Biochemical and Biophysical Research Communications 381 (2009) 96–101

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Resistin up-regulates fractalkine expression in human endothelial cells: Lack of additive effect with TNF-α

Ileana Manduteanu *, Elena Dragomir, Manuela Calin, Monica Pirvulescu, Ana Maria Gan, Daniela Stan, Maya Simionescu

Institute of Cellular Biology and Pathology "Nicolae Simionescu", 8 B.P. Hasdeu Street, P.O. Box 35-14, 050568 Bucharest, Romania

ARTICLE INFO

Article history:
Received 30 January 2009
Available online 11 February 2009

Keywords:
Endothelial cell
Resistin
Fractalkine
Cytokine
Inflammation
TNF-α
TNFR1

ABSTRACT

Resistin is a cytokine and fractalkine (Fk) a cell adhesion molecule and chemokine that contribute to human vascular inflammation by mechanisms not clearly defined. We questioned whether resistin induces Fk expression in human endothelial cells (HEC), compared the effect with that of the pro-inflammatory cytokine, TNF-α, and evaluated the consequences of co-stimulating HEC with both activators on Fk induction and on the signalling molecules involved. We found that resistin up-regulated Fk expression at comparable level to that of TNF-α by a mechanism involving P38 and JNK MAPK and NF-κB. Co-stimulation of cells with resistin and TNF-α did not increase Fk expression induced by every single inducer. Moreover resistin reduced the expression induced by TNF-α in HEC. The new data uncover Fk as a novel molecular link between resistin and inflammation and show that resistin and TNF-α have no additive effect in Fk up-regulation or on the signalling molecules implicated.

Introduction

The vascular endothelium plays a key role in the initiation of the inflammatory process associated with numerous diseases such as atherosclerosis and diabetes [1]. The inflammatory process entails the regulated expression of endothelial cell (EC) adhesion molecules and chemokines that elicit major roles in leukocyte recruitment. Fractalkine (Fk) has dual function acting both as a cell adhesion molecule and chemokine mediating direct capture, firm adhesion and transmigration of leukocytes such as monocytes, cytotoxic T lymphocytes or natural killer cells [2]. Fk is expressed by EC, vascular smooth muscle cells, dentritic cells and neurons [3–5] and Fk-mediated leukocyte adhesion was shown to play important roles in mediating inflammatory diseases [6,7]. Fk expression is enhanced in human atherosclerotic plaques [8], and is up-regulated by pro-inflammatory agents like TNF-α, LPS, IL-1 IFN-γ and CD40L while others such as IL-4 and IL-13 inhibit its expression [9,10].

Resistin is a newly described 12.5 kDa cytokine [11] with an unclear function in humans were it is assumed to be involved in inflammation [12]. Peripheral blood mononuclear cells and macrophages are the primary source of resistin in humans [12,13]. Serum resistin levels are elevated in patients with obesity-associated diabetes or cardiovascular disease [14–16]. Resistin which is present in both murine and human atherosclerotic lesions [17] was suggested to be an inflammatory marker of atherosclerosis in humans [14] and to promote atherogenesis by activating EC [18,19].

Since both molecules are involved in inflammation-associated with various pathologies, we hypothesised that a link may exist between them and questioned whether resistin modulates Fk expression in human endothelial cells (HEC) and compared its effects with TNF-α. Moreover given that resistin and TNF-α are often found together in the inflammatory areas, we evaluated the effect of co-stimulating HEC with both activators on Fk induction. We report here that resistin up-regulates significantly Fk expression at similar levels as TNF-α by a mechanism involving p38 and JNK MAPK and NF-κB. Furthermore we show that HEC exposed to both, resistin and TNF-α, do not exhibit an increase in Fk expression compared to that induced by every single activator and that during co-stimulation TNFR1 expression was reduced compared to the level of TNF-α acting alone.

Materials and methods

Cells. Human endothelial cells (HEC), the EAhy926 cell line was kindly donated by Dr. Cora Jean Edgell (Department of Pathology, University of North Carolina, Chapel Hill). The cells were grown in Petri dishes (60 mm diameter) or in tissue culture plates of 6, 24 or 96 wells.

Reagents. Human recombinant resistin, the monoclonal antibodies to p65 subunit of the transcription factor NF-κB and to p-JNK and p-p38 were from Santa Cruz Biotechnology. The monoclonal antibodies to fractalkine and TNFR1 were from R and

* Corresponding author. Fax: +40 21 3194519.
E-mail address: ileana.manduteanu@icbp.ro (I. Manduteanu).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.02.015

D Systems, the secondary antibodies and all the other reagents were from Sigma Aldrich Chemie GmbH (Germany). Proteins were detected using the Signal West Pico Chemiluminescent Substrate kit from Pierce (Rockford USA).

Cell culture and experimental design. HEC were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf bovine serum. The cells were exposed to resistin (R) (100 ng/ml), TNF-α (T) (10 ng/ml) or to both, resistin and TNF-α (RT) for 6, 18 and 24 h, and subsequently analyzed for Fk expression by RT–PCR and Western blot assays. To search for the mechanisms underlying the resistin effect on cells, 10 μM kinase inhibitors SB (inhibitor of p38 MAPK), and 25 μM SP600125 (inhibitor of JNK MAPK) and 50 μM of NF-κB transcription factor inhibitors (PDTC) were added to the culture medium 30 min before activating HEC and then the modulation of Fk expression was analyzed.

Reverse transcriptase–polymerase chain reaction (RT–PCR). Total cellular RNA was extracted from HEC using High Pure RNA isolation kit. First-strand cDNA synthesis was performed using 1 μg of total RNA and MMLV reverse transcriptase, according to the manufacturer's protocol. The PCR reaction was performed at 61 °C as annealing temperature using GoTaq polymerase, cDNA and the primers: for fractalkine sense 5′-AACTCGAAATGGCGGCAC CTT and antisense 5′-ATGAATTACTACCACAGCTCCG; for GAPDH: sense 5′-ACCACAGTCCATGCCATCAC and antisense 5′-TCCAC-CACCCTGTTGCTGTA. The DNA fragment was amplified in 35 cycles (30 s, 94 °C; 30 s, 61 °C; 45 s, 72 °C) followed by a final extension step (7 min, 72 °C). The products were subjected to electrophoresis on 1.5% agarose gel and analyzed on a gel analyzer system (Image Master VDS-Pharmacia Biotech). The Fk mRNA level was normalized to GAPDH mRNA level.

Detection of fractalkine protein expression in HEC by western blot. Cells were solubilised in 2× sodium dodecyl sulfate (SDS) gel sample buffer, boiled in a water bath (5 min), sonicated and diluted with 1× SDS sample buffer. 50 μg protein was subjected to 10% polyacrylamide gels electrophoresis, and transferred onto nitrocellulose membranes (using a Trans Blot Semi-Dry system). The blots blocked in 5% nonfat dry milk in TBS and 0.05% Tween (BB) were incubated with anti-human fractalkine mouse monoclonal antibody (1:500 dilution in BB), overnight, at 4 °C. After washing, the membranes were incubated with the secondary antibody (1:2000 dilution in BB) for 1 h at room temperature followed by washing in TBS-0.05% Tween (WB). The blots were incubated with enhanced chemiluminescence reagents (ECL) for 5 min and exposed to an X-ray film that was analyzed with a video system (Image Master from Pharmacia); the optical density was calculated with the Total Lab 1.11 software from Pharmacia.

Determination of P38 and JNK MAPK activation. This was done by assessing by western blot the phosphorylation of the p38, JNK and c-Jun proteins in HEC lysates. Briefly, samples boiled for 5 min were subjected to 10% SDS–PAGE and transfer to nitrocellulose membranes. After blocking for 2 h in BB, he blots were reacted with either (1) the affinity-purified rabbit anti-phospho-p38 MAPK antibody (0.5 μg/ml), (2) affinity-purified rabbit anti-phospho c-Jun (Ser 63/73) or (3) mouse monoclonal IgG-anti-phospho-JNK (2 μg/ml) overnight at 4°C, followed by HRP-conjugated goat anti-mouse or anti rabbit monoclonal antibody (1:2000) for 1 h. The detection was carried out by luminescence using the Super-Signal West Pico substrate from Pierce.

Evaluation of NF-κB activation. Western blot analysis of the p65 protein in nuclear fractions of HEC obtained as described in [20] was performed. Proteins (50 μg) were separated on 12% polyacrylamide gel, transferred to nitrocellulose membrane, blocked 1 h with BB, and incubated overnight with 2 μg/ml of anti-p65 subunit of NF-κB (primary antibody). After washing four times with WB, the membranes were incubated with the secondary antibody (1:3000) followed by ECL reagents for 5 min and exposed to an X-ray film. The films were analyzed with a video system (Image Master from Pharmacia) and the optical density of the bands was calculated with the Total Lab 1.11 software from Pharmacia.

Statistical analysis of the data. Statistical processing of the data was performed using the one-way analysis variance between groups (ANOVA) Program of Origin. All values were expressed as means ± SEM. p values of <0.05 were considered statistically significant.

Results

Resistin induces fractalkine expression in human endothelial cells

To establish the optimal experimental conditions we exposed HEC to different resistin concentrations (10, 25, 50 and 100 ng/ml) and for different time points (6, 18 and 24 h) and the mRNA and protein expressions of Fk were determined. The experiments showed that resistin up-regulated Fk expression in a dose and time dependent manner. Fk mRNA and protein level increased as a function of resistin concentration, a statistically significant value was obtained at 100 ng/ml (not shown). The maximum Fk mRNA was detected at 6 h then, decreased at 18 and 24 h at control value (Fig. 1A). The data corroborated well with the protein assay that revealed that resistin induced maximal Fk protein expression at 6 h, then decreased but it was still statistically significant expressed after 18 h (∼1,2 fold over control value) and reached the control level after 24 h of activation (data not shown). Based on these results all further experiments were done on confluent HEC exposed to 100 ng/ml Fk for 6 h.

Resistin induces fractalkine expression similarly to TNF-α and when acting together, they do not have additive effects

The potential of resistin to induce Fk expression in HEC was assessed by evaluating the Fk expression induced by resistin in comparison with TNF-α, and upon cell co-stimulation with both, resistin and TNF-α.

The RT–PCR data showed that resistin induced a significant (∼2.4-fold) increase in Fk mRNA expression above the control (unstimulated cells); likewise, a significant augmentation of Fk mRNA expression was detected after TNF-α stimulation (∼2.9-fold). Unexpectedly, the Fk mRNA detected in HEC exposed to both, resistin and TNF-α, did not exceed the level induced by each cytokine acting alone (∼2,2-fold increase above the control level) (Fig. 1B).

Western blot assays showed that resistin induced a significant increase in Fk protein level (∼2.3-fold over the control) similar to that induced by TNF-α. After co-stimulation with resistin and TNF-α, the Fk protein level did not exceed the mean value of each inducer acting separately (Fig. 1C).

Inhibition of p38, JNK and NF-κB reduces resistin-stimulated fractalkine expression

We carried out experiments to asses whether MAPK and NF-κB pathways are implicated in resistin-stimulated Fk expression in HEC. The cells were exposed to specific inhibitors (SB, SP600125, PDTC) before activation with resistin and then subjected to RT–PCR and Western blot. The results revealed that preincubation of HEC with inhibitors of p38 MAPK or JNK MAPK or with the NF-κB inhibitor (PDTC) reduced significantly the Fk mRNA and protein expression in resistin-stimulated HEC (Fig. 2A and B).

Resistin, activates JNK and p38 MAPK and the transcription factor NF-κB at similar levels as TNF-α

The above results suggested that JNK, P38 MAPK and the transcription factor NF-κB are involved in Fk induction by resistin in HEC. To

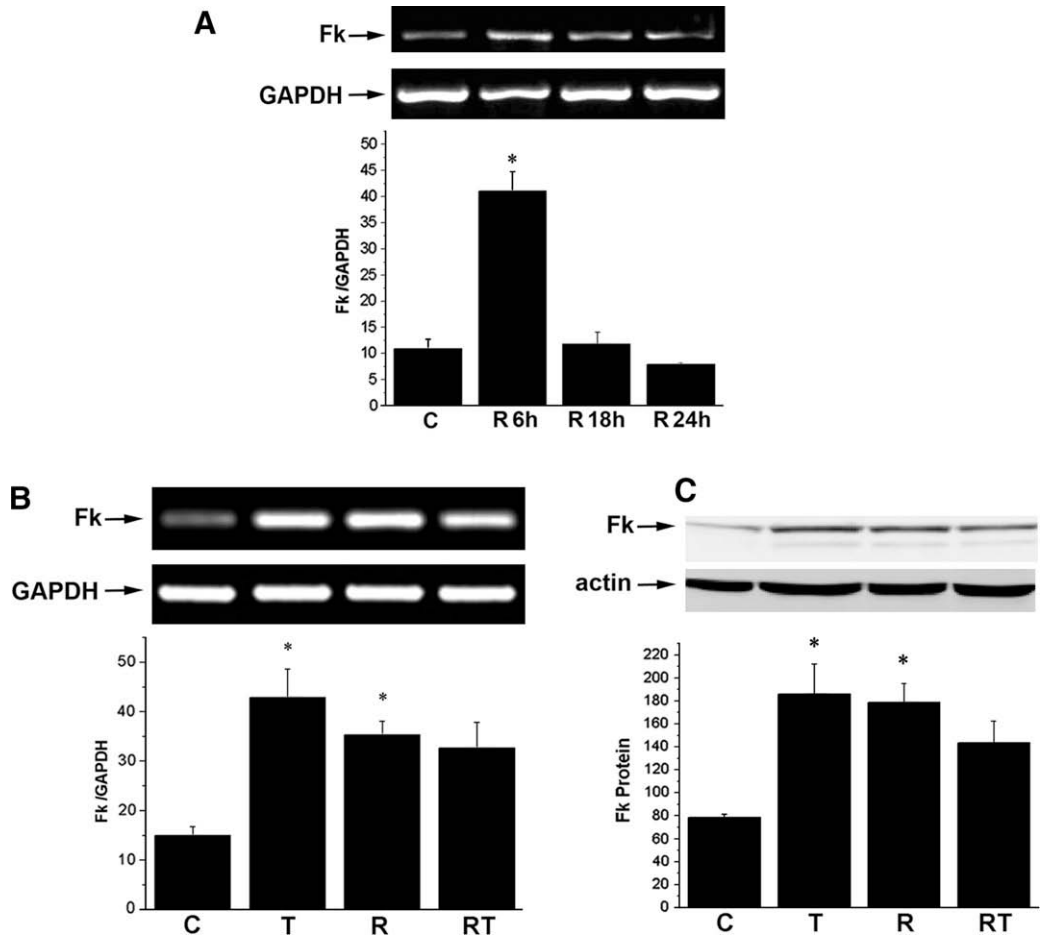

Fig. 1. Resistin induces fractalkine expression in HEC. (A) Fractalkine (Fk) mRNA expression as a function of time. The data are from one representative experiment (upper panel); densitometric analysis (lower panel). Bars represent the mean ± SE ($n = 3$). *Significantly different from control, $p < 0.05$. (B) Induction of Fk mRNA by resistin (R), TNF-α (T) or both (RT) in HEC (RT–PCR). A representative experiment (upper panel) and the densitometric analysis (lower panel). (C) Induction of Fk protein expression by R, T, or RT. A representative Western blot (upper panel) and densitometric analysis (lower panel). Bars: the mean ± SE ($n = 5$). *Significantly different from control (C), $p < 0.05$.

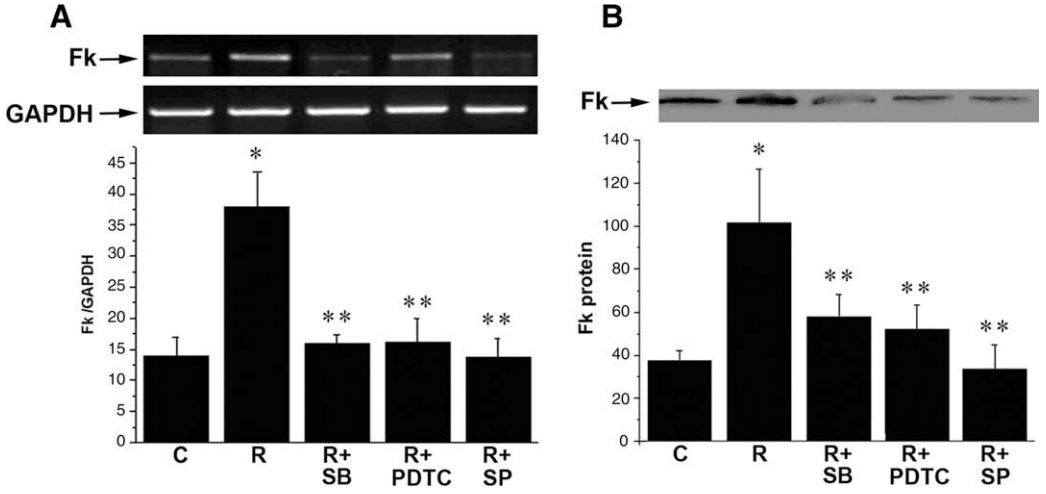

Fig. 2. Effect of inhibitors of p38 (R+SB) and JNK MAPK (R+SP) and NF-κB (R+PDTC) on resistin (R)-induced fractalkine expression in HEC. (A) Representative RT–PCR for fractalkine (Fk) mRNA (upper panel) and densitometric analysis (lower panel). (B) Representative Western blot (upper panel) and densitometric analysis (lower panel). Results are means ± SE ($n = 3$). *Significantly different from control (C); $p < 0.05$. **Significantly different from R.

better evaluate resistin-generated Fk up-regulation we performed experiments to assess the direct role of resistin in JNK, p38 MAPK and NF-κB activation in HEC by comparison to the effect of TNF-α.

HEC were stimulated with resistin, TNF-α or with resistin and TNF-α and then the activation of JNK and p38 MAPK, was examined (Western blot), by detecting the level of phosphorylation of JNK and c-Jun (considered a JNK-specific target) and of p38 MAPK. The results showed that resistin significantly phosphorylates JNK (Fig 3A) and c-jun (Fig 3B) at a level similar to that induced by TNF-α. Resistin and TNF-α induced a significant increase in p38

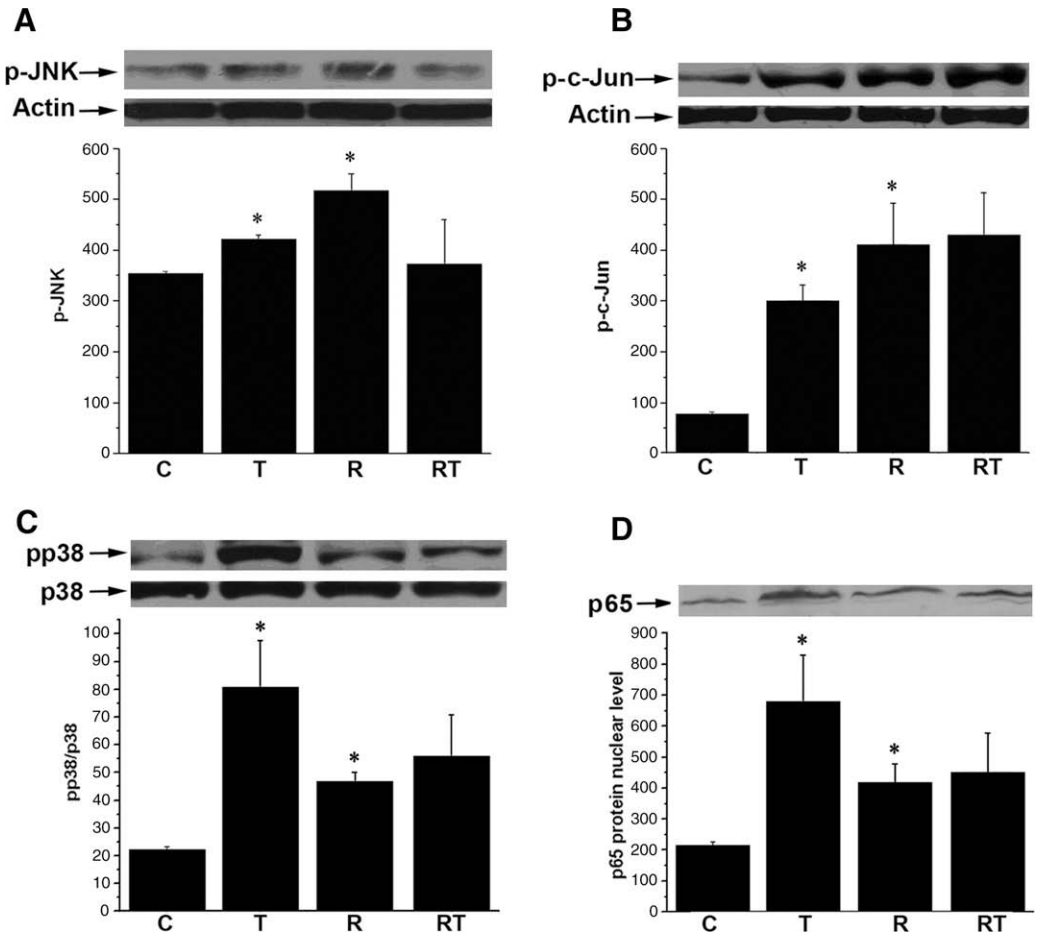

Fig. 3. Resistin induces activation of JNK, c-Jun, p38 and NF-κB similarly to TNF-α. (A–C) Detection by Western Blot of p-JNK (A, upper panel), p-c-Jun (B, upper panel) and p-p38 (C, upper panel). Densitometric analysis of p-JNK (A, lower panel), p-c-Jun (B, lower panel) and p-p38(C, lower panel). Data are expressed as means ± SE ($n = 3$). *Significantly different from control (C), $p < 0.05$. The data represent the means ± SE ($n = 3$). *Significantly different from control, $p < 0.05$. (D) NF-κB activation. The p65 protein level (upper panel) and the quantitative densitometric analysis (lower panel). Values are means ± SE ($n = 3$). $p < 0.05$. *Significantly different from control (C).

phosphorylation compared to unstimulated, control cells (Fig. 3C); although the effect of TNF-α appeared greater than that of resistin, there were no statistically significant differences between the two.

The role of NF-κB was estimated by Western blot analysis of p65 protein expression in nuclear lysates of HEC exposed to resistin, TNF-α or with resistin and TNF-α. The results showed that under normal conditions HEC exhibited a low level of expression of p65 whereas exposure of cells to resistin or TNF-α induced a significant increase in nuclear p65 level (Fig. 3D). Co-stimulation of cells with combined inductors significantly phosphorylated JNK, c-Jun and p38 at a level not exceeding that induced by each cytokine acting alone (Fig. 3A–C); the nuclear p65 protein level that was not statistically significant when compared to the effects of resistin or TNF-α acting alone (Fig. 3D).

Co-stimulation with resistin and TNF-α reduces the TNF-α induced TNFR1 expression

The data obtained in our experiments showed that resistin has a similar inflammatory effect to that of TNF-α, but upon co-stimulation, the two activators did not have additive effects on Fk expression or on the signalling molecules. To assess whether TNFR1 has a role in this process, we evaluated the effect of resistin, TNF-α, or both on TNFR1 expression in HEC. The results showed that after 6 h stimulation, resistin had no significant effect on the constitutive TNFR1 expression, whereas TNF-α up-regulated the receptor expression (~2.2-fold) over the constitutive level. Compared to the level of TNF-α, co-stimulation with resistin and TNF-α, led to a significant reduction in the mRNA (Fig. 4A) and protein expression of TNFR1 (Fig. 4B).

Discussions

Accumulated data designate resistin as a significant local and systemic modulator of inflammation [12,15,20] and Fk to be critically involved in inflammation [2,6,8]. Since both molecules are present in inflammatory areas, we searched for the direct effect of resistin on Fk expression in cultured HEC. We have found that resistin induces a significant increase in Fk expression at a maximal level after 6 h of HEC activation, similar to that induced by the potent inflammatory cytokine, TNF-α. However in our experimental conditions as in other similar studies [21], TNF-α had a more persistent effect and Fk mRNA and protein level were still significantly up-regulated at 24 h of cell activation. Furher experiments will elucidate the mechanisms involved.

Nevertheless, the multifaceted role of resistin in the up-regulation of fractalkine (this study), ICAM-1, VCAM-1 and MCP-1 [18,19] indicate its role in the initiation and evolution of the inflammatory process. Since it was reported that concomitant expression of fractalkine, ICAM-1, and VCAM-1 results in enhancement of leukocyte adhesion compared with each system alone [8], we may speculate that resistin-induced Fk expression may contribute to the enhancement of leukocyte adhesion and amplification of the inflammatory

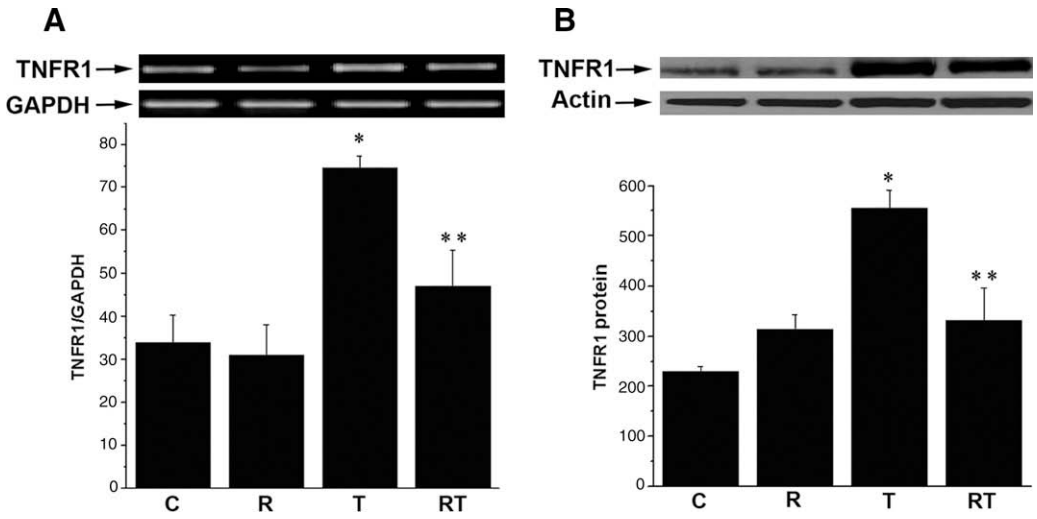

Fig. 4. Effect of resistin on TNFR1 mRNA (A) and protein expression (B) in HEC exposed to T, R, or the RT. TNFR1mRNA was detected by RT–PCR and protein expression by Western blot. Representative blots (upper panels) and densitometric analysis (lower panels) are shown. Data are expressed as means ± SE ($n = 5$). *Significantly different from control, $p < 0.05$. **Significantly different from T.

process. Investigation of the molecular mechanisms involved in resistin-induced increased in Fk expression in HEC revealed the involvement of p38 and JNK MAPK pathways in the process. Our data showed, similar to previous studies, that resistin activates directly the p38 and JNK MAPK [22]. To our knowledge this study is the first to compare resistin and TNF-α effect on JNK and p38 MAPK activation.

Moreover, resistin significantly increased c-Jun phosphorylation, which may indicate an enhanced transcriptional activity [23]. Since c-Jun is a component of the transcription factor AP-1 the data suggest that AP-1 may be involved in the process as reported for resistin-induced endothelin-1 in EC [19].

In addition, our data showed that NF-κB is implicated in resistin-induced Fk up-regulation in HEC. The data are in agreement and extend previous reports demonstrating the essential role of NF-κB in Fk induction by other cytokines [3,22], and in the resistin-induced ICAM-1 and VCAM-1 in HEC [18]. Interestingly, in our experiments on HEC, resistin activated NF-κB at a level similar to that of TNF-α signifying that resistin may be also considered a powerful inflammatory agent.

The relationship between resistin and TNF-α in human is not well known. Since it was reported that resistin and TNF-α may be found concurrently in inflammatory areas [24] and it is not known if their coexistence leads to a stronger inflammatory process, we examined the Fk expression in HEC stimulated concurrently with both, TNF-α and resistin. Unexpectedly, the data obtained showed that although both resistin and TNF-α induce Fk in HEC, the two cytokines do not have additive effects on Fk induction or in the activation of the signalling proteins involved in Fk up-regulation, suggesting that the simultaneous presence of resistin and TNF-α does not necessary lead to an increase in the inflammatory process.

Since in EC the TNF-α signalling events are dependent on the interaction of TNF-α with TNFR1 [25], we carried out experiments to assess if resistin influence the receptor expression in HEC We found that the constitutive expression of TNFR1was not significantly changed by resistin whereas in the case of co-stimulation of HEC with resistin and TNF-α, the receptor expression was reduced to a value lower than the level induced by TNF-acting alone; we may speculate that when both cytokines are present, resistin interferes with the signalling pathway of TNF-α.

In contrast it has been reported that resistin had additive effects with CD40L in MCP-1 production [19] without altering the CD40 receptor expression. Together, these data and our results indicate that resistin is an important modulator of inflammation acting as a function of specific inflammatory microenvironment.

In conclusion our results show that in HEC (1) resistin induces a significant increase in fractalkine expression at a level similar to that of TNF-α and that NF-κB and p38 and JNK MAPK participate in the process; (2) resistin and TNF-α do not have additive effects in the up-regulation of fractalkine; (3) resistin reduces the TNFR1 mRNA and protein expression during co stimulation with TNF-α. To the best of our knowledge, this is the first report on the effect of resistin on Fk expression and on the relationships between resistin and TNF-α in HEC. The study adds new data on the inflammatory potential of resistin, sheds new light in the cross talk between resistin and TNF-α in HEC, and uncovers Fk as a new molecular link between resistin and inflammation.

Acknowledgments

The authors are indebted for excellent technical help of Mrs. Gabriela Mesca and Ioana Manolescu, and to Mrs. Marilena Daju (graphic and editorial support).

The project was supported by the Romanian Academy and grants from the Ministry of Education and Research—CNMP Grant No. 41037, European Community-SSA Contract No 16873 and COST Action BM0602.

References

[1] M. Simionescu, Implications of early structural–functional changes in the endothelium for vascular disease, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 266–274.
[2] H. Umehara, E.T. Bloom, T. Okazaki, Y. Nagano, O. Yoshie, T. Imai, Fractalkine in vascular biology, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 34–40.
[3] S.Y. Ahn, C.H. Cho, K.G. Park, H.J. Lee, S. Lee, S.K. Park, I.K. Lee, G.Y. Koh, Tumor necrosis factor-alpha induces fractalkine expression preferentially in arterial endothelial cells and mithramycin A suppresses TNF-alpha-induced fractalkine expression, Am. J. Pathol. 164 (2004) 1663–1672.
[4] A. Ludwig, T. Berkhout, K. Moores, P. Groot, G. Chapman, Fractalkine is expressed by smooth muscle cells in response to IFN-gamma and TNF-alpha and is modulated by metalloproteinase activity, J. Immunol. 168 (2002) 604–612.
[5] W.J. Schwabe, C.M. Stover, T.J. Schall, et al., Neuronal expression of fractalkine in the presence and absence of inflammation, FEBS Lett. 439 (1998) 203–207.
[6] J.H. Ruth, M.V. Volin, G.K. Haines, et al., Fractalkine, a novel chemokine in rheumatic arthritis and in rat adjuvant-induced arthritis, Arthritis Rheum. 44 (2001) 1568–1581.
[7] B.W.C. Wong, D. Wong, B.M. McManus, Characterization of fractalkine (CXCL1) and CX3CR1 in human coronary arteries with native atherosclerosis, diabetes

mellitus, and transplant vascular disease, Cardiovasc. Pathol. 11 (2002) 332–338.
[8] C. Schulz, A. Schäfer, M. Stolla, S. Kerstan, M. Lorenz, M.L. von Brühl, M. Schiemann, J. Bauersachs, T. Gloe, D.H. Busch, M. Gawaz, S. Massberg, Chemokine fractalkine mediates leukocyte recruitment to inflammatory endothelial cells in flowing whole blood; a critical role for P-selectin expressed on activated platelets, Circulation 116 (2007) 764–773.
[9] P. Fraticelli, M. Sironi, G. Bianchi, D. D'Ambrosio, C. Albanesi, A. Stoppacciaro, M. Chieppa, P. Allavena, L. Ruco, G. Girolomoni, F. Sinigaglia, A. Vecci, A. Mantovani, Fractalkine (CX3CL1) as an amplification circuit of polarized Th1 responses, J. Clin. Invest. 107 (2001) 1173–1181.
[10] C.A. Bolovan-Fritts, R.N. Trout, S.A. Spector, Human cytomegalovirus-specific CD4+T-cell cytokine response Induces fractalkine in endothelial cells, J. Virol. 78 (23) (2004) 13173–13181.
[11] C.M. Steppan, S.T. Bailey, S. Bhat, E.J. Brown, R.R. Banerjee, C.M. Wright, H.R. Patel, R.S. Ahima, M.A. Lazar, The hormone resistin links obesity to diabetes, Nature 409 (2001) 307–312.
[12] I. Nagaev, M. Bokareva, A. Tarkowski, U. Smith, Human resistin is a systemic immune-derived proinflammatory cytokine targeting both leukocytes and adypocytes, PLOS One 1 (2006) 1–9.
[13] H.S. Jung, K. Park, Y. Cho, S. Chung, H. Cho, S. Cho, S. Kim, H. Lee, K. Park, Resistin is secreted from macrophages in atheromas and promotes atherosclerosis, Cardiovasc. Res. 69 (2006) 76–85.
[14] M.P. Reilly, M. Lehrke, M.L. Wolfe, A. Rohatgi, M.A. Lazar, D.J. Rader, Resistin is an inflammatory marker of atherosclerosis in humans, Circulation 111 (2005) 932–939.
[15] L. Michael, P.R. Muredach, C. Segan, Millington, I. Nayyar, J.R. Daniel, A.L. Mitchell, An inflammatory cascade leading to hyperresistinemia in humans, PLOS Med. 1 (2004) 161–168.
[16] Y. Takatai, H. Osawa, M. Kurata, M. Kurokawa, J. Yamauchi, M. Ochi, W. Nishida, T. Okura, J. Higaki, H. Makino, Hyperresistemia is associated with coexistence of hypertension and type 2 diabetes, Hypertension 51 (2008) 534–539.
[17] M. Burnett, C. Lee, T. Kinnaird, E. Stabile, S. Durrani, M. Dullum, J. Devaney, C. Fishman, S. Stamou, D. Canos, The potential role of resistin in atherogenesis, Atherosclerosis 182 (2005) 241–248.
[18] D. Kawanami, K. Maemura, N. Takeda, T. Harada, T. Nojiri, Y. Imai, I. Manabe, K. Utsunomiya, R. Nagai, Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-EC interactions, Biochem. Biophys. Res. Commun. 314 (2004) 415–419.
[19] S. Verma, S.H. Li, C.H. Wang, P.W. Fedak, R.K. Li, R.D. Weisel, D.A. Mickle, Resistin promotes EC activation: further evidence of adipokine–endothelial interaction, Circulation 108 (6) (2003) 736–740.
[20] I. Manduteanu, M. Voinea, F. Antohe, E. Dragomir, M. Capraru, L. Radulescu, M. Simionescu, Effect of enoxaparin on high glucose induced activation of endothelial cells, Eur. J. Pharmacol. 477 (2003) 269–276.
[21] S.O. Moon, W. Kim, M.J. Sung, S. Lee, K.P. Kang, D.H. Kim, S.Y. Lee, J.N. So, S.K. Park, Resveratrol suppresses tumor necrosis factor-α-induced fractalkine expression in endothelial cells, Molec. Pharmacol. 70 (2006) 112–119.
[22] Y.H. Shen, L. Zhang, Y. Gan, X. Wang, J. Wang, S.A. LeMaire, J.S. Coselli, X.L. Wang, Up-regulation of PTEN mediates p38 MAPK stress signal-induced inhibition of insulin signaling—a cross-talk between stress signaling and insulin signaling in resistin-treated human endothelial cells, J. Biol. Chem. 281 (12) (2006) 7727–7736.
[23] A. Minden, A. Lin, T. Smeal, B. Derijard, M. Cobb, R. Davis, M. Karin, C-Jun N-terminal phosphorylation correlates with activation of the JNK subgroup but not the ERK subgroup of mitogen-activated protein kinases, Mol. Cell. Biol. 14 (1994) 6683–6688.
[24] K. Migita, Y. Maeda, T. Miyashita, H. Kimura, M. Nakamura, H. Ishibashi, K. Eguchi, The serum levels of resistin in rheumatoid arthritis patients, Clin. Exp. Rheumatol. 24 (6) (2006) 698–701.
[25] D. Luo, Y. Luo, Y. He, H. Zhang, R. Zhang, X. Li, W.L. Dobrucki, A.J. Sinusas, W.C. Sessa, W. Min, Differential functions of tumor necrosis factor receptor 1 and 2 signaling in ischemia-mediated arteriogenesis and angiogenesis, Am. J. Pathol. 169 (5) (2006) 1886–1897.