Carboxyl-terminal cleavage of apolipoprotein A-I by human mast cell chymase impairs its anti-inflammatory properties

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Running title: Chymase blocks anti-inflammatory effects of apoA-I

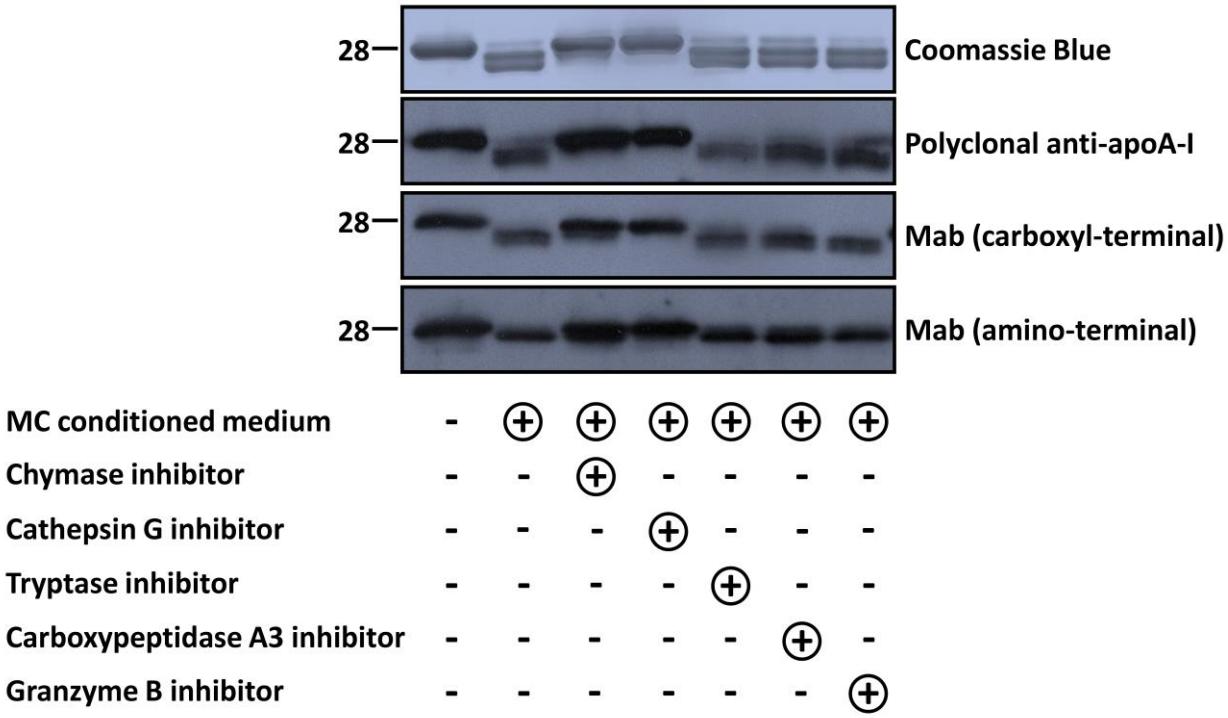
Supplemental Table I: Properties of (A-I)rHDLs

			Composition	
Type	Shape	Mobility	PC: cholesterol: apoA-I	Lipid: protein
			(molar ratio)	(mass ratio)
rHDL-1	Discoidal	Preβ	30:12.5:1	1:1
rHDL-2	Discoidal	Preβ	140:12.5:1	3.9:1
rHDL-3	Discoidal	Preβ	250:12.5:1	6.8:1
HDL_2	Spherical	α	ND	ND
HDL_3	Spherical	α	ND	ND

ND: Not determined

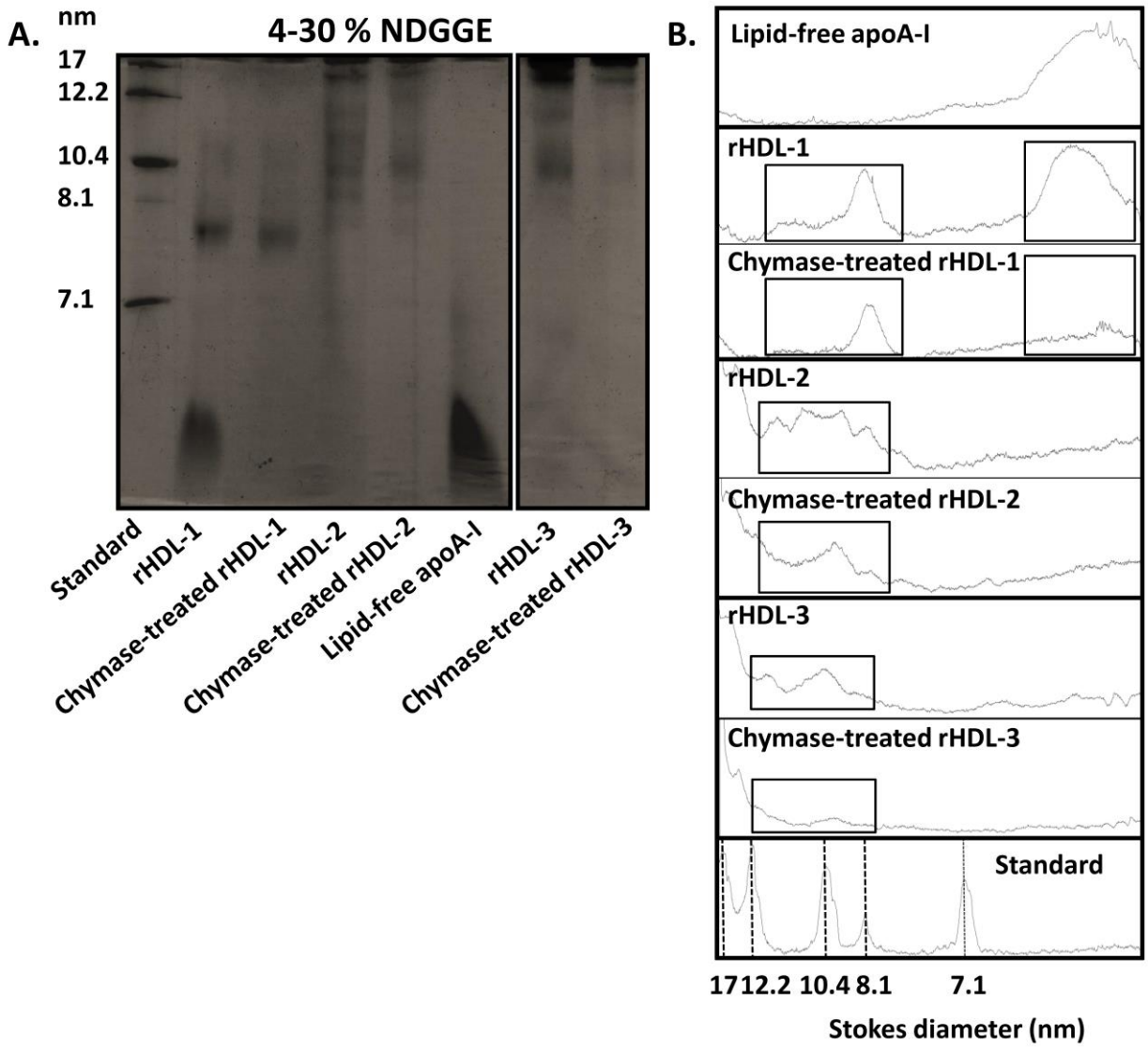
Supplemental Table II: Sequences of primers and fluorogenic probes used in qRT-PCR experiments

Gene	Forward Primers	Reverse Primers	Probes
VCAM-1	5’-CGCAAACACTTTATGTCAATGTTG-3’	5’-GATTTTCGGAGCAGGAAAGC-3’	5’-CCAGAGATACAACCGTCTTGGTCAGCC--3’
ICAM-1	5’-CCCTGATGGGCAGTCAACA-3’	5’-GCAGCGTAGGGTAAGGTTCTTG-3’	5’-CCTCACCGTGTACTGGACTCCAGAACG-3’
TNFα	5’-GCTGCACTTTGGAGTGATCG-3’	5’-GTTTGCTACAACATGGGCTACAG-3’	5’-CCCAGGCAGTCAGATCATCTTCTCGA-3’
IL-1β	5’-TTACAGTGGCAATGAGGATGAC -3’	5’-GTCGGAGATTCGTAGCTGGAT -3’	5’-AACAGATGAAGTGCTCCTTCCAGGACC-3’
IL-6	5’-GAACCTTCCAAAGATGGCTGA-3’	5’-CAAACTCCAAAAGACCAGTGATG-3’	5’-CAGGCAAGTCTCCTCATTGAATCCAGA-3’
IL-8	5’-CTCTTGGCAGCCTTCCTGA-3’	5’-GGTGGAAAGGTTTGGAGTATGTC-3’	5’-TGGCAAAACTGCACCTTCACACAGAG-3’
COX-2	5’-CGAGGGCCAGCTTTCAC-3’	5’-GGCGCAGTTTACGCTGTCTAG-3’	5’-TGATTTAAGTCCACCCCATGGCCC-3’
GAPDH	5’-CCACATCGCTCAGACACCAT-3’	5’-GGCAACAATATCCACTTTACCAGAG-3’	5’-CCAATACGACCAAATCCGTTGACTCC-3’



Supplemental Figure I. SDS-PAGE analysis of apoA-I after treatment with mast cell- conditioned medium.

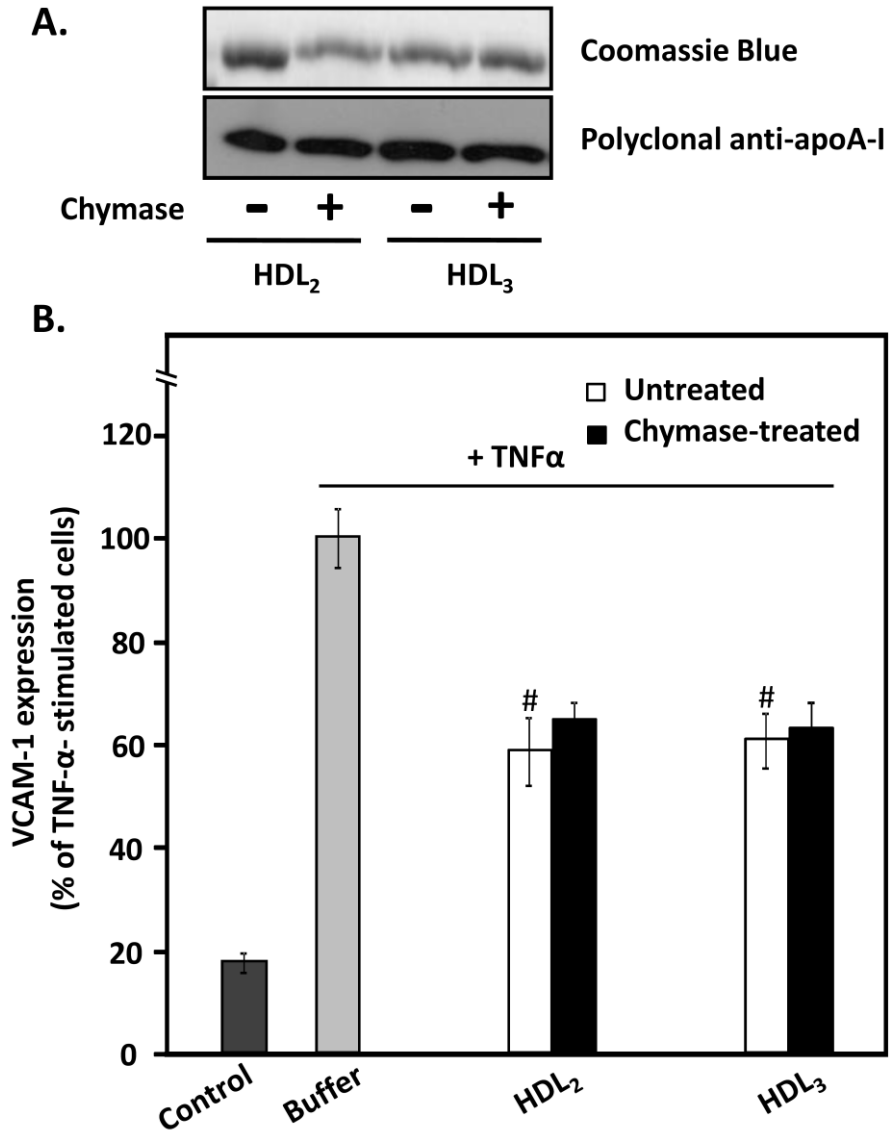
ApoA-I (1 mg/ml) and conditioned medium, in which mature human mast cells generated from circulating progenitors had been activated, were incubated at 37^{o}C for 6 h. Incubations were performed in the absence or presence of the indicated proteinase inhibitors, which were added at concentrations which fully inhibited the respective proteases. The conditioned medium contained 0.15 µg (36 BTEE units)/ml chymase and co-secreted mast cell granule neutral proteases (cathepsin G, tryptase, carboxypeptidase A3, and granzyme B). After incubation, proteins were resolved in NuPAGE Novex 4-12% Bis-Tris gels and detected by Coomassie Blue or resolved in 12.5% SDS-PAGE and immunoblotted with anti-human apoA-I polyclonal antibody or with anti-human apoA-I monoclonal antibodies recognizing either a carboxyl-terminal (amino acids 211-220) or an amino-terminal (amino acids 2-8) region of apoA-I. Similar results were obtained using conditioned medium from mature mast cells derived from progenitors of another human donor.



Supplemental Figure II. Non-denaturing polyacrylamide gradient gel electrophoresis (NDGGE) analysis of rHDLs upon chymase treatment

A, Aliquots of untreated and chymase-treated (A-I)rHDL were electrophoresed by 4-30% NDGGE. The gels were stained with Coomassie blue.

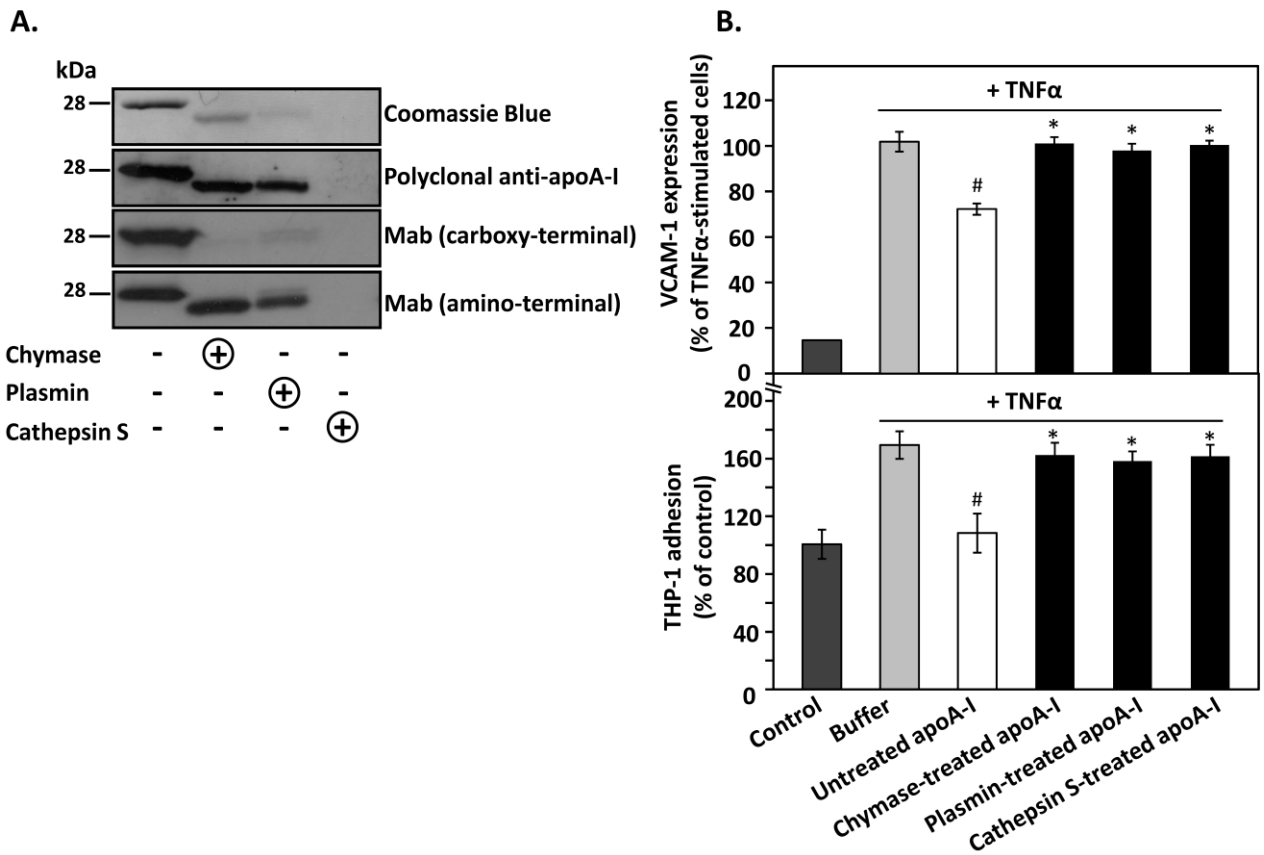
B, The profiles of particle size distribution were analyzed by NIH ImageJ software. The boxes show the areas in which the differences between each untreated and corresponding chymase-treated (A-I)rHDL are present.



Supplemental Figure III. Chymase treatment of mature HDL does not affect its ability to inhibit VCAM-1 expression on activated HCAECs.

A, HDL_2 or HDL_3 (1 mg/ml) were incubated in the absence (untreated) or presence of recombinant human chymase (40 BTEE units/ml) for 6 h. The proteins in the incubation mixture were resolved in 12.5% SDS-PAGE and detected by Coomassie Blue or immunoblotted with anti-human apoA-I polyclonal antibody.

B, HCAECs were preincubated for 16 h with untreated or chymase-treated HDL_2 or HDL_3 (50 µg/ml, each), and then stimulated with TNFα (10 ng/ml) for 5 h. Non-activated cells (control) and TNFα-activated cells preincubated in only medium (buffer) acted as references. VCAM-1 surface protein expression levels were analyzed by flow cytometry (% of buffer). Data represent the means ± SD from 3 independent experiments performed in duplicate. $^{\#}p< 0.01$ (untreated vs. buffer).



Supplemental Figure IV. Treatment of apoA-I with plasmin or cathepsin S impairs its ability to attenuate proinflammatory responses in activated HCAECs.

A, ApoA-I (1 mg/ml) was incubated for 1 h in the absence or presence of chymase (40 BTEE U/ml = 0.5 µg/ml), plasmin (0.1 U/ml = 50 µg/ml), or cathepsin S (0.15 U/ml = 5 µg/ml) in TNE buffer. The untreated and protease-treated apoA-I were resolved in 12.5% SDS-PAGE and detected by Coomassie Blue or immunoblotted with anti-human apoA-I polyclonal antibody or with anti-human apoA-I monoclonal antibody recognizing either a carboxyl-terminal (amino acids 211-220) or an amino-terminal (amino acids 2-8) region of apoA-I.

B, HCAECs were preincubated for 16 h with untreated or protease-treated apoA-I (50 µg/ml, each), and then stimulated with TNFα (10 ng/ml) for 5 h. Non-activated cells (control) and TNFα-activated cells preincubated in only medium (buffer) acted as references. Cell surface expression of VCAM-1 was determined by flow cytometry (top panel). Data represent the mean ± SD from three to four independent experiments performed in duplicate. In a separate experiment, HCAECs that had been treated as described above were incubated for 1 h with fluorescently labeled THP-1 macrophages. Nonadherent THP-1 cells were removed by gentle washing and the fluorescence of HCAECs-bound THP-1 cells was measured. The fluorescence signals of TNFα-stimulated HCAECs were expressed as percentages of the basal signal from the control cells. Data shown in the panels represent the means ± SEM from 3 independent experiments performed in triplicate wells. * $p < 0.01$ (untreated vs. protease-treated); # $p < 0.01$ (untreated vs. buffer).