Research article

Synovial microparticles from arthritic patients modulate chemokine and cytokine release by synoviocytes

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Abstract

Synovial fluid from patients with various arthritides contains procoagulant, cell-derived microparticles. Here we studied whether synovial microparticles modulate the release of chemokines and cytokines by fibroblast-like synoviocytes (FLS). Microparticles, isolated from the synovial fluid of rheumatoid arthritis (RA) and arthritis control (AC) patients (n = 8 and n = 3, respectively), were identified and quantified by flow cytometry. Simultaneously, arthroscopically guided synovial biopsies were taken from the same knee joint as the synovial fluid. FLS were isolated, cultured, and incubated for 24 hours in the absence or presence of autologous microparticles. Subsequently, cell-free culture supernatants were collected and concentrations of monocyte chemoattractant protein-1 (MCP-1), IL-6, IL-8, granulocyte/macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF) and intracellular adhesion molecule-1 (ICAM-1) were determined. Results were consistent with previous observations: synovial fluid from all RA as well as AC patients contained microparticles of monocytic and granulocytic origin. Incubation with autologous microparticles increased the levels of MCP-1, IL-8 and RANTES in 6 of 11 cultures of FLS, and IL-6, ICAM-1 and VEGF in 10 cultures. Total numbers of microparticles were correlated with the IL-8 (r = 0.91, P < 0.0001) and MCP-1 concentrations (r = 0.81, P < 0.0001), as did the numbers of granulocyte-derived microparticles (r = 0.89, P < 0.0001 and r = 0.93, P < 0.0001, respectively). In contrast, GM-CSF levels were decreased. These results demonstrate that microparticles might modulate the release of chemokines and cytokines by FLS and might therefore have a function in synovial inflammation and angiogenesis.

Introduction

Cell-derived microparticles, predominantly from platelets and erythrocytes, are present in human blood. The presence of such microparticles has been associated with the activation of coagulation [1-3]. We demonstrated recently that synovial fluid from the inflamed joints of rheumatoid arthritis (RA) and arthritis control (AC) patients also contains cell-derived microparticles. These microparticles originate from monocytes and granulocytes, and to a smaller extent from lymphocytes [4]. Synovial microparticles are strongly procoagulant via an initiation mechanism dependent on tissue factor and factor VII(a). We therefore proposed that such microparticles might contribute to the local formation of fibrin clots, the so-called rice bodies.

Fibroblast-like synoviocytes (FLS) have a key function in the development of sustained inflammation and angiogenesis in arthritic joints [5-8]. On activation in vitro by cytokines or bacterial lipopolysaccharides, FLS produce chemokines including monocyte chemoattractant protein-1 (MCP-1) [9,10], IL-8 [11-13] and RANTES [11,14], cytokines such as IL-6 [12,13] and granulocyte/macrophage colony-stimulating factor (GM-CSF) [13,15,16], and angiogenic factors such as vascular endothelial growth factor (VEGF) [17,18].
The presence of leukocyte-derived microparticles in blood has been associated with systemic inflammatory disorders, such as pre-eclampsia [19], sepsis with multiple organ failure [20], and meningococcal septic shock [21], and leukocyte-derived microparticles – but not platelet-derived microparticles – trigger the expression of IL-6 and MCP-1 by endothelial cells [22,23]. However, it is unknown whether leukocytic microparticles contribute to local inflammation. We therefore determined whether isolated synovial microparticles of arthritis patients trigger the release of (pro-) inflammatory and angiogenic mediators by cultured autologous FLS from inflamed joints of RA and AC patients.

Materials and methods

Patients

Paired synovial fluid, plasma and synovial tissue specimens were collected from eight RA and three undifferentiated AC patients. The diagnosis of AC patients stayed unchanged during 1 year of follow-up. The RA patients fulfilled the criteria of the 1987 Criteria of the American College of Rheumatology. The study was approved by the Medical Ethical Committee of the Academical Medical Center of the University of Amsterdam, and informed consent was obtained to participate in the present study. The demographic and clinical data are summarized in Table 1.

Reagents and assays

Anti-CD4 labeled with phycoerythrin (PE; CLB-T4/2 6D10, IgG1) and anti-CD66e-PE (CLB-gran/10 IH4Fc, IgG1) were obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB; Amsterdam, The Netherlands), anti-glycophorin A-PE (JC159, IgG1) was from DakoCytomation (Glostrup, Denmark). Anti-CD8-PE (Leu™-P9, IgG1), anti-CD14-PE (MφP9, IgG2a), anti-CD20-PE (L27, IgG1), anti-CD61-PE (VI-PL2, IgG1) and IgG1-PE (X40) were from Becton Dickinson (BD, San Jose, CA, USA). Anti-CD16a-PE (CLB-gran/10 HI4Fc, IgG1) and anti-CD66e-PE (CLB-gran/10 IH4Fc, IgG1) were obtained from Roche Diagnostics (Mannheim, Germany) and meningococcal septic shock [21], and leukocyte-derived microparticles – but not platelet-derived microparticles – trigger the expression of IL-6 and MCP-1 by endothelial cells [22,23]. However, it is unknown whether leukocytic microparticles contribute to local inflammation. We therefore determined whether isolated synovial microparticles of arthritis patients trigger the release of (pro-) inflammatory and angiogenic mediators by cultured autologous FLS from inflamed joints of RA and AC patients.

Collection of synovial fluid and blood samples

Immediately before the arthroscopy, we collected synovial fluid (4.5 ml) from the same joint and also venous blood (4.5 ml) into tubes containing 0.5 ml of 3.2% sodium citrate (BD). Immediately after collection, a further 0.5 ml of 3.2% sodium citrate was added to the synovial fluid to prevent clotting. Cells were removed from both blood and synovial fluid by centrifugation for 20 min at 1,550 g and 20°C. For all determinations, aliquots of cell-free plasma and synovial fluid were snap-frozen in liquid nitrogen for at least 15 min and stored at -80°C until use.

Incubation of FLS with microparticles

FLS were quiescent after incubation for 24 hours in medium containing 1% FCS. After 24 hours, this medium (1 ml) was replaced by culture medium containing 1% FCS without any other addition (1 ml; control), or by 975 µl of culture medium plus (1) 25 µl of IL-1β (125 pg/ml final concentration), (2) 25 µl of microparticle suspension or (3) 25 µl of microparticle-free synovial fluid that had been diluted 1:9 in PBS (that is, containing 2.5 µl of the original synovial fluid; this quantity was chosen arbitrarily to correct for both the onefold (unconcentrated) and threefold concentrated microparticle suspensions that, after washing of the microparticles, still contained about 0.7 and 2.1 µl of synovial fluid, respectively). Because individual FLS
cultures showed a considerable variation in (mediator) response to the positive control, namely IL-1β, we expressed the response of each FLS culture to microparticles as a percentage of the IL-1β-induced response.

**Flow-cytometric analysis**

Microparticles were measured by flow cytometry with a method that differed slightly from that used previously [4]. In the present study, the microparticles were not washed by centrifugation after being labeled with antibodies because this resulted in a selective loss of microparticle populations. In brief, 5 µl of the microparticle suspension was added to a mixture of PBS (45 µl) containing 2.5 mM CaCl₂ and 5 µl of PE-labeled mAb, and incubated for 15 min in the dark at ambient temperature (20 to 22°C). The following (final concentrations) of mAbs were used: anti-CD4-PE (0.5 µg/ml), anti-CD8-PE (0.25 µg/ml), anti-CD14-PE (0.25 µg/ml), anti-CD20-PE (0.5 µg/ml), anti-CD61-PE (0.5 µg/ml), anti-CD66e-PE (0.25 µg/ml) and anti-glycophorin A-PE (0.25 µg/ml). PE-labeled IgG1 and IgG2b (both at 0.5 µg/ml) were used as isotype-specific control antibodies. After incubation, 900 µl of PBS/CaCl₂ was added. Samples were analyzed on a FACSCalibur (BD) and data were analyzed with CellQuest™ Pro software (version 4.02; BD). Both forward scatter and side scatter were set at logarithmic gain. Microparticles were identified by forward scatter, side scatter and binding of cell-specific mAb. The number of microparticles per liter of plasma or synovial fluid was estimated by using the number of events (N) of cell-specific mAb-binding microparticles after correction for control antibody binding: number/liter = N × (150/5) × (955/67) × (10⁶/250). The lower detection limit of the particle count was previously established as 10⁷ microparticles per liter. In this formula, 150 (µl) is the final volume of the washed microparticle suspension, 5 (µl) is the volume of this suspension that is used for each labeling, 955 (µl) is the total volume of the microparticle suspension after labeling before fluorescence-activated cell sorting analysis, 67 (µl) is the average volume of the labeled microparticle suspension that is analyzed by the flow cytometer in 1 min, 10⁶ is the conversion from µl to liter, and 250 (µl) is the original volume of the plasma or synovial fluid sample used for microparticle isolation.

**Statistical analysis**

Data were analyzed with GraphPad Prism for Windows, release 3.02 (San Diego, CA, USA). Differences in the concentrations of chemokines, cytokines and VEGF between synovial fluid and plasma as well as in culture supernatants were analyzed with the Wilcoxon signed-rank test. Two-tailed significance levels were considered significant at P < 0.05. All data are presented as medians (range).

**Results**

**Cellular origin of synovial microparticles**

Previously, we found no differences between the numbers and cellular origin of microparticles in synovial fluid from RA and AC patients [4]. For all cell-specific antigens tested, the microparticle numbers of the three AC patients fell within the range of the RA patients, which is consistent with these earlier observations. The data in Table 2 therefore summarize the microparticle numbers for RA and AC patients together. Most microparticles originated from monocytes (CD14) and granulocytes (CD66e). Microparticles derived from platelets (CD61) and erythrocytes (glycophorin A) were below detection level (less than 10⁷/l) in synovial fluid from all patients, except in one RA patient who had a low but detectable number (1.7 × 10⁷/l) of platelet-derived microparticles. One
other RA patient had a relatively high number of erythrocyte-derived microparticles (3.1 × 10^6/l). Microparticles from CD4^+ cells were found in six RA patients and all AC patients. Microparticles from CD8^+ T cells were present in the synovial fluid of five RA patients and one AC patient. Microparticles from B cells were found in two RA patients only.

### Synovial microparticles stimulate FLS

FLS were quiescent after incubation for 24 hours in medium containing 1% FCS. The concentrations of all markers studied in the FLS culture supernatants are summarized in Table 3. In comparison with the control (unstimulated), IL-1 in the FLS culture supernatants are summarized in Table 3. In comparison with the control (unstimulated), IL-1

| Microparticle numbers in synovial fluid from patients with arthritic joints |
|---|---|---|
| **Origin** | **mAb** | **Synovial fluid** |
| CD4^+ cells | CD4 | 191 (<10–711) |
| CD8^+ cells | CD8 | <10 (<10–331) |
| Monocytic cells | CD14 | 1,315 (57–13,326) |
| B-cells | CD20 | <10 (<10–104) |
| Platelets | CD61 | <10 (<10–17) |
| Erythrocytes | Glycophorin A | <10 (<10–3,104) |
| Granulocytes | CD66e | 2,380 (<10–20,864) |

Results are medians, with ranges in parentheses. Data are the numbers (× 10^6/l) of marker-positive microparticles from all arthritic patients (n = 11).

Concentrations of MCP-1, IL-6, IL-8, RANTES, sICAM-1, VEGF and GM-CSF in vivo

For comparison, the concentrations of the various mediators were also determined in both synovial fluid and plasma from RA and AC patients. Because only 2 values (of 36) of the AC patients fell outside the RA range, namely MCP-1 in synovial fluid and sICAM-1 in plasma from the same AC patient, all data are summarized in Table 4. In comparison with plasma, levels of MCP-1 (P = 0.008), IL-6 (P = 0.002), IL-8 (P = 0.002) and VEGF (P = 0.002) were elevated in synovial fluid, those of RANTES and ICAM-1 were decreased (P = 0.001 and P = 0.006, respectively), and GM-CSF concentrations were similar (P = 0.125). Figure 2 shows that both the total number of microparticles (Fig. 2a; r = 0.91; P < 0.0001) and the numbers of granulocyte-derived microparticles (Fig. 2b; r = 0.89, P < 0.0001) were correlated with the IL-8 concentrations, whereas the numbers of monocyte-derived microparticles were not (Fig. 2c; r = 0.04; P = 0.89). In addition, concentrations of MCP-1 were correlated with total numbers of microparticles (r = 0.81, P < 0.0001) and numbers of granulocyte-derived microparticles (r = 0.93, P < 0.0001), but again not with the numbers of monocyte-derived microparticles (r = 0.06; P = 0.82; data not shown). No other correlations were found between microparticle numbers and concentrations of mediators.

Discussion

The present study shows that synovial fluid microparticles trigger FLS to release chemokines, cytokines and other mediators of inflammation. The extent to which these changes are solely induced by microparticles remains to be shown. We cannot exclude from our present data the possibility that the activation of FLS is due in part to synergistic actions of the microparticles with one or more mediators released by FLS themselves under these conditions. Neither can we exclude the possibility that microparticles activate FLS in synergy with one or more...
Responses of individual cultures of fibroblast-like synoviocytes from rheumatoid arthritis (RA; n = 8) and arthritis control (AC; n = 3) patients to their autologous synovial microparticles. All individual patient data for the markers studied are expressed as the concentration of the mediator in the presence of microparticles concentrated either onefold (black bars) or threefold (open bars) divided by the concentration of mediator in the presence of microparticle-free synovial fluid. ICAM-1, intracellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1.
### Table 3

**Effect of synovial microparticles on the release of inflammatory mediators by fibroblast-like synoviocytes from arthritic patients (n = 11)**

| Mediator | Control | Unstimulated | IL-1β |
|----------|---------|--------------|-------|
|          | MP-free synovial fluid | MP (1×) | Nx/Nt | MP (3×) | Nx/Nt | P* |
| MCP-1 (pg/ml) | 456 (355–1,292) | 469 (293–1,241) | 6/11 | 0.010 | 900 | 4/6 | 0.156 |
| sICAM-1 (ng/ml) | 0.09 (0–0.3) | 1.04 (0.34–1.84) | higher | (338–1,481) | higher | (0.91–11.75) |
| IL-8 (pg/ml) | 0 (0–564) | 26 (0–528) | higher | (30–1,176) | higher | (0–2,100) |
| IL-6 (pg/ml) | 74 (24–1,710) | 110 (1,870–22,797) | higher | (34–1,937) | higher | (44–3,766) |
| VEGF (pg/ml) | 48 (11–102) | 34 (7–141) | higher | (34–1,291) | higher | (2–716) |
| GM-CSF (pg/ml) | 32 (28–40) | 31 (26–70) | lower | (14–43) | lower | (14–25) |
| RANTES (pg/ml) | 0 (0–74) | 0 (0–58) | higher | (0–32) | higher | (0–32) |

Results are medians, with ranges in parentheses. Concentrations of mediators were determined in the culture supernatant of the fibroblast-like synoviocytes (FLS) by ELISA as described in the Materials and methods section. FLS were incubated for 24 hours with 1 ml of culture medium containing 1% FCS (negative control), 975 µl of culture medium supplemented with either (1) 25 µl of interleukin (IL)-1β (final concentration 125 pg/ml; positive control), (2) 25 µl (onefold (1×) or threefold (3×) concentrated) microparticles (MP), or (3) MP-free synovial fluid. P*, positive versus negative control; P†, MP (1×) versus MP-free synovial fluid; P‡, MP (3×) versus MP (1×). Nx/Nt, number of individual culture supernatants that contained elevated or decreased concentrations of mediators after incubation for 24 hours with isolated MP compared with MP-free synovial fluid, divided by the number of patients studied. GM-CSF, granulocyte/macrophage colony-stimulating factor; sICAM-1, soluble intracellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; VEGF, vascular endothelial growth factor.

### Table 4

**Concentrations of inflammatory mediators in synovial fluid and plasma from arthritic patients (n = 11)**

| Mediator | Concentration | Synovial fluid | Plasma | P |
|----------|---------------|----------------|--------|---|
| MCP-1 (pg/ml) | 134 (36–522) | 34 (15–62) | 0.008 |
| sICAM-1 (ng/ml) | 706 (226–1,085) | 871 (657–1,691) | 0.006 |
| IL-8 (pg/ml) | 614 (<50–24,630) | <50 | 0.002 |
| IL-6 (pg/ml) | 13,897 (35–43,131) | 11 (0–57) | 0.002 |
| VEGF (pg/ml) | 1,604 (528–2,506) | 23 (<5–69) | 0.002 |
| RANTES (pg/ml) | 7 (<5–35) | 3,086 (2,920–10,037) | 0.001 |
| GM-CSF (pg/ml) | <2 (<2–39) | <2 (<2–28) | 0.125 |

Results are medians, with ranges in parentheses. Concentrations of all mediators were determined by ELISA as described in the Materials and methods section. GM-CSF, granulocyte/macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein-1; sICAM-1, soluble intracellular adhesion molecule-1; VEGF, vascular endothelial growth factor.
mediators already present in the synovial fluid. Nevertheless, the release of IL-8 and MCP-1 was correlated directly to both the total number of microparticles and the number of granulocyte-derived microparticles. This suggests that microparticles might trigger FLS to release these mediators. Although no correlations were found between microparticle numbers and sICAM-1, IL-6, VEGF and RANTES, a threefold increased concentration of microparticles tended to induce a higher response.

On the basis of these data it is tempting to speculate that synovial fluid microparticles promote synovial inflammation and neoangiogenesis in arthritic joints. The FLS are localized in the intimal lining layer, which directly contacts the synovial fluid compartment. Thus, synovial fluid microparticles may interact directly with the FLS, thereby modulating the release of an array of proinflammatory cytokines and chemokines. This may lead to further cell activation, neoangiogenesis and cell recruitment, constituting a proinflammatory amplification loop. Consistent with this notion is the observation that the removal of synovial fluid by arthroscopic lavage has a positive therapeutic effect in RA [26]. In addition, it has previously been shown that intra-articular injection of corticosteroids is more effective after arthrocentesis [27]. This has been explained by the effects of removal of fluid containing various proinflammatory cytokines.

At present, we can only speculate how synovial microparticles trigger FLS to produce and/or release proinflammatory mediators. Synovial microparticles originate mainly from leukocytes [4]. In vitro, leukocytic microparticles trigger the release of IL-6 and MCP-1 from endothelial cells [22,23]. Microparticles can contain bioactive lipids such as oxidized phospholipids, arachidonic acid and lysophosphatidic acid [28,29]. In particular, both arachidonic acid and lysophosphatidic acid are present in microparticles previously exposed to secretory phospholipase A2 (sPLA2) [30]. Arachidonic acid is transferred directly from microparticles to endothelial cells, resulting in the production of IL-6 [29]. It is unknown whether lysophosphatidic acid, a multifunctional lipid mediator that induces cell proliferation, migration and survival, is also directly transferred [31]. Synovial microparticles have been exposed to high levels of sPLA2 in vivo and are therefore likely to contain elevated levels of bioactive lipids. Thus, we propose that synovial microparticles might directly transfer bioactive lipids to FLS, thereby modulating the production and/or release of proinflammatory mediators. For this transfer, a direct interaction between microparticles and the FLS is essential. Because microparticles expose an array of cell-type-specific adhesion receptors, a direct interaction is likely. Alternatively, we cannot exclude the possibility that synovial microparticles might also contain inflammatory cytokines, because monocyte-derived microparticles generated in vitro were recently demonstrated to contain IL-1β [32].

Finally, the present study again showed that elevated levels of microparticles from granulocytes, monocytes and lymphocytes are present in the synovial fluid of arthritic patients. At present it is unknown why such elevated numbers of microparticles occur under these conditions. Apoptotic cells expose phosphatidylserine. Macrophages expose phosphatidylserine receptors, which efficiently initiate the recognition and subsequent removal of apoptotic cells [33,34]. It is also likely that...
microparticles are removed from the circulation by means of such receptors. However, synovial microparticles bind less annexin V than microparticles from plasma [4]. This decreased binding is due either to a decreased exposure of phosphatidylserine or to the presence of high levels of sPLA2, which competes with annexin V for binding to phosphatidylserine [35,36]. The removal of microparticles by phagocytic cells might thus be impaired in inflamed joints, resulting in the prolonged presence of microparticles and therefore in the continued stimulation of the FLS.

Conclusion
The results of the present study suggest that microparticles modulate the release of chemokines and cytokines by FLS. However, their biological relevance, compared with or in synergy with other biological mediators in synovial fluid, remains to be determined. The beneficial effect of arthrocentesis and arthroscopic lavage in RA might be explained, at least in part, by the removal of synovial fluid microparticles.

Competing interests
The author(s) declare that they have no competing interests.

Authors’ contributions
RB wrote the manuscript, guided by RN and AS, with clinical input and final correction by PT, RB, RN and AS devised the experimental design. The selection of patients and collection of synovial biopsy and blood materials were performed by MK. All experiments were performed by RB and MS except the culture of synoviocytes, which was performed by DP and TS. Supervision was fulfilled by AS and PT, with daily supervision by RN. The manuscript was read and approved by all authors.

References
1. Holme PA, Solum NO, Brostad F, Roger M, Abdelnoor M: Demonstration of platelet-derived microvesicles in blood from patients with activated coagulation and fibrinolysis using a filtration technique and western blotting. Thromb Haemost 1994, 72:666-671.
2. Berckmans RJ, Nieuwland R, Boing AN, Romijn FP, Hack CE, Sturk A: Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. Thromb Haemost 2001, 85:839-846.
3. Katopodis JK, Kolodny LN, Jy W, Horstman LL, De Marchena EJ, Tao RN, Weyand PG, Haynes DH, Ahn YS: Platelet microparticles and calcium homeostasis in acute coronary ischemia. Am J Hematol 1997, 54:95-101.
4. Berckmans RJ, Nieuwland R, Tak PP, Boing AN, Romijn FP, Kraan MC, Breedveld FC, Hack CE, Sturk A: Cell-derived microparticles in synovial fluid from inflamed arthritic joints support coagulation exclusively via a factor VII-dependent mechanism. Arthritis Rheum 2002, 46:2857-2866.
5. Smith RS, Smith TJ, Bleden TM, Phipps RP: Fibroblasts as sentinel cells. Synthesis of chemokines and regulation of inflammation. Am J Pathol 1997, 151:317-322.
6. Paleolog EM, Miotia JM: Angiogenesis in arthritis: role in disease pathogenesis and as a potential therapeutic target. Angiogenesis 1998, 2:295-307.
7. Firestein GS: Invasive fibroblast-like synoviocytes in rheumatoid arthritis. Passive responders or transformed aggressors? Arthritis Rheum 1996, 39:1781-1790.
8. Pap T, Muller-Ladner U, Gay RE, Gay S: Fibroblast biology. Role of synovial fibroblasts in the pathogenesis of rheumatoid arthritis. Arthritis Res 2000, 2:361-367.
9. Villiger PM, Terkeltaub R, Lotz M: Production of monocyte chemotactrant protein-1 by inflamed synovial tissue and cultured synoviocytes. J Immunol 1992, 149:722-727.
10. Akahoshi T, Wada C, Endo H, Hirota K, Hosaka S, Takagishi K, Kondo H, Kashiwazaki S, Matsushima K: Expression of monocyte chemotactant and activating factor in rheumatoid arthritis. Regulation of its production in synovial cells by interleukin-1 and tumor necrosis factor. Arthritis Rheum 1993, 36:762-771.
11. Rathanasawari P, Hachicha M, Sadick M, Schall TJ, McColl SR: Expression of the cytokine RANTES in human rheumatoid synovial fibroblasts. Differential regulation of RANTES and interleukin-6 genes by inflammatory cytokines. J Biol Chem 1993, 268:5834-5839.
12. Nanki T, Nagasaka K, Hayashida K, Saita Y, Myasaka N: Chemokines regulate IL-6 and IL-8 production by fibroblast-like synoviocytes from patients with rheumatoid arthritis. J Immunol 2000, 167:5381-5385.
13. Georganas C, Liu H, Perlman H, Hoffmann A, Thimmapaya B, Pope RM: Regulation of IL-6 and IL-8 expression in rheumatoid arthritis synovial fibroblasts: the dominant role for NF-κB but not C/EBP β or c-Jun. J Immunol 2000, 165:7199-7206.
14. Volin MV, Shah MR, Takeuchi HT, Mon Y, Negishi M, Ide H, Adachi M: Vascular endothelial growth factor expression by activated synovial leukocytes in rheumatoid arthritis: critical involvement of the interaction with synovial fibroblasts. Arthritis Rheum 2001, 44:2512-2524.
15. Jackson JR, Minton JA, Ho ML, Wei N, Winkler JD: Expression of vascular endothelial growth factor in synovial fibroblasts is induced by hypoxia and interleukin 1beta. J Rheumatol 1997, 24:1253-1259.
16. VanVijk MJ, Nieuwland R, Boer K, van der Post JA, VanBavel E, Sturk A: Microvesicles from patients with rheumatoid arthritis that induces B-lymphocytes to secrete immunoglobulins. Ann NY Acad Sci 1989, 557:230-238.
17. Kasama T, Shiozawa F, Kobayashi K, Yajima N, Hanyuda M, Takeuchi HT, Mon Y, Negishi M, Ide H, Adachi M: Vascular endothelial growth factor expression by activated synovial leukocytes in rheumatoid arthritis: critical involvement of the interaction with synovial fibroblasts. Arthritis Rheum 1997, 44:2512-2524.
27. Weitoft T, Uddenfeldt P: Importance of synovial fluid aspiration when injecting intra-articular corticosteroids. Ann Rheum Dis 2000, 59:233-235.
28. Barry OP, Pratico D, Lawson JA, FitzGerald GA: Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. J Clin Invest 1997, 99:2118-2127.
29. Barry OP, Kazanietz MG, Pratico D, FitzGerald GA: Arachidonic acid in platelet microparticles up-regulates cyclooxygenase-2-dependent prostaglandin formation via a protein kinase C/mitogen-activated protein kinase-dependent pathway. J Biol Chem 1999, 274:7545-7556.
30. Fourcade O, Simon MF, Viode C, Rugani N, Leballe F, Ragab A, Fournie B, Sarda L, Chap H: Secretory phospholipase A2 generates the novel lipid mediator lysophosphatidic acid in membrane microvesicles shed from activated cells. Cell 1995, 80:919-927.
31. Moolenaar WH, van Meeteren LA, Giepmans BN: The ins and outs of lysophosphatidic acid signaling. Bioessays 2004, 26:870-881.
32. MacKenzie A, Wilson HL, Kiss-Toth E, Dower SK, North RA, Surprenant A: Rapid secretion of interleukin-1β by microvesicle shedding. Immunity 2001, 15:825-835.
33. Pradhan D, Krahling S, Williamson P, Schlegel RA: Multiple systems for recognition of apoptotic lymphocytes by macrophages. Mol Biol Cell 1997, 8:767-778.
34. Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM: A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature 2000, 405:85-90.
35. Hara S, Kudo I, Chang HW, Matsuta K, Miyamoto T, Inoue K: Purification and characterization of extracellular phospholipase A2 from human synovial fluid in rheumatoid arthritis. J Biochem (Tokyo) 1989, 105:395-399.
36. Buckland AG, Wilton DC: Inhibition of secreted phospholipases A2 by annexin V. Competition for anionic phospholipid interfaces allows an assessment of the relative interfacial affinities of secreted phospholipases A2. Biochim Biophys Acta 1998, 1391:367-376.