Revista Argentina de Clínica Psicológica
2021, Vol. XXX, N°1, 720-728
DOI: 10.24205/03276716.2020.2068

Investigation into the effects of IL-10 on the expression of indoleamine 2,3-dioxygenase and SOCS3 in chorionic villi and decidua during early stage of pregnancy

Minyan Yang[a], Jun Wang[b], Guanyou Huang[b], Fei Xia[a*]

Abstract
The present study aimed to evaluate the expression of indoleamine 2,3-dioxygenase (IDO) and cytokine signaling 3 (SOCS3) in chorionic villi and the decidua after interleukin-10 (IL-10) in early pregnancy. IDO and SOCS3 were expressed in chorionic villi and decidua. IL-10 at 0.1, 1 and 10 ng/ml concentration-dependently decreased the IDO expression in chorionic villi and the decidua tissues; while decreased the expression of SOSC3 in chorionic villi and the decidua tissues. IL-10 at 100 ng/ml transiently suppressed the IDO expression in chorionic villi and the decidua tissues; but had no effect on the SOCS3 expression in the chorionic villi and the decidua tissues. In addition, IL-10 at 100 ng/ml had effect on the IDO and SOCS3 expression at in the chorionic villi and the decidua tissues. IDO expression levels was negatively correlated with SOCS3 levels in chorionic villi and the decidua tissues. IDO expression was the weakest and SOCS3 expression was strongest at 12 h after treating with 10 ng/ml IL-10 in chorionic villi and decidua tissues. Our results indicated IL-10 may down-regulate IDO expression by up-regulating SOCS3 in chorionic villi and decidua tissues, and IL-10 may help prevent allogeneic fetal rejection.
Keywords: IL-10; Indoleamine 2, 3-dioxygenase; suppressor of cytokine signaling 3; maternal–fetal tolerance; chorionic villi; decidua

Introduction

Pregnancy is a unique and well-choreographed physiological process that involves intricate interplay of inflammatory and anti-inflammatory milieu, hormonal changes, and cellular and molecular events at the maternal-fetal interface [1]. Previous studies have suggested that the enzyme, indoleamine 2,3-dioxygenase (IDO), is a key protein in the maintenance of maternal-fetal tolerance[2]. IDO catalyzes the initial and rate-limiting step of tryptophan catabolism in a specific pathway, resulting in a series of extracellular messengers collectively known as kynurenines[2]. IDO has been recognized as an authentic regulator of immunity not only in mammalian pregnancy, but also in infection, autoimmunity, inflammation, allergy, transplantation and neoplasia. Accumulating evidence indicated that reduced activity or expression levels of IDO may contribute to pathological pregnancies[3].

Interleukin-10 (IL-10) is a multifunctional anti-inflammatory cytokine that is produced by various cells including monocytes, macrophages, B cells, T cells and mast cells [1]. In pregnancy, IL-10 is considered one of the major immunoregulatory cytokines important for a successful outcome[1]. A decreased production of IL-10 is associated with pregnancy loss and increase in preeclampsia[4,5]. Sytokine signaling 3 (SOCS3) is part of a protein family that binds cytokine receptors, thereby suppressing development since genetic deletion of SOCS3 in murine models resulted in embryonic lethality due to placental insufficiency[6].

In the present study, the expression of IDO and SOCS3 in chorionic villi and the decidua after IL-10 exposure in early pregnancy were evaluated, and the correlation of these proteins were also evaluated.

Minyan Yang[a] (email: ymyspring@163.com)
Jun Wang[b] (email: wangjunjessie@163.com)
Guanyou Huang[b] (email: guanyouhuang@hotmail.com)
Fei Xia[a] (email: xiafeisci@163.com)*
[a]Reproductive Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Soochow University, Suzhou 215006, China.
[b]Department of Obstetrics and Gynecology, Reproductive Medicine Center, The Affiliated Hospital of Guizhou Medical University, Guiyang 550004, Guizhou, China.
(Minyan Yang, Jun Wang) Both authors equally contributed to this work.
Correspondence to: Professor Fei Xia, MD, Reproductive Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Soochow University, Suzhou 215006, China. E-mail addresses: xiafeisci@163.com
Running title: IDO and SOCS3 expression in pregnancy

Materials and methods

Ethics statement

This study is based on the principles expressed in the Declaration of Helsinki. The study was approved by the Institutional Review Board of Taizhou People's Hospital (Taizhou, China). All patients provided written informed consent for the collection of samples and subsequent analysis.

Samples collection

A total of 30 normal pregnant women (Age: 28.35 ± 3.32 years; gestational age: 61.45 ± 6.20 days) who underwent legal termination at Taizhou People's Hospital between December 2019 and March 2020 were included in this study. Normal embryonic development was revealed by ultrasonic examination and those with abnormal reproductive and history of chronic diseases associated with

chronic hypertension, kidney disease and diabetes were excluded. Aseptic collection of chorionic villi and decidua (around 100 mg each) was followed by washing with phosphate-buffered saline (PBS) to remove red blood cells. Tissue samples were either preserved at -80°C prior or cut into small blocks respectively (no more than 1 mg of wet weight) for culture. Samples that were used for culture were processed within 40 min of collection.

Tissue Culture

Decidua were confirmed by one-side smooth surface and one-side fluff, chorionic villi tissues were identified by two-side fluff surface. The chorionic villi and decidua tissues were cut into small blocks (no more than 1 mg of wet weight), washed twice with F-12/DMEM (Invitrogen, USA), centrifuged at 1500 rpm for 30 min, and cultured in F-12/DMED (Invitrogen) containing 10% FBS (Gibco; Australia) and 1% penicillin–streptomycin (Invitrogen). Tissue explants were then placed in a CO_2 incubator (Thermo, USA) at 37°C with 5% CO_2. IL-10 was purchased from Sigma-Aldrich (USA).

Western blotting

Tissue stored at -80°C was placed in a mortar containing liquid nitrogen and ground to powder, after which radioimmunoprecipitation assay buffer (Sigma-Aldrich; Merck & Co., Inc., Whitehouse Station, NJ, USA) was added with protease inhibitors (P8340; Sigma-Aldrich; USA Merck & Co., Inc.). The lysate was centrifuged twice at 4°C for 30 min at 18,514 x g and the supernatants were collected. A bicinchoninic acid assay, protein assay kit (Beyotime Institute of Biotechnology, Haimen, China) was used to determine protein content separately. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 12.5% gels to separate the proteins, and gels were subsequently transferred onto polyvinylidene fluoride (PVDF) membranes (PerkinElmer, Inc., Waltham, MA, USA) by wet transfer method at 120 V(200 mA) for 2h. PVDF membranes were blocked in blocking buffer (Beyotime Institute of Biotechnology) overnight at 4°C. Blots were incubated with primary antibody (rabbit anti-human IDO and SOCS3 polyclonal antibody; 1:3000; cat. nos. 12006, 2932 and 3711, respectively; Cell Signaling Technology, Inc., Danvers, MA, USA) or human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) polyclonal antibody (1: 6,000; cat. no. GTX100118; GeneTex, Inc., Irvine, CA, USA) for 4 h at room temperature, then rinsed with tris-buffered saline with Tween-20 and incubated with secondary horseradish peroxidase (HRP)-labeled goat anti-rabbit immunoglobulin G antibody (PerkinElmer, Inc.). Protein bands were visualized by using the ECL kit (Thermo Fisher Scientific).

Statistical analysis

All experiments were performed at least in triplicate. All data are presented as the mean ± standard deviation of the mean (SD). All statistical analyses were performed using the SPSS statistical software package, version 13.0 (SPSS, Inc., Chicago, IL, USA) and the GraphPad statistical software package, version 6.0 (GraphPad Software, San Diego, CA, USA). Pearson's correlation analysis and the paired-samples t-test were used to analyze protein expression data from western blot analysis. For all analyses, P value < 0.05 was considered to indicate a statistically significant difference.

Results

IDO and SOCS3 in chorionic villi and decidual tissues of women in early pregnancy

The chorionic villi and decidual tissues were ground under liquid nitrogen, and then lysed for Western blot analysis. Western blot analysis showed that both IDO and SOCS3 were expressed in chorionic villi and decidua (Figure 1A and 1B). The expression of IDO in decidua was stronger than that in villi (Figure 1C). There was no significant difference in SOCS3 expression between villi and decidua (Figure 1D).

Effects of IL-10 with different concentrations on IDO and SOCS3 expression in chorionic villi and the decidua tissues

To study the effect of IL-10 on the expression of IDO and SOCS3, the concentrations of IL-10 used to treat chorionic and decidual tissues were 0.1, 1 and 10 ng/ml. Incubation duration was 12 h. Subsequently, tissue explants were lysed for Western blot analysis to evaluate the expression of IDO and SOCS3. IL-10 at 0.1, 1 and 10 ng/ml concentration-dependently decreased the IDO expression in chorionic villi and the decidua tissues; while decreased the expression of SOSC3 in chorionic villi and the decidua tissues (Figure 2A-2D). IDO expression levels was negatively correlated with SOCS3 levels in chorionic villi and the decidua tissues (Figure 2E and 2F).

In a further examination, IL-10 at 100 ng/ml transiently suppressed the IDO expression in chorionic villi and the decidua tissues (Figure 3A, 3C and 3E); but had no effect on the SOCS3 expression in the chorionic villi and the decidua tissues (Figure 3B, 3D and 3E). In addition, IL-10 at 100 ng/ml had effect on the IDO and SOCS3 expression at in the chorionic villi and the decidua tissues (Figure 3A-3F). IDO expression levels was negatively correlated with SOCS3 levels in chorionic villi and the decidua tissues (Figure 3G and 3H)

Effects of 10 ng/ml IL-10 with treatment duration on IDO and SOCS3 expression in chorionic villi and the decidua tissues

To investigate the relationship of IDO expression with the culture time, the chorionic villi and decidua tissues were cultured in a medium containing IL-10 at concentrations of 10 ng/ml for 6 h,12 h and 24 h respectively. The western blot results showed that, compared with culture for 6 h ,12 h and 24 h, IDO expression was the weakest and SOCS3 expression was strongest at 12 h after treating with 10 ng/ml IL-10 in chorionic villi (Figure 4A and 4C) and decidua (Figure 4B and 4D).

Discussion

Pregnancy represents a unique process, and the balance of locally produced pro-inflammatory and anti-inflammatory effectors is essential to a

successful pregnancy outcome[7-9]. It is now evident that the protection of the fetus against a harmful maternal immune response is based on a complicated mechanism, and the communication between the various steps in the cascade of events is accomplished via cytokines. Indoleamine 2, 3-dioxygenase (IDO) is a key immunomodulatory enzyme that functions to promote immune tolerance. Initial evidence for IDO-mediated immunosuppression was demonstrated at the maternal-fetal interface[10]. It has been previously reported that IDO is expressed in the chorionic villi and decidua of normal human pregnancies [11,12]. We used Western blot analysis to confirm that the expression of IDO in human decidua is stronger than that in chorionic villi, and also found that SOCS3 was present in the villi and decidua, and the expression of this protein was not significantly different in the villi and decidua. This is consistent with the above previous studies[13].

IL-10 functions not only as a potent immunosuppressive agent but also serves as a vascular protector and modulator of ER stress and autophagy at the maternal-fetal interface. IL-10 is a pivotal player involved in the maternal immune tolerance for survival of an allogeneic fetus[14]. The immunomodulatory effects of IL-10 were considered a key factor in the regulation of the Th1/Th2 balance required to maintain the immune privilege[15]. In humans, IL-10 deficiency has been found to be associated with many adverse pregnancy outcomes, such as recurrent spontaneous abortion (RSA), premature delivery and preeclampsia[16,17]. But its mechanism is not fully understood. We preliminarily explored the relevant signaling pathway mediating the effect of IL-10 for IDO. STAT3 is the only obligate factor required for IL-10-mediated anti-inflammatory signaling[18,19], The signaling results in sustained STAT3 activation, STAT3 has been described as a vital regulator for placentation and therefore to reproduction[20].

Some scholars have found that IL-10 has been shown to reduce pro-inflammatory cytokine expression through the induction of SOCS3 and IL10 may induce SOCS3 in a STAT3-dependent manner[21]. We found an inverse correlation between SOCS3 and IDO expression[22,23], and there is an inverse correlation between SOCS3 and IDO expression in chorionic villi and decidua from normal pregnant women[13]. It has been previously reported that IL-10 induces SOCS3 expression. SOCS3 protein acts as an intracellular inhibitory regulator of cytokine signaling through the STAT3 pathway, thereby reducing the production of inflammatory cytokines[24,25]. We also have found that IDO expression is negatively correlated with SOCS3, in chorionic villi and decidua, which suggests a possible role for SOCS3 in maintaining immunotolerance by regulating IDO expression at the maternal-fetal interface. SOCS3 elicits different intracellular effects including JAK1 inhibition that leads to a negative feedback of the JAK1/Tyk2/ STAT3 pathway [26]. SOCS3 is a major negative feedback regulator of signal transducer and activator of transcription (STAT)3-activating cytokines[27]. IL-10 induces the activation of STAT3[20,28,29]. Activated STATs not only drive transcription of many genes related to cell proliferation, function, and survival, but also induce the transcription of SOCS genes. It has been reported that SOCS3 is a negative regulator of the JAK-STAT signaling pathway of cytokine synthesis in PBMCs and neutrophils[26,30,31]. We suppose that SOCS3 protein may inhibit STAT3 phosphorylation by binding to its corresponding IL-10 receptor, acting as a feedback inhibitor of JAK / STAT3 pathway, and IL-10 may down regulate the expression of IDO via increasing the expression of SOCS3.

Conclusions

In conclusion, both IDO and SOCS3 are immunomodulatory molecules expressed in chorionic villi and decidual tissues. The large amount of IL-10 produced during pregnancy can increase the expression of SOCS3 to regulate the IDO expression to induce maternal and infant tolerance. This novel concept is worth to be investigated in experimental models with the final aim to create strategies for restoring the immune balance in those patients with spontaneous abortions owed to an incomplete immune tolerance.

Authors' contributions

MY, JW, GH and FX conceived and designed the study. MY analyzed the data. MY conducted the study. MY collected the chorionic villi and decidua tissue samples. MY and JW wrote the manuscript. All authors agreed with manuscript results, conclusions and approved the final manuscript. FX was responsible for the acquisition of funding.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

None.

Ethics approval and consent to participate

The procedures were approved the Ethics Committee of Taizhou Hospital.

Statement of Informed Consent

Each patient signed the informed consent.

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with guidelines of the Ethics Committee of Taizhou Hospital.

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Cubro H, Kashyap S, Nath MC, Ackerman AW, Garovic VD. The Role of Interleukin-10 in the Pathophysiology of Preeclampsia. Curr

Hypertens Rep. 2018;20(4):36.
[2] Chang RQ, Li DJ, Li MQ. The role of indoleamine-2,3-dioxygenase in normal and pathological pregnancies. Am J Reprod Immunol. 2018;79(4):e12786.
[3] Ban Y, Chang Y, Dong B, Kong B, Qu X. Indoleamine 2,3-dioxygenase levels at the normal and recurrent spontaneous abortion fetal-maternal interface. J Int Med Res. 2013;41(4):1135-1149.
[4] Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today. 1993;14(7):353-356.
[5] Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. J Immunol. 1993;151(9):4562-4573.
[6] Dela Justina V, San Martin S, López-Espíndola D, Bressan AFM, Alves de Freitas R, Lopes de Passos AM, Varas J, Lima VV, Giachini FR. Increased expression of STAT3 and SOCS3 in placenta from hyperglycemic rats. Eur J Histochem. 2019;63(4).
[7] Kalkunte S, Nevers T, Norris WE, Sharma S. Vascular IL-10: a protective role in preeclampsia. J Reprod Immunol. 2011;88(2):165-169.
[8] Thaxton JE, Sharma S. Interleukin-10: a multi-faceted agent of pregnancy. Am J Reprod Immunol. 2010;63(6):482-491.
[9] Sharma S. Natural killer cells and regulatory T cells in early pregnancy loss. Int J Dev Biol. 2014;58(2-4):219-229.
[10] Katz JB, Muller AJ, Prendergast GC. Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral immune escape. Immunol Rev. 2008;222:206-221.
[11] Hönig A, Rieger L, Kapp M, Sütterlin M, Dietl J, Kämmerer U. Indoleamine 2,3-dioxygenase (IDO) expression in invasive extravillous trophoblast supports role of the enzyme for materno-fetal tolerance. J Reprod Immunol. 2004;61(2):79-86.
[12] Kudo Y, Boyd CA, Spyropoulou I, Redman CW, Takikawa O, Katsuki T, Hara T, Ohama K, Sargent IL. Indoleamine 2,3-dioxygenase: distribution and function in the developing human placenta. J Reprod Immunol. 2004;61(2):87-98.
[13] Liu W, Huang Y, Huang G, Zhou C, Zeng X, Zhao S, Wu L, Zhou H, Wu Q, Dai L. Relationship of SOCS3 and TGF-β with IDO expression in early pregnancy chorionic villi and decidua. Exp Ther Med. 2017;14(5):4817-4824.
[14] Cheng SB, Sharma S. Interleukin-10: a pleiotropic regulator in pregnancy. Am J Reprod Immunol. 2015;73(6):487-500.
[15] Van Den Biggelaar AH, De Craen AJ, Gussekloo J, Huizinga TW, Heijmans BT, Frölich M, Kirkwood TB, Westendorp RG. Inflammation underlying cardiovascular mortality is a late consequence of evolutionary programming. Faseb j. 2004;18(9):1022-1024.
[16] Hanna N, Bonifacio L, Weinberger B, Reddy P, Murphy S, Romero R, Sharma S. Evidence for interleukin-10-mediated inhibition of cyclo-oxygenase-2 expression and prostaglandin production in preterm human placenta. Am J Reprod Immunol. 2006;55(1):19-27.
[17] Makhseed M, Raghupathy R, El-Shazly S, Azizieh F, Al-Harmi JA, Al-Azemi MM. Pro-inflammatory maternal cytokine profile in preterm delivery. Am J Reprod Immunol. 2003;49(5):308-318.
[18] Hutchins AP, Diez D, Miranda-Saavedra D. The IL-10/STAT3-mediated anti-inflammatory response: recent developments and future challenges. Brief Funct Genomics. 2013;12(6):489-498.
[19] Murray PJ. The JAK-STAT signaling pathway: input and output integration. J Immunol. 2007;178(5):2623-2629.
[20] Braun DA, Fribourg M, Sealfon SC. Cytokine response is determined by duration of receptor and signal transducers and activators of transcription 3 (STAT3) activation. J Biol Chem. 2013;288(5):2986-2993.
[21] Qin H, Wilson CA, Roberts KL, Baker BJ, Zhao X, Benveniste EN. IL-10 inhibits lipopolysaccharide-induced CD40 gene expression through induction of suppressor of cytokine signaling-3. J Immunol. 2006;177(11):7761-7771.
[22] Fallarino F, Orabona C, Vacca C, Bianchi R, Gizzi S, Asselin-Paturel C, Fioretti MC, Trinchieri G, Grohmann U, Puccetti P. Ligand and cytokine dependence of the immunosuppressive pathway of tryptophan catabolism in plasmacytoid dendritic cells. Int Immunol. 2005;17(11):1429-1438.
[23] Orabona C, Pallotta MT, Volpi C, Fallarino F, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Grohmann U, Puccetti P. SOCS3 drives proteasomal degradation of indoleamine 2,3-dioxygenase (IDO) and antagonizes IDO-dependent tolerogenesis. Proc Natl Acad Sci U S A. 2008;105(52):20828-20833.
[24] Sabat R, Grütz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J. Biology of interleukin-10. Cytokine Growth Factor Rev. 2010;21(5):331-344.
[25] Gadina M, Sudarshan C, Visconti R, Zhou YJ, Gu H, Neel BG, O'Shea JJ. The docking molecule gab2 is induced by lymphocyte activation and is involved in signaling by interleukin-2 and interleukin-15 but not other common gamma chain-using cytokines. J Biol Chem. 2000;275(35):26959-26966.
[26] Berlato C, Cassatella MA, Kinjyo I, Gatto L, Yoshimura A, Bazzoni F. Involvement of suppressor of cytokine signaling-3 as a mediator of the inhibitory effects of IL-10 on lipopolysaccharide-induced macrophage activation. J Immunol. 2002;168(12):6404-6411.
[27] Karlsen AE, Rønn SG, Lindberg K, Johannesen J, Galsgaard ED, Pociot F, Nielsen JH, Mandrup-Poulsen T, Nerup J, Billestrup N. Suppressor of cytokine signaling 3 (SOCS-3) protects beta -cells against interleukin-1beta - and interferon-gamma -mediated toxicity. Proc Natl Acad Sci U S A. 2001;98(21):12191-12196.
[28] Palmer DC, Restifo NP. Suppressors of cytokine

signaling (SOCS) in T cell differentiation, maturation, and function. Trends Immunol. 2009;30(12):592-602.
[29] Carow B, Rottenberg ME. SOCS3, a Major Regulator of Infection and Inflammation. Front Immunol. 2014; 5:58.
[30] Starr R, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA, Hilton DJ. A family of cytokine-inducible inhibitors of signalling. Nature. 1997;387(6636):917-921.
[31] Larsen L, Röpke C. Suppressors of cytokine signalling: SOCS. Apmis. 2002;110(12):833-844.

Figure Legends

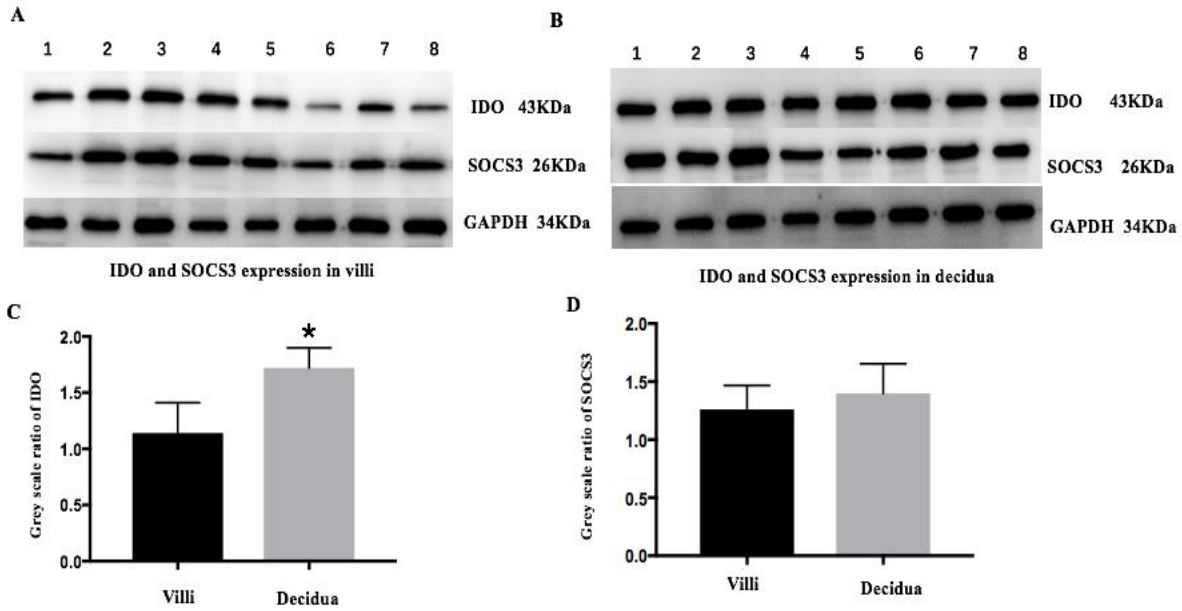

Figure 1. **Expression levels of IDO and SOCS3 in the chorionic villi and decidua. Expression levels of IDO and SOCS3 in the chorionic villi (A) and decidua (B), as detected by Western blot analysis. (C) The expression of IDO in villi was weaker than that in decidua. (D) The expression of SOCS3 in villi and decidua had no significant difference. *P < 0.05 compared to Villi group.**

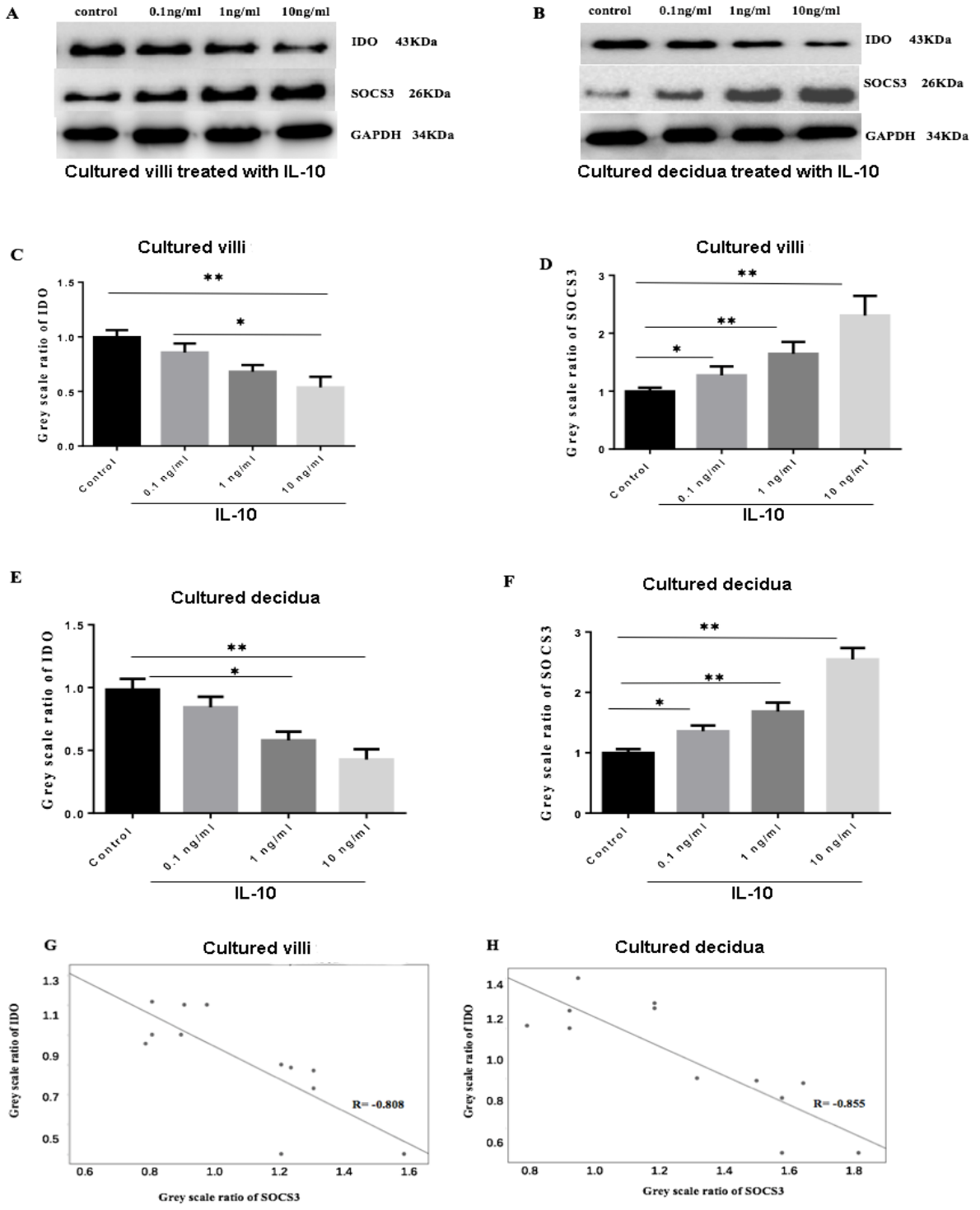

Figure 2. **The expression and correlation between SOCS3 expression and the expression of IDO in cultured tissues in the presence of IL-10 was analyzed. The expression of IDO and SOCS3 in tissues from chorionic villi (A, C, D and G) and decidua (B, E, F and H) incubated in medium containing IL-10 were analyzed. The concentrations of IL-10 were 0.1, 1 and 10 ng/ml, respectively. Data are the mean ± SE and are representative of three similar experiments. C: **P<0.01; *P <0.05 compared with the control group; D: **P<0.01; *P <0.05 compared with the control group. Data are representative of three independent experiments. Control: cultured tissues from chorionic villi or decidua without IL-10 added; Gray-scale ratio of SOCS3(IDO), the expression level of SOCS3(IDO) was assessed based on the ratios of the gray scale of the amplified SOCS3(IDO) band to that of the GAPDH band IDO, Indoleamine 2, 3-dioxygenase; SOCS3, suppressors of cytokine signaling 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-10, Interleukin-10.**

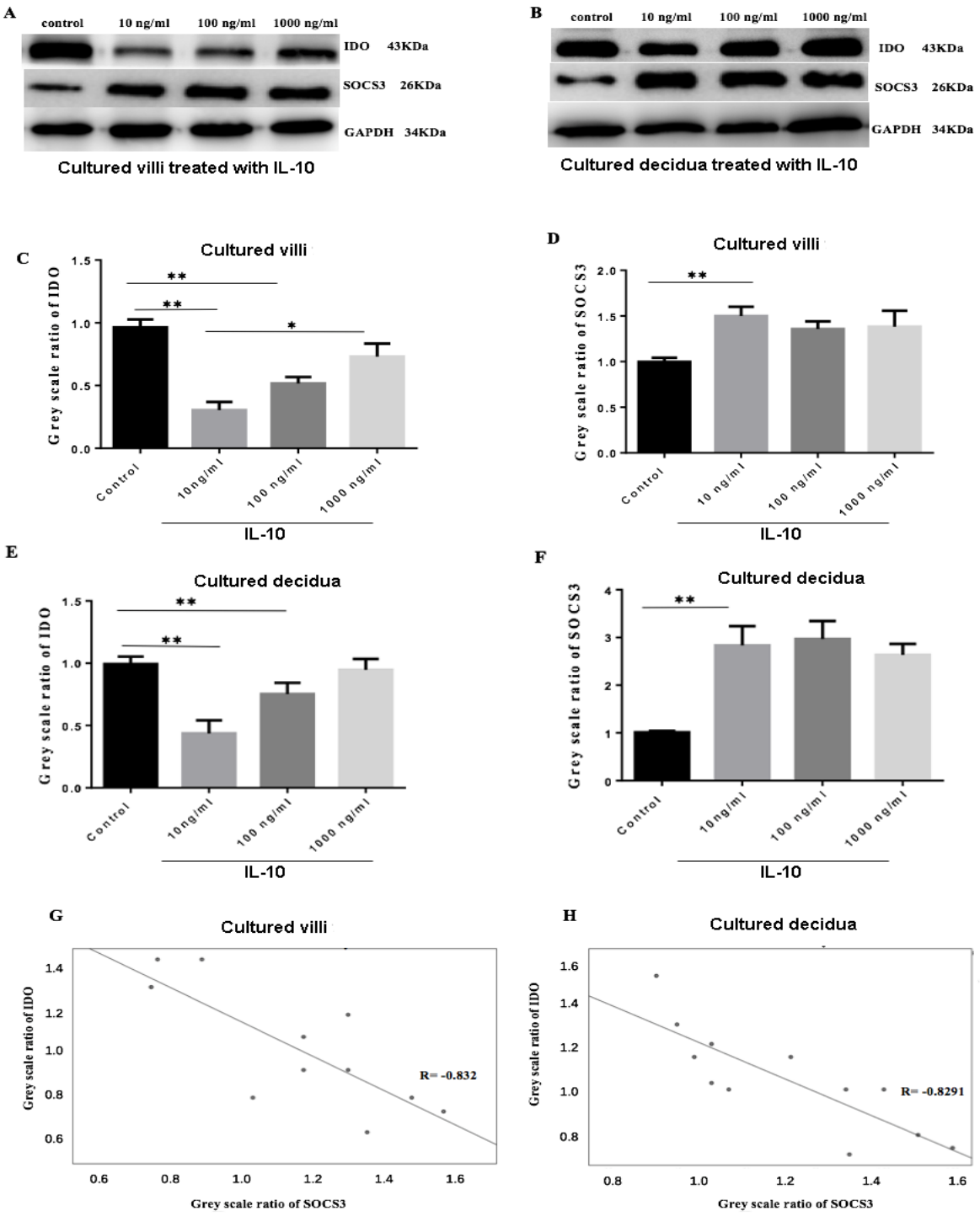

Figure 3. **The expression and correlation between SOCS3 expression and the expression of IDO in cultured chorionic villi tissues in the presence of IL-10 was analyzed. The expression of IDO and SOCS3 in tissues from chorionic villi (A, C, D and G) and decidua (B, E, F and H) incubated in medium containing IL-10 were analyzed. The concentrations of IL-10 were 10, 100 and 1000 ng/ml, respectively. C: **P<0.01, compared with the control group respectively; *P < 0.05, compared with the groups treated with10,100 and 1000 ng/ml, respectively. D: **P<0.01, compared with the control group; E: **P<0.01, compared with the control group respectively; F: **P<0.01, compared with the control group. Data are representative of three independent experiments. Control: cultured tissues from chorionic villi or decidua without IL-10 added; Gray-scale ratio of SOCS3(IDO), the expression level of SOCS3(IDO) was assessed based on the ratios of the gray scale of the amplified SOCS3(IDO) band to that of the GAPDH band IDO, Indoleamine 2, 3-dioxygenase; SOCS3, suppressors of cytokine signaling 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-10, Interleukin-10.**

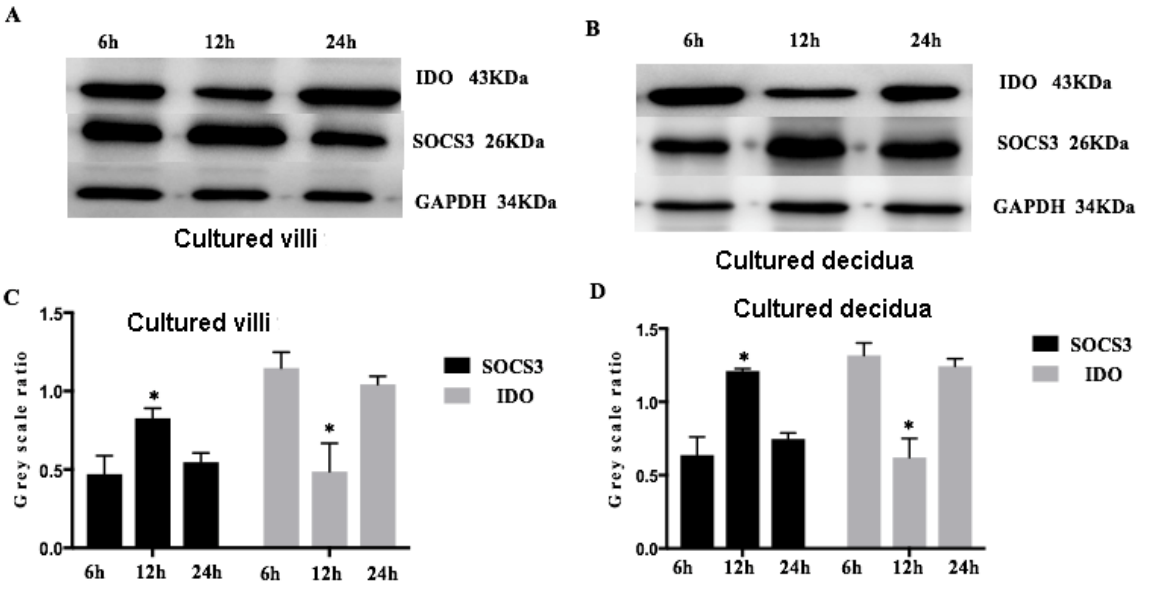

Figure 4. **The culture time on the expression of IDO in chorionic villi and decidua tissues treated with IL-10. The expression of IDO in chorionic villi (A and C) and decidua (B and D) incubated in medium containing IL-10 at concentrations of 10ng/ml for 6 h ,12 h and 24 h, respectively. *P<0.05 compared with chorionic villi incubated for 6 h and 24 h, respectively. Data are representative of three independent experiments. Gray-scale ratio of SOCS3(IDO), the expression level of SOCS3(IDO) was assessed based on the ratios of the gray scale of the amplified SOCS3(IDO) band to that of the GAPDH band IDO, Indoleamine 2, 3-dioxygenase; SOCS3, suppressors of cytokine signaling 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-10, Interleukin-10.**