Antigen-Independent Targeting of Long-Lived CD4+ Cytolytic T Effector Cells to Lesions of Atherosclerosis

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Acute coronary syndromes, most often characterized by fissuring or rupture of coronary lesions, are responsible for the majority of clinical manifestations of atherosclerosis.1–3 Studies of human atherosclerotic plaques after acute events have revealed that lesions that tend to rupture are rich in inflammatory cells and have a thin fibrous cap, implicating the inflammatory response as a key regulator of plaque stability.3–5 Activated inflammatory cells within lesions, particularly activated macrophages, have been considered to be prime candidates for regulating plaque stability by releasing matrix-degrading enzymes and apoptosis-inducing factors. Although CD4+ T cells are a recognized component of vulnerable plaques,3 studies have primarily focused on activities of their associated Th1 cytokines that cause activation of macrophages and other vascular cells.6 However, analysis of CD4+ T cells from patients with acute coronary syndrome have shown these T cells acquire unique properties including antigen-independent cytolytic capabilities that can kill endothelial cells and smooth muscle cells in vivo.7–9 In this issue of Circulation Research, trafficking of these CD4+ T effector cells into lesions is also shown to be independent of antigen stimulation,10 thus rendering them dangerous as they elude normal tolerance control.

Immune surveillance involves constant recirculation of lymphocytes through tissues. Naïve T cells traffic from the blood into the secondary lymphoid organs and then back into the blood via the afferent lymph, a cycle that continues until such time that they recognize antigens for which their T cell receptors are specific. Once activated, the T cells proliferate, generating short-lived effector cells that can migrate to B cell receptors are specific. Once activated, the T cells proliferate, generating short-lived effector cells that can migrate to B cell areas or to inflamed tissues. When antigen challenge recedes, a fraction of the primed cells will persist as circulating memory cells, a state that on secondary antigen stimulation allows them to again home to nonlymphoid tissues. The majority of CD4+ T cells within lesions are memory cells.11 Although memory cell regulation is still a very active area of investigation, two distinct subsets have been classified by Lanzavecchia and Sallusto.12,13 Central memory cells reside in secondary lymphoid tissue, have low effector functions, but can proliferate and expand in response to antigen. In contrast, effector memory cells have effector functions within peripheral inflamed tissues, but are short-lived and nondividing. As illustrated in the Figure, these distinct capabilities are reflected by the expression of different chemokine receptors whose ligands reside in secondary lymphoid organs or in peripheral inflamed tissues. Thus, the differential tissue localization is dependent on the presence of the required homing machinery.

The unusual CD4+ CD28null T cell subset described in this issue of Circulation Research resembles effector memory cells (Figure) as they produce high levels of interferon-γ, acquire cytolytic capability, and can effectively kill endothelial cells and smooth muscle cell in vivo.3–9,14 However, they acquire their cytolytic capabilities independent of the T cell receptor due to coexpression of APO2 ligand/tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) capable of inducing apoptosis9 and stimulatory killer immunoglobulin-like receptors that recognize HLA class I molecules and the adapter molecule DAP12 that leads to mitogen-activated protein kinase cascade and ultimately induces degranulation of cytotoxic granules and the release of cytokines.8 These CD4+ CD28null T cells also share features of central memory T cells (Figure) because they can undergo clonal expansion within lesions and persist for years in the circulation,15 but they functionally resemble natural killer cells.

An important property of the CD4+ CD28null T cell subset highlighted in the present study10 is expression of both subunits of the interleukin (IL)-12 receptor (R). In contrast, only low-level expression of IL-12Rβ1 receptor was detected on CD4+ CD28+ T cells, and these cells fail to respond to IL-12 administration. Treatment of the CD4+ CD28null T cell subset with IL-12 in vitro leads to increased expression of the chemokine receptor CCR5. CCR5 is the receptor for MIP-1α/CCL3 and RANTES/CCL5 that are both expressed within lesions of atherosclerosis and stimulate recruitment of T cells.16 This report10 further demonstrates that expression of CCL3 and CCL5 in human lesions correlates with levels of T cell receptor. The authors also show that IL-12 stimulation of the CD4+ CD28null T cell subset in vitro enhances chemotaxis and transendothelial cell migration to RANTES/CCL5. More significantly, recruitment of in vitro labeled human CD4+ CD28null T cells into carotid atheromas implanted in severe combined immunodeficiency (SCID) mice was observed, and was enhanced 2-fold by IL-12 treatment. Nondiseased tissue lacking expression of CCL3 and CCL5 did not attract T cell recruitment with or without IL-12 treatment. The recruitment to atheromas implanted in SCID mice was completely inhibited by an antibody to CCR5 and dependent on IL-12 receptor expression by CD4+ T cells.

IL-12 is a potent modulator of T cell and NK cell function that is produced by antigen-presenting dendritic cells and
macrophages in response to “danger” signals.\textsuperscript{17} Such signals include cell–cell interactions like CD40/CD40L and binding of pattern receptor ligands on pathogens as well as heat shock protein 60 and modified low-density lipoproteins to the Toll-like receptors (Figure), all of which have been identified in developing lesions of atherosclerosis.\textsuperscript{18} Optimal expression of IL-12 receptors normally requires prior stimulation through the T cell receptor, and subsequent IL-12 stimulation leads to interferon-$\gamma$ production and controls T cell differentiation into Th1 effector cells. The ability of IL-12 to circumvent the need for antigen triggering and to direct T effector trafficking to inflammatory lesions puts this cytokine in a different context. The consequence is that innate immune activation of IL-12, rather than antigenic stimulation, can control tissue migration of this unique CD4$^+$ CD28$^{\text{null}}$ T cell subset.

A challenge for research attempting to identify key molecular mechanisms involved in cardiovascular pathology is that mouse models only partially recapitulate the human disease and may not accurately reflect the behavior of the human immune system. The current study\textsuperscript{19} uses a novel xenograft model that combines an in vivo milieu with some of the species-specific features of human immunology. Human carotid lesions were implanted in immunodeficient NOD-SCID and NOD-Rag1 mice. Mice engrafted with carotid artery specimens lacking atherosclerotic plaques served as controls, and after 7 days full engraftment of the human tissue was achieved through formation of anastamoses between mouse and human capillaries on the graft surface. Human CD4$^+$ CD28$^{\text{null}}$ T cells were then adoptively transferred into the chimeric mice, and homing and effector function were characterized. Although this model offers many advantages, recruitment of T cells is limited to microvessels as the human tissue transplants do not have a functional lumen. It will therefore be important to confirm the observations in this study in macrovessels. However, the absence in this model of human monocytes and mouse monocyte migration into the human tissues, presumably attributable to poor recognition of
human chemokines and adhesion molecules, makes this approach a particularly sensitive way to study the homing of human T cells.

In healthy individuals, effector T cells do not express CCR7 and do not home to lymph nodes. However, the chemokine receptor expression profile of CD4+ CD28null T cells from acute coronary syndrome patients would be predicted to traffic to lymph nodes as recently shown for CD4+ CD28null T cells with similar properties isolated from patients with rheumatoid arthritis. Although the absolute significance of the CD4+ CD28null T cell subset that increases to 10% of the circulating T cells in acute coronary syndrome patients remains to be established, lymph node residence of this subset could lead to immune-mediated injury through their production of interferon-γ and their cytotoxic activity that is independent of T cell receptor signaling. Any immune stimulation that leads to lymphoid expression of IL-12 would also have the potential to redirect the migration pattern of CD4+ CD28null T cells to lesions and further promote lesion progression. The ability of CD4+ CD28null T cells to home to lymphoid organs may also contribute to their clonal dominance and may further promote the chronic inflammatory nature of the atherosclerotic process. Although the T cell subset is not found in patients with stable angina, it will be interesting to determine whether they are found in other patients at risk for acute events. Does this subset have a role in early stages of lesion formation, or are its actions limited to more advanced lesions? Does CCR5 expression contribute to retention of these effector cells within lesions? Answers to these questions will help determine the implications of potential intervention with IL-12-mediated trafficking of this effector CD4 T cell subset.

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