Investigation of the role of genetic and immunologic factors in the pathogenesis of acquired thrombotic thrombocytopenic purpura

PhD Theses

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1. INTRODUCTION

1.1. Thrombotic thrombocytopenic purpura (TTP)

Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy (TMA), thus it is characterized by pathologic changes and thrombosis of the small vessels (arterioles and capillaries), leading to thrombus formation. The generation of platelet-thrombi results in ischemic symptoms of the affected organs, and cause thrombocytopenia, whereas the mechanical injury of the red blood cells passing through thrombus-occluded vessels leads to the formation of fragmented erythrocytes, called schistocytes.

In TTP, any organ can be affected, thus the ischemic symptoms are diverse. Neurological symptoms occur in about 80% of the cases, with their severity spanning from mild headache and confusion through symptoms indicating focal abnormalities (hemiparesis, aphasia, visual disturbances) and epileptic seizures to coma. Symptoms indicating gastrointestinal, cardial, and renal involvement are also common; however, severe renal failure requiring dialysis is relatively rare.

TTP is an episodic disease. Most patients recover following appropriate treatment and reach remission. Remission can be permanent; however, approximately every third patient develops further acute episodes, called relapses.

The underlying cause of TTP is the deficiency of the ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motifs, member 13) enzyme. The role of ADAMTS13 is to cleave the ultra-large forms of von Willebrand factor (ULVWF) into smaller oligomeric forms, found in the plasma under normal circumstances and playing an important role in haemostasis. Because ULVWF is capable of binding platelets at shear forces normally present in the microvasculature, the prolonged presence of these macromolecules on the surface of endothelial cells and in the circulation, caused by their impaired cleavage, can lead to the adhesion and aggregation of platelets in the microvasculature, manifesting as a TMA.

ADAMTS13 deficiency is caused by bi-allelic (homozygous or compound heterozygous) mutations of the *ADAMTS13* gene in the rare, hereditary form of disease, and by anti-ADAMTS13 autoantibodies in its more common, acquired form, responsible for 95% of the TTP cases.

1.2. The anti-ADAMTS13 autoantibody-response in acquired TTP

In acquired TTP, most of the anti-ADAMTS13 autoantibodies are inhibitory autoantibodies, which are able to directly block the function of the enzyme; however, non-inhibitory autoantibodies have also been described, which may potentially contribute to the deficiency by facilitating the clearance of the enzyme from the circulation.

The majority of the inhibitory autoantibodies binds to a certain epitope of the spacer domain of the enzyme, but most acquired TTP patients produce autoantibodies against multiple domains of the enzyme (primarily against the C-terminal thrombospondin-like and CUB domains, besides the spacer domain).

Although IgM or IgA class anti-ADAMTS13 autoantibodies can also be present in a subset of acquired TTP patients, most of the anti-ADAMTS13 autoantibodies belong to the IgG class. IgG autoantibodies are primarily of IgG1 or IgG4 subclasses.

Whereas antibodies of the IgG1 subclass are able to effectively bind to the Fc receptors of leukocytes and to activate the complement system, the IgG4 antibodies are relatively inert and less capable of forming immune complexes because of their functional monovalence. The above differences may affect the clearance of the ADAMTS13 enzyme from the circulation. However, the association between the inhibitory potential and the subclass distribution of anti-ADAMTS13 autoantibodies has not yet been investigated.

The exact mechanism how anti-ADAMTS13 autoantibodies are generated is currently unknown. According to our present knowledge, certain genetic factors can influence the risk of acquired TTP, potentially by facilitating or interfering with the development of the anti-ADAMTS13 autoantibodies.

According to the results of previous association studies, the frequencies of the linked HLA-DRB1*11 and DQB1*0301 alleles are higher, whereas those of the linked DRB1*04 and DRB4 alleles are lower among acquired TTP patients from Western Europe compared to the general population. However, certain DR and DQ alleles are inherited in linkage, in the form of haplotypes – together with certain alleles of further genes of the immune system. Therefore, associations of disease risk with certain DR and DQ alleles do not necessarily indicate the causal role of the given allele, but that of the whole haplotype.

Nevertheless, molecular investigations have validated the role of the HLA-DR11 molecule in the pathogenesis of acquired TTP. Thus, the identification of further potential risk or protective haplotypes by association studies can pave the way for similar functional investigations.

As an association study is particularly suitable for the investigation of alleles that are frequent in a certain population, studying distinct populations can potentially identify more haplotypes associated with altered disease risk.

Acquired TTP affects more women than men, but why female sex is a risk factor for the disease remains to be explained. In some autoimmune diseases, different HLA-DR and DQ alleles have been observed to correlate with the risk of developing the disease. The effect of HLA-DR-DQ haplotypes on the risk of acquired TTP has not yet been investigated separately in women and men.

The *PTPN22* (protein tyrosine phosphatase, non-receptor type 22) gene codes a protein (Lyp) that is involved in the regulation of lymphocyte signalling. The *PTPN22* c.1858C>T polymorphism predisposes to a number of autoimmune diseases. The polymorphism was not found to be associated with the risk of developing acquired TTP in a previous study. However, the frequency of the minor allele shows considerable differences across geographic regions, so studying other populations with higher minor allele frequencies may help reveal hitherto unidentified disease associations. Furthermore, based on the results of animal studies, we hypothesised that the genetic background (e.g. HLA-DR-DQ haplotypes) can modify the effect of the *PTPN22* c.1858C>T polymorphism.

Besides its generation, the later evolution of the anti-ADAMTS13 autoantibody response is also relatively unexplored; in spite of the fact that during relapses, and – because of the constant presence of the antigen – even in remission, the autoantibody response can go through changes involving the quantity, subclass distribution, affinity and inhibitory potential of the autoantibodies.

Furthermore, it is still to be answered whether the genetic factors predisposing to acquired TTP can affect any quantitative or qualitative characteristics of the anti-ADAMTS13 autoantibody response.

2. AIMS

2.1. Investigation of genetic factors influencing the risk of acquired TTP

In our first study we aimed to:

1. Investigate the effect of HLA-DR-DQ haplotypes on the risk of developing acquired TTP.

   Therefore, we determined the proportions of individuals carrying certain HLA-DR-DQ haplotypes in a group of Hungarian acquired TTP patients and in healthy Hungarian control subjects, and compared these proportions.

2. Investigate the effect of HLA-DR-DQ haplotypes on the risk of developing acquired TTP in female and male individuals, separately.

   Accordingly, we made the above comparisons stratified by gender.

3. Investigate the effect of the *PTPN22* c.1858C>T polymorphism on the risk of developing acquired TTP in the Hungarian population.

   Consequently, we determined and compared the proportions of acquired TTP patients and healthy individuals carrying the minor c.1858T allele.

4. Investigate how the *PTPN22* c.1858C>T polymorphism and the HLA-DR-DQ haplotypes may interact in determining the risk of developing acquired TTP.

   Accordingly, we compared the proportions of acquired TTP patients and healthy individuals in groups stratified by the carriage of HLA-DR-DQ haplotypes and by that of the minor c.1858T allele.

2.2. Investigation of characteristics of the anti-ADAMTS13 autoantibody response

The majority of the anti-ADAMTS13 autoantibodies belongs to the IgG class; anti-ADAMTS13 IgG autoantibodies are present in virtually all acquired TTP patients in the acute phase of the disease, and in a subset of them even in remission.

Accordingly, in our second study we aimed to:

1. Determine the concentration, subclass distribution, and inhibitory potential of anti-ADAMTS13 IgG autoantibodies in samples of acquired TTP patients, and to compare these characteristics of samples from distinct disease stages – from the first acute episode, from relapse, and from remission.
2. Investigate the effect of the subclass distribution of anti-ADAMTS13 IgG autoantibodies on the inhibitory potential of patient samples.

   Consequently, we investigated the association between anti-ADAMTS13 IgG subclass concentrations and the strength of the ADAMTS13 inhibition. Furthermore, we directly compared the inhibitory potentials of samples with identical anti-ADAMTS13 IgG concentrations but with different subclass distributions.
3. Investigate the association between the carriage of HLA-DR-DQ haplotypes and the parameters of the anti-ADAMTS13 autoantibody response.

   Therefore, we compared the concentration and subclass distribution of anti-ADAMTS13 IgG autoantibodies in samples of patients carrying and not carrying risk or protective HLA-DR-DQ haplotypes.

3. METHODS

3.1. Patient and sample selection criteria

3.1.1. The TTP-HUS Registry

Our laboratory has been providing diagnostic services since August 2007 for patients with the suspicion of thrombotic microangiopathies. The samples sent to us for diagnostic purposes have since then been stored, and the data of patients and samples have been collected in a registry, called the TTP-HUS Registry.

The following diagnostic criteria were used to define acquired TTP: (1) thrombocytopenia (platelet count below 150 G/L), (2) microangiopathic haemolytic anaemia (decreased haemoglobin level, elevated LDH level, negative Coombs-test, schistocytes in the peripheral blood smear), (3) deficient ADAMTS13 activity (<10% by the FRET assay discussed below), (4) detectable ADAMTS13 inhibitors (by a functional mixing assay) or anti-ADAMTS13 IgG autoantibodies (by ELISA).

The patients in our registry were enrolled in the studies forming the basis of this doctoral thesis, based on the following criteria.

3.1.2. Patients and control subjects enrolled in the investigation of the role of HLA-DR-DQ haplotypes and the *PTPN22* c.1858C>T polymorphism

Out of the 84 acquired TTP patients in our registry in July 2015, 76 patients with available samples for genetic analysis were enrolled in our first study. One patient was excluded because of South-East Asian origin, all of the included 75 patients were Caucasians from Hungary (73 people) or from neighbouring European countries (2 people).

Two hundred and four healthy, non-related Hungarian individuals (106 parents of patients scheduled for bone marrow transplantation, and 98 parents from a family study) served as controls.

HLA-DR results were available for all (204) control subjects, whereas the HLA-DQ results were known in a subset of the control subjects (162 people). The samples for the analysis of the *PTPN22* c.1858C>T polymorphism were available in 169 control individuals.

3.1.3. Patients and samples enrolled in the investigation of the concentration, subclass distribution, and inhibitory potential of anti-ADAMTS13 IgG autoantibodies

One hundred and one samples of 81 acquired TTP patients were included in our second study. All available deficient (and some non-deficient) samples of the 113 acquired TTP patients in our registry in March 2017 were tested for the presence and concentration of anti-ADAMTS13 IgG autoantibodies. One hundred and nine samples gave positive results; the determination of the subclass distribution of anti-ADAMTS13 autoantibodies was successful in 101 of these samples. The functional testing (mixing assay) for ADAMTS13 inhibitors was performed in 97 of these samples. The specific inhibitory potential of autoantibodies were determined in acute samples with IgG4 proportions above 70% (19 samples) or below 30% (16 samples), and in all available remission samples (14 samples).

3.2. Methods

3.2.1. Determination of HLA-DR-DQ haplotypes

The low resolution typing of HLA-DRB1 and DQB1 alleles was performed at the Laboratory of Transplantation Immunogenetics by sequence specific oligonucleotide probe hybridization.

The HLA-DR-DQ haplotypes could be deduced from the inheritance pattern in the case of the 98 control subjects from the family study, whereas it was predicted by the PHASE (v2.1) software in the other 64 control subjects and in the 75 acquired TTP patients.

3.2.2. Investigation of the *PTPN22* c.1858C>T polymorphism

The *PTPN22* c.1858 genotypes were determined by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP).

3.2.3. Determination of ADAMTS13 activity and ADAMTS13 inhibition

ADAMTS13 activity was determined by a kinetic fluorescence resonance energy transfer (FRET) assay using the substrate FRETS-VWF73 (Peptides International Inc., Louisville, KY, USA).

The activities of the samples were compared to a pooled normal citrated plasma sample with a nominal activity of 100%.

The strength of the ADAMTS13 enzyme inhibition was investigated by a functional, so-called mixing assay. Prior to the activity measurement, the patient samples were mixed with normal pooled plasma in a 1:1 ratio, and were incubated for 2 hours at 37°C. The activities of the mixed samples were determined by the FRET assay described above. Lower ADAMTS13 activities of the mixed samples indicated stronger ADAMTS13-inhibition and vice versa.
The specific inhibitory potential was determined similarly, but the selected patient samples were diluted to identical anti-ADAMTS13 concentrations of 25 U/mL by PBS buffer (pH 7.4) before mixing with the normal pooled plasma samples.

3.2.4. Determination of the concentration and subclass distribution of anti-ADAMTS13 IgG autoantibodies

The concentrations of the anti-ADAMTS13 IgG autoantibodies were determined by ELISA, using the ADAMTS13-Inh® kit (Technoclone GmbH, Vienna, Austria). Following the manufacturer's instructions, antibody levels above 15 U/mL were regarded as positive.
The subclass distributions of anti-ADAMTS13 IgG autoantibodies were determined by an in-house ELISA method. The samples were incubated in ELISA plates coated with recombinant ADAMTS13, and were detected using subclass-specific secondary antibodies. Two columns of a separate microplate were coated by known concentrations of human antibodies of each subclass. Following a blocking step, the columns of this latter plate were incubated by the respective subclass-specific secondary antibodies, similarly to the samples. The wells of this second plate were used as calibrators, and the amount of bound antibodies of patient samples were determined separately for each subclass by comparing the signal to the subclass-specific standard curve. The proportion of bound antibodies per subclass indicated the subclass distribution of the patient sample. The absolute concentrations of each IgG subclass were calculated by multiplying the proportion of the subclass with the total anti-ADAMTS13 IgG concentration of the sample (determined with the ELISA kit).

4. RESULTS

4.1. Investigation of the frequencies of HLA-DR-DQ haplotypes and of the *PTPN22* c.1858C>T polymorphism in acquired TTP

4.1.1. The effect of HLA-DR-DQ haplotypes on the risk of acquired TTP

The proportions of individuals carrying the DRB1*11-DQB1*03 and DRB1*15-DQB1*06 haplotypes were higher (56.0% vs. 27.2% and 25.3% vs. 11.7%, respectively), whereas of those carrying the DRB1*07-DQB1*02 and DRB1*13-DQB1*06 haplotypes were lower (6.7% vs. 21.6% and 4.0% vs. 13.6%, respectively) in the group of acquired TTP patients (75 people, median age: 41 years, proportion of women: 80.0%) compared to that of healthy control subjects (162 people, 57 years, 53.1% women).

This suggests that the DRB1*11-DQB1*03 and DRB1*15-DQB1*06 haplotypes act as risk factors (odds ratio (OR), with interquartile range: 3.41 (1.93-6.05) and 2.55 (1.26-5.18), respectively) of acquired TTP, whereas the DRB1*07-DQB1*02 and DRB1*13-DQB1*06 haplotypes are protective (OR 0.26 (0.10-0.69) and 0.27 (0.08-0.92), respectively).

4.1.2. The effect of HLA-DR-DQ haplotypes on the risk of acquired TTP in women

The comparison of the groups of women with acquired TTP (60 people) and of healthy women (86 people) yielded similar results, however, even stronger associations were observed in spite of the lower number of individuals tested.

The proportions of individuals carrying the DRB1*11-DQB1*03 and DRB1*15-DQB1*06 haplotypes were significantly higher (61.7% vs. 26.7% and 30.0% vs. 12.8%, respectively), whereas those of individuals carrying the DRB1*07-DQB1*02 and DRB1*13-DQB1*06 haplotypes were much lower (5.0% vs. 22.1% and 1.7% vs. 14.0%, respectively) in the group of women with acquired TTP compared to that of healthy women.

The odds ratios were 4.41 (2.17-8.93) in the case of the DRB1*11-DQB1*03 haplotype, 2.92 (1.26-6.77) for DRB1*15-DQB1*06, 0.19 (0.05-0.66) for DRB1*07-DQB1*02, and 0.10 (0.01-0.83) for the DRB1*13-DQB1*06 haplotype.

The number of male patients with acquired TTP (15 people) was too low to perform statistic tests and to draw conclusions.

4.1.3. The effect of the *PTPN22* c.1858C>T polymorphism on the risk of acquired TTP

The proportion of acquired TTP patients carrying the rare allele of the *PTPN22* c.1858C>T polymorphism (20.1%) did not differ from the proportion of healthy carriers (21.3%). The above statement is also true if an analysis stratified by gender is performed.

These results suggest that the *PTPN22* c.1858C>T polymorphism in itself does not affect the risk of developing acquired TTP.

4.1.4. The role of interactions between the *PTPN22* c.1858C>T polymorphism and the HLA-DR-DQ haplotypes in affecting the risk of acquired TTP

The proportion of acquired TTP patients was significantly higher among people carrying both the *PTPN22* c.1858T rare allele and the DRB1*15-DQB1*06 haplotype (80.0%) than among those carrying the rare allele but not carrying the above haplotype (26.7%), whereas there was no difference between the two other groups (not carrying the rare allele, and carrying or not carrying the haplotype). On the other hand, the proportion of acquired TTP patients was significantly lower among people carrying the DRB1*07-DQB1*02 haplotype but not carrying the *PTPN22* c.1858T rare allele (7.7%) than among those carrying neither the DRB1*07-DQB1*02 haplotype nor the rare allele (40.4%), whereas there was no difference between the two other groups (carrying the rare allele, and carrying or not carrying the haplotype).

Our results suggest that the DRB1*15-DQB1*06 haplotype only acts as a risk factor for acquired TTP if the rare *PTPN22* c.1858T allele is present, whereas the DRB1*07-DQB1*02 haplotype is protective only if the c.1858T allele is absent.

4.2. Investigation of the concentration, subclass distribution and inhibitory potential of anti-ADAMTS13 IgG autoantibodies

4.2.1. Concentration and subclass distribution of anti-ADAMTS13 autoantibodies in different stages of acquired TTP

The median concentration of anti-ADAMTS13 IgG was 66.0 (interquartile range: 35.6-158.0) U/mL in all 101 tested samples (80 acute and 21 remission samples) of 81 individuals with acquired TTP (median age: 38 years, proportion of women: 76.5%).

The anti-ADAMTS13 antibodies were primarily of the IgG4 and IgG1 subclasses. The median proportion of IgG4 was 66.0% (34.5%-76.0%), and that of IgG1 was 32.0% (24.0-51.5%), whereas those of IgG3 and IgG2 were 0.0% (0.0-5.5% and 0.0-2.5%, respectively). IgG4 was the dominant subclass (i.e. with the highest relative proportion) in 70.3% of the samples, IgG1 in 27.7% and IgG3 in 2.0% them. Anti-ADAMTS13 autoantibodies of the IgG4 subclass were present in 96.0% of the samples, 93.1% contained IgG1, 50.5% IgG3, and 40.6% IgG2. All four subclasses were detectable in 23.8% of the samples, three subclasses could be detected in 36.6%, and two in 35.6% of them, only 4.0% of the samples contained autoantibodies of only one subclass.

Results of the comparison of independent samples from the first acute episode (57 samples of 57 patients, 41 years, 73.7% women) and from relapse (15 samples of 15 patients, 35 years, 80.0% women) showed that the concentrations of IgG1 and IgG3 were higher (35.7 (17.5-96.6) vs. 12.8 (6.0-19.9) and 1.6 (0.0-8.2) vs. 0.0 (0.0-0.1) U/mL, respectively) in the first acute episode than in a relapse. Consequently, the proportions of IgG1 and IgG3 were lower (24.3% (21.0%-28.8%) vs. 42.0% (30.6%-68.8%) and 0.0% (0.0%-0.2%) vs. 2.2% (0.0%-9.7%), respectively), whereas that of the IgG4 was higher (73.6% (65.0%-78.4%) vs. 42.0% (30.6%-68.8%)) in relapse than during the first acute episode.
While IgG1 was the dominant subclass in almost half (47.4%) of the samples from the first acute phase, IgG4 was dominant in all samples from a relapse. Accordingly, besides the independent samples, all relapse samples from patients with multiple samples were found to be IgG4-dominant.
IgG4 was the dominant subclass in 80.0% of the independent samples from the remission following the first acute episode (9 samples of 9 patients, 34 years, 88.9% women) and in 100% of the samples from a remission following a relapse (8 samples from 8 patients, 34 years, 100% women).

4.2.2. Association between the strength of the ADAMTS13 inhibition and the concentration and subclass distribution of anti-ADAMTS13 IgG autoantibodies

The total concentration of anti-ADAMTS13 IgG showed a negative correlation with the activities of the mixed samples in the functional inhibitor assay ($r = -0.326$; $p=0.011$), indicating a positive correlation with the strength of ADAMTS13 inhibition. However, a stronger correlation was observed between the activities of mixed samples and the concentrations of anti-ADAMTS13 autoantibodies of the IgG4 subclass ($r = -0.473$; $p<0.0001$). Interestingly, no

correlations were found with concentrations of anti-ADAMTS13 autoantibodies of any other subclass (IgG1, IgG2, or IgG3).

To assess the specific inhibitory potentials of samples from different disease stages with IgG4 proportions over 70% or below 30%, 35 acute and 14 remission samples were diluted to anti-ADAMTS13 IgG concentrations of 25 U/mL prior to mixing and incubation with pooled normal citrated plasma.
The specific inhibitory potentials of acute samples with high IgG4 proportions (19 samples, 8 from the first acute episode, 11 from relapse) were higher compared to samples with low proportions of IgG4 autoantibodies (16 samples, all from the first acute episode).
We observed no difference in the inhibitory potentials of samples with high IgG4 proportions from different disease stages. All available samples with low IgG4 proportions were taken in the first acute phase.

4.2.3. Associations between HLA-DR-DQ haplotypes and the characteristics of the anti-ADAMTS13 autoantibody response

The concentrations of anti-ADAMTS13 autoantibodies were higher (280.4 (142.8–687.0) vs. 65.7 (42.1–111.4) U/mL) in the first available acute sample of patients carrying protective haplotypes (DRB1*07-DQB1*02 or DRB1*13-DQB1*06, 9 patients) compared to patients not carrying these haplotypes (61 patients).
The anti-ADAMTS13 IgG concentrations did not differ in patients carrying (47 patients) and not carrying (23 patients) risk haplotypes (DRB1*11-DQB1*03 or DRB1*15-DQB1*06).
The subclass distribution of anti-ADAMTS13 IgG autoantibodies did not differ in groups stratified by the carriage of risk or protective haplotypes.

Moreover, we observed that in patients not carrying protective haplotypes, the anti-ADAMTS13 IgG concentration was lower in relapse samples than in samples from the first acute phase (42.3 (31.0–67.4) vs. 67.1 (43.6–152.0) U/mL). No such tendency could be observed in patients carrying protective haplotypes.

5. CONCLUSIONS

The main findings of the thesis are the following:

5.1. Investigation of the effect of HLA-DR-DQ haplotypes and their interactions with gender and the *PTPN22* c.1858C>T polymorphism on the risk of acquired TTP

1. We confirmed that the DRB1*11-DQB1*03 predisposes to acquired TTP, and observed that the DRB1*15-DQB1*06 haplotype increases, whereas the DRB1*07-DQB1*02 and DRB1*13-DQB1*06 haplotypes decreases the risk of developing acquired TTP.
2. We found that the above associations are especially strong in the case of women, in spite of the lower number of individuals.
3. We verified that the proportion of *PTPN22* c.1858T minor allele carriers does not differ between acquired TTP patients and healthy individuals.
4. We found that the *PTPN22* c.1858C>T polymorphism can modify the predisposing or protective effects of certain HLA-DR-DQ haplotypes on the development of acquired TTP.

   The DRB1*15-DQB1*06 increased disease risk only in patients carrying the minor c.1858T allele, whereas the DRB1*07-DQB1*02 haplotype decreased disease risk only in patients not carrying the minor allele.

5.2. Concentration, subclass distribution and inhibitory potential of anti-ADAMTS13 IgG autoantibodies

1a. Our results confirm the previous observations that the anti-ADAMTS13 IgG autoantibodies primarily belong to the IgG4 and IgG1 subclasses. Most samples were found to contain autoantibodies of multiple subclasses.

1b. We found that the subclass distribution of anti-ADAMTS13 autoantibodies shows certain characteristic changes.

   The amount and proportion of anti-ADAMTS13 IgG1 and IgG3 are lower, and the proportion of IgG4 is higher in relapse samples compared to samples from the first acute episode.

   Our results further suggest that IgG4 is the dominant subclass in all relapse samples and in samples taken in remission following a relapse.

2. We found that the inhibitory potentials of samples whose anti-ADAMTS13 autoantibodies belonged predominantly to the IgG4 subclass were stronger compared to samples with low proportions of IgG4 autoantibodies.

   We were the first to systematically investigate the association between the subclass distribution and inhibitory potential of anti-ADAMTS13 IgG autoantibodies. We base our above statement on the following observations. Firstly, the strength of inhibition correlated only with the concentration of IgG4 autoantibodies, and not with those of other subclasses. Secondly, diluted samples with a high proportion of IgG4 anti-ADAMTS13 autoantibodies exerted stronger ADAMTS13 inhibition compared to diluted samples with identical anti-ADAMTS13 IgG concentrations but lower proportions of IgG4 subclass autoantibodies.

3. We found an association between the HLA-DR-DQ haplotype background of acquired TTP patients and their anti-ADAMTS13 autoantibody response.

   Our results show that the concentration of anti-ADAMTS13 IgG autoantibodies is higher in patients carrying protective haplotypes (DRB1*07-DQB1*02 or DRB1*13-DQB1*06).

   Besides, we observed that in patients not carrying protective haplotypes, the anti-ADAMTS13 IgG concentration was lower in relapse samples than in samples from the first acute phase.

6. LIST OF THE CANDIDATE'S PUBLICATIONS

6.1. Publications related to the PhD thesis

Sinkovits G, Szilágyi Á, Farkas P, Inotai D, Szilvási A, Tordai A, Rázsó K, Réti M, Prohászka Z.
The role of human leukocyte antigen DRB1-DQB1 haplotypes in the susceptibility to acquired idiopathic thrombotic thrombocytopenic purpura.
HUMAN IMMUNOLOGY 78:(2) pp. 80-87. (2017)

IF: 1.994

Sinkovits G, Szilágyi Á, Farkas P, Inotai D, Szilvási A, Tordai A, Rázsó K, Réti M, Prohászka Z.
Concentration and subclass distribution of anti-ADAMTS13 IgG autoantibodies in different stages of acquired idiopathic thrombotic thrombocytopenic purpura.
FRONTIERS IN IMMUNOLOGY 9:1646. (2018)

IF: 4.716

Cumulative impact factor of the publications related to the thesis: 6.710.

6.2. Publications unrelated to the PhD thesis

Sinkovits G, Prohászka Z.
A komplementrendszer szerepe a thromboticus microangiopathiák patogenezisében.
ORVOSKÉPZÉS 88:(2) pp. 331-337. (2013)

IF: -

Mikes B, **Sinkovits G**, Farkas P, Csuka D, Schlammadinger Á, Rázsó K, Demeter J, Domján G, Réti M, Prohászka Z.
Elevated plasma neutrophil elastase concentration is associated with disease activity in patients with thrombotic thrombocytopenic purpura.
THROMBOSIS RESEARCH 133:(4) pp. 616-621. (2014)

IF: 2.447

Mikes B, **Sinkovits G**, Farkas P, Csuka D, Rázsó K, Réti M, Radványi G, Demeter J, Prohászka Z.
Carboxiterminal pro-endothelin-1 as an endothelial cell biomarker in thrombotic thrombocytopenic purpura.
THROMBOSIS AND HAEMOSTASIS 115:(5) pp. 1034-1043. (2016)

IF: 5.627

Leffler J, Prohaszka Z, Mikes B, **Sinkovits G**, Ciacma K, Farkas P, Réti M, Kelen K, Reusz GS, Szabó AJ, Martin M, Blom AM.
Decreased neutrophil extracellular trap degradation in Shiga toxin-associated haemolytic uraemic syndrome.
JOURNAL OF INNATE IMMUNITY 9:(1) pp. 12-21. (2017)

IF: 3.837

Farkas P, Csuka D, Mikes B, **Sinkovits G**, Réti M, Németh E, Rácz K, Madách K, Gergely M, Demeter J, Prohászka Z.
Complement activation, inflammation and relative ADAMTS13 deficiency in secondary thrombotic microangiopathies.
IMMUNOBIOLOGY 222:(2) pp. 119-127. (2017)

IF: 2.873

Garam N, Maláti É, **Sinkovits G**, Gombos T, Szederjesi A, Barabás L, Gráf L, Kocsis J, Prohászka Z.
Platelet count, ADAMTS13 activity, von Willebrand factor level and survival in patients with colorectal cancer: 5-year follow-up study.
THROMBOSIS AND HAEMOSTASIS 118:(1) pp. 123-131. (2018)

IF: 4.733

Horváth O, Kállay K, Csuka D, Mező B, **Sinkovits G**, Kassa C, Stréhn A, Csordás K, Sinkó J, Prohászka Z, Kriván G.
Early increase in complement terminal pathway activation marker sC5b-9 is predictive for the development of thrombotic microangiopathy after stem cell transplantation.
BIOLOGY OF BLOOD AND MARROW TRANSPLANTATION 24(5):989-996. (2018)

IF: 3.599

Trojnár E, Szilágyi Á, Mikes B, Csuka D, **Sinkovits G**, Prohászka Z.
Role of complement in the pathogenesis of thrombotic microangiopathies.
MAGAZINE OF EUROPEAN MEDICAL ONCOLOGY 11:3 pp. 227-234. (2018) (összefoglaló cikk)

IF: -

Trojnár E, Józsi M, Szabó Z, Réti M, Farkas P, Kelen K, Reusz GS, Szabó AJ, Garam N, Mikes B, **Sinkovits G**, Mező B, Csuka D, Prohászka Z.
Elevated systemic pentraxin-3 is associated with complement consumption in the acute phase of thrombotic microangiopathies.
FRONTIERS IN IMMUNOLOGY 10:240. (2019)

IF: 4.716*

Cumulative impact factor of the publications unrelated to the thesis: 27.832.

Total impact factor of the indicated publications: 34.542.