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Abstract

Galectins (Gal), which are β-galactoside-binding lectins, play a key role in the development and progression of Osteoarthritis (OA). With their carbohydrate-recognition domains and affinity to bind glycans they can bind to cell surfaces and extracellular matrix. Previous studies showed the involvement of galectins in pro-inflammatory and matrix-degrading processes. First, this thesis aimed to establish the methodology for the detection of anti-galectin autoantibodies in plasma of four healthy test persons. With ELISA tests, we successfully detected the presence of various anti-Gal autoantibodies in plasma samples of healthy donors. Additionally, higher levels of anti-Gal-1, -4, -9, -12 and anti-Gal-related protein autoantibodies were measured compared to anti-Gal-2, -3, -7, and -8. These experiments provide the basis for the upcoming evaluation of anti-galectin autoantibodies as biomarkers for diagnosis and therapy monitoring in patients with high risk of OA. Secondly, we investigated the mRNA levels of genes encoding for galectins in fibroblast-like synoviocytes (FLS) and THP-1 monocytes and macrophages and found predominant expression of LGALS1, LGALS3 and LGALS8 in FLS, as well as LGALS1, LGALS3, LGALS8 and LGALS9 in THP-1 cells. Then, marker gene expression in galectin-treated FLS and THP-1 monocytes and macrophages was assessed. We show that pro-inflammatory target genes such as TNFA, IL1B and IL8 were up-regulated in all three cell types. Interestingly, FLS showed greater reaction to galectins compared to THP-1 macrophages. Taken together, these results indicate that galectins are expressed by FLS and THP-1 monocytes and macrophages and that galectins induce pro-inflammatory marker genes in these cells of the OA synovium.

Zusammenfassung

Galektine, auch als β-Galaktosid-bindende Lektine bekannt, spielen eine Schlüsselrolle bei der Entwicklung und Progression der Osteoarthritis. Mit ihrer Erkennungsdomäne und Affinität zu Glykanen, sind sie in der Lage an Zelloberflächen und extrazellulärer Matrix zu binden. Frühere Studien zeigten die Beteiligung von Galektinen in pro-inflammatorischen und matrixabbauenden Prozessen. Daher war eines der Ziele dieser Arbeit, eine Methode zum Nachweis von Anti-Galektin-Autoantikörpern im Plasma gesunder Probanden zu etablieren (n=4 Spender). Mit ELISA Tests, zeigten wir die Anwesenheit von Autoantikörpern gegen ein breites Spektrum verschiedener Galektinen in Plasmaproben der Probanden. Verglichen mit Anti-Gal-2,-3,-7 und 8, wurden höhere Werte an Anti-Gal-1, -4, -9, -12 und Anti-Galectin-related protein-Autoantikörpern gemessen. Diese Experimente bilden die Grundlage für nachfolgende Evaluierungen von Anti-Gal-Autoantikörpern in Form eines Biomarkers für die Diagnose und Therapieüberwachung bei Patienten mit hohem OA Risiko.

Weiters wurden die mRNA-Spiegel von Genen, die für Galektine kodieren, in Fibroblasten-ähnlichen Synoviozyten (FLS) und THP-1-Monozyten und Makrophagen untersucht, und eine starke Expression von LGALS1, LGALS3 und LGALS8 in FLS, sowie in THP-1 Zellen, festgestellt. Daraufhin wurde die Markergenexpression in Galektin-behandelten FLS- und THP-1-Monozyten und Makrophagen bewertet. Es stellte sich heraus, dass pro-inflammatorische Gene wie TNFA, IL1B und IL8 in allen drei Zelltypen hochreguliert wurden. Interessant ist, dass FLS im Vergleich zu THP-1-Makrophagen eine stärkere Reaktion auf Galektine zeigten. Zusammenfassend deuten diese Ergebnisse darauf hin, dass Galektine durch FLS sowie THP-1-Monozyten und Makrophagen exprimiert werden, und dass Galektine pro-inflammatorische Markergene in diesen Zellen des OA-Synovialgewebes induzieren.

1 Introduction:

1.1 Anatomical features of the human knee joint

The knee joint, also called the tibiofemoral joint, is the largest joint in the human body. Generally, it is separated into three joints. First, the tibiofemoral joint is located laterally between the lateral condyle of the femur and lateral condyle of the tibia. A second tibiofemoral joint exist between the medial condyle of the femur and medial condyle of the tibia. The third joint, called patellofemoral joint is located between the patella and the femur and has been described as the most researched joint in the body. A series of different accessory ligaments, including the patellar ligament, the tibial and fibular collateral ligament and the anterior and posterior cruciate ligament are responsible for the strengthening of the whole knee joint. To compensate irregular shapes of spontaneous moves and supporting circulation of synovial fluid, two fibrocartilage discs (menisci) are located between the tibial and femoral condyles. So the knee joint is a complex of numerous parts that allows extension, flexion and few rotations (Nielsen, Mark T, Tortora 2009).

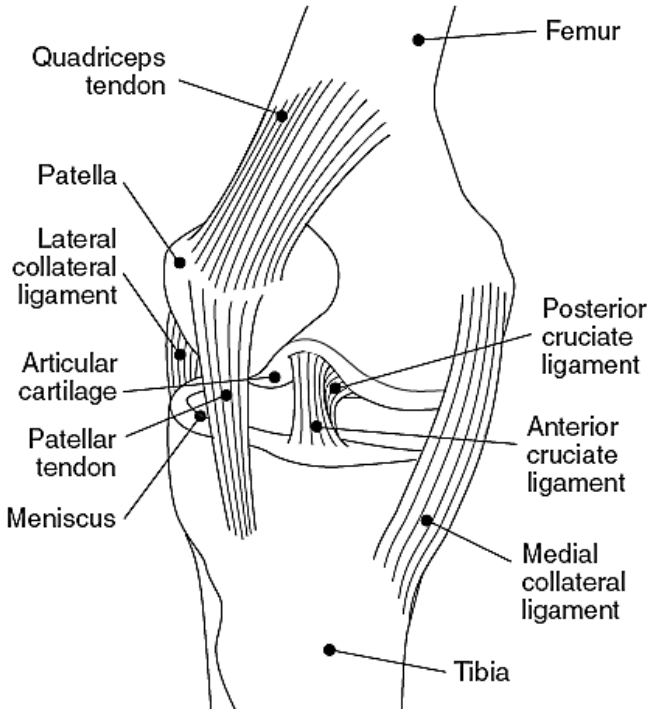


Figure 1 Medial view of the knee joint showing anatomical features.
(Image taken from National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), 30.05.2018)

1.1.1 Synovial joint anatomy and physiology

Synovial joints differ in their characteristics from other joints. One unique feature is the so called synovial cavity which is surrounded by a capsule that is connected with the articulating bones. Synovial joints are among the most mobile of all joints, The Bones are covered by a layer of articular cartilage Their range of mobility is huge, for example the movement of carpal bone is very limited while knee and shoulder are able to move in almost all directions (Nielsen, Mark T. ; Tortora 2009).

1.1.2 Pathology of the synovium

Synovium is a mesenchymal tissue which is essential for the specific function of the human locomotive system. The synovial membrane consists of two layers, the outer layer (subintima) and the inner layer (intima). More precisely, the s intima is a membrane which is one to three cell layers thick. Concerning the outer layer, three types are commonly found, the fibrous (rich of collagen), the adipose type (fatty) and the areolar (loose collagenous) type. The inner layer is responsible for secretion of lubricant fluid and only consists of a very thin cell layer. Generally, it is composed of two types of synovial cells, the fibroblast like-synoviocytes (FLS or Type-B cells) which are responsible for the production of synovial fluid to reduce friction during movements, and the macrophage like-synovial cells (Type-A cells), Particularly, Type-B cells are located in the deeper layer of the synovial intima and are very important in maintaining the joint homeostasis. Furthermore, Type-B cells are relevant in production of hyaluronic acid, collagen and fibronectin and form a regular network of the synovial membrane. In contrast, (Type-A cells) are non-fixed cells that can be found at the superficial layer and are relevant for the removal of endangering molecules via phagocytosis to keep the synovia protected. These cells play a key role in antigen presentation and immune response by expressing MHC II molecule and Ia antigen. Besides, many other cells can be found in the synovial fluid like lymphocytes, erythrocytes, monocytes, endothelial cells and neutrophils. It also may happen that cells from surrounded tissues like chondrocytes, osteoblasts or osteoclasts successfully migrate into synovial fluid (Iwanaga et al. 2000).

1.1.2.1 Macrophages in synovial inflammation

Synovial macrophages (M0) belong to one of the resident cell types in synovial tissue, (mentioned 1.1.2) and are responsible for secretion of pro-inflammatory cytokines. During inflammation, they become activated either by acetylation or de-acetylation of histones or by pro-inflammatory stimulation of cytokines and chemokines (e.g: TNFα, IL-10) and can secrete either pro- or anti-inflammatory cytokines. M0 macrophages are involved in many inflammatory processes, including fibroblast proliferation, angiogenesis and recruitment of leukocytes and lymphocytes. Commonly they are present in normal synovial tissue. M1 macrophages, activated via INF-γ in combination with the master cytokine TNF-α or lipopolysaccharide (LPS), are known to release pro-inflammatory cytokines such as TNF-α or Il-1. Macrophages (M2), alternatively are activated by anti-inflammatory cytokines like IL-10, and participate in few anti-inflammatory responses. Both M1 and M2 macrophages need specific stimuli to be activated and because of that they only exist in inflamed tissues. As such, the number of macrophages (M0) in synovial tissue correlates with the grade of joint destruction. In inflamed synovial tissue, the number of M0 macrophages is increased compared to non-affected joints (Kennedy et al. 2011).

It is known that macrophages are the main promotor in progression of rheumatoid arthritis (RA). Unfortunately, much less is known about the role and relevance of macrophages in OA. However, some studies suggested that synovial macrophages are mainly involved in OA by producing pro-inflammatory cytokines, vascular endothelial growth factor and metalloproteases (MMPs). It could be possible that fibroblasts up-regulate their expression of pro-inflammatory cytokines and MMPs in presence of macrophages (Bondeson et al. 2010). Accordingly, the number of synovial macrophages may correlate with the grad of inflammation. Perhaps there is also a potential that FLS get stimulated by oncostatin M and IL-6, released by macrophages (Bondeson et al. 2010). Especially the importance to understand the expression of TNFα and IL-1β via NF-kB pathway is important for further biomarker studies to find new options for currently incurable OA (Bondeson et al. 2010).

According to human derived macrophages, the immortalized human leukaemia-derived monocytic peripheral blood cell line, is the most commonly used cell line to investigate and simulate the function of primary human macrophages. In general, the main function of monocytes is the protection against infections with gram-negative bacteria. THP-1 cells normally express low levels of Cluster of differentiation (CD-14), which is known to build together with toll-like receptor 4 (TLR-4) the lipopolysaccharide-receptor (Bosshart and Heinzelmann 2016). One of the best ways to differentiate monocytes into macrophages is using phorbol 12-myristate 13-acetate (PMA) and so they acquire phenotypic and typical functions and characteristics which closely resemble those of human macrophages (Lund et al. 2016).

This phorbol ester has antineoplastic effects because of its potential impact on gene expression and protein kinase C (PKC). Besides, PMA also may exhibit tumor promoting activity (National Cancer Institute n.d.).

1.1.3 Composition and structure of articular cartilage

Articular cartilage is highly relevant to maintain the structure and flexibility of the human knee joint. Daily, the whole locomotive system is under stress and this can lead to deformation of the whole joint and natural wear. To reduce this, articular cartilage provides a smooth surface and minimizes peak stresses on subchondral bone. In Articular cartilage there are no blood vessels, nerves and metabolic activity can be found, compared to other tissues. Articular cartilage mainly consists of a collagen network and proteoglycans which provide mechanical stability to the tissue. Often, stability gets lost because of external load and further leads to a change in the proteoglycan patterns. Thus, it comes to viscoelastic responses, and proteoglycan concentration within the solid matrix increases. The outcome of this is exceeded osmotic swelling pressure. Collagen and proteoglycans, which are in conjunction with water, form the properties and inner structure of articular cartilage. The whole structure is divided in four main zones. The superficial zone with the gliding surface of the joint, the transitional zone and deep zone including active chondrocytes, and the

zone of calcified cartilage which divides subchondral bone from softer cartilage (James and Uhl 2001).

1.2 Osteoarthritis

Osteoarthritis (OA) is one of the most common, degenerative joint diseases which furthermore leads to limitations in the locomotive system. Patients who suffer from OA are restricted to do sports or practice their profession or hobbies. This still incurable disease is characterized by progressive degeneration of articular cartilage leading to pain, significant loss of mobility and quality of life. Nowadays, a lot of treatments are available but a real cure is still missing. Many studies investigated the deformation and deterioration of articular cartilage to find new ways and options for suffering patients. The articular cartilage with its chondrocytes, is mainly responsible for maintaining the structure and strength of the whole joint (Glyn-Jones et al. 2015).

During loading, chondrocytes are permanently under stress and this drives them to produce inflammatory cytokines such as IL-6, IL-1β, TNFα and MMPs and a disintegrin and metalloproteinase with thrombospondin-like motifs (ADAMTS). All these mentioned mediators have dispositive pathogenic effects on OA (Glyn-Jones et al. 2015). To compensate these processes, new bone material, called osteophytes are formed. However, a stronger formation of these cells can also promote typical painful symptoms. All these mentioned mediators have dispositive pathogenic effects on OA (Glyn-Jones et al. 2015). Mainly there exist two types of OA. Primary OA, which is more commonly diagnosed, is associated with aging and typical loadings during life. Usually, people tend to develop this type in later age around 55-60. In contrast, secondary OA results from different events such as injuries, obesity, inactivity, genetics or co-diseases. The treatment (see 1.4) for both types is the same (Marie Suszynski 2016). The causes for this disease are numerous. Several anatomical factors including high systemic bone mineral density, hip dysplasia and tibia and femoral bone changings increase the risk to suffer from OA. Besides, the main functional factor, loosing function of quadriceps could also increases the risk of OA (Glyn-Jones et al. 2015). Genetic predisposition and gender also play an important role. Besides,

several studies refer to the fact that adipokines (adiponectin, leptin, and visfatin) have an impact on joint disease. Often they can be found in synovial fluid of patients with OA and because of their pro-inflammatory activity they can lead to the typical symptoms of this malady. The grade of this disease is decisively important for the specific treatment (Calvet et al. 2016). Practice sport and maintain a "normal" weight still can be enough to reduce the risk of suffering OA (Heidari 2011).

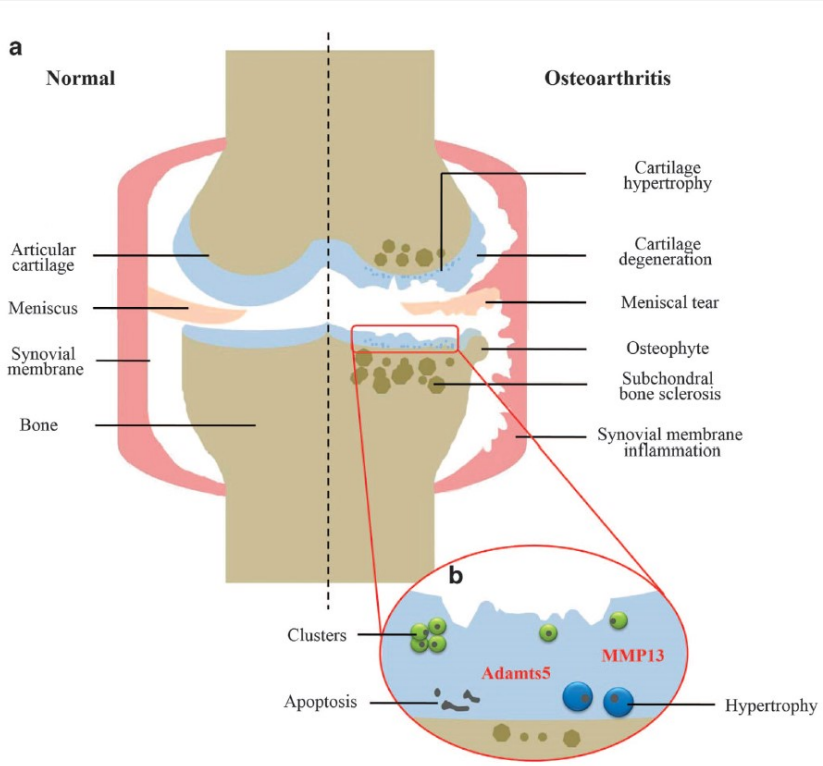


Figure 1 Comparison of healthy and OA cartilage. (a) Cellular process in OA cartilage. Image taken from (Zhang, Ouyang, Dass, & Xu, 2016) licensed under a Creative Commons Attribution 4.0 International License - https://creativecommons.org/licenses/by/4.0/

1.2.1 Synovitis in osteoarthritis

Particularly, synovial tissue also is affected in many patients with OA. Any abnormality or change of FLS such as synovial lining hyperplasia, neoangiogenesis, fibrosis and infiltration of macrophages can be causes for synovitis in OA. Typical catabolic cytokines such as TNF-α and IL-1 are produced by these macrophages and inflammation of the synovium continues. According to that, macrophage activation

and releasing MMPs damages not only the synovium but also the surrounding articular cartilage (Wenham and Conaghan 2010).

Furthermore, lower expression of the integrin laminin in synovial lining cells (SLC) (α6 and β1) is associated with chronic synovitis (Rinaldi et al. 1998). Normally, integrins interact with FLS to maintain the formation of a normally organized synovial inner lining. Moreover, the sublining layer can also be affected by invaded inflammatory cells including lymphocytes, macrophages and plasma cells. This great influx of cells may explain the increased glucose metabolism in synovial tissue (Bustamante et al. 2017).

Symptoms of synovitis are very similar to those of OA, such as moderate swelling, stiffness and loss of mobility but it is said that the pain is more intense measured by pain visual analog scale (VAS). With modern imaging methods such as magnetic resonance imaging (MRI) it is possible to detect synovitis in its initial phase. Different MRI markers are used to measure the synovial membrane thickness and synovial volume and viscosity. Another useful marker is the C-reactive protein (CRP) which correlates with inflammation status. Moreover, synovitis is substantially involved in OA and further clinical studies are needed (Wenham and Conaghan 2010).

1.3 Roles of cytokines

1.3.1 Inflammatory cytokines

This group of cytokines is mostly involved in the pathogenesis in OA. They are responsible for catabolic and destructive tissue processes via intracellular pathways. The most relevant mediators are: IL-1β, TNFα, IL-6, IL-15, IL-17 and IL-18. Especially IL-1β is one of the key cytokines leading to inflammatory reactions and degeneration of articular cartilage. Normally, the IL-1β level is increased in patients with OA. This cytokine binds to a TLR-receptor and affects on the metabolism of cells and the extracellular matrix (ECM). Furthermore, it increases the activity and synthesis MMPs and a ADAMTS, mainly MMP-3, MMP-13 and ADAMTS-5 (Wojdasiewicz, Poniatowski, and Szukiewicz 2014).

1.3.2 Anti-inflammatory cytokines

IL-4, IL-10 and IL-13 are the main anti-inflammatory cytokines regarding the pathogenesis of OA. Receptor dimerization of IL-4Rβα and IL-2Rγc is responsible for the attachment of IL-4. Then, phosphorylation induces an intracellular signal transduction to express several pro-inflammatory genes. This cytokine is often associated with chondroprotective and inhibiting effects concerning the degradation of proteoglycans by stopping MMP production. In addition, synthesis of IL-1β, TNF-α, and IL-6 decreases and inflammatory processes are reduced. The main functions and inhibition effects of IL-10 are similar to IL-4. Studies showed that IL-10 on the one hand down-regulated the mRNA level of IL-1β and on the other hand reduced the binding between IL-1β and its receptor in synovial fibroblasts. It also may inhibit the synthesis of cyclooxygenase-2 (COX-2) and this could lead to lower levels of prostaglandins (PGs) (Wojdasiewicz, Poniatowski, and Szukiewicz 2014).

1.4 Therapy concepts in OA

Unfortunately, there exists no real cure for patients with OA, only the symptoms can be regulated. First of all, patients with high risk to suffer from OA, should change their lifestyle. Reducing weight leads to decreased mechanical stress and is one of the most important first steps. Unfortunately, this step often is insufficient and therefore other pharmaceutical therapies, not to heal but to control this disease, are given (Wei Zhang et al. 2016).

1.4.1 Pharmacologic therapy

Pharmaceutical drugs can improve the life quality and alleviate pain and partially the inflammation. Acetaminophen (paracetamol) and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the treatment of OA. These pain-mitigating drugs also have anti-inflammatory effects which have long been used as an important remedy for OA. The advantage of non-steroidal anti-inflammatory drugs (NSAIDs) is

that they are more effective than paracetamol according to pain relief. However, a high risk of gastrointestinal bleeding and cardiovascular diseases is given because of the inhibition of COX-1 and the synthesis of prostaglandins. According to that, co-therapy with proton-pump inhibitors (PPIs) is indicated. In high doses, NSAIDs are less hepatotoxic but more nephrotoxic in contrast to paracetamol. According to Osteoarthritis Research Society International (OARSI) guidelines (W. Zhang et al. 2008), up to 4000 mg per day of paracetamol is an effective treatment especially for patients with mild-to-moderate symptoms. Patients with chronic liver disease clearly should not take this drug because it may be toxic to the liver. Patients with moderate-to-severe OA may also take opioids to relieve pain if NSAIDs are contraindicated or ineffective. Opioid analgesics do also have several side effects including nausea, vomiting, sleepiness and headache. These drugs are not for routine use only for second line therapy. Another possibility is intra-articular injections of corticosteroids and hyaluronic acid mainly for patients who do not respond to oral analgesics (Wei Zhang et al. 2016).

1.4.2 New drugs

The continuous search for new therapy concepts is important to minimalize side effect and improve the pharmacological properties of already known substances. Few of them received the approval for further clinical studies. In particular, the following candidates hold great promise for improvement of OA conditions: Bone morphogenetic protein-7 (BMP-7), Interleukin-1β receptor antagonists, monoclonal antibodies directed against beta-Nerve growth factor e.g Tanezumab, fibroblast growth factor and methotrexate which is normally used against RA (Wei Zhang et al. 2016).

1.4.3 Regenerative therapy

Implantation/transplantation of chondrocytes is a current method to repair or regenerate damaged cartilage. This process counts to the category tissue engineering. Bioactive factors should enhance cell migration, attachment and proliferation of

healthy FLS and chondrocytes and is widely used in clinical practice in OA. Recent studies investigated the reliability and efficacy of implantation mesenchymal stem cells (Wei Zhang et al. 2016). Moreover, a second promising method is gene therapy. Several preclinical studies showed its efficacy and safety, especially the transforming growth factor beta (TGF-β) gene therapy has been investigated (Wei Zhang et al. 2016). Further experiments have to be done to really establish these mentioned candidates (Wei Zhang et al. 2016).

1.5 Diagnosis of OA

1.5.1 Diagnostic biomarkers related to collagen metabolism

Ideally, diagnostic biomarkers are able to identify the period of OA where therapy concepts could be most effective. The early detection is the most important step to avoid damage and inflammation. This group of biomarkers can distinguish between people with and without OA with probability ratios including specificity, sensivity and area under the curve. Ideally, these biomarkers should identify early stages of OA, to quickly start with treatment. For instance, C-terminal telopeptide of collagen type II (CTX-II) could be a potential biomarker, because of its increased levels in urine in patients with OA. Some other potential diagnostic biomarkers are follistatin-like protein-1 (FSTL-1) or different fibulins (Fib3-1 and Fib3-2). Furthermore, the research for other great candidates should clearly be continued (Lotz et al. 2013).

1.5.2 Prognostic Biomarkers

In contrast to diagnostic biomarkers, they can be used for the determination of risk in patients with OA. As mentioned above, CTX-II could predict the loss of cartilage or rather the stage of degeneration of cartilage tissue. For bone turnover it is also a feasible marker but more data is needed. Moreover, inflammatory biomarkers could also be able to predict outcomes in patients with OA. A new collagen type-II specific neoepitope (C2C) has been discovered that might be promising for the prediction of cartilage loss. Definitely there is a huge potential for biomarkers in drug development

in OA and therefore it is necessary to continue research and find more attractive targets like CTX-II (Lotz et al. 2013).

1.5.3 Markers of Synovium Metabolism

FLS usually produce HA which is one of the most important molecules to maintain the structure and integrity of the cartilage surface. A small change of this molecule leads to loss of lubrication end potentially to matrix degradation. Higher levels of HA are normally detected in serum in OA patients and could possibly be a solid OA marker (Nguyen et al. 2017). Another biomarker, YKL-40, also secreted by FLS, increases the proteoglycan synthesis (De Ceuninck et al. 2001). Increased levels of this glycoprotein correlates with serum CRP, a typical inflammatory marker in blood (Conrozier et al. 2000). Levels of YKL-40, a chitinase-like protein, also correlate with MMP1, MMP3, Interleukin-6 (IL-6) and Interleukin-7 (IL-7) in OA patients with total knee replacement surgery (Väänänen et al. 2014).

1.6 Glycobiology

Glycobiology is understood as studying the biosynthesis, structure and biology of saccharides which are also called glycans and in a wider sense carbohydrates. Their relevance to biomedicine, biotechnology and especially basic research is great. Glycosylation is the reaction between carbohydrates (a so called glycosyl donor) and proteins, lipids or other biomolecular structures. The glycocalyx, a sugar coat of all vertebrate cells, primarily contains glycoproteins, glycolipids and proteoglycans. These glycans play an important role in the control of innate and adaptive immunity. We distinguish between two main types of glycosylation, the N-linked glycosylation and the O-linked glycosylation. Regarding to galectins, they are able to bind both glycan types (N-linked and O-linked), but stronger to N-linked glycans. (Schnaar 2016). To differ the two glycosylation types in their individual set of sugars see Figure 3.

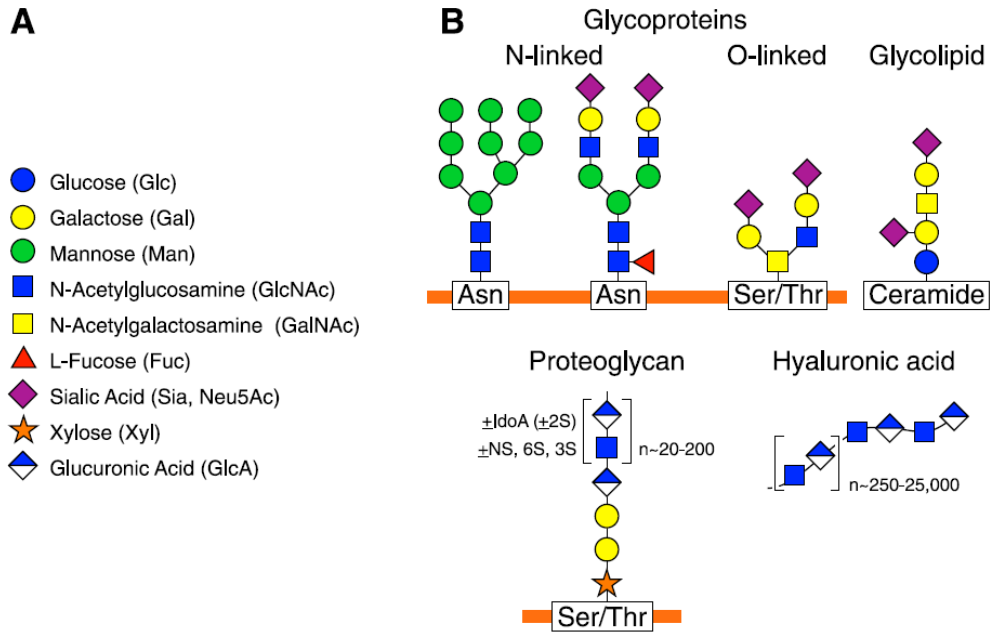
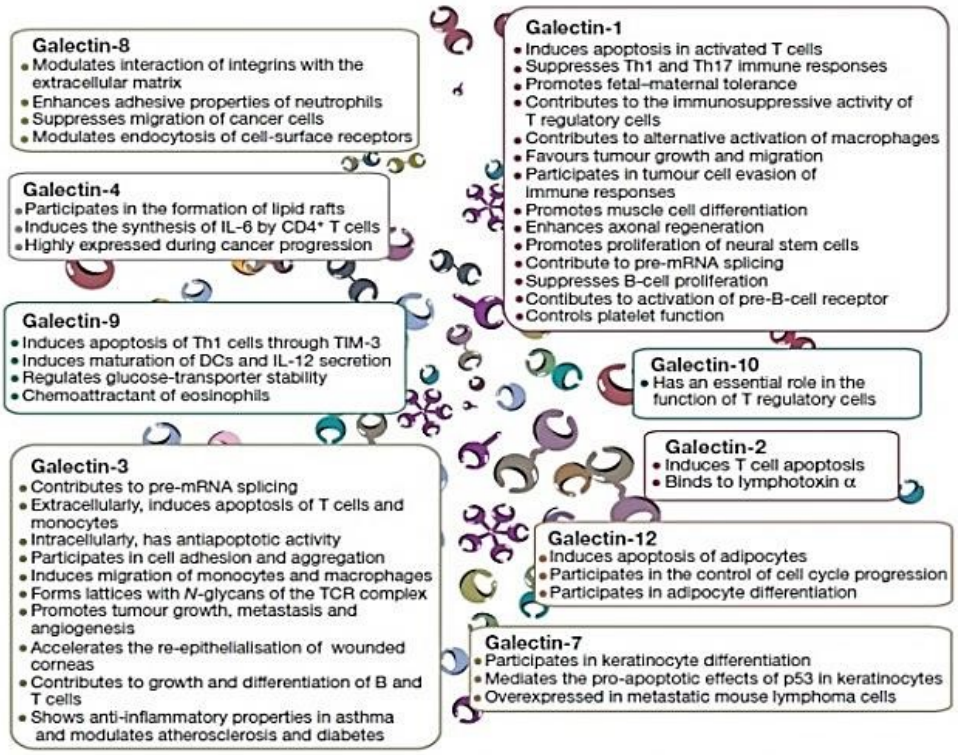


Figure 2 Major sugars of human glycome and linkage details. (A) Most common sugars. (B) Differences of the major classes of glycoproteins. Image taken from (Schnaar 2016).

1.7 Galectins

Galectins are proteins which are defined by their β-binding affinity for beta galactoside sugars. Currently, 15 different galectins from mammalia have been detected and synthetized. Each of them has different carbohydrates-recognition domains (CRDs) of about 130 amino acids they are very important and responsible for binding carbohydrates. Galectins distinguish from each other in terms of their carbohydrate-binding preferences. Especially the affinity to the saccharide ligand N-acetyllactosamine is very distinctive. Galectins affect a huge of different cellular reactions particularly immune responses and inflammatory processes (R. Y. Yang, Rabinovich, and Liu 2008). The figure below illustrates the individual functions of detected human galectins.



Selected biological functions assigned to different members of the galectin family

Figure 3 Essential biological functions from different members of the galectin familyTCR, T-cell receptor. (Image taken from Yang, Rabinovich, & Liu, 2008) Permission status granted see Page 76

1.7.1 Galectin-1

This lectin member counts to the homodimeric proteins and binds glycosylated ligands with his CRD. Often it can be detected at higher levels in different tumor cells. Binding to ECM gylcoproteins leads to adhesion and detachment of cancer cells. Gal-1 is involved in many metastatic processes by cross-linking integrins of tumor cell - ECM such as laminin and fibronectin (Cousin and Cloninger 2016). Mostly, it is detected on the outer cell wall and in the ECM of different tissues (Cousin and Cloninger 2016). It is well studied that binding glycans and its anti-inflammatory effector properties are associated with cartilage degeneration (Toegel et al. 2016). For differential diagnosis this lectin could be a great marker to distinguish between chondroblastic osteosarcoma and chondrosarcoma (Toegel et al. 2016). Besides, Gal-1 up-regulates MMP-13 gene transcription while type II Collagen gene transcription is

down-regulated at the same time. In summary this lectin member has been associated with cartilage degeneration in patients with OA and can be seen as a master regulator in inflammatory response-genes (Toegel et al. 2016).

1.7.2 Galectin-2

Gal-2 is normally expressed by monocytes and is similar in the structure comparing to Gal-1. It binds to the CD14, a co-receptor for toll-like receptor-4 (TLR-4) and induces the gene expression of IFN-β. The expression of Gal-2 induces a higher pro-inflammatory status in monocytes and macrophages. Relating to the biological processes it also can induce T-cell apoptosis like Gal-1. Also, this galectin is encoded by the LGALS2 gene which is associated with myocardial infarction (R. Y. Yang, Rabinovich, and Liu 2008). Furthermore, in arteriogenesis Gal-2 plays an important role because of its monocyte migration inhibition and should be further investigated (Yildirim et al. 2015).

1.7.3 Galectin-3

Gal-3 is a unique member of the lectin family because of its structure and folding. Only Gal-3 counts to the chimeric-type and shows unusual tandem repeats of proline and glycine which are linked to one CRD. In the presence of specific carbohydrate ligands it can oligomerise and is able to crosslink glycans on the cell surface. As the other lectins, Gal-3 induces T-cell apoptosis and activates several immune cells like neutrophils and monocytes. The role of Gal-3 in tumor development and progression is documented in many scientific researches (Song et al. 2014). According to that, Gal-3 was linked to prevent apoptosis (Liu and Rabinovich 2005), in contrast, the truncated version of Galectin-3 (Gal-tr) inhibits metastasis in breast tumors and showed apoptotic activity in a mouse model (John et al. 2003). Higher serum levels of Gal-3 are associated with few risk factors for cardiovascular diseases, including serum lipids, blood pressure and renal function. The function as a potential prognostic biomarker for metastasis and different cancer types may be given (Eliaz 2013).

1.7.4 Galectin-4

Gal-4, a two CRD lectin member, was first expressed and isolated in the intestines and the stomach in rats. The similarity of these two domains is 40% but they are able to bind different ligands. Experiments in vitro showed that the intracellular Gal-4 is responsible for cell proliferation and apoptosis, whereas extracellular Gal-4 regulates intercellular adhesion. It may induce or deteriorate intestinal inflammation and showed contradictory roles in different types of cancer. This lectin member also plays an important role in promotion of axons growth and may be crucial for our central nerve system (CNS). Gal-4 showed that it has the ability to enhance epithelial cell moving into the mucosal epithelial barrier in the intestinal lumen. In sera of cancer patients, the level of Gal-4 was significantly higher and therefore it might be a useful predictor for cancer patients (Cao and Guo 2016).

1.7.5 Galectin-7

Gal-7 is encoded by gene LGALS7 and is expressed by keratinocytes. Altered glycosylation is one of many biological outcomes of this galectin and leads to tumor pathogenesis. The main role is clearly the expression in different carcinoma cells. Many cancer types including breast cancer, head and neck cancer, cervical cancer, bladder cancer and many more are induced by this protein (Kaur et al. 2016).

1.7.6 Galectin-8

The identity of Gal-8 is 30-40% similar to the other tandem-repeat type galectins and four N-linked glycosylation sites can be found. This protein is secreted by several cell lines and via trypsinization it is released from the cell surface of lung, kidney, spleen and cardiac muscle cells. There exist two isoforms, which differ in linker length, called small Gal-8 (Gal-8S) and long Gal-8L (Si et al. 2016). It is known that the linker plays an essential role in mediating cell adhesion and regulating different functions.

Former studies showed, that Gal-8 is potentially involved in the development of various types of cancer such as prostate and lung cancer. However, Gal-8 is an essential mediator of cell adhesion especially for providing structural integrity to tissues. As some other galectins it modulates different tumor pathways and cellular processes too (Zick et al. 2002).

1.7.7 Galectin-9

Gal-9 differs from the two folded symmetric dimers (Gal-1 and Gal-2) in its three common isoforms with different length of its linker sequences. Potent chemoattractant properties to eosinophils and induction of Th-1cells have been reported. It has also been shown that the levels of Gal-9-positive cells in synovial tissue and synovial fluid are obviously higher in sera of patients with RA than in OA. In animal models Gal-9 alters the process of nephrotoxic nephritis. It may play a key role in the regulation of glucose homeostasis by modulating the retention of glucose transporter 2 (GLUT-2) (Zick et al. 2002).

1.7.8 Galectin-12

This lectin differs from the others in its main functions, particularly in lipid metabolism and energy homeostasis. It is expressed by adipocytes in mice and in leucocytes in humans. Regulating the lipolysis by its modulating activities of lipolytic protein kinase a (PKA) signaling and enhancing the lipid droplet protein perilipin 1 (PLIN1) counts to the main tasks. These two signaling molecules are the major regulators of lipolysis. Furthermore, it may function as a very interesting target for treatment of few hazardous diseases such as metabolic syndrome, type 2 diabetes and insulin resistance (R.-Y. Yang, Havel, and Liu 2012).

1.7.9 Galectin related protein (GRP)

This lectin member belongs to the network of chicken and human galectins. GRP is expressed in B cells, in bursal epithelium and vessels. The function of the N-terminal segment of Human GRP is still unknown and no ligands for its CRD have been detected. It's fundamental role and main functions have not been analyzed in details yet (Wälti et al. 2008).

1.8 Important functional receptors in humans

In case of Gal-1, it can bind a broad range of glycoprotein receptors including CD45, CD7, CD43, CD2, CD3 and many more (María T. Elola et al. 2005). One possible family of receptors are the toll like receptors (TLRs) are essential for our innate immunity system and are indispensable for recognizing invading microorganisms and pathogens. They belong to the group of Pattern-Recognition-Receptors (PRRs) with several signaling pathways. Stimulation of these receptors leads to the induction of pro-inflammatory cytokines such as TNF-α, IL-6, IL-12 and triggers expression of several genes which are important for immune responses. Every different TLR has his specific ligands and recognizes microbial components such as: lipoproteins, lipopolysaccharides (LPS), peptidoglycans and many more. Nowadays 11 Members of TLR are detected in Mammalia, but TLR 1-9 are mostly common for humans (Takeda and Akira 2005). Especially Gal-3 is an important sensor regulator of toll like receptors in synovial fibroblasts (Arad et al. 2015).

1.9 Aims of the study

Researching on biomarkers is very important for the early diagnosis and therapy monitoring in OA. Therefore, one aim of the study was to establish the methodology for anti-galectin autoantibody detection in our research lab in healthy test persons. This could form a base for further biomarker studies.

The second aim of the study was to analyze the activity of various galectins in THP-1 monocytes and macrophages and in human OA FLS (Passage 4). Therefore, THP-1 monocytes were differentiated with PMA into macrophages. Experiments were performed in non-starved and starved THP-1 cells. Synovial tissue was obtained and FLS were isolated and passaged to P4 to reach an homogenous FLS population without any macrophages. Finally, we treated all cell types with various recombinant galectins to further investigate the gene expression of typical pro-inflammatory target genes such as TNFA, IL1B and IL8 by qPCR.

2 Materials and Methods

2.1 Tissue collection

Synovial tissue was freshly obtained from OA patients during total knee replacement surgery. These surgical interventions were done at the Department of Orthopedics and Trauma Surgery, Medical University of Vienna. All patients signed documents of informed consent in accordance with the terms of the ethics committee of the Medical University of Vienna (EK-Nr.: 1822/2017). This also applies to the obtained plasma samples from healthy test persons.

2.2 Cell culture

2.2.1 Isolation of human FLS

Knee joint tissue, provided by orthopedic surgeons, was obtained in sterile physiologic salt (NaCl) solution to maintain the structure of the tissue. Synovial tissue was cut into small pieces on petri dishes (Corning, 100mm x 20mm Polystyrene) by the use of forceps (Wipak Steriking R41) and disposable scalpels (Medisafe, Surgical safety scalpels). After that, the minced synovial tissue was transferred into 50mL Polypropylene conical tubes (FALCON). To promote tissue digestion, sterile-filtered medium, containing 300Units/ml of lyophilized Collagenase Type II (Gibico by Life Technologies) was added and the tubes were sealed with PARAFILM (Benis). Afterwards the tubes were shaken overnight at 37°C at 70rpm/min (Benchmark, Incu-Shaker).

2.2.2 Separation and Cultivation

The separation of the FLS from the suspension was done with a 70µM cell strainer (FALCON) by using approximately 40ml Dulbecco's Phosphate Buffered Saline. The collected cell suspension was then centrifuged at 1000 rpm for 8min (AWEL Industries, Centrifuge CF 108-GR). The supernatant was aspirated and the pellet was resuspended with 3ml "Full Medium" made of Dulbecco´s Modified Eagle's Medium

(DMEM) containing 4.5g/l glucose and 10% fetal bovine serum (FBS) and 1% gentamycin (Gibco by Life Technologies). Then, cells were counted with IVO Piece Counter 130 and were seeded onto culture flasks in an incubator (RIEGER, Labotect Inkubator C200) at 37°C and 5% CO_2/90% Air.

2.2.3 Passaging of FLS

Normally, passaging starts from a T25 culture flask (TPP) when cells reach a confluency of approximately 80-90%. Full medium was removed and FLS were washed with PBS and 2ml of 0.25% Trypsin (Gibco by Life Technologies) was added and incubated for 3min. Then the suspension was checked under the microscope whether all cells were detached. The next step was stopping the enzymatic reaction by adding approx. 10ml full medium to the suspension. Centrifugation and removal of the cell suspension were done and afterwards the pellet was resuspended in full medium, so they were ready to count. Then, cells were seeded in T25 flasks and cultured with DMEM containing 4.5g/l glucose and FBS.

Cells were seeded in larger flasks (T75, T150, T225) when they have reached the optimum confluency in passage 0 (P0) In P0, the cell suspension consists of different cell types including FLS, erythrocytes, immune cells and endothelial cells. To obtain a pure population of FLS, the cell mixture was passaged to P4. Figure 5 shows different passages and morphological changes of FLS. All following experiments were performed in P4.

2.2.4 Cell cultivation of THP-1

First of all, THP-1 cells were rapidly thawed at 37°C in a water bath for approximately 2min. Then the vial content was put in a centrifuge tube containing 9.0mL “complete growth medium” and spinned at 800rpm for 5min. Complete growth medium was used for rapidly growing cells and contained RPMI 1640 (Gibco by life technologies), 5ml Penicillin-Streptomycin (Gibco by Life Technologies), 50ml FBS (Gibco by Life Technologies) and 250µL Amphotericin B. Then, the cell pellet was resuspended in

growth medium and cells were seeded onto a T-75 flask (TPP) and put in the incubator at 37°C and 90% air. For ideal growing it is important to keep the flask in vertical position. Furthermore, this cell line is particularly sensitive to movements, so the flask should be moved as little as possible. When exponential phase of growth is reached (it can take up to 7 days) and the culture is established, the serum concentration can be reduced to 10%. To keep cells in exponential growth, the quantity of 3-8 x 10^5 cells/ml should not be exceeded.

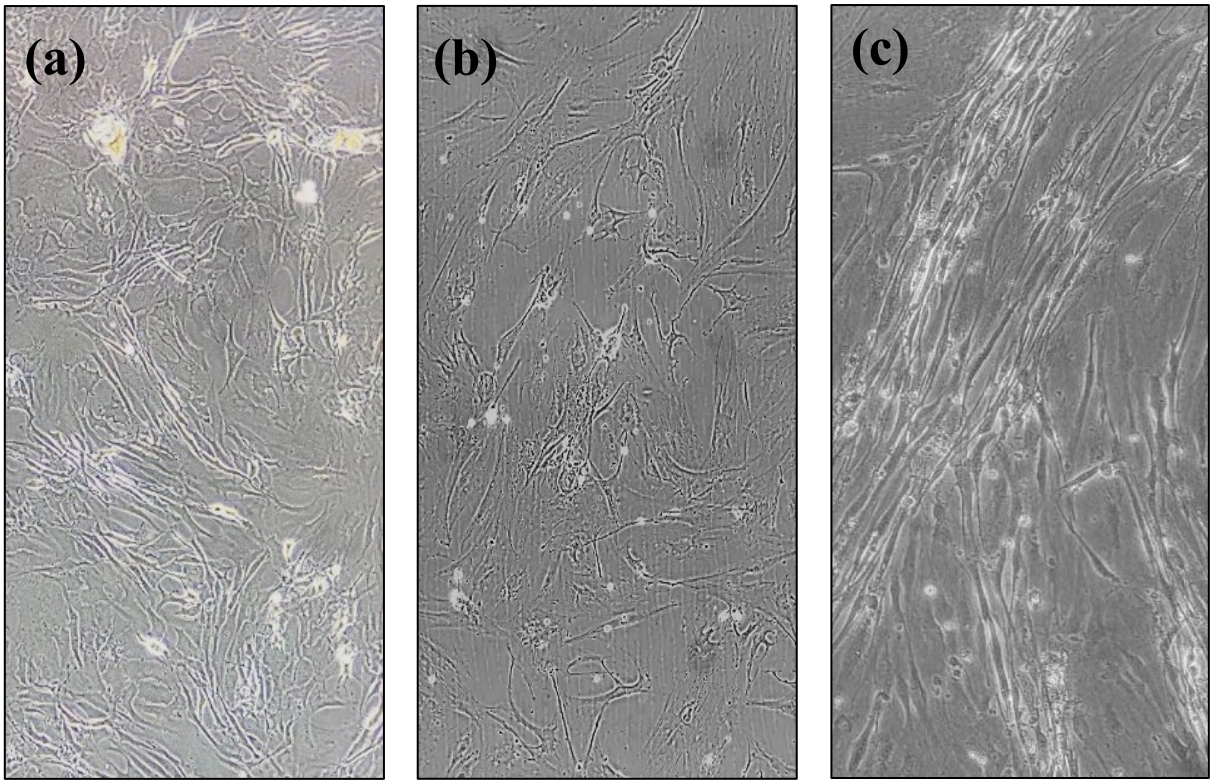


Figure 4 Overview of FLS and morphologic changes in different passages (x10).
(a) Passage 1 (b) Passage 2 (c) Passage 3

2.2.5 Counting cells

FLS were counted in a Neubauer hemocytometer (C-Chip, DHC-N01) Thus, to detect dead cells 10µL trypan blue and 10µL cell suspension were mixed. 10µL of this mixture were pipetted into a counting chamber. All four fields of the hemocytometer were counted to determine the living cells. The counting process was exactly done according to Figure 6.

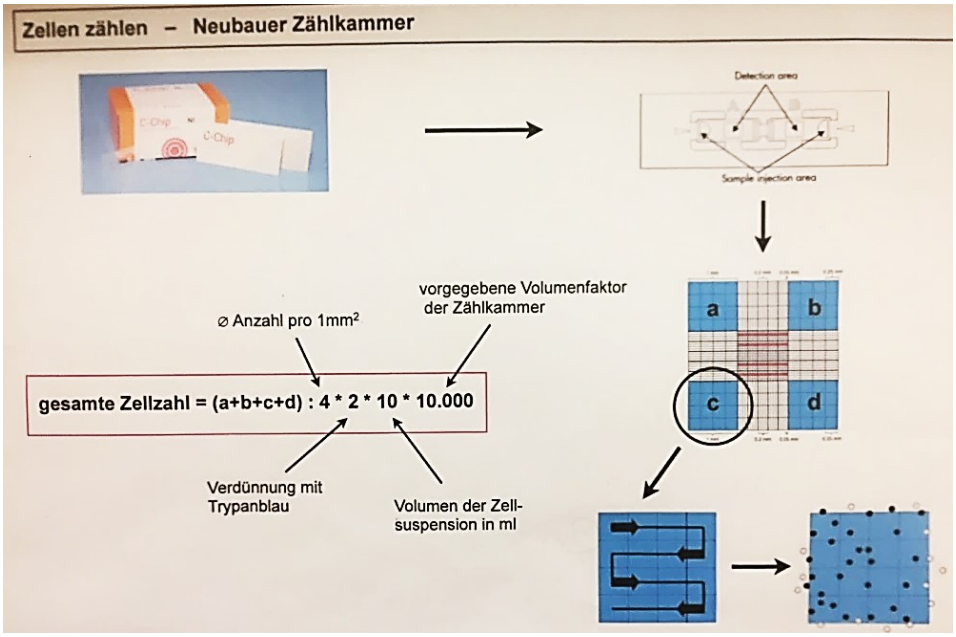


Figure 5 Schematic process of counting cells with Neubauer hemocytometer. Courtesy of Karl Chiari Lab for Orthopaedic Biology.

2.2.6 Starvation and treatment

Generally, serum starvation reduces basal cellular activity because of missing FBS for cell growth and proliferation and makes the cells more sensitive. This method is often used to study different molecular mechanisms and creates some kind of "stress situation" for the cells (Pirkmajer and Chibalin 2011). The composition of starvation medium is similar to the full medium but without FBS. FLS were starved overnight and were treated for 24h. THP-1 cells were treated with galectins either under starved or non-starved conditions. The following treatments were performed either in 6-Well or 12-Well plates with different concentrations of various galectins (see Figure 7). The used galectins were provided by Hans-Joachim Gabius´ Lab.

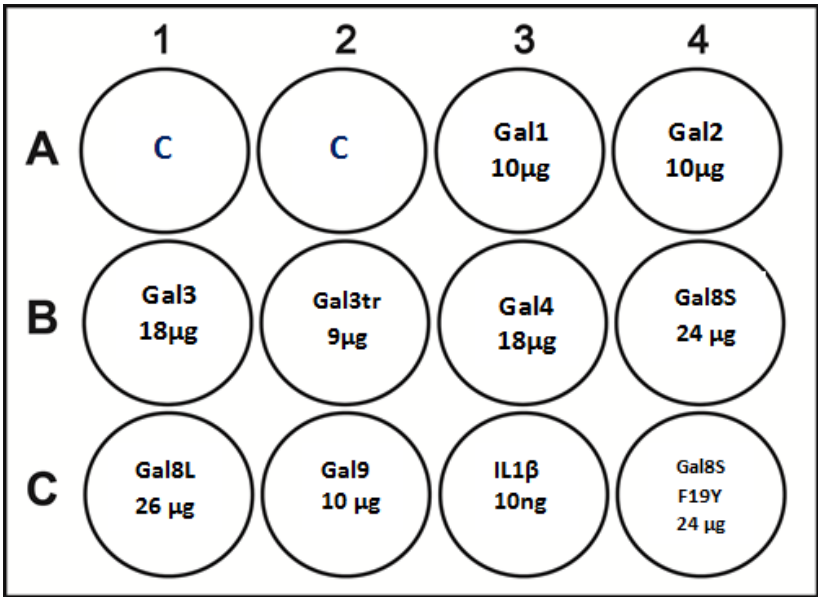


Figure 7 Galectin Treatment with different concentrations of galectins in FLS and THP-1 cells. Different concentrations of galectins including 10µg/ml Gal-1, 10µg/ml Gal-2, 18µg/ml Gal-3, 9µg/ml Gal-3tr, 18µg/ml Gal-4, 24µg/ml Gal-8S, 26µg/ml Gal-8L, 10µg/ml Gal-9, and 24µg/ml Gal_8SF19Y adjusted to their specific molecular weight were used. In addition, 10ng/ml IL-1β were used.

2.3 Lysis and RNA-Isolation

After treatment, cells were washed with PBS and then lysed by adding 350µl Lysis Solution RL per well. Then, up and down pipetting for several times was done to lyse all of the cells. For RNA isolation, innuPREP RNA Mini Kit from Analytik Jena was used.

At first, the lysate was filtered through innuPrep Spin Filter D, to remove selectively the gDNA and centrifuged for 2min at 11,000 rpm in an Eppendorf Centrifuge 5415D. Then the filtrate was saved because this contains the RNA and the filter was discarded. A new innuPrep Spin Filter R was prepared and 350µl of 70% ethanol was mixed with the filtrate and transferred onto the Spin Filter R. Then followed centrifugation for 2min at 11,000 rpm to bind the RNA on the filter and the filtrate was discarded again. The next step was to add 500µl of Washing Solution HS. Then the tube was centrifuged again for 1min at 11,000 rpm and the filtrate was discarded again. The remaining Spin Filter R was placed on a new Receiver Tube and 700µl Washing Solution LS were added followed by centrifugation for 1min 11,000 rpm. The RNA is still bound on the filter and the tube with the filtrate was discarded. The Spin Filter D

was placed again on a new Reveiver Tube and centrifuged for 2min 11,000 rpm to remove all traces of ethanol. Afterwards, the Filter R was placed on an Elution Tube and 40µl of RNase-free Water were pipetted onto the Spin Filter R, put into the centrifuge for 1min 11,000 rpm to elute the RNA. After this last step the RNA was frozen at -20°C or directly processed to complementary DNA (cDNA).

2.3.1 Quantification of RNA via Nanodrop

RNA concentration was determined using the NanoDrop 2000 (Thermo Scientific Pequlab Biotechnologie GmbH). First of all, the measuring surface was cleaned to avoid any contamination. After cleaning, molecular free water was loaded as blank, and the samples were measured by pipetting 1µl onto the measuring surface. The most important step is cleaning after every measurement to avoid contaminations.

2.4 Reverse transcription of RNA into cDNA

Transcription of the RNA to a single-stranded cDNA forms the base for quantitative polymerase chain reaction (qPCR). For this process, a special cDNA Reverse Transcription Kit (Applied Biosystems by Thermo Fisher Scientific) was used. At the beginning, all samples containing RNA, were diluted with RNase-free water to be sure that all of them have the same RNA concentration. Then, the final volume of every sample was 10µl. A reverse transcription Master Mix (see Table 1) was made and 10µl of it were mixed with the diluted RNA. All steps of this transcription were done on ice. The last step was the removal of air bubbles by centrifugation and placing the samples into a thermal cycler (Eppendorf Mastercycler Gradient). Detailed information on cycling conditions are given in Table 2.

Table 1 Schema of Master Mix composition.

Essential component	Volume (µl)
10x RT Buffer	2.0
25 x dNTP Mix (100mM)	0.8
10x RT Random Primers	2.0
MultiScribe® Reverse Transcriptase (50 U/µL)	1.0
Nuclease-free H_2O	4.2
Total Volume per Reaction	10.0

Table 2 Overview of thermal cycler program.

Step	Duration	Temperature
1	10 min	25°C
2	120 min	37°C
3	5 min	85°C
4	Hold	4°C

2.5 RT-qPCR

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is the most common real-time method for nucleic acids amplification based on the principal of polymerase chain reaction (PCR) and for quantification of mRNA levels. Detection is only possible by emission of fluorescence with the help of the dye SYBR Green,

which is measured after each cycle. This fluorescence reporter emits light when it binds to the double-stranded DNA. The more it binds, the more DNA is measured by its strong emission of bright light. Furthermore, it is safer than ethidium bromide regarding its toxicity and mutagenicity. One of the most important steps is using reference genes, such as succinate dehydrogenase (SDHA), whose expression level is unaffected by experimental factors such as galectin treatment.

The very first step was to dilute the thawed cDNA samples with RNase-free water. 8µl of H_2O, followed by 2µl per well of cDNA sample were pipetted into a small tube. A master mix, containing forward and reverse primer, SYBR green (SYBR Hi-ROX, Bioline) and nuclease-free water was necessary to carry out the complete reaction. Detailed information on its components and base sequences of primers are shown in the Table 3,4 and 5. respectively.

Table 3 Schema of Master Mix composition for RT-qPCR.

Essential component	Volume (µl)
Forward Primer	0.5
Reverse Primer	0.5
SYBR-Green	12.5
Nuclease-free water	10.5
Total per Reaction	24

Total volume (24µl) of master-mix were pipetted in 96-well plate (MicroAmp Thin-Walled Reaction Tube, Applied Biosystems) for each target and reference gene. Afterwards, 1µl of cDNA was added into the well. To control for pipetting errors, duplicates of each sample for target genes were made. Negative controls, one for each target gene, also were prepared to monitor potential contaminations. An adhesive film was put onto the plate which then was placed into the StepOnePlus Real-Time PCR instrument (Applied Biosystems).

Table 4 Detailed primer sequences of used genes for FLS.

	Forward Primer Sequence	Reverse Primer Sequence
SDHA	TGGGAACAAGAGGGCATCTG	CCACCACTGCATCAAATTCATG
IL1B	CTTATTACAGTGGCAATGAGGATG	AGTGGTGGTCGGAGATTCG
IL8	CAAACTTTCAGAGACAGCAGAG	CAGTGAGATGGTTCCTTCCG

Table 5: Detailed primer sequences used for THP-1 cells.

	Forward Primer Sequence	Reverse Primer Sequence
SDHA	TGGGAACAAGAGGGCATCTG	CCACCACTGCATCAAATTCATG
IL1B	CTTATTACAGTGGCAATGAGGATG	AGTGGTGGTCGGAGATTCG
TNFα	TCAGCAAGGACAGCAGAGG	CAGTATGTGAGAGGAAGAGAACC

Used samples and target genes were selected and the process was started by selecting the “run method”. Then the samples ran through 40 cycles and the completed file was saved. The specific information about the cycle program that was used is shown in Table 6.

Table 6 Temperature profile and cycling process of the RT-qPCR.

Cycle	Step	Temperature	Duration
Holding stage	Step1	95°C	10min
Melting Stage (40 Cycles)	Step1	95°C	00:30 min
	Step2	55°C	01:00 min
	Step3	72°C	01:00 min
Melt Curve Stage	Step1	95°C	00:15 min
	Step2	55°C	01:00 min
	Step3(Dissociation)	95°C	00:15 min

Denaturation at 95°C, to separate the double strands into single strands, primer annealing at 55°C and elongation at 72°C formed every of its 40 cycles. Normalization was done by means of using SDHA as reference gene. After completion of the run, melt curves were checked to verify the specific of the qPCR reaction and thresholds were set. Resulting C_T values are inversely correlated to the amount of nucleic acid in the samples, that means lower C_T values represent higher amount of mRNA, and vice versa.

2.6.1 Differentiation and treatment

THP-1 cells were used for experiments between passage numbers 5 and 10. When monocytes reached a good handling concentration, they were seeded onto 12-well plates at a concentration of $2x10^5$ cells/ml. For differentiation from monocytes to macrophages, 25nM of PMA in DMSO, diluted in media, was used. Differentiation process lasted for 48h and subsequently was stopped by replacing changing the medium to fresh medium without PMA. This step lasted 24h and is important to recover the cells. After recovery, starvation of cells for approximately 2h, followed by

treatment for 24h with different galectins has been done. Figure 8 shows the morphologic difference between monocytes and macrophages (magnification x10).

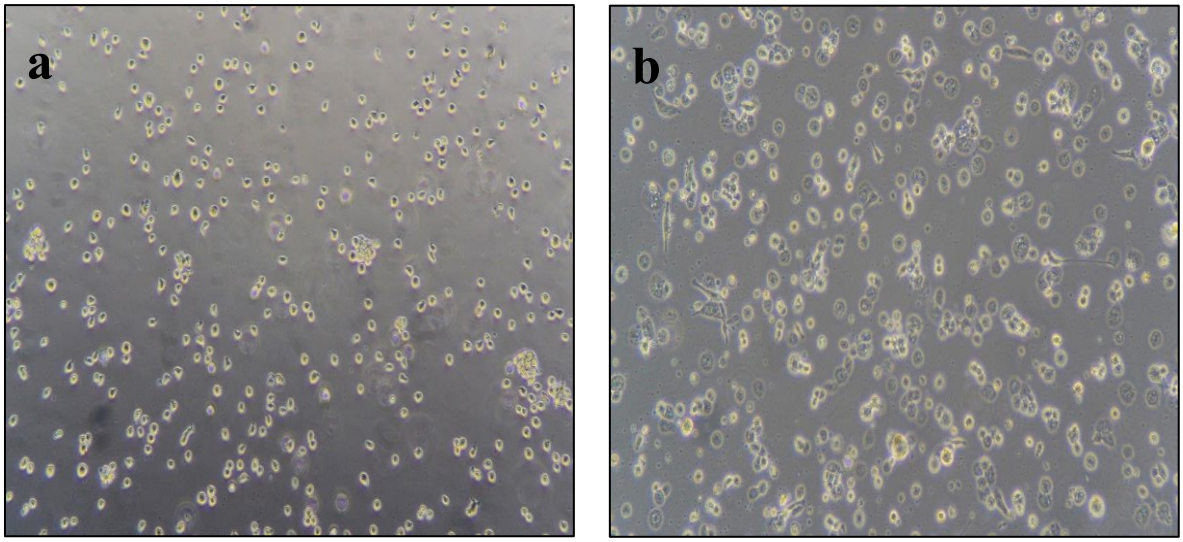


Figure 8 Microscopic view of the differentiation process of THP-1 cells.
(a) Shows monocytes without PMA after 48h (x10), (b) Shows macrophages with 25nM PMA after 48h (x10).

2.7 Enzyme-linked Immunosorbent assay (ELISA)

For detecting anti-galectin IgG autoantibodies in plasma from healthy individuals, home-made Sandwich-ELISA tests (see Figure 9) were performed. 96-well Maxisorb immunoplates (Nalgene Nunc, Sigma Aldrich), precoated with IgG antibodies, were used. Firstly, all buffers, washing and working solutions in the predetermined concentrations were prepared. Then 0.5µg of a specific galectin were mixed in 100µl coating buffer (15mM Na_2CO_3 and 35mM $NaHCO_3$) overnight at 4°C. Afterwards, all coated wells were thoroughly washed with phosphate-buffered saline (PBS)/0.05% Tween 20. In the next step, PBS/1% bovine serum albumin (BSA) was used for blocking and further to prevent non-specific binding between the antigen (galectin) and antibodies (IgG) of the Maxisorb immunoplate. After that, the plates were washed again three times with PBS/0.05% Tween 20. Next, a dilution series from 2µl to 0.125µl plasma was prepared (diluted in PBS/1% BSA/0.05% Tween 20) and added to each well, followed by incubation for exactly 1h at room temperature. Then, the plates were washed again with PBS/0.05% Tween 20 to remove all unbounded primary

antibodies. Afterwards, 100µl/well of secondary antibody, the horseradish peroxidase-labeled anti-human IgG F_{ab}-Fragment (Southern Biotech, Sigma Aldrich) diluted 1:10.000 in PBS/1%BSA, was added and incubated for 1h at room temperature. This antibody is conjugated to a peroxidase and is able to be activated by hydrogen peroxide (H_2O_2). Washing process with PBS/0.05% Tween 20 for three times was done again and 100µl of development solution was pipetted to each well and incubated for 15min at room temperate in the dark. During this time, the color of the samples turned into blue and the intensity of the color represents the quantity of autoantibodies. This is the decisive step for the detection of anti-galectin IgG autoantibodies. By freshly mixing 1mL of 3,3',5,5'-tetramethybenzidine (1mg/ml in DMSO) with 9mL of substrate buffer (0.1M Na_2HPO_4 and 0.05M citric acid monohydrate, ph 4.5-5.5) and 2µl of H_2O_2 the development solution is prepared. With the help of 100µl/well of 25% H_2SO_4, the enzymatic reaction was stopped and the color changed from blue to yellow. All plasma samples were tested in duplicates and the OD was measured at 450nm using the FLUOstar OPTIMA. The measurement at 650nm was served as a correction factor.

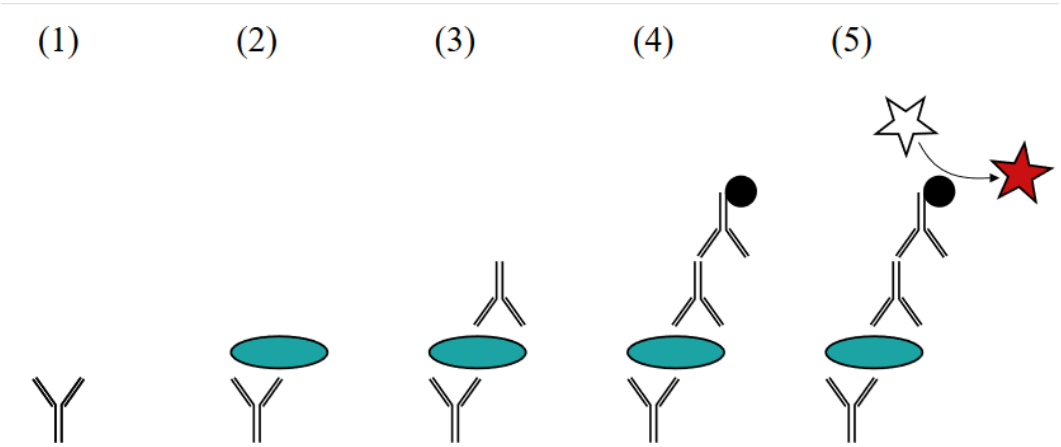


Figure 9 Typical procedure of a Sandwich-ELISA.
Image taken from https://commons.wikimedia.org/wiki/File:ELISA-sandwich.svg

(1) IgG antibody precoated on the maxisorb surface

(2) Antigen (Galectin) binds the IgG antibody

(3) Primary antibody (plasma) binds the galectin

(4) Secondary antibody (anti-human IgG Fab-Fragment) with linked enzyme binds the primary antibody

(5) Development solution transforms the substrate into a colored product

2.8 Statistical analysis

Results are expressed as mean ± standard deviation. T-test function from Microsoft Excel was used to examine statistical significances of the qPCR and ELISA results. For all results, two-tailed paired tests were used. The level of significance was set to $p<0.05$.

3 Results

3.1 Autoantibody ELISA: pre-tests

3.3.1 Plasma volume optimization

In order to define the optimal volume range of human plasma samples used in the ELISA, we performed pre-tests with Galectin-1. We observed that concentration dependency of results was given from 0.125µl to 2µl of plasma, whereas higher volumes of plasma (10µl, 50µl) did not yield higher absorbance values (Figure 10). Based on these results, we decided to perform ensuing tests with up to 2µl of human plasma.

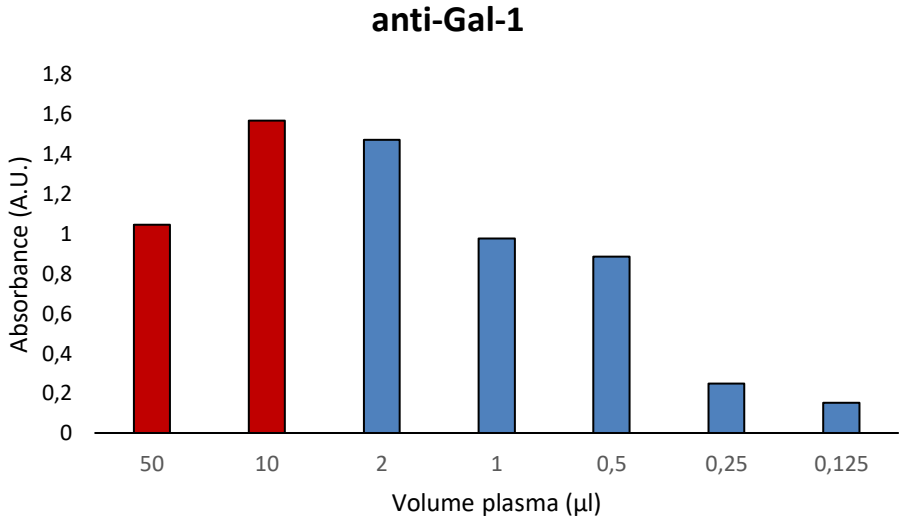


Figure 10 Pilot test targeting anti-Gal-1 autoantibodies using different volumes of plasma to determine the optimal sample volume for further tests. Absorbance was measured by ELISA at 450nm. The red bars demonstrate plasma volumes which were not used for further experiments. The blue bars show used plasma volumes in ELISA tests.

3.1.1 Serum and plasma yield comparable levels of autoantibodies

After having defined the optimal plasma volume for further tests, a comparison of serum and plasma samples was done to identify potential differences. Sera and plasma samples were taken from two healthy donors and ELISA tests were performed to detect anti-Gal-1 autoantibodies. In Figure 11, it is illustrated that there is no

difference in absorbance between serum and plasma at all concentrations tested. According to this finding, we decided to continue testing with plasma.

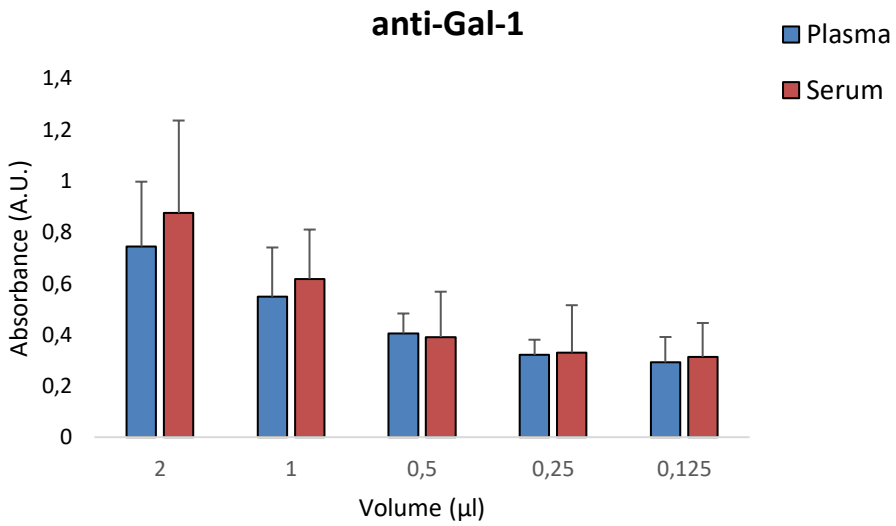
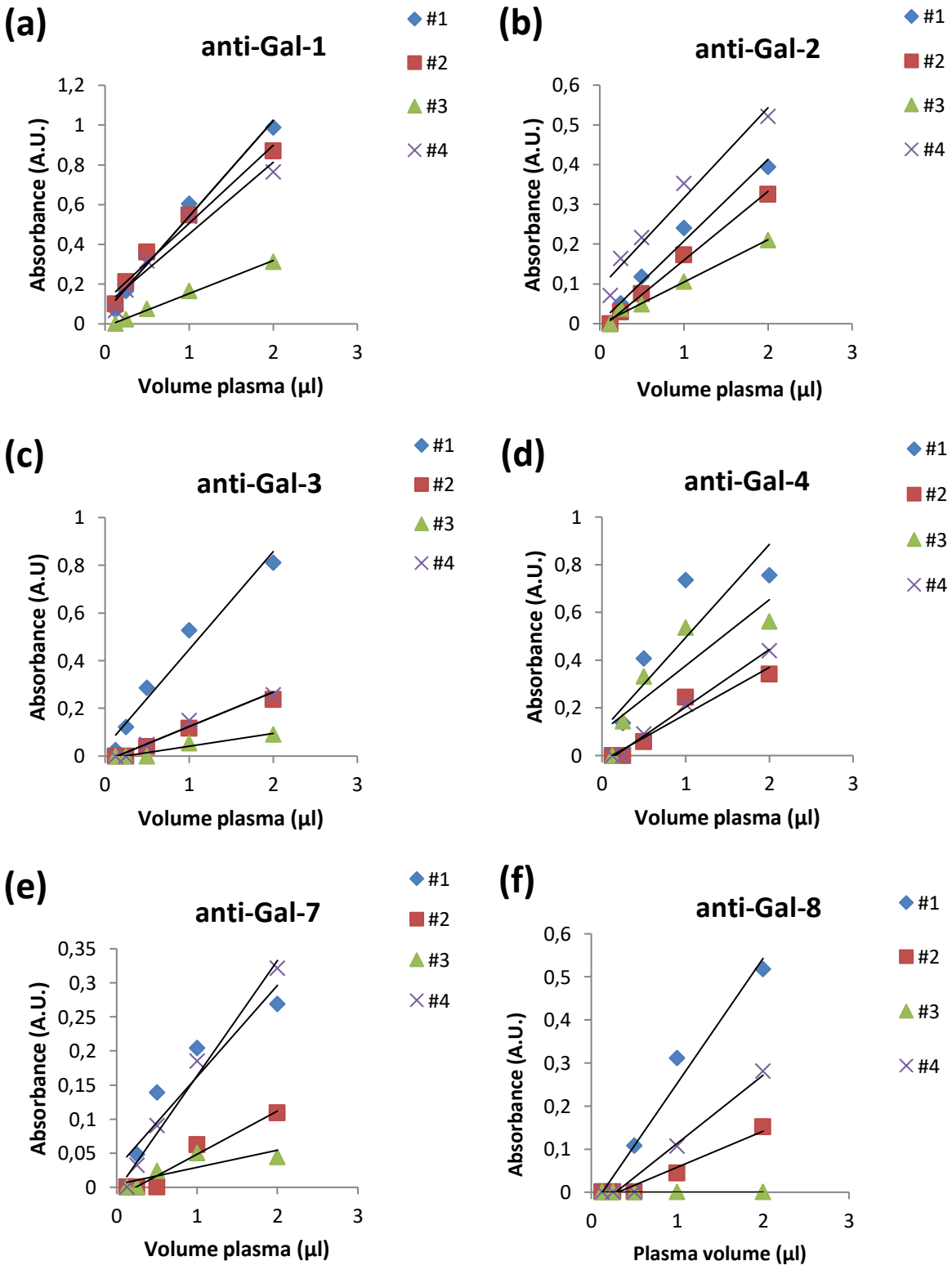


Figure 11 Levels of anti-Gal-1 autoantibodies measured in serum and plasma of healthy individuals (n=2 donors).

3.2 Levels of anti-Galectin autoantibodies in human plasma

The level of autoantibodies was measured in plasma of four donors with ELISA. In Figure 12, the levels of autoantibodies against (a) Gal-1, (b) Gal-2, (c) Gal-3, (d) Gal-4, (e) Gal-7, (f) Gal-8, (g) Gal-9, (h) Gal-12 and (i) galectin related protein (GRP) using five different concentrations of plasma are shown. Noteworthy, autoantibodies against all investigated galectins were found in the plasma of test person #1 (male). In comparison to donor #1, the plasma of test person #3 (female & vegetarian) yielded lower levels of almost all galectin autoantibodies. Test person #4 showed the highest level of anti-Gal-2. Furthermore, the levels of anti-Gal-3 autoantibodies in test person #1 were exceptionally higher than those in the other individuals. In summary, all analyzed anti-galectin autoantibodies were detectable in all healthy tested persons, except for anti-Gal-8 in donor #3 (Figure 12 (f)). The mean quantities of each anti-galectin autoantibody across all donors are presented in Figure 13, showing significantly higher levels of anti-Gal-1 and anti-GRP as compared to anti-Gal-3, -7 and -8 (Figure 13).


(a)
anti-Gal-1
Absorbance (A.U.)
Volume plasma (µl)
(b)
anti-Gal-2
Absorbance (A.U.)
Volume plasma (µl)
(c)
anti-Gal-3
Absorbance (A.U)
Volume plasma (µl)
(d)
anti-Gal-4
Absorbance (A.U.)
Volume plasma (µl)
(e)
anti-Gal-7
Absorbance (A.U.)
Volume plasma (µl)
(f)
anti-Gal-8
Absorbance (A.U.)
Plasma volume (µl)
#1
#2
#3
#4

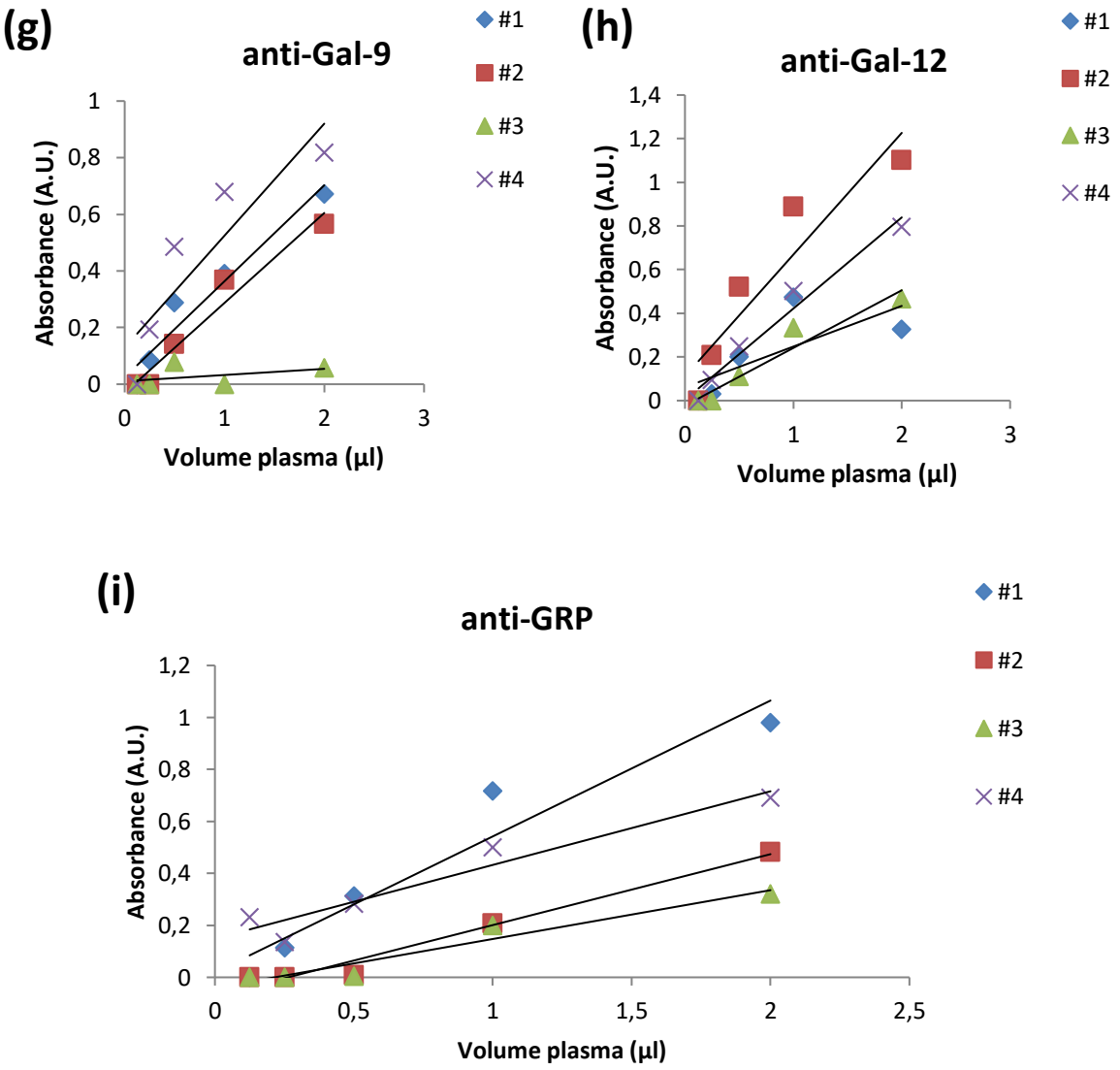


Figure 12 Detection of galectin autoantibodies in human plasma. Scatter plots show the levels of autoantibodies detected by ELISA in human plasma (n=4) against (a) Gal-1, (b) Gal-2, (c) Gal-3, (d) Gal-4, (e) Gal-7, (f) Gal-8, (g) Gal-9, (h) Gal-12, and (i) GRP (galectin related protein).

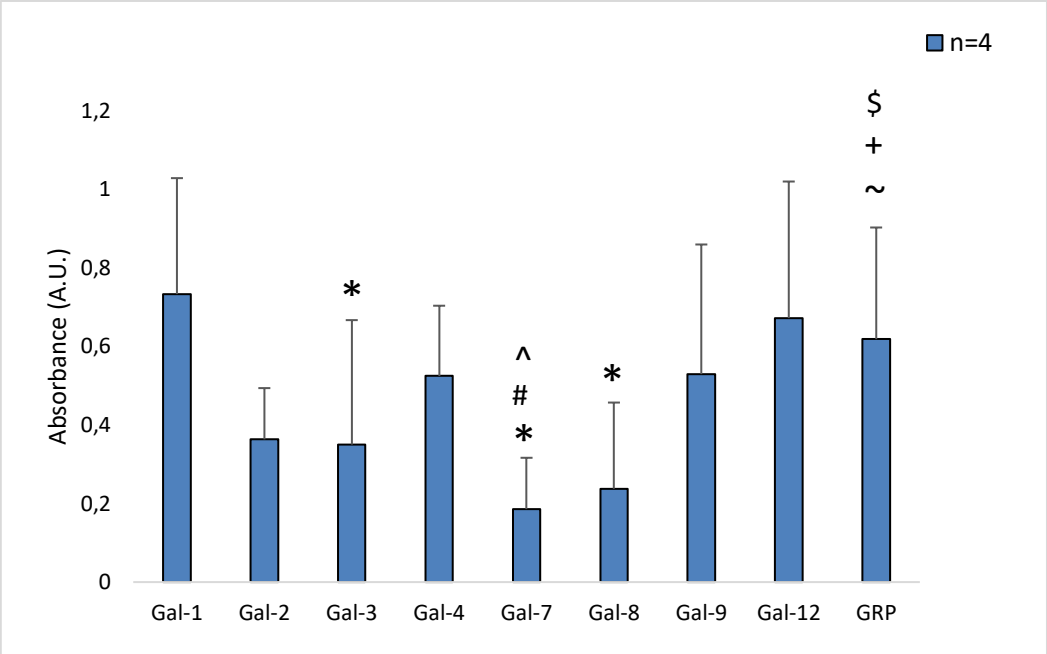


Figure 13 Comparison of various galectin autoantibodies in human plasma (n=4). Absorbance measured at 450nm. *p <0.05 (paired t-test vs Gal-1), #p<0.05 (paired t-test vs Gal-2), ~p<0.05 (paired t-test vs Gal-3), ^p<0.05 (paired t-test vs Gal-4), +p<0.05 (paired t-test vs Gal-7), $p<0.05 (paired t-test vs Gal-8).

3.2.1 Impact of gender and age on measured autoantibodies

Healthy test persons included men (n=2) and women (n=2). To show potential sex-related differences, male and female donors were analyzed separately and compared, showing that men tend to have higher quantities of anti-Gal-1, -3, and -8 autoantibodies. In contrast, all other anti-galectins tended to be similar (Figure 14). Furthermore, we were interested whether age is related to the amount of autoantibodies. Two test persons (23 and 25 years) and two (30 and 40 years) were compared. Results suggest that the test persons aged 23 and 25 had higher levels of all anti-galectin autoantibodies, except for anti-Gal-12, compared to the 30 and 40 aged test persons (Figure 15).

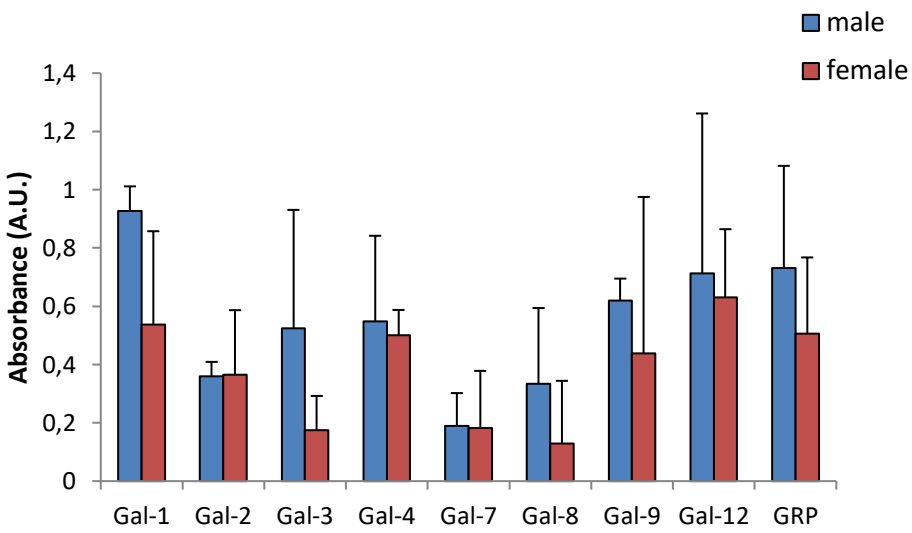


Figure 14 Overview of quantitative differences of anti-Gal antibodies.
2µl of plasma from healthy test persons (male n=2, female n=2) were used.

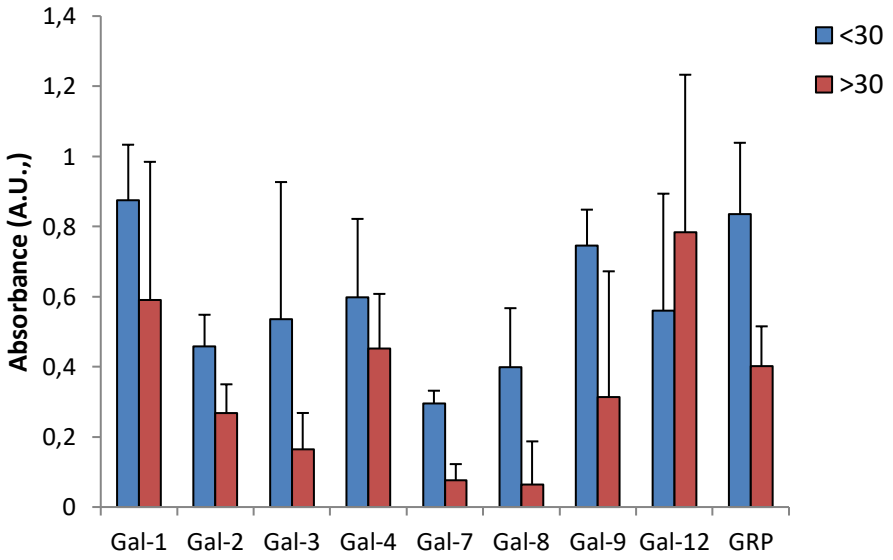


Figure 15 Anti-Gal autoantibodies of test persons under 30 (n=2) and over 30 years (n=2). 2µl of plasma from healthy test persons were used.

3.3 Impact of galectins on marker gene expression in FLS and THP-1 cells

To analyze the effect of different galectins on TNFA and IL1B gene expression in undifferentiated THP-1 monocytes, differentiated THP-1 macrophages and primary FLS, RT-qPCR experiments were performed. First, cells were treated with different concentrations of galectins (see 2.2.5) to adapt for their specific molecular weights. Following RNA isolation and reverse transcription, C_T values were measured by RT-qPCR and the common double delta C_t method was used for relative quantification. All data were normalized to the reference gene SDHA. The following concentrations of galectins were used for all experiments: 10µg/ml Gal-1, 10µg/ml Gal-2, 18µg/ml Gal-3, 9µg/ml Gal-3tr, 18µg/ml Gal-4, 24µg/ml Gal-8S, 26µg/ml Gal-8L, 10µg/ml Gal-9, and 24µg/ml Gal-8SF19Y. For comparison, cells were also treated with 10ng/ml IL-1β.

3.3.1 Marker gene levels in THP-1 galectin-treated macrophages

Figure 16 presents the alterations of target gene expression after galectin-treatment of differentiated (non-starved and starved) THP-1 macrophages. Apparently, this cell line showed reaction to all used galectins and IL-1β. The difference of mRNA expression of TNFA between non-starved and starved cells was minor in most cases. Clearly visible was that Gal-9 induced the strongest effect on TNFA up-regulation in THP-1 macrophages, whereas Gal-3 and Gal-3tr induced the lowest effect. In starved macrophages (TNFA) [Figure 16 (a)], Gal-9 activity was clearly higher than in non-starved cells. In contrast, IL1B seemed to be more up-regulated in non-starved than in starved macrophages following Gal-9 stimulation [Figure 16 (b)]. Furthermore, Gal-4 in non-starved macrophages and Gal-2 in starved macrophages induced higher mRNA levels of IL1B than the other galectins, but not than Gal-9. The truncated version of Gal-3 (Gal-3tr) had the lowest effects on IL1B and TNFA gene expression. Consistently, wild-type Gal-8S showed higher activity on both target genes than the single nucleotide polymorphism variant Gal-8S_F19Y.

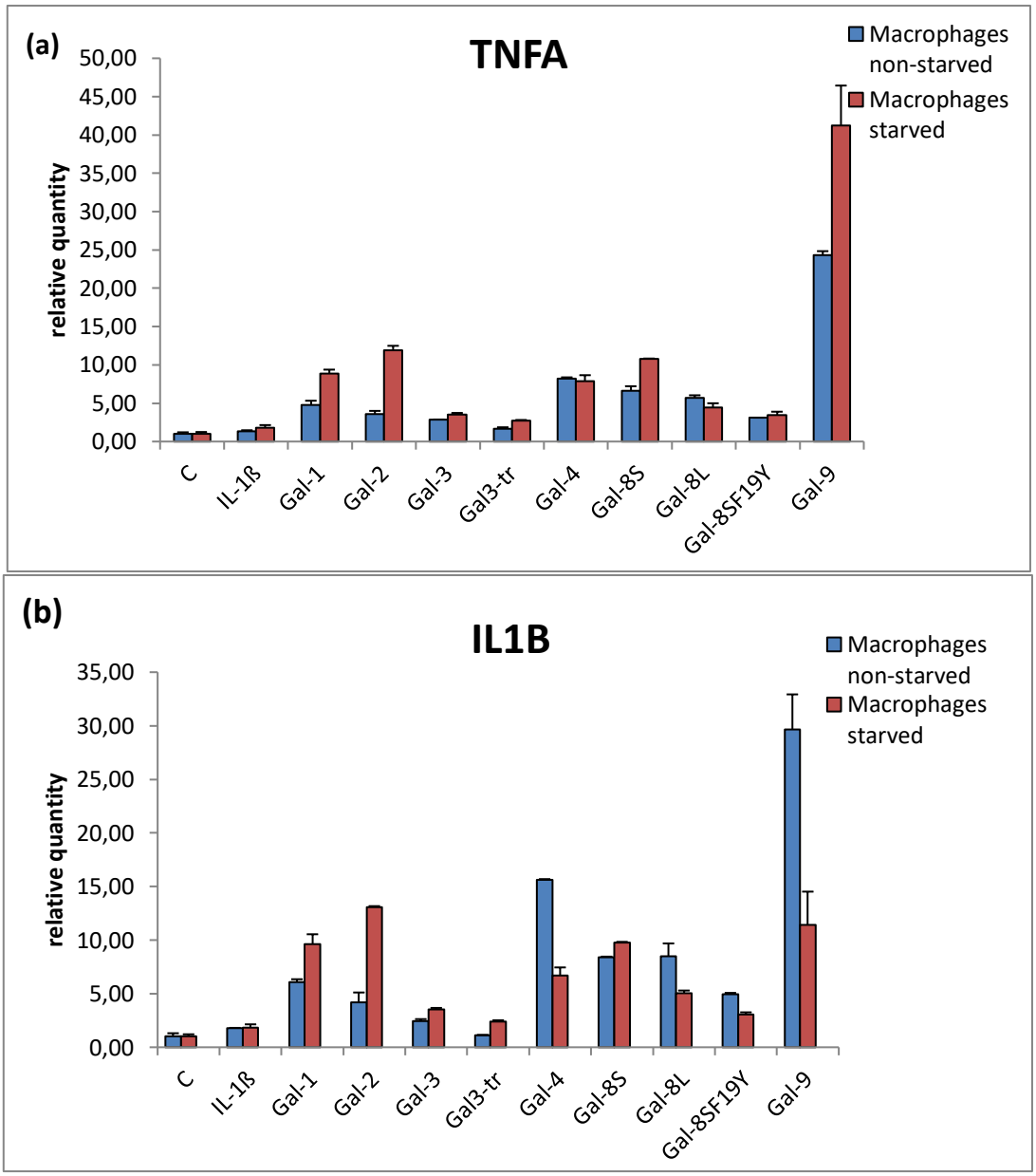


Figure 16 mRNA expression of target genes in non-starved and starved macrophages after galectin treatment. Different concentrations of galectins including 10µg/ml Gal-1, 10µg/ml Gal-2, 18µg/ml Gal-3, 9µg/ml Gal-3tr, 18µg/ml Gal-4, 24µg/ml Gal-8S, 26µg/ml Gal-8L, 10µg/ml Gal-9, and 24µg/ml Gal-8SF19Y adjusted to their specific molecular weight were used. In addition, 10ng/ml IL-1β were used. Shown is the relative quantity of target genes TNFA and IL1B measured with qPCR (n=1 experiment). (a) Comparison of TNFA gene expression between non-starved and starved macrophages following treatment with galectins. (b) Comparison of IL1B gene expression between non-starved and starved macrophages following treatment with galectins.

3.3.2 Gene expression in undifferentiated THP-1 monocytes

Figure 17 (a) illustrates that Gal-9 activity was remarkably higher in non-starved monocytes than in starved cells. Gal-1, Gal-2, and Gal-8S seemed to have a greater

impact on TNFA expression in non-starved monocytes compared to Gal-3, Gal-4, IL-1β, Gal-8S, Gal-8L and Gal-8F19Y in starved monocytes. In contrast, all galectins induced higher mRNA levels of IL1B compared to TNFA in non-starved monocytes. IL-1β, Gal-3 and Gal-3tr seemed to have the lowest effects on TNFA and IL1B. Additionally, Gal-9 treatment resulted in a 34-fold higher expression of IL1B compared to TNFA in non-starved monocytes.

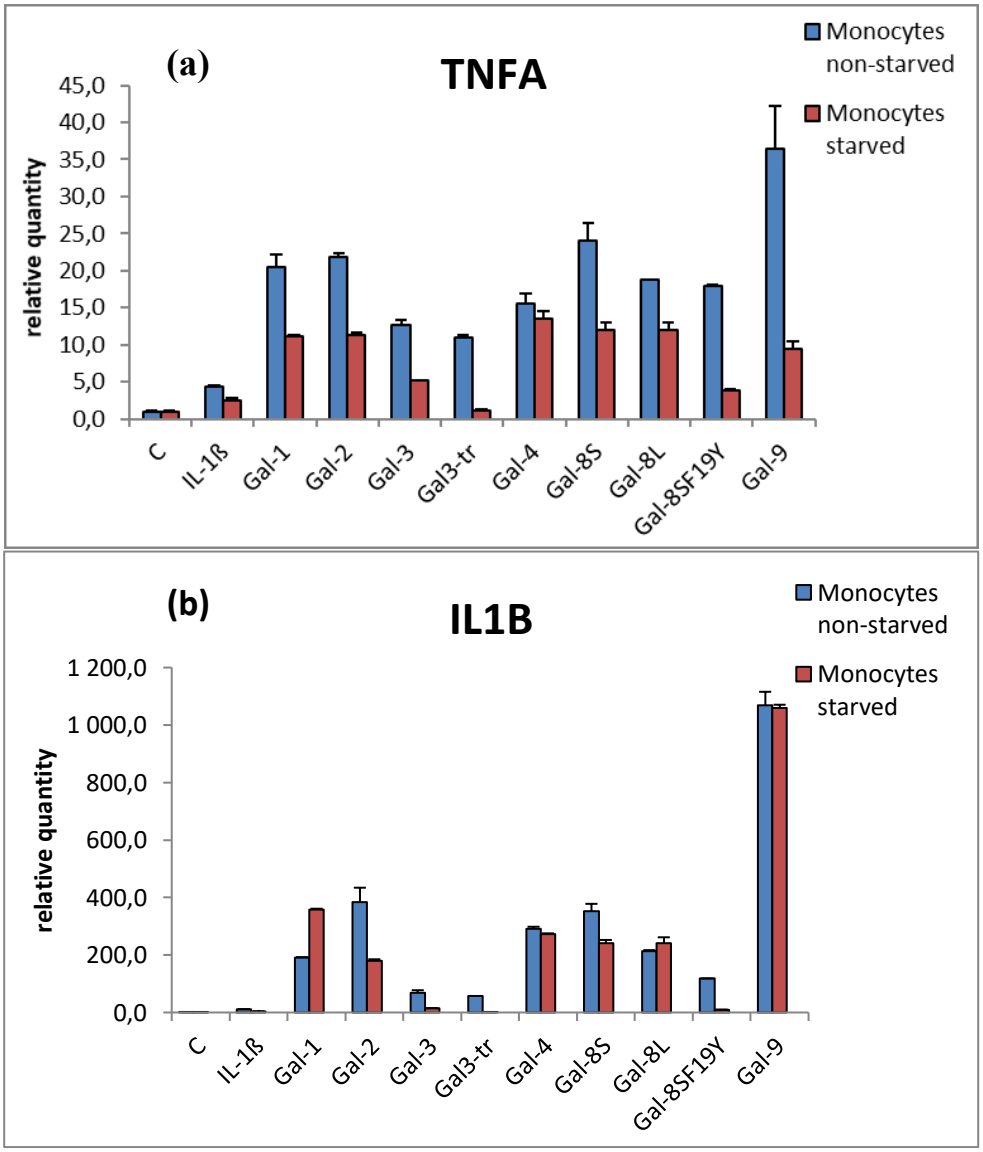


Figure 17 mRNA expression of starved and non-starved monocytes after treatment with different concentrations of galectin. Different concentrations of galectins including 10µg/ml Gal-1, 10µg/ml Gal-2, 18µg/ml Gal-3, 9µg/ml Gal-3tr, 18µg/ml Gal-4, 24µg/ml Gal-8S, 26µg/ml Gal-8L, 10µg/ml Gal-9 and 24µg/ml Gal-8SF19Y adjusted to their specific molecular weight were used. In addition, 10ng/ml IL-1β were used. (a) Comparison of TNFA gene expression between non-starved and starved monocytes following treatment with galectins. (b) Comparison of IL1B gene expression between non-starved and starved monocytes following treatment with galectins.

3.3.3 Comparison of mRNA levels in THP-1 macrophages and monocytes

Figure 18 (a) shows that non-starved monocytes exhibited higher TNFA gene expression levels compared to non-starved macrophages following stimulation with galectins. Under starvation conditions, reactivity of monocytes is reduced compared to macrophages (Figure 18 (b)).

Noteworthy, IL1B shows strongly up-regulated values compared to TNFA, in both starved and non-starved conditions of monocytes Figure 18 and 19. Furthermore, IL1B is stronger induced in monocytes (starved and non-starved) compared to macrophages (starved and non-starved) (Figure 19 (a and b)). Thus, monocytes show higher reactivity towards galectins than macrophages. We also observed that starvation leads to changes in reactivity dependent on cell type and the investigated target gene. In starved monocytes there is reduced reactivity regarding to the expression of TNFA compared to non-starved monocytes. Interestingly, we observed increased reactivity in starved macrophages compared to non-starved macrophages.

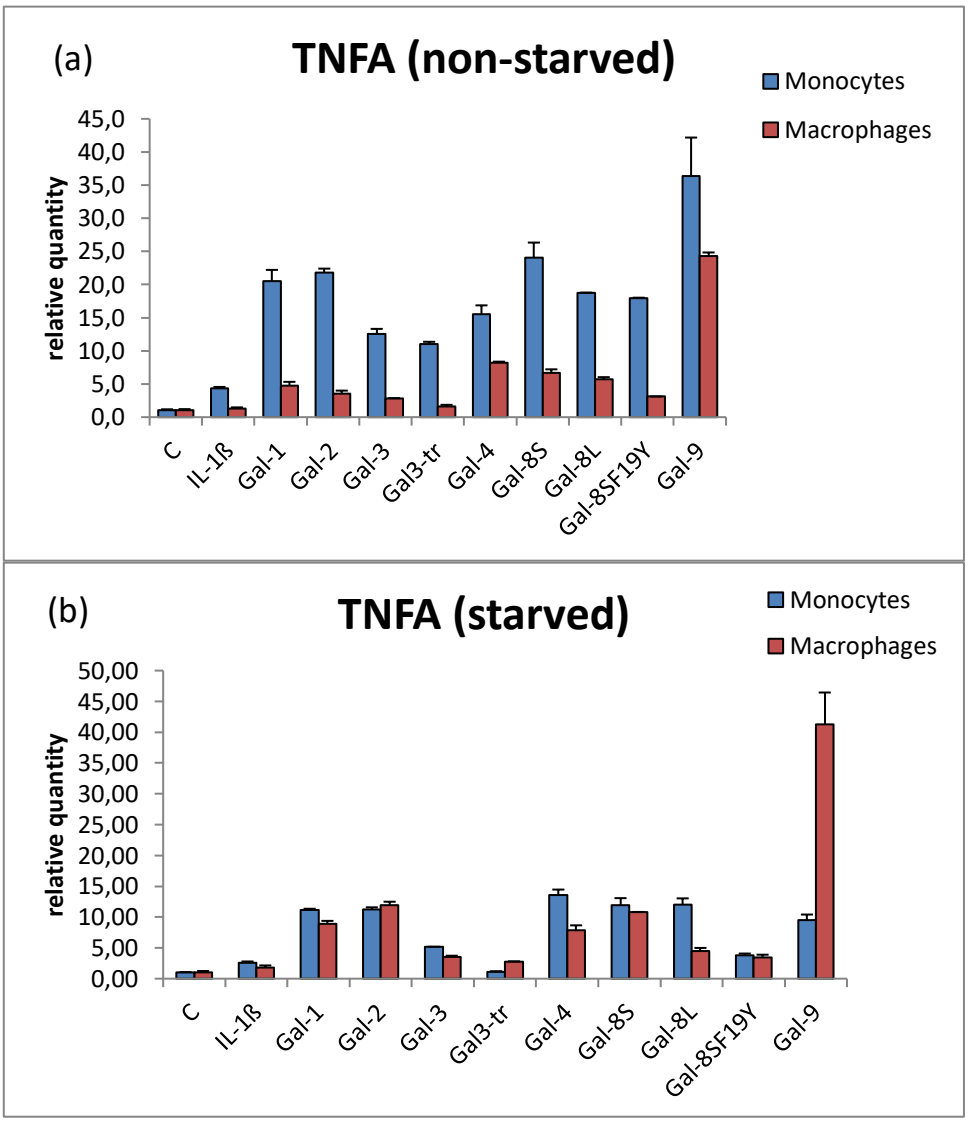


Figure 18 mRNA expression in non-starved and starved monocytes and macrophages after galectin treatment. Different concentrations of galectins including 10µg/ml Gal-1, 10µg/ml Gal-2, 18µg/ml Gal-3, 9µg/ml Gal-3tr, 18µg/ml Gal-4, 24µg/ml Gal-8S, 26µg/ml Gal-8L, 10µg/ml Gal-9 and 24µg/ml Gal-8SF19Y adjusted to their specific molecular weight were used. In addition, 10ng/ml IL-1β were used. Shown is the relative quantity of target gene TNFA measured with qPCR. (a) Comparison between non-starved monocytes and macrophages regarding TNFA expression. (b) Comparison between starved monocytes and macrophages regarding TNFA.

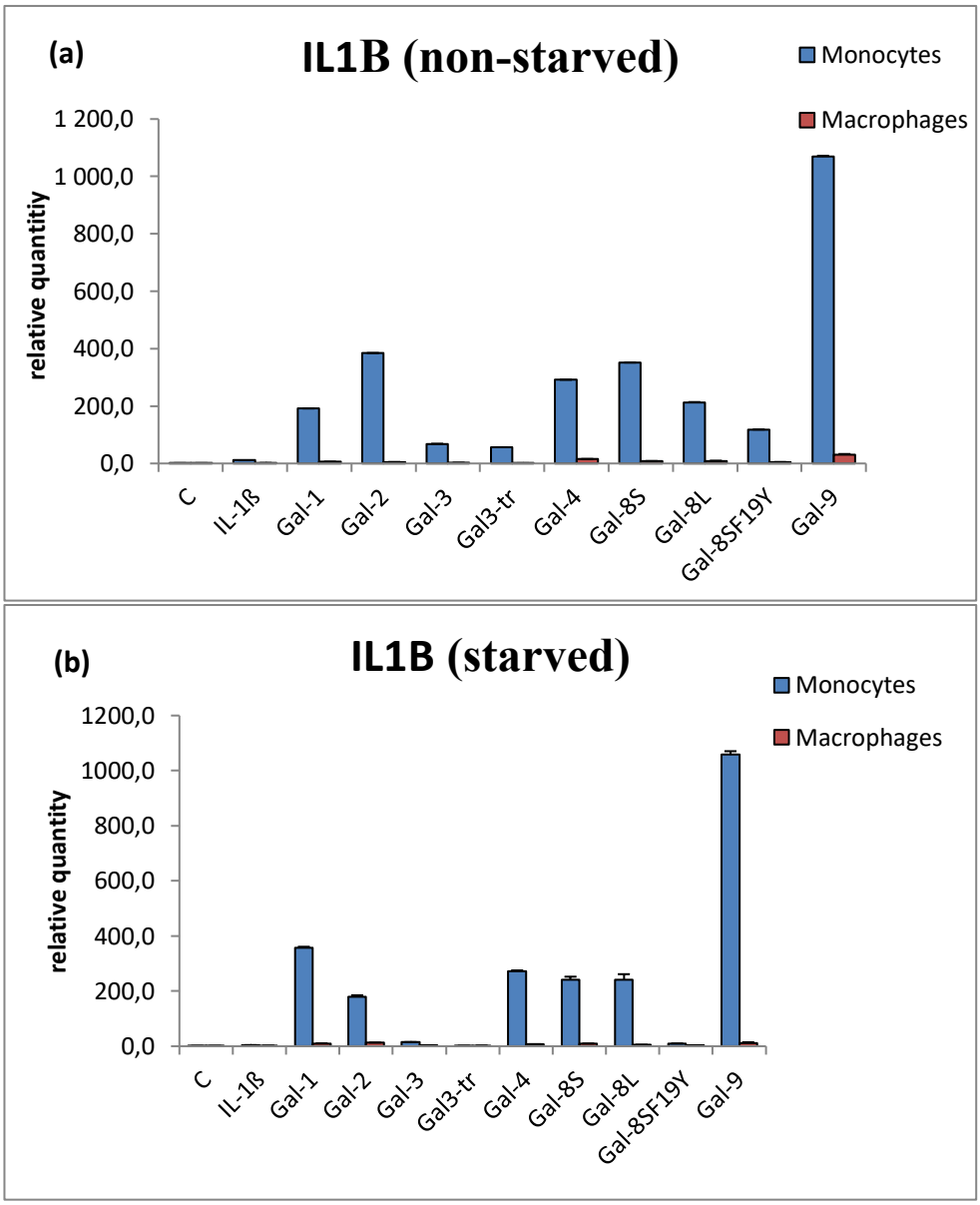
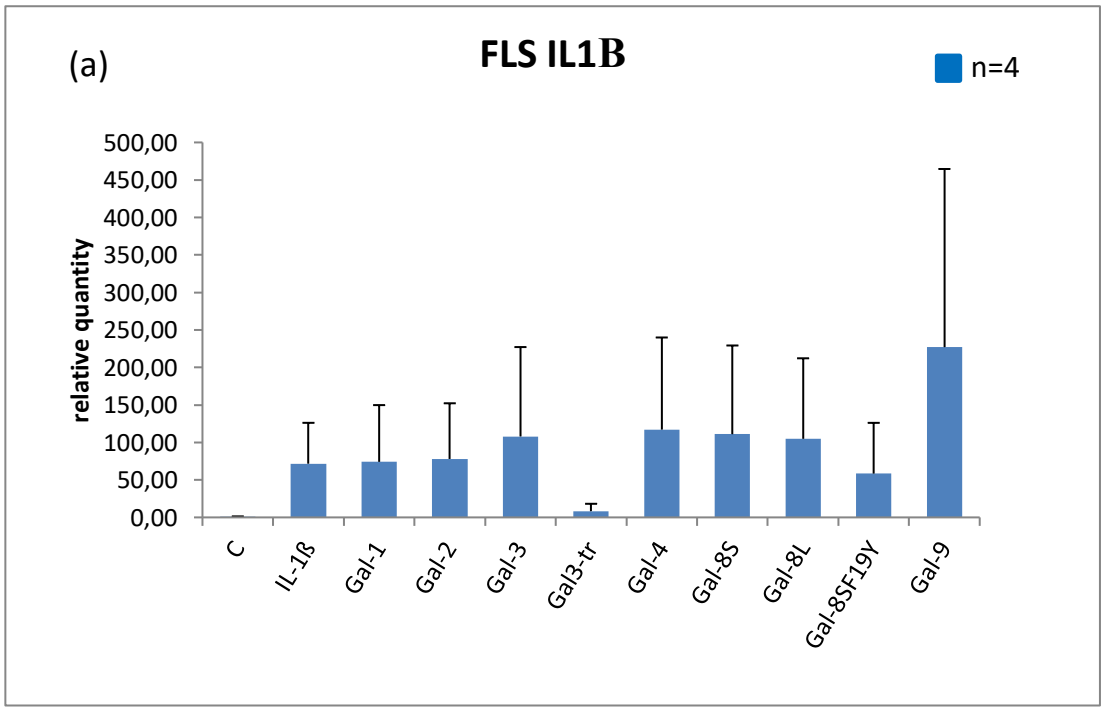


Figure 19 mRNA levels in non-starved and starved monocytes and macrophages after galectin treatment. Different concentrations of galectins including 10µg/ml Gal-1, 10µg/ml Gal-2, 18µg/ml Gal-3, 9µg/ml Gal-3tr, 18µg/ml Gal-4, 24µg/ml Gal-8S, 26µg/ml Gal-8L, 10µg/ml Gal-9 and 24µg/ml Gal-8SF19Y adjusted to their specific molecular weight were used. In addition, 10ng/ml IL-1β were used. Shown is the relative quantity of target gene IL1B measured with qPCR. (a) Comparison of non-starved monocytes and macrophages regarding IL1B expression. (b) Comparison of starved monocytes and macrophages regarding IL1B.

3.4 mRNA levels in galectin-treated FLS

We also investigated the role of galectins in gene expression of IL1B and IL8 in starved FLS. Figure 20 (a) and (b) illustrate that all galectins caused an up-regulation of IL1B. In contrast to Gal-3tr, Gal-9 showed an almost 28-fold higher induction of IL-1B expression. Similarly to experiments in THP-1 cells, Gal-3tr had the smallest effect on IL1B and IL8 in FLS. It is remarkable that IL-1β showed smaller effect to the target gene IL1B than to IL8. Furthermore, Gal-9 induced 20x higher gene expression of IL8 (approx. 4000-fold change) compared to IL1B (approx. 200-fold-change). Intriguingly, IL8 showed a striking up-regulation after IL-1β and Gal-9 treatment compared to a moderate up-regulation of IL1B.



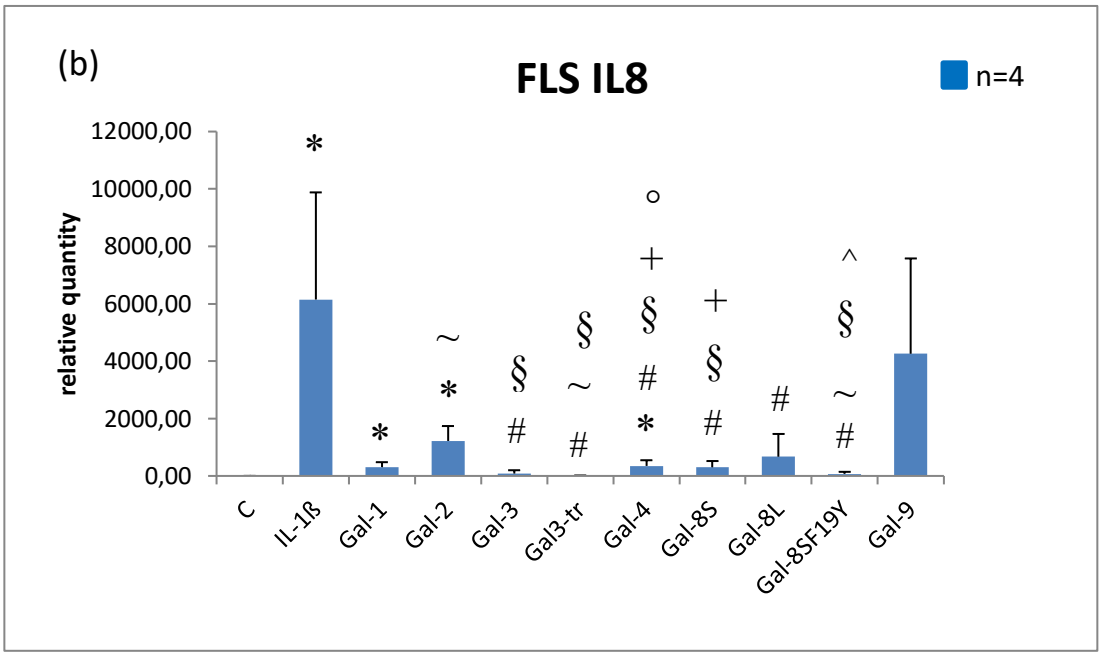


Figure 20 Gene expression of IL1B and IL8 in FLS measured by qPCR.
(a) Gene expression of IL1B in FLS. (b) Gene expression of IL8 in FLS. Different concentrations of galectins including 10µg/ml Gal-1, 10µg/ml Gal-2, 18µg/ml Gal-3, 9µg/ml Gal-3tr, 18µg/ml Gal-4, 24µg/ml Gal-8S, 26µg/ml Gal-8L, 10µg/ml Gal-9 and 24µg/ml Gal-8SF19Y adjusted to their specific molecular weight were used. In addition, 10ng/ml IL-1β were used. *p<0.05 (paired t-test vs control) ,#p<0.05 (paired t-test vs IL-1β), ~p<0.05 (paired t-test vs Gal-1), §p<0.05 (paired t-test vs Gal-2), +p<0.05 (paired t-test vs Gal-3), °p<0.05 (paired t-test vs Gal-3tr), ^p<0.05 (paired t-test vs Gal-4).

3.4.1 Comparison of galectin treated macrophages and FLS gene expression

We tested whether differentiated THP-1 macrophages react to galectins in the same extent as FLS. Therefore, we treated FLS (starved) and macrophages (starved and non-starved) with various galectins and evaluated IL1B expression. Figure 21 (a) and (b) illustrates the comparison of IL1B gene expression in galectin-treated FLS and THP-1 macrophages. Interestingly, FLS showed a stronger reactivity to galectins than starved and non-starved macrophages (Figure 21 (a) and (b), respectively). Furthermore, the truncated version of Gal-3 hardly showed any effect on IL1B transcription in both cell types.

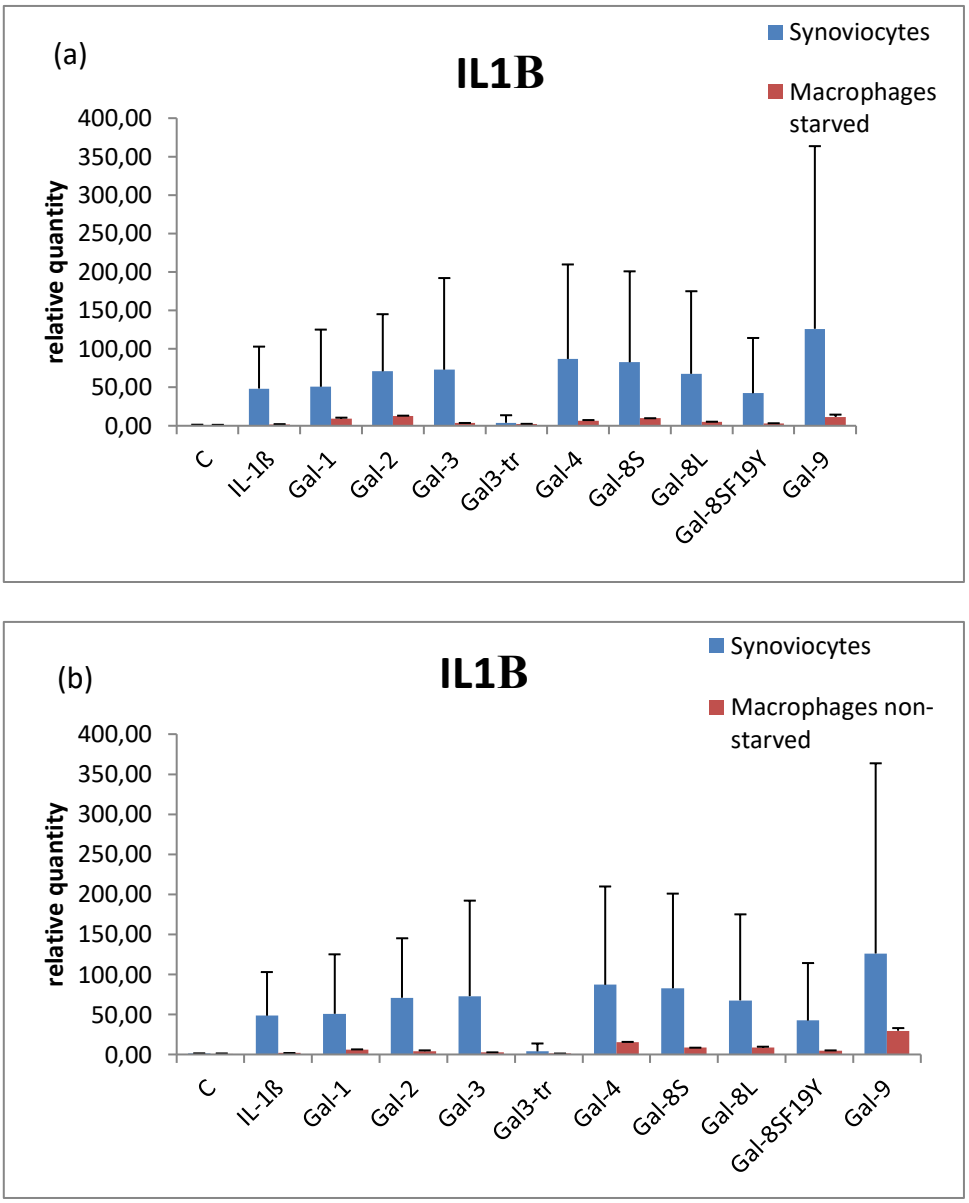


Figure 21 Overview of mRNA levels of IL1B in THP-1 macrophages and FLS. Shown is the relative quantity of target gene IL1B measured with qPCR. (a) Comparison between FLS and starved macrophages. (b) Comparison of FLS and non-starved macrophages. Different concentrations of galectins including 10µg/ml Gal-1, 10µg/ml Gal-2, 18µg/ml Gal-3, 9µg/ml Gal-3tr, 18µg/ml Gal-4, 24µg/ml Gal-8S, 26µg/ml Gal-8L, 10µg/ml Gal-9 and 24µg/ml Gal-8SF19Y adjusted to their specific molecular weight were used. In addition, 10ng/ml IL-1β were used.

3.5 Levels of galectin-type specific mRNA in FLS and THP-1 cells

FLS were isolated from four donors. mRNA levels of LGALS1, LGALS2, LGALS3, LGALS4, LGALS7, LGALS8, and LGALS9 were quantified in untreated non-starved cell cultures of FLS and THP-1 cells using RT-qPCR separately for each donor. All

samples were testes in duplicates to identify LGALS expression. Data denote relative copy numbers with respect to the transcription of the SDHA gene. Table 7 illustrates that LGALS1 and LGALS3 showed the greatest expression followed by LGALS8 in FLS. In contrast, THP-1 macrophages even seemed to show higher expression of LGALS1, LGALS8 and LGALS9. Furthermore, levels of LGALS9 were approximately 200 times higher in THP-1 macrophages compared to FLS. Also higher levels of LGALS1, LGALS3, LGALS9 could be measured in THP-1 macrophages than in THP1-monocytes.

Table 7 - mRNA levels of LGALS1, LGALS2, LGALS3, LGALS4, LGALS7, LGALS8, and LGALS9 in isolated FLS.

FLS P4

	LGALS1	*LGALS2*	*LGALS3*	*LGALS4*	*LGALS8*	*LGALS9*
OA patient 1	46,01	0,02	10,074	0,002	0,339	0,008
OA patient 2	47,62	0,02	21,940	0,001	0,316	0,015
OA patient 3	46,94	0,02	26,078	0,001	0,269	0,025
OA patient 4	33,11	0,01	12,630	0,000	0,241	0,016
Mean (n=4)	**43,42**	**0,02**	**17,68**	**0,00**	**0,29**	**0,02**
S.D.	**6,90**	**0,01**	**7,57**	**0,00**	**0,04**	**0,01**

Table 8 - mRNA levels of LGALS1, LGALS2, LGALS3, LGALS4, LGALS7, LGALS8, and LGALS9 in THP-1 cells.

THP-1 macrophages

	LGALS1	*LGALS2*	*LGALS3*	*LGALS4*	*LGALS8*	*LGALS9*
Passage 6	67,46	0,004	3,258	0,001	0,816	4,072

THP-1 monocytes

	LGALS1	*LGALS2*	*LGALS3*	*LGALS4*	*LGALS8*	*LGALS9*
Passage 6	16,08	0,01	1,340	0,002	0,845	2,644

4 Discussion

Synovitis plays a critical role in the progression of OA. Inflammatory processes can be caused by galectins (Liu and Rabinovich 2010),which thereby possess a strong capacity to degrade extracellular matrix proteins and induce intracellular signaling pathways (Laderach et al. 2010). As previous studies showed the impact of galectins in cartilage degeneration and OA (Toegel et al. 2014, Toegel et al. 2016, Weinmann et al. 2016, Weinmann et al. 2018), the first aim of the present study was to investigate the activity of galectins in cells of the OA synovium.

Toegel and coworkers successfully detected the expression of LGALS mRNA levels in OA chondrocytes (Toegel et al. 2014). According to these data, we further investigated LGALS expression in FLS and THP-1 cells. We observed high mRNA levels of LGALS1 and LGALS3 in FLS. In Figures 7 and 8, it is shown that higher levels of LGALS1, LGALS3, LGALS9 were measured in THP-macrophages than in THP-monocytes. Compared to FLS, levels of LGALS1, LGALS3, LGALS9 were also clearly higher in THP-macrophages. These results indicate that cells of the OA synovium express the relevant genes for galectin biosynthesis, suggesting that galectins might play a role in OA processes. Therefore, treatments with various galectins in FLS were performed in the following to measure mRNA levels of target genes IL1B and IL8.

Weinmann et al. showed that Gal-1 cooperates with Gal-3 to induce mRNA levels of TNFA and IL1B in human OA chondrocytes and that these effectors are substantially involved in the degeneration of cartilage and progression of OA (Weinmann et al. 2016). To investigate whether galectins have an impact on marker gene expression in human synoviocytes, we treated P4 FLS with a large panel of recombinant galectins such as Gal-1, -2, -3, -4, and -9, as well as with the selected galectin variants Gal-3tr, Gal-8S, Gal-8L, and Gal-8S_F19Y. We observed that all galectins induced the up-regulation of TNFA and IL1B mRNA in FLS. Compared to the previous studies (Weinmann et al. 2016, Weinmann et al. 2018), we showed that not only Gal-1, Gal-3 and Gal-8 are potent inducers of pro-inflammatory genes, but also that Gal-9 might be of interest for future investigations because of its strong activity in our study. Li and coworkers, in

their study, concluded that high amounts of Gal-1, -2, -3, -8, and -9 in FLS in vitro could play a critical role in the pathogenesis of RA (Li et al. 2014). In our study, these candidates and Gal-4, as well as the physiological variants of Gal-8, also showed great impact in the context of OA, especially in regard to the gene expression of IL1B. Gal-9 induced the strongest increase of IL1B expression among all galectins in all cell types. Furthermore, IL-1β, Gal-2, Gal-8L, and Gal-9 caused the strongest induction of IL8. In the context of IL-1ß activity, our findings are in line with former studies that also observed major alterations of IL8 gene expression in FLS stimulated with IL-1β (Hyun Mi Choi et al. 2010).

After having explored increased mRNA levels of marker genes in FLS, we further wanted to investigate the activity of galectins in THP-1 cells. It is known that macrophage depletion down-regulates secretion of several fibroblast-produced cytokines and MMPs in synovitis (Bondeson et al. 2010). This may illustrate the importance of interaction between synoviocytes and macrophages in synovial tissue. For this reason, treatments of undifferentiated THP-1 monocytes with different galectins were performed to identify target genes for further investigations with macrophages. In our pre-tests, TNFA and IL1B seemed to be the ideal target genes for further experiments with THP-1 macrophages. Using differentiated THP-1 cells is a common way to simulate human derived macrophages and to study the role of inflammation processes (Qin 2012).

Firstly, we discovered that non-starved monocytes were highly reactive to most of investigated galectins. For IL1B expression, fold-change values ranged from 56±0.69-fold (Gal-3tr) to 1070±47.05 (Gal-9). Subsequently, we decided to perform experiments with differentiated THP-1 macrophages. To the best of our knowledge, we are first to discover that THP-1 macrophages react to galectins with altered marker gene expression.

Unexpectedly, non-starved THP-1 monocytes showed greater reaction to galectins compared to starved cells (Figure 18). This was surprising since starved cells, in most cases, show greater reaction to different stimuli because starvation leads to a reduction of basal cell activity (Pirkmajer and Chibalin 2011). This behavior of THP1-monocytes should be taken into account when planning further studies on galectin

activity in these cells. In contrast, starved THP-1 macrophages showed greater reaction to galectins compared to non-starved THP-1 macrophages.

As shown in Figure 19, TNFA and IL1B levels in non-starved and starved THP-1 macrophages seemed reduced compared to non-starved and starved monocytes, respectively. This finding is in line with a report by Paclik and coworkers, who evaluated TNF-α levels in galectin-treated THP-1 monocytes and macrophages by FACS analysis (Paclik et al. 2011). There, it was shown that Gal-2 had a five times stronger impact on TNFA secretion in monocytes than in macrophages. As PMA treatment of THP-1 monocytes induces morphologic cell alterations, we speculate that the process of differentiation into THP-1 macrophages alters the characteristics of cell surface glycans, that act as counter-receptors for galectins. Similarly, changes in proteoglycan size and glycosaminoglycan chain length could play a role in reactivity to galectins and pro-inflammatory stimuli (Chang et al. 2012).

Taken together, galectins were shown to affect the gene expression of pro-inflammatory cytokines in FLS and THP-1 cells. Thus, these proteins may play an important role in inflammatory processes in the OA synovium that drive the progression of OA. Further studies should elucidate the molecular activity of selected galectins in cells of the OA synovium, characterize their counterreceptors and investigate their potential as therapeutic targets in OA conditions.

The second aim of this study was to establish the methodology for anti-galectin autoantibody detection in our research lab, following the technique described by Sarter et al. with minor modifications (Sarter et al. 2013). We performed these experiments because of the potential of anti-galectin autoantibodies as biomarkers for OA. In fact, galectins are often associated with the development and progression of different diseases (Liu and Rabinovich 2010). For example, the risk to suffer atherosclerosis, which is the major cause for cardiovascular disease (CVD), may correlate with higher levels of Gal-1, -3, and -9 (He et al. 2017). A previous study revealed the presence of anti-galectin-1, -2, -3, -4, -7, -8 and -9 autoantibodies in sera of patients with SLE, RA and sAPS (Sarter et al. 2013). Here, we describe the successful detection of various

anti-Gal autoantibodies in all test persons, except for anti-Gal-8 in test person #3. The results suggest that anti-Gal-1, -4,and -12 autoantibodies appear as the most abundant in human plasma (n=4), with age and gender being potential influencing factors.

We speculate that the higher production of galectins in osteoarthritic conditions leads to immune responses accompanied by autoantibodies at early phases in OA. Nevertheless, to elucidate the biological relevance of anti-galectin autoantibodies and their correlation with OA, more tests with OA patients and a larger set of healthy controls have to be done. Our feasibility study aimed to establish the methodology to detect autoantibodies in healthy test persons to provide a basis for such further biomarker studies in OA. Based on these preliminary experiments, a study is planned to investigate the levels of anti-galectin autoantibodies in OA patients and healthy controls, to determine their usefulness as biomarkers in the context of OA.

5 Conclusion

The results of this thesis indicate that all investigated galectins may play an important role in OA by up-regulating typical pro-inflammatory key genes such as TNFA, IL1B and IL8 in primary FLS and THP-1 macrophages. Among all galectins, Gal-9 showed the strongest effect in human macrophages and FLS. It could be concluded that galectins may be substantially involved in OA-related inflammatory processes including synovitis. Moreover, the reactivity of stimulated human THP-1 macrophages to galectins was investigated and could provide a basis for further studies using primary macrophages. In addition, successful detection of anti-galectin autoantibodies in healthy test persons was conducted. This small ELISA pilot study could provide a basis for further experiments with sera of OA patients to identify potential biomarker candidates for OA. Especially, early detection in form of natural biomarkers such as antibodies could be important for diagnosis and therapy monitoring in OA in its initial phase. In summary, the focus on continuing research on OA has to be maintained, to potentially find valuable biomarkers for patients with high risk for OA.

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7 Appendix

7.1 Abbreviations

APS	primary antiphospholipid syndrome
BMP-7	bone morphogenetic protein-7
C2C	collagen type-II specific neoepitope
CD-14	cluster of differentiation
cDNA	complementary Deoxyribonucleic acid
CNS	central nerve system
COX	cyclooxygenase
CRD	carbohydrate-recognition domain
C_T	cycle treshold
CTX-II	C-terminal telopeptide of collagen type II
DMEM	Dulbecco´s Modified Eagle's Medium
DMSO	dimethylsulfoxide
ELISA	Enzyme-linked Immunosorbent Assay
FBS	fetal bovine serum
Fib-3-1 & Fib3-2	fibulins
FSTL-1	follistatin-like protein-1
Gal	Galectin
gDNA	genomic Deoxyribonucleic acid
GLUT-2	glucose transporter 2
GRP	galectin-related protein
HA	hyaluronic acid
IFN-γ	Interferon gamma
IL	Interleukin
LGALS	Lectin, Galactoside Binding Soluble
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid

NSAID	non-steroidal anti-inflammatory drugs
OARSI	Osteoarthritis Research Society International
PBS	phosphate-buffered saline
PG	Prostaglandin
PKA	Protein kinase a
PKC	Protein kinase C
PLIN-1	lipid droplet protein PERILIPIN 1
PMA	phorbol 12-myristate 13-acetate
PPI	proton-pump inhibitors
PRR	Pattern-Recognition-Receptor
RT	reverse transcription; real time
RT-qPCR	quantitative real time PCR
RPMI	Roswell Park Memorial Institute
SDHA	succinate dehydrogenase
SLE	systemic lupus erythematosus
TGF-β	transforming growth factor beta
THP	human monocytic leukemia cell line
TLR	toll-like receptor
TNF-α/TNFA	tumor necrosis factor alpha

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