C-reactive protein exacerbates renal ischemia-reperfusion injury

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C-reactive protein exacerbates renal ischemia-reperfusion injury. Am J Physiol Renal Physiol 304: F1358–F1365, 2013. First published March 27, 2013; doi:10.1152/ajprenal.00476.2012.—Renal ischemia-reperfusion injury (IRI) is a common cause of acute kidney injury (AKI), occurring with hypotension and cardiovascular surgery and inevitably during kidney transplantation. Mortality from AKI is high due to incomplete knowledge of the pathogenesis of IRI and the lack of an effective therapy. Inflammation accompanies IRI and increases the blood level of C-reactive protein (CRP), a biomarker of worsened outcomes in AKI. To test if CRP is causal in AKI we subjected wild-type mice (WT) and human CRP transgenic mice (CRPtg) to bilateral renal IRI (both pedicles clamped for 30 min at 37°C then reperfused for 24 h). Serum human CRP level was increased approximately sixfold after IRI in CRPtg (10.62 ± 1.31 µg/ml at baseline vs. 72.01 ± 9.41 µg/ml at 24 h) but was not elevated by sham surgery wherein kidneys were manipulated but not clamped. Compared with WT, serum creatinine, urine albumin, and histological evidence of kidney damage were increased after IRI in CRPtg mice. RT-PCR analysis of mRNA isolated from whole kidneys of CRPtg and WT subjected to IRI revealed that in CRPtg kidneys 1) upregulation of markers of macrophage classical activation (M1 markers) was blunted, 2) downregulation of markers of macrophage alternative activation (M2 markers) was more robust, and 3) expression of the activating receptor FcγRI was increased. Our finding that CRP exacerbates IRI-induced AKI, perhaps by shifting the balance of macrophage activation and FcγR expression toward a detrimental portfolio, might make CRP a promising therapeutic target for the treatment of AKI.

ACUTE KIDNEY INJURY (AKI) can occur in any setting where renal ischemia reperfusion injury (IRI) is manifest, including during cardiovascular surgery (37) and kidney transplantation (50). In fact, AKI is a serious complication in ~1% of all hospitalizations and has a mortality rate as high as 80% (22, 37). Despite this risk to patients and its burden on the health care system, there is still no effective therapy for AKI. The pathogenesis of renal IRI is not completely understood, but it is recognized that it is always accompanied by a systemic inflammatory response (35). Damage to the kidney is thought to evoke the release of inflammatory cytokines like TNF-α and IL-6 that in turn foster renal infiltration of leukocytes, neutrophils, dendritic cells, and macrophages (1, 20). Exactly how this inflammatory cascade culminates in kidney damage and how this process is regulated are unknown.

In accordance with the inflammatory nature of AKI, people with increased urinary albumin, a biomarker of AKI, also tend to have increased blood levels of the acute phase reactant C-reactive protein (CRP; Refs. 15, 39). Blood CRP generally increases as kidney function decreases (26, 36), and CRP levels are positively associated with worse outcomes and increased mortality in AKI (48, 49). In the context of renal transplantation, elevated CRP levels in graft recipients associate with graft failure, and local expression of CRP mRNA in donated kidneys correlates with both acute and chronic rejection (14, 31, 43). There is evidence from animal models of chronic kidney disease suggesting that CRP actively increases inflammation (21, 23). Despite all of this evidence of association, it is not known if CRP is causal in AKI.

CRP is an acute phase protein in humans, wherein its blood levels can rise dramatically from a typical baseline of <3 µg/ml to levels approaching 1 mg/ml within days after an inflammatory insult (8). This dynamic expression is regulated at the level of transcription, with IL-6 being the main inducer of the CRP acute phase response (40). Because of these properties, the CRP blood level is useful in the clinical setting as a marker of inflammatory status in patients (8). Furthermore, CRP also has several biological actions that are of potential direct relevance to disease. The best understood of these is the ability of CRP to bind the membrane lipid phosphatidylcholine (44), a molecule expressed on the cell surface of apoptotic and necrotic cells. Also, CRP exerts many biological actions by binding to activating Fcγ receptors (FcγRI, FcγRIIA, and FcγRIII) and inhibitory ones (FcγRIIB; Refs. 24, 25, 27, 38), which are all widely expressed by cells resident in the kidney (38) and infiltrating it after injury (10, 34). Importantly, because CRP can bind to the different FcγRs with comparable affinities (24, 25, 38), the outcome of CRP signaling mediated via FcγR interactions in the kidney is predicted to be dictated by resident FcγR diversity and availability. Finally, although the liver is the major source of blood CRP (11), renal tubular epithelial cells also produce CRP (13, 41) and so CRP actions in the kidney may be manifest even during periods of restricted blood flow.

Of the many types of FcγR-expressing (and thus potentially CRP responsive) inflammatory cells that infiltrate the kidney during IRI, macrophages are thought to play a central role (16, 18, 33). Soon after the kidney is injured, monocytes are recruited into the organ where they undergo differentiation into macrophages. These become polarized and exhibit either a proinflammatory M1 (classically activated) phenotype or an anti-inflammatory M2 (alternatively activated) phenotype (2, 29). Once M1 macrophages are primed they are highly phagocytic and can produce high amounts of proinflammatory molecules such as TNF-α, IL-1, IL-6, and inducible nitric oxide synthase (iNOS) that together contribute to tissue injury (5, 28, 30). Once M2 macrophages are primed they exhibit increased efferocytosis and can produce beneficial molecules like IL-10, mannose receptor, andarginase that can foster a reparative phase of AKI (5, 28). In mice, M1 macrophages have been shown to predominate in the kidney in the hours immediately following renal IRI (19, 45) whereas days later there is a switch...
to M2 macrophages (19). Since CRP interaction with macrophage FcγRs has been shown to influence the outcome of both immune-complex-mediated nephritis in vivo (34) and macrophage polarization in vitro (7), it is possible that CRP (circulating through the kidney or expressed locally) could influence macrophage activity and thus the injury process during renal IRI (7).

We undertook the current study to ascertain the impact of CRP in a mouse model of AKI. We compared the outcomes of renal IRI in wild-type mice (WT; wherein CRP is present but is not a major acute phase reactant) and human CRP transgenic mice (CRPtg; wherein human CRP is expressed as an acute phase reactant; Ref. 6). The data obtained provide direct evidence that CRP exacerbates the injury process during renal IRI.

MATERIALS AND METHODS

Animals. Human CRP transgenic mice (backcrossed to C57BL/6) have been fully described elsewhere (6, 40). These carry a 31-kb ClaI fragment of human genomic DNA comprised of the CRP gene, 17 kb of 5′-flanking sequence containing the human CRP promoter and all the known CRP regulatory elements, and 11.3 kb of 3′-flanking sequence that includes the CRP pseudogene (6). Human CRP is present in the blood of CRPtg at concentrations relevant to humans, i.e., low levels under steady-state conditions (<1 to 30 µg/ml) and much higher levels during the acute phase response (100–500 µg/ml; Refs. 8, 40). In WT and in CRPtg mice, mouse CRP is not a major acute phase protein (46). All animals were housed at constant humidity (60 ± 5%) and temperature (24 ± 1°C) with a 12-h light cycle (6 AM to 6 PM) and maintained ad libitum on sterile bottled water and regular chow (Harlan Teklad). Only male mice were used in experiments as males are more susceptible to renal IRI (32) and male CRPgt express human CRP more robustly than females (40). All animals were 8- to 12-wk-old when used, all animal protocols were approved by the Institutional Animal Care and Use Committees at the University of Alabama at Birmingham, and all mouse experiments were consistent with the Guide for the Care and Use of Laboratory Animals (NIH publication 96-01, revised 1996).

Ischemia-reperfusion injury. To induce acute bilateral kidney IRI, mice were anesthetized with isoflurane 2.5% inhalation and, following flank incisions, the right and left pedicles were exposed, secured, and clamped with an atraumatic microserrefine vascular clamp (catalog no. 18055-05; Fine Science Tools) for 30 min. During this ischemia, the kidneys were kept moist and the mice were maintained at 37°C. The clamp was then removed to allow reperfusion, which was verified visually, and the kidneys were restored to their original position in the body cavity. The ischemia was divided into two parts, with the second ischemia lasting 30 min, and the mice were allowed to recover. Blood and urine samples were taken 24 h before surgery and again 24 h after surgery when the mice were killed and their kidneys harvested. Each separate renal IRI experiment included at least six mice (3 mice per genotype), and each experiment was repeated at least three times. Sham-operated mice (abdominal surgery with kidney displacement but no ischemia) served as controls.

Bilateral nephrectomy and CRP injections. Mice were anesthetized with isoflurane 2.5% inhalation, and a bilateral nephrectomy (BNs) was performed as described by Andres-Hernando et al. (3). Briefly, a midline incision was made and the right and left renal pedicles were exposed, tied off with sutures, and then cut distally. The ureters were pinched off with forceps, and each kidney was removed. Sham surgery involved the same procedure, but renal pedicles were not sutured and kidneys were not removed. The incisions were closed, and the mice were allowed to recover overnight before each received an intravenous injection of human CRP (1 mg/ml in phosphate-buffered saline; US Biological) estimated to achieve 8 mg CRP/kg body wt. Blood was collected via tail vein 2 and 4 h later for measurement of circulating CRP levels.

Measurement of biomarkers. Mouse CRP was measured using the mouse C-Reactive Protein kit (Life Diagnostics) and the manufacturer’s instructions. Human CRP was measured using an enzyme-linked immunosorbent assay (ELISA) developed in our laboratory (40). The latter does not detect mouse CRP and has a lower limit of detection of ~20 ng of human CRP per ml of mouse serum. Urine albumin was measured by ELISA (Bethyl Laboratories) that has a lower limit of detection of 80 ng/ml. Serum creatinine was determined by tandem mass spectrometry (LC-MS/MS) as described previously (51).

Histology. Kidneys were harvested, bisected transversely through the pelvis, formalin fixed, paraffin embedded, and cut into 5-µm-thick sections. Sections were stained with periodic acid-Schiff reagent and histological assessment of renal damage was performed in a blinded fashion (by M. Pegues and A. Zarjou). For representative thin sections prepared from each kidney, 10 high-powered (×400) nonoverlapping fields of both the cortex and outer stripe of the medulla were imaged and each image was examined for evidence of changes characteristic of AKI, i.e., the number of degenerating/necrotic tubules, the number of tubules with brush border loss, and the number of tubules containing casts was counted (51).

Protein and RNA extraction. To examine hepatic and renal proteins we obtained organs from transcardially perfused mice. Twenty-four hours after renal IRI or sham surgery, mice were deeply anesthetized with isoflurane 2.5% inhalation. The thorax and abdomen were exposed via midline incision, and an 18-gauge needle was inserted into the left ventricle. The right atrium was snipped open, and immediately thereafter sterile 0.9% saline was injected into the left ventricle. Saline perfusion continued until the liver became noticeably pale in color, at which time the livers and kidneys were harvested. Samples of each organ were digested in homogenization buffer containing 0.5% Triton X-100 and a protease inhibitor cocktail (Sigma) in PBS, and digests of each organ were used for subsequent determination of total protein (DC protein assay kit from Bio-Rad) and CRP content (ELISA). Hepatic and renal CRP was obtained from organs isolated 24 h after renal IRI or sham surgery (without perfusion) and treated with TRIzol reagent according to the manufacturer’s protocol (Invitrogen).

Quantitative RT-PCR. DNase 1 treated RNA was converted to cDNA using the RETROscript kit (Ambion), and each quantitative (q) real-time PCR was performed with SYBR Green Mastermix (Bio-Rad) and specific primers for human CRP and mouse FcγRI, FcγRII, FcγRIII, TNF-α, mannose receptor (MR), iNOS, and arginase (ARG). Briefly, CDNA was amplified in an iCycler for 40 cycles, and for each mRNA species of interest the expression level was calculated with iCycler software (Bio-Rad). The specificity of each PCR reaction was monitored using melting curve analysis, and each reaction was performed in triplicate. The primers used for detection of specific mRNAs were as follows: for human CRP, sense TTTA-CAGTGGGGTGGTCTGAA and antisense CCACCGAAAGGATC- CTG; for FcγRI, sense CTTCAAGTTGGAGGCTG and antisense AGCAGCTGGCGTGTAA; for FcγRII, sense CTGGTGCAGC- CATTGTAT and antisense CGATATTCCAGGCTGTAAGT; for FcγRIII, sense ACAACCCCTGGGGAACTTCT and antisense CTCCATTGTA- CACGATA; for arginase, sense CTCAAAGCCCAAGTCTGTAAGGA- GAG and antisense AGGAGCGCTCATTAGGGCATC; for mannose receptor, sense CCTGCTGTGACACACTTGTGTT and antisense GCACTGCTGACACACTTGT; for TNF-α, sense AGGGCCACATGATTTCCAT; for Arg, sense CTCAAGCCCTCATCTTCTCC and antisense CCTGA- GGAGGGCTGACACAGG. Expression of mRNA for each gene was normalized against mRNA for the housekeeping gene GAPDH (sense ATTCCTCCACCTTTGAGTAC and antisense TGGTCCAGGGTT- TTCACTT). Genotype and treatment effects on mRNA expression were estimated using the ΔΔCt method relative to sham treated WT mice.
Statistical analysis. All grouped data are presented as means with associated SE. Statistical analyses were performed using Graphpad Prism 3.02 and Statview 5.0.1. One-way ANOVA, post hoc protected least-squared difference tests, and Student’s t-tests were used for comparisons among and between genotypes. Linear regression analysis was used to test for correlations between biomarkers, in which case Fisher’s r to z test was used to calculate P values. In all analyses a P value of ≤0.05 was considered significant.

RESULTS AND DISCUSSION

Robust elevation of circulating CRP induced by renal IRI in CRPtg mice. In CRPtg mice subjected to renal IRI, serum human CRP was significantly increased from 10.62 ± 1.31 μg/ml at baseline to 72.01 ± 9.41 μg/ml at 24 h (P < 0.0001, Student’s t-test; Fig. 1, closed bars), but there was no significant change in human CRP level following renal surgery without IRI. Mouse CRP levels were not significantly affected by either treatment (Fig. 1, open bars). Since both human and mouse CRP are synthesized primarily in the liver (6, 8, 11) and only human CRP is a major acute phase reactant (6, 8, 11, 46), these blood CRP responses are consistent with the earlier proposition (9) that renal IRI-mediated signals communicating with the liver can evoke a systemic acute phase response. Human and mouse CRP were recovered from perfused kidneys of CRPtg mice subjected to both sham and IRI treatments; although the difference was not statistically significant, an IRI-associated increase in renal CRP of approximately threefold was evident for the human protein (Fig. 2). In a separate experiment (Fig. 2, inset) we found no evidence that the approximately threefold increased recovery of human CRP from injured kidneys was attributable to increased local expression of the human CRP transgene. In their sum, the data shown in Figs. 1 and 2 are consistent with accumulation of hepatically expressed/blood borne CRP in the kidney after IRI.

Despite the much higher level of human CRP than mouse CRP in the blood and in the kidneys after renal IRI (Figs. 1 and 2, respectively), the amount of human CRP detected in the voided urine (0.339 ± 0.061 μg/ml) was not increased compared with mouse CRP (0.405 ± 0.229 μg/ml). This finding suggested that reduced renal clearance was not likely a major reason for the observed increase in human CRP in the blood and kidney following AKI. To test directly if IRI-induced elevation of blood human CRP was due to reduced renal clearance, we compared the blood clearance of human CRP in the circulation 2–4 h after its intravenous administration, nor the amount of endogenously expressed mouse CRP, was significantly different between sham-operated and BNx animals. The combined data thus indicate that elevation of blood-borne human CRP after renal IRI (Fig. 1), and likely its appearance in increased amounts in the injured kidney (Fig. 2), are the consequence of increased hepatic expression of the protein and not because of reduced renal clearance after AKI.

Fig. 1. Serum C-reactive protein (CRP) response to renal ischemia-reperfusion injury (IRI). Human CRP (black bars) and mouse CRP (white bars) were measured by ELISA (see MATERIALS AND METHODS) in sera collected from CRP transgenic mice (CRPtg) 24 h before and 24 h after sham surgery (sham) or renal ischemia-reperfusion injury (IRI). Each bar and whisker indicates a group mean ± SE. *P < 0.0001 and #P > 0.05, for unpaired t-tests comparing 24-h vs. 24-h values for the bracketed groups. $P < 0.005$, for unpaired t-test comparing 24-h values of human CRP (sham treated vs. IRI treated). Sample sizes are indicated.

Fig. 2. Renal CRP response to renal IRI. Total protein and human and mouse CRP recovered from CRPtg kidneys 24 h after sham or IRI surgeries was measured by ELISA. Each bar and whisker indicate the group means ± SE. Inset: box-and-whisker plot showing expression of human CRP mRNA (relative to GAPDH) in kidneys of CRPtg subjected to IRI vs. sham surgery. Sample sizes are given.
Biomarkers of renal damage correlate to increased human CRP. Elevation of both serum creatinine and urine albumin following renal IRI was more pronounced in CRPtg than WT mice (Fig. 4, A and B, respectively), with the difference in serum creatinine levels achieving statistical significance \((P < 0.001,\) one-tailed t-test). Importantly, there was a strong positive association of human CRP serum levels measured 24 h after renal IRI with concurrently measured serum creatinine (Fig. 5A; \(\beta = 34.049; r^2 = 0.33; P = 0.0067\)) and urine albumin (Fig. 5B; \(\beta = 0.052; r^2 = 0.868; P = 0.001\)). In stark contrast the levels of these biomarkers 24 h after IRI was not associated to baseline levels of human CRP (Fig. 5). Thus, as in humans with AKI, in CRPtg with AKI serum human CRP level associates positively with biomarkers of renal injury. The fact that in CRPtg baseline human CRP does not correlate with biomarkers of kidney damage suggests that CRP might be a modifier of ongoing AKI rather than an initiator of AKI.

Histological evidence of kidney damage is more evident in CRPtg mice subjected to renal IRI. Periodic acid-Schiff-stained sections of kidneys were examined for histological evidence of renal damage. For all animals subjected to renal IRI, kidney damage was more pronounced in the outer medulla than in the cortex (Fig. 6). In alignment with the biomarker data, deleterious tissue changes in the medulla (tubular necrosis, casts, and brush border loss) were more evident in kidneys from CRPtg that had experienced renal IRI (Fig. 6E) than in kidneys from WT mice that had experienced renal IRI (Fig. 6D). Quantitation of the medullary changes verified that tissue necrosis, brush border loss, and the number of renal casts was increased for CRPtg compared with WT following renal IRI (Fig. 7), although the difference in numbers of casts did not achieve statistical significance. Like the human CRP acute phase response (Fig. 1), the worsening of pathology was associated with IRI per se, as the renal medulla in kidneys from CRPtg subjected to sham surgery without renal IRI was largely unaffected (Fig. 6F). These results show that CRP expression affects the tissue injury response to renal IRI.

Human CRP changes expression of Fc receptors and markers of macrophage activation. As a first step towards understanding how CRP overexpression might achieve its deleterious effect on renal IRI, we investigated the pattern of mRNA expression in whole kidneys of WT vs. CRPtg mice. Compared with the mRNA levels in kidneys from sham-treated WT mice, in kidneys from IRI-treated WT (Fig. 8, white bars) expression of the M2 macrophage markers mannose receptor and arginase was reduced \(2\text{-}\)fold and \(10\text{-}\)fold, respectively, whereas expression of the M1 macrophage markers iNOS and TNF-\(\alpha\) were increased \(9\text{-}\)fold and \(6\text{-}\)fold, respectively. In the same WT/IRI mice, expression of the inhibitory receptor Fc\#RIIB was reduced approximately sevenfold. This pattern is consistent with reports that the early response to renal IRI in mice is dominated by a proinflammatory M1 macrophage response (19, 45) and aligns with the notion that decreased expression of Fc\#RIIB should de-inhibit macrophage activation (4, 42, 47). In comparison, in kidneys of CRPtg subjected to IRI the upregulation of M1 markers was blunted or reversed.

Fig. 3. Renal clearance of human and mouse CRP. WT mice were given intravenous injections of human CRP after either sham or bilateral nephrectomy (BNx; see MATERIALS AND METHODS). Blood was taken 2 and 4 h later and human CRP and mouse CRP levels assessed by ELISA. Note the break in the y-axis.

Fig. 4. Biomarkers of acute kidney injury (AKI) after renal ischemia-reperfusion injury. A: serum creatinine 24 h before (basal) and 24 h after surgery (sham or IRI) as measured by tandem mass spectrometry (LC-MS/MS) for CRPtg and wild-type (WT) mice. B: urine albumin as measured by ELISA. *\(P < 0.001,\) one-tailed t-test, serum creatinine level was significantly greater in the CRPtg/IRI group than in WT/IRI. Sample sizes are indicated.

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and the downregulation of M2 markers more robust (Fig. 8, black bars), consistent with a weaker M2 response in CRPtg than WT. In the same CRPtg kidneys, downregulation of FcgRIIB and FcgRIII was more evident than in treatment-matched WT, but there was a approximately fourfold increase in expression of the activating receptor FcgRI that was not seen in WT kidneys. Notably, kidney expression of FcgRI was also much greater in sham-treated CRPtg than sham-treated WT (Fig. 8, inset). CRP is known to engage all three of the FcgRs whose expression we measured (24, 25, 27), but their expression in the context of AKI and their possible role in propagation of organ damage following AKI have never been investigated. We posit that if FcgRs expressed on macrophages or elsewhere are required for propagating the deleterious action of CRP in the injured kidney, then this could be a consequence of the enhanced expression of the activating FcgRI receptor in CRPtg. In this scenario, any damage propagated by CRP→FcgRI interaction would possibly be more potent because of the IRI-associated suppression of the counteracting FcgRIIB receptor (4, 12, 42, 47).

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**Fig. 5.** Association of human CRP with biomarkers of AKI. Blood and urine was collected from CRPtg mice 24 h before (● baseline) and 24 h after undergoing renal IRI (● post-IRI) and human CRP and creatinine and albumin levels were determined (see MATERIALS AND METHODS). Serum creatinine (A) and urine albumin (B) measured after IRI were positively associated with serum human CRP measured after IRI but not with human CRP baseline levels. The β coefficient and $r^2$ value for each regression line are shown.

**Fig. 6.** Histological changes after renal IRI. Histology [periodic acid-Schiff (PAS) staining] of the renal cortex (A–C) and outer medulla (D–E) of representative kidneys collected from WT and CRPtg mice 24 h after renal ischemia reperfusion injury (IRI) or sham-surgery (sham). Original magnification of A–F is ×40. Bottom: 4-fold magnifications of the areas indicated in D–F. Note the PAS-positive brush border is intact in F and either damaged or lost in D and E. Also note the extensive tubular casts and necrotic cells in E.
To our knowledge, this is the first study that indicates CRP might play an active role in AKI, with CRP worsening the damage caused by renal IRI. Our preliminary analysis indicates acute phase expression of human CRP is associated with diversion of macrophage activation away from an otherwise beneficial M2 phenotype, a process that itself might depend on the observed CRP-associated changes in the balance of activating and inhibitory FcγRs (12). In this AKI setting, increased expression of FcγRII possibly allows human CRP to promote a generally activating effect, which might be enhanced by the concomitant decrease in expression of the inhibitory receptor FcγRIIB. This is consistent with reports that in vitro human CRP polarizes macrophages towards an M1 phenotype and inhibits their transformation to an M2 phenotype in an FcγR-dependent manner (7). A recognized limitation of our study is that because it was limited to investigation of the early injury response, we were unable to compare any effect CRP might have on the later kidney repair process (19). This remains to be determined. Also not yet known is whether the observed changes in renal expression of M1 markers, M2 markers, and FcγRs reflect changes on a single cell type (macrophages) or multiple ones. Analysis of the communities of inflammatory cells recoverable from injured CRPtg vs. WT kidneys is required to address this question, which is currently ongoing.

Finally, not consistent with our “weakened M2” model of CRP action in AKI is our finding that TNF-α expression is significantly lower in kidneys from CRPtg mice (Fig. 8).

AKI is a prevalent and potentially lethal condition that arises in many health care settings. It is difficult to predict and diagnose AKI due to the use of biomarkers that lack sensitivity and specificity, and AKI is difficult to treat. Here we have provided evidence that CRP, a widely recognized blood marker of AKI, might exacerbate the injury response after renal IRI, perhaps by altering macrophage polarization and FcγR expression early during the course of injury. It is not yet known if our results will translate to humans, but if they do, targeting CRP (17) might be a potential therapeutic approach to limit tissue damage in AKI.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.A.P., A.Z., and A.J.S. conception and design of research; M.A.P. and M.A.M. performed experiments; M.A.P., A.Z., and A.J.S. analyzed data; M.A.P. and A.J.S. interpreted results of experiments; M.A.P. and A.J.S. prepared figures; M.A.P. and A.J.S. drafted manuscript;
REFERENCES


41. Whitehead AS, Zahedi K, Rits M, Mortensen RF, Lelias JM. Mouse C-reactive protein. Generation of cDNA clones, structural analysis, and


