Supplemental Data

α-Linoleic Acid Enhances the Capacity of α1-Antitrypsin to Inhibit Lipopolysaccharide-Induced IL-1β in Human Blood Neutrophils

Nupur Aggarwal,1 Elena Korenbaum,2 Ravi Mahadeva,3 Stephan Immenschuh,4 Veronika Grau,5 Charles A Dinarello,6,7 Tobias Welte,1 and Sabina Janciauskiene1

Online address: http://www.molmed.org

Supplementary Figure S1. A1AT (Prolastin) reduces LPS-induced IL-1β release and IL-β precursor expression Human neutrophils (5 × 10⁶) were incubated for 5 h in either medium alone or medium containing A1AT (Prolastin, 1 mg/mL) or LPS (20 ng/mL) in the absence or presence of Prolastin. Released IL-1β levels (A) and IL-1β gene expression (B) were determined. GAPDH was used as a housekeeping gene. Bars represent mean (SD) from 4 and 3 individual donors; n = indicates number of replicates for each experiment. P value indicates significant differences compared with the values seen in LPS-activated cells.
Supplementary Figure S2. Neutrophil viability as measured by the release of the cytoplasmic enzyme lactate dehydrogenase. Human neutrophils ($5 \times 10^6$) were incubated for 5 h in either medium alone or medium containing LPS (20 ng/mL) in the absence or presence of A1AT-0 (1 mg/mL) or A1AT-LA (1 mg/mL). Neutrophil culture supernatants were assessed for lactate dehydrogenase (LDH) release. The lysates from untreated neutrophils were used as a positive control. Percentage cytotoxicity in total cell lysates was calculated according to the formula: 

$$\frac{(\text{experimental value A490}) - (\text{spontaneous release A490})}{(\text{maximal release A490}) - (\text{spontaneous release A490})} \times 100\%$$

Bars represent percentage of cytotoxicity (mean ± SD) in comparison to positive control and are representative of 5 independent experiments.

Supplementary Figure S3. Effects of A1AT-0 and A1AT-LA on TLR2 and TLR4 surface expression. Human neutrophils ($5 \times 10^6$) were incubated for 5 h in either medium alone or medium containing LPS (20 ng/mL) in the absence or presence of A1AT-0 (1 mg/mL) or A1AT-LA (1 mg/mL). Surface staining of TLR2 and TLR4 was analyzed by flow cytometry. Cells were stained for 30 min at 4°C with anti-TLR4-Alexa fluor 488 or anti-TLR2-APC and with respective isotype antibodies. Gating strategy (A) and percentage of positive cells (B) in upper right quadrant determined by FACS Diva software. The respective dot plots are shown. Figures are representative of 4 individual donors, each performed in duplicate.
Supplementary Figure S4. Time-dependent complex formation of A1AT-0 and A1AT-LA with proteinase 3 and elastase. A1AT-0 or A1AT-LA was mixed with proteinase 3 or elastase (at a molar ratio 1.2:1 A1AT/enzyme) and incubated at room temperature for different time points. A1AT-0 and A1AT-LA alone were used as positive controls. Reaction was stopped by adding 2 × SDS sample buffer and boiling the samples for 2 min. Complex formation of A1AT-0 and A1AT-LA with proteinase 3 (A) and elastase (B) was analyzed on 12.5% SDS-PAGE gels. SDS-PAGE gels were stained with Coomassie Blue. Each figure is representative of 3 independent donors. (M-molecular size markers, kDa; PR3 = proteinase 3; NE = neutrophil elastase.)

Supplementary Figure S5. Myeloperoxidase release. Human neutrophils (5 × 10⁶) were incubated for 5 h in either medium alone or medium containing LPS (20 ng/mL) in the absence or presence of A1AT-0 (1 mg/mL) or A1AT-LA (1 mg/mL). Levels of peroxidase (MPO) were analyzed in cell supernatants by ELISA. Box plots represent data from 8 individual donors; n = number of replicates for each treatment.
Supplementary Figure S6. A1AT localizes in the cytosol of neutrophils. Human neutrophils (5 x 10⁶) were incubated for 5 h alone or with 1 mg/mL A1AT-0 or A1AT-LA. Subcellular fractions were analyzed by western blotting with mouse monoclonal anti-A1AT antibody. The blots were reprobed with anti-β-actin and anti-histone H1 antibodies as loading controls for cytosolic and nuclear fraction, respectively. Each blot is representative of 3 individual donors with similar results.