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Supporting Information

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Henriette Rudolph, Armelle Klopstein, Isabelle Gruber, Claudia Blatti, Ruth Lyck and Britta Engelhardt

Postarrest stalling rather than crawling favors $CD8^+$ over $CD4^+$ T-cell migration across the blood–brain barrier under flow in vitro

Supporting Information

Post-arrest stalling rather than crawling favors $CD8^+$ over $CD4^+$ T-cell migration across the blood-brain barrier under flow in vitro

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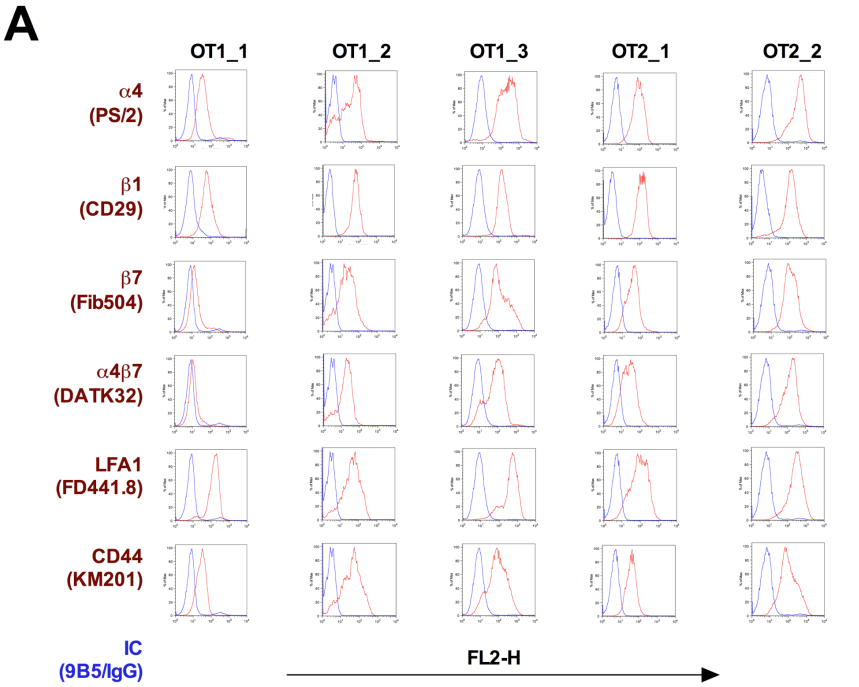
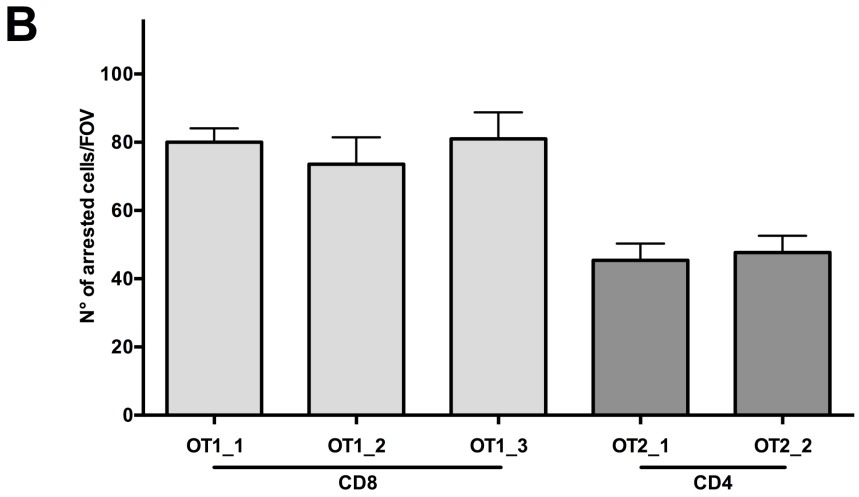
For information regarding the study please contact bengel@tki.unibe.ch

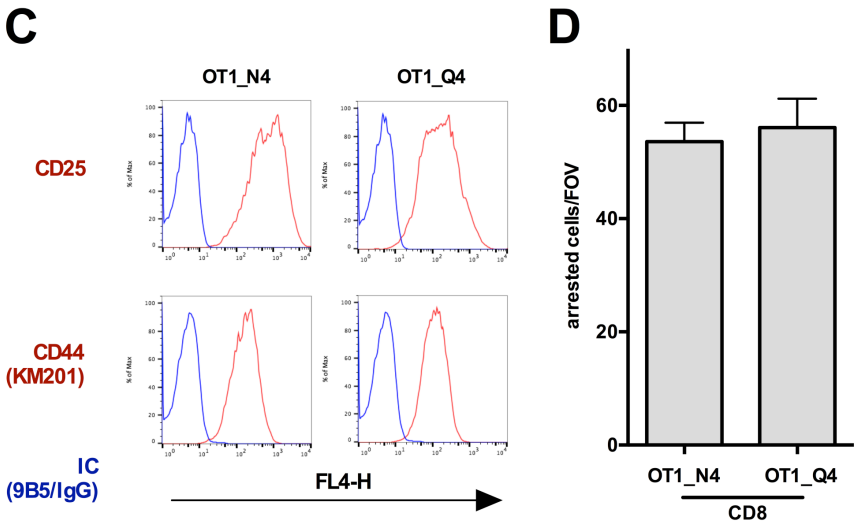
Supporting Information Figure 1: Comparable antigen-specific T-cell proliferation of $CD8^+$ and $CD4^+$ T cells

Proliferation of $CD8^+$ and $CD4^+$ T cells upon stimulation with OVA peptide 257-264 and OVA 323-339 respectively, was assessed via the incorporation of ^{3}H-Thymidine. Data are shown as mean ± SEM. One representative experiment out of 4 is shown with bars showing triplicates assessed with the cell preparations OT1_3 and OT2_2.

Supporting Information Figure 2







Supporting Information Figure 2: Comparable phenotype and shear resistant arrest of different $CD8^+$ and $CD4^+$ T-cell preparations

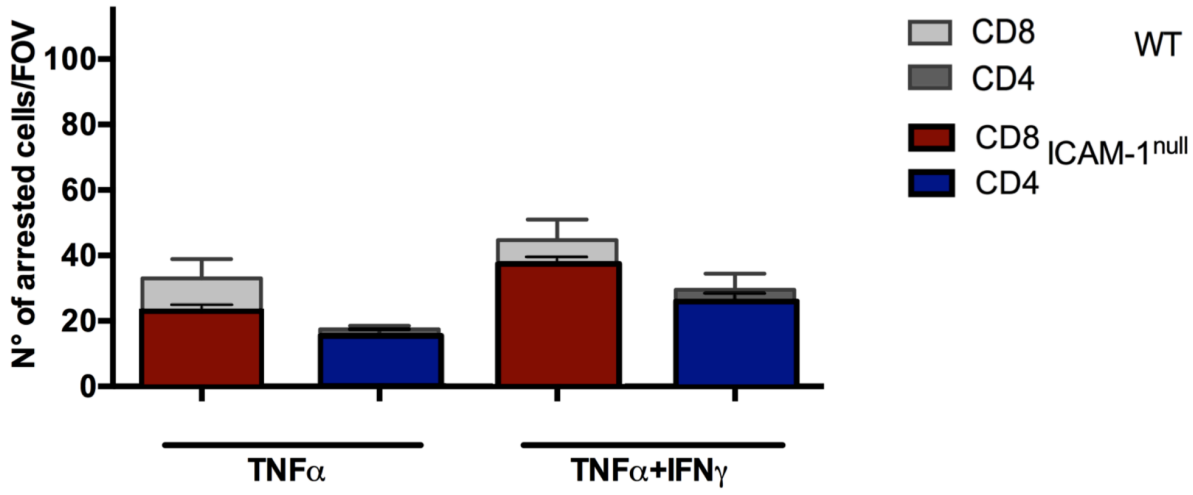
(A) Flow cytometry analysis of cell surface expression of LFA-1 (αL), α4-, β1-, β7- integrin subunits and α4β7-integrin as ligands for ICAM-1, ICAM-2 and VCAM-1, respectively and for CD44 as an activation maker (red lines) for different $CD8^+$ and $CD4^+$ T-cell preparations is shown. Isotype control staining is shown as blue lines. Data are from representative experiments out of five $CD8^+$ and four $CD4^+$ T-cell preparations used for the experiments. Histograms from light scatter gated live cells are shown. (B) Comparison of the shear resistant arrest of 3 different $CD8^+$ and 2 different $CD4^+$ T-cell preparations is shown. OT1 = $CD8^+$ T cells, OT2 = $CD4^+$ T-cell lines. Bars show mean numbers ± SEM of arrested $CD8^+$ or $CD4^+$ T cells per FOV (865.5 µm x 647.7 µm) perfused in a 1:1 ratio on TNF-α+IFN-γ stimulated pMBMECs. (C) Flow cytometry analysis of cell surface staining for CD25 and CD44 as activation markers of $CD8^+$ T cells stimulated with the high affinity OVA peptide N4 (OT1_N4) or the lower affinity peptide Q4 (OT1_Q4) (red lines). Isotype control staining is shown as blue lines. (D) Mean numbers OT1_N4 or OT1_Q4 per FOV (438 µm x 329 µm) perfused in a 1:1 ratio arrested on TNF-α+IFN-γ co-stimulated pMBMECs. Data are shown as mean ± SEM (n=10 experiments). The number of perfused T cells was $0{,}65x10^6$/ml in contrast to $0{,}85x10^6$/ml in the other movie experiments.

Supporting Information Figure 3: Gαi-dependent G-protein coupled receptor (GPCR) signalling is not required for shear resistant arrest and crawling of activated $CD4^+$ and $CD8^+$ T cells on pMBMECs

T cells were pre-treated with PTX for 1 hour in a concentration for 1µg/ml to inhibit Gαi dependent GPCR mediated inside-out integrin activation on their surface. As a control, T cells were pretreated with PTX-B which lacks Gi-inhibitory activity, but confers cell-surface binding specificity. (A) Mean numbers of PTX-pretreated $CD8^+$ versus $CD4^+$ T cells per FOV (438 µm x 329 µm) perfused in a 1:1 ratio arrested on TNF-α+IFN-γ co-stimulated pMBMECs. Data are shown as mean ± SEM (n=3 experiments). (B) Each T cell was assigned to one of 6 categories which are shown in the staple bars

as follows: Graphs to the left: Stalling with diapedesis (black), stalling (dark grey) and stalling with detachment (light grey); Graphs to the right: crawling with diapedesis (black), crawling (dark grey) and crawling with detachment (light grey). The number of arrested T cells per FOV was set to 100% and the 6 categories are displayed in percent as fraction of arrested T cells. Bars show means ± SEM. Differences between crawling PTX-pretreated $CD8^+$/ $CD4^+$ T cells versus crawling PTX-B-pretreated $CD8^+$/ $CD4^+$ T cells and stalling PTX-pretreated $CD8^+$/ $CD4^+$ T cells versus stalling PTX-B-pretreated $CD8^+$/ $CD4^+$ T cells were significant ($p=0,02$ for $CD8^+$ T cells /$p=0,0032$ for $CD4^+$ T cells). (C) Percentage of overall $CD4^+$ and $CD8^+$ T-cell diapedesis irrespective of prior crawling or stalling is shown. One-way ANOVA, followed by the Tukey multiple-comparison test. The diapedesis rates of crawling and stalling $CD4^+$ versus $CD8^+$ T cells pretreated with PTX or PTX-B did not differ significantly.

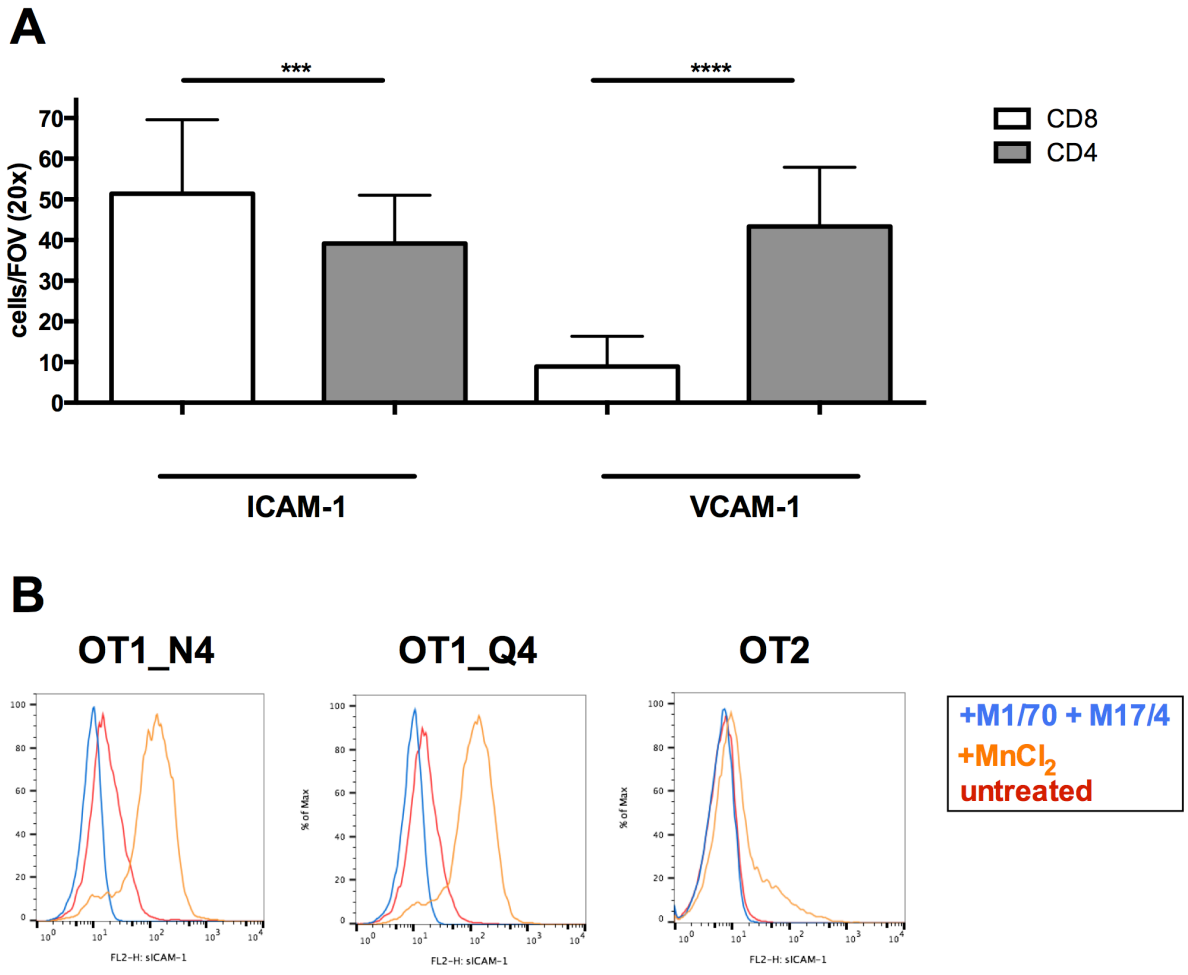
Supporting Information Figure 4



Supporting Information Figure 4: Involvement of endothelial ICAM-1 in increased shear resistant arrest of $CD8^+$ T cells compared to $CD4^+$ T cells on pMBMECs

Mean numbers of arrested $CD8^+$ T cells (red bars) and $CD4^+$ T cells (blue bars) per FOV on ICAM-1^{null} pMBMECs perfused in a 1:1 ratio are shown on TNF-α-stimulated (TNF-α) and TNF-α and IFN-γ co-stimulated (TNF-α+IFN-γ) ICAM-1^{null} pMBMECs. The respective T-cell arrest on WT pMBMECs (WT) always compared within the same assay is shown in the background in greyscale. Data are shown as mean ± SEM (n=11 for TNF-α, n=18 for TNF-α+IFN-γ). One-way ANOVA, followed by the Tukey multiple-comparison test. The number of perfused T cells on pMBMEC monolayers was $0{,}65x10^6$/ml in contrast to $0{,}85x10^6$/ml in the other movie experiments.

Supporting Information Figure 5



Supporting Information Figure 5: Binding of activated CD8$^+$ and CD4$^+$ T cells to immobilized ICAM-1 and VCAM-1 and soluble ICAM-1

(A) Bars show numbers ± SD of activated CD8$^+$ and CD4$^+$ T cells arrested on recombinant mouse ICAM-1 and VCAM-1 under static conditions assessed with the following T-cell lines: OT1_1, OT1_2 and OT2_1. ****p*= 0.0001; *****p*< 0.0001; One-way ANOVA, followed by the Tukey multiple-comparison test. (B) Flow cytometry analysis showing binding of soluble ICAM-1 on OT1_N4, OT1_Q4 and OT2 T cells. Untreated T cells are shown in red, T cells pretreated with the β2-integrin function blocking antibodies M1/70 and M17/4 are shown in blue (negative control) and T cells pretreated with $MnCl_2$ (positive control) are shown in orange.

Supporting Information video 1: Number of arrested T cells which are stalling or start crawling on NS wt pMBMEC.

Representative time-lapse movie that shows the interaction of activated $CD8^+$ and $CD4^+$ T cells on NS pMBMECs under flow over 20 minutes. Images were taken every 10 seconds and the movie is composed at 6 frames per second (fps). Arrows indicate direction of shear flow (original magnification 20x). The upper time displays the time after start of the movie whereas the lower displays the time of movie acquisition with the enhanced shear. The movie corresponds to experiments analyzed in Figures 1, 2 and 3. Fig. 1B shows one frame of this movie. $CD8^+$ T cells are labeled in green and $CD4^+$ T cells are labeled in red. Arrested T cells can be seen to stall or to start crawling.

Supporting Information video 2: Number of arrested T cells which are stalling or start crawling on wt pMBMEC stimulated with TNF-α.

Representative time-lapse movie that shows the interaction of activated $CD8^+$ and $CD4^+$ T cells on TNF-α stimulated pMBMECs. Arrows indicate direction of shear flow (original magnification 20x). The upper time displays the time after start of the movie whereas the lower displays the time of movie acquisition with the enhanced shear. Video acquisition was performed as described for Video 1. The movie corresponds to experiments analyzed in Figures 1, 2 and 3. Fig. 1B shows one frame of this movie. $CD8^+$ T cells are labeled in green and $CD4^+$ T cells are labeled in red. Arrested T cells either started to crawl or stalled. Diapedesis events can be seen when cells on top of the pMBMEC monolayer (visible as phase-contrast bright) change to below the monolayer (visible as phase-contrast dark). $CD8^+$ T-cell diapedesis can be observed in the lower right segment of the movie starting at minute 06:44 and is accomplished until minute 10:44 during acquisition of the movie. Quantification of the different T-cell behavioral categories is shown in Fig. 2B. Cell death of an endothelial cell can be observed but was only observed very rare during the live-time movies in the condition wt pMBMEC stimulated with TNF-α.

Supporting Information video 3 Number of arrested T cells which are stalling or start crawling on wt pMBMEC stimulated with TNF-α+IFN-γ.

Representative time-lapse movie that shows the interaction of activated $CD8^+$ and $CD4^+$ T cells on TNF-α+IFN-γ stimulated pMBMECs. Arrows indicate direction of shear flow (original magnification

20x). The upper time displays the time after start of the movie whereas the lower displays the time of movie acquisition with the enhanced shear. Video acquisition and analysis were performed as described for Videos 1 and 2. The following 6 categories were distinguished: i) Stalling T cells detaching during the observation period (dark blue), ii) T cells remaining stalled (green) and iii) stalling T cells that crossed the pMBMEC monolayer in the observation period (red), iv) T cells which crawled and detached (light blue), v) T cells which crawled for the entire observation period and finally (pink) vi) T cells which crossed the pMBMEC monolayer after crawling (yellow). The sequential appearance of the individual tracks is as follows: red (center, left), light blue (top right), pink (center), dark blue (center lower part), yellow (center) and green (top right). The movie corresponds to experiments analyzed in Figures 1, 2 and 3. Fig. 1B shows one frame of this movie. $CD8^+$ T cells are labeled in red and $CD4^+$ T cells are labeled in green.

Supporting information video 4: Paracellular diapedesis of activated $CD8^+$ T cells across the inflamed BBB. Representative time-lapse movie showing a paracellular diapedesis event of an activated $CD8^+$ T cell on pMBMECs stimulated with TNF-α+IFN-γ. Arrow indicates the site of diapedesis that correlates with a break of the GFP signal. Close-up of a 63x magnification with glycerol immersion. scale bar = 5µm.

Supporting information video 5: Transcellular diapedesis of activated $CD8^+$ T cells across the inflamed BBB. Representative time-lapse movie showing a transcellular diapedesis of an activated $CD8^+$ T cell on inflamed BBB stimulated by TNF-α+IFN-γ. Arrow indicated the site of diapedesis. Note that the GFP signal stays intact. Close-up of a 63x magnification with glycerol immersion. scale bar = 5µm.

Supporting Information video 6: Number of arrested T cells which are stalling or start crawling on NS ICAM-1^{null}/ICAM-$2^{-/-}$ pMBMECs.

Representative time-lapse movie that shows the interaction of activated $CD8^+$ and $CD4^+$ T cells on NS pMBMECs. Arrows indicate direction of shear flow (original magnification 20x). The upper time displays the time after start of the movie whereas the lower displays the time of movie acquisition with the enhanced shear. Video acquisition and analysis were performed as described for Videos 1 and 2.

The movie corresponds to experiments analyzed in Fig. 6. Fig. 6B is a preview of this movie. $CD8^+$ T cells are labeled in red and $CD4^+$ T cells are labeled in green.

Supporting Information video 7: Number of arrested T cells which are stalling or start crawling on ICAM-1^{null}/ICAM-$2^{-/-}$ pMBMEC stimulated with TNF-α.

Representative time-lapse movie that shows the interaction of activated $CD8^+$ and $CD4^+$ T cells on TNF-α stimulated pMBMECs. Arrows indicate direction of shear flow (original magnification 20x). The upper time displays the time after start of the movie whereas the lower displays the time of movie acquisition with the enhanced shear. Video acquisition and analysis were performed as described for Videos 1 and 2. The movie corresponds to experiments analyzed in Fig. 6. Fig. 6B is a preview of this movie. $CD8^+$ T cells are labeled in green and $CD4^+$ T cells are labeled in red.

Supporting Information video 8: Number of arrested T cells which are stalling or start crawling on ICAM-1^{null}/ICAM-$2^{-/-}$ pMBMEC stimulated with TNF-α+IFN-γ.

Representative time-lapse movie that shows the interaction of activated $CD8^+$ and $CD4^+$ T cells on TNF-α+IFN-γ stimulated pMBMECs. Arrows indicate direction of shear flow (original magnification 20x). The upper time displays the time after start of the movie whereas the lower displays the time of movie acquisition with the enhanced shear. Video acquisition and analysis were performed as described for Videos 1 and 2. The movie corresponds to experiments analyzed in Fig. 6. Fig. 6B is a preview of this movie. $CD8^+$ T cells are labeled in green and $CD4^+$ T cells are labeled in red.

Supporting Information Table 1: Adhesion molecule phenotype of different CD(and CD4 T cell preparations - Mean fluorescent intensities

	OT1_1	OT1_4	OT1_7	OT2_1	OT2_3
α4 (PS/2)	8.8; 36.8	2.83; 19.3	8.13; 190	4.71; 80.1	6.82; 321
β1 (CD29)	8.27; 60.5	2.22; 66.1	17; 157	2.95; 126	3.92; 121
β7 (Fib 504)	8.8; 15.6	2.83; 18.8	8.13; 123	4.71; 36.2	6.82; 112
α4β7 (DATK32)	8.8; 11.4	2.83; 14.6	8.13; 65.7	4.71; 23.6	6.82; 132
LFA1 (FD441.8)	8.8; 135	2.83; 38.6	8.13; 370	4.71; 83.6	6.82; 272
CD44 (KM201)	8.8; 27.4	2.83; 37.3	8.13; 83,7	4.71; 38.4	6.82; 107
IC (9B5/ IgG)					

Mean Flourescence intensity (MFI) values of flow cytometry analysis of cell surface expression of αL-, α4-, β1-, β7- integrin subunits and α4β7-integrin as ligands for ICAM-1, ICAM-2 and VCAM-1 respectively and for CD44 as an activation maker (red values) for different $CD8^+$ and $CD4^+$ T cell preparations are shown. MFI of the isotype control staining is shown as blue values. Data show of MFIs representative experiments out of a total of five $CD8^+$ and four $CD4^+$ T cell preparations used for the experiments. For further information please see also Supporting Information Fig.2.