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Previews

Tbx1 orchestrates an immune niche that safeguards a broken heart

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Cardiac lymphatics cooperate with the reparative immune response in myocardial healing after infarction. In this issue of *Immunity*, Wang and colleagues discover a mechanism underlying this cooperation, dependent on the transcription factor *Tbx1* and responsible for the creation of an immunosuppressive niche that mitigates autoimmunity.

Myocardial infarction (MI) is one of the leading causes of cardiovascular mortality. Susceptible patients are at risk of developing an autoimmune response evoked by the underlying ischemic injury that exposes cardiac antigens to T lymphocytes through dendritic cells (DCs). A sustained activation of this process hampers the regenerative phase typical of a favorable post-MI repair. After the first days following the ischemic injury, the inflammatory phase gives way to reparative processes, characterized by the disappearance of cells like neutrophils and proinflammatory monocytes, which are slowly replaced by reparative macrophages. The transition from inflammation to repair is driven by changes in the immune milieu that initiate a precise sequence of events aimed at preserving the residual cardiac function. To properly direct immune cell trafficking into the healing myocardium, it is necessary that a simultaneous process of blood and lymphatic vascular remodeling takes place.

Despite the known relevance of cardiac lymphatic vasculature, and data showing that lymphatics grow and expand in the infarcted myocardium, our understanding of the mechanisms that regulate lymphatics' restructuring lags behind that of the blood counterpart. An important advancement in the elusive role of cardiac lymphatics in myocardial repair is presented in this issue of *Immunity* by Wang and colleagues.[1] In this study, they discovered that lymphatic endothelial cells (LECs) of post-ischemic myocardial tissue have a previously unknown function that allows the establishment of an immunosuppressive microenvironment necessary to promote neo-lymphangiogenesis and reparative processes.

The hypothesis that the authors set out was based on the evidence that, usually, MI-induced autoimmunity is transient and resolved with a process of healing. Noticeably, the mechanisms allowing timely activation and the subsequent constraint of autoimmunity are unclear. Reasoning that an immunosuppressive mechanism should be operative during the post-acute MI phase, the authors focused their analysis on the expression dynamics of genes encoding for transcription factors during days 4–7 post-MI. The results showed *Tbx1* as the top-ranking gene, continuously upregulated during the reparative phase. Tbx1 is a T-box transcription factor involved in 22q11 deletion syndrome (22q11DS), known as DiGeorge syndrome.[2] Complex phenotypes have been described in patients with 22q11DS, ranging from major cardiovascular defects, craniofacial dysmorphism, immune deficiency, and behavioral and psychiatric manifestations.[2]

How does Tbx1 control post-MI immune milieu and contribute to the promotion of reparative processes? Tbx1 unveiled immunomodulatory functions that are performed by orchestrating an array of genes involved in the creation of a niche where tolerogenic DCs (tDCs) and regulatory T (Treg) cells are recruited while autoreactive $CD8^+$ T cells are hampered (Figure 1).

Wang et al. further found that the majority of *Tbx1*-expressing cells are $Vegfr3^+$ LECs, thus suggesting that this transcription factor is engaged to specifically shape the lymphatic vasculature and coordinate a proper immune response.[3] To better investigate the role of *Tbx1* expression in LECs during the post-MI reparative phase, the authors took advantage of the $Tbx1^{flox/flox}$ mouse model, crossed with *Fabp4-Cre* transgenic mice, to selectively delete the transcription factor in endothelial cells of vessels that arises through angiogenic sprouting of pre-existing vasculature. The investigators observed that adult *Fabp4-Cre*;$Tbx1^{flox/flox}$ mice subjected to MI had reduced lymphatic vessel density. This structural remodeling resulted in a larger infarcted area and decreased cardiac function, measured at the echocardiographic analysis. These results indicated that Tbx1 mediates neo-lymphangiogenesis by promoting LEC penetration into the infarcted myocardium, a phenomenon previously described as an important contributor to post-MI inflammation resolution and tissue repair.[4]

To understand which molecular pathways and cell functions were altered by the lack of Tbx1 in endothelial cells, the authors performed bulk RNA sequencing (RNA-seq) of cardiac $CD31^+$ cells, isolated from *Fabp4-Cre*;$Tbx1^{flox/flox}$ post-MI mice, to identify the differentially expressed genes. The analysis revealed that the absence of Tbx1 led to upregulation of 63 genes involved in immune activation and a concomitant downregulation of 310 genes involved in lymphangiogenesis and tissue repair. In this context, the authors found that one of the targets genes that was downregulated in mice

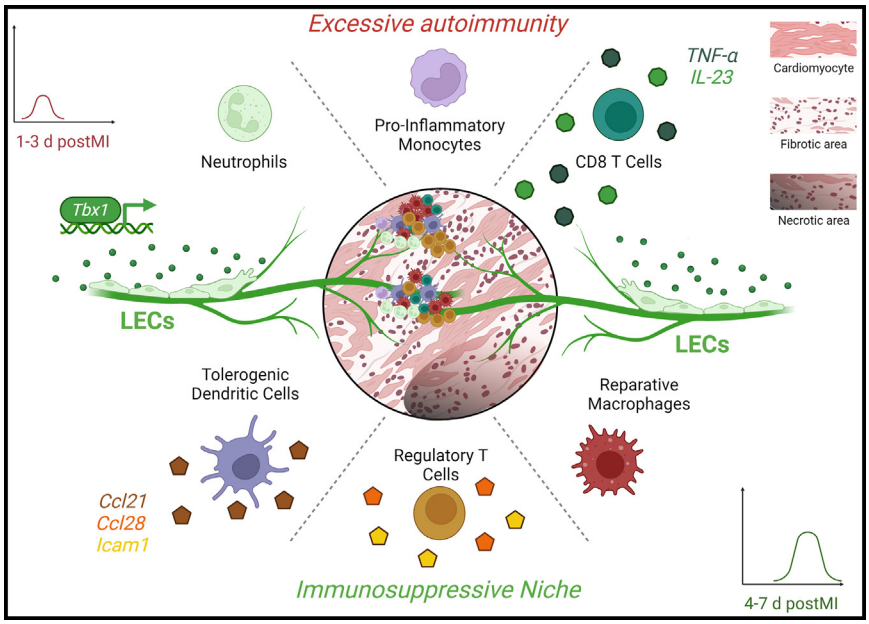

Figure 1. Tbx1-driven immunosuppressive niche in post-ischemic myocardial repair

Cardiac lymphatic vasculature is subjected to post-myocardial infarction (MI) remodeling through a gene expression program driven by the transcription factor *Tbx1* in lymphatic endothelial cells (LECs). During the post-acute MI phase, reparative mechanisms are established to avoid excessive autoimmunity. Tbx1 coordinates the transcription of chemokines (*Ccl21* and *Ccl28*) and adhesion molecules (*Icam1*) that allow the establishment of an immunosuppressive niche composed by tolerogenic dendritic cells and T regulatory lymphocytes that hamper cytotoxic $CD8^+$ T cell expansion. Created with BioRender.com.

with *Tbx1*-defective LECs was *Dtx1*, which encodes for a phylogenetically conserved ligase involved in lymphangiogenesis through an inhibitory effect on Notch1 signaling.[5] Notably, Wang et al. demonstrated that the *Tbx1*-defective LECs were characterized by low expression of Vegfr3, thus connecting to previous data showing that Notch1 inhibition is crucial for Vegfr3 upregulation and signaling activation.[6] Taken together, these results suggest that *Tbx1* promotes neo-lymphangiogenesis in ischemic myocardial tissue via a molecular sequence involving Dtx1-Notch1-Vegfr3. Because the molecular interactors of this axis have been previously identified as key players of lymphatic vasculature remodeling in other tissues, it is tempting to speculate that Tbx1 might utilize this molecular cascade to favor an immune-suppressive environment by recruiting anti-inflammatory immune cells through newly formed lymph vessels in other diseases.

Data mining of the differentially expressed genes in the absence of Tbx1 highlighted *Ccl21*, *Ccl28*, and *Icam1*, which are all involved in chemoattractive processes that promote a tolerogenic environment. On the other hand, a concomitant downregulation of *Tnfa* and *Il23*, which encode pro-inflammatory cytokines, was observed in *Tbx1*$^{-/-}$/LECs compared with LECs isolated from mice expressing *Tbx1*, suggesting a further contribution to induce an immunosuppressive microenvironment.

To characterize the subpopulations of leukocyte trafficking in the infarcted heart of *Fabp4-Cre;Tbx1*$^{flox/flox}$ mice during the reparative phase, the authors performed single-cell RNA-seq (scRNA-seq), identifying a prominent accumulation of cytotoxic $CD8^+$ T cells. In addition, by performing high-throughput sequencing of the T cell receptor, the authors found increased clonality and reduced diversity in the infarcted heart of mice deficient for Tbx1 in LECs, indicating that the absence of this transcription factor facilitates the activation of specific autoreactive $CD8^+$ T cells that contribute to the worsening of cardiac function. In more detail, the authors found that *Fabp4-Cre;Tbx1*$^{flox/flox}$ hearts had a significantly increased fraction of $CD8^+$ T cells that are autoreactive against α-myosin, a cardiomyocyte-specific protein. Interestingly, a recently published study on immunotherapy-related myocarditis demonstrated that autoreactive $CD8^+$ T cells were the culprit of cardiac function deterioration.[7] Similarly, in the context proposed by Wang and colleagues, it could be valuable to determine whether the clonally expanded $CD8^+$ T cells that are autoreactive against α-myosin are the effector cells responsible of cardiac function deterioration after MI. Testing whether autoreactive $CD8^+$ T cells isolated from *Fabp4-Cre;Tbx1*$^{flox/flox}$ mice subjected to MI are per se capable of aggravating post-ischemic remodeling in wild-type mice could help unravel this issue.

Interesting to note, the accumulation of $CD8^+$ T cells was associated with a decreased number of tDCs characterized by the expression of the chemokine receptor Ccr7, known to limit $CD8^+$ T cytotoxicity and promote peripheral tolerance. This observation fit well with the finding that Tbx1 absence causes downregulation of the chemokine Ccl21, which is a ligand of Ccr7.[8] Relevant to this, when the authors treated mice with an antibody neutralizing Ccl21, a significant reduction of $Ccr7^+$ tDCs and a concomitant increase of $CD8^+$ T cells were observed. At the functional level, this effect on immune cell composition led to loss of ejection fraction and heart failure progression, indicating that Tbx1 is a crucial regulator of tDC and $CD8^+$ T cell trafficking in the healing myocardium. Meanwhile, *Tbx1* additionally promoted Icam1 and Ccl28 production, known to participate in the recruitment of immunosuppressive Treg lymphocytes.[6,9] Further adding a piece of complexity to the myriad of cells participating in this tightly regulated response, the authors reconnected their data to previous findings showing that the second wave of the immune response receives a fundamental contribution from reparative macrophages that promote the repair of damaged cardiac tissue after MI. In fact, scRNA-seq analysis combined with flow cytometry revealed that Tbx1 promoted the expansion of a cluster of macrophages with reparative functions. Yet, how the molecular axis driven by Tbx1 governs the relationship between the expansion of reparative

macrophages and the inhibition of autoreactive CD8$^+$ T cells will need future investigation.

The key element of the findings shown by Wang and colleagues is the demonstration of a previously unknown role of Tbx1 in shaping the remodeling of lymphatic tissues to create tissue niches with specific immunomodulatory functions (Figure 1). Although these investigators have elegantly shown that Tbx1 drives the LEC transcriptional program in the healing myocardium by orchestrating the expression of chemoattractant molecules that constrain undesired excessive cytotoxic CD8$^+$ T cell activation, their study raises interesting questions regarding the possibility of managing this pathway to influence the immune milieu in a tissue- and disease-specific context.

As an example, the presence of niches with specific immunocompetent functions has been identified as a key mechanism of immune escape in tumor growth.[10] In fact, the failure to form these structures with a proper immune milieu has been observed in patients with progressive diseases, suggesting that niche breakdown may be a key mechanism in disease prognosis. The discovery of Tbx1 as a master regulator of the transcriptional program that guides the remodeling of lymphatic vasculature to shape the proper immune environment has great potential, especially in tissues where adequate immune surveillance might be hampered by a limited lymphatic drainage system, such as the brain. Yet, for now, we must take solace in the knowledge that Tbx1 can heal a broken heart.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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