Protein Cell 2011, 2(8): 672–679
DOI 10.1007/s13238-011-1084-4

Protein & Cell

RESEARCH ARTICLE

C-reactive protein functions as a negative regulator of macrophage activation induced by apoptotic DNA

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Received July 19, 2011 Accepted August 8, 2011

ABSTRACT

C-reactive protein (CRP), an acute-phase protein with an ability to bind to nuclear antigen, has been reported to regulate cytokine secretion and modulate immune responses. We previously reported that activated syngeneic lymphocyte-derived apoptotic DNA (apopDNA) could induce macrophage activation and contribute to the initiation and progression of lupus nephritis. It is reasonable to hypothesize that CRP might regulate apopDNA-induced macrophage activation. Herein, CRP was shown to promote macrophage-mediated apopDNA uptake by binding to apopDNA (CRP/apopDNA complex). Notably, CRP/apopDNA treatment inhibited the production of inflammatory cytokines and chemokines by macrophages which could be induced by apopDNA alone. Further coculture and transwell studies revealed that CRP/apopDNA-induced macrophages prohibited apopDNA-induced macrophage activation in an IL-10 dependent manner. These results provide insight into the potential mechanism of CRP regulatory activity in macrophage activation induced by apopDNA in the context of lupus nephritis and other autoimmune diseases.

KEYWORDS C-reactive protein (CRP), macrophage activation, autoimmunity, systemic lupus erythematosus (SLE), lupus nephritis

INTRODUCTION

Autoimmune response to antigens that are associated with nucleic acids is a hallmark of systemic autoimmune diseases such as systemic lupus erythematosus (SLE) (Rahman and Isenberg, 2008). It has become clear that dysregulation of apoptosis has been implicated in the pathogenesis of SLE by increasing the source of self antigens (Kaplan, 2004). During apoptosis which could be induced by infection, stress, and other danger signals, DNA was released from apoptotic cells, but not always provoking autoimmunity, suggesting that free apoptotic DNA could be cleared by intrinsic physiological mechanisms (Walport, 2000). Indeed, there is growing evidence that the binding and ingestion of apoptotic cells and the released nuclear antigens by monocytes/macrophages could influence immune responses remarkably by enhancing or suppressing inflammation.

In our previously study, by mimicking the amounts of DNA released from unremoved apoptotic cells in SLE patients, we immunized female BALB/c mice with activated syngeneic lymphocyte-derived apoptotic DNA (apopDNA) and established the SLE murine model which developed high levels of anti-dsDNA antibody, proteinuria, immune complex deposition, and lupus nephritis (Qiao et al., 2005; Wen et al., 2007). In this lupus murine model, we further revealed that activated macrophages induced by apopDNA infiltrated into kidneys of lupus mice and contributed to the initiation and progression of lupus nephritis, indicating that apopDNA might serve as an important self immunogen to trigger macrophage activation and autoimmune responses which eventually lead to the pathogenesis of SLE (Zhang et al., 2010).

Apart from overload of DNA released from unremoved apoptotic cells in SLE disease, insufficiency of DNA clearance represents the other side of the coin. Although the precise mechanism involved in triggering autoimmune

response to self antigens is largely unknown, there is now good experimental evidence for a link between the defective clearance of apoptotic cells, the released nuclear antigen or other debris and SLE disease associated with C1q or DNase deficiency (Taylor et al., 2000; Walport, 2000). More recently, C-reactive protein (CRP), a member of the pentraxin family with an ability to bind to nuclear antigens as well as to damaged membranes and microbial antigens (Du Clos, 1989), was found to play a key role in the protection from autoimmune disease (Ogden and Elkon, 2005; Rodriguez et al., 2005; 2006). Moreover, CRP was a prototypic acute phase protein produced mainly in response to inflammation, infection, or tissue damage (Garlanda et al., 2005; Bottazzi et al., 2010) and was reported to recognize nuclear autoantigens released from apoptotic cells, opsonize them through interacting with cell-surface FcγR, thereby activating macrophage-mediated phagocytosis (Mold et al., 2001; Bottazzi et al., 2010). In addition to activating phagocytosis through FcγR, emerging evidence demonstrated that CRP could also regulate immune responses (Marjon et al., 2009), indicating that CRP could modulate nuclear antigen-mediated immune response.

In this study, we examined the effect of CRP on macrophage-mediated apopDNA uptake and found that CRP could bind to apopDNA (CRP/apopDNA complex), which contributes to facilitating the uptake of apopDNA by macrophages. Moreover, CRP-mediated apopDNA clearance was found to inhibit macrophage activation which could be induced by apopDNA alone. CRP/apopDNA-induced macrophages were shown to prohibit apopDNA-induced inflammatory cytokine and chemokine production by macrophages, while this inhibitory effect was abolished when treated with neutralizing anti-IL-10 antibody, indicating that CRP/apopDNA could prohibit apopDNA-induced macrophage activation in an IL-10 dependent manner. These results shed a new light into the possible mechanism underlying CRP-mediated regulatory effect on apopDNA-induced macrophage activation in the context of SLE disease and other autoimmune diseases.

RESULTS

CRP binds to apopDNA and promotes macrophage-mediated apopDNA uptake

Accumulating data indicate that CRP has the capacity of binding to nuclear antigen (Garlanda et al., 2005; Bottazzi et al., 2010). To assess the binding ability of CRP to apopDNA, we performed dot blot analysis and found that CRP could bind to apopDNA (Fig. 1A and 1B). Further enzyme-linked immunosorbent (ELISA) assay confirmed that CRP could bind to apopDNA in a dose-dependent manner (Fig. 1C). In order to investigate whether the binding of CRP to apopDNA had any effects on the uptake of apopDNA by macrophages, flow cytometry was performed to determine the macrophage-mediated phagocytosis of apopDNA or CRP/apopDNA complex. It was found that CRP promoted the uptake of apopDNA by macrophages (Fig. 1D). These data indicate that CRP binding to apopDNA could efficiently promote apopDNA uptake by macrophages.

CRP binding to apopDNA inhibits macrophage activation

CRP has been reported to mediate several functions including cytokine secretion, opsonization of bacteria and altering or masking autoantigen from immune response (Rodriguez et al., 2007). To investigate the effect of CRP binding to apopDNA on macrophage activation, the expression of various activation markers in macrophages was analyzed. Similar to previous discovery, macrophages treated with apopDNA exhibited significantly enhanced expression of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1β, and monocyte chemoattractant protein-1 (MCP-1) (Zhang et al., 2010), whereas macrophages treated with CRP/apopDNA complex decreased their mRNA and protein levels as compared with apopDNA treatment (Fig. 2A and 2B). These results indicate that CRP/apopDNA treatment could prohibit macrophage activation.

The culture supernatants of CRP/apopDNA-induced macrophages inhibit apopDNA-stimulated macrophage activation

Since CRP/apopDNA treatment affected the cytokine and chemokine profile of macrophages, we further assessed the function of CRP/apopDNA-induced macrophages. Interestingly, enhanced expression levels of TNF-α, IL-6, and MCP-1 in macrophages stimulated with apopDNA were found to be attenuated by coculture with CRP/apopDNA-induced macrophages (Fig. 3A). To determine which components of CRP/apopDNA-induced macrophages inhibited apopDNA-stimulated macrophage activation, we performed transwell assay. Notably decreased levels of TNF-α, IL-6, and MCP-1 were detected when BMDMs (low chamber) were cultured with CRP/apopDNA-induced macrophages (upper chamber) in a dual-chamber transwell supplemented with apopDNA (Fig. 3B). Moreover, expression levels of TNF-α, IL-6, and MCP-1 in macrophages drastically decreased when the cultivation was performed with the culture supernatants (Culture-sup) of CRP/apopDNA-induced macrophages even in the presence of apopDNA (Fig. 3C). These data suggest that the culture supernatants derived from CRP/apopDNA-induced macrophages could inhibit apopDNA-induced macrophage activation.

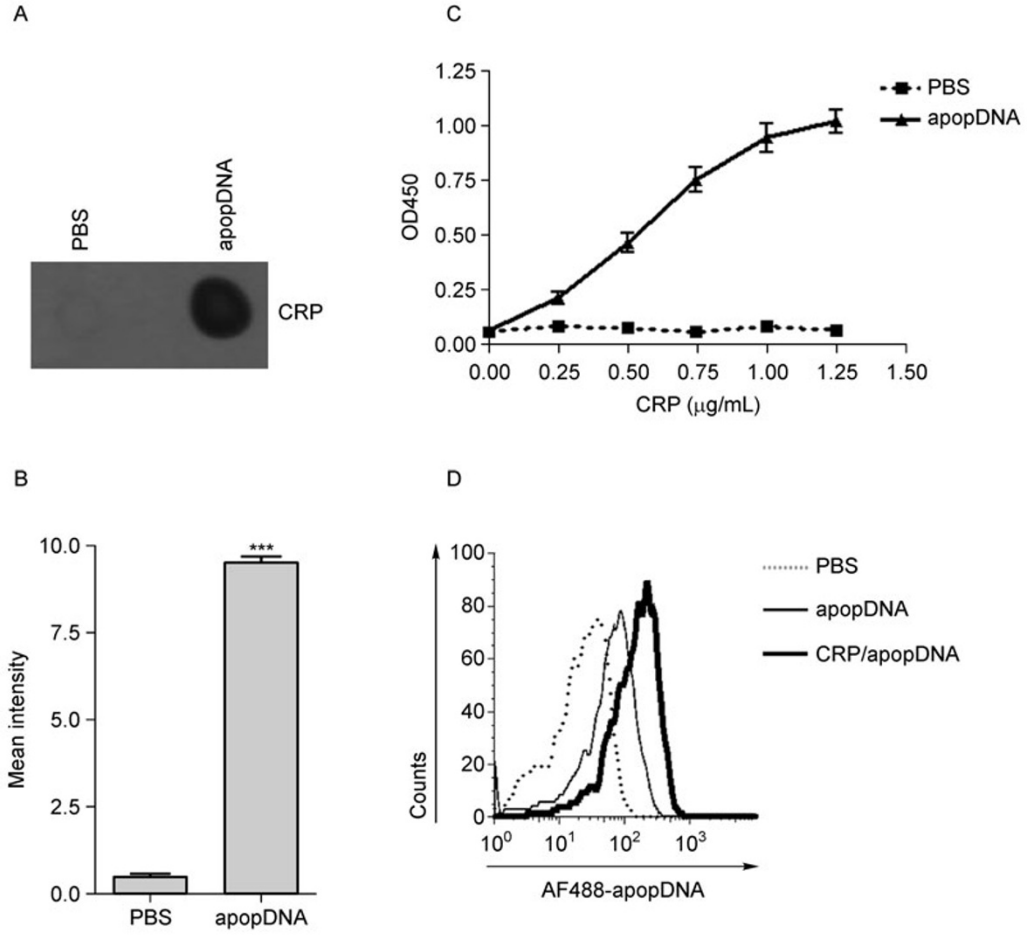

Figure 1. CRP binds to apopDNA and promotes the uptake of apopDNA by macrophages. (A) The binding ability of CRP to apopDNA was detected by dot blot. (B) The binding ability of CRP to apopDNA in (A) was represented as mean intensity. (C) The binding ability of CRP to apopDNA was detected by ELISA assay. (D) The phagocytosis of Alexa Fluor 488 labeled apopDNA (AF488-apopDNA) by macrophages treated with chloroquine was detected by flow cytometry. Data are representative of results obtained in three independent experiments or means ± SD of three independent experiments. *** $p < 0.001$.

IL-10 in the culture supernatants of CRP/apopDNA-induced macrophages inhibits apopDNA-induced macrophage activation

To further clarify the molecular mechanism involved in the inhibitory effect of CRP/apopDNA-induced macrophages, cytokine profiles in the culture supernatants of CRP/apopDNA-induced macrophages were screened and dramatically increased IL-10 was noticed (Fig. 4A and 4B). As one of the major inhibitory cytokines, IL-10 has been reported to be critical to ensure cellular homeostasis and suppression of inflammatory response (Saraiva and O'Garra, 2010). Treating the culture supernatants of CRP/apopDNA-induced macrophages with anti-IL-10 neutralizing antibodies abolished the inhibitory action of the culture supernatants, suggesting that IL-10 enabled CRP/apopDNA-induced macrophages to inhibit apopDNA-induced macrophage activation (Fig. 4C). Furthermore, increased expression levels of TNF-α, IL-6, and MCP-1 in the apopDNA-induced macrophages were efficiently inhibited by administration of exogenous IL-10 (Fig. 4C). Taken together, these data suggest that IL-10 secreted by CRP/apopDNA-induced macrophages could inhibit apopDNA-induced macrophage activation.

DISCUSSION

During the past decade, extensive evidence indicates that dysregulated apoptosis contributes to autoimmune response in SLE disease by increasing the source of self antigens (Mohan et al., 1993; Mevorach et al., 1998; Kaplan, 2004). Although numerous molecules responsible for recognition and uptake of apoptotic cells and their released nuclear antigen and other debris have been identified, carefully regulating the clearance of apoptotic cells and self nuclear

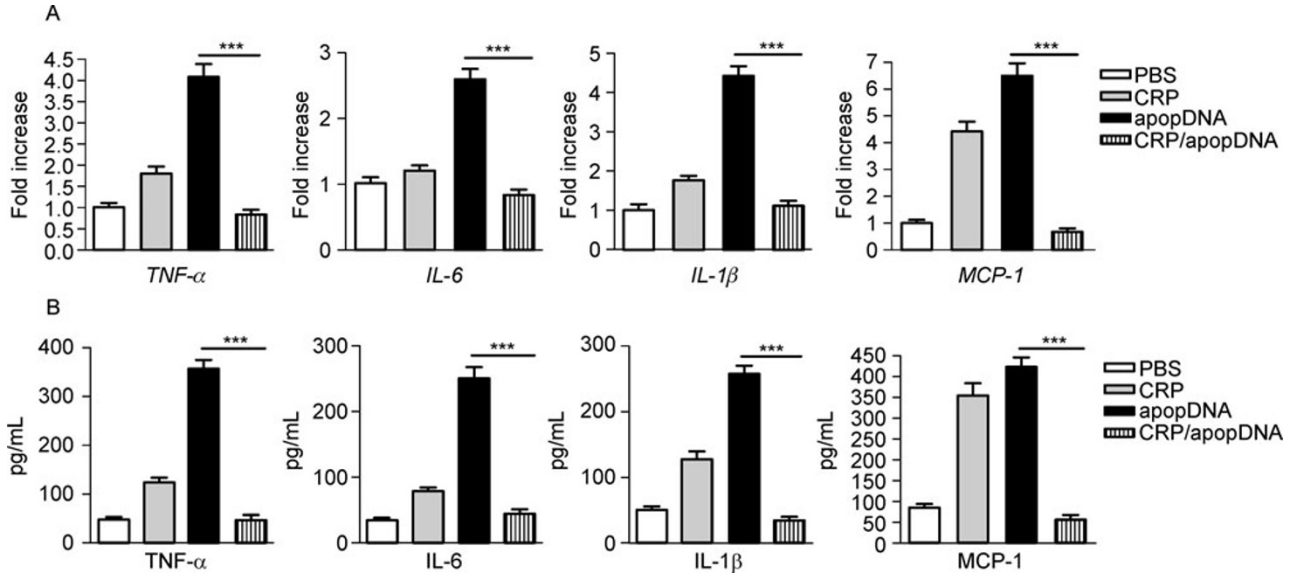

Figure 2. CRP binding to apopDNA inhibits macrophage activation. CRP was incubated with apopDNA (CRP/apopDNA) for 2 h. BMDMs were treated with PBS, CRP (50 μg/mL), apopDNA (50 μg/mL), or CRP/apopDNA (50 μg/mL). (A) 12 h later, levels of *TNF-α*, *IL-6*, *IL-1β*, and *MCP-1* in macrophages were measured by real-time PCR. (B) 24 h later, levels of TNF-α, IL-6, IL-1β, and MCP-1 in the culture supernatants of macrophages were measured by ELISA. Data are means ± SD of three independent experiments. *** $p < 0.001$.

antigens by macrophages is essential for prevention of autoimmune response in SLE disease (Mukundan et al., 2009). Our previous study revealed that apopDNA obtained from activated syngeneic lymphocyte-derived apoptotic cells could induce macrophage activation (Zhang et al., 2010), while the present study shows that CRP functions as a regulatory factor to modulate the phagocytic response and prohibit macrophage activation when macrophages are confronted with apopDNA.

It is known that self nuclear antigens released from apoptotic cells may be immunogenic in lupus mice, and the outcome of self nuclear antigen-induced autoimmune response appears to be regulated by many factors, including overload of self antigens and the clearance of antigen by macrophages. In our previous study, activated syngeneic lymphocyte-derived apopDNA immunization induced macrophage activation and subsequent autoimmune response to self apopDNA, leading to SLE disease, which mimics the findings that DNAs released from unremoved apoptotic cells accumulate in SLE patients and result in the onset of autoimmune response to self nuclear antigens (Qiao et al., 2005; Wen et al., 2007; Zhang et al., 2010). We also found that DNAs obtained from syngeneic lymphocyte-derived unapoptotic cells (referred as Un-apopDNA) could not induce macrophage activation and autoimmune response (Zhang et al., 2010; Chen et al., 2011). These results indicate that apopDNA serves as an important self nuclear antigen in SLE disease, and the immunogenicity of apopDNA may be due to its hypomethylation (Wen et al., 2007). Whether there are other factors cooperated with its hypomethylation contribute to apopDNA-induced macrophage activation and the following autoimmune responses remain to be elucidated.

As an important acute phase protein with an ability to bind to nuclear antigen, CRP has been reported to participate in modulating cytokine production and immune response mediated by macrophages in many diseases such as autoimmune disease, atherosclerosis, and immune thrombocytopenia (Rodriguez et al., 2005; Singh et al., 2006; Marjon et al., 2009). In the present study, we demonstrated that CRP functions as an important regulatory factor to modulate apopDNA-induced autoimmune response mediated by macrophages. We provided several lines of evidence that support this notion. First, CRP could bind to apopDNA and promote apopDNA uptake by macrophages. Secondly, CRP binding to apopDNA prohibits macrophage activation. More importantly, CRP/apopDNA-induced macrophages could block apopDNA-stimulated macrophage activation in an IL-10 dependent manner. Although CRP could also bind to Un-apopDNA, it could bind to apopDNA with a higher ability than Un-apopDNA (data not shown) and efficiently promote the uptake of apopDNA by macrophages. These results suggest CRP could modulate apopDNA-induced autoimmune response via regulating macrophage activation in the context of SLE disease. Whether other factors also participate in regulating macrophage activation and autoimmune response in SLE disease remains unclear.

Emerging evidence revealed that CRP could suppress inflammation, which has been well documented in studies on

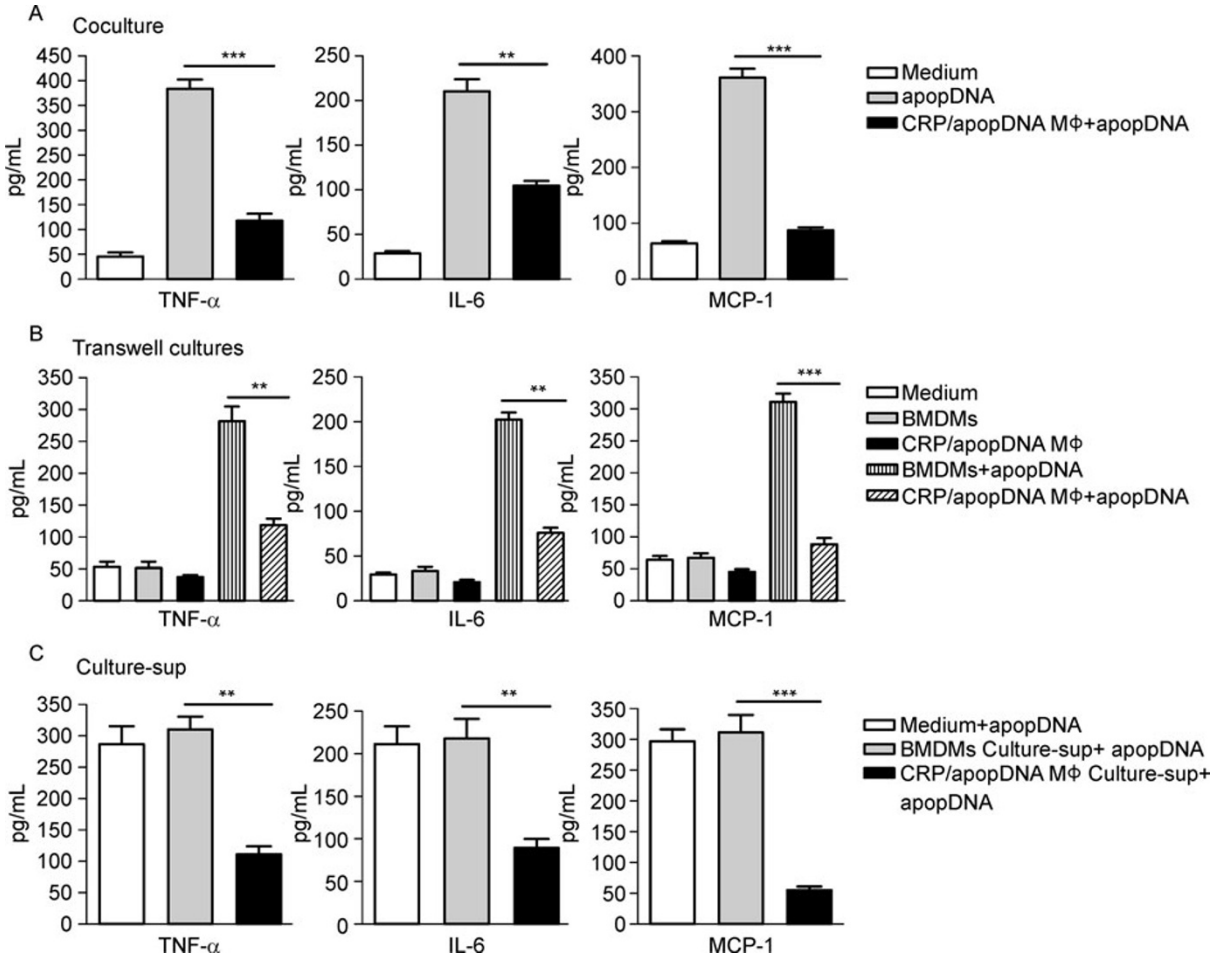

Figure 3. The culture supernatants of CRP/apopDNA-induced macrophages inhibit apopDNA-induced macrophage activation. CRP/apopDNA-induced macrophages (CRP/apopDNA MΦ) were the BMDMs treated with CRP/apopDNA (50 μg/mL) for 24 h. (A) Coculture: BMDMs were cocultured with medium, apopDNA, or CRP/apopDNA MΦ plus apopDNA. Levels of TNF-α, IL-6, and MCP-1 in the culture supernatants were measured by ELISA. (B) Transwell cultures: BMDMs (6×10^5 cells/well, low chamber) were cultured with medium, BMDMs (5×10^5 cells/well, upper chamber), CRP/apopDNA MΦ (5×10^5 cells/well, upper chamber), BMDMs (5×10^5 cells/well, upper chamber) plus apopDNA (50 μg/mL), or CRP/apopDNA MΦ (5×10^5 cells/well, upper chamber) plus apopDNA (50 μg/mL) in a dual-chamber transwell. 24 h after the initial cultivation, macrophages in the lower chamber harvested were washed and cultured for an additional 24 h. Levels of TNF-α, IL-6, and MCP-1 in the culture supernatants were measured by ELISA. (C) Culture-sup: in the presence of apopDNA (50 μg/mL), BMDMs were cultured with complete medium (Medium plus apopDNA group), complete medium supplemented with the culture supernatants from BMDMs (BMDMs Culture-sup plus apopDNA group), or complete medium supplemented with the culture supernatants from CRP/apopDNA-induced macrophages (CRP/apopDNA MΦ Culture-sup plus apopDNA group) at a ratio of 15% (*v/v*) for 24 h. The cells harvested were washed and cultured for an additional 24 h. Levels of TNF-α, IL-6, and MCP-1 in the culture supernatants were measured by ELISA. Data are means ± SD of three independent experiments. ** $p < 0.01$; *** $p < 0.001$.

rabbit CRP transgenic mice (Xia and Samols, 1997). Because of low levels of CRP (< 5 μg/mL) in mice, the murine model was shown to be useful to test the activity of transgenic or supplemental CRP *in vitro* and *in vivo* (Garlanda et al., 2005). Human and rabbit CRP could activate mouse complement and bind to Fcγ receptors on mouse macrophages (Rodriguez et al., 2007), so we chose human CRP to investigate its roles in modulating macrophage activation. Whether CRP/apopDNA could bind to Fcγ receptors on mouse macrophages remains to be investigated. In this study, we revealed that IL-10 in the culture supernatants of CRP/apopDNA-induced macrophages inhibited apopDNA-induced macrophage activation in the context of SLE disease, which is consistent with a previous study on the effect of CRP treatment in SLE disease (Rodriguez et al., 2005). However, discrepancies might also exist over CRP–IL-10 relationship between our study and other group's (Singh et al., 2006), which might be due to the disparation of cell types, the

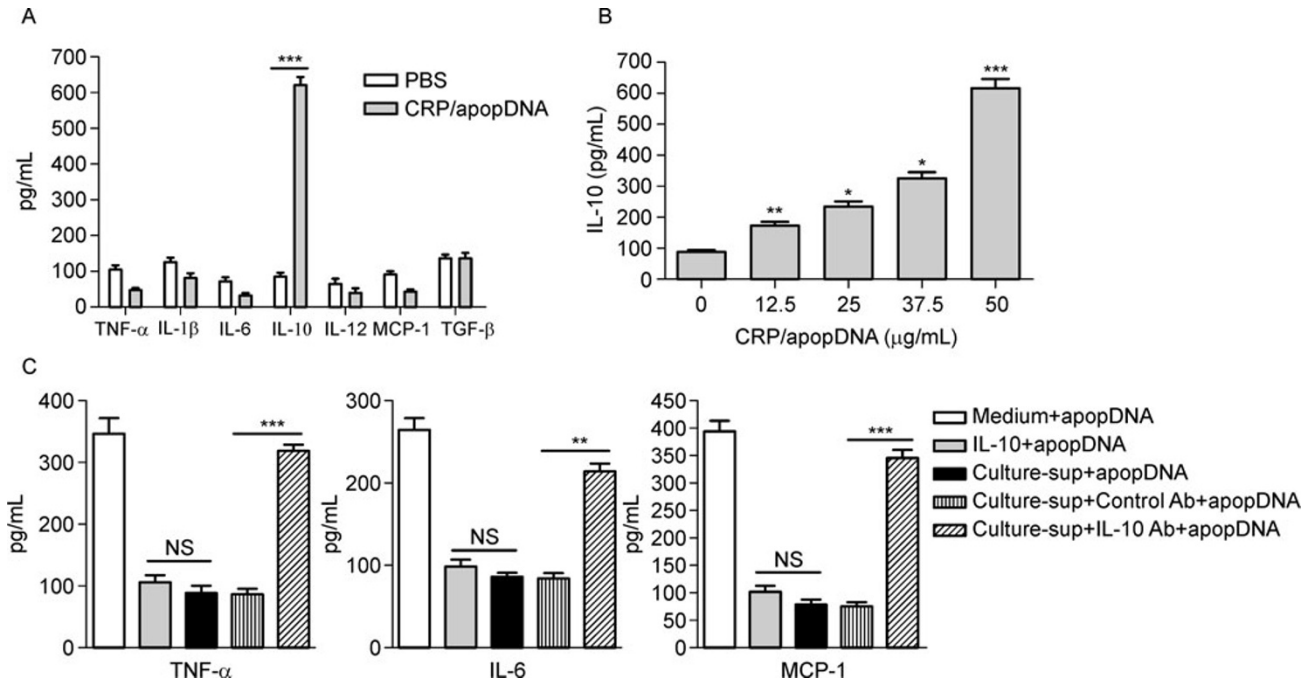

Figure 4. IL-10 in the culture supernatants of CRP/apopDNA-induced macrophages is the key factor to inhibit apopDNA-induced macrophage activation. (A) BMDMs were treated with PBS or CRP/apopDNA (50 μg/mL) for 24 h. Levels of TNF-α, IL-1β, IL-6, IL-10, IL-12, MCP-1, and TGF-β in the culture supernatants were measured by ELISA. (B) Levels of IL-10 in the culture supernatants of macrophages stimulated with increasing amounts of CRP/apopDNA for 24 h were determined by ELISA. (C) The culture supernatants of BMDMs which were treated with CRP/apopDNA (50 μg/mL) for 24 h (referred as Culture-sup) were collected. In the presence of apopDNA (50 μg/mL), macrophages were cultured with complete medium (Medium plus apopDNA group), complete medium supplemented with 20 ng/mL of IL-10 (IL-10 plus apopDNA group), complete medium supplemented with Culture-sup (Culture-sup plus apopDNA group), or complete medium supplemented with Culture-sup that had been pretreated with 50 ng/mL of anti-IL-10 neutralizing antibodies (Culture-sup plus IL-10 Ab and apopDNA group) or with 50 ng/mL of isotype control antibodies (Culture-sup plus Control Ab and apopDNA group). 24 h after cultivation, the cells harvested were washed and cultured for an additional 24 h. Levels of TNF-α, IL-6, and MCP-1 in the culture supernatants were measured by ELISA. Data are means ± SD of three independent experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, not significant.

microenvironment in different diseases, and other unknown factors. The precise molecular mechanisms involved in CRP/apopDNA-induced IL-10 production in macrophages need to be further investigated.

In summary, CRP promotes macrophage-mediated apopDNA uptake by binding to apopDNA. CRP/apopDNA complex inhibits apopDNA-induced macrophage activation in an IL-10 dependent manner. Our results might disclose the potential mechanism of CRP modulating self nuclear antigen-induced macrophage activation and the subsequent auto-immune response to self antigen in SLE disease and other autoimmune diseases.

MATERIALS AND METHODS

Mice

Six- to eight-week-old female BALB/c mice were purchased from the Experimental Animal Center of Chinese Academy of Sciences, Shanghai, China. Mice were housed in a specific pathogen-free room under controlled temperature and humidity. All mouse procedures were conducted according to the *Guide for the Care and Use of Medical Laboratory Animals* set up by the Ministry of Health, China, in 1998, and with the ethical approval of the Shanghai Medical Laboratory Animal Care and Use Committee as well as the Ethical Committee of Fudan University, Shanghai, China.

Cells and cell culture

L929 murine fibrosarcoma cells from ATCC were cultured in RPMI-1640 medium and maintained in a 5% CO_2 incubator at 37°C. For generation of bone marrow-derived macrophages (BMDMs), bone marrow (BM) cells were harvested from uninfected, normal BALB/c mice and filtered through nylon mesh. BM cells were cultured in L929 cell-conditioned medium at a density of 3×10^5 cells/mL of medium and maintained in a 5% CO_2 incubator at 37°C as described previously (Ito et al., 2009; Li et al., 2009). Six days after initial BM cell

culture, the medium was changed and the purity of $F4/80^+$ cells was more than 90%, as determined by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA).

Reagents

CRP was purified from human pleural effusion fluid as described previously (Du Clos, 1989). The purity of the CRP isolate was determined by SDS-PAGE and subsequent Coomassie brilliant blue staining. No additional bands other than the major band at ~23 kDa were seen. In addition, to exclude contaminations with LPS, sterile endotoxin-free plastic ware and reagents were used for CRP preparation. CRP samples were monitored for low level of endotoxin by the Limulus amoebocyte lysate assay (BioWhittaker, Walkersville, MD) according to the manufacturer's instructions. IL-10 neutralization antibody and relative isotype IgG were purchased from R&D System (Minneapolis, MN). Recombinant mouse IL-10 was purchased from PeproTech (Rocky Hill, NJ).

Preparation of apopDNA

ApopDNA was prepared with murine splenocytes which were generated from surgically resected spleens of six- to eight-week-old female BALB/c mice and cultured with Con A (Sigma-Aldrich, St. Louis, MO) *in vitro* (Qiao et al., 2005; Wen et al., 2007). In brief, splenocytes were seeded at 2×10^6 cells/mL in 75 cm^2 cell culture flask and cultured in the presence of Con A (5 μg/mL) for 6 days to induce apoptosis. The apoptotic cells were stained with FITC-labeled Annexin V (BD Biosciences) and propidium iodide (PI; Sigma-Aldrich), and sorted using a FACSAria (BD Biosciences). Genomic DNAs from syngeneic apoptotic splenocytes were treated with S1 nuclease (Takara Bio, Shiga, Japan) and proteinase K (Sigma-Aldrich), and then purified using the DNeasy Blood & Tissue Kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. To exclude contaminations with LPS, sterile endotoxin-free plastic ware and reagents were used for DNA preparation. DNA samples were also monitored for low level of endotoxin by the Limulus amoebocyte lysate assay (BioWhittaker) according to the manufacturer's instructions. The concentration of DNA was determined by detection of the absorbance (A) at 260 nm. The apoptotic DNA ladder of apopDNA was confirmed by agarose gel electrophoresis (AGE).

Binding ability of CRP to DNA

The binding ability of CRP to DNA was detected by dot blot analysis and ELISA analysis as previously described (Estabrook et al., 2004). For dot blot analysis, apopDNA (1 μg) was spotted on the nitrocellulose membranes. After the incubation of CRP protein (1 μg/mL), anti-CRP Abs, and peroxidase-labeled IgG Abs, the blots were developed with 3, 3'-diaminobenzidine (DAB) to measure the binding ability of CRP to apopDNA. Quantitative analysis of blots was done using Mini-Transilluminator (Bio-Rad Laboratories, Hercules, CA) equipped with molecular analysis software. The binding ability of CRP to apopDNA was reflected as mean intensity.

For ELISA analysis, ELISA plates (Costar) were pretreated with 0.5% protamine sulfate (Sigma-Aldrich) and then coated with apopDNA (1 μg/mL). The wells were blocked with 1% BSA and incubated with indicated mouse CRP protein. After incubating with anti-CRP Abs, the binding ability of CRP to apopDNA was detected with the HRP-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL). Tetramethylbenzidine (TMB) substrate (eBioscience, San Diego, CA) was used to develop colors and absorbance at 450 nm was measured on a microplate reader (Bio-Tek ELX800, Bio-Tek Instruments, Winooski, VT).

Real-time PCR analysis

Total RNA was isolated from cultured cells with TRIzol reagent (Invitrogen, Carlsbad, CA) and was reverse-transcribed (RT) using a cDNA synthesis kit (Ferments, Burlington, Ontario, Canada) according to the manufacturer's instructions (Xu et al., 2010). Subsequently, cDNA was subjected to quantitative real-time PCR using a Light-cycler480 and SYBR Green system (Roche Diagnostics Systems, Somerville, NJ) following the manufacturer's protocol.

ELISA assay

To assess protein levels of TNF-α, IL-1β, IL-6, IL-10, IL-12, MCP-1, and transforming growth factor-β (TGF-β) in the cell culture supernatants, ELISA assays were performed with relative ELISA kits (eBioscience) according to the manufacturer's instructions.

DNA uptake *in vitro*

ApopDNA was labeled with Alexa Fluor 488 (Invitrogen) according to the manufacturer's instructions. The labeled apopDNA (referred as AF488-apopDNA) was purified using Bio-Rad Micro Bio-Spin P-30 column (Bio-Rad, Hercules, CA), and then incubated with purified CRP protein (CRP/apopDNA) at 37°C for 2 h. BMDMs were treated with chloroquine (100 μg/mL) before apopDNA (50 μg/mL) or CRP/apopDNA (50 μg/mL) incubation. The intracellular AF488-apopDNA was determined by flow cytometry (FACSCalibur) as previously described (Chung et al., 2007). Data were acquired on a BD FACSCalibur (BD Biosciences) in CellQuest (BD Biosciences) and analyzed by FlowJo software (Tree Star, Ashland, OR).

Statistical analysis

All data are expressed as means ± SD of three independent experiments or from a representative experiment of three independent experiments. The statistical significance of the differences in the experimental data was examined by the Student's *t*-test. The statistical significance level was set at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China (Grant Nos. 30890141, 31100629, and 30671952), Shanghai STC grant (No. 10JC1401400), The Program for Outstanding Medical Academic Leaders of Shanghai (Grant No. LJ06011), Jiangsu "Pan-Deng" Project (Grant No. BK2010004), Postdoctoral Science Foundation Funded Project of China (Grant No. 20100480538) and Shanghai (Grant No. 11R21411000), The Research Fellow Fund and Startup Fund for Young Backbone Scholars of Shanghai Medical College, Fudan University.

ABBREVIATIONS

apopDNA, activated lymphocyte-derived apoptotic DNA; BM, bone marrow; BMDM, bone marrow-derived macrophage; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; FACS, flow cytometric; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; SLE, systemic lupus erythematosus; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; Un-apopDNA, lymphocyte-derived un-apoptotic DNA

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