EXTENDED REPORT

Activated complement components and complement activator molecules on the surface of cell-derived microparticles in patients with rheumatoid arthritis and healthy individuals

Éva Biró, Rienk Nieuwland, Paul P Tak, Loes M Pronk, Marianne C L Schaap, Augueste Sturk, C Erik Hack

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See end of article for authors' affiliations

Correspondence to: Éva Biró, Department of Clinical Chemistry, F-1-219, Academic Medical Center, University of Amsterdam, PO Box 22660, 1100 DD, Amsterdam, Netherlands; E.Biro@amc.nl

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Objectives: In vitro, microparticles can activate complement via the classical pathway. If demonstrable ex vivo, this mechanism may contribute to the pathogenesis of rheumatoid arthritis (RA). We therefore investigated the presence of activated complement components and complement activator molecules on the surface of cell-derived microparticles of RA patients and healthy individuals.
Methods: Microparticles from synovial fluid (n = 8) and plasma (n = 9) of 10 RA patients and plasma of sex- and age-matched healthy individuals (n = 10) were analysed by flow cytometry for bound complement components (C1q, C4, C3) and complement activator molecules (C-reactive protein (CRP), serum amyloid P component (SAP), immunoglobulin (Ig) M, IgG).
Results: Microparticles with bound C1q, C4, and/or C3 were abundant in RA synovial fluid, while in RA and control plasma much lower levels were present. Microparticles with bound C1q correlated with those with bound C3 in synovial fluid ($r = 0.961$, $p = 0.0001$), and with those with bound C4 in plasma (RA: $r = 0.908$, $p = 0.0007$; control: $r = 0.632$, $p = 0.0498$), indicating classical pathway activation. In synovial fluid, microparticles with IgM and IgG correlated with those with C1q ($r = 0.728$, $p = 0.0408$; $r = 0.952$, $p = 0.0003$, respectively), and in plasma, microparticles with CRP correlated with those with C1q (RA: $r = 0.903$, $p = 0.0021$; control: $r = 0.683$, $p = 0.0296$), implicating IgG and IgM in the classical pathway activation in RA synovial fluid, and CRP in the low level classical pathway activation in plasma.
Conclusions: This study demonstrates the presence of bound complement components and activator molecules on microparticles ex vivo, and supports their role in low grade complement activation in plasma and increased complement activation in RA synovial fluid.

Cell-derived microparticles are small vesicles released from cells upon activation or apoptosis. Via transfer of bioactive molecules or ligand-receptor interactions they activate endothelial cells and leucocytes, and thus promote inflammatory processes (for a recent review see Distler *et al*[1]). We demonstrated the presence of high concentrations of leucocyte-derived microparticles in synovial fluid of rheumatoid arthritis (RA) patients.[2] Subsequently, we demonstrated that microparticles from synovial fluid of arthritis patients induce monocyte chemoattractant protein (MCP) 1, interleukin (IL) 6, IL-8, RANTES, intercellular adhesion molecule-1, and vascular endothelial growth factor synthesis in synovial fibroblasts.[3] Distler *et al* have shown that in vitro, microparticles from stimulated T cells and monocytes induce the synthesis of matrix metalloproteinase 1, 3, 9, and 13 as well as of IL-6, IL-8 and MCP-1 and MCP-2 in fibroblasts.[4] These results suggest that microparticles play a part in the inflammatory processes in arthritic joints in several ways.

We hypothesise that cell-derived microparticles can also contribute to inflammation in RA by activation of the complement cascade. Many studies point towards a pathogenic role of the complement system in RA.[5–7] Among the functions of the complement system is the clearance of necrotic and apoptotic cells.[8 9] Such cells activate the complement system mainly via the classical pathway.[10–13] Since cell-derived microparticles share certain surface characteristics with necrotic and apoptotic cells—for example, exposure of phosphatidylserine (PS) and phosphatidylethanolamine (PE),[14 15] lysophospholipids,[16 17] or oxidised phospholipids,[18] they may also play a role in the activation of the complement system. In support of this, it has been demonstrated in vitro that microparticles derived from apoptotic Jurkat cells[19] or activated neutrophil granulocytes[20 21] can bind complement component C1q and activate the classical pathway of complement, as shown by the deposition of complement components C4 and C3. Nauta and colleagues also compared ex vivo microparticles isolated from plasma of healthy individuals and patients with systemic lupus erythematosus (SLE), but were unable to find any differences in C1q binding. Thus, there is to date no experimental data supporting complement activation by cell-derived microparticles in vivo.

We investigated the presence of bound complement components C1q, C4, and C3 on cell-derived microparticles isolated from synovial fluid and plasma of patients with RA, as well as on microparticles isolated from plasma of healthy individuals. Of these complement components, C4 and C3 (that is, their activation products C4b and C3b, respectively) bind covalently to their activating surfaces,[22 23] and are therefore especially suited as markers of complement activation on a given surface. To gain further insight into the mechanism of complement activation, we studied the presence of activator molecules on the surface of these microparticles (C-reactive protein (CRP), serum amyloid P component (SAP), immunoglobulin M and G

Abbreviations: CRP, C-reactive protein; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; Ig, immunoglobulin; IL, interleukin; MBL, mannan-binding lectin; MCP, monocyte chemoattractant protein; PBS, phosphate-buffered saline; PE, phosphatidylethanolamine; PS, phosphatidylserine; RA, rheumatoid arthritis; SAP, serum amyloid P component; SLE, systemic lupus erythematosus; $sPLA_2$, secretory phospholipase A_2

Table 1 Demographic and clinical data of the RA patients

	RA patients (n = 10)
Age (years)	57.7 (10.3)
Sex	4 males; 6 females
Disease duration (months)	145 (160)
Rheumatoid factor	9 positive; 1 negative
Tender joint count	9.5 (3.3)
Swollen joint count	11.9 (4.3)
ESR (mm in 1st hour)	55.3 (25.6)
Erosive disease*	8 positive; 2 negative
No of DMARDs	4.0 (1.9)

Data are presented as mean (SD), except where indicated otherwise. DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate.
*Erosive disease was established using x ray.

(IgM and IgG) molecules), which can bind C1q and thereby activate the classical pathway.[12 13 24–27]

MATERIALS AND METHODS

Patients and healthy individuals

We studied synovial fluid (n = 8) from inflamed knee joints and venous blood (n = 9) of 10 patients with RA, as well as venous blood of sex- and age-matched healthy individuals (n = 10) who had not taken any medication during the 10 days before blood collection. All patients fulfilled the criteria of the American College of Rheumatology for RA.[28] Their demographic and clinical characteristics are summarised in table 1.

This study was approved by the ethics committee of the Academic Medical Center of the University of Amsterdam and complies with the principles of the Declaration of Helsinki. All patients and healthy subjects had given their written informed consent.

Venous blood was collected into 0.1 volume of 105 mmol/l trisodium citrate. Synovial fluid from inflamed knee joints, because of its lower cell content, was collected into 0.1 volume of 210 mmol/l trisodium citrate.[2] Blood cells were removed by centrifugation (1550 g, 20 minutes, room temperature) immediately after sample collection, and the synovial fluid and plasma samples were snap frozen in liquid nitrogen and stored at –80°C.

Measurement of fluid phase complement activation products and complement activator molecules

Synovial fluid and plasma samples (250 µl aliquots) were thawed on melting ice, and made microparticle-free by centrifugation at 18 890 *g* for 60 minutes at 4°C. The upper 200 µl of the microparticle-free supernatants (synovial fluid or plasma) were removed and analysed for concentrations of the soluble complement activation products C4b/c (C4b, inactivated C4b and its further degradation product C4c) and C3b/c (C3b, inactivated C3b and its further degradation product C3c) as well as SAP, as described previously, by enzyme-linked immunosorbent assays.[29 30] CRP, IgM, and IgG concentrations were analysed on the Modular Analytics P800 using Tina-quant reagents (Roche Diagnostics, Basel, Switzerland).

Flow cytometric analysis of microparticles and bound complement components or complement activator molecules

Microparticles from synovial fluid and plasma were isolated as described previously.[31] Flow cytometric analysis was performed using an indirect staining procedure.[31] Since synovial fluid contains high levels of secretory phospholipase A_2 ($sPLA_2$), which hydrolyses (among others) the negatively charged phospholipids on the microparticle surface, we could not use annexin V as a general marker for microparticles in this study.[2 16] Microparticles were incubated for 30 minutes at room temperature in phosphate-buffered saline (PBS; 154 mmol/l NaCl, 1.4 mmol/l phosphate, pH 7.4) containing 2.5 mmol/l $CaCl_2$ (PBS/Ca, pH 7.4) and unlabelled mouse monoclonal antibodies against bound complement factors (C1q, C4, C3) or bound activator molecules (CRP, SAP, IgM, IgG), or the respective isotype-matched control antibodies (clones MOPC-31C (IgG_1) and G155–178 (IgG_{2a}) from Becton Dickinson Pharmingen, San Jose, CA, USA). The monoclonal antibodies against C1q, C4, C3, CRP, and SAP (clones C1q-2, C4-4, C3-15, 5G4, and SAP-14, respectively) were described previously.[30 32–34] Antibodies against the heavy chains of IgM and IgG molecules (clones MH15-1 and MH16-1, respectively) were obtained from Sanquin, Amsterdam, Netherlands. After incubation with the antibodies, the microparticles were washed with PBS/Ca. Next, rabbit anti-mouse $F(ab')_2$-phycoerythrin ($F(ab')_2$-PE; Dako, Glostrup, Denmark) was added, and the mixtures were again incubated for 30 minutes at room temperature. Subsequently, five volumes of PBS/Ca were added and the microparticles analysed on a FACSCalibur flow cytometer with CELLQuest 3.1 software (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Acquisition was performed for 1 minute per sample, during which the flow cytometer analysed approximately 60 µl of the suspension. Forward scatter and side scatter were set at logarithmic gain. To identify marker positive events, thresholds were set based on microparticle samples incubated with similar concentrations of isotype-matched control antibodies. Calculation of the number of microparticles per litre plasma was based upon the particle count per unit time, the flow rate of the flow cytometer, and the net dilution during sample preparation of the analysed microparticle suspension.

Statistical analysis

Data were analysed with GraphPad PRISM 3.02 (GraphPad Software, Inc, San Diego, CA, USA). Differences between groups were analysed with one way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test. Correlations were determined using Pearson's correlation test. In the correlation analysis of microparticles with CRP versus those with C1q on their surface, one outlier was removed. Differences and correlations were considered significant at $p<0.05$. Data are presented as mean (SD).

RESULTS

Concentration of fluid phase complement activation products and complement activator molecules

The fluid phase complement activation products C4b/c and C3b/c (table 2), as indicators of complement activation, were the highest in synovial fluid of the patients. In plasma of patients compared to healthy individuals, on average twice higher levels of complement activation products were present, although this difference did not reach significance.

As for the complement activator molecules (table 2), levels of CRP were about 15 times higher in plasma of the patients when compared to plasma of healthy individuals, while in synovial fluid CRP concentrations were about half of those found in plasma of the patients. Levels of SAP did not differ between plasma of the patients and controls, but were five to seven times lower in synovial fluid of the patients. Levels of IgM did not differ between the groups, and levels of IgG were the same in patient and control plasma but significantly lower in synovial fluid.

Microparticles with bound complement components on their surface

The total concentration of microparticles (fig 1) was on average highest in synovial fluid of RA patients ($8.9\ (10.2)\times10^9$/l). In

Table 2 Concentration of fluid phase complement activation products and complement activator molecules in synovial fluid and plasma of RA patients and plasma of healthy individuals

	RA synovial fluid	p Value†	RA plasma	p Value‡	Control plasma	p Value¶
C4b/c (nmol/l)	62.9 (67.4)	*	10.4 (6.7)	NS	5.8 (3.6)	**
C3b/c (nmol/l)	262.0 (232.6)	**	29.6 (8.2)	NS	16.3 (4.5)	**
CRP (mg/l)	18.0 (14.1)	NS	40.3 (29.2)	***	2.7 (2.6)	NS
SAP (mg/l)	13.8 (9.8)	***	91.6 (27.0)	NS	70.8 (16.5)	***
IgM (g/l)	0.3 (0.3)	NS	0.7 (0.5)	NS	0.8 (0.6)	NS
IgG (g/l)	5.1 (1.5)	**	9.7 (3.0)	NS	9.2 (2.3)	**

Data are presented as mean (SD). Differences were analysed with one-way ANOVA, followed by Bonferroni's multiple comparison test. Two-tailed significance levels are provided (p), which were considered significant at $p<0.05$. NS, not significant; *$p<0.05$; **$p<0.01$; ***$p<0.001$.
†Differences between RA synovial fluid and RA plasma. ‡Differences between RA plasma and control plasma. ¶Differences between RA synovial fluid and control plasma.

plasma of the patients the mean concentration of microparticles was 3.3 $(2.1)\times10^9$/l, and in plasma of healthy individuals 1.8 $(0.7)\times10^9$/l.

The presence of microparticles with bound C1q, C4, and/or C3 on their surface was especially pronounced in synovial fluid of RA patients, but could also be detected, albeit at lower levels, in plasma samples of several of the patients as well as controls (fig 2A). As shown quantitatively in figure 2B, the concentrations of microparticles binding C1q were 29-fold and 37-fold higher in RA synovial fluid when compared with RA plasma and plasma of healthy individuals. The concentrations of microparticles binding C4 were 23-fold and 19-fold higher in RA synovial fluid versus RA plasma and plasma of healthy individuals, and the concentrations of microparticles binding C3 were 38-fold and 21-fold higher in RA synovial fluid when compared with RA plasma and plasma of healthy individuals. There were no significant differences between plasma of the patients and healthy individuals regarding levels of microparticles binding C1q, C4, or C3.

The presence of activated C1q, C4, and C3 indicated classical pathway complement activation on the membrane surface of the microparticles. This was further corroborated by the fact that, as shown in table 3 and figure 4, the levels of microparticles binding C1q correlated significantly with those binding C3 in synovial fluid of the patients, and with those binding C4 in plasma of the patients as well as healthy individuals.

The concentration of microparticles with bound C4 did not correlate with fluid phase C4b/c in any of the three sample groups ($p>0.05$ for all; data not shown). Likewise, levels of microparticles with bound C3 did not correlate with fluid phase C3b/c ($p>0.05$ for all; data not shown). This can be attributed to the different clearance processes that fluid phase and microparticle-bound complement fragments undergo and to other possible contributors to complement activation besides microparticles.

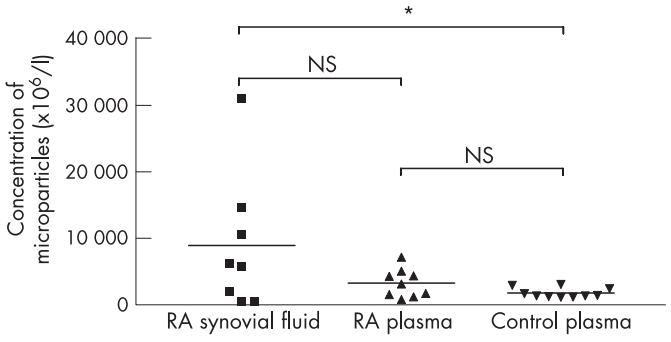

Figure 1 Total concentration of microparticles in synovial fluid and plasma of RA patients, and plasma of healthy individuals. Individual values are shown, with the horizontal lines representing the mean. Differences were analysed with one-way ANOVA, followed by Bonferroni's multiple comparison test. Two-tailed significance levels are provided (p), which were considered significant at $p<0.05$. NS, not significant; *$p<0.05$.

Microparticles with bound complement activator molecules on their surface

As for the microparticle-bound complement activator molecules (fig 3), microparticles with bound CRP on their surface were on average present at higher concentrations in synovial fluid and plasma of the patients, but the differences between the three groups of samples were not significant. Microparticles with bound SAP were present at similar concentrations in the three groups. On the other hand, microparticles with IgM and IgG on their surface were present at significantly higher levels in synovial fluid of the patients compared with plasma of the patients and healthy individuals.

Correlations between microparticles binding the activator molecules CRP, SAP, IgM, or IgG, and those binding C1q are shown in table 3 and figure 4. In synovial fluid of RA patients, the concentration of microparticles binding C1q correlated with the concentration of those binding IgG, and those binding IgM. In plasma of RA patients and in plasma of healthy individuals, the concentration of microparticles binding CRP correlated with those binding C1q.

DISCUSSION

In this study we demonstrated the presence of C1q, C4, and C3 on the surface of cell-derived microparticles isolated from synovial fluid and plasma of RA patients as well as plasma of healthy individuals. The levels of microparticles binding C1q correlated significantly with those binding C3 in synovial fluid of the patients, and with those binding C4 in plasma of the patients as well as healthy individuals. These results support the possible role of microparticles in complement activation in vivo via the classical pathway. We focused on classical pathway activation here because in previous studies performed in vitro on necrotic and apoptotic cells as well as neutrophil granulocyte-derived microparticles, a major role for the alternative pathway has been ruled out using Mg-EGTA, an inhibiting anti-C1q antibody, C1 inhibitor, and C1q or C2 deficient serum.[12 13 21] A role for the mannan-binding lectin (MBL) pathway in complement activation was also excluded in those studies, in line with a previous report that MBL binds to necrotic and apoptotic cells and cell blebs in vitro but does not initiate complement activation.[35] The alternative pathway of complement activation also functions as an amplification loop for the classical (as well as the lectin) pathway,[36] and possibly contributed to some extent to complement activation in the samples we studied here. A differing degree of alternative pathway activation may have accounted for the difference

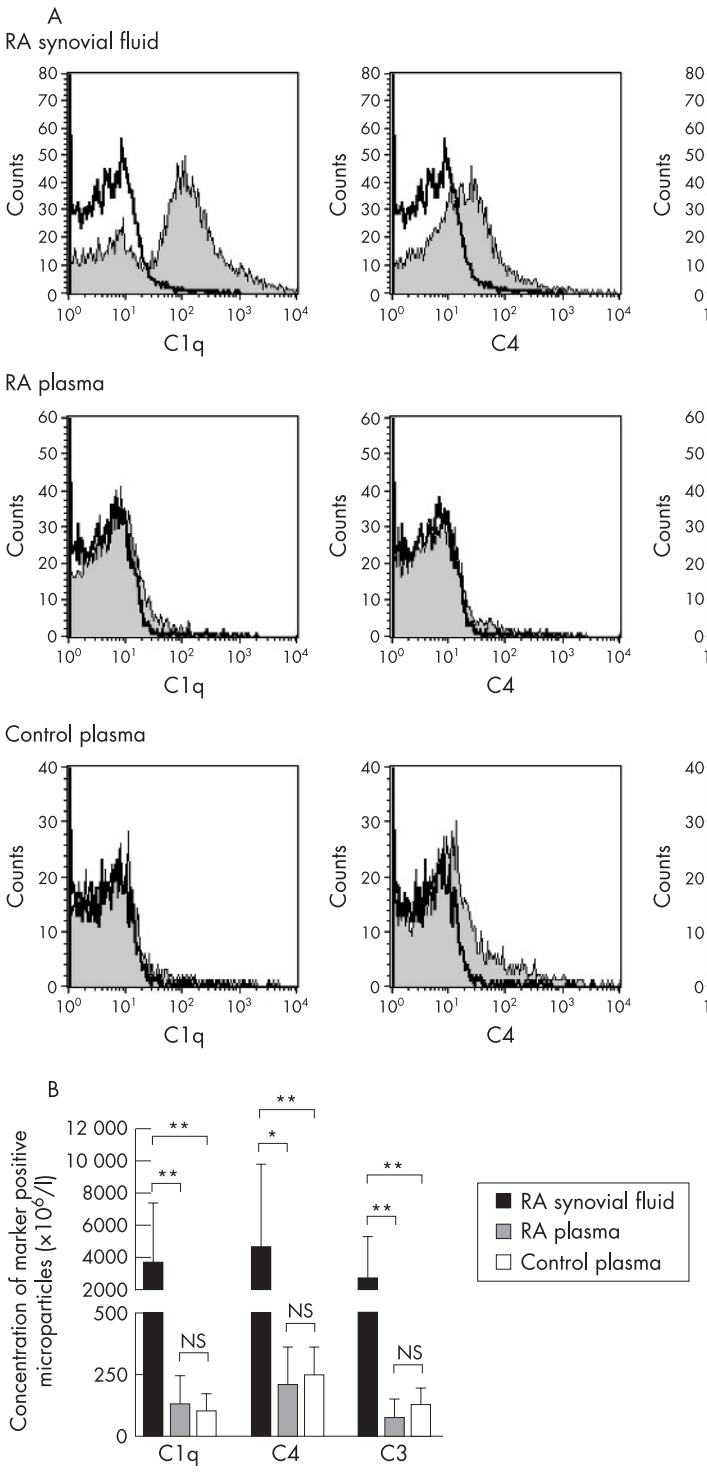

Figure 2 Complement components on the surface of microparticles. (A) Representative histogram plots of microparticles with bound complement components C1q, C4, or C3 in synovial fluid and plasma of an RA patient, and plasma of a healthy individual. Fluorescence intensity (x-axis) vs microparticle count (y-axis) is shown. Binding of the isotype-matched control antibody is depicted with the open histogram, and binding of the specific antibody with the shaded histogram. (B) Concentration of microparticles with bound C1q, C4, and C3 in the three groups of samples. Data are presented as mean (SD). Differences were analysed with one-way ANOVA, followed by Bonferroni's multiple comparison test. Two-tailed significance levels are provided (p), which were considered significant at $p<0.05$. NS, not significant; *$p<0.05$; **$p<0.01$.

between synovial fluid and plasma samples regarding correlation of microparticles with C1q on their surface to either microparticles with C3 (in synovial fluid) or C4 (in plasma) in the present study. Alternatively, different clearance rates of microparticles with bound C4 and C3 in synovial fluid versus plasma may be responsible for the observed discrepancy.

Although the total concentration of microparticles in synovial fluid of RA patients was on average only threefold higher than in plasma of these patients and fivefold higher than in plasma of healthy individuals, synovial fluid had on average 20-40-fold higher levels of C1q-, C4-, and C3- binding microparticles than plasma of the patients and healthy individuals, with no differences between the latter two groups. The numbers of microparticles in synovial fluid of the patients (with or without bound complement components) might even have been underestimated, given the high concentration of hyaluronan, a high molecular weight glycosaminoglycan, in synovial fluid,[37 38] which might "trap" some of the microparticles. Such high levels of C1q-, C4-, and C3- binding microparticles indicate a much higher level of complement activation on the membrane surface of microparticles in synovial fluid of RA patients than in plasma of the patients and healthy individuals. A contributing factor to these high levels of microparticles with activated complement components bound to their surface might again be

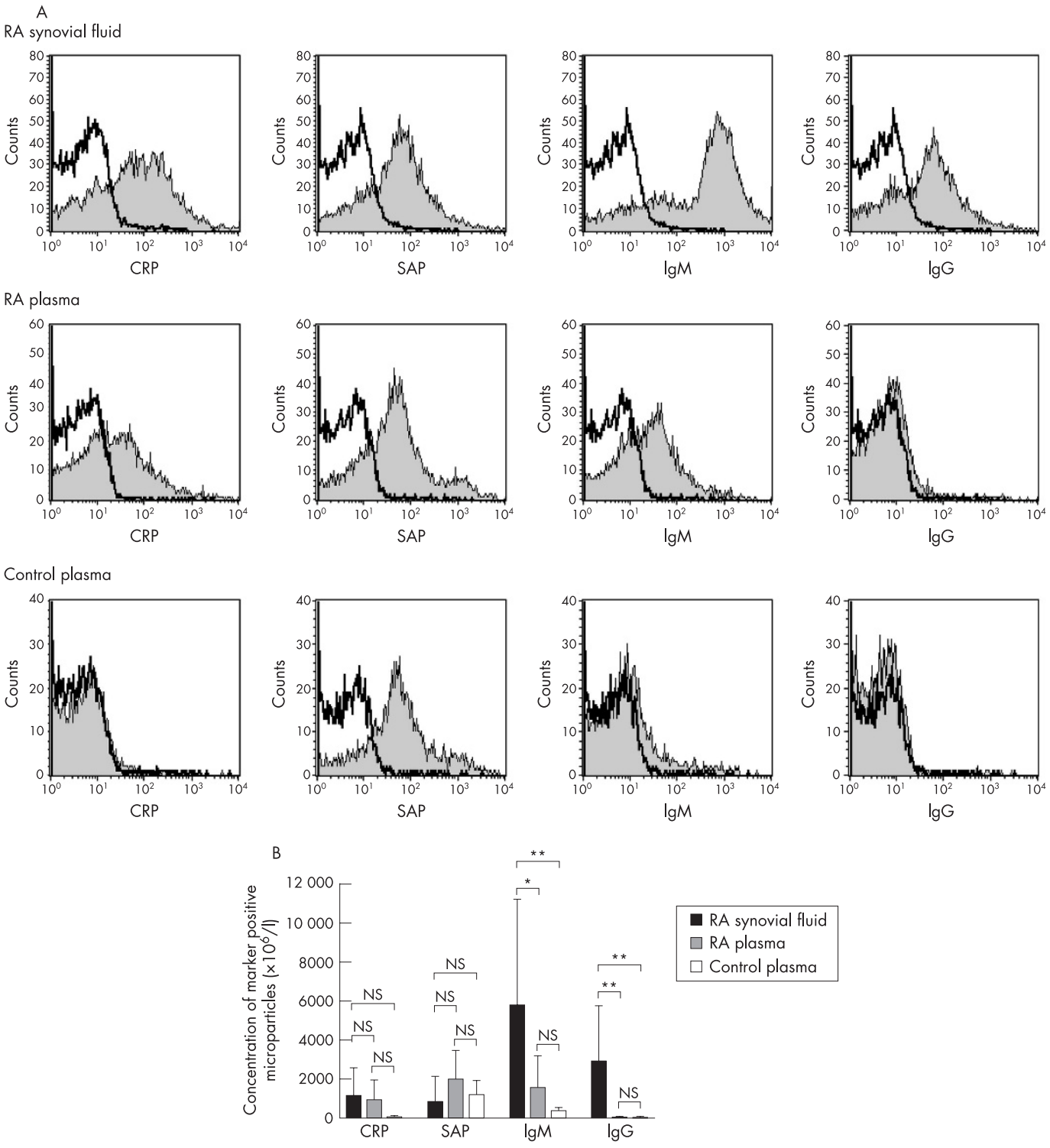

Figure 3 Complement activator molecules on the surface of microparticles. (A) Representative histogram plots of microparticles with bound complement activator molecules CRP, SAP, IgM, or IgG in synovial fluid and plasma of an RA patient, and plasma of a healthy individual. Fluorescence intensity (x-axis) vs microparticle count (y-axis) is shown. Binding of the isotype-matched control antibody is depicted with the open histogram, and binding of the specific antibody with the shaded histogram. (B) Concentration of microparticles with bound CRP, SAP, IgM, and IgG in the three groups of samples. Data are presented as mean (SD). Differences were analysed with one-way ANOVA, followed by Bonferroni's multiple comparison test. Two-tailed significance levels are provided (p), which were considered significant at $p<0.05$. NS, not significant; *$p<0.05$; **$p<0.01$.

a different (lower) rate of clearance compared to plasma of patients and healthy individuals. A lower clearance rate would, in return, be expected to result in higher rates of amplification of the complement cascade on the surface of the microparticles. Altogether, the higher levels of microparticles with activated complement components on their surface are expected to contribute to the proinflammatory state in the synovial compartment of RA patients.

The observed levels of microparticles with activated complement components on their surface in the different sample groups were in line with the levels of fluid phase complement activation products: the concentrations of C4b/c and C3b/c did

Table 3 Correlations between the concentrations of microparticles binding the various complement components or complement activator molecules in synovial fluid and plasma of RA patients and plasma of healthy individuals

	RA synovial fluid		RA plasma		Control plasma	
	r	p Value	r	p Value	r	p Value
C1q pos MP vs C4 pos MP	0.660	0.0533	0.908	0.0007	0.632	0.0498
C1q pos MP vs C3 pos MP	0.961	0.0001	0.205	0.5963	0.542	0.1057
CRP pos MP vs C1q pos MP	0.433	0.2841	0.903	0.0021	0.683	0.0296
SAP pos MP vs C1q pos MP	−0.015	0.9725	0.492	0.1786	0.280	0.4339
IgM pos MP vs C1q pos MP	0.728	0.0408	−0.175	0.6527	0.159	0.6611
IgG pos MP vs C1q pos MP	0.952	0.0003	0.420	0.2606	0.505	0.1364

Correlation analysis was performed using Pearson's correlation test (r, correlation coefficient; p, two-tailed significance level, considered significant at $p<0.05$).

not differ in patient and control plasma, but were significantly higher in synovial fluid of the patients. The fact that levels of microparticles with bound C4 and C3 activation products did not correlate with levels of fluid phase C4 and C3 fragments is not surprising, since the microparticle-bound and soluble forms of these complement components undergo different degradation and clearance processes. Furthermore, microparticles are probably not the only contributors to complement activation.

Regarding the role of activator molecules in complement activation on the surface of microparticles, in synovial fluid of the patients we found a significant correlation between the concentrations of microparticles with bound IgM and those with C1q, and an even stronger correlation between the concentrations of microparticles with IgG versus those with C1q. This suggests that the binding of C1q to IgG and IgM molecules on microparticles might be responsible for complement activation via the classical pathway in RA synovial fluid. Whether the binding of IgG molecules to microparticles occurs via F_c receptors or by specific binding of the F_{ab} regions, is as yet unknown. IgM molecules are known to bind to oxidised phospholipids and lysophospholipids,[27 39] both of which are likely to be exposed on microparticles in the inflamed synovial fluid as a result of oxidative processes[18] and increased $sPLA_2$ activity.[16] In plasma of both RA patients and healthy individuals, the concentrations of microparticles binding CRP correlated well with those binding C1q, implicating CRP in the initiation of the classical pathway of complement activation, albeit at relatively low levels, in plasma. CRP binds to

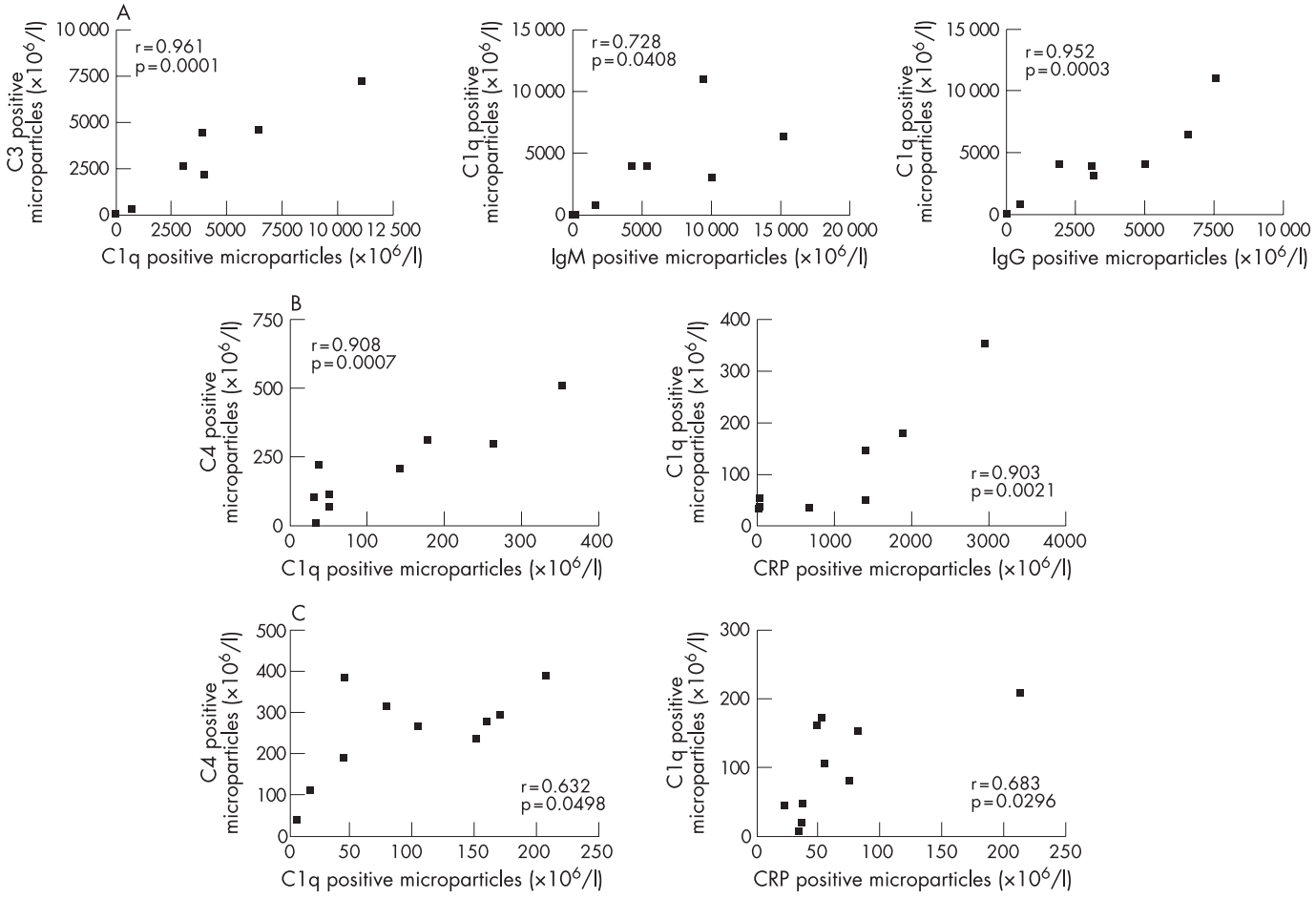

Figure 4 Correlations between the concentrations of microparticles binding various complement components or complement activator molecules. Only the statistically significant correlations (see table 3) are shown. (A) Correlations in synovial fluid of RA patients. (B) Correlations in plasma of RA patients. (C) Correlations in plasma of healthy individuals. Correlation analysis was performed using Pearson's correlation test (r, correlation coefficient; p, two-tailed significance level, considered significant at $p<0.05$).

phosphorylcholine in the outer leaflet of membranes in the presence of sufficient amounts of lysophosphatidylcholine,[40] or to oxidised phosphatidylcholine.[41]

Our finding that in synovial fluid of RA patients microparticle bound IgM and IgG, and in plasma of the patients and healthy individuals microparticle bound CRP can be implicated in complement activation on the surface of the microparticles, does not reflect the fluid phase levels of these complement activator molecules in the respective sample groups. In RA synovial fluid, the levels of fluid phase IgG molecules are actually lower than in plasma of the patients and controls, and levels of CRP are much higher in both plasma and synovial fluid of the patients than in plasma of healthy individuals. This may serve as additional evidence for our presumption that the microparticle bound molecules indeed play a part in complement activation. Here, we should point out that synovial fluid contains microparticles that are mainly of granulocytic and monocytic origin, with substantial numbers of microparticles derived from T cells as well. On the other hand, plasma of RA patients and healthy individuals contains microparticles derived mainly from platelets, in addition to considerable numbers of microparticles derived from erythrocytes.[2] The different cellular origin of the microparticles in synovial fluid versus plasma probably profoundly influences their ability to support complement activation on their surface, most likely via their ability to bind certain activator molecules.

Whether complement activation occurred on the surface of the microparticles themselves, or whether it had occurred on cells from which the microparticles had subsequently been released by blebbing of the surface membrane, is a question that still remains open. In experiments with apoptotic keratinocytes and endothelial cells in vitro, C1q was shown to bind specifically to surface blebs, regions about to be released as ''microparticles.''[10 42] On the other hand, Gasser *et al* have shown that isolated microparticles from in vitro activated neutrophil granulocytes are also capable of binding C1q, C4, and C3.[20 21] Based on these in vitro data, it is likely that both in vitro and in vivo, the processes of microparticle formation and complement activation overlap, with complement activation occurring both on the cell surface and on the released microparticles. Nevertheless, indisputably proving this in vivo will require further investigations. At the same time, the question also arises whether microparticles might be more or less potent complement activators compared to their mother cells. Such data are not yet available. We presume that not only the overall area of the membrane surface available (numbers of microparticles and their surface area), but also the differing antigenic and lipid composition of microparticles compared to their mother cells[43–46] influence their relative potency.

In conclusion, this study demonstrates for the first time the presence of bound complement components and complement activator molecules on the surface of microparticles ex vivo. Our data support the concept that cell-derived microparticles can activate the classical pathway of complement in vivo, and suggest that microparticles may contribute to the pathogenesis of RA by activation of the complement system, especially in the inflamed synovial compartment.

Authors' affiliations

Éva Biró, Rienk Nieuwland, Loes M Pronk, Marianne C L Schaap, Augueste Sturk, Dept. of Clinical Chemistry, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands
Paul P Tak, Dept. of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands
C Erik Hack, Crucell, Leiden, Netherlands

Competing interests: None.

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