Role of T-cells in myocardial infarction

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Innate immunity has been studied for several decades in the context of ischaemia-reperfusion injury, myocardial remodelling, and healing. In the last years, a number of experimental and clinical studies focused on adaptive immunity in these processes. Meanwhile, there is considerable evidence especially on the role of CD4⁺ T-cells in myocardial injury and healing, whereas their role in remodelling is less clear. Innate leukocytes are able to recognize a wide array of self and foreign molecular patterns, whereas the activation of adaptive immunity requires the highly specific cooperation of antigen-presenting cells and distinct antigen-specific receptors on lymphocytes. Relevant autoantigens have not yet been definitely identified but experimental evidence indicates that autoantigen recognition is necessary for T-cell activation after myocardial infarction. Non-antigen-specific modes of activation might also play a role especially during acute ischaemia and reperfusion of the myocardium. This review summarizes the current evidence from experimental studies and presents side-by-side recent clinical data on the role of T cells in the pathophysiology of myocardial reperfusion injury and post myocardial infarction healing.

Keywords

- Innate immunity
- Adaptive immunity
- Myocardial infarction
- Atherosclerosis
- Lymphocyte

Introduction

Atherosclerosis constitutes the pathophysiological basis for disabling diseases like stroke and myocardial infarction, both constituting the leading causes for morbidity and mortality in the Western World. Myocardial infarction is one of the most detrimental atherosclerosis related complications leading to considerable mortality and morbidity due to heart failure. Despite the encouraging effects of the current state-of-the-art primary and secondary prevention measures, there remains a substantial residual risk for ischaemic complications in individuals with sub-clinic or manifest coronary heart disease. Furthermore, it is now widely accepted that despite prompt reperfusion, which is today achieved in the majority of our patients, the myocardium is subject to additional injury induced by re-establishment of blood flow. The role of innate immunity has been a subject for experimental research in the field of atherosclerosis and myocardial ischaemia-reperfusion injury for decades whereas T-cells have only recently come into focus.

Therefore, the present review summarizes established knowledge regarding the role of T-cells in the pathogenesis of myocardial ischaemia-reperfusion injury and post myocardial infarction healing. A special focus will be on the juxtaposition of mechanistic concepts mainly derived from rodent studies and the relating recent data from human specimens. Current knowledge gaps, inherent problems in the transfer from small animal data to human pathophysiology, and the clinical significance regarding potential therapeutic implications will be discussed.

Basic principles of T-cell activation

For the better understanding of the data summarized below, we first would like to briefly envision some basic principles of T-cell generation, migration, and age-related changes in T-cell function. The central organ for the generation of T-cells is the thymus. The current nomenclature of T-cell subsets is based on several markers (Table 1) which are mostly analysed by fluorescence-activated cell sorting (FACS). The thymic release of naive CD4⁺ and CD8⁺ T-cells declines with age. The process of thymus involution starts in human childhood which is much earlier than observed in mice. This is associated with an age-dependent decline in the T-cell receptor diversity and the predominance of highly differentiated peripheral memory T-cells within an ‘aged’ T-cell compartment. The age-related changes in the memory T-cell repertoire underlies a strong modulation by chronic virus infection especially cytomegalovirus (CMV). Cytomegalovirus infection, having an age-dependent prevalence of ~70% in Western world, induces an expansion of the so-called effector-memory CD4⁺ and CD8⁺ compartment coming along with a shrinkage of the T-cell receptor diversity. The memory T-cell subsets in elderly are poised for proinflammatory cells which is aggravated by both CMV infection and traditional
cardiovascular risk factors. These processes are only poorly re-
lected in rodent models.

For adaptive immune responses, naïve T-cells have to recognize
their antigens in the context of major histocompatibility complex
surface molecules. This process takes place in secondary lymphatic
organs, mainly in the draining lymph nodes and the spleen. Besides
antigen recognition, additional stimuli are required for activation
of T-cells (‘costimulatory signals’). T-cell activation by contact with its
specific antigen is called T-cell priming. Primed CD4\(^+\) and CD8\(^+\)
T-cells can then both exert effector/proinflammatory and regula-
tory/anti-inflammatory function depending on their differentiation
profile which is defined by the signals the T-cell receives from
antigen-presenting cells and the environment in lymphatic organs.
So-called natural CD4\(^+\) regulatory T-cells constitute a particular
anti-inflammatory immune-regulatory T-cell subset which is gener-
ated in the thymus and is highly enriched for T-cells with autoantigen
specificity.

**Principles of T-cell migration**

In adults, memory T-cells predominate the peripheral T-cell com-
partment which upon antigen recognition are poised for rapid pro-
liferation and effector function execution. T-cells enter lymph nodes
either from the afferent lymphatics or from the blood and exit
lymph nodes via the efferent lymph which is mainly collected in
the thoracic duct where it is drained to the blood. Naïve T-cells rec-
circulate between blood and lymph nodes. Also different subsets of
the memory T-cell pool recirculate through the body in steady state.
Antigen-presenting cells collect antigens in peripheral tissues and
their antigens in the context of major histocompatibility complex
surface molecules. This process takes place in secondary lymphatic
organs, mainly in the draining lymph nodes and the spleen. Besides
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**Clinical evidence for the involvement of T-cells in acute coronary syndromes**

There is meanwhile a considerable number of studies that have ana-
lysed T-cells in peripheral blood in patients with unstable angina or
acute coronary syndromes. These data might partly reflect activa-
tion of T-cells secondary to coronary atherothrombosis and pre-
ceding coronary artery wall inflammation. However, they might
also represent system-wide changes in the T-cell activation status
and their migratory behaviour in response acute myocardial injury
and failure. Activation of the renin–angiotensin–aldosterone or
the sympathetic nervous system is also likely to influence the com-
position of the circulating T-cell subsets analysed in the periphery.
Hence, specific peripheral T-cell patterns observed in patients
with acute coronary events may not necessarily be informative on
the role of particular T-cell subsets in the pathophysiology of ather-
othrombosis or myocardial injury. For space restrictions, we would
like to refer the reader to a very recent comprehensive review on
clinical studies describing T-cell subsets in patients with atheroscler-
osis and acute coronary syndromes.\(^9\)

**Role of T-cells in ischaemia-reperfusion injury of the myocardium**

**Experimental evidence from mouse studies**

First experimental evidence that CD4\(^+\) T-cells contribute to myo-
cardial ischaemia-reperfusion injury came from a study analysing
wild-type and lymphocyte-deficient RAG1 KO mice. RAG1 KO

### Table 1 Key markers for immuno-phenotyping of T-cells referred in the text

<table>
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<th>CD</th>
<th>Cluster of differentiation nomenclature denotes surface markers for the immune-phenotyping of cells</th>
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<tr>
<td>CD4</td>
<td>CD4 antigen mainly identifies T-helper cells</td>
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<tr>
<td>CD8</td>
<td>CD8 antigen mainly identifies cytotoxic T-cells</td>
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<tr>
<td>CD25</td>
<td>Subunit of the interleukin-2 receptor, expressed on activated T-cells and CD4(^+) T-regulatory T-cells</td>
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<td>Foxp3</td>
<td>Forkhead-Box-Protein 3 transcription factor serves as marker for CD4(^+) T-regulatory cells in mice</td>
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<tr>
<td>CD39</td>
<td>Ectonucleoside triphosphate diphosphohydrolase, membrane bound enzyme hydrolyzes ATP and ADP to AMP</td>
</tr>
<tr>
<td>CD73</td>
<td>Ecto-5′-nucleotidase, membrane bound enzyme converts AMP to adenosine</td>
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mice revealed significantly smaller infarct sizes compared with control mice. Furthermore, CD4\(^+\) T-cell-depleted mice, but not CD8\(^+\)-depleted mice showed a significantly decreased infarct size compared with control mice. Reconstitution of RAG1 KO mice by adoptive transfer with CD4\(^+\) T cells reversed this protection seen in RAG1 KO mice. RAG1 KO mice reconstituted with CD4\(^+\) T-cells from IFN-\(\gamma\) KO mice did not increase myocardial infarct size, indicating that CD4\(^+\) T-cells promote ischaemia-reperfusion injury by IFN-\(\gamma\) expression.\(^{11}\)

The involvement of T-cells in ischaemia-reperfusion injury has been studied in animal reperfusion injury models in several organs but there are few data explaining how CD4\(^+\) T-cells become activated under these conditions.\(^{12}\) The timeframe in which myocardial injury occurs in the presence of CD4\(^+\) T-cells would rather indicate a non-T-cell receptor dependent way of T-cell activation, e.g. by recognition of ‘alarmins’\(^{13}\) from injured cell which are recognized by pattern recognition receptors, such as toll-like receptors.\(^{14}\)

**Figure 1** T-cells migration and recirculation pathways. The scheme depicts where particular T-cells subsets get accessible for analysis in experimental and clinical studies.

**Role of adenosine signalling and T-regulatory cells for protection against reperfusion injury**

In the reperfused myocardium, the ectoenzymes CD39 and CD73 are expressed by a broad range of leukocytes and cardiomyocytes.\(^{15}\) They promote the extracellular degradation of nucleotides released by cellular injury to anti-inflammatory adenosine. Treatment with an adenosine (A2A) receptor agonist blocked the increase in infarct size in Rag1 KO mice reconstituted with wild type but not A2A receptor deficient CD4\(^+\) T cells.\(^{11}\) This observation indicates that the injurious action of CD4\(^+\) T-cells can be mitigated by endogenous adenosine signalling.

Corresponding to the above-mentioned clinical data,\(^{16}\) CD4\(^+\) T-regulatory cells were recently reported to rapidly accumulate in murine hearts following ischaemia-reperfusion. The adoptive transfer of in vitro activated T-regulatory cells was able to decrease myocardial injury, whereas transfer of non-activated T-cells had no effect. Mechanistically, the protective effect of activated T-regulatory cells was related to ectonucleoidase (CD39) expression.\(^{17}\) From these data, one can speculate that recruitment of (in vitro) activated T-regulatory cells adds to the local CD39-mediated adenosine formation which counteracts the deleterious effects of other leukocytes subsets, including conventional T-cells in the context of myocardial ischaemia-reperfusion.

**Clinical evidence for the involvement of T-cells in myocardial reperfusion injury**

There are only few studies assessing the kinetics of blood T-cell subsets during early reperfusion. A novel approach using 13-parameter FACS immuno-phenotyping followed by hierarchical cluster analysis was applied to identify novel subsets of peripheral T-cells in patients with acute STEMI.\(^{18}\) This study reported a specific loss of CD4\(^+\) chemokine receptor CCR7\(^+\) T-cells during early reperfusion. These subsets include naive, central memory, and T-regulatory cells. At the moment, one can definitely not clarify whether this observation reflects a CCR7-mediated redistribution of this T-cell subset to peripheral (lymphatic) organs or a specific accumulation of these subsets in the reperfused microvascular bed of the myocardium.
However, for the interpretation of the data, it is important to mention that CCL19 and CCL21 are the ligands for CCR7. Both chemokines are reported to rise in plasma following coronary interventions. Thus, it is conceivable that these CCR7 ligands could play a role for the recruitment of T-cells to the reperfusion myocardium. The authors further concentrated their in-depth analysis on a subset of CD57+ T-cells likely resembling memory T-cells which have recently undergone proliferation. Unfortunately, T-regulatory cells which have been described to specifically accumulate in coronary thrombi were not further analysed in this study.

T-cell retention within the myocardium is reflected by transcoronary gradients. By such an experimental approach, a possible mechanistic hint for the injurious role of effector-memory T-cell retention in acutely reperfused myocardium was provided: the extent of T-cell retention in the coronary circulation correlated with microvascular obstruction as determined by cardiac magnetic resonance tomography imaging. One of the chemokines potentially mediating retention of T-cells in the reperfused microvascular bed might be fractalkine. Its receptor (CX3CR1) expression after reperfusion was especially regulated in the subset of CCR7+ effectort cells. 

Recently, the same group reported data on the CD8+ T-cell compartment in patients with reperfused MI. They found a particular early and long-lasting fall in the peripheral frequency of terminally differentiated effector-memory CD8+ T-cells in CMV-positive patients. The CD8+ subset that was depleted from peripheral blood included CMV-specific T-cells. Their data further indicate that the surface molecule PD-1 might be involved in the long-lasting loss of this subset which was specifically found in CMV-positive patients. Whether the putative preferential recruitment of this CD8+ T-cell subset to the reperfused myocardium impacts reperfusion injury and clinical outcome remains to be determined. Of note, a longitudinal cohort study found a significant association of coronary artery disease-related mortality and CMV status suggesting a potential mechanistic link between the changes in the CD8+ T-cell compartment induced by CMV infection and the pathophysiology of acute coronary events.

Collectively, there is consistent evidence from mouse studies that proinflammatory CD4+ T-cells contribute to ischemia-reperfusion injury whereas the role of CD4+ T-regulatory T-cells which were found to be enriched in coronary thrombi is less well established. However, the available experimental data indicate that they might constitute a therapeutic target to mitigate myocardial ischaemia-reperfusion injury. Data from clinical studies further indicate that besides CD4+ T-cells, CD8+ memory T-cell subsets become entrapped in the coronary microcirculation early after the onset of reperfusion and/or might even actively infiltrate the reperfused myocardium where they likely contribute to reperfusion.

Role of lymphocytes in nonreperfused myocardial infarction

Experimental evidence on CD4+ T-cells

First experimental evidence for the activation of T-cells with reactivity to myocardial autoantigens in response to experimental MI came from a study demonstrating that adoptive transfer of splenocytes from rats after acute myocardial infarction produced myocarditis in naïve recipient rats. Inspired by this study, we recently demonstrated in a mouse MI model that CD4+ T-cells get activated and proliferate in heart draining lymph nodes. The activation process takes place within days and requires an intact T-cell receptor repertoire. Absence of CD4+ T-cells in CD4 deficient and MHC class II deficient mice was associated with worse outcome. A similar phenotype was found in a mouse model harbouring a transgenic T-cell receptor for an in this context irrelevant ovalbumin-derived peptide (OT-II mouse) which showed no significant T-cell activation in mediastinal lymph nodes in response to MI. The most likely interpretation of the data is that the presence of CD4+ T-cells that can be activated by autoantigens presented by MHC class II molecules is a prerequisite for proper wound healing. The pivotal role of T-cells activated by interaction with antigen-presenting cells was indirectly proven in another experimental study where CD11c+ cells, mainly representing antigen-presenting cells, were ablated in a transgenic mouse model. After MI there was a strikingly similar phenotype as found in CD4+ T-cell deficient mouse models. Absence of CD11c+ cells resulted in deteriorated left ventricular function and remodelling after MI. However, the effect of CD11c+ antigen-presenting cells on the activation of CD4+ T-cells was not further explored in this study.

A major open question in the field is whether CD4+ T-cells respond to tissue-specific antigens or ‘universal’ tissue autoantigens. Mainly for methodological reasons it has not yet been experimentally addressed which antigens activate T-cells in experimental models of ischaemic tissue injury like MI.

Our experiments in mice showed that both conventional effector CD4+ T-cells having mainly a Th1 cytokine profile and Foxp3+ T-regulatory cells infiltrate the myocardium within a few days after MI. However, the exact pathophysiological role of conventional and regulatory T-cells in myocardial healing was initially not clear. Another group then reported in this context that T-regulatory cell function is compromised in mice that underwent experimental infarction. Therefore, we concentrated our studies on the differential effects of both CD4+ T-cell subsets.

Mechanistically, we found that CD4+ T-cells control the infiltration and most likely the differentiation of recruited proinflammatory monocytes, characterized by high surface expression of the antigen Ly6C. The absence of CD4+ T cells increased the number of Ly6-C(high)monocytes in the infarcted myocardium. Depletion of CD4+ T-regulatory cells induced a preferential M1-like phenotype, whereas activation of T-regulatory cells induced a M2-like phenotype in myocardial macrophages. Stimulation of M2-like macrophage differentiation by activation of T-regulatory cells induced expression of arginase-1, Interleukin-13, osteopontin, and TGF-β in macrophages. As these proteins are all involved in the extracellular matrix formation, the control of macrophage differentiation likely constitutes the main mechanism how T-regulatory cells improve myocardial healing. Accordingly, CD4+ T-cell deficient mice showed disturbed extracellular matrix formation in the scar and died from myocardial rupture. Another experimental study in the mouse MI model reported data fitting well to our concept that T-regulatory cells may modulate the fibroblast phenotype and function in the infarcted myocardium. Further adding to the concept of
a pivotal role of regulatory T-cell in myocardial post infarct healing, it was recently reported that expansion of T-regulatory cells in vivo by means of either adoptive transfer of Tregs or a CD28-superagonistic antibody attenuated myocardial proinflammatory cytokine expression and immune cell infiltration in a rat MI model. Accordingly another group reported that adoptive transfer of Treg cells in a mouse permanent MI model attenuates both the post-infarction inflammatory response and adverse remodeling. Collectively, endogenous T-regulatory cells emerged as pivotal cellular players regulating repair of the myocardium. The precise mechanism and location of their interaction with myeloid progenitors, monocytes, and macrophages represent major gaps in our understanding of these interaction processes. A schematic summary on the local action of CD4+ T-cells in infarcted myocardium is presented in Figure 2.

**Experimental evidence on CD8+ T-cells**

Concerning the role of CD8+ T-cells, a subset of angiotensin 2 receptor positive CD8+ T-cells was described in the peri-infarct zone of rats 7 days after MI. This subset of CD8+ T-cells differed from classical cytotoxic angiotensin 2 receptor negative CD8+ T-cells in their capacity to secret IL-10 in response to angiotensin 2 stimulation in vitro. Their functional significance in myocardial healing was demonstrated by transfer of these cells harvested from a donor after MI. This procedure significantly reduced infarct size after experimental MI. Correspondingly, the beneficial action of angiotensin 2 receptor expressing CD4+ T-cells which includes a subset of Foxp3+ T-cells was recently reported. Of note, animals with genetic CD8 deficiency showed no significant clinical phenotype after experimental MI compared with WT animals (own unpublished data).

**Evidence from human studies**

Histopathological analysis of myocardial specimen and non-invasive imaging modalities would be highly valuable to judge the relevance of the reported changes in the T-cell compartment in peripheral blood for myocardial injury and subsequent healing processes. However, at present there are no clinically applicable non-invasive imaging techniques to monitor T-cell migration. Histopathological studies reported T-cell infiltrates in both remote and peri-infarction myocardial regions and activated T cells within the coronary artery wall of both the infarct- and non-infarct-related arteries in necropsy specimen from patients suffering from MI. This gives a valuable hint that, in analogy to what was observed in mouse studies, myocardial injury in humans activates T-cells which home to the injured myocardium and likely modulate local inflammatory activity. Only one clinical study comparatively analysed thoracic lymph nodes from patients with acute coronary syndromes and control individuals. However, the authors could not find differences in the cellular composition of lymph nodes between the two cohorts. Thus, more data on T-cell activation and differentiation parameters from human tissue specimen would be highly valuable to decipher the role of T-cells post myocardial infarction.

Collectively, there is substantial experimental evidence showing that CD4+ T-cells, especially T-regulatory cells promote myocardial healing by interaction with macrophages and probably fibroblasts. Mostly due to methodological limitations, there are no clinical data to support this beneficial role of CD4+ T-cells which is obviously contrary to their immediate detrimental action during early reperfusion.

**Conclusion**

The available experimental data from animal models provide us a relative clear picture regarding the role of CD4+ T-cells: In the mouse ischaemia-reperfusion model CD4+ T-cells aggravate ischaemia-reperfusion injury. Subsequently, the activation of CD4+ T-cells, most likely by recognition of autoantigens, is required for proper healing. In this context, especially the subset of Foxp3+ CD4+ regulatory T-cells tunes the differentiation of macrophages towards a pro-healing phenotype preventing from left ventricular dilation and rupture. However, the role of newly primed effector T-cells, recirculating, and tissue resident memory T-cells has not
yet been addressed in this context. Also, the role of T-cells in later stages of remodelling is not clear. For identifying therapeutic approaches targeting T-cells to be further evaluated towards clinical application, we need to identify relevant antigens inducing T-cell activation and to understand which signals induce their recruitment to and retention in the infarcted myocardium (Figure 3).

However, regarding the particular differences in the T-cell compartment of humans and laboratory rodents we have to be cautious in translating the findings from experimental studies to the clinical scenario. The immune system of our patients shows phenomena subsumed under the term ‘immune-senescence’ that are not present in our laboratory animals. Further, subsets like CD4\(^+\) CD28\(^{null}\) T-cells which emerge in patients with chronic low-grade inflammation, e.g. associated with atherosclerosis\(^{36}\) can exert proinflammatory function without T-cell receptor ligation. These unconventional T-cells are widely missing in our laboratory mice but might especially contribute to atherothrombosis and reperfusion injury in humans. Thus, we further need a deeper understanding how, when, and which T-cell subsets are recruited to the human coronary microvasculature and subsequently to the reperfused myocardium. Also, we should not only focus on the ischemic myocardium but also on draining lymph nodes and other lymphatic organs including the spleen and bone marrow where interactions with other cellular components of immunity and haematopoiesis might take place in our patients. Therefore, a joint effort of cardiologists, immunologists, and imaging specialists is warranted for designing new approaches to be tested in translational and clinical studies. They should enable us to get deeper insights in the implications of T-cell activation in the clinical context and to validate new therapeutic approaches aiming at the modulation of T-cell immunity.

Authors’ contributions

S.F. handled funding and supervision; S.F., U.H. drafted the manuscript; S.F., U.H. made critical revision of the manuscript for key intellectual content.

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