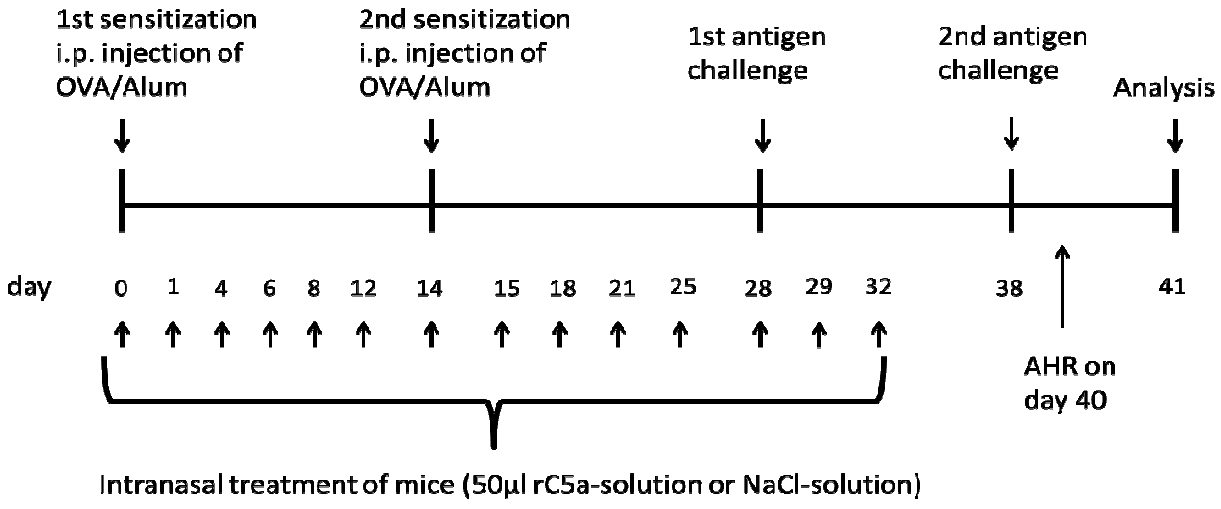
Online data supplement

Supplementary figure 1:

Murine model for the induction of allergic allergic airway inflammation.

Mice were sensitized by two intraperitoneal injections of 20 µg ovalbumin (OVA gradeV; Sigma, St Louis, MO, USA) emulsified in 2.2 mg aluminum hydroxide (ImjectAlum; Pierce, Rockford, IL, USA) in a total volume of 200 µl on days 0 and 14. On days 28 and 38 the mice were challenged via the airways with 1% OVA aerosol for 30 minutes using a PARI-Boy aerosol generator. During the sensitization period and around the first antigen challenge mice were treated intranasally for a total of 14 times with 50µl of either NaCl or different concentrations of rC5a (0,2µg, 2µg or 10µg rC5a per application, each application is indicated by an arrow in supplementary figure 1). Airway hyperreactivity was determined on day 40. Three days after the last challenge, mice were sacrificed and the different parameters of allergic sensitization and airway inflammation were investigated.

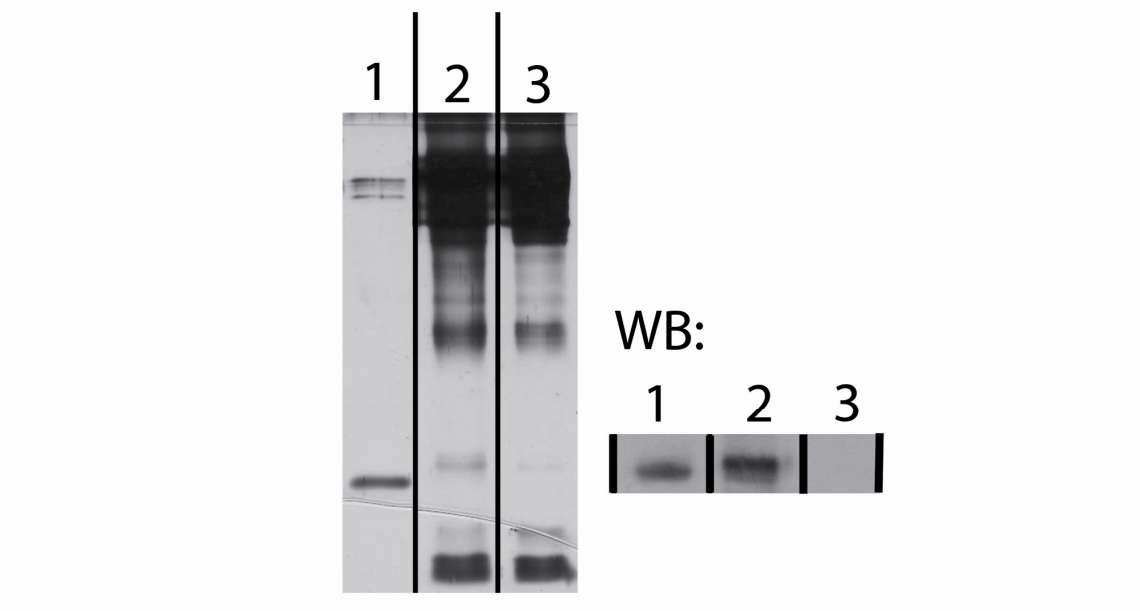


Supplementary figure 2:

Identification of C5a in murine BALs after incubation with CDE by SDS-PAGE (left picture) and Western-Blot analysis (right picture, WB= Western-Blot).

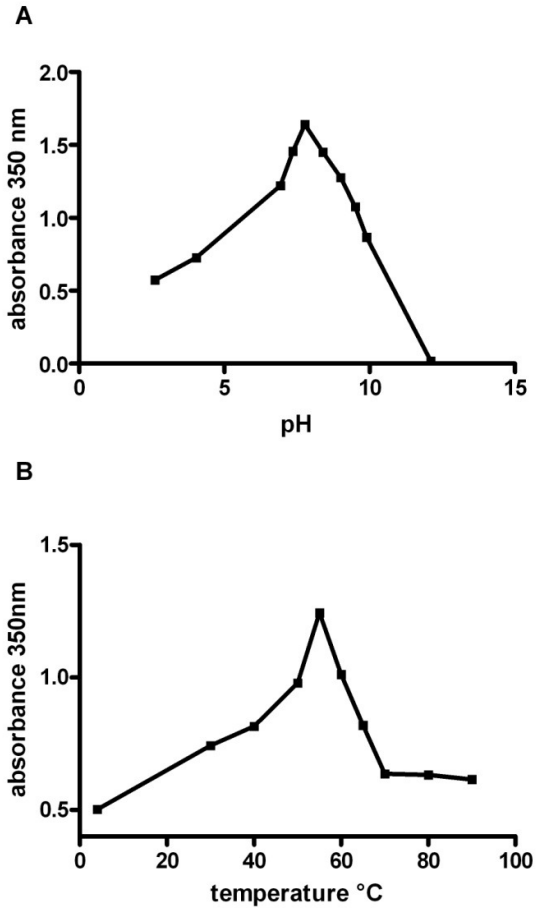
1: recombinant murine C5a (Hbt Biotech); 2: 150µl of BAL incubated with 10µl of 5mg/ml CDE for 30 minutes at 37°C 3: 150µl of BAL incubated with 10µl of PBS for 30 minutes at 37°C.

Proteins were transferred to a nitrocellulose membrane at a constant current of 0,4 A for 35 minutes .1: recombinant murine C5a (Hbt Biotech); 2: 150µl BAL with 10µl of a 5mg/ml CDE-solution; 3: 150µl of BAL incubated with 10µl of PBS.



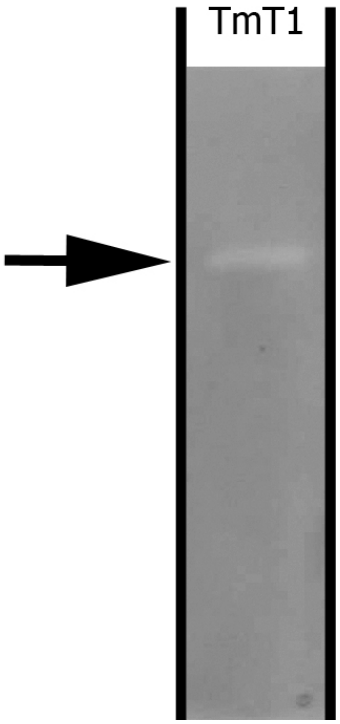
Supplementary figure 3:

Determination of pH and temperature optima of proteolytic activity in cowshed dust extract by the use of azocasein. To investigate the pH value for a maximum of proteolytic activity, a solution containing azocasein was adjusted to different pH values and incubated with cowshed dust extracts for 30 minutes. During this incubation time proteolytic acitivity in CDE degradates casein and thus releases the azo-groups. These azo-groups stay in solution whereas the casein fragments may be precipitated. Thus, measurement of the absorbance at 350nm in the supernatants correlates with the amount of proteolytic activity at different pH values. We also used different temperatures to investigate the temperature at which a maximal proteolytic activity was achieved.



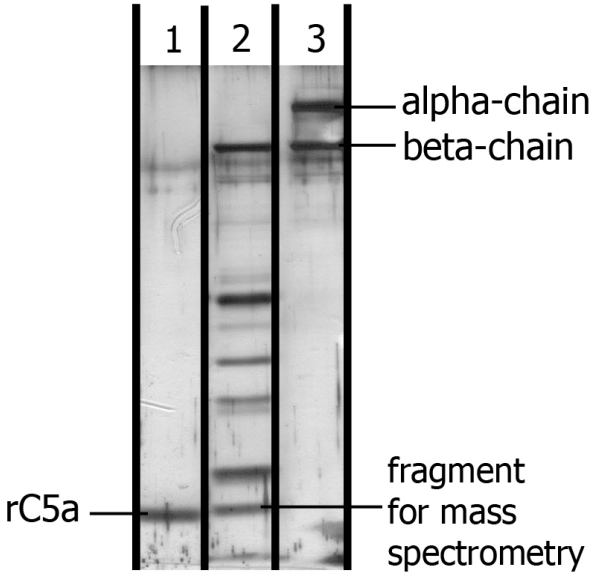
Supplementary figure 4:

Proteolytic activity of the isolated protease from the midgut of the yellow mealworm as shown by zymography. A 15% SDS gel was copolymerized with 1mg/ml BSA and electrophoresis was performed for 1,5 h at constant voltage (120V). Afterwards, the gel was incubated two times for a total of 30 minutes in 2,5% Triton-X 100 (v/v) dissolved in aqua dest. to remove SDS from the gel and allow the protease to renature. The gel was then equilibrated for 30 minutes in equilibration buffer (50mM Tris, 0,2mM NaCl, 5mM CaCl2, 0,02% Tween-20 (v/v) in aqua dest.) before the buffer was exchanged against fresh buffer. The gel was incubated in equilibration buffer over night at 37°C. Next, the gel was coomassie-stained and destained with standard methods. The unstained band occurring in the gel (arrow) represent the position of proteolytically active TmT1-protease in the gel.



Supplementary figure 5:

Digestion of human C5 (Sigma) with isolated TmT1 serine protease and identification of C5a by mass spectrometry. Purified human C5 (lane 3) was incubated with 0,18U/ml TmT1 serine protease for 30 minutes at 37°C and afterwards subjected to SDS-PAGE (lane 2). The two bands occurring in lane 3 represent the alpha and beta chain of human C5.Interestingly,as can be seen in lane 2, only the alpha-chain of C5 was cleaved whereas the beta-chain remained intact after digestion with TmT1-protease. In lane 2, the band occurring in the molecular mass range of murine rC5a (lane 1) was excised and subjected to mass spectrometry where it was identified as human C5a. 7 peptides from human C5a were identified, resulting in a sequence coverage of 28%.

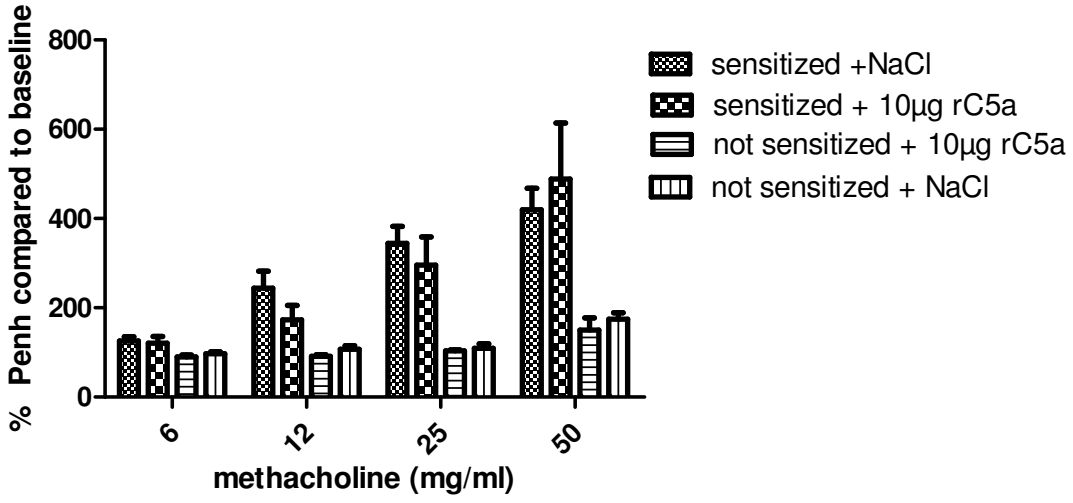


Protein description	Sequence Coverage [%)	Identified Peptides	Number of amino acids	MW [kDa]	Score
Human C5a	28,38	7	74	8,4	24,53

Peptide Sequences	Number of matches	Max probability
KIEEIAAK	2	46,26
YKHSVVK	2	23,85
IEEIAAK	1	43,28
DMQLGR	1	40,16
EEIAAK	1	28,96

Supplementary figure 6:

Airway hyperreactivity of mice that were sensitized to ovalbumine (injection of OVA/Alum for sensitization) or that were sham-sensitized (injection of PBS/Alum). Mice were furthermore either sham-treated intranasally with NaCl or treated with 10µg of rC5a during the sensitization phase and during the first antigen challenge. All groups were challenged with nebulized ovalbumine. AHR was determined by non-invasive measurement using whole-body plethysmography two days after the last provocation. Methacholine was applied to the mice in rising concentrations reaching from 6mg/ml to 50mg/ml. Percents of Penh-values compared to baseline values are shown. Baseline values for Penh are defined as those Penh values that were reached without methacholine provocation. No significant differences between the PBS treated and the C5a treated group were observed. Since the group which received 10µg rC5a was the only group that showed significant alleviation of parameters of allergic airway inflammation, we only compared this group to the sham treated group. Furthermore, groups of mice that were sham-sensitized (injection of Alum without ovalbumine, not sensitized) and challenged with ovalbumine did only show a weak response to methacholine when methacholine was given in a concentration of 50mg/ml. This result did not differ between mice that were treated with either NaCl (not sensitized + NaCl) or 10 µg rC5a (not sensitized + 10µg rC5a). n=6 in groups sensitized + NaCl and sensitized + 10µg rC5a; n=4 in groups not sensitized + NaCl and not sensitized + 10µg rC5a.



Supplementary table 1: Identification of the protease by LC/MS mass-spectrometry. Proteolytic active bands from anionexchange chromatography (figure 2b and 2c) were excised and used for mass spectrometry. The protein-accession number defines the databank-ID of the protein. The protein description is the name of the identified protein. The molecular mass of the protein (Protein mass (Da)) is calculated from the amino acid sequence of the protein. The peptide sequence defines those peptide sequences which have been identified by mass spectrometry and the peptide score (Mascot score) is the degree of the feasibility, that the identified peptide sequences are correct. The first three identified peptides belong to the serine protease TmT1 from *tenebrio molitor larvae*, whereas the last two peptides belong to trypsin, which has been used for in-gel digestion of the analyzed band.

Protein accession number	Protein description	Protein mass (Da)	Peptide sequence	Peptide score	Theoretical peptide mass (Da)	Measured peptide mass (Da)
gil61393532	posterior midgut digestive trypsin [Tenebrio molitor]	26401	GSGGQVVNVAR	92	1042,552	1042,525447
gil61393532	posterior midgut digestive trypsin [Tenebrio molitor]	26401	INQNPSYNDR	69	1219,5581	1219,539247
gil61393532	posterior midgut digestive trypsin [Tenebrio molitor]	26401	MLCAGVTGGGK	76	1049,4998	1049,474447
gil136429	RecName: Full=Trypsin; Flags: Precursor	25078	LSSPATLNSR	55	1044,5564	1044,584647
gil136429	RecName: Full=Trypsin; Flags: Precursor	25078	VATVSLPR	54	841,5022	841,5124471

Supplementary table 2: Identification of the protease from the midgut of *tenebrio molitor larvae* by mass-spectrometry. Bands were excised from SDS-PAGE and analysed by LC/MS spectrometry as described in material and methods. The identified peptide sequences, the quality of the identified peptides (peptide score) and the theoretical as well as the measured peptide mass calculated from the amino acid sequence of the peptides are shown.

Protein accession number	Protein mass (Da)	Peptide sequence	Peptide score	Theoretical peptide mass (Da)	Measured peptide mass (Da)
gil61393532	26401	GSGGQVVNVAR	63	1042,552	1042,551847
gil61393532	26401	INQNPSYNDR	68	1219,5581	1219,549647
gil61393532	26401	MLCAGVTGGGK	62	1065,4947	1065,431447