

# Imaging Apoptosis in Rheumatoid Arthritis

Quantitative imaging of apoptosis using  $^{99m}\text{Tc}$ -labeled annexin V in rheumatoid arthritis challenges our conventional thinking on how disease progression or remission should be monitored and reported. Until now, we imaged collagen vascular diseases using radiographs to study bone morphology and using MRI scans to measure articular cartilage. Apoptosis plays an important role in autoimmune diseases (1), but the potential for in vivo imaging has not been explored—at least not until the contribution of Post et al. (2) appeared in this issue of *The Journal of Nuclear Medicine*.

Apoptosis is an energy-dependent process for noninflammatory cell death. Programmed cell death is important in normal physiology and disease and may be induced by many stimuli, including stress, hypoxia, radiation, trauma, and cytotoxic drugs (3). Apoptosis as a common and universal mechanism of cell death, distinguishable from necrosis, is now a widely accepted concept after the landmark article by Kerr et al. in the early 1970s (4).

The process of programmed cell death, or apoptosis, has become one of the most intensively studied topics in the biologic sciences during the last 3 decades. Apoptosis research is growing, especially after the introduction of imaging agents that allow in vivo studies in humans. Between the mid 1980s and the late 1990s, the number of citations containing the words *apoptosis* or *programmed cell death* increased tenfold in MEDLINE, the bibliographic database of the U.S. National Library of Medicine (Bethesda, MD).

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Apoptosis-related drugs, assay kits, and now imaging agents have fueled this interest.

Numerous techniques are available to study apoptosis, resulting in the development of morphologic, immunocytochemical, and molecular genetic markers, including proteases, signal transduction molecules, and mitochondrial proteins (5).

Markers from signaling pathways involved in apoptosis include increased caspase-3 activity, poly(adenosine diphosphate-ribose) polymerase cleavage, chromatin condensation, DNA fragmentation, decreased cellular metabolism, compromised membrane permeability, and cleavage of nuclear envelope proteins (lamins) (5). Once the cell is committed to apoptosis, the caspase enzyme cascade is activated, with the early effect of rapid phosphatidylserine expression and externalization on the cell membrane (6,7).

Cell and nuclear shrinkage with chromatin condensation and margination followed by karyorrhexis were visualized in the early 1970s using electron microscopy (4,5). DNA dyes such as propidium iodide reveal apoptotic nuclear morphology and can be visualized directly by light, fluorescent, or confocal laser microscopy. In combination with annexin V techniques, apoptotic and necrotic cells can be distinguished (5).

## ANNEXIN V

Annexins are a family of structurally related eukaryotic proteins that demonstrate reversible calcium-dependent binding to membrane bilayers containing anionic phospholipids (8). The Nobel laureate Robert Huber solved the 3-dimensional structure of human annexin V in 1990 (9). The mammalian annexin family comprises at least 12 distinct genes, and all but 2 contain  $\text{Ca}^{2+}$ -binding sites. Annexins are char-

acterized by their ability to bind negatively charged phospholipids in the presence of  $\text{Ca}^{2+}$ . The human gene for the 35-kD annexin V protein is ANXA5, located on chromosome 4 at band 4q26–4q28.

Annexin V, a calcium-dependent binding protein specific for phosphatidylserine, binds to the surface of apoptotic cells. This labeling can be detected with flow cytometry or fluorescent microscopy. Double labeling of cells with annexin V and propidium iodide can readily distinguish apoptotic from necrotic cells.

## AUTOANTIBODIES TO ANNEXIN V

Autoantibodies to annexin V have been found in the sera of patients with autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and inflammatory bowel disease (10). This led to speculation that the autoantibodies may interfere with the putative functions of autologous annexin V, including collagen type II binding, inhibition of phospholipase A2 activity, and Fc receptor activity (10,11). Their clinical relevance remains unclear as a pathogenetic mechanism or merely a nonspecific aspect of autoimmunity (11,12,13). Standardized assays are not available for antibodies to annexin V.

## IMAGING OF APOPTOSIS

In vivo detection and imaging of phosphatidylserine expression during programmed cell death based on radiolabeled  $^{99m}\text{Tc}$ -recombinant human annexin V was introduced by Blankenberg et al. in 1998 (14,15). The biodistribution and associated radiation dose of  $^{99m}\text{Tc}$ -labeled annexin V has been evaluated in humans, demonstrating a shorter biologic half-life than physical half-life. The effective dose was found to be within the lower to middle range of values reported for

typical  $^{99m}\text{Tc}$ -labeled compounds (16,17). Technetium conjugation has been accomplished using  $^{99m}\text{Tc}$ -labeled 4,5-bis(thioacetamido)pentanoyl-annexin V (16),  $^{99m}\text{Tc}$ -(n-1-imino-4-mercapto-butyl)-annexin V (17), pegylated annexin V labeled with  $^{111}\text{In}$ -diethylene-triaminepentaacetic acid (18), and hydrazinonicotinamide (19). Iodination of annexin V for imaging apoptosis was recently described (20). Studies in humans demonstrated the feasibility of imaging cell death in acute myocardial infarction, in tumors with a high apoptotic index, and in response to antitumor chemotherapy of lung cancer, breast cancer, lymphoma, and sarcoma (21).

The role of apoptosis imaging in clinical care is being defined in clinical trials on the basis of the rationale that apoptosis is the likely mechanism behind the cytoreductive effects of many cytotoxic agents and radiotherapy, rejection of organ transplants, cellular damage in collagen vascular disorders, and delayed cell death due to hypoxic-ischemic injury in myocardial infarction and neonatal hypoxic-ischemic injury (21).

## PREDICTION OF RESPONSE TO THERAPY

In vivo and in vitro imaging measurement of apoptosis in breast cancer cells using  $^{99m}\text{Tc}$ -ethylenedicysteine-annexin V was developed and tested in assessments of response to radiation and chemotherapy. Significantly increased uptake was found after radiation (10–30 Gy) and paclitaxel treatment in vitro. In vivo biodistribution showed increased count density for tumor to blood, tumor to lung, and tumor to muscle (22).

Intramedullary apoptosis of hematopoietic tissue induced by cyclophosphamide treatment was evaluated in rats and showed that radiolabeled annexin V can be used to detect and directly quantify the degree of intramedullary and splenic apoptosis noninvasively (23).

Early prediction of tumor chemotherapy response and prognosis based on  $^{99m}\text{Tc}$ -annexin V imaging after 1 course of chemotherapy was reported for 15 subjects with lung cancer, lymphoma, and breast cancer (24,25). Recently, measurement of a neoepitope of cytokeratin 18 that appears after caspase cleavage during apoptosis was performed on the sera of chemotherapy patients using an enzyme-linked immunosorbent assay based on a monoclonal antibody, M30. This method has potential for therapeutic monitoring without imaging (26).

The Post et al. (2) contribution to this issue of *The Journal of Nuclear Medicine* demonstrates the potential for yet another application of radiolabeled annexin V. This new tool for noninvasively monitoring a common disease with high sensitivity to therapeutic interventions, especially before any clinical manifestations become evident, presents exciting opportunities for rapid translation of this bench research into clinical feasibility trials.

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