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# E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells *in vitro*

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## SUMMARY

Endothelial cells respond to several cytokines by a rapid increase in expression of the adhesion molecules E-selectin and intercellular adhesion molecule-1 (ICAM-1), followed by a gradual decline. The fate of these molecules, which was so far unknown, was studied. Specific sandwich ELISA for the detection of soluble (s)E-selectin and sICAM-1 were developed. In supernatant, centrifuged 3 hr at 100,000 ***g*** to remove microparticles, from human umbilical vein endothelial cells (HUVEC) activated with tumour necrosis factor (TNF), interleukin-1 (IL-1) or lipopolysaccharide (LPS), E-selectin and ICAM-1 molecules could be detected. Biochemical analysis revealed that sE-selectin migrated as a band of approximately 94,000 MW. The amount of soluble adhesion molecules released was directly correlated with cell surface expression. Maximal release of E-selectin was observed 6–12 hr after activation of HUVEC and decreased to below detection limit 24 hr after activation. After activation, release of ICAM-1 gradually increased with ICAM-1 cell surface expression, and reached a plateau after 24 hr, which was constant for 3 days. Since E-selectin and ICAM-1 are highly expressed at inflammatory sites, the resulting high concentrations of released E-selectin and ICAM-1 may affect interactions of leucocytes with endothelial cells. The physiological role, however, of the release of E-selectin and ICAM-1 remains to be elucidated.

## INTRODUCTION

Endothelial cells as well as leucocytes express adhesion molecules which mediate the adhesion and subsequent migration of leucocytes into tissue. *De novo* expression or enhanced expression of intercellular adhesion molecule-1 (ICAM-1) has been described on different cell types in inflammatory lesions, during rejection and on melanoma cells.[1-3] *De novo* expression of E-selectin in inflammatory areas,[4-6] and a generalized expression in the baboon and in cynomolgus monkeys in a septic shock model[7,8] strongly suggest that E-selectin is involved in the pathogenesis of acute inflammation. The regulation of expression of these adhesion molecules is considered to play a major role in the localization and development of an inflammatory reaction.

E-selectin and ICAM-1 are structurally unrelated adhesion molecules for granulocytes, monocytes and T lymphocytes.[9-15] The inflammatory cytokines tumour necrosis factor (TNF), interleukin-1 (IL-1), interferon-$\gamma$ (IFN-$\gamma$) and bacterial endotoxins [lipopolysaccharides (LPS)] are known inducers and enhancers of E-selectin and ICAM-1.[16,17] E-selectin expression *in vitro* can be rapidly induced reaching maximal expression after 4–6 hr of activation, followed by a rapid decline.[15,16] ICAM-1 is normally expressed at a low basal level on endothelial cells, but its expression can be enhanced and the expression remains maximal for about 48 hr.[17] The mechanism of down-regulation of surface E-selectin and ICAM-1 is so far unknown, but the down-regulation is an essential facet in the course of the immune response. In principle, surface molecules can be removed from the membrane by internalization or shedding, eventually by proteolysis. Release of functional adhesion molecules, leading to soluble adhesion molecules in plasma, may have physiological implications, such as inhibition of adhesion by competition. In this study we investigated whether release of E-selectin and ICAM-1 occurred from human umbilical vein endothelial cells (HUVEC) *in vitro*. It was found that concentrated supernatant of activated HUVEC inhibited specifically binding of monoclonal antibody (mAb) anti-E-selectin as well as anti-ICAM-1 to activated HUVEC, suggesting the presence of soluble (s)E-selectin and sICAM-1. The kinetics of appearance of sE-selectin and sICAM-1 was determined quantitatively with specific sandwich ELISA for sE-selectin and sICAM-1.

Abbreviations: HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1.

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## MATERIALS AND METHODS

*Cell cultures*

HUVEC were obtained by collagenase treatment of the umbilical vein as described previously.[16] The cells were cultured in fibronectin-coated (fibronectin kindly provided by Dr J. van Mourik, Red Cross Blood Trans. Service, CLB, Amsterdam, The Netherlands) tissue culture flasks (Costar, Cambridge, MA) in RPMI-1640 (Gibco, Paisley, U.K.), supplemented with 10% heat-inactivated human serum and 10% bovine calf serum (BCS), 50 $\mu$g/ml heparin, 30 $\mu$g/ml endothelial growth factor (Collaborative Research Incorporated, Bedford, MA), and antibiotics.

*Monoclonal antibodies*

Monoclonal antibodies ENA1 or ENA2 (both mIgG1) were directed against E-selectin.[9,18] Monoclonal antibodies MEM-111 (mIgG2a) and MEM-112 (mIgG1) directed against ICAM-1 were obtained by immunizing BALB/c mice with the human B-cell line Raji. The specificity of the mAb was determined in several ways. Both mAb (1) had an expression pattern characteristic for CD54 (ICAM-1), (2) immunoprecipitated from the lysate of Raji cells a glycoprotein of approximately 80,000 MW and (3) reacted specifically with COS cells transfected with the CD54 CDM8-cDNA clone (kindly provided by Dr B. Seed, Massachusetts General Hospital and Harvard Medical School, Boston, MA). Monoclonal antibody R/R1.1 (mIgG1) directed against ICAM-1 was kindly provided by Dr R. Rothlein (Boehringer Ingelheim, Ridgefield, CT).[19] Other mAb used were NKI-M9 (mIgG1, directed against the vitronectin receptor, kindly provided by Dr C. Figdor, NKI, Amsterdam, The Netherlands), mAb MOPC21 (mIgG1, kindly provided by Celltech, Slough, U.K.), and mAb 61E71 (mIgG1, directed against TNF).[20] Monoclonal antibodies ENA1, R/R1.1 and MOPC21 were radioiodinated using Iodobeads (Pierce, Rockford, IL) according to the manufacturer's instructions. Iodobeads were pre-loaded with 250 $\mu$Ci Na $^{125}$I (Amersham International, Amersham, U.K.) in 500 $\mu$l phosphate-buffered saline (PBS) for 5 min at room temperature. About 250 $\mu$g of purified mAb were added (100 $\mu$l) and the incubations were continued for 10 min. Subsequently, the Iodobeads were removed and labelled protein and free iodine were separated on a Sephadex G-50 column, equilibrated with 0·5% bovine serum albumin (BSA) in PBS. The radiolabelled protein (50–150 $\mu$Ci/mg) was stored at $-20°$.

*Reagents*

A truncated form of E-selectin ('soluble E-selectin'), which lacks a sequence (residues 534 to 588) containing the putative transmembrane domain, was constructed by means of oligonucleotide-directed mutagenesis, inserted into a modified form of the expression plasmid pMT2, and expressed in Chinese hamster ovary cells (G. R. Larsen, D. Sako, T. J. Ahern, M. Shaffer, J. Erban, S. A. Sajer, R. M. Gibson, D. D. Wagner, B. C. Furie and B. Furie, submitted for publication).

sICAM-1 standard was obtained from Bender MedSystems (Vienna, Austria). Recombinant TNF-$\alpha$ (rTNF-$\alpha$) was kindly provided by BASF Knoll AG (Ludwigshafen, Germany). rIL-1$\alpha$ was kindly provided by Dr S. Gillis (Immunex, Seattle, WA). LPS (*Escherichia coli* 055:B5) was purchased from Sigma (St Louis, MO). Biotin-X-NHS was used for the biotinylation of mAb (Calbiochem, La Jolla, CA).

*Monoclonal antibody binding assay*

Antibody binding to HUVEC in microtitre wells was quantified by use of $^{125}$I-labelled mAb ENA1, R/R1.1 and MOPC21 in quadruplicate. Specific binding was obtained by subtracting non-specific binding of MOPC21. Data were given as the mean c.p.m. and SD from quadruplicate wells.

*Preparation of supernatants of activated HUVEC*

HUVEC of passage 3 or 4 were grown to confluence in 75 cm$^2$ (approximately $3 \times 10^6$ cells/flask) cell culture flasks (Costar) or in 24 macro wells (Costar) and activated with TNF as indicated. After 4 hr of activation or otherwise as indicated, the cells were washed three times and further cultured with RPMI-1640 1% BCS as indicated. Supernatant was harvested, filtered (0·2 $\mu$m), for some experiments concentrated 10 or 15 times in 40% PEG 20,000 (Serva, Heidelberg, Germany), and stored at $-20°$ before use.

*Inhibition assay; ELISA on HUVEC*

Inhibition of reactivity of mAb anti-E-selectin and mAb anti-ICAM-1 with membrane-bound E-selectin and ICAM-1 by supernatants, derived from activated HUVEC, was measured in ELISA on E-selectin and/or ICAM-1 expressing HUVEC. A concentration range of mAb anti-E-selectin and mAb anti-ICAM-1 was preincubated for 3 hr with 50 $\mu$l supernatant. These mixtures were added to appropriately activated, glutaraldehyde fixed HUVEC for 1 hr.[9] After five washings, binding of mAb to membrane-bound E-selectin and ICAM-1 was quantified using goat anti-mouse peroxidase (Jackson, West Grove, PA), followed by *o*-phenylenediamine dihydrochloride (Sigma) as substrate. The plates were read on a microtitre ELISA reader at 492 nm. All data were given as the mean OD units from triplicate wells. SD were, unless stated otherwise, less than 10% of the mean.

*Sandwich ELISA for detection of sE-selectin and sICAM-1*

Ninety-six-well plates were coated with 100 $\mu$l ENA1 (protein-G purified) or MEM-111 (protein-A purified) overnight. Coating with 10 $\mu$g/ml ENA1 and 25 $\mu$g/ml MEM-111 gave optimal results. Non-specific binding was blocked by incubating the plates with 1% BSA (Sigma) in PBS. Samples of supernatants (50 $\mu$l/well) were added for 2 hr at 37°, followed by incubation with biotinylated mAb ENA2 or R/R1.1 for detection of sE-selectin and sICAM-1 respectively. After washing the wells were incubated with streptavidin–peroxidase conjugate (Dakopatts, Glostrup, Denmark) followed by addition of substrate. Coating with MOPC21, or using biotinylated anti-TNF mAb 61E71 as second antibody gave no signal.

*Biochemical methods*

HUVEC were cultured to confluence on fibronectin-coated 5-cm diameter plastic petri dishes. The cells were starved for 0·5 hr in methionine-free RPMI, followed by activation with TNF (10 ng/ml) for 4·5 hr in the presence of 100 $\mu$Ci/dish of [$^{35}$S]methionine (Amersham). Incorporation of label was terminated by addition of complete culture medium and cells were chased for 1 and for 20 hr at 37°. Supernatants were collected and cleared from debris by centrifugation (12,000 *g*, 10 min). HUVEC were

rinsed with ice-cold PBS and lysed for 30 min on ice in 1 ml 0·5% (v/v) Nonidet P-40 (NP-40) lysis mix, as described previously.[21] E-selectin in the supernatants and lysates was immunoprecipitated as described previously[22] with mAb ENA1. The precipitates were analysed by SDS–polyacrylamide gel electrophoresis (PAGE) (10% acrylamide) under reducing conditions as described previously.[22] Gels were fluorographed using DMSO/PPO and exposed to Kodak XAR-5 films (Rochester, NY).

## RESULTS

### Supernatants of activated HUVEC inhibit binding of anti-E-selectin and anti-ICAM-1 mAb to activated HUVEC

HUVEC were activated for 4 hr with TNF, and recultured for 16 hr. Supernatants were harvested, concentrated and tested for the presence of molecules, which are capable of binding mAb ENA1 (anti-E-selectin) and/or R/R1.1 (anti-ICAM-1), and thus inhibit binding of these mAb to activated HUVEC. Monoclonal antibodies ENA1 and R/R1.1 were preincubated at different concentrations either with concentrated supernatant of these activated cultures or with concentrated supernatant of cultures of unactivated HUVEC. The binding of mAb ENA1 to 4-hr activated, thus highly E-selectin expressing HUVEC in these mixtures was tested in an ELISA for detection of membrane-bound E-selectin. Binding of R/R1.1 in these mixtures was tested on 24-hr activated, thus highly ICAM-1 expressing HUVEC. As a control, mAb NKI-M9 (anti-vitronectin receptor) on HUVEC was tested. The data showed that reactivity of mAb ENA1 and R/R1.1, but not of mAb NKI-M9 was reduced when preincubated with supernatant of activated cells (Fig. 1). Half maximal signal was reached at 0·2 ng mAb ENA1 and 0·4 ng R/R1.1 per well, preincubated in control supernatant. However, if preincubated with supernatant harvested from activated HUVEC, half maximal signal was reached at a concentration of 5·3 ng ENA1 and 1·1 ng R/R1.1 per well. Binding of ENA2 and MEM-111 as alternative mAb anti-E-selectin and anti-ICAM-1 respectively was also inhibited by the appropriate supernatants (data not shown). The supernatants were ultracentrifuged (3 hr, 100,000 *g*) in order to investigate whether the inhibition was caused by microparticle-associated E-selectin or ICAM-1. The inhibitory effect, however, of supernatant before and after centrifugation was not different (data not shown). Five separately prepared supernatants were tested with similar results. We conclude that soluble (s)E-selectin and sICAM-1 are present in the supernatants of activated HUVEC. sE-selectin and sICAM-1 were found to be stable after freeze thawing, 2 months storage at −20°, and overnight incubation at 37°. Reactivity with mAb was partly abolished after incubation at 56° for 1 hr (data not shown).

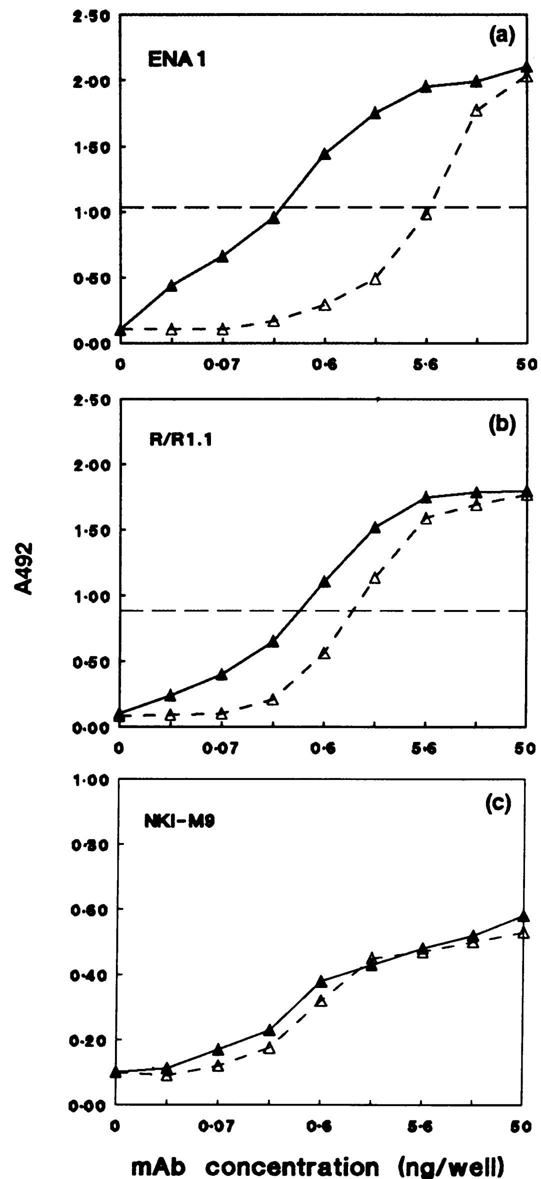


**Figure 1.** Presence of sE-selectin and sICAM-1 in supernatant of activated HUVEC. Effect of concentrated supernatant (△ – – △) from 16-hr-activated HUVEC on ENA1 (a), R/R1.1 (b) and NKI-M9 (c) binding to activated HUVEC as measured in ELISA. HUVEC was activated either for 4 hr (a,c) or for 24 hr (b). The intact lines (▲——▲) represent mAb binding after preincubation in 15 × concentrated control supernatant. The horizontal, dashed lines indicate the half maximal signal. Data are expressed as mean for four measurements (SD fall within the symbol).

### Quantification of sE-selectin and sICAM-1 by sandwich ELISA

To detect sE-selectin and sICAM-1 by sandwich ELISA, several mAb were tested for binding to E-selectin or ICAM-1 respectively without blocking each others binding. Monoclonal antibodies ENA1 and ENA2 recognize different epitopes on E-selectin, since binding to E-selectin expressing HUVEC of labelled ENA1 was not blocked by preincubation with ENA2 and vice versa as described earlier.[18] Monoclonal antibodies anti-ICAM-1 MEM-111 (mIgG2a), MEM-112 (mIgG1) and R/R1.1 (mIgG1) were tested for their capacity to inhibit each others binding to 24-hr activated HUVEC. The different mAb were detected by subclass-specific antisera, labelled with peroxidase. Binding of MEM-111 was inhibited by MEM-112 (Fig. 2a) and vice versa (data not shown). MEM-111 and R/R1.1 on the other hand recognized different epitopes on ICAM-1 (Fig. 2) and were therefore selected for the sandwich sICAM-1 ELISA. The sensitivity of the sandwich ELISA for sE-selectin and sICAM-1 was determined. Figure 3 gives the typical dose–effect

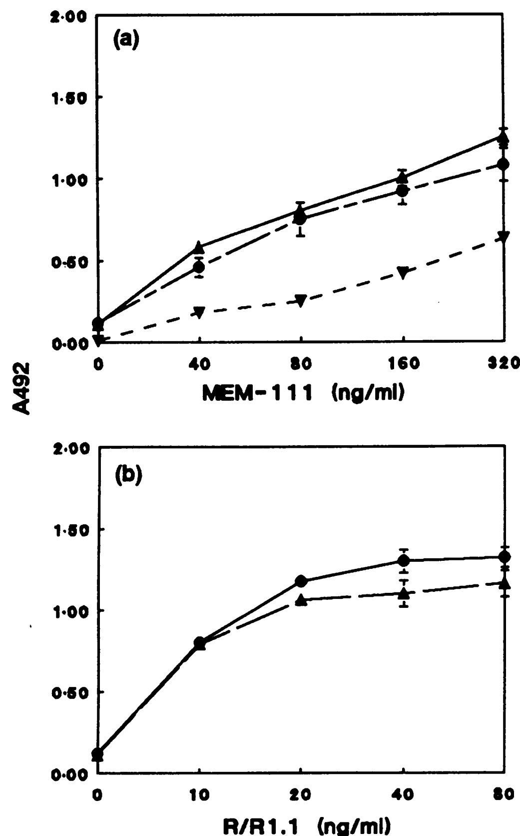


**Figure 2.** Reciprocal inhibition of mAb MEM-111, MEM-112 and R/R1.1 binding to ICAM-1. Effect of preincubation of anti-ICAM-1 mAb on reactivity pattern of MEM-111 (a) and of R/R1.1 (b) with 24-hr-activated HUVEC. The solid lines represent binding of different concentrations of mAb MEM-111 (▲-▲) and R/R1.1 (●-●) in the absence of additional mAb. The dotted lines represent the effect of (1) preincubation with R/R1.1 (● — — ●) or with MEM-112 (▼ - - - ▼) on MEM-111 binding (a); (2) preincubation of MEM-111 (▲ — — ▲) on R/R1.1 binding (b). Data are expressed as mean and SD of four measurements. SD bars which fall within the symbol are not given.

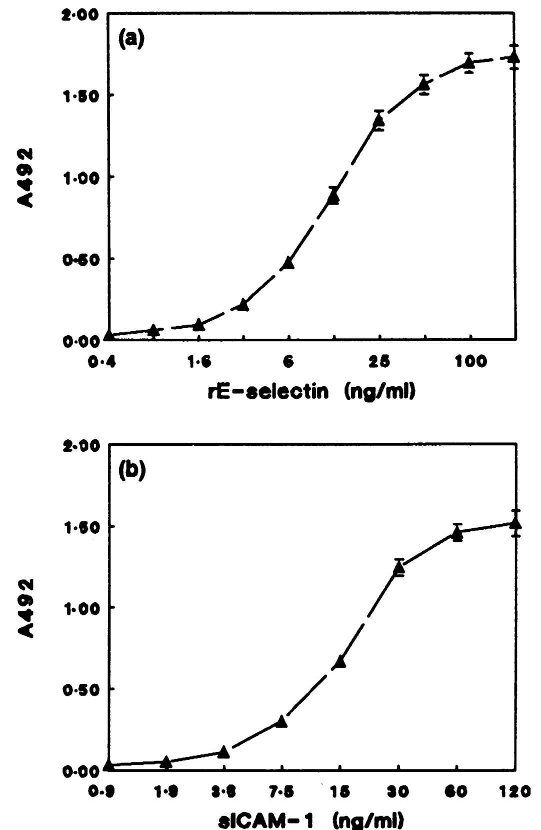


**Figure 3.** Standard titration curve of rE-selectin (a) and sICAM-1 (b) as measured in specific sandwich ELISA for sE-selectin and sICAM-1 described in Materials and Methods. In the sICAM-1 ELISA, a 10-fold concentrated supernatant from 24-hr-activated HUVEC, calibrated with a commercial available standard, was used. Data were expressed as mean and SD of four measurements. SD bars which fall within the symbol are not given.

curve of the E-selectin and ICAM-1 ELISA. For the sE-selectin ELISA, recombinant E-selectin was used as standard. The lower sensitivity level of the sE-selectin ELISA was about 1 ng/ml. For the sICAM-1 ELISA we used as standard a 10-fold concentrated supernatant from 24 hr-activated HUVEC culture, which was calibrated using a commercially available standard. The lower sensitivity levels of the sICAM-1 ELISA was approximately 2 ng/ml.

The sandwich ELISA for both sE-selectin and sICAM-1 appeared to be more sensitive than the inhibition ELISA on HUVEC, which permitted to omit concentration of supernatants in further experiments.

#### Kinetics of E-selectin and ICAM-1 release induced by different mediators

The kinetics of release of E-selectin and ICAM-1 after activation of HUVEC with TNF, IL-1 and LPS was investigated. After activation, HUVEC were washed at the onset of the indicated periods and supplied with new medium (also containing appropriate activator) for detection of E-selectin and ICAM-1, released in the interval. E-selectin release during time intervals of 3 hr (0–3, 3–6 hr, etc.), the last two being intervals of 0 hr, was determined. Released ICAM-1 was determined in the time intervals 0–6 and 6–12 hr, and subsequently in intervals of 12 hr. On equally treated HUVEC, cultured in micro-wells, E-selectin and ICAM-1 expression at the cell surface of HUVEC was determined using $^{125}$I-labelled mAb ENA1 and R/R1.1. Maximal release of E-selectin was observed 6–12 hr after activation (Fig. 4) and decreased below the detection limit 31 hr after activation. Release of ICAM-1 increased gradually after activation with TNF and reached a plateau level after 24 hr (Fig. 5). The release of sICAM-1 remained constant for at least 3 days. Similar results were obtained using IL-1 and LPS as activators (data not shown). The release of E-selectin and ICAM-1 was observed to follow cell surface expression with a lag period of c. 3–6 hr.

#### Biochemistry of sE-selectin

We biochemically analysed the release of E-selectin from activated HUVEC. From supernatants of activated, metabolically radiolabelled HUVEC radiolabelled sE-selectin of approximately 94,000 MW could be precipitated after a 1-hr chase (Fig. 6). After an overnight chase, this band was more pronounced.

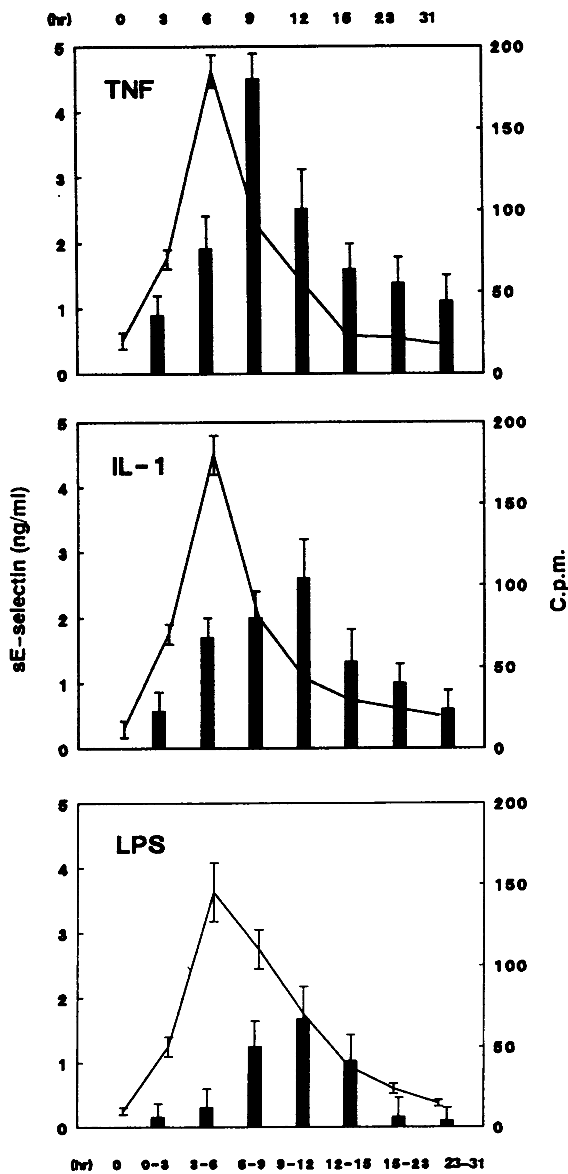


**Figure 4.** Correlation between E-selectin expression and E-selectin release. HUVEC, either grown on 96-micro well plates (for E-selectin expression) or on 24-macro well plates (for E-selectin shedding), were equally activated with TNF (10 ng/ml), IL-1 (10 U/ml) or with LPS (100 ng/ml), washed at the onset of the indicated periods and supplied with new medium and appropriate activator, for detection of released E-selectin. Released E-selectin (■) present in supernatant harvested at the end of each intermediate period (bottom *x*-axis) was assayed in the sandwich ELISA, using recombinant sE-selectin as standard. E-selectin expression (—) was determined using $^{125}$I-labelled mAb ENA1 (bound c.p.m.) at times indicated after activation (top *x*-axis).

The MW of sE-selectin is slightly lower than the MW of E-selectin recovered from the cell lysates (115,000, Fig. 6). The smaller band with a lower MW present in the lysates is an immunoreactive precursor of E-selectin. In the control precipitations with mAb 66Ig10 (reactive with the transferrin receptor),[23] transferrin receptor could only be recovered from cell lysates and not from supernatant (data not shown).

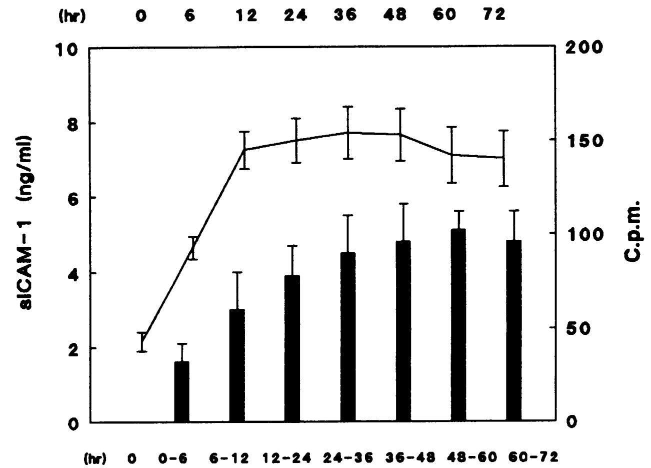


**Figure 5.** Correlation between ICAM-1 expression and ICAM-1 release. Like Fig. 3, HUVEC was activated with TNF and washed at the indicated time periods. sICAM-1 (■) was measured in supernatant, released by HUVEC during the indicated time intervals (bottom *x*-axis) in the sandwich ELISA using commercially sICAM-1 as standard. ICAM-1 expression (—) was measured using $^{125}$I-labelled R/R1.1 on the cells at times indicated (top *x*-axis).

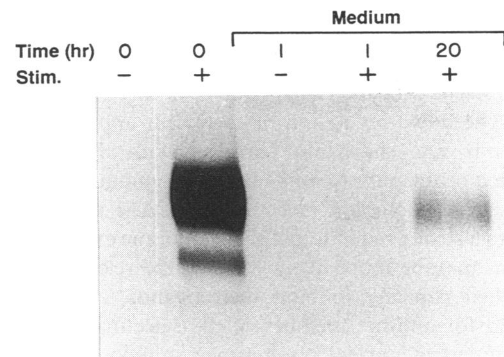


**Figure 6.** Release of E-selectin in the supernatant. HUVEC were activated (+) or not activated (−) and labelled with [$^{35}$S]methionine for 4·5 hr. Cells were lysed at 0 min of chase, or recultured for 1 or for 20 hr. Supernatants were harvested from cells, chased for 1 and 20 hr. E-selectin from cell lysate and from harvested supernatant was immunoprecipitated with mAb ENA1 and analysed on 10% SDS–PAGE. E-selectin was only expressed on activated HUVEC and a fraction of E-selectin was released in the medium.

## DISCUSSION

In this study, we demonstrated that E-selectin and ICAM-1 are released by activated endothelial cells as detected with specific sandwich ELISA. The MW of sE-selectin (94,000) was slightly lower than the MW of membrane-bound E-selectin, which suggests that only the extracellular part of E-selectin is secreted. However, alternative splicing of mRNA, resulting in a soluble, not membrane-bound form of E-selectin cannot be excluded. Recently, Rothlein *et al.*[24] described the presence of soluble ICAM-1 retaining its receptor-binding activity, in supernatants of the B-cell line JY, but in contrast to our results, not in culture supernatants of LPS-activated HUVEC. Furthermore, circu-

lating ICAM-1 in human serum with an apparent MW of approximately 85,000 has been detected.[25] Enhanced serum ICAM-1 levels were suggested to be a marker to monitor tumour burden in cancer patients,[25,26] or various inflammatory disorders.[26,27] The amount of soluble molecules could be correlated to the level of surface expression of E-selectin and ICAM-1. Whereas maximal surface expression of E-selectin was reached 4–6 hr after activation, maximal release was observed 6–12 hr after activation. Release of ICAM-1 reached a plateau level 24 hr after activation and remained constant for at least 3 days. In contrast to the shedding and/or release of a number of antigens such as L-selectin and P-selectin, which is instigated by specific activators,[28,29] the appearance of sE-selectin and sICAM-1 seemed not to be specifically triggered. However, Becker *et al.* described shedding of ICAM-1 from human melanoma cell lines induced by IFN-$\gamma$ and TNF.[30]

The nature of the mechanism of release of the adhesion molecules detected in the supernatant of the cultures, which may be either shed from the membrane or released without previous expression on the membrane has not been revealed as yet. Protease mediated shedding has been reported for a number of molecules among others L-selectin,[28,31] which is rapidly shed in contrast to sE-selectin and sICAM-1. Pilot experiments with the protease inhibitors PMSF and (chymo)trypsin inhibitor did not indicate a role of proteases in the shedding of E-selectin and ICAM-1 (data not shown).

Shedding of membrane molecules can be a process designed to reduce the density of the molecules present on the membrane. The decrease in expression of an adhesion molecule will reduce the adhesiveness of the cell for the cells carrying the relevant ligand. The down-regulation of L-selectin upon activation of neutrophils was shown to correlate with the inability of stimulated neutrophils to home to an inflammatory site.[31] Since the presence of soluble E-selectin and ICAM-1 is not directly related to a strong reduction of the membrane expression, other functions may be more likely. In case the released molecules retain their capacity to bind their ligands, as was recently described for another member of the selectins, i.e. P-selectin being present in normal plasma in a soluble and potentially functional form,[29] the release of these adhesion molecules may induce a decrease in the potential adhesiveness of the leucocytes by competing with the membrane-bound receptors for their ligands. The reactivity pattern of both sE-selectin and sICAM-1 with adhesion-inhibiting antibodies suggests that these soluble molecules retained their ligand-binding activity. Thus, the presence of locally high concentrations of soluble adhesion molecules E-selectin and ICAM-1 *in vivo* may have biological implications if these molecules are able to bind to their ligands on leucocytes and in this way inhibit the adhesion to activated, E-selectin or ICAM-1 expressing cells. Studies on the putative inhibition of adhesion of neutrophils to endothelial cells with sE-selectin or sICAM-1 present in supernatants of activated endothelial cells, were hampered most likely by co-effects of other mediators produced by the activated endothelial cells, such as IL-8 (present in supernatants of activated endothelial cells up to 150 ng/ml, data not shown). IL-8 was found to function as a leucocyte adhesion inhibitor (LAI),[32] although it has also been reported to promote adhesion via activation of CD18.[33]

Besides an inhibitory effect of soluble E-selectin on adhesion, this molecule could also play a role in activating neutrophils. The interaction of neutrophils with endothelial cells via E-selectin and its ligand(s) have been shown to lead to activation of CD18 of the neutrophil.[34,35] Soluble E-selectin may also play a role in activating CD18 on circulating neutrophils by binding to the ligand of E-selectin on these cells.[36] The physiological function of such an activation remains to be investigated.

As mentioned above, the presence of sE-selectin and sICAM-1 molecules is not related to a rapid decrease in membrane expression of the adhesion molecules. It is, therefore, not likely that shedding merely accounts for the down-regulation of the expression of the antigens. The presence of a tyrosine residue in the cytoplasmatic part of E-selectin,[37] but not in ICAM-1 suggests also an internalization process of E-selectin. Whether internalization also accounts for the decrease in expression is currently being investigated. Summarizing, we demonstrate that the adhesion molecules E-selectin and ICAM-1 are released *in vitro* from activated HUVEC. A relationship between induced and/or enhanced membrane expression upon activation by inflammatory cytokines and the release of both molecules was apparent. Further studies on the presence of E-selectin or ICAM-1 in plasma are warranted, since soluble adhesion molecules may present a clinical tool for diagnosis of tumours and acute inflammatory processes.

## ACKNOWLEDGMENTS

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