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Review

Molecular imaging: novel tools in visualizing rheumatoid arthritis

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Molecular imaging is a rapidly emerging field in biomedical research, aiming at the visualization, characterization and quantification of molecular and cellular processes non-invasively within intact living organisms. To sense biological processes such as gene expression, angiogenesis, apoptosis or cell trafficking *in vivo*, imaging reporter agents that interact specifically with molecular targets and appropriate imaging systems are currently under development. In rheumatoid arthritis, these novel tools will be used to evaluate physiological and pathophysiological processes, to facilitate diagnosis and monitor therapeutic regimens, to enable reliable prognosis and to support the development of new therapies. In this review, we summarize the basic principles of molecular imaging, such as the development of molecular imaging agents, the actual capabilities of different imaging modalities and the most recent advances in molecular imaging, demonstrating the potential of this technology. With regard to their applicability in rheumatic diseases, we discuss potential molecular targets, current experimental approaches and the future prospects for molecular imaging in rheumatoid arthritis.

KEY WORDS: Magnetic resonance tomography, Nuclear imaging, Optical imaging, Radiography, Ultrasound.

Background

Diagnosis and monitoring of rheumatoid arthritis (RA) by imaging techniques is difficult and restricted almost exclusively to the anatomical level. The majority of current imaging methods used in clinical medicine, especially radiography, X-ray computed tomography (CT), magnetic resonance tomography (MRT) and ultrasound, rely predominantly on energy/tissue interactions. Most contrast agents are unspecific and do not interact with specific molecular targets. Therefore, the contrast necessary for (computerized) visualization to differentiate pathological from normal tissue is based on anatomical, physiological or metabolic heterogeneity rather than identifying specific cellular or molecular events that underlie disease. In RA, articular bone erosion and joint space narrowing are relatively well delineated by conventional radiography. In the past, high-resolution ultrasound, and especially MRT, have improved substantially the ability to detect and visualize initial destruction during the first stages of RA, sometimes even preceding the clinical symptoms necessary to establish diagnosis, but still report relatively late in the course of the disease and do not provide any information about cellular and molecular mechanisms [1–3].

Recent advances in the molecular basis of disease, the development of target-specific imaging agents, and new developments in the field of imaging are increasingly allowing the non-invasive evaluation of cellular and molecular events, such as gene expression, angiogenesis, apoptosis and cell trafficking, within the context of physiologically authentic environments of living organisms. This technology, called molecular imaging, has its roots in nuclear medicine, which uses tracers that bind specifically to molecular targets [4–6].

Cellular and molecular changes often occur a long time before any signs of anatomical, physiological or metabolic changes become evident. Therefore, non-invasive visualization and quantitation of biological processes at the molecular level facilitates earlier detection, and furthermore it provides more detailed information about basic molecular events involved in disease compared with conventional imaging methods. Consequently, molecular imaging is a promising tool for the evaluation of physiology and pathophysiology. It enables a more fundamental understanding of key processes, for earlier and more reliable prognosis, earlier assessment of treatment response and the development of new therapies, which are urgently needed in many diseases. Molecular imaging technologies are currently being developed, based not only based on nuclear medicine techniques, but also on MRT, ultrasound and the emerging field of optical imaging. They will have a profound impact in research and the clinical management of numerous disorders, including cancer and cardiovascular, neurological and inflammatory diseases [4–6].

In RA, monitoring of specific biological processes, such as inflammatory and destructive pathways, in experimental arthritis models and in human disease would undoubtedly be very helpful for both basic research in arthritis and the clinical management of patients. The development of molecular imaging methods is a challenging multistep process, especially in RA. Of particular importance is the development of molecular imaging agents

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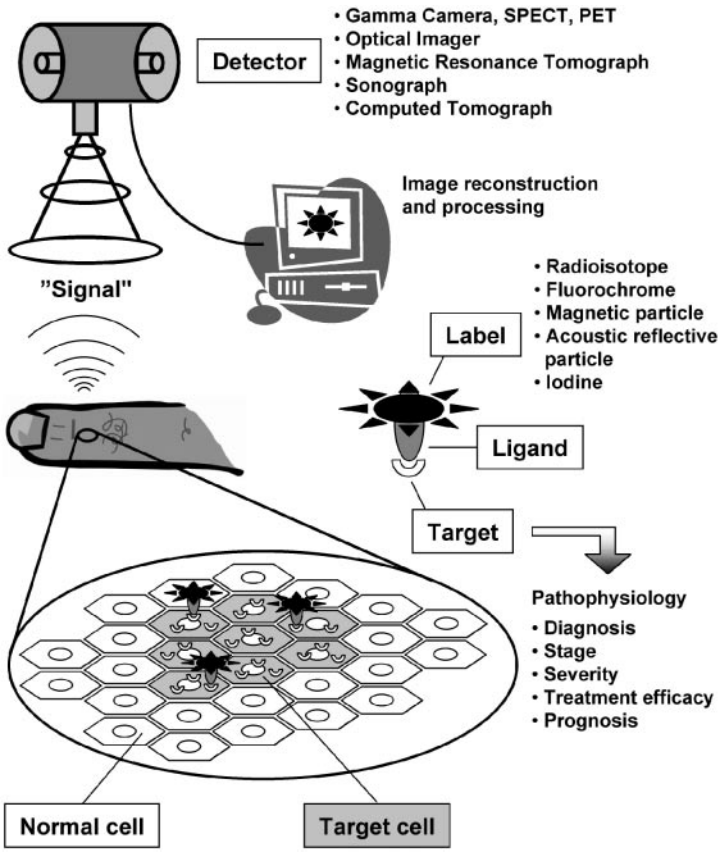

FIG. 1. Principle of molecular imaging and the development of molecular imaging agents. In order to visualize molecular events non-invasively, imaging agents need to be designed that interact specifically with appropriate molecular targets involved in the pathophysiology of disease. This includes markers for early diagnosis, the stage or the severity of disease, treatment efficacy or prognosis. Once a suitable target has been defined, a ligand that binds to the target with high affinity and specificity needs to be designed. Depending on the imaging modality, a signal molecule (label), such as a radioisotope for nuclear imaging, a fluorochrome for optical imaging, a magnetic particle for MRT, an acoustic reflective compound for ultrasound or a high-atomic-number atom that absorbs X-rays (e.g. iodine) for CT, has to be linked to the ligand, facilitating the sensitive detection of the imaging agent in a clinical setting. Finally, the acquired images need to be reconstructed and processed using computer systems.

enabling the analysis of pathophysiological events crucial in disease and the choice of an adequate imaging modality applicable in clinical settings [7, 8].

Development of novel molecular imaging agents

The design of molecular imaging agents is demanding because numerous critical issues need to be addressed: (i) of the multitude of extra- and intracellular processes in RA, the identification of a suitable molecular target by target screening and verification at the subcellular, cellular and tissue level is mandatory; (ii) a ligand that binds to this molecular target with high specificity and affinity by ligand screening needs to be identified; (iii) this ligand needs to be labelled with a signal molecule for visualization depending on the imaging modality used to sense the imaging agent; (iv) biological barriers for the imaging agents during on the way to the molecular target must be overcome not only *in vitro* but also *in vivo*; and (v) strategies for signal amplification in order to increase sensitivity need to be established. The final steps in the development of molecular imaging strategies are clinical trials to verify and validate clinical applicability and practical utilization in human disease (Fig. 1) [4–6].

Imaging agents can be subdivided into three categories: non-specific probes, targeted probes, and activatable probes (Fig. 2).

Non-specific imaging agents

These have compartmental distributions and do not interact with a specific molecular target. They accentuate dissimilarities between tissues, such as differences in permeability or perfusion, and are helpful in characterizing physiological processes such as blood volume, flow or perfusion [9]. In RA, the synovium of inflamed joints shows a large number of capillaries, enhanced capillary perfusion and permeability. After intravenous injection, higher amounts of unspecific contrast agents can therefore be detected in arthritic synovium compared with surrounding healthy tissue or normal synovium. The rate and magnitude of synovial enhancement on magnetic resonance images (MRIs) after intravenous injection of (non-specific) gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA) contrast agents has been shown to correlate with disease activity and is sensitive to therapy-induced changes. This demonstrates that non-specific contrast agents can be beneficial in assessing crucial parameters in clinical management [1–3].

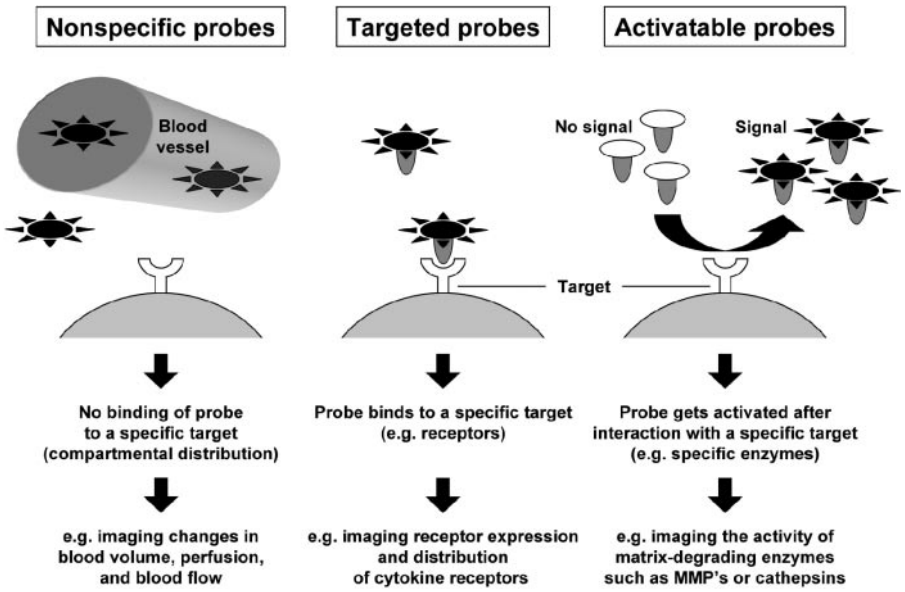

FIG. 2. Types of imaging agents. The most commonly used imaging agents are non-specific, i.e. they do not bind to a specific molecular target. These probes have compartmental distributions and can be used for imaging changes in blood volume, perfusion and blood flow. In contrast, targeted and activatable probes interact with specific targets and therefore make it possible to image biological processes at the cellular or molecular level. Targeted probes bind to specific targets, such as receptors, and can be used to image receptor expression and distribution (e.g. of cytokine receptors). Activatable probes are activated after interaction with a specific target. A number of activatable probes have been developed to non-invasively image, for instance, the activity of matrix-degrading enzymes such as MMPs or cathepsins. Adapted from [9].

Targeted imaging agents

These are usually created by coupling a signal molecule to affinity a ligand, such as an antibody, antibody fragment, peptide, a small molecule or a larger multivalent construct. This ligand binds with high specificity to a certain target which might be, for instance, a receptor [9]. In RA, this kind of imaging agent can be used to evaluate receptor expression and distribution (e.g. imaging of cytokine receptors involved in disease) [7, 8]. Shortcomings of targeted imaging agents are that both the unbound and the non-specifically bound fractions of an agent produce a signal, potentially resulting in high background noise. In addition, targeted imaging agents require a certain amount of time for washout. To improve the sensitivity of the imaging method, signal amplification strategies, such as pretargeting and avidin–biotin amplification, can be used to boost target/background ratios. Additional efforts to improve the attributes of an imaging agent include optimization of pharmacological properties [9].

Activatable imaging agents ('smart' or 'responsive' probes)

These undergo a physicochemical change after interacting with their intended target. In their native injected state, they are almost undetectable. Upon target interaction, the detectable signal increases substantially. Since one target molecule (enzyme) can activate several molecules of the imaging probe, an enormous signal amplification occurs, resulting in high signal-to-noise ratios through background suppression. Activatable imaging agents have been developed for MRT and fluorescence imaging. Examples of such agents include protease-sensitive imaging probes, which are activated upon enzymatic cleavage [9]. In RA, these probes can be used for monitoring the activity of matrix-degrading enzymes, such as matrix metalloproteinases (MMPs) and cathepsins [7, 9].

Imaging modalities

Various imaging modalities, such as conventional radiography, CT, ultrasound, MRT, nuclear imaging methods such as single photon emission computed tomography (SPECT) and positron emission tomography (PET), as well as other methods, including optical imaging methods such as fluorescence imaging and bioluminescence imaging, are available. In fluorescence imaging, light emitted by a fluorochrome (fluorescence) upon excitation by a light source is detected by sensitive cameras, whereas in bioluminescence imaging no excitation light is necessary. Bioluminescence imaging is based on the expression of luciferase by transfected cells, which catalyses the oxidation of luciferin and results in the release of photons. In case of fluorescence imaging, several modalities are available, e.g. fluorescence reflectance imaging (FRI; the light source and the detector are on the same side of the subject; 2-dimensional images) and fluorescence-mediated tomography (FMT; the subject is illuminated by different sources and the emitted light is captured by multiple detectors; 3-dimensional images) [4–6, 9].

The imaging methods differ with respect to spatial resolution, the ability to produce 3-dimensional images, depth limit, sensitivity, the possibility of quantification, the availability of imaging agents, the potential to gather information at the anatomical, physiological, cellular and molecular levels, costs and effort (Table 1). Therefore, the choice of imaging modality for molecular imaging depends on the kind and location of the cellular or molecular event that needs to be monitored, as well as the biological questions that need to be answered [4–6, 9].

Conventional radiography, CT, ultrasound and MRT provide high-resolution images at the anatomical level, whereas morphological information provided by nuclear and optical imaging methods are limited, because they are based on the detection of a signal coming from a radiolabelled or fluorescent imaging agent. Therefore, when nuclear or optical imaging methods are used it is beneficial to combine them with imaging modalities

TABLE 1. Main features of major imaging modalities and their role in RA

Imaging technique	Advantages	Disadvantages	Role in RA
Conventional radiography	High resolution (a few μm) No depth limit Fast, low costs	2-dimensional Low soft tissue contrast Shadowing Radiation Low sensitivity for label detection	Widely used Good delineation of bone erosions and joint space narrowing but limited to visualization of non-osseous features of disease
X-ray computed tomography (CT)	High resolution (tens of microns) No depth limit 3-dimensional	Low soft-tissue contrast Radiation Low sensitivity for label detection	Limited use in clinical practice
Ultrasound	High resolution (surface: tens of microns) Real time; repeatability Low costs High sensitivity for label detection	Low resolution (depth: centimetres) Depth limit Shadowing Documentation of findings Operator-dependent	Increasing use because of its superiority to clinical examination and CR in detecting joint erosions and its versatility
Magnetic resonance tomography (MRT)	High resolution (less than 100 microns) No depth limit 3-dimensional Good soft-tissue contrast	Limited to imaging osseous features of disease High costs, slow Medium sensitivity for label detection	Increasing use because of its superiority to clinical examination and CR in detecting joint erosions
Nuclear imaging (SPECT, PET)	No depth limit 3-dimensional Quantifiable High sensitivity for label detection Imaging at molecular level	Low resolution (a few mm) Limited information at anatomical level Radioactivity High costs, especially PET	Few studies
Fluorescence imaging (FRI, FMT)	3-dimensional (FMT) Fast, low costs Low costs High sensitivity for label detection Imaging at molecular level	Low resolution ($\sim$1 mm) Depth limit (FRI, a few mm; FMT up to 10 cm) 2-dimensional (FRI) Limited information at anatomical level	Currently limited to animal models
Bioluminescence imaging	Fast, low costs High sensitivity for label detection Imaging at molecular level	Low resolution ($\sim$1 mm) 2-dimensional Limited information at anatomical level	Animal models only

providing detailed information at the anatomical level in order to combine anatomical and functional information [4–6, 9].

The major limitation of conventional radiography and CT in molecular imaging is their low inherent sensitivity for label detection. In order to produce detectable contrast, large quantities of a contrast agent at the target site are necessary. Furthermore, the adverse effects of ionizing radiation can be restrictive, especially when repeated imaging is required. At present, the main value of X-ray-based methods for molecular imaging is the function of CT as a complementary modality to nuclear or optical imaging, as it provides high-resolution images at the anatomical level. In contrast to X-ray-based methods, ultrasound has been successfully advanced so that it can go beyond anatomical imaging: Doppler ultrasound and colour-coded duplex sonography enables measurements of blood flow, direction and speed. Furthermore, the development of targeted, acoustically reflective contrast agents has added molecular imaging capabilities, as evidenced by several experimental studies. Ultrasound is very sensitive for label detection, but achieving sufficient stability of such echogenic formulations is difficult (see the following section). Moreover, spatial resolution of ultrasound rapidly decreases with depth and therefore sonography does not provide as much detail about deeply located structures. MRT has a particular advantage for use in molecular imaging: information at the anatomical and molecular levels can be recorded simultaneously, as shown in numerous experimental approaches. Because MRT does not achieve the sensitivity of nuclear or optical methods, it necessitates higher amounts of contrast agent at the intended target to provide sufficient contrast. In addition to the high complexity of such probes (see the following section), this is one of the reasons why the design of MRT-based molecular imaging probes remains a challenge [4–6, 9].

Exquisite sensitivity, especially for PET, which is even more sensitive than SPECT, is the primary advantage of nuclear imaging methods. Also advantageous is their ability to provide quantitative measurements. The limiting factor is low spatial resolution, which can make it difficult to assign the signal to a specific anatomical–morphological structure, a disadvantage which can be partly compensated for by combining nuclear imaging techniques with CT. Like nuclear imaging techniques, optical imaging methods are very sensitive. Their major shortcoming is low spatial resolution and primarily limited depth penetration, due to light scattering and absorption in tissue. For imaging superficial tissues located at outer surfaces (e.g. skin lesions and arthritic finger joints) or at inner surfaces by using endoscopic devices (e.g. gastrointestinal lesions), optical imaging can provide a fast and inexpensive method, with relatively simple instrumentation requirements [4–6, 9].

In recent years, imaging instrumentation dedicated to small animal imaging has also become available for all major clinical modalities (microCT, small animal ultrasound systems, microMRI, microSPECT, microPET), as well as combinations of different imaging modalities (e.g. microSPECT/CT or microPET/CT). Since clinical systems are usually available in larger hospitals and small animal imaging systems often require

a high initial investment, there is also a parallel trend to adapt clinical scanners to the needs of small animal imaging, for instance by using dedicated animal coils in MRT [10]. Various instrumentations also exist for small animal optical-based imaging [11].

Recent advances in molecular imaging

Various imaging agents with molecular specificity (targeted and activatable probes) have been designed for ultrasound, MRT, nuclear and optical imaging. They can be used to image cellular and molecular events, such as gene expression, angiogenesis, apoptosis and cell trafficking [4–6, 9].

Targeted imaging agents

Targeted ultrasound imaging agents have been developed by conjugating affinity ligands to acoustically reflective particles, including microbubbles and liposomes. For clinical purposes, these agents have been developed for imaging, thrombosis, endothelial cell adhesion molecules, tissue factors, activated leucocytes and angiogenesis. Among the potential limitations of ultrasound-targeted contrast agents are their stability and the relatively large size of such formulations (>250 nm), limiting their ability to overcome biological barriers [12, 13].

Targeted MRT agents are based on superparamagnetic iron oxide nanoparticles or on lanthanide chelates such as gadolinium-DTPA. Superparamagnetic iron oxide particles usually consist of a crystalline iron oxide core containing thousands of iron atoms, surrounded by a polymer coating. The individual formulations vary in size (10–300 nm), coating (e.g. dextran or polyethyleneglycol) and magnetic properties (R1, R2, susceptibility). Efficient targeting also requires strategies such as caging of the dextran coat. Dextran caging has been achieved by cross-linking the coating and resultant particles (cross-linked iron oxide, CLIO), and is being used as a basis for targeting receptors, integrins or specific cells. Lanthanide chelates, such as gadolinium-DTPA, are also used as MRT contrast agents, but their lower intrinsic relaxivity requires larger-sized nanoparticle constructs, such as polymerized liposomes, dendrimers and perfluorocarbon nanoparticles, to achieve high magnetic payloads and longer intravascular lifetimes. However, these agents have been successfully used to image angiogenesis, progenitor cells and thrombotic events *in vivo* [14, 15].

Targeted nuclear imaging agents have been primarily developed for vascular processes and targets, including apoptosis and angiogenesis, viability, atherosclerosis and thrombosis. Targeted nuclear agents can also visualize gene expression, for example by targeting a gene-transcribed extracellular protein or by detecting expression of a reporter gene such as herpes simplex virus thymidine kinase (HSV-Tk). In mammalian cells, exogenous HSV-Tk phosphorylates acylguanosine residues, generating biological signal amplification when the phosphorylated radioisotopes are intracellularly trapped [16, 17].

A number of targeted optical imaging agents have been described, including those with specificity for receptors (e.g. for folate, somatostatin) or specific ligands primarily for imaging cancer cells or apoptotic cells. Using fluorochromes such as Cy5.5 or Cy7, which absorb and emit light in the near-infrared bandwidth, minimizes autofluorescence and maximizes tissue penetration because the major absorbers, haemoglobin and water, have their lowest absorption coefficients in the near-infrared region. Optical imaging is a rapid and inexpensive method of visualizing biological events. The use of spectrally different fluorochromes allows the concurrent imaging of multiple targets (multichannel imaging) [11].

Activatable agents

Activatable MRT agents are already detectable in their native form, but target interaction causes a change in relaxivity ('activation') which can be detected by MRT. Several probes based on lanthanide chelate and superparamagnetic nanoparticles with different molecular specificities have been developed in recent years, e.g. to visualize enzyme activity, different affinity targets such as E-selectin, or to evaluate molecular interactions (DNA–DNA, protein–protein, protein-small molecule) [18–20].

A number of activatable optical imaging agents have been developed for *in vivo* imaging of protease activity. The fluorescence of these probes is quenched until activation by enzymatic cleavage, which results in a marked increase in the fluorescence signal. Various probes with different specificities (e.g. cathepsins, MMPs, caspases) have been tested and characterized for the imaging of cancer invasiveness and apoptosis [11].

Molecular imaging in RA

Potential targets for molecular imaging in RA involve all aspects of its pathophysiology, inflammation and destruction operative in the different compartments of the human joint and in the respective animal models. In addition, the detection, tracking and monitoring of molecules driving or inhibiting the arthritic process are also of considerable interest for the development of novel therapeutic strategies. Studies using molecular imaging agents in RA are currently limited to nuclear and optical imaging methods.

Nuclear imaging approaches

Recent strategies to target and visualize specific processes in arthritic synovium using nuclear imaging approaches include targets that mark soluble factors, cells or surface molecules, or the activation of cells and apoptosis, or that indicate dynamic processes such as proliferation [21–30].

Interleukin-1 and TNF are key molecules driving arthritis and inflammation in RA synovium, and numerous groups have successfully used radiolabelled interleukin-1 receptor antagonist (123-J IL-1ra) [21] and anti-TNF monoclonal antibody [22] to localize these molecules in synovium. Using leucocytes labelled with 99mTc-hexamethylpropylene amine oxime (99mTc-HMPAO), synovial inflammation could be visualized, and estimation of disease activity was also possible [23]. In a similar approach, 99mTc-E-selectin binding peptide could localize this important adhesion molecule [24]. Of interest, 99mTc-anti-E-selectin-Fab can be used successfully to image synovitis with better specificity than 99mTc-HDP bone scanning [25].

Methyl-11C-choline is a marker of cellular proliferation. Based on the knowledge that choline is a precursor for the biosynthesis of phospholipids (in particular phosphatidylcholine), which is the essential component of all eukaryotic cell membranes, and that the proliferative changes occurring in arthritic synovium enhance membrane synthesis, which increases the demand for phospholipids, this molecular marker was able to indicate general proliferative activity in arthritic synovium [26]. Similarly, based on the fact that maintenance of arthritis requires a considerable amount of energy, enhanced glucose metabolism could be successfully imaged by 18F-fluorodeoxyglucose (FDG) PET imaging (Fig. 3) [26–29]. In addition, a recent study showed that 18F-FDG PET can assess the metabolic activity of synovitis and even measure disease activity in RA [29]. Moreover, the volume of enhancing pannus (VEP) was determined from fat-suppressed MRIs. VEP and FDG uptake were closely correlated, as were changes in VEP and standardized uptake volume. VEP and FDG uptake were

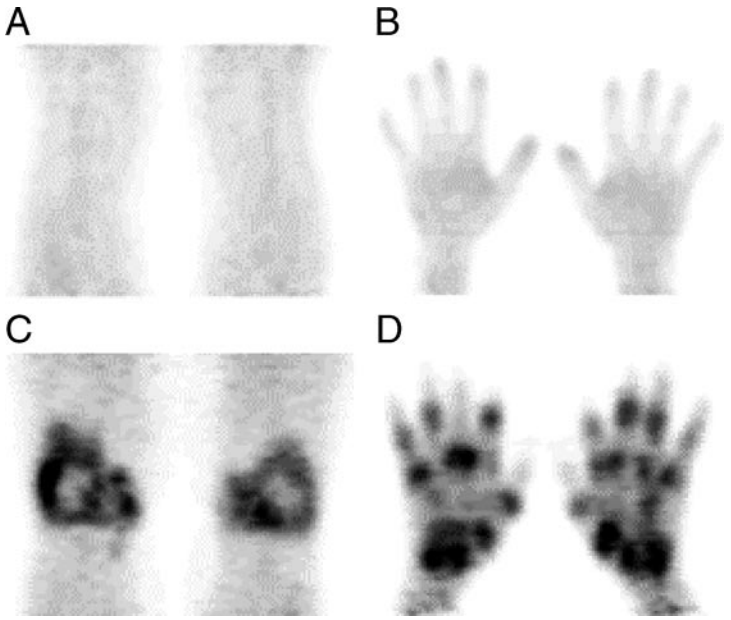

FIG. 3. 18F-Flurorodeoxyglocose (FDG) positron emission tomography (PET) imaging in arthritis. Images of a healthy control subject (A and B) and an RA patient with active RA (C and D). Normal tracer distribution in knee (A) and in hand and wrist (B). Tracer distribution in rheumatoid knee (C) and wrist (D). The figure shows that 18F-FDG PET can assess the high metabolic activity of inflamed joints. Reprinted from [29].

strongly associated with clinical findings in wrists but not with treatment outcomes. Contrast material-enhanced MRI and PET allow quantification of volumetric and metabolic changes in joint inflammation and comparison of efficacies of anti-inflammatory agents [28]. In contrast, the use of radiolabelled annexin V to image apoptosis, which may be used for monitoring therapies that induce reduced proliferation and apoptosis, is also feasible [30].

Optical imaging approaches

A number of recently published studies in experimental settings have shown the potential of optical-based imaging technologies (fluorescence reflectance imaging and bioluminescence imaging) to evaluate cellular and molecular events in arthritis. These approaches include non-invasive monitoring of inflammatory cells or transcription factors involved in disease, imaging gene transfer, and apoptosis or the activity of enzymes involved in joint destruction to monitor treatment response [31–36].

Using antimacrophage monoclonal antibodies (anti-F4/80) labelled with a near-infrared fluorochrome, Hansch and colleagues [31] demonstrated the feasibility of fluorescence reflectance imaging in visualizing inflammatory cells in arthritic joints *in vivo* in a murine arthritis model. Fluorescence reflectance imaging showed accumulation of the imaging agents in the inflamed knee joints and, to a lesser extent, in the contralateral (non-arthritic) knee joints. In this approach, monitoring of these inflammatory cells was optimal until 12 h after injection of the fluorochrome, but fluorescence measurements could be realized over a period of 7 days. Carlsen *et al.* have developed transgenic mice that express luciferase under the control of the transcription factor NF-κB, enabling non-invasive real-time bioluminescence imaging of NF-κB activity in intact animals. In a mouse model of RA, joints affected by arthritis produced strong NF-κB activity, resulting in a high signal intensity that could be imaged non-invasively. Since NF-κB is an important factor in the pathogenesis of RA, this model is a helpful tool in arthritis research [32].

Molecular imaging techniques can also be used for the visualization of disease- or therapy-related cells in living organisms. One of the most recent examples is the tracking of genetically altered cells in an adoptive gene therapy setting in which T cells were transduced in a murine model for RA. To be able to demonstrate the ability of injected cells to migrate preferentially into the inflamed articular tissue, *in vivo* bioluminescence imaging was applied. This technology allows longitudinal analyses; for example, adoptively transferred cells can be monitored in individual animals in real time on consecutive days for extended periods of time without the need to kill the animals. For this purpose, the cells of interest were transduced to express the enzyme luciferase prior to the transfer. Then, the animals received the luciferase substrate luciferin. Conversion of luciferin to oxyluciferin by luciferase from transferred cells generated light that could be detected sensitively by a camera system. Light intensity was visualized using a false-colour scale in which blue indicated low and red indicated high intensity, and the generated coloured spots were superimposed onto a grey-scale image of the animals that was obtained under weak illumination in the otherwise light-tight image chamber of the camera system. The coloured spots indicated the localization of the adoptively transferred cells (and the encoded gene products) within the different tissues of the animals. Figure 4 illustrates a bioluminescence images after adoptive gene transfer [33, 34].

In very recent approaches, we showed that near-infrared fluorescence imaging with a fluorescence reflectance imaging system can be used for non-invasive imaging of the treatment response in mouse models of RA using fluorescence-labelled annexin V as a biomarker of apoptosis and an enzyme-activatable fluorescent imaging probe as a marker of joint

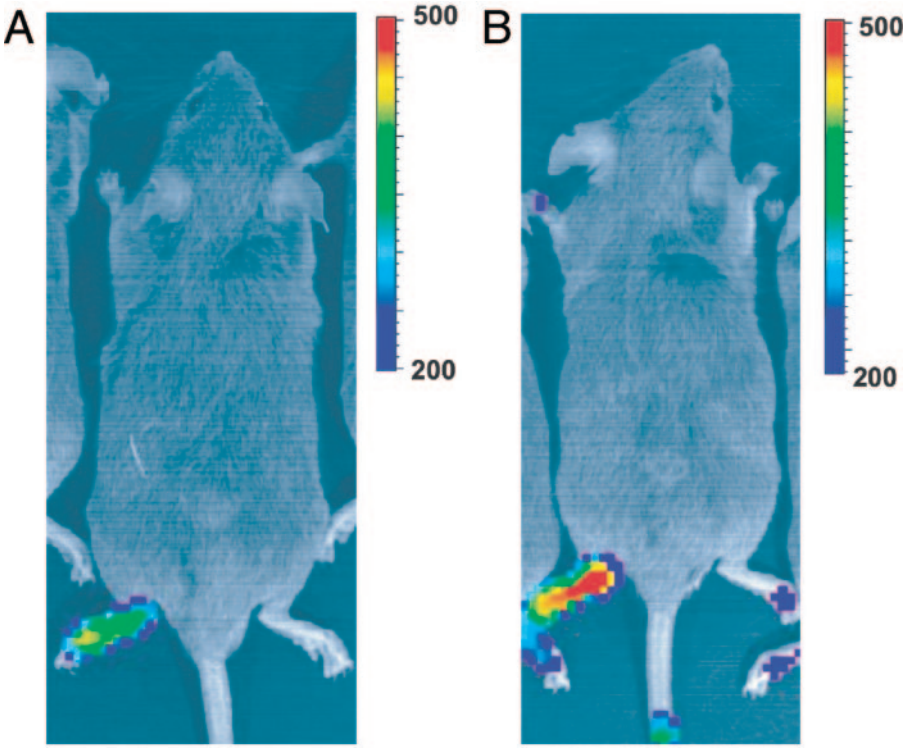

FIG. 4. Bioluminescence imaging of adoptive gene transfer *in vivo*. DBA/1 mice with collagen-induced arthritis (CIA) were injected intravenously with type II collagen-specific T-cell hybridomas retrovirally transduced to express the enzyme luciferase. After intraperitoneal injection of the luciferase substrate luciferin, the cells can be tracked by the resulting bioluminescence, which can be detected using a sensitive camera system. Detected light intensity is represented on a false colour scale, blue indicating low and red indicating high light intensity. This figure illustrates the accumulation of transferred cells in the severely inflamed left hind paw of an experimental animal on day 3 (A) and day 5 (B) after transfer. On day 5 (B), a weak signal is also detected in the right hind paw, which developed low grade inflammation over the course of the experiment. Adapted from [33].

destruction. Annexin V binds with high affinity to phosphatidylserine, a constitutive membrane phospholipid that is normally restricted to the inner leaflet of the plasma membrane lipid bilayer but is selectively exposed on the surface of cells as they undergo apoptosis. Arthritic paws of mice treated with methotrexate (MTX) showed a significantly higher fluorescence intensity than arthritic paws of untreated mice and non-arthritic paws of MTX-treated mice. Thus, monitoring the uptake of fluorescence-labelled annexin V in arthritic paws by fluorescence imaging may provide a method of assessing the treatment response to antirheumatic drugs such as MTX, a response which was readily quantitated with simple instrumentation and which occurred before conventional measurements of the treatment response [35].

In another approach, we used an enzyme-activatable near-infrared fluorescence imaging agent and fluorescence reflectance imaging to image protease activity for the non-invasive monitoring of the treatment response. Since matrix-degrading proteases such as cathepsins and MMPs are key players in joint destruction, we hypothesized that these enzymes would be suitable targets to visualize treatment effects *in vivo*. To test this principle, we used an imaging agent consisting of multiple Cy5.5 fluorochromes bound to a graft copolymer backbone of polylysine. Due to interactions between the fluorochromes, fluorescence quenching occurs. Therefore, the probe is relatively non-detectable in its native injected state. Enzymatic cleavage of the backbone *in vivo* by enzymes such as cathepsin B releases Cy5.5 and results in a significant increase in fluorescence (activation). After intravenous injection of the imaging probe, arthritic toes and paws showed a high fluorescence intensity. In MTX-treated animals, a significantly lower fluorescence signal was observed (Fig. 5). Using this experimental strategy, we could demonstrate not only that enzyme-activatable imaging probes can be used to visualize protease activity *in vivo*, but also that the reduction in activity following MTX therapy can be used to monitor the response to treatment [36].

Outlook

Parallel to our continuously increasing knowledge of molecular actions and interactions within the arthritic joint and its cellular and subcellular components, the various imaging techniques that have been developed in recent years will not only allow better identification, visualization and quantitation of key processes of the disease but most likely will also provide substantially improved capabilities for early diagnosis of RA, monitoring of treatment efficacy and more effective therapies.

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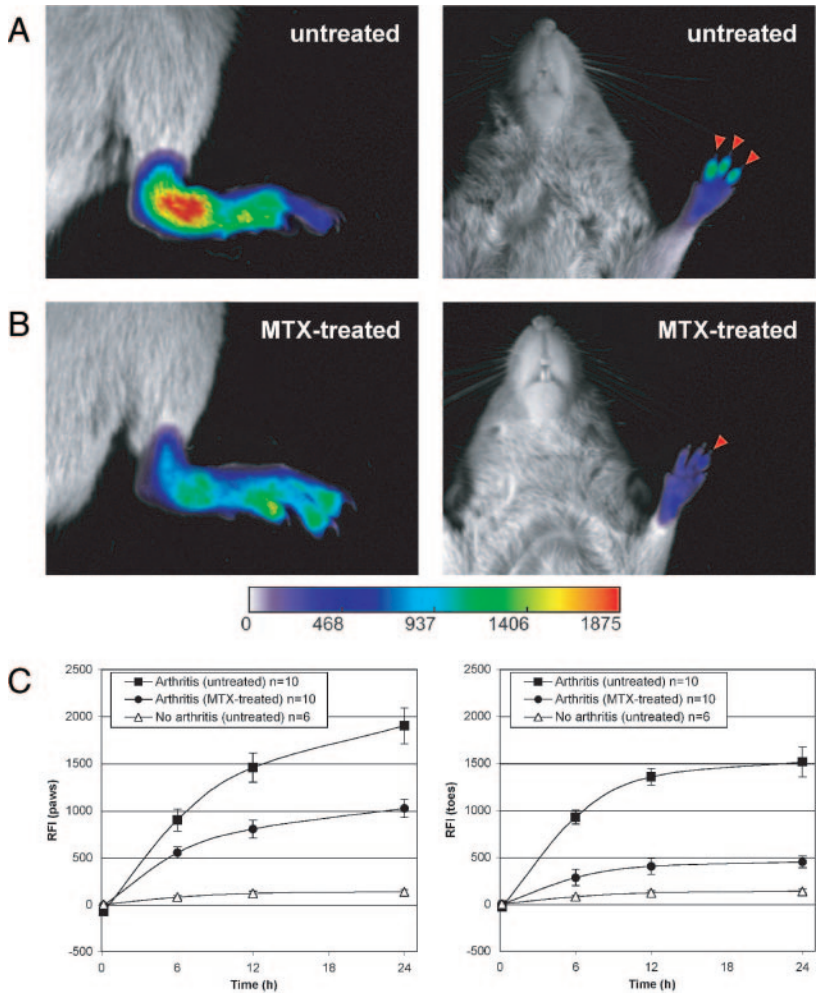

FIG. 5. Fluorescence reflectance imaging of protease activity for monitoring treatment response in arthritis non-invasively. Colour-coded near-infrared fluorescence images are superimposed on white light images of untreated (A) and MTX-treated (B) DBA/1 mice with collagen-induced arthritis (CIA) affecting the right hind paw (left) or toes (right; red arrowheads) 24 h after injection of a 'smart' imaging probe that is activated by proteases such as cathepsin B. MTX reduced fluorescence intensity from arthritic toes and paws compared with untreated mice. The time course of fluorescence intensities in arthritic and non-arthritic toes and paws of mice after probe injection is shown in panel C. Values are expressed as relative fluorescence intensity (RFI; mean ± S.E.M.). MTX treatment decreased fluorescence significantly 12 and 24 h after injection ($P < 0.001$). At 6 h, the level of significance was $P < 0.001$ for toes and $P = 0.01$ for paws. Inflamed toes of arthritic mice showed a 5-fold higher RFI than toes of healthy mice (paws, 7-fold) at 24 h ($P < 0.001$). Adapted from [36].

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