In vitro analysis of cytotoxic T cell recruitment mediated by the DC-derived chemokine CCL17

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Method Article

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Abstract

Introduction

Dendritic cell \(DC) licensing in cross-priming requires physical interaction of several rare immune cells, i.e. cytotoxic T cells \(CTL), and cross-presenting DCs. Here we describe a novel in vitro method of analyzing chemokine effects on complex recruitment events in a multi-cellular system. To study CTL recruitment towards CCL17-producing DCs, we established a co-culture system of murine splenic DCs with polyclonal splenic CTL from donor mice, which enables visualization of cell motility and cell-cell interactions at a high resolution over a period of several hours by use of multi-color live cell imaging. Differential cell labeling with plasma membrane-permeable cell tracker dyes was employed for the specific detection of each cell population. Mixed cell populations were placed on fibronectin-coated channel slides following recording of time-lapse video series with a fully automated inverted microscope. Motile CTL were individually tracked using ImageJ software with subsequent calculation of directionality and velocity of migrating CTL before physical contact to DC. The duration of cell-cell interactions was determined as well \(contact duration time). This procedure enables simultaneous analyses of cell velocity, migratory directionality and cell contact duration times in e.g. a three-partite system comprising DC, as well as wt and chemokine receptor null cytotoxic T cells.

Reagents

- RPMI cell culture medium \(Invitrogen, cat. no. 42401) - Fetal calf serum \(FCS, PAA, cat. no. A15-102) - 5,6-carboxyfluorescein diavetate, succinimidyl \(CFSE, Invitrogen, cat. no. C1157) - 5-\(and-6)-\(\(\(\(4cloromethyl)-enzoyl)amino)tetramethylrhodamine \(CMTMR, Invitrogen, cat. no. C2927) - FarRed \(Invitrogen, cat. no. C34553) - Phosphate buffered saline, 1x \(PBS, PAA, cat. no. H15-002) - Fibronectin \(Harbor, Bio-Products, cat. no. 2003)

Equipment

- Plastic channel µ-slides I \(IBIDI, cat. no. 80106) - Inverted confocal microscope \(FV1000, Olympus) - UPLSAPO20X/0.75 U Plan S Apo Objective \(Olympus) - Motorized xyz-microscope stage \(Märzhäuser, cat. no. 00-24-414) - Climate chamber for microscopes \(37°C, humidified, 5% CO2) - Laminar flow sterile hood - Cell culture incubator \(37°C, humidified, 5% CO2) - Manual Tracking Plugin of Image J - Chemotaxis and Migration Tool Plugin \(IBIDI) for Image J

Procedure

1. Coating of plastic channel slides Timing: 75 min To coat plastic channel slides, place 100 µl of Fibronectin-coating solution in the channel and incubate 60 min at room temperature. Subsequently aspirate the solution and wash once with 1 ml PBS. 2. Fluochrome labeling of cytotoxic T cells \(CTL) or dendritic cells \(DCs), respectively Timing: 20 min For fluorochrome labeling 1-2x107 cell/ml were

resuspended in PBS followed by addition of appropriate plasma membrane permeable cell tracker \(final concentrations and incubation times, see below). CFSE: 5 µM, 10 min CMTMR: 5 µM, 10 min FarRed 5 µM, 20 min 3. Seeding cells Timing: 15 min Mix CTL and DCs from spleen at a ratio of 4:1 in RPMI medium containing 2% FBS to a total cell number of 2x106/ml and apply 100 µl cell suspension into the channel of the µ-slide. Cover reservoirs loosely with supplied caps following live cell imaging. 4. Video microscopy Timing: 2-3 h Record time-lapse series with the help of a fully automated inverted widefield fluorescence respective confocal microscope equipped with motorized xyz-stage and a climate chamber \(37°C, humidified, 5% CO2). Record cell motility and cell-cell interactions over a period of 2-3h by capturing fluorescence and phase contrast or differential interference contrast \(DIC) images every minute. Stage inserts for more than one slides allows simultaneous time-lapse series of different approaches. 5. Analysis of cell motility and cell-cell interactions In each individual experiment track 100-300 motile CTL using the Manual Tracking and Plugin of Image J \(NIH). Calculate directionality of migrating CTL before physical contact to DC and duration of cell-cell interactions \(contact duration) by analysis with the Chemotaxis and Migration Tool Plugin \(IBIDI) for Image J.

Timing

4-5 hours

Troubleshooting

Step 3: Avoid tilting the slide in order to prevent cells from being flushed out of the channel. The day before cell seeding place the medium and the µ-slide into the incubator for equilibration. This will prevent the liquid inside the channel from producing air bubbles over the incubation time.

Anticipated Results

Examples for analysis of CTL-recruitment by DCs are given in Figures 1-2 and Ref. 1.

References

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Figures

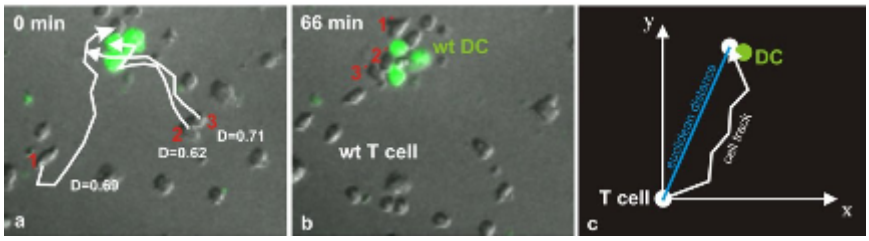

Figure 1

Calculation of directionality of motile CTL Tracks (white lines) of individual motile CTL (unlabeled) are followed (a) until they establish physical contact with a DC (green, a and b). Directionality of migration is calculated by the ratio of euclidean distance and accumulated distance (cell track) with the help of Image J software, i.e. maximal straightness of the cell path corresponds to the value "1" (c).

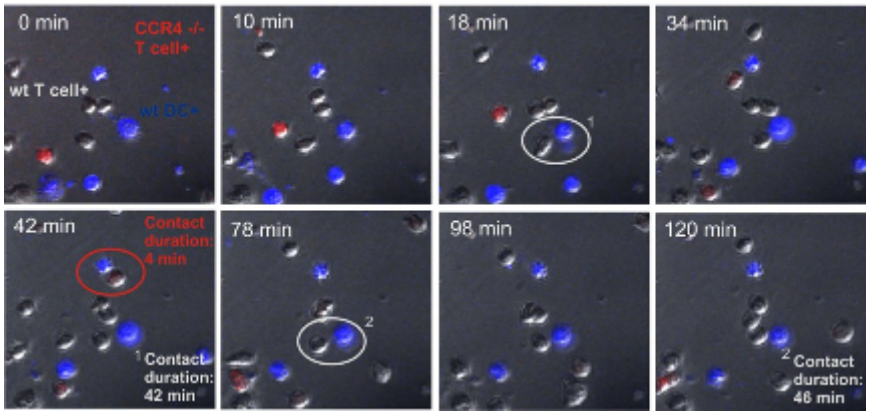

Figure 2

Analysis of CTL recruitment and contact duration time with chemokine-producing DCs Recruitment of motile CTL and physical contact with DCs is recorded by time-lapse videomicroscopy over 2 h (2 min/frame). Contact duration is calculated by using Image J software. The demonstrated example with mixed CTL populations shows that wt-CTL (unlabeled) bear prolonged contact duration with CCL17+ DC (FarRed-labeled, blue) compared to CCR4-/- CTL (CMTMR-labeled, red).