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Original article

Imaging fibroblast activation protein to monitor therapeutic effects of neutralizing interleukin-22 in collagen-induced arthritis

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Abstract

Objectives. RA is a chronic autoimmune disease leading to progressive destruction of cartilage and bone. RA patients show elevated IL-22 levels and the amount of IL-22-producing Th cells positively correlates with the extent of erosive disease, suggesting a role for this cytokine in RA pathogenesis. The purpose of this study was to determine the feasibility of SPECT/CT imaging with ^{111}In-labelled anti-fibroblast activation protein antibody (28H1) to monitor the therapeutic effect of neutralizing IL-22 in experimental arthritis.

Methods. Mice (six mice/group) with CIA received anti-IL-22 or isotype control antibodies. To monitor therapeutic effects after treatment, SPECT/CT images were acquired 24 h after injection of ^{111}In-28H1. Imaging results were compared with macroscopic, histologic and radiographic arthritis scores.

Results. Neutralizing IL-22 before CIA onset effectively prevented arthritis development, reaching a disease incidence of only 50%, *vs* 100% in the control group. SPECT imaging showed significantly lower joint tracer uptake in mice treated early with anti-IL-22 antibodies compared with the control-treated group. Reduction of disease activity in those mice was confirmed by macroscopic, histological and radiographic pathology scores. However, when treatment was initiated in a later phase of CIA, progression of joint pathology could not be prevented.

Conclusion. These findings suggest that IL-22 plays an important role in CIA development, and neutralizing this cytokine seems an attractive new strategy in RA treatment. Most importantly, SPECT/CT imaging with ^{111}In-28H1 can be used to specifically monitor therapy responses, and is potentially more sensitive in disease monitoring than the gold standard method of macroscopic arthritis scoring.

Key words: SPECT/CT, anti-FAP, therapy monitoring, anti-IL-22, experimental arthritis

BASIC SCIENCE

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Rheumatology key messages

- Neutralizing IL-22 might be an attractive new strategy in RA treatment.
- Fibroblast activation protein-based SPECT/CT can specifically monitor response to therapy in an objective, quantitative manner.
- Fibroblast activation protein-based SPECT/CT is more sensitive in disease monitoring compared with clinical arthritis scoring.

Introduction

RA is a chronic autoimmune disease affecting up to 1% of the world's population. The synovial inflammation characterizing RA leads to destruction of cartilage and bone, thereby disabling patients in their daily life. Increasing evidence suggests an important role for T cells in RA pathology [1–3], in particular for the Th17 cell subset. Th17 cells produce various pro-inflammatory cytokines such as IL-17, IL-21 and IL-22, thereby protecting the host from bacterial and fungal infections, but also promoting the development of autoimmune diseases when present at elevated levels [4–7]. In the synovium and in the circulation in RA patients, elevated levels of IL-22 are observed [8–10], which positively correlates with DAS28 [11], serum RF [9–11] and anti-CCP antibodies [9]. Interestingly, reports on the role of IL-22 in various arthritis mouse models show that this cytokine is not only pro-inflammatory, but also might exert anti-inflammatory activities during experimental arthritis. In CIA for instance, an IL-22 deficiency in the early phases of the disease reduced the susceptibility to arthritis development [12], whereas absence of the cytokine during the later phases aggravated joint pathology [13]. Further insights into the role of IL-22 in experimental and RA are crucial when evaluating the potency of this cytokine as a new therapeutic target in RA treatment.

Since RA is characterized by disease exacerbations, it is not only early diagnosis that is of utmost importance for an adequate treatment. In addition, monitoring of disease progression and early response monitoring after therapy are crucial for the prevention of progressive destruction. Considering that the response to therapy varies significantly among patients, personalized treatment based on accurate therapy response monitoring is essential. Molecular imaging of disease activity can be a valuable tool for accurate and timely monitoring of therapeutic efficacy. Specific tracer uptake can be quantified and can be assessed objectively. However, in most cases the tracer is not specific for arthritis at the molecular level [14, 15]. Terry *et al.* [16] demonstrated therapy response monitoring with tracers directed against targets that were specific biomarkers for arthritis. One of the tracers was the radiolabelled anti-fibroblast activation protein (FAP) antibody, 28H1. 28H1 is a non-internalizing antibody with high affinity for human and murine FAP. FAP is dominantly expressed by activated fibroblast-like synoviocytes in arthritic joints [17, 18]. Specificity of 28H1 for FAP was shown in *in vitro* binding assays and by immunohistochemistry in arthritic joints. ^{111}In-28H1 SPECT/CT imaging showed a significant correlation between the uptake of ^{111}In-28H1 in inflamed joints and the severity of arthritis [18]. In the present study, we investigated the feasibility of ^{111}In-28H1 SPECT/CT imaging of FAP-expressing synovium to monitor the therapeutic potential of neutralizing IL-22 during various phases of experimental arthritis, and compared its potency to our standard clinical, radiographical and histological disease scoring methods.

Methods

Mice

Male DBA-1/J mice were purchased from Janvier-Elevage (Le Genest Saint Isle, France). Mice, 10–12 weeks old, were housed in filter-top cages under specific pathogen-free conditions. Mice had unlimited access to a standard diet and water. All procedures performed on animals in this study were in accordance with established guidelines and regulations, and were reviewed and approved by the animal ethics committee of the Radboud University Nijmegen.

Study design

CIA was induced as described previously [19]. Arthritis development was macroscopically scored on a scale of 0–2 per paw, according to changes in redness and/or swelling of the paws. Animals were divided in six experimental groups (six mice/group): 1 and 2: no clinical arthritis ('before onset'); 3 and 4: arthritis score of 0.5–1.0 ('at onset'); 5 and 6: arthritis score of 1.5 or higher ('established disease'). Mice in groups 1, 3 and 5 were the controls, receiving rat IgG1 control antibodies (Pfizer, Cambridge, MA, USA), while mice in groups 2, 4 and 6 were treated with rat anti-mouse IL-22 antibody (Pfizer, Cambridge, MA, USA). Antibodies (8 mg/kg) were administered intraperitoneally three times per week for a total period of 12 days. This regimen was based on previous studies [20].

SPECT/CT images were acquired 12 days after start of treatment. After image acquisition, mice were euthanized, organs were isolated to determine tissue distribution of the tracer, and ankle joints were isolated for X-ray and histological analysis.

Antibody conjugation and radiolabelling

The humanized monoclonal anti-FAP antibody 28H1 (Roche, Schlieren, Switzerland) has a high picomolar affinity for both murine and human FAP. 28H1 was conjugated with the chelator isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (p-SCN-Bz-DTPA, Macrocyclics, Dallas, TX, USA) as described previously [16]. Radiolabelling with $^{111}InCl_3$ was performed as described previously [21].

Labelling efficiencies, determined by thin-layer chromatography, exceeded 99%.

Micro-SPECT/CT imaging

All mice were injected intravenously with 50 μg of anti-FAP antibody 28H1 labeled with the radioactive isotype indium (^{111}In-28H1) (15 MBq). At 24 h post injection, image acquisition with the U-SPECT-II/CT (MILabs, Utrecht, The Netherlands) was performed [22]. Mice were scanned under general anaesthesia (isoflurane and air) for 25 min while in prone position, using a 1.0 mm pinhole ultra-high sensitivity mouse collimator. SPECT scans were followed by CT scans for anatomical reference (spatial resolution of 160 μm, 65 kV, 615 μA). SPECT scans were reconstructed with software from MILabs (Utrecht, The Netherlands), using an ordered subset expectation maximization algorithm, with a voxel size of 0.375 mm. Reconstructed micro-SPECT scans were coregistered with micro-CT images using Inveon Research Workplace 4.1 software (Siemens Preclinical Solutions, Knoxville, TN, USA). To allow for quantitative SPECT analysis, 3D regions of interest were drawn on the CT images and transferred to the SPECT images to obtain mean voxel intensities of the SPECT data corresponding to those regions. Mean voxel intensity values were converted to percentage injected dose per gram tissue (%ID/g).

Histology

Murine joints were fixed for at least 4 days in 4% formaldehyde, decalcified in 5% formic acid, dehydrated and embedded in paraffin. Frontal sections of 7 μm were mounted on SuperFrost slides (Menzel-Gläser, VWR, Leuven, Belgium), and stained with haematoxylin and eosin for analysis of joint inflammation and bone erosion, or with Safranin O to determine cartilage damage (average of scored cartilage erosion, proteoglycan depletion and chondrocyte death). Joint inflammation and destruction was scored on an arbitrary scale of 0–3, as previously described [23]. Histopathological changes were scored on three semi-serial joint sections, in a blindfolded manner.

X-ray analysis of knee and ankle joints

X-ray images from knee and ankle joints were taken using a Faxitron MX20 instrument (Faxitron Bioptics, Tucson, AZ, USA) and analysed using Faxitron software. Bone destruction was scored on an arbitrary scale from 0 to 4 per paw, where joints without pathology were scored as 0, and with maximal pathology as 4.

FAP immunohistochemistry

Knee joint synovium pieces were fixed and frozen in Tissue-Tek O.C.T. Compound (Sakura, Torrance, CA, USA) upon isolation. Next, 7 μm sections were cut, dried on SuperFrost slides (Menzel-Gläser, VWR, Leuven, Belgium) and stained with human anti-mouse/human FAP Ab (28H1-IgG; 0.1 μg/ml; Roche, Schlieren, Switzerland), or human IgG control Ab (DP47GS; 0.1 μg/ml; Roche, Schlieren, Switzerland). Subsequently, sections were incubated with biotinylated goat-anti-human secondary antibodies (BA-3000; 1.5 mg/ml) and labelled with streptavidin-horseradish peroxidase (PK-6101) (both Vector Laboratories, Burlingame, CA, USA). Finally, peroxidase activity was developed with diaminobenzidine (D-5637; Sigma-Aldrich, St Louis, MO, USA) and counterstained with haematoxylin.

mRNA isolation and quantitative real-time PCR

Knee synovium biopsies were collected using a 3 mm biopsy punch (Stiefel, Wachtersbach, Germany), and subsequently disrupted and homogenized using the MagNA Lyser (Roche Diagnostics, Indianapolis, IN, USA). mRNA was isolated, treated with DNAse and reverse transcribed as previously described [20]. Gene expression levels were determined on the StepOnePlus sequence detection system using SYBR Green technology (both Applied Biosystems, Foster City, CA, USA). In the QPCR reaction, 2 μM of murine FAP primers (Biolegio, Nijmegen, The Netherlands) were used [5′-GTC-ACC-TGA-TCG-GCA-ATT-TGT-3′ (forward) and 5′-TCG-TAG-ATG-TAG-TAT-GTC-GCT-GT-3′ (reverse)]. Gene of interest expression levels were normalized for expression of the reference gene *GAPDH* [5′-GGC-AAA-TTC-AAC-GGC-ACA-3′ (forward) and 5′-GTT-AGT-GGG-GTC-TCG-CTC-CTG-3′ (reverse)]. To allow for statistical analysis, the $-\Delta C_t$ (the cycle threshold of the gene of interest corrected for the expression of the reference gene) value of samples with mRNA levels below detection limit was set at −30.

Statistics

To determine the level of statistical significance between the experimental groups, the Mann–Whitney or Spearman correlation test was applied, using GraphPad Prism (GraphPad Software, San Diego, CA, USA) version 5. $P < 0.05$ was considered significant. Results are expressed as bar graphs, Tukey boxplots or correlation plots.

Results

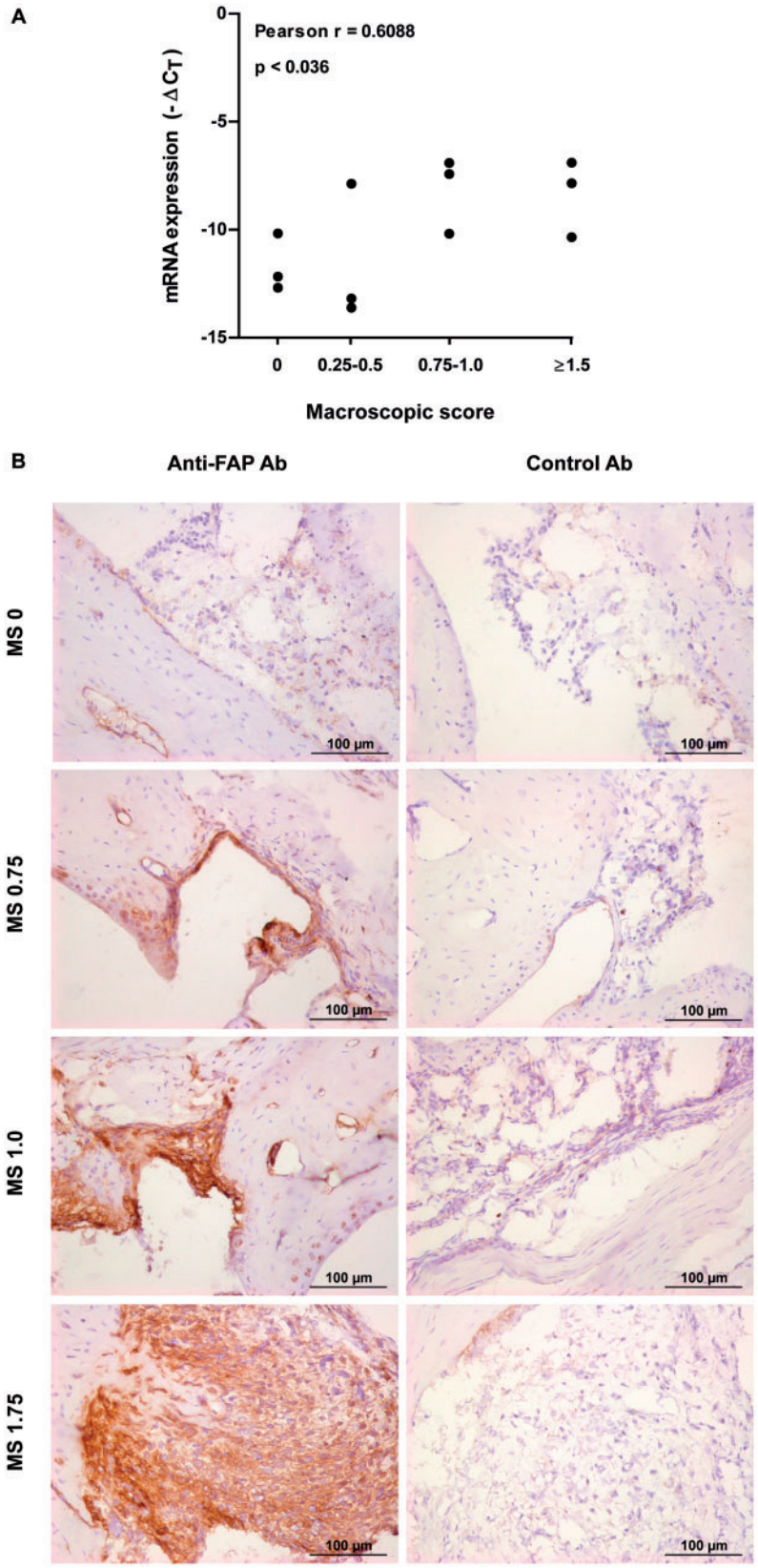
Severity of experimental arthritis correlates with synovial FAP expression

To determine the correlation between severity of experimental arthritis and FAP expression levels in the synovium, CIA was induced in DBA1/J mice. Mice were euthanized at various stages of the disease. Analysis of isolated synovium by qPCR and immunohistochemistry showed that both FAP mRNA and protein expression increased with progressive disease severity (Fig. 1A and B, respectively).

Decreased synovial FAP expression after early anti-IL-22 treatment

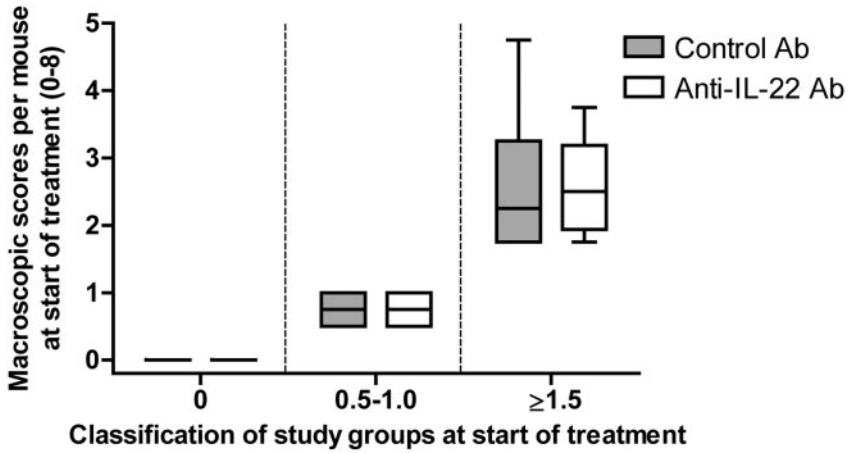
^{111}In-28H1 SPECT/CT is an excellent tool to monitor synovial FAP expression [18]. In order to test the potency of this tracer to monitor responses to arthritis therapy, mice in various stages of CIA (group divisions as depicted in Fig. 2) were treated with IL-22-neutralizing antibodies or a control treatment, and subsequently FAP expression was visualized using ^{111}In-28H1 SPECT/CT. Arthritic joints were specifically visualized with ^{111}In-28H1 in all groups (Fig. 3A–F). Images showed lower uptake of ^{111}In-28H1 when mice received anti-IL-22 treatment before onset of

FIG. 1 FAP expression increases in synovium of mice with more severe CIA



FAP mRNA expression levels depicted as $-\Delta C_t$ levels as determined using qPCR (**A**), and immunohistochemistry slides (**B**) of synovium isolated from mice with various severities of CIA, depicted as macroscopic scores (MS) on a scale of 0–2. Slides are stained with anti-FAP antibodies or appropriate isotype control antibodies.

Fig. 2 Macroscopic scores of the various experimental groups before treatment



Treatment with either IgG isotype control antibodies or anti-IL-22 antibodies was initiated when mice reached a macroscopic score of 0 (groups 1 and 2), 0.5–1.0 (groups 3 and 4) or higher than 1.5 (groups 5 and 6). Group division based on macroscopic scores at the time of treatment initiation is depicted as Tukey's boxplots (n = 6 mice/group).

macroscopic symptoms (Fig. 3D), as compared with mice in the control group (Fig. 3A). Interestingly, tracer uptake was unaffected in mice that were treated around onset (Fig. 3E) or during established disease (Fig. 3F) with this IL-22 neutralizing antibody, as compared with mice receiving the control antibody (Fig. 3B and C). Quantitative analysis of the SPECT images confirmed that tracer uptake in the joints was lower in joints after treatment with anti-IL-22 antibodies before onset of disease (Fig. 3G–I). For example, ankle joint uptake of ^{111}In-28H1 (Fig. 3G) was significantly higher ($P = 0.02$) in control-treated mice than in anti-IL-22 treated mice. Additionally, a significant reduction in tracer uptake ($P = 0.03$) was found in the knee joints of anti-IL-22-treated mice compared with control-treated mice, as depicted in Fig. 3H. No significant differences were found for ^{111}In-28H1 uptake in other joints, such as the wrists (Fig. 3I), or for tissue uptake in joints of mice that were treated in a later stage of disease.

Neutralizing IL-22 in an early phase reduces macroscopic arthritis incidence and severity, correlating with tracer uptake

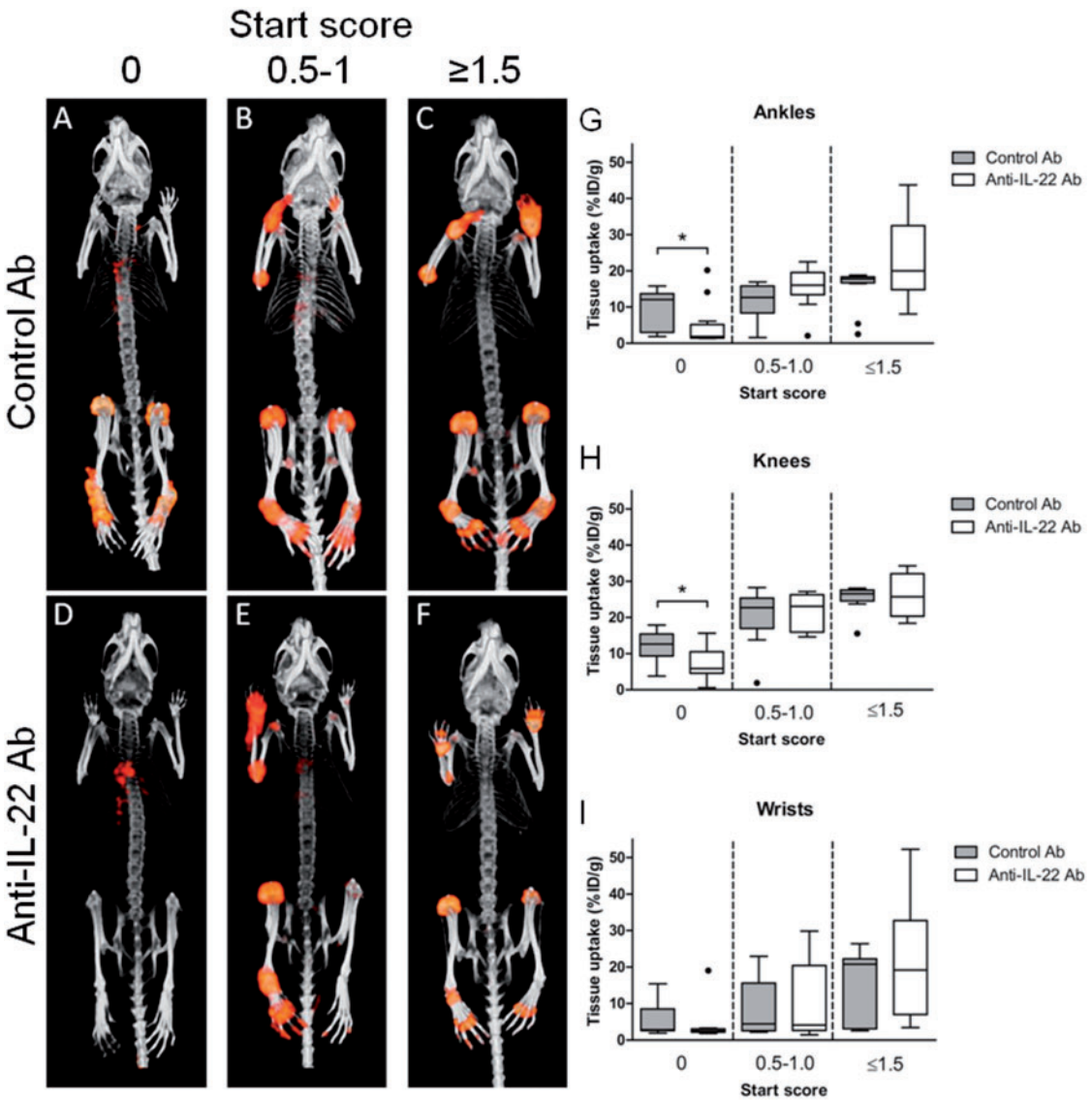
In line with the reduced levels of ^{111}In-28H1 uptake in synovium, we observed reduced clinical arthritis incidence (50 *vs* 100% at day 12 after start of treatment; Fig. 4A) as well as ankle (Fig. 4B), but not wrist (Fig. 4C), joint swelling in mice treated with anti-IL-22 antibodies before disease onset as compared with mice that received control antibodies. As with SPECT/CT measured joint tracer uptake, macroscopically scored ankle joint swelling was not inhibited in mice in which anti-IL-22 treatment was initiated during later phases of the disease in this arthritis model (Fig. 4B). It is noteworthy that a strong positive correlation was found between synovial uptake of ^{111}In-28H1 and macroscopic arthritis scores of the ankle joint (Spearman $r = 0.7937$, $P < 0.0001$; Fig. 4D). Interestingly, relatively high levels of FAP-tracer uptake were detected in ankle joints of several mice without clinical signs of arthritis (score 0; Fig. 4D). With macroscopic scoring, only the clinical signs of arthritis in wrists, ankles and digits of front and hind paws are included. As CIA is a systemic model also affecting larger synovial joints like the knee joints, hip, elbow and shoulders, not all affected joints can be scored using this system. Using FAP-based SPECT/CT *in vivo*, arthritis incidence and severity in those other affected areas can be detected (Fig. 3A–F).

Ankle and knee histopathology and radiographic ankle bone damage correlate with synovial tracer uptake after treatment

In agreement with SPECT/CT and macroscopic data, reduced inflammation and cartilage damage was observed on a histological level in the ankle joints of mice treated with anti-IL-22 antibodies before disease onset, as compared with the control group (Fig. 5A and B). In line with tracer uptake and clinical scoring, neutralization of IL-22 could not prevent histological damage in ankles of mice with starting or established disease. Interestingly, again a strong and highly significant correlation between joint damage and anti-FAP antibody uptake was apparent (Spearman $r = 0.7100$, $P < 0.0001$; Fig. 5C). Additionally, radiographic ankle bone damage, as measured using X-ray, was significantly reduced in the ankle joints after early anti-IL-22 antibody treatment as compared with control treatment (0.4 *vs* 1.5, respectively, on a scale of 0–4, $P < 0.05$; Fig. 5D and E). No effect of the IL-22-neutralizing antibody treatment was detected when treatment was initiated in mice with clinical signs of arthritis. Ankle tissue tracer uptake and radiological bone damage showed a strong, positive correlation (Spearman $r = 0.6993$, $P < 0.0001$; Fig. 5F).

In accordance with SPECT/CT images and observations in ankle joints, knee joint histopathology was reduced in mice treated with anti-IL-22 antibodies before

Fig. 3 Early anti-IL-22 treatment reduces tissue uptake of radiolabelled FAP-tracer, indicating reduced severity of CIA



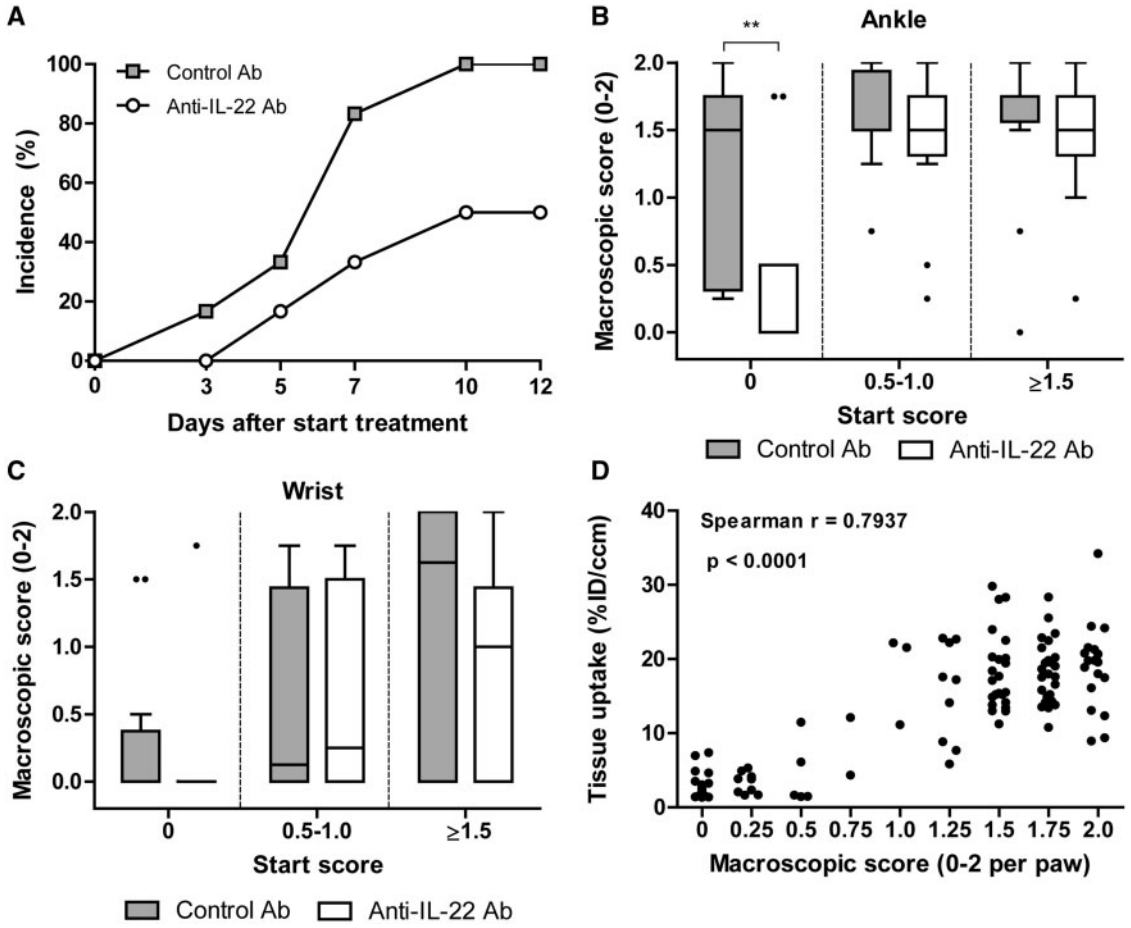
SPECT/CT images of mice injected with the FAP tracer ^{111}In-28H1 (**A**–**F**) and tissue uptake-based quantitative SPECT analysis in ankles (**G**), knees (**H**) and wrists (**I**). SPECT/CT imaging was performed after 2 weeks of treatment, with an isotype control antibody (**A**–**C**) or with the anti-IL-22 antibody (**D**–**F**). IL-22 signalling was neutralized before onset (**A** and **D**, macroscopic start score 0), around onset (**B** and **E**, macroscopic start score 0.5–1.0) or in established CIA (**C** and **F**, macroscopic start score $\geqslant$1.5). Tissue uptake (%ID/g) per ankle, knee and wrist joints (n = 6 mice/group) are shown (**G** and **H**; Tukey's boxplot; *$P < 0.05$; Mann–Whitney test).

disease onset, as compared with the control group (Fig. 6A and B). Histopathological scores positively correlated with FAP-tracer uptake in the knee joints (Spearman $r = 0.6710$, $P < 0.0001$; Fig. 6C). As opposed to the significantly reduced bone damage in the knees as observed during histological analysis, radiological scored knee bone damage was unaffected after early anti-IL-22 antibody treatment (Fig. 6D and E). An effect of the anti-IL-22 antibody was detected neither on a histological, nor on a radiographic level when treatment was initiated in mice with starting or established disease. When linking tissue uptake of the tracer with radiological ankle bone damage, again a strong positive correlation between the readout parameters was observed (Spearman $r = 0.7206$, $P < 0.0001$; Fig. 6F).

Discussion

We demonstrated, using ^{111}In-28H1 SPECT imaging, that IL-22 neutralization only in the early phase of disease effectively suppressed the development of CIA. Most

FIG. 4 Early anti-IL-22 treatment reduces macroscopically scored severity of CIA, correlating with tissue uptake of FAP-tracer



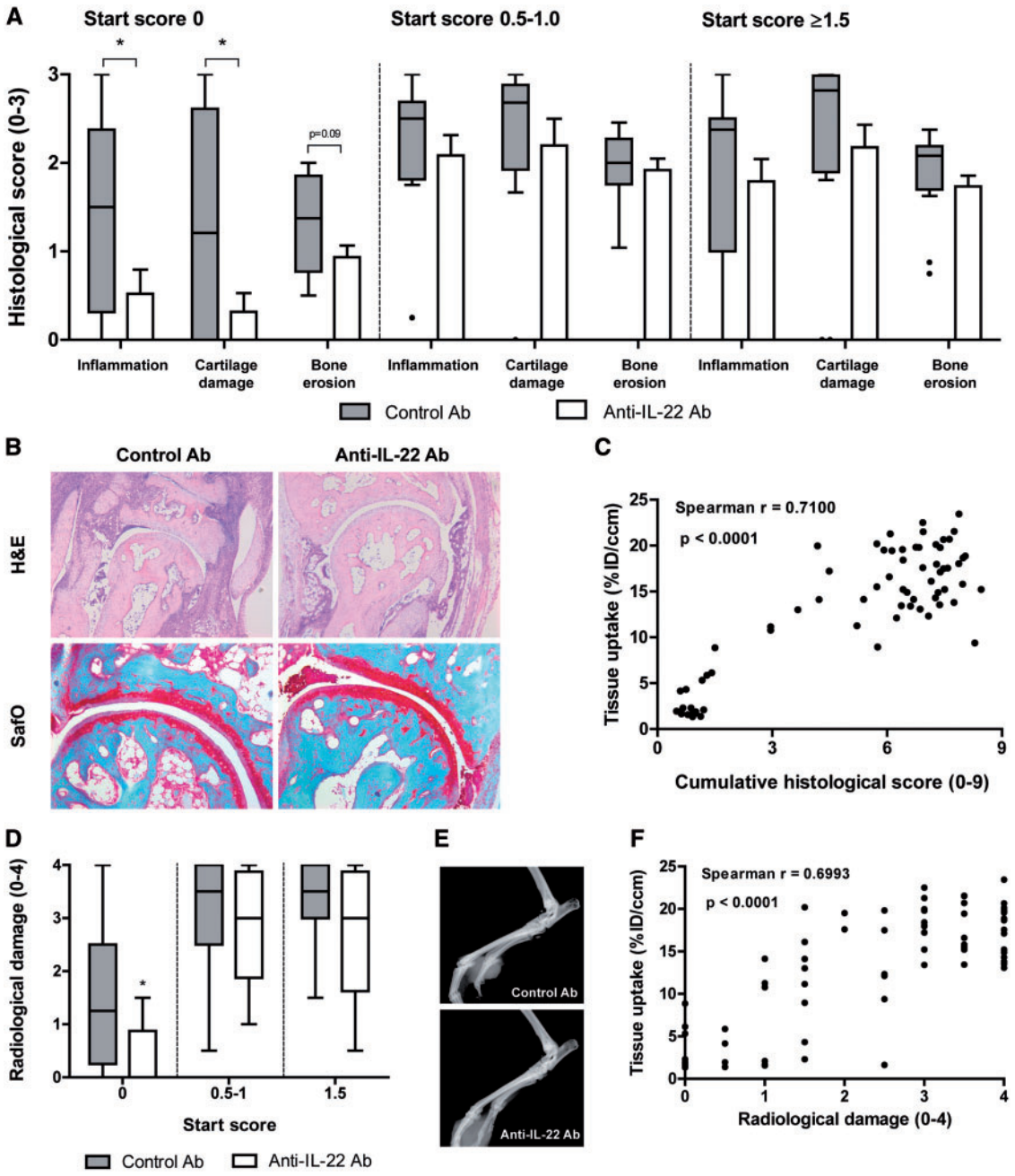
IL-22 signalling was neutralized in mice either before onset (macroscopic start score 0), around onset (macroscopic start score 0.5–1.0) or in established CIA (macroscopic start score $\geqslant$1.5). Disease incidence of mice treated before onset (**A**), and disease severity in ankle joints (**B**) and wrists (**C**) of all experimental groups at day 12, as scored macroscopically on a scale of 0–2 per paw (Tukey's boxplot; $^{**}P < 0.01$, Mann–Whitney test; $n = 12$ joints/group). (**D**) Correlation between FAP-tracer uptake by the tissue as measured using SPECT/CT and macroscopic arthritis severity scores ($n = 72$ ankle joints; Spearman correlation).

importantly, we showed that this response to anti-IL-22 treatment can be monitored non-invasively, specifically and objectively using quantitative SPECT/CT imaging of the inflamed synovium using ^{111}In-labelled anti-FAP antibody as a tracer.

Previous SPECT/CT studies with radiolabelled anti-FAP antibodies showed high-quality images [16, 18, 24]. Additionally, a better correlation between tissue uptake of the imaging tracer and severity of disease was found compared with the correlation found with the widely used Positron-emission tomograph imaging using fluorodeoxyglucose labelled with radioactive fluor (^{18}F-FDG-PET), which has been suggested as a tool to monitor therapeutic response in arthritis [14, 18, 25]. These studies also emphasized the feasibility of using ^{111}In-28H1 SPECT to specifically monitor therapy response at a molecular level.

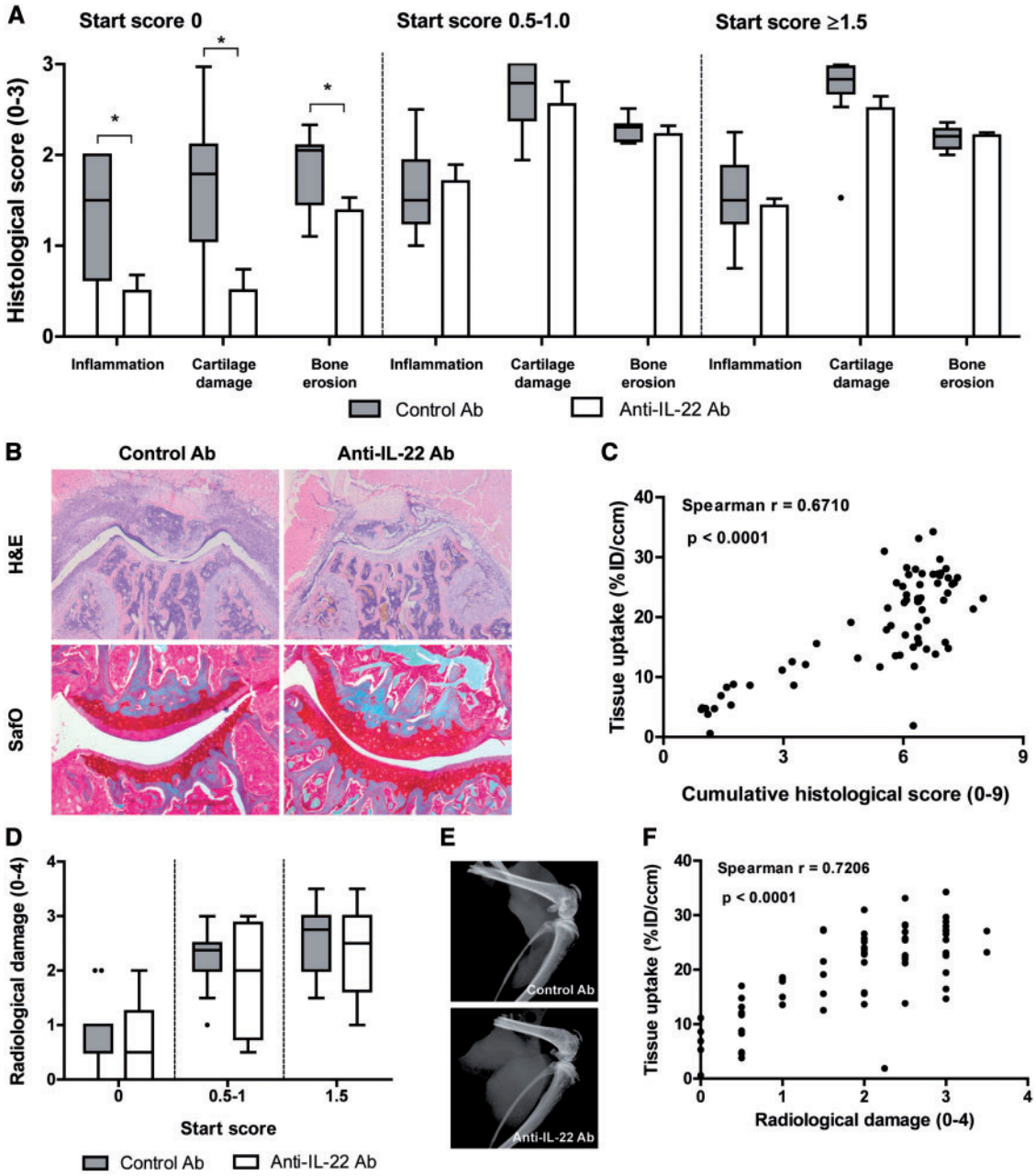
IL-22 is a cytokine to which both pro- and anti-inflammatory properties have been attributed. Even though most studies on the role of IL-22 in experimental arthritis hint towards a pro-inflammatory effect [12, 19, 26, 27], the exact mechanism by which it is involved in RA pathology remains unclear. The therapeutic potential of specifically neutralizing IL-22 in experimental arthritis was clearly shown by the highly efficient reduction in disease incidence when treatment was initiated early in disease development. Additionally, disease severity was lowered as demonstrated by significantly reduced clinical arthritis, histological damage and radiographic bone damage. Of note, a minor discrepancy between histological and radiographic scored bone damage in ankle and knee joints was observed. On the histological level, ankle joint damage was not significantly reduced after

FIG. 5 Reduced histopathology and radiographic damage of ankle joints after αIL-22 treatment before arthritis onset



Mice received anti-IL-22 antibodies either before onset (macroscopic score 0), around onset (macroscopic score 0.5–1.0) or during established CIA (macroscopic score $\geqslant$1.5). (**A**) Frontal sections of the ankle joint were stained with haematoloxylin & eosin (H&E) or with Safranin O (SafO) to analyse histological inflammation, cartilage damage and bone erosion, on a scale of 0–3 per parameter (Tukey's boxplot; *$P < 0.05$, Mann–Whitney test; $n = 12$ joints/group). (**B**) Images represent average group scores of mice treated before disease onset. (**C**) Correlation between ankle FAP-tracer uptake and the sum of the three histologically scored parameters ($n = 72$ joints; Spearman correlation). (**D**) X-ray images were acquired using the Faxitron, and bone damage was subsequently scored on a scale of 0–4 (Tukey's boxplot; *$P < 0.05$, Mann–Whitney test; $n = 12$ joints/group). (**E**) Images represent average group scores of mice treated before disease onset. (**F**) Correlation between ankle FAP-tracer uptake and radiological bone damage ($n = 72$ joints; Spearman correlation).

FIG. 6 Reduced histopathology of the knee joint



Mice received anti-IL-22 antibodies either before onset (macroscopic score 0), around onset (macroscopic score 0.5–1.0) or during established CIA (macroscopic score $\geqslant$1.5). (**A**) Frontal sections of the knee joint were stained with haematoloxylin and eosin (H&E) or with Safranin O (SafO) to analyse histological inflammation, cartilage damage and bone erosion, on a scale of 0–3 per parameter (Tukey's boxplot; *$P < 0.05$, Mann–Whitney test; $n = 12$ joints/group). (**B**) Images represent average group scores of mice treated before disease onset. (**C**) Correlation between knee FAP-tracer uptake and the sum of the three histologically scored parameters ($n = 72$ joints; Spearman correlation). (**D**) X-ray images were acquired using the Faxitron, and bone damage was subsequently scored on a scale of 0–4 (Tukey's boxplot; *$P < 0.05$, Mann–Whitney test; $n = 12$ joints/group). (**E**) Images represent average group scores of mice treated before disease onset. (**F**) Correlation between knee FAP-tracer uptake and radiological bone damage ($n = 72$ joints; Spearman correlation).

IL-22 neutralization, while this was the case on a radiographic level. Knee joints on the other hand, showed significantly less histological bone damage after anti-IL-22 treatment, while no effects were observed on a radiographic level. This might appear contradictory, but the two different methods visualize different aspects of bone damage. On a histological level, microscopic bone erosions can be detected, whereas bone density and larger areas of damage to the bone can clearly be observed using X-ray imaging.

As this study was designed to investigate whether SPECT/CT imaging with ^{111}In-labelled anti-FAP antibodies could be used to monitor response to anti-IL-22 therapy, we did not focus on deciphering the mechanism of IL-22 in experimental arthritis. Nevertheless, the difference in effectiveness of IL-22 neutralization in reducing arthritis severity between treatment initiation before disease onset *vs* at onset or during established disease is highly interesting. Based on the results of our previous study, which showed efficacy of IL-22 neutralization during established disease in IL-1Ra$^{-/-}$ mice [20], we hypothesize that the inadequacy of anti-IL-22 treatment in a later stage of disease is partly caused by the progressive and aggressive character of the arthritis during CIA. Although CIA is the most commonly studied animal model for RA and has become the 'gold standard' in pre-clinical rheumatology research over the past three decades, it is possible that disease development cannot be inhibited using IL-22-neutralizing antibodies once mice suffer from such a progressive form of autoimmune experimental arthritis. Another explanation might be the changing role of IL-22 during disease development. As suggested previously by Pineda *et al.* [13], the role of IL-22 might be pathogenic during early, but not during later phases of CIA. Hence, neutralizing this cytokine later in disease development would not affect joint pathology. Interestingly, Justa *et al.* [28] showed a protective role for IL-22 early in CIA development, and a pathogenic role during later phases of the disease. This is not only the opposite of what has been described by Pineda, but is also not in line with our current studies, where we observed a pathogenic role for IL-22 during the early stages of CIA development. Possibly, this is the consequence of differences in the inflammatory environment (e.g. cytokine milieu, level of inflammation), influencing the pro- or anti-inflammatory character of IL-22. IL-22 has been shown to be pathogenic in the presence, and protective in the absence, of IL-17 in an animal model for airway inflammation [29]; this cooperative mechanism with IL-17 or other pro- or anti-inflammatory cytokines might be present in experimental arthritis as well. This can provide a possible mechanism by which IL-22 exerts its pro- and anti-inflammatory activities, respectively. We conclude that IL-22 is an important mediator during the early phases of CIA and has potential as a target in treating early RA. Further studies into the exact mechanism of action are required before testing the druggability of anti-IL-22 treatment in the clinic.

Here, we showed the feasibility of ^{111}In-28H1 SPECT/CT as a specific and quantifiable tool to assess therapy response after IL-22 neutralization in experimental arthritis. As a result of the objectivity and specificity of this method, molecular imaging using radiolabelled anti-FAP antibodies as a tracer can be of additive value to existing methods of therapy monitoring in patients such as clinical macroscopic scoring (DAS28), MRI, US or conventional radiography. Additionally, the high sensitivity of ^{111}In-28H1 SPECT/CT to detect experimental arthritis was shown, due to the relatively high tissue uptake of ^{111}In-28H1 in joints that were macroscopically scored as not affected (score 0), and thus not showing any clinical signs. We suggest that the uptake of the tracer in the joints reflects increased FAP expression during subclinical synovitis and that these joints will show symptoms of inflammation upon disease progression, indicating a potential predictive value of ^{111}In-28H1 SPECT/CT.

In conclusion, ^{111}In-28H1 SPECT/CT imaging of the inflamed synovium can be used to specifically monitor response to therapy in an objective and quantitative manner, and is potentially more sensitive in disease monitoring compared with the standard methods of clinical arthritis scoring.

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