Detection of differentially expressed genes in synovial fibroblasts by restriction fragment differential display


Objective. To identify differentially expressed genes in synovial fibroblasts and examine the effect on gene expression of exposure to TNF-α and IL-1β.

Methods. Restriction fragment differential display was used to isolate genes using degenerate primers complementary to the lysophosphatidic acid acyl transferase gene family. Differential gene expression was confirmed by reverse transcription–polymerase chain reaction and immunohistochemistry using a variety of synovial fibroblasts, including cells from patients with osteoarthritis and self-limiting parvovirus arthritis.

Results. Irrespective of disease process, synovial fibroblasts constitutively produced higher levels of IL-6 and monocyte chemoattractant protein 1 (MCP-1) (CCL2) than skin fibroblasts. Seven genes were differentially expressed in synovial fibroblasts compared with skin fibroblasts. Of these genes, four [tissue factor pathway inhibitor 2 (TFPI2), growth regulatory oncogene β (GROβ), manganese superoxide dismutase (MnSOD) and granulocyte chemotactic protein 2 (GCP-2)] were all found to be constitutively overexpressed in synoviocytes derived from patients with osteoarthritis. These four genes were only weakly expressed in other synovial fibroblasts (rheumatoid and self-limiting parvovirus infection). However, expression in all types of fibroblasts was increased after stimulation with TNF-α and IL-1β. Three other genes (aggrecan, biglycan and caldesmon) were expressed at higher levels in all types of synovial fibroblasts compared with skin fibroblasts even after stimulation with TNF-α and IL-1β.

Conclusions. Seven genes have been identified with differential expression patterns in terms of disease process (osteoarthritis vs rheumatoid arthritis), state of activation (resting vs cytokine activation) and anatomical location (synovium vs skin). Four of these genes, TFPI2, GROβ (CXCL2), MnSOD and GCP-2 (CXCL6), were selectively overexpressed in osteoarthritis fibroblasts rather than rheumatoid fibroblasts. While these differences may represent differential behaviour of synovial fibroblasts in in vitro culture, these observations suggest that TFPI2, GROβ (CXCL2), MnSOD and GCP-2 (CXCL6) may represent new targets for treatments specifically tailored to osteoarthritis.

Key words: Rheumatoid arthritis, Inflammation, Differential expression, RFDD, Synoviocytes, Osteoarthritis.
trafficking and cell motility and are involved in regulating the production of proinflammatory cytokines in a number of cell types [10].

We have identified seven differentially expressed genes that, despite not being members of the LPAAT family, contained the shared consensus sequence PEGTRN. Three of these, GROβ, biglycan and MnSOD, have previously been identified as being differentially expressed in patients with RA, by differential subtraction [5, 10]. Altered expression of four additional genes, caldesmon, aggrecan-1, GCP-2 and TFPI2, is a novel finding. Four of the genes, GCP-2 (CXCL6), TFPI2, GROβ (CXCL2) and MnSOD, were significantly overexpressed in OA-derived fibroblasts compared with other synovial fibroblasts, representing a unique phenotype for OA rather than RA.

Methods

Cells and culture conditions

Synovial tissue was obtained at the time of joint replacement from four patients with RA, two with OA and one patient with proven Parvovirus arthritis. Skin fibroblasts were obtained by punch biopsy from the forearm from two patients with mechanical back pain treated with NSAIDs and one healthy volunteer on no treatment. Explant tissue was enzymatically dissociated and cultured through to a maximum of five passages in RPMI 1640 media (Life Technologies), supplemented with 20% fetal calf serum (Life Technologies), 1% penicillin/streptomycin, 1% L-glutamine, 1% sodium pyruvate and 1% non-essential amino acids (Life Technologies) as previously described [8]. Patient details are given in Table 1. Local ethical approval for this study was given (LREC number 5735). Where stated, cells were exposed to 10 ng/ml TNF-α and 1 ng/ml IL-1β (R & D Systems), in normal growth medium, for 24 h prior to harvesting. In line with current practice, informed consent was sought and obtained from all the patients taking part in this study.

RNA extraction, cDNA synthesis and TaqMan analysis

Total RNA was extracted from the cells by lysis in Trizol solution (Life Technologies), followed by purification on RNeasy columns (Qiagen). DNase I treatment was applied while the RNA was on the column. One microgram of RNA was reverse-transcribed for use in reverse transcription–polymerase chain reaction (RT-PCR) and Taqman (Perkin Elmer) assays for confirmation of differential expression of tissue factor pathway inhibitor 2 (TFPI2). Taqman assays were carried out on an ABI GeneAmp 5700 Sequence Detection System. Results were analysed as detailed in the protocol from the manufacturer. Briefly, values were first normalized to 18S RNA, to account for differences in amounts of starting material. The normalized value for a control sample (in this case untreated skin fibroblasts) was then subtracted from each value, and the result expressed as a fold increase using the formula 2^−ΔΔCT.

ELISA measurement of IL-6, CCL2 and IL-8 production

Cytokine production was measured in conditioned medium from fibroblasts by enzyme-linked immunosorbent assay (ELISA) as described [8]. Cells were plated at 2 × 10^5 cells/ml into a 24-well plate. Wells were treated with or without TNF-α and IL-1β, as described above. After a further 24 h the culture medium was removed and analysed for IL-6, IL-8 and CCL2 using an OptiEIA ELISA kits (Pharmingen, San Diego, CA, USA), according to the manufacturer’s instructions. The lower detection limits for each ELISA were 15 pg/ml for IL-6, 2 pg/ml for IL-8 and 2 pg/ml for CCL2.

Restriction fragment differential display

Signature motifs or regions of close conservation were first identified from the sequences of the LPAAT gene family and degenerate primers were designed to cover these regions. The functional PEGTRNX motif [12, 13] was used in the reverse orientation: 5’-CCHGARGVACHMGVAAYCA-3’ (degeneracy 648).

Single-stranded cDNA was made by reverse transcription, digested with the restriction endonuclease Taq1, and ligated to an adapter DNA (Display Systems). PCR was carried out on this template, using the degenerate primer and a specific primer complementary to the adapter, incorporating a fluorescent label. The PCR products were then electrophoresed on a 6% polyacrylamide gel (Zaxis) for 1.5 or 3 h at 60 W, in 1 × Tris–borate–EDTA (TBE) buffer, visualized on a phosphoimager (Typhoon 8600 variable mode imager; Amersham Pharmacia) and the band patterns were compared. The bands of interest were cut from the gel and re-amplified by PCR, using the same primers. After TOPO TA cloning (Invitrogen) of the PCR products, colony PCR and sequencing led to gene identification.

Confirmation of differential expression by RT-PCR

PCR primer sets were designed to amplify unique regions of the genes of interest (see Table 2 for sequences). Amplification was carried out in 25 μl reactions with 12.5 μM of each primer, and PCR mix from ABgene [1.5 mM MgCl2, 1.25 U Taq DNA polymerase, 75 mM Tris–HCL pH 8.8, 20 mM (NH4)2SO4, 0.01% Tween 20, 0.2 mM each of dATP, dCTP, dGTP and dTTP]. Template concentrations were normalized to β-actin levels, by PCR amplification using Clontech β-actin control primers. Amplification conditions were 94 °C for 5 min followed by 30, 32 or 35 cycles of 94 °C for 1 min, 55 °C for 40 s and 72 °C for 1 min.

Immunohistochemistry and microscopy

For live cell staining, 5000 fibroblasts were seeded into eight-well chamber slides (Falcon) and grown to 90–100% confluence. The cells were washed in phosphate-buffered saline (PBS), then rabbit anti-human fibronectin antibody (polyclonal, IgG1; Sigma F3648) was added in a 2% (w/v) solution of bovine serum albumin (BSA) in PBS for 30 min at room temperature. After washing, the secondary staining reagent, TRITC-conjugated goat anti-rabbit IgG1 [F(ab)2 fragment; Sigma], was added in a similar
way. The cells were then washed and a solution of DAPI (40 ng/ml) was added for 2 min at room temperature to stain cell nuclei. Cells were then washed and visualized. For staining of MnSOD expression, fibroblasts were seeded similarly, but cells were first fixed using acetone before washing and addition of primary (anti-MnSOD mouse monoclonal IgG1; Becton Dickinson 611580) and secondary (fluorescein isothiocyanate-conjugated goat anti-mouse pan IgG; Southern Biotechnology Associates 1031-02) antibodies. The cells were washed and treated with DAPI as before, then mounted in 1% (w/v) P-phenylene diamine, 90% (v/v) glycerol.

Fibroblasts were imaged by differential interference contrast and epifluorescence microscopy using a Zeiss Axiovert 200 microscope. Images were captured and merged using a Hamamatsu C4742-95 camera and Simple PCI software (Digital Pixel, Brighton, UK).

Results

Synovial fibroblasts produce high levels of IL-6 compared with skin fibroblasts irrespective of disease process

It has been well documented that rheumatoid fibroblasts constitutively produce large quantities of a variety of inflammatory cytokines and chemokines, such as IL-6, IL-8 and CXCL2 [14]. However, it is not clear whether this is a feature intrinsic to rheumatoid fibroblasts or more general to synovial fibroblasts irrespective of disease process. In order to examine this and to help choose representative cell lines for further study, we examined the morphology, production of fibronectin and secretion of IL-6, IL-8 and CCL2 from fibroblasts isolated from RA (four lines), OA (two lines) and self-limiting arthritis (one line) as well as skin (three lines) (Table 1). As described previously [8, 15] rheumatoid synovial fibroblasts exhibited a more heterogeneous stellate morphology compared with skin fibroblasts (Fig. 1A). Interestingly, the two fibroblasts from osteoarthritis synovia were more similar to rheumatoid fibroblasts, whereas the self-limiting fibroblast cell line was more similar to skin, with a more homogeneous spindle-shaped morphology. In general, skin fibroblasts were larger with larger nuclei. The production of fibronectin (measured by immunofluorescence on live unfixed cells) was variable between cell lines but in general was highest in the rheumatoid fibroblasts.

The production patterns of IL-6, IL-8 and CCL2 by the different fibroblasts confirmed our previous findings of high levels of IL-6 and CCL2 in rheumatoid compared with skin fibroblasts [8]. Interestingly constitutive IL-6 and CCL2 production was also high in fibroblasts from OA and self-limiting arthritis, suggesting that the production of these cytokines is a generic property of all synovial fibroblasts irrespective of disease state. As predicted from our previous studies [8], after 24 h stimulation with IL-1β and TNF-α the expression of IL-6 and CCL2 by all synovial fibroblasts remained higher than expression by skin fibroblasts (data not shown). Although basal IL-8 production was low in all fibroblasts, after stimulation with IL-1β and TNF-α synovial fibroblasts consistently expressed higher levels of IL-8 than skin fibroblasts, confirming our previous findings [8].

Identification of seven differentially expressed genes in synovial compared with skin fibroblasts by RFDD

Having demonstrated clear differences in phenotype between rheumatoid synovial fibroblasts and skin fibroblasts, we next looked for differences in mRNA expression patterns by RFDD, using both untreated and cytokine-stimulated cells. We specifically wanted to isolate new members of the LPAAT gene family, so primers were used to amplify potential conserved regions (see Methods). We chose RA-1 as a representative rheumatoid fibro-

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Table 2. Sequences of the primers used for RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5’ to 3’)</th>
<th>Reverse primer (5’ to 3’)</th>
</tr>
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<tbody>
<tr>
<td>Aggrecan</td>
<td>GGATCCGGGCTGCAAGCAAGGAC</td>
<td>CTGTGGTCGGGGCGGCTCTCCG</td>
</tr>
<tr>
<td>Biglycan</td>
<td>GGCCCAACACAGATCAGGATGATC</td>
<td>GAAGTTTGCTCTCTACTGAGATG</td>
</tr>
<tr>
<td>Caldesmon</td>
<td>GTATACAGTGCAATGTAGGACG</td>
<td>GGTGGAATGTCAGTCTGCG</td>
</tr>
<tr>
<td>GROβ</td>
<td>CGTCTGGTGCCACTGAGCCTGCG</td>
<td>GTTGTTCTGTTTCCACTGCG</td>
</tr>
<tr>
<td>GCP-2</td>
<td>CTTCCGTCAGCCGCGGCCGGCC</td>
<td>GTTGACCCAGAGGCGGGTCCG</td>
</tr>
<tr>
<td>MnSOD</td>
<td>GGTGACCCAGGGGCGGTTCTGGCGG</td>
<td>GGTGTGGGGCGGGTGGGTGCGG</td>
</tr>
<tr>
<td>TFPI2</td>
<td>CGACAGGTACAGCAGCGAGC</td>
<td>AGTCCTCTCATTTGGAGCT</td>
</tr>
</tbody>
</table>

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Fig. 1. Morphology and cytokine production from synovial and skin fibroblasts. (A) Fibroblasts were examined by immunohistochemistry as described in Methods. Nuclei are stained blue and fibronectin red (this figure may be viewed in colour as supplementary data at *Rheumatology* Online). (B) Conditioned medium from the fibroblasts shown in (A) was analysed for IL-6, MCP-1 (CCL2) and IL-8 by ELISA. The data are shown as a box plot extending from the 25th to the 75th percentile with a line at the median. The whiskers extend above and below the box to show the highest and lowest values. Data were pooled from all fibroblasts representing each condition, with a minimum of three data points for each fibroblast. This figure may be viewed in colour as supplementary data at *Rheumatology* Online.
Differential gene expression in synovocytes

blast cell line to compare against the skin fibroblast cell line SK-1. An example of a typical RFDD gel is shown in Fig. 2. Several bands showing distinct expression differences were selected for further analysis (red boxes; see figure in colour as supplementary data at Rheumatology Online).

DNA extraction, re-amplification, cloning and nucleotide sequencing of the differentially expressed bands revealed seven distinct genes. Comparison of the sequences with the GenBank database showed 100% homology with previously identified human genes. These were: tissue factor pathway inhibitor 2 (TFPI2), growth regulatory oncogene β (GROβ), caldesmon, manganese superoxide dismutase (MnSOD), biglycan, granulocyte chemotactic protein 2 (GCP-2) and aggrecan 1. These results are summarized in Table 3.

All of the differentially expressed genes identified using the LPAAT degenerate primers contained at least some of the amino acid PEGTR motif on which the primer sequence was based. Therefore, while this technique worked in recognizing specific motifs, the motif used here was not specific to LPAATs as no members of the LPAAT family were identified in this study.

Confirmation of differential expression by RT-PCR and immunohistochemistry

To confirm that the expression of these seven genes was differential between rheumatoid and skin fibroblasts and to extend our observations more generally, we used RT-PCR to assess the approximate expression level of each gene in our panel of fibroblasts. The PCR conditions were optimized so that amplification was semi-quantitative and the quantity of starting cDNA was normalized for β-actin. Figure 3 shows that these genes, identified by RFDD, show marked differential expression, both between tissue samples and with cytokine treatment.

TFPI2 was weakly expressed by all untreated skin fibroblasts, rheumatoid and parvovirus synoviocytes, but was constitutively expressed by both samples of OA synoviocytes. Treatment with TNF-α and IL-1β increased expression of TFPI2 in all samples except for the OA synoviocytes. In contrast, aggrecan-1 expression was highest in rheumatoid fibroblasts, TNF-α and IL-1β stimulation having little effect. Caldesmon and biglycan were expressed by all fibroblasts with no obvious effect of cytokine treatment.

The most striking finding was the enhanced expression of GCP-2, GROβ, MnSOD and TFPI2 in the untreated OA fibroblasts compared with other untreated fibroblasts. This increase was confirmed at the mRNA level by Taqman analysis of TFPI2 (Fig. 4A), as well as at the protein level for MnSOD (Fig. 4B). However, stimulation with IL-1β and TNF-α led to an increase in TFPI-1 mRNA and MnSOD in most of the fibroblasts, although this was variable between fibroblasts.

Discussion

In this study we have demonstrated two specific differential gene expression patterns in fibroblasts derived from synovium (RA, OA, parvovirus infection) and skin. First, there are genes which are more highly expressed in synovial fibroblasts compared with skin fibroblasts (i.e. in a site-specific manner). Secondly, there are genes which are differentially expressed between OA and RA synoviocytes and show a different response to cytokine treatment (i.e. disease-specific expression).

Using a panel of synovial fibroblasts, we found that, irrespective of disease process, synovial fibroblasts produce high levels of the cytokine IL-6 and the chemokine CCL2, although there was a trend towards higher levels of expression in RA and OA fibroblasts.

Table 3. Differentially expressed genes identified by RFDD-PCR

<table>
<thead>
<tr>
<th>Gene identified</th>
<th>Expression level (compared with skin)</th>
<th>Motif present</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROβ* (CXCL2)</td>
<td>Increased in stimulated RA cells</td>
<td>PEG</td>
<td>CXC chemokine (related to IL-8 and SDF-1)</td>
</tr>
<tr>
<td>Caldesmon</td>
<td>Only present in RA cells</td>
<td>PEG (out of frame)</td>
<td>Actin-binding protein that inhibits the actin activation of myosin ATPase</td>
</tr>
<tr>
<td>MnSOD*</td>
<td>Only in stimulated RA cells</td>
<td>PEGTR</td>
<td>An essential antioxidant that catalyses the dismutation of superoxide radicals</td>
</tr>
<tr>
<td>Biglycan*</td>
<td>Only in RA cells</td>
<td>PEG (out of frame)</td>
<td>Large proteoglycan, extensively degraded in diseased cartilage</td>
</tr>
<tr>
<td>GCP-2 (CXCL6)</td>
<td>Only in stimulated RA cells</td>
<td>PEG</td>
<td>A chemokine that uses the IL-8 receptors CXCR1 and CXCR2</td>
</tr>
<tr>
<td>Aggrecan-1</td>
<td>Only in RA cells</td>
<td>PEG</td>
<td>Large proteoglycan</td>
</tr>
<tr>
<td>TFPI2</td>
<td>Only in stimulated RA cells</td>
<td>No</td>
<td>A serine proteinase inhibitor</td>
</tr>
</tbody>
</table>

*These genes have been reported to be elevated in RA fibroblasts, as measured by subtractive hybridization [5, 11]. SDF-1/CXCL12, stromal cell-derived factor 1.
compared with fibroblasts from self-limiting arthritis. This suggests that constitutive IL-6 and CCL2 production from synovial fibroblasts represents a common synovial response to injury. Our findings are in keeping with the emerging view that OA can be considered a localized inflammatory disease compared with RA, which is a more systemic inflammatory syndrome. In line with this, a number of studies have shown that in patients with OA the extent of inflammation and particularly inflammatory cell infiltrate can reach that observed in RA [16–18]. Of note, both OA synovial fibroblast samples in this study were derived from patients with severe OA with histological evidence of heavy inflammatory infiltrates (data not shown).

The major cartilage proteoglycans are aggrecan, biglycan and decorin [19]. Biglycan is a small cellular or pericellular matrix proteoglycan, containing 11 tandem repeats of a leucine-rich 24 amino acid sequence [20]. It has been reported as being more abundant in proliferating cartilage, as would be expected in joint remodeling. This gene appeared to be differentially expressed between rheumatoid and skin fibroblasts in the RFDD analysis, we did not have additional data at Supplementary data Online.

Caldesmon is a high molecular weight protein that binds actin and calcium–calmodulin [22, 23]. It was initially purified as a component of smooth muscle thin filaments and was found to be able to inhibit the actin-tropomyosin ATPase. Non-muscle caldesmons regulate cell motility and cytoskeletal organization [24] and have also been implicated in leucocyte activation [25]. Although this gene appeared to be differentially expressed between rheumatoid and skin fibroblasts in the RFDD analysis, we did not observe any consistent differences in RT-PCR studies in a wider panel of fibroblasts.

In contrast, four other genes (GROβ, GCP-2, MnSOD and TFPI2) were all constitutively expressed in OA synoviocytes, showing no further increase in expression upon cytokine stimulation. These genes were expressed at very low levels in rheumatoid, parvovirus and skin fibroblasts but were up-regulated upon cytokine stimulation, suggesting that they can be regulated during

![Fig. 3. Confirmation of differential gene expression in a variety of fibroblasts by RT-PCR. (A) RNA was isolated from resting or 24-h stimulated fibroblasts (IL-1β and TNF-α) and subjected to RT-PCR (see Methods). SK, skin fibroblasts; RA, rheumatoid fibroblasts; OA, OA fibroblasts; parvovirus, parvovirus fibroblasts; negative, negative control. (B) Summary of mRNA expression by the different fibroblasts (− absent, ++ very strong).](image)

![Fig. 4. Confirmation of differential TFPI2 and MnSOD expression in OA synovial fibroblasts. (A) mRNA was isolated from the different fibroblasts and compared with skin fibroblasts (SK-1) before or after stimulation for 24 h with TNF-α/IL-1β. mRNA copies of TFPI2 were analysed by Taqman and expressed as fold increase compared with unstimulated SK-1 (see Methods). The experiment was carried out in triplicate and results are expressed as mean ± s.d. (B) Expression of MnSOD by immunohistochemistry in RA-1, OA-1, self-limiting and SK-1 cells before and after stimulation for 24 h with TNF-α/IL-1β. Nuclei are stained blue and MnSOD green (this figure may be viewed in colour as supplementary data at Rheumatology Online).](image)
the inflammatory response. Furthermore, we confirmed that the differential expression between resting OA and RA fibroblasts we observed occurred for TFPI2 using Taqman PCR and at the protein level for MnSOD using immunohistochemical studies of resting and activated fibroblasts.

In agreement with our results, constitutive expression of GROβ (CXCL2) in OA synoviocytes has been reported and treatment with anti-GROβ antibodies has been shown to delay the onset and reduce the severity of collagen-induced arthritis [26, 27]. GROα, β and γ are known to induce chemotaxis, changes in shape, a transient rise in cytosolic Ca2+ granule exocytosis and respiratory burst in neutrophils, thus acting as mediators in inflammation [28, 29]. Therefore, up-regulation of GROβ is likely to exacerbate the inflammatory response.

GCP-2 (CXCL6) is a member of the ELR-expressing CXC subfamily of chemokines, and acts as a potent chemoattractant of neutrophils in the course of acute inflammation [30, 31]. It binds and activates two receptors, CXCR1 and CXCR2, which are expressed on neutrophils. GCP-2 is the only ELR+ CXC chemokine, except for IL-8, that is an effective ligand for CXCR1 in addition to CXCR2. Intradermal injection of GCP-2 or GRO in normal rabbit skin provokes prominent granulocyte infiltration. Up-regulation of GCP-2 by cytokines has also been previously reported [30].

Superoxide dismutases (SOD), such as MnSOD, are metalloproteinases that convert oxygen radicals to hydrogen peroxide (H2O2). TNF-α has been shown to increase MnSOD expression in a variety of human cell lines. A number of potentially toxic elements are released at the site of inflammation, including oxygen radicals. Direct injection of superoxide dismutase into inflamed joints has been reported to partially relieve the symptoms of RA; this may be due either to removal of superoxide radicals or to a non-specific protein effect. Nivsarkar [32] found that in RA patients superoxide dismutase activity was significantly lower than in controls, but levels of circulating SOD were improved after NSAID therapy.

TFPI2 is a Kunitz-type serine proteinase inhibitor, isolated by virtue of its sequence homology to TFPI [33]. It is generally associated with the extracellular matrix (ECM) and is an inhibitor of trypsin, plasmin, plasma kallikrein, factor XIa, chymotrypsin, factor VIIa-tissue factor and cathepsin G [34]. TNF-α stimulation of HUVEC and human vascular smooth muscle cells has previously been shown to increase TFPI2 expression (and release) in a time and dose-dependent manner [34, 35]. TFPI2 may serve a protective function in inflammation, by inhibiting matrix metalloproteinase activation.

As a technique, RFDD compares favourably with other methods for the detection of differential gene expression, such as differential subtraction, hybridization and genome-wide microarray analysis. Differential subtraction and hybridization techniques are labour-intensive and require large quantities of RNA, which are often not easily obtained from diseased samples. In addition, these techniques cannot target specific gene sequences. Although microarray analysis using targeted arrays can identify specific gene families, these often require large amounts of sample, and will not detect novel members of specific gene families. RFDD does not suffer from these limitations and also allows fingerprints from several mRNA populations to be easily compared side-by-side on one gel [36]. Our work demonstrates the successful use of RFDD in identifying differentially expressed genes that contain a common motif. Although no differentially expressed members of the LPAAT family were identified in this study, six of the seven genes identified contained all or part of the LPAAT motif, amino acids PEGTRNX. TFPI2 did not show any part of the PTPase signature motif, and was presumably detected due to the high degeneracy of the primers used.

This paper reports the successful use of the RFDD technique to isolate seven genes which are differentially regulated in synovial and skin fibroblasts, and are further affected by TNF-α and IL-1β. The genes identified here allow a clear distinction to be made between fibroblasts derived from different anatomical sites (synovial vs skin) as well as different synovial diseases (OA vs RA). Of particular interest is the finding that proinflammatory genes such as GROβ (CXCL2) and GCP-2 (CXCL6), implicated in the recruitment of leucocytes to sites of inflammation, are constitutively expressed in resting OA but not rheumatoid fibroblasts, supporting the idea that in some circumstances OA can be considered an inflammatory disease [4]. While some of the differences we have observed between RA- and OA-derived fibroblasts may represent differences due to in vitro culture, we propose that these differences might facilitate a clearer understanding of those features of inflammation that are a common response to synovial inflammation (i.e. generic to OA and RA) from those that are more disease-specific and represent potential new targets for anti-inflammatory drugs tailored to OA.

**Rheumatology**

<table>
<thead>
<tr>
<th>Key messages</th>
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<tbody>
<tr>
<td>- Synovial fibroblasts spontaneously produce high levels of IL-6 and MCP-1 (CCL2) compared with skin fibroblasts.</td>
</tr>
<tr>
<td>- Synovial fibroblasts derived from patients with OA express high basal levels of proinflammatory cytokines/chemokines.</td>
</tr>
<tr>
<td>- Not all fibroblasts are the same.</td>
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**Acknowledgements**

We would like to thank Dr Craig Southern for advice and helpful discussions about the manuscript. This work was partially funded from grants from the ARC and MRC.

C. D. Buckley received funding for a PhD studentship from Yamamouchi Research Institute. The other authors have declared no conflicts of interest.

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