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Treatment with intravenous immunoglobulin increases the level of small EVs in plasma of pregnant women with recurrent pregnancy loss

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ABSTRACT

Extracellular vesicles (EVs), which are small cell-derived compartments, take part in numerous different physiological processes. The contents of EVs reveal the cell of origin and indicates pathophysiological states in different diseases. In pregnancy disorders, changes have been reported in the composition, bioactivity and concentration of placental and non-placental EVs. The purpose of this study was to monitor the effects on EVs in patients receiving intravenous immunoglobulin (IVIG) or placebo (albumin) treatment due to recurrent pregnancy loss (RPL).

In a placebo-controlled trial study of IVIG treatment, plasma collected from 39 women with RPL were investigated using the Extracellular Vesicle Array (EV Array). Plasma was sampled consecutively (from gestational week (GW) 5) and the protein phenotypes of the smaller EVs (sEVs) were analyzed for the presence of 34 markers. The levels of sEVs or changes in their levels in early pregnancy were correlated with treatment.

There was statistically significant increased levels of sEVs in patients who received IVIG versus placebo. In conclusion, the treatment with high-doses of IVIG clearly boosted the production and release of sEVs to the circulation; however, the biological role of this boost remains to be clarified in further studies.

1. Introduction

Pregnancy loss (PL) is the most common pregnancy complication happening in approximately 15 % of all pregnancies (Alijotas-Reig and Garrido-Gimenez, 2013). Recurrent pregnancy loss (RPL) is defined as either ≥3 consecutive pregnancy losses before gestational week 12 or as 2 pregnancy losses after normal ultrasound confirmation at week 12. RPL is estimated to have an incidence of 1–3 % of all couples (Alijotas-Reig and Garrido-Gimenez, 2013; Wang et al., 2016).

Since immunological disturbances seem to be a risk factor in many cases of RPL, various immune-therapeutic interventions have been tested in this context. Intravenous immunoglobulin (IVIG) exhibits a documented effect in many disorders caused by immunological abnormalities (Galeotti et al., 2018; Jolles et al., 2005). IVIG is a pooled preparation of normal IgG obtained from several thousand healthy donors. Besides IgG monomers (>96 %), a small percentage of IgG dimers, IgM and IgA can be found in IVIG preparations (Galeotti et al., 2018).

IVIG has also been tested in the treatment of women with RPL in several studies with very diverging results. In the late 1990's a randomized, double-blinded, placebo-controlled trial of IVIG was performed (Christiansen et al., 2002). The conclusion of the study was that IVIG might improve pregnancy outcome although a new placebo-controlled trial would be needed to confirm the results.

Since this study was published, the understanding of how cells communicate has undergone a paradigm shift with the recognition of the role of extracellular vesicles (EVs) in intercellular signaling. EVs are membrane-bound complexes secreted from cells under both

Abbreviations: EVs, extracellular vesicles; IVIG, intravenous immunoglobulin; RPL, recurrent pregnancy loss; GW, gestational week; PL, pregnancy loss; LB, live birth

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physiological and pathological conditions. They contain proteins, nucleic acids and lipids and act as messengers for cell–cell communication and signaling.

EV is a general term encompassing several different vesicle types, including exosomes, microvesicles, and apoptotic bodies, released by cells constitutively or in response to specific stimuli. The EVs are primarily distinguished on the basis of their size with exosomes, microvesicles and apoptotic bodies considered to be 30–100 nm, 100 nm – 1 μm and 1–5 μm, respectively (Raposo and Stoorvogel, 2013). Not only do these vesicles differ in size, but there are also differences with regard to their formation and protein content. Microvesicles are released from the plasma membrane by direct budding or shedding in response to *e.g.* cellular activation or stress, whereas exosomes are formed from internalized endocytic vesicles, and are constitutively secreted from the cell. Apoptotic bodies are released from blebbing cells undergoing apoptosis (Colombo et al., 2014).

EV research is a rapidly evolving and expanding field, and it appears that all biological fluids contain very large numbers of EVs; they are produced from all cells that have been studied to date, and are known to have roles in several reproductive processes. EVs have been demonstrated to be involved in modulating the immune system and their immunological effects comprise a broad range of mechanisms, including immune activation, immune suppression, and modulation of immune surveillance (Admyre et al., 2003; Gutiérrez-Vázquez et al., 2013; Pugholm et al., 2016; van der Vlist et al., 2012). Also during the early stages of the human reproductive process, the EVs from the ovarian follicle, seminal fluid, endometrium, embryo and trophoblast cells have shown potential to modulate maternal immune function locally (Redman and Sargent, 2007). During later pregnancy, the placenta is the primary source of EVs and the syncytiotrophoblast releases EVs directly into the maternal blood constituting a major signaling mechanism and maternal immune sensing of the fetus (Tannetta et al., 2014). Lately, it has also been found that EVs can cause functional inflammatory changes, and induce labor and delivery (Sheller-Miller et al., 2019).

Pregnancy is shown to cause an up to 50-fold increase in numbers of circulating exosomes, and placenta-derived exosomes are known to be present in maternal blood (Mincheva-Nilsson and Baranov, 2014; Mitchell et al., 2015; Salomon et al., 2014). In general, EVs are thought to be anti-inflammatory or tolerogenic upon uptake into recipient cells (Mincheva-Nilsson and Baranov, 2014). Therefore, placental EVs may play an important role in maternal-fetal tolerance and therefore also in relation to RPL (Stefanski et al., 2019). Pregnancy complications are hypothesized to have their origin in disorders of the early placentation (Mitchell et al., 2015). If these changes affect the placenta this would arguably affect EV production during early pregnancy. Women at risk of developing pregnancy complications, such as RPL patients, might benefit from identification of changes in the EV secretion and thereby provide an opportunity to develop clinically useful early pregnancy screening biomarkers.

In this study, plasma collected from 39 women with RPL from the study of Christiansen et al. (2002) were investigated using the Extracellular Vesicle Array (EV Array). The sampling of plasma was performed consecutively (from gestational week (GW) 5) and the protein phenotypes of the smaller EVs (sEVs) in plasma were analyzed for the presence of 34 markers of interest. The levels of sEVs or changes in their levels in early pregnancy were correlated with treatment groups (IVIG or placebo).

The Extracellular Vesicle Array (EV Array) is a sandwich ELISA-based method, which is optimized to catch and detect the smaller types of EVs, such as exosomes and exosome-like vesicles, with a diameter up to ~150 nm. The capturing of sEVs is performed with the use of an antibody panel targeting selected membrane- or membrane associated proteins (Jørgensen et al., 2013). The EV Array constitutes a fast, automated, economical and highly sensitive method for exploration of plasma-sEVs carrying CD9, CD63 and/or CD81.

2. Materials and methods

2.1. Patients included from the previous study

From the period June 1994 to June 1999, the placebo-controlled trial by Christiansen et al. (Christiansen et al., 2002) included a total of 58 patients with RPL who met the criteria eligible for participation. In short, the inclusion criteria were patients with four or more confirmed PLs before the end of GW26, of which the last three were consecutive.

After inclusion, all patients were randomly assigned to either IVIG or placebo treatment. These infusions were given double-blinded. For intervention, Nordimmun (HemaSure A/S, Copenhagen, Denmark) was used (human IgG prepared from plasma) containing human IgG, human albumin, sucrose and sodium chloride, whereas the placebo contained exclusively human albumin, sucrose and sodium chloride. The first infusion was given at GW5 and weekly infusions were given from GW5 to GW10, and afterwards every second week until GW26 or until time of miscarriage. Blood samples were drawn in EDTA tubes before each infusion of study drug, they were immediately centrifuged (standard protocol) and the extracted plasma was stored after at −80 °C.

For the current analysis, 39 patients from the study met the criteria set for data analysis in this study. Patients were included if at least two stored plasma samples were available, the first at GW5 before initiation of treatment and the next preferably from GW7. If patients did not have a GW7 plasma sample, another sample ±1 GW was chosen. Thereby, the interval for the second plasma sample became GW6-8.

2.2. Antibodies used

General EV markers: CD9 and CD81 (Ancell Corporation, MN, USA); CD63 (Bio-Rad, CA, USA); Flotillin-1 and TSG101 (Abcam, GB); Alix (clone 3A9, Biolegend, CA, USA).

Placental markers and hormone receptors: PLAP (clone 8B6, Santa Cruz Bio, TX, USA,); FSHR (clone 626717), Adenosine A2a R (clone 599717) (R&D Systems, MN, USA); LHR (clone 8G9A2), TSHR (clone 49), OPRL1 (Novus Biologicals, CO; USA); ADRB2 (clone 13A16), CHRM1, 3, 4 and 5 (LifeSpan Biosciences, WA, USA); CHRM2 (Boster Biological Technology, CA, USA).

Immune- and blood markers: CD4 (clone 34930), CD8a (clone 37006), CD45 (clone 2D1), MIC A/B (clone 159207), TRAIL (clone 75411)(R&D Systems); HLA ABC (clone W6/32, Biolegend); HLA DR/DP/DQ (clone HB-145, Loke Diagnostics Aps, DK); HLA-G (clone 87-G, Novus Biologicals); CD276 (Sdix, DE, USA); CTLA4 (clone ANC152.2/8H5, LifeSpan Biosciences); LFA1(clone HI111, Ab Biotec, CA, USA); ICAM-1 (clone R6.5, ThermoFisher Scientific, MA, USA); PD-L1 (Sino Biological, CH), FasL (clone 10F2, Bio-Rad, CA, USA).

Hypoxia markers: CAIX (Abcam), CAXII (clone 315601, R&D Systems).

2.3. EV Array analysis

Microarray epoxysilane-coated slides (Nexterion® Slide E, SCHOTT, DE) were customized by printing antibodies into micro-sized spots using a SciFLEXARRAYER S12 (Scienion, DE). All antibodies were printed in triplicates at a concentration of 200 μg/mL and for positive controls biotinylated human IgG at 100 μg/mL was used in a spotting buffer consisting of 5% glycerol in PBS, which were also used as negative control.

The analysis was performed as described by (Bæk and Jørgensen, 2017). First, the microarray slides were blocked with 50 mM ethanolamine, 0.1 M Tris, 0.1 % SDS, pH 9.0. The slides were mounted in multi-well hybridization cassettes (ArrayIt Corporation, CA, USA) before applying the plasma samples. sEVs from 10 μL of plasma (diluted 1:10 in washing buffer; 0.2 % Tween20 in PBS) were captured onto the microarray spot by an incubation at RT for two hours. Afterwards the incubation was continued overnight at 4 °C. The slides were removed

Table 1
Baseline characteristics of the women included in this study.

	IVIG treatment (n = 19)	Placebo (n = 20)
Age (years)[a]	31.6 (3.8)	31.5 (4.8)
Number of previous pregnancy losses		
4	12 (63.2 %)	16 (80.0 %)
5	5 (26.3 %)	1 (5.0 %)
6	1 (5.3 %)	2 (10.0 %)
8	1 (5.3 %)	
10		1 (5.0 %)
Outcome		
Livebirth	10 (52.6 %)	12 (60.0 %)
Pregnancy loss	9 (47.4 %)	8 (40.0 %)

[a] mean (SD).

from the multi-well cassettes and subjected to a washing procedure in washing buffer for 10 min in a high-throughput washing station (ArrayIt Corporation). To detect the captured sEVs the slides were then incubated with a cocktail of biotinylated detection antibodies (anti-human -CD9, -CD63 and -CD81, LifeSpan BioSciences) diluted in wash-buffer (1:1,500) for two hours at RT. Afterwards; the slides were washed for 10 min before adding streptavidin-Cy5 (ThermoFisher Scientific) diluted 1:1,500 in washing buffer. After a 30 min incubation, the slides were washed for 10 min in washing buffer, and then a 10 min wash in deionized water and lastly dried using a Microarray High-Speed Centrifuge (ArrayIt Corporation).

Scanning of the microarray slides and spot detection of the antibody markers by fluorescence readout was performed using an Innoscan 710AL (Innopsys, FR) as described by (Bæk and Jørgensen, 2017). Image analysis and calculation of total fluorescence intensity were performed using Mapix v. 8.3 (Innopsys).

2.4. Statistical analysis

Descriptive data were expressed as mean, standard deviation (SD), and percentage in Table 1.

Non-parametric Mann-Whitney tests were used to compare the influence of IVIG vs placebo treatment by analyzing the differences between the samples taken before (GW5) and after initiation of treatment (GW6-8). Differences between groups were assessed by the Mann-Whithey rank sum test. All p values were two-sided. To adjust for multiple comparisons, Bonferroni correction was applied and only p values less than 0.0015 were considered significant ($\alpha = 0.05/34$ EV markers = 0.0015).

Statistical calculations were performed using GraphPad Prism ver. 6.07 (GraphPad Software Inc. CA, US), and StataIC ver. 14.2 (StataCorp, TX, US).

3. Results

To characterize the phenotypes of sEVs an EV Array analysis was performed on plasma from 39 pregnant women with RPL. The characteristics of these women include age, number of previous pregnancy losses, treatment with IVIG or placebo and outcome of the pregnancy in the randomized trial; the distribution is presented in Table 1.

3.1. Influence of IVIG treatment on the contents of EVs in plasma

Thirty-four different antibodies were printed in triplicates and used to captures and analyze the content of sEVs in 10 µL of unpurified plasma. The protein profiles of 34 surface- or surface-associated markers on the sEVs were analyzed and the results are presented with reference to GW5 in Fig. 1 and Supplemental Figs. 1 and 2.

The study included 39 patients with RPL of which 19 were given

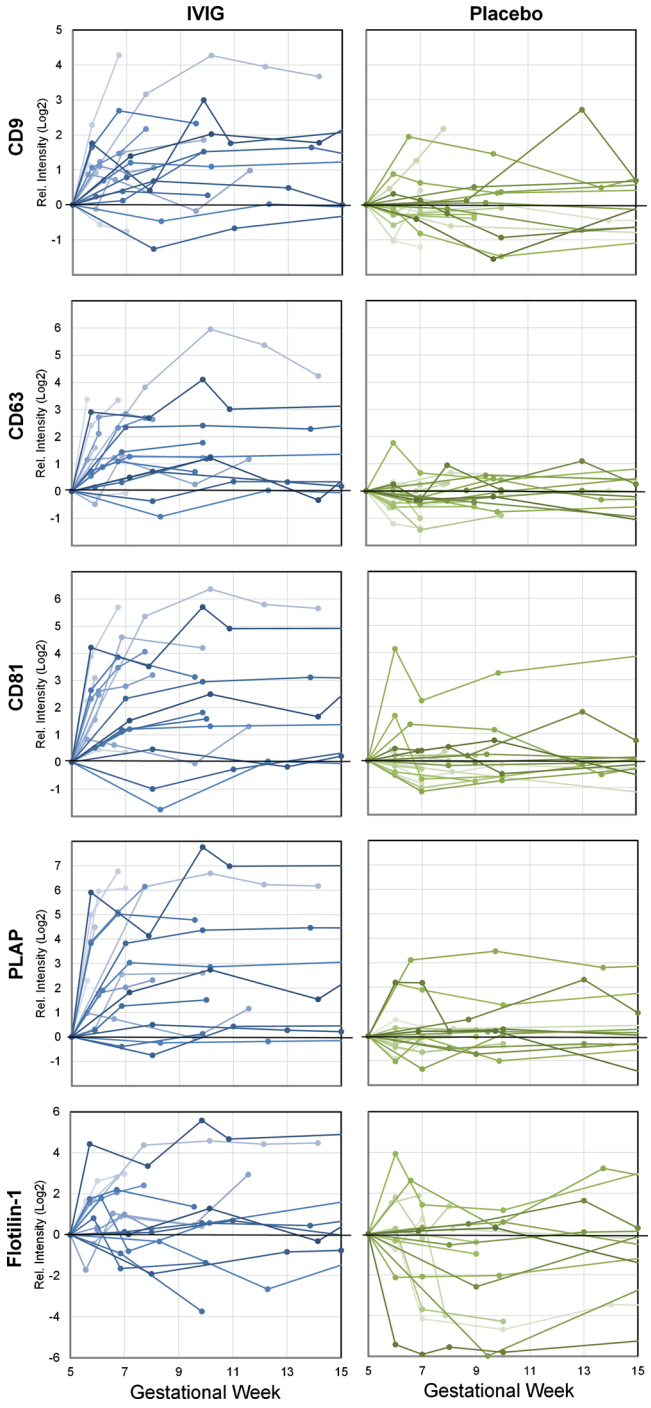

Fig. 1. The changes in sEV content for each individual patient during GW5 to GW15 with reference to GW5 (before initiation of treatment). The log2 transformed signal values are shown for individuals treated with IVIG (left panel, blue) or placebo (right panel, green). The profiles for the protein markers CD9, CD63, CD81, PLAP and Flotillin-1 are shown.

IVIG infusions and 20 were given placebo. Fig. 1 illustrates the effects of treatment on the plasma sEV contents throughout GW5 to GW15. Each patient had an individual response, but there is a clear tendency that the IVIG treatment contributes to an increase in the sEV contents rapidly after the first infusion, as seen for the EV markers CD9, CD63 and CD81. This effect was not seen for the patients treated with placebo, where the contents of several EV markers tended to decrease, as seen for Flotillin-1. The most pronounced increases (seen for the placental marker PLAP) and decreases are visibly seen within the very early

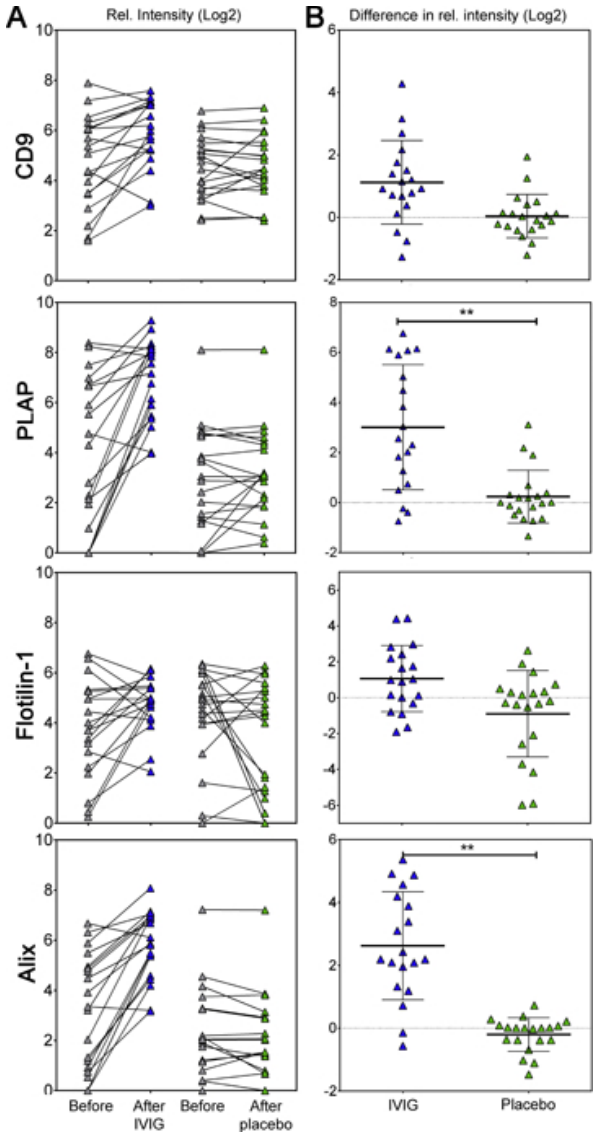

Fig. 2. The sEV contents of the protein markers CD9, PLAP, Flotillin-1 and Alix are shown as log2 transformed relative intensities and the differences between the samples taken before (GW5) and after (GW6-8) treatment. A) Log2 transformed relative intensities for the individuals before and after treatment (either IVIG (blue) or placebo (green)). B) Differences between the samples taken before and after treatment. Mean ± SD is shown and significant differences are marked with asterisks, $p < 0.0015$ (*); $p < 0.0003$ (**).

pregnancy (GW5-7). As the sampling of the blood were not consistently in time, it was chosen to perform the subsequent statistical analyses on the samples taken before initiation of treatment (GW5) and 1–3 weeks (GW6-8) after the first treatment. The influence of the infusions with IVIG is prominently seen as an increase in several sEV markers in Fig. 2A and for most of the markers (27 of 34) significant changes ($p < 0.0015$) were identified when performing a non-parametric comparison of the differences between the treatments (Fig. 2B and Table 2). Some of the general EV markers increased significantly (CD63, CD81 and Alix) whereas CD9 and Flotillin-1 did not. The placental marker PLAP showed the highest mean increase (6-fold higher) in patients treated with IVIG compared with patients treated with placebo.

4. Discussion

Investigating plasma sEVs during pregnancy can probably help to understand the different processes occurring during fetal development and the fetus-maternal interaction. To determine the levels and phenotypes of sEVs in plasma from patients with RPL, the established protein microarray-based analysis EV Array was used. The EV Array is optimized to catch and detect the smaller types of EVs, such as exosomes and exosome-like vesicles, with diameters up to ~150 nm.

Several studies have investigated the content of circulating EVs throughout pregnancy (Carp et al., 2004; Germain et al., 2007; Mitchell et al., 2015; Sabapatha et al., 2006; Salomon et al., 2014; Sarker et al., 2014; Sheller-Miller et al., 2019). All the studies identify that the plasma levels of EVs in general and in particular placenta-derived EVs, increases towards term. The used purification and detection methods differs between the studies, which makes it difficult to compare the results directly across the studies. A general conclusion though is that the increase in EV levels correlate with the mean uterine artery blood flow and at delivery with the placental weight. In the study of Miranda et al. (2018) they found that the proportion of circulating placental EVs compared to the total level of EVs was significantly reduced in small fetuses as compared to controls, and that the level of CD63 positive EVs is an indicator of fetal growth (Miranda et al., 2018).

The present study focused only on the early phase of the pregnancy (GW5 to GW15) and for the group of patients receiving placebo the levels of sEVs in plasma were not found to increase significantly, as seen in Fig. 1. This is partially in contrast to the above mentioned studies although the small dimensions of the fetus and the placenta this early in pregnancy should be taken into considerations.

We could not identify any studies investigating the relationship between sEVs and RPL, though several studies have investigated microparticles (MPs) in women with this complication (Alijotas-Reig et al., 2011; Carp et al., 2004; Kaptan et al., 2008; Laude et al., 2001). These studies used different methods such as flow cytometry and it can therefore be difficult to compare the findings as the use of non-standardized methods compromise comparative analysis.

Treatment with IVIG inhibits activation and functions of various innate immune cells such as DCs, monocytes, macrophages, neutrophils and NK cells. It neutralizes activated complement components. In addition, IVIG modulates B-cell functions and plasma cells (Pl), reciprocally regulates Treg cells and effector T cells such as Th 1 and Th 17 subsets and down-regulates the production of inflammatory cytokines (Galeotti et al., 2018). In this study, statistically significant higher levels of sEVs were found in the IVIG treated patients compared with the group receiving placebo with respect to 27 out of 34 markers. These findings suggest that treatment with IVIG contributes to an increased concentration of sEVs in plasma of pregnant patients with RPL.

This phenomenon of an overall increase in the levels of sEVs might be based on a systemic effect as IVIG has several immunomodulatory effects involving cytokines, the complement system and inflammatory cells, as well as the fact that one of the ways inflammatory cells exert immunomodulation is by secretion of EVs (Zhang et al., 2017). Although many studies have reported the anti-inflammatory function of IVIG therapy, the underlying mechanisms are not completely understood and the effect on the production of EVs has not been elucidated in detail. In patients with Kawasaki disease, Zhang et al. (2017) investigated the protein profiles of plasma exosomes before and after treatment with IVIG and found that the treatment reduced the acute-phase response and boosted complement activation, innate immune response, and antibacterial humoral response. Similar results are indicated in the present study, as it seems that treatment with IVIG boosts the production of sEVs in general, but also from the panel of immune markers investigated.

It could be questioned whether the increase in the levels of sEVs after IVIG is caused by sEVs contained in the IVIG product (Nordimmun) given. It has not been possible to test the product as it has unfortunately gone out of production, but during production, the IgG underwent an affinity purification after which it is believed that the product does not contain any sEVs.

The highest influence on the IVIG treatment were identified on sEVs

Table 2
Statistical summary for the 34 surface- and surface associated markers analyzed by the EV Array.

	Short name	Full name	Effect of IVIG vs placebo (p-values)	
EV markers	Alix	ALG-2-interacting protein X	<0.0001	**
	CD63	Cluster of differentiation 63; Tetraspanin 30	<0.0001	**
	CD81	Cluster of differentiation 81; Tetraspanin 28	<0.0001	**
	CD9	Cluster of differentiation 9; Tetraspanin 29	0.0024	
	Flot-1	Flotillin-1	0.0208	
	TSG101	Tumor susceptibility gene 101	<0.0001	**
Placental markers and hormone receptors	AA2a R	Adenosine A2a Receptor	<0.0001	**
	ADRB2	β-2 adrenergic receptor	0.0069	
	CHRM1	Muscarinic acetylcholine receptor M1	0.0046	
	CHRM2	Muscarinic acetylcholine receptor M2	<0.0001	**
	CHRM3	Muscarinic acetylcholine receptor M3	n.d.	
	CHRM4	Muscarinic acetylcholine receptor M4	0.0003	**
	CHRM5	Muscarinic acetylcholine receptor M5	0.0003	**
	FSHR	Follicle-stimulating hormone receptor	<0.0001	**
	LHR	Luteninzing hormone receptor	0.0001	**
	OPRL1	Opiod related nociceptive receptor 1	0.0032	
	PLAP	Placental alkaline phosphatase	0.0003	**
	TSHR	Thyroid stimulating hormone receptor	<0.0001	**
Blood- and Immune markers	CD276	B7-H3 Costimulatory B7 molecule	0.0147	
	CD4	Helper T-cell marker	0.0001	**
	CD45	Leukocyte marker, protein tyrosine phosphatase receptor type C	<0.0001	**
	CD8a	Cytotoxic T-cell marker	<0.0001	**
	CTLA4	Cytotoxic T-lymphocyte-associated antigen 4	0.0005	*
	FasL	Fas ligand	<0.0001	**
	HLA ABC	Human leukocyte antigens A, B and C	<0.0001	**
	HLA-G	Human leukocyte antigen G	<0.0001	**
	ICAM-1	Intercellular adhension molecule	<0.0001	**
	HLA DR/DP/DQ	Human leukocyte antigens DR, DP and DQ	0.0004	*
	LFA1	CD11a, Lymphocyte function-associated antigen	0.0002	**
	MIC A/B	MHC class I chain-related protein A and B	<0.0001	**
	PD-L1	Programmed death ligand 1	0.0009	*
	TRAIL	TNF-related apoptosis-inducing ligand, CD253, APO-2L	<0.0001	**
Hypoxia	CAIX	Carbonic Anhydrase 9	0.001	*
	CAXII	Carbonic Anhydrase 12	<0.0001	**

Asterisks indicate level of significance after Bonferroni correction for multiple comparisons. $p > 0.0015$ (*); $p > 0.0003$ (**).

carrying the placental marker PLAP, which showed a 6-fold increase. It could indicate that IVIG in particular binds to and interact with trophoblast cells when given to pregnant women causing a pronounced release of sEVs with placenta-specific markers.

The plasma samples used for this study have been stored at −80 °C for approximately 20 years. This long time storage should be taken into consideration, as there are no knowledge on how storage affects the sEVs. However, previous studies by Bæk et al., 2016 showed that the phenotypes of sEVs in plasma were not significantly affected by storage at −80 °C for several months (Bæk et al., 2016).

4.1. Conclusive remarks

Prevention of fetal rejection, in part by the suppression of maternal cell-mediated immune responses, has long been recognized. Evidence increasingly indicates that placental EVs form part of a range of immunosuppressive factors released by the placenta which are involved in this process (Tannetta et al., 2014). We used the EV Array microarray technology to analyze the protein profiles of sEVs in plasma of pregnant women with RPL. The findings in this study strongly demonstrate that treatment with high-doses of IVIG boosts the production and release of almost all types of EVs and in particular placental-derived sEVs into the circulation during pregnancy; however, the biological role of this boost remains to be clarified in further studies relating sEV protein profiles to subsequent pregnancy outcome.

Declaration of Competing Interest

The authors hereby declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jri.2020.103128.

References

Admyre, C., Grunewald, J., Thyberg, J., Gripenbäck, S., Tornling, G., Eklund, A., Scheynius, A., Gabrielsson, S., 2003. Exosomes with major histocompatibility complex class II and co-stimulatory molecules are present in human BAL fluid. Eur. Respir. J. 22, 578–583. https://doi.org/10.1183/09031936.03.00041703.

Alijotas-Reig, J., Garrido-Gimenez, C., 2013. Current concepts and new trends in the diagnosis and management of recurrent miscarriage. Obstet. Gynecol. Surv. 68, 119–129.

Alijotas-Reig, J., Palacio-Garcia, C., Farran-Codina, I., Zarzoso, C., Cabero-Roura, L., Vilardell-Tarres, M., 2011. Circulating cell-derived microparticles in women with pregnancy loss. Am. J. Reprod. Immunol. 199–208. https://doi.org/10.1111/j.1600-0897.2010.00972.x.

Bæk, R., Jørgensen, M., 2017. Multiplexed phenotyping of small extracellular vesicles using protein microarray (EV array). Methods Mol. Biol. 1545, 117–127. https://doi.org/10.1007/978-1-4939-6728-5_8.

Bæk, R., Søndergaard, E.K.L., Varming, K., Jørgensen, M.M., 2016. The impact of various preanalytical treatments on the phenotype of small extracellular vesicles in blood analyzed by protein microarray. J. Immunol. Methods 438, 11–20. https://doi.org/10.1016/j.jim.2016.08.007.

Carp, H., Dardik, R., Lubetsky, A., Salomon, O., Eskaraev, R., Rosenthal, E., Inbal, A., 2004. Prevalence of circulating procoagulant microparticles in women with recurrent miscarriage : a case-controlled study. Hum. Reprod. 19, 191–195. https://doi.org/10.1093/humrep/deg512.

Christiansen, O.B., Pedersen, B., Rosgaard, A., Husth, M., 2002. A randomized, double-

blind, placebo-controlled trial of intravenous immunoglobulin in the prevention of recurrent miscarriage : evidence for a therapeutic effect in women with secondary recurrent miscarriage. Hum. Reprod. 17, 809–816.
Colombo, M., Raposo, G., Théry, C., 2014. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu. Rev. Cell Dev. Biol. 30, 255–289. https://doi.org/10.1146/annurev-cellbio-101512-122326.
Galeotti, C., Kaveri, S.V., Bayry, J., 2018. IVIG-mediated effector functions in autoimmune and inflammatory diseases. Int. Immunol. 29, 491–498. https://doi.org/10.1093/intimm/dxx039.
Germain, S.J., Sacks, G.P., Soorana, S.R., Ian, L., Redman, C.W., Germain, S.J., Sacks, G.P., Soorana, S.R., Sargent, I.L., Redman, C.W., 2007. Systemic inflammatory priming in normal pregnancy and preeclampsia: the role of circulating syncytiotrophoblast microparticles. J. Immunol. 178, 5949–5956. https://doi.org/10.4049/jimmunol.178.9.5949.
Gutiérrez-Vázquez, C., Villarroya-Beltri, C., Mittelbrunn, M., Sánchez-Madrid, F., 2013. Transfer of extracellular vesicles during immune cell-cell interactions. Immunol. Rev. 251, 125–142. https://doi.org/10.1111/imr.12013.
Jørgensen, M., Bæk, R., Pedersen, S., Søndergaard, E.K.L., Kristensen, S.R., Varming, K., 2013. Extracellular Vesicle (EV) array: microarray capturing of exosomes and other extracellular vesicles for multiplexed phenotyping. J. Extracell. Vesicles 2. https://doi.org/10.3402/jev.v2i0.20920.
Jolles, S., Sewell, W.A.C., Misbah, S.A., 2005. Clinical uses of intravenous immunoglobulin. Clin. Exp. Immunol. 142, 1–11. https://doi.org/10.1111/j.1365-2249.2005.02834.x.
Kaptan, K., Beyan, C., Ifran, A., Pekel, A., 2008. Platelet-derived microparticle levels in women with recurrent spontaneous abortion. Int. J. Gynecol. Obstet. 102, 271–274. https://doi.org/10.1016/j.ijgo.2008.04.007.
Laude, I., Boyer-Neumann, C., Hugel, B., Freyssinet, J.M., Wolf, M., Eschwège, V., Frydman, R., Mairovitz, V., Meyer, D., Rongières-Bertrand, C., 2001. Circulating procoagulant microparticles in women with unexplained pregnancy loss: a new insight. Thromb. Haemost. 85, 18–21. https://doi.org/10.1055/s-0037-1612657.
Mincheva-Nilsson, L., Baranov, V., 2014. Placenta-derived exosomes and syncytiotrophoblast microparticles and their role in human reproduction : immune modulation for pregnancy success. Am. J. Reprod. Immunol. 72, 440–457. https://doi.org/10.1111/aji.12311.
Miranda, J., Paules, C., Nair, S., Lai, A., Palma, C., Scholz-romero, K., Rice, G.E., Gratacos, E., Crispi, F., Salomon, C., 2018. Placental exosomes profile in maternal and fetal circulation in intrauterine growth restriction - Liquid biopsies to monitoring fetal growth. Placenta 64, 34–43. https://doi.org/10.1016/j.placenta.2018.02.006.
Mitchell, M.D., Peiris, H.N., Kobayashi, M., Koh, Y.Q., Duncombe, G., Illanes, S.E., Rice, G.E., Salomon, C., 2015. Placental exosomes in normal and complicated pregnancy. Am. J. Obstet. Gynecol. 213, S173–S181. https://doi.org/10.1016/j.ajog.2015.07.001.
Pugholm, L.H., Bæk, R., Kristina, E., Søndergaard, L., Louise, A., Revenfeld, S., Jørgensen, M.M., Varming, K., 2016. Phenotyping of Leukocytes and Leukocyte-Derived Extracellular Vesicles 2016.
Raposo, G., Stoorvogel, W., 2013. Extracellular vesicles: exosomes, microvesicles, and friends. J. Cell Biol. 200, 373–383. https://doi.org/10.1083/jcb.201211138.
Redman, C.W.G., Sargent, I.L., 2007. Microparticles and immunomodulation in pregnancy and pre-eclampsia. J. Reprod. Immunol. 76, 61–67. https://doi.org/10.1016/j.jri.2007.03.008.
Sabapatha, A., Gercel-Taylor, C., Taylor, D.D., 2006. Specific isolation of placenta-derived exosomes from the circulation of pregnant women and their immunoregulatory. J. Reprod. Immunol. 56, 345–355. https://doi.org/10.1111/j.1600-0897.2006.00435.x.
Salomon, C., Torres, M.J., Kobayashi, M., Scholz-romero, K., Sobrevia, L., Dobierzewska, A., Illanes, S.E., Mitchell, M.D., Rice, G.E., 2014. A gestational profile of placental exosomes in maternal plasma and their effects on endothelial cell migration. PLoS One 9. https://doi.org/10.1371/journal.pone.0098667.
Sarker, S., Scholz-Romero, K., Perez, A., Illanes, S.E., Mitchell, M.D., Rice, G.E., Salomon, C., 2014. Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy. J. Transl. Med. 12, 1–19.
Sheller-Miller, S., Trivedi, J., Yellon, S.M., Menon, R., 2019. Exosomes cause preterm birth in mice: evidence for paracrine signaling in pregnancy. Sci. Rep. 1–18. https://doi.org/10.1038/s41598-018-37002-x.
Stefanski, A.L., Martinez, N., Peterson, L.K., Id, T.J.C., Treacy, E., Luck, M., Friend, S.F., Hermesch, A., Maltepe, E., Phang, T., Dragone, L.L., Id, V.D.W., 2019. Murine trophoblast-derived and pregnancy- associated exosome-enriched extracellular vesicle microRNAs: implications for placenta driven effects on maternal physiology. PLoS One 1–23.
Tannetta, D., Dragovic, R., Alyahyaei, Z., Southcombe, J., 2014. Extracellular vesicles and reproduction – promotion of successful pregnancy. Cell. Mol. Immunol. 548–563. https://doi.org/10.1038/cmi.2014.42.
van der Vlist, E.J., Arkesteijn, G.J.A., van de lest, C.H.A., Stoorvogel, W., Hoen, E.N.M.N., Marca, H.M., 2012. CD4' T cell activation promotes the differential release of distinct populations of nanosized vesicles. J. Extracell. Vesicles 1, 1–9.
Wang, N.F., Kolte, A.M., Larsen, E.C., Nielsen, H.S., Christiansen, O.B., 2016. Immunologic abnormalities, treatments, and recurrent pregnancy loss: what is real and what is not? Clin. Obstet. Gynecol. 59, 509–523. https://doi.org/10.1097/GRF.0000000000000215.
Zhang, L., Song, Q., Jin, J., Huang, P., Wang, Z., Xie, X., 2017. Differential Protein Analysis of Serum Exosomes Post-intravenous Immunoglobulin Therapy in Patients with Kawasaki Disease 1786–1796. https://doi.org/10.1017/S1047951117001433.