Fibroblast-Like Synoviocytes from Rheumatoid Arthritis Patients Have Intrinsic Properties of Follicular Dendritic Cells

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References
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Fibroblast-Like Synoviocytes from Rheumatoid Arthritis Patients Have Intrinsic Properties of Follicular Dendritic Cells

Ernst Lindhout,2* Marco van Eijk,* Melissa van Pel,* Jan Lindeman,† Huibert J. Dinant,‡ and Cornelis de Groot§

The production of IgG rheumatoid factors in the inflamed synovium of many patients with rheumatoid arthritis (RA) implies that local sites exist where plasma cell precursors undergo isotype switching and affinity maturation by somatic mutation and selection. Lymphonodular infiltrates of the synovium-containing germinal centers (GCs), are candidates to fulfill such function in the rheumatoid patient. It has been suggested that these GCs are organized around, obviously ectopic, follicular dendritic cells (FDCs). The present study attempts to find out whether these putative FDCs 1) are specific for RA, 2) have the same phenotype and functional capacity as FDCs in lymphoid organs, and 3) may locally differentiate from fibroblast-like synoviocytes (FLS). Synovial biopsies from patients with RA versus non-RA, yet arthritic backgrounds, were compared. Cells with the FDC phenotype were found in both RA and non-RA tissues as well as in single cell suspensions thereof. When FLS were cultured in vitro, part of these cell lines could be induced with IL-1β and TNF-α to express the FDC phenotype, irrespective of their RA or non-RA background. By contrast, the FDC function, i.e., stable binding of GC B cells and switching off the apoptotic machinery in B cells, appeared to be the prerogative of RA-derived FLS only. The present data indicate that FDC function of FLS in RA patients is intrinsic and support the idea that synovial fibroblast-like cells have undergone some differentiation process that is unique for this disease. The Journal of Immunology, 1999, 162: 5949–5956.

Germinal centers (GCs) of lymphoid follicles are the breeding places of memory B lymphocytes (1, 2). One of the hallmarks of GCs is the presence of follicular dendritic cells (FDCs), which are crucial in the selection process of memory B lymphocytes by their ability to switch off the apoptotic machinery in the candidate memory cells (3, 4). Under nonpathologic conditions, the formation of FDCs and GCs is restricted to peripheral lymphoid organs (3, 4). In patients with rheumatoid arthritis (RA), however, lymphonodular infiltrates containing lymphoid follicles with GCs are often seen in the inflamed synovial stroma (5, 6). Several authors have demonstrated the presence of cells expressing markers of FDCs, and some of these have claimed that this feature is specific for RA (6–10). Such ectopic GC formation may create the proper microenvironment in which B cells are maintained and selected that give rise to the local production of IgG rheumatoid factors in many of these patients (11, 12).

The precise origin of FDCs has not been elucidated completely. FDCs show phenotypic overlap with fibroblasts and proliferate poorly (13). Also, ontogenetic studies have indicated that FDCs originate from stromal cells (14). Moreover, in animals that are recovering from immune suppression, it has been shown that the local presence of B cells is a prerequisite for the induction of FDCs from stromal precursors (15). Recent studies with knockout mice support these findings and demonstrate an important role for lymphotoxin-α and lymphotoxin-β from bone marrow-derived cells and the involvement of the TNF-receptor 1 (TNFR1:p55) and the lymphotoxin-βR on stromal cells in the formation of GCs and FDC networks (16–23).

It is tempting to suppose that RA patients have synovial fibroblasts that are more prone to differentiate into FDC-like cells than patients that do not develop RA. Since TNF-α production is a common feature of the synovium in active RA (24–26), and also TNFRI (p55) is highly expressed in synovial tissues of RA patients (27), it is well conceivable that the basic conditions that lead to induction of FDCs in lymphoid organs are fulfilled in the rheumatoid synovium as well.

In this study, we have investigated whether the presence of cells with an FDC phenotype and FDC function (i.e., the capacity to bind GC B cells and to switch off their apoptotic machinery) is a specific feature of the synovium in RA patients or is a common phenomenon that rather reflects the inflammatory situation of the synovium. In addition, we have looked if and under what conditions cultured fibroblast-like synoviocytes (FLS) from RA and non-RA arthritic patients may express the phenotype and function of FDCs. Our data show that RA-derived FLS display intrinsic FDC function, whereas non-RA FLS do not.

Materials and Methods
Arthritis patients and biopsies

Biopsies were taken from the inflamed synovium of the knee joint of patients summarized in Table I using direct vision, low pressure arthroscopy.
A total of 10^5–10^6 synovial cells was obtained from each biopsy. The liberated cells were washed twice, resuspended in IMDM/g (1:5). Cultured FLS were used for experiments between passages 2 and 8. All FLS cell lines expressed the enzyme prolyl 4-hydroxylase (EC 1.14.11.2) and stained positive with the fibroblast-specific mAbs MAS516/1B10 and ASO2.

**Preparation of tissue sections of synovial biopsies**

Freshly obtained biopsies were frozen in liquid nitrogen and cut into 5-μm sections according to routine histopathologic procedures. For pathology, sections were stained with hematoxylin and eosin. Additional sections of the same biopsies were immunophenotyped, as described above.

**Isolation of synovial cells**

Biopsies were incubated twice for 60 min at 37°C under continuous shaking in 5 ml IMDM + 90 μg/ml gentamicin (IMDM/g) containing 800 U/ml collagenase type IV (Worthington, Freehold, NJ). This procedure led to virtually complete digestion of visible tissue fragments. Only blood vessels remained intact. These could readily be recognized and excluded from the final cell population.

**Immunoophtenotyping of synovial biopsies and synovial cells**

Immunophenotyping was done by indirect staining of cryosections, cyto- spin preparations, or chamber slide cultures. Preparations were incubated for 60 min with mAbs (Table II) or isotype-matched control Igs, followed by horseradish peroxidase-conjugated rabbit anti-mouse Ig (Dakopatts, Glostrup, Denmark). Isotype-matched control Igs used were MOPC21 (IgG1), UPC10 (IgG2a), and MOPC141 (IgG2b) (all from Sigma, St. Louis, MO), or normal mouse serum (Dako). No staining was found with any of these control Igs. Peroxidase activity was visualized by incubation with the substrate 3-amino-9-ethyl-carbazole (Sigma) in acetate buffer, pH 4.9, for 60 min at room temperature. Sections and cells were counterstained with hematoxylin.

**Generation of FLS**

Biopsies were cut into small pieces, placed into six-well culture plates (Costar, Cambridge, MA), and cultured in IMDM/g + 10% FCS. After 3 wk, FLS were harvested by incubating with 0.25% trypsin solution (Life Technologies, Paisley, Scotland) for 5–10 min at 37°C and transferred to 75-cm² culture flasks (Costar). Upon confluence, cultures were passaged (1:5). Cultured FLS were used for experiments between passages 2 and 8. All FLS cell lines expressed the enzyme prolyl 4-hydroxylase (EC 1.14.11.2) and stained positive with the fibroblast-specific mAbs MAS516 and ASO2.

**Stimulation of FLS in vitro**

FLS were harvested and cultured for 3 days in 16-well chamber slides (Nunc, Naperville, IL) in the presence or absence of human rTNFα (rhuTNF-α; Chiron, Emeryville, CA) and IL-1β (rhuIL-1β; CLB, Amsterdam, The Netherlands). Cytokines were added at 50 U/ml final concentration. After stimulation, the cells were fixed in acetone and immunophenotyped, as described above.

**Purification of GC B cells**

Tonsillar B lymphocytes were isolated according to the method described by Falkoff et al. (29). Briefly, tonsillar cell suspensions were depleted of T cells by rosetting with 2-aminoethylisothiouroniumbromide (AET; Sigma)-treated SRBC. The rosetted cells were removed by centrifugation on Lymphoprep (1077 mg/ml; Nycomed, Oslo, Norway). The final cell population

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**Table I. Patients included in this study**

<table>
<thead>
<tr>
<th>Diagnosis*</th>
<th>n</th>
<th>Age (years)</th>
<th>Male/Female</th>
<th>Duration of Disease (months)</th>
<th>RF +</th>
<th>Bone Erosions of Hands, Feet, and Knees</th>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>RA</td>
<td>10</td>
<td>22–59</td>
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<td>2–252</td>
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<td>5</td>
</tr>
<tr>
<td>Non-RA group</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>CPPD</td>
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<td>50–77</td>
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<td>3–50</td>
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<td>0</td>
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<tr>
<td>CPPD, OA</td>
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<td>51–65</td>
<td>0/2</td>
<td>8–12</td>
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<td>1/1</td>
<td>20–48</td>
<td>0</td>
<td>1</td>
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<tr>
<td>ReA</td>
<td>2</td>
<td>40–49</td>
<td>1/1</td>
<td>2–8</td>
<td>0</td>
<td>0</td>
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<tr>
<td>AS</td>
<td>3</td>
<td>25–64</td>
<td>2/1</td>
<td>8–348</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Otherc</td>
<td>3</td>
<td>40–49</td>
<td>0/3</td>
<td>2–124</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* RA, rheumatoid arthritis; CPPD, calcium pyrophosphate dihydrate crystal deposition disease; OA, osteoarthritis; Psor.A, psoriatic arthritis; ReA, reactive arthritis; AS, ankylosing spondylitis.

† One of these patients had polymyalgia rheumatica.

‡ One chronic monarthritis; one primary Sjögren’s syndrome; one TRO (transient regional osteoporosis).

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**Table II. Abs used in this study**

<table>
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<tr>
<th>Marker</th>
<th>mAb/Clone</th>
<th>Isotype</th>
<th>Source</th>
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<tr>
<td>CD3</td>
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<td>IgG2a</td>
<td>CLB</td>
</tr>
<tr>
<td>CD14</td>
<td>MO2</td>
<td>IgM</td>
<td>Coulter (Hialeah, FL)</td>
</tr>
<tr>
<td>CD20</td>
<td>NKI-H4</td>
<td>IgG1</td>
<td>CLB</td>
</tr>
<tr>
<td>CD21/CR2</td>
<td>IF8</td>
<td>IgG1</td>
<td>Dako</td>
</tr>
<tr>
<td>CD21L (long variant)</td>
<td>7D6</td>
<td>IgG1</td>
<td>Gift from Dr. Y.-J. Liu (Schering-Plough, Dardilly, France)</td>
</tr>
<tr>
<td>CD29/ VLA-β1</td>
<td>4B4</td>
<td>IgG1</td>
<td>Coulter</td>
</tr>
<tr>
<td>CD40</td>
<td>EA5</td>
<td>IgG1</td>
<td>Gift from Dr. T. LeBien (Minneapolis, MN)</td>
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<tr>
<td>CD49D/ VLA-α4</td>
<td>HP2/1</td>
<td>IgG1</td>
<td>Immunotech</td>
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<td>15.2</td>
<td>IgG1</td>
<td>CLB</td>
</tr>
<tr>
<td>CD55/ DAF</td>
<td>BRIC110</td>
<td>IgG1</td>
<td>CLB</td>
</tr>
<tr>
<td>CD106/ VCAM-1</td>
<td>4B9</td>
<td>IgG1</td>
<td>Gift from Dr. J. Harlan (Seattle, WA)</td>
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<tr>
<td>HLA-DR</td>
<td>L243</td>
<td>IgG2a</td>
<td>Becton Dickinson (San Jose, CA)</td>
</tr>
<tr>
<td>DRC-1</td>
<td>R4/23</td>
<td>IgM</td>
<td>Dako</td>
</tr>
<tr>
<td>Human fibroblast</td>
<td>MAS516/1B10</td>
<td>IgM</td>
<td>Seralab (Crawley Down, Sussex, U.K.)</td>
</tr>
<tr>
<td>Human fibroblast</td>
<td>ASO2</td>
<td>IgG1</td>
<td>Dianova (Hamburg, Germany)</td>
</tr>
<tr>
<td>Prolyl 4-hydroxylase (EC 1.14.11.2)</td>
<td>SB5</td>
<td>IgG1</td>
<td>Dako</td>
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<tr>
<td>CD31</td>
<td>EN-4</td>
<td>IgG1</td>
<td>Seralab</td>
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</tbody>
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contained >98% CD20-positive cells (B cells) and <1% CD3-positive cells (T cells), as analyzed by FACScan.

Low density B cell fractions were obtained according to the method described by Koopman et al. (30). Briefly, B cells were centrifugated (15 min, 1200 × g, 4°C) on a Percoll gradient, consisting of four layers (1077, 1067, 1056, and 1043 mg/ml). Cells at the 1043/1056 interface (low density B cells) were harvested. GC B cells were further purified by incubation of the LD B cell fraction with Abs against slgD (JA11; Oxoid, London, U.K.) and anti-CD39 (AC2; Immunotech, Luminy, France), followed by depletion of the labeled cells using sheep anti-mouse Ig-coated Dynabeads (Dynal, Oslo, Norway). Purified GC B cell fractions consisted of >98% CD38⁺ cells and <2% CD39⁺ and slgD⁺ cells.

**Cocultures of FLS with GC B cells**

Cultured FLS were seeded into six-well culture plates (Costar) and activated for 3 days with rhuTNF-α/rhuIL-1β, as described above. Next, the cultures were washed carefully and 2–4 × 10⁶ freshly isolated GC B lymphocytes were added. To study GC B cell apoptosis in FLS-B cell cocultures, the cultures were stained supravitally with Hoechst 33342 (Sigma) for 15 min and examined directly in their culture wells by fluorescence microscopy, as previously described (31), using a Leitz Orthoplan fluorescence microscope (Leitz, Wetzlar, Germany) with Ploem-Opak illumination. An NPL 50×/1.00 oil immersion objective was routinely used. In each individual coculture, 400–500 B lymphocytes were examined, and classified as single cells or cells binding to FLS as well as being apoptotic or not.

**DNA fragmentation assay of isolated nuclei**

To investigate the effect of FLS coculture on endonuclease activity in GC B cells, FLS-B cell clusters and single GC B cells were separated after 16 h of coculture. B cell nuclei were isolated according to Nieto et al. (32). Endonuclease activity in the nuclei of single and clustered B cells was assayed, as described previously (33).

**Results**

**Synovial cells with the phenotype of FDCs are not specific for RA**

Synovial biopsies taken from the inflamed knees of 10 RA patients were compared with biopsies taken from 16 non-RA arthritic patients (Table I). Cryosections revealed lymphonodular infiltrates in the deeper synovium containing B cells (CD20⁺, Fig. 1a), sometimes with clear GCs (CD21⁺, Fig. 1b) in part of the RA patients, but not in the non-RA group. FDC-specific staining (DRC-1⁺) was difficult to detect with indirect labeling, but enhancement with alkaline-phosphatase antialkaline-phosphatase (APAAP) revealed clear FDC networks inside some of these GCs (Fig. 1c). However, due to the limited size of the biopsies and the small size of these GCs, the incidence of such FDC networks was difficult to quantify. Our present data suggest that FDC networks are restricted to the RA patients, but it cannot be ruled out that due to their small size they have been missed in the non-RA group. Additional experiments, including representative numbers of synovial biopsies from every patient, will be more conclusive. In synovial tissues obtained from joint replacement surgery, we have found DRC-1⁺ FDC networks in occasional patients with osteoarthritis as well (not shown), indicating that the expression of DRC-1 is not RA specific.

As the sampling problems described above were recognized in an early stage of this study, additional biopsies were taken at further arthroscopies. These biopsies were digested with collagenase to produce single cell suspensions representative of a larger sample of the tissue.

As shown in Table III, the majority of the liberated cells have a fibroblast phenotype (MAMS16⁺), but also considerable amounts of macrophages (CD14⁺) were seen in all patients tested. T lymphocytes (CD3⁺) and B cells (CD20⁺) were seen in synovial cell suspensions of part of the patients. No obvious differences were seen between RA and non-RA patients. Cells expressing markers associated with activation/inflammation (HLA-DR, CD29, CD40, CD54, CD55, CD106) were seen in high amounts in almost every patient (Table III). In general, no differences were seen between both patient groups.

Some markers for FDCs (7D6, CD21) were found more often expressed in the RA group, but DRC-1 staining did not reveal differences between patient groups. In addition, in those patients that expressed FDC-specific markers (DRC-1, 7D6), the amounts of cells expressing each marker were comparable.

The present data indicate that cells with an FDC phenotype are seen more often in single cell suspensions prepared from synovial biopsies from RA patients than from non-RA patients. However, in a number of non-RA patients, these markers were found as well, suggesting that the possibility to express the FDC phenotype is a rather common feature of synoviocytes that may correlate with
local inflammatory conditions instead of being a specific hallmark of RA.

**FLS may express FDC-specific markers in vitro, irrespective of their diagnostic background**

In an attempt to identify putative precursors for FDCs, FLS were cultured and their phenotypes were studied directly after culture or after incubation with the proinflammatory cytokines IL-1β and TNF-α.

FLS cell lines were established from most patients. In our hands, these cell lines grew slowly in comparison with, for example, primary skin fibroblasts or tonsil fibroblasts. Their average doubling time was in the order of 1 wk.

All FLS cell lines were positive for the fibroblast markers MASS16, ASO-2 (34), and prolly4-hydroxylase, a key enzyme of collagen synthesis (not shown). Both in RA-FLS and in non-RA FLS, the basal expression of the tested markers was rather low (Table IV), except of ICAM-1 and VCAM-1, which were definitely expressed on the majority of the cell lines (Fig. 2, a and b). The same was seen in the non-RA FLS. CD55 expression was seen more often in unstimulated non-RA FLS than in RA-FLS (Fig. 2h). The expression of FDC-specific markers (DRC-1, 7D6) was virtually absent (Fig. 2g). A minority of the FLS cell lines showed faint DRC-1 staining in part of the cells.

After incubation with rhuIL-1β and rhuTNF-α, increased expression of ICAM-1, VCAM-1, and CD55 was seen in the vast majority of the FLS cell lines (Fig. 2e–g). No differences were mentioned when RA and non-RA FLS were compared (Table IV).

With the FDC-specific markers, it was seen that especially DRC-1 was up-regulated after incubation with rhuIL-1β and rhuTNF-α in a fraction of the FLS cell lines, irrespective of their diagnostic background. Although the expression of DRC-1 in RA-FLS (Fig. 2h) seems higher than in non-RA FLS, it remains unclear whether this difference must be considered as significant. The same holds true for the occasional expressions of the other FDC-specific marker, the 7D6 Ag (a long form of CD21 (35)).

To date, our data show that the FDC phenotype can be induced or up-regulated in a fraction of the synovial fibroblast-like cell lines from both RA and non-RA patients under conditions that mimic an inflammatory situation in vitro.

**FLS from RA patients can block apoptosis in GC B lymphocytes; non-RA FLS cannot**

To assess the capacity of FLS to display FDC function, we have tested the binding and survival of purified tonsillar GC B lymphocytes to FLS cell lines from RA and non-RA patients. All experiments were conducted with FLS cell lines precultured with or without rhuIL-1β and rhuTNF-α for 3 days. GC B cells readily bind to all FLS tested, leading to roughly 50% binding within 4 h (Fig. 3). After that moment, a difference was seen between RA and non-RA FLS. RA-FLS were capable of maintaining GC B cells on their surface for at least 48 h (Fig. 4a), whereas non-RA FLS could not (Fig. 4b). In addition to this, apoptosis was very low in GC B cells bound to RA FLS (Figs. 3c and 4a). With non-RA FLS, the percentage of apoptosis in the bound B cells was significantly

---

**Table III. Phenotype of freshly isolated synoviocytes**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Patients tested*</th>
<th>Patients positive</th>
<th>% positive cells (mean ± SD or range)</th>
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<tbody>
<tr>
<td>CD3</td>
<td>5</td>
<td>4</td>
<td>7 ± 2</td>
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<tr>
<td>CD20</td>
<td>5</td>
<td>2</td>
<td>2–10</td>
</tr>
<tr>
<td>CD14</td>
<td>2</td>
<td>2</td>
<td>20–26</td>
</tr>
<tr>
<td>MASS16</td>
<td>5</td>
<td>5</td>
<td>77 ± 17</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>5</td>
<td>5</td>
<td>54 ± 21</td>
</tr>
<tr>
<td>CD29</td>
<td>8</td>
<td>8</td>
<td>79 ± 21</td>
</tr>
<tr>
<td>CD40</td>
<td>3</td>
<td>3</td>
<td>48 ± 6</td>
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<tr>
<td>CD54/ICAM-1</td>
<td>5</td>
<td>5</td>
<td>28 ± 18</td>
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<td>CD106/VCAM-1</td>
<td>6</td>
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<td>CD55/DAF</td>
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<td>8</td>
<td>6</td>
<td>9 ± 7</td>
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<tr>
<td>DRC-1</td>
<td>8</td>
<td>3</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>7D6</td>
<td>8</td>
<td>3</td>
<td>6 ± 3</td>
</tr>
</tbody>
</table>

* Single cell suspensions were prepared by collagenase digestion of synovial biopsies. A total of 10^4 to 2 × 10^7 cells were used for each cytospin preparation.

**Table IV. Phenotype of FLS cell lines upon stimulation with IL-1β and TNF-α**

<table>
<thead>
<tr>
<th>Marker</th>
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<th>Stimulated*</th>
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<tbody>
<tr>
<td></td>
<td>Pos./tested</td>
<td>Expression</td>
</tr>
<tr>
<td>CD54/ICAM-1</td>
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</tr>
<tr>
<td>CD106/VCAM-1</td>
<td>4/6</td>
<td>+</td>
</tr>
<tr>
<td>CD55/DAF</td>
<td>2/6</td>
<td>±</td>
</tr>
<tr>
<td>DRC-1</td>
<td>1/6</td>
<td>±</td>
</tr>
<tr>
<td>7D6</td>
<td>0/6</td>
<td>–</td>
</tr>
</tbody>
</table>

* FLS were precultured on chamber slides with 50 U/ml IL-1β and 50 U/ml TNF-α for 72 h.

* Number of FLS cell lines found positive (Pos.) tested.

* Arbitrary units: expression ±, faintly positive in fraction of the cells; +, positive in all cells; +++++, strongly positive.
higher when compared with RA-FLS (Figs. 3d and 4b; p = 0.005 for unstimulated FL; p = 0.01 for FL stimulated with rhuIL-1β and rhuTNF-α). These data indicate that non-RA FLs do not rescue GC B cells from apoptotic cell death. By contrast, RA FLs have clear antiapoptotic function, and functionally behave as FDCs.

**FIGURE 2.** Induction of the FDC phenotype in RA FLS after 3 days of culture without (a–d) and with 50 U/ml rhuIL-1β and rhuTNF-α (e–f) on chamber slides. Stained for expression of CD54/ICAM-1 (a and e), CD106/VCAM-1 (b and f), CD55/decay-accelerating factor (DAF) (c and g), and DRC-1 (d and h). Bar represents 100 μm.

**FIGURE 3.** RA FLS can bind and rescue GC B cells from apoptosis; non-RA FLs cannot. Phase-contrast (a and c) and fluorescence microscopy (b and d) of purified tonsillar GC B lymphocytes adhering to RA-derived (a and b) and non-RA-derived FLs. GC B cells were incubated with FLs for 24 h. Hoechst 33342 staining. Note the condensed chromatin staining and often fragmented nuclei in apoptotic B cells. In d, the dimly stained nucleus of the FLs can be identified easily. Bar represents 100 μm.
Preincubation of the FLS with rhuIL-1β and rhuTNF-α did not significantly influence this cellular behavior, suggesting that the antiapoptotic action of RA FLS is an intrinsic property of these cells, independent of the presence of the cytokines used.

To investigate whether RA FLS, like FDCs, were able to switch off the apoptotic machinery in the nuclei of the bound GC B cells, an endonuclease activity assay (ENAA) was performed on B cell nuclei isolated from single B cells and from B cells harvested from the fraction bound to the FLS. As shown in Fig. 5, the ladder pattern typical of apoptotic DNA fragmentation was seen in nuclei of the cells that were not attached to FLS (single cells). In nuclei of B cells that had been bound to RA-FLS for 24 h, no sign of DNA-fragmentation activity could be detected, implying that their endonuclease activity has been switched off during contact with the FLS. Additional gel electrophoresis of high m.w. DNA confirmed the isolation of unfragmented DNA from these nuclei (not shown). By contrast, ENAA of nuclei from GC B cells bound for 24 h to non-RA FLS showed uninhibited DNA fragmentation in these nuclei.

Discussion

The presence of FDC in the inflamed synovium of RA patients has been studied by several different groups (5–10), and some of these have claimed that it would be a specific feature of the disease. This is an attractive idea, because it may explain why many of these patients have so many plasma cells in their synovium that produce IgG rheumatoid factors (11, 12). This idea was supported by Dechanet et al. (36), who showed that synoviocytes are able to support IgG production by B cells stimulated by Staphylococcus aureus Cowan I in vitro. Very recently, this was extended by the demonstration that nurse-like cells can be cultured from synovial tissues and bone marrow of patients with RA. These cells can bind peripheral B and T cells and support their survival (37).

However, the studies published to date do not point out whether these phenomena are specific features of RA only, or that certain functions are common to synovial cells in general. Also, it remains unclear what cell type may provide the precursors of putative FDCs in the RA synovium and under what conditions the phenotypic and/or functional differentiation has occurred. Elucidation of these issues may yield important new insights in the pathogenesis of RA. We have addressed these issues by studying arthroscopic synovial biopsies (and isolated cells thereof) from RA patients and comparing these with a variety of arthritic patients with a non-RA diagnosis.

Since FDCs have phenotypic overlap with a number of other cell types (reviewed in Ref. 13), we have used mAbs recognizing a long splice variant of CD21 (mAb DRC-1 and 7D6). These Abs are considered highly specific for FDCs (35). Cryosections of synovial biopsies indicated that GCs containing DRC-1+ networks of FDCs are present in a number of patients, especially in the RA group. These cryosections did not display DRC-1+ FDCs in every patient, even within the RA group. One explanation for this is that...
synovial GCs are rather small and, consequently, may be easily missed. Even if a GC was found in one section, the DRC-1 network appeared in only few subsequent sections (not shown). Also, it should be mentioned that DRC-1 expression is generally much lower in synovial tissues than in lymphoid organs. Therefore, although our cryosection data suggest that FDCs are more often found in the RA group, they are far from conclusive.

To obtain more information about the incidence of the FDC phenotype in synovial biopsies, we have made single cell suspensions by collagenase digestion of these biopsies and prepared cytospin preparations of the isolated cells. The incidence of cells with an FDC phenotype (DRC-1$^+$, 7D6$^+$) was higher in RA patients when compared with the non-RA group (Table III), but cells with the FDC phenotype were seen in some of the non-RA patients as well. This was not associated with any particular diagnosis (1 AS, 1 OA, and 1 ReA). In these patients, the percentages of FDC-like cells were very similar with those found in the RA group. The present data indicate that cells with the phenotype of FDCs are more often found in the RA group, but this may quite well reflect the higher inflammatory status in many of these patients rather than being an intrinsic property of this particular disease.

Recently, the group of Gay et al. has demonstrated that RA synovial fibroblasts after several passages of in vitro culture are autonomous and aggressive, i.e., tissue destructive toward human cartilage implanted in SCID mice (38). That study highlights the possibility that these cells may have undergone some RA-specific differentiation. Since it has been suggested that fibroblasts may serve as local precursors for FDCs, we have cultured FLS from each patient and searched for a putative aberrant propensity of RA-derived FLS to display the FDC-like phenotype (DRC-1$^+$ and 7D6$^+$) and function (the ability to block apoptosis in adherent GC B cells).

On cultured FLS, basal expression of FDC-specific markers was absent or marginal, both in the RA and in the non-RA group (Table IV). Incubation with the proinflammatory cytokines IL-1β and TNF-α clearly enhanced the expression of ICAM-1, VCAM-1, and CD55. In part of the FLS cell lines, DRC-1 was induced or up-regulated. Highest DRC-1 expressions were seen in RA-derived FLS to display the FDC-like phenotype (DRC-1$^+$ and 7D6$^+$) and function (the ability to block apoptosis in adherent GC B cells).

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The molecular mechanisms by which RA FLS bind GC B cells may differ from those by which, for example, tonsillar FDCs bind BC B cells. Previously, we have shown that tonsillar FDCs and GC B cells interact in vitro mainly by LFA-1/ICAM-1 and VLA-4/VCAM-1 interactions (30, 31, 40). To date, preliminary studies with blocking Abs have not revealed significant prevention of B cell binding to FLS, suggesting a different, yet unknown, cellular interaction (data not shown).

Additional experiments are now in progress to estimate the role of other cytokines in the induction of the FDC phenotype in FLS, to explain the discrepancies that are found between the phenotypic and the functional data, and to reveal the precise molecular mechanisms that enable RA FLS to behave as FDCs.

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