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STATE-OF-THE-ART PAPER

Radionuclide Imaging

A Molecular Key to the Atherosclerotic Plaque

Harald F. Langer, MD,* Roland Haubner, PHD,† Bernd J. Pichler, PHD,‡ Meinrad Gawaz, MD*

Tübingen, Germany; and Innsbruck, Austria

Despite primary and secondary prevention, serious cardiovascular events such as unstable angina or myocardial infarction still account for one-third of all deaths worldwide. Therefore, identifying individual patients with vulnerable plaques at high risk for plaque rupture is a central challenge in cardiovascular medicine. Several noninvasive techniques, such as magnetic resonance imaging, multislice computed tomography, and electron beam tomography are currently being tested for their ability to identify such patients by morphological criteria. In contrast, molecular imaging techniques use radiolabeled molecules to detect functional aspects in atherosclerotic plaques by visualizing their biological activity. Based upon the knowledge about the pathophysiology of atherosclerosis, various studies in vitro and in vivo and the first clinical trials have used different tracers for plaque imaging studies, including radioactive-labeled lipoproteins, components of the coagulation system, cytokines, mediators of the metalloproteinase system, cell adhesion receptors, and even whole cells. This review gives an update on the relevant noninvasive plaque imaging approaches using nuclear imaging techniques to detect atherosclerotic vascular lesions. (J Am Coll Cardiol 2008;52:1–12) © 2008 by the American College of Cardiology Foundation

Cardiovascular diseases are the most frequent causes of death in the Western world and represent a central challenge for modern research and medicine. Rupture of the atherosclerotic plaque accounts for approximately 70% of fatal acute myocardial infarctions and/or sudden coronary deaths (1). Thrombotic complications, which arise from rupture or erosion of a vulnerable plaque, may be clinically silent yet contribute to the natural history of plaque progression and ultimately luminal stenosis (1). Therefore, it seems to be essential to acquire information beyond the resulting degree of stenosis detected by angiography. Over the past years, profound knowledge about the mechanisms involved in atherogenesis was obtained due to ambitious and excellent research. In the future, it is our responsibility to extend this knowledge by utilizing the field of molecular plaque imaging to obtain new strategies to detect vulnerable atherosclerotic plaques that cause critical cardiovascular complications.

The "Vulnerable Plaque"

To identify apparently healthy subjects at risk for future cardiovascular events, a consensus of experts has recently defined criteria for the diagnosis of "vulnerable plaques" (1). Besides minor criteria like calcified nodules, yellow appearance of the plaque, or intraplaque hemorrhage, major criteria have been established that represent different aspects of the rupture-prone plaque (Fig. 1). Major criteria are active inflammation, thrombogenicity, and plaque injury. Further major criteria are related to plaque morphology and include a thin cap, a large lipid core, or severe luminal stenosis (1). Many groups have focused on anatomic imaging modalities such as intravascular ultrasound, magnetic resonance imaging (MRI), multislice computed tomography (MSCT), and electron beam tomography to view the vulnerable plaque (2–4).

Recent approaches using radionuclide imaging are based upon the pathophysiology of atherogenesis and have been evaluated to detect atherosclerotic lesions with a strong focus on the functional aspects of a plaque (Fig. 2). The development of atherosclerotic plaques is a process of complex, consecutive, and interacting steps involving chemokines, the up-regulation of adhesion molecules, recruitment of inflammatory cells to the arterial wall, transmigration of these cells, and the development of lipid-laden macrophages (the so-called foam cells) from invading monocytes (5). As a result of these processes, the early lesion of atherosclerosis—the fatty streak—appears. Subsequently, more complex lesions develop mediated by proliferation and migration of smooth muscle cells and excessive production of extracellular matrix proteins (5). Superficial erosions or fracture of the

From the *Medizinische Klinik III, Eberhard Karls Universität Tübingen, Tübingen, Germany; †Universitätsklinik für Nuklearmedizin, Medizinische Universität Innsbruck, Innsbruck, Austria; and the ‡Laboratory for Preclinical Imaging and Imaging Technology, Clinic of Radiology, University of Tübingen, Tübingen, Germany. Dr. Langer is currently affiliated with the Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

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Abbreviations and Acronyms

ApoE = apolipoprotein E
CT = computed tomography
FCH = fluorocholine
FDG = fluorodeoxyglucose
GPVI = glycoprotein VI
LDL = low-density lipoprotein
MCP = monocyte chemoattractant protein
MDA2 = malondialdehyde epitope on oxidized low-density lipoprotein
MMP = matrix metalloproteinase
MRI = magnetic resonance imaging
MSCT = multislice computed tomography
NIRF = near-infrared fluorescent
ox-LDL = oxidized low-density lipoprotein
PET = positron emission tomography
RGD = protein sequence "arginine-glycine-aspartic acid"
SPECT = single-photon emission computed tomography.

fibrous cap lead to plaque disruption followed by thrombus formation (6). Over the past years, distinct mediators and regulators involved in the cascade of atherosclerosis have been identified, which can be used to develop tools for the detection and characterization of atherosclerotic plaques by means of noninvasive molecular imaging (Table 1). The different approaches include radionuclide-labeled lipoproteins (7), components of the coagulation system (8), cytokines (9), mediators of the matrix-metalloproteinase (MMP) system (10), cell receptors (11), and even whole cells (12). This review gives an update on the relevant noninvasive approaches in plaque imaging with a focus on radionuclide-based techniques to detect vulnerable vascular lesions.

Nuclear Imaging Versus Other Noninvasive Modalities to Visualize the Atherosclerotic Plaque

Coronary angiography has so far been the gold standard to assess narrowing of the vessel lumen. Other invasive techniques have been introduced that provide additional information for plaque characterization, including intravascular coronary ultrasound, angioscopy, intravascular elastography, termography, or optical coherence tomography. All of these techniques have the disadvantage of being invasive. Toward the same end, increasing effort has been placed on noninvasive imaging techniques. The 3 major modalities used are MRI, MSCT, and nuclear imaging, each of them having advantages and disadvantages. In addition to stenosis detection, electron beam computed tomography (CT), dual source (dual energy), and MSCT are capable of picturing the coronary artery wall. By using MSCT, various components of the atherosclerotic wall may be distinguishable, allowing for identification of calcified areas or hypodensities, which correlate to echolucent areas seen with intravascular ultrasound (13,14). While such hypodense areas are suggestive of lipid-rich plaques, differential diagnosis of such areas can include other tissue types such as less dense fibrous tissue. Thus, while providing a better spatial resolution than nuclear imaging techniques, CT-based techniques may give less information on distinct plaque components aside from the level of calcification. Using MRI, T1- and T2-weighted imaging can provide in vitro information of the atheromatous core, collageneous cap, and calcifications (15). Although MRI provides good spatial resolution, results at the coronary artery level have not yet been convincing, as the arteries are small, tortuous vessels in continuous movement, which causes image aquisition difficulties. However, contrast-enhanced sequences have been tested and

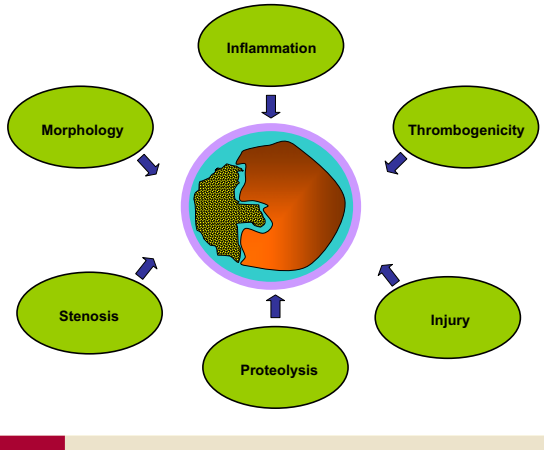

Figure 1 Major Criteria Defining the "Vulnerable Plaque"

Vulnerable atherosclerotic plaques are characterized by distinct attributes regarding morphology, stenosis, inflammation, thrombogenicity, injury, and enhanced proteinase activity.

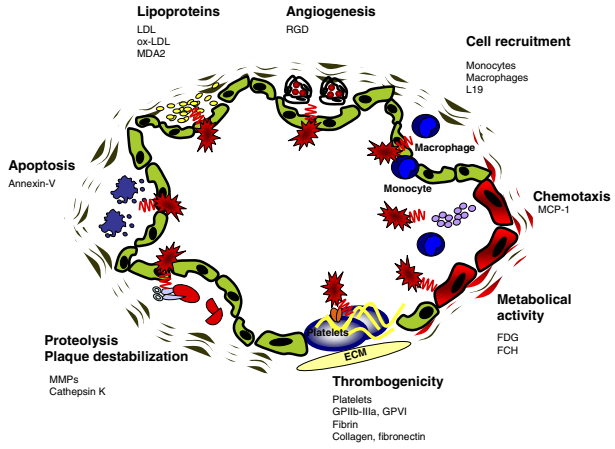

Figure 2 Molecular Principles to Detect Vulnerable Atherosclerotic Plaques

Based upon the increasing molecular knowledge regarding atherogenesis, different principles have been successfully used to image atherosclerotic plaques. One major complex is the molecular imaging of inflammation, which includes enhanced metabolic activity, chemotaxis, cell recruitment, and lipoprotein accumulation. Furthermore, mediators of angiogenesis, apoptosis, and matrix metalloproteinase (MMP) activity have been successfully applied. Another promising approach to detect vulnerable atherosclerotic plaque is the visualization of plaque thrombogenicity, including thrombosis and exposure of thrombogenic subendothelial matrix proteins. ECM = extracellular matrix; FCH = fluorocholine; FDG = fluorodeoxyglucose; GP = glycoprotein; LDL = low-density lipoprotein; L19 = antibody against the extra-domain B of fibronectin; MCP = monocyte chemoattractant protein; MDA2 = malondialdehyde epitope on oxidized low-density lipoprotein; ox-LDL = oxidized low-density lipoprotein; RGD = protein sequence "arginine-glycine-aspartic acid."

Table 1 Different Approaches to Visualize Atherosclerosis by Radionuclide Imaging

Underlying Plaque Biology	Radionuclide Tracer	Experimental Setting	Reference
Inflammation	^{99m}Tc-LDL	Human carotid/ileofemoral artery	Lees at al. 1988 (48)
Lipoprotein accumulation	^{123}I-LDL	Human carotid artery	Virgolini et al. 1991 (53)
	^{125}I-LDL	Rabbit aorta	Virgolini et al. 1991 (54)
	^{99m}Tc-ox-LDL	Human carotid artery	Iuliano et al. 1996 (57)
	^{123}I-MDA2 (Ab to ox-LDL epitope)	Rabbit arteries	Tsimikas et al. 1999 (49)
	^{125}I-IK17 (Ab to ox-LDL epitope)	Mouse aorta	Shaw et al. 2001 (50)
	^{123}I-SP4 (apoliprotein B fragment)	Rabbit aorta	Hardoff et al. 1993 (51)
	^{125}I-SP4	Rabbit aorta	Lu et al. 1996 (52)
Chemotaxis	^{125}I-MCP-1 (chemotactic molecule)	Rabbit aorta	Ohtsuki et al. 2001 (9)
Angiogenesis	^{125}I-c(RGD(I)yV) (peptide binding $\alpha_v\beta_3$)	Murine ischemic hindlimbs/HUVECs	Lee at al. 2005 (33)
	^{99m}Tc-(NC100692) (peptide binding $\alpha_v\beta_3$)	Murine ischemic hindlimbs	Hua et al. 2005 (47)
Monocyte recruitment/activity	^{111}In-monocytes	Human arteries	Virgolini et al. 1990 (17)
	^{18}F-FDG (metabolic activity)	Rabbit iliac artery	Lederman et al. 2001 (18)
	^{18}F-FDG	Human carotid artery	Rudd et al. 2002 (20)
	^{18}F-FDG	Human arteries	Ben Haim et al. 2004 (19)
	^{18}F-FDG in comparison with ^{18}F-FCH	Mouse aortae	Matter et al. 2007 (24)
	VHSPNKK-modified magnetofluorescent nanoparticle (VNP) (VCAM-1 expression)	Mouse carotid artery	Kelly et al. 2005 (31)
Apoptosis	^{99m}Tc-annexin V (phosphatidylserine expression)	Rabbit aorta	Kolodgie et al. 2003 (72)
	^{99m}Tc-annexin V	Human carotid artery	Kietselaer et al. 2004 (70)
	^{99m}Tc-annexin V	Swine coronary artery	Johnson et al. 2005 (71)
	^{99m}Tc-annexin V	Mouse aorta	Isobe et al. 2006 (75)
Proteolysis	^{123}I-HO-CGS27023 A (MMP inhibitor)	Mouse carotid artery	Schäfers et al. 2004 (10)
	GHPGGPQKC-NH_2 (cathepsin K substrate)—NIRF probe	Mouse aortae and human carotid arteries	Jaffer et al. 2007 (66)
Thrombogenicity and cell recruitment	^{111}In-platelets	Human carotid artery	Minar et al. 1989 (76)
	^{111}In-platelets	Human carotid artery	Moriwaki et al. 1995 (77)
	^{125}I-GPVI/^{123}I-GPVI (platelet collagen receptor)	Mouse carotid artery	Gawaz et al. 2005 (11)
	^{99m}Tc-DMP-444 (GPIIb-IIIa inhibitor)	Canine coronary artery	Mitchel et al. 2000 (78)
	^{99m}Tc-T2G1s Fab (fibrinogen binding)	Canine femoral/carotid artery	Cerqueira et al. 1992 (79)
	^{125}I-L19 (fibronectin binding)	Mouse aorta	Matter et al. 2004 (82)

FCH = fluorocholine; FDG = fluorodeoxyglucose; GP = glycoprotein; HUVEC = human umbilical vein endothelial cell; LDL = low-density lipoprotein; MCP = monocyte chemoattractant protein; MDA2 = malondialdehyde epitope on oxidized low-density lipoprotein; MMP = matrix metalloproteinase; NIRF = near-infrared fluorescent; ox-LDL = oxidized low-density lipoprotein; RGD = protein sequence "arginine-glycine-aspartic acid"; VCAM = vascular cell adhesion molecule.

shown to improve the sensitivity of the method (16). Computed tomography and MRI mainly provide information based on morphology or anatomy.

The strength of nuclear imaging is its ability to provide quantitative information on a functional level such as density of a specific receptor or the metabolic activity of a plaque. Nuclear imaging is based on radiolabeled biomarkers with a signal sensitivity in the pico-molar range, which is 1 million to 1 billion times above that of MRI or CT. Thus, nuclear imaging may be the most promising approach for vulnerable plaque detection. Currently, the leading modality in nuclear medicine is positron emission tomography (PET), which acquires images with a spatial resolution up to 4 to 5 mm, thereby improving on the performance of single-photon emission computed tomography (SPECT), which has a resolution of 10 to 15 mm. To obtain good-quality images, the radiotracer must have rapid clearance from the bloodstream and a good target-to-background ratio. Given the small size of the plaque, in which the radionuclide concentrates, a high background signal can impair the quality of PET or SPECT images. Thus, the identification of promising highly specific molecular targets is of central importance. Another problem that complicates imaging of coronary arteries is related to vessel anatomy and heart pulsatility. Although the fast imaging protocols of MRI or CT are advantageous over the relatively slow PET or SPECT imaging, modalities such as cardiac gating can improve motion artefacts in cardiac imaging. Current research in imaging technology centers on the development of multimodality scanners, such as PET/CT or more recently PET/MRI, to provide comprehensive morphological and functional information during 1 scan session. Such technical advance, in particular PET/MRI, has enormous potential in the field of cardiac imaging as it allows for simultaneous data acquisition, which is a huge improvement compared with the sequential scanning protocols of PET/CT. Although these approaches do not improve spatial resolution of the PET technique, they provide valuable information about the localization of the acquired signal.

Radionuclide Imaging of Plaque

Metabolic activity. Atherosclerotic plaques are characterized by an accumulation of cells with high metabolic activity. Therefore, various approaches addressed the prin-

ciple of metabolic activity to image atherosclerosis. Strategies to find a feasible molecular target included the imaging of metabolically active cells, metabolic substrates, or increased uptake of molecules in active cells. One of the earlier attempts to visualize atherosclerotic lesions used ^{111}In-labeled patient-derived monocytes, which were reinjected, and relied on the presumption that these monocytes become invasive plaque macrophages (17). A more practical way to image metabolically active macrophages and inflammation in general is the use of ^{18}F-radiolabled fluorodeoxyglucose (^{18}F-FDG) (18,19). Fluorodeoxyglucose competes with glucose for uptake into metabolically active cells such as macrophages, and it is already established in clinical practice for imaging of tumors or for assessment of myocardial viability. As this substance is therefore readily available, its use is an attractive strategy for imaging atherosclerotic lesions, and several FDG-based studies have been carried out. In one instance for example, a clinical study successfully employed autoradiography to visualize ^{18}F-FDG uptake in macrophage-rich lesions of carotid endarterectomy specimens ex vivo (20).

A recently published study suggested the use of alternative tracers that operate on a similar principle, namely fluorocholine (FCH). ^{18}F-labeled fluorocholine (^{18}F-FCH) has been introduced as a tracer for imaging both brain and prostate cancers. This choline derivative is taken up into cells by specific transport mechanisms, phosphorylated by choline kinase, metabolized to phosphatidylcholine, and eventually incorporated into the cell membrane (21,22). The increased choline uptake in highly proliferative cells such as tumor cells (23) and activated macrophages (22) has been related to an up-regulation of choline kinase as well as an increased activity of choline-specific transporters (23). Choline kinase expression and activity seem to be similar in normal and atherosclerotic murine aortae (24). Thus, enhanced ^{18}F-FCH uptake in activated murine plaque macrophages is not caused by changes in choline kinase, but rather by increased choline transport (24). The rapid uptake (within 30 min) of ^{18}F-FCH into inflammatory tissues supports this notion (25,26). To demonstrate the relative efficacy of ^{18}F-FCH versus ^{18}F-FDG, Matter et al. (24) injected apolipoprotein E (ApoE)-deficient mice intravenously with either tracer. En face measurements of aortae isolated 20 min after ^{18}F-FCH injections demonstrated a very good correlation between fat stainings and autoradiographies achieving a sensitivity of 84% to detect plaques while ^{18}F-FDG reached a lower sensitivity of 64% (24).

Chemotaxis. In the early atherosclerotic plaque, chemokines play an essential role, as they attract inflammatory cells to the lesion and keep the inflammation process progressing (5). One strategy to image atherosclerotic plaques is, thus, to visualize the expression of chemokines or their receptors within the vascular wall. Similar to chemokines, adhesion receptors mediate the recruitment of inflammatory cells to the atherosclerotic plaque. Thus, potential imaging strategies are to visualize chemokines, chemokine receptors or adhesion receptors, or ligands.

Monocyte chemoattractant protein (MCP)-1 is one of the strongest chemotactic agents, attracting inflammatory cells to the atherosclerotic plaque (5,27). Although MCP-1 is not expressed in healthy vessels, various stimuli (e.g., tumor necrosis factor and other cytokines) can induce the expression and secretion of MCP-1 in endothelial cells, vascular smooth muscle cells, or cardiomyocytes, which by triggering and sustaining leukocyte accumulation promote inflammation (28). Elevated levels of circulating MCP-1 have been found in patients with congestive heart failure and coronary artery disease and have been adversely correlated with disease progression (29). Ohtsuki et al. (9) analyzed the localization of its receptor by using ^{125}I-MCP-1 (half-time ~10 min) in atheromas of cholesterol-fed rabbits 4 weeks after arterial injury of the iliac artery and the abdominal aorta. The activity of ^{125}I-MCP-1 correlated with macrophage content per unit area as detected by immunohistochemistry using an antibody specific for rabbit macrophages. Furthermore, ex-vivo autoradiography revealed high ^{125}I-MCP-1 activity within the vascular lesions. Along with chemotaxis, the up-regulation of adhesion molecules on endothelial cells or leucocytes is also involved in atherosclerotic inflammation. Recently, an established receptor ligand pair, vascular cell adhesion molecule (VCAM)-1 (endothelial cells) and very late antigen-4 (leucocytes), has been used to visualize atherosclerotic plaques (30,31). Using a novel VCAM-1 peptide affinity ligand identified by phage display that was conjugated to a magnetofluorescent nanoparticle, atherosclerotic plaques were visualized by MRI as the ligand was actively internalized by VCAM-1–expressing cells (31). While the number and range of studies are limited, the use of chemokines may be a promising candidate for noninvasive plaque imaging in future clinical practice.

Angiogenesis/$\alpha_v\beta_3$ integrin. Accumulating evidence suggests that the extent of neovascularization is closely linked to the inflammatory reaction and infiltration of macrophages/foam cells within atherosclerotic plaques. A study of 269 advanced human atherosclerotic plaques concluded that microvessel formation is strongly correlated with both plaque rupture and the signature features of vulnerable plaques (32). So far, noteworthy studies have used 1 molecular target involved in the angiogenesis process, the $\alpha_v\beta_3$ integrin (33). This integrin is a heterodimeric transmembrane glycoprotein, which mediates cell/cell and cell/matrix interactions, but is also involved in signal transduction from inside as well as outside the cell.

The $\alpha_v\beta_3$ integrin is expressed on various cells originating from the mesenchyme and on a variety of cell types in the blood vessel (including endothelial cells, smooth muscle cells, fibroblasts, macrophages, and platelets). It is a promiscuous integrin that binds to many different ligands including a number of extracellular matrix proteins such as vitronectin, fibronectin, osteopontin, fibrinogen, and von

Willebrand factor, through the interaction with the Arg-Gly-Asp (RGD) motif (34,35). The $\alpha_v\beta_3$ integrin is highly expressed in angiogenic or activated endothelial cells but not, or to a much lower extent, in quiescent endothelial cells (36,37).

Radioactive-labeled $\alpha_v\beta_3$ antagonists and nuclear medicine imaging techniques may provide a helpful tool to study and quantify angiogenetic processes in plaque development. However, most studies concerning tracer development and evaluation were carried out using tumor models (38). The majority of compounds described are based on the α_v-selective cyclic pentapeptide with the amino acid sequence cyclo(-Arg-Gly-Asp-DPhe-Va-) (39). The type of tracer showed comparable affinity and selectivity as the lead structure in vitro and receptor-specific accumulation in the tumor in vivo (40). However, the predominantly hepatobiliary elimination of the tracer demonstrated high activity concentrations in the liver and intestine. Thus, several strategies to improve the pharmacokinetics of radiolabeled peptides have been undertaken. They include conjugation with sugar moieties, hydrophilic amino acids, and polyethylene glycol. The glycosylation approach (41,42), which is based on the introduction of sugar amino acids, resulted in tracers with good pharmacokinetic properties. In a murine tumor model, ^{18}F-Galacto-RGD (RGD: 1 letter code for the amino acids Arg-Gly-Asp, which are essential for binding to the receptor) allowed noninvasive determination of $\alpha_v\beta_3$ expression in the tumor vasculature (Fig. 3) (43). Currently, ^{18}F-Galacto-RGD has been evaluated in a first clinical trial and demonstrates rapid predominant renal excretion and high metabolic stability resulting in good tumor/background ratios and, thus, high-quality imaging (43,44). Moreover, this initial study indicates that tracer accumulation correlates with $\alpha_v\beta_3$ expression on blood vessels in the tumor lesions (Fig. 3). While a large set of studies demonstrating that the data concerning imaging tumor-induced angiogenesis can be translated to imaging, the pathogenesis of plaque formation has yet to be compiled. Initial findings using $\alpha_v\beta_3$-targeted nanoparticles and magnetic resonance (45) as well as $\alpha_v\beta_3$-targeted microbubbles and contrast ultrasound imaging (46) indicate that radiolabeled $\alpha_v\beta_3$-targeted tracer may be used to define the burden and evolution of atherosclerosis and the responsiveness of patients to corresponding therapies. Furthermore, a recent study employing a murine hindlimb ischemia model illustrated that a ^{99m}Tc-labeled peptide (NC100692) targeted to $\alpha_v\beta_3$ integrin selectively localized to endothelial cells in regions of increased angiogenesis and could be used for noninvasive serial "hot spot" imaging of angiogenesis (47).

Lipoproteins. Lipoproteins, particularly low-density lipoproteins (LDLs), contribute substantially to early lesion formation. These mainly oxidized lipoproteins initiate and sustain the inflammation process and are essential for foam cell generation (5). Strategies to utilize the involvement of lipoproteins in atherogenesis include the use of radiolabeled lipoproteins by themselves, detection of epitopes within the lipoproteins (e.g., by the use of radiolabeled antibodies) or radiolabeled endogeneous receptors for lipoproteins (48–52). In initial studies, foam-cell-rich early atherosclerotic lesions were detected by radiolabeled autologous LDL molecules in both human and animal studies (53,54). Using a rabbit model of balloon-induced atheroma, Virgolini et al. (54) found a positive correlation between ^{125}I uptake and the extent of foam cell infiltration. However, the use of radiolabeled LDL is not without difficulty as significant tracer activity appears within the blood pool even several hours after injection and hampers the detection of lipid-laden plaques. Further investigations have, therefore, focused on oxidized low-density lipoprotein (ox-LDL) or various epitopes of ox-LDL because of improved plaque-to-background ratio, the improved uptake into macrophage-rich tissues, and the rapid clearance from the blood pool. In addition, in this method there is no need to obtain autologous blood from the patients. Most of these studies have focused on malondialdehyde epitope on ox-LDL (MDA2), a radiolabeled murine monoclonal antibody, which binds to the malondialdehyde epitope on ox-LDL, and there is a significantly higher uptake of ^{125}I-MDA2 in lipid-rich lesions of atherosclerotic mice and rabbits compared with the uptake in healthy arteries (55,56). After dietary intervention, the same tracer was successfully used to track changes in macrophage foam cell density. In a clinical trial where ^{99m}Tc ox-LDL was used in humans (57), tomographic scintigraphy of the neck in patients suffering from transient ischemic attacks revealed accumulation of radiolabeled LDL preparations in the carotid artery affected by atherosclerotic lesions. We have followed a somewhat different approach. Mediating the uptake of LDL, particularly ox-LDL, into macrophages scavenger receptors (e.g., CD68, SR-A, CLA-1/SR-BI, CD36, LOX-1, and SR-PSOX) play a key role in atherosclerotic lesion formation (Fig. 4A) (58). Thus, we conjugated ^{124}I to the scavenger receptor CD68 (soluble CD68-Fc) thereby using this molecule as our imaging probe. CD68 has been recently described to be important for foam cell formation, as it binds ox-LDL, mediates its uptake (59), and may, therefore, be a useful tool for the detection of atherosclerotic plaques by the visualization of LDL (Fig. 4B). In our study, wild type or ApoE$^{-/-}$ mice (17 weeks old) were fed with a high cholesterol diet and injected with ~190 μCi (7MBq) ^{124}I-CD68-Fc, intravenously. After 48 h, animals were sacrificed and evaluated by ex vivo autoradiography (Fig. 4C). In addition, sudan red staining was performed to compare radioactivity with plaque formation (Fig. 4C). We found a good correlation of signal with the extent of the lesion. While preliminary, our data suggest that CD68 is a potent molecular marker for the detection of atherosclerosis. These findings have to be verified by further profound investigations.

Proteolysis. Through degradation of the extracellular matrix, proteases contribute to the progression and complica-

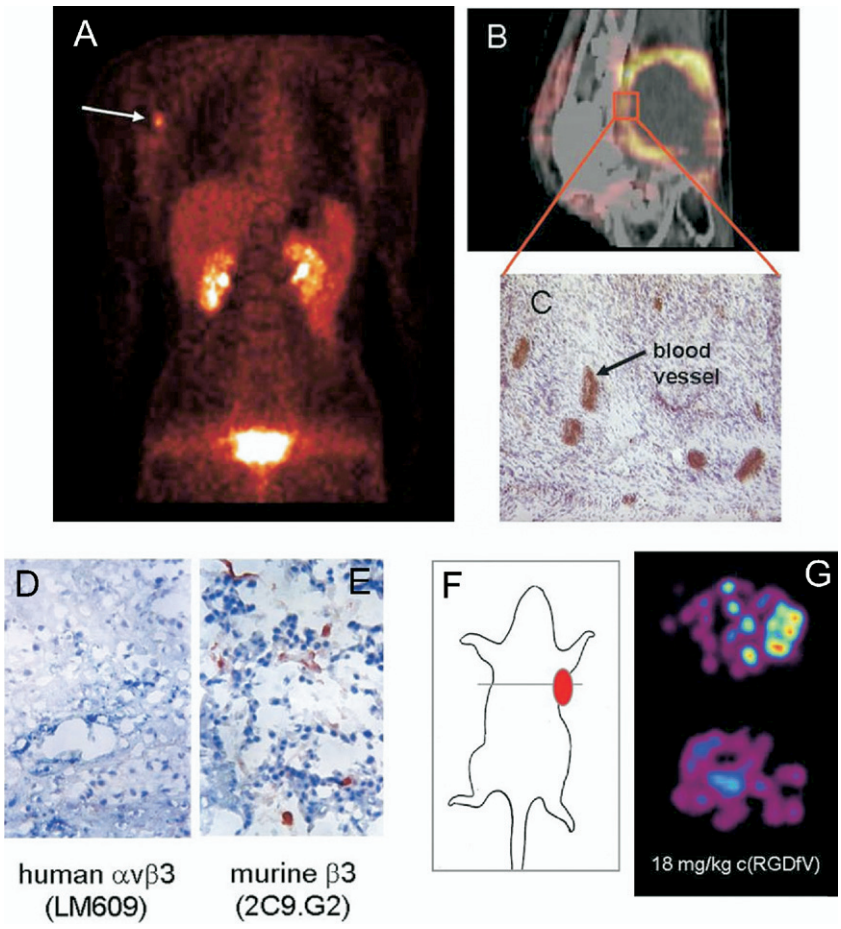

Figure 3 **Detection of Neoangiogenesis**

(Top) (A) Coronal image section from a ^{18}F-Galacto-RGD positron emission tomography of a patient with a malignant melanoma and a lymph node metastasis at the right axilla obtained 60 min after tracer injection. The image shows a clearly contrasting tumor and rapid predominately renal elimination with low background activity in almost all areas of the body. **(B and C)** Patient with a soft tissue sarcoma dorsal of the right knee joint. **(B)** The image fusion of the ^{18}F-Galacto-RGD positron emission tomography and the corresponding computed tomography scan shows that the regions of intense tracer uptake correspond with the enhancing tumor wall, whereas the nonenhancing hypodense center shows no tracer uptake. **(C)** Immunohistochemistry of a peripheral tumor section using the anti-$\alpha_v\beta_3$ monoclonal antibody LM609 demonstrates intense staining predominantly of tumor vasculature. **(Bottom) (D)** Immunohistochemical staining of tumor sections using the anti-$\alpha_v\beta_3$ mAb LM609 demonstrates that the squamous cell carcinoma of human origin do not express the $\alpha_v\beta_3$ integrin. **(E)** In contrast, staining of sections with an antibody against the murine β_3-subunit indicates that the tumor vasculature is $\alpha_v\beta_3$-positive. **(F and G)** Transaxial images of nude mice bearing a human squamous cell carcinoma at the right shoulder acquired 90 min after ^{18}F-Galacto-RGD injection show a clearly contrasting tumor. The signal reflects tracer accumulation due to $\alpha_v\beta_3$ expression exclusively in the tumor vasculature. Tracer accumulation can be blocked by injecting 18-mg cyclo(-Arg-Gly-Asp-DPhe-Val-) per kilogram mouse 10 min before tracer injection indicates receptor-specific accumulation. Adapted from Haubner et al. (43).

tions of atherosclerotic lesions. At later stages of atherosclerosis, a lesion core is covered by a fibrous cap, which consists mainly of smooth muscle cells and extracellular matrix and separates the lipid-rich core from the bloodstream (5).

Interstitial collagen molecules, adjacent to others like fibrin, confer most of the tensile strength on the fibrous cap, and several tightly regulated processes determine the levels of collagen required for stability of this structure (6). Certain proinflammatory cytokines, such as interferon-gamma, can inhibit collagen production by smooth muscle cells, the principle source of this extracellular matrix macromolecule in the arterial wall. Hence, the degree of plaque vulnerability is influenced by factors such as overall size, core size, cap thickness, cap inflammation, and cap fatigue.

Enhanced turnover of the extracellular matrix is known to be the reason for destabilization of the vulnerable fibrous cap. The cells mainly responsible for this process are monocytes, which differentiate to macrophages within the atherosclerotic lesion. The destabilization is mediated by MMPs, which can be released by activated macrophages, endothelial, and other vascular cells. Known mediators of such inflammatory processes are tumor necrosis factor-α and CD40L (60,61).

Strategies to image proteolytic activity included the use of radiolabeled MMP inhibitors, substrates of MMPs, MMP inhibitors, and cathepsins.

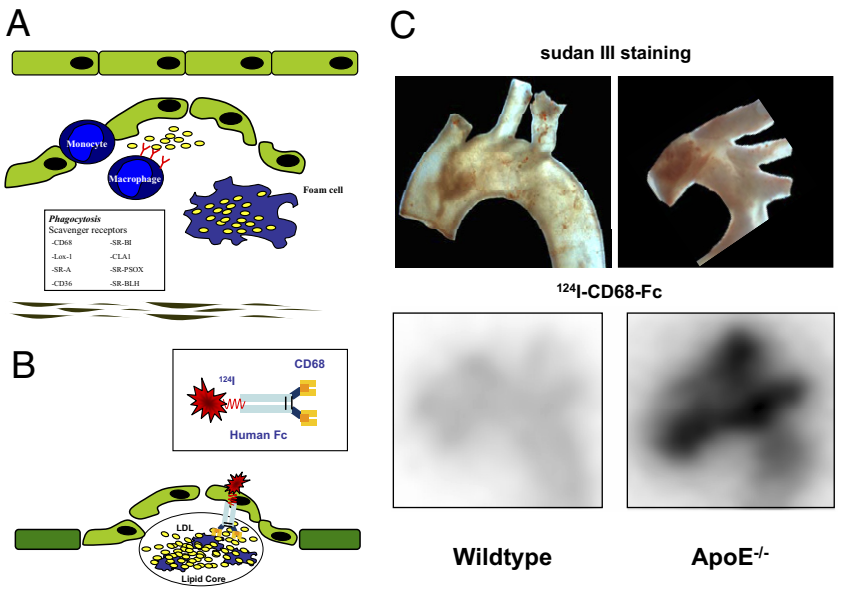

Figure 4 **Detection of Atherosclerotic Plaques by Radiolabeled CD68-Fc**

(A) (Oxidized) lipoproteins initiate and sustain the inflammation process in atherosclerotic lesions and are essential for foam cell generation. Scavenger receptors mediate the uptake of lipoproteins into macrophages and, thus, contribute substantially to foam cell formation. **(B)** A soluble dimeric form of the scavenger receptor CD68 conjugated to an Fc-fragment was radiolabeled with ^{124}I and used to detect atherosclerosis in vivo. **(C)** Apolipoprotein E $^{-/-}$ (ApoE$^{-/-}$) or wild type mice were fed a high cholesterol diet for 17 weeks. Subsequently, mice were injected intravenously with ^{124}ICD68-Fc and sacrificed after 48 h. Ex-vivo nuclear imaging was performed to evaluate tracer activity in the aortic arch. Activity of ^{124}ICD68-FC was enhanced in ApoE$^{-/-}$ compared with wild type mice **(bottom)** and correlated well with plaque extension as shown by sudan **red** staining in the aortic arch specimen **(top)**. LDL = low-density lipoprotein.

Schäfers et al. (10) used a broad-spectrum MMP inhibitor conjugated to ^{123}I to develop the radioligand ^{123}I-HO-CGS 27023A for in-vivo imaging of MMP activity. Four weeks after induction of arterial injury in the carotid artery of ApoE$^{-/-}$ mice fed a cholesterol-rich diet, the tracer was injected retro-orbitally, and uptake was measured. During the first 2 h, a steadily increasing uptake could be detected. Specificity was verified by predisposing cells with unlabeled CGS 27023A. A very elegant way to make use of enhanced MMP activity in tissues was recently published by Chen et al. (62), who used a novel long-circulating, quenched near-infrared fluorescent (NIRF) probe, that is activated by MMP-2 and -9, the essential proteases for the pathophysiology of atherosclerotic lesions. In vitro, the fluorescence signal changes can be detected after activation with MMP-2 or -9 at the specific wavelengths of the attached fluorochrome Cy5.5 by use of a fluorescence plate reader. For in vivo experiments, the authors chose a mouse myocardial infarction model to monitor MMP activity within the infarcted region. Near-infrared fluorescent imaging of MMP activity increased within 1 to 2 weeks after arterial ligation, a result that was confirmed by parallel in-vitro methods including zymography and reverse transcription-polymerase chain reaction analysis of MMP expression.

Choudhary et al. (63) used carotid endarterectomy specimen and multicontrast MRI to generate 3-dimensional reconstructions and evaluate spatial distribution of MMPs and their inhibitors (tissue inhibitors of metalloproteinases) in carotid atherosclerotic lesions. They found that their distribution is highly heterogeneous and reflects lesion location, size, and composition (63). Notably, the greater abundance of MMP-9 in the plaque area suggests that it is a major MMP mediating remodeling in this area. In grossly normal areas, the level of total MMPs is lower and dominated by MMP-2. The authors concluded that remodeling of atheroma and normal arterial wall are mediated by different MMPs (63). The results are consistent with previous observations that MMP-9, but not MMP-2, was necessary for organization of collagen by smooth muscle cells (64).

In addition to MMPs, the cathepsin family of proteases was tested for use in plaque imaging studies. Chen et al. (65) optically imaged cathepsin B activity in experimental atherosclerosis in vivo using a NIRF approach. Similarly, Jaffer et al. (66) were able to visualize atherosclerotic plaques very recently in both mice and humans using a NIRF imaging agent consisting of the cathepsin K peptide substrate (GHPGGPQKC-NH_2) linked to an activatable fluorogenic polymer. Taken together, MMPs represent a very promising target for noninvasive imaging, as they are essential for different pathophysiologic processes including tumor progression, inflammation, and atherogenesis.

Apoptosis. Apoptosis has been shown to be one of the characteristics of atherosclerotic plaques (67). Animal studies of hypercholesterolemia showed both proliferation and cell death of smooth muscle cells in early intimal lesions (68,69). Mentionable studies so far focused on annexin V using this molecule in a radiolabeled form (70). During the process of apoptosis, phosphatidylserine, a phospholipid normally residing on the inner cell membrane of viable cells, is externalized and, thus, available to affinity ligands such as annexin V.

In a model of porcine coronary atherosclerosis, apoptosis of cells in the vascular wall of coronary arteries was detected by SPECT using ^{99m}Tc-annexin V (71). Thirteen of 22 injured coronary vessels of animals receiving a high-fat diet showed focal ^{99m}Tc-annexin V uptake. Another group made use of the same marker to detect experimental atheroma in the aorta of balloon-injured rabbits in vivo (72). In this study, histologically verified macrophage apoptosis showed a positive correlation with tracer uptake. Several clinical studies using annexin V as a radionuclide tracer have been carried out so far. In a study with 18 cardiac allograft recipients, 5 patients had positive myocardial uptake of annexin V (13 negative) (73). As these 5 patients revealed at least moderate transplant rejection and caspase-3 staining in the biopsy specimen, the authors proposed the clinical feasibility of annexin V imaging for noninvasive detection of transplant rejection. Another group also tried to visualize myocardial infarction by ^{99m}Tc-annexin V uptake (74), and in this trial 6 of 7 patients showed increased uptake of the tracer in early and late SPECT images. Lastly, a recent study further underlined the hypothesis that atherosclerotic lesions induced by a high-cholesterol diet in ApoE$^{-/-}$ mice can be detected by apoptotic markers (75). Atherosclerotic plaques in the aorta were visualized by ^{99m}annexin A5 using microSPECT and the quantitative uptake of this tracer correlated with histological plaque extent and macrophage content (75). Despite these very promising results, further experimental and clinical studies have to be carried out to further establish the efficacy of employing nuclear imaging of programmed cell death for noninvasive detection of the vulnerable atherosclerotic plaque.

Thrombogenicity and cell recruitment. The majority of myocardial infarctions are caused by development of a thrombus in coronary vessels at the site of vascular lesions (erosion, plaque rupture) (Fig. 5A). Therefore, thrombogenicity is a central feature of plaque vulnerability. A prerequisite for the formation of a thrombus is the exposure of subendothelial matrix components resulting in enhanced cell recruitment. Plaque imaging approaches derived from this principle include the use of cells participating in thrombus formation, the detection of soluble coagulation factors, or the visualization of exposed subendothelial matrix.

Early approaches used radionuclide-labeled platelets or later radionuclide-labeled platelet inhibitors to detect vulnerable plaques (76–78). As the coagulation cascade results in the development of a fibrin-rich thrombus, Cerqueira et al. (79) used a radiolabeled monoclonal antibody fragment ^{99m}Tc-T2G1s Fab, which is specific for fibrin, in a canine carotid and femoral artery injury model. The tracer was shown to bind twice as strong to trauma-induced thrombotic arteries compared with arteries in sham-operated animals (79). Another group also demonstrated the feasibility of in vivo detection of acute and subacute thrombosis using a fibrin-binding contrast agent in an animal model of atherosclerosis by an MRI approach (80). A plaque imaging study was performed using a F(ab′)2 monoclonal antibody (TRF1) against the human fragment D dimer of cross-linked fibrin for atherosclerotic plaques. Atherosclerotic segments of carotid and femoral arteries of 6 patients and 5 control segments of atherosclerosis-free internal mammary arteries were drawn from 11 male patients undergoing bypass surgery (81). The ^{125}I-TRF1 antibody was used to distinguish atherosclerotic fragments, fatty streaks, and normal endothelium. The significantly higher binding of TRF to atherosclerotic plaques compared with that in normal vessels was specific, as a control antibody showed no binding (81).

Within vulnerable atherosclerotic lesions, extracellular matrix proteins like collagen or fibronectin are exposed that are highly thrombogenic to circulating platelets and coagulation but also represent potential targets for imaging techniques. Matter et al. (82) used an ^{125}I-labeled antibody L19, which targets the extradomain B of fibronectin and injected atherosclerotic ApoE$^{-/-}$ mice (82). By this approach, atherosclerotic plaques were detected after 3 days with signal-to-noise ratios of 105:1 at 3 days (82). Furthermore, increased expression of extradomain-B domain in human atherosclerotic plaques can be detected by immunohistochemical studies. Our group has followed a similar approach by visualizing collagen exposed to blood flow.

Detection of vulnerable plaques by radiolabeled platelet glycoprotein VI (GPVI). We made use of the major platelet collagen receptor GPVI, which plays a critical role in the process of thrombosis at sites of atherosclerotic lesions. As the main extracellular matrix protein of arteries, fibrillar collagen acts as a strong activator of platelets and supports platelet-dependent thrombus formation (83,84). Recently, platelet GPVI has been identified as the major platelet collagen receptor in vivo (85). GPVI is a 60 to 65 kDA type I transmembrane glycoprotein, which belongs to the immunoglobulin superfamily (83). The soluble dimeric form of human GPVI conjugated to an Fc-fragment, which was radioiodinated (Fig. 5B), was capable of detecting lesions of injured carotid arteries in mice through ex vivo and in vivo imaging (11). In 1 study, 5 min after induction of injury, ^{124}I-GPVI (or equivalent amounts of the radioiodinated Fc-fragment as a negative control) was injected intravenously. An experimental mouse model of vascular

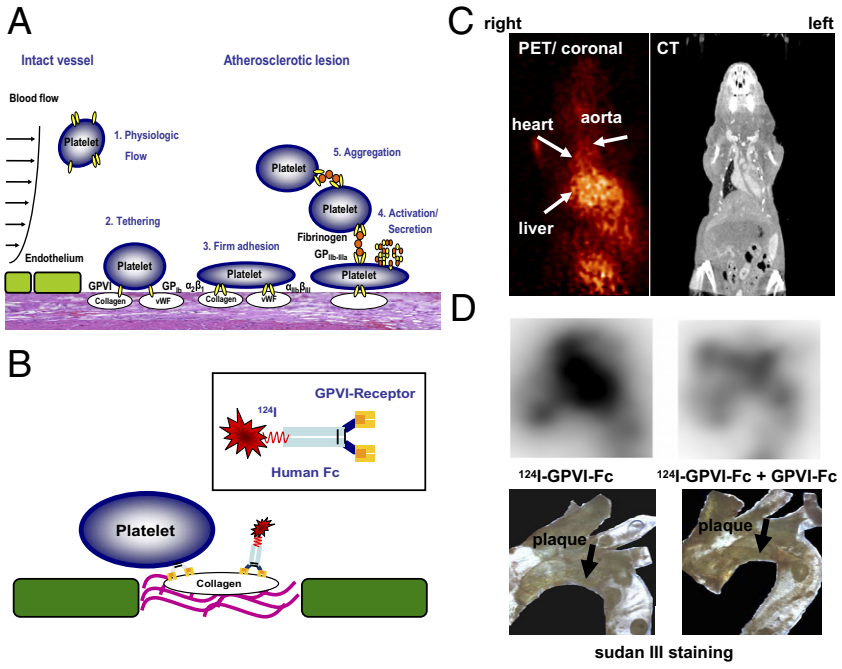

Figure 5 **Detection of Vulnerable, Thrombogenic Plaques by Radiolabeled Platelet GPVI**

(A) Pathophysiology of platelet adhesion, secretion, and thrombus formation at sites of injured vascular endothelium with exposed extracellular matrix. 1) Within the intact vessel, platelets do not adhere to the endothelial monolayer under physiological conditions. 2) At site of atherosclerotic lesions, subendothelial proteins such as von Willebrand factor (vWF) and collagen are exposed to blood flow. Platelet adhesion receptors glycoprotein Ib (GPIb) and glycoprotein VI (GPVI) mediate tethering of platelets. 3) After activation of the integrins $\alpha_2\beta_1$ (collagen receptor) and $\alpha_{IIb}\beta_3$ (fibrinogen receptor), platelets firmly adhere via interaction of these receptors with extracellular matrix proteins. 4) Subsequently, platelets get activated and secrete distinct mediators resulting in: 5) platelet aggregation via fibrinogen bridges between 2 $\alpha_{IIb}\beta_3$ receptors and thrombus formation. **(B)** A soluble dimeric form of human platelet GPVI conjugated to an Fc-fragment was used, which was radiolabeled with ^{124}I. Glycoprotein VI is essential to establish the first interaction of platelets with an exposed collagen surface. Therefore, we made use of this natural mechanism to detect thrombogenic and, thus, vulnerable plaques. **(C and D)** Apolipoprotein $E^{-/-}$ mice were analyzed with ^{124}IGPVI-Fc using an animal microPET scanner (MicroPET Focus 120, Siemens, Knoxville, Tennessee) **(left)**. Images were acquired 24 h after administration of the tracer and imaging time was 20 min. Positron emission tomography (PET) images were correlated with computed tomographic (CT) data **(right)** to verify anatomical structures. **(C)** Shows transverse sections of these experiments. **(D)** Ex vivo nuclear imaging was performed to evaluate tracer activity in the aortic arch **(top)**. The **bottom** shows the specimen after staining with sudan III. Activity of ^{124}IGPVI-Fc correlated well with plaque extension **(left)**. The signal could be nearly abolished, when nonlabeled GPVI-Fc was injected before application of ^{124}IGPVI-Fc **(right)**.

injury was used, and wire-induced injury of the carotid artery was performed as described previously (86). In this manner, vascular lesions were clearly detected with high resolution using a dedicated small-animal PET scanner (MicroPET Focus 120, Siemens, Knoxville, Tennessee) (Fig. 5C), and images were matched with CT data (Fig. 5C). The extent of the ex vivo autoradiography signal correlated with lesion formation as verified by lesion extent in the explanted artery specimen (Fig. 5D). These experimental data suggest that the soluble form of the platelet collagen receptor GPVI allows detection of thrombogenic and, thus, vulnerable arterial lesions.

Taken together, the thrombogenicity of atherosclerotic plaques is one of the most promising approaches to detecting vulnerable plaques.

Clinical Perspective

The strength of molecular imaging is based on the fact that most diseases have an underlying biological basis that is not visualized by traditional imaging methods. Furthermore, it will most likely contribute to a personalized medicine by helping to tailor drug selection to an individual's proteome and genome (87). Imaging of important molecular targets could transform clinical management for diagnosis, risk stratification, selection, and efficacy assessment of molecule-based therapeutics. If methods are optimized, we may be able to decide which patients harbor high-risk atherosclerotic plaques that will ultimately cause myocardial infarction or stroke. Other applications include determining which post-myocardial infarction patients will develop pathological ventricular remodeling and rapid progression to heart failure. Direct visualization of the underlying biology in the diseased tissue may identify patients at high risk for cardiovascular complications, allowing the clinician to tailor disease management on the basis of risk. Molecular imaging may not only identify patients at high risk for cardiovascular events (death, myocardial infarction, stroke) not identified by routine clinical evaluation (e.g., history, physical examination, electrocardiogram, lipid profile, C-reactive

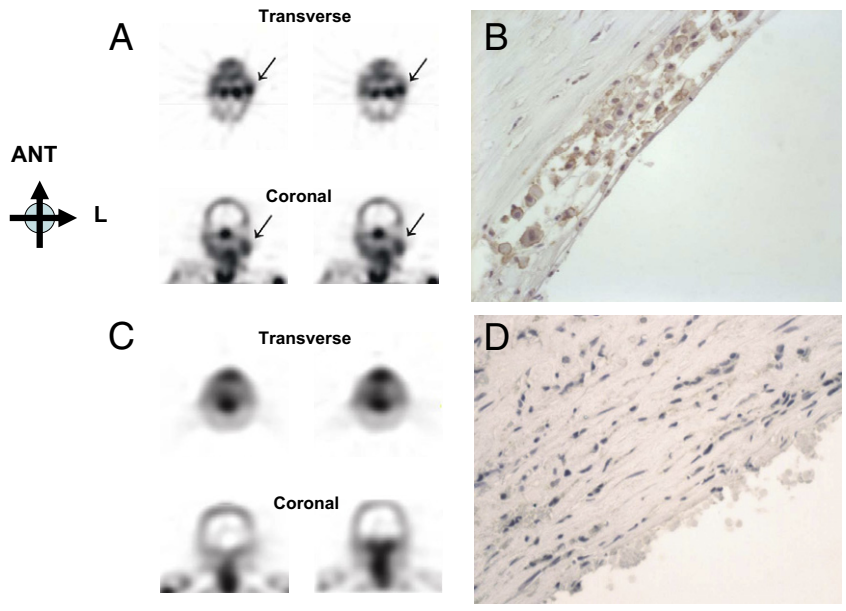

Figure 6 **Molecular Imaging of Apoptosis in Atherosclerosis Using Radiolabeled Annexin**

Technetium-99m labeled annexin A5 accumulates in unstable atherosclerotic plaques. **(A)** Single-photon emission computed tomography images of a patient with a recent transient ischemic attack. **(B)** Annexin A5 in sections of the resected plaque stains strongly in a macrophage-rich area. In contrast, a minimal annexin A5 signal **(C)** appears in the carotid artery of a patient with a transient ischemic attack 3 months before imaging. **(D)** Annexin A5 immunoreactivity is at a background level in the corresponding smooth-muscle-cell-rich lesion. Images provided courtesy of Dr. Bas Kietselaer and Dr. Leonard Hofstra, University Hospital of Maastricht, the Netherlands. Reprinted, with permission, from Kietselaer et al. (70).

protein, exercise treadmill testing), but also the characterization of lesion vulnerability in high-risk areas of the coronary vasculature (e.g., the proximal one-third of each of the coronary arteries [88]) may be possible. Once the lesion has been determined to be of particularly high risk, novel local therapies such as intracoronary drug-eluting stents or local drug delivery with suitable drug-delivery balloon catheters could be justified. At present, the selection of many target-specific therapeutics depends on population-based studies or randomized clinical trials. These approaches, however, do not routinely assess the biological variability of the disease process in individual patients. Thus, molecular plaque imaging will help to select individualized treatment strategies based on the molecular profile of vulnerable plaques identified in particular patients. In keeping with this, several promising atherosclerosis-targeted imaging agents have already undergone testing in the clinic or are on the clinical horizon (Fig. 6) (87).

Conclusions

Over the past years, clinical data and observations have emphasized the need for a more profound characterization of atherosclerotic plaques. It is essential to acquire information beyond the resulting degree of stenosis detected by angiography. Epidemiologic studies have shown that a considerable amount of patients with sudden cardiac events have no alarming symptoms before a serious event (89). Furthermore, acute coronary syndromes often result from plaque rupture at sites with no or only moderate luminal narrowing detected by angiography (90,91). Molecular imaging of atherosclerotic plaques offers new strategies to detect vulnerable atherosclerotic plaques that are prone to thrombus formation. Increasing our knowledge about the molecular biology of plaque vulnerability and identification of new mediators will prove most promising and challenging and will propel the field of plaque imaging to the forefront of cardiology.

Reprint requests and correspondence: Dr. Harald Langer, Medizinische Klinik III, Eberhard Karls Universität Tübingen, Otfried-Müllerstr. 10, 72076 Tübingen, Germany. E-mail: harald.langer@med.uni-tuebingen.de; currently affiliated with National Institute of Health/National Cancer Institute, Building 10, Room 5B17, Bethesda, Maryland 20852. E-mail: langerh@mail.nih.gov.

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