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Single-Cell Analysis Supports a Role for T cell Autoimmunity in Atherosclerosis and Helps Pave the Way for New Immunotherapies

The human immune system must constantly perform a delicate balancing act – weak immune responses can lead to infection and cancer, while overactive immune responses can lead to autoimmunity and the unintentional targeting of healthy tissues. Previous research has also suggested a role for autoimmunity in atherosclerosis, a chronic inflammatory disease of the arteries. Now, single-cell analysis has shed new light on this connection and opened up potential avenues for new therapeutic interventions.

By Katrina Woolcock

Original research: Wang, Z., Zhang, X., Lu, S. *et al*. Pairing of single-cell RNA analysis and T cell antigen receptor profiling indicates breakdown of T cell tolerance checkpoints in atherosclerosis. *Nat Cardiovasc Res* **2**, 290–306 (2023). https://doi.org/10.1038/s44161-023-00218-w

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New Ground article reviewed by: Andreas J. R. Habenicht

T cells, an important component of the adaptive immune system, recognize the peptides of antigen-presenting cells, e.g., dendritic cells and macrophages, by their T cell receptors. This recognition event triggers T cell activation and proliferation, or clonal expansion, launching an immune response. To ensure "tolerance of self" – i.e., tolerance of the body's own healthy cells and tissues – and thereby prevent autoimmunity, T cells are regulated through various "tolerance checkpoints." In certain cases, such as cancer, inhibiting these checkpoints can be therapeutically beneficial, as it boosts the immune response to e.g. tumor cells. However, when tolerance checkpoints break down, autoimmune diseases like multiple sclerosis, type 1 diabetes, and psoriasis can be the result.

T cell responses have also been observed in atherosclerosis, the leading cause of cardiovascular disease. Atherosclerosis begins as a condition in which lipids accumulate in the inner layer of arteries, but it can progress to the formation of atherosclerotic plaques that contain fatty

substances, cellular debris, and immune cells including macrophages and T cells. These plaques may eventually become unstable and rupture, leading to local blood clotting that can suddenly block an artery, causing a heart attack or stroke. Until recently, it was unclear where the T cell responses associated with atherosclerosis took place and whether autoimmune T cells were involved.

To address these questions, a team of researchers led by Changjun Yin and Andreas Habenicht together with Zhihua Wang and Christian Weber leveraged recent advances in single-cell technologies. Their study "Pairing of single-cell RNA analysis and T cell antigen receptor profiling indicates breakdown of T cell tolerance checkpoints in atherosclerosis," published in Nature Cardiovascular Research, shows that tolerance checkpoints are disrupted in atherosclerosis in tissue-specific and T cell-subtype-specific ways and strongly suggests a role for autoimmune T cells in atherosclerosis.

A role for adaptive immunity in atherosclerosis

Initially, it was believed that the inflammation observed in atherosclerosis resulted from activated innate immune responses, which can be mitigated by statin drugs that lower LDL cholesterol and thereby reduce the generation of plaque. However, many individuals still exhibit signs of inflammation despite taking statins. The notion that adaptive immunity, as opposed to innate immune responses, is involved in this residual inflammation emerged in the late 1980s, when it was discovered that human atherosclerotic plaques contained activated T cells. Helper T cells were found to be pro-atherogenic, whereas regulatory T (T_{reg}) cells were found to be anti-atherogenic. These studies sparked interest in developing immunomodulatory therapies to prevent cardiovascular disease by suppressing pro-atherogenic responses and promoting anti-atherogenic ones. However, our limited understanding of the dynamic changes and crosstalk between heterogeneous immune cell subsets in atherosclerosis has hindered progress in pursuing such therapeutic approaches.

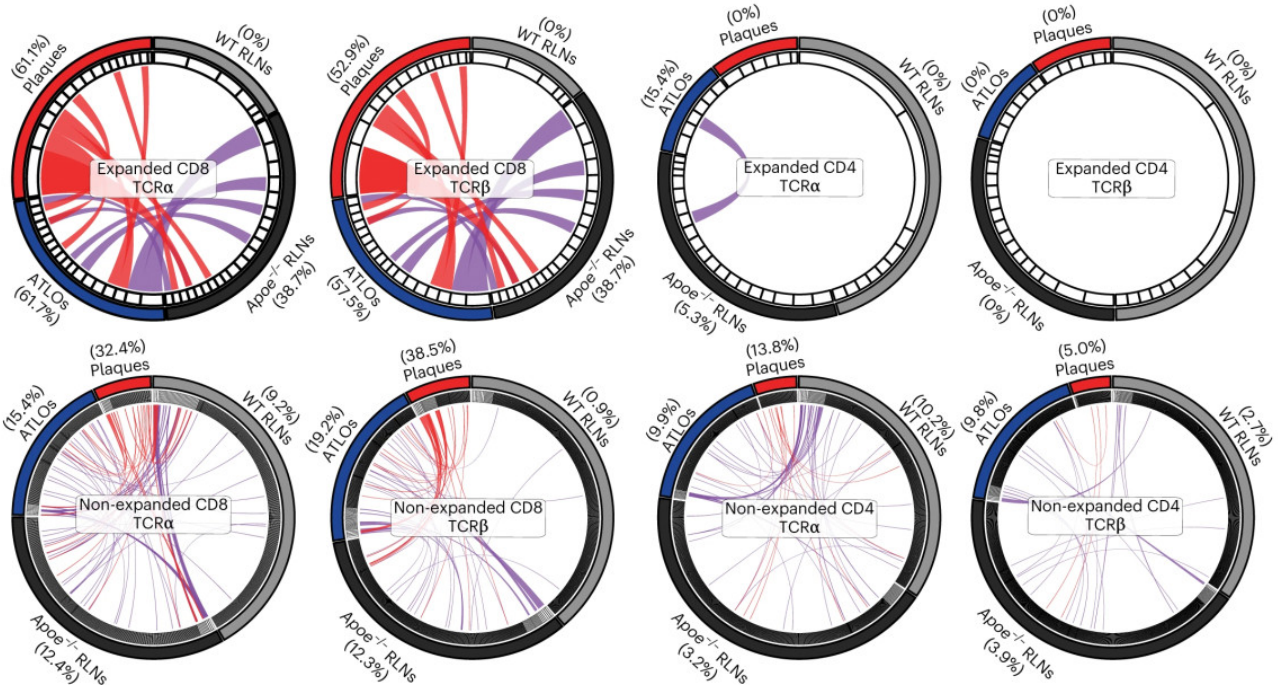
Tackling heterogeneity with single-cell analyses

The past decade has witnessed remarkable advances in single-cell technologies, including single-cell proteomics and single-cell RNA sequencing. These technologies hold the potential to identify new targets, pathways, and immune interactions, facilitating the development of precise immunotherapies. Drawing on them, the team investigated the coordinated activities of heterogeneous immune cells in $Apoe^{-/-}$ mice, a well-established model for studying atherosclerosis. Specifically, the researchers employed single-cell transcriptome sequencing and T cell receptor sequencing – scRNA-seq and scTCR-seq – to examine tolerance checkpoints in atherosclerosis. To determine the locations of immune responses, they analyzed blood, atherosclerotic plaques, local lymph nodes, and artery tertiary lymphoid organs (ATLOs) – aggregates of immune cells that form near plaques in response to inflammation. As controls, they used lymph nodes and blood from wild-type mice.

Breakdown of tolerance checkpoints

The research team discovered evidence of tissue-specific breakdown of multiple tolerance checkpoints in the $Apoe^{-/-}$ mice, with the most pronounced effects observed in plaques, followed by ATLOs and then lymph nodes. Compared to healthy tissue, they found increased or decreased populations of specific immune cells and observed the conversion of T_{reg} cells into inflammation-promoting helper T cells. They also found altered expression of tolerance-related RNA transcripts, and other anomalies like altered antigen presentation, all leading to an aberrant immune response. Importantly, T cell receptor sequencing revealed clonal expansion of three T cell subsets – $CD4^+$ T cells, T_{reg} cells and especially $CD8^+$ T cells – in ATLOs and plaques. Since the mice were kept under

sterile conditions, this clonal expansion could only have been triggered by self-antigens, indicating autoimmunity.



The T cell receptor (TCR) distribution among four tissues is shown in this plot: in atherosclerotic plaques, lymph nodes in wild type mice (renal lymph nodes in wild type mice, WT RLNs), lymph nodes in Apoe−/− mice (Apoe−/− RLNs), and artery tertiary lymphoid organs (ATLOs). Arcs in the outer layer of the circle represent the respective number of TCRs.

Finally, the team compared their findings with published datasets on human plaques and found that CD8⁺ T cells and macrophages were similar in terms of their cell numbers and transcript profiles between human and mouse plaques. Therefore, the mechanisms of tolerance breakdown involving these cells may also apply to human atherosclerotic plaques.

Future work will identify the self-antigens

To summarize, the study demonstrates that tolerance checkpoints are compromised at multiple levels in advanced atherosclerosis in mice, particularly within the plaques themselves. Furthermore, plaque-infiltrating T cells likely recognize atherosclerosis-relevant self-antigens, leading to the T cells' activation. These autoimmune T cells probably contribute to the control of plaque growth.

Interestingly, these findings may also explain why checkpoint inhibitors that have been successful in cancer treatment can actually worsen atherosclerosis. By unleashing T cells with prior antigen exposure, these inhibitors may activate immune responses to atherosclerosis-specific epitopes. Accordingly, identifying the distinct autoimmune T cell responses in both cancer and atherosclerosis will be of considerable interest.

As always, however, the findings have certain limitations. It is important to bear in mind that techniques used to isolate single cells may affect phenotypes so that the analyses might not exactly reflect the original cell state. Furthermore, the Apoe$^{-/-}$ mouse model does not fully reflect the processes in human atherosclerotic disease. Finally, the study did not identify the specific self-antigens recognized by the T cells; however, the identification of three T cell subsets as being clonally expanded in specific tissues paves the way for future investigations into the epitopes involved. For instance, prediction software could identify potential epitopes based on the T cell

receptor sequences.

Implications for immunotherapy

The findings hold significant translational potential. Future research will shed light on which tolerance checkpoints affect the progression of atherosclerosis, enabling the design of restorative therapies. In the case of self-antigens that bind to T_{reg} cells, the outcome may be anti-atherogenic. Therefore, engineered autoimmune T_{reg} cells could conceivably be used to dampen the immune response: T cells would be extracted from the patient, modified according to the T cell receptor sequences identified by the authors, expanded into millions, and infused back. By contrast, for self-antigens that bind to $CD4^+$ or $CD8^+$ cells, the outcome may be pro-atherogenic. In this case, tolerogenic vaccines could be developed to induce self-antigen-specific tolerance.

Such therapies would specifically target plaque inflammation, as opposed to using systemic anti-inflammatory treatments, which are associated with an increased risk of infection. However, immunotherapies may need to be tailored to specific patient groups based on their clinical status. In this regard, single-cell technologies like those used by Zhihua Wang et al. will play a vital role in guiding the design of future personalized immunotherapies.

In conclusion, the researchers have provided a rationale for future immunotherapies aimed at preventing the progression of human atherosclerosis driven by autoimmune T cells. These therapies could potentially reduce the burden of cardiovascular disease on public health. ●

The researchers

Zhihua Wang is a graduate student of Andreas Habenicht and Changjun Yin. He is currently working in Changjun Yin's group at Sun Yat-sen University, Guangzhou, China, to identify the self-antigens of the T cells identified by T cell receptor cloning as described in the article.

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