Peripheral blood leukocyte isolation
Blood was collected using EDTA-coated tubes (Vacuette, Grenier Bio-One, Austria). Polymorphonuclear leukocytes (PMNs) and peripheral blood mononuclear cells (PBMCs) were isolated by 1077/1119 histopaque double-gradient density centrifugation (Histopaque; Sigma-Aldrich, St. Louis, MO). For all experiments, the cell purity and viability were assayed by morphological evaluation of May-Grünwald-Giemsa–stained cytospin preparations (>98% pure) and trypan blue exclusion (>97% viable), respectively. The platelet contamination was <2 platelets/100 neutrophils. Moreover, no platelets adhering to neutrophils were found in the cytospins. Endotoxin contamination of all materials not assayed by their respective manufacturers was assessed by the Limulus amebocyte assay (Sigma-Aldrich).

Serum and plasma preparations
Blood samples collected into polypropylene tubes were stored at room temperature for 20 min to allow clotting to take place. Serum was isolated by centrifugation at 1400g for 15 min. Plasma was isolated from EDTA-collected blood by centrifugation at 1400g for 15 min at 4˚C.

RNA extraction, reverse transcription, and relative quantitative real-time PCR analysis
Total RNA was isolated from double gradient-purified PMNs and PBMCs with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was synthesized from 1 ug of isolated RNA using Superscript III reverse transcriptase (Invitrogen). To quantify the relative expression of a full-length isoform of TF (TF) and alternative spliced isoform (asTF), isoform-specific real-time PCR was performed. For each sample, TF, asTF, and GAPDH mRNA sequence-specific primers and probes were applied as described.[1] The 2^{-DDCT} method was used to quantify the target gene expression.[1]

Western blot analysis
Cell lysates were prepared from ~1×10^6 cells using a lysis buffer containing 1% Triton-X in 150 mM NaCl / 20 mM HEPES, pH 7.5, with protease inhibitors (Complete Protease Inhibitor Tablets, Roche, Indianapolis, IN, USA) and stored at −20˚C. Western blot analysis was performed as previously described.[1]

Flow cytometry analysis of peripheral blood leukocytes
The intracellular TF expression was evaluated in gradient-centrifugation isolated PMNs as described.[2] The TF expression in gradient centrifugation-isolated PBMCs was assayed on the surface of these cells using a FITC-conjugated TF Mab (Cat No. 4508CJ, American Diagnostica). A mouse IgG_1-FITC was used as an isotype control (Cat. No. 3458155; BD Biosciences, San Jose, CA, USA). PBMCs were gated on the basis of CD14 expression and scatter properties. For the *ex vivo* hemodialysis simulation experiments, the surface expression of CD11b in leukocytes was evaluated using PE-conjugated human CD11b mAb (Cat No. 555388; BD Biosciences) in whole blood preparations. After incubation with CD11b mAb or isotype control IgG (Cat No. 555749; BD Biosciences), red blood cells were lysed (BD FACS lysing solution, Cat No. 349202, BD Biosciences) and the whole-cell preparation was washed with PBS/1%BSA/0.02%NaN_3. The expression of C5aRs was analyzed either on the surface of leukocytes or in permeabilized cells after incubation with C5aR mAb (Cat No. 550494; BD Biosciences). IgG (Cat No 555749; BD Biosciences) served as an isotype control. To prevent any internalization of C5a receptors blood was collected in tubes prefilled with sodium azide

(0.02% final concentration). The stained cell suspensions were analyzed by flow cytometry (BD-FACS Canto II, BD Biosciences). PMNs were gated on the basis of their scatter properties. The amounts of CD11b, TF and C5aR in the PMNs were expressed as ratios of the mean fluorescence intensities of CD11b, TF and C5aR to their respective isotype controls.

Cytokine measurements

The Bio-Plex Pro Human Cytokine 27-plex Assay (Cat No. M50-0KCAF0Y; Biorad Laboratories, Inc., Hercules, CA, USA) was used, according to the manufacturer's instructions to measure: IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, basic FGF, Eotaxin, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1 (MCAF), MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF, and VEGF.

REFERENCES

1. Ritis K, Doumas M, Mastellos D et al. A novel C5a receptor-tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways. J.Immunol. 2006;177: 4794–4802.
2. Kambas K, Markiewski MM, Pneumatikos IA et al. C5a and TNF-alpha up regulate the expression of tissue factor in intra-alveolar neutrophils of patients with the acute respiratory distress syndrome. J.Immunol. 2008;180:7368–7375.

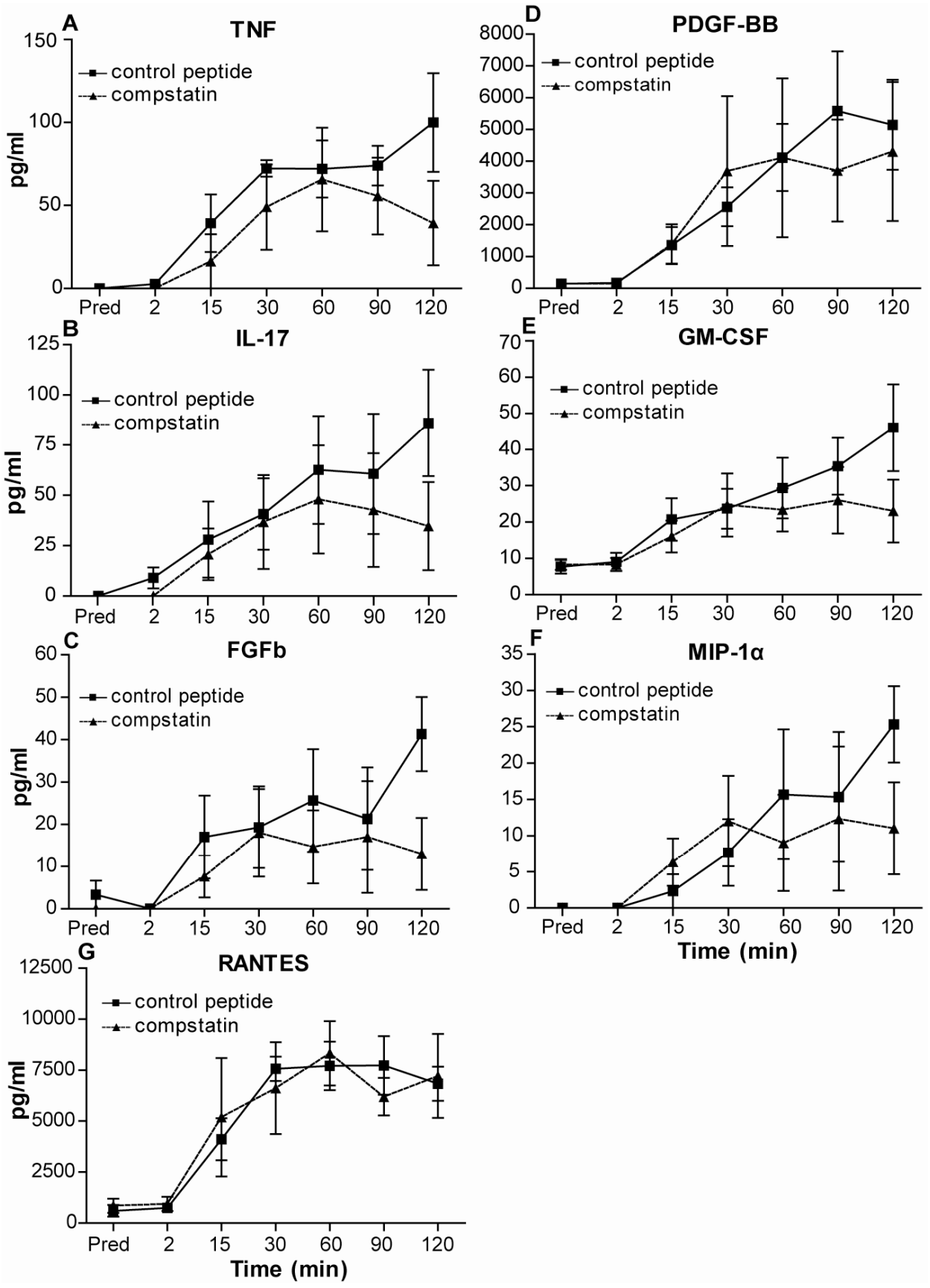
A
TNF
control peptide
compstatin
pg/ml
150
100
50
0
Pred
2
15
30
60
90
120
B
IL-17
125
100
75
50
25
C
FGFb
60
40
30
20
10
D
PDGF-BB
8000
7000
6000
5000
4000
3000
2000
1000
E
GM-CSF
70
F
MIP-1α
35
25
5
G
RANTES
12500
10000
7500
2500
Time (min)

Figure S1. Hemodialysis simulation increases the amounts of several cytokines in blood
The amounts of (A) TNF, (B) IL-17, (C) FGFb, (D) PDGF-BB, (E) GM-CSF, (F) MIP-1α, and (G) RANTES in blood during the recirculation through the hemodialysis circuit in the presence of compstatin analogues (▲) or the control peptide (■). Data are representative of three independent experiments (mean ± SEM). The two-way ANOVA test was used to assess the statistical significance over time. (A) $P = .0005$, (B) $P = .0237$, (C) $P = .0302$, (D) $P < .0001$, (E) $P = .0021$, (F) $P = .0179$, (G): $P < .0001$. However, the differences between the levels of cytokines in blood treated with compstatin or control did not reach a statistical significance.