Doctoral (PhD) Dissertation

The Immunological Role of the Spleen in Rheumatoid Arthritis

Esam Khanfar

Supervisor: Dr. Ferenc Boldizsár, MD, PhD

Department of Immunology and Biotechnology

Medical School, University of Pécs

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Supervisor: Dr. Ferenc Boldizsár, MD, PhD

Leader of the program:

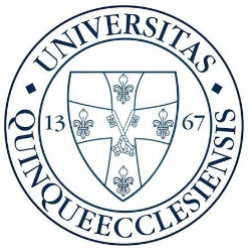
Prof. Dr. Tímea Berki, MD, PhD, DSc

Leader of the doctoral school:

Prof. Dr. Dóra Reglődi, MD, PhD, DSc

Department of Immunology and Biotechnology

Medical School, University of Pécs



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Summary

The immune system is a complex network of lymphatic and extra-lymphatic tissues, where various types of innate and adaptive immune cells intertwine to take part in, on one hand, protecting the body against foreign antigens, and on the other hand to maintain tolerance to self-antigens; thus, preventing the development of autoimmune diseases. The spleen is a major secondary lymphoid organ with special and diverse functions, such as participating in the homeostasis of the intraperitoneal cavity, innate immune response against foreign antigens, and adaptive immunity. Furthermore, this organ is crucial for B cell maturation, and it is considered as a harbor for the memory B cells. In the context of autoimmune diseases, specifically RA, spleen enlargement is commonly observed, indicating a direct involvement in RA pathogenesis in humans and mice.

In this thesis, our aim was to shed light, at least partially, on the immunological role of the spleen in the development and pathogenesis of RA using a mouse autoimmune arthritis model. First, we investigated autoimmune arthritis in mice with severely disturbed spleen structure due to Nkx2-3 deficiency, and we found that the anatomical defects of spleen affected the B cell activation, which might have resulted in ameliorated autoimmune arthritis in the Nkx2-3$^{-/-}$ mice. Second, we investigated the absence of the spleen in wild-type BALB/c mice, using surgical splenectomy prior to autoimmune arthritis induction and found that this way other components of the immune system compensated for the spleen and arthritis developed. Third, when we removed the spleen during the process of autoimmune arthritis induction, we observed that splenectomy in the early stages had a significant protective effect against the articular bone and cartilage damage.

Collective interpretations from the three parts indicate that the spleen highly influences the level of pro-inflammatory cytokines and autoantibodies in the blood serum. Also, it seems that the spleen's anatomical defects due to Nkx2-3 deficiency or its surgical removal shifts the T-helper cells polarization towards Th_2 while the presence of the spleen shifts the immune response towards Th_1. These data indicate that the spleen is indeed involved in the development of autoimmune arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a common systemic autoimmune disease that affects approximately 1% of the population [1]. The prevalence of RA is approximately three times higher in females than in males [2],[3]. RA patients develop symmetrical joint swelling, severe joint pain and stiffness, and ultimately bone erosion and joint deterioration [4]. Although RA is considered primarily as a pathology of the synovial joints, the systemic inflammation increases the risk of developing extra-articular manifestations in approximately 40% of the patients, such as vasculitis, cardiac-, kidney- and respiratory diseases, and Felty syndrome, thus increasing the morbidity and mortality rates [5]. Therefore, RA can significantly affect the patients' quality of life including physical activity and social life, and it also causes an economic burden to individuals and society [6].

Animal models of autoimmune arthritis like collagen-induced arthritis (CIA) and recombinant human G1 domain-induced arthritis (GIA) have proven to be very useful tools in studying the RA pathophysiology and its immunological processes [7],[8]. The translational significance of the GIA arthritis model lies in its ability to mimic many clinical and immunological aspects of human RA [9]. In the GIA mouse model there is a predominant Th1 and Th17 polarization during arthritis induction, which is accompanied by significant autoantibody production including anti-rhG1- and anti-cyclic citrullinated peptide antibodies (anti-CCP) [7].

The spleen is the largest secondary lymphoid organ which consists of two distinct areas: white pulp and red pulp [10]. The spleen takes part in maintaining homeostasis of the peritoneal cavity through mediating B cell migration between the peritoneum and the circulatory system [11]. Also, it participates in promoting T cell homeostasis and immune tolerance under normal conditions, as it induces the expression of autoimmune regulator gene (AIRE) [12]. Additionally, the spleen is crucial for B cell development and maturation including B1, marginal zone (MZ) and follicular (Fo) B cells [13].

Besides the above described physiological roles, the spleen is also involved in the development of autoimmune diseases, as for example splenomegaly is common in systemic sclerosis, SLE and RA [14],[15]. Similarly, in the CIA and GIA mouse models, spleen is involved in the development and pathogenesis of arthritis, as its size increases and more activated cells can be found there [16]. Accordingly, abnormalities in spleen development or deformities in its structure may lead to alterations in the immune response [17].

The homeodomain trascription factor Nkx2-3 is crucial for the development of the spleen, Peyer's patches and small intestine [17],[18]. Nkx2-3 is required for the expression and regulation of mucosal addressin cell adhesion molecule-1 (MADCAM-1) on high endothelial venules of the mLNs, and Peyer's patches and on the sinus lining of the spleen [17],[18]. Nkx2-3 is essential for normal immunological functions due to its role in spleen development and organization through establishing the correct microenvironment for B cell maturation and differentiation, and TD immune reaction [17].

Nkx2-3 deficient mice (Nkx2-3$^{-/-}$) are either asplenic or have a significantly reduced spleen size with the lack of the red pulp and MZ [17],[18]. The disturbed development of the spleen in the Nkx2-3$^{-/-}$ mice fails to provide the appropriate microenvironment that is necessary for the normal distribution of B and T cells [17],[18]. This results in disorganized germinal center (GC) formation leading to abnormal B cell development and decreased antibody response with failed affinity maturation [17].

2. Aims

The aim of this thesis was to investigate the immunological involvement of the spleen in the development and progression of RA. To this end, three different but interconnected studies were conducted:

1. We investigated the GIA in Nkx2-3$^{-/-}$ mice:

- The effect of Nkx2-3 deficiency (genetic malformation of the spleen) on the development of autoimmune arthritis (disease progression and severity).
- Investigate the effect of Nkx2-3 deficiency on the autoimmune response and serum parameters.
- Study the effect of Nkx2-3 absence on B cell signaling and activation.

2. We investigated the effect of splenectomy in GIA:

- Study the effect of splenectomy prior to autoimmune arthritis induction on the disease development and progression.
- Investigate the significance of the spleen in the development of autoimmune arthritis.
- Characterize the cellular- and humoral immune responses in arthritic splenectomized mice.
- Investigate the effect of splenectomy on the composition of circulating T- and B cells.
- Identify the immunological mechanisms by which splenectomized mice developed autoimmune arthritis similarly to the control.

3. We investigated whether splenectomy during the induction of GIA affected the autoimmune arthritis:

- Compare the effects of splenectomy performed after the first or the second immunization on the onset and severity in GIA.
- Evaluate the radiological- and histological changes of the affected joints in mice, which were splenectomized in early stages of arthritis.

- Find the correlation between the clinical picture, serum parameters and the cellular and humoral immune responses in the arthritic splenectomized and spleen preserved mice.
- Examine whether the surgical removal of the spleen in early stages of RA induction alters the T helper cell polarization.
- Assess the frequency of circulating Tregs at different stages of arthritis development.

3. Materials and methods

3.1. Mice

We used 2-6 months old female wild-type BALB/c and 4-5 months old female Nkx2-3$^{-/-}$ mice with a BALB/c genetic background. Mice were kept under conventional conditions at 24±2 °C with a controlled 12/12 h light/dark cycle, at the Department of Immunology and Biotechnology's Transgenic Mouse Facility. The mice were housed in groups of five and received acidified water and food ad libitum. All animal experiments were conducted following the University of Pécs, Animal Welfare Committee regulations (under licence numbers BA02/2000-23/2020 and BA02/2000-13/2022).

3.2. Surgical procedures

Mouse groups were splenectomized as follows: we anesthetized the mice using 100 mg/kg ketamine (Calypsol, Gedeon Richter, Budapest, Hungary) and 10 mg/kg xylazine (Sedaxylan, Eurovet Animal Health, Bladel, The Netherlands) ip. before the operation. Then the abdomen was shaved and disinfected, after which a skin incision was made laterally on the left side of the mice, followed by the opening of the abdominal cavity. First, the spleen vascular pedicles were ligated using a 6-0 silk suture, then the spleen was freed from the surrounding tissues and removed. The skin and abdominal incisions were closed using a 6-0 silk suture. The surgeries were performed under controlled temperature (37°C) to avoid hypothermia. After the surgeries, each mouse was provided with paracetamol (bene-Arzneimittel GMbH Munich, Germany) mixed with water for 7 days. Splenectomy did not influence the general health status, behavior, or the body weight of the mice.

3.3. Induction and assessment of recombinant human G1-induced arthritis

GIA was induced in 4-5 months old Nkx2-3$^{-/-}$or wild-type BALB/c mice. Mice were injected three times in 3-4 weeks interval intraperitoneally with a mixture of 40 µg rhG1 antigen and dimethyldioctadecyl-ammonium (DDA) adjuvant dissolved in PBS as described previously [7]. Arthritis severity and clinical signs were examined using a clinical scoring system as described previously [7]. Briefly, each mouse limb received a score between 0 and 4 based on swelling, redness and ankylosis of the joints of the paws (0= no swelling, 1= redness and mild swelling, 2= redness and moderate swelling, 3= severe swelling without joints' ankylosis and 4=

ankylosis of the joints of the paws), thus the highest severity score/mouse is 16. The diameter of inflamed limbs was measured using a digital caliper throughout the experiment. Mice were sacrificed three weeks after the third immunization, and blood sera, mLNs and inguinal lymph nodes (iLNs) and spleens were collected and later used for *in-vitro* studies.

3.4. Micro-Computed tomography

Mice were anesthetized with i.p 120 mg/kg ketamine (Calypsol, Gedeon Richter, Budapest, Hungary) and 6 mg/kg xylazine (Sedaxylan, Euro vet Animal Health, Bladel, The Netherlands). SkyScan 1176 *in-vivo* micro-CT system (Bruker, Kontich, Belgium) was used to scan the right hind paws of the mice. A 0.5 mm Al filter was used with a voxel size of 17.5 μm and a 50 Kv tube voltage, the tube current was fixed to 500 μA. CT Analyzer software was used to reconstruct the 3D scans. Furthermore, to highlight the osteophytes and the bone erosions, pseudo-color representative images were made.

3.5. Histology and immunohistology

Hind legs of arthritic mice were collected after sacrifice and fixed in 10% formalin. Then the specimens were decalcified in EDTA solution at 37 °C for one day. Following embedding in paraffin, 4 μm thick slides were made and stained with Mayer's hematoxylin and eosin (HE) solution using a Leica ST 4040 linear automatic stainer (Leica Biosystems, Germany). Finally, slides were scanned using Pannoramic MIDI Scanner (3DHistech, Hungary) and the images were analyzed using the Pannoramic View Software (3DHistech, Hungary).

The spleens of the control mice, and mesenteric- and inguinal LNs of both control and splenectomized mice were isolated and embedded in a cryostat embedding medium, then kept at -80 °C. Three different planes (150 μm apart) of 8 μm thick cryostat sections were cut. The slides were incubated overnight at room temperature, then fixed with cold acetone for 5 minutes.

For immunofluorescence, sections were blocked for 20 minutes. After that, a mixture of anti-B220-Alexa fluor 647 and anti-CD3-FITC antibodies was added to the sections and incubated for 45 minutes at room temperature, followed by washing three times with PBS.

For immunohistochemistry, sections were incubated with phenylhydrazine hydrochloride in PBS for 20 minutes, then washed with PBS, followed by blocking for 20 minutes. Next, the sections were incubated with anti-IgD and biotinylated PNA for 45 minutes, followed by 3 times washing with PBS. After that, sections were incubated with extravidine

alkaline phosphatase and goat anti-rat IgG-HRP for 45 minutes, then washed 3 times with PBS. For color development, DAB was used for HRP, and NBT with BCIP and levamisole (1 mg/mL) were used for AP detection, respectively. Sections were analyzed using an Olympus BX61 fluorescent microscope. Digital pictures were acquired with a CCD camera using the ZEN software. Images were analyzed using ImageJ Software to determine the average follicle size and germinal center size and number.

3.6. *In vitro* cell culture for rhG1 antigen-specific proliferation assay and cytokine production

Cells were isolated from the mesenteric LNs of the splenectomized and control mice, and the spleens of the Nkx2-3$^{-/-}$ and control mice and cultured in DMEM supplemented with 10% fetal calf serum (FCS). For proliferation assay $3x10^5$ cells/well were cultured with or without the rhG1 antigen in triplicates on 96-well plates for 5 days. The proliferation rate was assessed using Promega CellTiter96® Nonradioactive Cell Proliferation Assay (Promega, Madison, WI, USA) following the manufacturer's instructions. For cytokine production 1.8×10^6 cells/well were seeded on a 48-well plate in the presence or absence of the rhG1 antigen. After 5 days, supernatants were collected and kept at -20 °C and later used for cytokine ELISA measurements.

3.7. Cytokine- and antibody ELISA measurements

Blood sera and the supernatants of the *in vitro* cultured spleen and mesenteric LN cells were used to measure the level of IL-1β, IL-4, IL-6, IL-17, IL-23, IFNγ and TNFα using sandwich ELISA (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

The serum levels of the rhG1 antigen-specific antibodies (anti-rhG1) and anti-mouse proteoglycan (anti-mPG) were measured using indirect ELISA as described previously [7]. Levels of the serum anti-CCP IgG1 and -IgG2a antibodies were measured using the Immunoscan CCP Plus ELISA kit (SVAR, Malmö, Sweden) according to the manufacturer's instructions with slight modification. The reaction was developed using peroxidase-conjugated anti-mouse-IgG1 or IgG2 (BD Bioscience, San Jose, CA, USA) as secondary antibodies. RF levels (IgG and IgM) were measured using the FineTest Mouse RF-IgG or -IgM ELISA Kits (FineTest, Wuhan, China) according to the manufacturer's instructions.

3.8. Flow cytometric analysis

Blood-, spleen- and inguinal LN cells were isolated, then 1×10^6 cells/sample washed twice with flow cytometry washing buffer and then incubated with different cocktails of fluorochrome-conjugated monoclonal antibodies diluted in flow cytometry staining buffer for 30 min at RT in the dark. Finally, samples were washed twice and resuspended in flow cytometry fixation buffer. Data acquisition was performed using a FACS Canto II flow cytometer, and for data analysis FACS DIVA software (BD Biosciences) and FlowJo software v10 were used. We defined the following cell subsets based on surface and intracellular markers: $CD3^+$, T cells; $CD3^+CD4^+$, $CD4^+$ T cells; $CD3^+CD8^+$, $CD8^+$ T cells; $CD3^+CD4^+CD25^+$, activated $CD4^+$ T cells; $IgD^{hi}IgM^{low}CD23^+$, follicular B cells; $IgD^{low}IgM^{hi}CD23^-$, B1 and MZ B cells; $Ig^+CD73^+CD38^+$, memory B cells; $B220^-CD138^+$, plasma cells; $CD4^+FoxP3^+CD25^+$, natural regulatory T cells; $CD4^+FoxP3^+CD25^-$, induced regulatory T cells; $CD4^+T\text{-}bet^+$, T-helper-1 cells; $CD4^+GATA\text{-}3^+$, T-helper-2 cells; $CD4^+ROR\gamma^+$, T-helper-17 cells.

3.9. Ca^{2+} signaling measurements

To measure the intracellular calcium levels, single-cell suspensions of the inguinal- or mesenteric LNs were prepared and suspended in RPMI supplemented with 5% FCS and 4 mM $CaCl_2$ (1×10^6 cells/mL). Next, cells were loaded with Fluo-3-AM intracellular Ca^{2+} indicator fluorescence dye at 37°C in humidified air with 5% CO_2 for 30 minutes. Using BD FACS Calibur flow cytometer, the intracellular Ca^{2+} change was detected in the FL-1 channel. The baseline was measured for one minute, then the B cells were activated with anti-IgM or anti-IgG, while the T cells with anti-CD3 cross-linking. Cell Quest software (BD Biosciences, San Jose, CA, USA) was used to analyze the data.

3.10. Statistical analysis

Data analysis was performed with MS Excel. Data in the diagrams are presented either as mean ±standard error of the mean (SEM) or as box (representing the 25-75% interquartile range; wherein the median and average values are indicated with a horizontal line and "x", respectively) and whiskers (representing the minimum/maximum values) plots, indicating all data points. Student's t-test was used to compare the experimental groups, p-values ≤ 0.05 were considered statistically significant.

4. Results

4.1. Studying GIA in Nkx2-3$^{-/-}$ mice

In the first part of our studies, we aimed to investigate the effect of Nkx2-3 deficiency in RA pathogenesis. To that end, we induced autoimmune arthritis in Nkx2-3$^{-/-}$ mice in parallel with wild-type BALB/c using the GIA mouse model (Fig. 4.1). To our knowledge, this study was the first to investigate the effect of the Nkx2-3 deficiency in the context of autoimmune arthritis.

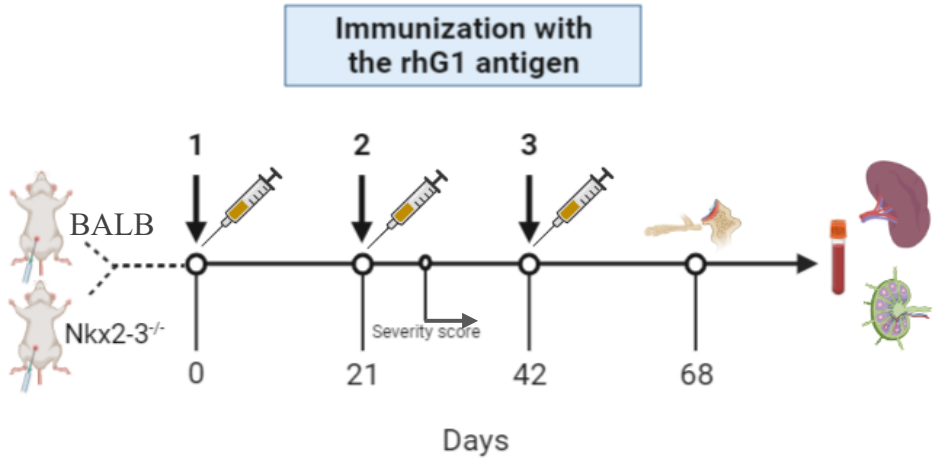

Figure 4.1. Schematic representation of the experimental design for GIA induction. Nkx2-3$^{-/-}$ and control BALB/c mice were immunized using the rhG1 antigen three times at 3-weeks interval

Main findings (Section 4.1):

- Autoimmune arthritis developed in Nkx2-3$^{-/-}$ mice despite the severely disturbed spleen structure.
- The severity and incidence of autoimmune arthritis was lower in Nkx2-3$^{-/-}$- compared to the control BALB/c mice.
- The rhG1 antigen-induced T cell proliferation and cytokine production decreased in Nkx2-3$^{-/-}$ mice.
- There was less bone damage in the Nkx2-3$^{-/-}$ mice.
- There was less anti-CCP-IgG2a, IL-17 and IFNγ, and more IL-4 in the sera of the Nkx2-3$^{-/-}$ mice.
- B cells isolated from the lymph nodes of Nkx2-3$^{-/-}$ mice showed decreased intracellular Ca^{2+} signalling.

4.2. Studying the effects of splenectomy prior to the induction of GIA

In this part, we were curious whether the decreased severity of autoimmune arthritis in the Nkx2-3$^{-/-}$ mice resulted from the anatomical defects of the spleen or if other cellular mechanisms played a role, too. Here, in this part of the thesis we followed a different approach to examine the spleen's involvement in the induction and pathogenesis of autoimmune arthritis: we investigated whether the complete absence of the spleen after surgical removal (splenectomy) in wild-type BALB/c mice could affect the development and severity of RA. To that end, we splenectomized normal BALB/c mice 4 weeks before the induction of autoimmune arthritis, and then we induced autoimmune arthritis in the splenectomized mice in parallel with control (spleen preserved) BALB/c mice using the GIA mouse model (Fig. 4.2).

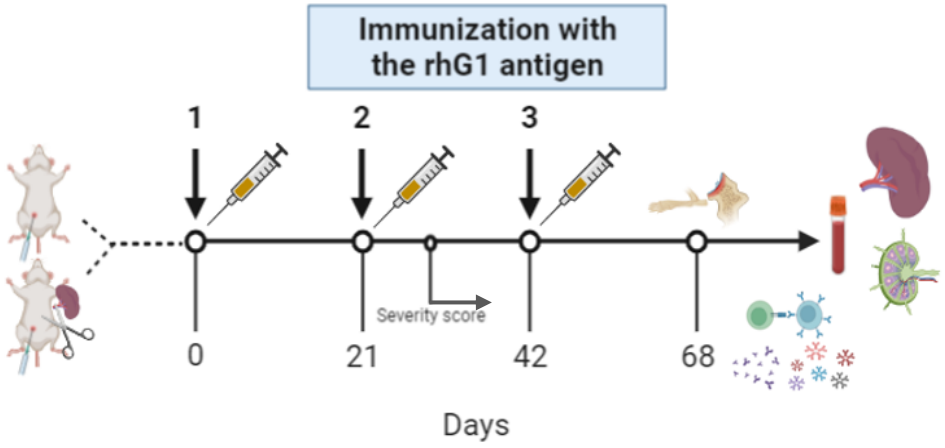

Figure 4.2. Schematic representation of the experimental design for 4.2. Splenectomized and control BALB/c mice were immunized using the rhG1 antigen three times at 3-weeks interval.

Main findings (Section 4.2):

- Autoimmune arthritis developed in splenectomized BALB/c mice despite the absence of the spleen.
- The severity and incidence of autoimmune arthritis was similar in splenectomised- and control (spleen preserved) BALB/c mice.
- The rhG1 antigen-induced proliferation and cytokine production by the mLN cells isolated from splenectomized mice was lower than the control.
- The levels of anti-rhG1 IgG1 and anti-CCP (IgG2a and IgG1) autoantibodies were higher in splenectomized compared to the control group.

- The iLNs of the splenectomized mice had markedly larger follicles and germinal centres than the control.
- B cells isolated from mLNs of splenectomized mice had lower Ca^{2+} signalling than control mice after anti-IgM or -IgG stimulation.

Table 1. Comparative summary of the main findings of Sections 4.1 and 4.2

	CTRL	Nkx2-3$^{-/-}$	SPE
Clinical Parameters			
Avg severity score*	~13.0	9.2±1.0	12.5±0.7
Incidence %**	>90%	~70%	100%
Level of Joints' Deterioration*†	Severe	Mild	Severe
Serum Parameters			
Cytokines††	↑↑IFNγ ↑↑IL-17, ↑↑IL-23 ↓↓IL-4	↑↓IFNγ ↓↓IL-17, ↑↓IL-23 ↑↑IL-4	↓↓IFNγ ↑↓IL-17, ↓↓IL-23 ↑↓IL-4
Autoantibodies††	↑↓ rhG1 IgG1 ↑↑ rhG1 IgM ↑↓ACPA & RF	↓↓rhG1 IgG1 ---- ↓↓ACPA	↑↑rhG1 IgG1 ↓↓rhG1 IgM ↑↑ACPA & RF
Ca^{2+} signaling			
B cells‡	Strong	<< CTRL	< CTRL
T cells‡	Strong	Strong	< CTRL

*Average severity scores of the experimental mouse groups at the end of the experiments. **Incidence percentages of mice developed GIA at the end of experiments. *†Results acquired from joints' CT-scan and H&E staining at the end of the experiments or after sacrificing the mice. ††Measurements performed after sacrificing the mice. ↑↓Measured value lies between the other two groups. ↑↑The highest measured value among all groups. ↓↓The lowest measured value among all groups. ‡Calcium signaling measurements acquired from the inguinal and mesenteric lymph nodes at the end of the experiments.

4.3. Studying the effect of splenectomy during the induction of GIA

In this part of the thesis, we wanted to investigate what if the spleen is removed during the induction period (corresponding to the early phase of RA) of arthritis in the GIA model. Therefore, during immunization with the rhG1 antigen, BALB/c mice were splenectomized at different time points (Fig. 4.3). We immunized all experimental groups three times on days 0,

28 and 56. Mice were randomized into three groups; i) group one was spleen preserved until the termination of the study (control group); ii) group two was splenectomized on day 7 (SPE1); iii) group three was splenectomized on day 35 (SPE2). We followed the clinical parameters, the joints' histological and radiological changes and serum parameters of all groups (Fig. 4.3).

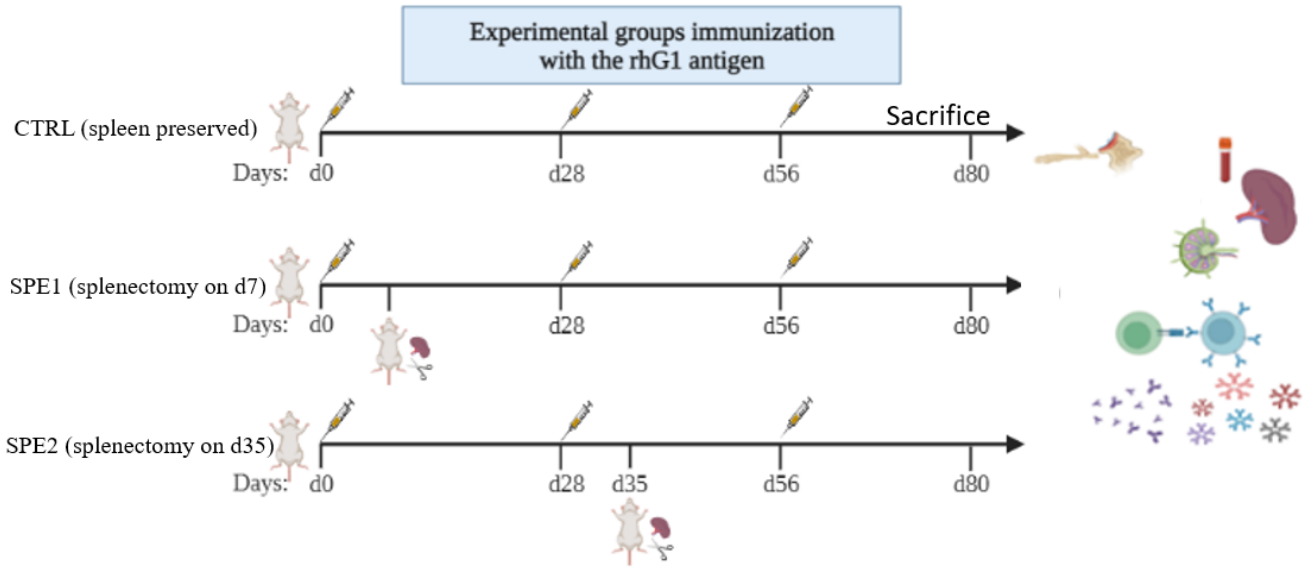

Figure 4.3. Schematic representation of the experimental design for 4.3. BALB/c mice were immunized using the rhG1 antigen (day 0). One week later (day 7), a group of mice were splenectomized (SPE1). On day 28 all mice received the 2nd immunization. One week later (day 35), another set of mice were splenectomized (SPE2). On day 56 all mouse groups received the 3rd immunization.

Main findings (Section 4.3):

- The development of autoimmune arthritis was delayed in mice, which were splenectomized after the first- or the second immunizations.
- There was significantly less bone- and cartilage destruction in those mice, which were splenectomized in the pre-clinical stage of RA.
- The concentrations of serum pro-inflammatory cytokines and autoantibodies were lower in the splenectomized mice than in the control.
- There was a shift towards the Th_2 direction in the splenectomised mice.
- The delayed inflammatory response in splenectomized mice corresponded with an increased circulating nTreg frequency.

Table 2. Comparison of the main observations of the experimental groups in Section 4.3

	CTRL	SPE1	SPE2
Clinical Parameter			
Avg severity score*	13.8±1.3	12.4±1	11.2±1.8
Incidence %**	~90%	~90%	~70%
Level of Joints' Deterioration*†	Severe	Mild	Moderate-Severe
Serum Parameters			
Cytokines ††	↑↑IFNγ ↑↑IL-17, ↑↑IL-23 ↑↑IL-4	↑↓IFNγ ↓IL-17, ↓IL-23 ↓↓IL-4	↓↓IFNγ ↓IL-17, ↓IL-23 ↑↓IL-4
Autoantibodies ††	↓↓rhG1 IgG1 ↑↑rhG1 IgM ↑↑Anti-mPG ↑↑ACPA IgG1 ↓↓ACPA IgG2a ↑↑RF	↑↓rhG1 IgG1 ↓rhG1 IgM ↓↓Anti-mPG ↓↓ACPA IgG1 ↑↑ACPA IgG2a ↓↓RF	↑↑rhG1 IgG1 ↓rhG1 IgM ↑↓Anti-mPG ↑↓ACPA IgG1 ↑↓ACPA IgG2a ↑↓RF
Frequencies of T-helper Cell Subsets			
Spleen ††	↑↑Th_1, ↑↑Th_{17} ↓↓Th_2	----	----
iLNs ††	↓↓Th_1, ↑↑Th_{17} ↑↓Th_2	↑↑Th_1, ↑↓Th_{17} ↑↑Th_2	↑↓Th_1, ↓↓Th_{17} ↓↓Th_2
Circulating nTregs‡‡	↓↓nTregs	↑nTregs	↑nTregs

*Average severity scores of the experimental mouse groups at the end of the experiments. **Incidence percentages of mice developed GIA at the end of experiments. *† Results acquired from joints' CT-scan and H&E staining at the end of the experiments or after sacrificing the mice. †† Measurements performed after sacrificing the mice. ↑↓ Measured value lies between the other two groups. ↑↑ The highest measured value among all groups. ↓↓ The lowest measured value among all groups. ↑ Measured values were similarly high in two groups. ↓ Measured values were similarly low in two groups. ‡‡ Frequencies of circulating nTregs on day 53.

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List of publications and communications related to this thesis

Research papers

E. Khanfar, K. Olasz, E. Gajdócsi, X. Jia, T. Berki, P. Balogh, F. Boldizsár, Splenectomy modulates the immune response but does not prevent joint inflammation in a mouse model of RA, *Clinical and Experimental Immunology*, (2022). *(IF: 5.732)*

E. Khanfar, K. Olasz, F. Gábris, E. Gajdócsi, B. Botz, T. Kiss, R. Kugyelka, T. Berki, P. Balogh, F. Boldizsár, Ameliorated Autoimmune Arthritis and Impaired B Cell Receptor-Mediated Ca2+ Influx in Nkx2-3 Knock-out Mice, *International Journal of Molecular Sciences*, **21** (2020) 6162. *(IF: 5.924)*

E. Khanfar, K. Olasz, S. Gál, E. Gajdócsi, B. Kajtár, T. Kiss, P. Balogh, T. Berki, F. Boldizsár, Splenectomy at Early stage of Autoimmune Arthritis Delayed Inflammatory Response and Reduced Joint Deterioration in Mice. (*Submitted)*

Oral presentations

E. Khanfar, K. Olasz, F. Gábris, E. Gajdócsi, B. Botz, T. Kiss, R. Kugyelka, T. Berki, P. Balogh, F. Boldizsár, Nkx2-3 controls autoimmune arthritis through modifying B cell activation, 40th European Workshop for Rheumatology Research (EWRR), Leuven, Belgium, 13-15/02/2020.

List of publications not related to this thesis

Research papers

K. Szabó, Á. Kemény, N. Balázs, **E. Khanfar**, Z. Sándor, F. Boldizsár, R. Gyulai, J. Najbauer, E. Pintér, T. Berki, Presence of TRPA1 Modifies CD4+/CD8+ T Lymphocyte Ratio and Activation, *Pharmaceuticals*, **15** (2022) 57. *(IF: 5.863)*

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