Autocrine stimulation of P2Y1 receptors is part of the purinergic signaling mechanism that regulates T cell activation

Tobias Woehrle, Carola Ledderose, Jessica Rink, Christian Slubowski, and Wolfgang G. Junger

SUPPLEMENTAL INFORMATION

Supplemental Methods

T cell migration

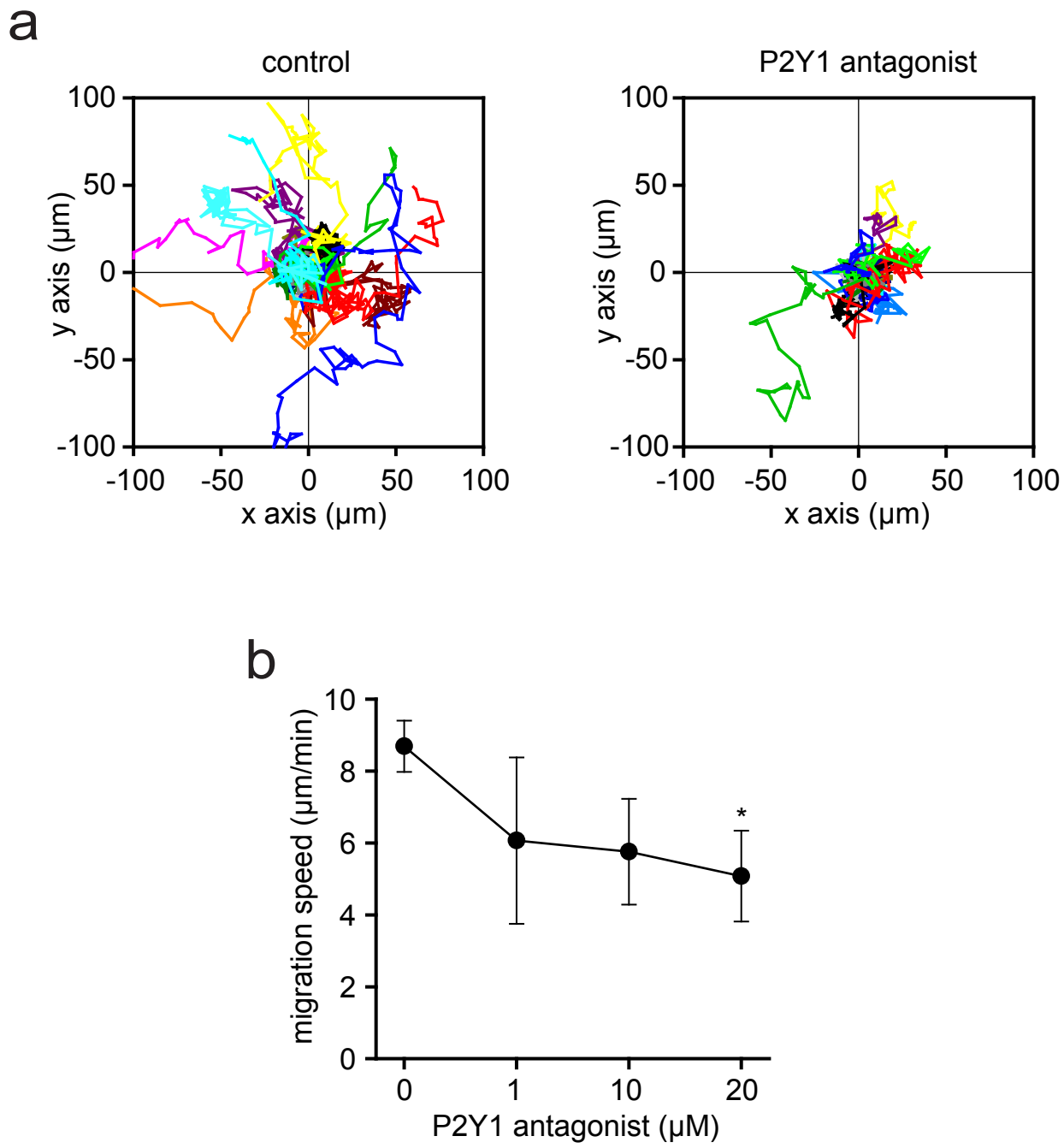
Purified human CD4 T cells were stimulated for 3 days with anti-CD3/CD28 antibody-coated beads to generate effector T cells. These cells were allowed to adhere to fibronectin-coated glass-bottom chamber slides (Lab-Tek, Rochester, NY), reconstituted with fully supplemented tissue culture medium, placed into a temperature-controlled (37°C) stage incubator and maintained in a humidified gas atmosphere containing 5% CO_2 and 21% O_2 (Live Cell Instrument, Seoul, South Korea). Cell migration in the presence or absence of the P2Y1 receptor antagonist MRS2279 was recorded by time-lapse microscopy using a Leica DMI RB inverted microscope and a 20x objective (NA 0.4; Leica, Wetzlar, Germany). Cells were monitored for 30 min and images were captured at 45-second intervals. Migration paths of individual cells were tracked with ImageJ software (NIH; MTrackJ plugin) and the lengths of individual cell tracks were used to calculate migration speeds. At least 20 randomly selected cells were analyzed for each experimental condition to calculate average migration speeds in each individual experiment.

Supplemental video

Video 1 P2Y1 receptor inhibition impairs T cell motility

Human CD4 effector T cells were attached to fibronectin-coated glass bottom dishes, treated or not (control) with the P2Y1 antagonist MRS2279 (20 µM), and migration was monitored with video microscopy. Images were captured at 45-second intervals through a x20 objective (NA 0.4). In the lower panels, superimposed images of the tracks of individual cells are shown.

Supplemental Figure 1



Supplemental Fig. 1 P2Y1 receptors are needed for T cell migration.
a Human CD4 effector T cells were treated or not (control) with the P2Y1 receptor antagonist MRS2279 (20 µM) and migration was observed over a period of 30 min with time-lapse video microscopy. Paths of individual cells alligned with their origins at x=y=0 are shown. Data are representative of 3 independent experiments. **b** Average migration speeds of cells treated as in **a**. Data represent means ± SD of 3 separate experiments comprising at least 20 individually analyzed cells each. *p<0.05 vs. controls without P2Y1 antagonist; Kruskal-Wallis test.