cells

MDPI

Supplementary Materials

The Posttraumatic Increase of the Adhesion GPCR EMR2/ *ADGRE2* on Circulating Neutrophils Is Not Related to Injury Severity

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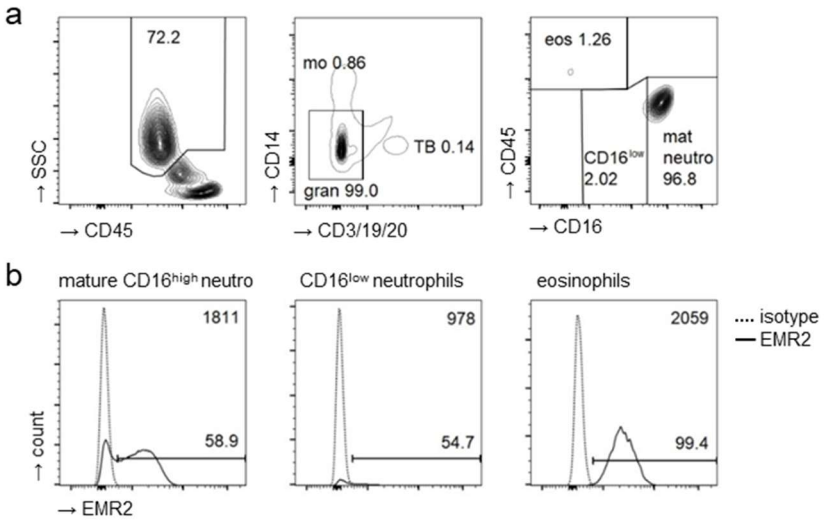

Figure S1. Flow cytometric analysis of EMR2 on peripheral leukocytes
a, b) Analysis of CD16^{high} and CD16^{low} neutrophils of an injured patient (ISS 32) 24 h after trauma. a) Main gating strategy for neutrophilic granulocytes (percentage of positive cells of all cells); mo monocytes, gran granulocytes, TB T- and B-cells, mat neutro mature neutrophils, eos eosinophils; b): Median fluorescence intensity (MFI; right upper corner) of isotype control- and EMR2 mAb-stained cells; the percentage of EMR2^{+} cells is indicated at the gate.

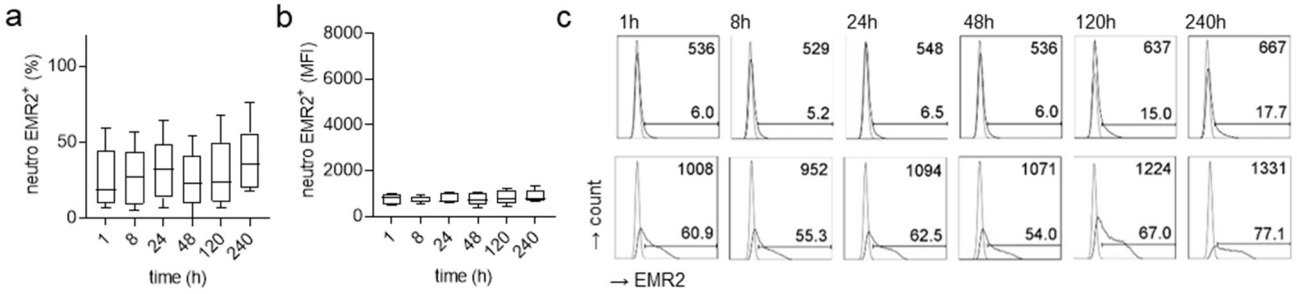

Figure S2. 240 h time course of EMR2 on circulating neutrophils of uninjured volunteers
(**a**) Percentage of EMR2⁺ neutrophils (a) and MFI of EMR2 on neutrophils (**b**), n= 5 volunteers.
(**c**) Time course of EMR2 on neutrophils in one typical volunteer (upper panel). In the lower panel a volunteer with a constantly high percentage of EMR2⁺ neutrophils is shown. The percentage of EMR2⁺ cells is indicated at the gate, MFI of EMR2⁺ cells is shown in the upper right corner.

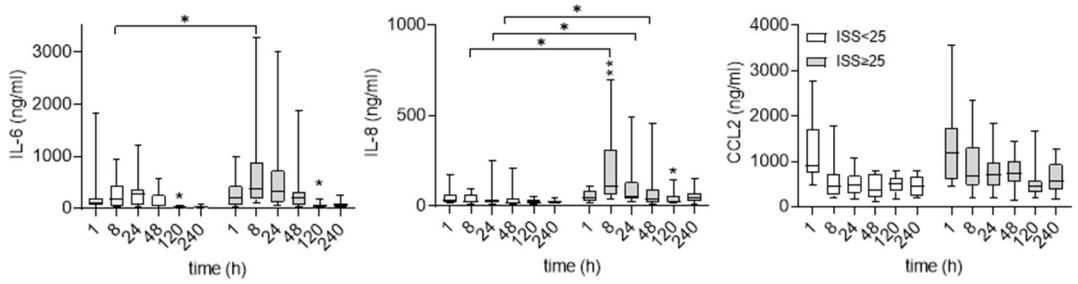

Figure S3. Posttraumatic time course of circulating cytokines
Comparison between patient groups ISS<25 (9-24) and ISS≥25, t-test (IL-6), U-test (IL-8, CCL2); comparison between consecutive time points in one patient group: ANOVA, only significant changes related to the previous time point are shown; *p<0.05).

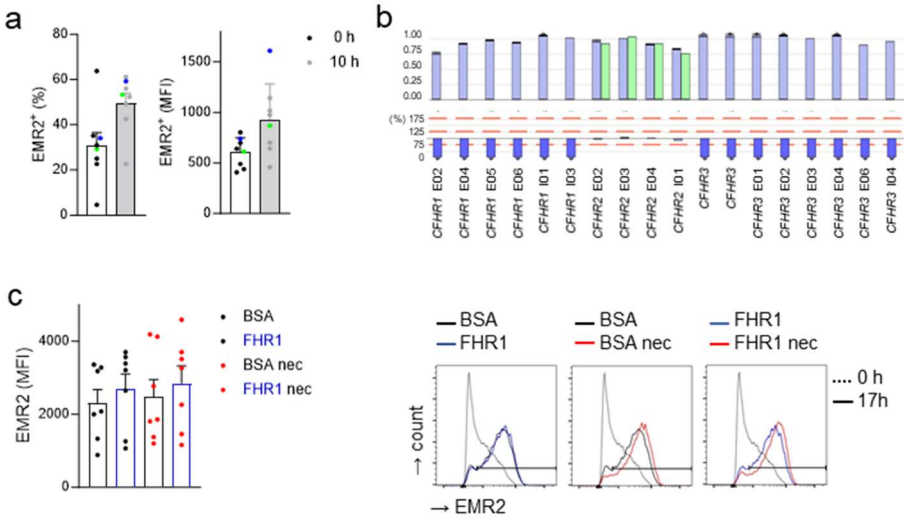

Figure. S4 FHR1 is not involved in the increase of EMR2 on neutrophils *in vitro*
(**a, b**) Leukocytes were cultured in RPMI1640/10% serum of the respective cell donor. EMR2 on neutrophils was quantified (**a**) before (0 h) and after 10 h by flow cytometry (n= 8 volunteers). 2 out of the 8 (2/8) volunteers, indicated by the colored dots in the graphs, were *CFHR1*-deficient, but they showed also a neutrophilic EMR2 increase; (**b**) MLPA analysis of the *CFHR1-3* target region. Upper trace: grey = mean value of 3 volunteers without, green = one volunteer with homozygous *CHFR1*/*CFHR3* deletion. Values between 0.75-1.25 display copy numbers of 2, as seen for the region *CFHR2*, while *CFHR1* and *CFHR3* revealed no signal for the target region, which means homozygous deletion. Lower trace: ratio between volunteers with/without deletion (%); 100 % means no difference between both. (**c**) Leukocytes were cultured either in BSA- or FHR1-coated wells with or without necrotic cells for 17 h. Afterwards the cells were stained and the expression of EMR2 on neutrophils was quantified by flow cytometry (n=7 donors). Right: One typical experiment of one donor is shown.

Table S1 Trauma classification based on the abbreviated injury scale (AIS)

Trauma group	Injuries	ISS<25 (n=9)	ISS≥25 (n=25)	p-value#
Neurotrauma	Traumatic brain injury	1	12	0.107
	Spinal cord injury	0	4	0.554
Facial trauma	Eye injury	1	3	1.000
	Fractured face	1	7	0.403
	Open wound face	3	1	0.048
Neck trauma	Esophageal trauma	0	1	1.000
	Tracheal trauma	0	1	1.000
Thoracic trauma	Internal organs injury	2	20	0.004
	Other injuries	4	17	0.254
Abdominal trauma	Internal organs injury	2	12	0.250
	Other injuries	0	6	0.162
Spinal trauma	Fracture	4	12	1.000
	Luxation/distortion	0	1	1.000
Upper extremities trauma	Fracture	4	11	1.000
	Luxation/distortion	1	1	0.465
Pelvic trauma	Fracture	2	16	0.052
Lower extremities trauma	Fracture	7	12	0.240
	Luxation/distortion	1	4	1.000

#Fisher's exact test

Table S2 Antibodies (Abs) and dyes used in flow cytometry

antigen, fluorophore	**clone**	**company, catalog number**
Abs for the clinical study		
CD14 BUV395	MφP9	Becton Dickinson GmbH (BD, Heidelberg, Germany) 563561
CD16 BUV737	3G8	BD, 612786
CD45 BB515	HI30	BD, 564585
EMR2 APC	2A1	Bio-Rad Lab GmbH (Feldkirchen, Germany), MCA2330A647T
CD3 BV510*	UCHT1	BD, 563109
CD19 BV510*	SJ25C1	BD, 562947
CD20 BV510*	2H7	BD, 563067
Abs and dyes additionally used for neutrophilic EMR2 analyses *in vitro*		
CD62L PE#	REA615	Miltenyi Biotec GmbH (Bergisch Gladbach, Germany) 130-114-151
CD11b Vio Bright B515#	REA713	Miltenyi, 130-131-818
CD11c PE-Cy7	B-Ly6	BD, 561356
EMR2 PE#	REA302	Miltenyi, 130-119-770
Fixable Viability Dye BV605		BD, 565694

*BV510 dump channel: CD3, CD19 and CD20 identify T and B cells, which are not of interest in this study; only $CD45^+$ $CD3/19/20^-$ cells are carried forward for analyses.