SPANDIDOS PUBLICATIONS

EXPERIMENTAL AND THERAPEUTIC MEDICINE 12: 1189-1195, 2016

Immunomodulatory effects of therapeutic plasma exchange on monocytes in antiphospholipid syndrome

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Received July 22, 2015; Accepted February 18, 2016

DOI: 10.3892/etm.2016.3441

Abstract. Antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by thrombosis and recurrent fetal loss, with the persistent presence of antiphospholipid antibodies (aPLs). aPLs exert their pathogenic effect via the overproduction of tissue factor and activation of complement and several cell types, including endothelial cells, platelets and notably monocytes. As a result, a hypercoagulable state develops leading to APS-associated obstetric complications and fetal loss. Despite being far from optimal, treatment of APS usually includes heparin and low dose aspirin. Recently, plasma exchange (PE) therapy was successfully used in patients with APS with obstetric complications who did not respond to the standard treatment. Therefore, the present study investigated the mechanism underlying PE action, and aimed to determine whether PE affects the functional activity of APS monocytes by examining the expression of 11 mRNA transcripts encoding cytokines, signaling molecules and transcription factors. Monocytes were collected prior to and following the PE treatment from women with APS who experienced recurrent pregnancy losses, as well as from healthy volunteers. Compared with control cells, APS monocytes showed deregulated expression of interleukin (IL)-1β, IL-6, IL-23, chemokine (C-C motif) ligand 2 (CCL2), C-X-C motif chemokine 10 (CXCL10), toll-like receptor 2, and signal transducer and activator of transcription 3. PE treatment resulted in increased IL-1β, IL-6, IL-23, CCL2, P2X7 and tumor necrosis factor-α mRNA transcripts in APS monocytes, restoring the mRNA expression levels to within normal ranges. Furthermore, PE therapy counterbalanced the expression levels of CCL2 and CXCL10, the levels of which are indicative of T helper cell 1/2 balance. The results of the present study indicate that the altered transcriptional profile in APS monocytes was restored by the immunomodulatory effect of plasmapheresis.

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Key words: therapeutic plasma exchange, monocytes, antiphospholipid syndrome, mRNA, cytokines

Introduction

Antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by recurrent arterial and/or venous thrombosis, pregnancy morbidity, thrombocytopenia and elevated titers of antiphospholipid antibodies (aPLs) (1,2). aPLs are a family of antibodies that target phospholipid-binding proteins, including lupus anticoagulant, anticardiolipin (aCL), and anti-β2-glycoprotein I (anti-β2-GPI) (2,3). APS displays a variety of clinical manifestations which are recognized to be associated with the presence of these aPLs (4). Thrombus formation is a key event in APS (5). A number of studies have been conducted in order to identify the pathogenesis of aPL-induced thrombosis, and it has been suggested that activation of monocytes (6,7), platelets (6) and endothelial cells (8) contribute to the thrombotic process. In particular, the procoagulant activity of monocytes and deregulated tissue factor expression were associated with an increased risk of thrombus formation. Although the association between monocytes and the development of thrombotic complications in patients with APS has been well-established (9), the mechanisms underlying aPL action on monocytes remain largely unknown.

Pregnant women with APS are at a high risk of complications, mainly due to thromboses in the placenta which can lead to miscarriages (10,11). The treatment of women with APS is predominantly focused on the prevention of further thrombotic events and subsequent fetal loss (12). Treatment strategies for patients with APS are individualized and usually require lifelong anticoagulant therapy (13,14). In the case of catastrophic aPL syndrome, a rare life-threatening variant of APS, plasma exchange (PE) therapy is recommended (15). Therapeutic PE (also termed plasmapheresis) is an extracorporeal blood purification method. The procedure includes the separation of blood plasma from cellular components, which are subsequently returned back to the patient, and the plasma is discarded and replaced with substitution fluids. This approach allows active and rapid body cleaning, and the elimination of circulating pathogenic autoantibodies, immunoglobulins, immune complexes and toxins (16-18).

Automated plasmapheresis is routinely used to collect plasma products from healthy donors for medical use (19,20). The results of previous studies have suggested that PE in combination with conventional methods of treatment appears to be effective in managing a range of neurological, hematological, renal, metabolic, infectious and immunological diseases (21,22). Furthermore, a previous study demonstrated that PE is able to prevent the deleterious effects on both mother and fetus in women with familial hyperlipidemia or systemic lupus erythematosus (23). Limited data is available regarding the effect of PE on pregnancy outcome in patients with APS. However, a number of clinical studies have reported promising results of PE therapy in APS, and recommended PE as an additional treatment modality during high-risk pregnancies in women with APS (24,25). The use of PE was effective in reducing the titers of aCL and anti-β2-GPI antibodies (24,25). Regardless of these data, few studies have investigated the immunological changes that occur following PE in APS. In consideration of the previous results indicating the involvement of monocytes in thrombotic complications, the present study aimed to investigate the effects of PE on the phenotype of peripheral blood monocytes in women with APS who experienced recurrent pregnancy losses. Monocyte transcriptional activity and the mRNA expression levels of 11 genes associated with signaling and recognition processes occurring in immune and inflammatory responses were investigated.

Materials and methods

Patients. A total of 11 women suffering from recurrent miscarriages and APS (mean age, 30±5.6 years) were selected for the present study. The patients with APS were classified at the Institute of Perinatology, Obstetrics and Gynecology according to the following criteria: i) Women with a history of two unexplained consecutive spontaneous pregnancy losses in the first and second trimesters; and ii) the presence of anti-cardiolipin antibodies [(immunoglobulin (Ig)G≥40 GPL units] and/or anti-β2-GPI (IgG≥40 GPL units) and/or positive lupus anticoagulant antibodies present in the plasma at two separate time points. None of the women met the criteria for systemic lupus erythematosus or any concomitant systemic autoimmune disease. Blood samples were obtained from the patients prior to and following PE therapy. All patients received a course of four PE sessions on alternating days. The first blood sampling was performed immediately prior to the PE procedure. The second blood sampling was obtained on day 3 following the last PE session. Nine healthy women (mean age, 29±8.5 years) without a positive family history of APS, autoimmune diseases, or thrombosis were enrolled as a control group at the Viola Blood Center and Diagnostics (Yerevan, Armenia). All participants of the present study provided written-informed consent. The present study was approved by the ethics committee of the Institute of Molecular Biology of the NAS RA (approval no. IRB IORG0003427).

PE therapy. The principle of plasmapheresis is to remove the plasma with retransfusion of the blood cells. The procedure is multistage and involves taking blood from a patient and placing it into a disposable plastic bag blood collection system (Baxter International, Inc., Deerfield, IL, USA). Separation of the plasma from the blood cells was achieved by centrifugation at 1,900 x g for 15 min at room temperature. The plasma was discarded and replaced with Ringer's solution (Liqvor Pharmaceuticals, Yerevan, Armenia), and the blood is then returned.

Preparation of peripheral/circulating monocytes. Fresh venous blood samples were obtained and placed into Vacutainer tubes containing ethylenediaminetetraacetic acid (BD Biosciences, Franklin Lakes, NJ, USA). Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density gradient centrifugation (Life Science Sweden, Sweden) according to the manufacturer's protocol. Monocytes were isolated from peripheral blood mononuclear cell (PBMCs) by plastic adherence. Briefly, $1x10^6$ PBMCs were distributed in plastic tissue culture flasks (Corning, Inc., Oneonta, NY, USA) and allowed to adhere at 37˚C for 2 h in 2 ml RPMI-1640 medium (Corning, Inc.) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA). Non-adherent cells were removed and the adherent cells were washed twice with phosphate-buffered saline. Following washing >75% of the cells were identified as monocytes, as assessed by FACS Calibur flow cytometry (BD Biosciences) using R-phycoerythrin-conjugated anti-human CD14 IgG antibody (BioLegend, Inc., London, UK) (26).

Monocytes ($1x10^6$ cells/ml) were cultured separately at 37˚C for 4 h in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mmol/l glutamine (Sigma-Aldrich), 5 mM HEPES (Sigma-Aldrich) in the absence or presence of 10 ng/ml lipopolysaccharide (LPS; *Escherichia coli* 026:B6; Sigma-Aldrich) or 10 ng/ml LPS + 100 μM ATPγ-S (Sigma-Aldrich) in a total volume of 1 ml. Following the culture period or directly following isolation, the cells were washed once with cold PBS, and stored in 150 μl RNAlater (Qiagen GmbH, Hilden, Germany) at -20˚C until use.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from monocytes samples using a High Pure miRNA Isolation kit (cat. no. 05080576001; Roche Diagnostics, Basel, Switzerland) according to the manufacturer's protocol. All samples were treated with RNaseOUT Recombinant Ribonuclease Inhibitor (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and maintained at -80˚C. cDNA synthesis and qPCR were performed as described previously (27). Briefly, cDNA synthesis was performed using a Transcriptor First Stand cDNA Synthesis kit (Roche Diagnostics) and stored at -20˚C prior to further use. RT-qPCR was performed using gene-specific primers designed using the Universal Probe Library system (Roche Diagnostics). The primer and probes sequences for the investigated genes (11 in total) are listed in Table I. Each qPCR reaction was performed in triplicate along with a negative template control (no cDNA), negative RT control (no reverse transcription) and positive template control (calibrator) in quadruplicates. Target gene expression levels were normalized against the reference gene ribosomal protein L32. Human universal reference RNA (Agilent Technologies, Inc., Santa Clara, CA, USA) was used as a calibrator at the concentration of 1.25 ng RNA/reaction in quadruplicates. qPCR reactions

Table I. Description of investigated genes and primers used in the present study.

Gene	Gene name (full)	GenBank accession no.[a]	Sense and antisense primers	LNA probe[b]
RPL32[c]	Ribosomal protein L32	NM_000994.3	F, 5'-GAAGTTCCTGGTCCACAACG-3' R, 5'-GCGATCTCGGCACAGTAAG-3'	#17
IL-1β[c]	Interleukin 1β	NM_000576.2	F, 5'-TACCTGTCCTGCGTGTTGAA-3' R, 5'-TCTTTGGGTAATTTTTGGGATCT-3'	#78
NLRP3[c]	Nod-like receptor family, pyrin domain containing 3	NM_001243133.1	F, 5'-TGTCCTCCCAAGCTCCTCT-3' R, 5'-AAGCAGCACTCATGCGAGA-3'	#27
CCL2[c]	Chemokine (C-C motif) ligand 2	NM_002982.3	F, 5'-AGTCTCTGCCGCCCTTCT-3' R, 5'-GTGACTGGGGCATTGATTG-3'	#40
TNF-α[c]	Tumor necrosis factor-α	NM_000594.3	F, 5'-CAGCCTCTTCTCCTTCCT-3' R, 5'-GCCAGAGGGCTGATTAGA-3'	#29
TLR2[d]	Toll-like receptor 2	NM_003264.3	F, 5'-CGTTCTCTCAGGTGACTG-3' R, 5'-CCTTTGGATCCTGCTTGC-3'	#14
IL-6[d]	Interleukin 6	NM_000600.3	F, 5'-GAAGCTCTATCTCGCCTCCA-3' R, 5'-AGCAGGCAACACCAGGAG-3'	#7
CXCL10[d]	Chemokine (C-X-C motif) ligand 10	NM_001565.3	F, 5'-GAAAGCAGTTAGCAAGGAAA-3' R, 5'-GACATATACTCCATGTAGGGA-3'	#34
STAT3[d]	Signal transducer and activator of transcription 3	NM_003150.3	F, 5'-TGATGCAGTTTGGAAATAATG-3' R, 5'-CATGTCAAAGGTGAGGGACTC-3'	#18
IL-23A[d]	Interleukin 23, α-subunit p19	NM_016584.2	F, 5'-GTTCCCCATATCCAGTGTGG-3' R, 5'-TCCTTTGCAAGCAGAACTGA-3'	#76
P2X7[e]	Purinergic receptor P2X, ligand-gated ion channel 7	NM_002562.5	Hs00175721_m1	
NF-κB p65[e]	Homo sapiens v-rel avian reticuloendotheliosis viral oncogene homolog A	NM_003998.3	Hs00153294_m1	

[a]Gene sequences available online at http://www.ncbi.nlm.nih.gov/. [b]Numbers of Locked Nucleic Acid (LNA) probes according to the commercially available library (www.universalprobelibrary.com). [c]Primers/probe sets (PrimeTime qPCR Assays) were from Metabion International AG (Planegg, Germany). [d]Primers/probe sets (PrimeTime qPCR Assays) were from Integrated DNA Technologies (Coralville, IA, USA). [e]Primers/probe sets (TaqMan® Gene Expression Assay) were from Applied Biosystems (Thermo Fisher Scientific, Inc., Foster City, CA, USA).

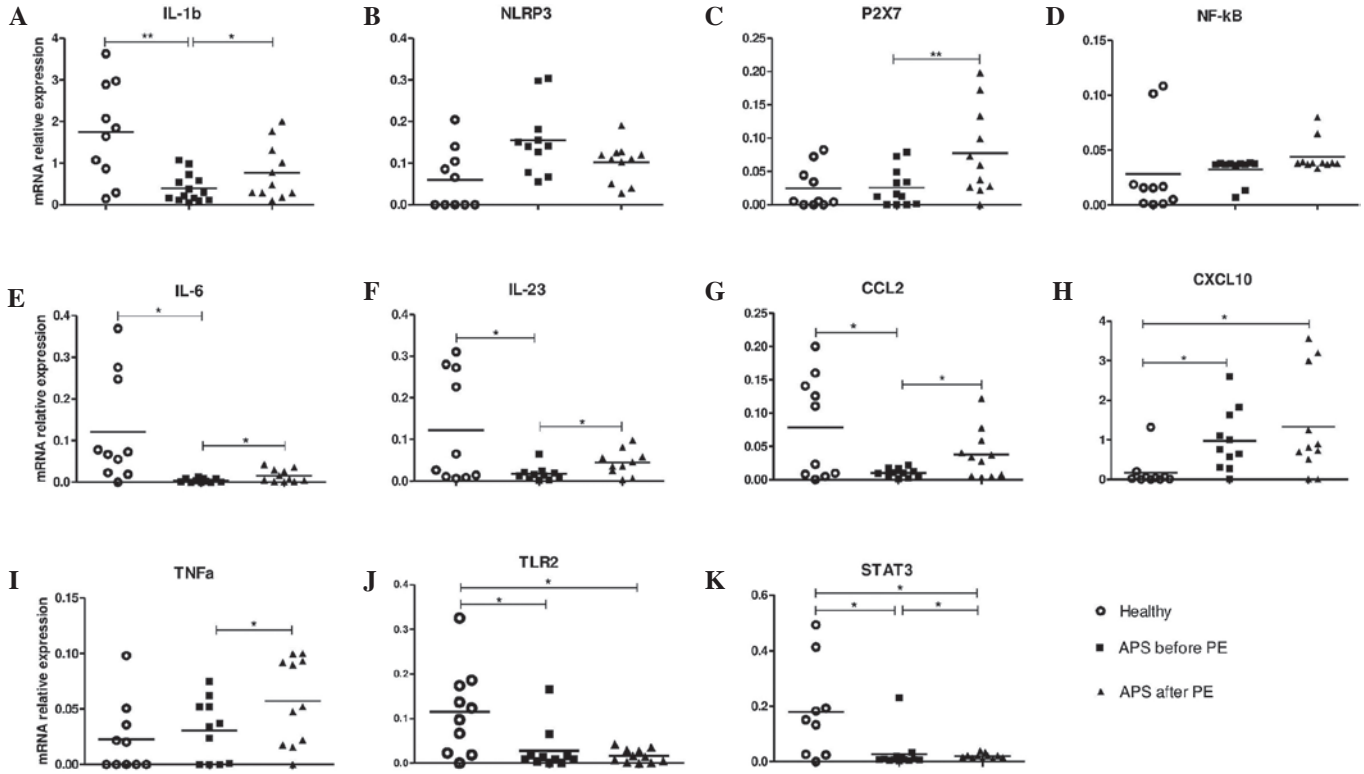

Figure 1. Relative mRNA expression levels of candidate genes in monocytes isolated from healthy subjects, patients with APS prior to PE therapy, and patients with APS on the third day following PE therapy. The mRNA expression levels of (A) IL-1b, (B) NLRP3, (C) P2X7, (D) NF-κB, (E) IL-6, (F) IL-23, (G) CCL2, (H) CXCL10, (I) TNF-α, (J) TLR2 and (K) STAT3 were normalized against those of RPL32. Horizontal bars represent the mean values for each group. *P<0.05 and **P<0.01. APS, antiphospholipid syndrome; PE, plasma exchange; RPL32, Ribosomal protein L32; IL, interleukin; NLRP3, Nod-like receptor family, pyrin domain containing 3; P2X7, purinergic receptor P2X, ligand-gated ion channel 7; NF-κB, nuclear factor-κB; CCL2, chemokine (C-C motif) ligand 2; CXCL10, chemokine (C-X-C motif) ligand 10; TNF-α, tumor necrosis factor-α; TLR2, toll-like receptor 2; STAT3, signal transducer and activator of transcription 3.

were performed using the Rotor-Gene 3000 system (Corbett Research, Mortlake, Australia) in a 0.1 ml strip tubes (Qiagen GmbH) and a final volume of 20 μl. The relative expression levels were calculated using the second derivative method (Rotor-Gene software 6.1.71; Corbett Research) (28).

Statistical analysis. The data are presented as means ± standard deviation. Statistical analyses were performed using Graph Pad Prism software, version 5.01 (GraphPad Software, Inc., La Jolla, CA, USA). Data were compared using paired (prior to PE therapy, vs. following PE therapy) and unpaired (all other comparisons) Student's t-tests or a Mann-Whitney U test if the data were not normally distributed. A P-value of <0.05 was considered to indicate a statistically significant difference.

Results

Subheading. Evidence for the clinical benefit of PE has been limited and the majority of previous investigations into the effectiveness of PE have been conducted in high-risk pregnancies in women with APS (24,25). However, it is not known whether the procedure itself affects the functional activity of monocytes. Therefore, the present study aimed to investigate the impact of PE therapy on gene expression by measuring the mRNA expression levels in the monocytes from women with APS who had experienced more than two pregnancy losses.

Baseline gene expression. Initially, the baseline gene expression levels of 11 genes [interleukin (IL)-1β, IL-6, IL-23, tumor necrosis factor-α (TNF-α), chemokine (C-C motif) ligand 2 (CCL2), C-X-C motif chemokine 10 (CXCL10), Nod-like receptor family, pyrin domain containing 3, purinergic receptor P2X, ligand-gated ion channel 7 (P2X7), nuclear factor (NF)-κB, toll-like receptor 2 (TLR2) and signal transducer and activator of transcription 3 (STAT3)] in monocytes were quantified in women with APS and compared with the expression levels in monocytes from healthy control women (Fig. 1). The results demonstrated that the baseline mRNA expression of six investigated genes were downregulated [healthy controls, vs. individuals with APS prior to PE (mean ± standard deviation)], specifically IL-1β; (1.690±0.467, vs. 0.394±0.09, P=0.002), IL-6 (0.107±0.046, vs. 0.004±0.001, P=0.023), IL-23 (0.125±0.068, vs. 0.017±0.005, P=0.039), CCL2 (0.076±0.027, vs. 0.010±0.002, P=0.028), TLR2 (0.107±0.033, vs. 0.029±0.016, P=0.045) and STAT3 (0.164±0.072, vs. 0.027±0.017, P=0.021); and one gene, CXCL10, was upregulated (0.203±0.161, vs. 0.960±0.258, P=0.03). P2X7, NF-κB and TNF-α mRNA expression did not differ between APS and healthy control monocytes (Fig. 1).

Effect of PE therapy on gene expression. The expression levels of all investigated genes prior to and following PE therapy were compared. A number of the investigated genes were upregulated following plasmapheresis. As shown in Fig. 1, the mRNA expression levels (prior to PE, vs. following PE therapy) of IL-1β

(0.394±0.09, vs. 0.718±0.213, P=0.038), IL-6 (0.004±0.001, vs. 0.017±0.005, P=0.025), IL-23 (0.017±0.006, vs. 0.037±0.014, P=0.047), CCL2 (0.010±0.002, vs. 0.047±0.016; P=0.030), P2X7 (0.026±0.008, vs. 0.081±0.026, P=0.029) and TNF-α (0.027±0.008, vs. 0.055±0.016, P=0.015) were increased in APS monocytes following PE therapy. STAT3 mRNA expression levels (0.027±0.017, vs. 0.022±0.003, P=0.049) were slightly decreased following PE therapy.

Expression levels. The expression levels (healthy controls, vs. APS following PE therapy) of TLR2 (0.107±0.034, vs. 0.015±0.005, P=0.015) and STAT3 (0.164±0.072, vs. 0.022±0.003, P=0.040) were significantly higher in the healthy monocytes compared with the APS monocytes following PE therapy. Furthermore, the mRNA expression levels of CXCL10 were significantly higher in APS cells (0.204±0.161, vs. 1.266±0.467, P=0.049).

Discussion

There has been considerable evidence that thrombosis is a common complication of APS, and monocytes are the primary cellular elements which are involved in the hypercoagulation observed in this pathology (29). Furthermore, the proinflammatory state of monocytes was demonstrated to be associated with predisposition to thrombosis (30). The present study examined whether monocytes from patients with APS displayed an altered or proinflammatory state. Contrary to expectations, low baseline expression levels of IL-1β, IL-6, IL-23, CCL2, TLR2 and STAT3 were observed, and only the expression of CXCL10 was upregulated in APS monocytes. Upregulated CXCL10 expression combined with downregulated CCL2 baseline expression in APS monocytes is of particular interest as these two chemokines possess unique roles in autoimmune diseases (31). CCL2 and CXCL10 regulate immune responses via the activation and recruitment of immune cells (32). CCL2/monocyte chemoattractant protein-1 is a crucial factor for the development of adaptive T helper cells (Th)2 responses, whereas CXCL10 is a strong chemoattractant for activated Th1 lymphocytes (33), and a reliable marker of Th1-mediated autoimmune diseases (34). The results of the present study demonstrated that a CCL2-low/CXCL10-high transcriptional profile of circulating APS monocytes, which may suggest a shift towards Th1 immune response in APS. A reduction in Th1 cellular immune response and a corresponding increase in Th2 cells are essential for successful pregnancy (35). An opposite tendency was present in women with recurrent miscarriages (36,37). In addition to a Th1-biased response in women with recurrent miscarriages, altered PBMC activity, prior to and following pregnancy loss, was also described (37). Transcriptional patterns of PBMCs that could account for the pathogenesis of primary APS have been studied by Bernales *et al* (38). Despite a wide diversity in the range of expression profiles, the authors of the study stated that altered cellular 'phenotype' was specific for APS (38). The reason for the low functional activity of APS monocytes is unknown. Further investigations are required in order to clarify whether the downregulation of gene expression in monocytes is due to an APS-associated, inherent feature. There is a possibility that this transcriptional profile of monocytes is associated with marked hormonal and immunological changes during pregnancy in addition to the biological changes that occur during miscarriage (39).

Notably, the present study indicated the immunomodulatory effect of plasmapheresis on APS monocytes. PE therapy resulted in increased mRNA expression levels in six of the 11 investigated genes, namely IL-1β, IL-6 IL-23, TNF-α, CCL2 and P2X7. Despite the fact that PE therapy increased the expression levels of a wide range of mediators, their expression levels did not exceed those in the cells from healthy subjects, and increased from low baseline expression levels to normal, physiological ranges. Although the exact mechanisms underlying this effect have yet to be elucidated, they likely involve multiple processes. In addition to the removal of IGs, increasing evidence suggests an immunomodulatory effect of PE. These include Th1/Th2 balance with a shift to Th2 cell response (40), as well as the suppression of IL-2 and interferon-γ production (41). Notably, PE therapy in the present study significantly increased CCL2 expression, whereas CXCL10 mRNA expression levels were not affected by the treatment procedure. The observed effect may be viewed as beneficial for Th1/Th2 balance restoration in APS patients following pregnancy loss.

It is well known that *ex vivo* manipulation of immune cells may induce their activation, and it is difficult to ensure that *in vitro* conditions will be the same as physiological conditions. In particular, the cell isolation procedure may affect gene expression and protein secretion by monocytes (42,43). In lung epithelial cells, centrifugation affects proliferation and expression of certain cytokines such as IL-1β (44). PE therapy involves a number of steps: Separation of blood components by centrifugation, erythrocytes washing and restoration of blood volume with replacement fluids. It may be hypothesized that *ex vivo* manipulation of blood cells during PE may have temporary and reversible effects on non-selective activation of blood cells. The half-life of circulating monocytes was estimated to be ≥3 days (45), and PE-activated circulating monocytes will be replenished with bone marrow-derived cells. At present, it is difficult to determine whether the observed changes in the expression levels of the candidate genes are associated with the unwanted side-effect of experimental manipulations or whether they indicate the positive effect of PE on functional recovery in APS monocytes. This limitation should be addressed in future studies of the impact of PE treatment on the immune system.

The therapeutic rationale for PE during APS has yet to be fully elucidated. Although PE is not routinely used in APS management, this treatment modality serves as a promising tool for novel and optimized treatment strategies. The present preliminary study demonstrated the immunomodulatory effect of plasmapheresis on monocytes obtained from APS patients. In a more general context, these observations should be considered with caution, as the activating effect of PE on immune cells may have dual consequences including adverse ones. In the case of APS, normalizing monocyte activity following PE is likely to be beneficial; however, treatment plans for PE therapy in other pathological conditions should be examined on a case-by-case basis with consideration of possible unwanted side-effects.

Acknowledgements

The authors of the present study are grateful to Dr Armen Blbulyan (Institute of Perinatology, Obstetrics and Gynecology, Yerevan, Armenia) for assistance with blood sampling. The present study was supported by a grant from the Czech government (grant no. CZ.1.07/2.3.00/30.00041; visegrad fund no. 51300184) and the State Committee Science MES RA (research project no. SCS13-1F236).

References

1. de Jesus GR, Agmon-Levin N, Andrade CA, Andreoli L, Chighizola CB, Porter TF, Salmon J, Silver RM, Tincani A and Branch DW: 14th International congress on antiphospholipid antibodies task force report on obstetric antiphospholipid syndrome. Autoimmun Rev 13: 795-813, 2014.
2. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, DE Groot PG, Koike T, Meroni PL, *et al*: International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 4: 295-306, 2006.
3. Gris JC and Bouvier S: Antiphospholipid syndrome: Looking for a refocusing. Thromb Res 131 Suppl 1: S28-S31, 2013.
4. Galli M, Borrelli G, Jacobsen EM, Marfisi RM, Finazzi G, Marchioli R, Wisloff F, Marziali S, Morboeuf O and Barbui T: Clinical significance of different antiphospholipid antibodies in the WAPS (warfarin in the antiphospholipid syndrome) study. Blood 110: 1178-1183, 2007.
5. Khamashta MA and Asherson RA: Hughes syndrome: Antiphospholipid antibodies move closer to thrombosis in 1994. Br J Rheumatol 34: 493-494, 1995.
6. Reverter JC, Tàssies D, Font J, Khamashta MA, Ichikawa K, Cervera R, Escolar G, Hughes GR, Ingelmo M and Ordinas A: Effects of human monoclonal anticardiolipin antibodies on platelet function and on tissue factor expression on monocytes. Arthritis Rheum 41: 1420-1427, 1998.
7. Kornberg A, Blank M, Kaufman S and Shoenfeld Y: Induction of tissue factor-like activity in monocytes by anti-cardiolipin antibodies. J Immunol 153: 1328-1332, 1994.
8. Branch DW and Rodgers GM: Induction of endothelial cell tissue factor activity by sera from patients with antiphospholipid syndrome: A possible mechanism of thrombosis. Am J Obstet Gynecol 168: 206-210, 1993.
9. López-Pedrera C, Cuadrado MJ, Herández V, Buendïa P, Aguirre MA, Barbarroja N, Torres LA, Villalba JM, Velasco F and Khamashta M: Proteomic analysis in monocytes of antiphospholipid syndrome patients: Deregulation of proteins related to the development of thrombosis. Arthritis Rheum 58: 2835-2844, 2008.
10. Ruiz-Irastorza G and Khamashta MA: Management of thrombosis in antiphospholipid syndrome and systemic lupus erythematosus in pregnancy. Ann N Y Acad Sci 1051: 606-612, 2005.
11. Di Prima FA, Valenti O, Hyseni E, Giorgio E, Faraci M, Renda E, De Domenico R and Monte S: Antiphospholipid syndrome during pregnancy: The state of the art. J Prenat Med 5: 41-53, 2011.
12. Meroni PL, Moia M, Derksen RH, Tincani A, McIntyre JA, Arnout JM, Koike T, Piette JC, Khamashta MA and Shoenfeld Y: Venous thromboembolism in the antiphospholipid syndrome: Management guidelines for secondary prophylaxis. Lupus 12: 504-507, 2003.
13. Erkan D, Ortel TL and Lockshin MD: Warfarin in antiphospholipid syndrome-time to explore new horizons. J Rheumatol 32: 208-212, 2005.
14. Tincani A, Branch W, Levy RA, Piette JC, Carp H, Rai RS, Khamashta M and Shoenfeld Y: Treatment of pregnant patients with antiphospholipid syndrome. Lupus 12: 524-529, 2003.
15. Marson P, Bagatella P, Bortolati M, Tison T, De Silvestro G, Fabris F, Pengo V and Ruffatti A: Plasma exchange for the management of the catastrophic antiphospholipid syndrome: Importance of the type of fluid replacement. J Intern Med 264: 201-203, 2008.
16. Ibrahim RB and Balogun RA: Medications in patients treated with therapeutic plasma exchange: Prescription dosage, timing and drug overdose. Semin Dial 25: 176-189, 2012.
17. Ibrahim RB and Balogun RA: Medications and therapeutic apheresis procedures: Are we doing our best? J Clin Apher 28: 73-77, 2013.
18. Kaplan AA: Therapeutic apheresis for renal disorders. Therap Apher 3: 25-30, 1999.
19. Bove LL, Bednall T, Masser B and Buzza M: Understanding the plasmapheresis donor in a voluntary, nonremunerated environment. Transfusion 51: 2411-2424, 2011.
20. Crocco I, Franchini M, Garozzo G, Gandini AR, Gandini G, Bonomo P and Aprili G: Adverse reactions in blood and apheresis donors: Experience from two Italian transfusion centres. Blood Transfus 7: 35-38, 2009.
21. Nguyen TC, Kiss JE, Goldman JR and Carcillo JA: The role of plasmapheresis in critical illness. Crit Care Clin 28: 453-468, 2012.
22. Nakanishi T, Suzuki N, Kuragano T, Nagasawa Y and Hasuike Y: Current topics in therapeutic plasmapheresis. Clin Exp Nephrol 18: 41-49, 2014.
23. Dittrich E, Schmaldienst S, Langer M, Jansen M, Hörl WH and Derfler K: Immunoadsorption and plasma exchange in pregnancy. Kidney Blood Press Res 25: 232-239, 2002.
24. Bortolati M, Marson P, Chiarelli S, Tison T, Facchinetti M, Gervasi MT, De Silvestro G and Ruffatti A: Case reports of the use of immunoadsorption or plasma exchange in high-risk pregnancies of women with antiphospholipid syndrome. Ther Apher Dial 13: 157-160, 2009.
25. Bontadi A, Ruffatti A, Marson P, Tison T, Tonello M, Hoxha A, De Silvestro G and Punzi L: Plasma exchange and immunoadsorption effectively remove antiphospholipid antibodies in pregnant patients with antiphospholipid syndrome. J Clin Apher 27: 200-204, 2012.
26. Ferrer DG, Jaldín-Fincati JR, Amigone JL, Capra RH, Collino CJ, Albertini RA and Chiabrando GA: Standardized flow cytometry assay for identification of human monocytic heterogeneity and LRP1 expression in monocyte subpopulations: Decreased expression of this receptor in nonclassical monocytes. Cytometry A 85: 601-610, 2014.
27. Manukyan G, Petrek M, Kriegova E, Ghazaryan K, Fillerova R, Fillerova R and Boyajyan A: Activated phenotype of circulating neutrophils in familial mediterranean fever. Immunobiology 218: 892-898, 2013.
28. Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29: e45, 2001.
29. Cuadrado MJ, López-Pedrera C, Khamashta MA, Camps MT, Tinahones F, Torres A, Hughes GR and Velasco F: Thrombosis in primary antiphospholipid syndrome: A pivotal role for monocyte tissue factor expression. Arthritis Rheum 40: 834-841, 1997.
30. Wakefield TW, Strieter RM, Wilke CA, Kadell AM, Wrobleski SK, Burdick MD, Schmidt R, Kunkel SL and Greenfield LJ: Venous thrombosis-associated inflammation and attenuation with neutralizing antibodies to cytokines and adhesion molecules. Arterioscler Thromb Vasc Biol 15: 258-268, 1995.
31. Godessart N and Kunkel SL: Chemokines in autoimmune disease. Curr Opin Immunol 13: 670-675, 2001.
32. Oghumu S, Lezama-Dávila CM, Isaac-Márquez AP and Satoskar AR: Role of chemokines in regulation of immunity against leishmaniasis. Exp Parasitol 126: 389-396, 2010.
33. Gu D, Chen Z, Zhao H, Du W, Xue F, Ge J, Sui T, Wu H, Liu B, Lu S, Zhang L and Yang R: Th1 (CXCL10) and Th2 (CCL2) chemokine expression in patients with immune thrombocytopenia. Hum Immunol 71: 586-591, 2010.
34. Rotondi M, Rosati A, Buonamano A, Lasagni L, Lazzeri E, Pradella F, Fossombroni V, Cirami C, Liotta F, La Villa G, *et al*: High pretransplant serum levels of CXCL10/IP-10 are related to increased risk of renal allograft failure. Am J Transplant 4: 1466-1474, 2004.
35. Wegmann TG, Lin H, Guilbert L and Mosmann TR: Bidirectional cytokine interactions in the maternal-fetal relationship: Is successful pregnancy a TH2 phenomenon? Immunology Today 14: 353-356, 1993.
36. Lissauer D, Goodyear O, Khanum R, Moss PA and Kilby MD: Profile of maternal CD4 T-cell effector function during normal pregnancy and in women with a history of recurrent miscarriage. Clin Sci (Lond) 126: 347-354, 2014.
37. Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M and Farhat R: Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. Hum Reprod 15: 713-778, 2000.
38. Bernales I, Fullaondo A, Marín-Vidalled MJ, Ucar E, Martínez-Taboada V, López-Hoyos M and Zubiaga AM: Innate immune response gene expression profiles characterize primary antiphospholipid syndrome. Genes Immun 9: 38-46, 2008.

39. Laird SM, Tuckerman EM, Cork BA, Linjawi S, Blakemore AI and Li TC: A review of immune cells and molecules in women with recurrent miscarriage. Hum Reprod Update 9: 163-174, 2003.
40. Goto H, Matsuo H, Nakane S, Izumoto H, Fukudome T, Kambara C and Shibuya N: Plasmapheresis affects T helper type-1/T helper type-2 balance of circulating peripheral lymphocytes. Ther Apher 5: 494-496, 2001.
41. Shariatmadar S, Nassiri M and Vincek V: Effect of plasma exchange on cytokines measured by multianalyte bead array in thrombotic thrombocytopenic purpura. Am J Hematol 79: 83-88, 2005.
42. Haskill S, Johnson C, Eierman D, Becker S and Warren K: Adherence induces selective mRNA expression of monocyte mediators and proto-oncogenes. J Immunol 140: 1690-1694, 1988.
43. Fuhlbrigge RC, Chaplin DD, Kiely JM and Unanue ER: Regulation of interleukin 1 gene expression by adherence and lipopolysaccharide. J Immunol 138: 3799-3802, 1987.
44. Yang J, Hooper WC, Phillips DJ, Tondella ML and Talkington DF: Centrifugation of human lung epithelial carcinoma a549 cells up-regulates interleukin-1beta gene expression. Clin Diagn Lab Immunol 9: 1142-1143, 2002.
45. Yona S and Jung S: Monocytes: subsets, origins, fates and functions. Curr Opin Hematol 17: 53-59, 2010.