ACADEMIC PRESS

Biochemical and Biophysical Research Communications 295 (2002) 1150–1155

BBRC

www.academicpress.com

Neutrophil capture by selectins on endothelial cells exposed to cigarette smoke

P.C.W. Stone,[a] A.C. Fisher,[b] G.E. Rainger,[a] and G.B. Nash[a,*]

[a] *Department of Physiology, The Medical School, The University of Birmingham, Birmingham B15 2TT, UK*
[b] *Department of Clinical Engineering, Royal Liverpool University Hospital, Liverpool L7 8XP, UK*

Received 1 July 2002

Abstract

We used a novel perfusion system to expose cultured human umbilical vein endothelial cells (HUVEC) to water-soluble components of cigarette smoke and study subsequent adhesion of flowing neutrophils. Neutrophils did not bind to HUVEC immediately after it had been exposed to cigarette smoke, but many adhered 90–150 min after exposure. The effect was reduced if the exposed medium was made serum-free, but this reduction was partially reversed if low density lipoprotein was added. Treatment of smoke-exposed HUVEC with antibodies against E-selectin or P-selectin reduced adhesion by ~50% or 75%, respectively; a combination of both antibodies essentially abolished adhesion. Enzyme-linked immunosorbent assay confirmed that exposure to smoke caused HUVEC to upregulate surface expression of E- and P-selectin. Thus, water-soluble constituent(s) of cigarette smoke cause efficient selectin-mediated capture of flowing neutrophils. This pro-inflammatory response may contribute to pathology associated with smoking, especially in tissues remote from the lung.

Keywords: Cigarette smoke; Endothelium; Neutrophils; Adhesion; Selectins

Cigarette smoking is an aetiological factor in inflammatory pathology of the lungs, remote organs, and arteries [1]. One mechanism contributing to such pathology may be increased leukocyte–endothelial adhesion. Cigarette smoking is known to alter functions of leukocytes and endothelial cells (e.g., [2–5]). In humans, smoking of cigarettes caused a delay in the transit of neutrophils through the lungs [6]. This has been attributed to an increase in neutrophil rigidity on smoke-exposure [4], while neutrophil adhesiveness itself was impaired by cigarette smoke [7]. On the other hand, smoking increased the adhesiveness of monocytes through activation of their integrin adhesion receptors [5]. In vitro, exposure to particulate condensate of cigarette smoke increased monocytes adhesion to endothelial cells by activating integrins on monocytes and upregulating adhesion receptors including E-selectin on the endothelial cells [3,8]. In animals, inhalation of cigarette smoke was associated with an increase in adhesion of leukocytes to arterial and venular endothelium within minutes [9,10]. These responses appeared to involve leukocyte aggregation with platelets, and changes in leukocyte and endothelial adhesiveness [9].

The constituents of cigarette smoke influencing endothelial function are uncertain, although oxygen free radicals have been implicated in modification of endothelial cell-mediated vasodilation [2]. In this context, it is interesting to note that oxidised low density lipoprotein (LDL) can increase leukocyte adhesion after treatment of animals [11] and of cultured endothelial cells [12], through upregulation of P-selectin. We sought to test whether water soluble components of cigarette smoke could directly induce endothelial cells to bind flowing leukocytes, without interference from effects on the leukocytes themselves. Such agents might influence responses in the lungs, but could also mediate effects in remote tissue to which they might be transported in the plasma. We chose to study adhesion of flowing neutrophils, as important contributors to inflammation, and as reporters of functional expression of endothelial selectin receptors capable of capturing flowing leukocytes [13].

* Corresponding author. Fax: +440-121-414-6919.
E-mail address: g.nash@bham.ac.uk (G.B. Nash).

0006-291X/02/$ - see front matter
PII: S0006-291X(02)00800-8

Methods

Isolation of neutrophils. Blood was collected from healthy volunteers into K_2EDTA, (Sarstedt, Leicester, UK) and used within 2 h of venepuncture. Neutrophils were isolated by centrifuging the whole blood at 800*g* for 30 min over a two-step density gradient consisting of equal quantities of Histopaques 1119 and 1077 (Sigma Chemical, Poole, UK) as described [14,15]. The neutrophil layer was aspirated from the density interface and washed twice in sterile PBSA: phosphate buffered saline (Invitrogen, Renfrewshire, Scotland) with 0.15% bovine serum albumin (Sigma). Neutrophils were counted using a Coulter counter (Coulter Electronics, Harpenden, Essex) and suspended at 10^6/ml in PBSA.

Endothelial cell culture. Endothelial cells from human umbilical veins (HUVEC) were isolated as previously described [16]. Primary cells were grown in 25 cm^2 flasks (Becton–Dickinson UK, Oxford) in culture medium containing M199 (Invitrogen) supplemented with 20% heat inactivated human serum, 50 U/ml sodium heparin (CP Pharmaceuticals, Wrexham), 1 mg/ml glutamine (Sigma) and 28 μg/ml gentamicin (David Bull Labs, Warwick, UK). The cells were grown to confluence before passaging onto one inner surface of microslides (flattened glass capillaries 50 mm long, with a rectangular cross-section of 0.3 × 3 mm; Camlab, Cambridgeshire, UK) as described [16]. Prior to culture the microslides were treated with 3-aminopropyltriethoxysilane (Sigma), autoclaved and coated with a collagen/gelatin mixture consisting of 200 μg/ml type IV collagen and 1% gelatin (Sigma) in PBS. This treatment facilitated adhesion of the endothelial cells to the glass surface. Microslides were connected to a pump system which allowed fresh medium to be perfused through them for 30 s each hour. HUVEC were seeded at a concentration sufficient to give a confluent monolayer 24 h after seeding.

Exposure of endothelium to water-soluble constituents of cigarette smoke. The apparatus for treating endothelium with cigarette smoke, and carrying out subsequent adhesion assays is shown schematically in Fig. 1. Four microslides containing confluent HUVEC were glued to a glass slide and placed on the stage of a video-microscope. The microslides, and a parallel purging line, were connected with silicon rubber tubing at one end to a manifold and thence to a Harvard syringe pump which drew medium through them. A system of electronically operated pinch valves controlled whether flow passed though a particular microslides, or the purge line. Upstream, the microslides and purge line were connected to another manifold, and thence to two electronic three-way valves which allowed smooth switching between three fluid reservoirs. The flow rate was maintained at 0.37 ml/min at all times, yielding a wall shear stress of 0.1 Pa in any perfused microslide.

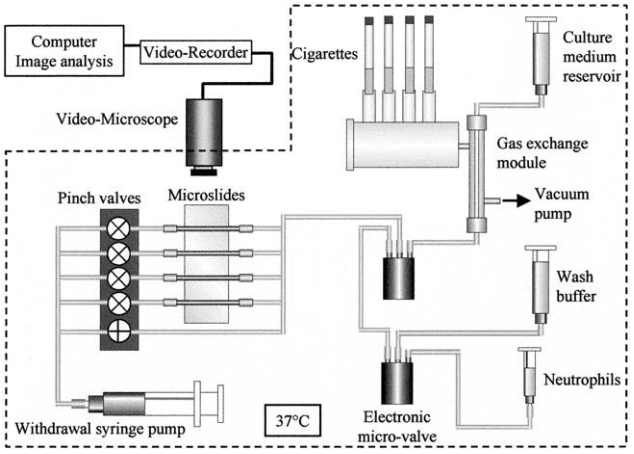

Fig. 1. Schematic of the flow system for perfusing HUVEC cultured in microslides with culture medium that had been exposed to water soluble components of cigarette smoke, and subsequently with neutrophils in suspension. Flow was induced by a withdrawal syringe pump. Electronically controlled pinch valves determined which microslide was perfused at any time, and fluid was either drawn from a culture medium reservoir, or reservoirs containing wash buffer (PBSA) or neutrophils in suspension. The culture medium was exposed to water soluble components of cigarette smoke in a gas exchange module (see text). Perfusion of neutrophils was viewed and recorded using a videomicroscope.

For cigarette smoke treatment, culture medium was drawn from one culture medium reservoir through a specially constructed gas exchange module. The module consisted of 48 hollow, microporous, polyethylene fibres with internal diameter of 200 μm and length of 60 mm, which were enclosed within an outer perspex cylinder and cemented into Luer connectors at each end. The medium was drawn through the fibres, while the outer perspex cylinder was flooded with smoke drawn by a rotating vacuum pump from upto four lighted cigarettes (medium tar, Embassy No.1) held in a manifold. Smoke was thus passed over the fibres and water-soluble constituents were taken up into the culture medium across the porous walls. The gas exchange modules were identical to those used previously to de-oxygenated medium in studies of effects of hypoxia on HUVEC [15]. The cigarette manifold was packed with cotton wool to remove particulates and some tar from the smoke. Cigarettes took approximately 5 min to be 'smoked' by the device. When not being perfused with smoke-exposed medium, flow could be switched instead to a wash reservoir containing PBSA, or another containing neutrophils in PBSA. Neither wash medium nor neutrophils were passed through the gas exchanger. The whole system (except pumps) was enclosed in a perspex box held at 37 °C. All reservoirs were discardable sterile syringe barrels. The barrels and all tubing were discarded after each experiment. Because the gas exchange module and valves could not be autoclaved at 250 °C, polymixin B (100 μg/ml) was added to all media to neutralise potential endotoxin contamination.

The basic treatment protocol was to flush all lines with culture medium and then light the cigarettes. A single microslide was then exposed to smoke/medium for 5 min, and then flow was switched to the purge line to clear the system. Exposure of three microslides continued in turn, commencing 5 min after the previous microslide. The fourth microslide (control) was exposed to medium alone, without exposure to smoke or the gas exchange module, because initial experiments showed some carryover of agents from the 'smoked' line. Smoke exposure could be repeated as desired, for instance, every 30 min, and/or desired periods allowed to elapse before perfusion of neutrophils. In some experiments, different media were exposed to cigarette smoke and perfused over HUVEC. Culture medium was compared to PBSA, M199 without serum or M199 without serum but with added LDL (100 μg/ml; derived from human plasma; Sigma). In these comparisons, exposure of HUVEC to one medium had to be completed before another one could be tested. Order of treatment was rotated between experiments.

Flow-based adhesion assay. After completion of chosen cycles of smoke exposure, neutrophil suspension was perfused across treated or untreated HUVEC for 5 min at a wall shear stress of 0.1 Pa. This stress is sufficient to ensure that binding of neutrophils to HUVEC requires selectin expression and cannot occur directly through integrin-mediated adhesion [15,17]. Non-adherent cells were washed out with PBSA for 2 min, and the number of adherent neutrophils was counted in at least 5 fields of known dimensions, along the centreline of the microslide. The count was expressed as cells/mm^2 and normalised for the number perfused. Video recordings were made of the behaviour of the adherent cells in the last minute of inflow. These were analysed off-line, and adherent cells were categorised as stationary (moving < one cell diameter in 10 s) or rolling (tumbling continually over the HUVEC at velocity >1 μm/s but much slower than free-flowing cells which were only visible as blurred streaks with velocity >500 μm/s).

In pilot studies, whole blood from a non-smoker was flowed through the gas exchange module and treated by the smoke from four cigarettes smoked simultaneously. The effluent blood showed an increase in carboxy-haemoglobin (% of total haemoglobin) from <1% to 8% when the blood was flowed at 0.37 ml/min. The latter carboxy-haemoglobin value is comparable to those found in habitual smokers.

Antibody treatments. After exposure to cigarette smoke, antibodies against E-selectin (1 μg/ml, ENA2 F(ab′)2; Bradshaw Biologicals, Chepshet, UK), P-selectin (20 μg/ml; polyclonal rabbit anti-human P-selectin; gift of Dr. Michael Berndt, Baker Institute, Melbourne) or both were perfused into microslides from the sample reservoir and incubated for 15 min. The antibodies were washed out and neutrophils perfused as before. Alternatively, neutrophils were treated with antibody against CD18 (16 μg/ml; MHM23; Dako, Ely, UK) for 20 min at room temperature before perfusion. Previous studies have shown that each of these antibodies is effective in blocking adhesive function of the receptors at the concentrations used here [17,18].

ELISA for surface expression of selectins on HUVEC in microslides. At the end of treatment with cigarette smoke, microslides with treated HUVEC and controls were disconnected from the flow system, and primary antibody against E-selectin (0.2 μg/ml, 1.2B6; Dako) or P-selectin (1 μg/ml; as above) was injected. Microslides were incubated for 60 min at room temperature. Antibody was washed out with PBSA and secondary horse radish peroxidase-conjugated goat-anti-mouse antibody (1/2000; Dako) was injected. After incubation for 60 min at room temperature, antibody was washed out and peroxidase substrate (1,2-phenylenediamine dihydrochloride; Dako) was injected and incubated for 5–10 min. The coloured reagent was flushed out with 200 μl of 1 M H_2SO_4 into a 96-well plate and the absorbance measured at 490 nm using a plate reader. As positive controls, HUVEC in microslides were treated with tumour necrosis factor-α (TNF; 100 U/ml) for 4 h [14] and subject to identical ELISA.

Results and discussion

Establishment of a smoking regime that could induce HUVEC to bind flowing neutrophils

Initial experiments aimed to test regimes for treating HUVEC with the water soluble products of cigarette smoke to see which might induce subsequent neutrophil adhesion. HUVEC were exposed to culture medium which had been treated by the smoke from four cigarettes smoked simultaneously. If neutrophils were perfused over the period 2–7 min afterwards, we found that there was no increase in neutrophil adhesion compared to untreated HUVEC (∼20 adherent cells/$mm^2/10^6$ perfused in each case in two experiments). Using the same dose of cigarette smoke, but waiting longer before perfusing neutrophils, we found a low but easily detectable level of adhesion occurred after 60 min (80 adherent cells/$mm^2/10^6$ perfused), and plentiful adhesion was observed after 90 or 120 min (mean of 330 adherent cells/$mm^2/10^6$ perfused in four experiments). In a further modification, HUVEC were exposed to an initial dose of smoke from four cigarettes, then treated to the smoke from one cigarette every 30 min for 120 min, before waiting a further 30 min and perfusing neutrophils. We found that neutrophil adhesion to the treated HUVEC was consistently greater than to untreated cells (505 ± 131 vs. 30 ± 7 adherent cells/$mm^2/10^6$ perfused, respectively; means ± SEM from 11 experiments; $p < 0.01$ by paired t test). Approximately half the cells adherent to smoke-treated HUVEC were rolling and half stationary ($55 \pm 3\%$ rolling; means ± SEM from seven experiments). This treatment regime was used henceforth.

Thus exposure of endothelial cells to water soluble components of cigarette smoke induced ability to capture flowing neutrophils, with adhesion detectable ∼60 min after treatment, and increasing upto ∼150 min. This is rather slower than the response of HUVEC to agonists such as histamine, which induce expression of P-selectin and capture of neutrophils within minutes [19]. It is closer to the time course for treatment with oxidised LDL, which induced expression of P-selectin over 60–120 min [12], and hydrogen peroxide, where we found P-selectin-mediated adhesion of flowing neutrophils after 60 min [20]. Responses of HUVEC to cigarette smoke condensate took ∼hours, judged by upregulation of E-selectin [8]. On the other hand, leukocyte and platelet adhesion was detectable on the walls of vessels in animals within 15 min of inhalation of cigarette smoke [10]. This response may have been as a result of changes in leukocyte and platelet adhesiveness rather than in the endothelial cells. The effect of smoke exposure in the present study was clearly on HUVEC and not on neutrophils, since neutrophils had no direct contact with treated medium, and did not bind when perfused immediately after HUVEC treatment. It is important to note that levels of adhesion were very low for control HUVEC indicating that attachment to the flow/smoking system for >2 h itself did not upregulate receptors.

Effect of medium used for perfusion

When PBSA was used for exposure to smoke and perfusion of HUVEC, and neutrophils perfused after 150 min, there was little increase in adhesion over controls (26 versus 11 adherent cells/ $mm^2/10^6$, respectively; means from three experiments). Culture with PBSA for this period caused some retraction of endothelial cells, and the failure in response may have been due to lack of nutrients, serum components, or both. Thus we compared culture medium with and without 20% human serum added. Using M199 without serum, there was a significant but lower induction of adhesion than when serum was present (Fig. 2). The proportion of adherent neutrophils rolling was similar (∼50%) for both media (data not shown).

We speculated that there might be a role for the low density lipoprotein fraction of serum, as these compounds form stable oxidation products which can cause activation of vascular endothelium [12]. We added purified LDL (100 μg/ml) to M199 instead of serum and

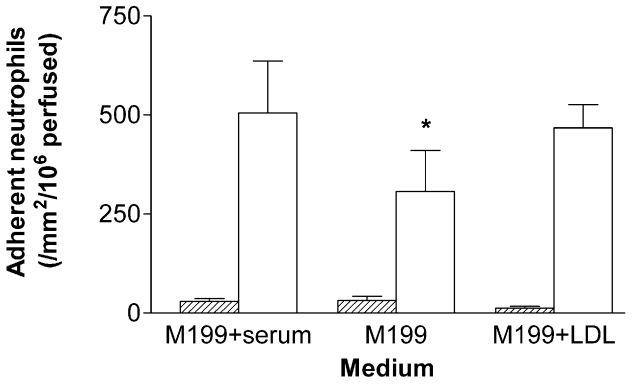

Fig. 2. Adhesion of flowing neutrophils to HUVEC which had been exposed to water soluble components of cigarette smoke in different media. Media were either untreated (▨) or exposed to smoke (□). M199 + serum = complete culture medium; M199 = culture medium without serum; M199 + LDL = culture medium without serum but with LDL (100 μg/ml) added. Data are means ± SEM from 4 to 11 experiments. Exposure to smoke caused significant increase in adhesion for each medium ($p < 0.01$ by paired t test in each case). *$p < 0.05$ compared to M199 + serum by paired t test.

compared this to M199 alone. Control HUVEC treated with M199 or M199 + LDL for 2 h without smoke exposure gave similar low levels of adhesion (Fig. 2). Smoke-exposed M199 + LDL tended to cause a greater level of adhesion, compared to smoke-exposed M199 alone (Fig. 2B). Thus addition of LDL returned the effect of smoke-exposure partway towards the level with serum. These results suggest that the effects on HUVEC were not necessarily directly mediated by substances in the smoke, but may have arisen from modification of compounds in the culture medium and serum. One possibility is that LDL in serum (or added to medium) was oxidised and effective in stimulating HUVEC. This cannot have been the only factor, as M199 alone did give a significant response when exposed to cigarette smoke.

Adhesion molecules supporting adhesion

Capture of flowing neutrophils by stimulated HUVEC is typically mediated by selectins (e.g., [14,15,19]). Thus, after exposure to cigarette smoke for 2 h, we treated the endothelial monolayer with function-blocking antibodies against E- and/or P-selectin during the 30 min before neutrophil perfusion. Individually, blockade of E-selectin caused a reduction in adhesion of about 50%, and blockade of P-selectin a reduction of about 75%, compared to control (Fig. 3A). Adhesion of neutrophils was returned to control levels when the HUVEC were treated with both antibodies together (Fig. 3A). Since these results indicate that cigarette smoke products can cause upregulation of both E- and P-selectin onto the surface of HUVEC, we carried out ELISA for these receptors on the HUVEC. ELISA

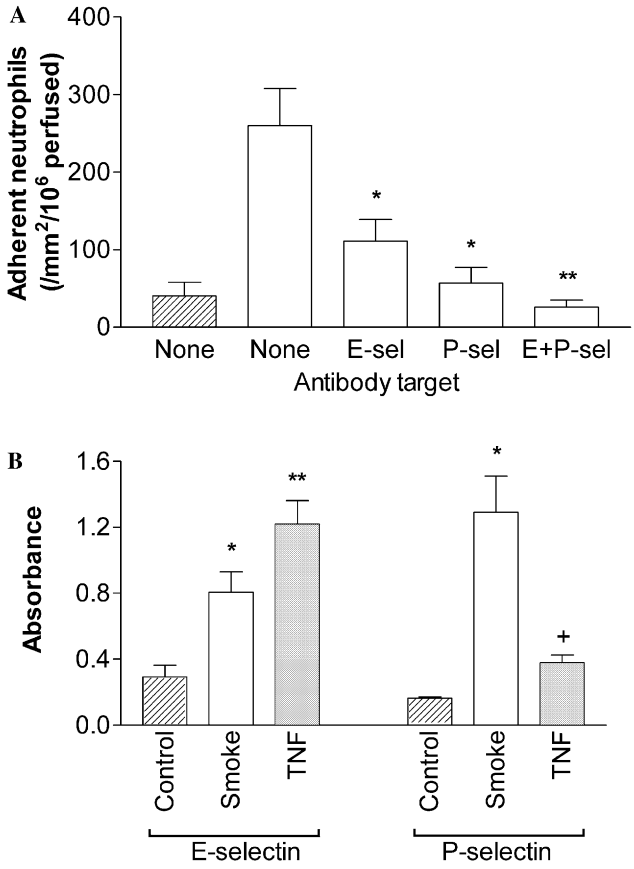

Fig. 3. Role of selectins in response of HUVEC to cigarette smoke. (A) Effects of antibody blockade on adhesion of flowing neutrophils to HUVEC which had been exposed to culture medium which was untreated (▨) or exposed to smoke (□). Subsequently, HUVEC were treated with antibodies against E-selectin (E-sel), P-selectin (P-sel), or both (E + P-sel), or no antibody (none). Data are means ± SEM from 3 to 7 experiments. *$p < 0.05$, **$p < 0.01$ compared to smoke exposed HUVEC without antibody. (B) Surface expression of selectins on HUVEC which had been exposed to culture medium which was untreated (control; ▨), exposed to smoke (smoke; □), or had 100 U/ml TNF added (TNF; ■). Data are absorbance values after ELISA carried out using antibodies against E-selectin or P-selectin (means±SEM from three experiments). +$p = 0.055$, *$p < 0.05$, **$p < 0.01$ compared to control HUVEC.

confirmed that there was significant increase in surface expression of both selectins after smoke exposure (Fig. 3B). Changes were compared to those induced by treatment of HUVEC in microslides with TNF for 4 h, which also causes adhesion of flowing neutrophils through E- and P-selectin [14]. The increase in expression of E-selectin induced by cigarette smoke was less than that induced by TNF, while the increase in P-selectin was greater.

Thus, after smoke exposure, P-selectin appeared to play the greater role in neutrophil capture but there was a contribution also from E-selectin. P-selectin is available in granule stores and can be exposed within an hour after treatment with oxidised LDL or hydrogen peroxide [12,20]. Expression of E-selectin in HUVEC requires

de novo gene expression [13] and this may explain the fact that adhesion took 1–2 h to reach high levels. It is notable that E-selectin was upregulated by cigarette smoke condensate, but not P-selectin [8], so that it is possible that different components of cigarette smoke induced the different receptors. For instance, short-lived oxidants may have modified medium constituents, which in turn upregulated P-selectin, while other soluble components of cigarette smoke may themselves have induced E-selectin expression. Further work is needed to define exactly what components and agents are involved in the response.

Since not all adherent cells rolled continually, and about 50% became stationary adherent, it is likely that agent(s) capable of activating neutrophils integrins were presented by the endothelial cells. We thus tested whether neutrophil immobilisation was mediated by β2-integrins, as in other models of HUVEC stimulation [14,15]. When neutrophils were treated with mAb against CD18 for 15 min immediately prior to the adhesion assay, we found that there was no reduction in number attached to the HUVEC, but most of the adherent cells now formed rolling attachments (88% rolling compared to 45% for untreated control neutrophils; mean from two experiments). This indicates that stationary adhesion could be largely attributed to activation of β2-integrins. However, it was notable that the stationary cells did not migrate through the HUVEC monolayer. Transmigrated cells are easily detectable as phase dark spread cells, rather than phase bright cells on top of the HUVEC [14,21]. For instance, we have previously found that ~50% of adherent cells transmigrate after treatment of HUVEC with 100 U/ml TNF [14,21]. It seems therefore that the water soluble fraction of cigarette smoke can not only induce selectin expression on HUVEC, but also expose activating agent(s) sufficient to cause some neutrophils to immobilise, but not migrate. HUVEC can potentially induce activation and migration of flowing neutrophils through a range of agents including CXC-chemokines and platelet-activating factor [15,21]. Further studies will be necessary to explore if any of these agents are indeed upregulated by smoke exposure.

Conclusions

Endothelial cells in tissues remote from the lungs do not come into contact with particulate or insoluble components of cigarette smoke. However, they will be exposed to substances exchanged into the blood from the lung air spaces. We modelled this process by perfusing culture medium through porous hollow fibres which allowed uptake of water soluble compounds. This method has worked previously in studies of response of HUVEC to hypoxic medium [15]. When endothelial cells were exposed to these components, we found a response that was apparent about 60 min later, when flowing neutrophils attached to the surface. Adhesion was more marked at 150 min and was attributable to upregulation of surface expression of P- and E-selectin. All major leukocyte subsets can bind to P-selectin, and monocytes and some T-cells also bind E-selectin, so that a comparable response in vivo would not necessarily be restricted to neutrophils. The implication is that cigarette smoking could induce a persistent inflammatory stimulus and thus contribute to long-term damage to vessel walls or surrounding tissue, or exacerbate other ongoing inflammatory disorders [1,22,23]. Exactly where this might occur would depend on local endothelial responsiveness, flow rates, and other environmental factors predisposing to inflammation.

Acknowledgment

This work was supported by a grant from The Leverhulme Trust.

References

[1] M. Bartal, Health effects of tobacco use and exposure, Monaldi Arch. Chest Dis. 56 (2001) 545–554.
[2] R.M. Pittilo, Cigarette smoking endothelial injury and cardiovascular disease, Int. J. Exp. Pathol. 81 (4) (2000) 219–230.
[3] Y. Shen, V. Rattan, C. Sultana, V.K. Kalra, Cigarette smoke condensate-induced adhesion molecule expression and transendothelial migration of monocytes, Am. J. Physiol. 270 (1996) H1624–H1633.
[4] E.M. Drost, C. Selby, M.M. Bridgeman, W. MacNee, Decreased leukocyte deformability after acute cigarette smoking in humans, Am. Rev. Respir. Dis. 148 (1993) 1277–1283.
[5] C. Weber, W. Erl, K. Weber, P.C. Weber, Increased adhesiveness of isolated monocytes to endothelium is prevented by vitamin C intake in smokers, Circulation 93 (1996) 1488–1492.
[6] W. MacNee, B. Wiggs, A.S. Belzberg, J.C. Hogg, The effect of cigarette smoking on neutrophil kinetics in human lungs, N. Engl. J. Med. 321 (1989) 924–928.
[7] C. Selby, E. Drost, D. Brown, S. Howie, W. MacNee, Inhibition of neutrophil adherence and movement by acute cigarette smoke exposure, Exp. Lung. Res. 18 (1992) 813–827.
[8] V.K. Kalra, Y. Ying, K. Deemer, R. Natarajan, J.L. Nadler, T.D. Coates, Mechanism of cigarette smoke condensate induced adhesion of human monocytes to cultured endothelial cells, J. Cell. Physiol. 160 (1994) 154–162.
[9] H.A. Lehr, B. Frei, K.E. Arfors, Vitamin C prevents cigarette smoke-induced leukocyte aggregation and adhesion to endothelium in vivo, Proc. Natl. Acad. Sci. USA 91 (1994) 7688–7692.
[10] H.A. Lehr, E. Kress, M.D. Menger, H.P. Friedl, C. Hubner, K.E. Arfors, K. Messmer, Cigarette smoke elicits leukocyte adhesion to endothelium in hamsters: inhibition by CuZn-SOD, Free Radic. Biol. Med. 14 (1993) 573–581.
[11] H.A. Lehr, A.M. Olofsson, T.E. Carew, P. Vajkoczy, U.H. von Andrian, C. Hubner, M.C. Berndt, D. Steinberg, K. Messmer, K.E. Arfors, P-selectin mediates the interaction of circulating leukocytes with platelets and microvascular endothelium in response to oxidized lipoprotein in vivo, Lab. Invest. 71 (1994) 380–386.

[12] V. Gebuhrer, J.F. Murphy, J.C. Bordet, M.P. Reck, J.L. McGregor, Oxidized low-density lipoprotein induces the expression of P-selectin (GMP140/PADGEM/CD62) on human endothelial cells, Biochem. J. 306 (1995) 293–298.
[13] G.S. Kansas, Selectins and their ligands: current concepts and controversies, Blood 88 (1996) 3259–3287.
[14] P. Bahra, G.E. Rainger, J.L. Wautier, N.-T. Luu, G.B. Nash, Each step during transendothelial migration of flowing neutrophils is regulated by the stimulatory concentration of tumour necrosis factor-alpha, Cell Adhes. Commun. 6 (1998) 491–501.
[15] G.E. Rainger, A. Fisher, C. Shearman, G.B. Nash, Adhesion of flowing neutrophils to cultured endothelial cells after hypoxia and reoxygenation in vitro, Am. J. Physiol. 269 (1995) 1398–1406.
[16] B.M. Cooke, S. Usami, I. Perry, G.B. Nash, A simplified method for culture of endothelial cells and analysis of adhesion of blood cells under conditions of flow, Microvasc. Res. 45 (1993) 33–45.
[17] G.E. Rainger, M.-P. Wautier, G.B. Nash, J.-L. Wautier, Prolonged E-selectin induction by monocytes potentiates the adhesion of flowing neutrophils to cultured endothelial cells, Br. J. Haematol. 92 (1996) 192–199.
[18] P.C.W. Stone, G.B. Nash, Conditions under which immobilised platelets activate as well as capture flowing neutrophils, Br. J. Haematol. 105 (1999) 514–522.
[19] D.A. Jones, O. Abbassi, L.V. McIntire, R.P. McEver, C.W. Smith, P-selectin mediates neutrophil rolling on histamine-stimulated endothelial cells, Biophys. J. 65 (1993) 1560–1569.
[20] G.E. Rainger, A.C. Fisher, G.B. Nash, Neutrophil rolling is rapidly transformed to stationary adhesion by IL-8 or PAF presented on endothelial surfaces, Am. J. Physiol. 272 (1997) H114–H122.
[21] N.T. Luu, G.E. Rainger, G.B. Nash, Differential ability of exogenous chemotactic agents to disrupt transendothelial migration of flowing neutrophils, J. Immunol. 164 (2000) 5961–5969.
[22] X. Guo, W.P. Wang, J.K. Ko, C.H. Cho, Involvement of neutrophils and free radicals in the potentiating effects of passive cigarette smoking on inflammatory bowel disease in rats, Gastroenterol 117 (1999) 884–892.
[23] F. Galeazzi, P.A. Blennerhassett, B. Qiu, P.M. O'Byrne, S.M. Collins, Cigarette smoke aggravates experimental colitis in rats, Gastroenterol 117 (1999) 877–883.