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Systemic and local regulation of joint inflammation

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Abbreviations

αCGRP	alpha calcitonin gene-related peptide
βCGRP	beta calcitonin gene-related peptide
ACAN	aggrecan
Acan	*aggrecan (encoding gene)*
Acp5/Trap	*tartrate resistant acid phosphatase 5 (encoding gene)*
ACR	American College of Rheumatology
ATP	adenosine triphosphate
CAIA	collagen II-antibody-induced arthritis
Calcr	*calcitonin receptor (encoding gene)*
Calcrl	*calcitonin receptor like receptor (encoding gene)*
CCL2	CC-chemokine ligand 2
Ccl2	*CC-chemokine ligand 2 (encoding gene)*
CD	cluster of differentiation
Cd14	*cluster of differentiation 14 (encoding gene)*
Cd68	*cluster of differentiation 68 (encoding gene)*
COL1A1	collagen type 1A1
COL2A1	collagen type 2A1
CRP	c-reactive protein
CT	calcitonin
Ctsk	*cathepsin K (encoding gene)*
DCA	dichloroacetate
DDP4	dipeptidyl-peptidase 4
DMARD	disease modifying anti-rheumatic drug
DMOAD	disease-modifying osteoarthritis drug
DNA	deoxyribonucleic acid
ECM	extra-cellular matrix
EMA	European Medicines Agency

ESR	erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FDA	U.S. Food and Drug Administration
FLS	fibroblast-like synoviocytes
FGF	fibroblast growth factor
GC	glucocorticoid
GLA:D®	Good Life with osteoArthritis in Denmark
GLP1	glucagon-like peptide 1
GMCSF	granulocyte-monocyte colony stimulating factor
IFN-γ	interferon gamma
IL	interleukin
Il1b	*interleukin 1 beta (encoding gene)*
Ihh	Indian Hedgehog
JAK	janus kinase
µCT	µ-computed tomography
Mmp13	*matrix metalloproteinase 13 (encoding gene)*
MSC	mesenchymal stromal cell
NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis
OARSI	Osteoarthritis Research Society International
OXPHOS	oxidative phosphorylation
PCT	procalcitonin
PDK3	pyruvate dehydrogenase kinase 3
PRP	platelet-rich plasma
PTH	parathyroid hormone
RA	rheumatoid arthritis
Ramp1	*receptor activity modifying protein 1*
RNA	ribonucleic acid

Sphk1	*sphingosine kinase 1 (encoding gene)*
TGF-β	transforming growth factor-beta
Tgfb1	*transforming growth factor-beta 1 (encoding gene)*
THY1+/-	thymus cell antigen 1 positive/negative
TNF-α	tumor necrosis factor alpha
Tnfa	*tumor necrosis factor alpha (encoding gene)*
WT	wild type
Wnt	wingless-related integration site

Content

1 Introduction

1.1 Joint inflammation – a link between rheumatoid arthritis and osteoarthritis

The immune system is characterized by a sophisticated network of cells, proteins and signaling molecules that effectively protects the body from pathogens. Collectively, the innate and adaptive immune system fight bacteria, viruses, fungi, parasites and noxins.

The immune system may however also respond to several conditions that are not directly induced by bacterial or viral infections. Inflammatory and degenerative joint diseases are progressive or chronic pathologies that often affect multiple joints and are initiated and maintained by a systemic or local immunoreaction. The most common inflammatory joint disease is rheumatoid arthritis (RA), which predominantly affects small joints in a symmetrical fashion, including the proximal interphalangeal and metacarpophalangeal joints (1). In contrast to RA, osteoarthritis (OA) was long perceived as a non-inflammatory, mechanically-induced degenerative joint pathology. In recent years this understanding has changed, and chronic low-grade inflammation is now recognized as one of the main disease components in OA (2-6). Although the origins of RA and OA are fundamentally different, intra-articular inflammatory changes drive and maintain disease activity in both pathologies.

1.1.1 Rheumatoid arthritis

RA is an autoimmune disorder characterized by inflammatory changes of the synovium that often affect multiple joints. Disease progression is commonly accompanied by rheumatic flares, and extra-articular manifestations are possible, most commonly affecting skin, eyes, and kidneys.

RA has a global prevalence of 0.5–1% with women being affected twice as often as men (7). RA diagnosis is given based on the 2010 American College of Rheumatology (ACR) / European League Against Rheumatism (EULAR) criteria, which include joint involvement (tender or swollen), serology for RA-specific antibodies, acute-phase reactants and persistence of symptoms for more than six weeks (8).

Although one single cause has not yet been identified, several risk factors seem to increase the risk of developing RA. Next to susceptibility genes, epigenetic and post-translational modifications as well as repetitive bacterial or viral infections, smoking and exposure to silica are associated with RA onset. These risk factors typically initiate a loss of tolerance of the immune system, causing immune cells, cytokines and auto-antibodies to progressively infiltrate the articular synovium which leads to symptomatic joint inflammation and, over time, concomitant cartilage and bone destruction (9). It has been suggested that the reactive systemic inflammation and autoimmunity may originally be triggered by immune cell contacts with antigens on various mucosal surfaces (10).

This so-called initiation phase, or pre-clinical RA, is often recognized by increased serum levels of auto-antibodies (11), cytokines (12, 13), and c-reactive protein (CRP) (14) years prior to detectable inflammatory changes in the joint. As soon as structural alterations are present in the joint, inflammation is maintained by a complex interplay between osteoblasts, osteoclasts, chondrocytes, fibroblasts, macrophages, mast cells, mesenchymal stromal cells (MSCs), and T and B cells, which accelerate synovial hyperplasia and neoangiogenesis (9).

The pro-inflammatory environment is further characterized by an interaction of cyto- and chemokines with endothelial cells, interleukins (ILs), tumor necrosis factor alpha (TNF-α) and granulocyte-monocyte colony stimulating factor (GMCSF), which are predominantly responsible for disease progression in RA (15).

1.1.2 Osteoarthritis

OA is the most common degenerative joint disease with a global prevalence of 15% in the adult population (16). Currently, OA affects around 500 million people, making it the third most rapidly growing disease with disability behind type II diabetes mellitus and dementia (17).

OA was long understood as a ‘wear and tear’ disease and thereby clearly separated from the inflammatory character of RA. However, through the works of Mary and Steve Goldring as well as Carla R. Scanzello, low-grade inflammation was fundamentally included in the pathogenesis of OA (3, 5, 6), which shares numerous risk factors with RA (18). OA can be

primary (without a known cause, and often associated with age) or secondary (due to injury, infection, or unbalanced joint loading).

As a response to traumatic or degenerative micro or macro lesions to cartilage, excessive repair processes dominated by pro-inflammatory signaling molecules cause new cartilage damage, thus initiating a vicious cycle (19). This explains why age, obesity and sports injuries are major risk factors for the development of OA. Nonetheless, genetic predispositions also exist in OA, where changes to the transforming growth factor-beta (TGF-β), wingless-related integration site (Wnt)/β-catenin, Indian Hedgehog (Ihh), Notch and fibroblast growth factor (FGF) pathways may lead to cartilage and extra-cellular matrix (ECM) degradation (20, 21). Chronic low-grade inflammation eventually leads to joint remodeling that can radiologically be observed as joint space narrowing, osteophytes, subchondral sclerosis and cysts (22). In 2015, the Osteoarthritis Research Society International (OARSI) defined OA as “a disorder involving movable joints characterized by cell stress and ECM degradation initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic, and/or physiologic derangements (characterized by cartilage degradation, bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness“ (19).

1.2 Principles of rheumatoid arthritis and osteoarthritis treatment

Non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids (GCs) are prescribed to reduce inflammation-induced pain and stiffness but are unable to halt or slow down RA progression. To decelerate disease activity and reduce joint destruction, disease modifying anti-rheumatic drugs (DMARDs) have proven to be safe and effective. In addition to conventional DMARDs (f.e. methotrexate, leflunomide, and sulfasalazine), biological DMARDs/biologics (f.e. TNF-, CD20-, CD80-, IL-6 receptor antibodies and janus kinase (JAK)-inhibitors) are applied to control disease activity and consecutively reduce pain. While the

introduction of biologics has revolutionized RA therapy since the 1990s by markedly reducing structural disease progression, complete remission is often not achieved (1).

Treatment of OA includes lifestyle adaptations, weight loss (if relevant), physical exercise, and oral or topic NSAIDs. Intra-articular GC and hyaluronic acid injections may be considered for short-term pain relief. Yet, the use of intra-articular injections has been debated in recent years due to reports of limited efficacy (23), a considerable placebo effect (24), and potential harm to the cartilage (25). If conservative care fails to provide sufficient symptom relief, surgical treatment – spanning from tibial or femoral osteotomies to partial and total joint replacement – is available for advanced disease stages of OA and in some cases for RA. Patient satisfaction after hip replacement surgery is commonly reported to be above 90% (26-28), but patients who have undergone knee replacement surgery are not satisfied in up to 20% of cases (27, 29). While patient satisfaction following surgery is multi-dimensional and expectation management and patient involvement can improve the quality of decisions for or against surgery (30), the need for disease modifying interventions that may prevent surgery and address OA at an early disease stage remains.

1.3 Limitations of current rheumatoid arthritis and osteoarthritis treatment

The introduction of DMARD therapies allowed disease control and improved joint function through a marked reduction of synovitis and systemic inflammation in patients with RA. Despite side effects that include severe infections, bone marrow suppression, and hepatoxicity (31), DMARD monotherapy or sophisticated DMARD combinations are increasingly prescribed and included in most RA treatment guidelines (32, 33). DMARDs are able to effectively reduce disease activity, but up to 40% of patients exhibit a poor clinical response, which is not yet mechanistically explained (34, 35). Further, especially bone erosions, a hallmark of RA, are often only insufficiently addressed by currently available DMARDs (36, 37).

To receive approval by regulators and advance to the market, a drug must have both symptomatic benefits (pain and function) and induce structural improvements (assessed

radiologically or by serum or synovial fluid biomarkers), and to date no agent has met those requirements for OA. This may be due to several trial design-related and drug-related shortcomings that are slowly being recognized and adapted in current clinical trial designs (38-40):

1. Preclinical OA animal models do often not adequately represent human disease and although disease-modifying osteoarthritis drugs (DMOADs) have often shown impressive structural and functional improvements in animal models, these results could often not be replicated in human trials. This may be because most preclinical *in vivo* research is conducted in post-traumatic OA models, which do not mimic primary OA, the most common form of OA and main target for DMOAD development (41).
2. A considerable number of human trials has focused on end-stage OA, where advanced structural changes are unlikely to be altered or reversed by biological agents.
3. OA is a chronic and slowly progressing disease, and tools currently applied are unable to detect meaningful improvements during typical trial follow-up periods of months to few years (42).

Following a white paper issued by OARSI in 2016 (43) and the subsequent recognition of OA as a so called ‘serious disease’ by the U.S. Food and Drug Administration (FDA) in 2018 (44), accelerated approval of DMOADs in combination with post-marketing confirmatory trials is now theoretically possible (38-40). This means that FDA approval may be granted faster than usual based on trials using a primary endpoint, or surrogate marker, that has not yet been defined, but is likely an imaging or molecular biomarker that can reliably predict clinical outcomes (45).

One of the challenges of developing a successful treatment for RA and OA includes targeting an abundance of immune cells and receptors, enzymes, cyto- and chemokines, signaling molecules and antibodies involved in these diseases. Targeting only one of the numerous pathways is likely not going to address the disease as a whole.

Nonetheless, current scientific efforts have been successful in identifying signaling pathways that may target more than one aspect of RA and OA pathology, including pain and bone and cartilage destruction (46). Table 1 summarizes RA- and OA-related signaling pathways explored in this thesis.

Table 1. Signaling pathways related to RA and OA researched in this thesis.

Signaling pathway	**Disease model**	**Target**
Alpha calcitonin gene-related peptide (αCGRP)	RA	Inflammation, cartilage, bone
Calcitonin (CT)	RA	Inflammation, cartilage, bone
Procalcitonin (PCT)	RA	Inflammation, cartilage, bone
Pyruvate dehydrogenase kinase 3 (PDK3)	OA	Inflammation, cartilage
Individual immune profiles in platelet-rich plasma (PRP)	OA	Inflammation, cartilage

1.4 *Calca*-derived peptides

We previously outlined distinct functions of *Calca*-derived peptides in bone metabolism (47-51). The *Calca* gene encodes the peptides alpha calcitonin gene-related peptide (αCGRP), calcitonin (CT), and procalcitonin (PCT) through alternative splicing. PCT is primarily produced in thyroid tissue and αCGRP in nervous tissue, and PCT is converted to CT through posttranscriptional cleaving (50) (Figure 1).

During inflammation and following trauma, tissue specificity is abrogated, and *Calca*-derived peptides are produced by various cell types in different tissues of the musculoskeletal system (52, 53).

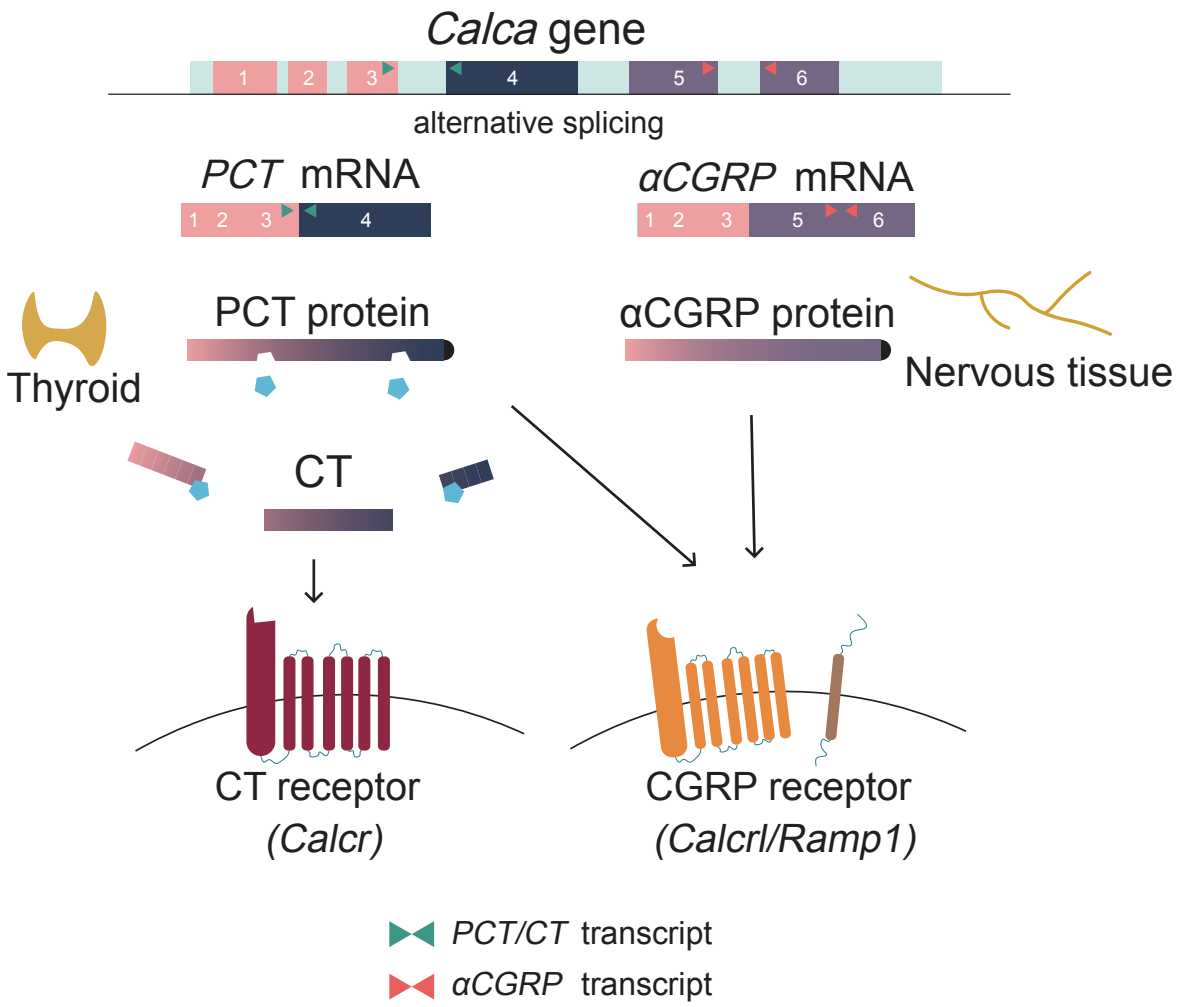

Figure 1. Alternative splicing of the *Calca gene*. The *Calca* gene has 6 exons of which 2-5 are coding exons. PCT mRNA is expressed in thyroid C cells where functional PCT is produced. Post-translational cleavage results in functional CT, and both PCT and CT are then released into the blood. αCGRP is translated, produced, and released into the blood by sensory neurons in the central and peripheral nervous systems. While αCGRP and PCT bind to the CGRP receptor encoded by the *Calcrl/Ramp1* gene, CT binds to the CT receptor encoded by the *Calcr* gene. Adapted from (50).

1.4.1 Calcitonin gene-related peptide

CGRP is a vasoactive neuropeptide and predominantly responsible for pain perception and modulation. It has two isoforms of which αCGRP is the principal form. The second isoform, βCGRP, is primarily expressed in the enteric nervous system and encoded by *Calcb* (54). CGRP is mainly released by afferent sensory neurons and enhances nociception through a combination of vasodilation and neurogenic inflammation (55). Due to its release in trigeminal

ganglion cells, CGRP has been directly tied to migraine (56), and anti-CGRP antibodies received FDA und European Medicines Agency (EMA) approval for the prevention and treatment of migraine in 2018/2019 (57).

As CGRP is also released from perivascular neurons in the musculoskeletal system, increased expression has been found in the synovial membrane (58-62), synovial fluid (61, 63, 64), and serum (63) of patients with painful joint pathologies, including RA and OA. These findings have raised questions about a potential independent and (patho)physiological role of CGRP in articular inflammation beyond pain perception and modulation.

1.4.2 Calcitonin

CT is produced by the parafollicular C-cells of the thyroid gland and naturally opposes the actions of parathyroid hormone (PTH) in regulating calcium and phosphate homeostasis. CT binds to the CT receptor and increases renal calcium secretion and inhibits osteoclasts, which both reduces net blood calcium levels.

Pharmacological CT (mainly in the form of teleost salmon or eel CT) was previously administered to prevent osteoporotic fractures in postmenopausal osteoporosis but almost vanished from the market due to limited efficacy (65) and accumulating reports of malignant adverse events (66, 67). However, as preclinical studies showed substantial cartilage- (68, 69) and bone-protective (70), anti-oxidative (71, 72), and anti-nociceptive effects (69, 70, 73) in experimental OA and RA, CT was discussed as a promising drug for human OA (74, 75) and RA (76, 77).

1.4.3 Procalcitonin

PCT is commonly employed as a serum biomarker for bacterial infections. In patients with RA, serum PCT can be helpful when distinguishing between septic arthritis and RA flares. In these cases, elevated serum PCT levels are more specific for septic arthritis than elevated CRP, prolonged erythrocyte sedimentation rate (ESR), and increased leukocyte count (78, 79). Beyond its role as a biomarker, little is known about the pathophysiological functions of PCT

in RA or OA. Previously an independent pro-inflammatory role was attributed to PCT in sepsis (80, 81), which raised the question whether PCT may also play a relevant role in inflammatory and degenerative joint diseases.

1.5 Pyruvate dehydrogenase kinase

The mitochondrial multienzyme complex pyruvate dehydrogenase links glycolysis to the tricarboxylic acid cycle to produce adenosine triphosphate (ATP) as part of the cellular energy metabolism. Pyruvate dehydrogenase

In healthy organisms, the pyruvate dehydrogenase complex is inhibited and regulated by pyruvate dehydrogenase kinase (PDK). An overexpression of PDK however causes a metabolic shift from mitochondrial respiration to glycolysis and is observed in cancer cells (82-84). PDK inhibition thereby helps to revert the metabolic shift observed in malignant cells and their proliferation (82).

PDK overexpression however also drives muscly hypotrophy (85), vascular inflammation in atherosclerosis (86), and has recently been discovered in contributing to intra-articular inflammation in RA (87, 88). This implies that PDK inhibition may be explored as a target to control disease activity in OA and RA.

1.6 Platelet-rich plasma

Counteracting intra-articular inflammation is a key aspect in the development of DMOADs and DMARDs. As DMARDs target systemic inflammation (which commonly translates into intra-articular changes in RA), they are commonly administered orally, subcutaneously, or intra-venously. Novel treatment strategies for OA, which is characterized by a local low-grade inflammation, are typically injected intra-articularly to reach target structures instantly and serve as a local depot.

Limited response to conventional and biologic DMARDs has been well documented for certain patient groups and disease stages (89-91). Similar challenges can be observed for novel intra-

articular treatment strategies for OA, including platelet-rich plasma (PRP) (92). PRP is an autologous blood product, containing a high yield of thrombocytes, leukocytes, growth factors, and cytokines. In OA therapy, PRP is injected intra-articularly to reduce inflammation, modulate local immune responses and thus reduce pain and improve function (93, 94). While recent research has focused on differences in PRP formulations determined by the manufacturer (mainly leukocyte-rich vs. leukocyte-poor PRP) (95, 96) and how they affect clinical outcomes, only little is known about how patients' individual immune profiles impact the composition of PRP and its efficacy.

Despite an increasing understanding of molecular patterns in RA and OA, current treatment strategies have limitations and rarely achieve disease remission. While a significant number of RA patients respond only inadequately to DMARDs, OA can currently only be treated symptomatically and ultimately by joint replacement surgery during late disease stages.

In this thesis we highlight signaling pathways and immune profile compositions that directly affect the development and progression of experimental RA and OA and that may be pharmacologically exploitable as targets in the development of novel disease-modifying agents.

2 Aims, hypotheses, and objectives

Ideally, addressing and modifying disease relevant cells, peptides, and molecules can improve structural damages (the disease), but subsequently also improve pain and function (the illness) in patients suffering from inflammatory and degenerative joint diseases. This work identified targets that collectively modulate articular inflammation, cartilage homeostasis, and bone integrity in experimental models of RA and OA.

To uncover the involvement of *Calca*-derived peptides in experimental RA, the first three studies explored the role of αCGRP (Study 1), CT (Study 2) and PCT (Study 3) signaling in antibody-mediated arthritis by employing three distinct gene inactivation mouse models (Figure 2).

Study	Model	αCGRP signaling	CT signaling	PCT signaling
1	αCGRP $^{-/-}$	✘	✓	✓
2	Calcr$^{-/-}$	✓	✘	✓
3	Calca$^{-/-}$	✘	✘	✘

Figure 2. Overview of gene deficiency models employed in Studies 1-3 and concomitant signaling pathway inactivations. Adapted from (50).

In Study 4, we explored if metabolic changes observed in fibroblast like synoviocytes (FLS) from OA patients may contribute to disease progression and if inhibiting this metabolic shift, may halt OA progression and restore a healthy joint metabolism. Finally, in Study 5 we assessed if individual immune profiles impact immunomodulatory and cartilage protective properties of different PRP products. Table 2 provides an overview of the aims, hypotheses and objectives of the studies included in this thesis.

Table 2. Aims, hypotheses, and objectives of Studies 1–5 included in this thesis.

Study 1	**Proinflammatory and bone protective role of calcitonin gene-related peptide alpha in collagen antibody-induced arthritis**
Aim	To determine the role of αCGRP in experimental RA in an αCGRP-deficient mouse model.
Hypothesis	αCGRP has a pro-inflammatory and cartilage destructive effect in antibody-mediated arthritis, but αCGRP is crucial to protect osseus structures from arthritis-induced bone loss.
Objectives	To evaluate the expression of αCGRP in serum and joints during experimental arthritis. To establish tissue specific gene expressions of αCGRP and βCGRP isoforms during arthritis. To assess arthritic inflammation, cartilage degradation and bone changes clinically, histologically, radiologically, and by gene expression analysis.
Study 2	**The calcitonin receptor protects against bone loss and excessive inflammation in collagen antibody-induced arthritis**
Aim	To understand whether endogenous CT signaling impacts murine antibody-induced arthritis in a model of CT receptor deficiency.
Hypothesis	Endogenous CT signaling has an anti-inflammatory and cartilage- and bone-sparing function in animals exposed to experimental arthritis.
Objectives	To assess serum levels of CT and identify intra-articular structures that express the CT receptor. To explore inflammation cartilage and bone integrity histologically, radiologically and by gene expression analysis. To compare clinical signs of arthritis and grip strength development during experimental RA.
Study 3	**Inactivation of the gene encoding procalcitonin prevents antibody-mediated arthritis**
Aim	To explore the pathophysiological function of PCT in murine antibody-mediated arthritis in a *Calca*-deficiency mouse model.
Hypothesis	PCT plays an independent pro-inflammatory and cartilage- and bone-destructive role in experimental RA.
Objectives	To evaluate PCT serum levels and detect intra-articular expression of PCT. To explore the clinical course of antibody-mediated arthritis during acute and chronic phases of inflammation. To assess cartilage degradation and bone changes histologically, radiologically, and by gene expression analysis.
Study 4	**Metabolic reprogramming of synovial fibroblasts in OA by inhibition of pathologically overexpressed pyruvate dehydrogenase kinases**
Aim	To understand if and how FLS can develop an osteoarthritic phenotype and whether this may be reversable and therefore exploitable as a target to modify OA disease progression.
Hypothesis	A shift between quiescent THY1- FLS and synovitis-driving THY1+ FLS phenotypes promotes OA development and progression. The proliferative THY1+ phenotype may be induced by a local metabolic imbalance which is

	controlled by PDKs. Inhibiting PDK activity may limit pro-inflammatory actions of THY1+ FLS in OA and serve as a pharmacologically exploitable target.
Objectives	To analyze metabolic, proteomic, and functional characteristics of THY1+ FLS from patients with OA. To compare FLS from ligament trauma patients to MSCs and perform mass spectrometry-based shotgun proteomics. To identify and inhibit PDK isoforms as disease driving targets in THY1+ FLS and confirm therapeutic effects by alterations in cytokine secretion.
Study 5	**Individual immune cell and cytokine profiles determine platelet-rich plasma composition**
Aim	To explore whether individual immune cell and cytokine profiles impact PRP compositions and to understand how different PRP products modulate cartilage homeostasis.
Hypothesis	High levels of immune cells and cytokines in whole blood samples can also be found in corresponding PRP products. There is a difference in immune cell and cytokine compositions dependent on the PRP product. Cytokines that are overexpressed in PRP products impact chondrocyte integrity and proliferation.
Objectives	To compare the cellular and cytokine composition of whole blood samples from healthy donors to three different PRP products derived from the same donors. To expose human OA chondrocytes to cytokines increased in specific PRP products and to test for proliferation and chondrogenic differentiation.

3 Results

3.1 Study 1: Calcitonin gene-related peptide alpha in experimental rheumatoid arthritis

Proinflammatory and bone protective role of calcitonin gene-related peptide alpha in collagen antibody-induced arthritis.

Maleitzke T, Hildebrandt A, Weber J, Dietrich T, Appelt J, Jahn D, Zocholl D, Baranowsky A, Duda GN, Tistsilonis T, Keller J

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The vasoactive neuropeptide CGRP was previously shown to modulate vasodilation and neurogenic inflammation in migraine (97, 98). While anti-CGRP antibody therapies have been successful in the treatment and prevention of pain, little is known about the role of αCGRP in inflammatory and degenerative joint diseases.

This study explored the short- and long-term effects of αCGRP in a preclinical model of RA. αCGRP-deficient ($\alpha CGRP^{-/-}$) mice were compared to wild type (WT) animals following induction of collagen II-antibody-induced arthritis (CAIA) (99). Animals were examined clinically, histologically, radiologically, and molecularly on day 10 or 48 following CAIA induction.

Following CAIA induction, an overexpression of αCGRP was noted intra- and periarticularly. βCGRP RNA was expressed in colon tissue, but not in articular tissues during arthritis. While WT CAIA animals exhibited a full arthritic phenotype, $\alpha CGRP^{-/-}$ CAIA mice showed markedly reduced signs of arthritis. Consistently, WT CAIA animals expressed increased intra-articular levels of *Tnfa*, *Il1b*, *Cd80*, and *Mmp13*, which was not the case for $\alpha CGRP^{-/-}$ CAIA mice. Increased bone turnover was also observed in WT CAIA animals, who recovered from

arthritis-induced bone changes. Interestingly, αCGRP$^{-/-}$ CAIA did not recover from arthritic bone destruction and showed relevant bone loss following long-term CAIA.

With this study, we uncovered a dual pro-inflammatory and bone-protective role of αCGRP in experimental RA. While αCGRP protected animals from arthritic bone loss, the neuropeptide increased articular inflammation during CAIA. As endogenous αCGRP was previously mainly discussed in the context of nociception, we were able to outline specific structural effects on cartilage, bone, and the synovium during experimental RA. These findings are especially relevant as anti-CGRP antibodies are increasingly employed in migraine therapy and other indications including peripheral nerve regeneration and Alzheimer's diseases are currently debated (100).

3.2 Study 2: Calcitonin signaling in collagen II-antibody-induced arthritis

The calcitonin receptor protects against bone loss and excessive inflammation in collagen antibody-induced arthritis

Maleitzke T, Hildebrandt A, Dietrich T, Appelt J, Jahn D, Otto E, Zocholl D, Baranowsky A, Duda GN, Tistsilonis T, Keller J

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After establishing a dual pro-inflammatory and bone-protective role of αCGRP in experimental RA (101), we examined the function of endogenous CT signaling in CAIA. While pharmacological CT was previously shown to exert chondro- and bone-protective effects in RA (76, 102), the properties of endogenous CT signaling in inflammatory joint disease are mostly unknown. Similar to αCGRP, CT is encoded by *Calca.* It binds to the CT receptor, which is a 7-pass trans-membrane protein predominantly expressed in kidney and bone (103).

In this study, CAIA was induced in mice deficient for the CT receptor ($Calcr^{-/-}$) and compared to WT animals. Clinical arthritis was assessed daily, and endpoint comparisons included histological, histomorphometrical, radiological, and molecular analyses on day 10 or 48 to study acute and chronic phases of CAIA.

Following CAIA induction, systemic CT was elevated in serum samples and the CT receptor was overexpressed in articular cartilage of WT CAIA mice. While there was no difference in clinical signs of arthritis between genotypes, histological signs of inflammation and cartilage degradation as well as radiological signs of systemic bone loss were markedly increased in CT receptor deficient animals. Consistently, markers for inflammation and immunomodulation (*Sphk1*, *Tgfb1*, *Il1b*, *Ccl2*, *Cd14*, *Cd68*), cartilage (*Mmp13*, *Acan*), and bone turn over (*Acp5*, *Ctsk*) were exclusively overexpressed in $Calcr^{-/-}$ mice.

More stable, potent, and bone specific CT prodrugs were recently introduced (104-106) that have not yet been tested in human RA and OA. However, given that the CT receptor is a g-protein-coupled receptor and therefore a convenient drug target, our data may help to exploit the specific anti-inflammatory and bone-protective properties of endogenous CT signaling present in experimental RA.

3.3 Study 3: Procalcitonin in antibody-mediated arthritis

Inactivation of the Gene Encoding Procalcitonin Prevents Antibody-Mediated Arthritis

Maleitzke T, Dietrich T, Hildebrandt A, Weber J, Appelt J, Jahn D, Otto E, Zocholl D, Shan J, Baranowsky A, Duda GN, Tsitsilonis T, Keller J

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After uncovering a pro-inflammatory and bone-protective role of αCGRP (101) and an anti-inflammatory and bone-protective role of the CT receptor in experimental RA (107), we explored the role of the third *Calca*-derived peptide, PCT, in a preclinical murine model of RA. PCT serves as a serum biomarker for the detection of bacterial infections, including septic arthritis. It is regularly assessed in RA patients to distinguish between rheumatic flares and septic arthritis (78, 79), which has an increased incidence in patients with RA (108, 109).
PCT was previously shown to promote pro-inflammatory cytokine expression in leukocytes (80) and drive bacterial sepsis (81). However, a pathophysiological function has not yet been explored for PCT in RA.

In this work, we studied the function of PCT in experimental arthritis by inhibiting the *Calca* gene as a whole ($Calca^{-/-}$) and comparing mutant animals to WT mice during CAIA. Arthritis was assessed daily using the semi-quantitative arthritis score and grip strength for 10 or 48 days. At these endpoints, acute and chronic arthritis was assessed by histology, immunohistochemistry, histomorphometry, gene expression analysis and and µ-computed tomography (µCT).

Following arthritis induction, PCT was elevated in serum samples and overexpressed in knee joint samples from WT CAIA animals. *Calca*-deficient animals showed no clinical and histological signs of arthritis and grip strength was maintained over time. While *Tnfa* and cartilage turnover markers were elevated in WT CAIA animals, this was not the case in

Calca$^{-/-}$ mice. Interestingly, *Il1b* was exclusively overexpressed in *Calca*-deficient animals. While WT animals exhibited decreased bone surface and increased subchondral bone porosity on day 10, Calca$^{-/-}$ animals were protected from systemic and local bone loss.

Inactivating PCT through the inactivation of *Calca* protected animals from clinical, histological, and molecular signs of experimental arthritis. These data, together with our previous works (101, 107), underline an independent pro-inflammatory role of PCT in preclinical RA.

3.4 Study 4: Pyruvat dehydrogenase kinase in experimental osteoarthritis

Metabolic reprogramming of synovial fibroblasts in osteoarthritis by inhibition of pathologically overexpressed pyruvate dehydrogenase kinases

Damerau A, Kirchner M, Pfeiffenberger M, Ehlers L, Nguyen DHD, Mertins P, Bartek B, **Maleitzke T**, Palmowski Y, Hardt S, Winkler T, Buttgereit F, Gaber T

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After investigating the effects of αCGRP (101), the CT receptor (107) and PCT (110) on inflammation and cartilage and bone metabolism in experimental RA, we aimed to uncover the impact of fibroblast-like synoviocytes (FLS) on arthritis progression. In both RA and OA, these cells have been identified as disease-driving by enhancing synovitis and intra-articular inflammation (111). When exposed to pro-inflammatory stimuli, FLS switch from a quiescent to a proliferative and pathological THY1+ FLS phenotype characterized by enhanced glycolysis and impaired oxidative phosphorylation (OXPHOS) (112, 113).

In this study, we harvested human synovium samples from end-stage knee OA patients, who underwent knee arthroplasty surgery, and analyzed metabolic, proteomic, and functional characteristics of THY1+ FLS. To identify cellular surface markers, FLS were compared to MSCs by flow cytometry. Through metabolic gene expression analysis, we identified pathologically elevated levels of pyruvate dehydrogenase kinase (PDK) 3 in FLS from OA patients, which we then inhibited by dichloroacetate (DCA). Cytokines were measured in cell culture supernatants. Additional analyses included glucose and lactate measurements, immunofluorescence staining, and RNA expression.

Phenotypical similarities were found between FLS from OA patients and MSCs, but FLS showed significantly higher proliferation rates than MSCs. Inhibiting PDK3 caused a metabolic shift from glycolysis to OXPHOS in FLS which reduced their proliferation rate and secretion of

pro-inflammatory cytokines. PDK3 overexpression was further shown to be specific for FLS located in OA-altered synovial tissue. Moreover, while inhibiting PDK reduced the inflammatory response observed in OA FLS, it did not compromise cell viability.

We identified PDK3 as a driver of OA by altering the cellular metabolism of FLS. Inhibiting PDK may therefore serve as a novel treatment strategy to reduce and halt OA disease activity by restoring a healthy FLS phenotype. PDK inhibition was previously proposed as a treatment for malignancies (114-117) and metabolic diseases such as obesity and diabetes (118, 119). OA has been discussed as a metabolic disease (120, 121), where hormonal and metabolic imbalances play a role in disease initiation and progression (122, 123). Therefore, PDK inhibitors represent a new group of OA therapies, that address metabolic pathways and challenge conventional target strategies.

3.5 Study 5: Immune profiles in platelet-rich plasma

Individual immune cell and cytokine profiles determine platelet-rich plasma composition

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Following our works on the contribution of specific proteins (101, 107, 110) and resident cells (124) on RA and OA progression, we explored how the innate and adaptive immune systems may impact novel therapies that aim to reduce local inflammation in OA.

Platelet-rich plasma (PRP) is an autologous biologic product derived from the plasma fraction of whole blood and characterized by an increased concentration of platelets. Platelets store an abundancy of growth factors and cytokines that possess immunomodulatory and tissue-restoring capacities, which makes PRP popular in the treatment of OA and cartilage injury (125, 126).

As PRP is derived from patients' whole blood, the individual immune cell and cytokine composition is likely to impact the final PRP product. In this study, we assessed leukocyte and cytokine patterns in different PRP products and compared them to whole blood samples from donors. In a second step, we identified pro-inflammatory cytokines that were overexpressed in PRP products and analyzed their effect on human OA chondrocytes.

Three different PRP products (ACP®, Angel™, and nSTRIDE® APS) were produced in this study and compared to whole blood samples from twelve healthy donors. Using flow cytometry, we assessed B and T cell compositions, and cytokine expressions were analyzed using the Meso Scale Discovery technique. Following identification of overexpressed pro-inflammatory cytokines in PRP products, human chondrocytes were treated with recombinant IFN-γ and TNF-α, and chondrogenic differentiation and proliferation were assessed.

Elevated leukocyte levels were detected in all PRP products compared to whole blood samples, and pro-inflammatory cytokines IFN-γ and TNF-α were overexpressed in nSTRIDE® APS samples compared to other products and whole blood. The distribution of other cytokines and immune cells was similar in whole blood samples and PRP products. When chondrocytes were treated with IFN-γ, proliferation decreased and when treated with TNF-α, it increased. Both cytokines compromised chondrogenic differentiation and cartilage formation, indicated by decreased proteoglycan contents, and decreased gene expressions of collagen type 1A1 (COL1A1), COL2A1, and aggrecan (ACAN).

This study demonstrated that individual immune cell and cytokine compositions are reflected in PRP products. Pro-inflammatory cytokine concentrations vary between PRP products, which may directly affect chondrocyte health. As pro-inflammatory cytokines have distinct and predominantly negative effects on chondrocytes, these data may explain heterogeneous treatment responses to PRP observed in OA patients. In summary, the patients' systemic immune profile as well as the manufacturing protocol seem to impact the respective PRP product which may thus affect regenerative effects on intra-articular tissues.

4 Discussion

This thesis investigates systemic and local signaling pathways involved in articular inflammation and their relevance to potential pharmacological interventions for RA and OA. Through *in vivo* and *in vitro* studies, we explored the impact of *Calca*-derived peptides (αCGRP (101), CT (107), PCT (110)), PDK3 (124), and individual leukocyte and cytokine compositions (127) on inflammation and cartilage integrity in preclinical models of RA and OA. Our data from Study 3 and 4 showed that PCT and PDK3 are relevant targets in RA and OA pathogenesis, and their inhibition may be effective in reducing inflammation and preserving joint integrity. Further, in Study 2 we identified endogenous CT signaling as joint protective during experimental RA. As the inhibition of αCGRP signaling reduced inflammation but impaired bone integrity in experimental RA in Study 1, αCGRP blockade could result in a complex tissue response that may not only be beneficial to the joint. Last, we demonstrated the impact of individual immune profiles and manufacturing protocols on the composition of different PRP products and chondrocyte health in Study 5.

4.1 Challenges in disease modifying drug development for rheumatoid arthritis and osteoarthritis

In joints, cartilage, subchondral bone, synovial tissue and fluid, ligaments, tendons, and neurovascular structures form a complex functional unit. If joints are exposed to pro-inflammatory stimuli or trauma, this commonly results in transient or chronic and progressive arthritis. Whether the initial trigger is systemic (in the case of RA) or local (in the case of OA), a complex activation of immune cells, cytokines, and signaling molecules disturbs the otherwise healthy joint metabolism.

Targeting and inhibiting signaling pathways linked to intra-articular inflammation has revolutionized treatment algorithms for patients with RA (128-130). Following the success of infliximab, a chimeric anti-TNF antibody, other antibody- and fusion protein-based therapies followed. These include rituximab (anti-CD20 antibody), tocilizumab (anti-IL6 receptor

antibody), and abatacept (T cell inhibition). While biologic DMARDs are effective in limiting pain and radiographic disease progression, a significant amount of patients show only limited treatment response (34, 35), and the increased risk of systemic and local infections may compromise compliance and cause premature treatment termination (131). To treat patients that do not respond adequately to available DMARDs, new monoclonal antibodies that address familiar targets but by modified modes of action such as the IL-6 antibody olokizumab, have recently been developed and clinically introduced (130, 132).

Current treatment strategies to reduce pain and improve function in OA comprise exercise, weight loss, topical and oral NSAIDs, and intra-articular GC injections (133). If structural damages are present and patients do not, or inadequately, respond to conventional treatment, tibial or femoral osteotomy, or joint replacement surgery may be considered (134). However, none of the conventional interventions can halt or reverse disease progression, and joint replacement surgery can only replace, not restore, joint integrity.

Data show that patients who are recommended non-surgical treatment for knee OA by their orthopaedic surgeon do generally not adhere to international recommendations due to a lack of standardization and education (135). Recently, guideline-based patient education and exercise therapy programs like Good Life with osteoArthritis in Denmark (GLA:D®) which include supervised neuromuscular exercises have been implemented nationwide in selected countries (Denmark, Canada, Australia). Adherence to GLA:D® has shown to improve function, reduce painkiller intake and sick leave, while being cost-effective (136-139). While programs like GLA:D® significantly increase the quality of conventional treatment for OA, pharmacological interventions that can reduce existing structural damages and thereby improve function are still missing. Although mechanistically plausible, biologic DMARDs used in RA therapy have not been successful in the treatment for OA (140), and no market-approved disease-modifying therapy exists for OA to date (38).

Methodological recommendations for how to best conduct clinical trials for knee OA have been published (39, 141). Still, various trial designs do not fully encompass the complexity of OA, probably explaining in part why phase III studies for DMOADs fail to meet their primary

endpoints. First, OA is a heterogenous disease entity with multiple proposed but not yet defined pheno- and endotypes (142, 143). Thus, some DMOADs may only work in certain OA phenotypes (f.e. inflammatory, metabolic, post-traumatic, etc.). Second, clinical trials employing biological therapies have so far focused on end-stage OA. These therapies are however probably unable to repair advanced structural alterations that cause pain mechanically and should be applied to early-stage OA patients. Third, it is still being debated whether treatment success should be defined by changes in structural parameters that characterize *the disease* OA, or by improved functional and pain parameters that characterize *the illness* OA – or a combination of both. Until consensus on surrogate markers for OA has been reached by scientists and regulators, trial designs and primary outcomes will vary between studies, which makes market approval of potential DMOADs complex and challenging (144).

4.2 The two-sided consequence of inhibiting calcitonin gene-related peptide

Although primarily researched in pain perception and modulation, αCGRP has been shown to be indispensable for bone formation (49, 145-147) and fracture healing (51), potentially in part due to αCGRP-regulated osteogenic differentiation of bone marrow-derived MSCs (148, 149). As CGRP also regulates vasodilation and neurogenic inflammation (55), we suspected an independent pathophysiological role of the neuropeptide in experimental arthritis beyond pain perception. By employing a murine αCGRP-deficiency model, we uncovered a dual pro-inflammatory and bone-protective role of αCGRP in antibody-mediated arthritis in Study 1 (101).

Interestingly, CGRP is also elevated in serum samples of patients following fractures (150, 151) and in synovial fluid and synovial membrane samples of patients with inflammatory and degenerative joint pathologies including RA and OA (59, 61, 64). Although an intra-articular overexpression of CGRP may in part be a result of pain (152), independent pro-inflammatory and catabolic effects on chondrocytes have been described in preclinical models of OA and RA (153-156). While the neutralization of CGRP had previously been shown to significantly

reduce pain in experimental OA (157), this effect could not be replicated in a randomized placebo- and celecoxib-controlled clinical trial (158).

In Study 1, we detected an independent pro-inflammatory role of αCGRP in experimental RA but also identified the neuropeptide as essential in maintaining bone integrity (101), which has previously also been shown for non-RA models (49, 51, 145-147). This dual role of αCGRP adds to the understanding of the pathophysiological function of this peptide in RA but makes it also difficult to exploit as a treatment target. However, as our findings are based on gene inactivation, which causes a lifelong deficiency of target proteins, follow-up studies will have to examine the effects of pharmacological αCGRP inhibition on RA, which is usually temporal. Further, CGRP seems to exert tissue-specific pro-inflammatory (55, 56, 101, 159) and anti-inflammatory effects (159-162), which prohibits a clear pro- or anti-inflammatory classification of the peptide. This dilemma was further underlined by the fact that some migraine patients receiving CGRP antibody therapy experienced inflammatory complications including autoimmune hepatitis, psoriasis arthritis, and urticarial eczema in a recent case series (163). As musculoskeletal and immunological side effects have not yet been in the focus of previous migraine studies (164), assessing bone and cartilage integrity in patient cohorts who have received anti-CGRP treatment will be detrimental in exploring the therapeutic potential for RA or OA.

4.3 New ways to exploit calcitonin signaling

CT has been shown to have anti-inflammatory (104, 165-169), anti-oxidative (71, 72), pain alleviating (69, 70, 73), and cartilage- and bone-protective (170-173) properties in preclinical models of OA and RA.

Similar to the results observed in clinical trials studying osteoporosis (65, 174), CT treatment produced ambiguous results in OA. On the one hand, CT improved pain and quality of life in women suffering from a combination of osteoporosis and OA (175) and reduced cartilage and bone resorption biomarkers in patients with OA (176). On the other hand, radiological and

functional improvements were not observed in two phase III studies 24 months following treatment (177).

In the treatment of human RA, the combination of CT and alendronate reduced bone turnover (102, 178) and improved bone density (76, 179), but market approval was never granted.

CT mainly binds to the CT receptor, which is primarily expressed in the central nervous system, the kidney, and on osteoclasts, where it halts bone resorption. This effect on osteoclasts has mainly been reported for teleost CT, which has a 50-fold higher potency than mammalian CT (180, 181). While pharmacological CT treatment has been studied extensively, little is known about the role of endogenous CT signaling in bone and cartilage.

To understand the role of endogenous CT signaling in experimental RA, CT receptor-deficient animals were exposed to arthritis and compared to WT animals in Study 2. We displayed an anti-resorptive and bone protective as well as anti-inflammatory effect of the CT receptor in antibody-mediated arthritis (107). While these effects were previously also described for pharmacological CT, we confirmed that intact endogenous CT signaling is essential to control inflammation and impede bone and cartilage loss in experimental RA.

The downsides of teleost CT treatment include the route of delivery (mainly nasal or subcutaneous), frequency of application (daily for osteoporosis), and reports of slightly increased malignancy rates (182, 183). To overcome some of these limitations for CT treatment, new formulations promising fewer side effects have been explored in recent years (104-106).

The anti-resorptive properties of CT may be most effective if the drug is tissue-specific and applied locally. Followingly, as an alternative to subcutaneous or nasal CT formulations, intra-articular formulations have been developed. As the clearance from the joint is relatively fast (5 hours), hyaluronic acid-conjugated forms of CT have been shown to increase the local bioavailability by delaying the passage into the blood stream while preserving the anti-catabolic properties of the drug (105). These findings were supported by data which showed that bisphosphonate-conjugated CT increased bone volume in rats suffering from osteoporosis or adjuvant-induced arthritis, while the unconjugated formulation showed no

such improvements (104). More recently, a novel phosphorylated human CT formulation has been developed. By adding a phosphate group, CT was protected from fibrillation which makes it significantly more stable (106).

Lastly, as an alternative to teleost CT, synthetic CT receptor agonists have been tested in preclinical models of RA (165) and OA (69), where they effectively reduced pain and improved cartilage quality.

Altogether, CT signaling is currently revisited as a relevant treatment target for OA and RA and endogenous CT signaling may be central to protect cartilage and reduce intra-articular inflammation (107). Clinical trials testing new CT formulations will likely follow with the aim to reproduce preclinical data.

4.4 Procalcitonin – more than a sepsis biomarker

In Study 3, we showed that PCT independently maintains inflammation during experimental arthritis (110). PCT has been established as a serum sepsis marker for the detection of bacterial infections and monitoring of antibiotic treatment (184-186). As RA patients have a 4–15-fold increased risk of septic arthritis (187), PCT is especially useful to distinguish between septic arthritis and RA flares (78, 79, 188, 189).

Interestingly, moderately elevated serum PCT levels were also found in RA patients without septic arthritis or other bacterial infections (190, 191) and a combination of elevated PCT and decreased CT levels was recently discovered in patients with early RA compared to healthy controls. Adding PCT and CT to traditional serum biomarker panels for RA could help to increase diagnostic sensitivity, which is known to be limited, while specificity is generally high (191).

PCT was previously shown to modulate immune responses. It is capable of inhibiting macrophage migration and early osteoclastogenesis (50) and of enhancing pro-inflammatory cytokine expression in innate immune cells (81). Based on our results in experimental RA, PCT also impairs articular cartilage and bone integrity during systemic inflammation (110).

Together, this suggests that PCT could serve as an exploitable therapeutic target in inflammatory diseases. In support of this, PCT-neutralizing antibody therapy has been shown to effectively reduce sepsis and mortality in rodents (192), but anti-PCT antibodies have not been tested clinically yet.

PCT binds to the CGRP receptor (81), and inhibiting PCT signaling can therefore also be achieved by anti-CGRP receptor antibody treatment, which has been market-approved for migraine treatment (f.e. olcegepant). When septic mice were treated with olcegepant, survival increased (81). Interestingly, intra-venous administration of PCT did not affect healthy hamsters, but doubled the mortality in septic animals, and, oppositely, prophylactic and therapeutic administration of goat PCT antiserum improved survival following sepsis induction (193).

Inhibiting dipeptidyl-peptidase 4 (DDP4) has been proposed as an alternative way to abrogate PCT signaling (194). DDP4 is an enzyme which coverts full-length 116–amino-acid PCT to N-terminally truncated 114–amino-acid PCT, the predominant form in serum. DDP4 inhibitors (also known as gliptins) have commonly been used to treat type II diabetes mellitus but have also been shown to reduce sepsis mortality in rodents (195-197). In OA, DDP4 has been identified as a marker of senescent chondrocytes, and DDP4 expression in synovial fluid has been associated with OA progression, cartilage degradation, and inflammation (198), and DDP4 inhibition ameliorated OA progression *in vivo* (199). The FDA issued a warning for DDP4 inhibitors following several cases of arthralgia and arthritis in patients with type II diabetes mellitus who received sitagliptin, saxagliptin, linagliptin, or alogliptin (200-202). However, larger cohort studies have later shown that DDP4 inhibitors were not associated with the development of RA (203) and potentially even decreased the risk of autoimmune disease and RA development (204).

Antibodies inhibiting the CGRP receptor, and thereby CGRP and PCT signaling, are already approved for the treatment and prevention of migraine. Using these drugs to treat RA and OA could be achieved via drug repurposing which includes lower development costs and shorter development timelines for regulatory approval (205, 206).

4.5 Osteoarthritis as a metabolic disease

In Study 4, we showed that PDK3 inhibition restored the altered cell metabolism present in FLS from OA patients. Inhibition of PDK3 reduced the secretion of pro-inflammatory cytokines IL6, IL8, TNF-α, GM-CSF, and CC-chemokine ligand 2 (CCL2) and the pathological proliferation of FLS (124). Congruently, growing evidence supports a central metabolic component in the pathophysiology of inflammatory and degenerative joint diseases. Circulating adipokines and lipid and glucose imbalances have been shown to chronically compromise cartilage integrity (207), which may explain how OA and RA are linked to type II diabetes and hypertension, independent of obesity (120, 121).

Another central link between metabolic diseases and OA is low-grade inflammation which is characterized by increased radical oxygen species and accumulating advanced glycation endproducts (AEGs), which drive tissue damage and disease progression in type II diabetes and OA (208-210). This may explain why drugs primarily developed for metabolic conditions, including DDP4 inhibitors (198, 199), glucagon-like peptide (GLP) 1 agonists (211-213), and PDK inhibitors (87, 124, 214) have been effective in the treatment of experimental OA and RA. Among others, the anti-inflammatory, anti-oxidative, and autophagy-regulating actions of metformin have been made responsible for reduced rates of knee replacement surgery and a decreased release of pro-inflammatory cytokines in type II diabetes patients with OA treated with metformin (215). DDP4 inhibitors, GLP1 agonists, and PDK inhibitors have not yet been tested in randomized clinical trials. To confirm previously reported joint preserving effects of these agents, they must be employed in controlled clinical settings.

4.6 Platelet-rich plasma as a highly individualized therapy

Previous reports on predictors for PRP treatment response in knee OA are inconsistent. Some suggest that high BMI (216) and OA severity (216, 217) compromise treatment success, while others could not confirm this (218, 219). One study found that PRP treatment efficacy is not

affected by age, sex, body weight, or platelet counts (217), while another study showed that only PRP from young donors, but not aged donors, had an anabolic and regenerative effect on chondrocytes and intra-articular cartilage (220). As both the innate (221) and adaptive (222) immune system drive OA development, the inconsistent reports on treatment success may be explained by inter-individual differences in immune profiles which affect treatment response, but were previously not assessed.

In Study 5, we showed that individual adaptive immune profiles, including leukocytes and cytokines with pro-inflammatory properties, observed in whole blood samples are maintained in PRP products (127). As sex (223), age (224), and disease stage in both OA (223) and RA (225) influence cytokine expressions in serum, this may explain, at least in part, the heterogenous treatment responses to PRP in OA patients. According to our results, pre-treatment screening of serum leukocytes and cytokines predict PRP composition, which seems to directly influence the effect of PRP on intra-articular structures and, in particular, cartilage (127). Thus, pre-treatment screening may be employed as a tool to predict individual treatment response to PRP in OA. We also showed that increased concentrations of pro-inflammatory cytokines IFN-γ and TNF-α were found in some but not all examined PRP products. Chondrocyte proliferation was impaired by IFN-γ and enhanced by TNF-α exposure, while proteoglycan content and cartilage turnover markers were reduced following exposure to both cytokines (127).

In accordance with our findings, increased levels of TNF-α and IFN-γ in synovial fluid were previously shown to be associated with OA progression and knee OA-related pain (226, 227). This highlights the relevance of choosing the appropriate PRP protocol and supports pre-treatment immune profile screening. Such individual immune screening is already in use for cancer patients to adjust immunotherapy and provide individualized treatment plans (228, 229).

In summary, patients receiving autologous therapies like PRP for OA may in the future undergo similar immune screenings to allow predictions about treatment success and the best suited manufacturing protocol.

5 Conclusion and outlook

Joint homeostasis is maintained by loading, synovial lubrication, and a plethora of resident and migrating immune cells, cytokines, and signaling molecules. If this balance is disrupted by either local or systemic factors, the body responds with articular inflammation. Persisting inflammation, like in the case of RA and OA, causes progressive joint destruction which often results in debilitating pain and reduced quality of life. This thesis outlined cellular and molecular pathways relevant in inflammatory and degenerative joint pathologies that contribute to disease progression, and which may be exploitable in the development of future therapeutics. *Calca*-derived peptides and PDKs play pivotal roles in arthritic inflammation and bone metabolism, which makes them potential targets for RA and OA therapy. To optimize treatment responses to PRP in OA patients, pre-treatment immune profile screenings may help to better identify suitable patients and products.

As relevant signaling pathways are being continuously uncovered, a more comprehensive understanding of RA and OA pathology is developing today. To allow development of more personalized treatments, current research efforts are focused on sub-classifying OA into different pheno- and endotypes (122, 123, 142, 143, 230, 231). One way of pheno- and endotyping is based on molecular serum biomarkers that are predominantly present in specific patient groups. Screening patients for these biomarkers prior to treatment may allow adjustment of treatment regimens early on. Currently, “inflammatory”, “catabolic/low repair”, and “metabolic” patterns are discussed as relevant molecular endotypes (142).

Although no DMOAD has obtained market approval yet, several intra-articular agents are now in advanced stages of clinical testing. Treatment with sprifermin, a recombinant human fibroblast growth factor 18, demonstrated long-term (5 years) structural and potential clinical benefits for knee OA (232-235). Lorecivivint, a Wnt pathway inhibitor, has also shown improvement in joint space width and pain reduction in a phase II clinical trial for knee OA (236, 237). For both agents, phase III results are currently awaited. Parallel to this, gene therapies based on adeno-virus vectors are being tested in pre-clinical and clinical trials.

These include vectors encoding the IL1 receptor antagonist (238-240) and plasmid DNA that carries the IL10 transgene (241, 242).

While pharmacological advances are central to finding disease modifying treatments in the years to come, advances in digital technology will be equally important to optimize diagnostic and therapeutic approaches. The RETRO study group has recently developed machine learning algorithms that can reliably predict RA flares following DMARD tapering during disease remission (243), and deep artificial neural networks can now detect arthritic changes on conventional radiographs (244). Moreover, virtual clinical trials, also known as *in silico* trials, are becoming relevant for regulators in preparation for actual clinical trials in RA and OA research (245, 246). Especially computer simulations that identify existing FDA-approved drugs as effective treatments of inflammatory and degenerative joint diseases (drug repurposing) are helpful in finding potential cures for RA and OA (247).

The overall success rate of drugs receiving market approval is 10–20% once applied in clinical trials. However, drug approval success rates are markedly decreased if the target or mechanism of action is unknown (248). This underlines the utmost importance of researching and identifying signaling pathways that are relevant targets in future RA and OA diagnostics and therapies.

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7 Appendix

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7.2 Eidesstattliche Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass - weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde, - die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden, - mir die geltende Habilitationsordnung bekannt ist. Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Datum Dr. med. Tazio David Maleitzke