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DEVELOPMENT OF IN VITRO MODEL SYSTEMS FOR DESTRUCTIVE JOINT DISEASES

Novel Strategies for Establishing Inflammatory Pannus

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Objective. **To establish a novel 3-dimensional (3-D) in vitro model for the investigation of destructive processes in rheumatoid arthritis (RA).**

Methods. **Two distinct culture systems were developed, consisting of RA synovial membrane and articular cartilage explants or interactive RA synovial cell/chondrocyte cultures embedded in 3-D fibrin matrices. The expression of proteolytic enzymes, chondrocyte matrix architecture, and matrix degradation parameters was analyzed by immunohistochemistry.**

Results. **Of 28 RA explant cultures, 16 displayed an invasion of synovial tissue into the cartilage explants, compared with 1 of 8 osteoarthritis explants. The expression of collagenase and vascular cell adhesion molecule 1 could be demonstrated at the cartilage–pannus junction. Of 20 interactive cell cultures, 18 revealed invasive behavior and remained vital for extended periods of time.**

Conclusion. **The models presented allow us to study distinct aspects of destructive joint diseases under in vitro conditions that resemble human pathology. Moreover, our model is able to supplement animal experiments in basic research and drug testing.**

In rheumatoid arthritis (RA), the physiologic balance between matrix deposition and degradation is disturbed by the chronic inflammation of the synovial joint, accompanied by infiltration of activated mononuclear cells (MNC) (1,2). Permanently activated synovial and recruited blood MNC form the hyperplastic synovial tissue (pannus), which invades cartilage and bone (3–7). The shift toward destruction of cartilage and bone is driven by a proinflammatory cascade of cytokines (8–10). Macrophage- and fibroblast-like cells of the synovial lining are the major source of these cytokines, such as tumor necrosis factor α (TNFα) and interleukin-1 (IL-1), and of various matrix-degrading enzymes, such as collagenase, stromelysin, and cathepsins (11,12).

The establishment of an experimental model system reflecting this complex pathogenetic interplay is difficult to achieve. The investigation of inflammatory joint diseases in animal models employs a variety of experimental strategies (13). Injection of arthritogenic antigens (e.g., type II collagen in Freund's complete adjuvant) into susceptible strains of mice induces synovitis and joint erosion (14,15). MRL-*lpr/lpr* inbred mice show a diminished expression of the apoptosis-triggering Fas protein that leads to the development of a severe autoimmune disease phenotype with symptoms resembling systemic lupus erythematosus and RA (16). Transgenic animals that constitutively express major pathogenetic factors such as TNFα (17) or the proto-oncogene c-*fos* (18) develop a destructive form of arthritis.

Another approach is to carry out in vivo studies by transplanting human synovial tissue into immunodeficient mice (19,20). Thus, inflammatory synovial membrane has been transplanted either directly into knee joints (21), subcutaneously together with human cartilage, or under the renal capsule (22). Although this approach results in cartilage invasion that resembles human arthritis, the limited comparability with the

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human in vivo situation remains a problem because of the lack of important components of the human environment. The divergence of methodologic approaches reflects the absence of a representative model of RA.

There are several groups currently working on the development of model systems which mimic the human joint microenvironment under in vitro conditions (23–26). Based on our experience with the development of 3-dimensional (3-D) chondrocyte cultures (27) in a perfusion system, we extended our efforts to study the process of joint erosion in vitro.

The major shortcoming of conventional culture systems is the lack of the complex network of cell–cell and cell–matrix interactions (28–30). In the usual monolayer cultures, cells undergo structural changes, nearly always accompanied by the loss of tissue-specific gene expression and by dedifferentiation (31). Cartilage cells, for instance, begin to transform into fibroblast-like cells after a few days on a plastic dish (32). Biochemical analysis reveals a switch of the collagen synthesis from cartilage-specific type II collagen to type I collagen, which is normally absent from cartilage (33).

Addressing these limitations, we focused on the development of 3-D culture models that would allow us to study the inflammatory joint process in the context of the extracellular matrix and to investigate the contribution of distinct cell populations such as chondrocytes (34,35). Having established a novel perfusion culture system (Figure 1) (36,37), we introduced 2 different technical approaches to cocultivating synovial cells and chondrocytes: we compared various forms of explant cultures with 3-D interactive gel cultures and show the specific advantages of each technique (Figure 2).

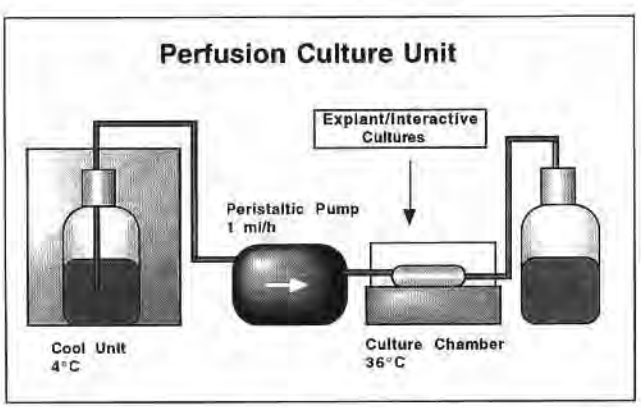

Figure 1. Continuous perfusion system used for long-term cultures. The tissue cultures in a special chamber were perfused by a continuous flow of medium at a rate of 1 ml/hour.

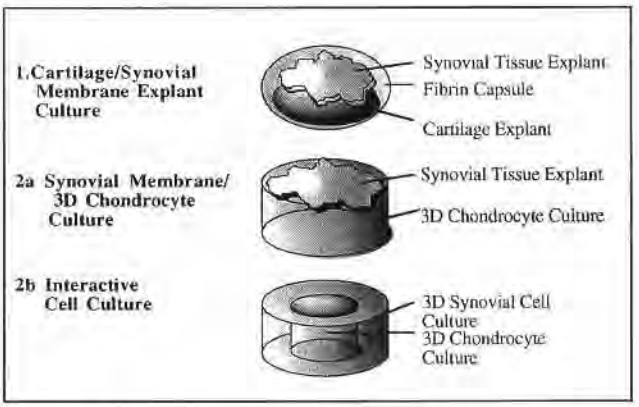

Figure 2. Different technical approaches used in the in vitro model systems: explant cultures with synovial tissue/cartilage (**1**) and 3-dimensional (3D) chondrocyte cultures with synovial membrane explants (**2a**) or interactive 3D synoviocyte cultures (**2b**).

PATIENTS AND METHODS

Synovial tissue. RA synovial membrane was obtained during orthopedic surgery from patients with definite RA (n = 15) according to the American College of Rheumatology (formerly, the American Rheumatism Association) criteria (38). In 4 cases, autologous cartilage could be obtained from the same joint and used for autologous explants and interactive cultures. For control samples, noninflammatory synovial tissue from patients undergoing reconstructive joint surgery (n = 4) was used. Active inflammation was confirmed by conventional staining with hematoxylin and eosin (H & E). One part of the synovial tissue was used for explant culture, with cartilage or with 3-D chondrocyte culture. We received sufficient material from 8 patients to establish both the explant culture and the interactive cell culture from the same donor. Depending on the amount of the tissue, 1–3 cultures were prepared from each tissue sample.

For cell isolation, synovial tissue was enzymatically dissociated with collagenase P (Boehringer, Mannheim, Germany) in supplemented RPMI medium (10% fetal calf serum [FCS], 25 m*M* HEPES, 100 units/ml of penicillin, 100 μg/ml of streptomycin; Seromed, Berlin, Germany) for 3–6 hours, depending on the amount of tissue. Isolated cells were plated into culture chambers and cultured (a) as primary cultures for 48 hours and (b) over 4 passages to obtain fibroblasts. Adherent cells were harvested by trypsin–EDTA digestion and used for interactive 3-D cultures.

Preparation of explant cultures. Human articular cartilage obtained at joint surgery (n = 6), nasal septum cartilage (n = 5), and bovine articular cartilage (n = 5) samples were cut into pieces with a diameter of 2–4 mm. Synovial membrane tissue was dissected into pieces of similar size. After histologic analysis, selected synovial tissue and cartilage explants were tightly fixed together and encapsulated in a fibrin gel matrix. Explants were cultured on 0.4-μm pore membrane inserts (Costar, Cambridge, MA) in 24-well tissue culture plates or in special culture chambers (Minucell and Minutissue, Bad

Table 1. Primary antibodies used in this study

Antibody	Origin*	Supplier†	Description
Type II collagen	R	Chemicon	Cartilage-specific matrix protein
Chondroitin sulfate	R	Chemicon	Cartilage-specific matrix protein
Cartilage proteoglycan	R	Chemicon	Cartilage-specific matrix protein
CD3	M	Dako	T cell antigen
CD22	M	Dako	B cell antigen
CD45	M	Dako	Leukocyte common antigen
CD44	M	Dako	Leukocyte common antigen, matrix-binding protein, and adhesion factor
CD68	M	Dako	Cytoplasmic macrophage marker
CD14	M	Dako	Monocyte/macrophage antigen
Fibroblast 5B5	M	Dako	Fibroblast enzyme for collagen synthesis
CD31	M	Dako	Platelet endothelial cell adhesion molecule 1
HLA–DR	M	Dako	HLA–DR complex on various cell types
CD25	M	Dako	Interleukin-2 receptor subunit
MMP-1	M	Dianova	Interstitial collagenase (matrix metalloproteinase 1)
PCNA	M	Dako	Proliferating cell nuclear antigen
VCAM	M	Dianova	Vascular cell adhesion molecule 1
COL2-3/4	M	A. Robin Poole, PhD	Type II collagen denaturation product

* R = rabbit (polyclonal); M = mouse (monoclonal).
† Chemicon, Temecula, CA; Dako, Hamburg, Germany; Dianova, Hamburg, Germany; Dr. Poole, Montreal, Quebec, Canada.

Abbach, Germany) in a continuous perfusion system for up to 10 weeks.

Preparation of 3-D chondrocyte cultures. To obtain native chondrocytes, we incubated slices of cartilage with 2 mg/ml of collagenase P (Seromed), 0.1 mg/ml of hyaluronidase (Serva, Frankfurt, Germany), and 1.5 mg/ml of type II DNase (Paesel, Frankfurt, Germany) in supplemented RPMI medium for 16 hours in a spinner flask. Cells were passed through a nylon filter (Reichelt Chemie, Heidelberg, Germany) and washed 3 times in Hank's solution (Seromed). The number of viable cells was determined by hemocytometer counting, using trypan blue exclusion staining.

Tissucol Duo S 1 Fibrin Glue System (Immuno, Heidelberg, Germany), consisting of a fibrinogen glue component (human plasma protein 80–120 mg/ml, fibrinogen 70–110 mg/ml, factor XIII 10–50 units/ml, fibronectin 2–9 mg/ml, plasminogen 0.02–0.08 mg/ml, and aprotinin 3,000 IU/ml) and a thrombin component (human thrombin 500 IU/ml and $CaCl_2$–$2H_2O$ 5.88 mg/ml) were mixed to obtain a gel structure. Chondrocyte-fibrin gels were prepared in separate wells of a 96-well tissue culture plate (Costar). Cells were suspended in Ham's F-12 medium (Seromed) and mixed with the fibrinogen component at a ratio of 3:1. A thrombin solution in a dilution of 1:10 with phosphate buffered saline (PBS) was added to the cell–fibrinogen suspension at a ratio of 1:20 to achieve gel polymerization. Cell density was $10 \times 10^6/cm^3$. Chondrocyte–gel cultures were subsequently cultured for 3 weeks to allow specific 3-D matrix formation and then transferred into 48-well tissue culture plates (Costar).

Preparation of interactive 3-D cultures. For 3-D synovial cell cultures, we used 2 defined synovial cell populations: 1) adherent synovial cells harvested on day 3 of monolayer culture, consisting of a mixed cell population of macrophage/monocytes, fibroblasts, and a small proportion of lymphocytes and endothelial cells; and 2) synovial fibroblasts obtained after 4 passages of monolayer culture. Isolated synovial cells were suspended in the fibrinogen solution in a ratio of 3:1 and transferred into separate wells of a 48-well tissue culture plate containing the prepared cylindrically shaped chondrocyte–gel culture (5 mm diameter; 5 mm height). Gel formation was achieved by the addition of 50 μl of thrombin/PBS solution into the synovial cell–fibrinogen suspension.

Cell culture conditions. Monolayer cultures were kept under standard conditions with supplemented RPMI medium (10% fetal calf serum [FCS], 100 units/ml of penicillin, 100 μg/ml of streptomycin; Seromed). Three-dimensional cell–gels and tissue explants were cultured in 96/24-well tissue culture plates with H12 medium, supplemented with 5% FCS and ascorbic acid (50 μg/ml) (Sigma, Deisenhofen, Germany), which was exchanged daily. For long-term cultures (>4 weeks), we used a continuous perfusion system with Minucell culture chambers (Minucell and Minutissue). Cultures (except long-term cultures) were monitored daily using a cell culture microscope.

Light microscopy. The invasive behavior of synovial tissue was determined by light microscopy. Cultures containing whole synovial tissue were evaluated by conventional H & E staining, whereas interactive cell cultures were examined by phase-contrast microscopy. Marked invasion was defined as synovial protrusions of >5 cell layers, whereas moderate invasion was defined as protrusions of 2–5 cell layers.

Immunohistochemistry. Cryosections (7 μm) were mounted on aminoalkylsilane-coated slides (Sigma), fixed for 5 minutes in acetone, and stored at −20°C. Synthesis of extracellular matrix compounds was analyzed by Safranin O and Azan staining (39). For immunohistochemistry, sections were incubated overnight at 4°C in a humid chamber with primary antibodies, diluted in 50 mmoles of Tris buffer solution (TBS), supplemented with 1% rabbit serum in a concentration recommended by the supplier. In all cases, control stainings were performed with species- and isotype-matched antibodies. Endogenous peroxidase was quenched by a peroxidase-blocking

reagent, containing 0.03% H_2O_2 and NaN_3 (Dako, Carpinteria, CA). Slides were incubated with a monoclonal biotin-labeled rabbit anti-mouse or anti-rabbit antibody, respectively, diluted 1:500 in 1% rabbit serum in TBS for 30 minutes. After subsequent washes, the sections were incubated either with avidin–biotin–horseradish peroxidase or with avidin–biotin–alkaline phosphatase–labeled antibodies. Color reaction was developed by diaminobenzidine or new fuchsin substrate kit, according to the instructions of the supplier (all immunohistochemical reagents from Dako). Counterstaining was performed with Mayer's hematoxylin (Sigma).

For the primary antibodies used, see Table 1.

RESULTS

Synovial membrane/cartilage explant cultures. A total of 28 cultures were established. Explant cultures with RA synovial tissue kept under conventional culture conditions (n = 15) remained viable for an average period of 4 weeks (minimum 15 days; maximum 50 days), and cultures in the continuous perfusion system (n = 13) for 10 weeks (minimum 24 days; maximum 85 days). Histologic analysis revealed the attachment and beginning invasion of the synovial membrane into the cartilage matrix after 4 weeks of culture (Figure 3A). Overall, in 16 of 28 RA cultures, a variable degree of cellular infiltration of the cartilage surface was observed. A pronounced invasion was detected in all of the autologous cultures. There was no significant difference in the degree of invasion between allogeneic and bovine articular cartilage and nasal septum cartilage. Control samples with noninflammatory synovial tissue and human articular cartilage (n = 8) revealed no significant invasion.

Immunohistochemistry showed the presence of fibroblasts (F5B+) and macrophages (CD68+, Mac3+, CD14+) in the hyperplastic lining layer and the sublining area, endothelial cells (CD31+), and scattered T cells (CD3+/CD4+/CD45RO+) organized in small aggregates (Figure 3B), whereas few of these cells revealed interleukin-2 receptor expression. These follicle-like structures were less prominent after 2 weeks in culture, compared with freshly prepared synovial membrane. Only scattered B lymphocytes (CD22+) were detected after 7 days in culture.

Approximately 70% of the synovial cells expressed the leukocyte common antigen (CD45+/CD44+), and between 40% and 60% of the cells showed HLA–DR expression. Matrix metalloproteinase 1 (MMP-1) was detected in the synovial lining and in several subintimal cells (Figure 3C). The marker for type II collagen degradation, COL2/3-4 (40), stained positive in a diffuse pattern within the cartilage explant (Figure 3D), whereas cartilage explants cultured with noninflammatory synovial tissue or without synovial tissue exhibited only weak or no staining (not shown).

Three-dimensional interactive cell culture. *Characterization of chondrocyte cultures.* Native chondrocytes were distributed homogenously within the fibrin gel and exhibited the round, chondrocyte-specific phenotype throughout the entire culture period. Bovine nasal septum cartilage chondrocytes were of more heterogenous shape and size, with ~10–20% hypertrophic cells. Safranin O and Azan staining of cryosections resulted in an intense staining of the newly synthesized glycosaminoglycans and proteoglycans after 3 weeks of culture.

Immunostaining with monoclonal antibodies to specific chondrocyte matrix components demonstrated the presence of type II collagen (Figure 4A), aggrecan, and chondroitin sulfate, whereas staining for type I collagen was negative (not shown). By directly comparing the staining intensity of 3-D cultures with freshly cut cartilage, we found a weaker staining for the respective matrix components, although the cultures stained markedly and homogeneously (data not shown).

Synovial membrane/3-D chondrocyte cultures. RA synovial tissue explants cocultured with the 3-D chondrocyte matrix (n = 17) remained viable for 4–6 weeks. After 2 weeks, local invasion into the chondrocyte matrix was observed. Compared with the synovial membrane/cartilage explants, these infiltrations were more pronounced and were detected more frequently (14 of 17) (Figure 4B). Only 3 of these 17 cultures showed no cellular infiltration, whereas in only 1 of 8 noninflammatory synovial tissue explants, a slight invasion was observed.

Invading cells were identified by immunohistochemistry as macrophage/monocytes (CD68+/CD14+) and fibroblasts (F5B+). Of the synoviocytes at the border zone, 60–80% stained positive for MMP-1 and HLA–DR. Scattered cells showed positive staining for the adhesion molecule vascular cell adhesion molecule 1 (VCAM-1) and proliferating cell nuclear antigen (PCNA) proliferation marker. The marker for type II collagen degradation, COL2/3-4, was found in a diffuse pericellular pattern within the cartilaginous matrix (not shown).

Interactive 3-D synoviocyte/chondrocyte cultures. In both cultures, a homogenous cell distribution was found. The chondrocyte–gel matrix, consisting of human articular chondrocytes, was clearly distinguishable from 3-D synovial cell culture by a sharp marginal zone

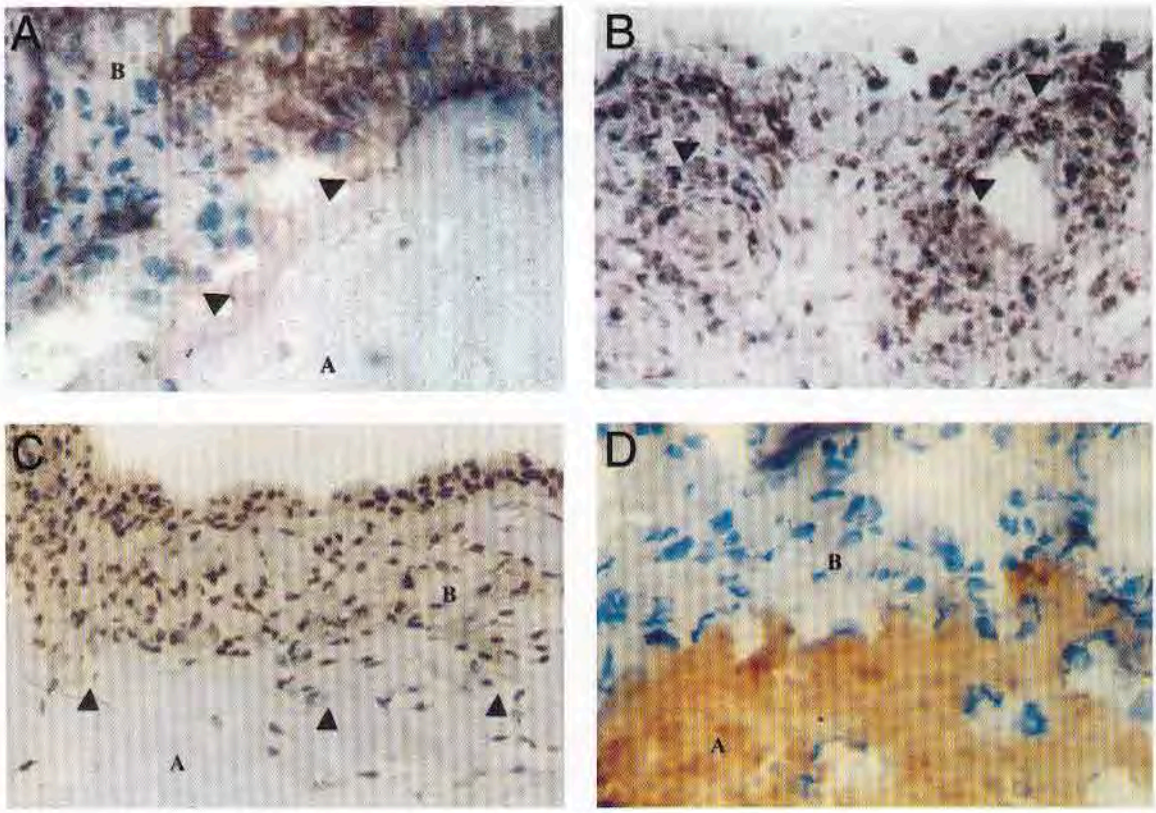

Figure 3. A–D, Explants of human articular cartilage **(A)** with autologous (panels **A–C**) or heterologous (panel **D**) rheumatoid arthritis (RA) synovial tissue **(B). A,** After 4 weeks, synovial cells attached to and began to invade the cartilage surface. Within the junction area, mainly synovial fibroblasts and CD14+/CD68+ macrophages (immunostained with horseradish peroxidase [HRP]) were seen. **Arrowheads** show the contact zone between explants. **B,** After 29 days, small aggregates of T cells within the RA synovial tissue explant **(arrowheads)** resembling the mononuclear perivascular and follicle-like infiltrates of the native RA synovial membrane were seen. (HRP immunostained for CD3.) These T cells were predominantly of the CD3+/CD4+/CD45RO+ subset (not shown). **C,** After 50 days, synovial cells at the contact zone **(arrowheads)** had invaded the cartilage surface. (HRP immunostained for interstitial collagenase matrix metalloproteinase 1, found predominantly in the synovial lining and several subintimal cells.) **D,** After 52 days, diffuse HRP immunostaining for type II collagen denaturation marker COL2/3-4 was seen within the articular cartilage adjacent to the contact zone of the RA synovial tissue. Cartilage explants with noninflammatory synovial tissue and without synovial tissue exhibited only weak or no staining (not shown).

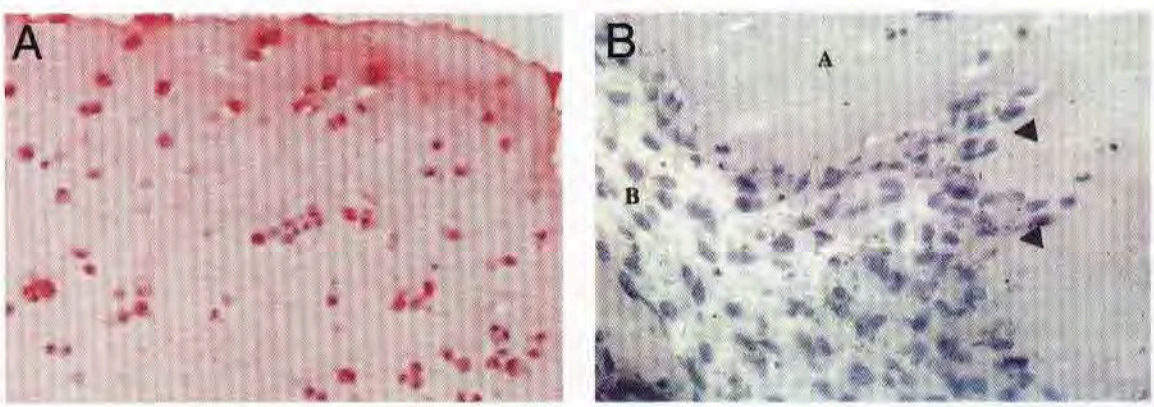

Figure 4. A, Three-dimensional (3-D) chondrocyte culture after 3 weeks, showing pronounced pericellular and weaker intercellular matrix staining for type II collagen (alkaline phosphatase–anti–alkaline phosphatase immunostained). Staining for type I collagen was negative (not shown). **B,** Contact zone between the rheumatoid arthritis synovial tissue explant **(A)** and the cartilaginous matrix of a 3-D chondrocyte culture **(B),** showing the tongue-like protrusion of invading synovial cells after 14 days in culture **(arrowheads)** (hematoxylin stained).

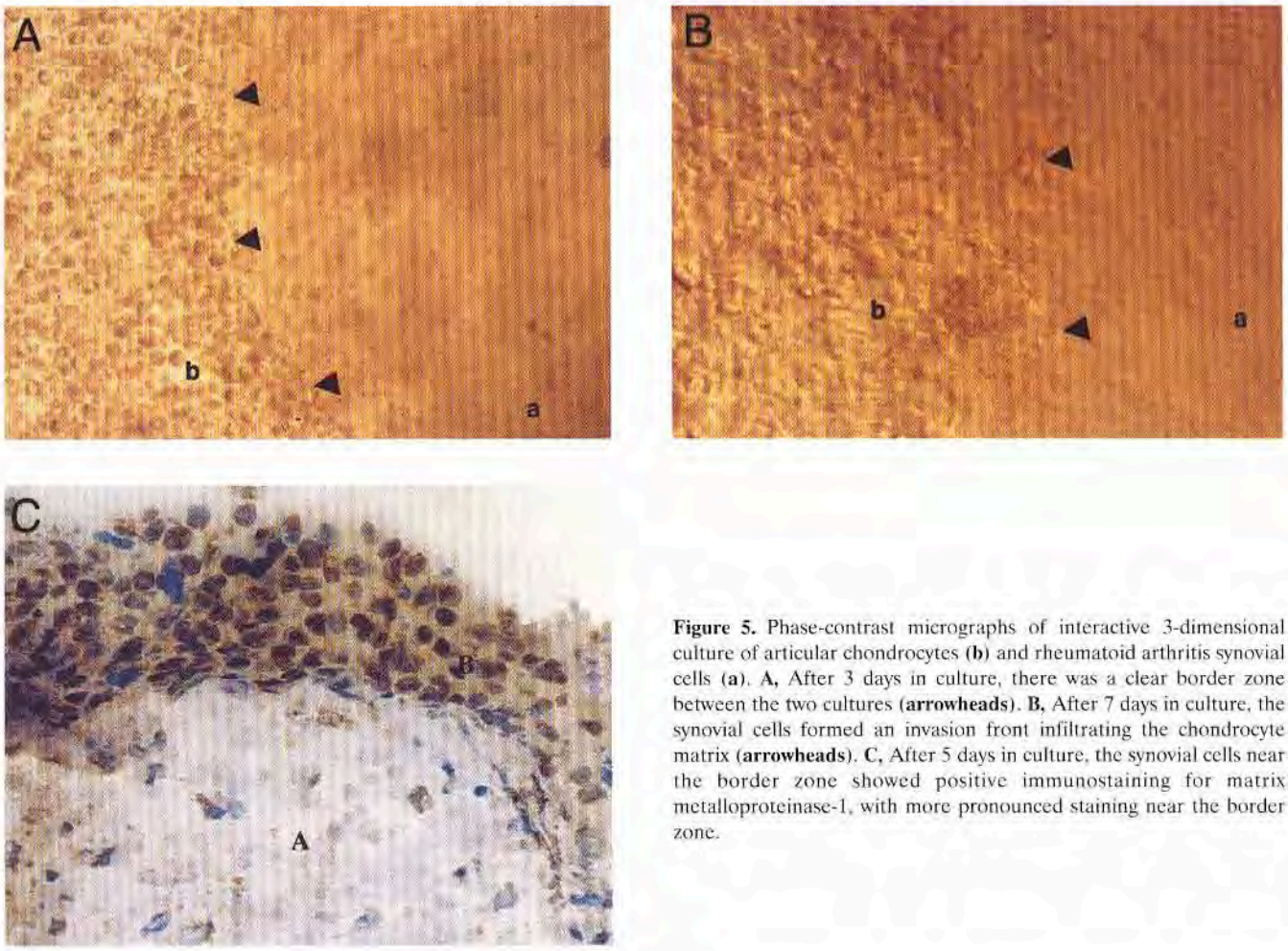

Figure 5. Phase-contrast micrographs of interactive 3-dimensional culture of articular chondrocytes **(b)** and rheumatoid arthritis synovial cells **(a)**. **A,** After 3 days in culture, there was a clear border zone between the two cultures **(arrowheads)**. **B,** After 7 days in culture, the synovial cells formed an invasion front infiltrating the chondrocyte matrix **(arrowheads)**. **C,** After 5 days in culture, the synovial cells near the border zone showed positive immunostaining for matrix metalloproteinase-1, with more pronounced staining near the border zone.

(Figure 5A). After 4–6 days, synovial cells at the junction area started to invade the cartilaginous matrix (Figure 5B). After 21 days of culture, no further matrix invasion took place. No significant difference in the dynamics of invasion was observed between primary cultures (n = 10) and synovial fibroblasts (n = 10). In contrast, neither 3-D cultures of primary synovial cells (n = 5) nor of synovial fibroblasts (n = 4) obtained from noninflammatory synovial tissue showed an invasive pattern. A pronounced expression of HLA–DR and CD45/44, and only a weak staining signal for the adhesion molecule VCAM-1 and the proliferation marker PCNA was seen, without regional preference. The proteolytic activity of synoviocytes was verified with monoclonal antibodies to the interstitial collagenase MMP-1 on both synovial cell types, with a more pronounced staining near the border zone (Figure 5C).

A summary of the data of the culture experiments, focusing on the degree of invasion of synovial cells into the cartilaginous matrix in the different experimental settings, is shown in Table 2.

DISCUSSION

The purpose of our model of inflammatory pannus was the in vitro simulation of the erosive process of inflammatory joint diseases and examination of the functional role of distinct synovial cell types and chondrocytes under different experimental conditions. Addressing the essential contribution of cell–cell and cell–matrix interactions, we searched for a model capable of reflecting the complexity of the joint environment. Two technical approaches were chosen: 1) a combined tissue explant culture with synovial membrane and cartilage; and 2) a novel 3-D chondrocyte culture cultivated with

Table 2. Degree of invasion of synovial cells into the cartilaginous matrix, by experimental condition*

Tissues/cells		Culture system	Degree of invasion			Mean days in culture (max./min.)
			None	Moderate	Marked	
1. RA synovial tissue/cartilage explant	(a)	Conventional culture (n = 15)	7	5	3	30 (50/15)
	(b)	Perfusion culture (n = 13)	4	3	6	72 (85/24)
Noninflammatory synovial tissue/ cartilage explant	(a)	Conventional culture (n = 4)	4	0	0	28 (32/24)
	(b)	Perfusion culture (n = 4)	3	1	0	72 (85/25)
2. RA synovial tissue/3-D chondrocyte culture	(a)	Conventional culture (n = 17)	3	5	9	36 (55/21)
Noninflammatory synovial tissue/ 3-D chondrocyte culture	(a)	Conventional culture (n = 8)	7	1	0	33 (37/28)
3-D interactive RA synovial cell/ chondrocyte culture	1.	Primary (conventional) culture (n = 10)	1	2	7	35 (50/18)
	2.	Fibroblast (conventional) culture (n = 10)	1	4	5	38 (56/20)
3-D interactive noninflammatory synovial cell/chondrocyte culture	1.	Primary (conventional) culture (n = 5)	4	1	0	31 (34/29)
	2.	Fibroblast (conventional) culture (n = 4)	4	0	0	38 (45/28)

* Marked invasion was defined as synovial protrusions of >5 cell layers; moderate invasion as protrusions of 2–5 cell layers. RA = rheumatoid arthritis; 3-D = 3-dimensional.

either a synovial membrane explant or an interactive 3-D gel culture consisting of synovial cells.

RA synovial membrane preserves its characteristic features and destructive potential when implanted into SCID mice (22). We were able to cultivate synovial membrane explants under conventional culture conditions for an average period of 4 weeks and to retain the structural integrity of the tissue without a detectable degree of necrosis. However, by employing a novel perfusion system which stabilized essential culture conditions such as pH and glucose concentration, we were able to extend this period for up to 10 weeks. Under these conditions, it was possible to reproduce the process of cartilage destruction in vitro by cocultivating inflammatory synovial tissue and cartilage.

Fibroblast-like cells as well as macrophages attached to and invaded the cartilage surface while secreting proteolytic enzymes such as MMP-1. The invasion of synovial cells into the cartilage matrix was paralleled by the degradation of cartilage-specific products, as demonstrated by immunohistochemistry for the type II collagen degradation product. With this antibody, a very weak, diffuse staining was also seen in the control samples. This staining, however, was markedly less pronounced compared with the coculture samples. It cannot be ruled out that a certain degree of degradation of the entire matrix occurred due to soluble proteinases secreted by synovial cells. After 4 weeks of culture, only scattered T cells were detected. This is consistent with the in vivo data presented by Rendt et al and Geiler et al (20,22).

Although our explant culture system does not address all aspects of RA, such as the influence of the circulation as well as the regional and systemic immune system, we are confident that it closely reflects the processes at the end of the inflammatory cascade which lead to cartilage and bone destruction. In order to disassemble the complex pathogenetic process of RA, we established a second model system that has the potential to separately study isolated cell populations. To achieve this goal, an "artificial cartilage" was first established: a 3-D culture of chondrocytes synthesizing their own cartilage-specific matrix (27,37,41). The advantage of the 3-D gels used is their flexibility and malleability, allowing us to rearrange the cell architecture in a 3-D orientation. This construct of relaxed cytoarchitecture favors differentiation and functional behavior (42–44). The gel guaranteed a homogenous diffusion of nutrients and retarded the release of matrix-bound factors. In contrast to the widely used monolayer cultures, chondrocytes in our 3-D system kept their specific morphology and started to produce cartilaginous matrix.

In a second step, investigations were performed to analyze whether synovial tissue explants were capable of invading these 3-D chondrocyte cultures. As seen in

Figure 4B, functionally active synovial cells started to invade the chondrocyte matrix after 2 weeks of culture. The invasion process was more intense compared with that of the cartilage explants. This may be explained by the fact that the chondrocyte matrix, although consisting of essentially the same components as native cartilage, was possibly not as dense. We believe this feature is rather advantageous, since it allows us to observe an invasion after only several weeks, compared with the conditions in the SCID mouse model, where up to 6 months are necessary to verify an invasion of the cartilage (22). It is not clear why the invasion slowed down after 21 days in the interactive RA synovial cell/chondrocyte culture. It can be speculated, however, that the contribution of other cells such as lymphocytes or endothelial cells is needed to maintain the invasion process (e.g., by the secretion of cytokines). However, this phenomenon also offers the chance to investigate the potential role of other cells or their respective products in the invasional process.

The reticular network of fibrin (45,46) provided the cells with a temporary matrix over a period of several weeks, and the optical transparency of the culture allowed continuous monitoring. The use of fibrin as a carrier for our cell cultures appears appropriate, since fibrin exudates are a prominent feature of rheumatoid inflammation (47,48).

In addition to the demonstration of an invasive process, our data demonstrate the expression of MMP-1 and the adhesion molecule VCAM-1. These factors have previously been shown to be predominantly expressed in RA synovium and to be of pathogenetic relevance (49–51). Our model systems offer several advantages compared with the currently established methods. Both of our approaches allow the investigation of human cells or tissues free of an animal carrier. Although our main focus is on interactive culture systems with autologous tissues, the use of allogeneic and xenogeneic chondrocytes is apparently feasible as well. However, this will have to be determined by more extended experiments.

Each of the in vitro systems developed has its specific advantages. Whereas the explant cultures can be established more readily and allow the investigation of whole human tissues, one can separately analyze the contribution of distinct cell populations to the inflammatory cascade in the interactive 3-D cultures. Our novel approach offers the opportunity to supplement the data obtained with animal systems and to study the effects of new therapeutic strategies in an experimental setting reflecting important features of human pathology.

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