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C-Reactive Protein Mediated FcγReceptor Signal Transduction Is Enhanced by Low Density Lipoprotein In Human Macrophages

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Abbreviations:

CRP: C-reactive protein

E-LDL: enzymatically modified low density lipoprotein

FcγR(s): Fcγ receptor(s)

FcγRI: Fcγ receptor I

FcγRIIa: Fcγ receptor IIa

IgG: Immunoglobulin G

LDL: low density lipoprotein

NaAz: Sodium azide

oxLDL: oxidized low density lipoprotein

PBMC: peripheral human blood monocyte(s)

1. Introduction:

Foam cells in atherosclerotic lesions are believed to derive from macrophages that have taken up aggregated low density lipoprotein (LDL) particles [14,25,32]. C-reactive protein (CRP), the prototypic acute phase reactant in humans [58], colocalizes with LDL and macrophages in atherosclerotic lesions [37,53]. Its role in atherogenesis, however, is still controversial.

Various gene knockouts and transgenic experiments have demonstrated the pivotal role of inflammation during all stages of atherogenesis, from the onset of endothelial cell dysfunction, adhesion of mononuclear cells to the incorporation of lipids by macrophages, which is the most important part of the initiation of fatty streak [14,19,25,43]. Among numerous inflammatory biomarkers, CRP is proposed to be an important risk marker for cardiovascular disease and is recommended for primary prevention [40]. Furthermore, CRP has been implicated to play a potential active role in promoting atherothrombosis [29,33,45,51,52,56]. The latter, however, is still under debate. Vast amounts of published data supports that CRP authentically participates in atherogenesis yet the molecular mechanisms are still poorly understood. The binding and interaction between CRP and LDL has been known for decades [12]. Since then, CRP has been reported to bind to different types of LDL, i.e., native LDL [49,60], oxidized LDL (oxLDL) [7] or enzymatically modified LDL (E-LDL) [3]. This binding has been suggested to be critical to the active role of CRP in atherogenesis.

Fcγ receptor I (FcγRI) [1,5,6,27] and Fcγ receptor IIa (FcγRIIa) [1,4,6,8,47] have been proposed to be the major receptors for CRP on phagocytes. FcγRI and FcγRII are naturally expressed on human monocytes and monocyte-derived macrophages. Fcγ receptors (FcγRs) mediate a number of responses crucial for host immunity. One of the most important functions of FcγRs on macrophages is their ability to promote phagocytosis [36]. Notably, phagocytic capacity and other effector

functions of mononuclear phagocytes change during differentiation/maturation of these cells [18]. After FcγR cross-linking, signaling events necessary for these responses are initiated by Src-family and Syk-family tyrosine kinases which become activated and associate with specific recognition sequences known as immunoreceptor tyrosine-based activation motifs (ITAMs), contained within the intracellular domains of some of the FcγR subunits [2,35]. Following FcγR engagement in macrophages, cytoplasmic tyrosine kinase Syk -the direct mediator of FcγR signaling is associated with the γ-chain and becomes phosphorylated on a tyrosine residue, and is enzymatically activated [9,21,28,46]. Thus, Syk couples the activated immunoreceptors to downstream signaling events that mediate diverse cellular responses and its phosphorylation is essential for FcγR-mediated phagocytosis on macrophages [9,21,28].

Recent studies by our groups have confirmed and manifested virtual binding and specific interactions between CRP and FcγRs [26,42,54,60].To further explore the mechanisms involved in atherogenesis, we investigated whether CRP binding to LDL may induce and enhance signal transduction of FcγR by activation of Syk kinase phosphorylation in monocytes and macrophages and whether this mechanism may contribute to the uptake and degradation of LDL during atherogenesis. Consequently, the focus of our study is to examine whether CRP can trigger Syk kinase phosphorylation on human monocytes and macrophages and whether this function can be enhanced by the involvement of LDL.

2. Materials and methods:

2.1. Reagents:

Native CRP from human ascites was purchased from Calbiochem, Merck (Darmstadt, Germany). Performa DTR gel filtration cartridges used for purifying human CRP and LDL were purchased from Edge Bio Systems (Gaithersburg, MD, USA) [26,42,46]. Native LDL from human plasma was purchased from Calbiochem, Merck (Darmstadt, Germany). IgG (Immunoglobulin G) from human serum was purchased from Biochemika, Fluka (Buchs, Switzerland). Na_3VO_4 was purchased from Sigma (St. Louis, MO, USA). Dulbecco's phosphate-buffered saline solution (PBS) with or without Ca^{2+} and Mg^{2+} were from PAA Laboratories (Pasching, Austria). Medium for monocyte culture consists of RPMI 1640 with L-glutamine obtained from PAA Laboratories (Linz, Austria) containing 5% human serum from PAA Laboratories (Pasching, Austria). Hank's balanced salt solution (HBSS) from PAA Laboratories (Linz, Austria) and lymphocyte separation medium 1077 from PAA Laboratories (Pasching, Austria) were used for monocyte isolation. Lysis buffer for macrophages was prepared with protease inhibitor cocktail tablets (EDTA -free) from Roche Diagnostics (Mannheim, Germany), Phosphatase inhibitor cocktail 1, 2 from Sigma (St. Louis, MO, USA) and DL-Dithiothreitol purchased from Sigma-Aldrich (St. Louis, MO, USA). NuPAGE Novex Bis-Tris mini gels were purchased from Invitrogen (Carlsbad, CA, USA). Precision Plus protein standards were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Phospho-Syk (Tyr525/526) (C87C1) rabbit mAb, Syk antibody and anti-rabbit IgG (HRP-linked antibody) were purchased from Cell Signaling Technology (Danvers, MA, USA). SuperSignal West Femto maximum sensitivity substrate and other ECL reagents used in immunoblotting were purchased from Thermo Scientific (Rockford, IL, USA).

2.2. Purification of CRP:

Purification of human CRP was performed with Performa DTR gel filtration

cartridges as described in previous reports [26,28,42,46,54]. IgG-contamination of final CRP-preparation was excluded by Western blot analysis.

2.3. Preparation of CRP-LDL complex:

Human CRP at various concentrations was coincubated with native LDL at 50 mg/L in PBS containing $CaCl_2$ (0.132 g/L) and $MgCl_2$ (0.1 g/L) at 37°C for 30 min in order to form CRP-LDL complexes [60].

2.4. Cell isolation and culture:

Peripheral blood monocytes were isolated from leukocyte-enriched buffy coats obtained from blood of healthy donors [53]. Cell suspensions were adjusted to densities of 1~2 × 10^6/mL and distributed in 12-well plates. Cells were seeded at 2~4 × 10^6 per well in culture plates and cultured in RPMI 1640 medium containing 5% human serum at 37°C in 5% CO_2 for 7 days to differentiate into macrophages [60]. Medium was renewed every 2 days.

2.5. Flow cytometry:

Flow cytometric analysis of human peripheral blood monocytes and macrophages was performed as described in our previous studies on transfected COS-7 cells [42] and Mono Mac 6 cells [54]. Briefly, cells were detached with Accutase (PAA Labs, Linz, Austria) and sedimented by centrifugation. The pellets were washed twice with PBS/0.1% BSA solution, resuspended in the same buffer and incubated with monoclonal FITC-conjugated anti-CD32, anti-CD64 (both at a dilution of 1:20) for 30 min at 4°C in the dark. The cells were then washed twice, resuspended in 500 µl of PBS and then subjected to analysis by flow cytometry with CellQuest software (BD Biosciences, Heidelberg, Germany). Forward and side scatter was used to gate cell population and to exclude cell debris. A total of 10 000 cells were gated for histogram plots. 97% of untreated cells were assessed as background (negative control).

2.6. Cell stimulation:

Freshly isolated monocytes and macrophages were serum-starved for 2 h and then stimulated with different concentrations of CRP (purified and unpurified), LDL and CRP-LDL complex, respectively, for 3 min. Human IgG at 100 μg/mL was set as a positive control in each group.

2.7. Protein preparation:

After stimulation, cells were immediately washed twice with cold PBS (4°C) without Ca^{2+} and Mg^{2+} to remove residual medium and kept on ice. Cells were solubilized in lysis buffer consisting of Tris HCl, NaCl, Glycerol, Triton (Dithiothreitol, phosphatase inhibitors 1/2, protease inhibitor and NaF were freshly added) and scratched off from the bottoms of culture plates. Suspensions were frozen in liquid N2 for 1 min. The thawed suspensions were centrifuged at 18000 × g at 4°C for 10 min to remove nuclei and large debris. The protein concentration of the supernatant was measured by the bicinchoninic acid method.

2.8. Immunoblotting:

After solubilization in lysis buffer, equal amounts of sample protein (50~80 μg/lane) were separated along with Precision Plus protein standards by SDS-PAGE gels at 200 V for 40 min and then transferred to nitrocellulose membranes at 30 V for 1 h. The membranes were saturated with 5% powdered milk (Biochemika, Fluka; Buchs, Switzerland) in TBS plus 0.1% Tween 20 for 1 h and then incubated with anti-phosphorylated-Syk mAb (1:1000) at 4°C overnight. The membranes were washed with 0.1% TBS/T and then incubated with HRP-labeled second reagent (1:2000) for 1 h. The assayed membranes were reprobed with anti-Syk mAb (1:1000) after stripping.

2.9. Analysis of phosphorylated signals:

The phosphorylated signals were detected by chemoluminescence and their relative intensities were quantitatively assessed by the Fuji image gauge program.

Autoradiograms were developed on film (Kodak BioMax; Rochester, NY, USA).

2.10. Statistical analysis:

We analyzed our data with Sigma v.3.5 program. Since we have 5-7 levels (various stimuli including human CRP, LDL, IgG etc.) with 1 factor (the same sort of cells: human macrophages or peripheral blood monocytes), we have chosen ANOVA method for our analysis. Results of ANOVA indicated statistical difference existed in the mean scores of the intensity of Syk kinase phosphorylation when $P<0.05$ and significant difference existed when $P<0.001$, suggesting that the intensities varied when different stimuli were given. No statistical differences were found between the compared scores when $P>=0.05$. Futhermore, the results of a series of LSD analysis revealed the most powerful and the weakest stimulus on the Syk phosphorylating effect among the stimuli.

3. Results:

3.1. FcγRI and FcγRII expression on human peripheral blood monocytes and macrophages:

According to previous reports, FcγRs are proposed to be the major receptors for CRP [1,4,5,6,8,26,27,42,47,54]. They appear to be the potential mediators of CRP -effects on macrophages. In order to examine the natural expression of FcγRI and FcγRII on human peripheral blood monocytes and macrophages, flow cytometry with respective FITC-labeled specific antibodies was performed. Staining with anti-CD32-FITC revealed 70% FcvRIIa and 61% FcγRI positivity for monocytes and 79% FcγRIIa and 62% FcγRI positivity for macrophages (Figure 1, 2). 97% of untreated cells from negative control were excluded as background.

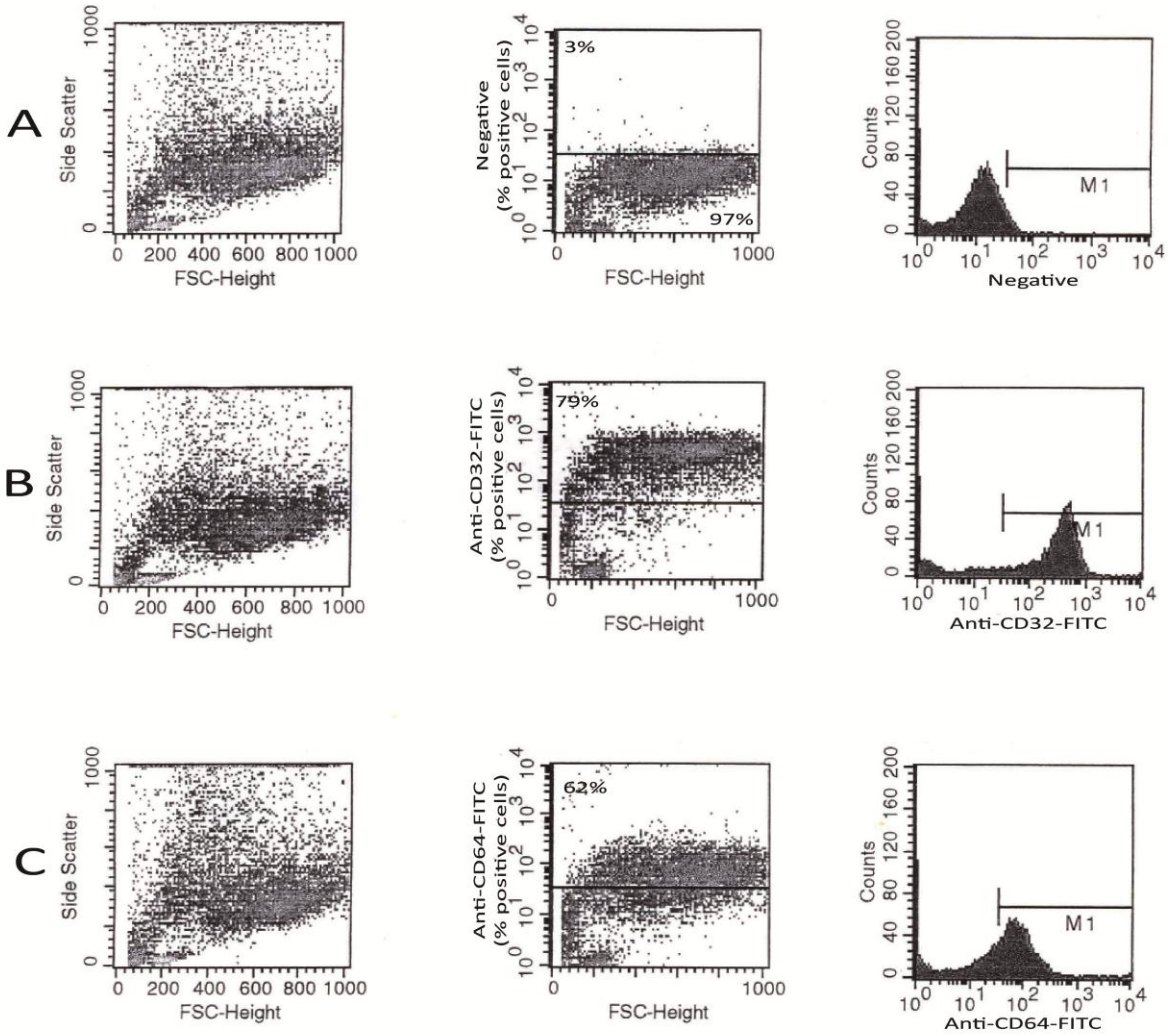


Figure 1. Flow cytometry analysis of natural FcγR expression on human macrophages

Flow cytometry analysis was performed on macrophages using anti-CD32-FITC and anti-CD64-FITC. A: Of untreated cells from negative control, 97% were excluded as background. B: Staining with anti-CD32-FITC revealed 79% positive cells for FcγRII. C: Staining with anti-CD64-FITC revealed 62% positivity for FcγRI.

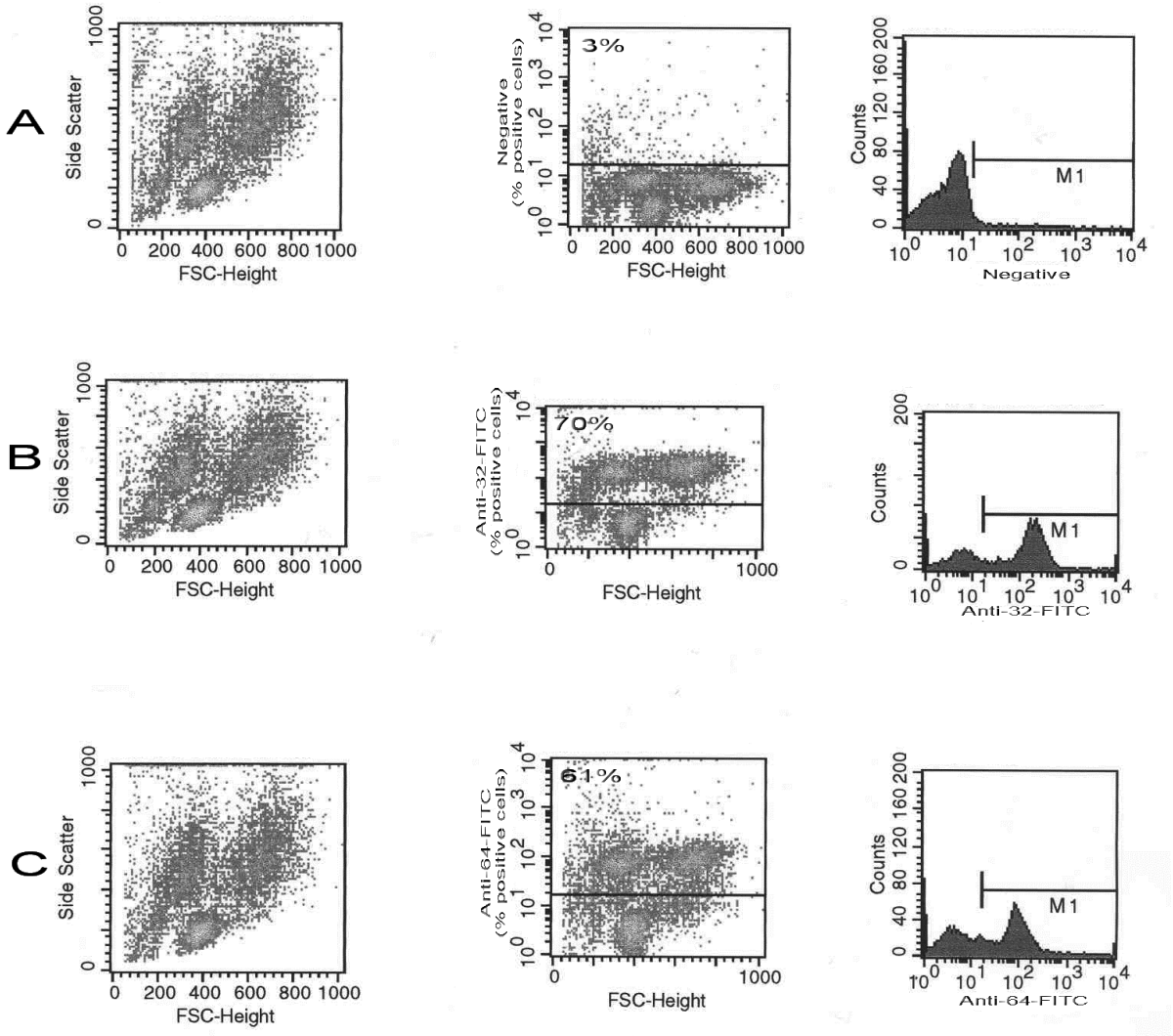


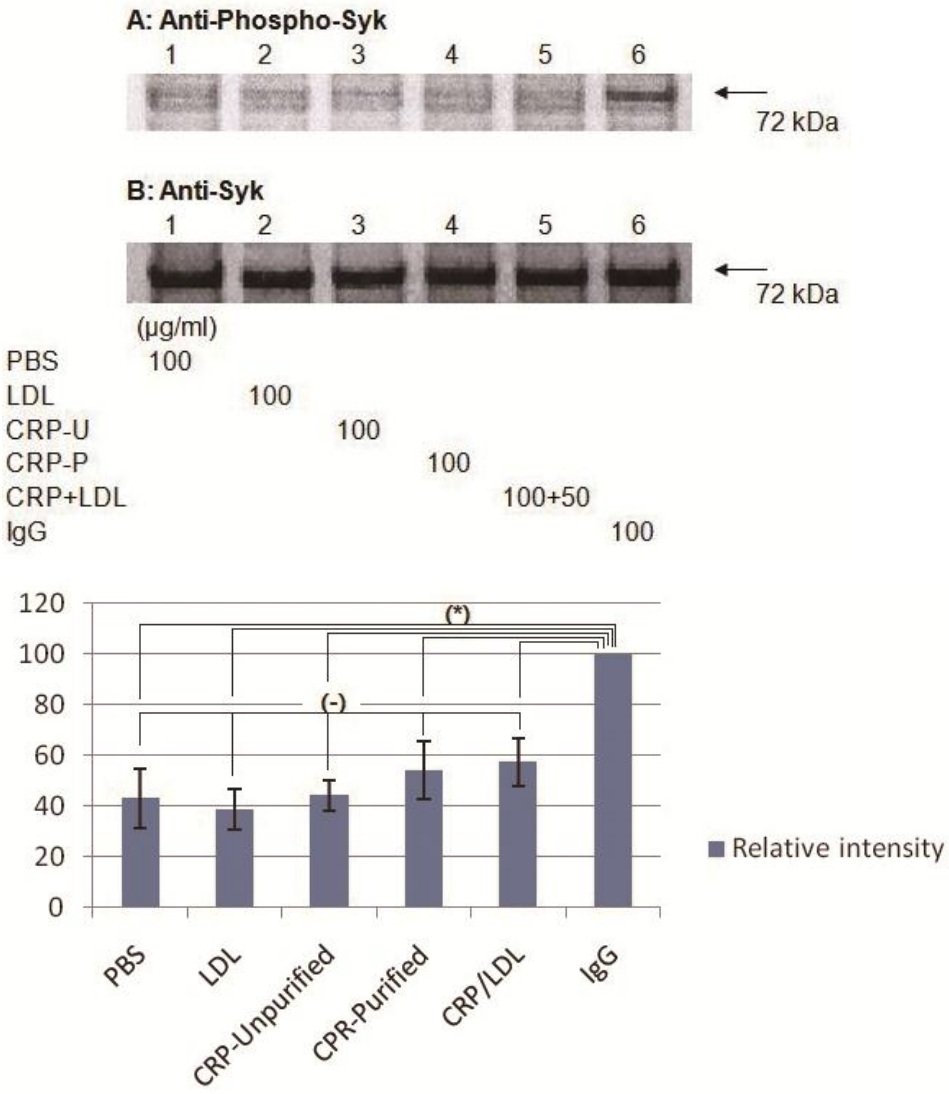
Figure 2. Flow cytometry analysis of natural FcγR expression on human peripheral blood monocytes

Flow cytometry analysis was performed on human peripheral blood monocytes using anti-CD32-FITC and anti-CD64-FITC. A: Of untreated cells from negative control, 97% were excluded as background. B: Staining with anti-CD32-FITC revealed 70% positive cells for FcγRII. C: Staining with anti-CD64-FITC revealed 61% positivity for FcγRI.

3.2. Effects of CRP on Syk phosphorylation:

Binding and specific interactions between CRP and FcγR have consistently been reported [1,4,5,6,8,27,47]. The phosphorylation of Syk kinase is essential for the activation of FcγR signaling and subsequent FcγR-mediated phagocytosis [9,21,28]. Macrophages in atherosclerotic tissue derive from blood monocytes that mature into macrophages during their migration into tissues, i.e., the prototype phagocyte. Therefore, we investigated and compared the effect of CRP on Syk phosphorylation by stimulating freshly isolated peripheral blood monocytes and macrophages with human CRP.

In the first series of experiments, cells were exposed to the following stimuli: CRP (purified and unpurified, 100 µg/mL), LDL (100 µg/mL) and CRP-LDL complex (100 µg/mL-50 µg/mL) for identical incubation intervals of 3 min at 37°C. The intensity of the phosphorylated signal in response to human IgG at 100 µg/mL was set as a positive control. PBS, NaCl and sodium azide (NaAz) (0.1%) served as negative controls. In freshly isolated peripheral blood monocytes, CRP and other stimuli yielded similarly trivial phosphorylation signals as compared to the signal intensity triggered by human IgG (Figure 3). Remarkably, CRP at 100 µg/mL triggers a notably higher signal intensity in macrophages similar to that induced by human IgG at identical concentration. The maximum intensity level of signals was reproducibly recorded in the lane of the CRP-LDL complex in macrophages (Figure 4).

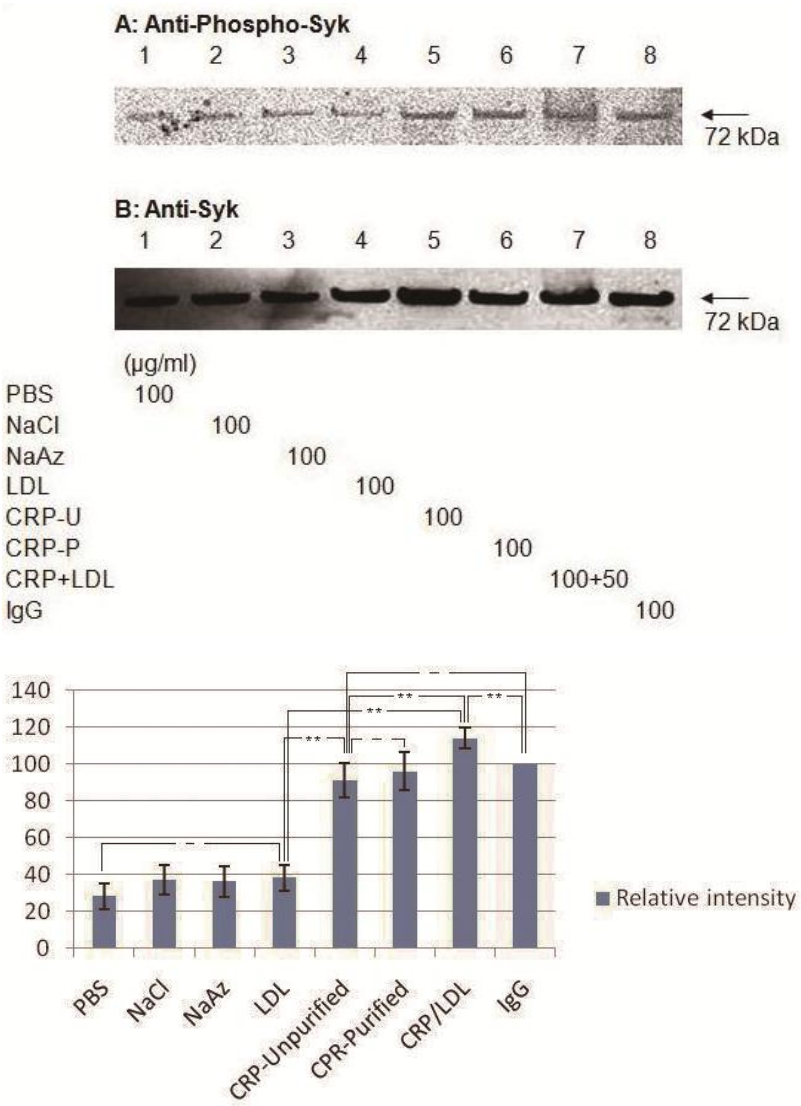


-: There is no statistical difference between groups. *: There is a statistical difference between groups ($p<0.05$). **:There is a statistically significant difference between groups ($p<0.001$)

Abbreviations: CRP: C-reactive protein; CRP/LDL: the complex of C-reactive protein and low density lipoprotein; IgG: Immunoglobulin G; LDL: low density lipoprotein; PBS: Dulbecco's phosphate-buffered saline solution

Figure 3. Western Blot and relative intensity of phosphorylated Syk

Freshly isolated peripheral blood monocytes at 2.5x106 /well (in 1 ml medium without serum) were exposed to various stimuli at 37°C for 3 min. Human IgG at 100 μg/mL was set as a positive control (100% relative signal intensity). A: Membranes were probed with anti-phospho-Syk and the relative intensity of induced signals was measured as shown. B: Membranes were reprobed with anti-Syk. Specific ~72 kDa bands are indicated by arrows. The blot is one of our four identical experiments.



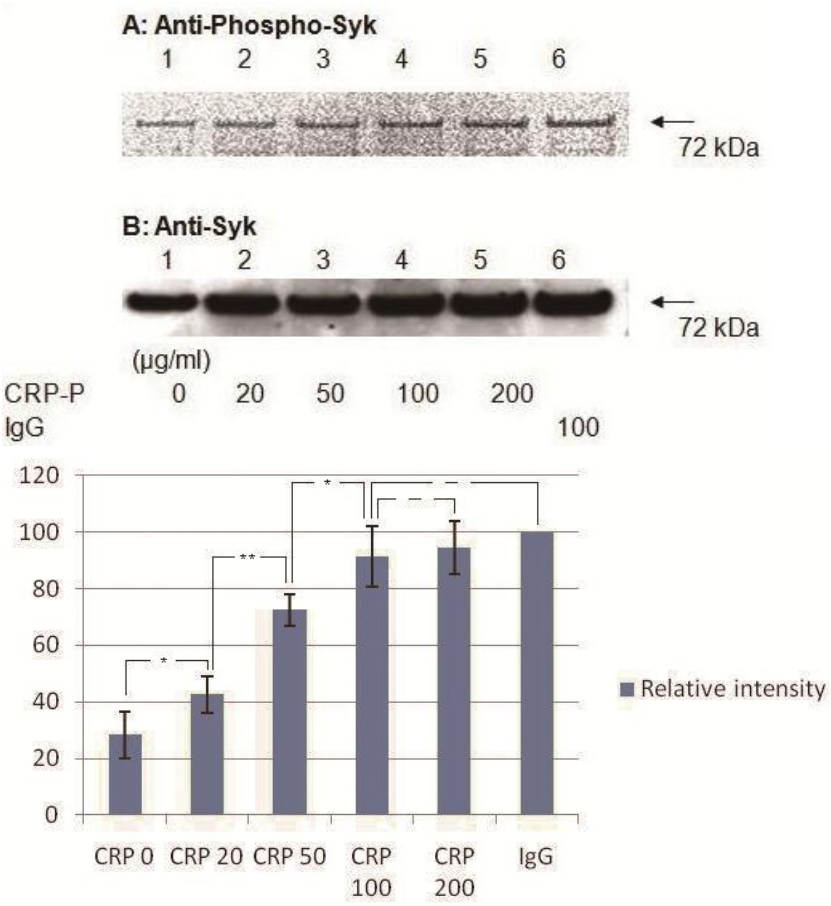
-: There is no statistical difference between groups. *: There is a statistical difference between groups ($p<0.05$). **: There is a statistically significant difference between groups ($p<0.001$)

Abbreviations: CRP: C-reactive protein; CRP/LDL: the complex of C-reactive protein and low density lipoprotein; IgG: Immunoglobulin G; LDL: low density lipoprotein; NaAz: Sodium azide; PBS: Dulbecco's phosphate-buffered saline solution

Figure 4. Western Blot and relative intensity of phosphorylated Syk

Macrophages at $3x10^6$ /well (in 1 ml medium without serum) were coincubated with the described stimuli at the concentration of 100 µg/mL at 37°C for 3 min. Human IgG at 100 µg/mL was set as positive control (100% relative signal intensity). A: Membranes were probed with anti-phospho-Syk and the relative intensity of induced signals was measured. B: Membranes were reprobed with anti-Syk. Specific ~72 kDa bands are indicated by arrows. The blot shown is representative for five identical experiments.

Next, macrophages were incubated with different concentrations of purified-CRP at 37°C for 3 min (Figure 5). The activating effect of CRP on Syk phosphorylation occurred in a concentration-dependent manner. Purified CRP at acute phase levels (≥ 100 μg/mL) induced phosphorylated signals at similar level as human IgG. Similar amounts of Syk protein were confirmed by membrane reprobing with anti-Syk mAb.



-: There is no statistical difference between groups. *: There is a statistical difference between groups ($p<0.05$). **: There is a statistically significant difference between groups ($p<0.001$))

Abbreviations: CRP: C-reactive protein; IgG: Immunoglobulin G

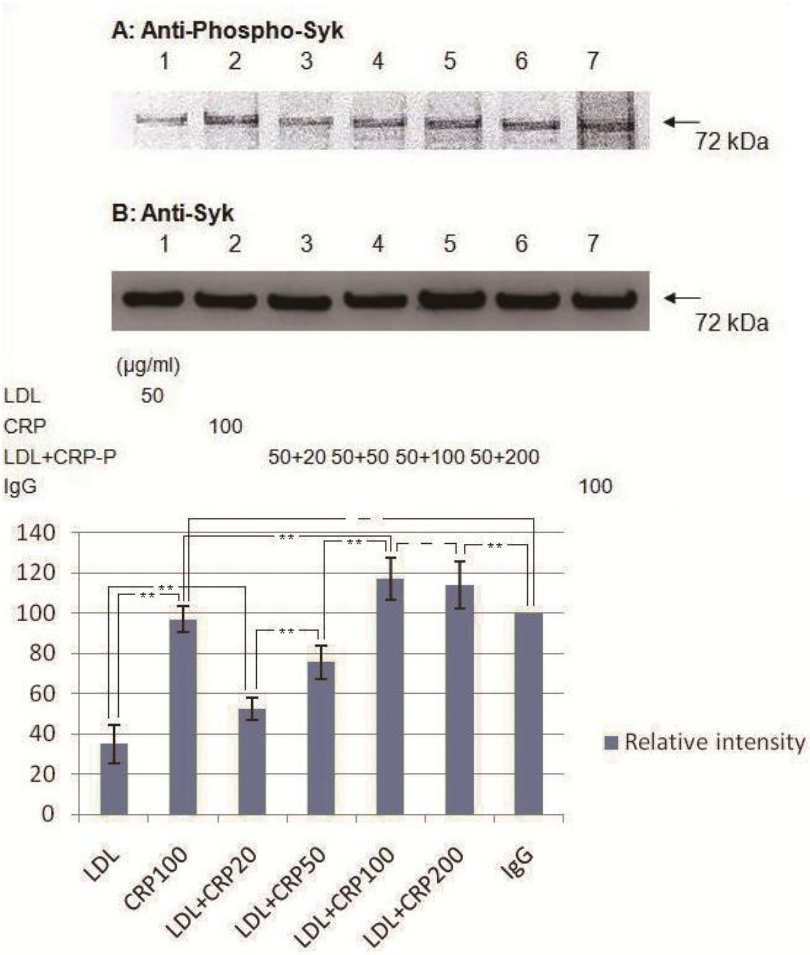
Figure 5. Western Blot and relative intensity of phosphorylated Syk

Macrophages at $4x10^6$ /well were incubated with various preparations of purified CRP with different concentrations at 37°C for 3 min. Human IgG at 100 μg/mL was set as positive control (100% relative signal intensity). A: Results from membrane probed with anti-phospho-Syk and relative intensity of signals are shown. B: The same membrane was reprobed with anti-Syk. Specific ~72 kDa bands are shown by arrows. This blot is one of five identical experiments.

3.3. Effects of CRP-LDL complex on Syk phosphorylation:

The opsonising property to biological particles and the binding to LDL are proposed as possible mechanisms by which CRP promotes uptake of LDL during atherogenesis. Opsonization of native LDL by CRP during LDL engulfment by macrophages has been proposed by Zwaka et al. [60].

Different doses of purified-CRP were coincubated with native LDL in order to yield various CRP-LDL complexes (Figure 6). The increasing doses of applied CRP markedly potentiate the promoting effect on Syk phosphorylation in macrophages. The most pronounced signals occurred in the lanes of complexes with CRP at acute phase concentrations. Besides, it is noteworthy that LDL itself only induced trivial phosphorylated signaling. Acute phase level CRP incubated with LDL induced a significantly stronger phosphorylated signal than that observed from LDL alone or CRP alone. We can therefore conclude that the binding of CRP to LDL evidently enhances the activation of Syk phosphorylation.



-: There is no statistical difference between groups. *: There is a statistical difference between groups ($p<0.05$). **: There is a statistically significant difference between groups ($p<0.001$)

Abbreviations: CRP: C-reactive protein; CRP100: C-reactive protein (100 μg/mL); IgG: Immunoglobulin G; LDL: low density lipoprotein; LDL+CRP20: the complex of C-reactive protein (20 μg/mL) and low density lipoprotein (50 μg/mL) ; LDL+CRP50: the complex of C-reactive protein (50 μg/mL) and low density lipoprotein (50 μg/mL) ; LDL+CRP100: the complex of C-reactive protein (100 μg/mL) and low density lipoprotein (50 μg/mL) ; LDL+CRP200: the complex of C-reactive protein (200 μg/mL) and low density lipoprotein (50 μg/mL)

Figure 6. Western Blot and relative intensity of phosphorylated Syk

Macrophages at $3x10^6$ /well were stimulated for 3 min with 50 μg/mL LDL, 100 μg/mL purified CRP and various preparations of CRP-LDL complex with different concentrations of purified CRP. Human IgG at 100 μg/mL was set as positive control (100% relative signal intensity). A: The membrane was probed with anti-phospho-Syk and the results of measured relative intensity of signals are shown in the figure below. B: The same membrane was reprobed with anti-Syk. Specific ~72 kDa bands are indicated. The blot shown is representative of five identical experiments.

4. Discussion:

Our present study on the effect of CRP on the phosphorylation of Syk kinase suggests that: (1) Human CRP at acute phase level concentration can trigger tyrosine phosphorylation of the immunoreceptor-tyrosine based activation motif of Syk kinase in human macrophages, but not in monocytes (2) The activating effect of CRP on FcγR-mediated phagocytosis is strongly correlated with the ascending concentration of CRP up to acute phase level and (3) The indicated effect of CRP is markedly enhanced in the presence of LDL.

Abundant experimental evidence supports a pivotal role for inflammation in the evolution of atherosclerosis [19,43]. Monocytes enter the atherosclerotic tissue from the blood stream and transform into macrophages. Macrophages engulf tissue-retained LDL to become foam cells and the lipid-laden macrophages accumulate in the arterial intima, forming fatty streak lesions. CRP, the prototypic inflammatory biomarker was found to deposit in atheroma along with LDL, macrophages [37,53] and terminal complement complex [50]. However, its biological role is unclear. Scientific interest was attracted by these findings and the role of CRP as a risk marker for the prediction of primary and secondary cardiovascular events (as confirmed by several large and well-controlled trials [11,20,22,38,39,40,41,59]) potentiated this interest. In addition to being a risk marker, there is indeed much evidence that CRP may be a culprit that worsens atherogenesis. Despite considerable research on this issue a general academic consensus has not yet been reached.

CRP is an ancient immune molecule that shares many functional properties with antibodies: it binds to a variety of ligands, activates complement, opsonizes biological particles and binds to and signals via FcγRs [1,4,5,6,8,27,47]. Thus, CRP may be the most primitive antibody in the evolution of the mammalian immune system. Many theories have been raised on potential mechanisms by which CRP

might contribute to cardiovascular disease at the molecular level. Over the last decade, various groups have provided in vivo evidence on the role of CRP in atherogenesis [10,16,17,23,31,48]. Human CRP (hCRP) has been shown to have the ability to promote thrombotic occlusion and to trigger development of larger infarct size in animal models [10,16,17,31]. In 2005, Jianglin Fan and his colleagues reported substantial correlation between plasma CRP and the degree of atherosclerosis in cholesterol-fed rabbits as well as deposition of the rabbit CRP in atherosclerotic lesions of these animals [31].

Of all these observations, the binding of CRP to LDL is the most consistent and is believed to lie at the core of a potential proatherogenic effect. Over the last two decades, many scientific efforts have been devoted to the study of the nature of this binding, preferably with different modifications of LDL. Furthermore, many published studies have shown binding of CRP to native LDL attached to a solid surface or collagen fibers [7,15,55]. The central role of Syk in FcγR-mediated phagocytosis on macrophages has been confirmed by studies of Syk-deficient murine macrophages [9,21]. In our study, combining our findings of the effects of CRP and CRP-LDL complex on Syk phosphorylation with the investigation of CRP-LDL binding and previous observations of the specific interactions between CRP and FcγRs [26,33,42,54], we make the assumption that CRP may contribute to the uptake of native LDL by macrophages in atherogenesis via both, binding to LDL and initiating FcγR-mediated phagocytosis by activation of Syk kinase phosphorylation.

By now, CRP has been proposed to contribute to atherosclerosis via its activating effects to monocytes/macrophages [53,60], endothelial cells [13,30] and vascular smooth muscles cells [57]. The results of our study again support the concept that CRP exerts its biological functions predominantly through activation of immune cells, especially through macrophages, which express FcγRs at high levels. Phagocytic capacity of mononuclear phagocytes is enhanced by cell differentiation.

This is a complex process governed by different soluble and micro-environmental factors, giving rise to the distinct phenotypic characteristics of the cells. Ortega and his colleagues [18] have observed a 10-fold increase in phagocytic capacity after calcitriol treatment, but it is not accompanied by an increase in FcγR expression. In contrast, the phosphorylation levels of Lyn and Syk after FcγRI or FcγRII crosslinking are increased. Their study demonstrates that signaling induced by FcγR in mononuclear phagocytes is not only dependent on the quantity of FcγRs aggregated by a stimulus, but it is also highly dependent on the cells' maturation state. We made the same observation in our experiments with human peripheral blood monocytes and mature macrophages. Whereas FcγR expression does not differ significantly between both cell types, the maturation state of these mononuclear cells determines the response to CRP/LDL complexes. The latter seems biologically very plausible because peripheral blood monocytes are in continuous contact with CRP and LDL in the blood stream. They do not respond to CRP and LDL by Syk activation. When LDL deposits the arterial wall in combination with CRP, monocytes enter the tissue, transform into macrophages, and begin to recognize tissue deposited CRP-opsonized LDL as a foreign antigen. Only tissue deposited LDL needs to be phagocytosed and degraded.

Recently, the JUPITER trial has refocused research interest on the role of CRP in cardiovascular disease [40]. The results from JUPITER demonstrated that cardiovascular events and mortality risk could be significantly lowered by treating patients who have normal LDL-C but elevated high-sensitivity CRP (hsCRP) with high-dose statin therapy. Some voices consequently suggest that CRP targeting may be a worthwhile approach for the primary and secondary prevention of cardiovascular disease [20,34].

In summary, our study has provided new insights into the question of how CRP, LDL, FcγRs, Syk and macrophages are orchestrated in the evolution of atherosclerosis. It may be interesting to hypothesize that, in the context of

cardiovascular disease, CRP may function similarly to an antibody that recognizes tissue-deposited LDL as a foreign antigen for macrophages.

5. Summary:

C-reactive protein (CRP) and low density lipoprotein (LDL) are risk markers for cardiovascular disease. In macrophages, Fcγ receptor I (FcγRI) and Fcγ receptor IIa (FcγRIIa) are proposed to be the major receptors for CRP. Syk kinase is essential for Fcγ receptor signaling in phagocytosis. CRP binds to LDL and both proteins deposit in atherosclerotic lesions. CRP has been implicated as a pivotal player in promoting atherothrombosis. However, it's role in atherogenesis is still controversial.

In order to explore the mechanisms involved in atherogenesis, we investigated whether CRP binding to LDL may induce and enhance signal transduction of FcγR by activation of Syk kinase phosphorylation in monocytes and macrophages and whether this mechanism may contribute to the uptake and degradation of LDL during atherogenesis. Consequently, the focus of our study is to examine whether CRP can trigger Syk kinase phosphorylation on human monocytes and macrophages and whether this function can be enhanced by the involvement of LDL.

Peripheral blood monocytes were isolated from buffy coats obtained from the blood of healthy donors. Cells were cultured for 7 days to induce transformation into macrophages. Fluorescence activated cell sorting analysis were performed respectively to peripheral human blood monocytes and mature macrophages. Peripheral human blood monocytes and mature macrophages were exposed to CRP and LDL. After cell lysis, equal amounts of protein were assayed by immunoblotting in order to investigate Syk kinase phosphorylation.

Fluorescence activated cell sorting analysis of monocytes and macrophages revealed 70% FcγRII and 61% FcγRI-, and 79% FcγRII and 62% FcγRI positivity, respectively. Syk kinase phosphorylation was assayed by immunoblotting.

Induction of Syk kinase phosphorylation was not observed in monocytes. In macrophages, however, Syk kinase phosphorylation in response to CRP (100 µg/mL) is equal to that induced by human IgG. Significant, and CRP dose-dependent, induction of the signal was observed in response to the CRP-LDL complex (50 µg/mL LDL). LDL by itself (50 µg/mL) only induced trivial Syk kinase signaling.

Our present study on the effect of CRP on the phosphorylation of Syk kinase suggests that: (1) Human CRP at acute phase level concentration can trigger tyrosine phosphorylation of the immunoreceptor-tyrosine based activation motif of Syk kinase in human macrophages, but not in monocytes (2) The activating effect of CRP on FcγR-mediated phagocytosis is strongly correlated with the ascending concentration of CRP up to acute phase level and (3) The indicated effect of CRP is markedly enhanced in the presence of LDL.

In summary, human CRP-mediated tyrosine phosphorylation of the immunoreceptor-tyrosine based activation motif of Syk kinase in human macrophages is enhanced by LDL. CRP may recognize human tissue-deposited LDL as a foreign antigen.

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