HHS Public Access

Author manuscript

Chembiochem. Author manuscript; available in PMC 2020 February 15.

Published in final edited form as:

Chembiochem. 2019 February 15; 20(4): 422–436. doi:10.1002/cbic.201800429.

Antibody-based Tracers for PET/SPECT Imaging of Chronic Inflammatory Diseases

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Abstract

Chronic inflammatory diseases are often progressive, resulting not only in physical damage to patients but also social and economic burdens, making early diagnosis of them very critical. Nuclear medicine techniques can enhance the detection of inflammation by providing functional as well as anatomical information when combined with other modalities such as magnetic resonance imaging, computed tomography or ultrasonography. While small molecules and peptides were mainly used for the treatment and imaging of chronic inflammatory diseases in the past, antibodies and their fragments have also been emerging for chronic inflammatory diseases since they show high specificity to their targets and can have various biological half-lives depending on how they are engineered. In addition, imaging using antibodies or their fragments can visualize the *in vivo* biodistribution of the probes or help monitor therapeutic responses, providing physicians a greater understanding of drug behavior *in vivo* and another means of monitoring their patients. In this review, we introduce various targets and radiolabeled antibody-based probes for molecular imaging of chronic inflammatory diseases in preclinical and clinical studies. Targets can be classified into three different categories: 1) cell adhesion molecules, 2) surface markers on immune cells, and 3) cytokines or enzymes, and limitations and future directions of using radiolabeled antibodies for imaging inflammatory diseases are also discussed.

Graphical Abstract

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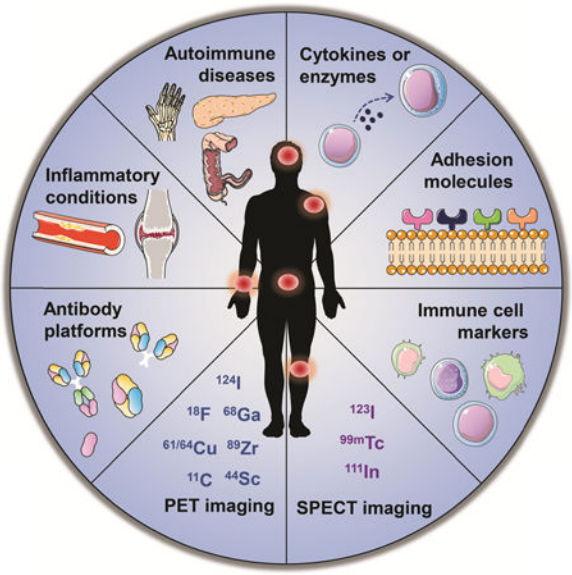

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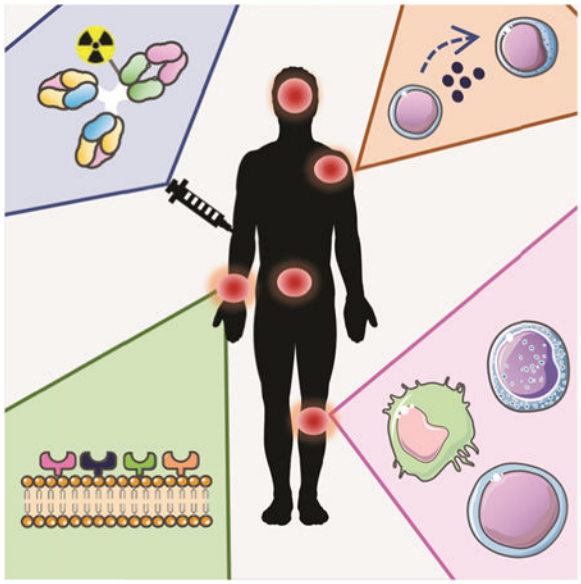

Chronic inflammatory diseases are often debilitating to their patients, and sometimes difficult to diagnose and monitor. To this end, molecular imaging techniques employing antibodies targeted to various inflammation-specific markers can aid in patient diagnosis and monitoring, as presented in this review.

Keywords

Antibodies; autoimmune diseases; inflammation; positron emission tomography (PET); single-photon emission computed tomography (SPECT)

1. Introduction

Chronic inflammatory diseases can progressively debilitate organs and body systems, and are often accompanied by comorbidities, such as cardiovascular problems, infectious diseases, or cancer.[1] Many chronic inflammatory diseases occur based on autoimmunity. Autoimmune diseases are characterized by the breach of immunological tolerance, which can be triggered by genetic predisposition and environmental factors.[2] In addition, both innate and adaptive immunity may also be involved in the pathophysiology of autoimmune diseases by producing autoantigens and inducing molecular mimicry, ultimately resulting in organ-specific or systemic tissue damages.[2] Rheumatoid arthritis (RA), inflammatory bowel disease (IBD), multiple sclerosis (MS), systemic lupus erythematosus (SLE), autoimmune thyroid disease (AITD) and autoimmune hepatitis are all chronic autoimmune diseases. On the other hand, chronic inflammatory diseases such as atherosclerosis or Alzheimer's disease are not derived from autoimmunity and are more likely related to aging and degenerative mechanisms.[3] Since diseases in both categories are mostly progressive and destructive, early and accurate detection of the diseases and stratification of patients by level of risk are essential to prevent complications and improve the patients' prognosis.

Typical diagnostic strategies for inflammatory diseases include physical examination, laboratory analyses, and endoscopy.[2] Furthermore, non-invasive imaging techniques including x-ray, ultrasonography, magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), and positron emission tomography (PET) are also widely used for the evaluation of disease activity and monitoring treatment response in a clinical setting.[4] In particular, radionuclide-based SPECT and PET imaging have several advantages over other modalities in that they can provide functional molecular information with high contrast and sensitivity in the nano- or pico-molar range.[5] Currently, several radiopharmaceuticals have been developed and used for the diagnosis of chronic inflammatory diseases in clinic. For example, radiolabeled white blood cells such as ^{99m}Tc-hexamethylpropyleneamine oxime (HMPAO)-leukocytes have been commonly used for monitoring the migration of immune cells into inflamed sites of IBD or RA.[6] Although ^{99m}Tc-HMPAO-leukocytes can detect the extent of disease with high sensitivity, this strategy has disadvantages since the *ex vivo* labeling techniques are cumbersome and it is hard to achieve long-term observation of cell viability with this method.[7]

^{18}F-fluorodeoxyglucose (FDG) is another radiopharmaceutical extensively used for various inflammatory diseases as well as malignancies by taking advantage of increased glucose metabolism in target cells. However, the usefulness of ^{18}F-FDG may be limited by its non-specificity and vulnerability to physiologic conditions such as glucose level or kidney functions.[8] In this sense, radiolabeled antibody-based molecular imaging has been emerging as a promising strategy not only for noninvasive detection of chronic inflammation but also selection of patients for antibody-based therapy. As a general method for synthesizing antibody-based radiotracers, antibodies are conjugated with chelators using the reactive amine or sulfhydryl groups on the antibodies, and then incubated with radiometal solutions. Detailed methods can be found in several other papers.[9] Since monoclonal antibodies (mAbs) are characterized by high specificity for target molecules and long *in vivo* circulation half-lives, mAbs labeled with radiometals may detect inflammation effectively

and quantitatively in patients with chronic inflammatory diseases.[10] In addition, intact mAb molecules can be engineered into other formats such as antibody fragments (e.g. $F(ab')_2$, Fab, nanobodies [Nb], and single chain variable fragments [scFv]) with lower molecular weights to achieve faster plasma clearance and potentially enhanced biodistribution.[11] In this review, we will discuss SPECT and PET imaging of chronic inflammatory diseases focusing on radiolabeled antibody-based probes and their targets in three different categories (adhesion molecules, surface markers of immune cells, and cytokines or enzymes) (Figure 1).

2. Targeting cell adhesion molecules

Interaction between cell adhesion molecules with their ligands regulates adherence of leukocytes to other cells or the extracellular matrix (ECM), and mediates migration of immune cells to sites of inflammation.[12] Since the activation of immune cells and subsequent cytokine production (e.g. Tumor necrosis factor-alpha (TNF-α), Interleukin-1, and Interferon-gamma) can increase the expression of adhesion molecules on lymphocytes and endothelial cells during inflammation, adhesion molecules have been promising targets for molecular imaging of autoimmune and inflammatory diseases.[12] Herein, we introduce molecular imaging of adhesion molecules based on radiolabeled antibodies in three different categories: the integrin family, immunoglobulin superfamily, and selectins.

2.1. Integrin family

Integrins are transmembrane molecules found on many cell types that mediate cell adhesion by coupling the ECM to intracellular cytoskeletons and influence cell signaling by activating various pathways including that of the mitogen-activated protein kinase.[12–13] Integrin is a non-covalently bound heterodimer consisting of α and β subunits. Among them, integrin β_7 is selectively expressed on lymphocytes in the Peyer's patches and mesenteric lymph nodes in inflamed guts and can pair with α_4 or α_E to form a heterodimer.[14] While integrin $\alpha_4\beta_7$ plays a role in the recruitment of lymphocytes by targeting mucosal addressin cell-adhesion molecule-1 present on endothelial cells of the intestine, the $\alpha_E\beta_7$ integrin is more likely to contribute to retention of lymphocytes by interacting with E-cadherin molecules.[14] In 2014, the US Food and Drug Administration (FDA) therefore approved vedolizumab, an anti-$\alpha_4\beta_7$ antibody, for the treatment of both moderate-to-severe ulcerative colitis and Crohn's disease.[15]

Dearling et al. employed ^{64}Cu-labeled mAbs (FIB504.64) against the β_7 molecule to evaluate the feasibility of immunodetection of lymphocytes in a mouse model of IBD.[14] The accumulation of ^{64}Cu-labeled FIB504.64 (average ± standard deviation, in percent of the injected dose per gram of tissue [%ID/g]) was higher in the gut of mice with colitis (6.49 ± 2.25) than in the mice without colitis (3.64 ± 1.12) or the ^{64}Cu-labeled non-specific antibody group (3.97 ± 0.48, $p < 0.05$, $n = 5–6$), indicating β_7 as a potential target for the diagnosis and therapy of colitis. Dearling and colleagues also extended their study to clarify target cell populations and to compare the pharmacokinetic properties of three different probes: the $F(ab')_2$ and Fab fragments of the previous FIB504.64 (anti-β_7 antibody), and DATK32 antibodies (anti-$\alpha_4\beta_7$ integrin) in mouse models of colitis.[16] While the uptake of

the DATK32 antibodies in the gut of colitis mice slightly increased at the last time point, 48 h post-injection (p.i.), the accumulation of FIB504.64-Fab fragments in the intestine was highest at 4 h p.i. and the uptake of FIB505.64-F(ab')$_2$ was obvious as early as 1 h p.i. Of note, the ratio of uptake between colitis and normal mice in the large intestine was lower for ^{64}Cu-labeled DATK32 (1.38) than for intact FIB504.64, F(ab')$_2$, or Fab fragments of FIB504.64 (1.78, 3.15, and 1.84, respectively). These results were also further confirmed by immunohistochemistry results showing a relatively smaller population of $\alpha_4\beta_7$ cells, implicating that targeting the entire population of cells expressing the β_7 molecule may be a better option for developing imaging tracers for colitis. Furthermore, the highest intestinal uptake ratio of the F(ab')$_2$ group suggests its potential as a promising tracer for immunodetection in colitis.

In addition to $\alpha_4\beta_7$, other integrin molecules such as $\alpha_4\beta_1$ (very late antigen-4 [VLA-4]), and $\alpha_L\beta_2$ (lymphocyte function-associated antigen-1 [LFA-1]) have also been related to autoimmunity.[12] VLA-4 is known to be associated with blood-brain barrier penetration of T cells in MS or experimental autoimmune encephalitis (EAE), an animal model of MS.[17] Natalizumab, a mAb against VLA-4, was accordingly approved in 2004 as an effective therapy against MS.[18] Similarly, the expression of LFA and its ligand ICAM-1 can promote infiltration of autoreactive lymphocytes into synovial tissues in RA or penetration of leukocytes into brain microvascular endothelial cells in MS.[19] Despite the involvement of VLA-4 and LFA-1 in autoimmunity, molecular imaging of autoimmune diseases with radiotracers targeting these integrin molecules has been limited to malignant and non-autoimmune inflammatory diseases. For example, VLA-4 mostly has been targeted for the molecular imaging of malignant diseases such as melanoma[20] or multiple myeloma.[21] Recently, ^{111}In-DOTA-butylamino-NorBIRT (^{111}In-DANBIRT) has been used for targeting LFA-1 on inflammatory cells in atherosclerosis and showed local uptake in atherosclerotic plaque lesions.[22] These integrin molecules certainly merit further investigation as promising targets for radionuclide-based imaging in autoimmune and inflammatory diseases.

2.2. Immunoglobulin superfamily adhesion molecules

Immunoglobulin superfamily adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were named after their structure containing immunoglobulin-like domains, and play a role in both the migration and adhesion of different types of immune cells at sites of inflammation by interacting with ligands including integrins (e.g. VLA-4).[12] Two peptide-based radiopharmaceuticals targeting VCAM-1 (^{99m}Tc-B2702-p,[23] and ^{18}F-4V[24]) were initially developed and the accumulation of the tracers in atherosclerotic plaques was observed in preclinical animal models. Even though they showed high affinity for VCAM-1 and good correlation with the expression levels of inflammatory genes such as CD68, a biomarker of macrophages in inflammatory atherosclerosis, clinical use of these tracers is still limited.[6]

Broisat et al. developed a radiolabeled Nb tracer, ^{99m}Tc-labeled cAbVCAM1–5, targeting VCAM-1 for the imaging of atherosclerotic lesions.[25] The tracer showed mouse and human cross reactivity in detection of VCAM-1 and higher lesion-to-control (VCAM-1-negative aortic walls) ratios (4.95 ± 0.85) compared to control cAbBcII10 antibodies (1.66

± 0.28, $p < 0.05$, n = 4–6) in ApoE-deficient (ApoE−/−) mice, an animal model of atherosclerosis. The same group also evaluated a potential use of this Nb tracer for monitoring response to statin therapy in mouse models of atherosclerosis.[26] *In vivo* SPECT/CT imaging showed uptake of ^{99m}Tc-cAbVCAM1–5 in the aorta, lymph nodes, and thymus, which correlated well with the expected distribution of VCAM-1 expression in mice with western food intake promoting atherosclerosis. In addition, a significant decrease in the uptake of ^{99m}Tc-cAbVCAM1–5 in aortic lesions was observed in 35-wk-old atorvastatin-treated mice (0.87 ± 0.06 vs. 1.11 ± 0.09 in the control group, in the injected dose per volume of tissues [%ID/cm^3]; $p = 0.035$, n = 9), suggesting this radiotracer as a promising agent for sensitive detection of atherosclerotic plaques and monitoring the effects of statin therapy in atherosclerosis.[26]

The same Nb has also been labeled with ^{18}F and used for PET/CT imaging of inflamed atherosclerotic lesions in ApoE$^{-/-}$ mice (n = 3).[27] The accumulation of the ^{18}F-labeled tracer in atherosclerotic plaques was significantly higher than that in the control group and showed good correlation with the extent of atherosclerosis as indicated by lesion-extension scores (Score 0 to 3, 3 is the most serious). Notably, while Nbs radiolabeled with ^{99m}Tc typically show high kidney retention, the uptake of ^{18}F-FB-cAbVCAM1–5 in the kidneys was much lower than that of ^{99m}Tc-cAbVCAM 1– 5,[26] implicating renal metabolization and excretion of this ^{18}F-labeled Nb.

A radiolabeled antibody against VCAM-1 has also been used with other imaging modalities. Liu et al. labeled a scFv targeting VCAM-1 with ^{99m}Tc (^{99m}Tc-scFv-VCAM1) and Cy5 (Cy5-scFv-VCAM1) for SPECT and optical imaging of atherosclerotic plaques in rabbit and mouse models of atherosclerosis.[28] In SPECT/CT imaging, uptake of the tracer in the aortic arch lesions of experimental rabbits was observed (Figure 2A) and the presence of atherosclerotic plaques was further confirmed by H&E staining. In fluorescence imaging, target-to-background ratios were significantly higher in atherosclerotic mice than control mice at 3 h (1.63 ± 0.09 vs 1.18 ± 0.08, $p = 0.003$, n = 5) and 6 h (1.39 ± 0.15 vs 1.06 ± 0.05, $p = 0.02$) while the accumulation was similar at 12 h and 24 h. Compared to a Nb tracer, the optimal *in vivo* imaging time required for this scFv probe may therefore be elongated since the size of a scFv is larger than that of a Nb (28 kDa vs 15 kDa) and the serum half-life is accordingly longer (194 ± 21 min vs 4.9 min).[28]

In addition to atherosclerosis, high VCAM and ICAM expression has also been found in many autoimmune diseases such as SLE,[29] RA,[30] and AITD.[31] In addition, Sans et al. performed a scintigraphy study with an ^{123}I-labeled anti-VCAM-1 mAb in animal models of colitis.[32] The radiotracer effectively visualized colonic inflammatory lesions and the extent of accumulation correlated well with histological damage, suggesting its potential use as an imaging probe for human IBD. Therefore, even though there have been few studies using VCAM-1 or ICAM-1 as molecular targets for imaging autoimmunity, immunoglobulin superfamily adhesion molecules can be promising markers for molecular imaging of autoimmune diseases.

2.3. Selectins

Selectins are single-chain transmembrane glycoproteins and can be classified into three subtypes (L-, E-, and P-selectins) depending on the cell types on which they are originate.[12] While L-selectin is expressed fundamentally on all leukocytes, E-and P-selectins are highly present on the endothelium and platelets at sites of inflammation induced by immunologic responses such as cytokines (e.g. Interleukin-1, TNF-α).[33] Similar to other adhesion molecules, selectins can mediate migration, localization, and activation of leukocytes at sites of inflammation after binding with glycosylated or sialylated ligands.[12] Therefore, the expression of E-selectin has been demonstrated in several inflammatory diseases[12] and has been targeted for many different imaging modalities including SPECT,[34] MRI,[35] and ultrasound.[36] ^{111}In-labeled anti-E-selectin F(ab')$_2$ fragments successfully visualized inflamed sites of patients with IBD, suggesting its potential use for evaluating and monitoring patients with IBD [37]. Studies performed by Jamar et al.[34] and Keelan et al.[38] also showed that ^{99m}Tc and ^{111}In-labeled mAbs against E-selectin (1.2B6) and its fragment (Fab or F(ab')$_2$) are promising molecular probes for imaging inflamed synovitis in preclinical RA models and human RA patients. Detailed information about E-selectin imaging with radiolabeled antibodies in RA has been covered in another review.[39]

P-selectin is located in the α-granules of platelets and Weibel-palade bodies of endothelial cells, and is also expressed by inflamed endothelium.[33] P-selectin can be a potential target for imaging atherosclerotic plaques and thrombi since it is involved in the recruitment of monocytes and lymphocytes and increases integrin expression on the arterial wall during inflammation.[40] One of the radiopharmaceuticals for imaging P-selectin is Fucoidan, a polysaccharide which has been utilized for SPECT[41] and PET[42] imaging of thrombi and vulnerable atherosclerotic plaques in preclinical models. Nakamura et al. utilized a ^{64}Cu-DOTA-anti-P-selectin mAb for imaging atherosclerotic plaques in low density lipoprotein receptor-deficient (LDLr$^{-/-}$) mice.[42b] PET/CT imaging showed selective accumulation of the radiotracer in the aorta of mice fed with a high cholesterol diet (Figure 2B). The results were further confirmed by autoradiography and *ex vivo* Oil Red O staining of plaques to visualize lipids, indicating that this ^{64}Cu-labeled probe can be useful in detecting atherosclerotic plaques. Furthermore, the presence of P-selectin in platelets allows it to be used as a potential thrombi diagnosis target. Xu et al. expressed the light chain of antibodies against P-selectin (SZ-51) in *P.pastoris* to generate radiolabeled recombinant proteins (SZ-LC) for imaging thrombi.[43] ^{99m}Tc-SZ-LC showed specific binding to activated human platelets and accumulation in the thrombus region induced in dog veins, indicating the feasibility of using this tracer for thrombi diagnosis. The selectin family is consequently a promising imaging target for autoimmune and chronic inflammatory diseases.

2.4. Vascular adhesion protein-1

Vascular adhesion protein-1 (VAP-1) is another glycoprotein on endothelial cells involved in inflammation responses by interacting with other adhesion molecules and producing inflammatory mediators.[44] Even though VAP-1 is one of the highly promising molecules targeted for both imaging and treatment of inflammatory diseases, most of the reported PET imaging studies of this target have been performed using radiolabeled peptides (e.g. ^{68}Ga-DOTAVAP-P1,[45] ^{68}Ga-DOTAVAP-PEG-P1 or 2,[46] and ^{68}Ga-DOTA-Siglec-9[47]). Details

of imaging with these probes have been covered by another review.[44] BTT-1023 is a human mAb against VAP-1 and has been used for the treatment of inflammatory diseases.[48] Synovitis was induced chemically in rabbits, and both PET/CT (^{124}I-BTT-1023) and SPECT/CT (^{123}I-BTT-1023) scans were performed.[48] Radiolabeled BTT-1023 was found to have high clearance and distributed to the liver and thyroid, which may be attributed to high VAP-1 expression on hepatic sinusoidal endothelia and the uptake of free iodine in thyroid (Figure 2C). Accumulation of the tracer was detected in inflamed lesions of rabbits with synovitis (Figure 2D), demonstrating the feasibility of using radiolabeled BTT-1023 for imaging of immune responses in inflammatory diseases.

3. Targeting surface markers on immune cells

3.1. Macrophages and monocytes

The activity of macrophages and monocytes is closely associated with the degree of inflammation and the severity of symptoms of autoimmune and inflammatory diseases. For example, in RA, infiltration of activated inflammatory macrophages into synovial tissues may cause matrix degradation, angiogenesis, and fibroblast proliferation.[49] In addition, infiltration of macrophages may result in cartilage and bone destruction, since they are the precursor cells of the osteoclasts responsible for bone resorption.[50] In MS, it is known that macrophages and microglia can induce neuroinflammation and neurodegeneration in the central nervous system (CNS) by secreting pro-inflammatory cytokines, causing oxidative stress, and changing the permeability of the BBB.[51] Currently, SPECT and PET imaging of macrophages and monocytes in inflammatory diseases are mostly based on radiolabeled cells (e.g. ^{99m}Tc-HMPAO-labeled monocytes[52]) or small molecules targeting markers of those immune cells (e.g. ^{11}C-PK11195 targeting translocator protein[53] and ^{99m}Tc-EC20 targeting the folate receptor[54]). More detailed information about these types of probes can be found in another review.[55] Here, antibody-based molecular imaging of macrophages and monocytes for autoimmune and inflammatory diseases will be described and radiolabeled antibodies for three different targets, including the macrophage mannose receptor, complement receptor of the Ig superfamily, and CD163, will be introduced.

Macrophage mannose receptor—Macrophage mannose receptor (MMR; CD206) is 175 kDa C-type lectin receptor mostly expressed on macrophages, dendritic cells, and selected endothelial cells.[56] Since MMR is involved in phagocytosis, processing, and presentation of self-derived peptides or proteins as well as unopsonized microorganisms or glycoproteins to T cells, expression of this receptor on macrophages may play a role in the pathogenesis of autoimmune diseases.[57] In addition, MMR is associated with the formation of osteoclasts by interacting with mannose-type oligosaccharides present on osteoclast precursor cells, which might affect bone resorption and erosion in RA.[58] Movahedi et al. developed a ^{99m}Tc-labeled Nb targeting MMR[59] and Put et al. utilized the probe for SPECT imaging to examine the expression level of MMR in macrophages and osteoclasts in collagen-induced arthritis (CIA), an animal model for RA.[60] The results of quantitative polymerase chain reaction and flow cytometry revealed that expression of MMR was found to be high in macrophages, and relatively low in osteoclasts.[60] The specificity of ^{99m}Tc-labeled Nb targeting MMR was confirmed by SPECT/CT imaging of both symptomatic and

naïve mice using a control Nb (^{99m}Tc-labeled BCII10). In addition, the accumulation of the radiolabeled tracer was significantly higher in arthritic paws than normal paws of mice (Figure 3A). Interestingly, the signal from the radiolabeled Nb was also detected in non-arthritic paws of mice whose other paws had arthritic symptoms, suggesting the potential utility of this radiotracer for early detection of RA before presentation of macroscopic clinical symptoms. Since there are also studies showing that MMR is involved in the pathogenesis of autoimmune thyroid diseases[61] and the expression of MMR is elevated in impaired mucosa of UC patients,[62] MMR also can be a potential imaging target for other autoimmune diseases.

Aside from autoimmune diseases, MMR has been investigated as a target for atherosclerosis since it is known that macrophages also play an important role in the formation of vulnerable plaques and foam cells in atherosclerosis.[63] For example, M1 macrophages (pro-inflammatory macrophages) are involved in lipid accumulation and inflammatory responses while M2 macrophages (alternatively activated macrophages) are associated with plaque stabilization and inflammation resolution.[63] Varasteh et al. successfully observed the expression of MMR on macrophages in atherosclerotic plaques in Apo E-knock out mice with ^{111}In-tilmanocept, a molecule targeting MMR.[64] However, another study performed by Bala et al. demonstrated that the uptake of ^{99m}Tc-labeled anti-MMR Nb in aortic lesions showed no significant difference between ApoE$^{-/-}$ mice and control C57BL/6 mice, requiring more consideration when using MMR for the detection of atherosclerosis.[65]

Complement receptor of the Ig superfamily—Complement receptor of the Ig superfamily (CRIg; also called CSIG4 or Z39Ig) is uniquely expressed on specific types of resident tissue macrophage subsets and plays a pivotal role in complement-mediated phagocytosis of C3-opsonized particles.[66] Tanaka et al. revealed that synovial tissues in RA patients include more cells expressing CRIg than normal synovium or tissues from other arthritic diseases such as osteoarthritis or psoriatic arthritis,[67] making CRIg a potential marker for imaging synovial tissues in RA. A CRIg-specific radiolabeled Nb (^{99m}Tc-NbV4m119) was developed by Zheng et al. and evaluated for *in vivo* biodistribution and detection of arthritic lesions in a mouse model of RA.[68] ^{99m}Tc-V4m119 was found to accumulate in CRIg-positive macrophages in the liver and inflamed joint lesions with good correlation with the severity of the symptoms (Figure 3B). Of note, ^{99m}Tc-NbV4m119 was detected in the knees of mice with arthritis even before the inflammatory symptoms appeared, indicating this tracer has potential for early detection in incipient lesions. The same tracer was also used for monitoring the efficacy of treatment with dexamethasone in arthritic mice.[69] The accumulation of ^{99m}Tc-NbV4m119 was observed in inflamed joints of mice with arthritis, and reduced after dexamethasone treatment in most joints, showing the potential of the tracer for monitoring therapeutic responses in autoimmune diseases.

CD163—CD163 is a 130-kDa macrophage-scavenger receptor that can eliminate plasma hemoglobin-haptoglobin complexes and is uniquely expressed on alternatively activated macrophages and a subset of monocyte lineage cells at sites of inflammation.[70] In addition, the soluble form of CD163, originating from the membrane bound receptor, is one of the promising biomarkers for diagnosis of many inflammatory diseases including type 2

diabetes, liver diseases, and sepsis.[71] Eichendorff et al. developed a radiolabeled anti-CD163 antibody, ^{68}Ga-ED2, and investigated the systemic biodistribution of the tracer and expression of CD163 homologues in rats with CIA through PET imaging.[72] They found high uptake of the tracer in the liver and spleen, which corroborated the findings that the expression level of CD163 is high in Kupffer cells, splenic red pulp macrophages, and bone marrow macrophages.[70] The authors found significantly higher uptake in the inflamed rear paw of rats with arthritis than that of normal rats (0.148 ± 0.023 vs 0.081 ± 0.050 %ID/g; $p = 0.021$, $n = 3–6$), suggesting increased macrophage infiltration and blood flow into the inflamed area. However, the accumulation of ^{68}Ga-ED2 in paws of rats was still low in both arthritic and normal rats in comparison to the liver or spleen, indicating the need for improvement of both metabolism and tissue penetration of the tracer.

3.2. T lymphocytes

T lymphocytes are highly involved in the initiation and development of autoimmune and inflammatory diseases. For instance, autoreactive T cells recognizing self-antigens and impaired regulatory T cells cause breakdown of tolerance and induce pro-inflammatory responses, resulting in the development of autoimmune diseases such as RA, MS, and type 1 diabetes.[73] Imaging of T lymphocytes with radiolabeled probes has been performed using various methods. For example, direct *in vivo* labeling of T lymphocytes or cytokines (e.g. ^{99m}Tc-HMPAO[74] or ^{111}In-oxine labeled T-lymphocytes[75], ^{99m}Tc labeled IL-2[76]), employing nucleoside metabolisms (e.g. ^{18}F-AraG[77], ^{18}F-FAC[78]) or reporter genes (e.g. ^{18}F-FSPG[79]), and targeting surface molecules on the immune cells can be effective strategies for molecular imaging of T lymphocytes. Detailed information about each of these approaches can be found in other reviews.[80] Here, molecular imaging of autoimmune and inflammatory diseases with radiolabeled antibodies targeting CD3 and CD4 will be introduced.

CD3—CD3 is a part of the T cell receptor complex and plays a key role in antigen recognition and T cell signal transduction.[81] CD3-specific monoclonal antibodies have been used for the treatment of various types of inflammatory diseases (e.g. RA, IBD, type 1 diabetes) since they can induce antigenic modulation and apoptosis of autoreactive T cells.[82] In addition, anti-CD3 mAbs can elevate the number and enhance the function of T_{reg} cells and anti-inflammatory cytokines such as TGF-β and IL-10.[83] OKT3 (Muromonab), developed by Kung et al.,[84] was the first monoclonal murine antibody recognizing CD3 molecules expressed on human T lymphocytes. However, due to its murine origin, OKT3 can elicit a high level of immunogenicity and various side effects in humans, requiring the development of more humanized genetically-engineered anti-CD3 mAbs such as visilizumab and teplizumab.[83] In terms of molecular imaging in autoimmune diseases, radiolabeled anti-CD3 mAbs targeting CD3 or T-cell receptor-CD3 complexes can be useful to monitor inflammation activity and therapeutic response, both of which can be associated with the presence of active T lymphocytes.[85] So far, imaging of radiolabeled anti-CD3 antibodies in inflammatory diseases has been mostly limited to SPECT. Several studies using ^{99m}Tc labeled anti-CD3 antibodies in RA patients were reviewed by another article.[80a] On the other hand, PET imaging with ^{89}Zr-labeled anti-CD3 mAbs in tumor models has been performed in several studies and successfully detected tumor infiltrating T cells.[86]

Therefore, PET imaging with radiolabeled CD3 antibodies in inflammation models merits further investigation.

CD4—$CD4^+$ T lymphocytes can interact with the major histocompatibility complex (MHC) II molecules on antigen-presenting cells, regulate both T and B cell function via helper T cells, and participate in intracellular signal transduction.[80a] It is known that $CD4^+$ T lymphocytes have at least four different subsets, T_H1, T_H2, T_H17 and T_{reg}.[87] Among them, T_H1 and T_H17 cells play a major role in autoimmunity by producing pro-inflammatory cytokines responsible for inflammation and tissue damage, while T_{reg} cells mainly suppress the immune system and play a role in tissue repair.[88] Since the CD4 molecule is expressed on the surface of helper T cells and macrophages in the synovial tissues of RA patients, there have been many trials using anti-CD4 antibodies for imaging RA.[80a] In 1990, Berker et al. published one of the first scintigraphy studies with ^{99m}Tc-labeled anti-CD4 antibodies (MAX.16H5) in RA patients.[89] Furthermore, Kinne et al. confirmed the specificity of a ^{99m}Tc-labeled anti-CD4 mAb against $CD4^+$ cells in arthritic lesions of RA patients[90] and also compared the ability to detect infiltrating $CD4^+$ T cells with anti-carcinoembryonic antigen mAbs using SPECT in another study.[91] More detailed information about these studies can be found in another review.[80a] On the other hand, the first clinical study using ^{99m}Tc-anti-CD4-Fab (^{99m}Tc-EP1645) was performed by Steinhoff et al. in 2014.[92] It was found that ^{99m}Tc-EP1645 showed good tolerability and could detect inflamed joints in arthritis patients with a 68% detection rate. As 8% of the positive scintigraphy scans were found in clinically negative joints, this tracer may have potential for early diagnosis of RA before clinical presentation. However, further investigation regarding correlation between clinical presentation and the expression of CD4 in synovial tissues still needs to be conducted.[92]

Radiolabeled anti-CD4 antibodies were also used for imaging $CD4^+$ T cells in inflamed mucosa in preclinical models. Kanwar et al. used ^{111}In-labeled anti-$CD4^+$ antibodies to visualize $CD4^+$ T cells in mice with dextran sulfate sodium (DSS)-induced colitis in SPECT/CT imaging.[93] The accumulation of the tracers in inflamed colon was significantly higher in the group of mice treated with 5% DSS compared to that in the healthy group ($p < 0.01$). In addition, the mean colon-to-muscle activity ratio showed good correlation ($p < 0.01$) with the histopathologic score, and with the number of total lymphocytes and $CD4^+$ T cells.[93] Freise et al.[94] developed an anti-CD4 Cys-diabody (GK1.5cDb) probe for immunoPET imaging of $CD4^+$ T cells, and employed it for the detection of DSS-induced colitis in mice.[95] ^{89}Zr-malDFO-GK1.5cDb was used to visualize and quantify $CD4^+$ T cells in the colons and mesenteric lymph nodes of colitis mice with immunoPET. The accumulation of the radiolabeled tracer in the colon of DSS-treated mice was significantly increased and confirmed by *ex vivo* results (DSS-treated group: 1.8 ± 0.4 vs control group: 0.45 ± 0.12 %ID/organ, $p < 0.005$, $n = 8$) (Figure 3C). Interestingly, %ID/g in the colon, cecum, and mesenteric lymph nodes did not show any significant difference between two groups, implicating the impact of the increased weight of the colons in colitis mice. In addition, even though the tracer enabled detection of $CD4^+$ T cells, it is still hard to distinguish each subset of $CD4^+$ T cells (e.g. T_H1, T_H17, etc), which may need further investigation.[95]

3.3. B lymphocytes

B lymphocytes as well as T lymphocytes are critical in the development of autoimmunity. Since B lymphocytes are involved in the production of autoantibodies, presentation of autoantigens, and activation of the complement cascade, depleting them with antibodies against surface markers on B lymphocytes has been one of the important approaches for the treatment of autoimmune diseases.[73b, 96] In a similar context, current molecular imaging of B lymphocytes in autoimmune and inflammatory diseases is mostly focused on targeting CD20 molecules on the surface of immune cells with anti-CD20 antibodies.[97] Here, we introduce the current status of antibody-based SPECT and PET imaging of B lymphocytes in autoimmunity and relevant diseases, mostly involving imaging of CD20 expression.

CD20—Rituximab is one of the most widely used drugs for the treatment of autoimmune diseases as well as non-Hodgkin lymphoma by targeting CD20. In 2006, the US FDA approved rituximab for the treatment of RA patients who are resistant to TNF-α therapy.[97] Furthermore, radiolabeled rituximab (e.g. ^{99m}Tc-labeled rituximab and ^{124}I-rituximab) has also been used for molecular imaging of autoimmune diseases in several scintigraphy and PET/CT studies, which were included in another review.[97] Recently, radiolabeled rituximab has further been employed for PET imaging of autoimmune diseases in preclinical models and patients. Bruijnen et al. utilized ^{89}Zr-radiolabeled rituximab in patients with RA to evaluate its biodistribution (Figure 4A) and find potential responders to rituximab therapy.[98] The authors found that the accumulation of the radiotracer in hand joints (Figure 4B) had significant correlation with response to rituximab therapy with 90% and 75% positive and negative predictive values, respectively, at target-to-background ratio ≥ 4.0. The probe was also found to accumulate in lymph nodes through PET imaging (Figure 4C) and the specificity of B cell-targeting was further confirmed by quantitative $CD22^+$ (a surrogate B cell marker) cell count in immunohistochemistry analysis of excised lymph node sections.[98] Since B cells are involved in the development of MS in both human and animals, James et al. employed ^{64}Cu-rituximab PET for non-invasive imaging of B cells in humanized mice with EAE.[99] The huCD20tg mouse model whose B cell is humanized was used since targeting human CD20 is more clinically relevant for evaluating rituximab as a molecular probe. The uptake of ^{64}Cu-rituximab was significantly higher in the lumbar spinal cord of huCD20tg mice than in normal groups as early as 1 h p.i. (5.44 ± 0.37 vs 3.33 ± 0.20 %ID/g, $p < 0.05$, $n = 4$) (Figure 5A). The results were also corroborated by elevated ^{64}Cu-rituximab accumulation in the spinal cord of huCD20tg EAE mice in *ex vivo* biodistribution studies and increased number of $B220^+$ (a marker for B cells) cells in immunostaining (Figure 5B, 5C).[99] However, even though the mice used in this study were humanized, the feasibility of using this probe in human MS patients and the range of imaging doses that can be used without inducing B cell depletion still need to be further investigated.

Anti-murine or chimeric (tositumumab) or -murine/human (rituximab) CD20 antibodies can cause side effects or problems when re-dosing. In this regard, a new generation of anti-CD20 antibodies such as obinituzumab (humanized) or ofatumumab (fully human) have been developed and recently approved by the FDA for the treatment of B cell lymphoma.[100] The potential of using these new generation drugs for the treatment of autoimmune diseases was described by another review.[101] While there are few studies using these new generation

antibodies for imaging autoimmune diseases, there are several studies showing positive results with humanized anti-CD20 antibodies for imaging in lymphoma models. For example, Zettlitz et al. used ^{89}Zr and ^{124}I-labeled engineered antibody fragments (cys-diabody and cys-minibody) of obinutuzumab and performed PET imaging to monitor CD20 expression and internalization after binding with antibodies in transgenic mice and a mouse model of lymphoma expressing human CD20.[102] Also, Yoon et al. utilized ^{89}Zr-labeled obinutuzumab and ofatumumab to observe tumor targeting and biodistribution of these antibodies, and to evaluate their feasibility of being used in anti-CD20 radioimmunotherapy of human lymphoma xenografts in mice.[100] Given these promising results, using these humanized anti-CD20 antibodies for molecular imaging of autoimmune diseases may also be worthwhile.

3.4. Other targets

In addition to macrophages, T and B lymphocytes, other immune cells have also been targeted with radiolabeled mAbs for molecular imaging of chronic inflammatory diseases. BW250/183 is an antibody against granulocytes targeting NCA95 epitopes and immunoscintigraphy with ^{99m}Tc-labeled BW250/183 has been performed in IBD patients.[103] However, a study performed by Gyorke et al. suggested that ^{111}In or ^{99m}Tc-labeled leukocytes should take priority over radiolabeled anti-granulocyte antibodies for the diagnosis of IBD patients as the method using anti-granulocyte antibodies is less sensitive and can cause patient reactions to the murine antibodies.[103b] Furthermore, class II MHC molecules and CD11b, which are expressed on various inflammatory cells including neutrophils and macrophages, have also been targeted for imaging of chronic inflammatory diseases. Rashidian et al. site-specifically labeled the variable domain of camelid heavy-chain only antibodies targeting class II MHC molecules and CD11b.[104] Both anti-MHC II and CD11b antibody fragments successfully visualized the myeloid cells in paws of mice with induced arthritis. In addition, a ^{99m}Tc-labeled anti-CD11b antibody has also been employed for SPECT/CT imaging of atherosclerotic plaques, suggesting the feasibility of this target for imaging of various chronic diseases.[105]

4. Targeting cytokines or enzymes

Targeting cytokines or enzymes unique to inflamed environments is another possible strategy for imaging of inflammatory diseases. There have been several methods for developing radiotracers targeting cytokines or enzymes such as direct labeling of cytokines[10] or employing radioligands targeting cytokine receptors.[106] Even though these peptide and protein-based probes can visualize inflamed lesions effectively, the direct labeling procedure is time-consuming and these probes have limited circulation properties. Therefore, we will focus on radiolabeled antibodies targeting cytokines and enzymes specific for inflammatory conditions, especially tumor necrosis factor-alpha (TNF-α), fibroblast active protein (FAP), and matrix metalloproteinase (MMP).

4.1. Tumor necrosis factor-alpha

Tumor necrosis factor-alpha (TNF-α) is one of the major proinflammatory cytokines involved in pathogenesis of many autoimmune diseases including RA,[39] IBD,[107] and

AITD.[108] Therefore, anti-TNF-α antibodies binding to membrane-bound or soluble TNF have been used for the treatment of inflammatory diseases by blocking TNF-α action and other inflammatory responses.[39] Furthermore, anti-TNF-α antibodies have also been used in molecular imaging and could potentially help physicians select patient populations for anti-TNF-α therapy and anticipate therapeutic responses to the therapy.[39] For example, both infliximab (chimeric anti-TNF-α antibody)[109] and adalimumab (fully human anti-TNF-α antibody)[110] were radiolabeled with ^{99m}Tc and successfully used for scintigraphy studies in RA patients. Detailed information about each study can be found in the review article by Malviya et al.[39] Etanercept is a fully human fusion protein of the TNF receptor and the constant portion of the IgG1 antibody, and particularly binds to soluble TNF-α resulting in its neutralization.[111] Several PET imaging studies have been performed with radiolabeled etanercept.[111–112] Cao et al. used ^{64}Cu-DOTA-etanercept to observe TNF-α levels in a mouse model of 12-O-tetradecanoyl-phorbol-13-acetate-induced acute and chronic inflammation.[111] The radiotracer successfully visualized ear inflammation and changes in TNF-α levels, which were dramatically increased in acute inflammation and lowered in chronic inflammation. Tobinick et al. observed the accumulation of ^{64}Cu-DOTA-etanercept in cerebral ventricles after peripheral perispinal administration of the tracer through microPET imaging in a rat.[112] Since it has been reported that administration of etanercept improved cognitive functions in patients with Alzheimer's disease,[113] imaging with radiolabeled etanercept may provide useful information about the distribution of the anti-TNF-α antibodies as well as the level of inflammatory cytokines in Alzheimer's disease.[112]

4.2. Fibroblast activation protein

Fibroblast activation protein (FAP) is a 170 kDa transmembrane glycoprotein that has dipeptidyl peptidase and endopeptidase activity.[114] While FAP expression is low in healthy organs, it shows high upregulation in epithelial carcinoma and sites of tissue remodeling including fibrosis and arthritis.[114] Several radiolabeled antibodies such as ^{131}I-F19 (murine),[115] ^{131}I-sibrotuzumab (humanized F19 mAbs),[116] and ^{177}Lu-labeled ESC11 and ESC14 were used for targeting and imaging malignant tissues.[117] In RA, FAP is mainly expressed on activated fibroblast-like synoviocytes and has been targeted for imaging inflamed tissue and disease activity.[4b] Laverman et al. developed an ^{111}In- and ^{89}Zr-labeled anti-FAP antibody, 28H1, for noninvasive imaging of mice with CIA.[4b] ImmunoSPECT imaging with ^{111}In-28H1 showed excellent targeting of inflamed joints with high resolution, suggesting that FAP is a promising target for the detection of arthritis. Similarly, PET/CT scans with ^{89}Zr-28H1 allowed quantification of the uptake of the tracer and showed good correlation with joint scores compared to ^{18}F-FDG; however, this ^{89}Zr-labeled tracer is less preferred than ^{111}In-28H1 due to increased uptake in the bone.[4b]

Several other studies have been performed with ^{111}In-28H1 to monitor treatment responses in preclinical RA models. For example, Terry et al. conducted a comparison study with different tracers which target fibroblasts (^{111}In-28H1), macrophages (^{111}In-anti-F4/80-A3–1), or integrin $\alpha_v\beta_3$ (^{111}In-RGD$_2$), to compare their capabilities for monitoring therapeutic responses to etanercept in experimental arthritis.[118] While the uptake of each tracer in inflamed joints before treatment with etanercept was 23 ± 15, 8 ± 4, and 2 ± 1 %ID/g for

^{111}In-28H1, ^{111}In-RGD$_2$, and ^{111}In-anti-F4/80-A3–1, respectively, it significantly decreased to 11 ± 11, 4 ± 4, and 1 ± 0.2 %ID/g after treatment (n = 1–3, 4 joints/mouse, $p < 0.001$). In addition, arthritis-to-blood ratios of ^{111}In-28H1, ^{111}In-RGD$_2$, and ^{111}In-anti-F4/80-A3–1 antibodies were also significantly higher than that of the control antibody, ^{111}In-DP47GS ($p = 0.002$), indicating specific uptake of the tracers in inflamed lesions. More recently, van der Geest et al. conjugated 28H1 with ^{99m}Tc-S-HYNIC for SPECT/CT imaging of mice with CIA to monitor responses to treatment with liposomes containing prednisolone.[119] ^{99m}Tc-S-HYNIC-28H1 was observed in inflamed joints and the uptake showed good correlation with arthritis scores (Figure 6A, 6B). The uptake of the tracer in each paw of liposome-treated mice was significantly lower than arthritic mice with no treatment at 5 and 9 days after treatment ($p < 0.02$) (Figure 6C). The same group also used the ^{111}In-28H1 antibody for monitoring response to neutralization of IL-22, which is an important cytokine for the development of RA.[120] Anti-IL-22 therapy effectively regulated the progression of arthritis in CIA models when initiated in the early phases of CIA. In SPECT/CT imaging with ^{111}In-28H1, significantly decreased uptake of the tracer was observed in the anti-IL-22 therapy group in a sensitive manner and further confirmed by histological and radiological analysis. Therefore, anti-FAP antibodies have potential to be used not only for imaging inflamed arthritic joints but also detecting changes in disease activity in response to various types of therapies.

4.3. Matrix metalloproteinase

Matrix metalloproteinases (MMPs) are a family of calcium-dependent zinc-containing endopeptidases with subtypes such as the collagenases, the gelatinases, and the membrane-type MMPs.[121] MMPs are involved in the progression of various inflammatory diseases as well as cancer by participating in degradation of the ECM, activation of biological molecules, and regulating the behavior of various cell types.[122] So far, the development of probes for SPECT or PET imaging has been mostly based on small molecules using broad spectrum MMP inhibitors. Gerwien and Hermann et al. demonstrated that MMP-9 derived from immune cells is essential in the development of EAE and is an important marker of leukocyte infiltration of the BBB.[123] PET imaging with ^{18}F-BR-351 allowed successful detection of MMP activity specific to early lesions and leukocyte penetration of the BBB, implicating usefulness of the tracer for monitoring disease activity during initial stages of MS development.[123] In addition, several other radioligands based on MMP inhibitors such as ^{123}I-HO-CGS-27023A[124] and ^{99m}Tc-RP805[125] enabled visualization of MMP activity in the arterial wall and MMP expression in atherosclerotic plaques in a mouse model of atherosclerosis.

Recently, ^{99m}Tc-labeled MMP-9 antibodies (^{99m}Tc-McAbs) were developed by Wang et al. for *in vivo* SPECT imaging of lesions in a mouse model of atherosclerosis [126]. The accumulation of ^{99m}Tc-McAbs in left common carotid lesions was detected 2 h p.i. of the radiotracer, and further confirmed by *ex vivo* autoradiography and immunostaining studies. While the probes mentioned above mainly target soluble MMPs such as MMP-2 and 9, Kuge et al. developed ^{99m}Tc-labeled mAbs targeting membrane-bound type 1 MMP (MT1-MMP), which is responsible for the activation of pro-MMP-2 and pro-MMP-13, and destabilization of atherosclerotic plaques.[127] The uptake of ^{99m}Tc-anti-MT1-MMP

antibodies in atherosclerotic aorta lesions in rabbit models was 5.4-fold higher than that in the control group and showed positive correlation with MT1-MMP expression. Importantly, the accumulation of ^{99m}Tc-anti-MT1-MMP antibodies was highest in the atheromatous lesions with high risk of rupture, indicating that this radiolabeled probe targeting MT1-MMP can be a potential marker for monitoring the risk of destabilization of atherosclerotic plaques.

5. Summary and outlook

Radiolabeled mAbs have high specificity, long circulation, and are easier to prepare than radiolabeled autologous immune cells.[10] By taking advantage of these properties, immunoSPECT and immunoPET can be used in chronic inflammatory diseases not only for evaluation of disease activity but also selection of patient populations for proper treatments or monitoring therapeutic responses.

Nonetheless, radiolabeled antibody-based molecular imaging of chronic inflammatory diseases has also its limitations. First, even though antibodies are molecular probes with high specificity toward their targets, it is difficult to develop tracers targeting molecules specific for chronic inflammatory diseases. Autoimmune diseases, non-autoimmune diseases, acute inflammatory diseases, and infectious diseases share many common mechanisms in their pathophysiology. In addition, general immune responses during inflammation such as increased blood flow also can increase uptake of non-specific IgG antibodies.[10] The combination of all of these factors indicates that finding highly specific biomarkers for autoimmune and inflammatory diseases and efficiently targeting them may be quite difficult. Second, although the accumulation of radiolabeled mAbs in inflamed lesions is significantly higher than that of non-inflamed lesions in many studies, the absolute uptake values of radiotracers when imaging inflammatory diseases are relatively low when compared to accumulations found in other models such as cancer, due to the relatively lower biomarker levels in these diseases. In addition, since antibodies have long circulation in the body, background radioactivity signals from antibodies are normally higher than small molecules, making high-contrast imaging difficult. Lastly, radiolabeled antibodies can face barriers for delivery to sites of inflammation. For example, even though the blood-brain barrier can be damaged during inflammation, delivery of antibodies into the brain is still limited compared to small molecules or peptides. Many studies have been performed to deliver antibodies into the brain via different routes;[128] however, molecular imaging of inflamed lesions in the central nervous system with radiolabeled antibodies still merits further investigation.

Given the studies described above, future exploration of radiolabeled antibodies for imaging chronic inflammatory diseases can take several directions. First, molecular probes targeting FAP at sites of inflammation showed relatively high accumulation compared to targeting other surface markers on immune cells or adhesion molecules, suggesting their high potential for further development and application in various chronic inflammatory diseases. [4b, 118−120]

Second, many antibodies have been modified to overcome their above-mentioned limitations. For example, engineering intact antibodies into fragments (e.g. $F(ab')_2$, Fab, Nb, scFv) can improve the pharmacokinetic properties of the molecules and expand the application of these probes by decreasing their size and weight. With these engineered agents, it will be important to choose appropriate radionuclides based on the adjusted biological circulation times of the tracers. For example, our group has used ^{44}Sc ($t_{1/2}$ = 4.0 h) with a relatively shorter physical half-life than ^{64}Cu ($t_{1/2}$ = 12.7 h) or ^{89}Zr ($t_{1/2}$ = 78.4 h) for the labeling of cetuximab Fab fragments.[129] In addition, we also employed ^{61}Cu ($t_{1/2}$ = 3.4 h) to label Fab[130] and $F(ab')_2$[131] fragments of TRC105, an antibody against CD105, and investigated the potential of the tracers for imaging angiogenesis in murine cancer model. Matching the biological and physical half-lives can not only provide higher-quality imaging, but also limit radiation dose to patients. The physical half-life is not the only property that should be considered when choosing a radionuclide; rather, other characteristics such as decay properties (emission type, energy, etc) need to be taken into account as well. Likewise, the behavior of the free element *in vivo* should be considered – for example, free zirconium accumulates in bone and may therefore confound imaging results in these tissues. Detailed information about each radiometal for imaging and therapy can be found in another review.[132]

Since murine or chimeric antibodies can induce anti-mouse antibodies in humans, a new generation of humanized or fully human antibodies are being developed.[83] It may also be worthwhile for researchers working in inflammatory diseases to take advantage of imaging tracers being developed for other applications, such as oncology. Indeed, with the rise of immunotherapy treatments for cancer patients, a great number of imaging agents for the immune system are being developed, which can certainly be translated to inflammatory and autoimmune diseases.[80b, 133] For example, our group successfully performed noninvasive PET imaging of lung cancer in mice by targeting CD30, which is expressed on several types of cancer cells as well as immune cells.[134] Since CD30 and its ligand play an important role in the differentiation of T_H1 and T_H17 cells and have been reported to be involved in the pathogenesis of chronic inflammatory diseases such as RA[135] or CNS autoimmunity,[136] they can be considered as targets for imaging chronic inflammatory diseases.

Given that chronic inflammation is associated with the development of cancer through mediation of DNA damage and neoplasia,[137] research on imaging agents for the detection of malignancy in the setting of inflammatory diseases may also help individuals with chronic inflammation and at high risk of cancer. The study performed by Turker et al. using ^{64}Cu-DOTA-cetuximab-$F(ab')_2$ for the detection of colonic tumors in colitis mice is an excellent example of this approach.[138]

Lastly, developing clinically-relevant animal models for chronic inflammatory diseases is also essential. Currently, animal models of autoimmune diseases such as CIA, EAE, and DSS-induced colitis are established mostly based on the symptoms shown in human diseases and therapeutic responses in a clinical setting. Animal models that can more accurately reflect the elaborate pathophysiology of human autoimmunity such as the presence of autoantibodies or autoreactive immune cells will certainly provide greater insight into these diseases. Models that mimic naturally-occurring autoimmune diseases will allow researchers

to develop better early detection imaging tools, as this stage of disease is often difficult to mirror in currently-existing animal models.

Overall, radiolabeled antibodies hold great potential for imaging of chronic inflammatory diseases, even though they do have some intrinsic limitations. Future studies aimed at overcoming these barriers will certainly give more power to immunoSPECT and immunoPET in autoimmune and inflammatory diseases, providing researchers with greater mechanistic understandings of these conditions, and physicians with greater power for diagnosing and monitoring their patients.

Acknowledgements

This work was supported, in part, by the University of Wisconsin - Madison and the National Institutes of Health (P30CA014520, T32GM008505, T32CA009206). Some figures were reproduced and modified under the Creative Commons Attribution License 3.0 from Servier Medical Art (https://creativecommons.org/licenses/by/3.0/).

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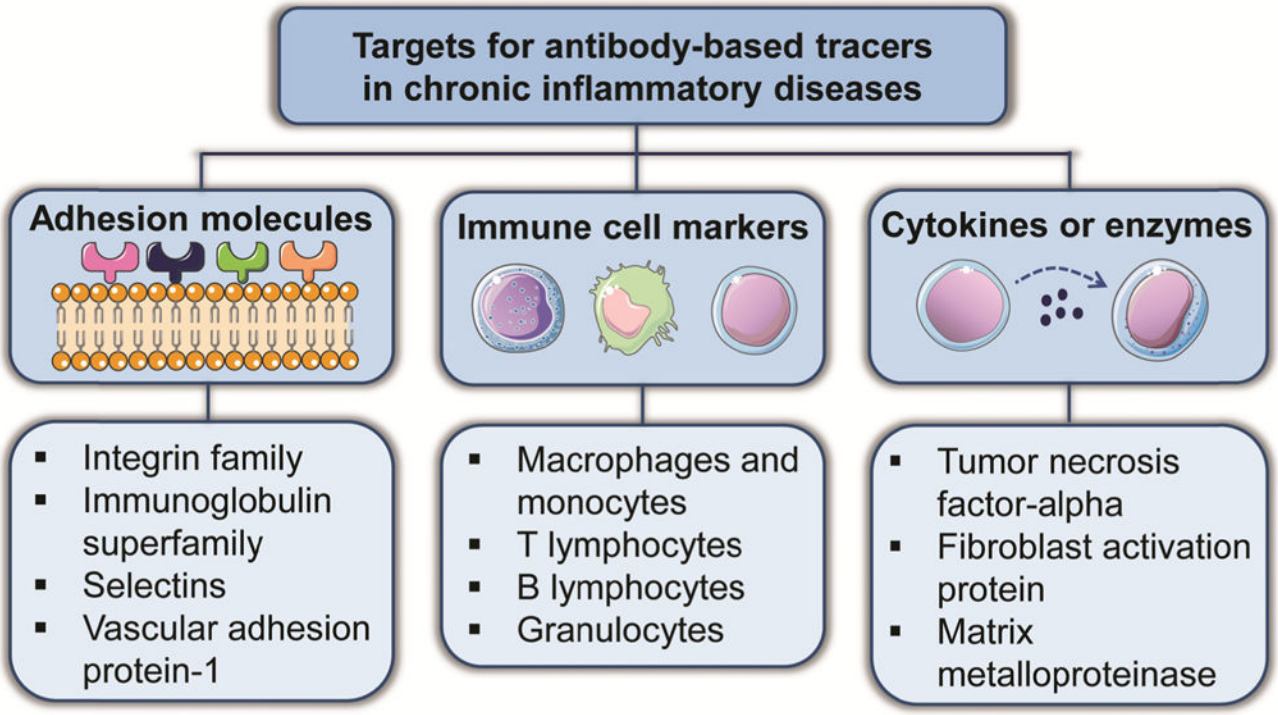

Figure 1.
Targets for antibody-based tracers in chronic inflammatory diseases.

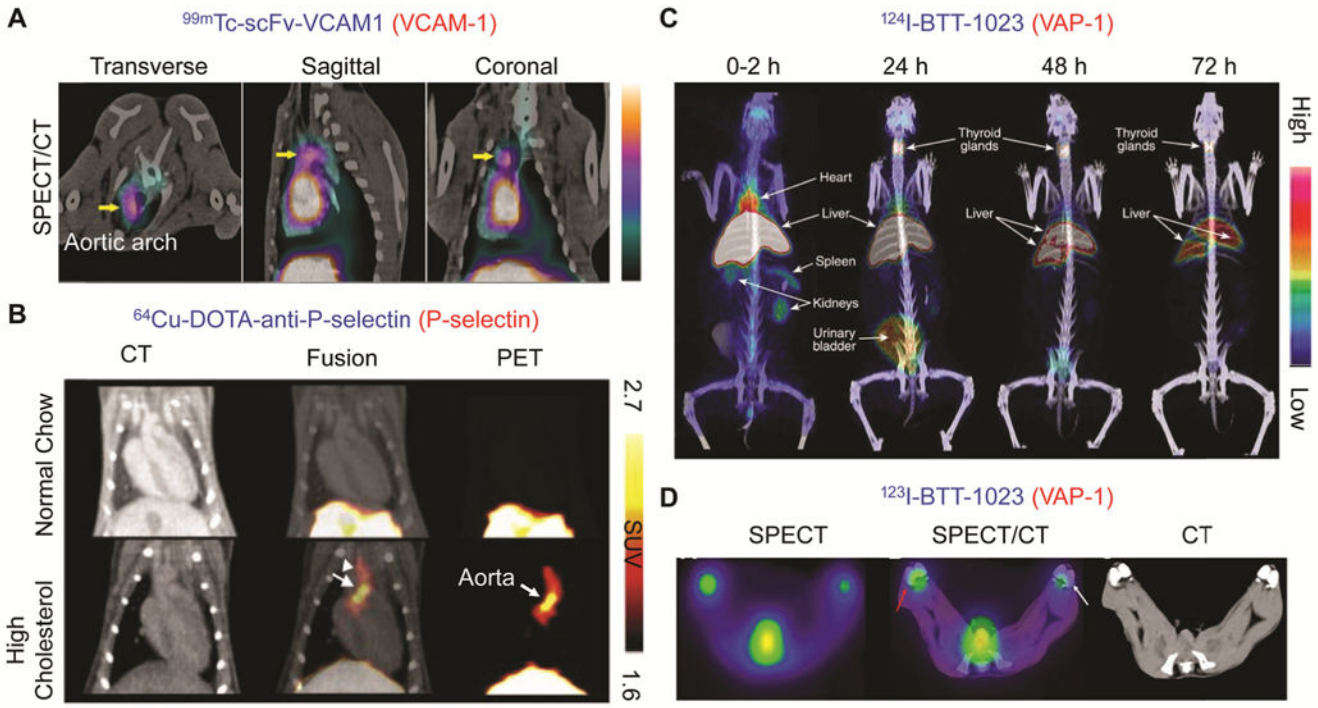

Figure 2.

(A) SPECT/CT images of ^{99m}Tc-scFv-VCAM1 in a rabbit model of atherosclerosis. The uptake of radiotracer in the aortic arch (yellow arrow) was observed.[28] (B) PET/CT images of ^{64}Cu-DOTA-anti -P-selectin mAbs in control mice (normal chow) and in atherosclerotic mice (high cholesterol).[42b] (C) Whole-body PET/CT images of ^{124}I-BTT-1023 in a healthy rabbit. The accumulation of radiotracer in the liver and thyroid glands was observed. (D) Transaxial SPECT/CT images of ^{123}I-BTT-1023 in rabbit knees at 2 h p.i. Red arrow and white arrow indicate inflamed and control joint, respectively.[48]

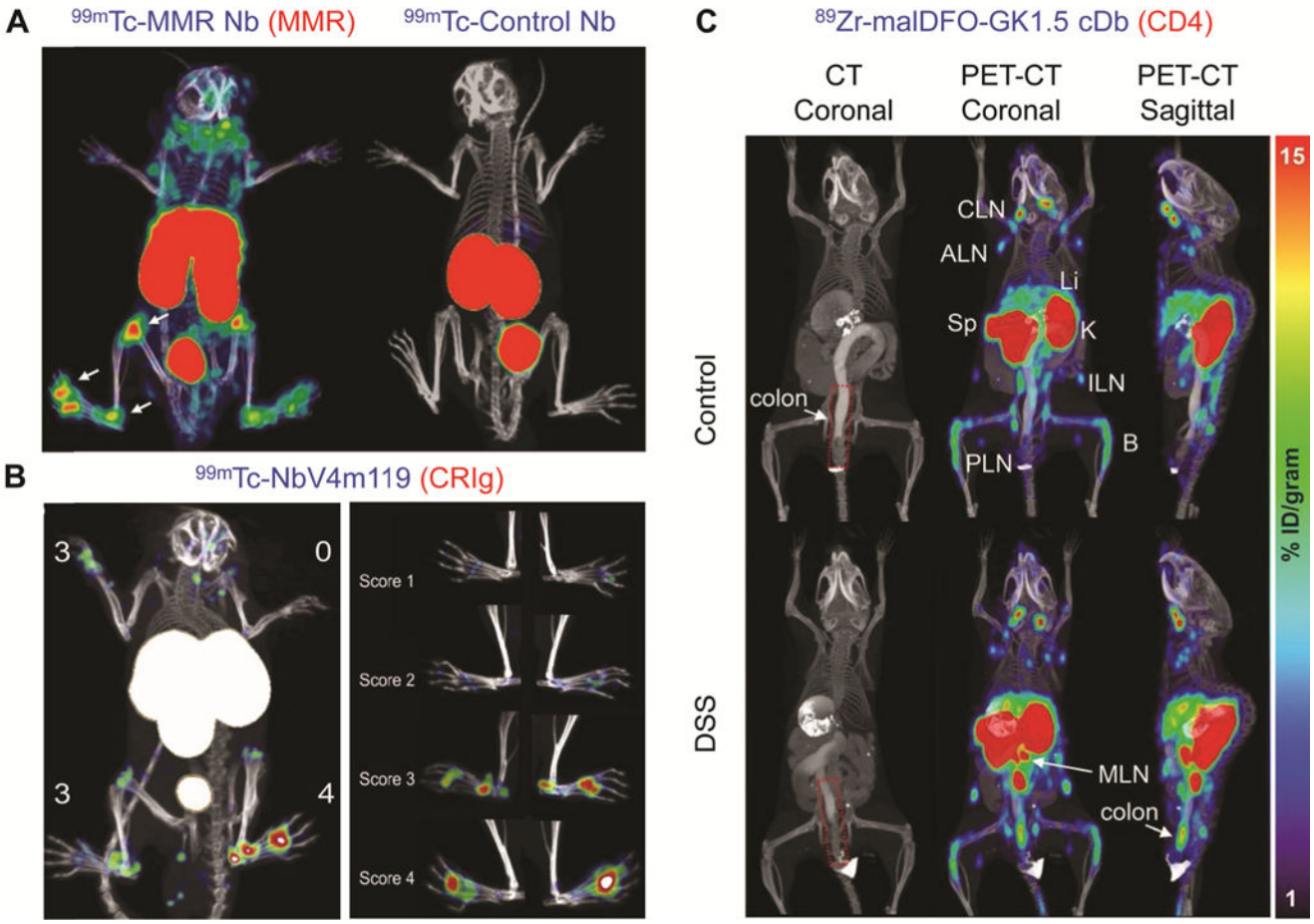

Figure 3.

(A) SPECT/CT images of symptomatic paws at 3 h after injection of ^{99m}Tc-labeled MMR Nb and control Nb in arthritic mice.[60] The white arrows indicate MMR-positive lesions. (B) SPECT/CT images of arthritic joints and individual limbs correlating with clinical scores at 3 h after injection of ^{99m}Tc-labeled NbV4m119.[68] (C) CT (coronal) and PET-CT (coronal and sagittal) images in DSS and control mice 20 h p.i. of ^{89}Zr-malDFO-GK1.5 cDb.[95] The red dotted lines indicate the colon ROI. Abbreviations: cervical lymph nodes (CLN), axillary lymph nodes (ALN), spleen (Sp), liver (Li), kidney (K), inguinal lymph nodes (ILN), popliteal lymph nodes (PLN), bone (B), mesenteric lymph nodes (MLN).

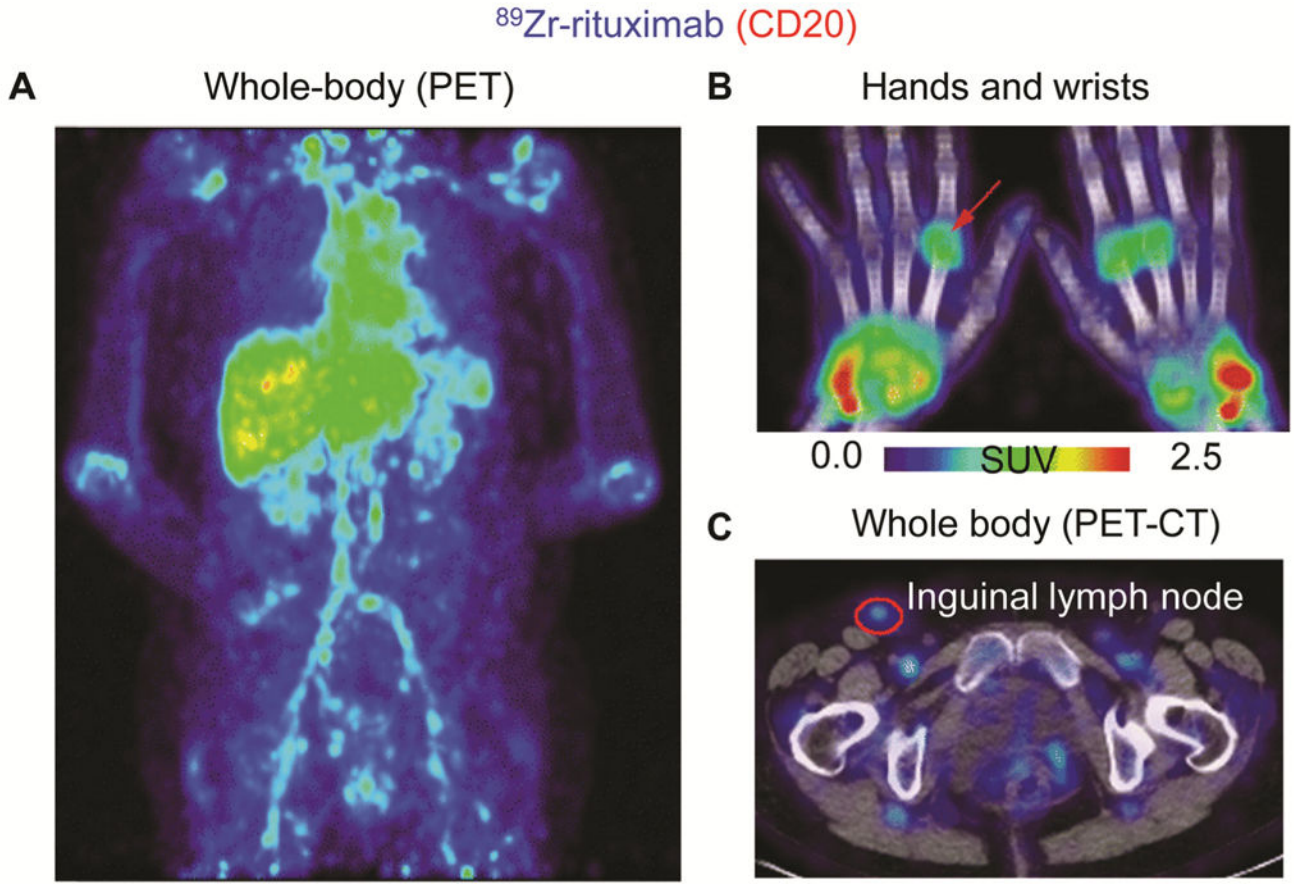

Figure 4.

PET-CT images of ^{89}Zr-rituximab in patients with rheumatoid arthritis (RA). (A) Whole-body (maximum intensity projection) PET image showing increased uptake of the tracer in the liver, spleen, and large joints including shoulders. (B) PET-CT image of an RA patient's hands/wrists indicating joints with positive uptake of the tracer (red arrow). (C) PET-CT image demonstrating uptake of ^{89}Zr-rituximab in an inguinal lymph node in a patient with RA (red circle).[98]

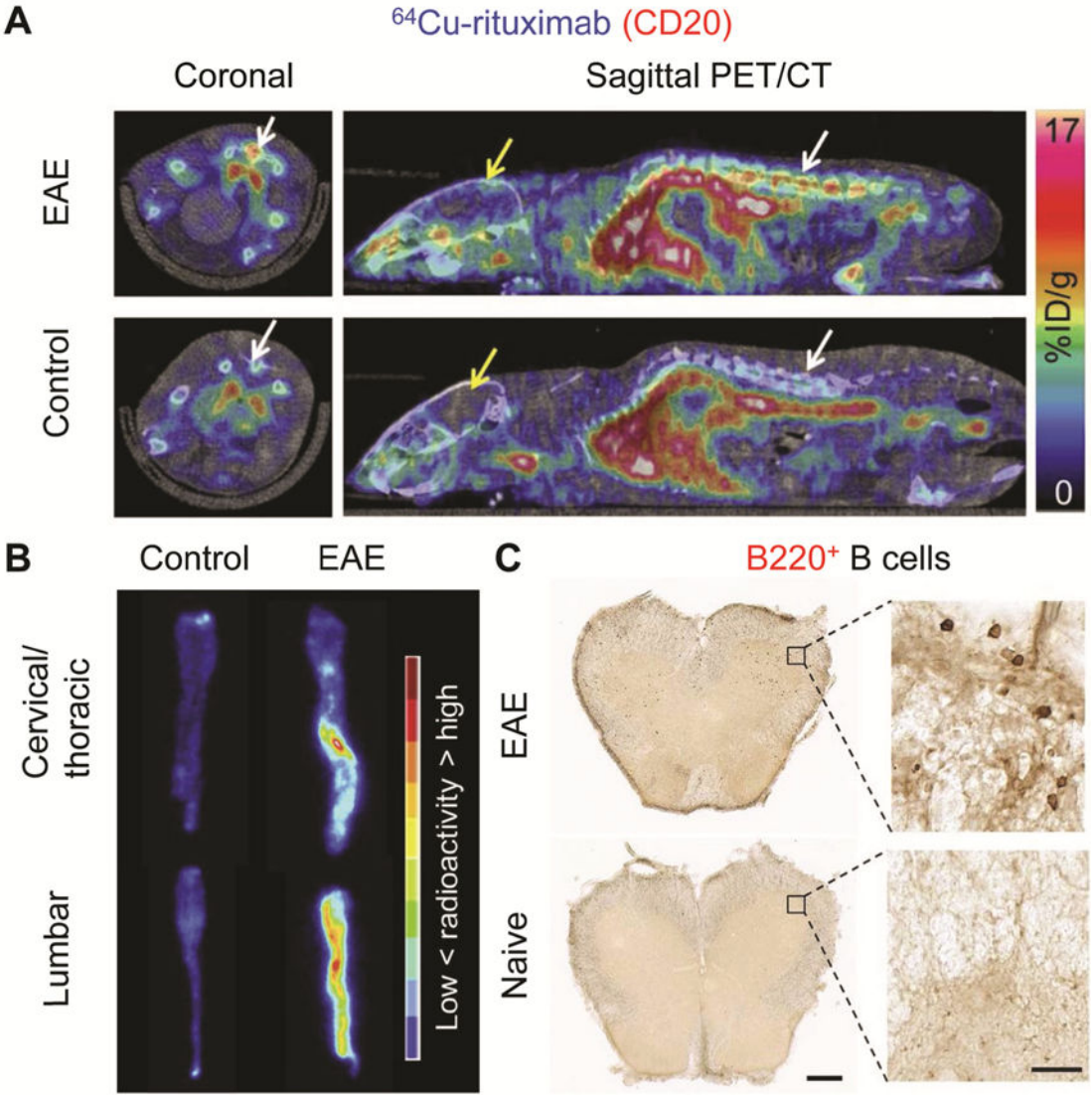

Figure 5.

(A) *In vivo* PET/CT images 1 hour p.i. of ^{64}Cu-rituximab in a mouse model of experimental autoimmune encephalitis (EAE) and control group. White and yellow arrows indicate spinal cord and brain, respectively. (B) Autoradiography images of the spinal cord in control and EAE mice. (C) Detection of B220^{+} B cells in the spinal cord of mouse with humanized B cells. The scale bar is 25 μm and 500 μm in high and low magnification images, respectively. [99]

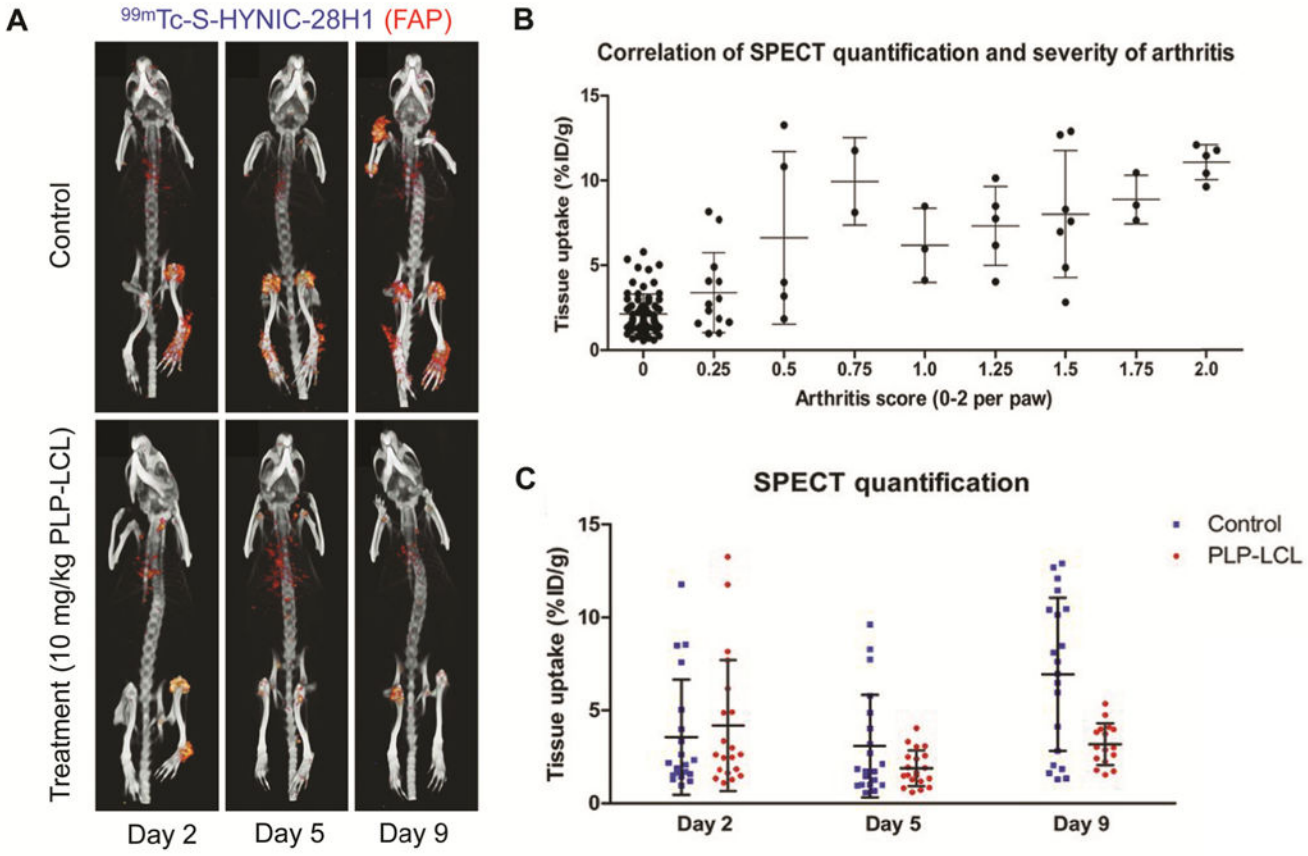

Figure 6.

Targeting FAP with ^{99m}Tc-S-HYNIC-28H1 in control and liposome-treated (PLP-LCL) mice with CIA. (A) SPECT/CT images of arthritic control and liposome-injected mice at 24 h p.i. of ^{99m}Tc-S-HYNIC-28H1. Images were obtained 2, 5, and 9 days after liposomal therapy. (B) Correlation between joint uptake (%ID/g) at 24 h p.i. of ^{99m}Tc-S-HYNIC-28H1 and arthritis score of each paw for individual mice. (C) Quantification of joint uptake (%ID/g) based on SPECT analysis 2, 5, and 9 days after PLP-LCL treatment in the control and treatment groups (n = 4 or 5 per group).[119]