Altered expression of SIRPγ on the T-cells of relapsing remitting multiple sclerosis and type 1 diabetes patients could potentiate effector responses from T-cells

Sushmita Sinha1*, Pranav S. Renavikar1, Michael P. Crawford1, Scott M. Steward-Tharp1,2, Ashley Brate1, Eva Tsalikian3, Michael Tansey3, Ezzatollah T. Shivapour4, Tracey Cho5, John Kamholz4, Nitin J. Karandikar1

1 Department of Pathology, University of Iowa Health Care, Iowa City, Iowa, United States of America, 2 Department of Oral Pathology, Radiology and Medicine, College of Dentistry, University of Iowa, Iowa City, Iowa, United States of America, 3 Department of Pediatrics, University of Iowa Health Care, Iowa City, Iowa, United States of America, 4 Department of Neurology, University of Iowa Health Care, Iowa City, Iowa, United States of America

* Sushmita-sinha@uiowa.edu

Abstract

Factors regulating self-antigen directed immune-responses in autoimmunity are poorly understood. Signal regulatory protein gamma (SIRPγ) is a human T-cell specific protein with genetic variants associated with type 1 diabetes (T1D). SIRPγ’s function in the immune system remains unclear. We show that T1D and relapsing remitting multiple sclerosis (RRMS) subjects have significantly greater frequency of rs2281808 T genetic variant, that correlates with reduced SIRPγ-expression in T-cells. Importantly, reduced SIRPγ-expression in RRMS and T1D subjects was not restricted to T variant, suggesting SIRPγ-expression is also regulated by disease specific factors in autoimmunity. Interestingly, increased frequencies of SIRPγlow T-cells in RRMS and T1D positively correlated with proinflammatory molecules from T-cells. Finally, we show that SIRPγlow T-cells have enhanced pathogenicity in vivo in a GVHD model. These findings suggest that decreased-SIRPγ expression, either determined by genetic variants or through peripherally acquired processes, may have a mechanistic link to autoimmunity through induction of hyperactive T-cells.

1. Introduction

Signal regulatory protein gamma (SIRPγ) is a human T-cell specific immunomodulatory protein encoded by the SIRPG gene [1, 2] with variants associated with autoimmunity in individuals with type 1 diabetes (T1D) in multiple GWAS studies [3–5]. Dysregulated SIRPγ expression has also been demonstrated in other autoimmune conditions such as systemic lupus erythematosus (SLE) [6], suggesting that SIRPγ may play a critical role in immune dysregulation in multiple different autoimmune diseases. However, SIRPγ’s potential mechanistic contribution to autoimmunity remains unclear due to a knowledge gap regarding its function.
in the immune system. There are only a handful of studies that have tried to address the biological function of SIRPγ. Binding of SIRPγ with its ligand CD47 has been shown to facilitate cell adhesion [2, 7]. Engagement of SIRPγ on T cells by CD47 on APCs has been shown to enhance antigen-specific T-cell proliferation [2].

SNP rs2281808 TT is an intronic SNP present between exons 5 and 6 of SIRPG and causes a C/T variant. Multiple GWAS studies have shown that SNP rs2281808 is associated with type 1 diabetes (T1D) [3–5]. We recently showed that the rs2281808-T allele is associated with a reduction in SIRPγ expression on human T-cells leading to a hyperactivated state with lower activation threshold in healthy donors (HD) [8], suggesting that perturbation in SIRPγ levels may lead to immune dysregulation in individuals with autoimmune diseases.

In light of these findings, we determined whether the T allele and/or reduced SIRPγ expression is an underlying feature in two T-cell mediated autoimmune diseases, including relapsing remitting multiple sclerosis (RRMS) and type 1 diabetes (T1D), and if this can have pathogenic consequences, presumably due to exaggerated effector responses from SIRPγ low T-cells. We also asked whether CD4 and CD8 T-cell SIRPγ expression levels in these autoimmune diseases was an exclusive function of the carrier associated intronic SNP or if there were other relevant disease-specific factors that contributed.

2. Material and methods

2.1. Patients and control subjects

After obtaining informed consent, 19 type 1 diabetes (T1D) and 33 treatment naïve relapsing remitting multiple sclerosis (RRMS) patients were recruited at the pediatric endocrinology and neurology clinics, University of Iowa, respectively. De-identified leukoreduction buffy coat samples from 145 healthy donors (HD) were obtained from the University of Iowa DeGowin Blood Center, Department of Pathology. All studies were approved by the University of Iowa IRB according to Declaration of Helsinki principles. Mean age of HD, RRMS and T1D subjects was 52±14, 47±10 and 20±3 respectively. Lower age range in T1D cohort was due to the fact that only newly diagnosed T1D subjects were enrolled for the study. The M:F sex distribution in HD, RRMS and T1D was 80:65, 12:21 and 10:9 respectively. In a large correlation study between age, sex and SIRPγ expression in HD, we did not find any correlation between age and sex with SIRPγ expression.

2.2. Cell preparation and genotyping for rs2281808 detection

PBMC were isolated from buffy coats using Ficoll Hypaque (GE Healthcare Biosciences, Pittsburgh, PA) density gradient. PBMC samples and sorted cells were stored in freezing media in liquid nitrogen until further use in multiple assays. DNA was isolated from PBMC samples using Qiagen mini DNA prep kit. Allelic discrimination PCR was done using TaqMan assay and probe as described previously [8].

2.3. Flow cytometric antibody staining

Anti-human antibodies used for extracellular multi-color flow cytometric analysis included: CD4-APC, CD8-BV786, SIRPγ-PE, CD3-Alexa700. Anti-human antibodies used for intracellular multi-color flow cytometric analysis included CD4- APC, CD8-BV786, SIRPγ-PE, CD3-FITC, IFNγ—Alexa700, TNFα PE-Cy7. All antibodies were obtained from either BD Biosciences (San Jose, CA), or Biolegend (San Diego, CA). PBMC samples were washed with 0.1% (w/v) sodium azide/ phosphate-buffered saline (Mediatech Cellgro) and stained with fluorescently labeled anti-human antibodies, then resuspended in 1% paraformaldehyde (Electron
Microscopy Sciences, Hatfield, PA). Flow cytometric data were acquired on a 4-Laser LSRII using FACSDiva software (Becton Dickinson). Data were analyzed using Flow Jo (TreeStar, Ashland, OR). gMFI was used to look at the MFI of SIRPγ.

2.4. PBMC stimulation and cytokine detection

As described previously [9], one million cells from HD were stimulated with PMA/Ionomycin/Brefeldin for 6 hours. Cells were washed with 0.1% (w/v) sodium azide/ phosphate-buffered saline and stained intracellularly for detecting IFN-γ or TNF-α.

2.5. Induction of xGVHD in NSG mice

We used a graft versus host disease (GVHD) model to test whether SIRPγlow T-cells display enhanced pathogenicity in vivo as compared to SIRPγhigh T-cells. From our pilot experiments, we determined that a minimum of 10 × 10⁶ SIRPγhigh T-cells are required to induce GVHD in NOD-SCID-gamma (NSG) mice. To test whether sub-optimal numbers of SIRPγlow T-cells will be pathogenic in vivo, we transferred 6 × 10⁶ sorted SIRPγlow or SIRPγhigh T-cells into NSG mice. The sample size of n = 3 in each group was based on the following power calculation: expected incidence of GVHD as 0% in SIRPγhigh vs 95% in SIRPγlow recipients, false positive rate of 0.05% and 95% confidence interval. SIRPγhigh and SIRPγlow T-cells were sorted from 3 different CC and TT healthy donors respectively using Miltenyi Biotech beads (Germany). The cell purity was >99% for each sample. Six million sorted cells from each CC and TT carrier were adoptively transferred into three different NSG mice and weight loss was monitored. At the end of the experiment, liver tissue was harvested, fixed in buffered formalin, paraffin embedded and H&E stained for histologic examination. Inflammation was scored as described previously [10].

2.6. Statistical analysis

The Chi-Square test was used to compare the rs2281808 genotype incidence between HD vs. RRMS and T1D patients and p<0.05 was considered significant. Data between the groups was analyzed with unpaired two-tailed Students t-test and p<0.05 was considered significant. One-way ANOVA with Tukey’s post-hoc test was performed to compare SIRPγ expression between the groups and p<0.05 was considered significant. Two-way ANOVA with Tukey’s post-hoc test was performed to compare SIRPγ expression between the genotypes and weight-loss in GVHD model and p<0.05 was considered significant. Correlation between SIRPγlow T-cells and proinflammatory molecules was done using Pearson’s test and p<0.05 was considered significant.

3. Results

3.1. Significantly greater preponderance of T allele in multiple sclerosis and type 1 diabetes (T1D) patients

Rs2281808 TT is an intronic SNP present between exons 5 and 6 of the SIRPG gene and causes a C/T variant. We have recently shown that the T allele is associated with hyperactivated T-cells with lower activation threshold in healthy donors (HD). Therefore, we asked whether the T allele may be overrepresented in two T-cell mediated autoimmune diseases, RRMS and T1D. Genotyping of 33 RRMS patients revealed that 10 (30%) and 18 (55%) patients showed CC and CT genotypes, respectively, whereas the TT variant was present in 5 (15%) patients (Fig 1). In T1D patients, 7 (37%) and 8 (42%) patients showed CC and CT genotype respectively, and the TT genotype was present in 4 (21%) patients (Fig 1). The rs22811808 genotype...
The distribution in RRMS and T1D patients was different than HD where CC genotype was present in 55%, CT in 42% and TT was present in only 4% HD (Fig 1). While comparing the three genotypes in HD and RRMS patients, CC genotype was predominant in HD vs. RRMS (HD vs. RRMS; 55% vs. 30%, p < 0.05). Interestingly, in RRMS subjects, CT and TT genotypes were significantly predominant vs. HD (HD vs. RRMS; CT: 41% vs. 55%, p < 0.05; TT: 4% vs. 15%, p < 0.05). Similarly in T1D subjects, CC genotype was predominant in HD vs. T1D (HD vs. T1D; 55% vs. 37%, p < 0.05). While the CT genotype was not different between HD and T1D subjects, the TT genotype was significantly over-represented in T1D subjects (HD vs. T1D; TT: 4% vs. 21%, p < 0.05). Collectively, the T allele (CT and TT) showed a significantly greater preponderance in both RRMS and T1D patients (T allele in HD vs. RRMS & T1D; 45% vs. 70% & 63%, p < 0.05). Overall, our results show that the T allele of SNP rs2281808 in SIRPG is associated with two T-cell mediated autoimmune diseases, RRMS and T1D.

3.2. SIRPγ expression in autoimmunity is regulated outside of the rs2281808 genotype

SIRPγ staining on the T-cells of HD and T1D subjects has already been published before [8, 11]. Representative staining of SIRPγ on T-cells of RRMS subjects including low vs. high SIRPγ gates is shown in Fig 2A. We found that overall SIRPγ expression on T-cells of RRMS and T1D patients was significantly lower than T-cells from HD irrespective of the rs2281808 genotype. Both, RRMS and T1D patients had significantly greater percentages of CD8- SIRPγlow T-cells as compared to HD (Fig 2B). Likewise, RRMS and T1D patients had significantly lower gMFI of SIRPγ on their CD4 T-cells as compared to HD (Fig 2B). Further analysis showed that the collective difference in SIRPγ expression between HD and autoimmune patients was driven by differences in SIRPγ expression on T-cells in CC carriers within the three groups. CC carriers from RRMS and T1D patients had significantly higher percentages of CD8- SIRPγlow T-cells as compared to HD (Fig 3). Similarly, CC carriers from RRMS and T1D subjects had significantly lower SIRPγ -gMFI on CD4-T-cells as compared to HD (Fig 3). Therefore, reduced SIRPγ expression on T-cells of RRMS and T1D subjects as compared to HD was not solely attributable to an increased frequency of the rs2281808 TT genotype, suggesting regulation of SIRPγ by certain unknown disease-specific factors.
3.3. Increase in SIRP\(\gamma\)\textsubscript{low} T-cells positively correlates with proinflammatory factors in RRMS and T1D patients

We have previously shown that SIRP\(\gamma\)\textsubscript{low} CD8 T-cells secrete greater amounts of effector molecules as compared to their SIRP\(\gamma\)\textsubscript{high} counterparts [8]. Since we found that RRMS and T1D patients have significantly greater SIRP\(\gamma\)\textsubscript{low} CD8 T-cells, we asked whether this increased frequency of SIRP\(\gamma\)\textsubscript{low} T-cells might contribute to increased proinflammatory factors in an
autoimmune setting. Indeed the percent of SIRPγ low CD8 T-cells positively correlated with the percent of IFNγ and TNFα producing CD8 T-cells, both in RRMS and T1D patients (Fig 4). Likewise, IFNγ producing CD4 T-cells positively correlated with lower SIRPγ gMFI on CD4 T-cells in RRMS and T1D patients (Fig 4).

3.4. Sub-optimal numbers of SIRPγ low T-cells are pathogenic in vivo

We tested activation of SIRPγ low vs. SIRPγ high T-cells in vivo in the development of xenoGVHD in NSG (NOD-SCID-gamma) mice. This model system has been used to induce disease mediated by human CD4 T-cells [12–15]. Suboptimal numbers of SIRPγ high or low T-cells, from three different CC and TT HD, were transferred individually into three different NSG mice and weight-loss was monitored. Interestingly, all the three mice that received SIRPγ low T-cells showed weight loss while all the SIRPγ high T-cell recipients remained healthy and exhibited no signs of GVHD (Fig 5A). Concomitantly, severe liver inflammation was detected only in the mice that received SIRPγ low T-cells from TT carriers (Fig 5B & 5C). Liver sections from the mice that received SIRPγ high T-cells from CC carriers were minimally involved with the exception of one mouse showing moderate inflammation. Importantly, no weight loss was detected in this mouse.

4. Discussion

The factors that exacerbate proinflammatory T-cell responses in autoimmunity are poorly understood. We have previously shown that reduced SIRPγ expression potentiates effector responses from human T-cells [8], suggesting that perturbed SIRPγ expression on T-cells may play a critical role in immune dysregulation of autoimmune diseases. The pathogenic effector role of T-cells, both in T1D and MS, is well established [16–32]. In both the diseases, autoreactive T-cells are thought to infiltrate the target organ and cause inflammation, leading to loss of insulin production (in T1D) or loss of nerve conduction (in MS). Here we report a novel association of reduced SIRPγ expression with two T-cell mediated autoimmune diseases including relapsing remitting multiple sclerosis (RRMS) and Type 1 diabetes (T1D). There is accumulating evidence in the literature to suggest that genetic variants in SIRPγ can lead to modulation of immune responses in humans. A recent study predicted that polymorphisms in SIRPG can interfere with transcription factors important in T-cell development [33]. A SNP in SIRPG was recently shown to be associated with the persistence of MenC-specific immunity following...
childhood immunization [34]. SNP rs2281808 in SIRPG has already been shown to be a risk factor for T1D by multiple GWAS studies [3–5]. Interestingly, early onset T1D patients provided more association evidence for rs2281808 [5]. We confirm the association of the rs2281808 TT genotype with T1D patients in our study population. Further, we found that RRMS patients also have a significantly greater preponderance of rs2281808 CT and TT allele as compared to healthy donors. We note that the difference in the prevalence of TT between RRMS vs. HD is smaller than that of T1D vs. HD. Therefore, this finding will benefit from corroboration in a larger sample size and we hope that this study will prompt investigators to study the prevalence of rs2281808 in other autoimmune diseases including RRMS.

We have recently shown that SIRPγ expression levels on CD4 and CD8 T-cells correlated with the genotype of the C/T polymorphism with expression being high in CC, intermediate in CT and low in TT subjects [8]. We found that this is also true for patients with RRMS and T1D. Additionally, we found that overall, SIRPγ expression on T-cells in patients with autoimmunity was significantly lower than healthy donors (HD), suggesting that SIRPγ expression...
may also be regulated by some as yet unknown disease-specific factors. Perturbed homeostasis of T-cells and constant exposure to pro-inflammatory cytokines in autoimmunity are plausible theories to be tested in future studies.

Positive correlation between SIRPγ\textsuperscript{low} T-cells and proinflammatory effector molecules from T-cells of RRMS and T1D patients suggest that reduced SIRPγ expression on T-cells could potentiate target organ-specific inflammation in autoimmunity. Indeed, we found that, unlike SIRPγ\textsuperscript{high} T-cells, suboptimal numbers of SIRPγ\textsuperscript{low} T-cells were enough to cause xGVHD in NSG mice. While the xGVHD experiment does not reflect the ability of T-cells to cause autoimmunity, it demonstrates that SIRPγ\textsuperscript{low} T-cells are hyperactive \textit{in vivo} and can infiltrate target organs and cause inflammation. Since SIRPγ\textsuperscript{low} vs. high T-cells even in the same individual produce significantly greater amounts of effector cytokines [8], we conclude that reduced SIRPγ expression functionally skews the T-cells toward potentiated effector responses. While CT and TT genotypes are associated with reduced SIRPγ expression on T-cells irrespective of the disease status, significantly reduced SIRPγ expression was seen selectively on the T-cells of CC carriers in RRMS and T1D cohort. Our findings suggest that both rs2281808 genotype and as yet unknown disease specific factors are associated with significantly reduced SIRPγ expression on the T-cells of autoimmune subjects. Therefore, in future studies it will be more informative to look at SIRPγ expression on T-cells particularly in the disease cohorts. Future studies on the role of SIRPγ in immune regulation and dysregulation may enlighten our understanding of the targetable pathways involved in autoimmunity.

Fig 5. Unlike SIRPγ\textsuperscript{high} T-cells, sub-optimal numbers of SIRPγ\textsuperscript{low} T-cells are sufficient to cause xenoGVHD in NSG mice. Three million SIRPγ\textsuperscript{high} T-cells or SIRPγ\textsuperscript{low} T-cells, from 3 separate CC or TT carriers, were transferred into NSG mice. Weight was monitored as shown in A. Representative H&E staining on liver tissues is shown in B. Liver inflammation was scored by a pathologist blinded to the experimental grouping, using a previously published, established scale. Two-way ANOVA with Bonferroni's multiple comparison test was performed to compare weight loss and p<0.05 was considered significant. An unpaired t-test was performed to compare inflammation scores and p<0.05 was considered significant.

https://doi.org/10.1371/journal.pone.0238070.g005
Acknowledgments
We thank Scott Lieberman and Ashutosh Mangalam for their inputs and discussion.

Author Contributions
Conceptualization: Sushmita Sinha, Nitin J. Karandikar.
Data curation: Sushmita Sinha.
Formal analysis: Sushmita Sinha, Scott M. Steward-Tharp.
Funding acquisition: Nitin J. Karandikar.
Investigation: Sushmita Sinha, Pranav S. Renavikar, Michael P. Crawford, Ashley Brate.
Methodology: Sushmita Sinha.
Project administration: Sushmita Sinha.
Resources: Eva Tsalikian, Michael Tansey, Ezzatollah T. Shivapour, Tracey Cho, John Kamholz.
Software: Sushmita Sinha.
Supervision: Sushmita Sinha.
Visualization: Sushmita Sinha.
Writing – original draft: Sushmita Sinha.
Writing – review & editing: Sushmita Sinha, Nitin J. Karandikar.

References
1. Barclay A.N., Brown M.H., The SIRP family of receptors and immune regulation, Nature reviews. Immunology, 6 (2006) 457–464. https://doi.org/10.1038/nri1859 PMID: 16691243
2. Piccio L., Vermi W., Boles K.S., Fuchs A., Strader C.A., Facchetti F., et al. Adhesion of human T cells to antigen-presenting cells through SIRPbeta2-CD47 interaction costimulates T-cell proliferation, Blood, 105 (2005) 2421–2427. https://doi.org/10.1182/blood-2004-07-2823 PMID: 15383453
3. Barrett J.C., Clayton D.G., Concannon P., Akolkar B., Cooper J.D., Erlich H.A., et al. Type 1 Diabetes Genetics, Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes, Nature genetics, 41 (2009) 703–707. https://doi.org/10.1038/ng.381 PMID: 19430480
4. Kiani A.K., John P., Bhatti A., Zia A., Shahid G., Akhtar P., et al. Association of 32 type 1 diabetes risk loci in Pakistani patients, Diabetes research and clinical practice, 108 (2015) 137–142. https://doi.org/10.1016/j.diabres.2015.01.022 PMID: 25661663
5. Reddy M.V., Wang H., Liu S., Bode B., Reed J.C., Steed R.D., et al. Association between type 1 diabetes and GWAS SNPs in the southeast US Caucasian population, Genes and immunity, 12 (2011) 208–212. https://doi.org/10.1038/gene.2010.70 PMID: 21270831
6. Kawasaki M., Sekigawa I., Nozawa K., Kaneko H., Takasaki Y., Takamori K., et al. Changes in the gene expression of peripheral blood mononuclear cells during the menstrual cycle of females is associated with a gender bias in the incidence of systemic lupus erythematosus, Clinical and experimental rheumatology, 27 (2009) 260–266. PMID: 19473566
7. Brooke G., Holbrook J.D., Brown M.H., Barclay A.N., Human lymphocytes interact directly with CD47 through a novel member of the signal regulatory protein (SIRP) family, Journal of immunology, 173 (2004) 2562–2570.
8. Sinha S., Borcherding N., Renavikar P.S., Crawford M.P., Tsalikian E., Tansey M., et al. An autoimmune disease risk SNP, rs2281808, in SIRPgamma and heightened effector state in human CD8+T-cells, Scientific reports, 8 (2018) 15440. https://doi.org/10.1038/s41598-018-33901-1 PMID: 30337675
9. Cunnusamy K., Baughman E.J., Franco J., Ortega S.B., Sinha S., Chaudhary P., et al. Disease exacerbation of multiple sclerosis is characterized by loss of terminally differentiated autoregulatory CD8+ T-cells, Scientific reports, 9 (2019) 4841. https://doi.org/10.1038/s41598-019-42752-7 PMID: 30902194
cells, Clinical immunology, 152 (2014) 115–126. https://doi.org/10.1016/j.clim.2014.03.005 PMID: 24657764

10. Cooke K.R., Hill G.R., Crawford J.M., Bungard D., Brinson Y.S., Delmonte J. Jr., et al. Tumor necrosis factor- alpha production to lipopolysaccharide stimulation by donor cells predicts the severity of experimental acute graft-versus-host disease, The Journal of clinical investigation, 102 (1998) 1882–1891. https://doi.org/10.1172/JCI4285 PMID: 9819375

11. Sinha S., Renavikar P.S., Crawford M.P., Rodgers J.W., Tsalikian E., Tansey M., et al. Autoimmunity-associated intronic SNP (rs2281808) detected by a simple phenotypic assay: Unique case or broader opportunity?, Clinical immunology, 198 (2019) 57–61. https://doi.org/10.1016/j.clim.2018.12.018 PMID: 30579937

12. Bezie S., Meistermann D., Boucault L., Kilens S., Zoppini J., Autrusseau E., et al. Ex Vivo Expanded Human Non-Cytotoxic CD8(+)CD45RC(low/-) Tregs Efficiently Delay Skin Graft Rejection and GVHD in Humanized Mice, Frontiers in immunology, 8 (2017) 2014. https://doi.org/10.3389/fimmu.2017.02014 PMID: 29443570

13. Bohana-Kashtan O., Morisot S., Hildreth R., Brayton C., Levitsky H.I., Civin C.I., Selective reduction of graft-versus-host disease-mediating human T cells by ex vivo treatment with soluble Fas ligand, Journal of immunology, 183 (2009) 696–705.

14. Ito R., Katano I., Kawai K., Yagoto M., Takahashi T., Ka Y., et al. A Novel Xenogeneic Graft-Versus-Host Disease Model for Investigating the Pathological Role of Human CD4(+) or CD8(+) T Cells Using Immunodeficient NOG Mice, American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons, 17 (2017) 1216–1228.

15. King M.A., Covassinn L., Brehm M.A., Raccki W., Pearson T., Leil J., et al. Human peripheral blood leucocyte non-obese diabetic-severe combined immunodeficiency interleukin-2 receptor gamma chain gene mouse model of xenogeneic graft-versus-host-like disease and the role of host major histocompatibility complex, Clinical and experimental immunology, 157 (2009) 104–118. https://doi.org/10.1111/j.1365-2249.2009.03933.x PMID: 19659776

16. Burrack A.L., Martinov T., Fife B.T., T Cell-Mediated Beta Cell Destruction: Autoimmunity and Alloimmunity in the Context of Type 1 Diabetes, Frontiers in endocrinology, 8 (2017) 343. https://doi.org/10.3389/fendo.2017.00343 PMID: 29259578

17. Knoop J., Gavrisan A., Kuehn D., Reinhardt J., Heinrich M., Hippih M., et al. GM-CSF producing autoreactive CD4(+) T cells in type 1 diabetes, Clinical immunology, (2017).

18. G.G. Pinkse, O.H. Tysma, C.A. Bergen, M.G. Kester, F. Ossendorp, P.A. van Veelen, et al. Autoreactive CD8+ T cells associated with beta cell destruction in type 1 diabetes, Proceedings of the National Academy of Sciences of the United States of America, 102 (2005) 18425–18430.

19. Pugliese A., Autoreactive T cells in type 1 diabetes, The Journal of clinical investigation, 127 (2017) 2881–2891. https://doi.org/10.1172/JCI94549 PMID: 28762987

20. Walker L.S., von Herrath M., CD4 T cell differentiation in type 1 diabetes, Clinical and experimental immunology, 183 (2016) 16–29. https://doi.org/10.1111/cel.12672 PMID: 26102289

21. Wallberg M., Cooke A., Immune mechanisms in type 1 diabetes, Trends in immunology, 34 (2013) 583–591. https://doi.org/10.1016/j.it.2013.08.005 PMID: 24054837

22. Fletcher J.M., Lalor S.J., Sweeney C.M., Tubridy N., Mills K.H., T cells in multiple sclerosis and experimental autoimmune encephalomyelitis, Clinical and experimental immunology, 162 (2010) 1–11. https://doi.org/10.1111/j.1365-2249.2010.03933.x PMID: 20682002

23. Hohlfeld R., Dormmair K., Meini E., Wekerle H., The search for the target antigens of multiple sclerosis, part 1: autoreactive CD4+ T lymphocytes as pathogenic effectors and therapeutic targets, The Lancet. Neurology, 15 (2016) 198–209. https://doi.org/10.1016/S1474-4422(15)00334-8 PMID: 26724103

24. Hohlfeld R., Dormmair K., Meini E., Wekerle H., The search for the target antigens of multiple sclerosis, part 2: CD8+ T cells, B cells, and antibodies in the focus of reverse-translational research, The Lancet. Neurology, 15 (2016) 317–331. https://doi.org/10.1016/S1474-4422(15)00313-0 PMID: 26724102

25. Huseby E.S., Kamimura D., Arima Y., Parello C.S., Sasaki K., Murakami M., Role of T cell-glial cell interactions in creating and amplifying central nervous system inflammation and multiple sclerosis disease symptoms, Frontiers in cellular neuroscience, 9 (2015) 255. https://doi.org/10.3389/fncel.2015.00295 PMID: 26300731

26. Salou M., Nicol B., Garcia A., Laplau D.A., Involvement of CD8(+) T Cells in Multiple Sclerosis, Frontiers in immunology, 6 (2015) 604. https://doi.org/10.3389/fimmu.2015.00604 PMID: 26635816

27. Yadav S.K., Mindur J.E., Ito K., Dhib-Jalbut S., Advances in the immunopathogenesis of multiple sclerosis, Current opinion in neurology, 28 (2015) 206–219. https://doi.org/10.1097/WCO.000000000000205 PMID: 25887768
28. Ramirez L., Hamad A.R., Status of autoimmune diabetes 20-year after generation of BDC2.5-TCR transgenic non-obese diabetic mouse, World journal of diabetes, 4 (2013) 88–91. https://doi.org/10.4239/wjd.v4.i4.88 PMID: 23961318

29. Itoh A., Ridgway W.M., Targeting innate immunity to downmodulate adaptive immunity and reverse type 1 diabetes, ImmunoTargets and therapy, 6 (2017) 31–38. https://doi.org/10.2147/ITT.S117264 PMID: 28580341

30. Marleau A.M., Sarvetnick N.E., IL-18 is required for self-reactive T cell expansion in NOD mice, Journal of autoimmunity, 36 (2011) 263–277. https://doi.org/10.1016/j.jaut.2011.02.005 PMID: 21414755

31. Theofilopoulos A.N., Kono D.H., Baccala R., The multiple pathways to autoimmunity, Nature immunology, 18 (2017) 716–724. https://doi.org/10.1038/ni.3731 PMID: 28632714

32. Christoffersson G., von Herrath M.G., A Deeper Look into Type 1 Diabetes—Imaging Immune Responses during Onset of Disease, Frontiers in immunology, 7 (2016) 313. https://doi.org/10.3389/fimmu.2016.00313 PMID: 27574523

33. Gabrielsen I.S., Amundsen S.S., Helgeland H., Flam S.T., Hatinoor N., Holm K., et al. Genetic risk variants for autoimmune diseases that influence gene expression in thymus, Human molecular genetics, 25 (2016) 3117–3124. https://doi.org/10.1093/hmg/ddw152 PMID: 27199374

34. O’Connor D., Png E., Khor C.C., Snape M.D., Hill A.V.S., van der Klis F., et al. Common Genetic Variations Associated with the Persistence of Immunity following Childhood Immunization, Cell reports, 27 (2019) 3241–3253 e3244. https://doi.org/10.1016/j.celrep.2019.05.053 PMID: 31189108