SUPPLEMENTAL MATERIAL

Supplemental Tables

S-Table 1. Antibodies used for the immunohistochemical staining

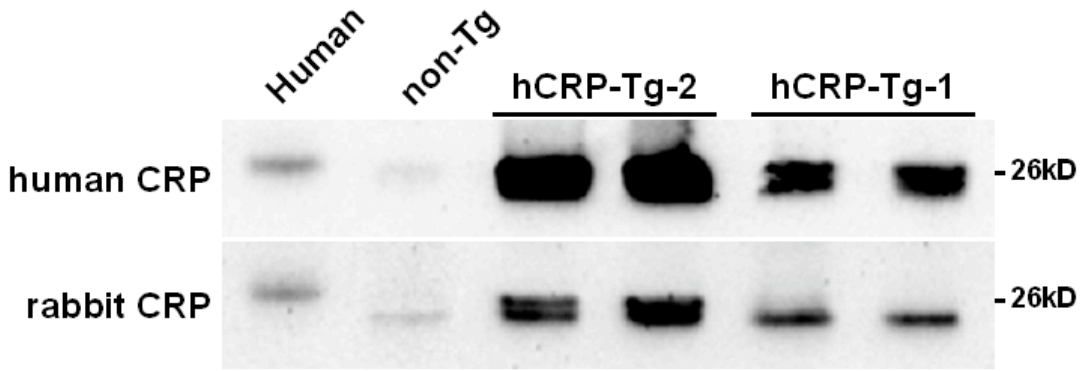
Antibodies	Working dilution	Species	Manufacturers
Human CRP	x 2,000	Mouse	Sigma-Aldrich Japan, Tokyo, Japan
Rabbit CRP	x 100	Chicken	Immunology Consultants Laboratory Inc., Newberg, OR
RAM11	x 400	Mouse	Dako Japan Inc., Tokyo, Japan
HHF35	x 300	Mouse	Enzo Biochemicals, NY

S-Table 2. Blood and biochemical analysis of cholesterol-fed Tg and non-Tg rabbits

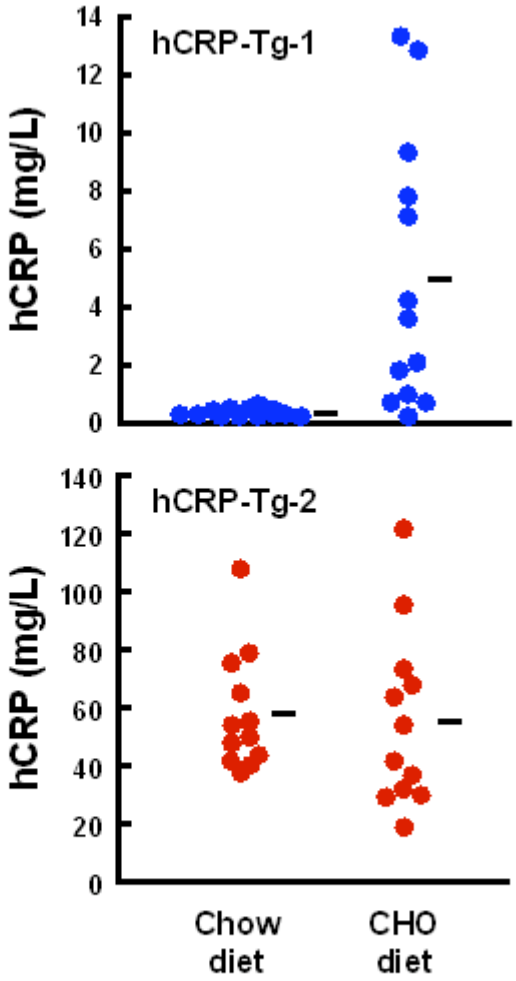
Variables examined	non-Tg (n=13)	hCRP-Tg-1 (n=13)	hCRP-Tg-2 (n=5)
WBC ($10^2/\mu L$)	113 ± 11	102 ± 5	95 ± 18
RBC ($10^4/\mu L$)	447 ± 44	471 ± 34	587 ± 13
HGB (g/dL)	9.6 ± 0.9	10.0 ± 0.7	12.3 ± 0.3
HCT (%)	31.1 ± 2.1	32.1 ± 1.7	36.8 ± 1.3
PLT ($10^3/\mu L$)	225 ± 25	193 ± 20	281 ± 27
NEUT (%)	36.7 ± 3.9	37.3 ± 2.7	32.3 ± 1.5
LYMPH (%)	56.0 ± 3.9	55.6 ± 2.9	62.0 ± 2.2
MONO (%)	3.2 ± 0.3	3.0 ± 0.5	2.3 ± 0.3
EO (%)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
BASO (%)	4.1 ± 0.4	4.1 ± 0.2	3.4 ± 0.6
TP (g/dL)	5.8 ± 0.1	6.1 ± 0.1	5.6 ± 0.1
Alb (g/dL)	0.9 ± 0.0	0.9 ± 0.1	1.0 ± 0.0
ALP (IU/L)	109 ± 9	127 ± 7	105 ± 12
LAP (IU/L)	69 ± 4	83 ± 2**	56 ± 3
γ-GT (IU/L)	9.8 ± 2.1	10.2 ± 2.2	7.0 ± 2.0
LDH (IU/L)	132 ± 22	110 ± 11	74 ± 6
AST (IU/L)	30 ± 7	22 ± 2	17 ± 3
ALT (IU/L)	32 ± 4	23 ± 2	26 ± 5
Cre (mg/dL)	1.3 ± 0.0	1. 3 ± 0.1	1.2 ± 0.0
CK (IU/L)	1568 ± 706	545 ± 60	390 ± 85
Amylase (IU/L)	453 ± 21	451 ± 19	538 ± 75
Glu (mg/dL)	99 ± 2	102 ± 2	89 ± 3*

WBC, white blood cells, RBC, red blood cells, HGB, hemoglobin, HCT, hematocrit, PLT, platelets, NEUT, neutrophils, LYMPH, lymphocytes, MONO, monocytes, EO, eosinophils, BASO, basophils, TP, total protein, Alb, albumin, ALP, alkaline phosphatase, LAP, leucine aminopeptidase, γ-GT, γ-glutamyl transferase, LDH, Lactate dehydrogenase, AST, aspartate aminotransferase, ALT, alanine aminotransferase, Cre, creatinine, Glu, glucose. The blood was collected from rabbits fed a cholesterol diet for 16 weeks. Rabbits were fasted for 16h before bleeding. Data are expressed as means ± SE. **P<0.01, *P<0.05 vs. non-Tg by ANOVA with Sheffe's F test.

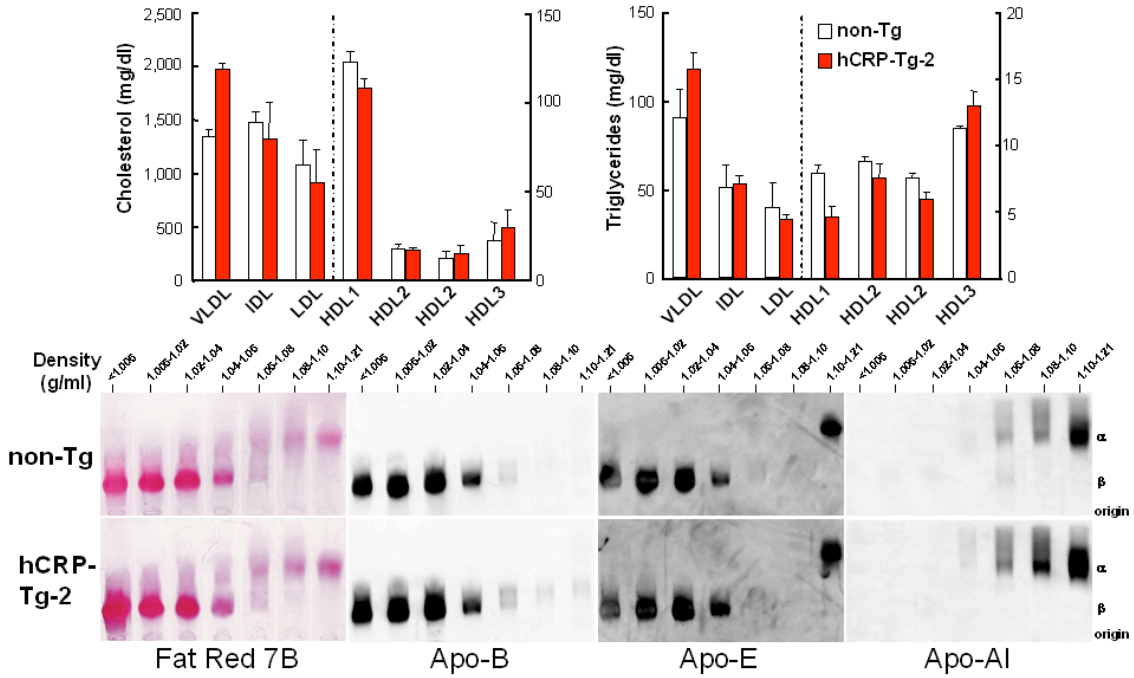
Supplemental Figures



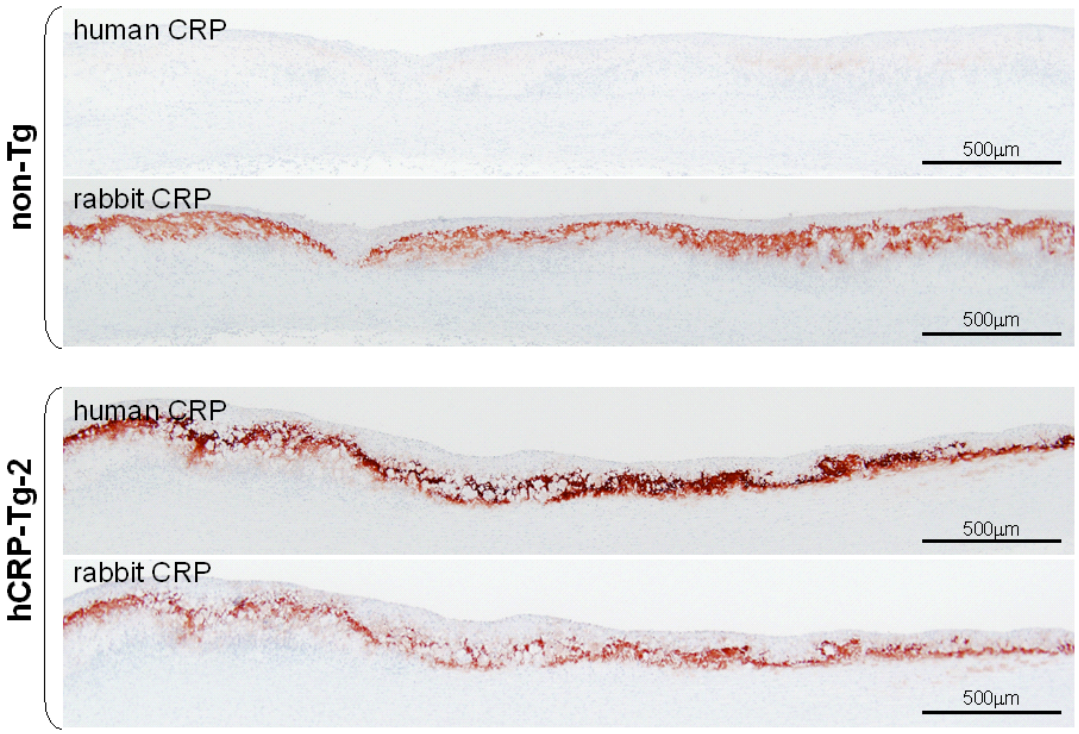
S-Fig.1


hCRP-Tg-1
hCRP (mg/L)
14
12
10
8
6
4
2
0
hCRP-Tg-2
hCRP (mg/L)
140
120
100
80
60
40
20
0
Chow diet
CHO diet

S-Fig.2


Cholesterol (mg/dl)
2,500
2,000
1,500
1,000
500
0
150
100
50
0
VLDL
IDL
LDL
HDL1
HDL2
HDL2
HDL3
Triglycerides (mg/dl)
150
100
50
0
20
15
10
5
0
non-Tg
hCRP-Tg-2
Density (g/ml)
<1.006
1.006-1.02
1.02-1.04
1.04-1.06
1.06-1.08
1.08-1.10
1.10-1.21
non-Tg
hCRP-Tg-2
α
β
origin
Fat Red 7B
Apo-B
Apo-E
Apo-AI

S-Fig.3


non-Tg
human CRP
500μm
rabbit CRP
500μm
hCRP-Tg-2
human CRP
500μm
rabbit CRP
500μm

S-Fig.4

Legends for Supplemental Figures

S-Figure 1. Comparison of antibodies against human and rabbit CRP by immunoblotting analysis
Plasma (1 μl) obtained from a healthy volunteer human, non-Tg, hCRP-Tg-1 and Tg-2 rabbits were electrophoresed by 10% SDS-PAGE and immunoblotted with hCRP mAb and rCRP polyclonal Ab as described in Methods. Note that human CRP mAb cross-reacted slightly with rabbit plasma CRP whereas rabbit polyclonal Ab showed cross-reactivity with human plasma CRP.

S-Figure 2. Plasma levels of hCRP of two lines of Tg rabbits either fed a chow diet or cholesterol (CHO) diet for 16 weeks.

S-Figure 3. Lipoprotein profiles and apolipoprotein distribution of Tg and non-Tg rabbits at 16 weeks of cholesterol diet feeding. Density gradient fractions were isolated from fasting rabbit plasma by ultracentrifugation, and total cholesterol and triglyceride levels were measured as described previously[1] (top). The combined recovery of cholesterol from each animal averaged ~80% of the total plasma level. Data are expressed as mean ± SE (n=4 for each group). Lipoproteins were resolved by electrophoresis in a 1% agarose gel and visualized with Fat Red 7B staining, and apolipoproteins were detected by immunoblotting with specific Abs against apo-B, apo-E, and apo-AI (bottom).

S-Figure 4. Detection of CRP immnoreactive proteins in the aortic lesions
Micrographs taken at lower magnification x4 show the both human and rabbit CRP immunoreactive proteins by immunohistochemical staining. Human CRP mAb showed slight cross-reactivity with rabbit endogenous CRP.

Supplemental Reference

1. Fan J, Ji ZS, Huang Y, de Silva H, Sanan D, Mahley RW, Innerarity TL, Taylor JM. Increased expression of apolipoprotein E in transgenic rabbits results in reduced levels of very low density lipoproteins and an accumulation of low density lipoproteins in plasma. *J Clin Invest.* 1998;101:2151-2164.