·Clinical Research·

Different expression pattern of serum soluble intercellular adhesion molecules-1 and neutrophilic expression of CD18 in patients with diabetic retinopathy

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Abstract

• AIM: To investigate the levels of serum soluble intercellular adhesion molecules-1 (sICAM-1) and neutrophilic expression of CD18 in patients with various stages of diabetic retinopathy and to determine their different expression pattern in the development of diabetic retinopathy(DR).

• METHODS: Levels of serum sICAM-1 and CD18 on the surface of neutrophile were measured in 41 DR patients, they were classified in three subgroups according to the stage of retinopathy as determined by fund's ophthalmoscopy; 10 control subjects were also studied. sICAM-1 were measured by enzyme-linked immunosorbent assay and CD18 by flow cytometry.

• RESULTS: The neutrophilic CD18 expression and serum sICAM-1 level were all significantly elevated in all diabetic subgroups compared to control subjects ($P<0.01$). The differences of CD18 and sICAM-1 among the diabetic subgroups were significant in CD18 but not in sICAM-1. The progression of retinopathy was associated with an increase both in CD18 and in sICAM-1 levels by simple correlation analysis ($\beta=0.74$, $P<0.001$; $\beta=0.38$, $P<0.01$, respectively). But stepwise multiple regression analysis revealed that only CD18 was independent determinant of retinopathy ($\beta=1.04$, $P<0.01$).

• CONCLUSION: Our results confirm the contribution of endothelial and neutrophilic activation in the development of DR as indicated by increased levels of CD18 and sICAM-1. However, a direct implication of CD18 and ICAM-1 in the progression of DR can be supported only in the CD18 but not ICAM-1. CD18 and ICAM-1 may play different role in the development of diabetic retinopathy.

• KEYWORDS: diabetic retinopathy; serum soluble intercellular adhesion molecules-1; neutrophilic; CD18

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INTRODUCTION

Diabetic retinopathy (DR) is a severe complication of diabetes in retinal microvasculature. It is a major cause of vision loss in the developing world [1] .The hallmark of this disease is endothelial cell death and capillary non-perfusion, which is considered a potentially critical event in the progression of DR to its more detrimental forms, proliferative diabetic retinopathy. Although the pathogenesis is unclear, DR may have aspects of low-grade chronic inflammation. Leukocyte-endothelial cell adhesion and entrapment (retinal leukostasis) in retinal capillaries is an early event associated with the development of diabetic retinopathy. This process has been shown to be critically dependent on interactions between ICAM-1 and β^2-integrins (CD18) in diabetic animal models, because treatment with blocking monoclonal antibodies against ICAM-1 or β^2-integrins (CD18) markedly decreases leukocyte adhesion and emigration at diabetic retina [2,3]. ICAM-1 is mainly expressed on endothelial cells and is the marker of activated endothelium in human microvascular endothelial cell [4], it binds to ligands on leukocytes and to mediate the adhesion and migration of leukocyte. Counter receptors for the

ICAM-1 belong to the integrin family and are expressed on leukocytes. Integrins are transmembrane receptors that consist of non-covalently bound heterodimers composed of α- and β-chains. The β^2-integrins are operative in leukocyte adhesion and include LFA-1 (lymphocyte function-associated antigen, CD11a/CD18), Mac-1 (leukocyte adhesion receptor, CD11b/ CD18) and p150/95 transmembrane polypeptide (CD11c/CD18). Each of the β^2-integrins has a common β-chain CD18 [5,6], β^2-integrin has been looked as the marker of leukocyte[7].

Increasing evidence comes from animal models of diabetic retinopathy show that, leukocyte, but not endothelium, plays a primary role in the development of DR [8-11]. Previous work has demonstrated that adherent leukocytes colocalized with dead and dying endothelial cells in the diabetic retina [11]. Another study demonstrates that adherent leukocytes are temporally and spatially associated with retinal endothelial cell injury and death within 1 week of streptozotocin-induced experimental diabetes in rats [8]. The adherence of leukocyte could produce temporary ischemia upstream and subsequent reperfusion, which may lead to endothelial cell injury and death through the formation of reactive oxygen species [10]. Further studies have implicated Fas-FasL-mediated apoptosis in these events. In the STZ-induced diabetic rat, FasL was found to be upregulated on neutrophils while its cognate ligand Fas was upregulated in retinal blood vessels [9]. This upregulation of Fas was accompanied by an enhanced capacity of the neutrophils to induce apoptosis of cultured endothelial cells[9].

In comparison, relatively few studies have examined the primary role of leukocyte in the development of DR in human with DR. In this study, we measured the serum level of ICAM-1 and CD18 on the surface of neutrophile in patients with diabetic retinopathy. We chose to study these adhesion molecules, because they have been linked with leukocyte-endothelial cell interactions and DR. Previous work has established the role of ICAM-1/CD18 in leukocyte adhesion in the pathogenesis of early diabetes-induced leukostasis and blood-retinal barrier breakdown [2,3]. The association of these adhesion molecules with the increasing severity of diabetic retinopathy was assessed.

MATERIALS AND METHODS

Subjects This study included 41 cases of DR patients recruited from outpatients of the Department of Ophthalmology of Xi'an No. 4 Hospital during a 10-month period from November 2006 to September 2007. All were referred from the Department of Endocrinology and examined for DR. Ten (M/F=5/5) age-matched, non-diabetic healthy subjects were selected as controls. Ethical approval for the study was received from the local ethics committee at the Hospital and oral consent obtained from all participants.

Measurement of height, weight and waist and hip girth were made using standard procedures [12]. The body mass index (BMI, kg/m^2) and waist-to-hip ratio (WHR) were calculated. Blood pressure was measured in all subjects in a sitting position on the right arm with a standard mercury sphygmomanometer.

Methods The presence of retinopathy was determined by fundus ophthalmoscopy by an expert ophthalmologist and was graded as stage1, stage2-4 and stage 5 according to the proposed International Clinical Classification of DR and Diabetic Macular Edema[13].

Serum samples were obtained early in the morning from control subjects and DR patients. Neither the controls nor the diabetic patients had allergies, inflammatory disease, malignancy or hepatic disease. No control subjects had diabetes mellitus.

Hemoglobin A1c (HbA1c), and serum glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) were measured. Serum samples were stored at -80 C until assays for sICAM-1 were performed.

Enzyme linked immunosorbent assays were used for the analysis of sICAM-1 (R&D Systems, Minneapolis, Minn., USA). Flow cytometry were used for the measurement of the levels of CD18 on the neutrophile from all the participants. Cells were isolated using density gradient centrifugation and stained with phycoerythrin-labeled CD18 antibody as previously described [14]. Neutrophils were manually gated on the basis of their characteristic forward and side light-scattering properties. Surface expression was presented as the mean channel fluorescence (MCF) on a logarithmic scale.

Statistical Analysis All analyses were performed using the SPSS statistical package for Windows version 13.0. All results were expressed as means±SD or SE. The stage of DR was entered into the model using continuous variable. One-way analysis of variables (ANOVA) was used for between-group comparisons of continuous variables, and the χ^2 test was used for categorical variables.

The comparisons of the levels of adhesion molecules were first made without any adjustment and then were repeated following adjustment. The variables were analyzed using a one-way analysis of covariance (ANCOVA) with post-hoc test, which gave P values for comparisons among all diabetic and control subjects before and after adjustments for BMI and other biological variables. The relationships between sICAM-1, CD18 concentrations and other variables were

Table 1 Clinical and biochemical characteristics of the control group and DR group

Variables	Controls group	DR stage 1	DR stage 2-4	DR stage 5	*P*
n (M/F)	10(5/5)	12(7/5)	14(8/6)	15(7/8)	0.91*
Age (years)	51.18±6.37	51.30±3.86	50.93±4.92	52.86±4.91	0.70
BMI (kg/m^2)	20.86±1.48	22.30±1.89	22.08±1.68	22.92±1.32	0.03
WHR	0.86±0.11	0.95±0.06	0.92±0.10	0.94±0.03	0.07
FPG(mmol/L)	4.72±0.50	8.83±1.33	8.36±0.88	8.82±1.23	0.00
SBP (mmHg)	104.10±14.69	126.00±16.35	125.50±7.30	131.86±9.21	0.00
DBP (mmHg)	74.40±10.10	82.40±5.66	84.07±10.29	86.64±7.33	0.08
HbA1c%	5.68±0.29	8.21±1.42	8.65±0.79	8.48±1.30	0.00
Cholesterol (mg/dl)	167.00±12.83	189.60±29.46	191.86±19.72	197.21±19.19	0.01
HDLc (mg/dl)	47.14±6.37	45.88±7.08	45.78±4.52	47.50±4.21	0.80
LDLc (mg/dl)	100.25±13.87	106.90±20.80	100.07±17.28	112.57±15.96	0.20
TG(mg/dl)	130.40±12.20	211.10±50.86	236.21±68.84	225.21±45.82	0.00
VLDLc (mg/dl)	19.70±6.46	41.50±16.24	45.21±11.89	42.85±11.31	0.00

*P-value by χ^2 test; DR: diabetic retinopathy; BMI: body mass index; WHR: waist-to-hip ratio; FPG: fasting plasma glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1c: glycosylated hemoglobin; HDLc: high-density lipoprotein; LDLc:low-density lipoprotein cholesterol; VLDLc: very-low-density lipoprotein cholesterol; TG: triglycerides.

Table 2 Mean CD18 MCF and sICAM-1 levels (ng/mL) according to stage of DR

	Non-diabetic control group	DR stage 1 group	DR stage 2-4 group	DR stage 5 group	*P*
Crude CD18	1.41±0.20	2.99±1.45	3.20±0.69	4.44±0.78	0.00
Adjusted CD18	2.61±0.25	2.82±0.23	3.46±0.66	3.61±0.26	0.003
Crude sICAM-1	32.63±6.64	113.25±6.69	156.06±86.48	132.42±49.49	0.01
Adjusted sICAM-1	-84.42±60.09	140.26±22.81	177.85±21.24	174.95±23.98	0.01

estimated by Pearson's correlation coefficients. Multiple stepwise regression analysis was used to determine the relationships between retinopathy and the levels of adhesion molecules. P<0.05 was considered statistically significant.

RESULTS

We studied the levels of sICAM-1 and CD18 in 41 cases of type 2 diabetic patients and in 10cases of control subjects. According to the proposed International Clinical Classification of DR and Diabetic Macular Edema[13], the 45 cases of diabetic patients were classified as stage 1 group (n= 12, M/F=7/5), stage 2-4 group (n=14, M/F=8/6) and stage-5 group(n=15, M/F=7/8).The clinical and biochemical characters of the patients and control subjects were showed in Table 1; the 4 groups were matched for age, gender, WHR, LDL, and TG.

The levels of sICAM-1 and CD18 were significantly higher in all three diabetic groups compared to control subjects (Table 2). After adjusting for BMI, fasting plasma glucose, systolic blood pressure, diastolic blood pressure, HbA1, cholesterol and HDLc using ANCOVA, the sICAM-1 level and CD18 were still uncomparable among the four groups.

According to the simple correlation analysis, the levels of CD18 and sICAM-1 were all significantly associated with the development of DR (Table 3). There were no significant correlation between CD18 and sICAM-1 (Table 3). Multiple stepwise regression analysis identified CD18 as the only independent factor that determined the development of DR. (β=1.04, t=7.45, P<0.001, Table 4).

Table 3 Simple correlation analysis between CD18, ICAM-1 and other clinical parameters

	CD18		ICAM-1	
	γ	*P*	γ	*P*
DR	0.74	0.00	0.38	0.01
BMI	0.52	0.00	0.09	0.54
WHR	0.23	0.12	0.22	0.12
FPG	0.79	0.00	0.48	0.001
SBP	0.69	0.00	0.31	0.03
DBP	0.61	0.00	0.41	0.004
HbA1c%	0.58	0.00	0.42	0.002
Cholesterol (mg/dl)	0.44	0.002	0.28	0.05
HDLc (mg/dl)	0.31	0.03	0.03	0.98
LDLc (mg/dl)	0.28	0.054	0.02	0.85
TG(mg/dl)	0.52	0.00	0.41	0.004
VLDLc (mg/dl)	0.47	0.001	0.39	0.01

DISCUSSION

It is reported that the number of people worldwide at risk of developing vision loss from diabetic retinopathy is predicted to double over the next 30 years [15], so it is imperative to develop better means to identify, prevent, and treat this vision-threatening disease. Recently, DR has been considered as a low-grade chronic inflammatory disease. As we know

Table 4 Multiple regression analysis between DR and other clinical parameters

	β	*P*
CD18	1.04	0.00
Icam-1	0.13	0.24
BMI	-0.09	0.46
WHR	-0.03	0.81
FPG	0.08	0.65
SBP	0.11	0.45
DBP	0.22	0.08
HbA1c%	0.08	0.52
Cholesterol (mg/dl)	0.04	0.72
HDLc (mg/dl)	-0.19	0.06
LDLc (mg/dl)	-0.01	0.90
TG(mg/dl)	0.03	0.78
VLDLc (mg/dl)	0.05	0.68

that the key step in inflammation is leukocyte adhesion to the vascular endothelium, which involves three steps in leukocyte sequestration: rolling, firm adhesion and transmigration. The adhesion of leukocytes to the vascular endothelium is largely dependent upon interactions between endothelial cell and leukocyte expressed adhesion molecules[16].

Endothelial cell surface adhesion molecules such as, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) facilitate the attachment of leukocytes to the endothelium and are key mediators of the low-grade inflammation [17, 18]. Compared with VCAM-1, ICAM-1 is the better marker of activated endothelium in human microvascular endothelial cell [4]. It has been shown that proteolytic cleavage from transmembrane proteins results in liberation of a circulating form of ICAM-1 (sICAM-1) from activated endothelium[19], and the amount of sICAM-1 correlates with surface expression [20].

The ligand of ICAM-1 expressed on leukocyte were LFA-1 and Mac-1, these heterodimers all have the invariable portion of the CD18 [21]. Leukocytes use CD18 to tether themselves to ICAM-1 on the surface of microvascular endothelial cell. CD18 has been looked as a leukocyte marker mainly expressed on monocytes and neutrophils [7].

Evidence from diabetic rats shown that the expression of CD18 on the surface of neutrophile was higher than that in non diabetic control, and retinal leukostasis was significantly reduced by intravenous administration of an antibody to CD18 [2]. In the present study, we found that the neutrophilic CD18 expression was significantly elevated in all diabetic subgroups compared to control subjects. The progression of retinopathy was associated with an increase in neutrophilic CD18 expression even by multiple regression analysis. Theses findings were coincident with our previous report[14]. From these findings, we could induce that neutrophile play important role in the development of DR.

It was shown that increased numbers of neutrophils are activated in human diabetic subjects [22]. Additionally, increased leucostasis in the retinal microvasculature is also well documented in diabetic rat models [11]. Activation of leukocytes leads to functional activation of integrins constitutively expressed on the surface of the cells (CD11a/CD18) [23] and translocation to the surface membrane of integrin molecules stored in granules (CD11b/CD18) [6]. Therefore, increased β^2-integrin (CD18) activation/expression in the diabetic state might potentially contribute to enhance leukocyte recruitment.

Another adhesion molecule that has been proved crucial in leukocyte recruitment is ICAM-1. Immunoneutralization of this adhesion molecule has a potent blocking effect on leukocyte recruitment at retina of diabetic rats [3]. On the other hand, increased expression of ICAM-1 has been documented by immunohistochemistry in diabetic human retina and choroids [24]. We have shown in this paper that serum concentrations of sICAM-1 are higher in patients with DR than in normal controls, but the concentration of it among the DR subgroups are comparable. Stepwise multiple regression analysis revealed that sICAM-1 was not associated with the development of DR. As far as we know, only there studies [25-27] have examined the levels of sICAM-1 in the patients with DR. Two studies [26, 27] showed that serum sICAM-1 levels were higher in diabetic patients with retinopathy than that in control. The third study, by Boulbou *et al* [25], is the only one that analyzed the relationship between the sICAM-1 levels and the development of DR, these authors found that sICAM-1 was not involved in the development of retinopathy in type 2 diabetic patients. These findings were similar with ours in this study. In ocular, Hughes *et al* [28] also found that ICAM-1 was not involved with the development of DR. In a comparative immunohistochemical study, they compared the expression of ICAM-1 in the retinal vasculature of 41 eyes obtained from diabetic people with 19 eyes from 19 non-diabetic controls. Serial cryosections of postmortem posterior tissue from diabetic non-diabetic eyes were stained with the monoclonal antibodies ICAM-1. They found that a similar pattern of vascular ICAM-1 staining was observed between diabetic and non-diabetic eyes. These results indicated that ICAM-1 was constitutively expressed on retinal and choroidal vasculature of non-diabetic, control subjects and that this level of expression was not significantly altered by

the diabetic environment.

From the results of present study, we know that CD18 and ICAM-1 have different expression pattern, which indicated that these two adhesion molecule played different role in the development of DR. In the experimental diabetic model, Salas *et al* found that β^2-integrin and ICAM-1 played different role in exacerbation of ischemia- reperfusion-induced inflammatory response in diabetes. They studied leukocyte-endothelial cell interactions in mesenteric venules, the expression of ICAM-1 in mesenteric venule and β^2-integrin on rat leukocytes were also studied. They found that ischemia-reperfusion elicited significantly larger increases in leukocyte adhesion and emigration in diabetic rats than in control rats; β^2-integrin expression was higher in leukocytes from diabetic animals. Endothelial ICAM-1 in mesentery and in intestine did not differ between diabetic and control rats. These results indicate that diabetes is associated with an enhanced response to ischemia-reperfusion. An increased β^2-integrin, but not ICAM-1 expression, may account for the exaggerated inflammatory response to ischemia-reperfusion in diabetes[29].

Because CD18 and ICAM-1 are involved in the adhesion of leukocyte during the process of DR, meanwhile, they are the markers of leukocyte and endothelium, respectively, so the discrepancy of their expression in the present study indicates that leukocyte, but not endothelium, may play primary role in the development of DR in human. Some possible mechanisms primary to increased expression of ICAM-1 could be the narrowing of capillary lumens as a result of endothelial cell hypertrophy [30] and increased plasma levels of the vasoconstrictor endothelin-1[31]. In these situations it is feasible that even constitutive levels of ICAM-1 would be enough to adhere slower moving or partially obstructed leucocytes to the endothelium causing increased leucostasis.

Our results confirm the contribution of endothelial and neutrophilic activation in the development of DR as indicated by increased levels of CD18 and sICAM-1. However, a direct implication of CD18 and ICAM-1 in the progression of diabetic retinopathy can be supported only in the CD18 but not ICAM-1. CD18 and ICAM-1 may play different role in the development of diabetic retinopathy. These findings are especially relevant for the development of new therapeutic strategies to prevent retinal damage and indicate that in the diabetic condition, agents that block the action of CD18 can be particularly beneficial.

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REFERENCES

1 Kempen JH, O'Colmain BJ, Leske MC, Haffner SM, Klein R, Moss SE, Taylor HR, Hamman RF; Eye Diseases Prevalence Research Group. The prevalence of diabetic retinopathy among adults in the United States. *Arch Ophthalmol* 2004; 122:552-563

2 Barouch FC, Miyamoto K, Allport JR, Fujita K, Bursell SE, Aiello LP, Luscinskas FW, Adamis AP. Integrin-mediated neutrophil adhesion and retinal leukostasis in diabetes. *Invest Ophthalmol Vis Sci* 2000;41: 1153-1158

3 Miyamoto K, Khosrof S, Bursell SE, Rohan R, Murata T, Clermont AC, Aiello LP, Ogura Y, Adamis AP. Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. *Proc Natl Acad Sci U S A* 1999; 14;(96): 10836-10841

4 Videm V, Albrigtsen M. Soluble ICAM-1 and VCAM-1 as markers of endothelial activation. *Scand J Immunol* 2008; 67:523-531

5 Anderson DC, Springer TA. Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1 and P150, 95 glycoprotein. *Annu Rev Med* 1987; 38:175-194

6 Carlos TM., Harlan JM. Leukocyte-endothelial adhesion molecules. *Blood* 1994; 84: 2068-2101

7 Diamond DS, Springer TA. A subpopulation of Mac-1 (CD11b/CD18) molecules mediates neutrophil adhesion to ICAM-1 and fibrinogen. *J Cell Biol* 1993; 120:545-556

8 Joussen AM, Murata T, Tsujikawa A, Kirchhof B, Bursell SE, Adamis AP. Leukocyte-mediated endothelial cell injury and death in the diabetic retina. *Am J Pathol* 2001; 158:147-152

9 Joussen AM, Poulaki V, Mitsiades N, Cai WY, Suzuma I, Pak J, Ju ST, Rook SL, Esser P, Mitsiades CS, Kirchhof B, Adamis AP, Aiello LP. Suppression of Fas-FasL-induced endothelial cell apoptosis prevents diabetic blood-retinal barrier breakdown in a model of streptozotocin-induced diabetes. *FASEB J* 2003;17:76-78

10 Kohner EM, Patel V, Rassam SM. Role of blood flow and impaired autoregulation in the pathogenesis of diabetic retinopathy. *Diabetes* 1995; 44:603-607

11 Wierusz-Wysocka BH. Wysocki H. Siekierka A. Wykretowicz A. Szczepanik, Klimas R. Evidence of polymorphonuclear neutrophils (PMN) activation in patients with insulin dependent diabetes mellitus. *J Leukoc Biol* 1987; 42: 519-523

12 Ramachandran A, Snehalatha C, Latha E, Vijay V, Viswanathan M. Rising prevalence of NIDDM in urban population in India. *Diabetologia* 1997; 40:232-237

13 Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, Dills D, Kampik A, Pararajasegaram R, Verdaguer JT, Global DRP Project Group. Proposed international clinical DRP and diabetic macular edema disease severity scales. *Ophthalmology* 2003; 110:1677-1682

14 Song H, Wang L, Hui Y. Expression of CD18 on the neutrophils of patients with diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2007; 245:24-31

15 Antonetti DA, Barber AJ, Bronson SK, Freeman WM, Gardner TW, Jefferson LS, Kester M, Kimball SR, Krady JK, LaNoue KF, Norbury CC, Quinn PG, Sandirasegarane L, Simpson IA; JDRF Diabetic Retinopathy Center Group. Diabetic retinopathy: seeing beyond glucose-induced microvascular disease. *Diabetes* 2006; 55:2401-2411

16 Gonzalez-Amaro R, Sanchez-Madrid F. Cell adhesion molecules: selectins and integrins. *Crit Rev Immunol* 1999; 19: 389-429

17 Harrison AA, Stocker CJ, Chapman PT. Expression of vascular cell adhesion molecule-1 by vascular endothelial cells in immune and nonimmune inflammatory reactions in the skin. *J Immunol* 1997; 159: 4546-4554

18 Szekanecz Z, Koch AE. Endothelial cells in inflammation and angiogenesis. *Curr Drug Targets Inflamm Allergy* 2005; 4: 319-323

19 Sultan S, Gosling M, Nagase H, Powell JT. Shear stress-induced shedding of soluble intercellular adhesion molecule-1 from saphenous vein endothelium. *FEBS Lett* 2004; 564:161-165

20 Leeuwenberg JMF, Smeets EF, Neefjes JJ. E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells *in vitro*. *Immunology* 1992; 77:543-549

21 Joussen AM, Poulaki V, Le ML, Koizumi K, Esser C, Janicki H, Schraermeyer U, Kociok N, Fauser S, Kirchhof B, Kern TS, Adamis AP. A central role for inflammation in the pathogenesis of diabetic retinopathy. *FASEB J* 2004; 18:1450-1452

22 Wierusz-Wysocka BH. Wysocki H. Siekierka A. Wykretowicz A. Szczepanik, Klimas R. Evidence of polymorphonuclear neutrophils (PMN) activation in patients with insulin dependent diabetes mellitus. *J Leukoc Biol* 1987; 42: 519-523

23 Smith, CW, Marlin SD, Rothlein R, Toman C, and Anderson DC. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils *in vitro*. *J Clin Invest* 1989; 83: 2008-2017

24 McLeod, DS, Lefer DJ, Merges C, and Lutty GA. Enhanced expression of intracellular adhesion molecule-1 and P-selectin in the diabetic human retina and choroid. *Am J Pathol* 1995;147:642-653

25 Boulbou MS, Koukoulis GN, Petinaki EA, Germenis A, Gourgoulianis KI. Soluble adhesion molecules are not involved in the development of retinopathy in type 2 diabetic patients. *Acta Diabetol* 2004;41:118-122

26 Fasching P, Veitl M, Rohac M, Streli C, Schneider B, Waldhäusl W, Wagner OF. Elevated concentrations of circulating adhesion molecules and their association with microvascular complications in insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1996;81:4313-4317

27 Matsumoto K, Sera Y, Ueki Y, Inukai G, Niiro E, Miyake S. Comparison of serum concentrations of soluble adhesion molecules in diabetic microangiopathy and macroangiopathy. *Diabet Med* 2002; 19(10): 822-826

28 Hughes JM, Brink A, Witmer AN, Hanraads-de Riemer M, Klaassen I, Schlingemann RO. Vascular leucocyte adhesion molecules unaltered in the human retina in diabetes. *Br J Ophthalmol* 2004; 88:566-572

29 Salas A, Panès J, Elizalde JI, Casadevall M, Anderson DC, Granger DN, Piquè JM. Mechanisms responsible for enhanced inflammatory response to ischemia-reperfusion in diabetes. *Am J Physiol* 1998;275(5 Pt 2): H1773-1781

30 Hofman P, van Blijswijk BC, Gaillard PJ. Endothelial cell hypertrophy induced by vascular endothelial growth factor in the retina: new insights into the pathogenesis of capillary nonperfusion. *Arch Ophthalmol* 2001; 119:861-866

31 Takahashi K, Ghatei MA, Lam HC. Elevated plasma endothelin in patients with diabetes mellitus. *Diabetologia* 1990;33:306-310