Role of Lymphocytes in Myocardial Injury, Healing, and Remodeling After Myocardial Infarction

Ulrich Hofmann, Stefan Frantz

Abstract: A large body of evidence produced during decades of research indicates that myocardial injury activates innate immunity. On the one hand, innate immunity both aggravates ischemic injury and impedes remodeling after myocardial infarction (MI). On the other hand, innate immunity activation contributes to myocardial healing, as exemplified by monocytes’ central role in the formation of a stable scar and protection against intraventricular thrombi after acute infarction. Although innate leukocytes can recognize a wide array of self-antigens via pattern recognition receptors, adaptive immunity activation requires highly specific cooperation between antigen-presenting cells and distinct antigen-specific receptors on lymphocytes. We have only recently begun to examine lymphocyte activation’s relationship to adaptive immunity and significance in the context of ischemic myocardial injury. There is some experimental evidence that CD4+ T-cells contribute to ischemia–reperfusion injury. Several studies have shown that CD4+ T-cells, especially CD4+ T-regulatory cells, improve wound healing after MI, whereas depleting B-cells is beneficial post MI. That T-cell activation after MI is induced by T-cell receptor signaling implicates autoantigens that have not yet been identified in this context. Also, the significance of lymphocytes in humans post MI remains unclear, primarily as a result of methodology. This review summarizes current experimental evidence of lymphocytes’ activation, functional role, and crosstalk with innate leukocytes in myocardial ischemia–reperfusion injury, wound healing, and remodeling after myocardial infarction. (Circ Res. 2015;116:354-367. DOI: 10.1161/CIRCRESAHA.116.304072.)

Key Words: antigen ■ B-cell ■ dendritic cell ■ myocardial infarction ■ T-cell

Myocardial infarction (MI) is the prevailing cause of heart failure in the Western world. Necrotic myocardial tissue is first replaced by a capillary-rich granulation tissue. In adult mammals, myocardial tissue has negligible regenerative potential. Consequently, post-MI healing leaves a collagenous scar that is—at best—a stable noncontractile substitute for cardiac muscle. However, large MI typically leads to progressive remodeling of the viable myocardial tissue, decreased contractile function, and manifest heart failure over time. The immune system plays a critical role in healing and remodeling after MI.

Immunity has evolved as a defense system that protects the organism from injury. Historically, immunity was conceived as an armamentarium that protected against infectious diseases. Today we know that, in addition to pathogen elimination, the immune system is a central component in the wound healing processes. Immunity’s defensive armory and healing functions span 2 distinct yet interconnected systems known as innate immunity and adaptive immunity.

The cellular components of innate immunity are myeloid cells, including monocytes, macrophages, dendritic cells (DC), natural killer (NK) cells, as well as neutrophilic, basophilic, and eosinophilic granulocytes. Innate leukocytes bear germline-encoded receptors (pathogen recognition receptors) and can sense pathogen-derived molecular patterns. Once detected, pathogens are eliminated by, for example, phagocytosis or secretion of toxic substances, such as reactive oxygen species. Similarly, pattern recognition receptors enable innate recognition of so-called danger-associated molecular patterns released from injured tissue. Innate leukocytes, especially monocytes and monocyte-derived macrophages, have been extensively studied in the contexts of myocardial injury, wound healing, and regeneration. Monocyte recruitment from the bone marrow and spleen during infarct healing in the adult mouse heart has been of particular interest in recent years. Besides their pivotal function in modulating wound healing, monocytes also protect against ventricular thrombi formation early after MI. Broadly speaking, we now have a firm understand of innate leukocyte, especially monocyte-derived cells, activity after MI.

The central cellular components of adaptive immunity are T- and B-cells that arise from lymphoid progenitor cells in the bone marrow. In contrast to innate immunity, adaptive immunity responds relatively slowly, imprints an immunologic
memory, and is highly antigen-specific. T- and B-cells bear unique antigen receptors (T-cell receptors [TCRs] and B-cell receptors) generated by random somatic recombination of receptor encoding gene segments. Adaptive immunity activates (primes) T-cells in response to major histocompatibility complex (MHC)–encoded antigen-presenting molecules. Dedicated antigen-presenting cells include DCs, monocytes, macrophages, and B-cells. These cells internalize the antigen, degrade it in lysosomes, and present the antigen-derived peptides on both MHC class I and MHC class II molecules. After priming, conventional T cells secrete significant amounts of interleukin-2 (IL-2), a central T-cell growth and differentiation factor. Eventually, differentiated effector T-cells leave the lymph node and migrate to inflammatory sites where they exert specific effector functions. CD4+ effector T-cells are classified into Th1, Th2, Th17, and Treg subsets based on their distinct cytokine repertoire.

Regulatory CD4+ T cells (Treg cells) are specialized cells that actively suppress immune reactions. They were initially identified as a fraction of suppressive CD4+ T cells that constitutively express CD25. The transcription factor forkhead box P3 (Foxp3) was than identified as a key regulatory gene for the regulatory T-cell development, thereby allowing more precise identification of this subset, at least in mice. The development of autoimmune disorders in neonatally thymectomized mice indicated that these cells originate in the thymus and constitute a central mechanism in preventing adaptive immunity from mounting an immune response against self-antigens. Because natural CD4+ Treg cells are highly enriched for T-cells recognizing self-antigens, these cells might rapidly respond to sterile tissue injury on autoantigen release, as will be discussed in more detail below.

To date, research on cardiac adaptive immunology has mainly focused on autoimmunity in the context of autoimmune myocarditis. In such context, adaptive immunity directed against myocardial antigens is categorically detrimental and inevitably leads to myocardial tissue destruction. Meanwhile, there is also experimental evidence that lymphocytes, especially T-cells, determine both myocardial ischemia–reperfusion injury and healing after MI. These findings, which will be summarized below, indicate that lymphocyte activation by myocardial autoantigens, recognized by either their respective TCR or B-cell receptor or, alternatively, by pattern recognition receptors, plays a significant role in the response to ischemic myocardial injury. However, we still need to deepen our understanding of both cardiac autoimmunity containment in the steady and the role of lymphocytes in wound healing and myocardial tissue remodeling in response to MI. This review should therefore serve as a call for further research into the role of lymphocytes in myocardial injury and repair.

Myocardial Lymphocyte Subsets in Steady State and After MI

To date, human myocardial lymphocyte subsets in steady state and after injury remain poorly characterized, and most data are based on the mouse MI model. In comparison to skeletal muscle, the adult mouse heart contains a considerable number of leukocytes, macrophages being the most prevalent subset. There are <10,000 lymphocytes, and B-cells are twice as prevalent as T-cells per milligram of tissue in steady state. After MI, induced by permanent coronary artery occlusion, B- and T-cell levels increase 5- to 10-fold, most likely as a result of recruitment because there is no reported lymphocyte proliferation in the myocardium. Both B- and T-cell levels peak around day 7 after MI. Flow cytometry shows that myocardial B220+ B-cells were CD19+IgD+IgMlow, indicating that MI triggers the infiltration of circulating mature B-cells. T-cell response to MI include both conventional and Foxp3 regulatory CD4+ T-cell activation and proliferation in heart-draining lymph nodes. The absolute number of Foxp3+ T-cells in lymph nodes and myocardium peaks on day 7, whereas their relative level remains nearly doubled until at least day 56. Conventional CD4+ T-cells in infarcted myocardium are mainly Th1 polarized, as indicated by their preferential expression of IFN-γ. IFN-γ secretion by both CD8+ and CD4+ T-cells considerably increases after depletion of Foxp3 regulatory CD4+ T-cells. Th17- and Th2-differentiated T-cells are barely detectable in infarcted myocardium. Reperfusion reduces the total number of leukocytes recruited to the myocardium, probably because of reduced infarct volume and temporarily shifts the entire array of recruited leukocytes, including lymphocytes, to earlier time points, with absolute cell numbers peaking around day 3.

Role of Lymphocytes in Myocardial Ischemia–Reperfusion Injury

CD4+ T-Cells Enhance Ischemia–Reperfusion Injury

There is solid experimental evidence that CD4+ T-cells contribute to myocardial ischemia–reperfusion injury. Using lymphocyte-deficient RAG1 KO mice and wildtype (WT) controls, researchers examined myocardial infarct size after transient coronary artery occlusion. RAG1 KO mice had significantly smaller infarcts than WT mice. Furthermore, CD4+...
T-cell depleted mice, but not CD8+ T-cell depleted mice, had significantly smaller infarcts than WT mice. Accordingly, reconstituting RAG1 KO mice by adoptively transferring CD4+ T-cells reversed the protection seen in RAG1 mice in the ischemia–reperfusion model, but RAG1 KO mice reconstituted with CD4+ T-cells from IFN-γ KO mice did not have larger myocardial infarcts.23 These experiments strongly indicate that CD4+ T-cells, but, rather unexpectedly, not CD8+ T-cells, contribute to myocardial ischemia–reperfusion injury involving IFN-γ expression.

Adenosine Modulates CD4+ T-Cell Function in Hypoxic Microenvironments

Treatment with the adenosine (A2A) receptor agonist ATL146e prevented increased infarct size in Rag1 KO mice reconstituted with WT but not A2AR KO CD4+ T-cells. Indeed, A2A adenosine receptor signaling on T-cells protects against CD4+ T-cells’ detrimental effects in the setting of myocardial ischemia–reperfusion. Hypoxic tissue injury modulates local adenosine signaling to recruit leukocytes, partly because inhibited adenosine kinase in hypoxic cells increases the cellular accumulation of adenosine.21 Furthermore, CD73 (5′-ectonucleotidase) is expressed by 2 distinct mouse CD4+ T-cell populations: Treg cells and CD25− uncommitted primed precursor T-cells. On both T-cell subsets, CD73 converts extracellular 5′-adenosine-monophosphate to adenosine that suppresses proliferation and cytokine secretion of Th1 and Th2 effector T-cells.24 A recent study investigating the role of adenosine metabolism by CD73 in myocardial ischemia–reperfusion demonstrated that cardiac adenosine release is augmented over 7 days after ischemia–reperfusion in WT mice but reduced by 90% in CD73 KO mice. Myocardial function in CD73 KO mice declined more severely and was associated with enhanced myocardial edema formation, as determined by MRI.25 However, this study did not fully assess the contribution of CD73 on infiltrating T-cells versus other leukocytes. Adenosine-mediated regulation of microvascular permeability and direct anti-inflammatory effects on effector T-cells and other leukocytes were particularly noted for A2A receptor signaling, which is the predominant adenosine receptor subtype on lymphocytes.26 Yet A2B adenosine receptor signaling can promote inflammation on other leukocyte subsets, and the significance of this receptor is not yet defined in this context.

Modes of CD4+ T-Cell Activation in Ischemia–Reperfusion

Although T-cell involvement in ischemia–reperfusion injury has been studied in animal models, there are few data explaining how CD4+ T-cells are activated during ischemia–reperfusion.27 Additionally, studies of CD4+ T-cell contributions to myocardial reperfusion injury did not address whether the CD4+ T-cells’ deleterious effects require classical TCR-dependent activation by an autoantigen presented in the context of MHC-II molecules, though the timeframe in which injury occurs in the presence of T-cells indicates nonclassical T-cell activation, for example, by pattern recognition receptor, such as toll-like receptors28 or mediated alarmin recognition.29 Indeed, it was recently reported that the stimulation of toll-like receptor 2 directly induced IFN-γ expression without TCR stimulation in mouse Th1 cells.30 One molecule that is released by ischemic tissues and known to activate toll-like receptor 2 is high-mobility group box-1.31 High-mobility group box-1 is also sensed by the RAGE receptor, which resembles another kind of pattern recognition receptor that leads to inflammasome activation.32 Inflammasome activation after tissue injury induces strong IL-1β expression that significantly amplifies the inflammatory response by recruiting inflammatory cells and directly effects leukocytes, as in stimulation of cytokine expression and matrix-metalloproteinase activity.33 Thus, pattern recognition receptor activation on T-cells might be a relevant mechanism in the rapid proinflammatory lymphocyte activation seen in ischemia–reperfusion. The significance of T-cell activation by autoantigens that might be presented by MHC class II molecules after ischemia–reperfusion has not yet been sufficiently investigated.

Significance of T-Regulatory Cells in Ischemia–Reperfusion

The roles of effector versus Tregs remain unclear because no study has yet assessed the contribution of conventional versus Tregs in myocardial ischemia–reperfusion injury. A recent study indirectly indicated that Tregs contribute to rosuvastatin-induced cardioprotection against myocardial reperfusion injury.34 Statins like rosuvastatin increase both CD4+CD25+ Treg numbers and Foxp335 expression levels; however, the mechanism behind this protection is not clear. Treg cells and autoreactive conventional T-cells use overlapping pools of self-reactive TCRs, but Treg cells have considerably lower self-antigen activation thresholds.36 Several lines of evidence, for example, induction of autoimmunity by Foxp3+ Treg ablation in mice, indicate that self-reactive T-cells are constitutively suppressed by Tregs.37 After tissue injury, early Treg activation via released self-antigens might be part of the physiological machinery that contains autoimmunity. Given that T-cells are activated early after ischemia–reperfusion by autoantigen recognition, Tregs are more likely than conventional T-cells to become rapidly activated in this context. Tregs’ broad anti-inflammatory properties may limit reperfusion injury by attenuating local proinflammatory innate-immune activation. Indeed, there are reports that Treg activation protects against experimental ischemia–reperfusion injury in other organs, such as the liver38 and kidney.39 Most of these studies do not address the exact mode of Treg activation and interaction with innate immune cells, though these phenomena are generally associated with increased Treg levels in the injured organ.

In addition to identifying multiple ways of modulating adaptive immune responses, some experimental data indicate that activated Tregs may interfere with innate immunity. Recent in vitro studies suggest that Treg cells may play a direct role in controlling innate immune responses by modulating neutrophil activity.40 Neutrophils, which are the most numerous leukocyte subset during the first hours after ischemia–reperfusion in the myocardium, critically influence ischemia–reperfusion injury depending on their degree of recruitment and local activity.41 Collectively, CD4+ T-cells were shown to contribute to ischemia–reperfusion injury, especially by secreting IFN-γ, which
might be mediated by a nonclassical activation mechanism rather than TCR-mediated autoantigen recognition. CD4+ T-cells might rather play a protective role, at least at later stages when being concomitantly activated by autoantigen recognition via their TCR, although their precise role in myocardial ischemia–reperfusion has not yet been experimentally defined.

Roles of Other Lymphocyte Subsets
In addition to classical CD4+ αβ T-cells, reperfused myocardium also contains γδ T-cells, albeit in low absolute numbers. γδ T-cells contribute to local IL-17A secretion in myocardium more than CD4+ T cells.41 IL-17A mediates cardiomyocyte apoptosis by regulating the Bax/Bcl-2 ratio. γδ T-cells play a largely unrecognized role on the border between adaptive and innate immunity. γδ TCRs are not restricted to peptide recognition in the context of MHC molecules, which distinguishes them from classical αβ T-cells. Because many γδ T-cells directly migrate to tissues, these cells may recognize either pathogen-encoded antigens encountered in specific tissues or self-molecules. γδ T-cells known capacity for recognizing antigens that rapidly seem following tissue injury and responding in considerable numbers without requiring extensive clonal expansion predisposes them toward participating in the early immune response to ischemic injury. However, γδ T-cells’ activation mechanism and contribution to myocardial ischemia–reperfusion injury remains incompletely explored.

B-cells are also recruited to reperfused myocardium in I/R, but their local contribution has not yet been studied in detail. Yet we do know that circulating natural immunoglobulin M (IgM) plays a significant role in reperfusion injury of several organs. Innate-like B-cells can rapidly acquire immune regulatory activities after innate activation by the secretion of natural IgM and IL-10. Natural IgM constitutively produced by innate-like B1-cells targeting a specific self-antigen (nonmuscle myosin heavy chain [MyHC] II) is pathogenic in myocardial ischemia–reperfusion as well. Synthetic peptide mimetopes or monoclonal antibodies designed to prevent specific IgM binding to the self-antigen are protective in a mouse model of myocardial ischemia–reperfusion. Blocking IgM reduces leukocyte recruitment. This indicates that natural IgM triggers early activation of innate immunity in the reperfused myocardium. Binding of natural IgM likely initiates an innate immune response by complement activation that subsequently leads to the formation of complement fragments. C3a and C5a, the so-called anaphylatoxins, are particularly potent specific leukocyte attractants and activators that might be relevant to enhancing reperfusion injury.

Role of Lymphocytes in Myocardial Healing and Remodeling After Infarction
Role of CD4+ T-Cells
It is well established that wound healing after MI is centrally modulated by innate immunity. Several recent studies unambiguously demonstrate that monocytes become mobilized from the bone marrow and the spleen and constitute the central effector leukocyte population in the healing myocardium. In contrast, experimental evidence for the role of lymphocytes is still limited. The first experimental in vivo study of adaptive autoimmunity triggered by MI showed that adoptive splenocyte transfer after acute MI in rats produced myocarditis in naïve recipients. The levels of infiltrate and necrosis in the recipient rats appeared to correlate with donor animal infarct size. The study did not identify which leukocyte population was activated by MI in donors, but the authors speculate that clonal T-cell activation in response to a myocardial tissue antigen was the underlying mechanism.

We recently used a permanent MI mouse model to show, for the first time, that myocardial ischemic injury induces CD4+ T-cell activation in heart-draining lymph nodes. This activation process takes place in mediastinal lymph nodes within days after MI and requires an intact TCR repertoire for self-antigen recognition. Interestingly, CD4 KO or MHC class II KO mice, which are characterized by the absence of CD4+ T-cells, showed a similar detrimental phenotype as was observed in a mouse model using a transgenic TCR for an ovalbumin-derived peptide that is irrelevant in this setting (OT-II mice). Our studies show that CD4+ T-cells activated by TCR signaling must be present for proper collagen scar deposition, which protects against left ventricular dilation and rupture after MI. An experimental study that ablated CD11c+ cells, mainly representing antigen presenting, namely DCs and macrophages, in a transgenic mouse model indirectly proved that T-cells are specifically activated by interaction with antigen-presenting cells. The authors report a phenotype that is strikingly similar to CD4+ T-cell-deficient mouse models, resulting in deteriorated left ventricular function and remodeling. CD11c ablation enhances expression of inflammatory cytokines and recruitment of proinflammatory Ly6C+ monocytes, prolongs extracellular matrix degradation, and diminishes endothelial cell proliferation. Unfortunately, the effect of CD11c cells on T-cell activation was not explored in this study because the authors mainly focused on local effects of CD11c+ cells in myocardium.

CD4+ T-Cells Interact With Innate Immunity and Improve Wound Healing After MI
CD4+ T-cells regulate the infiltration of proinflammatory monocytes, which are characterized by high surface expression of the antigen Ly6C. Absence of CD4+ T-cells increases the density of Ly6C+ monocytes, impaired neovascularization, and collagen deposition 7 days after MI. Two independent studies conclusively indicate that CD4+ T-cells reduce left-ventricular dilation and mortality after MI, most likely by modulating local innate immune activation, especially monocyte infiltration. It remains to be clarified how CD4+ T-cells activated during myocardial injury interact with monocytes. Further unanswered questions include which antigens activate T-cells and whether activated T-cells must infiltrate the myocardium to be beneficial.

Our results indicate that both conventional effector CD4+ T-cells, showing mainly a Th1 cytokine profile, and, to a lesser extent, Foxp3+ Treg cells infiltrate the myocardium within days after MI. Yet the differential roles played by conventional T-cells versus Treg cells that were concurrently activated in heart-draining lymph nodes remains unclear in the context of myocardial healing and remodeling.
Several studies indicate that especially Tregs play a beneficial role after MI. Attenuated Treg cell recruitment in global CCR5-deficient mice correlates with increased expression of inflammatory mediators in the infarct zone, in line with deteriorated left-ventricular dilation. Further, stimulated Tregs could beneficially modify the outcome after experimental MI in a rat model. Expanding Treg cells in vivo, by either adoptive transfer of Tregs or a CD28-superagonistic antibody, attenuates myocardial proinflammatory cytokine expression and immune cell infiltration post MI. In vitro data also indicate that Tregs might directly protect against apoptosis. Accordingly, a single adoptive Treg transfer in a permanent MI mouse model attenuates both the postinfarction inflammatory response and adverse remodeling. However, none of these results define how Tregs exert their beneficial effects. Unanswered questions of particular importance include how therapeutically activated/transfered Tregs interfere with the protective activation of adaptive T-cell immunity as demonstrated in response to MI and how Tregs interact with innate leukocytes.

We recently showed that Tregs are a prerequisite for proper extracellular matrix formation and angiogenesis during wound healing post MI. Ablating Tregs using anti-CD25 antibodies or diphtheria toxin (in mice expressing the diphtheria toxin receptor under control of the Foxp3 promoter) impaired left ventricular dilation and survival early after permanent MI in mice. Treg-cell–depleted animals had significantly increased neutrophil numbers in the infarct zone. Furthermore, in comparison with control mice, Treg-cell–ablated mice had proportionally elevated inflammatory Ly-6Chigh monocyte levels in the infarct zone. This indicates that Tregs limit proinflammatory myeloid cell infiltration. Recently, another study has also reported that depletion of Tregs enhances the number of macrophages in myocardium. Monocytic cells sorted from Treg-depleted mice showed significantly higher expression of inducible nitric oxide synthase 5 days post-MI when compared with cells sorted from control mice. Moreover, the expression of M2-associated anti-inflammatory cytokines IL-10 and transforming growth factor β1 was downregulated. Our data therefore indicate that Tregs contribute to the switch in the local cytokine milieu during the first week after MI. By secreting factors, including IL-10, IL-13, and transforming growth factor-β, Tregs promote the differentiation of recruited Ly6C<sup>neg</sup> monocytes toward anti-inflammatory M2-like macrophages in myocardium. Soluble factors derived from activated Tregs might also directly stimulate fibroblasts in myocardium. As Tregs have low absolute numbers in myocardium, the more relevant mechanism might be the much more prevalent monocye-derived cells’ induction of scar formation promoting and wound stabilizing factors like IL-13, osteopontin, and coagulation factor XIII in the myocardium (Figure 1). The interaction between activated Tregs and monocytes likely constitutes an amplification loop that creates an anti-inflammatory micro-milieu and promotes extracellular matrix de novo deposition in infarcted myocardium. According to this model, treating mice with a CD28 superagonistic antibody that preferentially stimulates Tregs enhances an M2-like monocyte differentiation pattern in the myocardium. This was associated with increased collagen de novo expression within the scar correlating with decreased rates of left-ventricular ruptures.

In addition to shaping myeloid cell differentiation and recruitment, Treg depletion also modifies T-cell infiltration, probably indirectly via the locally enhanced proinflammatory milieu. Absolute numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T cells increased in the healing infarcts of Treg-cell–ablated mice after permanent MI. Though it has not yet been explicitly shown, Tregs should also suppress the concomitantly activated Th1 cells, which constitute the predominant conventional CD4<sup>+</sup> T-cell phenotype in myocardium, favoring MI-like differentiation.

Like extracellular matrix formation, angiogenesis is vitally important during healing. We showed that absence of CD4<sup>+</sup> T-cells reduces the capillary density within the healing infarct zone. This reflects that CD4<sup>+</sup> T-cells have a particular role in angiogenesis, which might also be mediated by M2 macrophages, for example, by their secretion of vascular endothelial growth factor.

Collectively, there is good evidence that CD4<sup>+</sup> T-cells promote myocardial healing particularly through their effects on monocyte recruitment and differentiation.

**T-Cell Subsets Other Than CD4<sup>+</sup> T-Cells**

Several recent studies explored other T-cell subsets in the context of MI. A subset of CD8<sup>+</sup> T-cells that express angiotensin 2 receptor has been observed in the peri-infarct zone of rats 7 days after MI. These CD8<sup>+</sup> T-cells differ from cytotoxic angiotensin 2 receptor–negative CD8<sup>+</sup> T-cells in their capacity to secrete IL-10 in response to angiotensin-II stimulation in vitro. Transferring these cells, harvested from a donor rat 7 days after MI, reduced infarct size 2 weeks after MI, thereby indicating CD8<sup>+</sup> T-cells’ potential significance in myocardial wound healing.

Along with conventional αβ CD4<sup>+</sup> T-cells, γδ T-cells, NK T-cells, and B-cells are found in the mouse myocardium early after MI. γδ T-cells are recruited to the myocardium within days after MI in the mouse. They constitute the major source of IL-17A in myocardium, meaning that T17 polarization of CD4<sup>+</sup> T-cells does not play a significant role in myocardial remodeling post MI. IL-17A–deficient mice showed no difference in infarct size compared with WT mice on day 1. However, improved survival and attenuated left ventricular dilation in IL-17A KO mice over the course of 28 days post MI. Non-Th17–derived IL-17A promotes infiltration of neutrophils and monocytes and stimulates them to produce pro-inflammatory cytokines, thereby aggravating cardiomyocyte death and enhancing fibroblast proliferation and profibrotic gene expression. γδ T-cells are activated by combined toll-like receptor and IL-23/IL-1β stimulation, which indicates that they contribute to the innate immune response after myocardial injury. Similarly, iNKT cells also take part in the innate response to myocardial injury. They are activated by either antigens, presented by the MHC class I-like molecule CD1d or cytokines. Identified antigens to date include endogenous danger signals released after tissue injury. Also, the glyco-sphingolipid α-galactosylceramide is widely used as a ligand to activate iNKT cells. Injecting α-galactosylceramide after MI reduced left ventricular dilation in a permanent MI mouse model. Thus, iNKT cells have a protective function post MI.
via enhanced expression of anti-inflammatory cytokines such as IL-10. However, the specificity of α-galactosylceramide treatment in vivo for stimulating anti-inflammatory iNKT cell activation, and especially the physiological role of iNKT cells, remain undetermined.

Role of B-Cells After MI
A pilot study showed that intramyocardial injection of B-cells into early post-ischemic myocardium preserved cardiac function, whereas unfracotated bone marrow cells showed no effect. This study could not delineate a definite mechanism for B-cells’ beneficial influence. The role of B-cells after MI was recently investigated using several B-cell ablation strategies in a permanent MI mouse model. Applying an antibody against the CD20 antigen ablated both follicular and marginal B-cells but not B1 cells. This treatment reduced infarct size, lessened left ventricular dilation, and improved recovery of left ventricular function, as determined 14 days after MI. B-cell activating factor (Baff) receptor KO mice, which have profoundly reduced follicular and marginal zone B-cells, also showed improved left ventricular function post MI. Anti-CD20 treatment, Baff receptor KO mice, and Baff neutralization by an antibody all reduced levels of the chemokine CCL7, which is associated with lower proinflammatory Ly6Chigh monocyte infiltration, probably because of increased retention of these cells in bone marrow. However, the study did not address B-cells’ role in monocyte trafficking to and from the spleen, which is the major reservoir and source of monocytes recruited to the myocardium after MI. The significance of B-cells activation by toll-like receptors for CCL7 expression in this MI model was further underlined by myeloid differentiation primary response 88 (MyD88) KO and adaptor protein Toll/IL-1 receptor (TIR)-domain containing inducing IFN-β (Trif) KO mice, which cannot properly respond to extracellular TLR stimulation and bone marrow chimera harboring CCL7 KO bone marrow cells all showing reduced CCL7 expression after MI. Although B-cells are clearly recruited to the myocardium, the study does not identify the responsible factors. Furthermore, the local effects of B-cells recruited to the injured myocardium, their possible interactions with innate inflammation, and their relationship to activation of adaptive immunity all merit further investigation. Also, the potential role of autoantibody formation against myocardial epitopes has not yet been studied in this context.

In summary, CD4+ T-cells and B-cells both modulate early myocardial healing and progressive detrimental remodeling after MI, though the latter is less clearly determined in current research. Regulation of their recruitment from bone marrow and spleen along with local monocyte differentiation were identified as underlying mechanisms (Figure 2).

Homeostatic Maintenance of Tolerance Against Myocardial Tissue Antigens: Possible Autoantigens and Mechanism for Activation of Self-Reactive T-Cells
Cardiomyocyte Proteins as Prototypical Myocardial Autoantigens
As discussed earlier, lymphocyte activation requires the highly specific recognition of an antigen via the B-/TCR. Relevant autoantigens have not yet been unequivocally identified in the setting of myocardial ischemic injury. A key paper recently ascribed an outstanding immunologic function to the cardiac protein α-MyHC, which is encoded by the gene Myh6. Immunization with α-myosin–derived peptides is an established model for creating autoimmune myocarditis in susceptible mouse strains, such as BALB/c mice. The study...
identified α-MyHC as a pathogenic autoantigen for CD4+ T-cells in a mouse model of spontaneous myocarditis. The authors showed that mouse medullary thymic epithelial cells lacked Myh6 transcripts. Transgenic expression of α-MyHC in thymic epithelium conferred tolerance to cardiac myosin.59 The study neatly mapped how a hole in central tolerance to a highly tissue-specific antigen can lead to spontaneous autoimmunity in the related organ.

This autoantigen for myocardial autoimmunity is also clinically relevant: thymic α-MyHC expression was only barely detectable in humans. Notably, α-MyHC–specific T-cells were found in peripheral blood even in healthy subjects without heart disease. These results indicate that human central tolerance against α-MyHC is leaky, and some other peripheral mechanism likely induces tolerance against this self-antigen in steady state. Obviously, when α-MyHC–specific T-cells encounter their antigen in DQ8− mice, DQ8+ mice with background other than nonobese diabetic, or healthy humans, organ-specific autoimmunity does not occur. However, antigen binding by specific MHC class II alleles, such as DQ8, together with additional costimulatory signals, such as local inflammation, might be able to transiently break tolerance and induce autoimmunity against this myocardial antigen in mice and humans. This concept is supported by evidence that α-myosin is a major T-cell autoantigen in humans with chronic myocardial Trypanosoma cruzi infection (Chagas cardiomyopathy).61 Also, patients have more prevalent antimyosin antibodies after MI.62

### Regulation of Tolerance to Myocardial Autoantigens Beyond Acute Myocardial Injury

Interestingly, constitutive MHC presentation of MyHC-derived peptides in the myocardium of rodents from different genetic backgrounds was shown years ago.63 Low-level cardiac myosin turnover likely allows local antigen-presenting cells to encounter, process, and present myosin-derived peptides constitutively. In humans, constitutive MHC class II expression also appears in endothelial cells in myocardium.64 This might be part of a peripheral tolerance mechanism against organ-specific autoimmunity. However, the role of antigen-presenting cells65 and their constitutive low-level autoantigen presentation for homeostasis in healthy myocardial tissue remains unclear.

The role of CD4+ Treg cells for peripheral tolerance under homeostatic conditions also needs further exploration. Tregs and DCs crosstalk, using an array of mechanisms, to maintain immune tolerance.66 The constitutive expression of MHC class II molecules in myocardial tissue, and especially the constitutive presentation of myocardial autoantigens in the context of MHC class II molecules, suggests that the myocardial milieu induces a tolerogenic phenotype, characterized by low expression of costimulatory molecules, in antigen-presenting cells. This tolerogenic phenotype should also apply to DCs in heart-draining lymph nodes under homeostatic conditions. In the absence of innate stimuli, immature DCs likely migrate to lymphoid tissues and present myocardial antigens for recognition by naive self-antigen–specific T-cells. The continued encounters between circulating naive self-reactive T-cells and immature DCs presenting myocardial antigens may lead to peripheral tolerance by inducing T-cell anergy toward myocardial tissue antigens. On myocardial injury, antigen-presenting cells should receive potent maturation signals by damage-associated molecular patterns released from the necrotic tissue. DCs presenting myocardial antigens might thus induce T-cell activation via a multistep activation process that uses MHC class II upregulation to display cognate peptides to TCRs, surface expression of costimulatory signals, and production of cytokines for effector T-cell activation and polarization. As

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### Figure 2. Lymphocytes control monocytes recruitment and differentiation post-myocardial infarction (MI).

MI triggers mobilization of monocytes from spleen and progenitor cells from bone marrow. T- and B-cells regulate the recruitment of Ly6Chigh monocytes from blood to the injured myocardium, for example, by CCL7 expression in B-cells. CD4+ T-cell–derived cytokines modulate the differentiation of monocyte–derived cells within the myocardium.
discussed earlier, we have shown that ischemic myocardial injury activates Tregs early after MI in heart-draining lymph nodes, and this may prevent sustained tissue-destructive autoimmunity after cardiac injury.20

Especially in patients with dilative cardiomyopathy (DCM), a high prevalence of autoantibodies against MyHC isoforms was found, which further underlines the particular role of this protein for cardiac autoimmunity.67 Autoantibodies against a variety of unrelated proteins expressed by cardiomyocytes occur in patients with heart failure of various etiologies,68 which suggests that other antigens may also induce autoimmunity when peripheral tolerance is broken in diseased myocardium. The proportion of DCM patients with inflammatory cardiomyopathy of autoimmune or infectious pathogenesis may be underestimated because of a lack of histological analysis. However, the relatively high prevalence of autoantibodies against contractile proteins in patients with coronary heart disease strongly indicates that noninfectious cardiac injury in immune-competent individuals can break tolerance and induce autoimmunity. Among others, autoantibodies against tropomysins show some prognostic effect after MI.69 Cardiomyocyte surface antigens, especially the constitutively expressed β1-adrenoreceptor, are also targets for autoantibodies, especially in DCM patients, and can directly interfere with cardiomyocyte function.70 Their prevalence and prognostic implication is currently under study for patients after MI.71

Overall, the data summarized above indicate that lymphocyte activation contributes to acute ischemia–reperfusion injury by not fully elucidated mechanism. However, after the early phase of ischemic injury especially CD4+ T-cells promote wound healing in the myocardium. How this particular Janus-faced form of autoimmunity is regulated, normally preventing a transition into a persistent form of adverse autoimmunity leading to an inflammatory dilated cardiomyopathy-like phenotype, remains to be explored. Antigen presenting cells and Treg cells might be key regulators in this process (Figure 3). Also, the physiological role of lymphocytes found in healthy myocardial tissue and peripheral mechanism conferring tolerance against autoimmunity in steady state remains to be determined.

Evidence for Lymphocyte Activation After MI in Humans

Evidence for T-Cell Activation

The vast majority of data that will be discussed below comes from clinical studies that phenotyped blood lymphocytes. However, only a minority of lymphocytes circulating in peripheral blood represent an ongoing adaptive immune reaction because the antigen-specific primed lymphocytes are kept in lymphatic organs and are partially recruited to the inflamed target organ. As coronary artery disease (atherosclerotic macroangiopathy) is the most relevant underlying cause for MI, patients with stable coronary heart disease are the best control group for observing lymphocyte activation by MI/acute coronary syndromes (ACS). Patients having ACS have significantly increased peripheral blood Th17/Th1 and Th1 T-cell levels,73–75 Furthermore, the peripheral number and anti-inflammatory function of Tregs may be transiently impaired.75,76 The pathological cause of MI is atherosclerosis within the coronary arteries, characterized by both local inflammation in the diseased vessel wall and systemic inflammation. In patients with cardiovascular risk factors or already established atherosclerosis, the frequency of circulating CD4+CD28null T-cells has prognostic relevance.77 CD4+CD28null T-cells are terminally differentiated and have proinflammatory functions leading to an inflammatory dilated cardiomyopathy-like phenotype, remains to be explored. Antigen presenting cells and Treg cells might be key regulators in this process (Figure 3). Also, the physiological role of lymphocytes found in healthy myocardial tissue and peripheral mechanism conferring tolerance against autoimmunity in steady state remains to be determined.

Figure 3. Regulation of T-cell activation in steady state and post-myocardial infarction (MI). There is a constitutive presentation of myocardial antigens in the context of major histocompatibility complex (MHC)-II in steady state in myocardium. One can speculate that resting/tolerogenic antigen–presenting cells prevent CD4+ T-cell activation and subsequent induction of autoimmunity in heart draining lymph nodes. Activation of antigen-presenting cells, for example, by ligands of pattern recognition receptors released from injured myocardium or expression of proinflammatory cytokines leading to upregulation of costimulatory molecules, transiently brakes the tolerance against myocardial antigens. CD4+ T-cells have been shown to aggravate ischemia–reperfusion injury. Otherwise, they beneficially modulate wound healing, especially inducing the formation of a stable scar. Adapted from Lichtman et al72 with permission of the publisher.
characterized by the production of interferon-γ and tumor necrosis factor-α. In addition, they share features of cytotoxic CD8+ T-cells, such as expression of perforin and granzyme, enabling them to effectively kill, for example, endothelial cells. This uncommon T-cell subset is expanded in the elderly and especially in individuals with autoimmune disease, like rheumatoid arthritis. In the context of cardiovascular disease, the CD4+CD28null T-cell subset is larger in patients with unstable angina than in patients with stable coronary disease, with clonality within the T-cell compartment identified by TCR spectrotyping and subsequent sequencing. CD4+CD28null T-cell frequency was also an independent predictor of future acute coronary events. CD4+CD28null T-cells include a large monoclonal population, and the T-cell clonotypes from different patients with unstable angina used antigen receptors with similar sequences. Their proinflammatory and cytotoxic functions were inhibited by experimental blocking of OX40 and 4-1BB costimulatory receptors. In blood of patients with ACS and in atherosclerotic plaque specimen, OX40 and 4-1BB expression levels were significantly higher in circulating CD4+CD28null T-cells compared with classical CD4+ T-cells.

The oligoclonal expansion of the CD4+CD28null T-cell subset suggests that repetitive stimulation by a limited number of antigens triggers the generation and accumulation of these cells in patients with vascular disease. Interestingly, CD4+CD28null T-cell clones generated from peripheral blood of patients with coronary artery disease recognize heat shock protein 60, an antigen present in atherosclerotic plaques and released by cell necrosis. Patients with ACS have more CD4+CD28null T-cells in the peripheral blood than patients with chronic stable angina. This indicates that release of plaque or tissue antigens stimulates this T-cell subset. Because the frequency and clonal identity of CD4+CD28null T-cells remains stable months after MI, one might speculate that this subset is also involved in healing and myocardial remodeling after MI, but the subset’s contributions to myocardial injury and healing have not yet been determined, due in large part to the lack of a comparable subset in laboratory animals.

FACS-based T-cell phenotyping of peripheral blood from patients after acute ST-elevation MI showed that CCR7+ CD4+ T-cells were depleted during the first 30 minutes of reperfusion. The chemokine receptor CCR7, which directs T-cell migration to and within secondary lymphoid tissues and coordinates both homeostatic proliferation of Tregs and conventional T-cells during ongoing immune response in lymphatic organs, might contribute to T-cells either redistributing to lymphatic tissues or migrating to the infarcted heart during ischemia/reperfusion. However, mechanistic data on the CCR7 receptor and its chemokines ligands from animal MI models are not yet available. It must also be pointed out that, in human studies, phenotyping peripheral lymphocytes in stable versus unstable post-MI patients cannot definitely distinguish between lymphocyte activation in the context of local coronary inflammation and lymphocyte activation by myocardial injury. Using either histopathology or imaging to monitor lymphocyte trafficking would be highly desirable, but such data are limited because of lack of specific noninvasive imaging techniques to monitor lymphocyte activation and traffic.

As in mice, necropsy specimen from patients having MI showed T-cells in both remote and peri-infarction myocardial regions. Also, there are activated T-lymphocytes within the epicardial coronary artery walls, including both the infarct-related and noninfarct-related arteries. This corroborates blood lymphocyte analyses and further supports the idea that myocardial injury activates lymphocytes that migrate to the injured myocardium and likely modulate local inflammatory activity. A comparative analysis of thoracic lymph nodes from patients with ACS and stable angina undergoing cardiac bypass surgery failed to find differences between the 2 cohorts’ lymph node cellular composition. Thus, more data on T-cell activation and differentiation parameters from human tissue specimen would be highly valuable in deciphering the role of lymphocytes after MI.

**Myocardial Autoantibodies**

The role of B-cells and autoantibodies against myocardial proteins has not yet been systematically analyzed. After MI, patients have increased autoantibodies that recognize cardiac myosin in patients, which indicates an adaptive humoral immune response against myocardial proteins after MI. The presence and titer of antitroponin I autoantibodies shows some prognostic potential for MI. Patients with a negative total IgG antibody titer against troponin I, measured during hospitalization for an index MI, showed a modestly increased ejection fraction over 6 to 9 months, in contrast to patients with total IgG antibody titers ≥1:160. Note that low titers (>1:40) of antitroponin T antibodies were present in 28% of a cohort of patients with ischemic cardiomyopathy, and antitroponin I antibody levels were much lower. The presence of antitroponin I autoantibodies in plasma is associated with improved survival in patients with chronic DCM, but not ischemic cardiomyopathy. Patients with ischemic cardiomyopathy also have autoantibodies against the second extracellular receptor domain of β-adrenergic receptors. These activate the sympathetic transmembrane signaling cascade, thereby increasing sarcoplasmatic cAMP and calcium concentrations, potentially leading to cardiomyocyte apoptosis. The prevalence of autoantibodies against β-adrenergic receptors was 1% in healthy subjects, 10% in patients with ischemic cardiomyopathy, and 26% in DCM.

Taken in sum, the experimental and clinical data summarized above indicate that antibodies against cardiac epitopes may play a causal role in adverse remodeling, leading to chronic ischemic cardiomyopathy. As for the disease progression of nonischemic cardiomyopathies, the connections between natural IgM and adaptive, mostly IgG, autoantibodies against myocardial proteins and the development of ischemic cardiomyopathy has not yet been fully established. Consequently, we do not currently know whether they causally contribute to the pathophysiology of cardiac healing and remodeling.

**Future Perspectives**

**Lymphocyte Activation in the Clinic: Comorbidities and Beyond**

Research dealing with adaptive immunity in myocardial pathologies faces fundamental challenges. First, mechanistic
studies rely heavily on animal models, as lymphocyte priming takes place in lymphatic organs, and their effector function likely goes beyond the myocardium to other lymphatic organs, including bone marrow. However, human tissue specimens from all these organs are not widely accessible. Second, besides general inevitable differences between mouse and human immunity, laboratory animals are housed in a more or less sterile environment and have a defined genetic background. We now know that the gut microbiome, which fundamentally influences adaptive immunity, is highly dependent on both environmental and genetic factors. The limited genetic background and standardized low-pathogen environment likely narrow the immunologic repertoire in our laboratory animals as compared with humans. Furthermore, laboratory animals lack the comorbidities seen in patients having coronary heart disease. Patients with coronary heart disease are, prototypically, obese smokers having hypertension, diabetes mellitus, and hypercholesteremia. All these comorbidities and some of the drugs they necessitate, like angiotensin-converting enzyme–inhibitors and statins, have been shown to influence lymphocyte function. Additionally, most experimental studies are conducted in young animals. In patients, however, cardiovascular pathologies emerge primarily in immuno-senescent elderly with decreased immune response capacity. Age significantly affects immune activation and function in myocardial pathologies. Therefore, extrapolating experimental findings from young healthy mice to middle-aged or elderly human patients may be problematic.

Our brief noncomprehensive discussion of the genetic components, environmental factors, comorbidities, medications, and age of typical MI patients is meant to illustrate the particular challenges of developing therapies to modify the adaptive immune response during acute myocardial ischemic injury, subsequent wound healing, or remodeling post MI. At best, a yet to be defined immuno-modulating therapeutic approach will tackle chronic systemic lymphocyte activation, induced by the aforementioned comorbidities, to limit atherosclerotic progression as beneficially as it modifies myocardial injury and healing in response to ischemia.

T-Cells as Therapeutic Targets
Manipulating T-cell function and abundance is an attractive therapeutic strategy for modulating immune responses in a variety of clinical settings. The immunosuppressant Ciclosporin is widely used to treat autoimmune disease and transplant rejection. Ciclosporin suppresses the activation of the calcium-dependent phosphatase calcineurin, thereby inhibiting T-cell activation. Calcineurin also inhibits the opening of mitochondrial permeability-transition pores, which was the rationale for clinically testing Ciclosporin’s capacity to reduce myocardial injury during reperfusion for MI. The drug’s positive effect on infarct size may stem from inhibition of the early detrimental effect of T-cells during reperfusion, though this analysis was not included in the study.

Tregs are another therapeutic target, and infusing ex vivo–generated or expanded Tregs is being investigated for treating autoimmunity and graft versus host disease and for preventing allograft rejection. Adoptively transferring Tregs showed efficacy in some animal models of cardiovascular disease. More specifically, Treg transfer attenuated fibrosis in response to experimental aortic constriction and improved remodeling after experimental MI. The mechanism behind these results is not clear, especially because the number of transferred cells only modestly adds to the endogenous Treg pool. Furthermore, most clinical trials of adoptive Treg therapy fail to achieve long-term cell engraftment or substantial clinical benefit, particularly for treating graft versus host disease and type 1 diabetes mellitus.

There are several other approaches to activating and expanding Tregs in vivo that work well in animal models. CD28-superagonistic antibodies activate and expand Tregs. Therefore, they attenuate proinflammatory cytokine expression and immune cell infiltration post MI. Our results indicate strongly modulated innate immunity, especially the induction of an M2-like differentiation of heart-infiltrating monocytes as the basis for the protective effect of superagonist-mediated Treg stimulation after MI. Anti-CD28 superagonistic antibodies differ from conventional CD28-specific antibodies by their ability to activate T-cells without TCR ligation. Superagonistic anti-CD28 antibodies have been used for polyclonal in vivo Treg stimulation, which led to not only their numeric expansion but also a dramatic increase in IL-10 production. In mice, polyclonally expanded Tregs shift their migration receptor pattern after activation from CCR7+ CCR5− lymph node-seeking to CCR7− CCR5+ inflammation-seeking, which induces their recruitment to sites of ongoing immune responses. Despite its promise, however, the first Phase I clinical trial of superagonistic anti-CD28 antibody in humans produced catastrophic effects for several reasons.

Meanwhile, a different, reduced-dose antibody application regimen proved to be safe and effective in humans. Other antibody approaches activating Tregs in humans for treating autoimmune disease, including anti-CD3 antibodies, have been developed and tested in preclinical studies, but there are no data on their efficacy in cardiac disease models.

IL-2 plays a critical role in both Treg homeostatic maintenance and activation. Low-dose IL-2 in vivo leads to expanded CD4+ CD25+ FOXP3+ Tregs and is safe for clinical use. However, administering IL-2 to patients, though clinically approved for kidney cancer, can have severe side effects, including severe pulmonary edema. IL-2 side effects might be avoided by administration of IL-2/anti-IL-2 antibody complexes, which efficiently stimulates Tregs. Experimentally, IL-2/anti-IL-2 antibody complex therapy attenuated atherosclerotic progression in apolipoprotein E-deficient mice. To date, no published studies explore this approach for clinical use in myocardial disease.

All the above approaches will systemically activate Tregs, which might cause considerable off-target effects, induce immunosuppression, and increase susceptibility to infection. Antigen-specific approaches may obviate these adverse outcomes. Mucosal application of antigens is able to potently induce Tregs, with some specificity against the applied antigen. Several different Treg subsets can be induced via mucosal surfaces. Th3 type Tregs are transforming growth factor–β–dependent and express latency-associated peptide. Tr1 Tregs (IL-10 dependent) are preferentially induced by nasal antigen,
and Foxp3+ iTregs are preferentially induced by oral antigen. Furthermore, anti-CD3 monoclonal antibody acts similarly on mucosal surfaces. Both oral and nasal anti-CD3 monoclonal antibody applications are particularly effective in inducing LAP+ Tregs.22 Numerous clinical studies have evaluated different protocols of mucosal tolerance induction for autoimmune disease, with mixed results.113

An interesting technique based on the concept of administering disease-specific antigens mucosally to induce Tregs involves applying a heart homogenate by gavage before isoproterenol-induced myocardial injury. This technique mitigated the inflammatory infiltrate within myocardium and collagen deposition, improved cardiac performance, and decreased lymphocyte proliferation in mediastinal lymph nodes.114 It is not clear, however, whether T-cell activation in this model was in response to isoproterenol-induced cardiac injury or, alternatively, T-cells also become directly activated by isoproterenol and might amplify the detrimental effects of chronic isoproterenol exposure to the myocardium. Nevertheless, nasal application of the myocardial antigen troponin reduces infarct size induced by myocardial ischemia–reperfusion injury, thereby providing further support for the mucosal tolerance induction approach.115 In the myocardial ischemia–reperfusion model, CD4+ T-cells’ contribution to myocardial injury has been established more precisely.22 This protocol may have generated tolerance against T-cell activation induced by an array of antigens released during reperfusion. Furthermore, intranasal troponin applied 7 days before MI reduces inflammation and improves heart function in a model of chronic MI.116 These results indicate that mucosal tolerance induction against myocardial antigens might also beneficially modify chronic remodeling. For clinical translation, in addition to unresolved issues regarding optimal antigen, adjuvant, route, and dosing schedule, it remains to be seen whether the approach is effective when instituted post-MI during an already ongoing autoimmune response.

<table>
<thead>
<tr>
<th>Genetic Ablation</th>
<th>Phenotype</th>
<th>I/R Injury/Infarct Size Outcome</th>
<th>Healing Phase Outcome/Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAG KO</td>
<td>Absence of functional adaptive immunity/lymphocytes</td>
<td>Reduced infarct size, reversal of protection by transfer of CD4+ and IFN-γ T-cells22</td>
<td>Increased proinflammatory monocyte infiltration, impaired scar formation and ventricular dilation19</td>
</tr>
<tr>
<td>CD4 KO</td>
<td>Absence of functional CD4 T cells</td>
<td></td>
<td>Impaired scar formation, impaired survival20</td>
</tr>
<tr>
<td>MHCI KO</td>
<td>Absence of functional CD4 T cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT-II</td>
<td>CD4 T cell incapable of recognizing autoantigens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-17A KO</td>
<td>Absence of IL-17A</td>
<td>Heterogenous effects on infarct size41,56</td>
<td>Improve survival, attenuated LV dilation56</td>
</tr>
<tr>
<td>CCR5 KO</td>
<td>Absence of CCR5</td>
<td></td>
<td>Enhanced inflammation, reduced Treg recruitment, increased ventricular dilation27</td>
</tr>
<tr>
<td>Foxp3+/- DEREGR</td>
<td>Conditional ablation of Foxp3+ cells</td>
<td></td>
<td>Preferential M1-like monocyte differentiation, increased infarct size, impaired LV function27</td>
</tr>
<tr>
<td>Anti CD20 AB</td>
<td>Ablation of follicular and marginal zone B-cells</td>
<td></td>
<td>Improved LV function, reduced proinflammatory monocyte infiltration19</td>
</tr>
<tr>
<td>Anti CD28 superagonist AB</td>
<td>Treg stimulation</td>
<td></td>
<td>Reduced proinflammatory monocytes infiltration, reduced infarct size and LV dilation, improved LV function15</td>
</tr>
<tr>
<td>Anti-CD25 AB</td>
<td>Ablation of Treg</td>
<td></td>
<td>Increased proinflammatory monocytes, increased LV dilation, impaired survival21</td>
</tr>
</tbody>
</table>

AB indicates for antibody; Baff, B-cell activating factor; KO, genetic deficiency; LV, left ventricle; MHC, major histocompatibility complex; and MI, myocardial infarction.

Summary and Conclusion
There is considerable experimental evidence that lymphocytes play a significant role in the response to ischemic myocardial injury. Experimental mouse models of MI reveal that both T- and B-lymphocytes activate and modify myocardial ischemia–reperfusion injury, wound healing, and remodeling (Table). More specifically, CD4+ T-cells add to immediate ischemia–reperfusion injury but are also required for proper healing and might also attenuate chronic remodeling after MI. B-cells seem to have detrimental effects: they both impair healing and potentially contribute to autoantibody formation in chronic ischemic cardiomyopathy. The lack of clinical data confirming that lymphocyte activation in humans after MI is not just an epiphenomenon or consequence of other comorbidities but rather causally contributes to myocardial injury is mostly because of methodological shortcomings. Future experimental research will concentrate on modes of lymphocyte activation, identification of tissue autoantigens for immunotherapy, the staged role of different lymphocyte effector mechanisms in myocardial disease, and the regulatory crosstalk of lymphocytes with innate leukocytes.

Given the expanding body of knowledge, broad clinical experience, and rapidly expanding field of new therapeutics...
designed for immunomodulation in autoimmune and hema-
tologic disease, a deeper understanding of lymphocytes’
pathophysiological role in the network of innate and adap-
tive immunity activated by ischemic myocardial injury
will open new directions in immunomodulatory treatment
options for post-MI patients. We hope to find therapeutics
that can benefit coronary artery inflammation, thereby
impeding recurrent ischemic events, and improve myocardial
healing to prevent ensuing chronic remodeling of the myo-
cardium after MI.

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None.

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