Tregs and Human Atherothrombotic Diseases
Toward a Clinical Application?

Giuseppina Caligiuri, Antonino Nicoletti

Immunology has recently penetrated the field of atherothrombosis. A number of human and experimental studies have documented that both cellular and molecular immune effectors are involved at various stages of the pathological process. This has directed our attention toward the use of immunomodulatory strategies in atherothrombosis; these strategies are commonly used for the treatment of other chronic inflammatory diseases. Notably, the administration of immunosuppressive drugs is the most common approach for treating immune-mediated diseases. However, the long-term administration of non–antigen-specific agents that cannot distinguish between beneficial and destructive immune responses is a major drawback of this strategy.

Interesting alternatives consist of strategies aimed at potentiating the physiological regulatory mechanisms, if defective. In the field of atherothrombosis research, experimental studies have clearly shown that a defect in regulatory CD4+ T cells (Tregs) favors atherogenesis. Based on their potent immunosuppressive activity, the expansion of the Treg compartment by cell transfer or the administration of stimulating immunosuppressive activity, the expansion of the Treg compartment is associated with the progression of atherosclerotic disease and the occurrence of atherothrombosis.

These findings have provided a strong incentive to perform clinical studies with the aim of assessing whether a defective Treg compartment by cell transfer or the administration of stimulating molecules can be envisaged as a new therapeutic strategy. These findings have clearly shown that a defect in regulatory CD4+ T cells (Tregs) favors atherogenesis. Based on their potent immunosuppressive activity, the expansion of the Treg compartment favors atherothrombosis.

In the present issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, a larger clinical study reports interesting challenges to these findings; a second study explores the role of Tregs in stenotic and dilative pathological remodeling of atherosclerotic aortas.

Before opening the debate on the role of Tregs in clinical atherothrombosis, it is important to reach a consensus on the phenotypic markers of Tregs. Indeed, the definition of these suppressive T cells can be equivocal because of their heterogeneous origin and consistent differences in their characteristic markers between humans and mice. Initially, Tregs were defined as CD4+CD25+high. Because this population may also include activated effector T cells, it is not surprising that the number of CD4+CD25+ Tregs can be highly variable and does not differ between healthy individuals and patients diagnosed as having coronary artery disease or an abdominal aortic aneurysm. The demonstration that Tregs require the fork head box P3 transcription repressor has allowed discrimination of Tregs among CD4+CD25+ T cells. However, this requires a complex (intracellular) staining technique that is suitable for experimental studies but difficult to use routinely in longitudinal clinical studies. To overcome this technical hurdle, (low) CD127 expression can be used as a surrogate marker of Tregs.

By using this identification strategy, Ammirati et al have identified Tregs as CD3CD4CD25highCD127low in patients with carotid or coronary atherosclerosis and have evaluated the diagnostic/prognostic value of measuring their circulating levels. Although the previous studies reported a significant decrease in the percentage of blood Treg levels in patients diagnosed as having a myocardial infarction, Mor et al found that these levels were, on the contrary, significantly increased (Table) in the blood of these patients.

Even though the method used to identify Tregs was different, the patient population and the time of sampling were similar in the 4 studies; therefore, methodological issues cannot be used to explain the different results. This means that the evidence for a role for Tregs in myocardial infarction requires further clarification. Furthermore, it is mandatory to establish the biological significance of circulating Tregs. Indeed, a variation in the number of Tregs in the blood may reflect either a global change of the pool or that, at the time of sampling, they have been redistributed between the blood and sites of inflammation. Recent experimental work suggests that Tregs in the vascular compartment infiltrate the inflamed tissue; migrate to draining lymphoid organs, where they suppress the generation of immune effectors; and then recirculate back to the inflamed tissue, where they contribute to the downregulation of local immune responses. Therefore, the dynamics of Treg trafficking hamper the interpretation of their peripheral blood count at a given time point.

Nonetheless, in unstable angina, the 4 clinical studies consistently found that the percentage of circulating Tregs is decreased, suggesting that this characterizes patients with acute coronary syndromes without evidence of myocardial necrosis. How can we interpret this finding? We could hypothesize that these patients have a global Treg defect, possibly favoring the destabilization of coronary atherosclerosis. However, we can also envisage that Tregs of these
patients are reduced in the bloodstream because they have been massively recruited at the sites of unstable coronary arteries, where they might attempt to control the inflammatory reaction. If we extrapolate this concept to the whole acute coronary syndrome cohort, this could explain the difference from patients diagnosed as having a myocardial infarction. The relative increase in Tregs in the blood of the latter, reported by Ammirati et al., may indeed reflect an insufficient recruitment of these cells in the inflamed arterial and/or myocardial tissue. Consequently, unrestrained local inflammation would precipitate the transition from unstable angina toward myocardial infarction. If this hypothesis is correct, Treg-based therapeutic strategies will have to overcome the mechanisms that prevent Treg from entering the target tissue in these patients.

The 2 articles published in this issue also point to another interesting aspect: The role of Treg in long-term manifestations of atherosclerosis may depend on the type of vascular remodeling. Indeed, both studies show that in the case of inward remodeling, leading to stenosis of either coronary or carotid arteries or the aorta, the blood Treg count is normal. Thus, the clinical value of peripheral Tregs in patients with stable occlusive atherosclerotic disease appears rather limited, both in diagnosis and as a prognostic marker. Indeed, the blood Treg level was not able to predict either the presence or the progression of carotid atherosclerosis in the 6-year follow-up study of Ammirati et al. In a pathophysiological perspective, the fact that peripheral Tregs are normal in these patients can be interpreted in at least 2 ways: either human atherogenesis does not involve a Treg defect or the Treg compartment is efficient in maintaining tolerance and preventing the inflammatory events that precipitate the occurrence of atherothrombotic events.

In contrast, in patients with dilative vascular remodeling of atherosclerotic aortas, Yin et al. found that blood Treg levels are reduced and display a defective suppressive function in vitro. Taken together, the results of these 2 studies suggest that Tregs may be particularly important for the control of the immune effectors that are involved in arterial wall dilation and thrombosis, 2 pathological features common to atherosclerotic aortas and acute coronary syndromes.

Future experimental and clinical studies are warranted to establish the clinical value of quantifying Tregs in atherothrombosis. Interventional experimental studies should aim at elucidating the causes underlying the Treg defects in atherosclerotic diseases. In addition, large prognostic clinical studies should serve to evaluate the Treg threshold that can identify patients at risk of developing life-threatening atherothrombotic diseases.

As for the therapeutic perspective, despite the data generated in preclinical animal models that successfully show that Tregs can prevent or cure several T-cell–mediated diseases, many questions remain to be addressed for the translation of this approach to the clinic. A major obstacle is technical and relates to cell manipulation. The phenotype used by Ammirati et al. (CD4+CD25+CD127low) allows for the isolation and purification of viable autologous Tregs from the blood; because of their limited number, they need to be further expanded in vitro. However, Tregs are poorly proliferating cells, and the ex vivo expansion of Tregs remains an unresolved issue, especially when antigen-specific Tregs are desired. In fact, current expansion protocols generate polyspecific Tregs that may cause “panimmunosuppression” if transferred in vivo. Presently, the only clinical trials of immunotherapy based on Tregs are performed in patients undergoing bone marrow transplantation for the prevention or cure of graft-versus-host disease.

Finally, although it appears certain that Tregs critically control atherosclerotic diseases, the translation of this knowledge into clinical practice will require extensive future studies.

**References**


**Table. Treg Count Variations in the Blood of Patients With CAD**

<table>
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<th>No.</th>
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<td>↓</td>
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<td>↑↑</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; CSA, chronic stable angina; MI, myocardial infarction; NCA, normal coronary artery; ND, not determined; UA, unstable angina.

1, 2, or 3 arrows indicate the degree and direction of significant differences; the equal sign indicates the absence of difference.

*Data are given as number of patients unless otherwise indicated.

**Key Words:** regulatory T cells ■ acute coronary syndromes ■ abdominal aortic aneurysm ■ biomarkers
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