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Mechanical Forces in Endothelial Cells during Firm Adhesion and Early Transmigration of Human Monocytes

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Abstract—Transmigration of leukocytes across the endothelial barrier is a tightly controlled process involving multiple steps, including rolling adhesion, firm adhesion, and then penetration of leukocytes through the endothelial monolayer. While the key molecular signals have been described in great detail, we are only just beginning to unveil the mechanical forces involved in this process. Here, using a microfabricated system that reports traction forces generated by cells, we describe forces generated by endothelial cells during monocyte firm adhesion and transmigration. Average traction force across the endothelial monolayer increased dramatically when monocytes firmly adhered and transmigrated. Interestingly, the endothelial cell that was in direct contact with the monocyte exhibited much larger traction forces relative to its neighbors, and the direction of these traction forces aligned centripetally with respect to the monocyte. The increase in traction force occurred in the local subcellular zone of monocyte adhesion, and dissipated rapidly with distance. To begin to characterize the basis for this mechanical effect, we show that beads coated with anti-ICAM-1 or VCAM-1 antibodies bound to monolayers could reproduce this effect. Taken together, this study provides a new approach to examining the role of cellular mechanics in regulating leukocyte transmigration through the endothelium.

Keywords—Transmigration, Endothelial cells, Mechanical forces, Mechanotransduction, Microposts, MEMS.

INTRODUCTION

The primary function of the endothelium is to act as a living barrier between blood and tissue that can, among many other functions, control the passage of molecules and leukocytes into and out of the bloodstream. This ability to dynamically prevent or allow cells within the bloodstream to transit into tissues plays a critical role in tissue maintenance and repair, and inflammation.[28] The migration of cells across the endothelial barrier, termed trans-endothelial migration (TEM), extravasation, or diapedesis, is a tightly controlled, multistep process that has been best characterized in the context of leukocytes. Initially, in response to inflammatory cytokines such as tumor necrosis factor-α (TNF-α), leukocytes in the bloodstream slow down and start rolling on the endothelium apical surface via the selectins.[23] The expression of intercellular adhesion molecule-1 (ICAM-1) and vascular endothelial cell adhesion molecule (VCAM-1) on the activated endothelium subsequently triggers a transition from rolling to firm adhesion via leukocyte integrins.[6,7,22,30] This firm adhesion allows the leukocytes to actively migrate on the endothelium, often exploring at or near cell–cell junctions, and finally locate a site to transmigrate through the endothelium at both junctional and nonjunctional locations.[27] Failure of the appropriate control of these steps is associated with many pathological conditions, such as chronic inflammatory disorders and atherosclerosis.[8,17]

Among various types of leukocytes, monocytes are thought to be important in particular for a variety of chronic inflammatory responses, most notably in the development of atherosclerotic lesions.[14] As a result, much effort has focused on studying monocyte transmigration in a variety of experimental models. Such studies have led to a detailed understanding of the molecular signals involved in rolling, adhesion, and transmigration.[24,37]

It is largely thought that the key transition for the transmigration process is firm adhesion of monocytes to the endothelial surface, which ends the rolling process and starts the positioning of the monocytes to the final locations on the endothelial cells for transmigration. Interestingly, evidence is mounting that endothelial cells also actively adapt themselves to assist the transmigration process. Firm adhesion-mediated clustering of ICAM-1 and VCAM-1 on the endothelium can activate the Rac1/ROS and Rho/ROCK GTPases signaling pathways, which are critical cytoskeletal signaling regulators that induce stress fiber formation and disassembly of endothelial cell–cell junctions to assist transmigration.[10,11,13,20,31,38] Blocking these cytoskeletal changes in the endothelium appears to

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suppress leukocyte transmigration, suggesting a critical role for these mechanical effects. Despite the apparent importance of changes in endothelial cell mechanics to the transmigration process, such mechanical effects remain largely uncharacterized. One recent study has described traction forces exerted by neutrophils during transmigration,[26] and adds to the body of literature focused on neutrophil motility itself.[12,25]

Herein, we describe a method to measure changes in cytoskeletally generated mechanical forces in the endothelial cell monolayer during firm adhesion and early transmigration of human monocytes. Briefly, endothelial cells were grown to form confluent monolayers with predefined geometries on substrates containing arrays of vertical elastomeric microposts whose deflections report cellular traction forces. The use of predefined geometries allowed us to consistently characterize and compare changes in mechanical force between monolayers. We primed endothelial cells by exposure to TNF-α, introduced monocytes, and subsequently allowed them to firmly adhere and transmigrate through the endothelial monolayers. By comparing mechanical forces in endothelial cells before and after leukocyte adhesion, we observed an increase in the traction force of the whole endothelial monolayer during firm adhesion of monocytes. The endothelial cell in direct contact with the monocyte responded with much larger increases in traction force relative to its neighbors, and the direction of the traction force aligned more centripetally with respect to the location of the monocyte. Moreover, engagement of ICAM-1, or VCAM-1 to a lesser degree, using beads was sufficient to increase endothelial cell traction forces. This report describes a novel approach to characterizing the mechanical effects of monocyte interactions with the endothelium.

MATERIALS AND METHODS

Preparation of Substrates

Elastomeric microposts array substrates were fabricated via polydimethylsiloxane (PDMS; Sylgard 184, Dow-Corning, Midland, MI)-based replica-molding and patterned with fibronectin by microcontact-printing as described previously.[32] The fibronectin (BD Biosciences, San Jose, CA) patterns consisted of square-shaped regions each with a total area of 10,000 μm^2 and coverage of ~120 microposts. Subsequently, microposts were fluorescently labeled with 5 μg/mL Δ^9-DiI (1,1′-dioleyl-3,3,3′,3′-tetramethylindocarbocyanine methanesulfonate; Invitrogen, Carlsbad, CA). Cell adhesion was restricted to the squares by blocking the unprinted surface with 0.1% Pluronics F127 (BASF, Mount Olive, NJ).

Cell Culture and Reagents

Human pulmonary artery endothelial cells (HPAECs, Lonza, Basel, Switzerland) were cultured in EGM-2 complete medium (Lonza) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO). Cells were seeded onto substrates in normal serum-containing media and allowed to spread and grow to form confluent monolayers on post arrays with predefined geometries for 36 h before fixation or further treatment. Human monocytic THP-1 cells were purchased from American Type Culture Collection (Rockville, MD) and grown in RPMI-1640 medium (Sigma) with supplements. To activate HPAECs for adhesion of THP-1 cells, we incubated them with TNF-α (25 ng/mL, Roche, USA) for 6 h prior to introduction of THP-1 monocytes. For fluorescence imaging, prior to being introduced to endothelial monolayers, THP-1 cells were treated with Cell Tracker™ Green (Invitrogen, Carlsbad, CA) at 1:5000 dilution for 30 min for fluorescently labeling. All images were taken from Zeiss microscope with Apotome Z-stack imaging acquisition system. Antibodies used for immunofluorescence labeling (and sources) included: mouse anti-β-catenin (BD Biosciences, San Jose, CA), β-catenin (BD Biosciences, San Jose, CA). Recombinant adenovirus encoding GFP-tagged VE-cadherin was a generous gift of Dr. Sunil Shaw.[29]

Immunofluorescence and Image Analysis

Cells were fixed in 4% paraformaldehyde, blocked in goat serum, incubated with antibody against β-catenin (BD Biosciences, San Jose, CA) and then detected with fluorophore-conjugated isotype-specific anti-IgG antibodies (Invitrogen, Carlsbad, CA). Cells were also labeled with AlexaFluor-488-conjugated Phalloidin and Hoescht33342 (Invitrogen, Carlsbad, CA). Fluorescence images were acquired on a Zeiss Axiovert 200 M with 40× oil objective (Zeiss MicroImaging, Thornwood, NY) and processed in Matlab to quantify mechanical force.

Measurement of Traction Forces

Traction forces were analyzed as described previously.[19] Briefly, fluorescence images were taken of the microposts at focal planes passing through the tips and base, using an Axiovert 200 M (Zeiss MicroImaging, Thornwood, NY) equipped with an Apotome module to remove out-of-focus fluorescence signals. The centroids of the microposts at both planes were determined by localized thresholding using an automated Matlab program (Mathworks, Natick, MA). After performing image registration on the tip and base centroids, the force on each post was computed by multiplying the deflection

by the spring constant of the post, which is 64 nN/μm. Adherens Junction staining was used to identify which microposts were attached to each cell in a monolayer.

Preparation of ICAM-1- and VCAM-1-Coated Beads

Beads were prepared as previously described.[2] Briefly, anti-ICAM-1, anti-VCAM-1, and IgG control mAbs were purchased from R&D Systems. Three-micron polystyrene beads were purchased from Polysciences and were pretreated overnight with 8% glutaraldehyde, washed five times with PBS, and incubated with 300 μg/mL ICAM-1 or VCAM-1 mAb according to the manufacturer's protocol, and then washed before use.

Statistical Analysis

Data are expressed as mean ± SEM or mean ± SD as indicated in the figure legends. Linear regression and Student's *t*-test were performed for statistical analysis. Zonal analysis was performed using an automated program coded in MATLAB (Mathworks, Natick, MA). Briefly, the zones were generated by dilation from the microposts underneath the monocytes in "local" to "distant" zonation, and erosion from the outermost layer of microposts in "edge" to "interior" zonation, respectively. Due to the square shape of monolayers, 8-nearest neighbor dilation/erosion was used to generate the zones. To compare "local" to "distant" zones with the Ctrl and TNF conditions, ghost monocyte locations were generated using Matlab to compute a uniform distribution across the endothelial monolayers. Applying a Kolmogorov–Smirnov Test indicated no significant differences between the sample and ghost monocyte distributions ($p = 0.99$ and 0.43 for comparisons on x- and y-coordinates, respectively). The histograms in relative angle analysis in Figs. 2d and 2f were fit with a Gamma distribution with nonlinear least square methods for TEM conditions, or with an average line for Ctrl and TNF conditions. The pseudo-color plot for traction forces in Fig 3a was filtered and smoothed with a bi-cubic 2D spatial filter in Matlab.

RESULTS

Approach to Measure Traction Force in Endothelial Monolayers During Monocyte Adhesion and Early Transmigration

To measure traction forces generated by endothelial cells during monocyte adhesion and transmigration, we developed an approach based on a previously described system of microfabricated post array detectors (mPADs) that report traction forces.[32] Endothelial cells were seeded and allowed to spread and grow to form confluent monolayers with predefined geometries on substrates containing arrays of vertical elastomeric microposts whose deflections report cellular traction forces (Fig. 1a). To be consistent in the comparisons between different conditions and treatment, we fixed the geometry of endothelial monolayers by microcontact-printing of fibronectin (FN) on 100 μm × 100 μm square regions on posts for all subsequent experiments.

Endothelial cells were allowed to form mature cell–cell junctions and fully spread into the square FN-coated regions on posts for 36 h, and then treated with TNF-α to induce expression of the ICAM-1 and VCAM-1 required for monocyte adhesion. After 6 h of incubation with TNF-α, we then introduced monocytes (THP-1 cells) into the system and allowed them to roll on, firmly adhere to, and finally transmigrate across the activated endothelial monolayers. After initial adhesion, the monolayers were rinsed to remove monocytes not firmly bound, fixed, and processed for immunofluorescence imaging. In the absence of TNF-α treatment, monocyte adhesion was virtually undetectable, while in the presence of TNF-α, monocyte adhesion was robust and reproducible. Using optical sectioning to image samples, the vertical position of monocytes with respect to the associated endothelial monolayers could be measured. Monocytes were found at multiple z-axis planes including slightly above, co-planar, and slightly underneath the endothelial plane, consistent with the presence of firmly adherent as well as transmigrating stages of monocytes (Fig. 1b). We restricted all subsequent studies to cases in which the z-plane with the strongest fluorescent signal for monocytes were in the same plane with the endothelial cells (0 μm) or underneath endothelial cells (-3 μm) (Fig. 1c). Operationally, we defined firm adhesion as the population of monocytes that remain attached after a washing step but do not protrude below the endothelial monolayer. Because firm adhesion is followed by transmigration and this transition is gradual, we defined firm adhesion (TEM-FA) when the main monocyte body was above the monolayer (0 and $+3$ μm), even though it can still protrude partially into the endothelial monolayer. We defined early transmigration (TEM-ET) conversely, when a larger portion of the cell was one layer below the monolayer plane (0 and -3 μm).

By carefully controlling the density of monocytes and the timing of washing-off and fixation, we focused only on monolayers exhibiting either one or zero monocyte firmly adhered/transmigrating on it before fixation (Fig. 1d and 1e), which allowed us to consistently compare the forces in endothelial monolayers with or without monocytes on it.

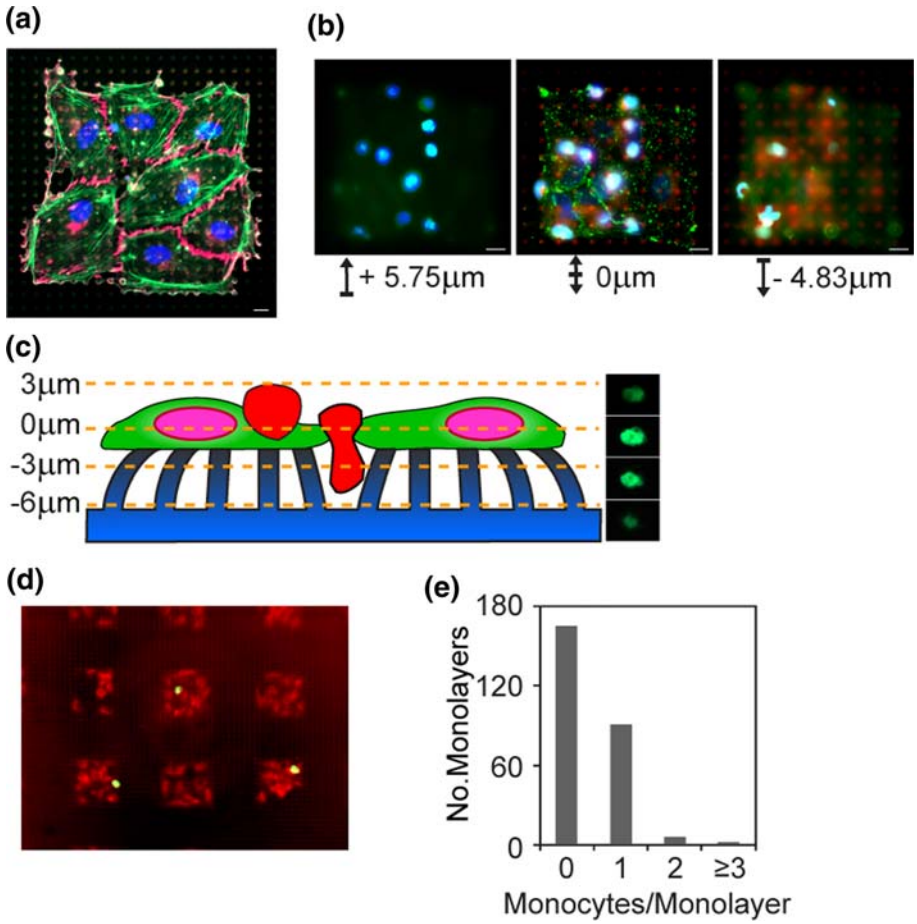

FIGURE 1. Approach to measure traction force in endothelial monolayers during monocyte adhesion and early transmigration. (a) Endothelial cells grown on mPADs (monolayer size: 150 μm × 150 μm). Immunofluorescence staining indicates cell nucleus (*blue*), actin filaments (*green*), microposts (*gray*), and β-catenin (*magenta*), respectively. (b) Endothelial cell monolayers grown on mPADs with monocytes transmigrating through them. Images taken from focal plane 5.75 μm above, 0 μm, and 4.83 μm below the monolayer. Immunofluorescence staining indicates endothelial cell nucleus (*dark blue*), monocytes (*bright cyan*), microposts (*red*), and β-catenin (*green*) (monolayer size: 100 μm × 100 μm). (c) Schematic figure for monocyte firm adhesion and early transmigration on endothelial cells on posts. The fluorescence images on the right are cell-tracker green staining at different focal planes of the monocyte transmigrating on an endothelial monolayer. (d) Array of endothelial monolayers showing each monolayer has 1 or 0 monocytes transmigrating on it. (e) Histogram showing most of the endothelial monolayers have 1 or 0 monocyte on it. Scale bars indicate 10 μm.

Traction Forces Reported During Firm Adhesion and Early Transmigration

Traction forces were obtained for endothelial monolayers in untreated control, TNF-α-treated, and transmigration conditions (Fig. 2a). By quantifying the average magnitude of traction forces across each monolayer as a measure of average endothelial contractility, we observed a slight increase in TNF-α-treated cells and a large increase in the monolayers with monocytes in either firm adhesion or early transmigration stages (Fig. 2b, Supplementary Fig. 1a). Because we did not observe significant differences in endothelial mechanics between firm adhesion and early transmigration conditions, we did not distinguish these two cases and instead refer to both as "trans-endothelial migration" (TEM), treating them as one group in all subsequent quantifications. These data indicated that the monocytes appear to induce a change in the mechanics of the monolayer.

We postulated that one possible effect of monocyte adhesion would be to either induce endothelial cells to generate traction forces to pull away from the monocyte (allowing retraction and opening of a hole for monocytes pass through the monolayer) or to pull centripetally toward the monocyte (perhaps acting to anchor and stabilize monocyte adhesion with additional actin stress fiber connections). To investigate these possibilities, we quantified the degree of re-alignment of traction forces in the endothelial cells, with respect to the position of the monocyte on the

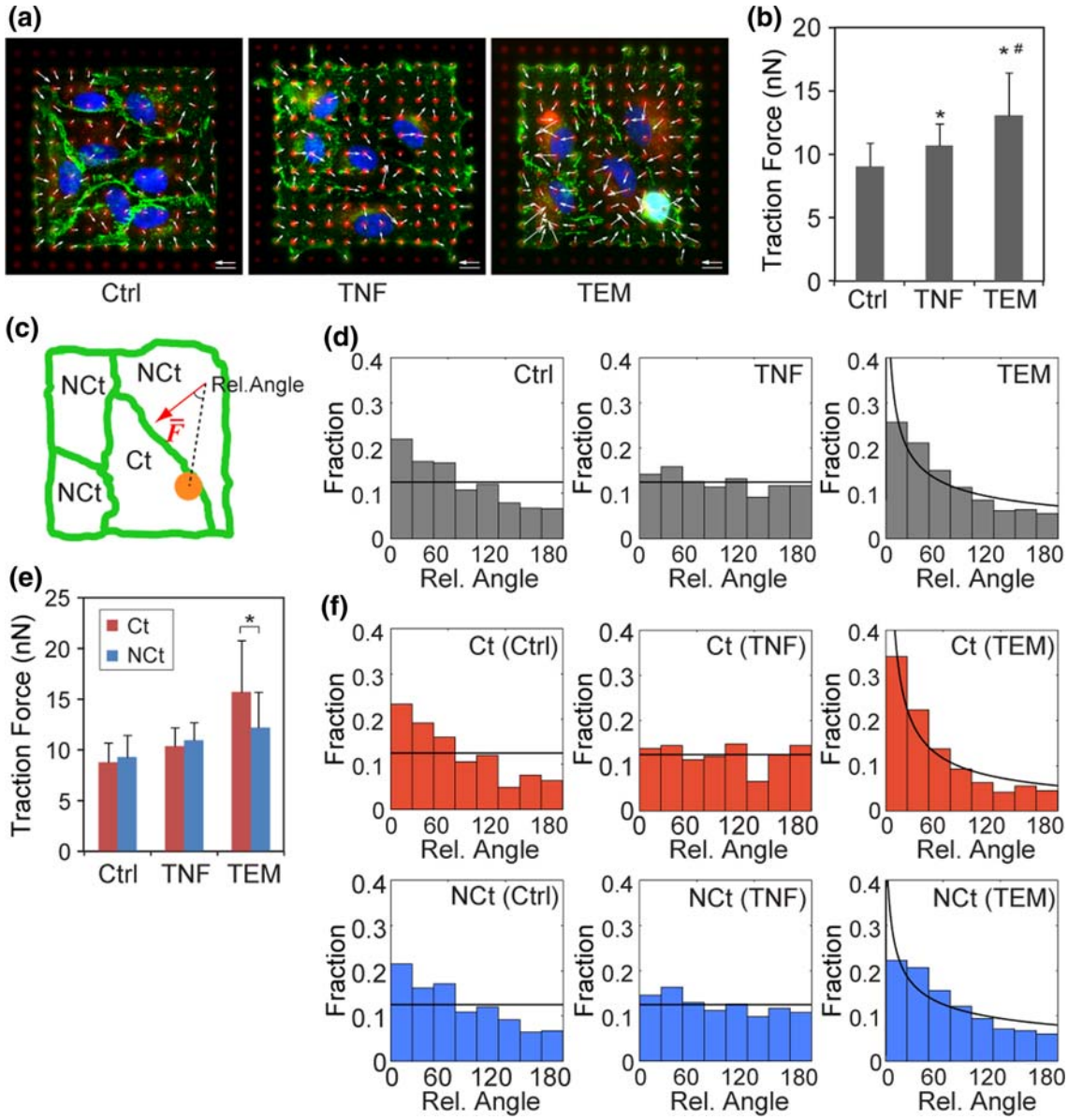

FIGURE 2. Traction forces reported during firm adhesion and early transmigration. (a) Fluorescence images showing endothelial monolayers at baseline (Ctrl), TNFα-treated (TNF), and with monocyte transmigrating on it (TEM), respectively. Immunofluorescence staining indicates β-catenin (*green*); and monocyte (*bright cyan*); nucleus (*blue*); microposts (*red*). Scale bars indicate 10 μm; *white arrows* in figures indicate the vector of traction forces with scaled arrow bar indicating 32 nN. (b) Bar graph indicating increase in average traction force in endothelial monolayers with monocytes transmigrating on them. *$p < 0.05$, indicates comparison against Ctrl; # $p < 0.05$, indicates comparison against TNF. (c) Definition of the relative angle (Rel. Angle): the angle (absolute value, in degrees) between the traction force vector of each location on the endothelial monolayer and the centripetal line connecting the center of the monocyte to that location; and also the definitions of Ct Endo and NCt Endo, as the endothelial cell directly contacting the monocyte, or not, respectively. For monocytes spanning more than one endothelial cell, we defined the Ct cell as the one with the largest contacting area with the monocytes. (d) Histograms showing the distribution of Rel. Angle for Ctrl, TNF, and TEM conditions. The TEM condition was fit with gamma distribution (see "Materials and Methods" section). See "Materials and Methods" for how ghost monocyte locations were generated for Ctrl and TNF conditions. (e) Bar graph indicating a significant difference in average traction forces between Ct and NCt cells in the TEM condition. *$p < 0.05$, indicates comparison against Ct. (f) Histograms showing the distribution of Rel. Angle in both Ct and NCt cells for Ctrl, TNF, and TEM conditions. Ctrl and TNF are fit with uniform distribution and TEM is fit with gamma distribution. All error bars indicate standard deviation.

monolayer. We defined a relative angle (Rel. Angle) between the traction force vector for any particular micropost and the centripetal line connecting the centroid of the monocyte to the micropost location (Fig. 2c). Here, zero degree denotes a force vector pointing toward the monocyte. The histogram of the relative angles for all of the monolayers indicated a small bias at lower angles in the nontreated

monolayers (Ctrl), which reflected an expected intrinsic centripetality of forces toward the centroid of the non-treated monolayer. Interestingly, this intrinsic bias was lost when monolayers were treated with TNF-α, and introduction of monocytes led to a pronounced peak near zero angle (Fig. 2d), which indicated a re-direction of traction forces in the endothelial monolayer guided toward the centroid of monocyte adhesion.

Previous studies have indicated that after firm adhesion of monocytes, the endothelial cell directly contacting the monocyte weakens their cell–cell junctions to prepare for the monocytes to transmigrate through between them.[3,4,16,18,29,36] Thus, we categorized the endothelial cells in our dataset into two subgroups: the endothelial cell directly contacting the monocyte (Ct) and all of the rest of the endothelial cells not directly contacting the monocyte (NCt) (Fig. 2c). For monocytes spanning more than one endothelial cell, we defined the Ct cell as the one with the largest contacting area with the monocytes. We then performed the analysis of traction forces as above (Figs. 2e–2f, Supplementary Fig. 1b). We observed much larger and more centripetal traction force in the monocyte-contacting endothelial cells, though we still observed a significant but smaller increase in magnitude and re-orientation of traction forces in the rest of the endothelial cells not contacting the monocytes (Figs. 2e–2f). Together, these results indicated that monocytes transmigrating on endothelium induce substantial increases in traction forces within the endothelial monolayer. These effects are most pronounced in the endothelial cell in direct contact with the monocyte, but importantly are also propagated to the surrounding endothelial cells within the same monolayer.

Spatial Distribution of Traction Forces in Endothelial Monolayer During Firm Adhesion and Early Transmigration

Although there was a more pronounced stimulation of traction force in the endothelial cell in direct contact with the monocyte, we considered the possibility that the stimulatory mechanical effect was even more localized than just defined by cell boundaries: Replotting Fig. 2a revealed local peaks in contractile force that were not defined by the boundaries of cells, but much smaller regions. Indeed, it appeared that there was a peak in force in a small subcellular region directly underneath the monocyte (Fig. 3a).

In order to extend this observation across our entire dataset, we refined the force analysis by segmenting the monolayer into zones from "local" to "distant" relative to the location of monocyte (Fig. 3b). Then, we obtained the average traction forces for every zone in the monolayer and compared it across different conditions. This analysis revealed a significant increase in local contractility in the endothelial cells with respect to the transmigrating monocyte (Fig. 3c), and is consistent with reports of actin remodeling locally around the position of monocyte firm adhesion.[13,34]

We also tested whether there might be differences in average traction forces near the edges vs. interior of monolayers as has been suggested by previous studies,[21] by segmenting the monolayer into zones from "edge" vs. "interior" zones (Fig. 3d). Slightly larger traction forces appeared to exist at the edges as compared to the interior zone for both baseline and transmigrating monolayers, but the trend was not significant ($p > 0.10$) (Fig. 3e).

Activation of Endothelial ICAM-1/VCAM-1 is Enough to Trigger Increase in Traction Forces

ICAM-1 and VCAM-1 appear to be critical players during firm adhesion-induced transmigration.[1,2,33,36] It has been reported that ICAM-1-coated beads were sufficient to mimic ICAM-1 engagement during firm adhesion and trigger downstream intracellular signal pathways required for subsequent TEM.[2] To test whether engagement of either receptor might be involved in the observed changes in endothelial mechanics observed with monocytes, we exposed endothelial monolayers with polystyrene beads coated with anti-ICAM-1 or anti-VCAM-1 mAb (Fig. 4a). Engagement of ICAM-1 but not VCAM-1 significantly increased average traction force in the monolayer (Fig. 4b), although the increase was not as high as when exposed to monocytes. When comparing traction forces of cells in contact vs. noncontact with beads, there appeared to be a slight trend toward increased traction but the effect was not significant (Fig. 4c). However, when using local zones to segment the dataset, one observed a high traction force level in the local zones near either ICAM-1- or VCAM-1-coated beads (Fig. 4d). Together, these data suggest that both receptors are involved in the mechanical response of endothelium to monocytes.

DISCUSSION

With the use of a microfabricated force measurement system, we report the first characterization of mechanical forces in endothelial monolayers induced by monocyte adhesion and transmigration.

Our results demonstrate an increase in traction forces in endothelial monolayer during firm adhesion and early transmigration. Previous studies have indicated that after firm adhesion of monocytes, the

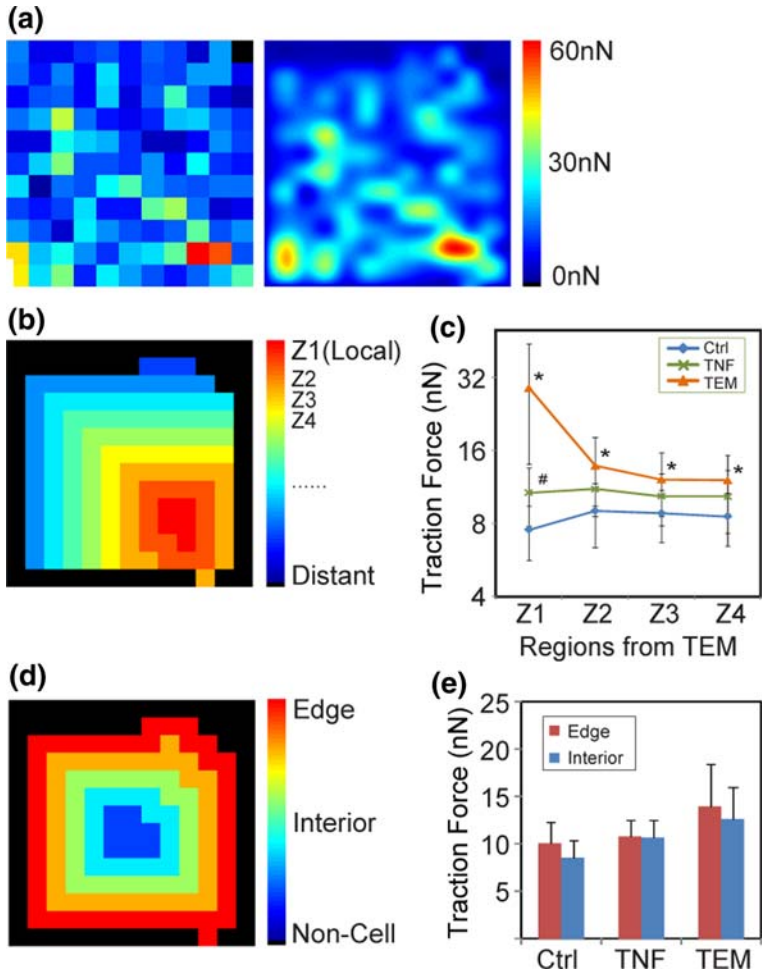

FIGURE 3. Spatial distribution of traction forces in endothelial monolayer during firm adhesion and early transmigration. (a) The magnitude of traction forces in the transmigration monolayer in Fig. 2a was re-plotted in pseudo-color, filtered and smoothed with a bi-cubic 2D spatial filter. (b) Zones from "Local" to "Distant" relative to the location of monocyte. Z1 is the zone of posts in the closest vicinity around the monocyte, Z2 next closest, Z3… and so on. The segmentation was performed on the transmigrating monolayer in Fig. 2a. (c) Average traction force in each zone as defined in (b) compared across all conditions. * or #, $p < 0.05$, indicates comparison against Ctrl at each zone. (d) Zones from "Edge" to "Interior." "Edge" is defined as the outmost zone, and "Interior" is defined as the combination of all the rest of the zones. (e) Bar graph indicating no significant difference in average traction forces between "Edge" and "Interior" zones as defined in (d) in all conditions. All error bars indicate standard deviation.

endothelial cell directly contacting the monocyte weaken their cell–cell junctions to prepare for the monocytes to transmigrate through between them.[3,4,16,18,29,36] Other studies have shown that vasoactive agents like thrombin induce a rapid and transient activation of RhoA, accompanied by an increase in myosin light chain phosphorylation, the generation of F-actin stress fibers, and a prolonged increase in endothelial permeability.[35] In this process, endothelial cells change their cytoskeleton to allow small gaps forming between neighboring cells, potentially to allow molecules and cells to cross through. It is possible that leukocytes can usurp this same pathway for inducing transmigration, by initiating Rho-dependent signaling that in turn activates contractility to promote gap formation.[15,24,30,39]

Consistent with this link between RhoA signaling and permeability is also the local nature of the effect: transmigrating T-lymphocytes appear to be surrounded by a microvillus-like docking structure whose formation appears to involve RhoA/ROCK signaling.[5,9] Importantly, abrogation of these structures appears to inhibit transmigration but not firm adhesion. Because ROCK can also induce contractile forces, these observations are consistent with our results of a local increase of traction force in the monolayer at the point of monocyte contact. Understanding the spatiotemporal dynamics of these localized traction forces may provide additional insights into how these mechanochemical signals ultimately impact transmigration.

There are number of studies showing that during firm adhesion, the engagement of endothelial ICAM-1

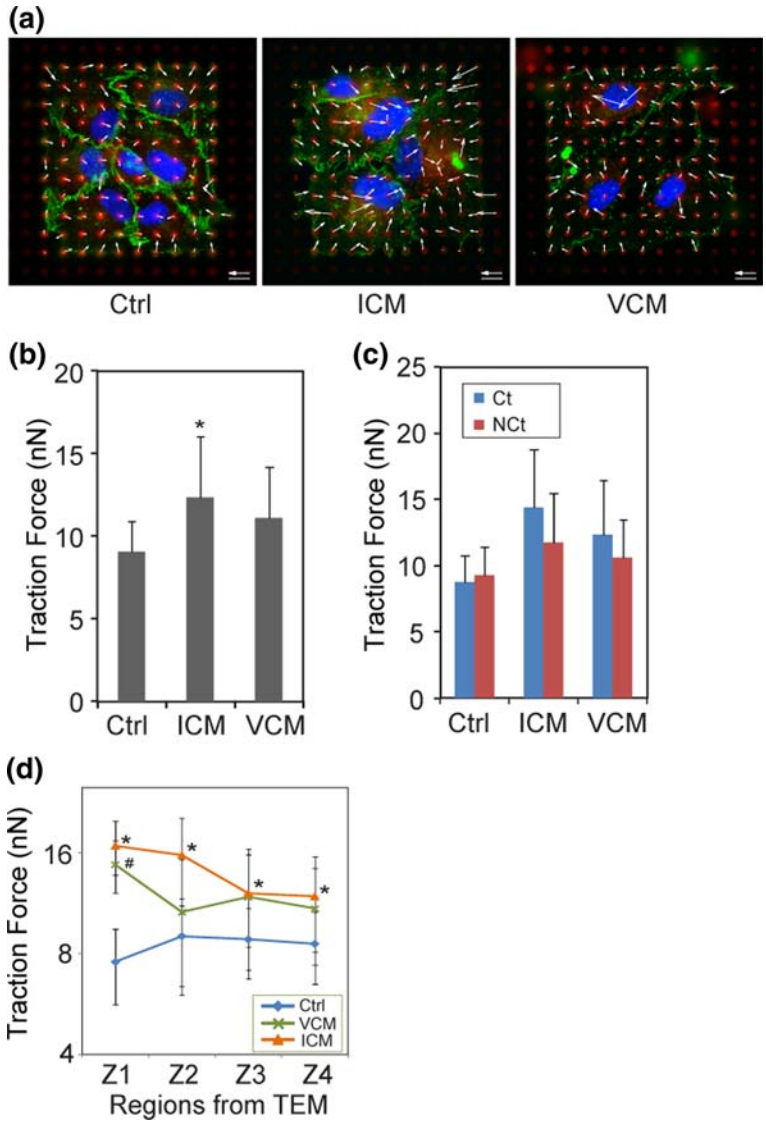

FIGURE 4. Activation of endothelial ICAM-1/VCAM-1 is enough to trigger increase in traction forces. (a) Fluorescence images showing endothelial monolayers at baseline (Ctrl), TNFα-treated (TNF), and with monocyte arrested on it (TEM), respectively. Immunofluorescence staining indicates beta-catenin (*green*); and monocyte (*bright cyan*); nucleus (*blue*); microposts (*red*). In the last two images, the bright green circular dots are the ICAM-1 and VCAM-1-coated beads. Scale bars indicate 10 μm; *white arrows* in figures indicate the vector of traction forces with scaled arrow bar indicating 32 nN. (b) Bar graph indicating increase in average traction force in ICAM-1-treated endothelial monolayers. *$p < 0.05$, indicates comparison against Ctrl. (c) Bar graph indicating no significant difference in average traction forces between Ct and NCt cells in ICM or VCM condition. (d) Average traction force in each zone from ''Local'' to ''Distant'' relative to the location of ICAM-1/VCAM-1 beads, as defined in Fig. 3b, compared across all above conditions. * or #, $p < 0.05$, indicates comparison against Ctrl at each zone. All error bars indicate standard deviation.

triggers Rho/ROCK signaling and stress fiber assembly.[13,33,34] Our results showed an increase in traction force after inducing ICAM-1 but not VCAM-1. One possibility is that VCAM-1 was suboptimally activated using antibody-coated beads. The difference between ICAM-1 and VCAM-1 also could result from the differential involvement of these two factors in different steps of transmigration. VCAM-1 is thought to be involved earlier, including both the rolling and adhesion stages, while ICAM-1 is more restricted to the firm adhesion and transmigration process.[20] The difference in the induced traction force may reflect different requirements for mechanical changes within endothelial cells for these different stages of transmigration.

In summary, the studies reported here highlight the intimate, highly dynamic, and spatially localized mechanical interactions between leukocytes and the endothelial monolayer, that are likely critical to the biophysical process of transmigration. As such, the

development of new tools to characterize the mechanics of these events is likely to play a critical role in elucidating the mechanisms by which this dynamic barrier known as the endothelium operates.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (doi:10.1007/s12195-010-0105-3) contains supplementary material, which is available to authorized users.

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