Research Article

Umbilical Serum sHLA-G Levels in Preeclamptic Pregnancies with and without Intrauterine Fetal Growth Restriction: A Comparison with Normotensive Pregnancies with Isolated IUGR and Healthy Controls

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The aim of this study was the analysis of the umbilical cord serum sHLA-G levels in pregnancies complicated by severe preeclampsia and/or IUGR. *Patients and Methods.* The study was carried out on 12 preeclamptic patients with appropriate-for-gestational-age weight infants, 14 pregnant patients with severe preeclampsia complicated by IUGR, 7 normotensive patients with isolated IUGR, and 22 healthy controls with uncomplicated pregnancies. *Results.* Our study revealed higher umbilical serum levels of sHLA-G in preeclamptic pregnancies complicated by IUGR and decreased in the preeclamptic women with the normal fetal growth. The pregnant normotensive patients with isolated IUGR revealed the same levels of sHLA-G as observed in healthy pregnant controls. *Conclusions.* Lower levels of sHLA-G observed in preeclamptic pregnancies without IUGR may lead to excessive maternal immune reaction against the fetus. Higher levels of sHLA-G in preeclamptic pregnancies with IUGR may reflect the excessive releasing of this molecule to protect fetus, but it may be insufficient to balance disturbed fetomaternal immune relationship. The lack of association with normotensive pregnancies complicated by isolated IUGR may suggest that a different pathomechanism is involved in the impairment of intrauterine fetal growth in both patient groups with pregnancies complicated by isolated IUGR and in the course of preeclampsia.

1. Introduction

Preeclampsia is a severe clinical disorder manifested after the 20th week of gestation, but its onset is at an early stage of gestation [1]. It is manifested by both systemic and local anomalies with increased maternal and fetal morbidity and mortality, and it was suggested that this condition is associated with abnormal placentation.

According to the theory of genetic conflict, genetic difference between mother and fetus plays a role in pregnancy success [2]. It appears that the maternal immune system must recognize trophoblast cells as foreign in order to remodel the placental bed and create an environment to nourish the developing fetus [3]. There is growing evidence that decidual natural killer (NK) cells supply factors necessary for the development and arterial modification of the maternal-fetal interface [4]. These interactions prevent the attack of the fetus by the maternal immune system and also regulate production of cytokine and angiogenic factor by uterine NK, favouring implantation and placental vascularization and development processes [5], and they have been recently attributed to the HLA-G molecule secreted by trophoblast [6].

Carosella et al. [7] found that HLA-G is a key tolerogenic molecule which plays a beneficial role in pregnancy and transplantation or immune disease, in which it acts by reducing immune reaction against the fetus, allograft or self components.

The precise physiological role of HLA-G has yet to be defined [8]. The nonclassical MHC class I molecule HLA-G may have nonimmune functions relating to angiogenesis and

placentation, but most evidence suggests that HLA-G may be a part of the mechanism that enables trophoblasts to invade without being attacked by the lymphocyte population, decidual NK cells [3, 8, 9], and protects fetal cells from lysis by maternal uterine NK cells [8, 9].

It is possible that trophoblasts with defective HLA-G expression are prone to attack by decidual NK cells and that it results in shallow trophoblast invasion and abnormal non physiological spiral artery remodelling [3].

The expression of HLA-G is normally restricted to the placenta during pregnancy, where it is found on trophoblasts, fetal endothelial and epithelial cells, and noninvasive cytotrophoblsats cells, specifically those at the maternal/fetal interface [8, 9].

Soluble form of HLA-G molecule (sHLA-G) has a potentially greater range of activity than membrane-bound HLA-G [10]. The soluble isoforms could perform the same functions not only locally but also systemically [10].

Elevated serum sHLA-G levels have been reported during acute rejection episodes following organ transplantation and severe graft-versus-host disease after transplantation [11]. It was also demonstrated that HLA-G is also involved in the implantation and acceptation by maternal immune system in normal pregnancy [12, 13].

Decreased HLA-G RNA and protein have been found in placental sections of patients who subsequently developed preeclampsia [14–16]. Trophoblasts which lack expression of HLA-G are susceptible to lysis by the decidual NK cells and would be prevented from invading deeply into deciduas and spiral arteries. This reduced expression of HLA-G found in some preeclampsia may be a primary cause of shallow trophoblast invasion [15].

The aim of the present study was to investigate the relationship between umbilical cord soluble human leukocyte antigen-G (sHLA-G) and the incidence of pregnancy-specific disorders such as preeclampsia and/or intrauterine fetal growth restriction. A particular objective was the comparison of umbilical serum sHLA-G levels in pregnancies complicated by intrauterine fetal growth restriction in the course of preeclampsia and in normotensive pregnancies complicated by isolated IUGR. We also compared the serum umbilical levels of sHLA-G in pregnancies complicated by preeclampsia and/or IUGR with those in healthy normotensive pregnancies with normal intrauterine fetal growth and development.

2. Patients and Methods

This study was carried out at the Department of Obstetrics and Perinatology Medical University Hospital in Lublin, Poland. The study design was approved by the institutional ethics committee. Consent for participation in the study was obtained from the patients on admission.

The diagnosis of preeclampsia was based on the presence of elevated systolic blood pressure of at least 140 mmHg and diastolic blood pressure of at least 90 mmHg after the 20th week of gestation, in association with proteinuria of at least 300 mg/24 h in absence of urinary tract infection. Severe preeclampsia was diagnosed based on the following criteria:

systolic blood pressure >160 mmHg, diastolic blood pressure >110 mmHg and proteinuria >5 g/in 24 h period. In addition patients with one or more of the following clinical manifestations: renal abnormalities (oliguria), hematologic abnormalities (thrombocytopenia and microangiopathic hemolysis), or HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count, and right-upper quadrant pain), or neurologic symptoms (headache, visual disturbances, and seizures) was considered to have severe preeclampsia.

None of the pregnant patients with preeclampsia was affected by chronic hypertension or renal disorders and/or proteinuria before pregnancy, and all were normotensive before the 20th week of gestation.

The diagnosis of intrauterine growth restriction was based on the following criteria: clinical evidence of suboptimal growth when the weight of the fetus was lower than expected in relation to gestational age as determined by standard curves characteristic of the Polish population, ultrasonographic evidence of deviation from an appropriate growth percentile, and individualized weight ratios. The exclusion criteria for women whose pregnancies were complicated by IUGR fetuses were the presence of a congenital malformation or chromosomal abnormality in the fetus, recent cytomegalovirus infection, or drug or alcohol abuse during pregnancy.

Pregnant women with multiple pregnancies were also excluded from this study.

The study was carried out on 7 normotensive pregnant patients with pregnancy complicated by intrauterine growth-restricted fetuses (the IUGR group), 14 patients with preeclampsia complicated by fetal intrauterine growth restriction (the PI group) and 12 preeclamptic patients with appropriate-for-gestational-age weight infants (the P group). The control group consisted of 22 healthy normotensive pregnant patients with singleton uncomplicated pregnancies, without any renal, cardiac, and vascular diseases and with normal laboratory tests and appropriate-for-gestational-age weight infants (the control group).

All arterial blood pressure measurements in the control group and in the IUGR group of pregnant patients were normal and did not exceed 135/85 mmHg. None of the patients from any of these both groups suffered from proteinuria. All patients in the study were nonsmokers.

Samples of five milliliters of umbilical blood were taken immediately after the delivery and collected in sterile tubes. They were centrifuged for 15 min at $500 \times g$ immediately after sampling. Each sample of obtained serum was frozen until assayed.

2.1. Determination of sHLA-G. The umbilical serum soluble human leukocyte antigen-G levels were determined using an enzyme-linked immunosorbent assay technique, according to the manufacturer's instructions (Human, a double monoclonal sandwich enzyme immunoassay, BioVendor Laboratorni Medicina a.s., Turnov, Czech Republic). A standard curve was performed to calculate the protein concentration of sHLA-G molecules. The minimum detectable sHLA-G concentration was 1 Unit/mL, the intraassay and interassay variations were <5% and <10%, respectively.
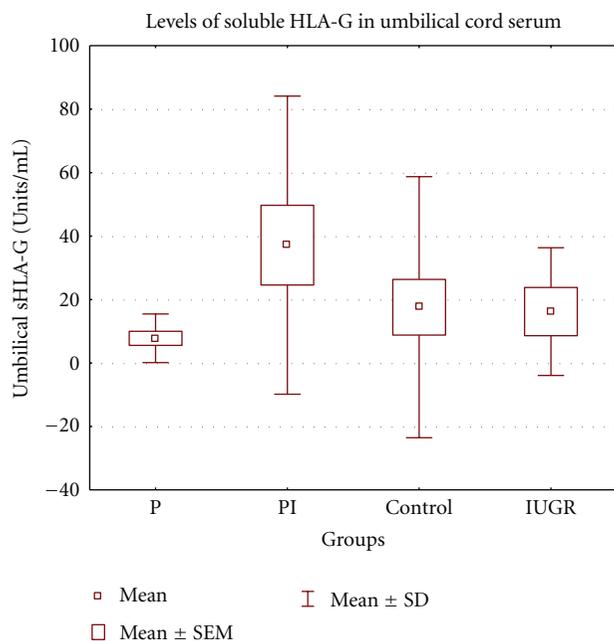
Figure 1

2.2. Statistical Analysis. Data were expressed as mean ± SD and were statistically analyzed with the computer program "Statistica 8." The level of statistical significance was established as $P < 0.05$.

3. Results

Table 1 summarizes the characteristics of the patients enrolled in the study.

Creatinine and urea levels were normal in all patients. Significant severe proteinuria (more than 5 g in 24 h urine) was found only in patients from both preeclamptic groups.

There were no statistically significant differences in age and height in patient profiles between groups. Maternal weight and body mass index (BMI) were higher in both preeclamptic groups. The gravidity and parity were higher in the preeclamptic patients with normal intrauterine fetal growth.

As expected, preeclamptic patients with and without IUGR (the PI and the P groups) presented significantly higher systolic and diastolic blood pressure than the IUGR and the control groups.

The mean systolic blood pressure values were 115.714 ± 13.281 mmHg in the group of women with pregnancy complicated by isolated intrauterine growth-restricted fetuses (the IUGR group), 166.333 ± 13.567 mmHg in the preeclamptic group without IUGR (the P group), 176.143 ± 16.459 mmHg in patients with IUGR in the course of preeclampsia (the PI group), and 117.762 ± 16.266 mmHg in the control group. The mean diastolic blood pressure values were 81.429 ± 11.931 mmHg in the group of normotensive women with pregnancy complicated by isolated IUGR, 117.083 ± 27.848 mmHg in the P group, 110.857 ±

9.965 mmHg in the PI group, and 74.905 ± 10.839 mmHg in the healthy controls.

Lower gestational age and lower birth weight of infants were found in both preeclamptic groups (the P and the PI groups) and in the group of normotensive women with isolated IUGR (the IUGR group). The mean age of gestation was 36.347 ± 4.209 weeks in the IUGR group, 36.155 ± 3.863 weeks in the P group, 33.071 ± 3.880 weeks in the PI group, and 38.728 ± 0.983 weeks in the control group.

The mean birth weight of infants was 2179.29 ± 809.83 g in the IUGR group, 2890.91 ± 952.13 g in the P group, 1638.93 ± 702.72 g in the PI group, and 3369.05 ± 403.68 g in the control group.

The gestational age and the birth weight of infants were the lowest in the PI group and were statistically significantly lower than in the P group, in the IUGR group, and in the control subjects. However birth weight of infants in the group of patients with pregnancy complicated by isolated intrauterine fetal growth restriction (IUGR group) was also lower than in group P and in the control subjects but higher than in pregnancies complicated by IUGR in the course of preeclampsia, in spite of significantly higher age of gestation in the IUGR group in comparison with both preeclamptic groups of studied women.

The levels of soluble HLA-G in umbilical serum were elevated in infants born by mother with pregnancy complicated by intrauterine fetal growth restriction in the course of preeclampsia (the PI group) and lower in preeclamptic patients with appropriate-for-gestational-age fetuses (the P group) in comparison with pregnancies complicated by isolated IUGR and in comparison with healthy control subjects. The difference between both preeclamptic groups was statistically significant.

The pregnant normotensive patients with isolated IUGR revealed the same levels of sHLA-G as observed in healthy pregnant controls. The observed mean umbilical serum values of sHLA-G levels were 16.198 ± 20.133 Units/mL in the IUGR group, 7.778 ± 7.664 Units/mL in the P group, 31.128 ± 46.972 Units/mL in the PI group, and 17.578 ± 41.136 Units/mL in the healthy controls.

The results of this analysis are presented in Figure 1 and Tables 1 and 2.

4. Discussion

Pregnancy is characterized by the presence of sHLA-G [17], whose secretion in early embryos is crucial for the successful implantation [18, 19]. Hunt et al. [10] postulated that sHLA-G is synthesized at the maternal-fetal interface by trophoblasts and possibly also by macrophages and that this substance targets to different types of leukocytes at the maternal-fetal interface but not to trophoblasts.

Also maternal-derived sHLA-G seems to support early implantation, because women with intact pregnancies after IVF revealed preovulatory significantly higher sHLA-G levels than women who experienced early abortion [19]. These findings indicate that sHLA-G molecules derived from both fetal and maternal cells may have a crucial effect on the process of suppression of fetal allograft rejection [20].

TABLE 1: Analysis of obtained results.

Data	The control group ($n = 22$)	Statistical analysis Control-IUGR	The IUGR group ($n = 7$)	Statistical analysis Control-P	The P group ($n = 12$)	Statistical analysis Control-PI	The PI group ($n = 14$)
Gravidity	1.286 ± 0.561	$P = 0.560220$	1.429 ± 0.534	$P = 0.041323^*$	2.000 ± 1.342	$P = 0.613156$	1.429 ± 1.089
Parity	1.238 ± 0.436	$P = 0.352426$	$1.1.429 \pm 0.534$	$P = 0.049840^*$	1.818 ± 1.162	$P = 0.910206$	1.214 ± 0.802
Maternal age (years)	30.200 ± 4.501	$P = 0.435645$	28.625 ± 3.182	$P = 0.737581$	29.509 ± 6.236	$P = 0.654183$	29.493 ± 4.184
Maternal height (cm)	166.929 ± 7.457	$P = 0.670774$	165.400 ± 3.847	$P = 0.510086$	164.600 ± 2.608	$P = 0.438557$	164.625 ± 4.502
Maternal weight (kg)	81.593 ± 15.537	$P = 0.639047$	78.100 ± 7.284	$P = 0.565824$	86.450 ± 9.618	$P = 0.148973$	91.357 ± 9.983
Maternal BMI (kg/m^2)	29.175 ± 4.381	$P = 0.759907$	28.531 ± 2.238	$P = 0.095018$	33.918 ± 2.557	$P = 0.053344$	33.348 ± 4.364
Systolic blood pressure (mmHg)	117.762 ± 16.266	$P = 0.874032$	115.714 ± 13.281	$P < 0.000001^*$	166.333 ± 13.567	$P < 0.000001^*$	176.143 ± 16.459
Diastolic blood pressure (mmHg)	74.905 ± 10.839	$P = 0.301856$	81.429 ± 11.931	$P = 0.000001^*$	117.083 ± 27.848	$P < 0.000001^*$	110.857 ± 9.765
Age of gestation (weeks)	38.728 ± 0.983	$P = 0.019867^*$	36.347 ± 4.209	$P = 0.006425^*$	36.155 ± 3.863	$P < 0.000001^*$	33.0711 ± 3.880
Birth weight of infant (g)	3369.05 ± 403.68	$P = 0.000021^*$	2179.29 ± 809.83	$P = 0.054141^*$	2890.91 ± 952.13	$P < 0.000001^*$	1638.93 ± 702.72
Umbilical sHLA-G levels (Units/mL)	17.578 ± 41.136	$P = 0.933070$	16.198 ± 20.133	$P = 0.422740$	7.778 ± 7.664	$P = 0.197061$	37.128 ± 46.972

Data presented as a mean $\pm$ SD.
*Statistical significance ($P < 0.05$).
Groups of studied pregnant women: *Control*—healthy normotensive pregnant women, *IUGR*—normotensive women with pregnancy complicated by isolated intrauterine growth restriction, *P*—preeclamptic women without IUGR, *PI*—women with IUGR in the course of preeclampsia.

TABLE 2: Analysis of umbilical levels of sHLA-G in studied groups of pregnant women.

Data	The IUGR group ($n = 7$)	Statistical analysis IUGR/P	The P group ($n = 12$)	Statistical analysis P/PI	The PI group ($n = 14$)	Statistical analysis PI/IUGR
Umbilical serum sHLA-G (Units/mL)	16.198 ± 20.133	$P = 0.205734$	7.778 ± 7.664	$P = 0.043250^*$	37.127 ± 46.972	$P = 0.277821$

Data presented as a mean $\pm$ SD.
*Statistical significance ($P < 0.05$).
Groups of studied pregnant women: *IUGR*—normotensive women with pregnancy complicated by isolated intrauterine growth restriction, *P*—preeclamptic women without IUGR, *PI*—women with IUGR in the course of preeclampsia.

Hackmon et al. [17] observed the strong correlation of sHLA-G levels between cord serum and maternal serum and suggest that sHLA-G in maternal and umbilical serum has the same origin in these two compartments. Puppo et al. [21] suggest that elevated amounts of sHLA-G molecules in amniotic fluid, which is continuously ingested by the fetus, may be of particular relevance for the induction of tolerance and may play a major role in the immune interplay between mother and fetus.

This study was conducted to investigate whether umbilical serum sHLA-G concentrations are altered in women with pregnancy complicated by preeclampsia with and without intrauterine growth-restricted fetus. The control group consisted of healthy normotensive patients without any cardiac,

renal, or vascular diseases and with normal intrauterine fetal growth.

The umbilical serum levels of soluble HLA-G were also compared with their levels in infants delivered by normotensive patients with pregnancy complicated by isolated IUGR.

It is noteworthy that in our study women with pregnancies complicated by severe preeclampsia with and without IUGR and normotensive pregnant women with isolated intrauterine fetal growth restriction were investigated separately.

The present study revealed different levels of soluble HLA-G in the umbilical cord serum in both groups of preeclamptic women with and without IUGR. The umbilical cord serum levels of sHLA-G were increased in pregnancies

complicated by intrauterine fetal growth restriction in the course of preeclampsia and decreased in the preeclamptic women with the appropriate-for-gestational-age fetuses. The difference between these both preeclamptic groups was statistically significant.

Contrary to both preeclamptic groups, the pregnant normotensive patients with isolated IUGR revealed the same levels of sHLA-G as observed in healthy pregnant controls.

Our findings support the idea that sHLA-G may play a significant role in pathogenesis of preeclampsia with and without IUGR, but pathophysiological mechanisms of disturbances in these two groups of preeclamptic women are different.

The lack of such association with normotensive pregnancies complicated by isolated intrauterine fetal growth restriction may suggest that sHLA-G does not play a significant role in isolated IUGR and that the pathophysiological mechanism inducing intrauterine fetal growth restriction in normotensive and preeclamptic pregnant women is different, and that abnormal umbilical serum levels of sHLA-G is associated with preeclampsia.

Both membrane-bound and soluble forms of the human leukocyte antigen-G protect the fetus from maternal immune attack [20]. The sHLA-G has potentially greater range of activity than membrane-bound form of HLA-G, could bind to the same sets of leukocytes, and perform exactly the same functions not only locally but also systematically [10]. But the results from the studies on circulating HLA-G are not consistent [21, 22].

It was also suggested that placenta may be a significant source of sHLA-G, but the levels of sHLA-G in nonpregnant and pregnant women were very similar [20]. It was reported that the concentration of sHLA-G does not increase during normal pregnancy [21, 23]. These findings suggest that the majority of the sHLA-G molecules detected in maternal circulation are produced by immunocompetent cells of mother.

Lila et al. [24] found that the presence of HLA-G in biopsies and higher levels of sHLA-G in serum from patients after heart transplantation are associated with the reduced number of acute graft rejection episodes. These authors expressed the opinion that HLA-G and soluble HLA-G favour graft tolerance [24]. It was also suggested that the expression and release of HLA-G protects tissue from the deleterious allospecific effects of infiltrating T cells and NK cells [25].

Measurable levels of sHLA-G can be detected in maternal and cord blood, as well as in amniotic fluid, which suggests that HLA-G may protect the fetal trophoblasts from mother NK lysis and confer immune tolerance of fetus versus mother [21, 26, 27].

Carosella et al. [25] observed that HLA-G plays a significant role as a tissue-protective molecule in inflammatory processes and may be an important factor for limitation of the inflammation. It was also suggested that HLA-G influences the Th cytokine production by promoting the secretion of Th2 cytokines and down-regulation of production of IFN-γ and TNF-α [28–30]. Higher levels of sHLA-G promote Th2 response and are associated with normal pregnancies. It was suggested that the development of a Th2-type cytokine response is associated with the exposure to high concentra-

tions of soluble HLA-G and the maintenance of pregnancy, whereas low levels of HLA-G increase the expression of Th-1-type cytokines [29, 31].

Steinborn et al. [20] observed higher levels of sHLA-G in pregnant patients with HELLP syndrome and concluded that clinical HELLP syndrome may reflect the condition of acute graft rejection and that this syndrome may reflect the disturbed fetomaternal immune balance. These authors found that in pregnancy complicated by HELLP syndrome sHLA-G may be produced by activated maternal CD4 positive T helper cells and result in excessive allogenic reaction against fetal antigens [20]. It was also presented by Steinborn et al. [20] that the soluble form of HLA-G is probably released by not only maternal but also by fetal immune cells and may have an important function in the pathophysiology of some pregnancy disorders. These authors suggest that higher levels of sHLA-G may be due to inflammatory reaction of the fetus which results in the increased release of fetally derived sHLA-G into maternal circulation [20].

Given these findings, higher levels of umbilical sHLA-G in preeclamptic pregnancies complicated by IUGR may reflect disturbances in maternal tolerance of the fetal antigens and reaction of chronic graft rejection. Contrary to results from patients with IUGR in the course of preeclampsia, no difference was found in normotensive patients with isolated IUGR.

Lower levels of sHLA-G observed in preeclamptic pregnancies with normal fetal growth may lead to impaired placentation and decreased apoptosis on activated immune cells, which results in an excessive maternal immune reaction against the fetus and finally results in maternal syndrome of preeclampsia. It seems possible that our results may also reflect a condition of excessive inflammation.

Higher levels of sHLA-G in preeclamptic pregnancies with IUGR may reflect the excessive releasing of this molecule to protect fetus, but it may be insufficient to balance disturbed fetomaternal immune relationship and may result in maternal syndrome of clinically diagnosed preeclampsia and fetal syndrome called IUGR in the course of preeclampsia.

5. Conclusions

Our findings suggest that the umbilical cord serum soluble HLA-G may be involved in the pathogenesis of preeclampsia. Lower levels of sHLA-G observed in preeclamptic pregnancies with appropriate-for-gestational-age fetal growth may be associated with excessive maternal immune reaction against the fetus. Higher levels of sHLA-G in preeclamptic pregnancies with IUGR may reflect the excessive releasing of this molecule to protect fetus, but it may be insufficient to balance disturbed fetomaternal immune relationships. Contrary to both preeclamptic groups of women, no differences were found in the umbilical serum levels of soluble HLA-G in normotensive patients with pregnancies complicated by isolated fetal growth restriction. It may suggest different pathomechanism of the fetal growth disturbances in normotensive patients with isolated IUGR and in pregnancies complicated by fetal growth restriction in the course of preeclampsia.

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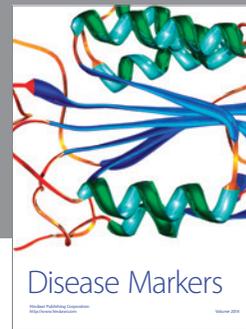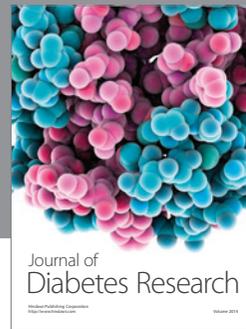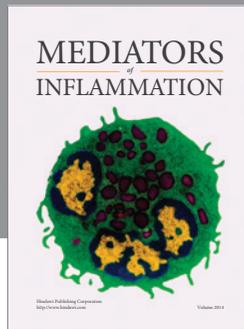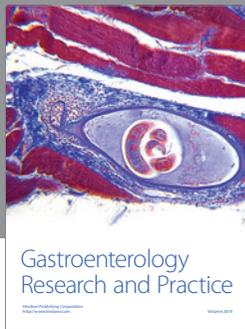
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