Over a Hump for Imaging Atherosclerosis
Nanobodies Visualize Vascular Cell Adhesion Molecule-1 in Inflamed Plaque

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The “Holy Grail” in molecular imaging of cardiovascular disease is sensitive, specific, economic, and radiation-free detection of atheromata prone to produce thrombotic complications. In this issue of Circulation Research, Broisat et al describe another step on the path toward noninvasive molecular imaging of inflammation in atherosclerotic plaque. Although still unproven, the early detection of trouble looming ahead could trigger steps for intervention, possibly involving the aggressive modulation of risk factors. Imaging might even help to define individual risk and to guide appropriate local therapy.

The field has provided proof-of-concept studies for a variety of molecular targets. While searching for suitable approaches, imaging scientists have perused the work of molecular biologists and immunologists working in atherosclerosis. The recognition that inflammation drives the development and complication of atherosclerosis offers several potential imaging targets. Adhesion molecules, innate immune cells (monocytes/macrophages), extracellular matrix, oxidized lipids, and proteases all have received scrutiny, but vascular cell adhesion molecule-1 (VCAM-1) has gained considerable attention in this regard. Expressed at low levels in nonatherosclerotic arteries, hypercholesterolemia rapidly induces VCAM-1 expression by endothelial cells in regions prone to atheroma formation. Arterial muscle cells and even macrophages can express VCAM-1 in hypercholesterolemic animals. In vitro, proinflammatory cytokines readily augment the expression of VCAM-1 in endothelial cells and in other cell types relevant to atherosclerosis. VCAM-1 binds just those cell types recruited to the nascent atherosclerotic lesion—namely, monocytes and T lymphocytes—via engagement of the integrin very late antigen-4. VCAM-1 expression precedes leukocyte recruitment during experimental atherogenesis, and genetic interference with VCAM-1 function retards atheroma formation in mice. VCAM-1 expression characterizes the extensive microvascular network found in established human atherosclerotic plaques. These properties render VCAM-1 an attractive target for the molecular imaging of inflammation in atherosclerosis.

Previous studies on the imaging of VCAM-1 have used antibodies or peptides as affinity ligands detected by ultrasound (coupled to microbubbles), by MRI (attached to iron oxide particles or using fluorine-19 for detection), or by nuclear imaging (derivatized with fluorine-18). This earlier work provided evidence for the feasibility in several modalities, addressed the imaging of therapeutic efficiency, and explored a variety of applications.

Broisat et al chose nanobodies as affinity ligands. Nanobodies, single-domain antibody fragments, occur naturally in sharks and camelids. Despite their low molecular weight—they are approximately one-tenth the size of more common antibodies—they selectively bind to antigens with very high affinity. The small molecular weight of nanobodies translates into much more rapid pharmacokinetics when compared with full-size antibodies, which may circulate for days. These considerations have particular importance when molecular targets localize in proximity to the blood pool, because long clearance times would yield unfavorable target-to-background ratios for antibodies. The short blood half-life reported by Broisat et al allowed single-photon emission computed tomography imaging with Tc-labeled nanobodies 3 hours after injection, which is reasonable timing for the isotope half-life (6 hours) and practical from a clinical perspective. Nanobodies retain the specificity and outstanding affinity in the low nanomolar range characteristic of antibodies.

Broisat et al present single-photon emission computed tomography/computed tomography data acquired in apolipoprotein E–/– and wild-type mice controlled by appropriate competition experiments. The next steps toward clinical translation will involve toxicity studies and will explore whether the nanobodies are immunogenic, which could limit their use for repetitive imaging.

Broisat et al pursued an elegant targeting ligand discovery approach (Figure). Making use of the natural occurrence of nanobodies in camelids, they immunized a dromedary with murine and human recombinant VCAM-1. The animal had development of natural nanobodies, which are produced by the expanded B-cell clone. RNA was retrieved from the B cells of the dromedary, and the derived sequences were incorporated into a phage library panned against immobilized VCAM-1. The selected phage clones were then tested for the production of nanobodies in Escherichia coli. This sophisticated discovery process successfully enriched for nanobodies...
that were cross-reactive with mouse and human proteins and could prove suitable for imaging in human patients.

Molecular imaging could transform clinical medicine by uniting advances in the biological understanding of disease with progress in imaging technology. However, many obstacles must be overcome to achieve this promise. Although experimental studies like that of Broisat et al serve as “proof of principle,” translation to humans will require overcoming barriers, including toxicology studies and the production of clinical-grade materials. Ultimately, as with all biomarkers, imaging approaches to improving the management of patients at risk for atherosclerosis will require demonstration of clinical benefit and cost-effectiveness. Only multidisciplinary efforts will help us move the molecular imaging of atherosclerosis from the laboratory into the clinic.1

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References

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