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).

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

<table>
<thead>
<tr>
<th>ApoE−/-</th>
<th>TCRα−/-</th>
<th>CD3+</th>
<th>CD4+</th>
<th>CD8+</th>
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<tbody>
<tr>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td></td>
<td>0.14</td>
<td>35.6</td>
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<tr>
<td>CD3+CD4−</td>
<td></td>
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Sudan IV Aorta Staining

Plaque Quantification

Plaque Composition

Discussion

Deletion of αβ T cells reduces the amount of plaque, though it does not completely abrogate it. Thus, while αβ T cells are not the sole drivers of plaque formation, they are still key in promoting atherosclerosis. Absence of αβ T cells also appears to influence the composition of plaques, suggesting that the products of activated αβ T cells may be crucial in the differentiation and phenotypic modification of cells within these lesions. Reduction in overall collagen content seen in ApoE−/- TCRα−/- lesions, suggests that αβ 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 IFNγ by pro-atherogenic CD4+ T 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 αβ 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 αβ T cells are implicated in atherosclerosis pathogenesis. Further, the absence of αβ 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 αβ T cell subsets (i.e., Th40 and CD8+) to the ApoE−/- TCRα−/- mice.

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