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ORIGINAL ARTICLE

Oxidized laminin-1 induces increased monocyte attachment and expression of ICAM-1 in endothelial cells

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Summary

Atherosclerosis has been associated with increased oxidative stress and monocyte recruitment by endothelial cells. Sub-endothelial basement membrane proteins, such as laminins that play a central role in cell adhesion, are exposed to reactive oxygen species. In the present study monocyte attachment on human umbilical cord vein endothelial cells (HUVEC) that were preattached to oxidized or native laminin, was investigated. Intracellular cell adhesion molecule-1 (ICAM-1) expression by HUVEC was estimated by an enzyme-linked immunosorbent assay. HUVEC attachment to oxidized or native laminin-1 was examined using the Hemacolor kit. Anti-alphaL, anti-alphaM, anti-alpha2 and anti-beta2 integrin subunit antibodies were used in order to further investigate the above phenomena. HUVEC that were preattached to oxidized laminin expressed higher levels of ICAM-1 and monocytes attached at a higher degree to these cells as compared to HUVEC that were preattached to native laminin. Incubation of monocytes with monoclonal antibodies against the alphaM and beta2 integrin subunits equalized the above mentioned differences. Moreover, HUVEC attached to oxidized laminin at a higher degree as compared to native laminin. This difference was equalized after incubation with the antibody against the alpha2 integrin subunit. These results indicate a modified interaction between HUVEC and the basement membranes in cases where laminin is oxidatively modified. This modified interaction results in increased ICAM-1 expression by endothelial cells and consequently increased monocyte recruitment capacity.

Keywords
attachment, human umbilical cord vein endothelial cells, laminin-1, monocytes, oxidation

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Basement membrane (BM) proteins can be oxidized after their exposure to reactive oxygen species such as superoxide anions, hydrogen peroxide and hydroxyl radicals (Ahmed *et al.* 2003). This oxidation may alter protein configuration and properties, thus affecting its interactions with several types of cells, including monocytes and endothelial cells and

therefore may represent a novel mechanism in the initiation of atherosclerosis.

Laminins are multidomain and multifunctional cross-shaped glycoproteins which are key structural and functional components of the basement membranes. Moreover, they play a central role in many biological functions such as cell adhesion, differentiation and migration of several cell types (Castronovo 1993; De Arcangelis *et al*., 1996). Previous studies performed in our laboratory indicated that laminin as well as collagen IV carbonylation strongly influenced its interactions with monocytes (Kostidou *et al*. 2007, 2008). Accordingly, laminin oxidation/carbonylation may also change its interactions with endothelial cells affecting their subsequent interactions with monocytes and contributing in this way to the formation of the atheromatic lesion.

Monocyte attachment to and migration through the activated endothelium represents the first crucial step which leads to the initiation of atherosclerosis (Osterud & Bjorklid 2003). Firm adhesion between monocytes and endothelial cells is mediated by the interactions between the supergene immunoglobins (ICAM-1, ICAM-2, VCAM-1) located on endothelial cells with their ligands, the beta2 integrins (β2, CD18) located on most leukocytes (Anderson 1995). Intracellular cell adhesion molecule-1 (ICAM-1) promotes monocyte attachment to endothelial cells through interaction with the beta2 (CD11/CD18) integrins. CD11 subunits, alphaL (aL, CD11a) and alphaM (aM, CD11b) are expressed on monocytes and bind to different domains on ICAM-1 (Anderson 1995; Huo & Ley 2001). ICAM-1 release and expression is induced by several cytokines (Carley *et al*. 1999) or ROS, such as hydrogen peroxide (Lo *et al*. 1993) and is regulated transcriptionally (Panes *et al*. 1995). It has been reported that chemotactic factors, ROS and phorbol esters induce increased attachment of neutrophils to endothelial cells by the involvement of the beta2 (CD11/CD18) integrins (Anderson *et al*. 1986; Seliak *et al*. 1994). However, the involvement of the endothelial cell BM interaction to the above phenomenon has never been studied.

In the present study, monocyte attachment to HUVEC that were preattached to either native or oxidized laminin has been investigated.

Materials and methods

Materials

Laminin-1 was isolated from EHS (Engelbreth-Holm-Swarm) tumour. Medium Earle 199, basal Iscove medium (IMDM) plus $NaHCO_3$ and Percoll were purchased from Biochrom (Cambridge, UK). 3,3′,5,5′-Tetramethylbenzidine dihydrochloride (TMB) tablet, anti-mouse antibody HRP-conjugated IgG and o-dianisidinedihydrochloride tablet were from Sigma (St. Louis, MO, USA). Hydrogen peroxide (30%), ascorbic acid and Hemacolor staining kit were from Merck (Darmstadt, Germany). Anti-ICAM-1 monoclonal antibody [Mouse anti-human IgG1, epitope FL (h)] was purchased from Santa Cruz Biotechnology, Inc (Heidelberg, Germany). Ferrous ammonium sulphate and hexadecyltrimethylammonium bromide were from Fluka (Seelze, Germany). Acetic acid was from Applichem (Darmstadt, Germany) and anti-CD49b-FITC (Mouse anti-human IgG 2ak, clone HAS6) was from Ancell (Bayport, MN, USA). Anti-CD11a (Mouse anti-human IgG2a, clone 38), anti-CD11b (Mouse anti-human IgG1, clone ICRF44) and anti-CD18 (Rat anti-human IgG2b, clone YFC 118,3) were from AbD Serotec (Düsseldorf, Germany). All other reagents were of analytical grades and were obtained from commercial sources.

Participating subjects

Endothelial cells were isolated from the umbilical cords of 15 healthy newborns. Monocytes were prepared by differential centrifugation and solid face attachment from blood samples taken from 15 healthy volunteers. All samples were tested for each parameter/experiment.

Laminin oxidation

Laminin-1 was isolated from EHS (Engelbreth-Holm-Swarm) tumour and it was oxidized as described elsewhere (Alamdari *et al*. 2005). In brief, laminin in PBS was incubated with 100 mM EDTA, 833 mM ascorbic acid and 100 mM ferrous ammonium sulphate for 1 h at 37 °C. An overnight dialysis against PBS at 4 °C with two buffer changes followed and then oxidized laminin was stored at −80 °C. For the estimation of laminin oxidation, a high-sensitivity ELISA (enzyme-linked immunosorbent assay) method first established in our laboratory was used (Alamdari *et al*. 2005), while the level of *in vitro*-produced oxidized laminin was quantified using a colorimetric carbonyl assay (data not shown) (Alamdari *et al*. 2005).

Endothelial cell preparation and culture

Endothelial cells were isolated from the umbilical cords of healthy newborns by the collagenase perfusion method as previously described by Jaffe *et al*. with minor modifications

(Jaffe *et al.* 1973). In brief, the umbilical cord veins were carefully washed with sterile phosphate buffered saline (PBS) (0.9% NaCl, pH 7.4) and were then filled with Earle 199 medium that contained 0.5 μg/ml collagenase, for 10 min at room temperature. The content was then emptied in a clean, sterile falcon and the umbilical cord vein was washed with Earle 199 medium (without collagenase). The total content was then centrifuged for 10 min at 260 ***g*** and finally resuspended in complete Earle 199 medium (20% FCS, 25 mM HEPES, 50 μl Pen/Strep, 0.25 μg/ml fungizon, 50 μg/ml gentamycin, 90 μg/ml heparin). The cells were then placed in flasks precoated with 0.2% w/v gelatin in PBS and cultured at 37 °C until they reach confluence. Before use, the cells were trypsinised with 0.05% trypsin and 0.02% EDTA, centrifuged for 10 min at 260 ***g*** and finally resuspended in complete Earle 199 medium.

Monocyte preparation

Monocytes were isolated from blood samples drawn in the morning before breakfast from healthy donors through a modification of a previously described method (Seager Danciger *et al.* 2004). In brief, heparinized whole blood was diluted with PBS 1× (1 mM EDTA, pH 7.2) and underlayered with the use of an 18-gauge spinal needle with Ficoll-Paque Plus (1.077 g/ml) in 50 ml falcon tubes. After centrifugation (400 ***g***/20 min/RT/no brake) the peripheral blood mononuclear cell (PBMC) layer was collected and three washes with PBS 1× (1 mM EDTA, pH 7.2) followed. PBMCs were then diluted with IMDM and overlayered on 46% Percoll in 50 ml falcon tubes. After centrifugation (550 ***g***/30 min/-RT/no brake) the monocyte layer was collected, diluted with PBS 1× (1 mM EDTA, pH 7.2) and washed with cold PBS 1× before use in experiments. The number of the isolated monocytes was estimated by measurement on a Neubauer plate after a 1:1 dilution with 0.4% trypan blue solution. A total of 3–4 million cells/ml were isolated each time with a viability of at least 98%. Flow cytometry using a CD14 monoclonal antibody labelled with phycoerythrin revealed that more than 85% of the cells were monocytes.

Endothelial cell adhesion assay

Endothelial cell attachment was measured on microwells. 80,000 cells resuspended in Earle medium (10% FCS) were placed in each well of polystyrene plates that had been precoated with 37.5 μg/ml oxidized or native laminin-1. The cells were incubated in the wells for 30 min at 37 °C and after incubation, non-adherent cells were discarded by aspiration and the wells were rinsed three times with sterile PBS (pH 6.0). Endothelial cells that attached to laminin were stained with the Hemacolor staining kit, immersed in 200 μl 10% acetic acid added to each well in order to remove the bound dye and incubated on an orbital shaker for 3–4 min. The optical density of the stained solution was finally measured at 590 nm.

Endothelial cell attachment to laminin in the presence of the anti-human alpha2 (a2, CD49b) integrin subunit

One to two million HUVEC were washed and preincubated on ice for 5 min with 20 μl of 250 μg/ml human IgG in order to block non-specific binding and were then incubated for 45 min on ice and in the dark with 80 μl of a monoclonal anti-alpha2 integrin subunit (initial concentration: 0.25 mg/ml) at 1:50 dilution. Cells that were incubated with 250 μg/ml human IgG in the absence of the anti-alpha2 integrin subunit used as controls. Cells were then washed three times with a specific buffer that contained 50 mM sodium phosphate pH 7.5, 100 mM potassium chloride, 150 mM NaCl, 5% glycerol and 0.2% BSA. For the dilution of the antibody the same buffer was used.

Human umbilical cord vein endothelial cells attachment to native and oxidized laminin was then estimated as described above.

ELISA assay for measuring ICAM-1 expression in endothelial cells

One hundred microliters of cell suspension (80,000 cells in Earle 199 medium, 10% FCS) were placed in each well of polystyrene plates that had been precoated with 37.5 μg/ml either native or oxidized laminin-1. Cells were then incubated in the wells overnight at 37 °C. In pilot experiments the number of cells attached to either oxidized or native laminin under these conditions (overnight incubation) was estimated using the Hemacolor kit as described above and was found equal.

After incubation, culture medium was discarded by aspiration and the wells were rinsed one time with 100 μl sterile PBS 1×. ICAM-1 expression in endothelial cells attached to native or oxidized laminin was estimated through modifications of a previously described method (Shingu *et al.* 1994). In brief, 50 μl of 0.2 μg/well mouse anti-ICAM MoAb in Earle 199 medium were added to each well and incubated for 1 h at 37 °C. After incubation, the wells were washed three times with PBS 1× and 100 μl of a horseradish peroxidase-conjugated anti-mouse IgG 1:10,000 diluted in Earle 199 medium were added to each well and incubated for 1 h at 37 °C. The wells were then washed four times with PBS 1×

and 100 μl of a 3,3′,5,5′-tetramethylbenzidine (TMB) solution in substrate buffer (1.455 g Na_2HPO_4, 1.91 g citric acid, pH 5 and 2 μl 30% H_2O_2, final volume: 200 ml) were added to each well and incubated for 50 min at 37 °C. The optical density was finally read at 450 nm using an ELISA reader.

Monocyte adhesion assay

Monocyte attachment was measured on microwells. 80,000 cells in IMDM, (10% FCS) were placed in each well of polystyrene plates precoated with endothelial cells (10^6/ml) that had been preattached to 37.5 μg/ml oxidized or native laminin-1 as described above. Monocytes were incubated in the wells for 30 min at 37 °C and after incubation, non-adherent cells were discarded by aspiration and the wells were rinsed three times with sterile PBS (pH 6.0). Monocyte attachment was quantified using the myeloperoxidase (MPO) assay as previously described (Koliakos *et al.*, 2004). In brief, monocytes were lysed with 0.5% (w/v) hexadecyltrimethylammonium bromide in PBS (pH 6.0) for 30 min at 37 °C. After lysis, 50 μl 0.2 mg/ml dianisidinedihydrochloride in PBS (pH 6.0) containing 0.4 mM H_2O_2 were added to each well. After 15 min, the MPO activity of the lysate was measured spectrophotometrically at 405 nm, using an ELISA reader.

Monocyte attachment to HUVEC that had been preattached to either oxidised or native laminin, in the presence of the anti-human alphaL (aL, CD11a), alphaM (aM, CD11b) and beta2 (b2, CD18) integrin subunits

One to two million monocytes were washed and preincubated on ice for 5 min with 20 μl of 250 μg/ml human IgG in order to block non-specific binding and were then incubated for 45 min on ice and in the dark with 10 μl of 0.1 mg/ml anti-human alphaL (CD11a), or 0.1 mg/ml anti-human alphaM (CD11b) or 0.1 mg/ml anti-human beta2 (CD18) integrin subunits. Cells were then washed three times with PBS. Monocyte attachment to HUVEC that had been preattached to either oxidized or non-oxidized laminin was then estimated as described above.

Statistical analysis

For the statistical evaluation, the statistical software GraphPad InStat version 3.00 for Windows was used (GraphPad Software Inc., San Diego, CA, USA). Values are expressed as means ± standard error of means (SEMs). The statistical significance of the differences between the sets of data was estimated by the Student's t-test (paired or un-paired). In addition, when appropriate, one-way ANOVA with Bonferroni or Dunnett post-test was used. The two-tailed $P < 0.05$ was used as the significance level.

Results

Endothelial cell attachment to oxidized and native laminin-1. The role of the alpha2 integrin subunit

In an attempt to investigate whether laminin oxidation could affect its interactions with endothelial cells, endothelial cell attachment to oxidized and non-oxidized laminin after 30 min of incubation was studied. Our results indicated that HUVEC attach to oxidized laminin at a higher degree in relation to the non-oxidized molecule after 30 min of incubation ($P \leq 0.05$) (Figure 1). Moreover, incubation of HUVEC with the antibody against the alpha2 integrin subunit equalized the previously observed differences ($P \leq 0.05$) (Figure 1).

On the other hand, after overnight incubation all the cells attached to the matrix either native or oxidized. Therefore in the following experiments preattached endothelial cells were used after overnight incubation.

ICAM-1 expression in endothelial cells attached to oxidized and native laminin-1

In order to quantitate any differences in relation to ICAM-1 expression between endothelial cells attached to oxidized laminin and those attached to the native molecule we used a sensitive ELISA assay (Shingu *et al.* 1994). ICAM expression was also estimated to HUVEC attached to plastic

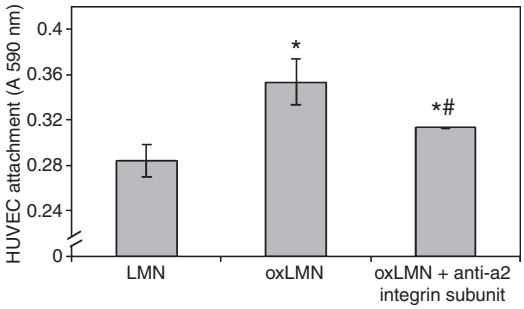

Figure 1 Endothelial cell attachment to oxidized or native laminin-1 in the presence or absence of the antibody against the alpha2 (a2) integrin subunit (anti-CD49b). Bars represent mean value ± standard error of means of at least 15 independent experiments. * indicates significant difference with the value obtained in endothelial cells that attached to non-oxidized laminin-1. # indicates significant difference with the value obtained in endothelial cells that attached to oxidized laminin-1.

surface. Plastic surface is referred to the bottom of the well of the ELISA plate used to estimate ICAM expression of HUVEC attached to either oxidized or native laminin. These samples were used as negative controls to estimate HUVEC attachment to a non-protein substrate. HUVEC attachment to plastic surface was estimated to 0.316 ($A_{450\ nm}$).

Our results indicated that endothelial cells that attached to oxidized laminin-1 expressed higher levels of ICAM-1 as compared to those attached to the non-oxidized molecule ($P < 0.0001$) (Figure 2).

Monocyte attachment to endothelial cells that were attached to oxidized or native laminin-1

As we found that HUVEC attached to oxidized laminin expressed higher levels of ICAM-1, therefore we investigated whether their monocyte recruitment capacity was increased accordingly. For the quantification of unknown monocyte samples attached to HUVEC that were preattached to oxidized or native laminin, a standard curve used, based on the absorbance ($A_{405\ nm}$) of different proportions of MPO, which reflects the different amount of cells (100,000 = 100% MPO) (Figure 3a).

According to our results, monocytes attached at a higher degree to endothelial cells that were attached to oxidized laminin, as compared to those attached to the native molecule ($P = 0.005$) (Figure 3). Moreover, as monocyte attachment to endothelium is mainly mediated by ICAM-1 interaction with the integrins alphaLbeta2 (a_Lb_2) and alphaMbeta2 (a_Mb_2), we studied monocyte attachment on HUVEC that were attached to oxidized or native laminin, in the presence or absence of anti-alphaM and anti-beta2 integrin subunit antibodies. Our results indicated that incubation of monocytes with the antibody against the alphaM

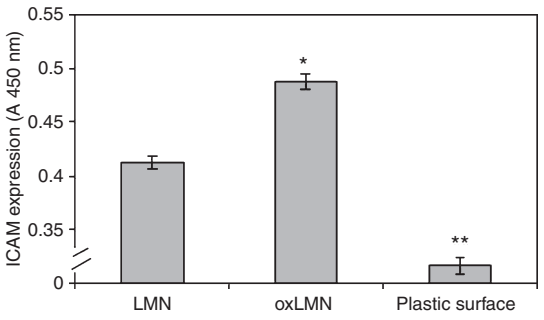

Figure 2 ICAM-1 expression in endothelial cells that attached to either oxidized or native laminin-1. Bars represent mean value ± standard error of means of at least 15 independent experiments. * indicates significant difference with the value obtained in endothelial cells that attached to non-oxidized laminin-1. ** indicates significant difference with the value obtained in endothelial cells that attached to either oxidized or native laminin-1.

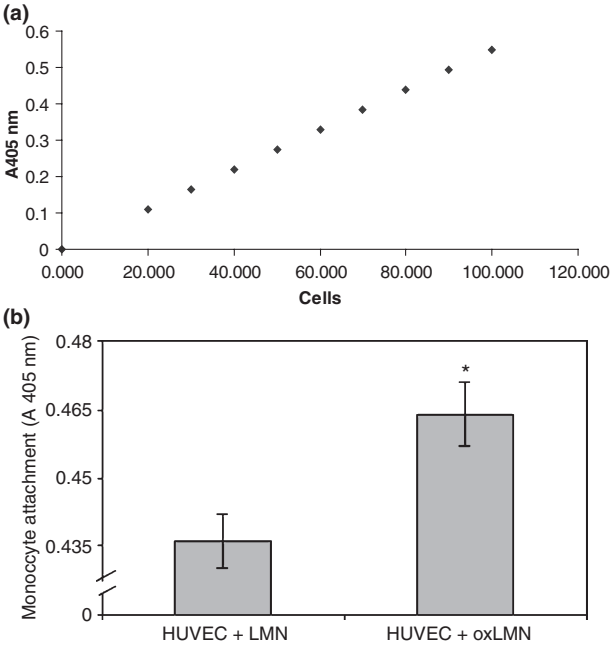

Figure 3 (a) Standard curve for the quantification of unknown monocyte samples attached to HUVEC that were preattached to oxidized or native laminin. (b) Monocyte attachment to endothelial cells that had been preattached to either oxidized or non-oxidized laminin-1. Bars represent mean value ± standard error of means of at least 15 independent experiments. * indicates significant difference with the value obtained in monocytes that attached to endothelial cells that had been preattached to native laminin-1.

Table 1 Monocyte attachment on HUVEC that were attached to oxidized or native laminin, in the presence of the anti-alphaL (CD11a), or alphaM (CD11b) or beta2 (CD18) integrin subunits

Treatment	Number of cells ± SD[#] ($\times 10^3$)
LMN	91.7 ± 3.6
LMN + anti-alphaL (CD11a)	95.3 ± 9*
LMN + anti-alphaM (CD11b)	90.5 ± 0.2
LMN + anti-beta2 (CD18)	91.7 ± 3.7
oxLMN	95.2 ± 0.26*
oxLMN + anti-alphaL (CD11a)	95.8 ± 0.2
oxLMN + anti-alphaM (CD11b)	75.4 ± 1.5**
oxLMN + anti-beta2 (CD18)	90.1 ± 0.3**

[#]Mean of at least 10 independent experiments.
*Significant difference with the LMN value ($P < 0.05$).
**Significant difference with the oxLMN value ($P < 0.05$).

and beta2 integrin subunits equalized the previously observed differences ($P < 0.0001$ in both cases), while incubation with the antibody against the alphaL integrin subunit did not affect HUVEC attachment to oxLMN (Table 1) (Dunnett post-test: $P < 0.0001$, Bonferroni post-test: $P < 0.0001$). Moreover, our results showed that the differences on monocyte attachment were not attributed to differences in the number of endothelial cells attached to either oxidized or native laminin, as under our experimental conditions HUVEC attached at the same degree to either oxidized or native laminin (data not shown).

Discussion

The results of the present study indicate an increased monocyte attachment rate to surface of the endothelial cells (HUVEC) that were attached to the oxidized laminin as compared to the same cells attached to native laminin. This is the first time that increased attachment of monocytes to endothelial cells is associated with laminin oxidation.

Incubation of monocytes with antibodies against the alphaM and beta2 integrin but not alphaL integrin subunits equalized the previously observed differences. In accordance with our results, previous studies showed that stimulated monocyte adherence to endothelial cells was mediated by the alphaM (CD11b) (Prieto *et al.* 1988; Hassall *et al.* 1991), but not the alphaL (CD11a) integrin subunits (Prieto *et al.* 1988) when specific antibodies against the above integrins were used.

Moreover, it has been reported that monocyte affinity to the surface of endothelial cells is dependent on ICAM-1 expression as ICAM-1 is one of the major monocyte attachment molecules on the endothelium cell surface (Huo & Ley 2001).

Accordingly, the data of the present study indicate that ICAM-1 expression was higher on the surface of HUVEC cells attached to the oxidized laminin in comparison to HUVEC attached to the native molecule. For the estimation of monocyte attachment and ICAM-1 expression on the surface of HUVEC, overnight incubation of HUVEC to laminin was performed in order to achieve the attachment of all the cells (100%) to either oxidized or native laminin. When monocytes were added to the endothelial cell substrate, the endothelial cells had already completely attached to either oxidized or non-oxidized laminin. According to our knowledge, after overnight incubation, no differentiation of HUVEC cells has been reported. Furthermore, the population of the HUVEC cells used was homogenous, while the cells added to either oxidized or native laminin were derived from the same sample.

Accordingly, the differences observed could not be attributed to the number of cells attached to laminin, but to the oxidative modification of the protein itself.

A further finding of the present study was that HUVEC cells attach to oxidized laminin with a higher affinity as compared to the native molecule after 30 min of incubation. To our knowledge this is the first time that laminin oxidation is associated with endothelial cell attachment. It should be noted that previous studies performed in our laboratory showed that monocytes attach at a higher degree to oxidized laminin as compared to the non-oxidized molecule (Kostidou *et al.* 2007; Kostidou *et al.*, 2009). For the estimation of differences in HUVEC affinity to laminin, the cells were incubated with laminin for only 30 min. It is conceivable that HUVEC require more time to completely attach to laminin. Accordingly, after overnight incubation all seeded cells attached to both forms of laminin, native and oxidized. Based on the above, it could be conjectured that there is time dependency on the initial HUVEC attachment to laminin that reflects differences in the affinity between cells and oxidized or native laminin.

The increased affinity of endothelium to oxidized laminin as compared to the native molecule may reflect an *in vivo* tendency for faster coverage of lesions exposed to oxidative stress by endothelial cells. Oxidative stress is a common feature of atherosclerosis and therefore, extracellular matrix oxidation may be also common in atherosclerosis.

Human umbilical cord vein endothelial cells interact with extracellular matrix mainly through various integrin receptors, each one recognizing specific extracellular sequences (Cines *et al.* 1998; Garmy-Susini & Varner 2008). The differences on HUVEC attachment to laminin observed between the oxidized and the native molecule could be attributed to cell-laminin interactions via different integrin receptors. Accordingly, it could be assumed that the addition of negative charged residues by laminin oxidation could change the shape and conformation of the molecule leading to modified interactions with the cells. Oxidation could release some new integrin binding sites on laminin's surface, which were cryptic under non-oxidative conditions.

Our data also indicate that the a2 integrin subunit plays a specific role for the attachment of HUVEC to the oxidized but not to the native laminin. Alpha2beta1 integrin is known to be involved in atherosclerosis (Grenache *et al.* 2003), and was found increased in diabetes mellitus and metabolic syndrome (Jin *et al.* 1996; Kostidou *et al.* 2007; Kostidou *et al.*, 2009) however apart from the a2 integrin subunit our data do not exclude the involvement of other cell surface molecules on the described phenomenon.

Laminin-1, used in the present paper, is considered the prototype laminin because it can be easily isolated from the matrix of the Engelbreth-Holm-Swarm (EHS) tumour (Timpl *et al.* 1979). The vascular-specific laminin isoforms that encounter with monocytes during extravasation are laminin-8 and laminin-10 (Pedraza *et al.* 2000). Interestingly, laminin-1, laminin-8 and laminin-10 share the same beta and gamma chains (beta1-gamma1) (Miner *et al.* 1997). Accordingly, it could be assumed that the above chains, which are prone to phosphorylation (Trachana *et al.* 2005), may also be prone to other types of post-translational modification, such as oxidation.

Summarizing our data, it is suggested that the oxidatively modified laminin activates endothelial cells, possibly via alpha 2 integrins and triggers them to express increased levels of ICAM-1 and other cell surface molecules, recruiting blood monocytes on the endothelium surface. Upregulation of ICAM-1 expression on endothelial cell surface has been also reported after endothelial cell binding on oxidized fibrinogen (Shcheglovitova *et al.* 2006).and fibronectin (Orr *et al.* 2005). An increased production of ICAM by dysfunctional endothelial cells was reported to cause an increased attachment of monocytes and T lymphocytes in atherosclerosis (Gimbrone & Topper 1999). However, this is the first time that increased ICAM expression by endothelium and monocyte attachment is associated with oxidized laminin. Activated endothelial cells are known to release a variety of molecules including chemokines, which have been reported to activate integrins that are required for a more sufficient binding of mononuclear cells to endothelium (Weber *et al.* 1996). So, activated endothelium could induce in this way alphaMbeta2 (CD11b/CD18) integrin activation in monocytes, while increased ICAM-1 expression could recruit monocytes through interaction with the activated alphaM-beta2 (CD11b/CD18) integrins.

Our data do not exclude the involvement of other adhesion molecules, such as VCAM-1 and selectins in the above phenomenon. It is noteworthy that VCAM-1 was found to play a key role in the initiation of the atherosclerotic process in mice, while its expression in luminal endothelium has been reported to precede subendothelial accumulation of macrophages (Dansky *et al.* 2001). However, the present study was focused on the ICAM-1 expression in endothelial cells that attached to oxidized laminin.

In conclusion, our data indicate for the first time that in cases where basement membrane proteins such as laminins are oxidatively modified the interaction between endothelia and the basement membranes can be also modified with the involvement of the alpha2 integrin subunit. This modified interaction can result in increased monocyte recruitment capacity attributed to increased ICAM-1 expression with the involvement of the alphaM and beta2 integrin subunits. These initial observations, further substantiated with future studies, may lead to novel insights on the initiation and progress mechanism of atherosclerotic lesions.

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