Supplementary Material:

Videos 1A to 4A: Initial contact of human T cells with the inflamed spinal cord microvasculature

Each movie shows at the beginning the inflamed spinal cord microvasculature as visualized by TRITC-dextran in the circulation of a SJL mouse (Videos 1A and 2A: during EAE, Videos 3A and 4A: 4 hours after TNF-α injection) within one field of view. Above the large spinal cord collecting vein, always seen at the bottom of each video frame, the spinal cord white matter microvasculature is visualized. After switching the fluorescence filter at the microscope, infusion of 1 aliquot (1.3 $\times 10^6$ cells/100µL) calcein-AM labeled human T cells is started and T cells can be observed either passing through the corresponding vascular beds or interacting and firmly adhering to the microvasculature in real time (objective x10). Each of the videos shows the real time observation of the initial interaction of human T cells with the inflamed spinal cord microvessels by rolling or capturing during the infusion of one aliquot of T cells (1.3 $\times 10^6$ cells/100µL) via the right carotid artery within one field of view. Circles are drawn to highlight examples of T cells rolling or capturing in each video.

Videos 1B to 4B: Adhesion of human T cells to the inflamed spinal cord microvasculature

Each movie (Videos 1B and 2B: during EAE, Videos 3B and 4B: 4 hours after TNF-α injection) shows the scanning of several fields of view within the entire spinal cord window 10 minutes after infusion of the total amount of T cells (4×10^6 cells) to quantify those T cells that firmly adhere to the spinal cord microvasculature.

Video 1A: Initial contact of human T cells with the spinal cord microvasculature during EAE – control condition (human IgG4):

The inflamed spinal cord white matter microvasculature within one field of view as visualized by TRITC-dextran is shown (time 05:18:14.18 to 05:18:17.42). Infusion of one aliquot of fluorescent T cells (time: 05:18:25:42 until the end) follows. A T cell capturing, i.e. abruptly stopping in a post-capillary venule wall is seen at the top left within the white circle (at 11 o’clock) at time 05:18:29.06. Starting at time 05:18:31.06 several human T cells can be observed to roll along the vascular wall within the postcapillary venule located in the center of the white circle.

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Video 1B: Firmly adherent human T cells with the spinal cord microvasculature during EAE – control condition (human IgG4):

Scanning of the spinal cord window 10 minutes after T cell infusion is shown from time 05:33:03:51 to the end.

Video 2A: Initial contact of natalizumab-pretreated human T cells with the spinal cord microvasculature during EAE:

The inflamed spinal cord white matter microvasculature within one field of view as visualized by TRITC-dextran is shown (time 03:50:23:02 until 03:50:26:62). Infusion of one aliquot of fluorescent T cells (time: 03:50:36:18 until the end) follows. At time 03:50:48.47 one T cell is captured to the vascular wall as seen in the center of the right white circle. Within the same blood vessel a second T cell follows rolling along the vascular wall and briefly arrests at the same spot (03:50:54.59). At 03:50:59.59 both T cells continue to roll along the vascular wall. Another T cell rolling along the microvascular wall can be observed within the left white circle starting at time 03:50:48.83. T cells firmly adhering remain visible within the spinal cord microvessels.

Video 2B: Firm adhesion of natalizumab-pretreated human T cells in the spinal cord microvasculature during EAE:

Scanning of the spinal cord window 10 minutes after T cell infusion is shown from time 04:04:50:28 to the end. The number of Natalizumab-pretreated T cells found to firmly adhere to the inflamed spinal cord microvasculature during EAE is visibly lower when compared to the number of human T cells found to firmly adhere to the spinal cord microvascular wall in the presence of control antibody (see Video 1B from time 05:33:03:51 until the end).

Video 3A: Initial contact of human T cells with the TNF-α stimulated spinal cord microvasculature – control condition (human IgG4):

The inflamed spinal cord white matter microvasculature within one field of view as visualized by TRITC-dextran is shown (time 05:12:11:56 until 05:12:12:80). Infusion of one aliquot of fluorescent T cells (time: 05:12:22:00 until the end) follows. At time 05:12:23.49 (lower white circle), one cell captures - abruptly stops – at the post-capillary vessel wall right behind a T cell already adhering to the vascular wall. Two T cells rolling along the vascular wall can be observed starting at time

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05:12:27.01 and 05:12:34.89 within the upper white circled area, while other T cells pass through this vessel.

Video 3B: Firm adhesion of human T cells to the TNF-α stimulated spinal cord microvasculature – control condition (human IgG4):

Scanning of the spinal cord window 10 minutes after T cell infusion is shown from time 05:25:30.19 to the end.

Video 4A: Initial contact of natalizumab-pretreated human T cells with the TNF-α stimulated spinal cord microvasculature:

The inflamed spinal cord white matter microvasculature within one field of view as visualized by TRITC-dextran is shown (time 01:27:32:76 until 01:27:37:24). Infusion of one aliquot of fluorescent T cells (time: 01:27:45:12 until the end) follows. At time 01:27:49.37 a T cell that first captures and then rolls along the microvascular wall can be seen in the middle of the white circle. At time 01:27:59.53, within the left circle, a single T cell can be observed rolling along the microvascular wall.

Video 4B: Firm adhesion of natalizumab-pretreated human T cells to the TNF-α stimulated spinal cord microvasculature:

Scanning of the spinal cord window 10 minutes after T cell infusion is shown from time 1:45:00.60 to the end. The number of Natalizumab-pretreated T cells found to firmly adhere to the TNF-α stimulated spinal cord microvascular wall can be seen to be lower, when directly compared to the number of human T cells found to firmly adhere to the TNF-α stimulated spinal cord microvessels under control conditions (Video 3B from time 05:25:30:19 until the end).

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Supplementary methods for intravital microscopic image analysis

Hemodynamic parameters

The hemodynamic parameters of the blood flow were evaluated for each analysed post-capillary venule based on the Hagen-Poiseuille law on laminar flow through cylindrical tubes as follows: the mean blood flow velocity (V_{blood}) represents $V_{blood} = V_{max}/2\text{-}(D_L/D_V)^2)$ (μm/s), where V_{max} is the velocity of the fastest non-interacting DC. D_L represents the DC diameter and D_V the vessel diameter (1, 2).

To quantify the force acting on rolling or adherent leukocytes, the wall shear rate (γ) was evaluated for each venule as $\gamma = V_{blood} \times 8/D_V$ (s^{-1}). The wall shear stress (τ), dependent on blood viscosity (assumed to be 0.0025 poise) was calculated as: $\tau = \gamma \times 0.0025$ ($dyne.cm^{-2}$) (2).

Quantitative analysis of the initial contact of T cells within postcapillary venules (∅ 20-60 μm) of the spinal cord in mice with EAE

This analysis was performed exactly as described before (3). The total number of T cells passing through a given postcapillary venule was recorded for 1 minute and noted as total cellular flux (TF). Next the number of human T cells initiating contact with the vascular wall by rolling or capturing was determined and noted as initiating contact flux (IF). The fraction of T cells initiating contact within a postcapillary venule in the given obervation time is then calculated as follows: IF/TF x 100 % and thus represents the percentage of T cells initiating contact with the vascular wall from all T cells passing through this blood vessel within 1 minute.

Quantitative analysis of firm adhesion

This analysis was performed exactly as described before (3). Permanently adherent T cells were defined as cells that stuck to the vessel wall without moving or detaching from the endothelium for a period of at least 20 seconds. Permanent T cell adhesion at 10 minutes, 30 minutes, 1 hour, and 2 hours after cell injection were expressed as number of adherent T cells per field of view (FOV) using the ×10 objective. Per mouse, 4-6 FOV can be defined within the spinal cord window. The numbers of firmly adherent T cells per FOV from different mice were pooled to generate a mean ± standard deviation.

1. Ley, K., and P. Gaehtgens. 1991. Endothelial, not hemodynamic, differences are responsible for preferential leukocyte rolling in rat mesenteric venules. *Circ Res* 69:1034-1041.
2. Von Andrian, U. H., P. Hansell, J. D. Chambers, E. M. Berger, I. Torres Filho, E. C. Butcher, and K. E. Arfors. 1992. L-selectin function is required for beta 2-integrin-mediated neutrophil adhesion at physiological shear rates in vivo. *American Journal of Physiology* 263:H1034-1044.

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3. Bauer, M., C. Brakebusch, C. Coisne, M. Sixt, H. Wekerle, B. Engelhardt, and R. Fassler. 2009. {beta}1 integrins differentially control extravasation of inflammatory cell subsets into the CNS during autoimmunity. *Proc Natl Acad Sci U S A* 106:1920-1925.

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Supplementary Figures:

Supplementary Figure 1: **Human and mouse VCAM-1 bind equally well and specifically to α4-integrins on human T cells *in vitro***

A: Binding of human soluble recombinant VCAM-1/Fc fusion proteins to human Jurkat T cells sorted for high α4-integrin expression was determined by FACS analysis. Geometrical means of fluorescence intensity obtained after incubation with increasing concentrations of VCAM-1-Fc fusion proteins is shown. Binding of the human VCAM-1-Fc fusion protein to Jurkat cells was mediated by α4-integrins as demonstrated by complete inhibition of binding by the anti-human α4-integrin antibody 21/6, but not by antibodies directed against CD3 or CD18. **B:** Binding mouse soluble recombinant VCAM-1/Fc fusion proteins to human Jurkat T cells was determined by FACS analysis. Geometrical means of fluorescence intensity obtained after incubation with increasing concentrations of VCAM-1-Fc fusion proteins is shown. Binding of the mouse VCAM-1-Fc fusion protein to Jurkat cells was mediated by α4-integrins as demonstrated by complete inhibition of binding by the anti-human α4-integrin antibody 21/6, but not by antibodies directed against CD3 or CD18.

Supplementary Figure 2: α4-integrin suface expression of human peripheral blood T cells

Cell surface expression of α4-integrins as regularly observed on purified human peripheral blood T cells by Natalizumab is shown. T cells were scatter gated, overlay compares suface expression of α4-integrin as detected by Natalizumab (pink line) versus control staining with human IgG4 (light blue line) versus unstained T cells (black line).

Supplementary Figure 3: Microhemodynamic parameters in the spinal cord post capillary venules in SJL mice during EAE and after injection of TNF–α. For EAE 18 post-capillary venules in 6 mice and 19 post-capillary venules in 6 mice were analysed for IgG4 and Natalizumab conditions, respectively. For TNF-α conditions 20 post-capillary venules in 6 mice were analysed for both IgG4 and Natalizumab conditions. **a** : Microvessel diameters. **b** : Evaluation of the mean blood flow velocity (μm/s). **c** : The wall shear rate (s^{-1}). **d** : The wall shear stress (dyne/cm $^{-2}$).

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