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IL (Interleukin)-2 Therapy in Cardiovascular Disease: The Potential to Regulate Innate and Adaptive Immunity

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ABSTRACT: Regulatory T cells and type-2 innate lymphoid cells represent 2 subsets of immune cells, which have been shown in preclinical models to be important in atherosclerosis and myocardial repair. Regulatory T cells play a crucial role in immune homeostasis and tolerance via their interactions with effector T cells, dendritic cells, and monocytes/macrophages. They also utilize and secrete inhibitory cytokines, including interleukin 10 and transforming growth factor β, to regulate or suppress pathogenic immune responses. Type-2 innate lymphoid cells have an important role in type-2 immune responses and tissue repair through secreting interleukins 5 and 13, as well as a variety of biological mediators and growth factors. Intriguingly, interleukin-2 has emerged as a common cytokine, which can be harnessed to upregulate both cell types, and also has important translational consequences as clinical trials are ongoing for its use in cardiovascular disease. Here, we briefly review the biology of these regulatory immune cell types, discuss the preclinical and clinical evidence for their functions in cardiovascular disease, examine the prospects for clinical translation and current ongoing trials, and finally, postulate how overlap in the mechanisms of upregulation may be leveraged in future treatments for patients.

Key Words: atherosclerosis ■ cardiovascular disease ■ cytokines ■ interleukin ■ myocardial infarction

Ischemic heart disease remains an ongoing health issue with the 2017 Global Burden of Disease survey placing it as the top cause of early death and disability. Furthermore, the World Health Organization expects annual deaths from cardiovascular disease (CVD) to increase from 18.1 million in 2010 to 24.2 million in 2030 globally. There is now overwhelming evidence that inflammation and the immune system play a key role in atherosclerosis, and the condition is recognized as a chronic inflammatory disease. Furthermore, the clinical consequences of disease progression, namely plaque disruption, vessel occlusion and, myocardial infarction (MI) or stroke, are also driven by inflammation.

INFLAMMATION AND ATHEROSCLEROSIS

The link between inflammation and atherosclerosis in patients is seen in various forms. For example, the systemic inflammatory biomarker CRP (C-reactive protein) correlates closely with future cardiovascular risk. Furthermore, the driver of its production, IL (Interleukin)-6, is itself linked to atherosclerosis. In patients with stable coronary artery disease, increased levels of IL-6 were associated with increased subsequent MI or sudden death on follow-up. Genetic Mendelian randomization studies showed that the Asp358Ala variant in the IL-6 receptor gene caused cleavage of the membrane-bound receptor, leading to its reduction and consequently higher circulating concentrations of the soluble form of the IL-6 receptor, which decreases membrane IL-6 signaling on leukocytes and hepatocytes. This was associated with a reduced risk of coronary heart disease of 3.4% and 5.0% per allele in 2 large studies, therefore, suggesting a causal link. Autoimmune diseases—like rheumatoid arthritis and systemic lupus erythematosus (SLE) are associated with accelerated development of atherosclerosis. It has been shown that rheumatoid arthritis is associated with enhanced proinflammatory cytokine expression and increased circulating CRP levels.
Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>DAMPs</td>
<td>damage associated molecules patterns</td>
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<td>GvHD</td>
<td>graft versus host disease</td>
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<td>ICOS</td>
<td>inducible T-cell costimulatory</td>
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<td>IFN</td>
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<td>IL</td>
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<td>ILC2s</td>
<td>type 2 innate lymphoid cells</td>
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<td>STAT</td>
<td>signal transducer and activator of transcription</td>
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<td>TCR</td>
<td>T-cell receptor</td>
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<td>TNF</td>
<td>tumor necrosis factor</td>
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arthritis is an independent risk factor for CVD, with the amount of inflammation correlating with atherosclerosis burden and increased cardiovascular mortality for these patients. Similarly, patients with SLE have been shown to have more atheroma on imaging compared to controls, which leads to up to 7× increased risk of having angina, MI, or heart failure.

REGULATORY T CELLS AND TYPE-2 INNATE LYMPHOID CELLS IN THE CONTROL OF THE IMMUNE MECHANISMS OF ATHEROSCLEROSIS

The initiation of atherosclerosis involves endothelial dysfunction, which can be caused by many systemic (e.g., hyperlipidemia, hypertension, smoking, or diabetes mellitus) and local (e.g., low shear stress) factors. This results in increased permeability and active transportation of lipids by endothelial cells to the subendothelial space, where their interaction with proteoglycans causes retention. Modification of lipoproteins causes them to act as DAMPs (damage-associated molecules patterns), which further activate endothelial cells, vascular smooth cells, and resident macrophages, as well as stimulating the recruitment of immune cells from the circulation. Monocytes infiltrate from the blood and differentiate into macrophage subtypes, which engulf the lipoproteins and produce inflammatory mediators. With increasing amounts of lipoproteins, macrophages are overloaded and form foam cells with dysfunctional metabolisms, decreased cholesterol efflux and efferocytosis, and increased cell death, which forms the basis of a necrotic core. Vascular smooth cells migrate to the area of inflammation, proliferate, and can help with plaque healing by switching to a fibrocyte-like phenotype. However, under some circumstances, they may switch to a more detrimental macrophage-like phenotype. The adaptive immune system is also activated by antigen presentation via dendritic cells and macrophages. These cells have been shown to present MHC Class II–restricted CD4+ T-cell peptides from apo B100, which initiates antigen-specific T-cell responses. T cells can differentiate into several types, including the helper T cells (Th1, Th2, Th17, and T follicular helper), of which Th1, Th17, and Tfh are mostly atherogenic, and regulatory T cells (Tregs) which maintain immune homeostasis and are atheroprotective. In patient’s carotid atherosclerotic plaques, T cells are more activated, differentiated, and exhausted compared with their blood counterparts. Furthermore, plaques of symptomatic patients show expansion of an activated effector memory CD4+ T-cell subset. Innate and adaptive B cells are also involved in the inflammatory process through both cellular and humoral responses. More recently other immune cell types, for example, type-2 innate lymphoid cells (ILC2), have been shown to have significant effects in atherosclerosis.

In this focused review, we address the roles of Tregs and ILC2 in CVD and specifically how IL-2 may modulate their response. Although their absolute numbers in blood, lymph organs, arterial plaque, and tissue are low, they represent 2 major adaptive and innate counter-regulatory pathways, respectively, and their impact on the immunologic milieu in disease is substantial. Indeed, a better understanding of the role of these immune cells may lead to a therapeutic breakthrough in CVD treatment.

Regulatory T Cells and Atherosclerosis

Sakaguchi et al identified CD4+ T cells, which express the IL-2 receptor molecule CD25 as responsible for the maintenance of peripheral tolerance. It was shown that if this population is depleted in mice, systemic autoimmune pathologies were unleashed. Subsequent
research in scurfy mice identified the transcription factor (Foxp3) as central for Treg function\(^{28}\) and its expression is a requirement for Treg mediated suppression.\(^{29}\) The lineage can arise in either the thymus or the periphery, Thymic Tregs (also known as natural Treg) differentiate from CD4\(^+\) single-positive precursors after high-affinity TCR (T-cell receptor) stimulation with self-antigen, surviving clonal deletion due to the context of the antigen presentation,\(^{25,31}\) before migration into peripheral tissues. When they originate in the periphery, they are described as induced Tregs, and they are differentiated from naive CD4\(^+\) T cells (ie, already undergone negative selection) subjected to TGF (transforming growth factor) β-initiated transcription at the Foxp3 locus.\(^{32}\) This is stabilized by concurrent IL-2 signaling through STAT5 (signal transducer and activator of transcription 5), which commits the cell to the T regulatory program and prevents switching to other undesired lineages, such as Th17.\(^{33}\)

Tregs exert their regulatory function through secretory factors like IL-10 and TGFβ as well as via direct cell contact, arbitrated by coinhibitory receptors, such as CTLA4 and ICOS (inducible T-cell costimulatory; see Figure). Unsurprisingly, natural or experimental Treg deficiency unleashes systemic inflammation and autoimmunity, and given their capacity for maintaining this inflammatory balance, Tregs are obvious targets for cardiovascular research.\(^{34}\)

Preliminary work interrogated the role of immunoregulatory cytokines IL-10 and TGFβ in plaque development. IL-10 was known to be expressed in the lesion in vivo, it reduced monocyte secretion of IL-12, after ox-LDL (oxidized low-density lipoprotein) activation,\(^{36}\) and importantly, it was shown to be critical at reducing plaque burden in vivo.\(^{36}\) T cells that produce high levels of IL-10, called T regulatory 1 cells, were then shown to limit atherogenesis.\(^{37,38}\) Additionally, disruption of TGFβ signaling resulted in larger plaques with a more vulnerable phenotype.\(^{39,40}\) Subsequently, genetic or antibody-mediated deletion of CD4\(^+\)CD25\(^-\)Foxp3\(^+\)Tregs in mice was shown to accelerate atherosclerosis, in part through TGFβ-dependent mechanisms, whereas adoptive transfer of Tregs was atheroprotective.\(^{23,41}\) It has since been extensively demonstrated that Treg number and function have direct influence on the progression of atherosclerosis, through immune and lipid dependent mechanisms.\(^{42}\)

Tregs from athero-prone strains of mice have diminished regulatory capacity compared to wide-type and are fewer in number.\(^{43}\) Although abundant during the early stages of atheroma development, they progressively decrease as the plaque matures with prolonged hyperlipidemia,\(^{44}\) possibly influenced by ox-LDL levels, an observation which has also made in patients with acute coronary syndromes (ACS).\(^{45}\) Depletion of the pool may be exacerbated by population instability or plasticity within a proatherogenic environment, skewing them towards inflammatory IFN (interferon) γ secreting cells,\(^{46}\) or Th17-like cells.\(^{47}\)

### ILC2 and Atherosclerosis

ILC2 are a rare population of lymphocytes that lack recombined antigen receptors at their surface. They do not express any of the conventional lineage markers (eg, CD3, CD4, CD19), and identification relies on coexpression of defined markers (eg, CD127, ICOS, KLRG1, CD25) in cells expressly lineage-negative. They function as helper-like cells, bridging the innate and adaptive responses through the enhanced production of type-2 cytokines, such as IL-5 and IL-13, or through cell-cell contact regulation (eg, via ICOS on their surface).\(^{48}\) Conventionally, they are activated by cytokines and alarmins, such as IL-25 and IL-33, but more recently, a body of evidence has emerged suggesting there is also a neuroimmune aspect of ILC2 regulation.\(^{49,50}\) They are found in the primary immune organs (eg, bone marrow) as well as resident in tissues, such as the lung, gut, and adipose tissue, and their location relates to their subtype.\(^{51}\)

The role of ILC2 in atherosclerosis and the impact of its signature cytokines on disease development is becoming clearer. IL-5 and IL-13 were previously shown to affect atherosclerosis through various mechanisms, including IL-5-dependent stimulation of innate B1b cells to secrete atheroprotective (natural) IgM,\(^{52}\) and IL-13-dependent control of endothelial inflammation and monocyte maturation into the less inflammatory alternatively activated macrophage phenotype.\(^{53}\) Couple these observations with the atheroprotective effect of IL-33\(^{54}\) and IL-25,\(^{55}\) and it became apparent that there was an ILC2-shaped hole in atherosclerosis (see Figure). This was then confirmed when our own work demonstrated that mice with genetic deficiency of ILC2 have larger plaques and less favorable macrophage phenotype. Crucially, we showed that it is the expression of ILC2 signature cytokine IL-13, which favorably skews the plaque macrophage phenotype towards the wound healing alternative activation program. In this context, the plaques were smaller and had a more mature collagen cap.\(^{26}\) The ILC2 reside in fat-associated lymphoid clusters (complex structures containing a distinct lymphoid population) in the peri-aortic adipose tissue adjacent to the developing lesions, where they would have a modulating effect on the macrophage and B1b cells in their proximity.

### REGULATORY T CELLS AND ILC2 IN THE CONTROL OF THE IMMUNE MECHANISMS OF MI

Progression of atherosclerosis can cause plaque disruption, blockage of the coronary vessel, and MI. Modern
treatment pathways focus on the early restoration of blood flow to the ischemic region. However, the inevitable myocyte damage causes release of intracellular molecules, such as DAMPs, high-mobility group box-1, heat shock proteins, nucleic acids, and mitochondrial molecules, which activate the immune system. The first systemic immune cells to respond to injury are neutrophils which infiltrate the area and are likely to have initially inflammatory, and later inflammatory resolution, actions within the myocardium (reviewed in Wang). Shortly after neutrophils infiltration, inflammatory Ly-6Chigh monocytes are attracted to the area mostly by CCL2 expression in injured tissue. After a few days, the heart switches to a CX3CL1-mediated recruitment of reparative Ly-6Clow monocytes, which support angiogenesis, myofibroblast accumulation, and extracellular matrix synthesis in part through VEGF (vascular endothelial growth factor) and TGF-β secretion. Some of the infiltrating Ly-6Chigh monocytes turn into classically activated macrophages which secrete high levels of proinflammatory cytokines IL-12, IL-23, IL-1, and IL-6, whereas Ly6Clow monocytes differentiate into alternatively activated macrophages and promote tissue healing and angiogenesis. The classically/alternatively activated paradigm represent 2 extremes of macrophage phenotype, although the reality is that the population is much more heterogeneous. Furthermore, it has been shown that resident tissue CCR2+ macrophages promote monocyte recruitment via an MYD88 dependent mechanism, whereas tissue-resident CCR2+ macrophages inhibit monocyte recruitment. Interestingly, depletion of CCR2+ macrophages before MI, resulted in improved left ventricular (LV) systolic function, smaller LV chamber dimensions, whereas depletion of CCR2- macrophages resulted in decreased LV systolic function and larger LV dimensions.

B-lymphocytes infiltrate the mouse myocardium after experimental MI and are activated by DAMPs and damaged myocardial tissue. Antibody-mediated depletion of CD20 or BAFF (B-cell activating factor) in experimental MI models reduced monocytes recruitment and improved cardiac function.

Regulatory T Cells and MI

During normal myocardial homeostasis, CD4+ T cells recognize cardiac antigens, like MYHCA (myosin heavy chain alpha), presented by dendritic cells and differentiate into Tregs to ensure continued immune-tolerance.
After an MI, T-lymphocyte numbers increase significantly, and they are primed to differentiate into inflammatory Th1 and Th17 cells. Interestingly, however, the infarcted myocardium remains conducive to the recruitment and differentiation of (MYHCA-specific) Tregs, where they act to resolve inflammation and promote repair via decreased cardiomyocyte apoptosis and increased proliferation, decreased accumulation of inflammatory macrophages and T cells, increased alternative macrophage activation, and modulation of fibroblast activation and extracellular matrix deposition. It is essential that the Tregs are appropriately activated. Reports of dysregulated Treg function highlight that in these situations, the loss of an effective Treg pool has adverse effects leading to ischemic cardiomyopathy and adverse LV remodeling. In patients with MI, several studies have shown a perturbation of the T-cell repertoire with expansion of the effector T-cell subset, including antigen-specific effector T cells and increased ratio of Th1 helper T cells. Along with the activation of effector T cells, the number and function of Tregs appear to be decreased in patients with ACS. This balance of effector and regulatory T cells seems to be important in orchestrating the immune response in atherosclerotic disease. Indeed, in a prospective study, low levels of circulating baseline CD4+Foxp3+ Tregs were associated with an increased risk for acute coronary event. Thus, maintaining an appropriately activated Treg pool presents a clear clinical target for stabilizing the chronic inflammation in atherosclerotic lesions as well as improving the outcome of MI.

ILC2 and MI

The function of ILC2 in MI is less clear currently but is starting to emerge. Recently, Bracamonte-Baran et al detected tissue-resident ILC precursors maintained in steady-state conditions in the cardiac muscle, which expand and differentiate into natural ILC2 during periods of myocarditis and ischemic cardiomyopathy. This was replicated in mouse models of these conditions, and the expansion was dependent on fibroblast-derived IL-33. The presence of ILC2 during the early stages of ischemic injury is an important observation as it expands our knowledge on the surveillance function that these cells perform and suggests a time frame in which they start to orchestrate the tissue repair and wound healing during recovery. It is also worth considering other niches adjacent to the heart as a source of ILC2 during MI, in particular, the adipose tissue of the pericardium. Theoretically, ILC2 may modulate postischemic cardiac remodeling through several mechanisms. For example, they could promote type-2 alternative activation of macrophages, and they could interact with mast cells and Tregs to preserve cardiomyocyte contractility and promote better tissue repair. ILC2 have also been shown to produce amphiregulin, a member of the epidermal growth factor family, which may have a protective role during myocardial ischemia-reperfusion injury. In turn, amphiregulin may maintain the suppressive function of Tregs through stabilization of Foxp3 expression.

Finally, it is of interest that ILC2 have also been shown to be altered in other infarct-driven pathologies. Patients with atherosclerotic cerebral infarction may have modified ILC1/ILC2 ratios in their peripheral blood, possibly due to the expression of type-1 cytokines. The authors also observed that ILC2 proportion decreased as ox-LDL levels increased in a dose-dependent manner, mirroring our own observation that ILC2 are less abundant in mice maintained on a high-fat diet. Mechanistically, this is an important finding as it may suggest a link between cholesterol levels, type-1 inflammation, and the abundance of a cardiovascular protective cell population.

IL-2 THERAPY TO EXPAND REGULATORY T CELLS AND ILC2

The unique protective roles of Tregs and ILC2 in atherosclerosis and tissue functional recovery after ischemic injury suggest that a therapy that simultaneously targets those 2 pathways may be highly beneficial to limit atherosclerotic disease (see Figure). Interestingly, both Tregs and ILC2 are activated by IL-2, and here, we present IL-2 as a prototype of such a therapeutic strategy.

Low-Dose IL-2

The dose of IL-2 may be the key to using this cytokine as a successful treatment for CVD through augmenting these 2 protective immune cell types. It is often commented in the literature that IL-2 has a paradoxical effect within T-cell biology. On the one hand, it can augment the proliferation and survival of effector T cells, whereas at the same time, it is essential for the development of Tregs. The key to this conundrum seems to be the activation threshold for each respective cell type. Work by Yu et al demonstrated that Tregs have a lower threshold for activation by IL-2 compared with effector cells (reviewed in Klatzmann and Abbas). They argue that uniquely in Tregs, the phosphorylation of STAT5 downstream of the IL-2 receptor complex saturates at concentrations of IL-2 hundred-fold lower than the equivalent effector cells. By utilizing these lower doses, Tregs can, therefore, be safely expanded in vivo.

In the case of ILC2, IL-2 can be thought of as a costimulatory cytokine that functions to support the proliferation and survival of the cell both in vitro and in vivo. Indeed, early identification of a subset of ILC2 then named the natural helper cell, demonstrated that IL-2 could maintain viable ILC2 cultures for an extended period of time. It appears to be less capable at independently inducing
type-2 cytokines, and although the literature suggests that IL-5/IL-13 secretion is possible after IL-2 stimulation, others maintain that this effect is due to increasing the ILC2 population rather than the cytokine production of individual cells. However, it is clear that IL-2 functions to greatly increase type-2 cytokine secretion in cells when co-stimulated with the activatory cytokines IL-33 or IL-25. Interestingly, it also allows the ILC2 to modify their secretory repertoire to include the production of the atheroprotective IL-10.

Other Strategies that Target IL-2

Several IL-2-based approaches that target Tregs (and potentially ILC2) have been employed recently.

Complex With Antibodies
The IL-2 receptor has 3 component parts, IL2Rα (CD25), IL2Rβ (CD122), and IL2Rγ (CD132). By generating immunocomplexes of recombinant IL-2 and antibodies which interfere with the binding to select parts of the IL-2 receptor, it is possible to direct the expansion of preferred cell types. An example is the IL-2/Jes6-1 immunocomplex. This monoclonal antibody binds to a site on the IL-2 molecule required for interaction with CD122 (CD122). At the same time, it also lowers the binding affinity of the IL-2/Jes6-1 complex to CD25, preferentially selecting for cells which express high amounts of IL2Rγ (CD132). By generating immunocomplexes of recombinant IL-2 and antibodies which interfere with the binding to select parts of the IL-2 receptor, it is possible to direct the expansion of preferred cell types. An example is the IL-2/Jes6-1 immunocomplex. This monoclonal antibody binds to a site on the IL-2 molecule required for interaction with CD122 (CD122). At the same time, it also lowers the binding affinity of the IL-2/Jes6-1 complex to CD25, preferentially selecting for cells which express high amounts of IL2Rγ (CD132).

IL-2 Fusion Proteins
This requires the production of chimeric proteins containing an active component of IL-2 conjugated to a second protein. Variations of this theme include CD25 fusion proteins, which improved half-life and expanded Tregs, or improved atheroma targeting through IL-2 coupling with an antifibronectin antibody, allowing local expansion of Tregs in atherosclerotic plaques. A chimeric protein containing IL-2 and IL-33 domains could be of particular interest. This protein retains the activities of both cytokines and thus expands Treg and ILC2 simultaneously.

Bioengineered IL-2
This involves direct modification of IL-2 protein structure to manipulate receptor signaling. The approach attempts to control IL-2 receptor binding dynamics through point mutations in the loop regions of the IL-2 molecule. Early work used directed evolution to increase the binding affinity with CD25 increasing T-cell growth. More recently, Peterson et al engineered an IL-2 molecule with reduced binding affinity to CD122, reducing by 30- to 80-fold the activation of T effectors and natural killer (NK) cells and favoring the expansion of Tregs.

IL-2 THERAPY IN AUTOIMMUNE AND INFLAMMATORY DISEASES
A recombinant form of IL-2 was first licensed in 1990s under the trade name Proleukin (aldesleukin) to treat metastatic renal cell carcinoma and, in certain markets, metastatic melanoma. However, it had limited success due to toxicity at high doses, a short half-life, and an expansion of Tregs. Indeed, relatively low doses of IL-2 were initially shown to increase CD4+CD25+Foxp3+ Treg numbers in cancer patients. Accordingly, the focus shifted to its use in autoimmune or inflammatory conditions with the first 2 major publications in this field being in graft versus host disease (GVHD) and hepatitis C virus vasculitis. The former article used 3 doses of 0.3, 1, and 3×10⁶ IU/m² per day subcutaneously for 8 weeks in total, whereas the latter used 1.5×10⁹ IU/d for 5 days, followed by three 5-day courses of 3×10⁶ IU/day at weeks 3, 6, and 9. They both significantly increased Treg numbers in blood with limited side effects. Although both were open-label and small trials, they demonstrated proof-of-concept and promising clinical data. Following on, ld-IL-2 was trialed in diabetes mellitus. Twenty-four patients with type-1 diabetes mellitus were randomized to placebo or 3 dose groups (0.33, 1, or 3×10⁶/day subcutaneously) for a 5-day course. The authors found the drug to be well tolerated; however, the top dose caused expansion of T effector cells and NK cells with mild-moderate side effects recorded. Crucially no metabolic disturbances were seen. Ld-IL-2 has also been trialed in early phase studies in SLE, thrombocytopenia, alopecia areata, polymyositis, healthy volunteers, and a trial which included 11 different autoimmune conditions. Recently, larger phase 2 studies have been completed in SLE and GVHD. The SLE trial was double-blind and placebo-controlled with 60 patients. Although it failed to meet its primary endpoint of a difference in response rate comparing ld-IL-2 and placebo (55.17% versus 30.00%; P=0.052) at 12 weeks, it did show a difference at 24 weeks (65.52% versus 36.67% ld-IL-2 versus placebo; P=0.027). The GVHD trial was open-label and included 35 patients with steroid-refractory chronic GVHD. Of the 35 patients, 20 of the patients had clinical responses in multiple organ systems, whereas 3 had clinical progression.

POTENTIAL OF IL-2 THERAPY IN CVD
Low-Dose IL-2 in the Treatment of Coronary Artery Disease
Despite CANTOS demonstrating proof-of-concept that treating inflammation can improve patient’s cardiovascular outcomes, there remains a lack of approved therapies to target this process. Although the IL-1β pathway seems causative in CAD, it has been argued...
that targeting this pathway chronically may be problematic. The absolute risk of cardiovascular death may not be high enough in the general CAD population to balance the potential risk of severe or life-threatening infection, especially when the necessary treatment period in secondary prevention is poorly defined. Targeting the adaptive immune response and especially Tregs is attractive as it is pivotal in the resolution of inflammation and repair mechanisms. Furthermore, evidence from autoimmune clinical trials targeting Tregs, B cells, and T cells seem to suggest that any infection risk can be minimized.

Therefore, we wanted to test 2 hypotheses: first, we can safely and effectively increase the Treg population in patients with ACS, and second, whether this might be beneficial for clinical outcomes. To do this, we have set out to perform 2 clinical trials sequentially. LILACS (Low-Dose Interleukin-2 in Patients With Stable Ischemic Heart Disease and Acute Coronary Syndrome) is a randomized, placebo-controlled, dose-escalation, double-blind, phase 1/2a clinical trial. Two populations were tested, first patients with stable ischemic heart disease (part A) and then patients with ACS (part B). The primary outcome was to assess the safety and tolerability of Id-IL-2 in both patient groups and to calculate the optimal dose to increase Tregs by 75% without increasing T effector cells in the ACS group. The dose range we tested in LILACS was 0.3 to 3 × 10^6 IU/d subcutaneously for 5 days. In part A, the starting dose group was 0.3 × 10^6 IU/d, and subsequent groups had increasing doses. Part B took the safety and pharmacodynamic information from part A and focused on the optimal dose for the targeted ACS group of patients. In addition, we accessed the secondary end points like the change in effector T cells, ILC2, B cells, and NK cells.

With a suitable dose discovered in LILACS and adequate safety data (unpublished data), we plan to progress to IVORY (Low-Dose IL-2 for the Reduction of Vascular Inflammation In Acute Coronary Syndromes). This is a randomized, placebo-controlled, double-blind, parallel-group, phase 2 clinical trial where 60 patients will be allocated to Id-IL-2 or placebo with daily subcutaneous injections for the first 5 days and then weekly injections at the same dose for a total of 8 weeks. The primary end point is the change in vascular uptake of glucose using ^18^F-fluorodeoxyglucose positron emission tomography/computed tomography as a measure of vascular inflammation. Although in vitro macrophages appear to uptake additional glucose during hypoxia and not with cytokines stimulation, the hypoxic microenvironment in vivo is inflammatory, and inflammatory stimuli may feedback through the induction of hypoxic signaling pathways. For example, TLR4 stimulation in human macrophages induces HIF1α expression, which promotes macrophage inflammation and is associated with inflammatory plaques. Indeed, this may, in part, explain the close correlation seen between macrophage content and fluorodeoxyglucose–positron emission tomography signal in patients and animal models. As an exploratory end point, we will also assess the effect of Id-IL-2 on ILC2s and its correlation with vascular inflammation.

IL-2 Therapy in other CVD Settings

IL-2 Therapy in Heart Failure

One of the signatures of chronic heart failure is elevated cytokines, including TNF (tumor necrosis factor)-α, IFN-γ, IL-1β, IL-6, IL-17, and IL-18. TNF-α was a promising early therapeutic target; however, 3 randomized controlled trials showed no benefit and possible harm. Other immune-modulating therapies have been attempted, including celecide, pentoxifylline, intravenous immunoglobulins, and methotrexate. Disappointingly, all showed negative or inconclusive results. Canakinumab has been shown to decrease the incidence of hospitalization and mortality related to heart failure in a population of patients after MI and with hsCRP >2 mg/L. However, no difference was found in outcomes in patients with heart failure at baseline.

A role for Tregs in heart failure is emerging. Preclinical mouse models have shown that they are expanded and activated and that increasing their numbers with the IL-2/JES6-1 complex before transverse aortic constriction caused a markedly reduced lung and right ventricular weight and improved LV ejection fraction and LV end-diastolic pressure. However, in a chronic heart failure model, depletion of Tregs seem to reverse ischemic cardiomyopathy LV remodeling and dysfunction, suggesting that they can phenotypically switch to a dysfunctional proinflammatory role. In patients with heart failure, circulating numbers and suppressive function of Tregs seem to be decreased and may be associated with outcomes. Therefore, we can hypothesize that using Id-IL-2 to increase Treg numbers early after an MI and before the spiraling downward cycles of end-stage heart failure, might be beneficial. Furthermore, congestive heart failure is associated with multiple organ dysfunction, including kidney, liver, and skeletal muscle, which might also benefit from increased Tregs. Interestingly, in muscle tissue, Treg regulation is mediated via IL-33, which provides a common link between Tregs and ILC2s. One can, therefore, hypothesize that treating with Id-IL-2 may have a holistic effect on the syndrome of heart failure through multiple organ systems.

IL-2 in Transplant Rejection and Arteriosclerosis

In preclinical experiments, ex vivo expanded human Tregs have controlled rejection in human allografts in humanized mouse models and furthermore prevented transplant-associated atherosclerosis. Several small clinical
studies have reported use of ex vivo expanded Tregs in kidney and liver transplantation with limited success, and there are a number of ongoing clinical trials (reviewed in[144]). Currently, there are no trials testing Id-IL-2 in transplant disease. However, it is tempting to hypothesize a beneficial effect as adjunctive therapy, especially in transplant-associated arteriosclerosis.

**IL-2 Therapy in Stroke**

In preclinical mice models of stroke, Tregs appear beneficial with acute depletion causing increased brain damage and deteriorated functional outcomes,[161] whereas increasing their numbers using the JES6-1 IL-2/ab complex reduced infarct volume, inhibited neuroinflammation, and improved sensorimotor functions.[142] However, there are concerns that Tregs may cause microvascular dysfuntion in a mouse model of stroke and may actually increase infarct size.[143]

**POTENTIAL LIMITATIONS TO THE USE OF IL-2 IN THE TREATMENT OF CVDS**

Although it is potentially beneficial to utilize IL-2 to expand Tregs (and potentially ILC2), the possibility for off-target effects should also be considered. In patient trials using Id-IL-2 to treat hepatitis C virus–induced vasculitis or GvHD, NK cells increased during the treatment period.[106,144] Although NK cells are a potential source of inflammatory cytokines, such as IFN-γ and TNF-α as well as containing cytolytic granules, in experimental models of atherosclerosis they do not appear to exacerbate disease.[145]

The synergistic effect IL-2 has on IL-5 secretion from ILC2 is thought to contribute to moderate eosinophilia.[146] The potential impact of the latter on the progression of atherosclerosis is currently unknown. Although hypereosinophilia has been linked to rare cases of myocarditis (reviewed in Dinis[147]), recent data suggest that eosinophils may contribute to myocardial repair after ischemic injury.[148] Finally, B-cell numbers have been seen to decrease with Id-IL-2. Mature B cells have been extensively investigated in both atherosclerosis (reviewed in Sage et al[25]) and MI[63] where their role in preclinical models seems to be detrimental. Indeed, an early phase clinical trial is currently looking at B-cell depletion in acute MI (URL: https://www.clinicaltrials.gov; Unique identifier: NCT03072199). Therefore, the decrease seen in B cell numbers with Id-IL-2 may prove to be beneficial.

**CONCLUSIONS**

We have long known the essential role the immune system plays in CVD; however, our inability to effectively target this with pharmacological therapy is partially due to its complexity and vast interconnections. With our increased understanding of the disease process and as we untangle this complex Web, there are exceptional opportunities to leverage this to our advantage. As discussed, the effect of Id-IL-2 is not specific to Tregs, and the possibility of also recruiting ILC2, which may have additional atheroprotective effects, is tantalizing. Furthermore, there is the possibility to combine immunotherapies to simultaneously target complementary pathways; for example, chimeric IL-2/IL-33, IL-2 and B-cell depletion, and IL-2 combined with Treg-promoting vaccination against atherosclerosis. We, therefore, propose that the future of immune-modulation therapy in CVD is bright.

**ARTICLE INFORMATION**

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**Disclosures**

None.

**REFERENCES**

Zhao et al IL-2 Therapy in Cardiovascular Disease


Zhao et al. IL-2 Therapy in Cardiovascular Disease


91. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, Furusawa J, Klatzmann D, Abbas AK. IL-2 Therapy in Cardiovascular Disease


125. Coletta AP, Clark AL, Banarjee P, Cleland JG. Clinical trials update: Anti-TNF Therapy Against Congestive Heart Failure Investigators. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-α, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation. 2003;107:3131–3140. doi: 10.1161/01.CIR.0000077913.03634.02


128. Coletta AP, Clark AL, Banarjee P, Cleland JG. Clinical trials update: Anti-TNF Therapy Against Congestive Heart Failure Investigators. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-α, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation. 2003;107:3131–3140. doi: 10.1161/01.CIR.0000077913.03634.02


