Differential expression and functional behaviour of the \( \alpha v \) and \( \beta 3 \) integrin subunits in cytokine stimulated fibroblast-like cells derived from synovial tissue of rheumatoid arthritis and osteoarthritis in vitro

Nadia Rinaldi, D Weis, B Brado, M Schwarz-Eywill, M Lukoschek, A Pezzutto, U Keilholz, T F E Barth

Abstract

Objective—The aim of this study was to investigate in situ the expression of the classic vitronectin (VN) receptor consisting of the \( \alpha v \) and \( \beta 3 \) subunits in synovial lining cells (SLC) of chronic synovitis occurring in osteoarthritis (OA) and in rheumatoid arthritis (RA). The expression and function of \( \alpha v \) and \( \beta 3 \) as VN receptor in cultured fibroblast-like synoviocytes (FBS) derived from patients with OA and RA was also compared.

Methods—Expression of \( \alpha v \) and \( \beta 3 \) was examined immunohistochemically in normal synovial tissue and in synovial tissue from patients with OA and RA. The effect of proinflammatory cytokines and of a synovial fluid of a patient with RA on the expression of the \( \alpha v \) and \( \beta 3 \) subunits of cultured FBS was determined by flow cytometry. Binding of OA and RA-FBS to VN was quantified using adhesion assays and the effect of interleukin 1\( \beta \) (IL1\( \beta \)) and tumour necrosis factor \( \alpha \) (TNF\( \alpha \)) on adhesion was measured. The specificity of the adhesion was tested by inhibition studies using monoclonal antibodies to integrin subunits.

Results—In situ studies normal SLC showed a parallel distribution of \( \alpha v \) and \( \beta 3 \) subunits. OA-SLC strongly and uniformly expressed \( \alpha v \) whereas RA-SLC showed heterogeneous expression of \( \alpha v \). In situ both OA-SLC and RA-SLC lacked the expression of the integrin subunit \( \beta 3 \). In in vitro studies, OA-FBS and RA-FBS did not differ as regards expression of \( \alpha v \) and \( \beta 3 \), and VN attachment. Binding of RA-FBS to VN was partially blocked by antibodies against \( \alpha v \), \( \beta 1 \), and \( \beta 3 \) subunits, whereas only antibodies against \( \alpha v \) and \( \beta 3 \) inhibited the binding of OA-FBS to VN. The proinflammatory cytokines TNF\( \alpha \) and IL1\( \beta \) increased the expression of \( \alpha v \) and \( \beta 3 \), and the VN binding of OA-FBS, whereas \( \alpha v \) and \( \beta 3 \) expression, and VN binding were downregulated in RA-FBS. Similar effects were found when the synovial fluid of an RA patient was used.

Conclusion—The integrin subunit \( \beta 3 \) seems to be one partner but not the major one with which the subunit \( \alpha v \) forms functional vitronectin receptors in OA-FBS and RA-FBS. The interaction between synovial cells and inflammatory cytokines seems to be different for OA and RA; the basis for this difference, however, remains to be established.

(Vitronectin (VN) is distributed like fibronectin and laminin broadly among loose connective tissues showing particularly high concentrations in vascular subendothelium. It is also found around synovial lining cells in normal synovial membranes. VN is a cell spreading factor and a cell adhesion promoting factor. The cell attachment activity of VN resides in the arg-gly-asp (RGD) sequence. \( \alpha v \beta 3 \) is the best known VN receptor; however, it is highly promiscuous in recognising RGD in several adhesive proteins, including fibronectin. Conformational changes of VN that occur during remodelling processes can lead to exposure of the RGD sequence, which is then recognised by integrin \( \alpha v \beta 3 \). This could support a role for \( \alpha v \beta 3 \) during wound healing and tissue remodelling processes, which may be important in diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA).

As immunohistochemical studies have shown that VN staining is increased in chronically inflamed synovial membranes, we have investigated the expression of \( \alpha v \) and \( \beta 3 \) in lining synoviocytes (SLC) of OA and RA synovial membranes compared with normal synovia by in situ staining.

This expression was also evaluated in cultured fibroblasts like synoviocytes (FBS) derived from OA and RA synovial tissue. The capacity of SLC to interact with VN through \( \alpha v \beta 3 \) was studied by adhesion assays in vitro. To study the regulation of expression and function of \( \alpha v \beta 3 \), we evaluated the effect of inflammatory cytokines and of a synovial fluid derived from an arthritic joint of a patient with RA on \( \alpha v \) and \( \beta 3 \) expression, and VN attachment.)
Table 1  Inflammation score and expression of the αv and β3 subunits in synovial lining cells of normal synovial membranes, OA synovia, and RA synovia

<table>
<thead>
<tr>
<th>Normal synovial membranes</th>
<th>OA synovia</th>
<th>RA synovia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>3</td>
<td>1+ (−)</td>
</tr>
<tr>
<td>Sample 2</td>
<td>3</td>
<td>1+ (−)</td>
</tr>
<tr>
<td>Sample 3</td>
<td>5</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 4</td>
<td>8</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 5</td>
<td>7</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 6</td>
<td>11</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 7</td>
<td>4</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 8</td>
<td>4</td>
<td>3+ (−)</td>
</tr>
<tr>
<td>Sample 9</td>
<td>6</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 10</td>
<td>4</td>
<td>3+ (2 (+))</td>
</tr>
<tr>
<td>Sample 11</td>
<td>7</td>
<td>1+ (−)</td>
</tr>
<tr>
<td>Sample 12</td>
<td>8</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 13</td>
<td>5</td>
<td>3+ (−)</td>
</tr>
<tr>
<td>Sample 14</td>
<td>7</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 15</td>
<td>6</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 16</td>
<td>4</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 17</td>
<td>7</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>OA synovia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 18</td>
<td>7</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 19</td>
<td>11</td>
<td>3+ (−)</td>
</tr>
<tr>
<td>Sample 20</td>
<td>15</td>
<td>3+ (−)</td>
</tr>
<tr>
<td>Sample 21</td>
<td>9</td>
<td>3+ (2 (+))</td>
</tr>
<tr>
<td>Sample 22</td>
<td>9</td>
<td>4+ (1 (+))</td>
</tr>
<tr>
<td>Sample 23</td>
<td>11</td>
<td>4+ (1 (+))</td>
</tr>
<tr>
<td>Sample 24</td>
<td>6</td>
<td>2+ (−)</td>
</tr>
<tr>
<td>Sample 25</td>
<td>12</td>
<td>4+ (3 (+))</td>
</tr>
<tr>
<td>Sample 26</td>
<td>9</td>
<td>4+ (1 (+))</td>
</tr>
<tr>
<td>Sample 27</td>
<td>8</td>
<td>1+ (−)</td>
</tr>
<tr>
<td>Sample 28</td>
<td>13</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 29</td>
<td>4</td>
<td>1+ (−)</td>
</tr>
<tr>
<td>Sample 30</td>
<td>6</td>
<td>4+ (1 (+))</td>
</tr>
<tr>
<td>Sample 31</td>
<td>15</td>
<td>4+ (1 +)</td>
</tr>
<tr>
<td>Sample 32</td>
<td>10</td>
<td>4+ (−)</td>
</tr>
<tr>
<td>Sample 33</td>
<td>12</td>
<td>3+ (−)</td>
</tr>
<tr>
<td>Sample 34</td>
<td>15</td>
<td>− (−)</td>
</tr>
<tr>
<td>Sample 35</td>
<td>15</td>
<td>3+ (−)</td>
</tr>
<tr>
<td>Sample 36</td>
<td>10</td>
<td>2 (2 (+))</td>
</tr>
</tbody>
</table>

Expression of the αv and β3 subunits in synovial lining cells of normal osteoarthritic, and rheumatoid synovia in situ. Scoring of cell reaction: 4+, 75–100% cells positive, 3+, 50–75% cells positive, 2+, 25–50% cells positive, 1+, 0–25% cells positive, (−), low level of antigen expression, −, all cells negative.

Methods

SYNOVIAL TISSUE

Synovial tissues were obtained at arthroplasty from the hip, knee or wrist of 22 patients with RA and 18 patients with OA (according to the criteria of the American College of Rheumatology, formerly, the American Association of Rheumatology),[10][11] Normal specimens were obtained at necropsy from two patients without arthriti who died of unrelated causes. Necropsy specimens were taken within six hours after death. Nineteen synovia specimens of patients with RA and 15 synovia specimens of patients with OA were quick frozen in liquid nitrogen and stored at −70°C, whereas the other specimens (from three patients with OA and of three patients with RA) were processed immediately after excision and prepared for FBS culture. A synovial fluid sample was collected from a patient with RA into tubes containing EDTA (Biochrom, Berlin, Germany) and stored at −70°C.

FBS ISOLATION

In vitro cultures of FBS were obtained by standard methods,[12] as previously described.[13] The tissue was minced into small pieces and digested with collagenase type Ia (Sigma Chemical Co, St Louis, MO, USA) in serum free basal Iscove medium (Seromed-Biochrom, Berlin, Germany). The samples were washed and suspended in basal Iscove medium, supplemented with 10% fetal calf serum (FCS, Bio Pro, Karlsruhe, Germany) and with penicillin-streptomycin-amphotericin B (10 units/ml, 10 mg/ml, and 0.25 mg/ml). Finally, isolated cells were cultured in a humidified 5% carbon dioxide atmosphere. After overnight culture, non-adherent cells were removed. Fresh medium was added and the incubation continued. At confluence, cells were trypsinised, split at 1:3 ratio, and recultured. The medium was changed twice each week. FBS from passages 3 through 9 were used in these experiments. Each of the three cultures of OA-FBS and RA-FBS were detached with EDTA (0.02%) and centrifuged at 1000 rpm for five minutes. The cultured cells comprised a homogeneous population of FBS with respect to morphological and immunocytochemical criteria (<1% CD11b+, <1% CD11a+, <1% CD11c+, <1% CD53+, <1% CD3+, and <1% Factor VIII+).

STIMULATION OF FBS

FBS were cultured for 72 hours in the presence of the cytokines. Pilot studies were performed to test the concentration of cytokines showing the most stimulating effect on the expression of αvβ3. On the basis of these data we used the following concentrations: interleukin 1β (IL1β) (Genzyme; Cambridge, MA): 40 U/ml; tumour necrosis factor α (TNFα) (Knoll; Ludwigshafen, Germany): 10 ng/ml. In four experiments FBS cultures were cultured for 72 hours in the presence of basal Iscove's medium, supplemented with 10% supernatant of synovial fluid from a patient with RA. At the end of the incubation, cells were washed with PBS, and detached with EDTA. Cell viability was measured by the trypan blue exclusion test, which was >95%. Before analysis, cells were washed twice in PBS and resuspended with RPMI 1640 (Gibco, Paisley, Scotland, UK).

REAGENTS

Human VN was purchased from Biomol (Hamburg, Germany). The monoclonal primary antibodies (mAbs) AMF7 (anti-αv; IgG1), SZ.21 (anti-β3; IgG1), HP2/1 (anti-α4; IgG1), Gi9 (anti-α2; IgG1), and SAM-1 (anti-α5; IgG2β) were supplied by Dianova (Hamburg, Germany). The monoclonal primary antibodies P4C10 (anti-β1; IgG1), and P1B5 (anti-α3; IgG1) were obtained from Telios (San Diego, USA). An anti-cytomegalovirus antibody (clone CCH2; IgG1) was used as a negative control and was obtained from Dako (Glostrup, Denmark). A polyclonal biotinylated sheep antibody to mouse Ig (reactive with all mouse isotypes), and a streptavidin-biotinylated peroxidase complex, were purchased from Amersham (High Wycombe, UK). 3-amino-9-ethylcarbazole (AEC) and N’N-dimethylformamide (DMF) were obtained from Sigma.
IMMUNOSTAINING PROCEDURE
Serial frozen sections of about 1 cm² in area and 4–6 µm in thickness were air dried overnight, fixed in acetone for 10 minutes at room temperature, and immunostained immediately or after storage at −20°C for one to three weeks. Immunostaining procedures were carried out according to standard methods as previously described.14 Briefly, for immunohistochemistry after rehydration with phosphate buffered saline solution (PBS; pH 7.5), the frozen sections were incubated for one hour with primary mAbs. The primary mAbs were used in a protein concentration of about 5 µg/ml PBS. The sections were then incubated with biotinylated antimouse (20 µg/ml protein) and streptavidin/biotin-peroxidase complex (10 µg/ml protein) for 30 minutes, respectively. All incubation steps were carried out in a humid chamber at room temperature. Between each incubation step the sections were rinsed twice in PBS. Using AEC as the chromogen (0.4 mg/ml in 0.1 mol/l acetate buffer, pH 5.0, with 5% DMF and 0.01% hydrogen peroxide for about 20 minutes), the peroxidase reaction caused an intense red precipitate. The sections were then rinsed in tap water, counterstained with Harris’ haematoxylin, and mounted with glycerol gelatin. Each series of frozen section contained a control without the primary reagent. In addition, a negative control was carried out using an isotype matched mAb against cytomegalovirus in a number of tissue sections to exclude a non-specific binding. No staining was observed except for scattered granulocytes whose endogenous peroxidase was not blocked to permit optimal antigenicity. This reactivity was disregarded during evaluation. In tissue sections, strongly stained endothelial cells, lymphocytes, or stromal cells, or all three, always present in combinations characteristic of the respective antigen under study, served as intrinsic positive controls.

CONTROLS AND EVALUATION OF ANTIGEN EXPRESSION
SLC staining in tissue sections was evaluated in a semiquantitative manner: +, indicates strong intensity of the detected antigen; (+), is regarded as weak intensity of antigen.

Figure 1 Expression of αv and β3 integrin subunits in SLC of normal synovia (A and B), in SLC of OA synovia (C and D), and in SLC of RA synovia (E and F). Normal synovia (serial sections, sample 1): (A) Normal, non-inflamed SLC are strongly positive for αv in a subset of SLC. (B) SLC in a non-inflamed synovia are weakly positive in a minor population of SLC for the β3 integrin subunit. Osteoarthritis (sample 3): (C) SLC form a multicellular layer and strongly express the αv integrin subunit. (D) SLC of OA are negative for β3. Endothelial cells are strongly positive for β3. Rheumatoid arthritis (sample 34): (E) SLC and lymphoid aggregates are negative for the αv integrin subunit; αv is expressed on endothelial cells of a small vessel serving as a positive control of the immune reaction. (F) SLC and lymphoid cells are β3 negative while endothelial cells are strongly positive for β3; original magnification × 150.
expression. The absence of antigen was symbolised as \( - \). Whenever the staining intensity within FBS washeterogeneous, a simple semiquantitative statement was made: 4+, meaning 75–100% cells positive; 3+, meaning 50–75% cells positive; 2+, meaning 25–50% cells positive; 1+, meaning 0–25% cells positive.

**DEGREE OF INFLAMMATION**

Haematoxylin and eosin stained sections from each synovial specimen were scored for the degree of inflammation by two independent observers, according to Rooney et al. Synovial lining cell depth was determined, and the results were recorded as 0 (1–3 cell layers), 1+ (4–5 cell layers), 2+ (6–8 cell layers), and 3+ (>9 cell layers). Proliferating blood vessels were determined by immunostaining using anti-von Willebrand factor (Dako, Glostrup, Denmark) and recorded as 1+ (0–5 vessels/high power field (HPF) (100×), 2+ (5–10 vessels/HPF), 3+ (10–20 vessels/HPF), and 4+ (>20 vessels/HPF). The size of lymphoid aggregates on stained sections was recorded as 0+ (no aggregates), 1+ (1–20 cells/HPF), and 2+ (>20 cells/HPF). The number of vessels showing perivascular infiltrates was determined for the final score and regarded as 1+ (1–25% of the vessels involved), 2+ (25–50% of the vessels involved), 3+ (50–75% of the vessels involved), and 4+ (>75–100% of the vessels involved). The degree of fibrosis and the presence of necrosis was assessed. Sections containing <10% fibrous tissue in the membrane sublining layers were considered normal and graded 0. Sections containing 10–50% fibrous tissue were scored as 1+, whereas sections with >50% fibrous tissue were graded as 2+. The absence of necrosis was scored 0. The presence of necrosis was scored 2+. The scores of the individual items were added, and the sum was used as an estimate of the overall intensity of inflammation by histological criteria (range of possible values 2–17).

**FLOW CYTOMETRIC ANALYSIS**

FBS \((1 \times 10^6)\) were suspended in RPMI 1640, 10% FCS, 0.1% NaH3 (Merck, Darmstadt, Germany), and 2% HEPES buffer (Seromed-Biochrom). The expression of \( \alpha \) and \( \beta \) was assayed by flow cytometry, performed on a FACSscan (Becton Dickinson) using the Lysis II Software. W6/32, a monoclonal antibody that reacts with the HLA-A,B,C/\( \beta \)2m complex, served as a positive control. The mAb CD21

### Table 2: Flow cytometric analysis of surface expression on untreated (medium) and stimulated FBS from OA and RA patients

<table>
<thead>
<tr>
<th>Antigen</th>
<th>OA</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Positive (SD)</td>
<td>mF</td>
</tr>
<tr>
<td>Medium</td>
<td>( \alpha )</td>
<td>42 (9.1)</td>
</tr>
<tr>
<td></td>
<td>( \beta )</td>
<td>20.6 (10.4)</td>
</tr>
<tr>
<td>IL1( \beta )</td>
<td>( \alpha )</td>
<td>75.9 (5.4)</td>
</tr>
<tr>
<td></td>
<td>( \beta )</td>
<td>38.7 (11.9)</td>
</tr>
<tr>
<td>TNF( \alpha )</td>
<td>( \alpha )</td>
<td>72.1 (9.8)</td>
</tr>
<tr>
<td></td>
<td>( \beta )</td>
<td>52.5 (12.5)</td>
</tr>
<tr>
<td>IL1( \beta ) + TNF( \alpha )</td>
<td>( \alpha )</td>
<td>63 (10.4)</td>
</tr>
<tr>
<td></td>
<td>( \beta )</td>
<td>62.4 (11.5)</td>
</tr>
<tr>
<td>SF from RA</td>
<td>( \alpha )</td>
<td>75.2 (5)</td>
</tr>
<tr>
<td></td>
<td>( \beta )</td>
<td>55.3 (4.4)</td>
</tr>
</tbody>
</table>

mF, mean fluorescence. SF from RA, medium with SF from an RA patient.

![Figure 2](http://ard.bmj.com/)

**Figure 2** Histograms of flow cytometric analysis for the \( \alpha \) integrin subunit on FBS from OA (upper panels) and from RA (lower panels). The fluorescence obtained with isotypic negative control antibodies is plotted as a plain line, the results with anti-\( \alpha \) as a bold line.
Expression of the αv and β3 integrin subunits in cytokine stimulated fibroblast-like cells

Patients, as determined by adhesion assay. Patient on the binding of FBS cultures derived from two OA patients and from two RA patients, as determined by adhesion assay.

Figure 3  Effect of cytokines and synovial fluid (SF) from an arthritic joint of a RA patient on the binding of FBS cultures derived from two OA patients and from two RA patients, as determined by adhesion assay.

ADHESION ASSAYS
Flat bottomed 96 well plates (Titertek, Amstelstad, Netherlands) were coated with VN (10 µg/ml) at 4°C overnight. Control wells were only treated with PBS. Plates were emptied and unspecific binding blocked by 100 µl of RPMI 1640 containing 1% bovine serum albumin (BSA). Control FBS (cultured only with medium) and stimulated FBS were harvested with EDTA (0.02%), pelleted, and resuspended in 1% BSA/RPMI 1640 at a concentration of 5 × 10^5 cells/ml. For inhibition of cell attachment, FBS were pre-incubated with the following antibodies: P1B5 was used at a 1:300 dilution and PAC10 at a 1:200 dilution, mAbs SAM-1, AMF7, Gi9, and SZ.21 were used at a 1:20 dilution, which corresponded to an mAb concentration of 10–20 µg/ml. Cells were added to microtitre wells (100 µl/well) and incubated for 20 minutes at 37°C. After this time the plates were turned upside down so that the fluid formed a hanging drop inside the wells. After a further five minutes of upside down incubation, plates were gently flicked off and washed once with PBS. Adherence was quantified by measurement of hexosaminidase activity according to Landegren. Briefly, 60 µl of a p-nitrophenol-n-acetyl-β-D-glucosaminide (Sigma) solution were added to each well, incubated at 37°C, and the reaction stopped after three hours with 100 µl of glycine buffer. One hundred per cent references were obtained by spinning equal volumes of cell suspension in Eppendorf tubes, performing hexosaminidase reaction, and transferring the developed colour solution to the plate. Absorption was measured using a Titertek multiscan at 405 nm. Background adhesion was always less than 2% of the cells plated.

STATISTICAL ANALYSIS
For the comparison of the histological scores of inflammation of the two groups of chronic synovitis the Mann-Whitney non-parametric test was applied. Correlation between the expression of αv and β3 subunits and the inflammation score was calculated using the rank correlation Spearman test. Data concerning the expression of αv and β3, and vitronectin binding were reported as mean (SD).

Results
INFLAMMATION SCORES AND IMMUNOSTAINING DATA
Histological scores of inflammation and immunostaining data are summarised in table 1(A) and 1(B). In OA synovia the mean inflammation score was higher (mean (SD) 6.2 (1.9)) in comparison to normal synovia. Only in one of 15 synovia samples was the inflammation score >10. In comparison, the mean inflammation score of RA synovia was significantly (p < 0.005) higher (9.8 (3.1)). Inside this group eight of 16 synovia samples had an intermediate inflammation scoring value <10, the remaining synovial samples exhibited a high inflammation score ≥10. In serial immunostained sections of normal synovia a small proportion of SLC (1–25%) expressed αv at high levels in two of two samples (fig 1A), while the β3 integrin subunit was weakly positive in a small SLC subset in two out of two synovia samples (fig 1B).

In contrast, high expression of αv was found in SLC of OA synovia. In 11 of 15 samples all SLC strongly expressed αv (fig 1C). In three of 15 samples 50–75% SLC displayed strong expression of αv. Only in one of 15 samples the expression of αv was limited to 1–25% of the SLC. The integrin subunit β3 was expressed only in a subset of the SLC in two of 15 samples.
To investigate the expression of \( \text{EXPRESSION OF THE } \alpha \text{V AND } \beta \text{3 SUBUNITS ON STIMULATED FBS} \)

To examine whether cytokines influence expression and function of \( \alpha \text{V} \) and \( \beta \text{3} \), OA-FBS and RA-FBS were stimulated in the presence of IL1\( \beta \), TNF\( \alpha \) or the combination of both and were analysed for \( \alpha \text{V} \) and \( \beta \text{3} \) expression (table 2). IL1\( \beta \) and TNF\( \alpha \) induced an increase in the \( \alpha \text{V} \) expression on OA-FBS (fig 2). Combining IL1\( \beta \) and TNF\( \alpha \) increased \( \alpha \text{V} \) surface staining on OA-FBS. However, the effect of the cytokine combination was less than that of single cytokines. The \( \beta \text{3} \) integrin subunit was present at low levels in cultured OA-FBS. In the presence of IL1\( \beta \), and particularly, of TNF\( \alpha \) an increase in the \( \beta \text{3} \) expression was observed. A moderate synergism was observed with the two cytokines. TNF\( \alpha \) and IL1\( \beta \) had completely different effects on RA-FBS: the expression of \( \alpha \text{V} \) was considerably reduced in the presence of IL1\( \beta \), both alone and in combination with TNF\( \alpha \) (fig 2). To reproduce in our in vitro model the effect of factors that play a part in the inflammatory process in vivo, FBS were also cultured in the presence of the supernatant of one RA synovial fluid. This led to a pronounced increase in the expression of \( \alpha \text{V} \) and \( \beta \text{3} \) on OA-FBS, while the expression was strongly reduced by the RA synovial fluid on RA-FBS.

ADHESION TO VITRONECTIN OF CULTURED FBS

To examine the interaction between VN and FBS, adhesion assays were carried out on VN coated microtitre wells. FBS adhered well to VN. No significant difference in VN binding was observed between OA-FBS and RA-FBS (fig 3). To investigate further the role of \( \alpha \text{V} \) and \( \beta \text{3} \), and of other integrin subunits in FBS binding to VN, inhibition assays were performed (fig 4). For blocking studies we used monoclonal antibodies against \( \alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha \text{V}, \beta \text{1}, \) and \( \beta \text{3} \) integrin subunits. Binding to VN of OA-FBS was blocked by approximately 40% using anti-\( \alpha \text{V} \) antibody, whereas this antibody blocked VN binding of RA-FBS by about 60%. Anti-\( \beta \text{3} \) blocked the adhesion of RA-FBS and OA-FBS by approximately 20%. Adhesion to VN was blocked by about 40% with anti-\( \beta \text{1} \) antibodies only in RA-FBS, whereas this antibody showed no inhibiting effect on VN adhesion of OA-FBS. The other monoclonal antibodies against \( \alpha_2, \alpha_3, \alpha_4, \) and \( \alpha_5 \) had no effect on VN attachment in our cellular system.

BINDING TO VN OF CYTOKINE TREATED FBS

VN attachment of OA-FBS was increased by TNF\( \alpha \) while IL1\( \beta \) alone had no effect (fig 3). The addition of IL1\( \beta \) partially inhibited the TNF\( \alpha \) effect. Culturing of OA-FBS with RA synovial fluid induced an increase in VN binding. In analogy to the effects on the expression, cytokines and RA synovial fluid inhibited the binding of RA-FBS to VN. In particular, TNF\( \alpha \) alone or in combination with IL1\( \beta \) decreased the VN binding of RA-FBS, whereas IL1\( \beta \) used as a single agent had no effect. The major inhibiting effect on VN attachment was seen by treatment of RA-FBS with the inflammatory synovial fluid.

EXPRESSION OF THE \( \alpha \text{V} \) AND \( \beta \text{3} \) SUBUNITS ON CULTURED FBS

To investigate the expression of \( \alpha \text{V} \) and \( \beta \text{3} \) in long term cultures derived from OA and RA synovia, flow cytometric analysis was performed (table 2). OA-FBS and RA-FBS expressed \( \alpha \text{V} \) at intermediate levels. The integrin subunit \( \beta \text{3} \) was expressed at low levels in FBS cultures. There was no relevant difference in the expression of \( \alpha \text{V} \) and \( \beta \text{3} \) between OA-FBS and RA-FBS.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure4}
\caption{Inhibition of adhesion of FBS from OA and RA to VN. Monoclonal antibodies (mAb) against \( \alpha_3, \alpha_5, \alpha_6, \alpha_5, \beta_1, \) and \( \beta_3 \) were used; however only mAbs that blocked FBS binding are shown in the figure. Inhibition through the mAb is given as relative percent adhesion compared with adhesion without antibodies (100%). Data represent the means of assays of three OA-FBS cultures and of three RA-FBS cultures.}
\end{figure}
Expression of the αv and β3 integrin subunits in cytokine stimulated fibroblast-like cells

Discussion

This study showed that SLC in normal, non-infamed synovial membranes show a similar distribution pattern of the αv and β3 integrin subunits according to the view that these subunits may associate to form receptors for ECM proteins. In contrast with the parallel distribution of these molecules in SLC of normal synovial membranes, the expression of αv is much stronger than β3 in SLC of OA and RA synovia. This is in agreement with other authors. A possible explanation for this finding may be that during chronic synovitis αv preferentially associates with other integrin subunits like β1 and β5. The expression of αv in SLC during chronic synovitis was heterogeneous in RA compared with OA. In an RA synovial tissue specimen with severe inflammatory changes SLC were evenly devoid of the expression of αv, which is in accordance with previous data. Although αv expression by SLC is generally increased in chronic synovitis, disease specific mechanisms (as yet undefined) seem to regulate this expression, eventually leading to downregulation. The integrin receptor αvβ3 has been shown to mediate adhesion to the complement complex SC5b-9, protecting cells against complement lysis. The decrease in the expression of αv in RA-SLC might be related to low levels of complement lysis inhibitors. In the rheumatoid process this might facilitate bystander lytic attacks on SLC and other joint cells. Attacked SLC may be stimulated to produce collagenase, contributing to synovial tissue damage.

No difference in the expression of the αv and β3 subunits or in the ability to bind VN was found between cultured RA-FBS and OA-FBS. We have found that the capacity of RA-FBS to adhere depends on the expression of αv and to a lesser extent β3 of αv and β3. On the contrary, binding of OA-FBS to VN was partially inhibited only by the mAb to αv and β3. These data suggest that αv may associate with β1 and β3 to form the functional receptor for VN on RA-FBS. It is possible that αv also heterodimerises with β5 to serve as a VN receptor in our cellular system.

To investigate further the functional behaviour of FBS we studied the effect of proinflammatory cytokines that have been shown relevant during chronic synovitis processes on the expression of αv and β3, and the VN attachment of cultured FBS from OA and RA patients. The cytokine effect on αv and β3 expression and on VN attachment was dependent on the source of the cultured FBS. In particular, IL1β and TNFα increased the expression of αv and β3, and increased the VN attachment of OA-FBS, whereas they exerted an inhibitory effect on both expression of αv and β3, and the VN binding of RA-FBS. This differing susceptibility to the same cytokines suggests a different functional state of OA-FBS and RA-FBS. This finding is supported by studies showing that (long term) cultured RA-FBS differ functionally from non-rheumatoid FBS in their capacity to synthesise spontaneously IL1β and PGE2. Recently, it has been shown that RA-FBS can spontaneously produce the zinc finger gene Z-225/Egr-1, a transcription factor expressed in the immediate early events of cellular activation. Cytokines and other inflammatory factors have been largely detected in the synovial fluid of inflamed joints. Therefore, to substantiate the effect of inflammatory factors displaying a role in the rheumatoid process in vivo, we kept FBS of OA and RA in culture in the presence of a synovial fluid derived from a patient with RA. In analogy to the results obtained by cytokine treatment, the synovial fluid increased the expression of αv and β3, and the binding to VN in FBS of OA, whereas its presence determined a decrease in αv and β3 staining and VN attachment of RA-FBS. Down regulation of integrin subunits has been also observed in cultured FBS " and in other cellular systems such as mesothelial cells by TGFβ and IFNγ, keratinocytes by TGFβ3, and endothelial cells by IFNγ and TNFa. Furthermore, a decrease in the expression of αvβ3 was induced by bFGF in endothelial cells.

Taken together, in SLC αv seems to heterodimerise with β3 to form VN receptors. Nevertheless, β3 seems not to be the major partner for αv in OA-FBS and RA-FBS. This is supported by the data (a) showing that αv and β3 staining only rarely in SLC of chronic synovium in comparison with the frequent expression of αv and in vitro data (b) showing that anti-β3 antibodies have only minimal effect on VN binding of cultured OA-FBS and RA-FBS. Furthermore, our in situ and in vitro findings show differences in αv expression and VN binding related to the aetiology of the chronic synovitis. RA-SLC seem to abrogate the expression of αv, whereas OA-SLC show a constant strong αv expression. In vitro, treatment by cytokines led in RA-FBS to a decrease in αv and β3 expression and VN binding and in OA-FBS to an increase in the expression of αv and β3, and VN binding.

As differences in the αv distribution of SLC and in the functional behaviour of cultured FBS were related to two joint diseases of different aetiology, it can be suggested that the interaction between inflammatory effectors and SLC in vivo may be specific for a certain inflammatory process.

We would like to thank U Fabian and E Michalek for technical assistance and K Koretz from the Institute of Pathology at the University of Ulm for methodological advice.


21 Jahn B, Von Kempis J, Kramer KL, Filsinger S, Hansch GM. Interaction of the terminal complement components CSβ-9 with synovial fibroblasts: binding to the membrane surface leads to increased levels in collagenase-specific mRNA. Immunology 1993;78:329–34.


Differential expression and functional behaviour of the \( \alpha v \) and \( \beta 3 \) integrin subunits in cytokine stimulated fibroblast-like cells derived from synovial tissue of rheumatoid arthritis and osteoarthritis in vitro

Nadia Rinaldi, D Weis, B Brado, M Schwarz-Eywill, M Lukoschek, A Pezzutto, U Keilholz and T F E Barth

Ann Rheum Dis 1997 56: 729-736
doi: 10.1136/ard.56.12.729

Updated information and services can be found at:
http://ard.bmj.com/content/56/12/729

These include:

References
This article cites 27 articles, 9 of which you can access for free at:
http://ard.bmj.com/content/56/12/729#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Connective tissue disease (4253)
- Degenerative joint disease (4641)
- Immunology (including allergy) (5144)
- Inflammation (1251)
- Musculoskeletal syndromes (4951)
- Rheumatoid arthritis (3258)
- Osteoarthritis (931)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/