Short communication

A STUDY OF THE INTERACTION OF THE C-REACTIVE PROTEIN MONOMER WITH THE U937 MONOCYTE

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Abstract: C-reactive protein (CRP) has two structurally distinct isoforms, the CRP pentamer and the CRP monomer. A role for the CRP monomer in atherosclerosis is emerging, but the underlying mechanisms are only beginning to be understood. Monocytes are an important contributor to atherosclerosis, and foam cell formation is the hallmark of atherogenesis. However, whether the CRP monomer can directly interact with the monocytes and modulate their responses remains unknown. Furthermore, although FcγRIII (CD16) has been identified as the receptor for the CRP monomer on neutrophils, its role in mediating the CRP monomer’s biological effects in other cell types has been questioned. In this study, we investigated the interaction of the CRP monomer with the monocytes using the U937 monocytic cell line. The CRP monomer specifically binds to U937 cells. This binding is unique in that it is independent of FcγRs and insensitive to protease digestion of the cell surface proteins. Further assays revealed that the CRP monomer directly incorporates into the plasma membrane. Interestingly, the presence of the CRP monomer efficiently retards oxidized low-density lipoprotein-induced foam cell formation of PMA-differentiated U937 macrophages and peripheral blood monocyctic cell-derived macrophages. These findings provide additional evidence for the notion that the CRP monomer is an active CRP isoform that plays a role in atherogenesis via the direct modulation of the behavior of the monocytes.

Key words: C-reactive protein, Monocyte, Low-density lipoprotein, Foam cell

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Abbreviations used: CRP – C-reactive protein; FcγR – Fc gamma receptor; LDL – low-density lipoprotein; ox-LDL – oxidized low-density lipoprotein; PBMC – peripheral blood monocyteic cell; TBARS – thiobarbituric acid reactive substances
INTRODUCTION

Human C-reactive protein (CRP) is a typical acute phase protein composed of five identical subunits [1]. Increasing evidence indicates that in addition to the classical pentamer isoform, CRP can also exist as a monomer [2, 3]. These two isoforms exhibit distinct conformations, bioactivities and \textit{in vivo} distribution patterns [2, 3]. It has emerged that both the CRP monomer [2, 3] and the CRP pentamer [4, 5] play roles in the development of atherosclerosis. It has been proposed that the tightly regulated interplay between these two isoforms endows CRP with its competence as a fine modulator of inflammation [6].

The CRP monomer has only recently been recognized as a naturally occurring CRP isoform, after considerable controversy, so the mechanisms whereby it participates in the underlying process of cardiovascular disease are just beginning to be understood. The CRP monomer may promote the endothelial dysfunction associated with the initiation and progression of atherosclerosis by inducing pro-inflammatory responses in endothelial cells [7]. Moreover, the CRP monomer may play a role in acute events of atherosclerosis through its capability to enhance platelet aggregation [8] and neutrophil activation [9-11].

Monocytes are an important contributor to the development of atherosclerosis [12], but whether the CRP monomer can directly interact with monocytes and modulate their responses remains unclear. Furthermore, although FcγRIII (CD16) has been identified as the receptor for the CRP monomer on neutrophils [13], this receptor appears not to be the major mediator of the CRP monomer’s actions on endothelial cells [7] and platelets [8]. In this study, we investigated the interaction of the CRP monomer with the U937 monocytic cell line, finding evidence that the CRP monomer can directly regulate the behavior of monocytes in a CD16-independent manner.

MATERIALS AND METHODS

Reagents

CRP pentamer was purchased from Calbiochem. CRP monomer was generated from CRP pentamer via conventional urea-chelation [14]. The structural homogeneity of the CRP isoforms was verified via native electrophoresis and silver staining. FITC labeling was performed according to the manufacturer’s instructions (Pierce). Native low-density lipoprotein (n-LDL) was purchased from Sigma. To prepare the oxidized LDL (ox-LDL), 1 mg/ml n-LDL was first dialyzed into EDTA-free buffer, and then dialyzed against 5 µM CuSO₄ at 37°C for 18 h, followed by addition of 1 mM EDTA [15]. Heat aggregated IgG (HAG) was prepared by incubating 10 mg/ml human IgG at 63°C for 60 min. The degree of LDL oxidation was assessed using the thiobarbituric acid reactive substances (TBARS) assay [16]. Heparan sulfate, chondroitin sulfate A and chondroitin sulfate B were purchased from Sigma. Anti-CD32 mAb, anti-CD16 mAb and isotype controls were purchased from BD Pharmingen.
**Cellular binding**

U937 monocytic cells were cultured in RPMI 1640 with 10% FBS, 5% CO₂. To evaluate binding of the CRP isoforms with U937 cells, 2 × 10⁶ cells were incubated with different concentrations of FITC-labeled CRP pentamer or monomer in a binding buffer (1 mg/ml BSA and 10 mM NaN₃ in PBS with calcium and magnesium) for 30 min at 37°C. The inclusion of NaN₃ efficiently blocked the cell internalization of the surface-bound FITC-labeled CRP monomer. Incubation with NaN₃ under these conditions did not result in appreciable changes in cell viability as evaluated by trypan blue staining. After binding, the cells were washed twice and transferred to a new tube to avoid the introduction of non-specific protein binding to the plastic tube wall during lysis. After two additional washes in the new tube, the cells were lysed by 0.1 M NaOH plus 0.1% SDS. The CRP isoform bound in ng was quantified by fluorescence intensity measurement according to a standard curve constructed using serial dilutions of the labeled protein. The total cellular protein was determined using BCA kits from Pierce. The final protein binding was expressed as ng CRP isoform bound/μg total cellular protein.

In the competition experiments, U937 cells were incubated with unlabeled CRP isoforms, 20 µg/ml of anti-FcγR mAbs or isotype IgG controls in the binding buffer for 30 min at 37°C, followed by addition of 50 µg/ml of the FITC-labeled CRP monomer. In some experiments, 50 µg/ml of the FITC-CRP monomer was pre-incubated with 100 µg/ml of various glycosaminoglycan ligands for 30 min before being added to the cells. To remove the receptors on the cell surface, the U937 cells were treated with 15 µg/ml trypsin for 30 min before being used in the binding assays. In the post-binding treatments, after incubation with FITC-labeled CRP monomer, the cells were treated for 5 min with high salt (2 M NaCl), acid (50 mM glycine, pH 3.0) [17] or alkaline (pH 11.3) buffer [18], to evaluate the forces involved in CRP monomer-cell interaction. To determine the localization of the CRP monomer incorporated into the plasma membrane, post-binding extraction with 1% triton X-100 at 4°C for 15 min was also conducted [18].

**LDL uptake and foam cell formation**

The differentiation of the U937 monocytes into macrophages was performed as described previously [19]. Rat PBMC were isolated, and non-adherent cells were removed after a 2-h culture. The adherent cells were cultured for 5 days to differentiate them into macrophages. The detached cells were seeded for 2 h at 37°C (2 × 10⁵ cells/well). After being washed, the cells were incubated with LDL or ox-LDL with or without the CRP monomer in RPMI 1640 at 37°C, 5% CO₂ for 16 h. Foam cell formation was visualized by Nile red staining [20] and observed with fluorescence microscopy (Leica DMIL). In some experiments, the fluorescence intensity was quantified with a fluorescence spectrometer. 25 µg/ml polymyxin B was co-present during all of the experiments to neutralize the possible effects of LPS [21].
**FcγR expression**
The U937 expression of FcγR was evaluated via RT-PCR. The primers used are listed in Tab. 1.

Tab. 1. Primers for the amplification of FcγR genes.

| Protein | Primer sequence sense | Primer sequence anti-sense |
|---------|-----------------------|---------------------------|
| CD64    | 5’-AGGTGTCAATCGGCTGAAAG-3’ | 5’-CCTGAGCAATGGTAGGTG-3’ |
| CD32    | 5’-AGGTGTCAATCGGCTGAAAG-3’ | 5’-CCTGAGCAATGGTAGGTG-3’ |
| CD16    | 5’-ACAAGCAGCAGGAACATAGAAC-3’ | 5’-GATTACCATCCCTAGCCTGTATT-3’ |
| GAPDH   | 5’-ACCACAGTCATGCCCATC-3’ | 5’-CCTGAGCAATGGTAGGTG-3’ |

**Statistical analysis**
All of the measurements were performed in duplicate and repeated 3-6 times. The data is shown as the means ± SE. Student’s t-test and ANOVA were respectively used to assess the differences between two and more than two groups.

**RESULTS**
The positive expression of FcγRI (CD64) and FcγRII (CD32) in the U937 monocytes [22, 23] was confirmed via RT-PCR (Fig. 1A). These two receptors have been reported to mediate the cellular binding and biological responses of the CRP pentamer [23]. Accordingly, obvious binding of the CRP pentamer to U937 cells was observed (Fig. 1B) in agreement with the previous reports [23, 24]. By contrast, U937 does not express CD16 [22, 23], the putative receptor of the CRP monomer [13]. However, binding assays revealed strong binding of the CRP monomer to U937 cells with an apparent $K_d$ of 88 ± 13 μg/ml (Fig. 1B). Pre-incubation with unlabeled CRP monomer efficiently suppressed the cellular binding of its labeled counterpart (Fig. 1C), indicating that the interaction of the CRP monomer with U937 monocytes is specific.

Additional experiments were performed to elucidate the nature of the interaction between the CRP monomer and U937 monocytes. Since FcγRs have been identified as the major receptors for the two CRP isoforms, we first tested the involvement of FcγRs using specific mAbs and a high affinity ligand (i.e. HAG) of FcγRs as the competitors. Although these reagents efficiently suppressed pentamer binding, only slight effects could be noticed on monomer binding (Fig. 2). Interestingly, removing the cell surface receptors via trypsin digestion merely resulted in a moderate decrease (~25%) in the level of CRP monomer binding (Fig. 2), suggesting the interaction of the CRP monomer and U937 monocytes is independent of protein receptors.
Fig. 1. The specific binding of the CRP monomer to U937 monocytes. A – U937 expression of FcγRI (143 bp), FcγRII (180 bp), FcγRIII (221 bp) and GAPDH (452 bp) by RT-PCR. B – Dose curves of FITC-labeled CRP pentamer and monomer binding to U937 monocytes. C – The competition of FITC-labeled CRP monomer binding with increasing concentrations of unlabeled CRP monomer.

Fig. 2. The FcγRs-independent binding of the CRP monomer to U937 monocytes. U937 monocytes were pre-incubated with 20 μg/ml anti-FcγRII mAb, 20 μg/ml anti-FcγRIII mAb or 50 μg/ml HAG for 30 min, followed by the addition of 50 μg/ml FITC-labeled CRP monomer. The U937 monocytes were also pre-treated with 15 μg/ml trypsin for 30 min before the binding assays.

Glycosaminoglycan is a class of non-protein surface receptor, but several major cellular glycans, including heparan sulfate and chondroitin sulfate A and B, failed to inhibit CRP monomer binding (Fig. 3A). To gain further insight into the forces underlying mCRP monomer interaction with U937 monocytes, cells with bound CRP monomer were treated with high levels of salt, acid or alkaline (Fig. 3B). High salt and acid rinses disrupt weak electrostatic interactions and strong
ligand-receptor binding, while an alkaline wash can reveal whether a protein is a membrane protein, because such harsh treatment will extract most of the peripheral proteins from the plasma membrane. However, these treatments yielded at best moderate inhibition, i.e. a ~35% decrease in CRP monomer binding due to the alkaline wash, suggesting that the cell-bound CRP monomer is incorporated into the plasma membrane. Cold triton extraction is routinely used to differentiate raft and non-raft resident membrane proteins. Interestingly, ~50% of the cell-associated CRP monomer, representing ~80% of the membrane-incorporated CRP monomer, is resistant to cold triton extraction. This indicates that membrane-inserted CRP monomer primarily locates in the cholesterol-enriched lipid raft microdomain.

Fig. 3. The incorporation of the CRP monomer into the plasma membrane. A – U937 monocytes were pre-incubated with 100 μg/ml heparan sulfate, chondroitin sulfate A or chondroitin sulfate B for 30 min followed by the addition of 50 μg/ml FITC-labeled CRP monomer. B – After incubation with FITC-labeled CRP monomer, the cells were treated for 5 min with a high salt (2 M NaCl), acid (pH 3.0) or alkaline (pH 11.3) buffer. Alternatively, CRP monomer-bound cells were extracted using cold triton for 15 min (1% triton X-100, 4ºC). The residue binding of CRP monomer was assessed as described in the Materials and Methods section. *p < 0.05 compared to the control.

We further investigated whether the CRP monomer can influence foam cell formation. U937 monocytes were first transformed into macrophages with PMA. A similar binding pattern was observed for the CRP monomer binding to the differentiated macrophages exhibiting upregulated CD32 and negative CD16 (not shown). As shown in Fig. 4A, incubating n-LDL with U937 macrophages for 16 h did not result in foam cell formation. No apparent difference was observed when n-LDL was added together with the CRP monomer. By contrast, adding ox-LDL lead to dramatic foam cell formation as evidenced by the massive accumulation of lipid droplets in the cytoplasm, showing punctuates and exceptionally bright Nile red staining [20]. The presence of the CRP monomer significantly reduced ox-LDL-induced intracellular lipid accumulation (~65% decrease in fluorescence intensities). In addition, the CRP monomer was also able to inhibit ox-LDL-induced lipid droplet accumulation in PBMC-
derived macrophages in a dose-dependent manner (Fig. 4B). These findings suggest that the CRP monomer was able to prevent foam cell formation.

![Image of Fig. 4](image)

**Fig. 4.** The inhibition of ox-LDL-induced foam cell formation by the CRP monomer. A – U937 macrophages were incubated with 20 μg/ml native LDL (n-LDL), 20 μg/ml LDL + 20 μg/ml CRP monomer (mCRP), 20 μg/ml ox-LDL or 20 μg/ml ox-LDL + 20 μg/ml CRP monomer for 16 h. The levels of intracellular lipid droplet accumulation and foam cell formation were determined by Nile red staining. One representative result is shown. B – PBMC-derived macrophages were incubated with or without 20 μg/ml ox-LDL in the presence of CRP monomer at the indicated concentrations for 16 h. The fluorescence of Nile red staining of each sample was then quantified. *p < 0.05 compared to the control.
DISCUSSION

Although the CRP monomer evokes cellular responses from neutrophils through CD16, the existence of additional receptors or pathways on neutrophils [10, 11] and other cell types [7, 8] has been proposed. In this study, using a CD16-negative U937 cell line, we demonstrated a specific cellular binding of the CRP monomer in a FcγRs-independent manner, consistent with the previous findings using endothelial cells [25]. By contrast, the association of the CRP monomer with U937 monocytes seems to be mediated by incorporation into the plasma membrane rather than by binding to a cell surface ligand. This conclusion is supported by three lines of evidence:

1. enzymatic digestion of the surface protein by trypsin was less efficient at suppressing CRP monomer binding, suggesting a protease-insensitive mechanism;
2. non-proteinous glycosaminoglycan ligands failed to inhibit CRP monomer binding;
3. cell-bound CRP monomer could not be released by high salt, acid or alkaline extraction, again excluding the involvement of ligand-receptor interactions.

It is still possible that the CRP monomer may bind to a trypsin-insensitive transmembrane receptor with exceptionally strong affinity and thus be resistant to harsh acidic dissociation. Nevertheless, our findings highlight that the CRP monomer may directly interact with monocytes independently of CD16. The uptake of modified LDL by monocyte-derived macrophages in plaque is the major route for foam cell formation, a critical event in atherogenesis [12]. Interestingly, we found that ox-LDL-induced accumulation of intracellular lipid droplets could be effectively inhibited by the CRP monomer. As the membrane binding of the CRP pentamer would lead to rapid conversion of the CRP monomer [6, 26], our data can be well reconciled with a recent report that the CRP pentamer can block foam cell formation induced by enzymatically modified LDL [27]. Together with the possible beneficial effects of the CRP monomer on complement regulation in plaques [28], our results suggest a protective role of the CRP monomer in atherogenesis, in agreement with the finding that the CRP monomer inhibits early progression of atherosclerosis in an ApoE−/− mice model [29]. Whereas these may be seemingly incompatible with the various pro-inflammatory actions of the CRP monomer on endothelial cells [7], platelets [8] and neutrophils [10], it would not be surprising for one protein to play different or even opposite roles at different stages of a disease. The balance of pro- and anti-atherosclerotic actions of the CRP monomer may be fine-tuned by the local microenvironment associated with the disease. Thus, extensive investigations will be required for a full understanding of the context-dependent contributions of the CRP monomer.

The CRP monomer was reported to form a complex with ox-LDL, and to inhibit its uptake by monocytes [30]. However, it is not likely that the < 30%
suppression in ox-LDL uptake [30] could account for the profound inhibition of the intracellular lipid droplet accumulation demonstrated herein. Rather, because the CRP monomer can incorporate into both the lipid shell of LDL particles [30] and the plasma membrane of macrophages, as shown in this study, these interactions may retard the cellular adsorption of lipids from LDL either at the cell surface or in the endocytic vesicles. Alternatively, the CRP monomer may directly change the profile of lipid metabolism in the monocytes. These interesting possibilities certainly deserve further investigation.

REFERENCES

1. Pepys, M.B. and Hirschfield, G.M. C-reactive protein: a critical update. J. Clin. Invest. 111 (2003) 1805-1812.
2. Boncler, M. and Watala, C. Regulation of cell function by isoforms of C-reactive protein: a comparative analysis. Acta Biochim. Pol. 56 (2009) 17-31.
3. Schwedler, S.B., Filep, J.G., Galle, J., Wanner, C. and Potempa, L.A. C-reactive protein: a family of proteins to regulate cardiovascular function. Am. J. Kidney Dis. 47 (2006) 212-222.
4. Verma, S., Devaraj, S. and Jialal, I. C-reactive protein promotes atherothrombosis. Circulation 113 (2006) 2135-2151.
5. Casas, J.P., Shah, T., Hingorani, A.D., Danesh, J. and Pepys, M.B. C-reactive protein and coronary heart disease: a critical review. J. Intern. Med. 264 (2008) 295-314.
6. Ji, S.R., Wu, Y., Zhu, L., Potempa, L.A., Sheng, F.L., Lu, W. and Zhao, J. Cell membranes and liposomes dissociate C-reactive protein (CRP) to form a new, biologically active structural intermediate: mCRP(m). FASEB J. 21 (2007) 284-294.
7. Khreiss, T., Jozsef, L., Potempa, L.A. and Filep, J.G. Conformational rearrangement in C-reactive protein is required for proinflammatory actions on human endothelial cells. Circulation 109 (2004) 2016-2022.
8. Molins, B., Pena, E., Vilahur, G., Mendieta, C., Slevin, M. and Badimon, L. C-Reactive Protein Isoforms Differ in Their Effects on Thrombus Growth. Arterioscler. Thromb. Vasc. Biol. 28 (2008) 2239-2246.
9. Khreiss, T., Jozsef, L., Potempa, L.A. and Filep, J.G. Opposing effects of C-reactive protein isoforms on shear-induced neutrophil-platelet adhesion and neutrophil aggregation in whole blood. Circulation 110 (2004) 2713-2720.
10. Khreiss, T., Jozsef, L., Potempa, L.A. and Filep, J.G. Loss of pentameric symmetry in C-reactive protein induces interleukin-8 secretion through peroxynitrite signaling in human neutrophils. Circ. Res. 97 (2005) 690-697.
11. Khreiss, T., Jozsef, L., Hossain, S., Chan, J.S., Potempa, L.A. and Filep, J.G. Loss of pentameric symmetry of C-reactive protein is associated with delayed apoptosis of human neutrophils. J. Biol. Chem. 277 (2002) 40775-40781.
12. Li, A.C. and Glass, C.K. The macrophage foam cell as a target for therapeutic intervention. Nat. Med. 8 (2002) 1235-1242.

13. Heuertz, R.M., Schneider, G.P., Potempa, L.A. and Webster, R.O. Native and modified C-reactive protein bind different receptors on human neutrophils. Int. J. Biochem. Cell Biol. 37 (2005) 320-335.

14. Potempa, L.A., Maldonado, B.A., Laurent, P., Zemel, E.S. and Gewurz, H. Antigenic, electrophoretic and binding alterations of human C-reactive protein modified selectively in the absence of calcium. Mol. Immunol. 20 (1983) 1165-1175.

15. Taskinen, S., Kovanen, P.T., Jarva, H., Meri, S. and Pentikainen, M.O. Binding of C-reactive protein to modified low-density-lipoprotein particles: identification of cholesterol as a novel ligand for C-reactive protein. Biochem. J. 367 (2002) 403-412.

16. Nagano, Y., Arai, H. and Kita, T. High density lipoprotein loses its effect to stimulate efflux of cholesterol from foam cell after oxidative modification. Proc. Natl. Acad. Sci. USA 88 (1991) 6457-6461.

17. Olsson, U., Camejo, G., Hurt-Camejo, E., Ehsber, K., Wiklund, O. and Bondjers, G. Possible functional interactions of apolipoprotein B-100 segments that associate with cell proteoglycans and the ApoB/E receptor. Arterioscler. Thromb. Vasc. Biol. 17 (1997) 149-155.

18. Bickel, P.E., Scherer, P.E., Schnitzer, J.E., Oh, P., Lisanti, M.P. and Lodish, H.F. Flotillin and epidermal surface antigen define a new family of caveolae-associated integral membrane proteins. J. Biol. Chem. 272 (1997) 13793-13802.

19. Fu, T. and Borensztajn, J. Macrophage uptake of low-density lipoprotein bound to aggregated C-reactive protein: possible mechanism of foam-cell formation in atherosclerotic lesions. Biochem. J. 366 (2002) 195-201.

20. Greenspan, P., Mayer, E.P. and Fowler, S.D. Nile red: a selective fluorescent stain for intracellular lipid droplets. J. Cell Biol. 100 (1985) 965-973.

21. Ryu, J., Lee, C.W., Shin, J.A., Park, C.S., Kim, J.J., Park, S.J. and Han, K.H. FegammaRIIa mediates C-reactive protein-induced inflammatory responses of human vascular smooth muscle cells by activating NADPH oxidase 4. Cardiovasc. Res. 75 (2007) 555-565.

22. Looney, R.J., Abraham, G.N. and Anderson, C.L. Human monocytes and U937 cells bear two distinct Fc receptors for IgG. J. Immunol. 136 (1986) 1641-1647.

23. Bharadwaj, D., Stein, M.P., Volzer, M., Mold, C. and Du Clos, T.W. The major receptor for C-reactive protein on leukocytes is fegamma receptor II. J. Exp. Med. 190 (1999) 585-590.

24. Crowell, R.E., Du Clos, T.W., Montoya, G., Heaphy, E. and Mold, C. C-reactive protein receptors on the human monocytic cell line U-937. Evidence for additional binding to Fc gamma RI. J. Immunol. 147 (1991) 3445-3451.

25. Ji, S.R., Ma, L., Bai C.J., Shi, J.M., Li, H.Y., Potempa, L.A., Filep, J.G., Zhao, J. and Wu, Y. Monomeric C-reactive protein activates endothelial
cells via interaction with lipid raft micro-domains. **FASEB J.** 23 (2009) 1806-1816.

26. Eisenhardt, S.U., Habersberger, J., Murphy, A., Chen, Y.C., Woollard, K.J., Bassler, N., Qian, H., von Zur Muhlen, C., Hagemeyer, C.E., Ahrens, I., Chin-Dusting, J., Bobik, A. and Peter, K. Dissociation of pentameric to monomeric C-reactive protein on activated platelets localizes inflammation to atherosclerotic plaques. **Circ. Res.** 105 (2009) 128-137.

27. Singh, S.K., Suresh, M.V., Prayther, D.C., Moorman, J.P., Rusinol, A.E. and Agrawal, A. C-reactive protein-bound enzymatically modified low-density lipoprotein does not transform macrophages into foam cells. **J. Immunol.** 180 (2008) 4316-4322.

28. Ji, S.R., Wu, Y., Potempa, L.A., Liang, Y.H. and Zhao, J. Effect of modified C-reactive protein on complement Activation. A possible complement regulatory role of modified or monomeric C-reactive protein in atherosclerotic lesions. **Arterioscler. Thromb. Vasc. Biol.** 26 (2006) 935-941.

29. Schwedler, S.B., Amann, K., Wernicke, K., Krebs, A., Nauck, M., Wanner, C., Potempa, L.A. and Galle, J. Native C-reactive protein increases whereas modified C-reactive protein reduces atherosclerosis in apolipoprotein E-knockout mice. **Circulation** 112 (2005) 1016-1023.

30. Ji, S.R., Wu, Y., Potempa, L.A., Qiu, Q. and Zhao, J. Interactions of C-reactive protein with low density lipoproteins: implications for an active role of modified C-reactive protein in atherosclerosis. **Int. J. Biochem. Cell Biol.** 38 (2006) 648-661.