Molecular imaging techniques for rheumatology: paving the way for novel molecular therapies

Anatomical imaging using radiological techniques have underpinned the investigation and monitoring of treatment in modern clinical practice. Molecular imaging of inflammatory targets and pathways offers a unique insight into *in vivo* disease pathogenesis and the discovery and evaluation of novel therapeutic targets. This review summarizes current clinical and preclinical imaging strategies in inflammatory joint disease, in particular rheumatoid arthritis, and focuses on optical imaging as an example of a promising novel molecular imaging modality. Optical imaging has already been developed for *in vitro* and *ex vivo* applications in molecular and cellular biology (e.g., fluorescence confocal microscopy), but is still at an early stage of development as a technique for *in vivo* imaging of arthritis. This innovative technique, therefore, has significant potential to provide noninvasive molecular imaging of arthritis and serves as a demonstration of the role that *in vivo* imaging techniques can play in evaluating arthritis.

KEYWORDS: arthritis ■ fluorescence ■ imaging ■ molecular ■ optical ■ selectin

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Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease, primarily characterized by inflamed synovial tissue in multiple joints leading to localized destruction of cartilage and bone. It is a severe disabling disease that affects approximately 1% of the population on a worldwide basis [1]. RA is characterized by hyperplasia of the synovial lining layer and marked infiltration of synovium by lymphocytes, macrophages and plasma cells. Locally produced inflammatory mediators and the subsequent upregulation of adhesion molecules at sites of inflammation are pivotal to RA pathogenesis. Normal synovium is a highly vascular tissue that protects the joint, and provides oxygen, and nutrients to the synovial tissue, and to the relatively avascular cartilage. The normal synovium consists of an intimal lining, which is composed of macrophage- and fibroblast-like cells that are loosely associated without a definite underlying basement membrane, and a relatively acellular sublining, which merges with the joint capsule and contains loose connective tissue containing fibroblasts, macrophages, adipocytes and vascular endothelial cells. In RA, the synovium is altered to a thickened and invasively growing tissue several cell layers thick, which covers and erodes the adjacent cartilage, bone and tendon. Histologically, the inflamed synovium shows pronounced angiogenesis, cellular hyperplasia and influx of inflammatory cells.

Significant advances in understanding the underlying etiology of RA have been made. For example, a predominant role for major histocompatibility complex class II-dependent immune activation is supported by powerful evidence for antibody reactivity to proteins modified by citrullination [2]. Epidemiological and genetic studies of RA in relation to anti-citrulline immunity have demonstrated significant differences in subsets of patients with and without the presence of antibodies to citrullinated protein antigens (ACPA). Antibodies to citrullinated proteins can be found in approximately 60% of RA patients but only in 2% of the normal population, making them highly specific for RA [3–5]. The occurrence of ACPA is linked to the HLA-DRB1 shared epitope alleles [6]. In addition to the previously established risk posed by smoking, there are further associations between those who are HLA-DRB1 positive and a close segregation with those who are ACPA positive [6]. Such progress in our understanding of the pathogenesis of RA has led to the introduction of novel biological therapies in RA, such as those that target TNF-α and other specific biological targets [7,8].

Why do we need novel molecular imaging modalities in RA?

Nevertheless, despite such advances in understanding disease pathogenesis and hence in the identification of new therapeutic options, a significant proportion of patients do not respond positively to treatment. In addition, the pattern of disease in patients may change over time, and alternative therapy may be required at different times [8]. New *in vivo* imaging techniques, such as those described in this review, would be useful for elucidating pathogenesis and quantifying

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the effects of therapy. Furthermore, new imaging modalities, as well as providing invaluable information on the effects of therapy, are desirable in terms of RA diagnosis. The early diagnosis and treatment of RA is challenging. This is because there is significant heterogeneity in its clinical presentation and lack of sufficiently specific and sensitive laboratory tests. Conventional imaging strategies, such as plain x-ray imaging, are still widely used clinically to assess disease, and to measure progression and response to treatment [9]. X-rays rely on relatively late features such as bone erosion and joint space narrowing. These may only show significant changes many months after the onset of disease [10]. Greater joint damage at baseline is associated with poorer physical function and less improvement after treatment, underlying the importance of early detection and intervention to slow the progression of joint destruction since early treatment significantly improves long-term outcomes [11].

Various imaging modalities are available, such as computed tomography (CT), ultrasound, MRI and nuclear imaging methods, such as single-photon emission computed tomography (SPECT) and positron emission tomography (PET). These methods differ with respect to spatial resolution, ability to produce 3D images, depth limit and sensitivity, the possibility of quantification and availability of imaging agents that can be coupled to specific molecular perturbations. Classical approaches, such as ultrasound and bone scintigraphy, are useful techniques for assessing RA and can provide high-resolution images at the anatomical level. Ultrasound is useful for rapidly assessing multiple joints and is well established in the clinical assessment of synovitis and tenosynovitis; and may be combined with power Doppler ultrasound to provide further information on disease activity [12]. MRI can directly visualize the bone and soft tissues in 3D and has the potential to measure inflammatory activity and joint destruction. Synovitis volume, bone marrow edema and bone erosions are all potentially suitable for serial measurement, but none of these scoring systems is yet in routine clinical practice. MRI scanning is the current gold standard modality in clinical practice for imaging synovitis and tenosynovitis in patients with inflammatory arthritis. Prior studies have indicated that, using MRI, synovitis, bone edema and erosion can be detected within weeks of the onset of symptoms [13], and of these features, bone edema has repeatedly been shown to be the most important predictor of future erosions at the wrist [14–16]. Studies have shown correlations between the degree of inflammation and vascularity of synovium obtained at biopsy and postcontrast enhancement on matching dynamic MRI scans. MRI-observed synovitis is almost invariable in those with active RA, but recent studies have also demonstrated its presence in patients in clinical remission, emphasizing the sensitivity of this technique and the importance of subclinical joint inflammation [17]. Bone scintigraphy has been used for many years to detect inflammatory arthritis, but while it is a relatively sensitive technique it lacks specificity, has poor anatomical resolution and cannot accurately detect erosions [18]. As a consequence, information on the character of the initial destructive events in RA is limited, since the affected structures are not directly accessible in early disease and because the above techniques do not offer insight into the cellular and molecular processes involved early in the cause of disease pathogenesis. Sensitive and specific methods of imaging are, therefore, required for the detection of early inflammatory changes to the synovium in patients with arthritis to determine the best available therapy and for monitoring individual responses of differing patients to their tailored therapy.

As our understanding of the molecular basis of RA has improved, it has become possible to develop more sophisticated and sensitive techniques for imaging disease and detecting pathological change at an early stage. Predicting prognosis in any patient with newly diagnosed RA is also of key importance so that high-cost therapies can be tailored to the needs of the individual, particularly since, as mentioned earlier, a significant proportion of patients do not respond positively to treatments such as TNF-α inhibitors. Molecular imaging enables the noninvasive visualization of molecular processes and the follow-up of cellular function in living organisms [19]. Molecular imaging differs from classical traditional imaging in that probes known as biomarkers are used to help image particular targets or pathways. Biomarkers interact chemically with their surroundings and in turn alter the image according to molecular changes occurring within the area of interest. This process is markedly different from previous methods of imaging, which primarily imaged differences in qualities, such as density of bony tissue or water content. This ability to image molecular changes *in vivo* offers a number of exciting

possibilities for medical application, including early detection and treatment of disease and rational drug development [20]. There are many different imaging modalities that are highly applicable to rheumatic disease in addition to other diseases. This technique also contributes to improving potential novel therapy by optimizing the preclinical and clinical therapy of new medication and offering further information about pathobiology. Molecular imaging techniques are also expected to have a major impact in preclinical diagnosis of inflammatory disease, and prediction of individual responses to targeted therapy. In particular, molecular imaging may also provide insight into disease mechanisms and elucidate the mode of action of therapeutic agents, particularly using animal models, which are of vital importance in developing these techniques [21]. Relevant animal models help us to both understand the mechanism of action of novel therapies and to validate their use in ameliorating arthritis, by developing reproducible objective measures of scoring disease activity and monitoring disease activity *in vivo* [22]. Furthermore, molecular imaging can provide quantitative results, for objective testing of therapeutics [21].

A range of novel imaging approaches have been developed that combine the pathological imaging techniques described above with methods of detecting molecular peturbations. As a development of conventional ultrasound imaging, optoacoustic molecular imaging holds possible potential as a technique that can provide spatially resolved information about the presence of molecular markers *in vivo*. A recent study explored the photoacoustic properties of gold nanorods as nanoprobes for molecular optoacoustic imaging in arthritis. In this study, elongated gold nanorods were synthesized that had an absorption maximum in the range of 1064 nm that were then conjugated with anti-TNF antibodies for targeting TNF-α to detect inflammation in arthritic mouse knees. This showed a differential enhancement of optoacoustic signal by using a fast-scanning optoacoustic imaging platform based on a pulsed Nd:YAG laser and a single focused ultrasound transducer. The excellent photoacoustic properties of the gold nanorods confirmed the overexpression of TNF-α in arthritic knees. Due to the uncomplicated coupling chemistry and the scalability of ultrasound-based imaging approaches, these results potentially allow a transfer to various preclinical and clinical applications [23].

With MRI, for example, while bone edema has a significant prognostic value it can also be a comparatively nonspecific finding, hence attempts have been made to utilize other new contrast agents as molecular imaging probes for MRI. As an example of a targeted imaging technique, E-selectin expression on activated vascular endothelium has been evaluated using MRI in an *in vivo* mouse model. This study utilized the conjugation of ultra-small superparamagnetic iron oxide nanoparticles as a contrast agent on an oxazolone-induced contact hypersensitivity model and effectively depicted activated vascular endothelium in murine inflammation *in vivo* with MRI [24]. A further example is the use of nanoSPECT/CT imaging in the severe combined immunodeficiency mouse engrafted with human synovial tissue, using a radiolabeled anti-E-selectin antibody to give quantifiable 3D images of E-selectin targeted signal following stimulation with TNF-α [25]. High-resolution SPECT imaging of bony pathology has also recently been performed in patients with early RA and images can be overlaid with MRI to generate anatomically localized images [26]. Finally, optical imaging, using fluorescent or bioluminescent molecules, has already been developed for *in vitro* and *ex vivo* applications in molecular and cellular biology (e.g., fluorescence confocal microscopy). Optical imaging is still at an early stage of development as a technique for *in vivo* imaging of arthritis, and as an example of an emerging molecular imaging technique it will be discussed in more detail in the following sections.

Fluorescence imaging studies *in vivo*

Fluorescence is defined as the emission of light following the excitation of a molecule (fluorophore) by light to a higher energy state, and its subsequent decay, resulting in emission light of a different wavelength. This difference is termed the 'Stokes shift' named after Sir George Stokes who first described fluorescence in 1852. Some small biological molecules (e.g., NADH and tryptophan) and proteins (e.g., phycoerythrin or GFP) are naturally fluorescent (intrinsic fluorophores), whereas extrinsic fluorophores are derived from many common dye families, such as xanthenes, cyanine and coumarin derivatives. Extrinsic fluorophores may be attached to proteins of interest by chemical modification of specific functional groups, such as amino, carboxyl or thiol groups, thereby enabling them to track specific molecular changes [27].

Fluorescence is visualized by excitation at an appropriate wavelength and capture of the emitted photons usually by a charge-coupled detector (CCD). Advances in CCD design have enabled accurate detection of low levels of light. Early fluorescence imaging in animals was invasive, with intravital microscopy being the most common tool. Intravital fluorescence microscopy in inflammatory arthritis was first performed in 1993. This technique allows measurement of microhemodynamic parameters as well as leukocyte–endothelial cell interactions [28]. Blood vessel diameter [29,30] and capillary density [31] were measured in the synovial microcirculation of mouse knees following surgical exposure of the area of interest. The inhibitory effect of antileukoproteinase, a physiological inhibitor of granulocyte serine proteases on blocking the interactions of leukocytes on the endothelial lining was investigated by microscopic imaging of leukocyte rolling and adhesion *in vivo* [32]. This technique, however, does require local dissection of the area for direct microscopy. By contrast, fluorescence reflectance imaging is noninvasive and *in vivo* real-time images of the whole animal can be taken. The high degree of absorption and scattering of light by biological samples also limits the penetration of both the excitation and resulting emission signal [33]. Studies have suggested that fluorescent signals may be reduced by up to tenfold per centimeter of depth, although this will vary with tissue type, wavelength and dye brightness [34]. Another limitation is that images from a planar CCD are 2D and therefore lack tissue depth information. In addition to the absorption and scattering of light [33], biological samples autofluoresce at the wavelengths used for traditional extrinsic dye families (300–500 nm). Newer dyes better suited for *in vivo* studies fluoresce at higher wavelengths, close to the infrared range (700–900 nm) have been developed. The intrinsically fluorescent protein, GFP, has a peak emission wavelength of 509 nm and is therefore subject to significant autofluorescence from surrounding tissue if used for *in vivo* fluorescence imaging. However, several mutants of the monomeric red fluorescent protein that fluoresce at wavelengths above 600 nm have been produced [35]. Advances in CCD technology and use of near infrared (NIR) fluophores will continue to allow for better image resolution [36]. This has the potential to provide noninvasive imaging of molecular targets, and physiological process *in vivo*. Other advantages of *in vivo* fluorescence imaging are that there is no exposure to harmful radiation, it is relatively cheap and images can be acquired rapidly [37].

In RA, disordered new vessels have increased vascular leakiness to macromolecules compared with normal vessels, and that this may affect interpretation of results from *in vivo* fluorescence imaging studies [38]. Multiple factors influence the leakage of substances from blood vessels. The major physiological mechanisms include the luminal surface area, permeability of the vessel wall and concentration of driving forces (hydrostatic and osmotic gradients) as well as the blood flow across the endothelium [39,40]. Early pathological studies identified vasodilation and increased vessel permeability from biopsies of arthritic joints [41,42]. As the vascular permeability increases this leads to plasma extravasation, edema formation and swelling of the joint. Pathologic newly formed vessels also have increased leakiness compared with normal vessels. This effect is well documented in neoangiogenic tumor tissue and is thought to also occur in RA due to increased blood perfusion and vascular permeability as part of both the ongoing inflammatory process and disordered architecture of new vessels. This may lead to as much as a 40-fold increase in macroglobulin permeability in the inflamed joint [38,43].

Enhanced localized deposition of free dye (not bound to a disease-specific antibody or other target) has been demonstrated in the arthritic joints of animals with collagen-induced arthritis in a number of studies. For example, the NIR fluorophore Cy5.5 was visualized in arthritic knee joints following induction of antigen-induced arthritis in mice [44]. This demonstrated an approximate 1.5-fold increase in signal in arthritic compared with nonarthritic joints. Two other NIR dyes were evaluated in a murine *Borrelia*-induced Lyme arthritis model [45]. These techniques rely on the generalized increased blood perfusion and 'vascular leakiness' that are recognized to be present around the inflamed joint [43]. In such cases native dye most likely accumulates in the inflamed synovial membrane following phagocytosis by activated macrophages. Another study has demonstrated increased levels of fluorescence in inflamed paws with a fluorescently labeled PEGylated anti-TNF-α agent compared with a non-PEGylated TNF-α inhibitor [46]. This study again highlights that there is significant nonspecific diffusion of molecules of all sizes into areas in inflamed areas with, in this case increased local deposition of

the anti-TNF antibody with PEGylated anti-TNF. PEGylation of a molecule therefore does not confer specific molecular targeting but rather an increased local diffusion of molecule into an inflamed space. It is also worth noting that no isotype control antibody targeting compared with anti-TNF or with PEGylated anti-TNF was included in this study. There is also a recognized nonspecific autofluorescence change in the absorption emission spectra of inflamed tissue [47], although this phenomenon is likely to be more limited at wavelengths in the NIR spectrum. This may also interfere with the specific signal detectable from sites of inflammation.

Targeting of specific molecular changes in arthritis

Understanding the key components of the inflammatory cascade in RA has been pivotal for the development of novel molecular targets and treatment paradigms [48]. *In vivo* molecular imaging may also help to increase specificity in assessing disease activity levels and differentiating subsets of arthritis for altered treatment protocols.

Macrophages targeted with Cy5.5-labeled anti-F4/80 antigen have been delineated in the arthritic joint of antigen-induced arthritic mice. The F4/80 antigen is expressed on the macrophages when they accumulate in inflamed joints [49] and specific deposition could be detected. There was, however, a small increase in signal in the uninflamed contralateral knee joints as well as increased uptake in inflamed knee joints with the Cy5.5-labeled isotype control antibody. Folate receptors on activated macrophages have also been detected in a similar study. This demonstrated a 2.3-fold increase in signal in inflamed joints compared with uninflamed joints [50]. This increase was greater than that obtained following injection of free dye. Allogenic leukocytes labeled *ex vivo* with fluoresecent dye and reinjected into arthritic mice have also been detected. In this study, however, treatment with steroid reduced the signal intensity obtained. However, the degree of quantitative analysis was hampered by a relatively small change in signal intensity compared with the background signal.

Amplification of fluorescent signal at the site of inflammation using protease-activatable probes may be another method that could be deployed for *in vivo* imaging of arthritis. This may be especially required for molecular imaging of osteoarthritis because the local perturbations in proteolytic activity within the arthritic joint may be comparatively small when compared with inflammatory arthritis. Cathepsins have been associated with arthritis and bone degeneration in osteoarthritis [51]. A cathepsin B-activatable NIR fluorescent probe was used in a mouse model of osteoarthritis. This demonstrated that a threefold difference in signal intensity could be achieved between normal and osteoarthritic joints [52,53]. This particular probe has also been applied to tumor detection [54] as well as the localization of atherosclerotic plaques *in vivo* [55]. The effect of methotrexate therapy was also investigated using a similar probe and this demonstrated that methotrexate treatment could abrogate some of the increased signal found in arthritic joints [56]. A further study also demonstrated that specific signal could be reduced and correlated with a decrease in clinical disease indices, such as redness and measurable paw swelling [57]. However, these studies did not examine whether an uncleavable form of peptide demonstrated similar signal enhancements or whether, for example, any specific inhibitor of the protease could prevent cleavage in the arthritic joint.

As an example of how a specific molecular imaging technique can be developed, we here describe a molecular target for fluorescent labeling that appears to have excellent potential. This is E-selectin (ELAM-1), a 115-kDa glycoprotein induced on endothelial cells in response to proinflammatory cytokines, such as IL-1 and TNF-α, which are involved in RA. As part of a complex multistep process, selectins promote the initial attachment (tethering) and subsequent moving (rolling) of leukocytes on endothelium, where they become activated as a result of locally produced chemokines [58]. The endothelium in human RA synovium has been demonstrated to express E-selectin [59]. Furthermore, previous imaging studies have assessed E-selectin expression *in vivo* [24,25]. Scintography utilizing a radiolabeled anti-E-selectin antibody has been used to successfully image synovitis in patients with RA, with a ^{99m}Tc-anti-E-selectin-Fab demonstrating improved specificity compared with a conventional tracer for bone and joint inflammation, ^{99m}Tc-oxidronate [60–62]. This study also demonstrated particular specificity for targeting active joint inflammation [62]. Acute paw swelling caused by local injection of TNF-α was initially developed as a reproducible acute model of joint inflammation. Injection of TNF-α into the mouse foot-pad produced a transient

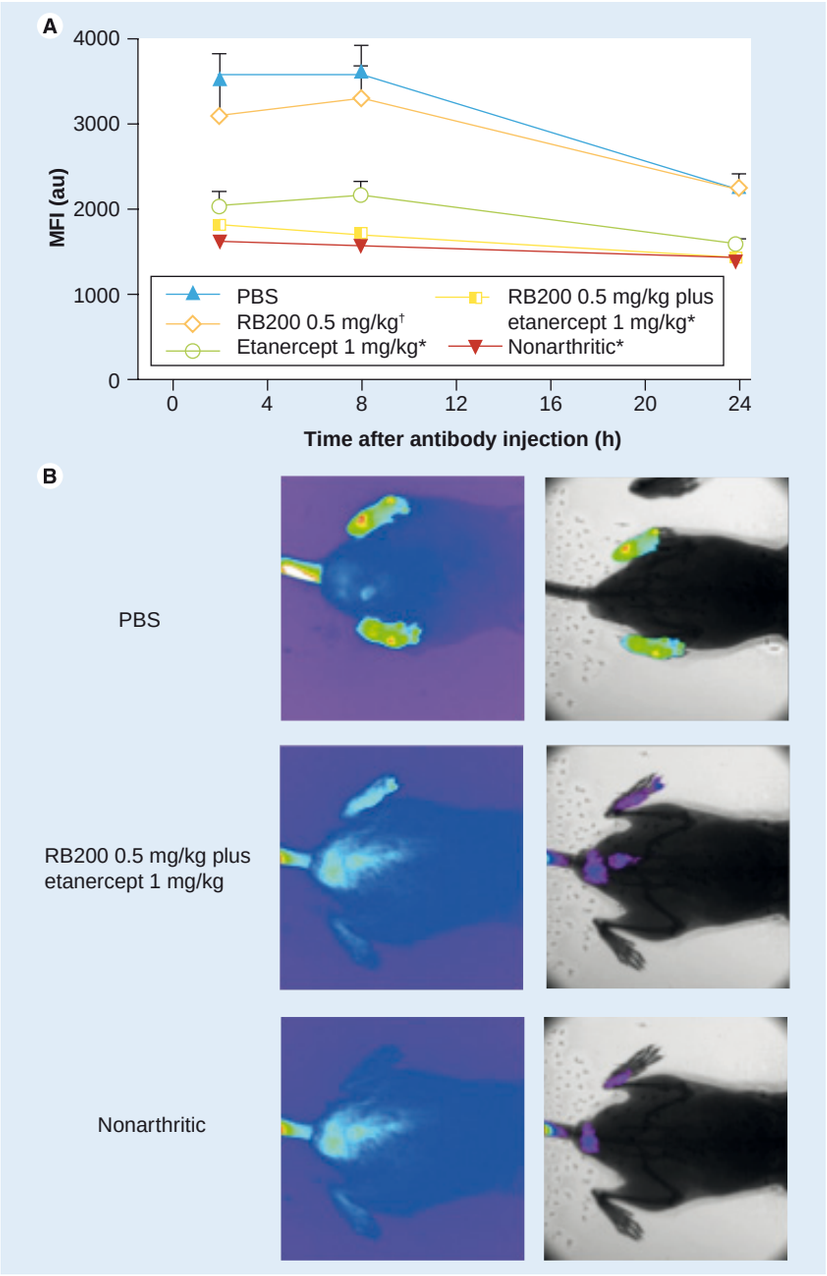

Figure 1. Additive effect of low-dose etanercept and RB200 on E-selectin-targeted *in vivo* fluorescent imaging in collagen-induced arthritis. (A) Following onset of arthritis induced by bovine collagen, mice were treated intraperitoneally on the day of disease onset (day 1) and then on days 4 and 7 of disease, with RB200 at a dose of 0.5 mg/kg (n = 6), 1 mg/kg of etanercept (n = 7) or a combination of RB200 plus etanercept (n = 6). As controls, mice received equivalent volumes of PBS (n = 7) intraperitoneally For imaging, mice were injected intravenously with NIR dye labeled anti-E-selectin antibody (5 μg). Images were obtained at 2, 8 and 24 h following injection of the dye–antibody conjugate. Results are au MFI for both paws (±SEM), and were analyzed using two-way ANOVA versus PBS-treated mice: †not significant, *$p < 0.001$. **(B)** Representative images were obtained 8 h following injection of dye–antibody conjugate. Left hand panels depict representative fluorescent images following application of a color wheel to depict signal intensity. Right hand panels demonstrate corresponding image coregistered with x-ray following subtraction of background fluorescence. au: Arbitrary unit; MFI: Mean fluorescence intensity; PBS: Phosphate-buffered saline.
Adapted from [63].

dose-dependent paw swelling that peaked at 4 h post-injection and resolved within 24 h. To study anti-E-selectin antibody trafficking *in vivo*, fluorescence images were obtained at different times following induction of paw swelling with TNF-α and injection of NIR fluorophore-labeled anti-E-selectin or control antibody. Our results demonstrate that E-selectin antibody localized selectively to the TNF-α-injected paws. Quantification of fluorescence intensity levels from regions of interest of inflamed paws demonstrated that fluorescence signal was significantly higher for anti-E-selectin-injected animals than for control antibody-injected mice. Furthermore, fluorescence signal was lower in the contralateral left paws (not injected with TNF-α). This initial work helped validate the use of E-selectin-targeted imaging as a powerful optical imaging modality for arthritis. Specifically, this approach could be applied for the detection of early inflammation in RA, to quantify disease and investigate the local effects of new therapies. Importantly, this methodology may also be useful for the detection of subclinical disease and systemic endothelial activation in RA.

We have therefore demonstrated specific localization of NIR-labeled anti-E-selectin antibody to activated endothelium in mice using fluorescence imaging *in vivo* and have shown this can be specific for detecting subclinical levels of arthritis [63]. This technique was then used to assess the responses of a novel medication. The highly unique compound RB200 is a bispecific ligand trap that targets all four members of the EGF receptor family that have been demonstrated to be effective in the abrogation of collagen-induced arthritis in mice. We were able to explore the therapeutic effects of this compound as well as its effect in combination with the TNF receptor blocker etanercept. By using low-dose RB200 and etanercept in combination, complete abrogation of collagen-induced arthritis was demonstrated [63,64]. This included the subclinical arthritis that could be detected with *in vivo* E-selectin-targeted fluorescence imaging (Figure 1). This serves as an example of the potential for molecular imaging and in particular optical imaging to assess the therapeutic response of novel medications.

Future perspective

As optical imaging becomes more targeted through the use of specific antibodies, activatable probes, as well as brighter, more photostable dyes, targeted fluorescence optical imaging

in humans is becoming a realistic possibility. Indeed, nonspecific optical imaging of patients with RA has already taken place with some initial optical imaging studies undertaken following the injection of indocyanine green (ICG) into patients with RA. ICG is already routinely used in the clinic for retinal angiography [65] and liver function testing [66,67]. Most recently, ICG has been tested for use during liver cancer surgery by two groups to identify small and macroscopically unidentifiable liver cancers in real time, which were confirmed microscopically [68,69]. ICG has also been tested in the real-time identification of sentinel lymph nodes in small numbers of breast cancer patients [70–72]. As ICG is nontargeted, however, a small number of false positives were detected [68]. Conjugation of ICG to a molecule that would enable specific targeting would reduce this. However, the use of ICGs as a conjugated NIR probe is limited as upon protein binding, fluorescence is lost. In an animal model, this property was exploited by conjugation with antibodies to CD25, which upon CD25-positive tumor cell binding become internalized, leading to catabolism in lysosomes, with release of fluorescence from unbound ICG [73]. ICG unbound to a target has been used to detect arthritis in a rat model of arthritis. This demonstrated that native dye was able to provide a significant increase in the fluorescent signal of arthritic joints compared with baseline values [74]. Several translational optical imaging devices have recently been introduced to the clinic. This includes a custom-built ICG-enhanced optical imaging device that has recently been shown by Fischer *et al.* to correlate well to MRI changes in the detection of pathological change in RA [75]. Five patients and a corresponding number of normal controls were studied using 0.1 mg/kg by body weight of ICG as an unconjugated nonspecific contrast agent. Fluorescence images were acquired continuously over a 15-min period. Findings were also compared directly to contrast-enhanced magnetic resonance images taken concurrently. This demonstrated that ICG levels were increased in inflammatory joints although sensitivity and specificity was not clearly defined. They did conclude, however, that NIR imaging was limited to the finger and foot joints because the strong scattering and absorption of light would preclude imaging beyond a few centimeters of tissue depth.

A further study has compared ICG-enhanced fluorescence optical imaging with clinical examination, ultrasound and MRI in 252 patients with arthritis and allied conditions. This has demonstrated that ICG-targeted fluorescent imaging is a valid imaging technique that is able to sensitively detect synovitis when compared with 1.5 T MRI and ultrasound [76]. It may be possible that specificity could be amplified beyond this by conjugating fluorescent dye to a particular target, such as E-selectin, or by using a method of enhancing the signal by using an enzyme-linked probe as discussed in the above sections.

In summary, the addition of TNF-α blockade to the therapeutic armamentarium for patients with RA has had a revolutionary impact on the treatment of both RA and other inflammatory diseases with differing molecular approaches used to target TNF-α. This has paved the way for many other agents other than TNF-α blockade to be validated as therapeutic agents, but there still remains considerable uncertainty about which individual patients will respond to any particular treatment. Increasingly as further agents become clinically available the complexity of individual treatment plans for patients with RA is likely to increase. Biomarkers are therefore required that enable both the early diagnosis of RA and help to identify particular patients that may respond positively to tailored biological therapy. Furthermore, early synovitis destined to develop into RA is characterized by a distinct and transient cytokine profile that may be different to that in established disease. There is much need therefore, to determine how levels of cytokines and other inflammatory mediators change during the evolution of disease both systemically and in local sites of inflammation. It is the latter that will be most amenable to examination by novel bioimaging techniques such as optical imaging. With the added molecular perspective to diagnosis, this may translate into improved targeting of treatments to particular patients depending on the phenotype of the disease thereby helping to determine appropriate treatment.

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EM Paleolog has worked as a consultant for Receptor Biologix Inc. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Executive summary

Rheumatoid arthritis

- Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that affects approximately 1% of the population worldwide.
- It is primarily characterized by inflamed synovial tissue in multiple joints leading to localized destruction of cartilage and bone. RA is a severe disabling disease.

Why do we need molecular imaging modalities in RA?

- The early diagnosis of RA is made problematic by heterogeneity in its clinical presentation and lack of sufficiently specific and sensitive laboratory tests.
- Conventional imaging strategies, such as plain x-ray imaging, are limited in their ability to assess early disease progression.
- Molecular imaging enables the *in vivo* visualization of molecular processes and follow-up of cellular function in living organisms without perturbing them.

Fluorescence imaging

- Near-infrared fluorescence imaging is an *in vivo* modality for monitoring biologic processes at the cellular and molecular level based upon the targeting of molecules with fluorochrome-labeled carriers.
- Modern dyes that fluoresce close to the near-infrared range (700–900 nm) are better suited for *in vivo* studies because they avoid the significant tissue autofluorescence that occurs at lower wavelengths.
- There is no exposure to harmful radiation, it is relatively cheap and images can be acquired rapidly.

Targeting of specific molecular changes in arthritis

- E-selectin, an adhesion molecule expressed during the inflammatory cascade, represents a well-validated target for *in vivo* optical molecular imaging.
- This can be used as a novel molecular imaging tool for assessing novel forms of inflammation.

Summary

- Targeted optical imaging of arthritis represents a novel molecular imaging technique that has significant potential for future insights into disease pathogenesis, and targeted therapeutic development.

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