Importance of C-Reactive Protein in Regulating Monocyte Tissue Factor Expression in Patients with Inflammatory Rheumatic Diseases

HONG CAI, CHANGJIE SONG, IRWIN GEOK SAN LIM, STEVEN A. KRILIS, CAROLYN L. GECZY, and H. PATRICK McNEIL

ABSTRACT. ***Objective.*** To determine the relationship between plasma C-reactive protein (CRP) concentrations and monocyte tissue factor (TF) expression induced *in vitro* by combinations of CRP, ß$_2$-glycoprotein I (ß$_2$-GPI), and lipopolysaccharide (LPS).

Methods. Peripheral blood mononuclear cells (PBMC) from 26 healthy individuals and 31 patients with inflammatory rheumatic diseases (IRD) were cultured with combinations of CRP, purified or recombinant ß$_2$-GPI, and LPS and monocyte TF procoagulant activity, TF antigen, and TF mRNA were measured. Results were examined against plasma CRP levels.

Results. Monocytes from patients with IRD expressed significantly more TF when stimulated with CRP compared to normal monocytes ($p = 0.002$). An incremental positive correlation was observed between plasma CRP levels and TF induced by CRP or ß$_2$-GPI. Significantly more TF was induced with CRP combined with ß$_2$-GPI, compared to ß$_2$-GPI alone, either with costimulation or CRP priming. Conversely, when combined with LPS, ß$_2$-GPI suppressed TF induction in a dose-dependent manner on normal PBMC but not on PBMC from patients with IRD. The loss of suppression correlated strongly with plasma CRP levels.

Conclusion. This study shows a remarkably consistent effect of CRP on monocyte TF expression. Systemic inflammation associated with elevated plasma CRP conferred a phenotype on PBMC, whereby incremental priming with respect to TF expression (induced by CRP itself or ß$_2$-GPI) was apparent, and ß$_2$-GPI-mediated inhibition of TF expression induced by LPS was incrementally lost. CRP regulation of monocyte TF could contribute to the higher than expected atherosclerotic vascular disease seen in patients with IRD. (J Rheumatol 2005;32:1224–31)

Key Indexing Terms:
RHEUMATOID ARTHRITIS THROMBOPLASTIN C-REACTIVE PROTEIN

Inflammation and coagulation are inextricably linked[1,2], even though they have generally been studied separately. Fibrinogenesis, one outcome of coagulation activation, is a long-recognized prominent feature of synovial inflammation[3,4], and recent studies have started to define the relevant molecular pathways[5]. Fibrin formation *in vivo* occurs predominantly via activation of the extrinsic coagulation pathway, initiated by tissue factor (TF), a transmembrane receptor for plasma factor VII (FVII). FVII/TF ligation results not only in activation of factor VII (FVIIa), which generates FXa, leading to thrombin formation, but also in cellular activation via intracellular signaling[6]. Although fibroblast, epithelial, and endothelial cell TF expression is relevant to healing and repair processes in tissue injury, induction of TF expression on monocytes and macrophages is considered the dominant process responsible for enhanced TF activity and fibrin deposition in inflammatory and infectious diseases[7]. The acute phase reactant C-reactive protein (CRP) induces monocyte TF *in vitro*[8] and CRP-transgenic mice have higher rates of thrombosis occlusion following arterial injury[9]. Moreover, elevated plasma CRP concentrations are associated with thrombosis, acute coronary syndromes, and death in atherogenic cardiovascular disease[10,11]. Atherosclerosis is considered to be a chronic inflammatory disease[12-14] with evidence for roles for proinflammatory cytokines such as interleukin 1ß (IL-1ß), tumor necrosis factor, and interferon-γ[15], and for monocyte TF[16].

Patients with chronic inflammatory rheumatic diseases (IRD) such as rheumatoid arthritis (RA) and systemic lupus

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erythematosus (SLE) generally have sustained elevations of acute phase proteins in plasma, and these patients also have higher than expected rates of atherogenesis and thromboses[17-20]. Thus CRP-mediated monocyte/macrophage TF may be an important link between synovial inflammatory pathways and vascular disease. In SLE, both atherogenesis and thromboses are associated with the presence of antiphospholipid (aPL) antibodies[21], the primary antigen of which is a phospholipid-binding plasma protein, $ß_2$-glycoprotein I ($ß_2$-GPI)[22,23]. There is evidence for enhanced monocyte TF expression in patients with the aPL syndrome (APS)[24], and although we previously proposed that autoimmunity to $ß_2$-GPI may contribute to monocyte TF expression in APS[25,26], initial results supporting this hypothesis have not been sustained[27].

In this study, we investigated the complex interactions between CRP and $ß_2$-GPI in induction of TF *in vitro* on monocytes from healthy subjects and patients with IRD. The results emphasize a key role for CRP in influencing the ability of $ß_2$-GPI to either induce or inhibit monocyte TF expression.

MATERIALS AND METHODS

Patients, blood samples, and materials. The study group consisted of 57 individuals. There were 26 healthy persons and 31 patients with IRD. Ten patients had either SLE or the APS or both; 3 of these had SLE without anticardiolipin (aCL) antibodies and no histories of APS clinical events; 7 had APS including elevated levels of aCL antibodies and/or presence of lupus inhibitor (data not shown). Of the remaining 21 patients, 14 had RA (American College of Rheumatology criteria[28]) and 7 had either acute or chronic gout. Peripheral venous citrated blood was collected from all subjects and used to prepare peripheral blood mononuclear cells (PBMC). Plasma CRP levels were measured using a sensitive turbidometric immunoassay (Dade Behring Inc., Dearfield, IL, USA).

Details of the study groups are shown in Table 1. The normal group was slightly younger than the patient group but differences between these groups and between patient subgroups were not statistically significant. The gender ratio was 58% female in both normal (15/26) and patient (18/31) groups. Patients with IRD had variably elevated plasma CRP levels (range 1–277 µg/ml), whereas all but one normal individual had levels < 5 µg/ml. Patients with APS with or without SLE had only modest elevations of plasma CRP levels compared to the inflammatory arthritis group.

Purified CRP from human plasma and lipopolysaccharide (LPS) were purchased from Sigma (St. Louis, MO, USA). Human $ß_2$-GPI was purified from pooled human plasma as described[25]. Recombinant $ß_2$-GPI (r$ß_2$-GPI) was generated as described[29,30], using baculovirus pBacPak6-mediated expression of the human $ß_2$-GPI cDNA in Sf 9 cells grown in serum-free medium. The recombinant protein was purified from conditioned medium using affinity chromatography with a nickel column or a rabbit polyclonal anti-$ß_2$-GPI antibody. Endotoxin levels of purified and r$ß_2$-GPI were determined by Limulus amoebocyte lysate (LAL) assay (Associates of Cape Cod Inc., East Falmouth, MA, USA). Reagents containing > 0.125 EU/ml endotoxin were not used.

Table 1. Details of the study group. Data represent mean ± SEM.

Group	n	Age, yrs	Plasma CRP, mg/l
Healthy controls	26	44.5 ± 3.8	2.7 ± 0.7
Patients	31	53.9 ± 3.0	39.5 ± 13.7
APS and/or SLE	10	50.8 ± 5.0	9.8 ± 4.4
Inflammatory arthritis	21	55.3 ± 3.7	53.6 ± 19.4

CRP: C-reactive protein, APS: antiphospholipid syndrome, SLE: systemic lupus erythematosus.

Cell culture. PBMC from all subjects were isolated by density gradient centrifugation as described[25], washed twice in Hanks' balanced salt solution (Sigma), resuspended (1.5×10^6/ml) in AIM V serum-free medium (Gibco, Invitrogen Corp., Auckland, New Zealand), and cultured (1.5×10^5/100 µl/well) in duplicate in 96 well flat-bottom plates (Nunc, Roskilde, Denmark). CRP (25 µg/ml final concentration), $ß_2$-GPI (12.5 µg/ml), LPS (100 ng/ml), or their combinations were incubated in a final volume of 200 µl AIM V medium, at 37°C in 5% CO_2 in air for 16 h. For CRP priming, PBMC were cultured with AIM V or with CRP (25 µg/ml) for 6 h, then $ß_2$-GPI (12.5 µg/ml) was added and cells cultured for a further 16 h.

Measurement of procoagulant activity. At the end of culture, plates were centrifuged at 1400 rpm for 10 min, supernatants removed, and cells resuspended in 200 µl/well AIM V and subjected to 2 cycles of freeze-thawing (–70°C/37°C). After the final thaw, procoagulant activity was measured using a 1-stage plasma recalcification test as described[31] using an automatic coagulometer (Diagnostica Stago, Asnieres, France). Duplicate measurements were made for each sample. Activity was calculated from a TF standard curve[32] and results expressed as stimulation index (TF-SI: mU TF of stimulated PBMC/mU TF of unstimulated PBMC). Earlier studies confirmed that, as with LPS, the majority of procoagulant activity induced by CRP and CRP in combination with other stimulants is TF[8,33].

Flow cytometry. PBMC (2×10^6) from healthy subjects were incubated in AIM V or AIM V containing $ß_2$-GPI (12.5 µg/ml), LPS (100 ng/ml), or $ß_2$-GPI (12.5 µg/ml) plus LPS (100 ng/ml) for 16 h in minisorb tubes (Nunc), then tubes were gently vortexed and centrifuged for 5 min at 1300 rpm and supernatants discarded. Cells were stained with FITC-labeled mouse anti-human CD14 monoclonal antibody (Mab; Becton Dickinson Pharmingen, Mountain View, CA, USA), FITC-conjugated mouse anti-human TF Mab (American Diagnostic Inc., Hauppauge, NY, USA) or FITC-conjugated mouse IgG isotype control antibody (Serotec, Kidlington, UK). Flow cytometry was performed using a FACScan (Becton Dickinson). Populations were gated on CD14-positive monocytes for analysis. Fold increases in mean channel fluorescence relative to control IgG were calculated.

Measurement of TF mRNA in PBMC. After stimulation for the times indicated, total RNA was extracted using the single-step guanidinium thiocyanate-phenol-chloroform method[34]. TF mRNA was quantified using the ABI-Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) and expressed relative to mRNA levels of the internal positive control, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH).

Statistical analysis. Data are expressed as mean ± SEM and analyzed by unpaired or paired t test when appropriate. Data not normally distributed were converted to their squared roots for analysis. Statistical significance was set at $p < 0.05$. Correlations between TF-SI values and levels of CRP (log) were examined using the 2-tailed Pearson r test.

RESULTS

CRP-induced TF is enhanced in patients with IRD and correlates with plasma CRP levels. When PBMC from patients with IRD (n = 31) were stimulated with CRP, monocyte TF activity increased markedly above basal levels (mean TF-SI 19.2 ± 3.1). In contrast, only modest induction was observed with PBMC from healthy individuals (n = 26, mean TF-SI 7.6 ± 1.3; p = 0.0006; Figure 1A). Of the 26 healthy individuals, plasma from 18 had CRP levels ≤ 2 µg/ml, and 25 had ≤ 5 µg/ml. Most patients with IRD had elevated plas-

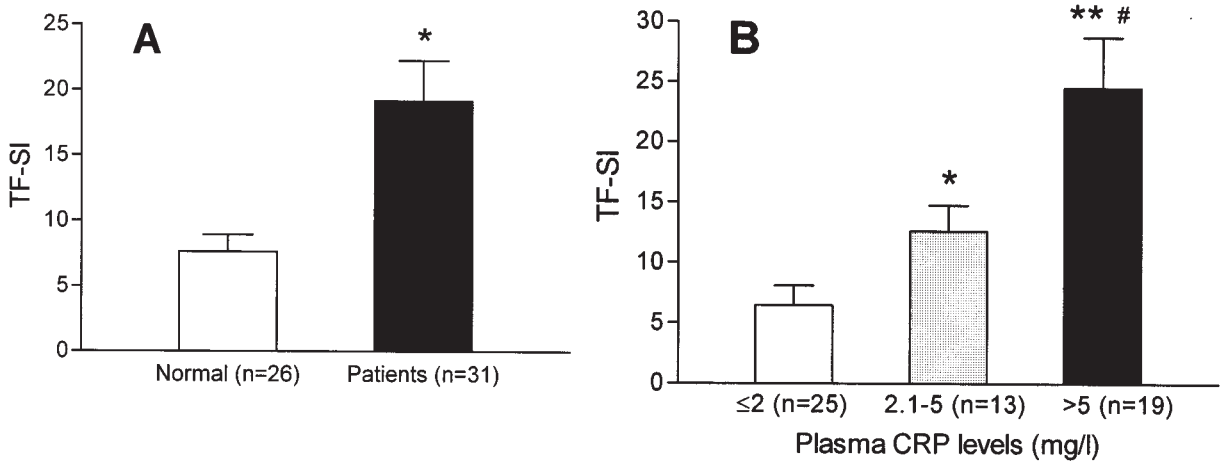


Figure 1. (A) Tissue factor (TF) expression (TF-SI) after stimulation with 25 µg/ml CRP on PBMC from healthy controls (n = 26) and patients with IRD (n = 31). *p = 0.0006. In (B), the data from both groups (n = 57) are stratified according to plasma CRP levels. *p = 0.03 (CRP 2.1–5 vs CRP ≤ 2 mg/ml), **p = 0.04 (CRP > 5 vs CRP 2.1–5 mg/ml), #p < 0.0001 (CRP > 5 vs CRP ≤ 2 mg/ml).

ma CRP levels across a wide range (mean 39.5 ± 13.7, range 1–277; Table 1); levels in patients with APS and/or SLE were ~80% lower than those with RA or gout, although procoagulant responses induced by CRP, on PBMC from the latter, were significantly greater than activity induced on cells from control samples. When all patients with IRD and healthy controls were considered together and stratified according to plasma CRP concentrations, an incremental relationship between plasma CRP level and monocyte TF activity stimulated by CRP *in vitro* was apparent (Figure 1B). Activity of cells from individuals with CRP levels > 5 mg/l was some 4-fold greater than cells from individuals with plasma CRP levels < 2 mg/l, after stimulation with CRP ($p < 0.0001$).

To examine this relationship further, plasma CRP levels in all subjects (n = 57) were logarithmically transformed to normalize the distribution of data and were examined against CRP-induced TF. A statistically significant correlation ($r = 0.60$, $p < 0.0001$) was evident (Figure 2), and was apparent both at the low levels of plasma CRP found in the normal group ($r = 0.66$, $p < 0.001$) and with the higher plasma CRP levels in the patient group ($r = 0.46$, $p = 0.009$). In contrast to the differences in magnitude of TF activity induced by CRP, PBMC from controls and patients expressed high but not statistically different levels of TF activity in response to LPS (TF-SI 171 ± 44.6 vs 207 ± 30.2, respectively).

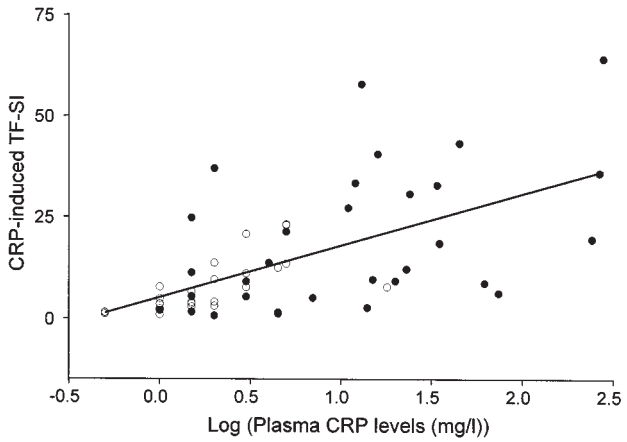


Figure 2. Correlation between TF expression on PBMC induced by 25 µg/ml CRP (y axis) and normalized plasma CRP levels (x axis) in the entire study group.

TF induced by ß$_2$-GPI is highest on cells from individuals with high CRP levels. When incubated with purified ß$_2$-GPI, PBMC from healthy controls showed a mild dose-dependent induction of TF that was optimal at 12.5 µg/ml (data not shown). There was no significantly higher induction on PBMC from patients with APS and/or SLE than on cells from controls (Table 2). Further, no differences were apparent when the APS/SLE group was separated into those with or without APS. Monocytes from patients with inflammato-

Table 2. Monocyte tissue factor (TF) activity following stimulation with purified or recombinant ß$_2$-GPI. Data represent mean ± SEM of TF stimulation index (TF-SI) on PBMC incubated with purified or recombinant ß$_2$-GPI (12.5 µg/ml) for 16 h.

Group	TF Induction (TF-SI) Purified ß$_2$-GPI	rß$_2$-GPI
Healthy controls	2.2 ± 0.4 (n = 26)	5.0 ± 1.1 (n = 8)
Patients with IRD	4.5 ± 1.1 (n = 31)	8.8 ± 3.1 (n = 10)
APS and/or SLE	3.1 ± 0.6 (n = 10)	6.9 ± 2.2 (n = 7)
SLE without APS	3.1 ± 1.6 (n = 3)	13.4 ± 5.9 (n = 2)
APS with or without SLE	3.1 ± 0.7 (n = 7)	4.2 ± 0.9 (n = 5)
Inflammatory arthritis	5.1 ± 1.6* (n = 21)	13.2 ± 9.7 (n = 3)

* p = 0.006 vs controls. IRD: inflammatory rheumatic disease.

ry arthritis expressed slightly higher TF activity in response to ß$_2$-GPI than cells from controls, and although levels were low, differences reached statistical significance (p = 0.006). Similar results were obtained with rß$_2$-GPI, although TF-SI levels were higher than values obtained with purified ß$_2$-GPI, but the differences obtained with the 2 reagents were not significant.

When all patients with IRD (n = 31) and controls (n = 26) were considered together and stratified according to plasma CRP levels, an incremental relationship between plasma CRP levels and TF induced by ß$_2$-GPI was evident (Figure 3). In individuals with plasma CRP levels > 5 mg/l, ß$_2$-GPI induced significantly higher TF (~2.5-fold) than on monocytes from individuals with plasma CRP levels ≤ 2 mg/l (p = 0.04), suggesting that CRP *in vivo* may enhance the sensitivity of PBMC to *ex vivo* ß$_2$-GPI stimulation.

To examine the role of CRP in upregulation of TF induction by ß$_2$-GPI further, PBMC from 16 controls and 13 patients with IRD were stimulated with the combination of CRP plus ß$_2$-GPI. This induced more TF activity than CRP or ß$_2$-GPI alone, on monocytes from healthy controls (p = 0.007 and p = 0.001, respectively). In the 13 patients (7 APS/SLE and 6 inflammatory arthritis), CRP + ß$_2$-GPI induced greater TF activity than ß$_2$-GPI alone (p = 0.007), but the difference compared to CRP alone was not significant (p = 0.4; Figure 4). In addition, CRP + ß$_2$-GPI induced more TF activity on monocytes from patients with IRD than on monocytes from healthy individuals (p = 0.009).

To determine whether CRP priming could enhance TF induced by ß$_2$-GPI, PBMC from 6 healthy individuals and 4 patients with APS/SLE were precultured with CRP prior to stimulation with ß$_2$-GPI. ß$_2$-GPI-induced TF on PBMC from controls was enhanced in a dose-dependent manner by CRP priming (Figure 5A). PBMC from controls and patients primed with 25 µg/ml CRP had significantly enhanced ß$_2$-GPI-induced TF activity (TF-SI 13.7 ± 4.2 vs 1.2 ± 0.1, and 8.9 ± 3.0 vs 1.8 ± 0.2; Figure 5B). To test whether CRP priming enhanced responses to other stimulants, or was specific for ß$_2$-GPI, primed PBMC were tested with LPS. Figure 5C shows that responses of cells from patients primed with 25 µg/ml CRP were approximately doubled (TF-SI 208.6 ± 32.0 vs 113.0 ± 21.4). Responses of PBMC from controls were also enhanced by CRP priming (TF-SI 108.6 ± 31.1 vs 61.7 ± 15.2; Figure 5C).

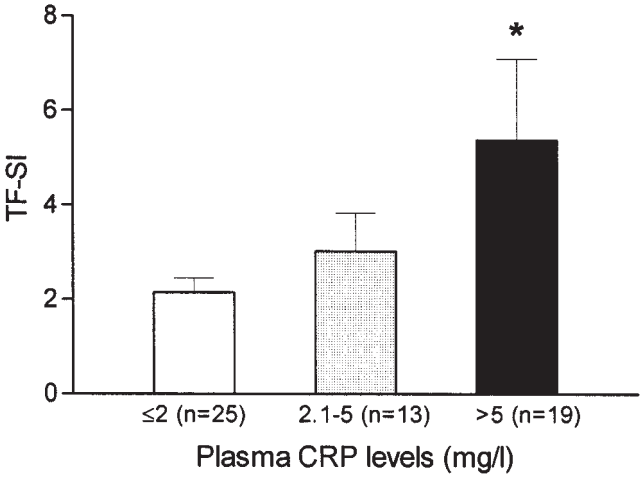


Figure 3. TF expression (TF-SI) after stimulation with 12.5 µg/ml ß$_2$-GPI on PBMC from the entire study group (n = 57) stratified according to plasma CRP levels. *p = 0.04 (CRP > 5 vs CRP ≤ 2 mg/ml).

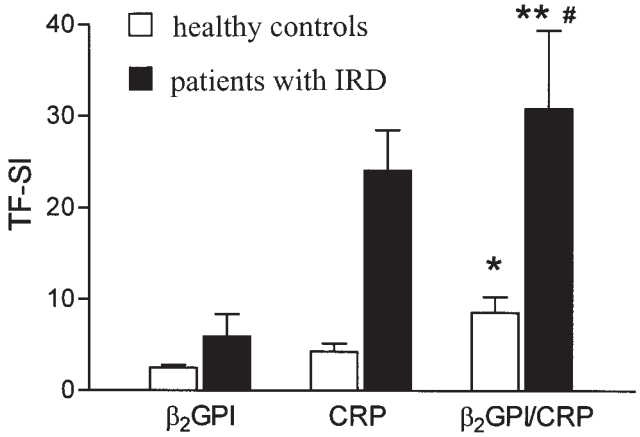
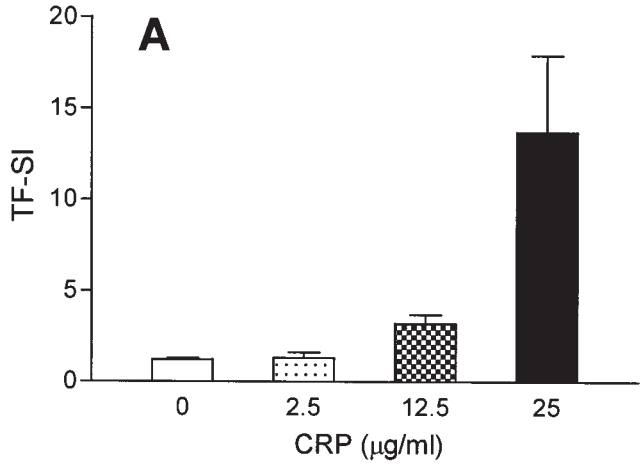


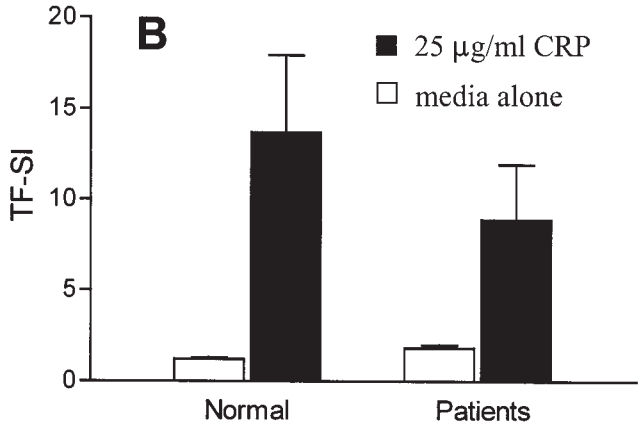
Figure 4. TF expression (TF-SI) after stimulation with 12.5 µg/ml ß$_2$-GPI, 25 µg/ml CRP, or the combination on PBMC from healthy controls (n = 16) and patients with IRD (n = 13). *p = 0.007 and p = 0.001 (CRP + ß$_2$-GPI vs CRP, and CRP + ß$_2$-GPI vs ß$_2$-GPI); **p = 0.01 (CRP + ß$_2$-GPI vs ß$_2$-GPI); #p = 0.009 (patients vs controls).

ß$_2$-GPI inhibition of LPS-induced TF is dependent on plasma CRP levels. When combined with LPS (100 ng/ml), ß$_2$-GPI suppressed TF induction. Inhibition was dose-dependent, and was maximal with 12.5 µg/ml of purified or rß$_2$-GPI (Figure 6A). More extensive testing using PBMC from controls (n = 26) showed that purified ß$_2$-GPI consistently inhibited LPS-induced TF, with statistically significant differences (p = 0.001). When tested on cells from a subgroup of healthy individuals (n = 8), rß$_2$-GPI was more inhibitory (p = 0.003; Figure 6B).

To determine if the inhibitory effect of ß$_2$-GPI was due to a reduction in TF antigen, levels on PBMC were compared using flow cytometry. The relative expression of LPS-induced TF antigen on monocytes from 3 of 3 healthy controls was reduced by ß$_2$-GPI (5.2 ± 0.6 vs 4.5 ± 0.6). Moreover, using real-time reverse transcription-polymerase chain reaction (RT-PCR) to assess steady-state TF mRNA levels, purified ß$_2$-GPI inhibited TF gene transcription induced by LPS in 3 of 3 controls (relative TF mRNA, 32.4 ± 10.2 vs 26.1 ± 8.8).

In contrast to the reduction in TF levels observed on PBMC from healthy controls, purified ß$_2$-GPI did not significantly inhibit LPS-induced TF activity of PBMC from patients with IRD (n = 31; Figure 6C). In addition, the weak inhibitory effect of rß$_2$-GPI on TF expression of PBMC





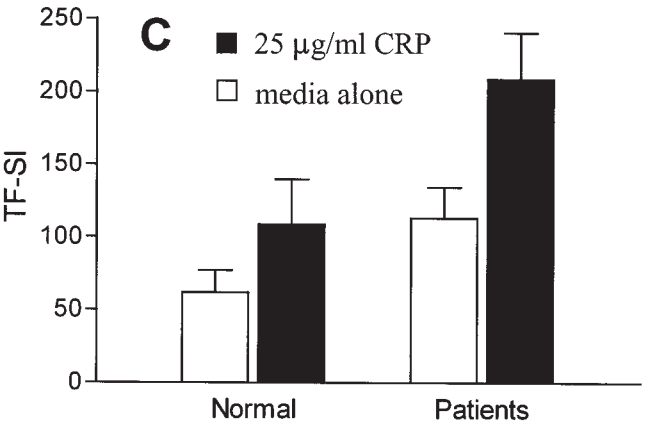
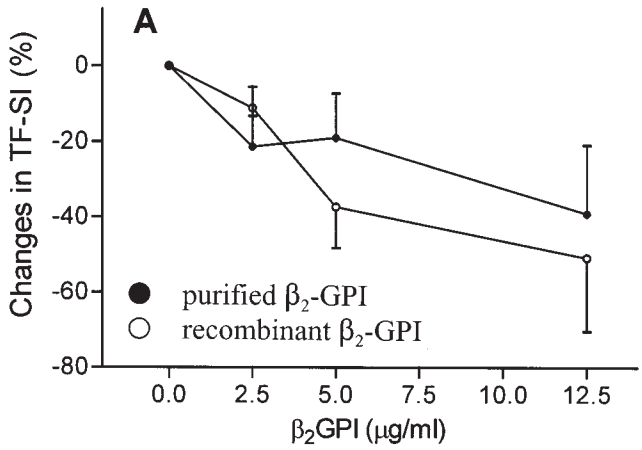
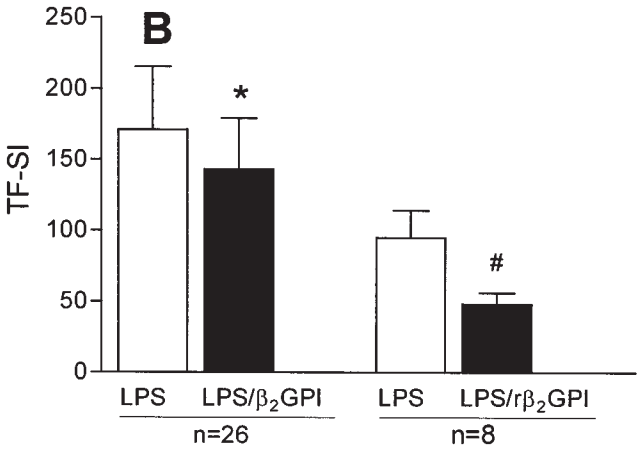


Figure 5. TF expression (TF-SI) on PBMC from healthy controls and patients with IRD after stimulation with 12.5 µg/ml ß$_2$-GPI after priming with 0–25 µg/ml CRP for 6 h. (A) Dose-dependent enhancement of ß$_2$-GPI-induced TF expression by CRP on PBMC from healthy controls (n = 6). (B) ß$_2$-GPI-induced TF expression after 6 h priming with 25 µg/ml CRP vs media alone on PBMC from healthy controls (n = 6) and patients with IRD (n = 4). (C) LPS-induced TF expression after 6 h priming with 25 µg/ml CRP vs media alone on PBMC from healthy controls (n = 6) and patients with IRD (n = 4).





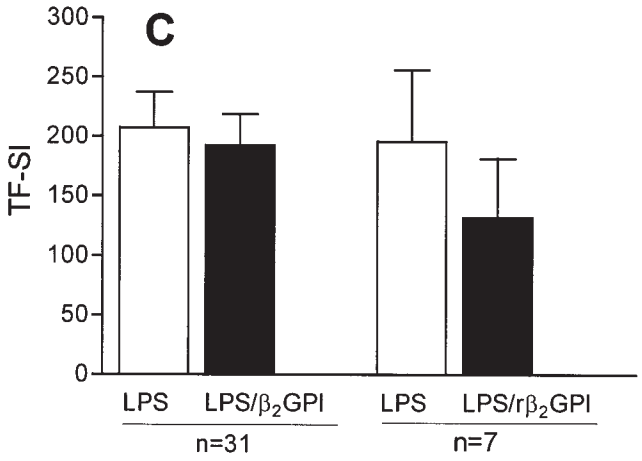


Figure 6. (A) Reduction in TF expression induced by 100 ng/ml LPS by the addition of 0–12.5 µg/ml purified ß$_2$-GPI or recombinant ß$_2$-GPI on PBMC from healthy controls. Results are expressed as percentage reduction in TF-SI and represent the means of duplicates of 3–4 separate experiments. (B and C) LPS-induced TF expression (TF-SI) with or without 12.5 µg/ml purified ß$_2$-GPI (left side of graphs) or 12.5 µg/ml recombinant ß$_2$-GPI (right side of graphs), on PBMC from (B) healthy controls (*p = 0.001, #p = 0.003) or (C) patients with IRD.

from patients with IRD (n = 7) was not statistically significant (p = 0.15).

Because healthy individuals and patients with IRD differed in their plasma CRP levels, the relationship of these to $ß_2$-GPI-mediated inhibition of LPS-induced TF activity was examined. When all patients with IRD (n = 31) and controls (n = 26) were considered together and stratified according to plasma CRP levels, an incremental loss of inhibitory activity of $ß_2$-GPI was observed as plasma CRP levels increased (Figure 7). The differences were significant when TF activity on cells from individuals with CRP levels > 5 mg/l were compared to those with CRP levels ≤ 2 mg/l (p = 0.02).

DISCUSSION

Inflammation and immune activation are linked with thrombosis associated with rheumatoid disorders, and proposed mechanisms include modulation of TF expression, enhanced procoagulant binding to platelets, and interference with antithrombotic mechanisms. For example, antiphospholipid antibodies against the phospholipid-binding protein $ß_2$-GPI have been reported to increase thrombin generation by enhancing procoagulant reactions on TF-bearing cells[35]. CRP is a marker of inflammation and has numerous proinflammatory effects on monocytes/macrophages, including upregulation of TF activity[8]. We showed previously that synergy between CRP and mediators such as LPS and interferon-γ exhibits a striking positive correlation between age and TF induction on monocytes from healthy individuals[33], and suggested that this may be an important amplification mechanism triggering thrombosis. Furthermore, transgenic mice overexpressing human CRP exhibit a prothrombotic phenotype following vascular injury[9], confirming that CRP can directly modulate vascular function *in vivo.* High serum concentrations of CRP in apparently healthy individuals are associated with increased risk of cardiovascular events[36-38], and in patients with unstable coronary syndromes are associated with increased risk of infarction or death[10]. CRP is increased in most inflammatory disorders, including IRD, and although patients with RA and SLE have higher than expected rates of atherogenesis and thromboses[17-20], the responsiveness of monocytes from these patients to CRP has not been reported.

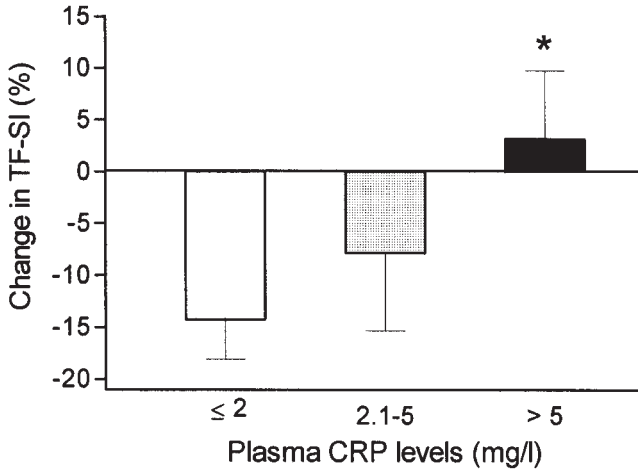


Figure 7. Change in TF expression induced by 100 ng/ml LPS by the addition of 12.5 µg/ml purified $ß_2$-GPI on PBMC from the entire study group (n = 57) stratified according to plasma CRP levels. *p = 0.02, CRP > 5 vs CRP ≤ 2 mg/ml.

A major finding of this study is that plasma CRP levels, a measure of inflammatory activity *in vivo*, correlated positively with the degree of TF induced by CRP on monocytes from healthy controls or individuals with IRD *in vitro* (Figures 1 and 2). Similarly, $ß_2$-GPI weakly induced TF on PBMC from controls with low plasma CRP levels, and greater induction was observed on cells from patients with inflammatory arthritis (Table 2). Elevated plasma CRP levels were associated with incremental increases in TF expression induced by $ß_2$-GPI (Figure 3).

Importantly, PBMC expressed significantly more TF *in vitro* when CRP was combined with $ß_2$-GPI compared to $ß_2$-GPI alone (Figure 4). Moreover, CRP was also able to enhance the sensitivity of PBMC to express TF when added prior to subsequent $ß_2$-GPI stimulation (Figure 5). CRP binds monocytes via FcγRII and activates the extracellular signal-related kinase (ERK) pathway[39]. LPS also employs the ERK/nuclear factor-κB pathway to induce TF gene expression in monocytes[40], and it is probable that the ability of CRP to enhance TF expression by other stimulants occurs at the intracellular signal transduction level. Together, these results provide strong evidence that monocytes from patients with IRD are activated or "primed" with respect to TF induction, and that CRP may mediate this, since it can be replicated *in vitro* using CRP, and *in vivo*, plasma CRP levels correlated with the priming effect.

Initial interest in $ß_2$-GPI as a possible inducer of TF arose after the description of cellular immunity to $ß_2$-GPI in patients with APS[25], a condition associated with a prothrombotic diathesis. We observed that purified or recombinant $ß_2$-GPI induced similar levels of TF on PBMC from patients with APS and on PBMC from healthy individuals (Table 2). However, $ß_2$-GPI partially suppressed TF induced by LPS on PBMC from healthy individuals, but not on PBMC from patients with IRD (Figure 6). Flow cytometry confirmed that the suppression was likely a diminution in TF antigen expression and the effect was at the level of gene transcription, as $ß_2$-GPI suppressed LPS induction of TF mRNA assessed by quantitative RT-PCR. This ability of $ß_2$-GPI to partially inhibit LPS-induced TF on monocytes from healthy individuals is a novel finding, and may suggest a regulatory role for $ß_2$-GPI in expression of monocyte procoagulant activity *in vivo*. The importance of CRP in modulating this suppression was evident by the correlation between plasma CRP levels and the incremental loss of $ß_2$-GPI-mediated suppression of LPS-induced TF (Figure 7).

The IRD group was relatively heterogenous, comprising

patients with acute and chronic joint inflammation, with variable levels of disease activity, and who were taking a range of medications. The small size of these various subgroups within the overall IRD group did not allow subgroup analysis. Nevertheless, the results show a remarkably consistent effect of CRP on monocyte TF expression. Systemic inflammation associated with elevations of plasma CRP appears to confer a phenotype on monocytes, whereby incremental priming with respect to TF induced either by CRP itself or by/with ß$_2$-GPI was apparent, and ß$_2$-GPI-mediated downregulation of TF expression induced by LPS was incrementally lost.

Our results strengthen studies proposing CRP as an important inducer of monocyte TF *in vitro*[8]. Because patients with IRD have a higher than expected incidence of atherosclerotic vascular disease (most thoroughly documented in RA and SLE), CRP induction of monocyte TF could be an important mechanism that contributes to vascular disease *in vivo*.

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