Medical University of Vienna

Department of Internal Medicine III

Division of Rheumatology

Elucidating the role of T cells in the murine modified OVA-induced Arthritis (mOIA) model

Bacherlorarbeit / Bachelor's Thesis

University of Veterinary Medicine Vienna

verfasst von / submitted by

Lina Breit

Vienna, July 2022

External supervision:

Priv.-Doz. Dr.med.univ. Michael Bonelli

Teresa Preglej, PhD

Internal supervision:

Ass. Prof. Dr.med.vet. Martina Patzl

Reviewer:

Kerstin Mair, M.rer.nat. PhD.

Acknowledgement

Writing this thesis would not have been possible without the support of my colleagues, friends and family. First, I want to thank Teresa Preglej, for the best supervision I could have asked for. Not only was the time working together a lot of fun, but I was able to learn a lot from Teresa. Thank you for making this such a great experience. Next, I want to thank my Principal Investigator Michael Bonelli for the time he invested and his support along the writing process. Also, for giving me the opportunity to work on this project with all the amazing people in the department of Rheumatology. Thank you to all the lab technicians, who supported me with my work and helped me with their expertise. Further, I am very grateful for my colleagues Marie Brinkmann, Maximilian Kugler and Mirjam Dellinger, including Teresa Preglej, who not only supported me on a professional level but also became my friends.

Last but not least I want to thank my family and friends who were always there for me throughout this journey.

Table of Contents

1 Introduction

1.1 Rheumatoid arthritis

Rheumatoid arthritis (RA) is a common chronic autoimmune disease, causing systemic and local inflammation, which leads to irreversible destruction of the joints. Studies show that 0.5-1.0 % of the Western population is affected by RA (Smolen et al., 2018). Genetics, epigenetics, sex and environmental factors are well known risk factors in RA (Aletaha & Smolen, 2018). Since genetic and epigenetic predispositions increase the risk of RA, early diagnosis is essential for optimal treatment (**Figure 1**). However, RA is a very heterogenous disease which for example differs in clinical presentations and response to therapy (Guo et al., 2018). RA can be characterized by synovial inflammation, swelling, autoantibody production (rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA)) as well as destruction of cartilage and bone (Smolen, 2020). Poorly treated or patients under severe disease courses can develop keratitis, pulmonary granulomas and other non-specific extra-articular symptoms (Guo et al., 2018). Taking a closer look into the pathogenesis of RA, cytokines such as tumor necrosis factor α (TNFα) and interferon γ (IFNγ) are important for the development of inflammation and bone destruction (Kollias et al., 1999; Smeltz et al., 2002). Although crucial insights into the disease were gained over the last two decades, the pathogenic pathways as well as the role of T cells still remain unclear (Smolen, 2020).

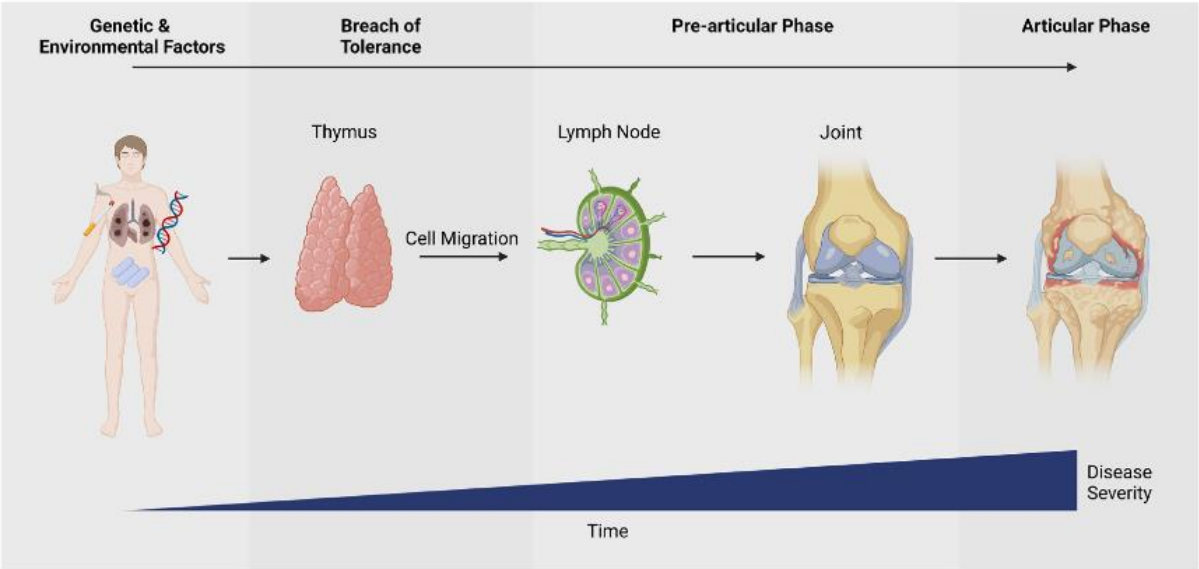

Figure 1. Disease progression of rheumatoid arthritis (Meehan et al., 2021).

1.2 Cytokines in rheumatoid arthritis

Cytokines are humoral mediators adapting the immune response and play an important role in a broad range of inflammatory processes. They can be classified into pro- and anti-

inflammatory factors, which describes the respective effect on immune cells and the surrounding tissue.

TNFα, for example, is a pro-inflammatory cytokine and plays a major role in RA disease progression. It is produced by macrophages, monocytes, fibroblast-like synoviocytes (FLS), B and T cells as well as natural killer (NK) cells (McInnes & Schett, 2007).

Transforming growth factor β (TGFβ) has multiple functions and can for example promote or inhibit T cell activation, differentiation and proliferation (Gonzalo-Gil & Galindo-Izquierdo, 2014). Moreover, this anti-inflammatory cytokine plays a role in differentiation of T_H17 cells, and thereby also stimulates osteoclastogenesis. However, it can also decrease NK cell functions (McInnes & Schett, 2007).

Interleukin (IL) 17 mainly shows pro-inflammatory functions and is primarily produced by a specific T cell subset and thus considered the signature cytokine for T_H17 cells (Lubberts, 2010; Merola et al., 2018; van den Berg & Miossec, 2009). In RA, IL17 can enhance osteoclastogenesis and promote the survival of FLS, resulting in synovial hyperplasia (Benedetti & Miossec, 2014). IL17 blockage in animal models showed promising results supressing inflammation and joint erosion in RA (van den Berg & Miossec, 2009). However, clinical trials did not achieve expected therapeutic efficiency (Merola et al., 2018).

IL6 is produced by monocytes and neutrophils and exerts a pro-inflammatory function by activating innate and adaptive immune pathways. It induces release of acute phase proteins which is a clinical parameter for RA disease activity (Aletaha & Smolen, 2018; McInnes & Schett, 2007). Moreover, IL6 is involved in T cell proliferation, differentiation and cytotoxicity (McInnes & Schett, 2007).

Although IL4 is a known driver of T_H2-mediated disease, it can act as an anti-inflammatory cytokine inhibiting osteoclastogenesis and preventing degradation of cartilage and bone erosions (McInnes & Schett, 2007).

To the contrary, members like Type II interferon (IFNγ) can initiate T_H1 differentiation and promote inflammation in RA (McInnes & Schett, 2007). Furthermore studies show that IFNγ is associated with RANKL expression, a key factor for osteoclastogenesis (Aletaha & Smolen, 2018). Maintenance of pro and anti-inflammatory cytokines is key for immune homeostasis and a disruption can promote autoimmunity, inflammation and joint destruction in RA patients (McInnes & Schett, 2007).

1.3 Cellular and molecular drivers of synovitis and joint destruction

Overtime many cell types have been discovered to be important for the development of inflammation. Cluster of differentiation 4 $(CD4)^+$ T cells for example have been described as important key players for RA. They are activated by antigen presenting cells, evolve to T helper (T_H) or regulatory T cells (T_{reg}) and can further differentiate into T cell subsets (described in 0) which stimulate macrophages and B cells. B cells can differentiate to autoantigen-producing plasma cells which promote the formation of immune complexes, which can trigger local inflammation by activation of macrophages, which are producers of pro-inflammatory cytokines such as TNFα or IL6.

T cells are able to activate macrophages by cell-to-cell interaction or releasing cytokines such as IL17 or IFNγ. This pro-inflammatory environment leads to activation of FLS which can produce receptor activator of nuclear factor κB ligand (RANKL). RANKL promotes the development of osteoclasts from monocytes which are bone degrading cells and therefore important contributors to bone erosions (**Figure 2**) (Aletaha & Smolen, 2018; Puchner et al., 2018; Strand et al., 2007). The complex interactions between T cells, fibroblasts mediating the development of osteoclasts is shown in **Figure 3**.

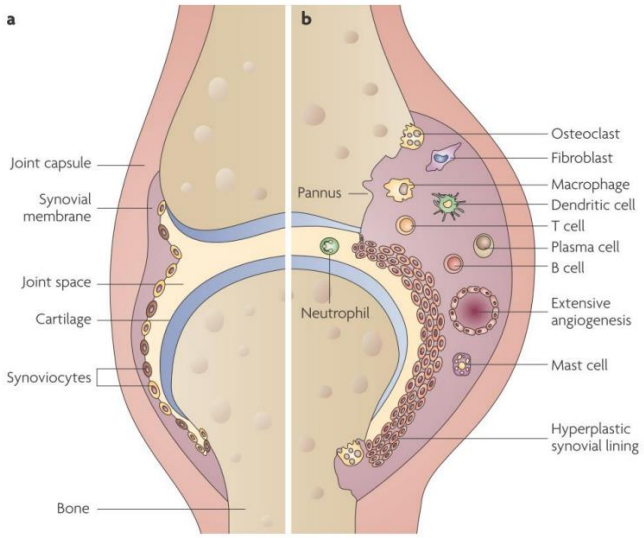

Figure 2. The joint in a healthy state (a) compared to rheumathoid arthritis (b).
In the healthy joint (a), the synovial membrane consists of a thin layer of synoviocytes and an intact periosteum (outer layer of the bone). In a rheumatoid arthritis joint (b), the synovial membrane is hyperplastic and along with the joint space infiltrated by different immune cells. Here the periosteum is destructed by osteoclasts (Strand et al., 2007).

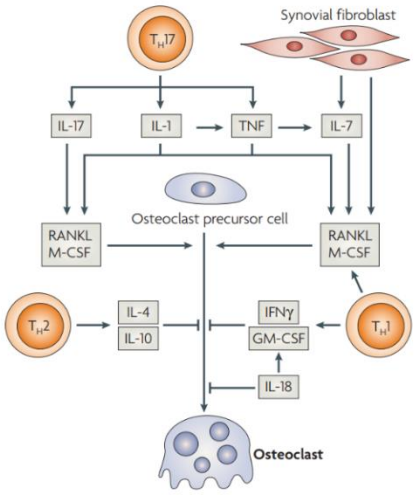

Figure 3. A systematic overview of involved cells in osteoclastogenesis.
Receptor activator of nuclear factor κB ligand (RANKL) as one of the essential cytokine mediators, which is expressed by synovial fibroblasts and T helper 1 (T_H1) cells. Osteoclast differentiation is driven by tumor necrosis factor (TNF), interleukin-1 (IL-1) and -17 (IL-17) proliferated by T_H17 cells. In contrast, IL-4, IL-10 as well as interferon γ (IFNγ) inhibit osteoclast differentiation (McInnes & Schett, 2007).

1.4 $CD4^+$ T cells in rheumatoid arthritis

During the development of an inflammation T cells, monocytes and B cells play a major role. B cells interact with T cells, dendritic cells and FLS and produce autoreactive antibodies such as RF and ACAP which contribute to the disease. Moreover, they release cytokines which drive cellular activation and synovitis (Edwards et al., 2004). Besides B cells, T cells play a central function in RA pathogenesis (Chemin et al., 2019).

T cells develop in the thymus, then migrate into the periphery where they exert their effector functions. They can be classified into two subclasses depending on the expression of their surface marker CD4 or CD8. $CD4^+$ cells are defined as T helper cells whereas $CD8^+$ cells are defined as cytotoxic T cells. The T cell receptor (TCR) can recognize antigen when presented on major histocompatibility complex (MHC), thus mediating cell interaction with other leukocytes. MHC can also be classified into two subtypes named class I and class II. MHC class I is expressed on all nucleated cells allowing intracellular antigen presentation to $CD8^+$ T cells. Throughout this process $CD8^+$ T cells are able to exert their primary function which is destruction of infected cells by releasing cytotoxic granula. MHC class II is expressed on antigen presenting cells like dendritic cells, macrophages and B cells. These cell types can process extracellular antigens presenting them on MHC class II. The TCR of $CD4^+$ T cells can specifically recognize the cognate antigen bound to the MHC class II and therefore induces activation. Some of the activated T cells differentiate towards immunosuppressive regulatory T cells (Tregs) upon the presence of TGFβ and absence of IL6. However, most of the T cells exert their function as T helper (T_H) cells. They can be subdivided into T_H1, T_H2 or T_H17 depending on their cytokine production. Each subset is characterized by lineage defining transcription factors and specific cytokine patterns. T_H1 cells are characterized by their signature cytokine IFNγ, T_H2 cells by IL4, IL5 or IL13 and for T_H17 cells by IL17 and IL22 production (Murphy & Weaver, 2017).

Further classifications depend on the respectively expressed cluster of differentiation (CD). These surface markers contribute to the specific response of T cells (Cibrián & Sánchez-Madrid, 2017). For instance, naïve T cells express surface markers, such as CD62L but lack CD25 and CD44 expression (Eagar & Miller, 2019). Further, activated T cells with surface expression of CD44 are linked to memory T cells (Murphy & Weaver, 2017).

Today we know that immune regulatory factors play a key role in the pathogenesis of RA (MacGregor et al., 2000). In particular, the human leukocyte antigen (HLA)-D related locus that forms the major histocompatibility complex (MHC) has been identified as an sever risk factor (McInnes & Schett, 2007). Moreover studies suggest that the formation of immune complexes

during infection triggers the production of RF (Wegner et al., 2010). In autoimmune diseases, as it is in RA, an infiltration of $CD4^+$ T cells at the site of inflammation is common. These findings show the high potential of T cells as a target for therapy (Chemin et al., 2019).

1.5 Animal models

Animal models have shown promising results by elucidating novel therapeutic strategies to supress inflammation and joint erosion in RA (van den Berg & Miossec, 2009). These require a specific, susceptible strains of mice to induce autoimmunity. Numerous models have been described in the field of RA such as collagen-induced arthritis (CIA), proteoglycan (PG)-induced arthritis (PgIA) or antigen-induced arthritis (AIA) (Pan et al., 2004). CIA and PgIA models are induced by an autoantigen and adjuvant which creates an environment for immunogenicity (Tong et al., 2018). In further models RA is caused by inducing serum-transfer arthritis (STA) or transfer of $CD4^+$ T cells and further immunization with a specific endogenous peptide, such as ovalbumin leading to (OVA)-induced arthritis (OIA) (Maffia et al., 2004).

1.5.1 Collagen-induced arthritis

In CIA an emulsion of complete Freund's adjuvant (CFA) and type II collagen (CII) is injected into the tail of RA susceptible mouse strains. The development of anti-CII autoantibody along with a specific B cell and T cell response can be observed in this model (Brand et al., 2007; Holmdahl et al., 1986). This and other parallels to the immunopathogenesis of RA such as synovial hyperplasia, mononuclear cell infiltration and cartilage degradation occur in this model. The CIA is a T_H17 driven disease model which is used to investigate therapies that target TNFα, a major driver of inflammation in RA (Brand et al., 2007; Schurgers et al., 2011). However, effects of IFNγ are deviating in the CIA model. Considering the fact that those mechanisms which are driving the disease are not necessarily equal to those occurring in RA patients a breach of auto-immunity is aspired (Meehan et al., 2021).

1.5.2 K/BxN serum-transfer arthritis

In this model, arthritis is induced by transferring serum or the purified specific disease inducing immunoglobulins (IgG) of immunized K/BxN mice (Kouskoff et al., 1996). The transfer of serum or IgGs from arthritic K/BxN mice into different mouse strains such as BALB/c or C57BL/6 as well as T and B cell-deficient mice leads to a reproducible course of arthritis (Ji et al., 2001; Korganow et al., 1999). Arthritis is driven by activation of T cells that recognize the self-antigen glucose-6-phostphate isomerase (G6PI) leading to the aspired break of tolerance as mentioned before (Matsumoto et al., 1999). Although G6PI is not essential in progression of RA, the K/BxN STA model is relevant to gain better understanding of general mechanisms of autoantibodies during the development of arthritis (Christensen et al., 2016).

1.5.3 OVA-induced arthritis

Proinflammatory cytokines such as IL1 or TNFα, chemokines, and growth factors are characteristic appearances in synovitis. The dysregulation of cytokines is assumed to contribute to the pathogenesis of RA (Feldmann & Maini, 2001). Further T cell-mediated autoimmune responses play a crucial role in the pathogenesis of RA. This was shown in SCID mice where T cells from RA patients caused inflammatory arthritis (Mima et al., 1995). Within this thesis, the aim is to characterize disease-driving T cells within a murine model of OVA-induced arthritis (OIA).

In OIA the essential antigen is not an autoantigen but the cognate antigen ovalbumin. For immunisation OVA is intra-articular injected into RAG1 KO mice carrying antigen-specific T cells. This leads to the activation, differentiation and thus migration of antigen-specific T cells to the OVA-injected joints which further leads to the development of activated T cells (Maffia et al., 2004). In the OIA model high INFγ production can be observed suggesting a T_H1 driven course of disease. The described role of T_H1 cells as an upstream regulator mediating pathogenic mechanisms puts these cells into the spotlight of research (CM et al., 2008). This among other reasons makes the OIA an attractive model for studying immunological mechanisms and therapeutic responses.

1.6 Hypothesis

Despite enormous efforts to develop new therapeutic strategies for the treatment of rheumatoid arthritis (RA), a large number of non-responding patients to available drugs still urges the need to identify new treatment targets. Several leukocyte subsets drive inflammation in the joints of RA patients resulting in joint damage. In this context, T-helper cells are major contributors to the chronic autoimmune responses in RA patients. However, T cell-targeted therapies are limited so far. Within this thesis, we aim to characterize disease-driving T cells within a murine model of OVA-induced arthritis (OIA). In the OIA, activated T cells are enriched in the synovial cavity of the knee and - supported by other pathogenic immune cell subsets - drive inflammation and bone erosion. Although histological stainings of knees show the influx of immune cells into the synovial cavity, the characteristics of the resident T cells are still illusive. The analysis of this pathogenic T cells, will help to better understand the role of T cells in the development and progression of rheumatoid arthritis.

2 Material and Methods

2.1 Prepared solution

2.1.1 Lysate buffer

Lysate buffer for DNA-extraction consisted of 1 part Proteinase K (20 g/L) and 9 parts Lysate buffer without Proteinase K with 100 mM Tris-HCl pH 8.5, 5 mM EDTA pH 7.5-8, 0.2 % SDS, 200 mM NaCl.

2.1.2 Cell culture complete medium

RPMI medium (Thermo Fisher Scientific, Massachusetts, USA) used for isolated mouse cells contained 10 % fetal bovine serum (FBS) (Sigma-Aldrich, Missouri, USA), 1 % PenStrep (Thermo Fisher Scientific, Massachusetts, USA), 1 % 2 mM L-Glutamax (Sigma-Aldrich, Missouri, USA) and 50 µM β-Mercaptoethanol (Sigma-Aldrich, Missouri, USA).

2.2 Cell counting

To count cells Z Series Coulter Counter (Beckman Coulter, California, USA) was used. Therefore 20 µL of single cell suspension from isolated spleen and knee (2.6, 2.7) was diluted in 10 mL of counting buffer. Murine cells were counted at a 5 – 15 µm range.

2.3 Polymerase Chain Reaction (PCR) for genotyping

2.3.1 DNA extraction

Mice of interest were clipped, then the tissue was lysed using 100 µL Lysate buffer (2.1.1) in a 1.5 mL Reaction tube and put in the Thermomixer (Thermomixer comfort, Eppendorf, Hamburg) at 37 °C, 850 rpm for 1 h 30 min. The reaction was stopped by heating the sample for further 30 min at 90 °C mixing at 1400 rpm. Afterwards, the extracts were put on ice for 3 min and centrifuged at 400 g for 3 min at 4 °C. Then 60 µL of the supernatant were mixed with 500 µL deionized water (dH_2O) (Aqua ad iniectabilia, B.Braun, Melsungen) and stored at 4 °C.

2.3.2 OTII transgene PCR

To perform the PCR detecting the OTII transgene, first a master mix was prepared containing 10x Buffer -MgCl2, MgCl2 (50mM), dNTPS (2mM), Taq-Polymerase (5U/µL), dH_2O and the following primers: oIMR1880 (10 µM), oIMR1881 (10 µM), oIMR7338 (10 µM) and oIMR7339 (10 µM) (Table 1). 1 µL of DNA-extract per sample was mixed with 24 µL of master mix and amplified with the following PCR program (Table 2).

Table 1. Primer used in PCR for genotyping of OTII transgenic mice.

Primer	Sequence (5'–3')	Supplier
oIMR1880	AAA GGG AGA AAA AGC TCT CC	The Jackson Laboratory
oIMR1881	ACA CAG CAG GTT CTG GGT TC	The Jackson Laboratory
oIMR7338	CTA GGC CAC AGA ATT GAA AGA TCT	The Jackson Laboratory
oIMR7339	GTA GGT GGA AAT TCT AGC ATC ATC C	The Jackson Laboratory

Table 2. PCR program used for genotyping of OTII transgenic mice.

Temperature [°C]	Time	
94	2 min	
94	20 sec	Repeat steps for 10 cycles
65	15 sec	
68	10 sec	
94	15 sec	Repeat steps for 28 cycles
55	15 sec	
72	10 sec	
72	2 min	hold

2.3.3 Agarose gel electrophoresis

To identify the size of the individual PCR products 4 µL 6x Loading Dye (Thermo Fisher Scientific, Massachusetts, USA) were added to the amplified samples and a volume of 18 µL loaded onto a 2 % agarose gel containing 0.01 % SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, Massachusetts, USA). As reference for fragment length, 3 µL GeneRuler DNA Ladder (0.1 µg/µL) (Thermo Fisher Scientific, Massachusetts, USA) was used. The gel ran for 35 min at room temperature and 70 V. For imaging, the ChemiDoc™ Imaging System (Bio-Rad, California, USA) was used.

2.4 Mice

Mice used for experimental procedures were about 8-12 week old OTII transgenic (Tg) (B6Cg-Tg(TcraTcrb)425Cbn/J) and RAG1 KO (B6.129S7-*Rag*^{tm1Mom}/J) mice. OTII Tg mice express a murine alpha and beta-chain T cell receptor, that is specific for ovalbumin peptide (OVA 323-339), on $CD4^+$ T cells. Therefore, $CD4^+$ T cells from these mice primarily recognize ovalbumin peptide presented by MHC class II molecules. To the contrary, RAG1 KO mice do not produce mature T or B cells and are therefore described as immune-deficient. In the described experiments, OTII Tg mice were utilized as donors for transfer to the immune-deficient RAG mice.

2.5 Induction of OIA

Cells from OTII Tg mice were isolated from spleen and lymph nodes. Naïve $CD4^+$, $CD8^-$, $CD25^-$ $Vα2^+$ $CD44^{low}$ $CD62L^{high}$ T cells were isolated by fluorescence-activated cell (FACS) sorting. Sorted cells were then injected intravenously (i.v.) into anesthetized RAG1 KO mice. To induce arthritis in this model, cationized OVA protein (kindly provided by the group of Prof. Dr. Hyun-Dong Chang, Deutsches Rheuma-Forschungszentrum Berlin) was injected intra-articular (i.a.) into the knee of RAG1 KO mice 7 days after transfer of naïve $CD4^+$ T cells. Mice were monitored for clinical signs of arthritis and sacrificed mice were analyzed for histological signs of arthritis. In parallel, splenocytes and single cell suspensions from synovial tissue were isolated one, three, 6, 9 and 13 days after the OVA injection and analysed using flow cytometry and histology. The experimental setup is shown in **Figure 4**.

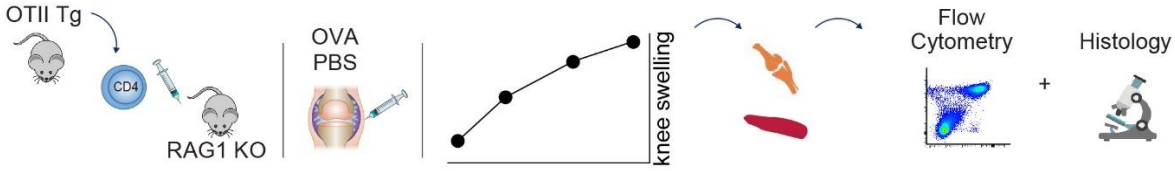

Figure 4. Schematic illustration of the workflow of OIA model and assessment of the experimental kinetics. Day 0: Sort of naïve $CD4^+$ T cells and i.v. transfer into RAG1 KO mice. Day 7: OVA i.a. injection. Day 8, 10, 13, 16 and 21: Sacrificing of mice and isolation of spleen and knee followed by analysis using flow cytometry and histology, respectively.

2.6 Isolation of cells from spleen and lymph nodes

Spleen, axillary brachial and inguinal lymph nodes were isolated from sacrificed mice and mashed through a 70 µm filter (Cell Strainer, Falcon, New York, USA) in a 6-well-plate (Tissue culture testplate, Buch & Holm, Hovedstaden, Denmark) containing 5 mL 2% Buffer (PBS (G) supplemented with 2 % FBS (Sigma-Aldrich, Missouri, USA)). Remaining cells were rinsed with another 5 mL of 2 % Buffer. Then the suspension was transferred into a 15mL Falcon tube and centrifuged at 1200 rpm, 4 °C for 6 min. For red blood cell lysis, the pellet was resuspended in 1 mL of BD Pharm Lyse (BD Biosciences, New Jersey, USA, 1:10 diluted in deionized water) and incubated for 1 min at room temperature. To stop the reaction 9 mL 2 % Buffer was added and again centrifuged as described above. The remaining pellet was resuspended in the appropriate amount of 2 % Buffer according to requirements.

2.7 Isolation of single cell suspensions from knees

The knee was isolated and incubated at 37 °C and 300 rpm for 30 min in an incubator (IKA, Germany) in a 6-well-plate with 3 mL PBS (Thermo Fisher Scientific, Massachusetts, USA) and 200 µL 10x Collagenase D (Sigma-Aldrich, Missouri, USA). To each digestion 2 mL

complete medium (2.1.2) were added, transferred to Miltenyi C tube (Miltenyi Biotec, Germany) and homogenized with gentleMACS™ Octo Dissociator (Miltenyi Biotec, Germany) (Table 3). Then, 5 mL complete medium were added to the Miltenyi C tube and the digested joints transferred and filtered through a 70 µm filter (Cell Strainer, Falcon, New York, USA) into a 50 mL Falcon tube. The tissue remaining on top of the filter was further mashed. Afterwards, the suspension was centrifuged at 1200 rpm, 4°C for 8 min and the pellet resuspended in the appropriate amount of 2 % Buffer according to requirements.

Table 3. Joint Dissociation Program on gentleMACSTM Octo Dissociator.

Joint Dissociation Program	
Time [s]	**RPM**
15	0
14-12	+ 3000
11	- 2800
5-10	+ 3000
4	- 2800
1-3	+ 3000
0	0
end	

2.8 Purification of naïve CD4+ T cells

To purify $CD4^+$ T cells from the single cell suspensions from spleen and lymph nodes (as described in 2.6), a magnetic-activated cell sorting (MACS) depletion was performed for granulocytes, B cells, cytotoxic T cells, mature erythrocytes, erythroid precursor, natural killer, and myeloid cells. Cells were incubated on ice for 20 min with 500 µL 2 % Buffer containing an antibody cocktail (Gr-1, B220, Ter119, NK1.1, CD11b, CD8α) (Table 4). After incubation, cells were washed with 9 mL of 2 % Buffer and then cells were pelleted for 5 min at 1200 rpm. The pellet was resuspended in 150 µL 2 % Buffer and 15 µL Magnetic beads (Invitrogen) and again incubated on ice for 20 min. After adding 3 mL of 2 % Buffer per mouse, cells were transferred into a Polypropylene tube (Falcon, New York, USA) and applied to a magnet (R & D Systems, Minnesota, USA) for 3 min. Supernatant (containing purified $CD4^+$ T cells) was collected in another Polypropylene tube (Falcon, New York, USA) and Magnetic beads were washed twice with 1 mL of 2 % Buffer. To remove remaining beads the cells were again applied to the magnet for 6 min. The supernatant was centrifuged at 1200 rpm, 4 °C for 5 min and the pellet resuspended in 500 µL antibody mix for FACS sorting (see chapter 2.9.2).

Table 4. List of antibodies used for MACS depletion

Antibody	Stock concentration [mg/mL]	Dilution	Supplier	Clone	Catalog
Gr-1	0.5	1:125	BioLegend	RB6-8C5	108404
B220	0.5	1:125	BioLegend	RA3-6B2	103204
Ter119	0.5	1:500	BioLegend	TER-119	116204
NK1.1	0.5	1:500	BioLegend	PK136	108704
CD11b	0.5	1:500	BioLegend	M1-70	101204
CD8α	0.5	1:166	BioLegend	53-6.7	100704

2.9 Flow cytometry and cell sorting

2.9.1 Extracellular and intracellular stainings

For extracellular stainings antibodies against TCRβ (clone: H57-597), CD4 (clone: GK1.5), CD44 (clone: IM7), CD69 (clone: H1.2F3), RANKL (clone: IK22/5), CD62L (clone: MEL-14), CD25 (clone: PC61.5) and Vα2 (clone: B20.1) were diluted in PBS. Dead cells were excluded using LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific, Massachusetts, USA) according to the manufacturer's protocol.

For intracellular cytokine staining cells were re-stimulated with 25 ng/mL PMA (1 mg/mL, Sigma-Aldrich, Missouri, USA), 750 ng/mL Ionomycin (1 mg/mL, Sigma-Aldrich, Missouri, USA), GolgiStop (1:800, BD Biosciences, New Jersey, USA) and GolgiPlug (1:800, BD Biosciences, New Jersey, USA) in complete medium for 4 h at 37 °C. Two million cells were transferred into FACS tube and surface stained for 20 min on ice. For staining antibodies against TCRβ (clone: H57-597), CD4 (clone: GK1.5), CD44 (clone: IM7), CD69 (clone: H1.2F3), RANKL (clone: IK22/5), CD62L (clone: MEL-14), CD25 (clone: PC61.5) and Vα2 (clone: B20.1) were diluted in PBS. Dead cells were excluded using LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific, Massachusetts, USA) according to the manufacturer's protocol. For intracellular stainings, cells were fixed with 100 µL Cytofix Fixation Buffer (BD Biosciences, New Jersey, USA) for 2 min on ice. After 2 washing steps with 1 mL 2 % Buffer, cells were permeabilized in 500 µL Perm/Wash Buffer (BD Biosciences, New Jersey, USA, 1:10 diluted in deionized water) for 10 min at 4 °c. Then cells were pelleted and stained with the following antibodies: IL17a (clone: eBio17B7), IFNγ (clone: XMG1.2), TNFα (clone: MP6-XT22), RORγT (clone: Q31-378), T-bet (clone: eBio4B10) diluted in Perm/wash Buffer for 1 h at 4 °C. Cells were again washed with 1 mL Perm/Wash Buffer and subjected to flow cytometry. Anti-mouse antibodies for flow cytometry are listed in Table 5.

Stained cells were analysed with FACSVerse Flow Cytometer (BD Biosciences, New Jersey, USA) and analysed using FlowJo 10.2 software. For graphical presentation GraphPad Prism 6.01 was used.

Table 5. List of antibodies, cytokines and surface marker used for intra- and extracellular staining.

Reagent	Fluorochrome	Stock concentration [mg/mL]	Dilution	Supplier	Clone	Catalog
IL17a	V450	0.2	1:100	Thermo Fisher Scientific	eBio17B7	48-7177-82
IFNg	FITC	0.5	1:100	Thermo Fisher Scientific	XMG1.2	11-7311-41
Vα2	PE	0.2	1:100	Thermo Fisher Scientific	B20.1	12-5812-82
CD4	PerCP-Cy5.5	0.2	1:400	Thermo Fisher Scientific	RPA-T4	45-0049-42
CD4	Pe-Cy7	0.2	1:400	Thermo Fisher Scientific	GK1.5	25-0041-82
RORγT	PerCP-Cy5.5	0.2	1:100	BD Biosciences	Q31-378	15812149
Tbet	APC	0.2	1:100	Thermo Fisher Scientific	eBio4B10	17-5825-82
TCRb	APC-Cy7	0.2	1:200	Thermo Fisher Scientific	H57-597	109219
CD44	V450	0.2	1:200	Thermo Fisher Scientific	IM7	48-0441-82
CD69	FITC	0.5	1:200	Thermo Fisher Scientific	H1.2F3	11-0691-82
RANKL	PE	0.2	1:100	Thermo Fisher Scientific	IK22/5	510005
CD62L	APC-Cy7	0.2	1:200	Thermo Fisher Scientific	MEL-14	47-0621-82
CD62L	PerCP-Cy5.5	0.2	1:200	BD Biosciences	MEL-14	553148
CD25	APC	0.2	1:200	Thermo Fisher Scientific	PC61.5	17-0251-82
CD8	APC	0.2	1:200	Thermo Fisher Scientific	53-6.7	17-0081-82
TNFα	PE	0.2	1:100	Thermo Fisher Scientific	MP6-XT22	14-7321-81

2.9.2 Sort

To obtain naïve $CD4^+$ T cells, cells were stained for the required surface markers. Stained cells were gated for lymphocytes, single and live cells. $CD25^-CD44^{lo}CD62L^+CD8^-Vα2^+$ T cells were sorted using a BD FACSAria (BD Biosciences, New Jersey, USA). The exact gating strategy used for cell sorting is shown in **Figure 5**.

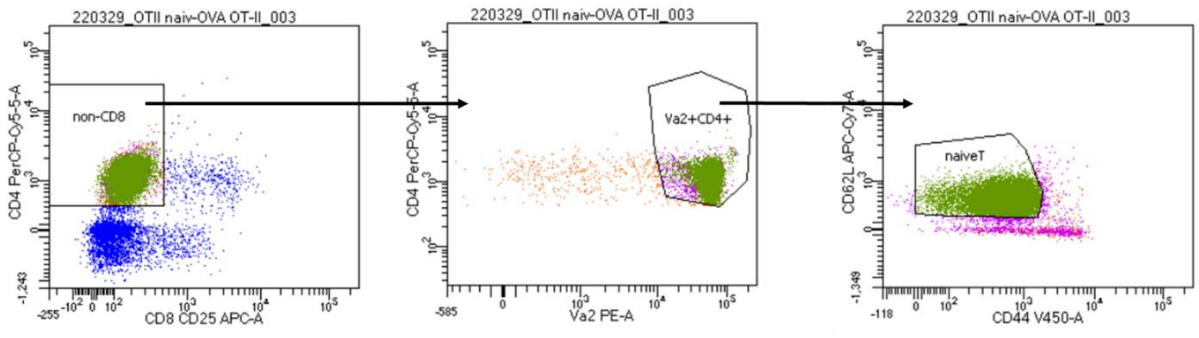

Figure 5. Sorting strategy for naïve CD4⁺ T cells using BD FACSAria.
The first plot shows single live lymphocytes gated on $CD25^{-}CD8^{-}CD4^{+}$. The second plot shows further gating for $V\alpha2^{+}CD4^{+}$ T cells. The third plot shows sorted $CD44^{low}$ $CD62^{high}$ naïve $CD4^{+}$ T cells.

2.10 Histology of knees

2.10.1 H&E and TRAP-staining

To deparaffinise the sections were treated two times with xylene for 5 minutes at room temperature (RT), followed by rehydration. For rehydration a graded series of 100 %, 96 % and 70 % ethanol and distilled water (each 5 min at RT) was used. Isolated knees were stained with hematoxylin and eosin (H&E) for general assessment of inflammation and structural damage. Therefore hemalum, a complex formed from haematoxylin and alum, was applied (10 min), rinsed with distilled water and differentiated with 1 % HCl in 70 % ethanol to colour the nuclei which appear blue. Afterwards a counterstaining with eosin (15 s) and dehydration in 96 % then 100 % ethanol (each 5 min) was performed, colouring eosinophilic structures as cytoplasm and erythrocytes pink or red. All steps were conducted at RT. To identify bone-resorbing osteoclasts a tartrate-resistant acid phosphatase (TRAP)-staining was performed. Therefore, the section was incubated 1 hour in a freshly prepared tartrate solution (0.25 mL Naphthol AS-BI phosphoric acis, 0.1 mL acetate solution, 0.5 mL tartrate, 45 mL distilled water) at 37 °C in a water bath, protected from light. Then the freshly prepared substrate solution (0.25 mL fast garnet GBC base solution, 0.25 mL sodium nitrite solution) was added to preincubate the slides. Afterwards sections were incubated at 37 °C for 2 min. After development samples were rinsed with distilled water, followed by counterstaining the nuclei with hemalum as previously described.

2.10.2 Histological scoring of inflammation and erosion

To score the severity of inflammation and erosion, H&E stained histological sections from the murine knees were evaluated. Each knee section was divided into 4 quadrants, and for each section, a semi-quantitative score for inflammation and erosion, respectively, ranging from 0 to 3 was applied. In this context, a score of 0 describes the control knee, displaying no infiltrates

of immune cells into the synovium and no signs of bone erosion. A score of 3 defines an inflamed knee, where the entire joint cavity is filled with inflammatory cells and bone and cartilage are highly degraded. In the described scoring scheme, a knee section with a severity score of ≥2 is defined as indicative of arthritis.

2.11 Statistics

Statistical analysis of data and summary of biological replicates was done with GraphPad Prism 6. Experiments with multiple time points and n >1 data was tested for statistical significance using a paired t test.

3 Results

3.1 Clinical disease activity in OIA

Swelling of the synovial joint is known to be one of the clinical manifestations of rheumatoid arthritis (Smolen et al., 2018). Thus the diameters of the PBS and OVA injected knee of RAG1 KO mice were measured at different time points during conducting the OIA model (**Figure 6A**). Measurements in the PBS injected knee did not show any changes overtime. However, knee swelling occurred 6 days after injection of OVA over an observational period of 13 days (**Figure 6B-C**).

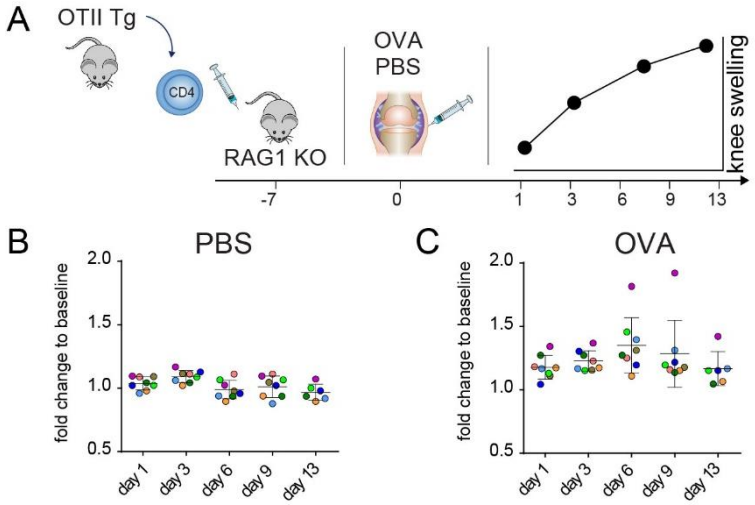

Figure 6. OIA leads to knee swelling in RAG1 KO1 mice.
(A) Schematic illustration of the workflow of OIA model and assessment of the experimental kinetics. Summary dot plots show fold change to baseline of the PBS injected knee (B) and OVA injected knee (C). Each colour represents a biological replicate. The paired t-test was used to test statistical significance. Only significant p-values are shown.
KO — knockout; OIA: ovalbumin-induced arthritis; OVA: ovalbumin; PBS — phosphate buffered saline

3.2 Histological disease severity in OIA

Synovial inflammation, bone erosion and cartilage degradation are common histological features of inflammatory arthritis (Hayer et al., 2021). To determine synovial inflammation in the OIA model, sections of PBS and OVA injected knees of RAG1 KO mice were stained for hematoxylin and eosin (H&E) (**Figure 7A**). While no signs of inflammation could be observed on day one after injection, increased inflammation and erosion were detected after day 3 onwards. In addition to bone erosion synovial inflammation can be seen on day 13 (**Figure 7B-C**). To quantify the histological signs of inflammation and erosions, a histological scoring was applied (152.10.2). A strong increase in inflammation in the joint, reaching an approximate score of 0.5 on day 3, a score between 2 and 3 at day 9 and a score of 3 at day 13, was calculated in 4 biological replicates (**Figure 7C**). In line, erosions in the OIA-model frequently reached the bone marrow cavity by day 13.

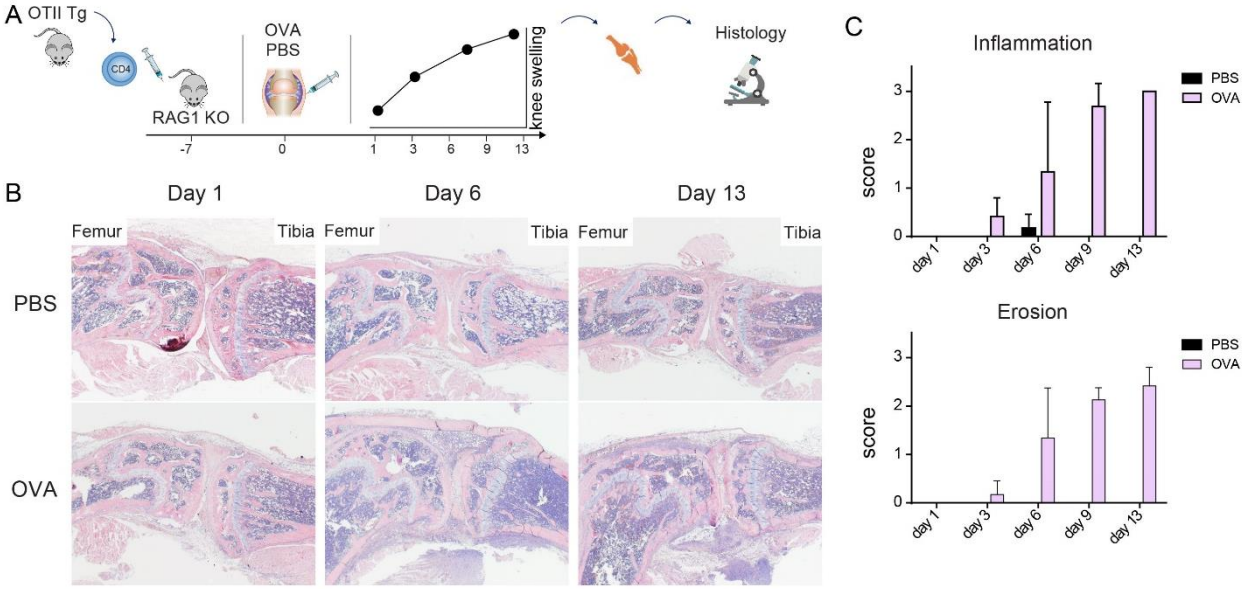

Figure 7. OIA leads to erosive arthritis in RAG1 KO1 mice.
(A) Schematic illustration of the workflow of OIA model and assessment of the experimental kinetics. (B) H&E stainings illustrate erosion and inflammation in the joint one, 6 and 13 days after PBS and OVA injection. (C) Histological results gained by semi-quantitative scoring showing a severity score erosion and inflammation in the joint in a time course. KO — knockout; HE — hematoxylin and eosin; OIA — ovalbumin-induced arthritis; OVA — ovalbumin; PBS — phosphate buffered saline

3.3 Development of activated CD4⁺ T cells

During the pathogenesis of RA, cytokines and intracellular signalling molecules contribute to the activation of naïve CD4⁺ T cells (McInnes & Schett, 2011). Naïve T cells are characterized by the expression of CD62L and lack of expression of CD44 (Eagar & Miller, 2019). Activated T cells which produce IFNγ are characterized as T_H1 cells and surface expression of CD44 is considered a marker for memory T cells (Murphy & Weaver, 2017; Schumann et al., 2015). To analyse and characterize the development of injected CD4⁺ T cells, splenocytes were isolated at different time points and stained with different surface and intracellular markers (**Figure 8A**). Within the living cell population numbers of CD4⁺ T cells increased until day 9, while a reduction of CD4⁺ Cells was observed on day 13 (**Figure 8B, C**). For further characterization, CD4⁺ T cells were stained with antibodies against CD62L, CD25, CD44, IFNγ and T-bet. While numbers of naïve CD62L⁺ CD25⁻ T cells decreased, memory T cells or number of Th1 cells (defined as T-bet⁺ IFNγ⁺ CD4⁺ T cells) increased over time (**Figure 8D**).

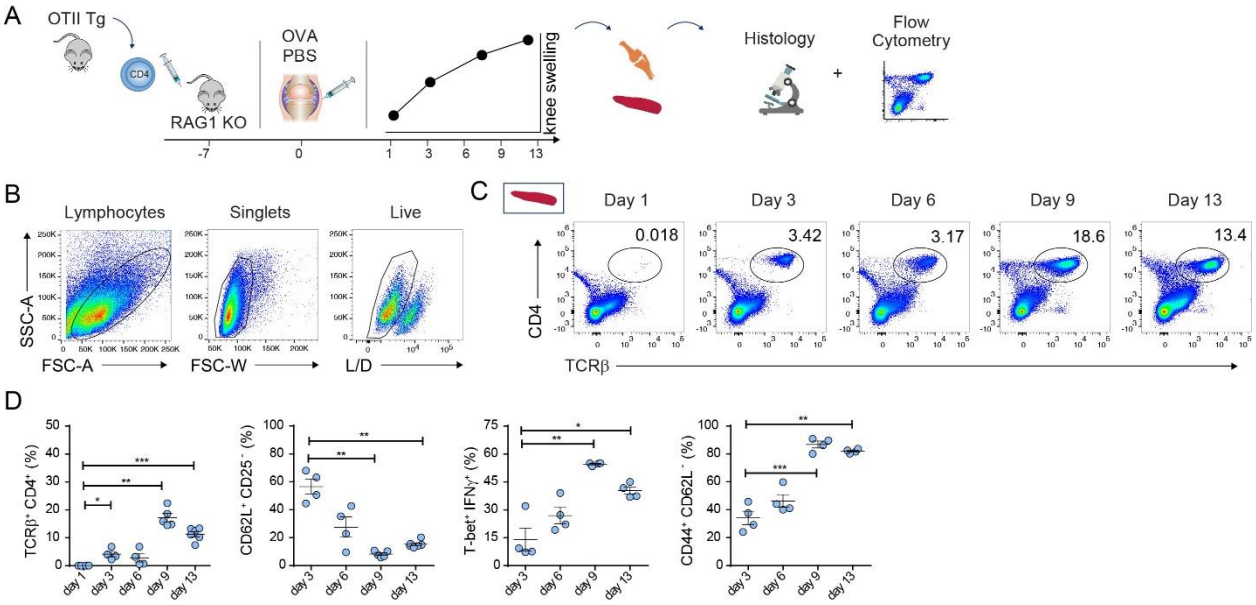

Figure 8. CD4⁺ T cells in spleens of immunized RAG1 KO mice during the course of OIA.
(A) Schematic illustration of the workflow of OIA model and assessment of the experimental kinetics. (B) Cells are gated for lymphocytes while excluding noise in the SSC-A against FSC-A plot. Further gated for singlets in SSC-A against FSC-W plot and for live cells against live/dead staining. (C, D) Cells are stained with different activation markers as well as cytokines and transcription factors for flow cytometry. (C) T cells are identified as CD4⁺ TCRβ⁺ cells. Representative plots show percentage of T cells at different time points. (D) Summary dot plots show percentage of different cell populations during course of time. Naïve T cells are identified as CD62L⁺ CD25⁻ cells. T_H1 cells are identified as T-bet⁺ IFNγ⁺ cells. Memory T cells are identified as CD44⁺ CD62L⁻ cells. Each point represents a biological replicate. Mean value and SD of 4 replicates is shown for each time point. The asterisks represent the significance levels compared to day 1 or 3 (paired t-test), * — $p<0.05$, ** — $p<0.005$, *** — $p<0.0005$
KO — knockout; CD — cluster of differentiation; OIA — ovalbumin-induced arthritis; SSC — side scatter; FSC — forward scatter; -A — area; -W — width; TCR — T cell receptor; T_H — T helper; SD: standard deviation

3.4 Infiltration of CD4⁺ T cells after OIA induction

Cell infiltration into the synovium is a prototypical inflammatory process in the pathogenesis of RA (Bradfield et al., 2003; Szekanecz et al., 2009). To analyse infiltration of i.v. injected T cells, cells were isolated from the joints and analysed by flow cytometry. PBS injection knees did not reveal any infiltration of CD4⁺ T cells. However, i.a. OVA injection resulted in infiltration of CD4⁺ T cells into the synovium after day 6 onwards (**Figure 9**).

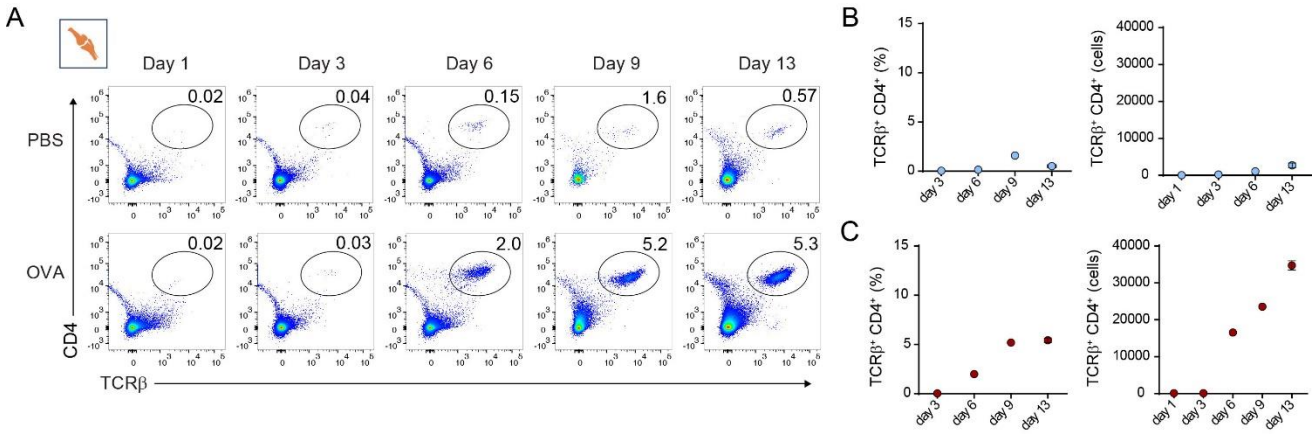

Figure 9. CD4⁺ T cell migration in synovial tissue of immunized RAG1 KO mice during course of OIA.
(A) T cells are identified as CD4⁺ TCRβ⁺ cells. Representative plots of flow cytometric analysis show percentage of migrated T cells in PBS as well as OVA injected joint at different time points. (B, C) Dot plots show percentages and total cell number of CD4⁺ T cells in the PBS (B) and OVA (C) injected joint. 1 biological sample or mean value and SD of 2 biological replicates of each time point are shown. In detail, at most time point only one replicate is shown.
KO — knockout; CD — cluster of differentiation; OIA — ovalbumin-induced arthritis; TCR — T cell receptor; OVA — ovalbumin; PBS — phosphate buffered saline

3.5 T_H1 driven inflammation in the OIA model

TNFα and IFNγ are described as T_H1 cytokines and play an important role in the pathogenesis of RA (McInnes & Schett, 2007). The transcription factor T-bet is a known regulator of T_H1 development (Stolarczyk et al., 2014). After OIA induction cells were stained with antibodies against T-bet, TNFα and IFNγ. Pro-inflammatory cytokine production, such as TNFα and IFNγ, could be observed in cells isolated from in spleen and knee respectively at day 6, suggesting a Th1 driven inflammatory process (**Figure 10A, B**).

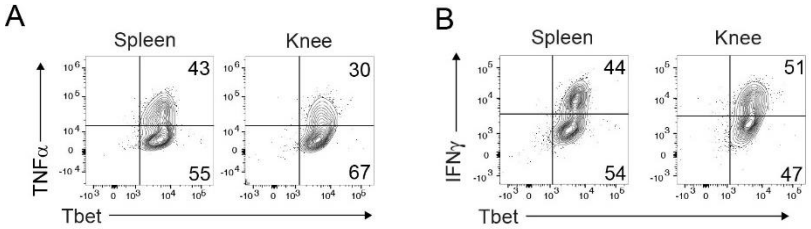

Figure 10. TNFα and IFNγ production by CD4⁺ T cells in spleen and knee of RAG1 KO mice on day 6.
Representative plots of flow cytometric analysis show percentages $CD4^+$ $TCR\beta^+$ T cells further gated on TNFα against T-bet (A) and IFNγ against T-bet (B) in spleen and knee of RAG1 KO mice 6 days after OVA injection.
KO — knockout; CD — cluster of differentiation; TCR — T cell receptor; TNF — tumor necrosis factor; IFN — interferon; OVA — ovalbumin

3.6 RA characteristic osteoclast differentiation in the OVA injected knee

RANKL interaction promotes maturation and activation of osteoclasts, which are bone-degrading cells and can be observed in the pathogenesis of RA (McInnes & Schett, 2011). Isolated cells of knee and spleen were stained with antibodies against the activation marker CD44 and the RANK-Ligand. In the OVA injected knee an increase of $CD44^+$ RANKL-producing $CD4^+$ T cells could be detected, while there was no difference in the $CD4^+$ T cell population in the spleen of RAG1 KO mice (**Figure 11A, B**). The upregulation of RANKL suggests the presence of osteoclasts. TRAP-stained sections 13 days after OVA injection show a clear degradation of the bone and the osteoclasts involved in this pathological process (**Figure 11C**).

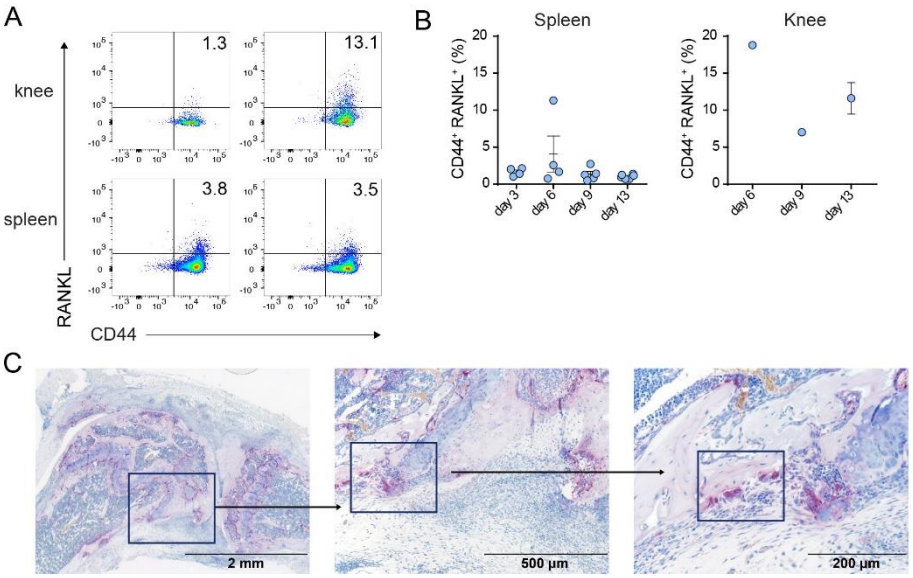

Figure 11. Manifestation of osteoclastogenesis in the knee of RAG1 KO mice.
(A) Representative plots of flow cytometric analysis show percentages of $CD4^+$ $TCR\beta^+$ $CD44^+$ $RANKL^+$ T cells 3 and 13 days after OVA injection. (B) Summary dot plots show percentages of $CD44^+$ $RANKL^+$ $CD4^+$ T cells. Mean value and SD of biological replicates or one biological sample on each time point is shown, respectively. (C) TRAP stainings of joint 13 days after OVA injection show osteoclasts in the highlighted magnified area.
CD — cluster of differentiation; TCR — T cell receptor; RANKL — receptor activator of nuclear factor κB ligand; TRAP — tartrate-resistant acid phosphatase

4 Discussion

We could successfully establish a T cell dependent arthritis model, which allows to study pathogenic T cells during the development of arthritis. Flow cytometric analysis revealed the proliferation of $CD4^+$ T cells, which differentiate from a naïve state to IFNγ producing effector T cells. In line, histological analysis shows cell infiltration, bone erosion and cartilage degradation.

RA is a common autoimmune disease, which primarily affects the joints. Among the different factors that drive the development of the disease, T cells are one important element that can also be targeted by different treatment strategies. Different T cell subsets have been shown to be important in the pathogenesis of RA. Among them T_H17 cells have been discussed as major drivers due to high levels of IL17 producing cells in the synovial fluid of RA patients. In addition, T_H17 cells were found to enhance osteoclastogenesis leading to bone erosions (Yang et al. 2019) However, in clinical trials no clear beneficial effects of IL17 blockade could be observed.(Merola et al., 2018)

Within the OIA model we could show that injected T cells start to proliferate and gain features of T_H1 cells, such as expression of T-bet and IFNγ or TNFα secretion.

T_H1 cells can activate macrophages leading to increased production of TNFα. In patients activated macrophages, IFNγ and therefore T_H1 cells are found in synovial tissue and fluid.(Chemin et al., 2019) In this context, typical features of RA such as increasing inflammation and erosion as well as the infiltration of $CD4^+$ T cells at the site of inflammation(Chemin et al., 2019) in the OVA injected knee can be observed in our model.

It is proposed that the imbalance of T_H1 and T_H2 cells is a driver of a chronic immune response. Therefor T_H1 cells play a crucial role in the pathogenesis of RA. They release the proinflammatory cytokines IL2, IFNγ and TNFα, leading to an inflammatory environment in the joint. IL2 secretion plays a role in osteoclastogenesis and promotes IFNγ production. Further IFNγ contributes to T_H1 differentiation and induces inflammation. High levels of IFNγ as well as TNFα were found to be associated with RANKL expression in osteoclastsogenesis and activation of dendritic cells which leads to T cell activation. These characteristic effects of the T_H1 lineage further promotes cartilage damage and bone erosion. Therefore, T_H1 cells and their effector cytokines directly contribute to the progression of RA.(Luo, Pan, Wang, Peixu, Xu, Jiawen, Hou, 2022)

In summary we could establish for the first time a purely T cell dependent arthritis model. Animal models provide valuable insights on the development of human disease. However, they do not achieve full translatability.(Meehan et al., 2021) Whilst mechanisms like cross-reactions

between antibodies and antigen are well understood, present models cannot fully reflect how autoimmunity develops in patients with RA.(Maffia et al., 2004) The OIA model described in this thesis shows the development of pathogenic T cells during the progression of RA. It provides the opportunity to investigate the role of different T cell subsets during the development of RA. Ongoing analysis aim to define transcriptional and chromatin changes of injected T cells, which will allow to gain better knowledge on pathogenic T cells and define potential treatment targets. The OIA model will also provide a perfect tool to study the role of specific target genes in the context of rheumatoid arthritis by performing gain-of-function and loss-of-function experiments. In addition, T cell targeting drugs can be tested in the OIA model and therefore be validated.

5 Abstract

Despite enormous efforts to develop new therapeutic strategies for the treatment of rheumatoid arthritis (RA), a large number of non-responding patients to available drugs still urges the need to identify new treatment targets. As one of the most common inflammatory joint diseases, development of RA has been associated with alterations in human leukocyte antigen (HLA) class II genes, which encode for essential molecules for activation and differentiation of $CD4^+$ T cells. Furthermore, several studies demonstrate the presence of effector $CD4^+$ T cells in the inflamed joints of RA patients, concluding that $CD4^+$ T cells are implicated in the development and progression of this disease. However, T cell-targeted therapies are limited so far. Within this thesis, the aim is to characterize disease-driving T cells within a murine model, the OVA-induced arthritis (OIA). In this model, naïve $CD4^+$ T cells are transferred into immune deficient host mice that lack endogenous T cells as well as B cells. Host mice are then challenged by intra-articular injection of cationized OVA, 7 days after cell transfer. First analysis of the flow cytometry data shows an increase of percentages and cell numbers of $CD4^+$ T cells overtime in the spleen and knee of OVA-injected mice. Isolated $CD4^+$ T cells produce high levels of the inflammatory cytokines IFNγ and TNFα suggesting a mainly Th1-driven disease model. Synovial $CD4^+$ T cells possess a highly activated phenotype, characterized by the expression of CD44, CD69 and CD25. In addition, histological staining of the knees show that immune cells are enriched in the synovial cavity of the knee, and drive inflammation and bone erosion. However, further characteristics of the resident T cells are still illusive. Transcriptomic analysis of re-isolated peripheral and synovial $CD4^+$ T cells within the OIA model offer a unique possibility to understand the development of pathogenic T cells and identify factors that drive inflammation.

6 Zusammenfassung

Trotz kontinuierlicher Entwicklungen der therapeutischen Strategien für die Behandlung der rheumatoiden Arthritis (RA), existiert eine große Anzahl an Patient/innen, die nicht auf die verfügbaren Medikamente ansprechen. Dadurch entsteht eine dringende Notwendigkeit, neue Therapieansätze zu finden. Als eine der häufigsten inflammatorischen Autoimmunerkrankungen wurde die Entstehung von RA mit Veränderungen in humanen Leukozytenantigenen (HLA) der Klasse II in Verbindung gebracht, welche für wichtige Moleküle in der Aktivierung und Differenzierung von $CD4^+$ T-Zellen kodieren. Darüber hinaus belegen mehrere Studien das Vorhandensein von $CD4^+$ T-Zellen in den entzündeten Gelenken von RA-Patient/innen, was darauf schließen lässt, dass $CD4^+$ T-Zellen an der Entstehung und dem Fortschreiten dieser Krankheit beteiligt sind. Bisher gibt es jedoch nur wenige Therapien, die direkt T-Zellen beeinflussen. Ziel dieser Arbeit ist es, die pathogenen T-Zellen in einem Mausmodell der OVA-induzierten Arthritis (OIA) zu charakterisieren. In diesem Modell werden naive $CD4^+$ T-Zellen in immun-defiziente Mäuse übertragen, denen sowohl endogene T-Zellen als auch B-Zellen fehlen. Die Wirtsmäuse werden 7 Tage nach dem Zelltransfer durch intraartikuläre Injektion von kationisiertem OVA immunisiert. Eine erste Analyse der durchflusszytometrischen Daten zeigt einen Anstieg des Prozentsatzes und der Zellzahl der $CD4^+$ T-Zellen in der Milz und im Knie der OVA-injizierten Mäuse. Isolierte $CD4^+$ T-Zellen setzen hohe Mengen an entzündlichen Zytokinen wie IFNγ und TNFα frei, was auf ein hauptsächlich Th1-gesteuertes Krankheitsmodell hindeutet. Synoviale $CD4^+$ T-Zellen besitzen einen stark aktivierten Phänotyp, der durch die Expression von CD44, CD69 und CD25 gekennzeichnet ist. Darüber hinaus zeigen histologische Färbungen der OVA-injizierten Knie, dass sich Immunzellen in dem Synovialgewebe des Knies anreichern und Entzündungen und Knochenerosionen induzieren. Weitere Merkmale der involvierten T-Zellen sind jedoch noch nicht bekannt. Das murine OIA-Modell bietet die Möglichkeit durch Analysen des Transkriptoms und Epigenoms von isolierten peripheren und synovialen $CD4^+$ T-Zellen die Entstehung pathogener Zellen besser zu verstehen und neue Faktoren zu identifizieren, welche die Entzündung vorantreiben.

7 References

Aletaha, D., & Smolen, J. S. (2018). Diagnosis and Management of Rheumatoid Arthritis. *JAMA*, *320*(13), 1360. https://doi.org/10.1001/jama.2018.13103

Benedetti, G., & Miossec, P. (2014). Interleukin 17 contributes to the chronicity of inflammatory diseases such as rheumatoid arthritis. *European Journal of Immunology*, *44*(2), 339–347. https://doi.org/10.1002/eji.201344184

Bradfield, P. F., Amft, N., Vernon-Wilson, E., Exley, A. E., Parsonage, G., Rainger, G. E., Nash, G. B., Thomas, A. M. C., Simmons, D. L., Salmon, M., & Buckley, C. D. (2003). Rheumatoid fibroblast-like synoviocytes overexpress the chemokine stromal cell-derived factor 1 (CXCL12), which supports distinct patterns and rates of CD4+ and CD8+ T cell migration within synovial tissue. *Arthritis & Rheumatism*, *48*(9), 2472–2482. https://doi.org/10.1002/art.11219

Brand, D. D., Latham, K. A., & Rosloniec, E. F. (2007). Collagen-induced arthritis. *Nature Protocols*, *2*(5), 1269–1275. https://doi.org/10.1038/NPROT.2007.173

Chemin, K., Gerstner, C., & Malmström, V. (2019). Effector Functions of CD4+ T Cells at the Site of Local Autoimmune Inflammation—Lessons From Rheumatoid Arthritis. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.00353

Christensen, A. D., Haase, C., Cook, A. D., & Hamilton, J. A. (2016). K/BxN Serum-Transfer Arthritis as a Model for Human Inflammatory Arthritis. *Frontiers in Immunology*, *7*. https://doi.org/10.3389/fimmu.2016.00213

Cibrián, D., & Sánchez-Madrid, F. (2017). CD69: from activation marker to metabolic gatekeeper. *European Journal of Immunology*, *47*(6), 946–953. https://doi.org/10.1002/eji.201646837

Reilly C, Thomas M, Gogal R, Olgun, Santo A, Sodhi R, Samy E, Peng S, Gilkeson G, Mishra N. (2008). The histone deacetylase inhibitor trichostatin A upregulates regulatory T cells and modulates autoimmunity in NZB/W F1 mice. *Journal of Autoimmunity*, *31*(2), 123–130. https://doi.org/10.1016/J.JAUT.2008.04.020

Eagar, T. N., & Miller, S. D. (2019). *Clinical Immunology* (5th editio).

Edwards, J. C. W., Szczepański, L., Szechiński, J., Filipowicz-Sosnowska, A., Emery, P., Close, D. R., Stevens, R. M., & Shaw, T. (2004). Efficacy of B-Cell–Targeted Therapy with Rituximab in Patients with Rheumatoid Arthritis. *New England Journal of Medicine*, *350*(25), 2572–2581. https://doi.org/10.1056/NEJMoa032534

Feldmann, M., & Maini, R. N. (2001). Anti-TNFα Therapy of Rheumatoid Arthritis: What Have We Learned? *Annual Review of Immunology*, *19*(1), 163–196. https://doi.org/10.1146/annurev.immunol.19.1.163

Gonzalo-Gil, E., & Galindo-Izquierdo, M. (2014). Papel del factor de crecimiento transformador-beta (TGF-β) en la fisiopatología de la artritis reumatoide. *Reumatología Clínica*, *10*(3), 174–179. https://doi.org/10.1016/j.reuma.2014.01.009

Guo, Q., Wang, Y., Xu, D., Nossent, J., Pavlos, N. J., & Xu, J. (2018). Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone Research*, *6*(1), 15. https://doi.org/10.1038/s41413-018-0016-9

Hayer, S., Vervoordeldonk, M. J., Denis, M. C., Armaka, M., Hoffmann, M., Bäcklund, J., Selva Nandakumar, K., Niederreiter, B., Geka, C., Fischer, A., Woodworth, N., Blüml, S., Kollias, G., Holmdahl, R., Apparailly, F., & Koenders, M. I. (2021). “SMASH” recommendations for standardised microscopic arthritis scoring of histological sections from inflammatory arthritis animal models. *Ann Rheum Dis*, *0*, 1–13. https://doi.org/10.1136/annrheumdis-2020-219247

Holmdahl, R., Jansson, L., Larsson, E., Rubin, K., & Klareskog, L. (1986). Homologous type II collagen induces chronic and progressive arthritis in mice. *Arthritis & Rheumatism*, *29*(1), 106–113. https://doi.org/10.1002/art.1780290114

Ji, H., Gauguier, D., Ohmura, K., Gonzalez, A., Duchatelle, V., Danoy, P., Garchon, H.-J., Degott, C., Lathrop, M., Benoist, C., & Mathis, D. (2001). Genetic Influences on the End-Stage Effector Phase of Arthritis. *Journal of Experimental Medicine*, *194*(3), 321–330. https://doi.org/10.1084/jem.194.3.321

Kollias, G., Douni, E., Kassiotis, G., & Kontoyiannis, D. (1999). On the role of tumor necrosis factor and receptors in models of multiorgan failure, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease. *Immunological Reviews*, *169*(1), 175–194. https://doi.org/10.1111/j.1600-065X.1999.tb01315.x

Korganow, A.-S., Ji, H., Mangialaio, S., Duchatelle, V., Pelanda, R., Martin, T., Degott, C., Kikutani, H., Rajewsky, K., Pasquali, J.-L., Benoist, C., & Mathis, D. (1999). From Systemic T Cell Self-Reactivity to Organ-Specific Autoimmune Disease via Immunoglobulins. *Immunity*, *10*(4), 451–461. https://doi.org/10.1016/S1074-7613(00)80045-X

Kouskoff, V., Korganow, A.-S., Duchatelle, V., Degott, C., Benoist, C., & Mathis, D. (1996). Organ-Specific Disease Provoked by Systemic Autoimmunity. *Cell*, *87*(5), 811–822. https://doi.org/10.1016/S0092-8674(00)81989-3

Lubberts, E. (2010). Th17 cytokines and arthritis. *Seminars in Immunopathology*, *32*(1), 43–53. https://doi.org/10.1007/s00281-009-0189-9

Luo, Pan, Wang, Peixu, Xu, Jiawen, Hou, W. (2022). Immunomodulatory role of T helper cells in rheumatoid arthritis. *Bone & Joint Research*. https://doi.org/https://doi.org/10.1302/2046-3758.117.BJR-2021-0594.R1

MacGregor, A. J., Snieder, H., Rigby, A. S., Koskenvuo, M., Kaprio, J., Aho, K., & Silman, A. J. (2000). Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis & Rheumatism*, *43*(1), 30–37. https://doi.org/10.1002/1529-0131(200001)43:1<30::AID-ANR5>3.0.CO;2-B

Maffia, P., Brewer, J. M., Gracie, J. A., Ianaro, A., Leung, B. P., Mitchell, P. J., Smith, K. M., McInnes, I. B., & Garside, P. (2004). Inducing Experimental Arthritis and Breaking Self-Tolerance to Joint-Specific Antigens with Trackable, Ovalbumin-Specific T Cells. *The Journal of Immunology*, *173*(1), 151–156. https://doi.org/10.4049/jimmunol.173.1.151

Matsumoto, I., Staub, A., Benoist, C., & Mathis, D. (1999). Arthritis Provoked by Linked T and B Cell Recognition of a Glycolytic Enzyme. *Science*, *286*(5445), 1732–1735. https://doi.org/10.1126/science.286.5445.1732

McInnes, I. B., & Schett, G. (2007). Cytokines in the pathogenesis of rheumatoid arthritis. *Nature Reviews Immunology*, *7*(6), 429–442. https://doi.org/10.1038/nri2094

McInnes, I. B., & Schett, G. (2011). The Pathogenesis of Rheumatoid Arthritis. *New England Journal of Medicine*, *365*(23), 2205–2219. https://doi.org/10.1056/NEJMra1004965

Meehan, G. R., Thomas, R., Al Khabouri, S., Wehr, P., Hilkens, C. M., Wraith, D. C., Sieghart, D., Bonelli, M., Nagy, G., Garside, P., Tough, D. F., Lewis, H. D., & Brewer, J. M. (2021). Preclinical models of arthritis for studying immunotherapy and immune tolerance. *Ann Rheum Dis*, *80*, 1268–1277. https://doi.org/10.1136/annrheumdis-2021-220043

Merola, J. F., Espinoza, L. R., & Fleischmann, R. (2018). Distinguishing rheumatoid arthritis from psoriatic arthritis. *RMD Open*, *4*(2), e000656. https://doi.org/10.1136/rmdopen-2018-000656

Mima, T., Saeki, Y., Ohshima, S., Nishimoto, N., Matsushita, M., Shimizu, M., Kobayashi, Y., Nomura, T., & Kishimoto, T. (1995). Transfer of rheumatoid arthritis into severe combined immunodeficient mice. The pathogenetic implications of T cell populations oligoclonally expanding in the rheumatoid joints. *Journal of Clinical Investigation*, *96*(4), 1746–1758. https://doi.org/10.1172/JCI118220

Murphy, K., & Weaver, C. (2017). *Janeway's Immunobiology* (9th editio). 2017: Garland

Science.
Pan, M., Kang, I., Craft, J., & Yin, Z. (2004). Resistance to Development of Collagen-Induced Arthritis in C57BL/6 Mice Is Due to a Defect in Secondary, but Not in Primary, Immune Response. *Journal of Clinical Immunology*, *24*(5), 481–491. https://doi.org/10.1023/B:JOCI.0000040919.16739.44
Puchner, A., Saferding, V., Bonelli, M., Mikami, Y., Hofmann, M., Brunner, J. S., Caldera, M., Goncalves-Alves, E., Binder, N. B., Fischer, A., Simader, E., Steiner, C.-W., Leiss, H., Hayer, S., Niederreiter, B., Karonitsch, T., Koenders, M. I., Podesser, B. K., O'Shea, J. J., … Blüml, S. (2018). Non-classical monocytes as mediators of tissue destruction in arthritis. *Annals of the Rheumatic Diseases*, *77*(10), 1490–1497. https://doi.org/10.1136/annrheumdis-2018-213250
Schumann, J., Stanko, K., Schliesser, U., Appelt, C., & Sawitzki, B. (2015). Differences in CD44 Surface Expression Levels and Function Discriminates IL-17 and IFN-γ Producing Helper T Cells. *PLOS ONE*, *10*(7), e0132479. https://doi.org/10.1371/journal.pone.0132479
Schurgers, E., Billiau, A., & Matthys, P. (2011). Collagen-Induced Arthritis as an Animal Model for Rheumatoid Arthritis: Focus on Interferon-γ. *Journal of Interferon & Cytokine Research*, *31*(12), 917–926. https://doi.org/10.1089/jir.2011.0056
Smeltz, R. B., Chen, J., Ehrhardt, R., & Shevach, E. M. (2002). Role of IFN-γ in Th1 Differentiation: IFN-γ Regulates IL-18Rα Expression by Preventing the Negative Effects of IL-4 and by Inducing/Maintaining IL-12 Receptor β2 Expression. *The Journal of Immunology*, *168*(12), 6165–6172. https://doi.org/10.4049/jimmunol.168.12.6165
Smolen, J. S. (2020). Insights into the treatment of rheumatoid arthritis: A paradigm in medicine. *Journal of Autoimmunity*, *110*, 102425. https://doi.org/10.1016/j.jaut.2020.102425
Smolen, J. S., Aletaha, D., Barton, A., Burmester, G. R., Emery, P., Firestein, G. S., Kavanaugh, A., McInnes, I. B., Solomon, D. H., Strand, V., & Yamamoto, K. (2018). Rheumatoid arthritis. *Nature Reviews Disease Primers*, *4*(1), 18001. https://doi.org/10.1038/nrdp.2018.1
Stolarczyk, E., Lord, G. M., & Howard, J. K. (2014). The immune cell transcription factor T-bet. *Adipocyte*, *3*(1), 58–62. https://doi.org/10.4161/adip.26220
Strand, V., Kimberly, R., & Isaacs, J. D. (2007). Biologic therapies in rheumatology: lessons learned, future directions. *Nature Reviews Drug Discovery*, *6*(1), 75–92. https://doi.org/10.1038/nrd2196
Szekanecz, Z., Pakozdi, A., Szentpetery, A., Besenyei, T., & Koch, A. E. (2009). Chemokines and angiogenesis in rheumatoid arthritis. *Frontiers in Bioscience (Elite Edition)*, *1*(1), 44–51. https://doi.org/10.2741/E5
Tong, D., Lönnblom, E., Yau, A. C. Y., Nandakumar, K. S., Liang, B., Ge, C., Viljanen, J., Li, L., Bãlan, M., Klareskog, L., Chagin, A. S., Gjertsson, I., Kihlberg, J., Zhao, M., & Holmdahl, R. (2018). A Shared Epitope of Collagen Type XI and Type II Is Recognized by Pathogenic Antibodies in Mice and Humans with Arthritis. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.00451
van den Berg, W. B., & Miossec, P. (2009). IL-17 as a future therapeutic target for rheumatoid arthritis. *Nature Reviews Rheumatology*, *5*(10), 549–553. https://doi.org/10.1038/nrrheum.2009.179
Wegner N, Wait R, Sroka A, Eick S, Nguyen K-A, Lundberg K, Kinloch A, Culshaw S, Potempa J, Venables P. (2010). Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and α-enolase: Implications for autoimmunity in rheumatoid arthritis. *Arthritis & Rheumatism*, *62*(9), 2662–2672. https://doi.org/10.1002/art.27552

Yang F, Qian M, Zhang A, Xu X, Wang B, Jiang L, Zhou P. 2019. *Th17 cell pathogenicity and plasticity in rheumatoid arthritis*. https://doi.org/10.1002/JLB.4RU0619-197R

8 List of Figures

9 List of Tables

10 List of Abbreviations

-A	area
ACAP	anti-citrullinated protein antibody
AIA	antigen-induced arthritis
APC	Allophycocyanin
APC-Cy7	Allophycoyanin-cyanine 7
CD	cluster of differentiation
CFA	complete Freund's adjuvant
CIA	collagen-induced arthritis
CII	type II collagen
FACS	flurescence-activated cell sorting
FBS	fetal bovine serum
FITC	Fluorescein
FLS	fibroblast-like synoviocytes
FSC	forward scatter
G6PI	glucose-6-phosphate
H_2O	water (Dihydrogenmonoxid)
HCl	Hydrochloric acid
H&E	hematoxylin and eosin
HLA	human leukocyte antigen
IFN	interferon
IL	interleukin
KO	knockout
MACS	magnetic-activated cell sorting
$MgCl_2$	Magnesium chloride
MHC	major histocompatibility complex
NK	natural killer
OIA	ovalbumin-induced arthritis
OVA	ovalbumin
PBS	phosphate buffered saline
PE	Phycoerythrin
PE-Cy7	Phycoerythrin-cyanine 7
PerCP-Cy5.5	Peridinin chlorophyll protein-Cyanine 5.5
Pg	proteoglycan
PgIA	proteoglycan-induced arthritis
RA	rheumatoid arthritis
RANKL	receptor activator of nuclear factor κB ligand
RF	rheumatoid factor
SD	standard deviation
SDS	sodium dodecyl sulfate
SSC	side scatter
STA	serum-transfer arthritis
TCR	T cell receptor
Tg	transgenic
TGF	transforming growth factor

T_H	T helper
TNF	tumor necrosis factor
TRAP	tartrate-resistant acid phosphatase
T_{reg}	T regulatory
-W	width