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Profile of VEGF Secretion in Human Peripheral Blood Mononuclear Cells *In vitro*

Fatemeh Hajighasemi
Department of Immunology, Faculty of Medicine, Shahed University, Tehran, Iran

Abstract: Peripheral Blood Mononuclear Cells (PBMCs) are key regulators of inflammation. Vascular Endothelial Growth Factor (VEGF) is a cytokine which is produced by a number of cells and has an important role in inflammation. In this study, the patterns of VEGF expression by human PBMCs were assessed *in vitro* using Phytoheamagglutinin (PHA), Phorbol Myristate Acetate (PMA) and Lypopolysaccharide (LPS) as VEGF inducers. Human PBMCs were cultured in complete RPMI-1640 medium. Then, the cells were seeded at a density of 10^6 cells mL^{-1} and subsequently were incubated with PMA (0-25 ng mL^{-1}), PHA (0-10 μg mL^{-1}) or LPS (0-4 μg mL^{-1}) for 48 h. The level of VEGF secreted in the cell culture supernatants was measured with enzyme-linked immunosorbent assay kits (R and D systems). Human PBMCs cultured without any stimulus, produced detectable amount of VEGF after 48 h incubation. PMA significantly reduced while PHA/LPS significantly increased VEGF production in human PBMCs dose-dependently. The results show that PMA, PHA and LPS have different effects on VEGF production by PBMCs.

Key words: VEGF, profile, human, PBMCS, therapeutic

INTRODUCTION

Inflammation plays an important role in various diseases, such as multiple sclerosis, rheumatoid arthritis and some cardiovascular disorders (Abrahamsson *et al*., 2013; Van Herwijnen *et al*., 2013; Yardim-Akaydin *et al*., 2013). Peripheral Blood Mononuclear Cells (PBMCs) are key regulators of inflammation (Downer *et al*., 2013; Santer *et al*., 2012). The inflammatory effects of PBMCs are accomplished through some factors especially inflammatory cytokines (Yougbare *et al*., 2013; Gola *et al*., 2013; Azimi-Nezhad *et al*., 2013). Vascular Endothelial Growth Factor (VEGF) is a cytokine which is produced by a number of cells and has a key role in inflammation (Shaik-Dasthagirisaheb *et al*., 2013; Yuan *et al*., 2013). Also, the increased serum level of VEGF in inflammatory diseases has been reported (Karatolios *et al*., 2012; Park *et al*., 2012). The expression of VEGF-A mRNA from PBMCs of thromboangitis obliterans has been shown (Fazeli *et al*., 2013).

Moreover, expression the of VEGF-145 isoform mRNA and its relations to cellular adhesion molecules has been discovered (Azimi-Nezhad *et al*., 2013).

Furthermore, an association between the increase in risk of Multiple Sclerosis (MS) exacerbation and enhanced disease activity with VEGF production by CD4+T cells has been revealed (Correale *et al*., 2012). In addition, high VEGF Levels in PBMCs and pulmonary edema fluid of acute hantavirus pulmonary syndrome patients has been detected (Gavrilovskaya *et al*., 2012). Besides conditioned medium of oral carcinoma tumor cells induced production of VEGF by normal human PBMCs (Franca *et al*., 2011).

Moreover, the elevated expression of VEGF in PHA-activated T cells by human dental pulp-derived stem cells has been shown (Demircan *et al*., 2011).

Also, intrauterine administration of PBMCs in pregnant mouse increased the endometrial VEGF expression and pregnancy rate (Yu *et al*., 2013).

Effect of some anti-inflammatory agents has in part attributed to their suppressive effect on VEGF secretion (Palmieri *et al*., 2011; Gallelli *et al*., 2013). Furthermore, the preventive effect of a number VEGF-inhibitors in autoimmunity has been shown (Seabrook *et al*., 2010).

Due to important role of PBMCs and also VEGF in a number of physiologic and pathologic conditions, such as inflammatory and autoimmunity states (Sendergaard *et al*., 2013; Sohn *et al*., 2013), determination the expression profile of VEGF in PBMCs could be valuable in screening of VEGF enhancers/inhibitors, selection the ideal drugs with modulatory effects on VEGF and designing of novel drugs to target VEGF response in related disorders. In this study, the patterns of VEGF expression by human PBMCs were assessed *in vitro* using Phytoheamagglutinin (PHA), Phorbol Myristate Acetate (PMA) and Lypopolysaccharide (LPS) as VEGF inducers.

Corresponding Author: Fatemeh Hajighasemi, Department of Immunology, Faculty of Medicine, Shahed University, No. 31, Shahid Abdollahzadeh Street, 1415635111 Keshavarz Blvd., P.O. Box: 14155-7435, Tehran, Iran

MATERIALS AND METHODS

Reagents: RPMI-1640 medium, penicillin, streptomycin, Ficoll-hypaque, Lypopolysaccharide (LPS), Phorbol Myristate Acetate (PMA) and Trypan Blue (TB) were from sigma (USA). Fetal Calf Serum (FCS) was from Gibco (USA). Microtiter plates, flasks and tubes were from Nunc (Falcon, USA). VEGF standard ELISA kit was obtained from R and D company (USA).

PBMCs isolation: PBMCs from the venous blood of healthy adults volunteers were isolated by ficoll-hypaque-gradiant centrifugation. Subsequently, the cells were washed three times in Phosphate Buffer Saline (PBS). Then the cells resuspended in RPMI-1640 medium supplemented with 10% FCS and were incubated in 5% CO_2 at 37°C.

Cell culture and treatment: The method has been described in detail elsewhere (Hajighasemi and Mirshafiey, 2010). Briefly, the human PBMCs were cultured in RPMI-1640 medium supplemented with 10% FCS, penicillin (100 IU mL^{-1}) and streptomycin (100 μg mL^{-1}) at 37°C in 5% CO_2. The cells were seeded at a density of 10^6 cell mL^{-1} and then incubated with different concentrations of PMA (0-25 ng mL^{-1}), PHA (0-10 μg mL^{-1}) or LPS (0-4 μg mL^{-1}) for 48 h. The supernatants of cell cultures were collected, centrifuged and stored at -80°C for next experiments. All experiments were done in triplicate.

Evaluation of VEGF production by ELISA: The amount of VEGF secreted in the cell culture supernatants by human PBMCs was measured with the Quantikine human VEGF ELISA kits (R and D systems) according to the manufacturer's instructions. This assay uses the quantitative sandwich enzyme immunoassay technique. Complete RPMI medium was used as control and human recombinant VEGF165 was employed as standard for drawing the standard curves.

Statistical analysis: VEGF level quantification in cell-conditioned media was performed in three independent experiments and the results were expressed as mean±SEM. Statistical comparisons between groups were made by Analysis of Variance (ANOVA). The $p<0.05$ was considered significant. Test of multiple comparison of Tukey was applied (5%) for statistically significant differences. The software SPSS 11.5 and Excel 2003 were used for statistical analysis and graph making, respectively.

RESULTS

Profile of VEGF production in PMA-stimulated human PBMCs: Human PBMCs produced a noticeable amount of VEGF without any stimulus after 48 h incubation and PMA significantly reduced VEGF production by human PBMCs dose-dependently as was shown in Fig. 1 ($p<0.05$).

The human peripheral blood mononuclear cells (1×10^6 cells mL^{-1}) were cultured in complete RPMI-1640 medium and then were stimulated with different concentrations of Phorbol Myristate Acetate (PMA) (0-25 ng mL^{-1}) for 48 h. At the end of incubation, VEGF concentration in conditioned medium was measured by ELISA. Data are mean±SEM of three independent experiments. *$p<0.05$ was considered significant.

Profile of VEGF production in PHA- stimulated human PBMCs: Human PBMCs cultured without any stimulus, produced detectable amount of VEGF after 48 h incubation. PHA significantly increased VEGF production in human PBMCs dose-dependently as was shown in Fig. 2 ($p<0.05$).

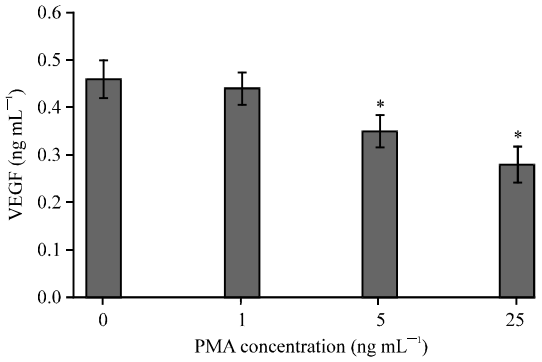

Fig. 1: Effect of PMA on VEGF production by human peripheral blood mononuclear cells

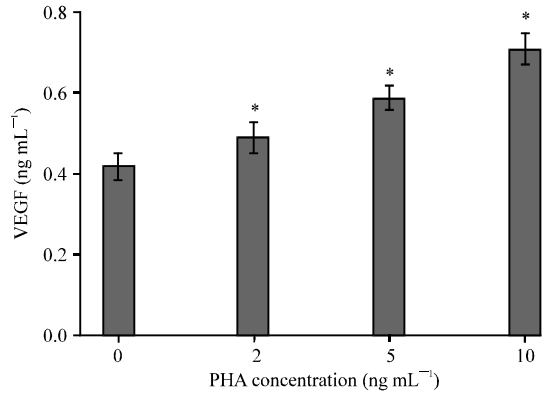

Fig. 2: Effect of PHA on VEGF production by human peripheral blood mononuclear cells

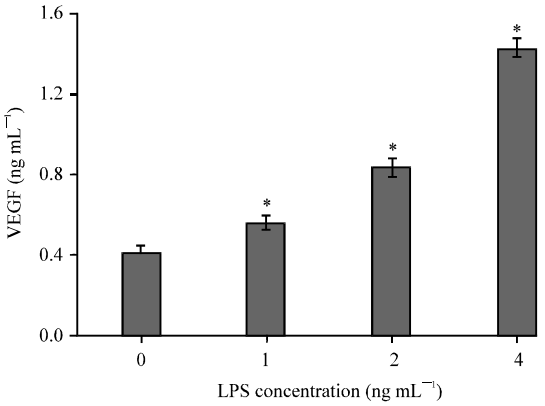

Fig. 3: Effect of LPS on VEGF production by human peripheral blood mononuclear cells

The human peripheral blood mononuclear cells (1×10^6 cells mL^{-1}) were cultured in complete RPMI-1640 medium and then were stimulated with different concentrations of Phytoheamagglutinin (PHA) (0-10 μg mL^{-1}) for 48 h. At the end of incubation, VEGF concentration in conditioned medium was quantified by ELISA. Data are mean±SEM of three independent experiments. *$p<0.05$ was considered significant.

Profile of VEGF production in LPS-stimulated human PBMCs: Human PBMCs cultured alone (without any inducer), produced evident amount of VEGF after 48 h incubation. LPS significantly increased VEGF production in human PBMCs dose-dependently as was shown in Fig. 3 ($p<0.05$).

The human peripheral blood mononuclear cells (1×10^6 cells mL^{-1}) were cultured in complete RPMI-1640 medium and then were stimulated with different concentrations of Lypopolysaccharide (LPS) (0-4 μg mL^{-1}) for 48 h. At the end of incubation, VEGF concentration in conditioned medium was quantified by ELISA. Data are mean±SEM of three independent experiments. *$p<0.05$ was considered significant.

DISCUSSION

In this study, researchers found out that human PBMCs could potentially produce VEGF. Reseaechers, also found that PHA/LPS increase whereas PMA decreases VEGF production in PBMCs. Different patternsof VEGF are expressed in different normal and cancer cells (Kaczmarek *et al*., 2011). In this study, researchers used PMA, PHA and LPS as inducers of VEGF expression.

Converse to the results, induction or increase of VEGF expression in PMA-stimulated cancer and normal cells have been shown by a number of studies. By Young, PMA increased the VEGF expression and secretion by retinal pigment epithelial cells in high glucose and hypoxia. The discrepancy between the results and Young may be in part due to the origin of cells and condition of the study. Young used retinal pigment epithelial cells in high glucose and hypoxia while researchers used PBMCs in normal condition. Also by Karroum, PMA induced VEGF expression in MCF-7 breast cancer cells. Once again the inconsistency between the results and Karroum may be somewhat due to the origin of cells. Karroum used breast cancer cells while researchers used normal PBMCs.

Moreover, researchers have shown the inhibitory effect of PMA on Matrix Metalloproteinase-9 (MMP-9) production in human PBMCs in previous study (Hajighasemi and Hajighasemi, 2011). As both of VEGF and MMP-9 have important role in angiogenesis (Tang *et al*., 2013), it seems that the decrease of VEGF production in PBMCs by PMA may have some relation to PMA-mediated suppression of MMP-9.

Similar to the findings, induction or increase of VEGF expression in a number of cells by LPS has been reported (Lin *et al*., 2006; Kim *et al*., 2008; Bassols *et al*., 2009; Van Dooren *et al*., 2013). In Kim *et al*. (2008), LPS increased VEGF mRNA expression in rat lung pericytes dose-dependently. In Van Dooren *et al*. (2013), whole blood, PBMCs and polymorphonuclear cells were stimulated with LPS and then the cytokine secretion was determined. In Van Dooren *et al*. (2013), VEGF only was produced by PBMCs. In the present study, LPS enhanced the VEGF production by PBMCs dose dependently. Taken together Lps-stimulated PBMCs are good tools for studies on VEGF inducers/regulators.

Additionally in the study, PHA increased VEGF production in PBMCs dose-dependently. The elevated expression of VEGF in PHA-activated T cells by human dental pulp-derived stem cells has been shown (Demircan *et al*., 2011).

Besides the role of mitogen-activated protein kinases, as key mediators of VEGF production has been shown (Kim *et al*., 2011). Also, intervention with mitogenic signal transduction pathways reduced the expression of VEGF (Rizzo *et al*., 2012).

The results show that PMA, PHA and LPS have different effect on VEGF production by PBMCs. PMA decreased whereas PHA/LPS increased the VEGF production in PBMCs. Moreover, it seems that LPS is a more potent inducer of VEGF production by PBMCS than PHA. Because for example as it can be seen in Fig. 2 and 3, the amount of VEGF production at 2 μg mL^{-1} of LPS is about 1.7 fold of the same dose of PHA.

PBMCs and VEGF are key regulators of inflammation (Downer *et al*., 2013; Santer *et al*., 2012; Yuan *et al*., 2013;

Shaik-Dasthagirisaheb *et al*., 2013). Also, it was reported that VEGF release in mononuclear cells is of particular importance as is useful in prognosis of autoimmune disease (Correale *et al*., 2012). Accordingly, an association between the increase in risk of Multiple Sclerosis (MS) exacerbation and enhanced disease activity with VEGF production by CD4+T cells has been revealed (Correale *et al*., 2012). In addition, high VEGF levels in PBMCs and pulmonary edema fluid of acute hantavirus pulmonary syndrome patients has been detected (Gavrilovskaya *et al*., 2012).

Targeting the VEGF may be precious in control of inflammation, autoimmunity and angiogenesis (Szekanecz *et al*., 2009).

The therapeutic effect of some anti-inflammatory agents has in part attributed to their suppressive effect on VEGF secretion (Palmieri *et al*., 2011; Gallelli *et al*., 2013). Furthermore, the preventive effect of a number VEGF-inhibitors in autoimmunity has been shown (Seabrook *et al*., 2010).

Important role of PBMCs and also VEGF in a number of physiologic and pathologic conditions, such as angiogenesis (neovascularization), inflammation and autoimmunity states (Sendergaard *et al*., 2013; Sohn *et al*., 2013) has been reported. Different profile of VEGF production by different stimulators makes PBMCS valuable screening tools for angiogenesis inducers/suppressors, VEGF enhancers/inhibitors and also study of regulatory mechanisms of VEGF secretion. Furthermore, PMA, PHA or LPs-stimulated PBMCs may be good models for study and planning the useful therapeutic approaches for inflammatory mediated diseases, selection the ideal drugs with modulatory effects on VEGF and designing of novel regulators of VEGF response in related disorders.

CONCLUSION

It seems that PBMCs provide valuable screening tools for VEGF enhancers/inhibitors and also study the regulatory mechanisms of VEGF secretion. Furthermore, PMA, PHA or LPS-stimulated PBMCs may be good models for study and planning the useful therapeutic approaches for inflammatory mediated diseases, such as autoimmunity.

REFERENCES

Abrahamsson, S.V., D.F. Angelini, A.N. Dubinsky, E. Morel and U. Oh *et al*., 2013. Non-myeloablative autologous haematopoietic stem cell transplantation expands regulatory cells and depletes IL-17 producing mucosal-associated invariant T cells in multiple sclerosis. Brain, 136: 2888-2903.

Azimi-Nezhad, M., M.G. Stathopoulou, A. Bonnefond, M. Rancier *et al*., 2013. Associations of vascular endothelial growth factor (VEGF) with adhesion and inflammation molecules in a healthy population. Cytokine, 61: 602-607.

Bassols, J., F.J. Ortega, J.M. Moreno-Navarrete, B. Peral, W. Ricart and J.M. Fernandez-Real, 2009. Study of the proinflammatory role of human differentiated omental adipocytes. J. Cell Biochem., 107: 1107-1117.

Correale, J., M.F. Farez and M.C. Ysrraelit, 2012. Increase in multiple sclerosis activity after assisted reproduction technology. Ann. Neurol., 72: 682-694.

Demircan, P.C., A.E. Sariboyaci, Z.S. Unal, G. Gacar, C. Subasi and E. Karaoz, 2011. Immunoregulatory effects of human dental pulp-derived stem cells on T cells: Comparison of transwell co-culture and mixed lymphocyte reaction systems. Cytotherapy, 13: 1205-1220.

Downer, E.J., R.S. Jones, C.L. McDonald, E. Greco and S. Brennan *et al*., 2013. Identifying early inflammatory changes in monocyte-derived macrophages from a population with IQ-discrepant episodic memory. PLoS One., Vol. 8 10.1371/journal.pone.0063194.

Fazeli, B., H. Rafatpanah, H. Ravari, R.F. Hosseini and S.A. Rezaee, 2013. Investigation of the expression of mediators of neovascularization from mononuclear leukocytes in thromboangiitis obliterans. Vascular.

Franca, C.M., F.M. Barros, M.A. Lotufo, K.P. Fernandes and R.C. Borra, 2011. Response of peripheral blood mononuclear cells to conditioned medium from cultured oral squamous cell carcinomas. Braz. Oral. Res., 25: 414-420.

Gallelli, L., O. Galasso, D. Falcone, S. Southworth and M. Greco *et al*., 2013. The effects of nonsteroidal anti-inflammatory drugs on clinical outcomes, synovial fluid cytokine concentration and signal transduction pathways in knee osteoarthritis. A randomized open label trial. Osteoarthritis Cartilage, 21: 1400-1408.

Gavrilovskaya, I., E. Gorbunova, F. Koster and E. Mackow, 2012. Elevated VEGF levels in pulmonary edema fluid and PBMCs from patients with acute hantavirus pulmonary syndrome. Adv. Virol. 10.1155/2012/674360.

Gola, H., H. Engler, A. Sommershof, H. Adenauer and S. Kolassa *et al*., 2013. Posttraumatic stress disorder is associated with an enhanced spontaneous production of pro-inflammatory cytokines by peripheral blood mononuclear cells. BMC Psychiatry., Vol. 13. 10.1186/1471-244X-13-40.

Hajighasemi, F. and A. Mirshafiey, 2010. Propranolol effect on proliferation and vascular endothelial growth factor secretion in human immunocompetent cells. J. Clin. Immunol. Immunopathol. Res., 2: 22-27.

Hajighasemi, F. and S. Hajighasemi, 2011. Zymographic analysis of gelatinases patterns in peripheral blood mononuclear cells. J. Med. Med. Sci., 2: 904-909.

Kaczmarek, M., A. Nowicka, M. Kozlowska, J. Zurawski, H. Batura-Gabryel and J. Sikora, 2011. Evaluation of the phenotype pattern of macrophages isolated from malignant and non-malignant pleural effusions. Tumor Biol., 32: 1123-1132.

Karatolios, K., R. Moosdorf, B. Maisch and S. Pankuweit, 2012. Cytokines in pericardial effusion of patients with inflammatory pericardial disease. Mediators Inflamm. 10.1155/2012/382082

Kim, C.O., A.J. Huh, M.S. Kim, B.S. Chin and S.H. Han *et al*., 2008. LPS-induced vascular endothelial growth factor expression in rat lung pericytes. Shock, 30: 92-97.

Kim, H.N., H. Kim, J.M. Kong, S. Bae and Y.S. Kim *et al*., 2011. Vitamin C down-regulates VEGF production in B16F10 murine melanoma cells via the suppression of p42/44 MAPK activation. J. Cell Biochem., 112: 894-901.

Lin, C.M., H. Chang, Y.H. Chen, S.Y. Li, I.H. Wu and J.H. Chiu, 2006. Protective role of wognin against lipopolysaccharide-induced angiogenesis via VEGFR-2 not VEGFR-1. Int. Immunopharmacol., 6: 1690-1698.

Palmieri, D., B. Pane, C. Barisione, G. Spinella and S. Garibaldi *et al*., 2011. Resveratrol counteracts systemic and local inflammation involved in early abdominal aortic aneurysm development. J. Surg. Res., 171: e237-e246.

Park, H.Y., C.R. Hahm, K. Jeon, W.J. Koh and G.Y. Suh *et al*., 2012. Serum vascular endothelial growth factor and angiopoietin-2 are associated with the severity of systemic inflammation rather than the presence of hemoptysis in patients with inflammatory lung disease. Yonsei. Med. J., 53: 369-376.

Rizzo, A., M. Spedicato, M. Mutinati, G. Minoia, R. Patruno, G. Ranieri and R.L. Sciorsci, 2012. The pharmacological pathways of GnRH mediating the inhibition of mammary tumours: implications in humans and domestic animals. Curr. Med. Chem., 19: 982-991.

Santer, D.M., A.E. Wiedeman, T.H. Teal, P. Ghosh and K.B. Elkon, 2012. Plasmacytoid dendritic cells and C1q differentially regulate inflammatory gene induction by lupus immune complexes. J. Immunol., 188: 902-915.

Seabrook, T.J., A. Littlewood-Evans, V. Brinkmann, B. Pollinger, C. Schnell and P.C. Hiestand, 2010. Angiogenesis is present in experimental autoimmune encephalomyelitis and pro-angiogenic factors are increased in multiple sclerosis lesions. J. Neuroinflammation., 7: 95-95.

Sendergaard, H.B., D. Hesse, M. Krakauer, P.S. Serensen and F. Sellebjerg, 2013. Differential microRNA expression in blood in multiple sclerosis. Mult. Scler. 10.1177/1352458513490542

Shaik-Dasthagirisaheb, Y.B., G. Varvara, G. Murmura, A. Saggini and G. Potalivo *et al*., 2013. Vascular endothelial growth factor (VEGF), mast cells and inflammation. Int. J. Immunopathol. Pharmacol., 26: 327-335.

Sohn, H.J., D.H. Han, D.Y. Lee and D.H. Nam, 2013. Changes in aqueous cytokines after intravitreal triamcinolone versus bevacizumab for macular oedema in branch retinal vein occlusion. Acta Ophthalmol.

Szekanecz, Z., T. Besenyei, G. Paragh and A.E. Koch, 2009. Angiogenesis in rheumatoid arthritis. Autoimmunity, 42: 563-573.

Tang, L., X. Ma, Q. Tian, Y. Cheng, H. Yao, Z. Liu, X. Qu and X. Han, 2013. Inhibition of angiogenesis and invasion by DMBT is mediated by downregulation of VEGF and MMP-9 through Akt pathway in MDA-MB-231 breast cancer cells. Food Chem. Toxicol., 56: 204-213.

Van Dooren, F.H., N.W. Duijvis and A.A. Te Velde, 2013. Analysis of cytokines and chemokines produced by whole blood, peripheral mononuclear and polymorphonuclear cells. J. Immunol. Methods., 396: 128-133.

Van Herwijnen, M.J., R. Van Der Zee, W. Van Eden and F. Broere, 2013. Heat shock proteins can be targets of regulatory T cells for therapeutic intervention in rheumatoid arthritis. Int. J. Hyperthermia., 29: 448-454.

Yardim-Akaydin, S., E. Caliskan-Can, H. Firat, S. Ardic and B. Simsek, 2013. Influence of gender on C-reactive protein, fibrinogen and erythrocyte sedimentation rate in obstructive sleep apnea. Antiinflamm. Antiallergy Agents Med. Chem.

Yougbare, I., G. Boire, M. Roy, C. Lugnier and E. Rouseau, 2013. NCS 613 exhibits anti-inflammatory effects on PBMCs from lupus patients by inhibiting p38 MAPK and NF-êB signalling pathways while reducing proinflammatory cytokine production. Can. J. Physiol. Pharmacol., 91: 353-361.

Yu, N., J. Yang, Y. Guo, J. Fang and T. Yin *et al*., 2013. Intrauterine administration of Peripheral Blood Mononuclear Cells (PBMCs) improves endometrial receptivity in mice with embryonic implantation dysfunction. Am. J. Reprod. Immunol. 10.1111/aji.12150.

Yuan, J., Q. Guo, A.R. Qureshi, B. Anderstam and M. Eriksson *et al*., 2013. Circulating vascular endothelial growth factor (VEGF) and its soluble receptor 1 (sVEGFR-1) are associated with inflammation and mortality in incident dialysis patients. Nephrol. Dial. Transplant., 28: 2356-2363.