Figures and figure supplements

Different TCR-induced T lymphocyte responses are potentiated by stiffness with variable sensitivity

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Figure 1. T cell migratory properties and morphology are modulated by substrate stiffness. (A) Mean instantaneous velocities of migrating T cells on ICAM-1-coated PA-gels of varying stiffness (n_cells: 50–100 for each condition from n_Donors: 4). (B) Maximum distance travelled by T cells on PA-gels of varying stiffness for a duration of 5 min (n_cells: 50–100 for each condition from n_Donors: 4). Boxes and whiskers for minimum and maximum are shown. For statistical analysis, unpaired parametric t-tests were performed: ****p-value<0.0001, ***p-value<0.001, **p-value<0.01. (C) Percentage of arrested cells on aCD3+aCD28+ICAM-1-coated PA-gels of varying stiffness. T cell response on glass coated with aCD3+aCD28+ICAM-1 is shown for comparison. Mean values and number of cells per condition are shown (n_Donors: 2). (D) Scanning electron microscopy pictures of T cells (representative of n_cells: 5 per condition from n_Donors: 2) on aCD3+aCD28+ICAM-1-coated substrates for two magnifications (5000x and 20000x). Black scale bars: 2 μm, white scale bars: 500 nm. DOI: 10.7554/eLife.23190.004
Figure 1—figure supplement 1. Characterization of PA-gels and additional data on migration. (A) Measurement of the elastic modulus $G'$ of a PA-gel containing 5% acrylamide and 0.5% bis-acrylamide. The value associated to a given sample corresponds to the maximum of $G'$ as a function of the distance between the rheometer plates. The dotted line shows the mean value of the elastic shear modulus $G' = 2212 \pm 79$ Pa for $n = 15$ different samples of PA-gels at 5% acrylamide and 0.5% bis-acrylamide. (B) Coating of PA-gels and glass coverslips by biotinylated (b-fibronectin) or non biotinylated fibronectin quantified by immunofluorescence labeling. (C) Biotinylated antibody coating on streptavidin containing PA-gels of varying stiffness and neutravidin-coated glass coverslips quantified by immunofluorescence labeling ($n_{samples}$: 14). (D) 5 min tracks of individual CD4$^+$ T lymphoblasts on the 100 kPa gels coated with aCD3+aCD28+ICAM-1. Arrows indicate migrating cells and the arrowhead indicates an arrested cell. Scale bar: 10 μm. (E) Mean instantaneous velocities of migrating T cells on PA-gels of varying stiffness, coated with either ICAM-1 or aCD3+aCD28+ICAM-1, for a duration of 5 min ($n_{cells}$: 50–100 for each condition from $n_{Donors}$: 4). Boxes and whiskers for minimum and maximum are shown. For statistical analysis, unpaired parametric t-tests were performed: ****p-value<0.0001.

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Figure 2. Gene expression of CD4⁺ T cells shows a graded response to stiffness. (A) Principal component analysis reveals that gene expression is modulated by T cell substrate stiffness only in presence of aCD3 (n=Donors: 4). (B) Number of genes that displayed differential expression between the
Figure 2 continued on next page
conditions with and without aCD3 on PA-gels of varying stiffness. ‘Exclusive’ indicates the genes that are found Up- or Down-regulated only at a given stiffness value. (C) Relative expression of T cell related genes following Affymetrix microarray analysis. Asterisks indicate the presence of these genes in the differential analysis: *** for 0.5 to 100 kPa, ** for 6.4 to 100 kPa, * for 100 kPa only.

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Figure 3. Stiffness potentiates TCR/CD3-induced transcriptional response. (A) Enrichment of gene sets on PA-gels of varying stiffness for p-values lower than 0.05, false discovery rates lower than 0.25 and NES values higher than 1.75. (B) Pathway analysis with the GO – BP database for differentially expressed genes between the conditions with and without aCD3 on PA gels of varying stiffness. The number of different pathways (pie-chart) and the top 2 hits of the enriched pathways, along with their negative log adjusted p-value (table), are shown. (C) K-means clustering of genes with different expression profiles on PA-gels of varying stiffness demonstrates three different clusters: one with strong up-regulation in the presence of aCD3 (containing 1022 probes), one with weak down-regulation (containing 4412 probes) and one with weak or no up-regulation (containing 5928 probes). (D) Comparison of the relative changes in gene expression in the presence of aCD3 for the strong up-regulation cluster. The x-axis shows the difference in gene expression for the transition of 0.5 to 6.4 kPa, the y-axis for the transition of 6.4 to 100 kPa and the colour gradient for the transition of 0.5 to 100 kPa.

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Figure 3—figure supplement 1. Pathway analysis with the KEGG database for differentially expressed genes between the conditions with and without αCD3 on PA gels of varying stiffness.
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Figure 4. Cytokine production is sensitive to a wide range of stiffness. Production of (A) IFNγ \((n_{\text{donors}}: 13)\) and (B) TNFα \((n_{\text{donors}}: 10)\) on PA-gels of varying stiffness. In the presence of αCD3, the αCD3:αCD28 coating ratio was 1:10. (C) FACS plot of CD25 staining. A representative experiment is shown. (D) Mean fluorescence intensity of CD25-stained cells \((n_{\text{donors}}: 5)\). Mean values with standard error are shown. For statistical analysis, paired parametric t-tests were performed: *** \(p\text{-value}<0.001\), ** \(p\text{-value}<0.01\), * \(p\text{-value}<0.05\).

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Figure 4—figure supplement 1. Cytokine production on PA-gels: additional data. (A) Production of IFNγ on PA-gels of varying stiffness; in the presence of αCD3, the αCD3:αCD28 coating ratio was 1:100 (n_Donors: 5).

(B) Production of IFNγ on PA-gels of varying stiffness for non-biotinylated soluble αCD3+αCD28 antibodies at concentrations of 1 + 10 μg/mL respectively (n_Donors: 4). (C) Production of IFNγ and TNFα on PA-gels of varying stiffness coated with αCD3+αCD28 only. The αCD3:αCD28 coating ratio was 1:10 (n_Donors: 5). (D) Production of IFNγ and IL-2 and percentage of CD69+ cells for memory CD4+ T cells cultured on PA-gels of varying stiffness (n_Donors: 3). Mean values with standard error are shown. For statistical analysis, paired parametric t-tests were performed: *p-value<0.05.

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Figure 5. T cell metabolism is modulated by increased stiffness. (A) Lactate production in the supernatant of T cell cultures on PA-gels of varying stiffness (n\textsubscript{Donors}: 3). (B) Percentage of phospho-rpS6\textsuperscript{+} T cells cultured on PA-gels of varying stiffness (n\textsubscript{Donors}: 4). (C) Overall glycolytic capacity of T cells cultured on PA-gels of varying stiffness for 48 hr. The extracellular acidification rate is normalized to the number of cells per well. Mean values with standard error are shown (n\textsubscript{Donors}: 3). (D) Maximal mitochondrial respiration of T cells following culture on PA-gels of varying stiffness for 48 hr. The oxygen consumption rate is normalized to the number of cells per well. Mean values with standard error are shown (n\textsubscript{Donors}: 3). For statistical analysis, paired parametric t-tests were performed: **p-value<0.01, *p-value<0.05.

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Figure 5—figure supplement 1. Phospho-rpS6 and metabolism: additional data. (A) Representative FACS analysis of phospho-rpS6+ T cells at different culture times on aCD3+aCD28+ICAM-1 coated PA-gels of varying stiffness. (B) Overall glycolytic capacity of T cells following culture on PA-gels of varying stiffness for 24 hr. The extracellular acidification rate is normalized to the number of cells per well. Mean values with standard error are shown (n=Donors: 3). (C) Maximal mitochondrial respiration of T cells following culture on PA-gels of varying stiffness for 24 hr. The oxygen consumption rate is normalized to the number of the cells per well. Mean values with standard error are shown (n=Donors: 3).

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Figure 6. Proliferation and cell cycle progression are potentiated by stiffness in response to TCR/CD3 induced activation. The percentages of cells in G₀/G₁, S phase and G₂/M are shown for (A) 24 hr (n=Donors: 4) and (B) 72 hr (n=Donors: 4). (C) Percentage of proliferating T cells following 72 hr culture on PA-gels of varying stiffness. (n=Donors: 4). Mean values with standard error are shown. For statistical analysis, paired parametric t-tests were performed: **p-value<0.01, *p-value<0.05.
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Figure 6—figure supplement 1. Cell cycle and proliferation: additional data. (A) Representative FACS analysis of propidium iodide staining of T cells following 24 hr culture on PA-gels in the presence of aCD3. (B) Representative dot plot of T cell proliferation following 72 hr culture on PA-gels in the presence of aCD3. DOI: 10.7554/eLife.23190.020
Figure 7. T cell activation is potentiated by APC mechanical properties. (A) HeLa-CIITA cells were grown at confluence on fibronectin coated PDMS gels of varying stiffness and were stained with phalloidin (F-Actin, in red) and cell trace violet (CTV, in cyan) (scale bar: 10 μm). (B) Area of HeLa-CIITA cells cultured on 1.5 kPa (453 ± 14 μm², n_cells = 254) and 28 kPa (569 ± 25 μm², n_cells = 215) PDMS gels. Boxes and whiskers for minimum and maximum are shown. For statistical analysis, unpaired t-tests with Welch’s correction were performed: ****p-value<0.0001. (C) Young’s modulus of HeLa-CIITA cells cultured on 1.5 kPa (1.43 ± 0.15 kPa, n_cells = 13) and 28 kPa (1.72 ± 0.2 kPa, n_cells = 15) PDMS gels. Boxes and whiskers for minimum and maximum are shown. Production of (D) IFNγ and (E) TNFα by T cells interacting with HeLa-CIITA on PDMS gels of varying stiffness in the presence of different TSST-1 superantigen concentrations. The response for individual donors is shown (n_donors = 8). For statistical analysis, paired parametric t-tests were performed: *p-value<0.05.
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**Figure 7—figure supplement 1.** Characterization of APC mechanical properties and T cell activation: additional data. 

(A) Young’s modulus measurement of HeLa-CIITA cells. Left: principle of measurement and image of the HeLa cell monolayer with the glass probe on top of it (scale bar: 10 μm). When the probe is lowered by a distance D, its tip indents the top of the cell leading to a tip displacement d < D. Thus, the probe is deflected and exerts an elastic force F = k×D−d, where k is the calibrated probe stiffness. Right: force-indentation curve for an individual HeLa cell from a confluent layer, fitted following the Hertz model. 

(B) FACS plots of HeLa-CIITA cells stained for the APC markers HLA-DR and ICAM-1. Dotted lines display isotype antibody. 

(C) Mean fluorescence intensity of CD25 stained cells, which were activated for 24 hr by HeLa-CIITA cells grown at confluence on PDMS of varying stiffness in the presence of TSST-1 superantigen (1 ng/mL). The response for individual donors is shown (n=Donors: 8). 

(D) Area of HeLa-CIITA cells cultured on 1.5 kPa (453 ± 14 μm², n_cells = 254), 28 kPa (569 ± 25 μm², n_cells = 215) PDMS gels or glass (473 ± 19 μm², n_cells = 140). Boxes and whiskers for minimum and maximum are shown. 

(E) Production of IFNγ by T cells interacting with HeLa-CIITA on PDMS gels of varying stiffness or glass in the presence of different TSST-1 superantigen concentrations. The response for individual donors is shown (n=Donors: 4). For statistical analysis, paired parametric t-tests were performed: *p-value<0.05.

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