Analysis of B-cell function mediated by the Toll-like receptor homologue CD180 in systemic sclerosis

PhD Thesis

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1. Introduction

Systemic sclerosis (SSc) is a complex systemic autoimmune rheumatological disease characterized by vascular damage, immunological abnormalities and fibrosis of the skin and internal organs. Diffuse cutaneous SSc (dcSSc) is a more severe form of the disease with a worse prognosis and is characterized by extensive skin lesions and severe cardiac, pulmonary, renal and gastrointestinal symptoms [1]. B cells play a role in the pathogenesis of SSc through several functions, such as the production of autoantibodies and cytokines, the regulation of T cell and dendritic cell functions and altered antigen presentation [2]. It is also known that B-cell activation is an early event in the development of the disease [3].

In SSc, the most obvious sign of impaired B-cell tolerance is the high levels of circulating autoantibodies in the serum of patients. Anti-nuclear autoantibodies (ANA) directed against various nuclear components, including anti-topoisomerase I (anti-topo I), anti-centromere (ACA) and anti-RNA polymerase III, are typical and they correlate with disease severity [2]. In SSc, increased production of IL-6 and TGF-β by B cells has been described, which contribute to antibody production and the development of fibrosis [4,5]. Furthermore, decreased numbers and impaired function of IL-10 producing regulatory B cells were observed in SSc [6].

In addition to altered B-cell homeostasis, a different distribution of B-cell compartments was also observed in SSc. Naive B cells are increased, whereas memory B cells are decreased but activated in the peripheral blood of patients [7]. Our research team has previously shown that reduced memory B-cell levels in patients with SSc may be due to a reduction in the number of non-switched memory (NS) B-cells, which are thought to be able to produce natural autoantibodies [8].

Natural antibodies are low-affinity, polyreactive, mostly IgM isotype antibodies directed against various evolutionarily conserved antigens, and play an important role in defense against pathogens and infections, modulating innate and acquired immune responses and regulating inflammatory and autoimmune processes [9]. Our research team has previously shown that natural autoantibodies against the mitochondrial inner membrane enzyme citrate synthase (CS) and the F4 epitope of topoisomerase I are present in the serum of healthy individuals and autoimmune patients [10,11].

B cell function is known to be regulated by innate immune molecules such as Toll-like receptors (TLRs) [12]. TLRs are able to recognize pathogen-associated molecular patterns

(PAMPs) and contribute to the development of an effective immune response by serving as a first line of defense and early recognition of the invading pathogen [13]. Altered expression and function of several TLRs have been observed in autoimmune diseases [14].

CD180 is a TLR homologue membrane protein lacking the intracellular TIR signaling domain [15]. CD180 was originally identified as a B-cell surface molecule capable of inducing polyclonal B-cell activation, proliferation and robust immunoglobulin (Ig) production [16]. In patients with systemic lupus erythematosus (SLE), an increase in the proportion of CD180-negative B cells was observed, which was associated with disease severity [17].

Phosphatidylinositol 3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) signaling pathway appear to play a key role in the pathogenesis of SSc, in particular in two typical features of SSc, fibrogenesis and B-cell activation [18,19]. TLR and B-cell activating factor (BAFF)-mediated effects also act through the PI3K/Akt/mTOR pathway [20].

BAFF plays an important role in B-cell survival and homeostasis, its increased level have been observed in SSc, contributing to the breakdown of B-cell tolerance by protecting autoreactive B-cells [21,22]. Increased levels of anti-BAFF autoantibodies have been observed in SLE, which may regulate BAFF activity [23]. BAFF is able to bind to several receptors on B cells, such as the BAFF receptor (BAFF-R), which is a positive regulator of B cell homeostasis providing a continuous survival signal, and the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), which stimulates Ig isotype switching and plasma cell differentiation [24,25].

2. Aims

Our main objective was to investigate the possible role of the TLR homologue CD180 mediated B-cell activation in the pathogenesis of dcSSc.

Our specific objectives:

1. To determine the expression of CD180 molecule in peripheral blood B cells from early dcSSc patients compared to healthy individuals.
2. To investigate the effect of stimulation via CD180 on the phosphorylation of Akt, S6 and NF-κB molecules,which are related to signaling pathways that play a crucial role in both SSc pathogenesis and B cell activation, in B cells of dcSSc patients compared to healthy individuals.
3. To measure anti-BAFF autoantibody levels in serum samples from dcSSc patients compared to healthy individuals.
4. To determine the expression of BAFF receptors in B-cells and B-cell subsets from dcSSc patients compared to healthy individuals and to investigate the effect of stimulation via the CD180 molecule on BAFF receptor expression.
5. To model the possible role of CD180 in the pathogenesis of SSc using tonsillar B cells:
    a. To investigate B cell activation, cytokine and antibody production after anti-CD180 antibody treatment.
    b. To study whether the presence of another TLR ligand (CpG) affects the effect of CD180 ligation on these B cell functions.

3. Materials and methods

3.1. Samples

We used peripheral blood samples from 30 early dcSSc patients for our studies on peripheral blood B-cells. All patients fulfilled the 2013 ACR/EULAR SSc classification criteria. Controls (n=36) were age and sex-matched healthy individuals (HC). For our studies on tonsillar B cells, we used tonsillar samples from asymptomatic children who had undergone routine tonsillectomy.

3.2. Mononuclear cell isolation, B cell separation and cell stimulation

Mononuclear cells were isolated from peripheral blood samples or from manually homogenized tonsillar cell suspensions by Ficoll density gradient centrifugation, followed by purification of B cells by magnetic bead-based negative selection. The natural ligand of the CD180 molecule is unknown, therefore monoclonal anti-CD180 antibody was used to stimulate B cells via the CD180 molecule. Anti-human CD180 antibody and TLR9 ligand CpG were both used at a concentration of 1 ug/ml.

3.3. RNA isolation, cDNA Synthesis and qPCR

After RNA isolation from the isolated B-cells and cDNA generation, the expression of CD180 and BAFF-R mRNA was determined by qPCR using GAPDH as a reference.

3.4. ELISA and MAGPIX measurements

The levels of natural autoantibodies (anti-CS and anti-topo I antibodies) were measured from the supernatants of stimulated tonsillar B cells using in-house ELISA tests, and the levels of cytokines (IL-6 and IL-10) were determined using commercial ELISA kits. Luminex MAGPIX method was used to quantify anti-BAFF autoantibodies in the serum of dcSSc patients and HCs.

3.5. Flow cytometry

In our flow cytometry studies, B cells were identified using the CD19 cell line marker and the CD27 and IgD markers were used to identify the following 4 B cell subsets: CD27+IgD+ non-switched memory (NS), CD27+IgD- switched memory (S), CD27-IgD- double negative (DN) and CD27-IgD+ naive B cells. Furthermore, flow cytometry assays were also used to determine CD180, BAFF-R and TACI protein expressions. Phosphorylation assays of Akt, S6 and NF-κB signaling molecules were performed using the BD Phosflow protocol and flow cytometry.

4. Results and conclusions

4.1. Investigation of the effects of stimulation via the CD180 molecule on peripheral blood B cells in dcSSc patients

Studies on the involvement, the contribution of innate immune molecules to B-cell dysfunction in SSc are scarce. The TLR homologue CD180 is able to activate the majority of B cells and induce phenotypic and functional changes. Elevated levels of CD180-negative B cells have been described in SLE patients and in a mouse model of SLE. We were the first to determine that peripheral blood B cells of dcSSc patients showed diminished expression of the CD180 molecule at both protein and mRNA levels, suggesting a possible role for the CD180 molecule in B cell dysfunction in SSc.

PI3K/Akt/mTOR signaling contributes to the development of fibrosis in SSc and inhibition of the pathway prevents fibrosis development in a bleomycin-induced SSc mouse model. In addition, the PI3K/Akt/mTOR pathway plays an important role in B-cell activation and differentiation and B cells are known to play a key role in the pathogenesis of SSc. Furthermore, TLRs also act through this signaling pathway and stimulation via CD180 has been described to increase the phosphorylation of Akt in B cells from B-CLL patients. We showed that stimulation via CD180 increased phosphorylation of Akt and S6 in both dcSSc and HC B cells, however, we observed lower levels of Akt activation in dcSSc than in HC, suggesting an impairment of CD180 signaling in the PI3K/Akt pathway in B cells from dcSSc patients.

Previously, decreased phosphorylation of mTOR was observed in B cells from SSc patients. In contrast, we found no difference in S6 phosphorylation between dcSSc and HC B cells, suggesting that alterations in mTOR pathway in SSc B cells may influence other down-stream molecules than S6. NF-κB is an important central pathway that interacts with several other upstream and downstream signaling pathways, including the PI3K/Akt/mTOR pathway. Furthermore, it is known that stimulation via TLRs can trigger NF-κB activation. In our studies, anti-CD180 antibody treatment increased NF-κB phosphorylation in B cells both in dcSSc and HC, but to a lesser extent in dcSSc, suggesting an impairment of CD180 signaling in the NF-κB pathway in B cells from dcSSc patients.

The PI3K/Akt/mTOR pathway also plays an important role in the BAFF-mediated B-cell activation and differentiation. BAFF is essential for B cell survival, maturation and homeostasis. Increased BAFF may contribute to the development of systemic autoimmune

diseases by disrupting B-cell tolerance. Elevated levels of BAFF have been observed in patients with SSc, which correlated with disease severity and activity. Although autoantibodies against BAFF have also been detected in the serum of healthy individuals, elevated levels have been observed in patients with SLE. Examining the levels of anti-BAFF autoantibodies in patients with SSc, we obtained similar results to those observed in patients with SLE, levels of anti-BAFF autoantibodies were higher in dcSSc patients than in HCs. This suggests that the increased levels of anti-BAFF autoantibodies may be part of a regulatory mechanism in response to the elevated BAFF levels observed in SSc patients.

BAFF is able to bind to multiple receptors on B cells thus exert different effects. BAFF-R is involved in normal B cell development and survival, while TACI stimulates Ig isotype switching and plasma cell differentiation. Our studies have shown decreased expression of BAFF-R at protein and mRNA levels and increased expression of TACI at protein level in B cells from dcSSc patients compared to HCs. The decreased expression of BAFF-R and increased expression of TACI in B cells from SSc patients may contribute to the differentiation of autoreactive B cells into antibody-producing plasma cells and it is well known that autoantibodies play a key role in the pathogenesis of SSc.

Differences in BAFF-R and TACI expression observed in B cells from dcSSc patients and HCs may be due to different expression of these molecules in naive B cells, since examining BAFF-R and TACI expression of B cell subsets identified by CD27 and IgD labelling, we found decreased BAFF-R and increased TACI expression in naive B cells in dcSSc. The significance of this finding is highlighted by the increased proportion of naive B cells observed in SSc patients and these results provide further evidence that differences in the distribution of B cell subsets, in particular an increase in the number of naive B cells, may play an important role in the development of SSc.

TLR signaling can influence the expression of BAFF receptors, therefore we investigated the effect of activation via CD180 on the expression of BAFF-R and TACI of B cells and B cell subsets defined by CD27 and IgD labeling, in dcSSc and HC. We showed that CD180 ligation resulted in decreased expression of BAFF-R and increased expression of TACI in HC B cells, reaching levels observed in dcSSc. This change may also be due to differences in naive B-cells, as stimulation via CD180 increased BAFF-R and decreased TACI expression in naive B-cells from HCs, reaching the levels observed in naive B-cells from dcSSc patients. Our results raise the possibility of an impairment of CD180 signaling in B cells in dcSSc patients, which may contribute to a pathological shift in BAFF signaling, particularly in naive B cells.

4.2. Investigation of the effects of stimulation via CD180 on tonsillar B cells

In our studies on tonsillar B cells, we have shown that CD180 expression in tonsillar B cells is downregulated following stimulation via the CD180 molecule. It is known that in SLE, CD180-negative B cells are highly activated and that CD180 can be internalized following anti-CD180 antibody binding. These findings suggest that increased B-cell activation via CD180 may be a possible explanation for the reduced CD180 expression observed in B-cells from SSc patients.

First, we examined the expression of CD180 molecule in the B-cell subsets identified by CD27 and IgD labelling and showed that the proportion of CD180+ cells was highest in the NS B-cell subset. Anti-CD180 antibody treatment significantly decreased the proportion of CD180+ cells in all investigated B-cell subsets, suggesting that CD180 ligation stimulates CD180 internalization. In addition, we showed that anti-CD180 antibody stimulation decreased the expression of CD180 mRNA supporting the possibility of CD180 autoregulation by B cells.

Stimulation via CD180 can activate marginal zone (MZ) B cells. NS B-cells represent the MZ-derived B-cells in human peripheral blood and our results showed that NS B-cells were the most activated among B-cell subsets following anti-CD180 antibody stimulation when examined for expression of CD69, an early activation marker.

Our research team has previously shown that the proportion of NS B cells is decreased in SSc. Like B1 B cells, NS B cells may also be able to produce natural autoantibodies. Natural IgM autoantibodies are polyreactive and play an important role in the clearance of damaged molecules and cells and in the regulation of inflammatory and autoimmune processes. We investigated the effect of stimulation via TLRs on natural autoantibody production by tonsillar B cells and found that simultaneous administration of anti-CD180 antibody and TLR9 ligand, CpG, significantly increased the production of anti-CS and anti-topo I IgM autoantibodies, suggesting a synergistic effect of anti-CD180 antibody and TLR9 ligand on natural autoantibody production. Anti-CD180 antibody treatment alone increased the amount of produced anti-topo I IgM antibody, but did not affect anti-CS IgM antibody production. The CS molecule is not a target for disease-specific pathological antibodies, whereas natural anti-topo I autoantibodies are directed against topo I, which is the target antigen for SSc-specific pathological autoantibodies (anti-Scl-70). Activation of B cells via CD180 may play a role in regulating the levels of natural IgM antibodies directed against the target antigen of pathological antibodies.

Elevated serum levels of IL-6 have been observed in patients with SSc and SLE, and in addition, The IL-6 production production by B cells promotes the formation of autoimmune germinal centers and thereby the development of the disease in a mouse model of SLE. In addition, IL-6 plays a role in plasma cell differentiation and survival. In our studies, anti-CD180 antibody treatment stimulated IL-6 production by tonsillar B cells, which was increased by the addition of CpG. This suggests that B cell activation via CD180 alone, or in combination with TLR9 ligand may contribute to plasma cell differentiation and antibody production.

Decreased number and impaired function of regulatory B cells have been described in patients with SSc, therefore we also examined the effect of anti-CD180 antibody treatment on IL-10 production by tonsillar B cells. IL-10 production was only enhanced only by anti-CD180 antibody and CpG co-treatment suggesting a synergistic effect of the two TLRs.

Our results suggest that CD180-mediated B-cell function may play a role in the development of SSc, but also suggest that anti-CD180 antibody therapy, which has been implicated in the treatment of SLE patients, may have an adverse effect on B-cell function in SSc.

5. Summary of the new scientific results

5.1. Investigation of the effect of stimulation via the CD180 molecule on peripheral blood B cells in patients with dcSSc:

1. We found reduced protein and mRNA expression of the CD180 molecule in peripheral blood B cells of early dcSSc patients compared to healthy individuals.

2. Upon stimulation via CD180, we observed a lower increase in Akt and NF-κB and a similar increase in S6 phosphorylation in B cells from dcSSc patients compared to healthy controls.

3. Elevated serum levels of anti-BAFF autoantibodies were detected in patients with dcSSc compared to healthy individuals.

4. We found decreased BAFF-R and increased TACI expression in B cells from dcSSc patients compared to healthy individuals, which was due to differences observed in naive B cells.

5. CD180 ligation reduced BAFF-R expression and increased TACI expression in B cells, specifically in naive B cells of healthy controls to the levels observed in dcSSc patients.

5.2. Investigation of the effect of stimulation via the CD180 molecule on B-cell function in tonsillar B-cells:

6. The expression of CD180 protein and mRNA in tonsillar B cells and B cell subsets was reduced following anti-CD180 antibody treatment.

7. Anti-CD180 antibody treatment activated all investigated tonsillar B-cell subsets.

8. The expression of CD180 was highest in non-switched memory B cells and this tonsillar B-cell subset was activated to the highest extent after anti-CD180 antibody treatment.

9. Stimulation via CD180 induced the IL-6 production of tonsillar B cells, but it did not affect their IL-10 production.

10. The anti-CD180 antibody treatment itself increased the production of anti-topo I but not the production of anti-CS IgM natural autoantibodies by tonsillar B cells.

11. We observed a synergistic effect of anti-CD180 antibody and TLR9 ligand CpG on cytokine and natural autoantibody production of tonsillar B cells.

6. References

1. Allanore, Y.; Simms, R.; Distler, O.; Trojanowska, M.; Pope, J.; Denton, C.P.; Varga, J. Systemic sclerosis. *Nat. Rev. Dis. Prim.* **2015**, *1*.
2. Bosello, S.; De Luca, G.; Tolusso, B.; Lama, G.; Angelucci, C.; Sica, G.; Ferraccioli, G. B cells in systemic sclerosis: A possible target for therapy. *Autoimmun. Rev.* **2011**, *10*, 624–630.
3. Skaug, B.; Khanna, D.; Swindell, W.R.; Hinchcliff, M.E.; Frech, T.M.; Steen, V.D.; Hant, F.N.; Gordon, J.K.; Shah, A.A.; Zhu, L.; et al. Global skin gene expression analysis of early diffuse cutaneous systemic sclerosis shows a prominent innate and adaptive inflammatory profile. *Ann. Rheum. Dis.* **2019**, *79*, 379–386.
4. Sato, S.; Hasegawa, M.; Takehara, K. Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. *J. Dermatol. Sci.* **2001**, *27*, 140–146.
5. J, V.; ML, W. Transforming growth factor-beta in systemic sclerosis (scleroderma). *Front. Biosci. (Schol. Ed).* **2009**, *1*, 226–235.
6. Mavropoulos, A.; Simopoulou, T.; Varna, A.; Liaskos, C.; Katsiari, C.G.; Bogdanos, D.P.; Sakkas, L.I. Breg Cells Are Numerically Decreased and Functionally Impaired in Patients with Systemic Sclerosis. *Arthritis Rheumatol.* **2016**, *68*, 494–504.
7. Sato, S.; Fujimoto, M.; Hasegawa, M.; Takehara, K. Altered blood B lymphocyte homeostasis in systemic sclerosis: Expanded naive B cells and diminished but activated memory B cells. *Arthritis Rheum.* **2004**, *50*, 1918–1927.
8. Simon, D.; Balogh, P.; Bognár, A.; Kellermayer, Z.; Engelmann, P.; Németh, P.; Farkas, N.; Minier, T.; Lóránd, V.; Czirják, L.; et al. Reduced non-switched memory B cell subsets cause imbalance in B cell repertoire in systemic sclerosis. *Clin. Exp. Rheumatol.* **2016**, *34*, 30–36.
9. Maddur, M.S.; Lacroix-Desmazes, S.; Dimitrov, J.D.; Kazatchkine, M.D.; Bayry, J.; Kaveri, S. V. Natural Antibodies: from First-Line Defense Against Pathogens to Perpetual Immune Homeostasis. *Clin. Rev. Allergy Immunol.* 2020, *58*, 213–228.
10. Czömpöly, T.; Olasz, K.; Simon, D.; Nyárády, Z.; Pálinkás, L.; Czirják, L.; Berki, T.; Németh, P. A possible new bridge between innate and adaptive immunity: Are the anti-mitochondrial citrate synthase autoantibodies components of the natural antibody network? *Mol. Immunol.* **2006**, *43*, 1761–8.
11. Simon, D.; Czömpöly, T.; Berki, T.; Minier, T.; Peti, A.; Tóth, E.; Czirják, L.; Németh, P. Naturally occurring and disease-associated auto-antibodies against topoisomerase I: A fine epitope mapping study in systemic sclerosis and systemic lupus erythematosus. *Int. Immunol.* **2009**, *21*, 415–422.
12. Kremlitzka, M.; Mácsik-Valent, B.; Erdei, A. Regulation of B cell functions by Toll-like receptors and complement. *Immunol. Lett.* **2016**, *178*, 37–44.
13. Vidya, M.K.; Kumar, V.G.; Sejian, V.; Bagath, M.; Krishnan, G.; Bhatta, R. Toll-like receptors: Significance, ligands, signaling pathways, and functions in mammals. *Int. Rev. Immunol.* **2018**, *37*, 20–36.
14. Farrugia, M.; Baron, B. The Role of Toll-Like Receptors in Autoimmune Diseases

through Failure of the Self-Recognition Mechanism. *Int. J. Inflam.* **2017**, *2017*.

15. Schultz, T.E.; Blumenthal, A. The RP105/MD-1 complex: molecular signaling mechanisms and pathophysiological implications. *J. Leukoc. Biol.* **2017**, *101*, 183–192.
16. Chaplin, J.W.; Kasahara, S.; Clark, E.A.; Ledbetter, J.A. Anti-CD180 (RP105) Activates B Cells To Rapidly Produce Polyclonal Ig via a T Cell and MyD88-Independent Pathway. *J. Immunol.* **2011**, *187*, 4199–4209.
17. Koarada, S.; Tada, Y.; Ushiyama, O.; Morito, F.; Suzuki, N.; Ohta, A.; Miyake, K.; Kimoto, M.; Nagasawa, K. B cells lacking RP105, a novel B cell antigen, in systemic lupus erythematosus. *Arthritis Rheum.* **1999**, *42*, 2593–2600.
18. Liang, M.; Lv, J.; Chu, H.; Wang, J.; Chen, X.; Zhu, X.; Xue, Y.; Guan, M.; Zou, H. Vertical inhibition of PI3K/Akt/mTOR signaling demonstrates in vitro and in vivo anti-fibrotic activity. *J. Dermatol. Sci.* **2014**, *76*, 104–111.
19. Limon, J.J.; Fruman, D.A. Akt and mTOR in B cell activation and differentiation. *Front. Immunol.* **2012**, *3*, 228.
20. Werner, M.; Hobeika, E.; Jumaa, H. Role of PI3K in the generation and survival of B cells. *Immunol. Rev.* 2010, *237*, 55–71.
21. Matsushita, T.; Hasegawa, M.; Yanaba, K.; Kodera, M.; Takehara, K.; Sato, S. Elevated serum BAFF levels in patients with systemic sclerosis: Enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum.* **2006**, *54*, 192–201.
22. Thien, M.; Phan, T.G.; Gardam, S.; Amesbury, M.; Basten, A.; MacKay, F.; Brink, R. Excess BAFF Rescues Self-Reactive B Cells from Peripheral Deletion and Allows Them to Enter Forbidden Follicular and Marginal Zone Niches. *Immunity* **2004**, *20*, 785–798.
23. Price, J. V.; Haddon, D.J.; Kemmer, D.; Delepine, G.; Mandelbaum, G.; Jarrell, J.A.; Gupta, R.; Balboni, I.; Chakravarty, E.F.; Sokolove, J.; et al. Protein microarray analysis reveals BAFF-binding autoantibodies in systemic lupus erythematosus. *J. Clin. Invest.* **2013**, *123*, 5135.
24. Schiemann, B.; Gommerman, J.L.; Vora, K.; Cachero, T.G.; Shutga-Morskaya, S.; Dobles, M.; Frew, E.; Scott, M.L. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science (80-.).* **2001**, *293*, 2111–2114.
25. Zhang, Y.; Li, J.; Zhang, Y.M.; Zhang, X.M.; Tao, J. Effect of TACI signaling on humoral immunity and autoimmune diseases. *J. Immunol. Res.* **2015**, *2015*.

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8. Publications

8.1. Publications related to the thesis

1. **Szabina Erdő-Bonyár**, Judit Rapp, Tünde Minier, Gábor Ráth, József Najbauer, László Czirják, Péter Németh, Timea Berki, Diána Simon: Toll-Like Receptor Mediated Activation of Natural Autoantibody Producing B Cell Subpopulations in an Autoimmune Disease Model. International journal of molecular sciences, 2019, 20(24), 6152. https://doi.org/10.3390/ijms20246152 *IF: 4,556*

2. Diána Simon, **Szabina Erdő-Bonyár**, Judit Rapp, Péter Balogh, Tünde Minier, Gábor Gabriella Nagy, László Czirják, Timea Berki: Analysis of PI3K Pathway Associated Molecules Reveals Dysregulated Innate and Adaptive Functions of B Cells in Early Diffuse Cutaneous Systemic Sclerosis. International journal of molecular sciences, 2021, 22(6), 2877. https://doi.org/10.3390/ijms22062877 *IF: 5,923*

3. **Szabina Erdő-Bonyár**, Judit Rapp, Dávid Szinger, Tünde Minier, Gábor Kumánovics, László Czirják, Timea Berki, Diána Simon: Ligation of TLR Homologue CD180 of B Cells Activates the PI3K/Akt/mTOR Pathway in Systemic Sclerosis and Induces a Pathological Shift in the Expression of BAFF Receptors. International Journal of Molecular Sciences, 2022, 23(12), 6777. https://doi.org/10.3390/ijms23126777 *IF: 6,208*

8.2. Other publications

4. Hayden, Z., **Erdő-Bonyár, S.,** Bóné, B., Balázs, N., Bodó, K., Illes, Z., Berki, T., & Simon, D. (2021). Toll-Like Receptor Homolog CD180 Expression Is Diminished on Natural Autoantibody-Producing B Cells of Patients with Autoimmune CNS Disorders. Journal of immunology research, 2021, 9953317. https://doi.org/10.1155/2021/9953317 *IF: 4,818*

5. Böröcz, K., Simon, D., **Erdő-Bonyár, S**., Kovács, K. T., Tuba, É., Czirják, L., Németh, P., & Berki, T. (2021). Relationship between natural and infection-induced antibodies in systemic autoimmune diseases (SAD): SLE, SSc and RA. Clinical and experimental immunology, 203(1), 32–40. https://doi.org/10.1111/cei.13521 *IF: 4,330*

6. Olmos Calvo, I., Kuten-Pella, O., Kramer, K., Madár, Á., Takács, S., Kardos, D., Simon, D., **Erdö-Bonyár, S**., Berki, T., De Luna, A., Nehrer, S., & Lacza, Z. (2021). Optimization of Lyophilized Hyperacute Serum (HAS) as a Regenerative Therapeutic in Osteoarthritis. International journal of molecular sciences, 22(14), 7496. https://doi.org/10.3390/ijms22147496 *IF: 5,923*

7. Schranz, D., Molnar, T., **Erdo-Bonyar, S.,** Simon, D., Berki, T., Nagy, C., Czeiter, E., Buki, A., Lenzser, G., & Csecsei, P. (2021). Increased level of LIGHT/TNFSF14 is associated with survival in aneurysmal subarachnoid hemorrhage. Acta neurologica Scandinavica, 143(5), 530–537. https://doi.org/10.1111/ane.13394 *IF: 3,902*

8. Simon, D., Balogh, P., **Erdő-Bonyár, S**., Böröcz, K., Minier, T., Czirják, L., & Berki, T. (2021). Increased Frequency of Activated Switched Memory B Cells and Its Association With the Presence of Pulmonary Fibrosis in Diffuse Cutaneous Systemic Sclerosis Patients. Frontiers in immunology, 12, 686483. https://doi.org/10.3389/fimmu.2021.686483 *IF: 7,561*

9. Schranz, D., Molnar, T., **Erdo-Bonyar, S**., Simon, D., Berki, T., Zavori, L., Szolics, A., Buki, A., Lenzser, G., & Csecsei, P. (2021). Fatty Acid-Binding Protein 3 and CXC-Chemokine Ligand 16 are Associated with Unfavorable Outcome in Aneurysmal Subarachnoid Hemorrhage. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association, 30(11), 106068. https://doi.org/10.1016/j.jstrokecerebrovasdis.2021.106068 *IF: 2,136*

10. Hau, L., Tényi, T., László, N., Kovács, M. Á., **Erdö-Bonyár, S**., Csizmadia, Z., ... & Csábi, G. (2022). Anti-Neuronal Autoantibodies (Cell Surface and Onconeural) and Their Association With Natural Autoantibodies in Synthetic Cannabinoid-Induced Psychosis. Frontiers in Psychiatry, 13. doi: 10.3389/fpsyt.2022.850955 *IF: 4,157*

11. Schranz, D., Molnar, T., **Erdo-Bonyar, S**., Simon, D., Berki, T., Nagy, C., Czeiter, E., Buki, A., Lenzser, G., & Csecsei, P. (2021). A magasabb LIGHT/TNFSF14 szint összefügg a subarachnoidalis vérzésben szenvedők túlélésével. FOCUS MEDICINAE 23 : 1 pp. 6-13. , 8 p.

Cummulative impact factor of publications related to the thesis: ***16,687***

Cummulative impact factor of all publications: ***48,821***

9. Conference presentations and posters related to the thesis

1. A Magyar Immunológiai Társaság (MIT) 48. vándorgyűlése. 2019.10.16-18. Bükfürdő: Flow cytometric analysis of tonsillar B cells activated via CD180

2. MEDPÉCS (Medical Conference for PhD Students and Experts of Clinical Sciences), 9th of November 2019, Pécs: Flow cytometric analysis of tonsillar B cells activated via CD180

3. EWRR (European Workshop for Rheumatology Research), 13-16th of February 2020, Leuven, Belgium: Activation of B cells via toll-like receptor analogue CD180 shift B cells to natural autoantibody production in a systemic sclerosis disease model

4. A Magyar Immunológiai Társaság (MIT) 49. vándorgyűlése, 2020.10.7-8, Online: A TLR-analóg (CD180) megváltoztatja a B-sejtek aktivációját és eloszlását szisztémás sclerosisban

5. MEDPÉCS (Medical Conference for PhD Students and Experts of Clinical Sciences), 17th of November 2020, Online: TLR analogue (CD180) alters B cell activation and distribution in systemic sclerosis

6. A Magyar Immunológiai Társaság (MIT) 50. vándorgyűlése 2021.10.20-22, Kecskemét:

    a. B-sejtek funkcionális vizsgálata a PI3K jelátviteli útvonalhoz kapcsolódó molekulák elemzésével szisztémás sclerosisban

    b. A CD19/IgD/CD27/CD38/CD95 markerekkel definiált memória B-sejt alcsoportok megoszlásának eltérései szisztémás sclerosisban

7. Az "Intelligens szakosodás megvalósítása a Pécsi Tudományegyetemen" című online rendezvény, 2022.03.21-23: B-sejtek funkcionális vizsgálata a PI3K jelátviteli útvonalhoz kapcsolódó molekulák elemzésével szisztémás sclerosisban

8. 51. Membrán-transzport konferencia, 2022.05.17-20, Sümeg: B-sejtek funkcionális vizsgálata a PI3K jelátviteli útvonalhoz kapcsolódó molekulák elemzésével szisztémás sclerosisban

9. PTE helyi UNKP rendezvény, 2022.06.02, Pécs: A TLR homológ CD180 molekula szerepe a B-sejtek szignalizációjában, citokin- és antitesttermelésében