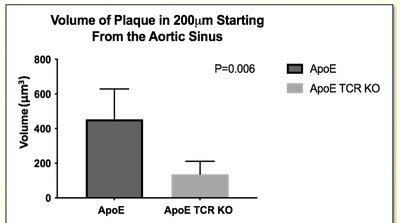

Figure 6. Substantial decrease (p=0.006) in volume of plaque (measured beginning when the three leaflets of the aortic valve are in view through 200μm into the aortic outflow tract) in the double KO as compared to the ApoE-/- model.

Plaque Composition

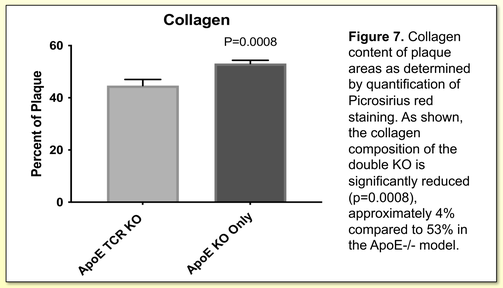

Figure 7. Collagen content of plaque areas as determined by quantification of Picrosirius red staining. As shown, the collagen composition of the double KO is significantly reduced (p=0.0008), approximately 4% compared to 53% in the ApoE-/- model.

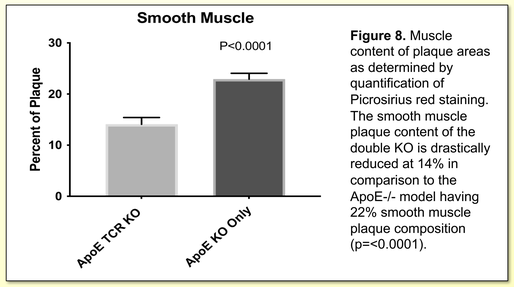

Figure 8. Muscle content of plaque areas as determined by quantification of Picrosirius red staining. The smooth muscle plaque content of the double KO is drastically reduced at 14% in comparison to the ApoE-/- model having 22% smooth muscle plaque composition (p=<0.0001).

Discussion

Deletion of $\alpha\beta$ T cells reduces the amount of plaque, though it does not completely abrogate it. Thus, while $\alpha\beta$ T cells are not the sole drivers of plaque formation, they are still key in promoting atherogenesis. Absence of $\alpha\beta$ T cells also appears to influence the composition of plaques, suggesting that the products of activated $\alpha\beta$ T cells may be crucial in the differentiation and/or phenotypic modification of cells within these lesions. Reduction in overall muscle content seen in ApoE-/- TCRα-/- lesions, suggests that $\alpha\beta$ T cells are critical in inducing a phenotypic change within nearby smooth muscle cells, thereby impacting plaque composition. This can be explained by T cell-driven modification of plaque in the production of the pro-inflammatory cytokine IFN-γ by pro-atherogenic CD4+ T_H1 cells, serving to induce expression of MHC class II antigens, such as HLA-DR on smooth muscle cells (SMCs). Further, SMCs are known to produce the majority of collagen within arteries. Therefore, with the aforementioned reduction in overall SMCs, the reduction in overall collagen content seen in our model is expected. Despite this, the ratio of collagen to SMCs is greater in the ApoE-/- TCRα-/- mouse model, a finding which has been seen in previous studies in which CD40 signaling has been interrupted. Provided these similarities in plaque compositional changes brings to question whether or not $\alpha\beta$ T cells bearing these markers are the primary basis of this transformation.

Conclusion

Alpha-beta T cell-deficient ApoE-/- mouse models demonstrate a decreased plaque burden in comparison to their ApoE-/- counterparts illustrating that $\alpha\beta$ T cells are implicated in atherosclerosis pathogenesis. Further, the absence of $\alpha\beta$ T cells impacts the composition of plaque, bearing some resemblance to models in which CD40 signaling has been interrupted.

Future Aims

- Characterize the specific T cell populations within the plaques, particularly exploring the population of cells expressing the CD40 marker.
- Perform 'add-back' experiments of $\alpha\beta$ T cell subsets (i.e., Th40 and CD8$^+$) to the ApoE-/-TCRα-/- mice.

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