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KEYNOTE PAPER

STUDYING INFLAMMATORY RESPONSES OF ENDOTHELIAL CELLS AND LEUKOCYTES IN PERFUSED MICROCHANNELS

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ABSTRACT

Circulating leukocytes must adhere to the endothelial cells (EC) that form the lining of blood vessels, and migrate through them to carry out their protective immune functions. During inflammation this recruitment is typically controlled by cytokines released from tissue that act on the EC. The endothelial cells respond by increasing the expression of adhesion molecules on their surface (to capture flowing leukocytes), and also by presenting chemotactic agents (to induce the captured cells to migrate). This recruitment process is influenced by the local haemodynamic milieu in several ways: interactions with red cells modify the distribution of leukocytes in the blood stream; flow velocity and shear stress influence the formation and breakage of adhesive bonds; flow forces act on EC and modify their responses to inflammmatory cytokines. Microchannels have been widely used to study these processes, especially the specific receptors required for capture of isolated flowing leukocytes and their ability to support adhesion as a function of fluid shear stress. We developed a versatile system based on pre-fabricated glass capillaries with rectangular cross-section (microslides) in which we cultured EC, and which could also be coated with purified adhesion receptors for reductive studies. We also developed fluoresence-microscope-based systems for using these microslides to observe adhesion in flowing whole blood, and multiple parallel cultures for studying the effects of conditioning the EC by growth at different levels of shear stress before investigations. The microslides are available in various dimensions, and smaller versions can be used to generate high circulatory stresses when small volumes of materials (such as blood from genetically modified mice) are available. With these systems, we have for instance, been able to show how varying the concentration and aggregability of red blood cells alters leukocyte adhesion, and how expression levels of endothelial genes which underly inflammatory responses are modified by culture at a range of shear stresses mimicking different regions of the circulation.

INTRODUCTION

Inflammation is a protective response to tissue injury and infection which requires recruitment of flowing leukocytes from the blood, onto the vessel wall and into tissue. The process is regulated through the endothelial cells (EC) which form a monolayer lining the blood vessels[1,2]. The EC respond to chemical signals from the underlying tissue (such as cytokines released by resident macrophages) by expressing specialist adhesion receptors on their surface (including members of the selectin family) which enable capture of fast-moving leukocytes. Typically, the initial attachments are unstable but allow leukocytes to bind stimulatory agents also presented on the endothelial surface (such as chemotactic cytokines or 'chemokines'). These induce a rapid activation of different adhesion molecules (integrins) on the leukocytes which support firm adhesion and enable the cells to migrate over and then through the endothelial monolayer. While these steps are essential for immune destruction of pathogens and tissue remodelling, uncontrolled or inappropriate recruitment can also be damaging to tissue, and contributes to a variety of important pathologies, including atherosclerosis, rheumatoid arthritis and vasculitis. Thus, the processes of adhesion and migration, and the regulation of endothelial responses have been widely studied.

Experimentally, however, a considerable challenge is presented by the fact that the system operates in a dynamic flow environment which physically constrains cell-cell contacts and adhesive interactions[3]. The fluid forces can also modify the behaviour of the endothelial cells and leukocytes through 'mechanotransduction'. For realistic studies the choice has been either to use animals and intravital microscopy of the circulation, or to develop more controllable in vitro models where human blood or isolated leukocytes are perfused over surfaces coated with purified adhesion molecules or cultured EC. The in vitro approach has typically involved the use of

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microchannels, with the aim to reproduce some aspects at least of the in vivo milieu.

DESIGN REQUIREMENTS FOR MICROCHANNEL STUDIES

Reproduction of critical aspects of adhesion and migration in the circulation is not easy. During inflammation, most adhesion occurs in post-capillary venules with circular cross-section and diameter ~ a few tens of µm, and absolute volumetric flow rates of only ~nl/s. On the other hand, pathogenic inflammation of artery walls in atherosclerosis may occur in vessels with diameter ~cm and flow ~10ml/s. The fluid itself, blood, is particulate and non-newtonian, with viscosity decreasing with shear rate[3]. In small vessels the blood tends to undergo phase separation, with red cells flowing centrally and a relatively cell-depleted layer of plasma near the wall (i.e., plug flow). The flow is thus complex, and indeed the wall shear rate (which will determine how rapidly cells are flowing immediately before capture) and the wall shear stress (which will determine the force applied to cells which have adhered) cannot be predicted precisely even when blood is perfused at known rate through a microchannel with known dimensions.

Practically, most studies have used dilute suspensions of leukocytes in physiological saline (with newtonian viscosity, η ~ water), and rectangular channels because of their good optical qualities[4,5]. The channel width (w) is made >> height (h), so that side-wall effects can be ignored and flow can be modelled as two-dimensional between infinite parallel plates. The wall shear rate (γ_w) and stress (τ_w) along the centre-line are then well-approximated, as functions of volumetric flow rate Q, by: $\gamma_w = 6.Q/w.h^2$; $\tau_w = \gamma_w.\eta$ (see Figure 1). Clearly, if a dilute suspension is used instead of blood, the wall shear rate and stress cannot both be matched to the situation in vivo, and typically the wall shear stress is chosen experimentally to be in the range found in venules. This is not ideal because the presence of red cells affects the stress rate and stress, and also transport of leukocytes to the wall. The phase separation and plug flow causes the wall shear rate to be higher than expected for simple parabolic flow, and it is hard to define the wall shear stress because the local fluid viscosity is lower than expected for homogenous, mixed blood. Margination is a term used for non-uniform distribution of leukocytes driven by a form of facilitated diffusion through collisions with red cells, and encouraged at low shear rate by tendency of red cells to aggregate and more strongly undergo axial migration toward the centre. Thus, overall, use of whole blood may be preferable for mechanistic studies but it is problematic because of opacity of the fluid, the need for anti-coagulation, and the presence of mixed populations of leukocytes. As a result, blood has not been used commonly to date, although we and others have carried out microchannel studies of leukocyte adhesion using whole blood[6,7].

The primary goals of such studies may be to determine how haemodynamic and rheological factors influence contact between leukocytes and the vessel wall, and to define the cell velocities and shear forces which influence the efficiency of attachment of flowing leukocytes and the survival of the receptor-ligand bonds which support this attachment[8]. In addition, the molecular mechanisms underlying adhesion need to be disected in realistic models, if results are to reflect accurately the processes which occur in vivo. It also appears that the behaviour of leukocytes after they adhere to the wall of vessels are modulated by shear forces acting on them, manifest e.g., as preferential migration in the direction of flow[9]. Finally, the local haemodynamic forces can modify the expression of genes by the endothelial cells that line vessels, and hence influence their intrinsic ability to support leukocyte recruitment[10]. Thus, endothelial cell cultures have been incoporated in microchannels, to study how their responses are conditioned by flow over time, as well as for adhesion testing per se. In this context, it should also be borne in mind that ‘resting’ unstimulated endothelial cells do not generally support recruitment of leukocytes, and that to induce ‘inflammation’ in vitro, cytokines (such as tumour necrosis factor-α, TNF, or interleukin-1β, IL-1) or faster-acting agents such as histamine or thrombin need to be added to cultures.

LEUKOCYTE RECRUITMENT IN MICROCHANNELS - HISTORICAL OVERVIEW

Methodology

Microchannels for adhesion studies have typically been constructed of a coated-glass base-plate, overlaid by a sealing gasket with an excised slot that defines the flow channel (width, w~cm, depth h<mm), an upper plate, and inlet and outlet machined to allow perfusion (see e.g., Figure 1). In early biomechanical studies, such chambers were used to study the shear-dependent deformation of red blood cells[11] or for perfusing blood over surfaces in studies of thrombosis[12]. Early work with leukocytes (specifically the neutrophilic granulocytes, neutrophils) analysed adhesion of flowing cells to protein-coated glass surfaces[13]. The chambers were later adapted to incoporate cultured endothelial cells grown on the base-plate, to study abnormal adhesiveness of red cells from patients with sickle cell disease[14], and endothelial attachment of neutrophils[4,15]. Specific purified adhesion receptors embedded in lipid layers were used to study better-defined capture processes soon after[16]. Initially we used parallel plate flow chambers to study adhesion of malarial parasitised red cells to endothelial cells[17], but then used preformed glass channels (microslides; with w=3mm and h=0.3mm; Figure 1b) coated with platelets to analyse leukocyte adhesion processes relevant to inflammation and thrombosis[18]. We developed a system for culture of endothelial cells inside the microslides[5], and adapted this for studies of prolonged exposure to different levels of flow[19], allowing studies of the leukocyte adhesion to endothelial monolayers and migration through them for differently conditioned endothelium. Microslides with smaller dimensions (w=1mm and h=0.1mm) have been used for studies of platelet rather than leukocyte adhesion, making it possible to apply high shear rates using relatively small volumes of blood[20]. Even smaller microfluidic channels have recently been used to study adhesion and migration of leukocytes on proteins[21]. The smaller scale channels have not been used with endothelial cells to date. In a different development, we returned to use of parallel plate systems but incorporated porous filters in the base (Figure 1c). This allowed us to study adhesion to co-cultures of endothelial cells and stromal cells (such as smooth muscle cells

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or fibroblasts) grown on the oppositie surfaces of the filters[22,23], and to study the ability of leukocytes to migrate away from underneath of the endothelial cells after they have been recruited[24].

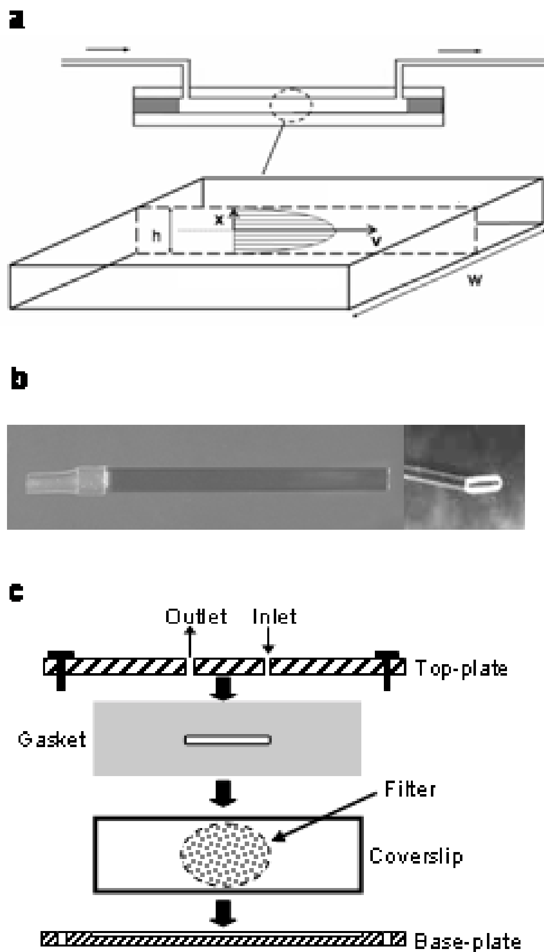

Figure 1. Microchannels used for adhesion studies.
a. Basic parallel plate construction (channel width w, height h)[17]. b. Pre-fabricated microslide; glass capillary with internal width 3mm, height 0.3mm[5]. c. Parallel plate flow chamber used to incorporate filter into flow channel[24].

In all of the above, the transparent channels are attached to perfusion systems and mounted on the stage of a microscope (preferably all held at 37°C), so that isolated leukocytes or blood can be delivered, and interactions between cells and surface directly viewed and recorded. Phase-constrast microscopy may be used to visualise the unstained, live cells, although if blood is used, fluorescence microscopy and dyed cells must be used to observe attachments as they happen. Using the above, many studies of leukocyte recruitment have been made, broadly divisible into those evaluating the capture phase, and those investigating the activation and subsequent migration of the captured cells.

Leukocyte capture from flow in microchannels

In early studies of adhesion of flowing neutrophils to unstimulated endothelial cells, 'resting' neutrophils did not bind. Activated neutrophils attached through their integrin receptors but only at wall shear stress <0.1Pa, a range rather lower than expected in vivo[15]. However, when cytokine stimulated endothelial cells were used, unstimulated neutrophils bound efficiently at stress ~0.2Pa, a more realistic value[4,25]. Some cells rolled on the surface while others clearly became activated there, and attachment required engagement of selectins on the endothelium. The situation was clarified in studies with purified rececptors, where P-selectin supported capture and rolling adhesion of neutrophils, again upto about 0.2Pa, and stationary adhesion could be induced if the neutrophils were treated with exogenous agents (such as bacterial toxin analog fMLP or the chemokine, IL-8) that caused integrin activation[16]. Subsequent studies with purified E-selectin[26], and P-selectin expressed on activated immobilised platelets[18] confirmed that using isolated suspensions, neutrophil capture from flow was possible upto a maximum wall shear rate ~400s^{-1} or wall shear stress 0.3Pa. Interestingly, while selectins were also capable of capture of flowing lymphocytes[27,28], a member of the immunoglobulin superfamily, VCAM-1 was found to be capable of fulfilling the role as well, through binding to the lymphocyte $\alpha4\beta1$-integrin not found on human neutrophils[29]. Immobilisation as well as rolling of lymphocytes was possible on VCAM-1 if the cells were activated[30]. Monocytes have also been shown to be capable of capture through selectins[31].

The picture that arose from such studies was that specialised receptors (selectins and, in some cases, VCAM-1) could support capture and rolling of unstimulated leukocytes on activated, inflammed endothelium. However, immobilisation of leukocytes would require presentation of some signal, presumably on the endothelium, so that integrins would undergo 'activation' and engage their counter-receptor(s).

Leukocyte activation and migration on endothelial cells

Flow studies using endothelial cells that had been treated with IL-1 or TNF, or exposed to hypoxia and reoxygenation, clearly dissected the sequential steps of neutrophil capture and rolling, followed by firm adhesion and onward migration[25,32,33]. Activating agents presented by the endothelium included chemokines such as IL-8, and the phospholipid-derived, platelet-activating factor, PAF. Indeed, in separate experiments, when purified IL-8 or PAF were added to the surface of endothelial cells which had been stimulated only to express selectins, they caused transformation of unstable rolling attachment to stationary adhesion[34]. This transformation could be signalled in only 0.25-0.5s depending on the agent. Examining the kinetics of adhesion and migration on TNF-treated endothelial cells in more detail, we found that on average, ~40% of captured neutrophils became activated in <1s while others responded more slowly after roling for ~20s[35]. Activated cells migrated for 1-2min on the EC surface before taking ~1min to migrate through it. In later experiments it became evident that cells preferentially migrated in the direction of flow on the endothelial surface, guided by mechanotransduction of signals through specific adhesive signals[9]. In studies by others, when activating agents were added to the surface of endothelial cells, the transmigration process was more efficient in the presence of flow than under static conditions[36]. Thus, using dynamic microchannel models

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it has been shown that after capture, leukocytes can respond rapidly and become immobilised, or alternatively integrate signals over time. Having received signal(s) to stablise adhesion and engage motile apparatus, migration is guided and perhaps made more efficient by flow.

BUILDING FLOW-BASED MODELS OF VASCULAR INFLAMMATION

Building on the basic models of leukocyte adhesion and migration on purified proteins or endothelial cell monolayers, other elements can be included to model the vasculature more accurately or introduce features relevant to vascular pathology.

Use of whole blood and of disturbed flow

Perfusion of whole blood through microchannels makes observation of adhesion and migration more difficult. While residual adherent cells can be observed after 'washout' of the blood, direct evaluation of capture requires use of fluorescent dyes to label the leukocytes. Use of whole blood reveals that leukocytes capture can occur at higher stresses than in suspensions of isolated cells[7]. The numerous red cells not only modify the leukocyte velocities near the wall by blunting the expected parabolic velocity profile, but also act to stabilise adhesion[8]. Another complication in vivo is that while flow is laminar (albeit not simply parabolic), with low Reynold's number in microvessels where leukocyte adhesion typically occurs, this is not the case in all regions. In larger arteries, flow is disturbed near bends and bifurcations, and vortices may be set up which fluctuate with the cardiac output. Discontinuities can be built into the wall of microchannels, to mimic this situation. These studies indicate that adhesion of leukocytes is promoted in disturbed flow, associated with a velocity component normal to the wall and regions of low shear stress in an otherwise high-shear conduit[37].

Conditioning endothelial cells by different flow regimes

In vivo, endothelial cells are exposed continually to forces exerted by the circulation, which are generally higher in the arterial than venous circulation, and may vary in magnitude between vascular beds in different organs. The shear stress applied to the vessel wall by the flowing blood may be quite uniform spatially or temporally, or have local gradients and/or oscillatory components (e.g., provided by the cardiac cycle and near junctions in vessels). Endothelial cells cultured in microchannels have been used to study the effects of different flow regimes, such as abrupt initiation or cesssation of flow[38,39], or more prolonged conditioning at different levels of steady or oscillatory shear stress[10,40]. Readouts of these experiments have often been at the level of signal transduction, gene expression or production of a specific protein, although we have allied flow culture with flow-based adhesion assays to provide direct functional readouts[41]. Broadly speaking, steady laminar flow of increasing shear stress tends to reduce the propensity of endothelial cells to respond in a pro-inflammatory manner, while oscillation or disruption of flow appears to pro-inflammatory per se. These differential responses may be relevant to the physiological conditioning of the vasculature in different regions, as well as the propensity of pathologies, such as atherosclerosis, to develop at particular sites.

Modelling the effects of the stromal environment

In vivo, endothelial cells grow on a distinct proteinaceous basement membrane, in close association with stromal cells such as smooth muscle cells, pericytes and fibroblasts. This stromal environment is widely believed to influence the responses of the endothelial cells. Indeed, when we cultured endothelial cells for prolonged periods, to develop a basemement membrane, and incorporated them into a flow channel, adhesion and migration of flowing neitrophiols was enhanced compared to more-conventional short-term cultures[42]. In addition, coculture of endothelial cells with smooth muscle cells or fibroblasts had marked effects on their ability to recruit different subsets of flowing leukocytes[22,23]. These experiments required culture of the different combinations of cells on either side of porous filters held in 'Transwell' inserts and the construction of a special parallel-plate flow-chamber to hold these inserts and allow the perfusion studies[43].

Models for migration out of the sub-endothelial compartment

To date, studies using microchannels have analysed capture from flow, activation at the surface, and migration over and through endothelial surfaces. In vivo, the next stage is for leukocytes to penetrate the basement membrane and move into the tissue, e.g., to engulf and destroy invading pathogens. This stage is not open to study when using typical cultures grown on solid substrates. Adapting the filter studies noted above, we recently described a system where endothelial cells were grown on filters with 3μm pores, and these filters were cut out and mounted onto glass plates[24]. The plates were incoporated into a flow channel, so that we could directly observe adhesion and migration of flowing neutrophils, including their movement away from the endothelial monolayer and through the filter. Thus, regulation of this step is now open to mechanistic studies in a circulatory milieu including human tissue.

In conclusion, perfused microchannels have proved useful and adaptable for studying the adhesion and migration of leukocytes, in processes directly relevant to human physiology and pathology. The conditions mimicked can be relevant to protective immunity, or to disrupted processes which underly pathologies as diverse as atherosclerosis, vasculitis, liver disease or rheumatoid arthritis. Studies can take advantage of available pre-fabricated channels such as microslides, or be based on modifications of widely-used parallel plate constructions. Specific protein coating can be used to answer narrowly-defined questions regarding adhesion mechanisms, or endothelial cells can be cultured under a variety of conditions to reproduce aspects of the complex environment that operates at and regulates the interface between the blood and tissue.

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REFERENCES

1. Springer TA. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. Ann Rev Physiol, 57, 827-872, 1995
2. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, Pober JS, Wick TM, Konkle BA, Schwartz BS, Barnathan ES, McCrae KR, Hug BA,

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Schmidt AM, Stern DM. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood, 91, 3527-61, 1998
3. Goldsmith HL, Turitto VT. Rheological aspects of thrombosis and haemostasis: basic principles and applications. Thrombos Haemost, 55, 415-35, 1986
4. Lawrence MB, McIntire LV, Eskin SG. Effect of flow on polymorphonuclear leukocyte/endothelial cell adhesion. Blood, 70, 1284-90, 1987
5. Cooke BM, Usami S, Perry I, Nash GB. A simplified method for culture of endothelial cells and analysis of adhesion of blood cells under conditions of flow. Microvasc Res, 45, 33-45, 1993
6. Reinhardt PH, Kubes P. Differential leukocyte recruitment from whole blood via endothelial adhesion molecules under shear conditions. Blood, 92, 4691-9, 1998
7. Abbitt KB, Nash GB. Characteristics of leucocyte adhesion directly observed in flowing whole blood in vitro. Br J Haematol, 112, 55-63, 2001
8. Abbitt KB, Nash GB. Rheological properties of the blood influencing selectin-mediated adhesion of flowing leukocytes. Am J Physiol, 285, H229-H240, 2003
9. Rainger GE, Buckley CD, Simmons DL, Nash GB. Neutrophils sense flow-generated stress and direct their migration through alphaVbeta3-integrin. Am J Physiol, 276, 858-64, 1999
10. Sampath R, Kukielka GL, Smith CW, Eskin SG, McIntire LV. Shear stress-mediated changes in the expression of leukocyte adhesion receptors on human umbilical vein endothelial cells in vitro. Ann Biomed Eng, 23, 247-256, 1995
11. Hochmuth RM, Mohandas N. Uniaxial loading of the red-cell membrane. J Biomech, 5, 501-509, 1972
12. Turitto VT, Baumgartner HR. Platelet interaction with subendothelium in a perfusion system: physical role of red blood cells. Microvasc Res, 9, 335-344, 1975
13. Forrester JV, Lackie JM. Adhesion of neutrophil leucocytes under conditions of flow. J Cell Sci, 70:93-110., 93-110, 1984
14. Barabino GA, McIntire LV, Eskin SG, Sears DA, Udden M. Endothelial cell interactions with sickle cell, sickle trait, mechanically injured and normal erythrocytes under controlled flow. Blood, 70, 152-157, 1987
15. Lawrence MB, Smith CW, Eskin SG, McIntire LV. Effect of venous shear stress on CD18-mediated neutrophil adhesion to cultured endothelium. Blood, 75, 227-37, 1990
16. Lawrence MB, Springer TA. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. Cell, 65, 859-73, 1991
17. Nash GB, Cooke BM, Marsh K, Berendt A, Newbold C, Stuart J. Rheological analysis of the adhesive interactions of red blood cells parasitized by Plasmodium falciparum. Blood, 79, 798-807, 1992
18. Buttrum SM, Hatton R, Nash GB. Selectin-mediated rolling of neutrophils on immobilized platelets. Blood, 82, 1165-1174, 1993
19. Sheikh S, Rainger GE, Gale Z, Rahman M, Nash GB. Exposure to fluid shear stress modulates the ability of endothelial cells to recruit neutrophils in response to tumor necrosis factor-alpha: a basis for local variations in vascular sensitivity to inflammation. Blood, 102, 2828-2834, 2003
20. Jones KL, Hughan SC, Dopheide SM, Farndale RW, Jackson SP, Jackson DE. Platelet endothelial cell adhesion molecule-1 is a negative regulator of platelet-collagen interactions. Blood, 98, 1456-1463, 2001
21. Long A, Mitchell S, Kashanin D, Williams V, Prina MA, Shvets I, Kelleher D, Volkov Y. A multidisciplinary approach to the study of T cell migration. Ann N Y Acad Sci, 1028, 313-319, 2004
22. Rainger GE, Nash GB. Cellular pathology of atherosclerosis: smooth muscle cells prime cocultured endothelial cells for enhanced leukocyte adhesion. Circ Res, 88, 615-622, 2001
23. Lally F, Smith E, Filer A, Stone M, Shaw J, Nash GB, Buckley CD, Rainger GE. A novel mechanism of neutrophil recruitment in a coculture model of the rheumatoid synovium. Arth Rheum, 52, 3460-3469, 2005
24. Chakravorty S, McGettrick HM, Butler LM, Buckley CD, Rainger GE, Nash GB. An in vitro model for analysing neutrophil migration into and away from the sub-endothelial space: roles of flow and CD31. Biorheology, in press, 2006
25. Smith CW, Kishimoto TK, Abbassi O, Hughes B, Rothlein R, McIntire LV, Butcher E, Anderson DC, Abbass O. Chemotactic factors regulate lectin adhesion molecule 1 (LECAM-1)-dependent neutrophil adhesion to cytokine-stimulated endothelial cells in vitro. J Clin Invest, 87, 609-618, 1991
26. Lawrence MB, Springer TA. Neutrophils roll on E-selectin. J Immunol, 151, 6338-6346, 1993
27. Jones DA, McIntire LV, Smith CW, Picker LJ. A two-step adhesion cascade for T cell/endothelial cell interactions under flow conditions. J Clin Invest, 94, 2443-2450, 1994
28. Lalor P, Nash GB. Adhesion of flowing leucocytes to immobilized platelets. Br J Haematol, 89, 725-32, 1995
29. Alon R, Kassner PD, Carr MW, Finger EB, Hemler ME, Springer TA. The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. J Cell Biol, 128, 1243-53, 1995
30. Lalor PF, Clements JM, Pigott R, Humphries MJ, Spragg JH, Nash GB. Association between receptor density, cellular activation, and transformation of adhesive behavior of flowing lymphocytes binding to VCAM-1. Eur J Immunol, 27, 1422-6, 1997
31. Luscinskas FW, Ding H, Tan P, Cumming D, Tedder TF, Gerritsen ME. L- and P-selectins, but not CD49d (VLA-4) integrins, mediate monocyte initial attachment to TNF-alpha-activated vascular endothelium under flow in vitro. J Immunol, 157, 326-35, 1996
32. Rainger GE, Fisher A, Shearman C, Nash GB. Adhesion of flowing neutrophils to cultured endothelial cells after hypoxia and reoxygenation in vitro. Am J Physiol, 269, 1398-406, 1995
33. Bahra P, Rainger GE, Wautier JL, Luu N-T, Nash GB. Each step during transendothelial migration of flowing neutrophils is regulated by the stimulatory concentration of tumour necrosis factor-alpha. Cell Ad Commun, 6, 491-501, 1998
34. Rainger GE, Fisher AC, Nash GB. Neutrophil rolling is rapidly transformed to stationary adhesion by IL-8 or PAF

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presented on endothelial surfaces. Am J Physiol, 272, H114-H122, 1997

35. Luu NT, Rainger GE, Nash GB. Kinetics of the different steps during neutrophil migration through cultured endothelial monolayers treated with tumour necrosis factor-alpha. J Vasc Res, 36, 477-85, 1999
36. Cinamon G, Shinder V, Alon R. Shear forces promote lymphocyte migration across vascular endothelium bearing apical chemokines. Nature Immunol, 2, 515-522, 2001
37. Skilbeck C, Westwood SM, Walker PG, David T, Nash GB. Dependence of adhesive behavior of neutrophils on local fluid dynamics in a region with recirculating flow. Biorheology, 38, 213-27, 2001
38. Orr AW, Sanders JM, Bevard M, Coleman E, Sarembock IJ, Schwartz MA. The subendothelial extracellular matrix modulates NF-kappaB activation by flow: a potential role in atherosclerosis. J Cell Biol, 169, 191-202, 2005
39. Manevich Y, Al-Mehdi A, Muzykantov V, Fisher AB. Oxidative burst and NO generation as initial response to ischemia in flow-adapted endothelial cells. Am J Physiol, 280, H2126-H2135, 2001
40. Chappell DC, Varner SE, Nerem RM, Medford RM, Alexander RW. Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. Circ Res, 82, 532-539, 1998
41. Sheikh S, Gale Z, Rainger GE, Nash GB. Methods for exposing multiple cultures of endothelial cells to different fluid shear stresses and to cytokines, for subsequent analysis of inflammatory function. J Immunol Methods, 288, 35-46, 2004
42. Butler LM, Rainger GE, Rahman M, Nash GB. Prolonged culture of endothelial cells and deposition of basement membrane modify the recruitment of neutrophils. Exp Cell Res, 310, 22-32, 2005
43. Rainger GE, Stone P, Morland CM, Nash GB. A novel system for investigating the ability of smooth muscle cells and fibroblasts to regulate adhesion of flowing leukocytes to endothelial cells. J Immunol Methods, 255, 73-82, 2001

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