

Supplemental Materials

IgE stimulates human and mouse arterial cell apoptosis and cytokine expression and promotes atherogenesis in *Apoe*^{-/-} mice

Jing Wang, Xiang Cheng, Mei-Xiang Xiang, Mervi Alanne-Kinnunen, Jian-An Wang, Han Chen, Aina He, Xinghui Sun, Yan Lin, Ting-Ting Tang, Xin Tu, Sara Sjöberg, Galina K. Sukhova, Yu-Hua Liao, Daniel H. Conrad, Lunyin Yu, Toshiaki Kawakami, Petri T. Kovanen, Peter Libby, Guo-Ping Shi

Supplemental Tables

Supplemental Table 1. Clinical data and serum IgE comparisons between patients with and without coronary heart disease (CHD) from Eastern China.

Variables	non-CHD (n=93) (Mean ± S.E.)		CHD (n=147) (Mean ± S.E.)		<i>t</i> value	<i>P</i> value*
Age (year)	58.45±1.12		65.79±0.81		5.408	<0.001
Body-mass index (kg/mm ²)	23.28±0.41		23.70±0.26		0.591	0.357
Fasting glucose (mg/dL)	98.02±2.03		101.17±2.37		1.011	0.313
Total cholesterol (mg/dL)	161.60±3.28		163.01±3.80		0.281	0.779
Triglyceride (mg/dL)	156.94±10.84		170.63±12.46		0.765	0.445
High-density lipoprotein (mg/dL)	51.05±1.41		47.24±1.01		-2.245	0.026
Low-density lipoprotein (mg/dL)	91.15±2.81		94.48±3.06		0.803	0.423
Immunoglobulin E (IU/mL)	62.21±5.69		99.55±9.84		3.286	0.001
	Non-CHD (n=93)		CHD (n=147)		<i>P</i> value**	
	0***	1***	0***	1***		
Sex	40	53	42	105		0.026
Smoking	62	31	91	56		0.493
Hypertension	34	59	48	99		0.577
Diabetes mellitus	84	9	116	31		0.022

*Independent sample *t* test. **Fisher's exact test. *P*<0.05 was considered statistically significant. ***Sex: 0-female, 1-male; Smoking: 0-non-smoker, 1-smoker; Hypertension: 0-no, 1-yes; Diabetes mellitus: 0-no, 1-yes.

Supplemental Table 2. Clinical data and serum IgE comparisons among CHD subgroups and non-CHD subjects from Eastern China.

Variables	non-CHD (n=93)		AMI (n=33)		UAP (n=83)		SAP (n=31)		P value ^d
Age (year)	58.45 ± 1.12		69.88 ± 1.83**		65.23 ± 0.96**#		62.94 ± 1.95###		0.000 ^a
Body-mass index (kg/mm ²)	23.45 ± 0.35		22.75 ± 0.53		23.63 ± 0.34		24.92 ± 0.60		0.059 ^a
Fasting glucose (mg/dL)	98.02 ± 2.03		116.17 ± 6.52		97.23 ± 2.57#		95.06 ± 4.58#		0.004 ^b
Total cholesterol (mg/dL)	161.60 ± 3.28		155.85 ± 5.27		164.55 ± 5.49		167.18 ± 9.27		0.843 ^b
Triglyceride (mg/dL)	156.94 ± 10.84		129.88 ± 8.42		176.21 ± 13.01		203.11 ± 48.81		0.233 ^a
High-density lipoprotein (mg/dL)	51.05 ± 1.41		47.64 ± 2.07		46.65 ± 1.30		48.39 ± 2.54		0.145 ^a
Low-density lipoprotein (mg/dL)	91.15 ± 2.81		90.85 ± 4.45		96.14 ± 4.45		94.14 ± 7.20		0.889 ^a
Immunoglobulin E (IU/mL)	62.21 ± 5.69		133.63 ± 26.28**		97.72 ± 12.41*▲		68.18 ± 15.76##		0.003 ^a
	non-CHD (n=93)		AMI (n=33)		UAP (n=83)		SAP (n=31)		P value ^{c,d}
	0	1	0	1	0	1	0	1	
Sex	40	53	13	20	21	62	8	23	0.058
Smoking	62	31	19	14	52	31	20	11	0.816
Hypertension	34	59	12	21	26	57	10	21	0.884
Diabetes mellitus	84	9	25	8	67	16	24	7	0.121

a: one way ANOVA LSD test (normal distribution and homogeneity of variance) ; b: Kruskal-Wallis test (abnormal distribution or heterogeneity of variance); c. Pearson Chi-Square test. d: $P < 0.05$ was considered statistically significant between the groups.

* $P < 0.05$ vs. non-CHD; ** $P < 0.01$ vs. non-CHD; # $P < 0.05$ vs. AMI; ## $P < 0.01$ vs. AMI; ▲: $P < 0.05$, vs. SAP

Supplemental Table 3. Variables associated with serum IgE in all subjects (n=240) from Eastern China.

	Correlation Coefficient	t value	P value
Age (year)	0.027		0.676*
Sex	-1.669	0.096**	
Body-mass index (kg/mm ²)	0.000		0.996*
Hypertension	0.334	0.739**	
Diabetes mellitus	-1.292	0.203**	
Smoking	-0.772	0.441**	
Fasting glucose (mg/dL)	0.218		0.001*
Total cholesterol (mg/dL)	0.108		0.104*
Triglyceride (mg/dL)	-0.037		0.575*
High-density lipoprotein (mg/dL)	0.027		0.686*
Low-density lipoprotein (mg/dL)	0.121		0.068*

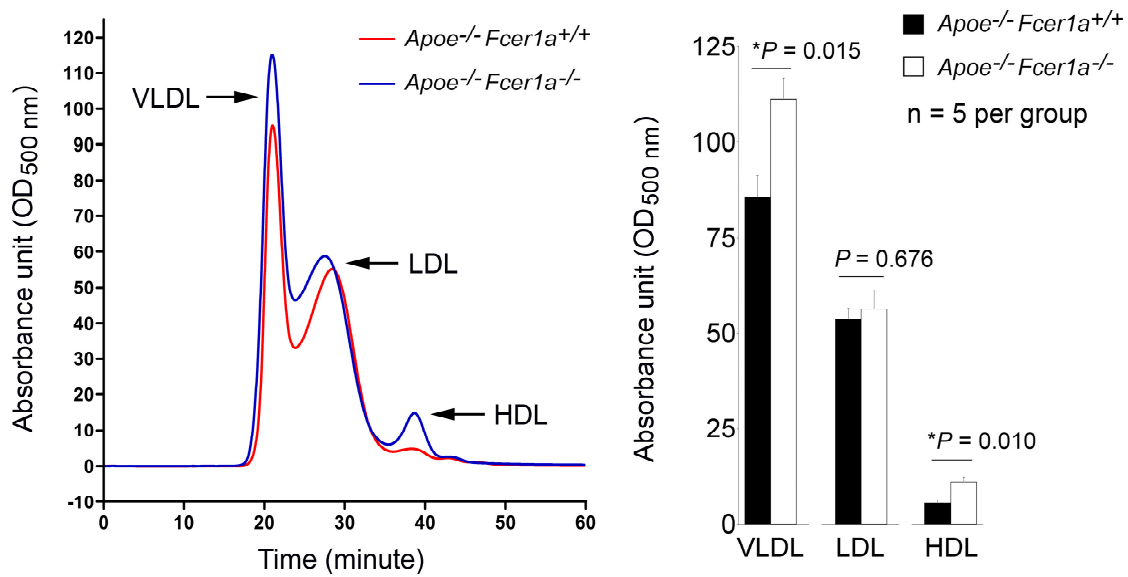
* Pearson's correlation test; **Independent sample t test.

Supplemental Table 4. Mouse serum lipid profiles.

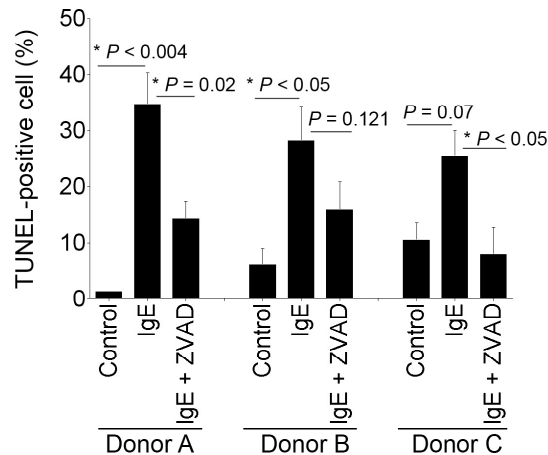
Genotype	Total cholesterol (mg/dL)	HDL (mg/dL)	Triglyceride (mg/dL)	LDL (mg/dL)
<i>Apoe</i> ^{-/-} <i>Fcer1a</i> ^{+/+} (n=18)	1449.49 ± 85.65	35.38 ± 1.33	230.77 ± 17.89	1367.96 ± 82.69
<i>Apoe</i> ^{-/-} <i>Fcer1a</i> ^{-/-} (n=11)	1512.44 ± 140.25	41.08 ± 1.85	282.42 ± 30.57	1414.88 ± 135.61
P value*	0.589	0.019*	0.080	0.653

*Mann-Whitney U test, $P < 0.05$ is considered statistically significant.

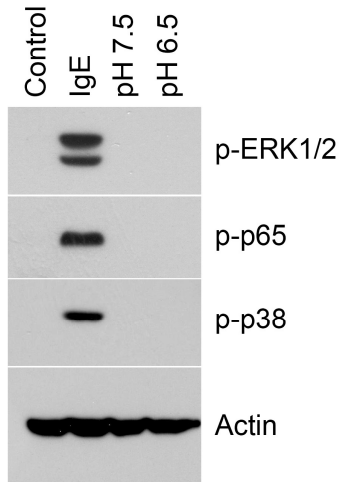
Supplemental Figures



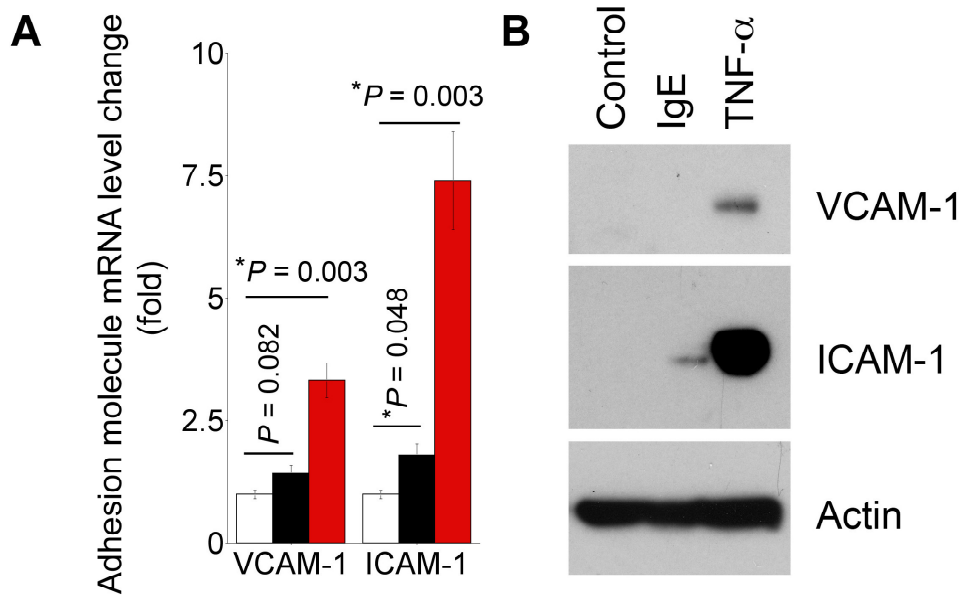
Supplemental Figure 1. FPLC analysis of serum samples from *Apoe*^{-/-} *Fcer1a*^{+/+} mice and *Apoe*^{-/-} *Fcer1a*^{-/-} mice after 12 weeks on a Western diet. $P < 0.05$ was considered statistically significant, Mann-Whitney *U* test. Representative data are shown to the left.



Supplemental Figure 2. IgE induces human monocyte-derived macrophage apoptosis. Macrophages from three donors respond to IgE (SPE-7, 50 μ g/mL) and undergo apoptosis. Caspase inhibitor ZVAD-FMK (20 μ M) efficiently blocks IgE-induced macrophage apoptosis, although macrophages from different donors respond differently. * $P < 0.05$ is considered statistically significant, non-parametric Mann-Whitney test.



Supplemental Figure 3. pH effects on macrophage signaling molecule activation. Culturing human macrophages in pH6.5 or pH7.5 media did not cause MAPK (ERK1/2 and p38) or MF-kB phosphorylation, as detected by immunoblot analysis. IgE (50 $\mu\text{g}/\text{mL}$) was used as a positive control, and actin blot was used as a protein loading control.



Supplemental Figure 4. Human endothelial cell adhesion molecule expression after stimulation with purified IgE (50 $\mu\text{g}/\text{mL}$) or recombinant TNF- α (10 ng/mL). **A.** RT-PCR to detect mRNA levels. **B.** Immunoblot analysis to detect protein levels. TNF- α was used as a positive control, and actin blot was used to ensure equal protein loading.