Migration of T Cells on Surfaces Containing Complex Nanotopography

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Abstract

T cells navigate complex microenvironments to initiate and modulate antigen-specific immune responses. While recent intravital microscopy study revealed that migration of T cells were guided by various tissue microstructures containing unique nanoscale topographical structures, the effects of complex nanotopographical structures on the migration of T cells have not been systematically studied. In this study, we fabricated surfaces containing nanoscale zigzag structures with various side lengths and turning angles using UV-assisted capillary force lithography and motility of T cells on zigzag patterned surfaces was studied. Motility of T cells was mostly affected by the turning angle, not by the side length, of the zigzag structures. In particular, motility behaviors of T cells near interfaces formed by turning points of zigzag patterns were significantly affected by turning angles. For obtuse turning angles, most of the T cells smoothly crossed the interfaces, but as the turning angle decreased, a substantial fraction of the T cells migrated along the interfaces. When the formation of lamellipodia, thin sheet-like structures typically generated at the leading edges of migrating cells by actin polymerization-driven membrane protrusion, was inhibited by an Arp2/3 inhibitor CK-636, a substantial fraction of T cells on those surfaces containing zigzag patterns with an acute turning angle were trapped at the interfaces formed by the turning points of the zigzag patterns. This result suggests that thin, wide lamellipodia at the leading edges of T cells play critical roles in motility of T cells in complex topographical microenvironments.

Introduction

T cells are immune cells playing a central role in antigen-specific immune responses. To successfully mount antigen-specific immune responses, T cells must navigate to the right place and encounter their partners [1]. For example, they become activated by interacting with antigen-presenting cells presenting antigens specific for their T cell receptors in secondary lymphoid organs such as a spleen and lymph nodes, and they perform effector functions by contacting pathogen-harboring cells or transformed cells in peripheral tissues. Therefore, how quickly T cells find their interaction partners may determine the overall efficacy of immune responses [1,2].

Multi-photon microscopy performed over the last decade has allowed us to understand how T cells migrate in search for their interaction partners in vivo [3,4]. Overall, they migrate rapidly with a peak velocity of 25 μm/min in a rather random fashion to maximize the scanning area [5]. At the same time, their motility is guided not only by soluble factors such as chemokines [6], but also by many cellular/extracellular structures such as collagen fibers [7], specialized lymph node stromal cells called fibroblastic reticular cells [8], and fibrous structures formed by infection [9], which typically have unique nanoscale topographical structures. While the effect of soluble factor on directional migration of T cells has been extensively studied using various in vitro model systems such as agarose gel [10], Boyden chambers [11], and microfluidic channels [12], relatively less attention has been paid to the effects of nanotopography on motility of T cells.

Recently, we investigated how motility of T cells is affected by nanoscale topographical structures mimicking fibrous structures of ECMs using polymer surfaces containing straight lines of nanoscale topographical structures [13]. Compared with epithelial and mesenchymal cells, which have been extensively studied using nanostructured surfaces [14–17], T cells exhibit a completely different mode of migration, so called amoeboid migration: T cells only weakly adhere to the substrates, generate weak traction forces and migrate 10–100 times faster than epithelial cells and fibroblasts [18]. As a result, the behavior of T cells on nanogrooved surfaces was different from that of epithelial/mesenchymal cells. While epithelial/mesenchymal cells aligned almost perfectly and migrated along the nanogroove direction, migration of T cells were close to a biased random walk with increasing directional persistence with increasing adhesiveness [13]. Lamellipodia, a thin sheet-like membrane protrusion, at the leading edge appeared to be guided toward the direction of the nanogrooves when adhesive substrates were used, but the role of lamellipodia on topography sensing of T cells has not been fully elucidated. Moreover, straight nanoscale ridge/groove structures may not fully represent the complex topographical structures T cells encounter in vivo.

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To address the aforementioned issues, we fabricated surfaces containing nanoscale zigzag structures with various side lengths and turning angles, and then investigated the effects of these zigzag nanotopographical structures on the motility of T cells. The roles of lamellipodia in T cell migration on complex nanotopographical surfaces were studied by treating T cells with a pharmacological inhibitor targeting Arp2/3, a key regulator for lamellipodia formation [19], and comparing the motility of Arp2/3 inhibitor-treated T cells with that of untreated T cells.

Results and Discussion

Preparation of the Nanoscale Zigzag Structures

To study how the motility of T cells is affected by complex nanotopography, we fabricated nanoscale zigzag structures using UV-assisted capillary force lithography (CFL) with UV curable polymer PUA on glass coverslips as shown in Fig. 1A [20]. Scanning electron microscope images of successfully fabricated nanostructured surfaces are shown in Fig. 1B. The width of ridges, grooves, and the height of the nanoscale zigzag structures were 350 nm, 700 nm, and 300 nm, respectively, which mimic the dimensions of fibrous extracellular matrix (ECM) structures [17,21]. Three different lengths of sides (L = 15, 30, and 60 μm) and three different turning angles (θ = 45°, 90°, and 135°) of nanoscale zigzag structures were fabricated (Fig. 1C). The average length and breadth of the T cells used for the experiments were 16.2 μm and 10.8 μm, respectively, close to the shortest length of the fabricated zigzag structures. Nanostructured PUA surfaces were coated with 10 μg/mL of ICAM-1, a ligand for lymphocyte function-associated antigen 1 (LFA-1) that is one of major integrins of T cells.

Migration of T cells on the Surfaces Containing Zigzag Nanotopography Structures

To quantitatively analyze the motility of the T cells on the nanoscale zigzag structures, zigzag patterns were mounted on a microscope stage with the orientation schematically shown in the left panel of Fig. 2A so that all the sides of the zigzag patterns will have identical angles, ± half of the turning angle θ, to y-axis. Then, cell movements were recorded by time-lapse microscopy, and the trajectories of individual T cells were tracked by image processing, and analyzed. Δx and Δy are displacements of T cells along the x- and y-axis, respectively. From the displacement information, mean velocity (Vmean) and directionality of migration along the x-axis (dx) were calculated using the equations shown below:

\[ V_{\text{mean}} = \frac{\sqrt{\Delta x^2 + \Delta y^2}}{\Delta t} \]

\[ d_x = \frac{V_x}{V_x + V_y}, \text{ where } V_x = \frac{\sum |\Delta x|}{\Delta t} \text{ and } V_y = \frac{\sum |\Delta y|}{\Delta t} \]

The dx value is larger or smaller than 0.5 when T cells migration is biased either to the direction of x-axis or y-axis, respectively, and it is close to 0.5 if the T cell migration is not biased to any directions.

First, we assessed the effect of turning angle θ on the velocity and directionality of T cell migration. The Vmean and dx values of T cells on zigzag patterns with fixed side length L = 60 μm and various turning angles were plotted in Fig. 2B and C, respectively. The average Vmean value of T cells on zigzag patterns with θ = 45° was 16.2 μm and 25 μm, n = 42 for all cases). Data are representative of two independent experiments. (line: average, Mann-Whitney test, N.S.: not significant, **p<0.001, ***p<0.0001). doi:10.1371/journal.pone.0073960.g002
was significantly lower than that of T cells on zigzag patterns with \( \theta = 90^\circ \) or \( 135^\circ \), meaning that motility of T cells was hampered by nanoscale zigzag topographical structures with a sharp turning angle. The average values of \( d_x \) of T cells on the zigzag patterns with \( 45^\circ, 90^\circ \), and \( 135^\circ \) was 0.39, 0.51, and 0.69 respectively, meaning that the migration of T cells on the zigzag patterns with \( 45^\circ \) and \( 135^\circ \) of turning angles was biased along the y- and x-axis, respectively, while the migration the T cells on the zigzag patterns with \( 90^\circ \) turning angle was not biased to x or y directions. These results indicate that T cell migration was guided by nanoscale topographical structures as previously demonstrated [13] because the sides of the zigzag patterns with \( 45^\circ \) and \( 135^\circ \) of turning angles are oriented toward y-axis and x-axis, respectively while sides of zigzag patterns with \( 90^\circ \) of turning angles are not biased toward any axis. Then, we assessed whether the lengths of sides of the zigzag patterns affected the migration of T cells by using zigzag patterns with various Ls. Both \( V_{\text{mean}} \) and \( d_x \) values were not affected by L even at the sharpest turning angle (Fig. 2D and E). Indeed, similar results were obtained with all the turning angles examined (data not shown), meaning that the length of the sides of the zigzag patterns had minimal effect on T cell motility. Therefore, we fixed the value of L at 60 \( \mu \)m for the rest of the study and primarily focused on the effect of the turning angles.

While overall motility and directionality of T cells can be described by \( V_{\text{mean}} \) and \( d_x \), they contain limited information about the local behaviors of T cells, in particular near the turning points of the zigzag patterns. Interfaces composed of turning points of zigzag patterns can easily be detected by differential interference contrast (DIC) mode of imaging, which uses polarized light, because the amount of transmitted light differs depending on the angle between the polarized light and the sides of the zigzag patterns. As a result, clear boundaries were visible at interfaces formed by the turning points of the zigzag patterns, and when the surface containing the zigzag surface was rotated \( 90^\circ \) clockwise, the dark and bright areas were altered (Fig. 3A). When we carefully examined the T cells entering interfaces defined by the turning points of zigzag patterns, we observed two distinct populations of T cells. Some T cells crossed the interface with minimal residence time at the interfaces denoted as ‘crossing’ (Fig. 3B and Movie S1), and some T cells migrated along the interfaces denoted as ‘migrating along’ (Fig. 3C and Movie S2). The percentage of each population was measured for zigzag patterns with different turning angles and plotted in Fig. 3D. Most of the T cells on the zigzag patterns with \( 135^\circ \) turning angle crossed the peaks smoothly, but as the turning angles became sharper, more and more T cells migrated along the interfaces, and about 30% of the T cells on the zigzag patterns with \( 45^\circ \) turning angle migrated along the peaks parallel to the y-axis.

**Roles of Lamellipodia on Motility Behavior of T cells on the Surfaces Containing Zigzag Nanotopography Structures**

Migrating T cells exhibit a characteristic hand-mirror shape with wide lamellipodia at the leading edge (Fig. 4A). In our previous study, we demonstrated that actin polymerization driven leading edge protrusion is critical for contact guidance of T cells [13], but the role of the lamellipodia, which form at the leading edges of migrating T cells, on nanotopography-guided migration of T cells has not yet been elucidated. To address this, we inhibited Arp2/3, which nucleates actin branching to form lamellipodia [19], using CK-636, a recently developed pharmacological inhibitor [22]. To assess whether CK-636 inhibits formation of lamellipodia in T cells, we fixed CK-636-treated T cells on nanostructured surfaces and examined their ultrastructures using SEM. For a control, T cells treated with DMSO, a carrier used to dissolve CK-636, were also examined. While the DMSO-treated T cells exhibited characteristic hand mirror-shaped morphology with wide and thin sheet-like lamellipodia at the leading edges (Fig. 4A), the CK-636-treated T cells exhibited elongated morphology with sharp pseudopodia at the leading edges (Fig. 4B). When we quantitatively analyzed length and breadth of the T cells, the length of the CK-636-treated T cells were comparable to those of the DMSO-treated T cells (Fig. 4C), while the breadth of the CK-636-treated T cells was about 30% less than that of DMSO-treated T cells (Fig. 4D).

With this clear inhibition of lamellipodia formation at the leading edge by CK-636-treated T cells, we next performed migration assays of CK-636-treated T cells on the surfaces containing nanoscale zigzag structures with various turning angles. The \( V_{\text{mean}} \) and \( d_x \) values of CK-636-treated T cells were calculated and plotted in Fig. 5A and 5B, respectively. To compare the motility of CK-636-treated T cells with the untreated T cells, the relative \( V_{\text{mean}} \) or \( d_x \) values were defined by the ratio between \( V_{\text{mean}} \) or \( d_x \) values of CK-636-treated T cells and the average of \( V_{\text{mean}} \) or \( d_x \) values of untreated T cells on the same type of surfaces. Calculated relative \( V_{\text{mean}} \) and \( d_x \) values of CK-636-treated T cells were plotted in Fig. 5C and 5D, respectively. Similar to the case of untreated T cells (Fig. 2B), the average \( V_{\text{mean}} \) value of the CK-636-treated T cells on surfaces with \( \theta = 45^\circ \) was significantly lower that of CKC-636-treated T cells on surfaces with \( \theta = 90^\circ \) and \( 135^\circ \) (Fig. 5A). In addition, the relative \( V_{\text{mean}} \) value of CK-636-treated T cells was slightly above 0.6 for all the turning angles examined (Fig. 5C), meaning that CK-636 treatment significantly reduced the velocity of T cells on the surfaces regardless of turning angles. In contrast, normalized \( d_x \) values of CK-636-treated T cells on zigzag surfaces with \( \theta = 45^\circ \) was about 1.2 while normalized \( d_x \) values of CK-636-treated T cells on zigzag surfaces with \( \theta = 90^\circ \) and \( 135^\circ \) was about 0.2.
cells on zigzag surfaces with $\theta = 90^\circ$ and $\theta = 135^\circ$ were close to 1, indicating that abrogation of lamellipodia formation at the leading edges of T cells by the treatment of CK-636 had profound impact on the directionality of T cells on zigzag patterns with an acute turning angle, but minimal effect for T cells on zigzag patterns with right or obtuse turning angles.

To gain further information, we analyzed the behaviors of CK-636-treated T cells near interfaces where the direction of the sides changed. Interestingly, a few T cells stayed at the interfaces for more than two minutes with minimal net translocation longer than 2 min. (Fig. 6A and Movie S3). This population of T cells was denoted as ‘trapped’, and T cells encountering interfaces formed with turning points of zigzag patterns were classified into three categories and plotted in Fig. 6B. As expected, the behavior of CK-636-treated T cells on zigzag patterns with $\theta = 90^\circ$ or $135^\circ$ were not much different from those of untreated T cells; more than 95% of T cells crossed the interfaces when $\theta = 135^\circ$ while the population of T cells migrating along the interfaces increased when $\theta$ became smaller. In contrast, more than 25% of CK-636-treated T cells were trapped at the interfaces when $\theta = 45^\circ$.

These results suggest that wide and thin lamellipodia formed at the leading edge of T cells are important for maintaining the motility of T cells on complex topography. Coordinated membrane protrusion and focal adhesion formation via lamellipodia have recently been shown to be important for directed migration of fibroblasts toward biochemical cues [23,24]. Our previous study of T cell migration on straight nanogrooves coated with ICAM-1 also showed that T cells generated aligned protrusion of lamellipodia toward the direction of nanogrooves [13]. Thus coordinated leading edge protrusion via lamellipodia may also be important for nanotopography-guided migration of T cells. In addition to coordinating leading edge protrusion of migrating cells to augment directed migration of cells, lamellipodia may also promote motility of cells under complex nanotopographical microenvironments, which may be formed by interwoven fibrous bundles of ECMs in vivo, by allowing smooth direction change as demonstrated in this study.

Conclusions

In summary, surfaces containing various nanoscale zigzag structures were fabricated by CFL and the effects of complex nanotopography on the motility of T cells were studied. Motility of T cells was mostly affected by the turning angle, not by the side length, of the zigzag structures. T cells on zigzag patterns with an acute turning angle exhibited significantly reduced migration speed and altered migration direction compared with T cells on zigzag patterns with right or obtuse angles. Lamellipodia formation at the leading edges of migrating T cells could be inhibited by treating T cells with CK-636, a pharmacological inhibitor targeting Arp2/3. Overall, CK-636-treated T cells
exhibited reduced velocity compared with untreated T cells. In particular, a substantial fraction of CK-636-treated T cells on zigzag patterns with an acute turning angle were trapped near the interfaces formed by the turning points of zigzag patterns, suggesting that lamellipodia play essential roles in rapid migration of T cells under complex topographical microenvironments.

Materials and Methods

Fabrication of Nanoscale Zigzag Structured Surfaces
A silicon master of nanoscale zigzag structures was fabricated at the Korea Advanced Nano Fab Center (KANC). To fabricate nanoscale structures on large areas of silicon wafer, K motel Fluoride (KF) stepper was used in photolithography. Nanostructures on the silicon master were replicated using poly(urethane acrylate) (PUA) by UV-assisted capillary force lithography (CFL), and by replicating the PUA nanostructured surfaces one more time on thin glass coverslips by CFL. In this way, nanostructures identical to the original silicon master were formed on coverslips [20]. The glass coverslips were cleaned using ethanol and deionized (DI) water and dried in a vacuum oven. Next, the dried coverslips were coated with adhesion promoter (Minuta Tech) and baked in the oven at 120°C for 15 min. The PUA precursor (Minuta Tech) was drop-dispensed onto the surface, and a PUA mold containing the engraved nanoscale zigzag patterns was placed directly on each coated surface. The PUA precursor spontaneously moved into the cavity of the mold by means of capillary action and was subsequently cured by exposure to UV light (λ = 250–400 nm, 100 mJ/cm²) for ~30 s through the transparent backplane. After the curing process, the molds were peeled from the surfaces.

T cell Preparation

DO11.10 T cell receptor transgenic mice were purchased from Jackson Laboratories and bred in the animal care facility in POSTECH Biotech Center (PBC) under pathogen-free conditions. All experiments involving mice were approved by the Institutional Animal Care and Use Committee at PBC. Next, DO11.10 CD4+ T cell blasts were prepared by stimulating cells from the spleens and lymph nodes of DO11.10 T cell receptor transgenic mice with 1 mg/mL OVA323–339 peptide (ISQAVHAAHAEINEAGR, Peptron, Inc. Korea). Then, DO11.10 blasts were cultured in R-10 (RPMI media with 10% fetal bovine serum (Gibco), 100 U/mL penicillin, 100 mg/mL streptomycin (Invitrogen)) with 1–2 U/mL interleukin-2 (Peprotech), and the T cell precursor (Minuta Tech) was drop-dispensed onto the surface, and a PUA mold containing the engraved nanoscale zigzag patterns was placed directly on each coated surface. The PUA precursor spontaneously moved into the cavity of the mold by means of capillary action and was subsequently cured by exposure to UV light (λ = 250–400 nm, 100 mJ/cm²) for ~30 s through the transparent backplane. After the curing process, the molds were peeled from the surfaces.

Migration Assays

For migration assays, the nanoscale zigzag structured surfaces were coated with 10 µg/mL ICAM-1 (R&D systems) by incubating the ICAM-1 solution in PBS for 1 h at 37°C after about 60 s air plasma treatment (200–500 W, Femto Science, Korea). T cell blasts labeled with 10 µM M 5-(and-6)-(((4-chloro-para-methylene))amino)tetramethylrhodamine (CMTMR, Invitrogen)) with 1–2 U/mL interleukin-2 (Peprotech), and the T cells were used 5–7 days after stimulation.

Supporting Information

Movie S1 Representative movie of a T cell ‘crossing’ the interfaces of the nanoscale zigzag structures (L = 60 µm, θ = 45°). Time stamp = min:sec, Scale bar = 20 µm. (AVI)

Movie S2 Representative movie of a T cell ‘migrating along’ the interfaces of the nanoscale zigzag structures (L = 60 µm, θ = 45°). Time stamp = min:sec, Scale bar = 20 µm. (AVI)

Movie S3 Representative movie of a CKC-636-treated T cell ‘trapped’ at the interfaces of the nanoscale zigzag structures (L = 60 µm, θ = 45°). Time stamp = min:sec, Scale bar = 20 µm. (AVI)

Author Contributions

Conceived and designed the experiments: KWK HP JD. Performed the experiments: KWK HP. Analyzed the data: KWK HP. Wrote the paper: KWK JD.

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