In vitro effects of azide-containing human CRP isoforms and oxLDL on U937-derived macrophage production of atherosclerosis-related cytokines

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Abstract. Atherosclerosis is an inflammatory chronic disease of the arterial wall. Monomeric (m) and pentameric (p) C-reactive protein (CRP) and oxidized low density lipoproteins (oxLDL) seem to affect the pattern of cytokine production by macrophages, thus playing an important role in atherogenesis. Azide, the commercial preservative of CRP, may influence its action *in vitro*. The present study aimed to determine the effects of both isoforms of azide-containing CRP (mCRP and pCRP) with and without oxLDL on cytokine production by U937-derived macrophages. U937 monocytes were cultured and differentiated into macrophages and treated with mCRP, pCRP, oxLDL and azide individually and in combination. ELISA were performed to measure the levels of interferon-γ (IFN-γ), interleukin (IL)-4, IL-6, IL-10 and tumor necrosis factor (TNF)-α in culture supernatants collected from U937-derived macrophages following their respective treatments. Most single and combined treatments, especially in triple combination, were able to downregulate the levels of IFN-γ and IL-6 compared with control untreated cells, whilst the combination of mCRP and pCRP increased IL-4 levels. Regarding IL-10, except for an increase induced by mCRP, no significant effect was caused by any treatment compared with the control. On the other hand, the levels of TNF-α were not significantly affected by any treatment except for a decreasing trend that was observed with mCRP/oxLDL treatment compared with control. By contrast, double azide caused a significant decrease in the levels of IFN-γ and IL-6. The results of the present study indicated that mCRP, pCRP, oxLD and possibly azide, individually or in different combinations, had the tendency to upregulate the expression of IL-4 and to downregulate that of the pro-atherogenic cytokines, IFN-γ and IL-6, suggesting that the intima microenvironment serves a crucial role in atherogenesis.

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Introduction

Cardiovascular diseases such as ischemic heart disease and stroke have been the leading cause of death globally for the last 15 years. In 2016, 17.9 million deaths were attributed to cardiovascular disease. High levels of cholesterol and triglycerides, insulin resistance, obesity, smoking, unhealthy diets and lack of exercise are the most important risk factors for a number of cardiovascular diseases (1).

Atherosclerosis, a cause of most cardiovascular diseases, is a chronic inflammatory process occurring at the artery wall as a response to modified structures, particularly oxidized low-density lipoproteins (oxLDL), stimulating both innate and adaptive immune responses (2). Following oxLDL migration to the subendothelial space of the intima (the innermost layer of the artery), monocytes differentiate into macrophages, which then ingests oxLDL via upregulated scavenger receptors such as scavenger receptor A and CD36, leading to the formation of 'foam cells' particularly at injury-prone areas such as aortic bifurcations (3). Subsequently, the nascent atheroma typically progresses into a more complex lesion called a fibrous plaque via accumulation of connective tissue with an increased number of smooth muscle cells and lipid-laden macrophages (3). These activated macrophages secrete numerous cytokines such as interleukin (IL)-12, IL-1β and tumor necrosis factor (TNF)-α, leading to the recruitment of monocytes and T lymphocytes (4). However, during early atherogenesis, a predominant infiltration of M2 macrophages induces small atherosclerotic lesions, suggesting that this macrophage subset may favor an atheroprotective state mainly via the secretion of IL-4 (5). In advanced lesions, M1 macrophages cause plaque vulnerability by degrading the fibrous layer matrix via metalloproteinases. This might lead to sudden ruptures inside the artery (5,6), allowing thrombi to form and therefore disrupt blood flow, leading to life-threatening conditions such as myocardial infarction and stroke (7,8).

Recruited T lymphocytes differentiate into different effector cells which affect atherogenesis (9). Type 1 T helper (Th1)-related cytokines such as interferon gamma (IFN)-γ and TNF-α are known to be pro-inflammatory and promote

the development and progression of atherosclerosis (2). On the other hand, type 2 T helper (Th2) anti-inflammatory cytokines, especially IL-4 and IL-10, seem to exert anti-atherogenic effects by inhibiting IFN-γ production (9). Although the role of Th17 cells, which mainly secrete IL-17A, in atherosclerosis has not yet been fully elucidated, most studies (2,9) attribute the pro-inflammatory effects of IL-17 to the activation of NF-κB, a hallmark transcription factor associated with the induction of pro-inflammatory agents, including IL-1, IL-6, TNF-α and nitric oxide synthase 2 (10). In addition, IL-17, which is mainly released by visceral adipose tissue, seems to play a role early in atherogenesis by stimulating smooth muscle cells of the atheromatosus arteries to secrete the chemokine eotaxin (11). Macrophages treated with IL-17 express high levels of toll-like receptor (TLR) 2 and TLR4 and secrete high levels of inflammatory cytokines such as TNF-α and low levels of IL-10 in the presence of oxLDL, which is a profile similar to that of M1 macrophages, which are known to be proatherogenic (12).

T regulatory (Treg) cells constitute another subset of lymphocytes that play a role in atherogenesis. Treg-related cytokines such as IL-10 and TGF-β are anti-inflammatory and promote anti-atherogenic activities (2).

C-reactive protein (CRP) is produced by the liver in response to tissue injury or inflammation (13). CRP exists in two isoforms: The pentameric isoform (pCRP) and the modified monomeric isoform (mCRP). Studies have shown that mCRP is dissociated from pCRP when it is exposed to endothelial cells (14), neutrophils (15), cell membranes, liposomes (16) and activated platelets (17,18).

To the best of our knowledge, few studies have taken the difference between the two isoforms of CRP into consideration (19). The pCRP isoform has been reported to play an anti-inflammatory role and stop the progression of atherosclerosis, while the mCRP isoform has pro-inflammatory roles and enhances the activation of different pathways for the production of numerous inflammatory cells and cytokines (20). This demonstrates that the mechanisms of mCRP dissociation could potentially be the target of anti-inflammatory chemotherapeutics.

CRP has been shown to bind to oxLDL and promote its uptake into the U937-derived monocyte/macrophage cell line (21). OxLDL are a class of lipoprotein particles, consisting of triglycerides and cholesterol esters which form the hydrophobic core surrounded by a hydrophilic shell formed of phospholipids, apolipoproteins and free cholesterol. CRP, in its two isoforms, and oxLDL form a complex with glycoproteins and are localized with macrophages in human atherosclerotic lesions. OxLDL and CRP levels are directly correlated in patients suffering from acute coronary syndromes (22). The oxLDL/CRP/lysophosphatidylcholine complex has low pro-inflammatory activities *in vitro*, and is known to be anti-atherogenic (23).

The present study aimed to investigate the single and combinatory effects of oxLDL and human CRP containing azide, which is usually used as a preservative with CRP in its two isoforms (mCRP and pCRP) on U937-derived macrophage release of cytokines. The results clarified the optimal effects of CRP and oxLDL combinations, which might help in identifying their role in atherosclerosis.

Materials and methods

Materials and reagents. RPMI-1640 medium, PBS, L-glutamine, penicillin/streptomycin, polymyxin B sulfate salt, FBS, and phorbol-myristate-acetate (PMA) were purchased from Sigma-Aldrich; Merck KGaA. CRP purified from pleural human fluid (purity >99%) containing 0.09% sodium azide as a preservative was obtained from Lee Biosolutions, Inc. Human oxLDL and sodium azide were purchased from Kalen Biomedical, LLC and Sigma-Aldrich; Merck KGaA, respectively.

Differentiation of U937 cells into macrophages. Human monocytic U937 cells (kindly provided by Dr Marwan El Sabban at the American University of Beirut) were cultured in RPMI-1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 1% penicillin/streptomycin and 20 μg/ml polymexin B (referred to as complete growth medium hereafter). The cultures were maintained in a humidified 5% CO_2 incubator at 37˚C. Cells were counted for viability using the Trypan blue exclusion method (24). For differentiation into macrophages, U937 cells were treated with 100 nM PMA for 24 h. Following treatment, media were removed and adherent cells were washed twice with PBS and cultured in PMA-free complete growth medium for 24 h. To collect adherent U937-derived macrophages, cells were washed twice with PBS, incubated on ice for 30 min and gently detached using a cell scraper. Detached cells were centrifuged at 250 x g at 4˚C for 5 min. Cells were then seeded at a density of $7x10^5$ cells/well in a 24-well plate and allowed to adhere for 24 h prior to treatment.

CRP monomerization. Human mCRP was prepared by heating human pCRP at 80˚C for 70 min, as previously described (25). To confirm CRP monomerization, mCRP and pCRP samples were mixed and loaded on a 12.5% polyacrylamide gel. The gel was then stained with Coomassie brilliant blue for 1 h at room temperature, and incubated in destain solution overnight at room temperature. SDS-PAGE broad range protein standard (6.5-200 kDa; Bio-Rad Laboratories), was used as a marker.

Treatment of U937-derived macrophages. U937-derived macrophages were counted and seeded in a 24-well plate at a density of $7x10^5$ cells/well and cultured in a humidified 5% CO_2 incubator at 37˚C for 24 h to adhere. Subsequently, cells were washed with PBS and incubated in complete growth medium containing the corresponding treatment reagents for 24 h. Each well contained one of the following treatments at a concentration of 25 μg/ml for each component: mCRP, pCRP, mCRP/pCRP, oxLDL, mCRP/oxLDL, pCRP/oxLDL, mCRP/pCRP/oxLDL, 0.09% sodium azide solution (SA1x), 0.18% sodium azide (SA2x), SA1x, SA2x, SA1x/oxLDL, with an additional control well with no treatment added. SA1x and SA2x served as single and double concentration controls for sodium azide present in mCRP and mCRP/pCRP, respectively. The adopted concentrations were chosen based on previous literature (26).

Flow cytometry. U937 monocytes and untreated U937-derived macrophages were collected, washed in cold PBS at and

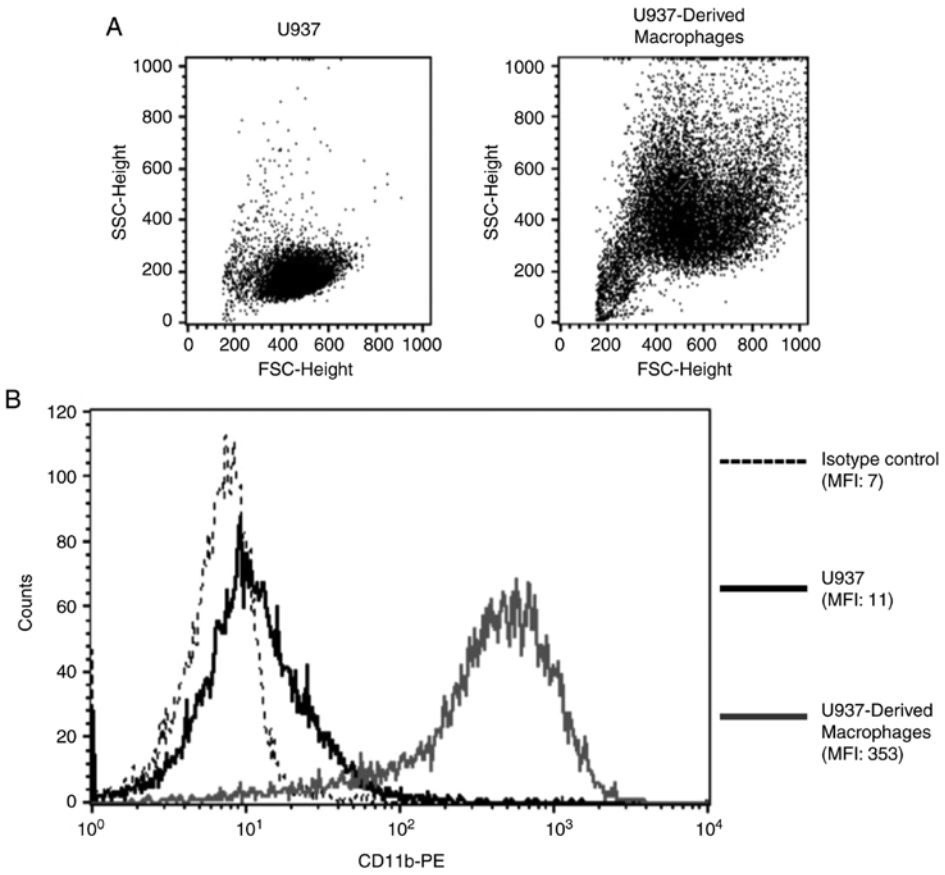

Figure 1. Phenotypic changes associated with the differentiation of U937 monocytes to macrophages. (A) Representative flow cytometry dot plot showing the SSC and FSC properties of U937 monocytes and U937-derived macrophages. (B) Representative histogram plot demonstrating the surface expression of CD11b on U937 monocytes and U937-derived macrophages. Numbers in parentheses indicate the geometric MFI of CD11b. FSC, forward scatter; SSC, side scatter; MFI, mean fluorescence intensity; CD11b, CD11 antigen-like family member B; PE, phycoerythrin.

incubated at a density of $1x10^5$ cells per 100 μl PBS containing 10% human serum AB (Gibco; Thermo Fisher Scientfic, Inc.) for 30 min at 4˚C to block non-specific Fc receptors. The cells were then stained with 1 μg/ml of phycoerythrin (PE)-conjugated mouse anti-human CD11 antigen-like family member B (CD11b) antibody (5 μl/$1x10^5$ cells; cat. no. 555388; BD Biosciences) or PE-conjugated mouse immunoglobulin G1 isotype control antibody (5 μl/$1x10^5$ cells; cat. no. 555749; BD Biosciences) for 15 min at 4˚C. Cells were then washed with cold PBS supplemented with 1% human serum AB) and finally resuspended in PBS containing 1% human serum AB. Samples were kept on ice and CD11b expression was analyzed within 30 min by a BD FACSCalibur™ flow cytometer and CellQuest software (version 5.1; BD Biosciences). U937 cells and U937-derived macrophages were identified based on their forward scatter (FSC) and side scatter (SSC) properties. Results are expressed as geometric mean fluorescence intensity.

ELISA. Culture supernatants from untreated and treated U937-derived macrophages were collected and stored at -80˚C for later cytokine analysis. Commercial ELISA kits (PeproTech, Inc.) were used to measure the levels of IFN-γ (cat. no. 900-K27), IL-4 (cat. no. 900-K14), IL-6 (cat. no. 900-K16), IL-10 (cat. no. 900-K21), and TNF-α (cat. no. 900-K25) in culture supernatants. ELISA was performed according to the manufacturer's instructions.

Statistical analysis. Statistical analysis was performed using GraphPad Prism software (version 6.0; GraphPad Software, Inc.) by conducting one-way ANOVA followed by Tukey's multiple comparison post hoc test. Data are presented as the mean ± SEM. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Confirmation of U937 differentiation into macrophages. In order to validate PMA-induced differentiation of U937 monocytes into macrophages, FSC/SSC properties and CD11b surface expression were analyzed for both cell populations. U937-derived macrophages were more granular and larger in size when compared with U937 monocytes, indicative of a macrophage phenotype (Fig. 1A). Additionally, there was a 32-fold increase in the surface expression of the macrophage differentiation marker, CD11b, upon the differentiation of U937 monocytes into macrophages (Fig. 1B).

Confirmation of CRP monomerization. Gel electrophoresis showed that unheated pCRP showed slight migration at 200 kDa (lane B) while heated pCRP migrated further (40 kDa, lane C), confirming the effective monomerization of pCRP to mCRP (Fig. 2).

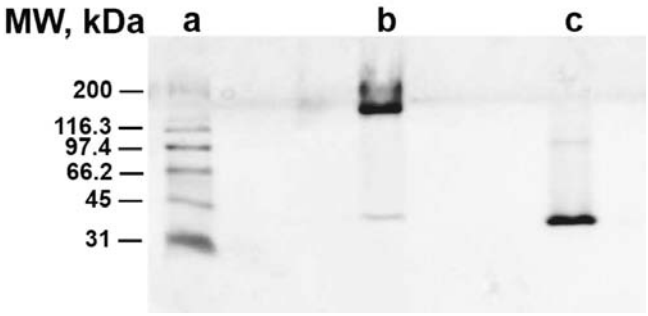

Figure 2. Gel electrophoresis for confirmation of CRP monomerization. Gel image shows (A) Protein ladder, (B) unheated and (C) heated CRP loaded into SDS-PAGE. The gel was later stained with Coomassie brilliant blue for visualization of different protein bands. MW, molecular weight; CRP, C-reactive protein.

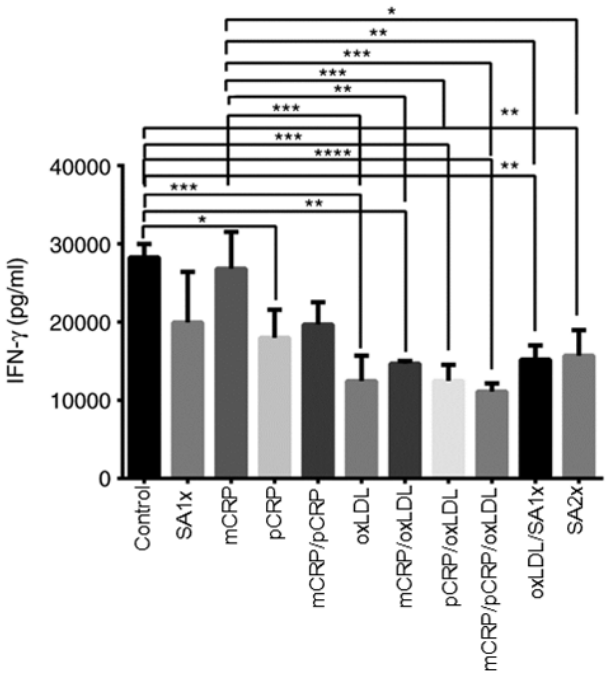

Figure 3. IFN-γ release by U937-derived macrophages. U937-derived macrophages were cultured for 24 h in the presence or absence of mCRP, pCRP oxLDL, SA1x and SA2x as indicated. Data are presented as the mean values of cytokine levels ± SEM of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. mCRP, monomeric C-reactive protein; pCRP, pentameric C-reactive protein; oxLDL, oxidized low-density lipoprotein; SA1x, 0.09% sodium azide solution; SA2x, 0.18% sodium azide solution; INF-γ, interferon-γ.

IFN-γ. Although mCRP alone had no effect on IFN-γ production compared with control, other treatments (oxLDL, mCRP/oxLDL, pCRP/oxLDL, mCRP/pCRP/oxLDL) reduced IFN-γ production when compared with those of control and mCRP treatment. IFN-γ levels for samples treated with both CRP isoforms combined with oxLDL was significantly reduced when compared with control and mCRP treatment. Treatment with oxLDL or a combination of pCRP/oxLDL significantly decreased the amount of secreted IFN-γ to half, as compared to control and mCRP treatment (P<0.001). IFN-γ was also significantly reduced (P<0.01) in samples treated with mCRP/oxLDL and oxLDL/SA1x when compared with

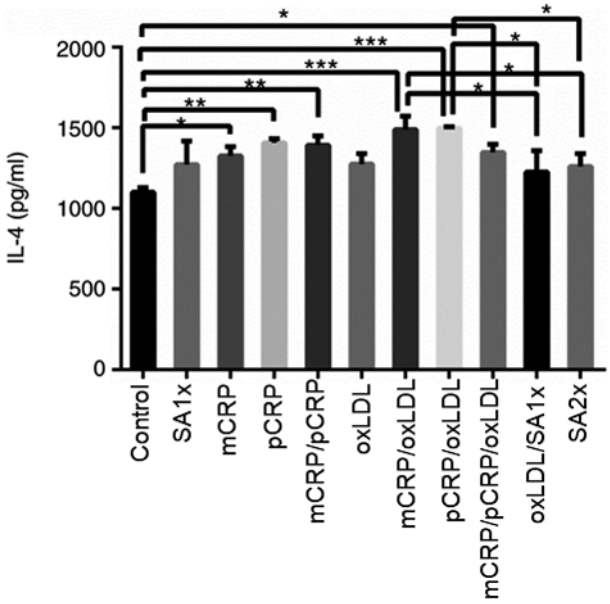

Figure 4. IL-4 release by U937-derived macrophages. U937-derived macrophages were cultured for 24 h in the presence or absence of mCRP, pCRP oxLDL, SA1x and SA2x as indicated. Data are presented as the mean values of cytokine levels ± SEM of three independent experiments. *P<0.05, **P<0.01 and ***P<0.001. mCRP, monomeric C-reactive protein; pCRP, pentameric C-reactive protein; oxLDL, oxidized low-density lipoprotein; SA1x, 0.09% sodium azide solution; SA2x, 0.18% sodium azide solution; IL, interleukin.

levels measured in mCRP alone and control. pCRP treatment resulted in a significant decrease in IFN-γ levels when compared with control (P<0.05; Fig. 3).

IL-4. IL-4 secretion showed significant increase when macrophages were treated with pCRP, mCRP, or a combination of both isoforms, as compared with control macrophages (P<0.05 and P<0.001). Furthermore, IL-4 was highly secreted when samples were treated with mCRP/oxLDL when compared with control (P<0.001). A similarly significant increase in IL-4 production was observed in macrophages treated with mCRP (P<0.05), mCRP/pCRP/oxLDL combination (P<0.05) and pCRP/oxLDL (P<0.001), compared with control. In addition, treatment with SA2x or oxLDL/SA1x resulted in a slight decrease in IL-4 secretion compared with mCRP/oxLDL and pCRP/oxLDL treatments (P<0.05; Fig. 4).

IL-6. IL-6 release was reduced by almost 3-fold when macrophages were treated with mCRP/pCRP/oxLDL and SA1x as compared to untreated macrophages (P<0.0001). Similarly, treatment with pCRP and pCRP/oxLDL downregulated IL-6 production to half the levels measured in control cells (P<0.01). IL-6 levels were significantly reduced upon oxLDL, mCRP/oxLDL, oxLDL/SA1x and SA2x treatment when compared with control group (P<0.001). mCRP treatment resulted in a significant decrease in IL-6 levels, as compared with control cells (P<0.05). In addition, IL-6 levels were further reduced by half when macrophages were treated with oxLDL, pCRP/oxLDL or mCRP/pCRP/oxLDL (P<0.001), as compared with the levels secreted by mCRP-treated macrophages. Secretion levels of IL-6 in samples treated with mCRP/pCRP were significantly higher in comparison with IL-6 levels

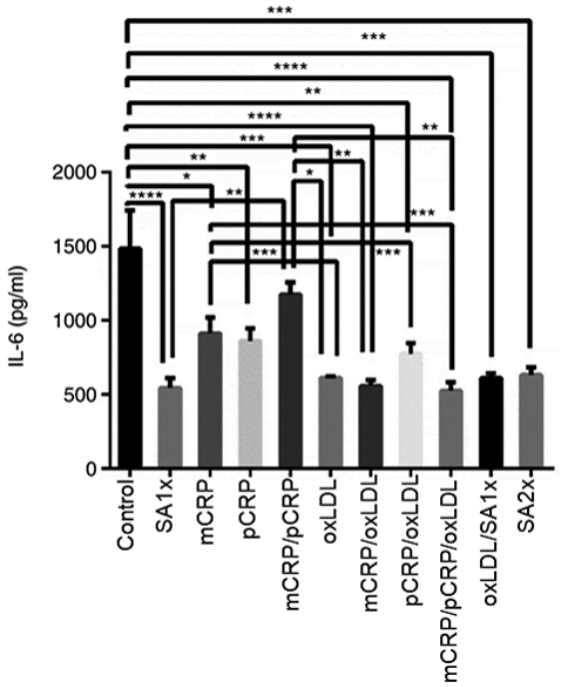

Figure 5. IL-6 release by U937-derived macrophages. U937-derived macrophages were cultured for 24 h in the presence or absence of mCRP, pCRP oxLDL, SA1x and SA2x as indicated. Data are presented as the mean values of cytokine levels ± SEM of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. mCRP, monomeric C-reactive protein; pCRP, pentameric C-reactive protein; oxLDL, oxidized low-density lipoprotein; SA1x, 0.09% sodium azide solution; SA2x, 0.18% sodium azide solution; IL, interleukin.

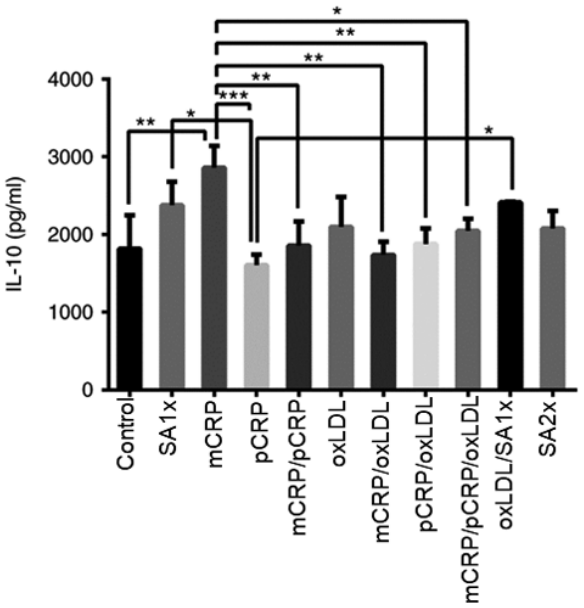

Figure 6. IL-10 release by U937-derived macrophages. U937-derived macrophages were cultured for 24 h in the presence or absence of mCRP, pCRP oxLDL, SA1x and SA2x as indicated. Data are presented as the mean values of cytokine levels ± SEM of three independent experiments. *P<0.05, **P<0.01 and ***P<0.001. mCRP, monomeric C-reactive protein; pCRP, pentameric C-reactive protein; oxLDL, oxidized low-density lipoprotein; SA1x, 0.09% sodium azide solution; SA2x, 0.18% sodium azide solution.

after treatment with mCRP/oxLDL and mCRP/pCRP/oxLDL (P<0.01; Fig. 5).

IL-10. IL-10 release increased by 50% when macrophages were treated with mCRP compared with controls (P<0.01). By contrast, a 2-fold reduction in IL-10 production was detected in cells treated with pCRP as compared to mCRP treatment (P<0.001). IL-10 levels significantly decreased after treatment with mCRP/pCRP, mCRP/oxLDL and pCRP/oxLDL compared with mCRP alone (P<0.01). The combination of mCRP/pCRP/oxLDL significantly reduced IL-10 levels compared with mCRP treatment. SA1x treatment and oxLDL/SA1x resulted in a higher release of IL-10 compared with pCRP treatment (P<0.05; Fig. 6).

TNF-α. TNF-α levels showed an almost 3-fold decrease when macrophages were treated with the mixture of mCRP/oxLDL compared to the levels of TNF-α in samples treated with oxLDL alone (P<0.01). A two-fold increase was observed in macrophages treated with SA1x and SA2x) compared with TNF-α levels measured in macrophages treated with mCRP/oxLDL (P<0.05; Fig. 7).

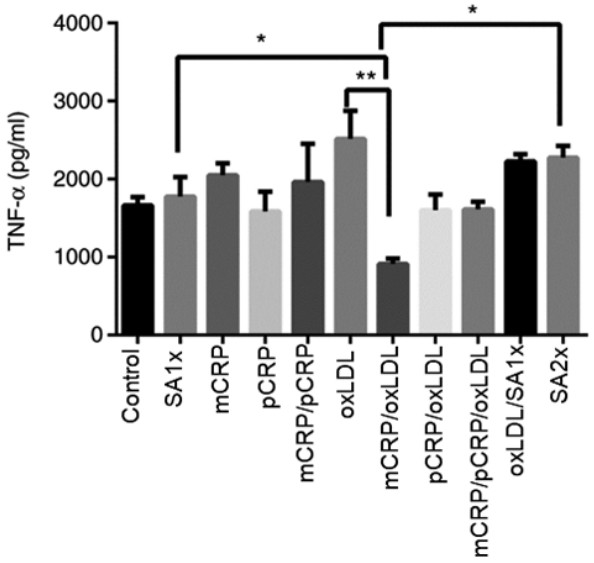

Figure 7. TNF-α release by U937-derived macrophages. U937-derived macrophages were cultured for 24 h in the presence or absence of mCRP, pCRP oxLDL, SA1x and SA2x as indicated. Data are presented as the mean values of cytokine levels ± SEM of three independent experiments. *P<0.05 and **P<0.01. mCRP, monomeric C-reactive protein; pCRP, pentameric C-reactive protein; oxLDL, oxidized low-density lipoprotein; SA1x, 0.09% sodium azide solution; SA2x, 0.18% sodium azide solution.

Discussion

In order to have a better understanding of the factors affecting the activity of macrophages, which are known to play a pivotal role in atherogenesis, the current study assessed the single and combined effects of monomeric and pentameric CRP azide-containing isoforms and oxLDL on the release of selected atherosclerosis-related cytokines, including IFN-γ, IL-4, IL-6, IL-10 and TNF-α, by U937-derived macrophages.

IFN-γ is known to be a pro-atherogenic cytokine mainly by activating macrophages to produce other pro-inflammatory cytokines, such as TNF-α and IL-6, via the JAK/STAT

signaling pathway (23). The present study showed that all treatments, with the exception of mCRP alone, caused a marked decrease in IFN-γ levels secreted by macrophages when compared with the control group. The combination of oxLDL, mCRP and pCRP resulted in the most significant decrease in IFN-γ levels. This indicated that neither oxLDL or pCRP alone nor in combination with mCRP can induce atherogenesis by upregulating IFN-γ secreted by macrophages. On the contrary, treatments involving oxLDL might have anti-atherogenic effects as oxLDL treatment induced a decrease in the secretion of IFN-γ by macrophages. Such effects might be mediated by the downregulation of cytokines known to induce IFN-γ production, including IL-12 and IL-18 (27,28). This was further supported by the increased levels of IL-4, which has been previously demonstrated to suppress IL-12/IL-18 secretion (25,26), under most single and combined treatments, the least effect being observed following treatment with oxLDL.

Although it was hypothesized that IL-4 acts as an anti-atherogenic cytokine, experimental data from the present study alone are not sufficient to fully support this assumption. However, it can be assumed that IL-4 is an immunoregulatory molecule controlling inflammatory response by downregulating TNF production (29). The tendency to downregulate pro-inflammatory cytokines (especially IFN-γ) and upregulate IL-4 suggest that U937-derived macrophages tend to acquire the M2 profile (5). This was further confirmed by the decreased IL-6 production under most treatments especially those involving oxLDL in combination with mCRP.

This suggested scheme (acquisition of M2 profile) is complemented by the inability of most treatments to affect the levels of the anti-inflammatory cytokine IL-10, which suppresses the expression of certain inflammatory cytokines, including IL-6, IL-1 and TNF-α (23) and has been revealed to reduce atherogenesis (30). In addition, mCRP induced a significant upregulation of IL-10 levels when compared with control and most other treatments. TNF-α is another potent pro-inflammatory cytokine mainly secreted by activated macrophages. Plasma levels of TNF-α are increased in pathologies such as atherosclerosis. This cytokine enhances the expression of other pro-inflammatory cytokines via NF-κB activation (31).

In line with previous studies, the present results indicated that the secretion of TNF-α by U937-derived macrophages increased when cells were exposed to oxLDL (26,32). However, when oxLDL was combined with mCRP, TNF-α production significantly decreased. This demonstrated that the effect of oxLDL on the activity of macrophages may depend on the microenvironmental composition.

Another important factor that might affect the microenvironment, and therefore the results, is the presence of azide which is usually used as a preservative for CRP. Especially at higher doses, azide caused a significant decrease in the secretion of IFN-γ by U937-derived macrophages. This tendency did not appear to inhibit the effect of all other treatments (except for mCRP) in reducing the secretion of this cytokine and even may enhance this downregulatory effect. The presence of azide did not appear to significantly affect the activity of macrophages in secreting IL-4, IL-10 and TNF-α when compared with control. However, the present study showed that azide alone at both concentrations significantly reduced IL-6 secretion by macrophages. In a previous study, when azide-free CRP was used, pCRP was able to enhance IL-6 release by U937-derived macrophages which is contradictory to the results from the present study (30).

In the present study, mCRP did not have any marked effect on the levels of IFN-γ and TNF-α but it had the ability to upregulate IL-4 and IL-10 levels and to downregulate IL-6 levels secreted by U937-derived macrophages. Therefore, future studies assessing the role of mCRP is atherosclerosis are required, as a recent study has shown that mCRP might have contradictory proangiogenic and antiangiogenic effects (33).

pCRP was found to be able to downregulate most pro-inflammatory cytokines, mainly IFN-γ and IL-6 and to upregulate the anti-inflammatory cytokine, IL-4, whilst having no notable effects on IL-10 secretion. A similar trend was observed with the combination of mCRP and pCRP. A significant reduction in the secretion of the proatherogenic cytokines (IFN-γ and IL-6) was found in samples treated with oxLDL alone and in combination with mCRP and/or pCRP. However, no statistically significant differences in the levels of IL-10 and TNF-α were detected between oxLDL-, mCRP/oxLDL- or pCRP/oxLDL-treated and control macrophages. Therefore, it was shown in the present study that, oxLDL combined with mCRP may have an anti-atherogenic effect by modulating cytokine production by U973-derived macrophages.

In conclusion, mCRP, pCRP and oxLDL, either individually or in combination, can upregulate IL-4 and IL-10 secretion and downregulate IFN-γ and IL-6 secretion by U937-derived macrophages, thus favoring M2 macrophage. Therefore, the microenvironment of the intima, involving especially atherosclerosis-related cytokines as IL-6, TGF-β, IL-10 and IL-17, may be a critical factor affecting atherogenesis. Further studies should attempt to mimic the *in vivo* medium of the intima to investigate the type of macrophages, which is heavily associated with atherogenesis, and other cell types in addition to their behavior under different conditions.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DJ and IK performed the experiments. DJ, IK, SB and MK analyzed and interpreted the data. DJ wrote the first draft of the manuscript. SB and MK reviewed and edited the

manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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