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Kinetics of leukocyte-induced changes in endothelial barrier function

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1 Extravasation of polymorphonuclear leukocytes (PMN) and associated plasma leakage are key events in the inflammatory process. The kinetics of PMN-induced changes in endothelial barrier function were studied by means of confluent monolayers of bovine aorta or human umbilical vein endothelial cells (EC), cultured on permeable membranes and mounted in a two-compartment diffusion chamber. The model permitted continuous measurement of transendothelial electrical resistance (TEER), and analysis of protein efflux and PMN migration across the EC monolayer.

2 Transendothelial chemotactic stimulation (fMLP or LTB_4) of PMN resting on EC in the upper compartment induced a prompt decline in TEER, followed by an increase in protein flux and transmigration of PMN. Adding the chemoattractant together with PMN in the upper compartment provoked adhesion of PMN, fall in TEER and increase in protein permeability, but no transmigration of PMN, whereas inhibition of PMN adhesion to EC by pretreatment with anti-CD18 mAb prevented all responses to chemotactic stimulation.

3 Chemoattractant-induced adhesion of PMN to the EC monolayer induced a rapid rise in EC cytosolic free Ca^{2+}, similar to that obtained by direct stimulation of EC with histamine, indicating an active response of EC to PMN activation and adhesion.

4 In summary, continuous recording of transendothelial electrical resistance in the *in vitro* model described permits rapid and sensitive analysis of leukocyte activation-induced effects on EC barrier function. The kinetics and specificity of the EC and PMN responses to chemoattractant stimulation suggest that activated PMN, *via* adhesion-dependent events, have a direct effect on EC junctional integrity independent of whether transmigration occurs or not.

Keywords: Inflammation; leukocyte extravasation; endothelial cells; vascular permeability; plasma leakage

Introduction

The host defense reaction to tissue trauma or infection involves a series of adaptive changes in the microcirculation induced by locally released inflammatory mediators acting on the endothelium and the circulating blood cells. Increased vascular permeability for macromolecules and extravasation of polymorphonuclear leukocytes (PMN) are key events in this reaction. The increased permeability and associated plasma leakage may be evoked by mediators (e.g. histamine and leukotriene C_4) acting directly on the endothelial cells (EC), but may also appear as a consequence of leukocyte activation and extravasation (Wedmore & Williams, 1981; Dahlén *et al*., 1981; Björk *et al*., 1982; Arfors *et al*., 1987). It is well established that enhanced macromolecular permeability in response to directly-acting mediators is attributable to conformational changes in the EC cytoskeleton in turn leading to cell contraction and opening of interendothelial junctions (Majno *et al*., 1969; Rotrosen & Gallin, 1986; Joris *et al*., 1987). Similar structural changes in EC shape have been suggested to accompany PMN adhesion and migration across the endothelium (Marchesi & Florey, 1960; Furie *et al*., 1987), but the particular mechanism behind the PMN-dependent change in EC permeability remains unknown.

A variety of whole-organ and intravital microscopic models have been used to study permeability changes in the microvasculature following PMN activation and adhesion to EC (Wedmore & Williams, 1981; Dahlén *et al*., 1981; Arfors *et al*., 1987). On the other hand, *in vitro* analysis of EC barrier function employing cultured EC monolayers grown on permeable substrates represents a tool for studies of PMN induced effects on EC permeability independent of other tissue components. To this end, determination of transendothelial macromolecular efflux and/or electrical resistance has been used to monitor EC monolayer integrity (Furie *et al*., 1984; Garcia *et al*., 1986; Huang *et al*., 1988; Albelda *et al*., 1988). In this report, the kinetics of PMN activation-induced changes in EC barrier function have been characterized in a model allowing continuous and precise measurement of the electrical resistance across bovine aorta and human umbilical vein EC monolayers together with analysis of transendothelial macromolecular efflux and PMN migration. The transendothelial electrical resistance was found to be a sensitive measure of EC monolayer integrity and effectively reflected rapidly occurring changes in EC permeability well ahead of any measurable change in macromolecular or PMN transport across the monolayer.

Methods

Reagents

Medium-199, RPMI-1640 (containing L-glutamine), foetal bovine serum (FBS), trypsin-EDTA, phosphate buffered saline (PBS) and Hanks balanced salt solution (HBSS) were from Life Technologies (Gaithersburg, MD, U.S.A.). Gelatin, collagenase, penicillin, streptomycin, catalase, bovine serum albumin (BSA), N-formyl-methionyl-leucyl-phenylalanine (fMLP), Evans blue dye, hexadecyltrimethylammonium bromide and tetramethylbenzidine were from Sigma Chemical

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Co. (St. Louis, MO, U.S.A.). H_2O_2 was from E. Merck (Darmstadt, Germany). Biomatrix I was from Biomedical Technologies Inc. (Stoughton, MA, U.S.A.), and Dextran 70 (Macrodex) and Ficoll-Paque were from Pharmacia Biotech AB (Uppsala, Sweden). The basal culture medium consisted of a 1 : 1 mixture of M-199 and RPMI-1640 supplemented with 20% heat inactivated FBS, penicillin (100 U/ml) and streptomycin (100 μg ml^{-1}). The monoclonal antibody IB4 against the common β-chain (CD18) of β_2-integrins was provided by Dr Claes Lundberg, Pharmacia & Upjohn (Uppsala, Sweden) by courtesy of Dr Samuel D. Wright, Rockefeller University.

Endothelial cell isolation and culture

Human umbilical vein endothelial cells (HUVEC) were isolated from fresh umbilical cords as described (Jaffe *et al*., 1973) and bovine aorta endothelial cells (BAEC) were isolated according to the method of Booyse *et al*. (1975) with a few modifications. Briefly, the lumen of the umbilical vein or bovine aorta was incubated with a 0.25% collagenase solution for 10 min, and detached endothelial cells washed out with PBS. Harvested cells were pelleted and resuspended in culture medium, seeded in tissue culture flasks and incubated under standard conditions. Medium was changed every 48 h until cultures had reached confluence (3–5 days). HUVEC and BAEC in the first to fifth passages were used in the experiments. Identification and purity determination of EC was based on cobblestone morphology and characterization by factor VIII/vwf antigen (Booyse *et al*., 1975). Staining for factor VIII/vwf factor was homogeneous in the cells.

Seeding of EC on permeable membranes

Cultured EC were detached by brief (2 min) trypsin-EDTA treatment (0.25% trypsin/0.01% EDTA) and replated onto either 0.2 μm pore size Anopore inorganic membrane or 3.0 μm pore size polycarbonate filters (Tissue Culture inserts, 10 mm; NUNC, Roskilde, Denmark). In order to promote cellular differentiation and enhance attachment of EC, the filters were treated with 50 μl Biomatrix I (167 μg ml^{-1}) and left to air-dry. EC were seeded at a density of 2×10^5 cells per filter and incubated in culture medium at 37°C in a humidified atmosphere of 5% CO_2 in air. The EC were grown to confluent monolayers, as assessed by daily microscopic observation and measurement of the electrical resistance across the monolayer.

Measurement of transendothelial electrical resistance (TEER)

For measurement of electrical resistance across the EC monolayer, the filter insert was transferred to a resistance measurement chamber (ENDOHM-12; World Precision Instruments, Sarasota, FL, U.S.A.) modified so as to assure exact positioning of the electrodes in relation to each other and to the filter insert during repeated measurements. The chamber (lower compartment) and the filter insert (upper compartment) were filled with 2 ml and 400 μl culture medium, respectively. All measurements were made at 37°C with the equipment placed in a cell culture incubator. Direct readings of the electrical resistance were given by an ohmmeter (EVOM; World Precision Instruments, Sarasota, FL, U.S.A.) connected to the electrodes of the resistance chamber. The electrical resistance of individual EC monolayers was obtained by subtracting the resistance of the corresponding naked filter coated with Biomatrix as measured prior to seeding of the endothelial cells.

Determination of albumin clearance

Evans blue dye-conjugated albumin (EBA) was used as a marker for macromolecular permeability of the EC monolayers (Patterson *et al*., 1992). The dye at a final concentration of 0.67 mg ml^{-1} was mixed with culture medium containing 4% BSA. In experiments where albumin permeability was analysed, the culture medium in the upper compartment was exchanged for medium containing EBA. The EBA concentration in fluid samples of the upper and lower compartment was determined spectrophotometrically through measurement of absorbency at 620 nm (Titertek Multiskan MCC; Flow Laboratories, Solna, Sweden). Albumin clearance was calculated according to the relationship: $V1 = A2 \times V2 \times 1/A1$, where V1 is the clearance volume (i.e. the theoretical volume of the apical medium cleared from albumin by diffusion to the basolateral compartment), V2 is the basolateral volume, and A1 and A2 is the absorbency of the apical and basolateral medium, respectively. The baseline clearance in absence of any stimulus, averaging 0.08 ± 0.01 μl min^{-1}, was subtracted from the clearance volume obtained in response to respective specific stimulus.

PMN isolation and quantification

Leukocyte-rich plasma, obtained from human whole blood by dextran sedimendation, was carefully layered onto Ficoll-Paque and centrifuged at $400 \times g$ for 30 min. The PMN-containing pellet was resuspended and washed twice (400 g, 7 min) in ice cold PBS. The PMN were resuspended in culture medium at a final concentration of $2-5 \times 10^7$ cells ml^{-1}. Purity of PMN was >98% and viability by Trypan blue exclusion was >95%. In some experiments PMN were incubated with the anti-CD18 mAb IB4 (6 μg per 2×10^6 cells) for 20 min at room temperature before being added to the filter insert (see below).

PMN adhesion to EC and transendothelial migration were quantified by assaying the PMN-specific enzyme myeloperoxidase (MPO). In brief, PMN were lysed in 0.5% hexadecyltrimethylammonium bromide and centrifuged, and the enzyme activity in the supernatant determined spectrophotometrically as the change in absorgency at 650 nm that occurs in the redox reaction of H_2O_2-tetramethylbenzidine catalyzed by MPO (Suzuki *et al*., 1983). MPO activity of adherent and transmigrated PMN were related to that of the total number of PMN added to the EC monolayer.

Experimental procedures

Before transferring the filter inserts to the resistance measurement chamber (kept at 37°C), the medium in the insert and in the chamber was replaced with fresh culture medium (37°C) supplemented with 10 mM HEPES. PMN (2×10^6), with or without mAb IB4 (final conc. 15 μg ml^{-1}), were added to the upper compartment (PMN : EC ratio = 10 : 1) and allowed to sediment for 10 min. Activation of PMN was induced by addition of fMLP (10^{-7} M) or LTB_4 (10^{-9} M) to the lower or the upper compartment. In other experiments, histamine ($10^{-5}-10^{-6}$ M) was used to stimulate the EC monolayer in the absence of PMN. Transendothelial electrical resistance was measured before and every minute after start of stimulation until a plateau phase in the resistance change was reached, and then at 5-min intervals. In experiments where albumin permeability and PMN migration across the EC monolayer were studied, the medium in the insert contained EBA. During the experiment the filter

insert was moved at 5–10 min intervals to new wells containing fresh medium with or without chemotactic stimulus. All incubations were performed at 37°C. After the experiment, the lower wells were centrifuged at $425 \times g$ and 4°C for 20 min and the supernatant was analysed for EBA content. PMN that had migrated across the monolayer were quantified through analysis of the MPO-activity of the remaining pellet at the bottom of the wells. The medium in the filter insert was aspirated for quantification of non-adherent PMN, and the filter membrane was removed from the insert to quantify the adherent fraction of PMN.

Determination of EC intracellular [Ca^{2+}]

The Ca^{2+} sensitive fluorescent probe fluo-3/AM (Molecular Probes Europe BV, Leiden, The Netherlands) was used for determination of changes in EC intracellular free Ca^{2+}. Confluent BAEC monolayers cultured on Biomatrix I coated filters were incubated with a fluo-3/AM solution (3 μM in HBSS containing 2% FCS and 10 mM HEPES), added to both the apical and basolateral surface, for 30 min at 37°C. The monolayers were washed three times and incubated with fresh HBSS in the dark for 20 min at room temperature before use to allow complete hydrolysis of the dye ester to its calcium sensitive free acid form. EC were stimulated either with histamine (10^{-5} M) or by activated PMN added to the apical surface of EC. In the latter case, PMN were allowed to sediment onto the monolayer before activation with fMLP (10^{-7} M). Changes in EC $[Ca^{2+}]_i$ in response to these stimuli were measured through continuous registration of fluorescence intensity with a laser-scanning confocal imaging system (Insight Plus: Meridian Instruments Inc., Okemon, Michigan, U.S.A.).

Results

Endothelial cells from bovine aorta (BAEC) and human umbilical vein (HUVEC) were grown on permeable filters to confluent monolayers, as assessed by daily microscopic examination of cell growth on the 0.2 μm pore size filters (transparent when wet). In addition, sterile measurement of transendothelial electrical resistance was performed everyday. Maximal resistance was observed at day 7 (Figure 1) indicating that this time was required for monolayers to develop well-formed intercellular junctions. Accordingly, all experiments were performed on filters 7 days postseeding. At this time, the resting transendothelial resistance was 19 ± 3 ohm $\times$ cm^2 and 15 ± 4 ohm $\times$ cm^2 (mean $\pm$ s.d.) in BAEC and HUVEC, respectively, and did not change when followed for at least 1 h. Data presented in the following refer to results obtained with BAEC. Although there was no qualitative difference between the responses of BAEC and those of HUVEC to any of the experimental manoeuvres tested, the results obtained with BAEC were more consistent.

Addition of histamine (10^{-5} M) to either the lower or upper compartment elicited a marked decrease in TEER, which was manifested within 30 s, reached its maximum (53 ± 6% of control) after 4 min, and remained at this level throughout the observation period (Figure 2). Challenge with histamine also caused an increase in EC macromolecular permeability, as reflected by a progressive rise in albumin clearance yielding a net clearance volume of 18.7 ± 4.3 μl (mean $\pm$ s.d.) in 30 min corresponding approximately to ten times that in the absence of histamine (baseline clearance). The responses to histamine stimulation were dose-dependent. Challenge with histamine, 10^{-6} M, caused less pronounced changes in both resistance and albumin clearance (Figure 2).

Having established the capacity of histamine to induce decreases in transendothelial resistance and increased albumin clearance, indicating a contractile response of EC, we next investigated the effect of PMN activation on the EC barrier function. Adding PMN to the upper compartment and fMLP (10^{-7}) to the lower compartment 10 min later resulted in a fall in TEER, which was clearly visible within 1 min after fMLP administration, reached its maximum, 45 ± 14% of control (mean $\pm$ s.d.), after 15 min, and then remained depressed during the observation period (Figure 3a). Closely the same pattern was obtained with LTB_4 (10^{-8} M) instead of fMLP as the chemotactic stimulus. In this case, resistance decreased to 52 ± 7% of control (mean $\pm$ s.d.; $n = 13$). Adding PMN to the EC monolayer in the absence of a chemotactic stimulus did not result in any change in TEER, nor did administration of fMLP or LTB_4 alone effect EC resistance (data not shown), indicating that the presence of both PMN and chemoattractant was required to induce the EC response.

In experiments where the effect of PMN activation on albumin clearance was studied, PMN were allowed to settle for

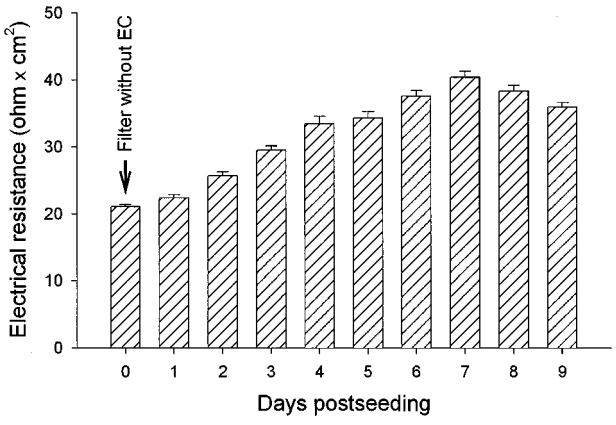

Figure 1 Increase in electrical resistance of BAEC monolayers after seeding on permeable filters. Maximal resistance was obtained at day seven postseeding. Day zero depicts resistance of matrix-coated filters before seeding of EC (mean $\pm$ s.d., $n = 11$).

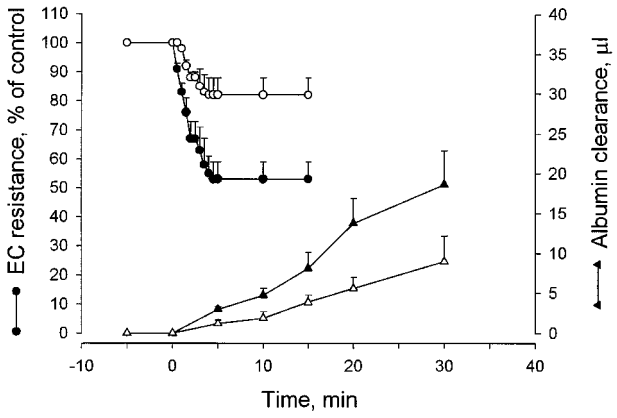

Figure 2 Effect of histamine on electrical resistance and macromolecular permeability (albumin clearance) of BAEC monolayers separating an upper and a lower compartment. Histamine, 10^{-5} M (filled symbols) and 10^{-6} M (open symbols), was added to the lower compartment at time zero (mean $\pm$ s.d.; $n = 6$–12).

10 min before addition of EBA. The PMN/EBA containing filter inserts were then moved at regular time intervals to new wells containing control medium or medium plus fMLP (10^{-7} M). In the presence of fMLP, there was an increased clearance of albumin in parallel with leukocyte transmigration (Figure 3a). The net albumin clearance rose from 0.5 ± 0.2 μl during the first 5-min period to a total of 18.9 ± 2.7 μl (mean $\pm$ s.d.) in 60 min. During the first 15 min of fMLP challenge transmigrated PMN were few in numbers (less than 1%) and not different from control in the absence of chemotactic stimulation. However, during the subsequent 45 min there was a progressive rise in PMN transmigration, totaling 14 ± 3% (mean $\pm$ s.d.) of added PMN 60 min after start of challenge (Figure 3a).

In further experiments, PMN and fMLP were added to the upper compartment and changes in transdendothelial resistance, albumin clearance, and PMN transmigration were monitored over the next 60 min. The electrical resistance fell to 34 ± 10% (mean $\pm$ s.d.) of control, with a slope similar to that of transendothelial chemotactic challenge (fMLP added to the lower compartment), and then remained at this low level (Figure 3b). With a lag time of approximately 20 min there was also a continuous and marked clearance of albumin (19.7 ± 2.1 μl after 60 min), but no measurable transmigration of PMN (Figure 3b). Analysis of MPO activity in the different compartments after the 60 min incubation period revealed that 60 ± 12% (mean $\pm$ s.d.) of added PMN adhered to the EC monolayer, with the remainder left free in the medium of the upper compartment.

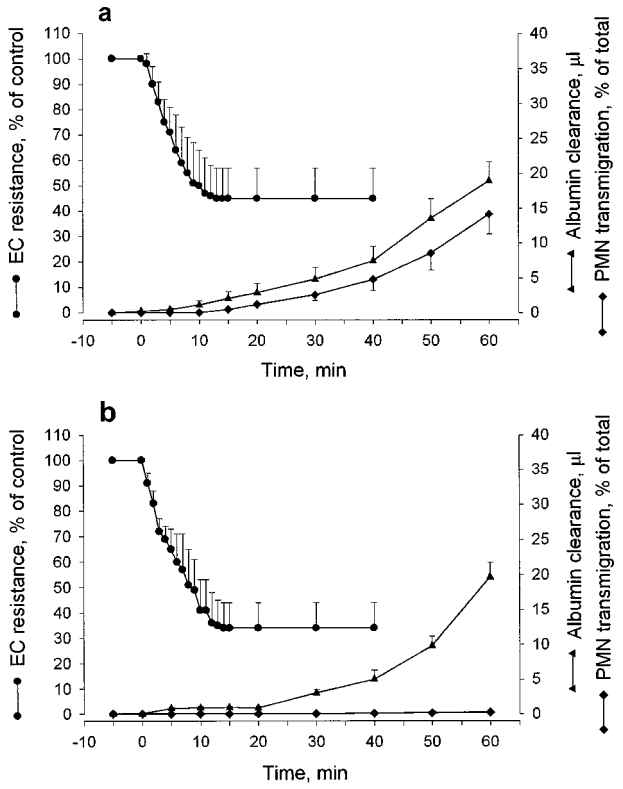

Figure 3 Effect of PMN activation on electrical resistance and macromolecular permeability (albumin clearance) of BAEC monolayers separating an upper and a lower compartment, and on transendothelial migration of PMN. PMN in upper compartment were stimulated with fMLP (10^{-7} M) added at time zero either to the lower compartment (a), or the upper compartment (b) (mean $\pm$ s.d.; $n = 8-24$).

The observation that chemotactic stimulation elicited increased EC permeability also in the absence of PMN transmigration prompted us to investigate the significance in this process of β_2 integrin-mediated firm adhesion of the leukocytes to the endothelial monolayer. In the presence of the anti-CD18 mAb IB4, the change in TEER was totally prevented and there was no increase in albumin clearance in response to chemotactic stimulation of PMN with fMLP (10^{-7} M), indicating that β_2 integrin-dependent adhesion of PMN to EC is required for these processes. Accordingly, the added PMN remained non-adherent in the upper compartment and no transmigrated cells were recovered (data not shown).

In separate experiments, EC monolayers were labeled with the Ca^{2+}-sensitive fluorophore fluo-3, and monitored for stimulus-induced changes in EC $[Ca^{2+}]_i$, using laser-scanning confocal microscopy. Addition of histamine (10^{-5} M) provoked an almost instantaneous increase in cytosolic $[Ca^{2+}]$, which peaked within 20 s (Figure 4). Closely similar results were obtained in experiments where PMN added to fluo-3-labeled EC monolayers were stimulated with fMLP (10^{-7} M). Thus, the endothelial cells reacted with a clear-cut increase in cytosolic $[Ca^{2+}]$, reaching the same peak level as after histamine stimulation after approximately 50 s (Figure 4). Stimulation with fMLP of EC alone caused no change in EC $[Ca^{2+}]_i$ (data not shown). In additional experiments histamine was added to EC monolayers after omitting calcium in the medium. In this case, histamine elicited increased cytosolic $[Ca^{2+}]$ with the same amplitude and time to peak as in the presence of calcium containing medium, suggesting that the initial calcium response was due to mobilization from intracellular stores.

Discussion

In acute inflammation, circulating PMN respond to locally released chemotactic mediators and extravasate into surround-

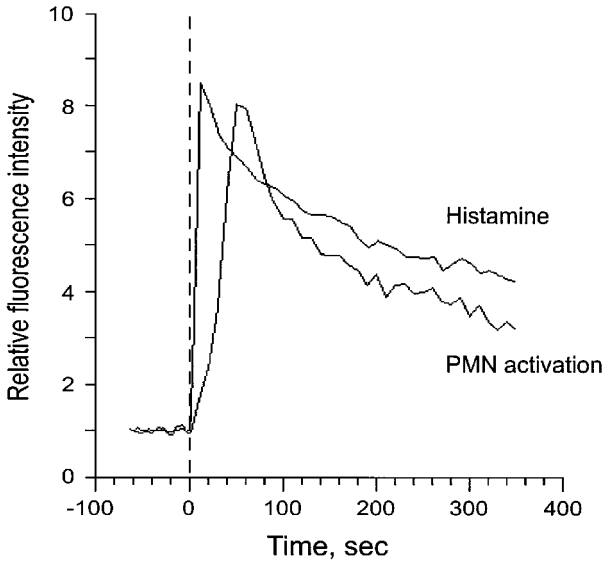

Figure 4 Effect of histamine and PMN activation on EC cytosolic free Ca^{2+}. Stimulation of fluo-3 loaded BAEC monolayers was induced at time zero either directly with histamine (10^{-5} M) or through fMLP-induced (10^{-7} M) activation of PMN. In the latter case PMN were added to the EC monolayer 10 min before start of Ca^{2+} measurements. Tracings are representative of >7 separate experiments and illustrate average fluorescence intensities of five to eight endothelial cells normalized to the initial fluorescence intensity.

ing tissue. In this multi-step reaction, β2 integrin (CD11/CD18)-mediated firm adhesion to the venular endothelial lining is critical for subsequent transmigration across the vessel wall (Arfors *et al*., 1987). In conjunction with the PMN extravasation, increased vascular permeability for macromolecules is induced, leading to plasma exudation and edema formation (Issekutz, 1981; Wedmore & Williams, 1981; Arfors *et al*., 1987). The plasma leakage occurring in response to chemoattractant stimulation is entirely dependent on the presence of PMN (Björk *et al*., 1982). Although this relationship has been recognized since long, the exact mechanisms behind the leukocyte dependent permeability change remain largely unresolved.

Here, we used an *in vitro* model system to simulate leukocyte interactions with the vessel wall and to study kinetics of PMN activation-induced effects on EC barrier function. The model allowed continuous measurement of transendothelial electrical resistance (TEER) together with analysis of macromolecular efflux and PMN migration across the EC monolayer. Through the TEER measurements rapid changes in EC permeability could be detected. Following chemoattractant-induced PMN activation, the EC resistance started to decline already within the first minute after stimulus induction, clearly preceding any visible change in albumin efflux or PMN transmigration. At 10 min, when the resistance change was close to its maximum, albumin transport across the monolayer had just began to rise from the low basal level and there was still no measurable transmigration of PMN. Although temporally dissociated, the change in resistance was invariably accompanied by an increase in macromolecular permeability. Moreover, there was a dependence on stimulus strength in the responses observed, as illustrated by the dose-related effects on these variables induced by histamine.

The kinetics of the EC and PMN responses to transendothelial chemotactic stimulation suggest that increase in EC permeability induced by activated PMN is unrelated, at least initially, to PMN transmigration. On the other hand, this endothelial response is dependent on a physical interaction of PMN with EC *via* β2 integrins, inasmuch as anti-CD18 mAb treatment of PMN totally abolished the effects observed. Thus, PMN adhesion to, but not migration through, the EC monolayer is required for induction of the permeability change. This conclusion is supported also by the observation that activation of PMN by administration of the chemoattractant to the apical surface of the EC monolayer (no chemotactic gradient) resulted in adhesion-dependent increase in permeability but no transmigration. Both coherent (Huang *et al*., 1993) and conflicting (Rosengren *et al*., 1991) views with regard to dependency of protein leakage on transmigration *per se* have been forwarded based on findings in similar model systems.

Certain specific inflammatory mediators like histamine and the cysteinyl leukotriences (i.e. LTC_4, LTD_4 and LTE_4) exert their permeability increasing effect *via* a direct action on the endothelial cells independent of leukocyte activation and adhesion (Majno & Palade, 1961; Dahlén *et al*., 1981). In the present model, histamine stimulation of EC elicited a similar, however more rapid, response as for PMN activation with regard to changes in both resistance and albumin permeability. Histamine (and other soluble agonists) is known to stimulate, *via* Ca^{2+} signaling events, conformational changes in the EC cytoskeleton leading to opening of interendothelial junctions (Rotrosen & Gallin, 1986; Lum *et al*., 1989). A prompt rise in EC cytosolic free Ca^{2+} in response to histamine stimulation was confirmed in our model. A strikingly similar change in EC $[Ca^{2+}]$ was generated by activated PMN, together with F-actin formation (unpublished data), suggesting that PMN activation-induced changes in EC permeability may occur through similar mechanisms as those involved in the responses to direct acting inflammatory mediators. Thus, in agreement with previous findings (Huang *et al*., 1993), PMN activation and adhesion to the endothelium induces an instantaneous active response of EC, as intimated by the rapid changes in $[Ca^{2+}]_i$ and electrical resistance, leading to structural rearrangement of the EC cytoskeleton and impairment of barrier function.

Measurements of electrical resistance across EC monolayers to indicate EC permeability characteristics have been previously reported (Langeler & van Hinsbergh, 1988; Albelda *et al*., 1988; Sill *et al*., 1992). TEER values presented in the literature exhibit large variations between studies. This variability may be due, in part, to the type, number, and passage of endothelial cells used, the membrane and its coating, as well as the number of days post-seeding before use (Shasby, 1992). Moreover, different methods and instrumentation employed to measure resistance across the cell monolayer may render comparisons difficult between studies. In this study, a resistance chamber based in principle on a commercially available device was used. However, in order to obtain strict reproducibility in the readings, it was found important to modify the device so that exact positioning of the opposing electrodes in relation to each other and to the EC monolayer was assured. This was of concern when different filters were to be compared or when individual filters were analysed at different occasions. Moreover, we found considerably higher consistency in the TEER measurements when EC from bovine aorta were used compared with HUVEC. Even though our TEER readings from confluent HUVEC monolayers agreed with previously reported data (Huang *et al*., 1993), and the results obtained with HUVEC with respect to PMN activation-induced effects did not differ significantly from those with BAEC, there was typically a larger variability in the data when HUVEC were used. Therefore, BAEC were considered advantageous over HUVEC in the present model. Despite a heterologous system with respect to origin of leukocytes and EC thus was introduced we found no evidence to indicate that the responses observed were distinct from those obtainable with homologous cells. The electrical resistance of the BAEC monolayers increased daily after seeding onto the permeable membrane and correlated with progressive growth of EC and development into confluent monolayers as microscopically deduced in transparent filters. Resistance peaked at day seven post-seeding at values within the range of previously reported data for BAEC (Sill *et al*., 1992). The presence of tight junctions in cultured BAEC has been previously characterized (Haudenschild, 1984), and it was found important to run the experiments when these junctional structures were fully developed. The accuracy of the TEER measurements with the use of BAEC in the present model is reflected also in the small variability within the different groups depicted in Figure 1.

Taken together, an *in vitro* model system for analysis of leukocyte activation-induced effects on endothelial barrier function has been characterized. Continuous measurement of transendothelial electrical resistance permits detection of rapid changes in EC permeability. The kinetics and specificity of the EC and PMN responses to chemoattractant stimulation suggest that increase in EC permeability induced by activated PMN is closely linked to initial adhesion-dependent events independent of subsequent transmigration of the PMN. The model will be used for further investigations of EC

responsiveness to PMN activation with regard to inductive mechanisms for PMN dependent changes in EC barrier function.

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